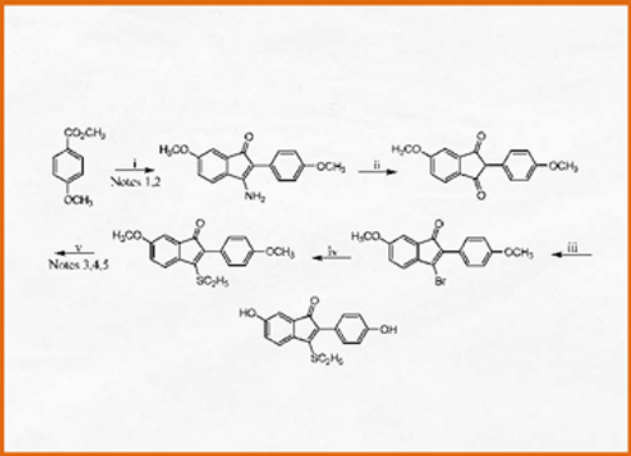
Significant Pharmaceuticals Reported in US Patents

Thomas F. DeRosa



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Dedicated to the Loving Memory of

William A. Schuster
May 31, 1920–May 3, 2006

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Introduction

The objective of this review has been to identify and communicate to academic, government, and industrial researchers the Next Generation Pharmaceuticals reported in 2005–2006 US Patents.

This text also provides explicit laboratory methods for preparing these Next Generation Pharmaceuticals as well as biological testing protocols used to evaluate them. The significance of the current treatment agent over previous methods is also highlighted.

The text format has been designed to be used as both a reference and a synthetic guide for medicinal and organic chemists and graduate students. However, the text is not restricted to medicinal chemistry. In many instances and with few modifications, intermediate(s) or the Next Generation Pharmaceutical itself are readily convertible into other industrial agents including anti-oxidants, chemical additives, herbicides, polymer precursors, and water purification agents. To underscore this point, structural depictions of reagents and chemical transformations have been supplied to permit the chemist to visually identify other/future applications.

Treatment using Next Generation Pharmaceuticals was limited to the following 27 human pathologies:

- Acquired Immune Deficiency Syndrome
- Addiction Disorders
- Alzheimer's Disease
- Analgesics
- Antibacterial Agents
- Anti-inflammatory Agents
- Autoimmune Disorders
- Cardiovascular Disorders
- Diabetes
- Diagnostics
- Epilepsy
- Gastrointestional Disorders

- Hepatitis C
- Hormonal Disorders
- Immunosuppressants
- Improved Synthetic Methods
- Incontinence
- Irritable Bowel Syndrome
- Osteoporosis
- Malaria
- Migraine Headaches
- Obesity
- Proliferative Disorders
- Skin Disorders
- Sleep Disorders
- Thyroid Disorders
- Tinnitus

Next Generation Pharmaceuticals were included in this review only if at least two of the four criteria were met including:

1. Effectiveness
2. Innovative
3. Ease of Preparation
4. Synergy with Existing Medications

'Compounds Used to Treat Alcoholism' by B. Lojo *et al*., US Patent 7,026,514 certainly warranted consideration:

1. The data provide overwhelmingly support for the effectiveness of using *N*,*N*′-bis(3-aminopropyl)-cyclohexane-1,4-diamine tetramethanesulfonate monohydrate as an alcohol suppressant.
2. The literature does not report agents remotely similar or effective.
3. The chemical agent is readily synthesized in batch quantities.

Finally, I thoroughly enjoyed compiling this review and trust the Reader will find it helpful.

Thomas F. DeRosa
October, 2006

CHAPTER I

Acquired Immune Deficiency Syndrome

I. Antioxidants, Preparation Methods, and Uses

Title Antioxidants, Preparation Methods and Uses
J. Oiry *et al.*, US Patent 6,989,372 (January 24, 2006)

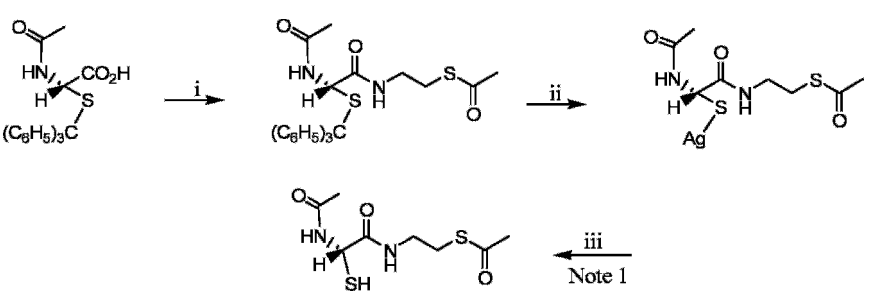
Assignee Centre National de la Recherche Scientifique *and* Commissariat a l'Engergie Atomique

Utility Treatment of Pathologies Associated with Glutathione Depletion

Invention Significance Reactive oxygen species play an important role in human pathologies and are particularly associated with retroviral infections such as human immunodeficiency virus (HIV). Intracellular defense against oxidative species is controlled by glutathione. To contain these reactive oxygen species effects, free radical scavengers have been prepared that increase glutathione reserves.

Reaction

Eq. 1



i- *N*-Methylmorpholine, isobutyl chloroformate, EtOAc, S-acetylcysteamine·HCl
ii- Methyl alcohol, $CHCl_3$, silver nitrate, pyridine
iii- $CHCl_3$, hydrochloric acid

Experimental

1. **Preparation of *N*-(*N*-acetyl-S-trityl-L-cysteinyl)-S-acetylcysteamine**

A solution of *N*-acetyl-S-trityl-L-cysteine (0.71 mmol) and 80 µl *N*-methylmorpholine dissolved in 5 ml of EtOAc was stirred at −15°C, then treated with 93 µl isobutyl chloroformate. After 15 minutes, S-acetylcysteamine hydrochloride (0.71 mmol) and an additional 80 µl *N*-methyl-morpholine were added and the mixture stirred for 15 minutes at −15°C and then 3 hours at ambient temperature. *N*-Methylmorpholine hydrochloride was then filtered off and the mixture was washed twice with 2.5 ml of EtOAc and concentrated. The gummy residue was purified by flash chromatography with silica gel using EtOAc/30% petroleum ether and the product isolated in 55% yield as a colorless powder, mp = 111–113°C.

$\boldsymbol{R_f} = 0.41$, EtOAc/petroleum ether, 9:1
$[\alpha]_D^{20} = +10.5°$ (c. 0.8, $CHCl_3$
^{1}H NMR ($CDCl_3$) δ (ppm) 1.90 (s, 3H, $NCOCH_3$), 2.29 (s, 3H, $SCOCH_3$), 2.48 (dd, $J = 5.7$, 12.9 Hz, 1H, β Ha cys), 2.82 (dd, $J = 6.4$, 12.9 Hz, 1H, β Hb cys), 2.92–3.01 (m, 2H, NCH_2CH_2S), 3.32–3.42 (m, 2H, NCH_2CH_2S), 4.07–4.20 (m, 1H, α H cys), 5.70 (d, $J = 7.6$ Hz, 1H, NH cys), 6.34 (t, $J = 5.5$ Hz, 1H, $NHCH_2$), 7.19–7.35 and 7.40–7.47 (2m, 15H, aromatic H)
MS (FAB+/NBA+K+) *m/z* 545 $(M + K)^+$, 507 $(M + H)^+$; (FAB−/NRA) *m/z* 505 $(M-H)^-$

Analysis Calc. for $C_{28}H_{30}N_2O_3S_2$ (506): C, 66.40; H, 5.93; N, 5.53. Found: C, 66.17; H, 6.00; N, 5.81

2. **Preparation of *N*-(*N*-acetyl-L-cysteinyl)-S-acetylcysteamine, silver**

While protected from light, a saturated solution of the Step 1 product (2.49 mmol) in 20 ml methyl alcohol and 1.5 ml of $CHCl_3$ stirred at ambient temperature was treated with a light-protected mixture of silver nitrate (2.64 mmol) and 213 µl pyridine in 13 ml methyl alcohol. When a precipitate was formed, stirring was stopped and mixture left overnight at ambient temperature. The solid was isolated, then washed twice with 10 ml apiece methyl alcohol and $CHCl_3$, and the product isolated.

Analysis Calc. for $C_9H_{15}N_2O_3S_2Ag$ (371): Ag, 29.11. Found: Ag, 29.16

3. **Preparation of *N*-(*N*-acetyl-L-cysteinyl)-S-acetylcysteamine**

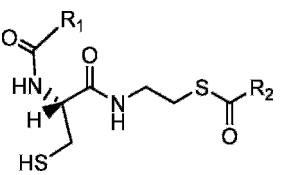
While protected from light, a stirred suspension of the Step 2 product in 15 ml $CHCl_3$ at ambient temperature was treated with 400 µl 12 M HCl, then stirred 2 hours

at ambient temperature and 2 minutes at 30–35°C. The mixture was diluted with 70 ml $CHCl_3$, then filtered to remove AgCl, and the combined organic phase washed three times with 10 ml ice-cold water. The solution was then dried with Na_2SO_4 and concentrated. A semicrystalline paste was collected, which was recrystallized using EtOAc/petroleum ether, and the product isolated in 56% yield as colorless microcrystals, mp = 121–122°C.

$[\alpha]_D^{20} = -39.1°$ (c. 0.9, $CHCl_3$).

Derivatives

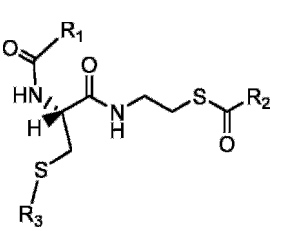
Table 1 Selected Step 3 cysteamine derivatives and their associated mass spectral data



Entry	R_1	R_2	MS $(M+H)^+$
I-152	CH_3	CH_3	247
I-188	CH_3	$CH(CH_3)_2$	293
I-198	CH_3	C_6H_5	327
I-203	$CH(CH_3)_2$	C_6H_5	293
I-208	$CH(CH_3)_2$	$CH(CH_3)_2$	321
I-219	$CH(CH_3)_2$	C_6H_5	355

[1]H MS data for products and intermediates supplied by author.

Table 2 Selected S-acetated cysteamine derivatives and associated physical properties



Entry	R_1	R_2	R_3	MS $(M+H)^+$
I-189	CH_3	$CH(CH_3)_2$	$COCH_3$	335
I-197	CH_3	$C(CH_3)_3$	COC_6H_5	411
I-204	$CH(CH_3)_2$	CH_3	$COCH_3$	335
I-207	$CH(CH_3)_2$	CH_3	COC_6H_5	397
I-210	$CH(CH_3)_2$	$CH(CH_3)_2$	$COCH(CH_3)_2$	391
I-223	$CH(CH_3)_2$	C_6H_5	COC_6H_5	459

^{1}H NMR data for products and intermediates supplied by author.

Testing

I. *Antiviral Effectiveness of I-152 in Spleen Monocytes and Macrophages*

The spleen was dissected, sieved, and mononuclear cells isolated using a density gradient. Monocytes/macrophages were isolated by adherence to plastic and differentiated into macrophages for 7 days. Thereafter, they were infected with HIV-1/Ba-L and evaluated. Testing results are provided in Table 3.

Table 3 Antiviral activity of the preferred experimental agent, I-152, *N*-(*N*-acetyl-L-cysteinyl)-S-acetylcysteamine, in spleen MDMs infected with 10 000 $TCID_{50}$s of the HIV-1/Ba-L isolate at 50, 70 and 90% effective doses

Entry	ED_{50} (μM)	ED_{70} (μM)	ED_{90} (μM)
I-152	38	54	97

II. *Anti-HIV Effectiveness Using Macrophages Derived from Human Blood Monocytes*

Synergistic effects of anti-oxidant agents when used in conjunction with AZT using macrophages derived from human blood monocytes infected by the HIV-1/Ba-L strain were evaluated according to the method of Chou (1). Testing results are provided in Table 4.

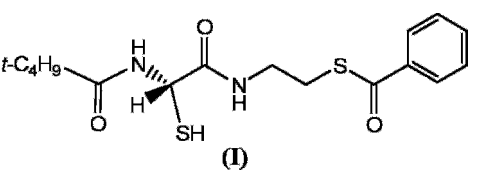
Table 4 Comparison of the antiviral activities of selected derivatives in MDM cultures infected with 10 000 $TCID_{50}$s of the HIV-1/Ba-L isolate at 50, 70, and 90% effective doses

Entry	ED_{50} (μM)	ED_{70} (μM)	ED_{90} (μM)
I-152	43	58	141
I-203	74	93	132
I-204	74	91	127
I-207	43	63	112
I-208	36	50	88
I-209	51	71	118

The preferred agent is I-152, *N*-(*N*-acetyl-L-cysteinyl)-S-acetylcysteamine.

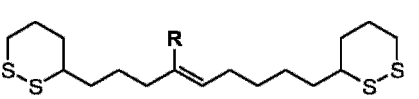
Notes

1. Additional S-acetylcysteamine derivatives, (**I**), were prepared by the author (2) and were effective in increasing the intracellular and/or extracellular glutathione levels.



(I)

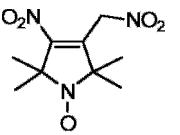
2. The use of glutathione as a free radical scavenger in treating oxidative stress improved fetal and neonatal outcome of preterm birth and is described by Buhimschi (3).
3. Lipolal aldol condensation derivatives, (**II**), prepared by Haj-Yehia (4) were effective as reactive oxygen species scavengers and used in treating conditions associated with oxidative stress or free radical injury including mitochondrial cytopathies and HIV infection.



R = CO_2H, $CONH_2$, CH_2OH, CH_2NH_2

(II)

4. Piperidinyl and pyrrolidinyl nitric oxide derivatives, (**III**), prepared by Anggard(5) were capable of acting both as nitric oxide donors and as scavengers of superoxide ions and used in the treatment of conditions associated with oxidative stress such as neurological disorders, drug- and disease-induced nephropathies, and reproductive disorders.



(III)

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1. H. Zee-cheng *et al.*, J. Med. Chem., 13, 414 (1970)
2. J. Oiry *et al.*, US Patent 6,979,747 (December 27, 2005)
3. I.A. Buhimschi *et al.*, US Patent 6,852,698 (February 8, 2005)
4. A. Haj-Yehia, US Patent 6,900,338 (May 31, 2005)
5. E.E. Anggard *et al.*, US Patent 6,759,430 (July 6, 2004)

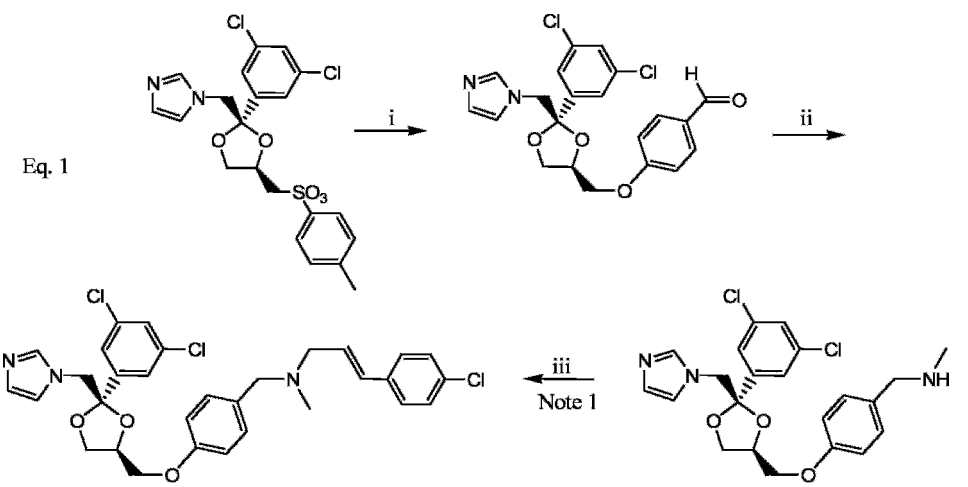
II. Azole-based Fungicides

Title Compounds
D. Babin *et al.*, US Patent 6,946,460 (September 20, 2005)
Assignee Aventis Pharma S.A.
Utility Treatment of Candida or Aspergillus Strains

Invention Significance Antifungal agents effective against Candida or Aspergillus strains have been prepared with improved bioabsorbability. These azole-based fungicides are particularly useful for treating fungal-resistant strains in immunodeficient patients.

Reaction

Eq. 1



i- 4-Hydroxybenzaldehyde, DMF, sodium hydride
ii- Methylamine hydrochloride, sodium cyanoborohydride, methyl alcohol
iii- Tris(3-sulphonatophenyl) phosphine tetrahydrate sodium, palladium(II) acetate, 4-chloro-(*E*)-cinnamyl acetate, acetonitrile

Experimental

1. **Preparation of 4-[*cis*-[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]-benzaldehyde**

   A solution of 4-hydroxybenzaldehyde (0.0376 mol) dissolved in 80 ml DMF was added to a suspension of sodium hydride (1.723 g) in 150 ml DMF, then stirred 30

minutes at ambient temperature. The mixture was then treated with (*cis*-[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl)methyl] 4-methyl-benzenesulphonate (20 g), then heated overnight at 90°C, and then poured into 500 ml water. It was extracted four times with 300 ml CH_2Cl_2 and the combined extract washed with brine, dried with $MgSO_4$, and then concentrated. The residue was dissolved in 40 ml diethyl ether, whereupon crystallization occurred. The crystals were washed four times with 20 ml diethyl ether and 20.32 g product isolated.

$R_f = 0.18$ CH_2Cl_2/MeOH, 98:2
^{1}H NMR (300 MHz, $CDCl_3$) 3.35 (dd) and 3.77 (dd) 2H, 3.80 (dd) and 3.91 (dd) 2H: O−C$\underline{H}_2$−CH−C$\underline{H}_2$−O; 4.41 (m, 1H O−CH_2−C$\underline{H}$−CH_2−O); 4.45 and 4.55 (AB, 2N, N−CH_2−Cq); 6.42 and 7.85 (AA′BB′) 9.90 (s, 1H, C$\underline{H}$O); 7.03 (dl, 2H, H_4 and H_5); 7.29 (dd, 1H, Hb); 7.49 (d, 1H, Ha); 7.61 (d, 1H, Hc); 7.70 (s, 1H, H2)

2. **Preparation of 4-[*cis*-[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]-*N*-methyl-benzenemethanamine**

Methylamine hydrochloride (0.131 mol) and $NaBH_3CN$ (0.026 mol) were added to a solution of the Step 1 product (0.0131 mol) dissolved in 170 ml methyl alcohol and the mixture stirred 19 hours at ambient temperature. The mixture was then concentrated and the residue dissolved in water/CH_2Cl_2/2 M NaOH, 50 ml:20 ml:70 ml, respectively. The aqueous phase was further extracted with 70 ml CH_2Cl_2 and combined extracts were washed with brine, dried, reconcentrated, and 6.1 g crude product obtained. This material was purified by chromatography with silica using CH_2Cl_2/methyl alcohol, 95:5, and then CH_2Cl_2/methyl alcohol, 87:13, containing 1% of TEA and 4.2 g product isolated.

$R_f = 0.46$ CH_2Cl_2/MeOH, 90:10
^{1}H NMR (300 MHz, $CDCl_3$) 2.44 (s, 3H, N−C$\underline{H}_3$); 3.33 (dd) and 3.76 (dd) 2H; 3.73 (dd) and 3.88 (dd) 2H: O−C$\underline{H}_2$−CH−C$\underline{H}_2$−O; 4.35 (m, 1H, O−CH_2−C$\underline{H}$−CH_2−O); 3.68 (s, 2H, N−C$\underline{H}_2$−Cq); 4.45 (AB, 2H); 6.78 and 7.23 (AA′BB′); 7.46 (d, 1H, Ha); 7.26 (dd, 1H, Hb); 7.58 (d, 1H, Hc); 7.50 (s, 1H, H2); 6.98 (d, 2H, H4 and H5)

3. **Preparation of *cis*-4-[[2-(2,4-dichlorophenyl)-2-[(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]-*N*-(3-phenyl-2(*E*)-propenyl)]-4-chloro-benzenemethanamine**

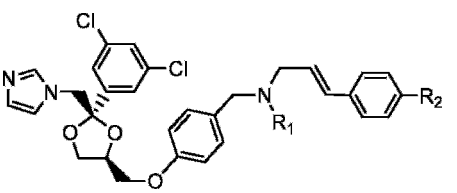
Tris(3-sulphonatophenyl) phosphine tetrahydrate sodium salt (42 mg) and palladium(II) acetate (18 mg) were mixed in 2 ml water for 1 hour at ambient temperature, then treated with the Step 2 product (0.7 mmol), and 4-chloro-(*E*)-cinnamyl acetate (0.5 mmol) dissolved in 2 ml acetonitrile. The mixture was stirred 90 minutes at 50°C and was then cooled to ambient temperature and diluted with water. It was then extracted several times with CH_2Cl_2 and extracts dried and concentrated. The residue was purified by chromatography using CH_2Cl_2/methyl alcohol, 90:10, and 154 mg product isolated.

$R_f = 0.15$ CH_2Cl_2/MeOH, 9:1

^{1}H NMR (300 MHz, $CDCl_3$) 2.32 (bs, 3H, $C\underline{H}_3-N$); 3.30 (m, 2H, $N-C\underline{H}_2-CH=CH-$); 6.32 (dt, **1**H, $J = 16$, 7 Hz, $N-CH_2-C\underline{H}=CH-$); 6.52 (d, 1H, $J = 16$ Hz, $N-CH_2-CH=C\underline{H}-$); 3.30 (m) and 3.75 (m) 2H, 3.75 (m) and 3.89 (dd) 2H: $O-C\underline{H}_2-CH-CH_2-O$; 4.36 (m, 1H, $O-CH_2-C\underline{H}-CH_2-O$); 3.64 (bs, 2H, $Cq-C\underline{H}_2-N$); 6.81 and 7.31 (AA′ BB′, Ph−O); 7.47 (d, 1H, Ha); 7.25 (masked, 1H, Hb); 7.60 (d, 1H, Hc); 7.31 (AA′BB′, Ph); 7.53 (bs, H, H2); 6.92 (bd, 2H, H4, and H5)

Derivatives

Table 1 Selected azole-based fungicides and corresponding retention factors in solvent mixtures. ^{1}H NMR data supplied by author. All derivatives were biologically active against *Candida albicans*



Entry	R_1	R_2	R_f
1	CH_3	H	0.40 (CH_2Cl_2/CH_3OH, 9:1)
4	H	H	0.20 ($CH_2Cl_2/CH_3OH + 1\%$ H_2O, 9:1)
5	CH_3	OPO_3H	0.24 (CH_3CN/H_2O (containing 0.03% TFA), 6:4)
7	CH_3	OCH_2CH_2OH	0.30 (CH_2Cl_2/H_2O, 93:7)
8	$CH_2CH_2NH_2$	H	0.68 (CH_3CH/H_2O (containing 0.03% TFA), 6:4)
9	$CH_2CH_2NH_2$	Cl	0.09 (CH_2Cl_2/CH_3OH, 85:15)

Testing

I. *Minimum Inhibitory Concentration Assay*

Candida albicans cells were prepared and used immediately to determine the minimum inhibitory concentration (MIC). RPMI-1640 was used as medium with L-glutamine buffered to pH 7 with 0.15 M 3-(*N*-morpholino)propane sulphonic acid solution. *Candida albicans* cells, 1.5×10^3 cells/ml, were added to the wells of a 96-well plate containing RPMI-1640 and treated with selected experimental

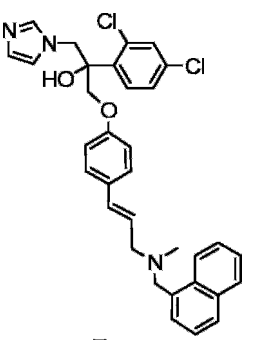
agents. Testing results were read 48 hours after incubation at 35°C and the MIC determined.

Results

All experimental agents provided in Table 1 demonstrated activity at $<100\,\mu g/ml$ in the MIC test.

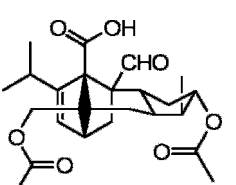
Notes

1. Additional azole derivatives, (**I**), effective against *C. albicans* and related fungal disorders were prepared by the author (1) and used as fungicides for treating resistant strains in immunodeficient patients.



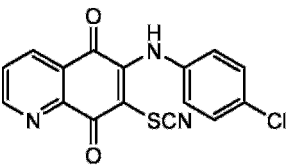
(I)

2. Sordarin derivatives, (**II**), prepared by Balkovec (2) were effective in treating fungal infections caused by *C. albicans* and used in treating infections in immunocompromised patients.



(II)

3. 5,8-Quinolinediones derivatives, (**III**), and lupeol, (**IV**), prepared by Ryu (3) and Gibson (4), respectively, were effective in treating opportunists infections caused by *Candida albican*s pathogens.



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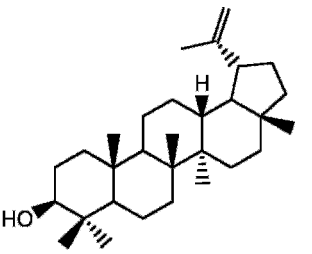

(IV)

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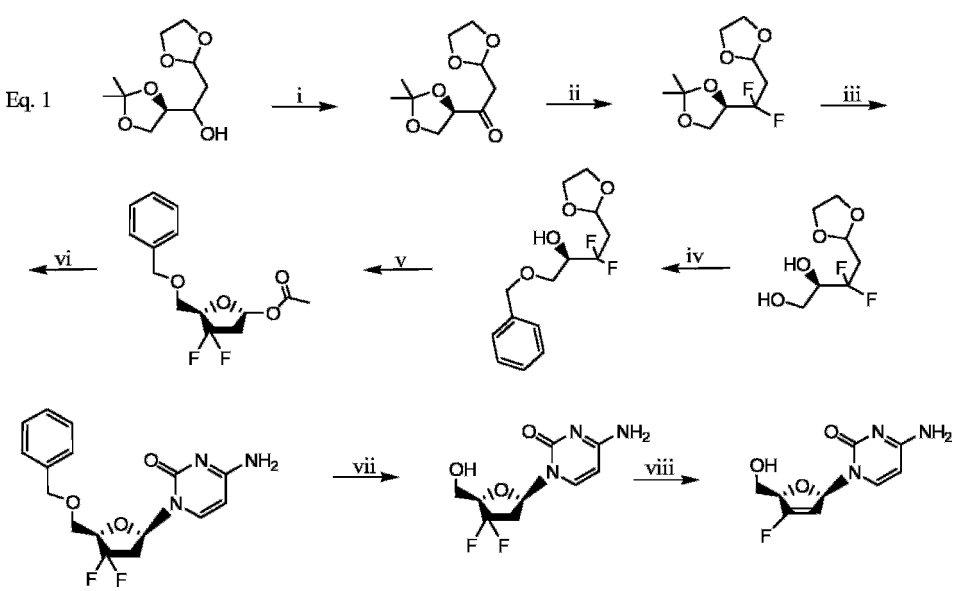
1. D. Babin *et al.*, US Patent 6,960,600 (November 1, 2005) and US Patent 6,900,161 (May 31, 2005)
2. J.M. Balkovec *et al.*, US Patent 6,864,278 (May 8, 2005)
3. C.K. Ryu, US Patent 6,818,653 (November 16, 2004)
4. D.J. Gibson *et al.*, US Patent 6,951,847 (October 4, 2005)

III. Human Immunodeficiency Virus Replication Inhibitor

Title β-2′ or 3′-Halonucleosides
M.J. Otto *et al.*, US Patent 6,949,522 (September 27, 2005)
Assignee Pharmasset, Inc, The University of Georgia Research Foundation, Inc. *and* Emory University
Utility Method of Treatment for HIV

Invention Significance Although synthetic nucleosides effective in the treatment of the human immunodeficiency virus exist, their use is associated with a variety of side effects including headaches, fever, fatigue, loss of appetite, and nausea. Medicaments consisting of β-2′- or β-L-3′-halonucleosides have been prepared which are effective in inhibiting HIV while having only marginal side effects.

Reaction



i- CH_2Cl_2, oxalyl chloride
ii- Diethylaminosulfur trifluoride, CH_2Cl_2
iii- Dioxane, hydrochloric acid
iv- Pyridine, benzoyl chloride
v- Methyl alcohol, diethyl ether, hydrochloric acid

vi- N^4-Benzoylcytosine, ammonium sulfate,
1,1,1,3,3,3-hexamethyldisilazane, acetonitrile,
trimethylsilyl trifluoromethanesulfonate
vii- Ammonium hydroxide, methyl alcohol
viii- Sodium methoxide, DMF, Dowex 50 WX8 (H^+) resin

Experimental

1. **Preparation of 1-[(*S*)-2,2-dimethyl-(1,3)-dioxolan-4-yl]-4-(1,3-dioxolan-2-yl)-2-one**

   Oxalyl chloride (5.25 ml) dissolved in 8.58 ml DMSO and CH_2Cl_2 was cooled to −78°C, then treated with 1-[(*S*)-2,2-dimethyl-(1,3)-dioxolan-4-yl]-4-(1,3-dioxolan-2-yl)-2-ol (54.9 mmol) followed by 38.2 ml triethylamine after 15 minutes. The solution was warmed, concentrated, the residue purified by flash chromatography with silica gel using 20% EtOAc/hexanes, and the product isolated in 95% yield as yellow oil.

   ^{1}H NMR ($CDCl_3$) δ 1.354 (S, 3H), 1.453 (S, 3H), 2.953 (m, 2H), 3.83 (m, 2H), 3.95 (m, 2H), 4.027 (m, 1H), 4.154 (m, 1H), 4.417 (m, 1H), 5.268 (m, 1H)
   MS (FAB) *m*/*z* 216 (M^+)

2. **Preparation of 1-[(*S*)-2,2-dimethyl-(1,3)-dioxolan-4-yl]-2-difluoro-4-(1,3-dioxolan-2-yl)-pentane**

   The Step 1 product (39.4 mmol) dissolved in CH_2Cl_2 was treated with 15 ml diethylaminosulfur trifluoride, dissolved in CH_2Cl_2 at 0°C, and then stirred 24 hours at ambient temperature. The mixture was poured into saturated $NaHCO_3$ solution at 0°C, then washed, dried, and concentrated. The residue was purified by flash chromatography using 10% EtOAc/hexanes and the product isolated in 53% yield as yellow oil.

   ^{1}H NMR ($CDCl_3$) δ 1.340 (s, 3H), 1.424 (s, 3H), 2.23–2.41 (m, 2H), 3.85 (m, 2H), 3.97 (m, 2H), 4.09 (m, 2H), 4.24–4.33 (m, 1H), 5.121 (t, 1H)
   MS (FAB) *m*/*z* 238 (M^+)

3. **Preparation of 3-difluoro-5-(1,3-dioxolan-2-yl)-2S-1,2-diol**

   The Step 2 product (20.2 mmol) dissolved in 1,4-dioxane at 0°C was treated with 2.5% aqueous HCl, then stirred overnight at ambient temperature, and neutralized with $NaHCO_3$ solution. The mixture was washed, dried, and then concentrated. The residue was purified by flash chromatography using 60% EtOAc/hexanes and the product isolated in 90% yield as colorless oil.

   ^{1}H NMR ($CDCl_3$) δ 2.30–2.52 (m, 2H), 3.577 (d, $J = 5.88$ Hz, D_2O exchangeable, 1H), 3.83–4.10 (m, 7H), 5.090 (q, 1H)
   MS (FAB) *m*/*z* 198 (M^+)

4. **Preparation of 1-benzoxy-3-difluoro-5-(1,3-dioxolan-2-yl)-2S-ol**

A solution of the Step 3 product (3.1 g) in 5 ml pyridine was treated with 1.82 ml benzoyl chloride at 0°C, then stirred 60 minutes at ambient temperature, and concentrated. The residue was dissolved in CH_2Cl_2, then washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$, and reconcentrated. The residue was purified by flash chromatography using 30% EtOAc/hexanes and the product isolated in 85% yield as colorless oil.

^{1}H NMR ($CDCl_3$) δ 2.43 (m, 2H), 3.84 (m, 2H), 3.95 (m, 2H), 4.03 (m, 1H), 4.19 (m, 1H), 4.48 (m, 1H), 4.59 (m, 1H), 5.08 (m, 1H), 7.38 (m, 2H), 7.51 (m, 1H), 8.01 (m, 1H)
MS (FAB) *m*/*z* 302 (M^+)

5. **Preparation of 1-acetyl-5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-D-ribofuranose**

A solution of the Step 4 product (5.3 mmol) in 15 ml methyl alcohol was treated with 15 ml 1 M HCl in diethyl ether, then refluxed 60 minutes, and neutralized with $NaHCO_3$. The mixture was then filtered, washed, dried, and concentrated. The residue was then treated with 40 μL 18 M ice-cold sulfuric acid, 3 ml acetic anhydride, and 15 ml acetic acid and stirred 30 minutes at ambient temperature. It was then poured into ice-cold 100 ml saturated $NaHCO_3$ solution and extracted three times with 100 ml CH_2Cl_2, then washed, and concentrated. The residue was purified by flash chromatography using 10% EtOAc/hexanes and the product isolated as 2:1 anomeric mixture in 95% yield as yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02, 2.10 (s, 3H), 2.53–2.65 (m, 1H), 2.74–2.88 (m, 1H), 4.46–4.66 (m, 3H), 6.442, 6.399 (d, $J = 5.85, 5.76$ Hz, 1H), 7.38–7.51 (m, 2H), 7.57 (m, 1H), 7.98–8.12 (m, 2H)
MS (FAB) *m*/*z* 301 (MH^+)

6. **Preparation of N^4-Benzoyl-1-(5-*O*-benzoyl-2,3-dideoxy-3,3-difluoro-β-L-ribofuranosyl) cytosine**

A mixture of N^4-benzoylcytosine (4.20 mmol) and ammonium sulfate (0.212 mmol) dissolved in 30 ml 1,1,1,3,3,3-hexamethyldisilazane was refluxed 4 hours, then concentrated, and treated with the Step 5 product (2.1 mmol) dissolved in 15 ml acetonitrile. After cooling the mixture to 0°C, it was treated with 0.60 ml trimethylsilyl trifluoromethanesulfonate, then stirred 3 hours at ambient temperature. It was then poured into 25 ml $NaHCO_3$ solution at 0°C, the organic phase was washed with 5 ml brine, dried with $MgSO_4$, and concentrated. The residue was purified by flash chromatography using 50% EtOAc/hexanes and the product isolated in 36% yield as a white solid, mp = 166 – 167°C (dec).

$[\alpha]_D^{24} = 80.50°$ (c. 0.50, $CHCl_3$)
UV (MeOH) λ_{max} 259.5, 301.0

^{1}H NMR ($CDCl_3$) δ 2.57 (m, J = 15.6, 10.5, 5.3 Hz, 1H, $H_{2'\beta}$), 3.26 (m, J = 15.6, 12.8, 7.0 Hz, 1H, $H_{2'\alpha}$), 4.57 (ddd, J = 18.0, 9.7, 4.8 Hz 1H, $H_{4'}$), 4.71 (dd, J=12.5, 5.5 Hz 1H, $H_{5'b}$), 4.75 (dd, J = 12.5, 4.3 Hz, 1H, $H_{5'a}$), 6.30 (t, J = 6.1 Hz, 1H, $H_{1'}$), 7.65–7.44 (m, 7H), 8.10–7.88 (m, 5H), 8.81 (bs, 1H)
HRMS (FAB) Obs., m/z 456.137 7; calc. for $C_{23}H_{20}F_2N_3O_5$, m/z 456.137 1 (MH^+)
Analysis Calc. for $C_{23}H_{19}F_2N_3O_5$: C, 60.66; H, 4.21; N, 9.23. Found: C, 60.50; H, 4.19; N, 9.00

7. **Preparation of 1-(2,3-dideoxy-3,3-difluoro-β-L-ribofuranosyl)cytosine**

A mixture consisting of the Step 6 product (0.30 mmol) in 10 ml saturated ammonia/methyl alcohol solution was stirred 4 hours at ambient temperature and then concentrated. The residue was purified by flash chromatography using 10% methyl alcohol/$CHCl_3$ and the product isolated in 100% yield as a white solid, mp = 194 − 196°C (dec).

$[\alpha]_D^{24} = 45.56°$ (c. 1.0, MeOH)
UV (MeOH) λ_{max} 276.5 (ε 18 160) (pH 2), 268.0 (ε 13 280) (pH 7), 268.5 (ε 13 580) (pH 11)
^{1}H NMR (CD_3OD) δ 2.51 (m, 1H, $H_{2'}$), 2.90 (m, 1H, $H_{2'}$, 3.83 (m, 2H, $H_{5'}$), 4.17 (m, 1H, $H_{4'}$), 5.93 (d, J = 7.3 Hz, 1H, H_5), 6.27 (t, J = 6.8 Hz 1H, $H_{1'}$), 7.97 (d, J = 7.3 Hz, 1H, H_6)
HRMS (FAB) Obs., m/z 248.084 1, calc. for $C_9H_{12}F_2N_3O_3$, m/z 248.084 7 (MH^+)
Analysis Calc. for $C_9H_{11}F_2N_3O_3$•0.1 H_2O: C, 43.41; H, 4.53; N, 16.88. Found: C, 43.45; H, 4.50; N, 16.54

8. **Preparation of 1-(2,3-dideoxy-3-fluoro-β-L-glycero-pent-2-eno-furanosyl) cytosine**

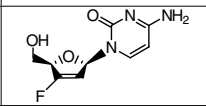
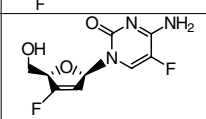
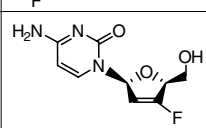
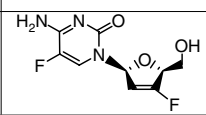
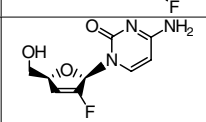
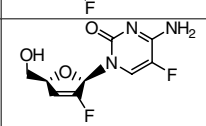
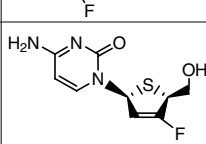
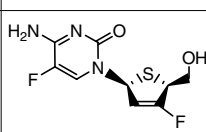
The Step 7 product (0.36 mmol) was treated with sodium methoxide (1.1 mmol) dissolved in 3 ml DMF, then stirred overnight at ambient temperature, and neutralized with Dowex 50 WX8 (H^+) resin. After filtration and concentration, the residue was purified by chromatography with 10% methyl alcohol/ $CHCl_3$ and the product isolated in 60% yield as a white solid, mp = 182−183°C (dec).

$[\alpha]_D^{24} = -5.34°$ (c. 0.39, MeOH)
UV (MeOH) λ_{max} 276.0 (ε 11 990) (pH 2), 267.5 (ε 8010) (pH 7), 264.5 (ε 8060) (pH 11)
^{1}H NMR (CD_3OD) δ (ppm) 3.766, 3.771 (m, 2H, H5′), 4.733 (m, 1H, $H_{4'}$), 5.438 (m, 1H, $H_{2'}$), 5.887 (d, J = 7.5 Hz, 1H, H_5), 6.975 (m, 1H, $H_{1'}$), 8.137 (d, J = 7.5 Hz, 1H, H_6)
HRMS (FAB) Obs., m/z 228.077 6, calc. for $C_9H_{11}FN_3O_3$, m/z 228.078 4 (MH^+)
Analysis Calc. for $C_9H_{10}FN_3O_3$: C, 47.58; H, 4.44; N, 18.50. Found: C, 47.34; H, 4.45; N, 18.27

Derivatives

Selected derivatives are provided in Table 1.

Table 1 Anti-HIV-1 activity of selected experimental nucleosides used in the HIV-1 Q-RT-PCR assay in human PBM cells. All concentrations are expressed in micromolar units. ^{1}H NMR and elemental analysis of experimental agents of selected intermediates supplied by author

Entry	Structure	LAI[a] EC_{90}	xxBRU[a] EC_{90}	184V[a] EC_{90}	FI[a,b]	LAI[c] EC_{90}	xxBRU[c] EC_{90}	184V[c] EC_{90}	FI[b,c]
10		25.3	35.5	572	16.1	50.6	35.4	>100	ns[d]
10-F		34.5	37.5	494	13.2	36.3	34.8	>100	ns[d]
23		0.67	0.14	82.0	586	0.92	0.60	68.4	115
23-F		0.12	0.14	67.0	479	0.09	0.16	53.0	333
D-17		11.9	5.0	125	25.0	24.6	5.2	65.8	12.8
D-18		>100	119	>100	ns[d]	>100	73.9	>100	ns[d]
L-17		3.3	1.4	1.569	1.121	4.1	2.1	>100	>50
L-18		2.6	1.1	>100	>100	4.3	0.94	>100	>100

[a] Q-RT-PCR HIV-1 assay
[b] FI is the fold increase (EC_{90} HIV-1_{184V}/EC_{90} HIV-1_{xxBRU})
[c] Endogenous viral RT assay
[d] Not significant

Testing

I. *HIV-1 Q-RT-PCR Assay in Human PBM Cells*

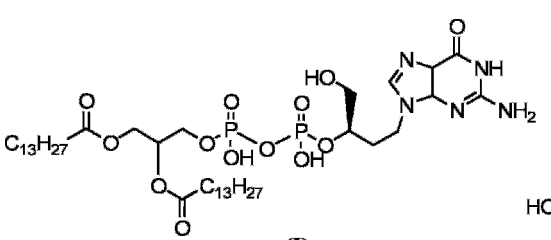
Viral RNA was detected in real time by monitoring increases in fluorescence signal that resulted from degradation of a quenched fluorescent probe molecule following the hybridization of the probe to the amplified viral DNA. TaqMan probe and primers were used covering highly conserved sequences complementary to an 81-base pair long fragment from the HIV-1 RT gene between codons 230 and 257 of the group MHIV-1 genome as indicated below:

i- *Sequence 1* Probe 5′-6FAM-TTTCTGGCAGCACTATAGGCTGTACTGTCCATT-TAMRA-3′
ii- *Sequence 2* Sense primer: 5′-TGGGTTATGAACTCCATCCTGAT-3′
iii- *Sequence 3* Antisense primer 5′-TGTCATTGACAGTCCAGCGTCT-3′

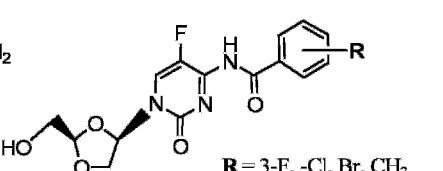
HIV-1 present in culture supernatant fluids was quantified by Q-RT-PCR with readout in log copies per milliliter and the endogenous viral RT assay on cell supernatant with readout in log counts per minute per milliliter. Although the two methodologies measured different parameters, i.e., viral RNA versus active RT enzyme, test results were not markedly different from each other. A summary of these data expressed as effective concentration to reduce the viral RNA or the RT activity by 90%, EC_{90}, for the three HIV-1 viral strains is provided in Table 1.

Notes

1. (*R*)-Peniciclovir phosphates, (**I**), prepared by Vere Hodge (1) and N^4-acylcytosine-1,3-dioxolane nucleoside derivatives, (**II**), prepared by Du (2) and Watanabe (3) were effective in the treatment of both HIV-1 and hepatitis B virus infections in mammals.

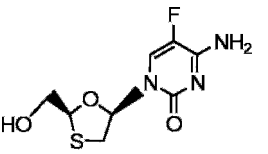


(I)



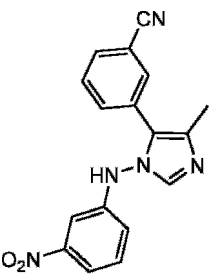
(II) **R** = 3-F, -Cl, Br, CH_3
4-F, -Cl, Br, I, CH_3

2. 1,3-Oxothiolane derivatives, (**III**), prepared by Painter (4) were effective in inhibiting the replication of HIV in vivo or in vitro.



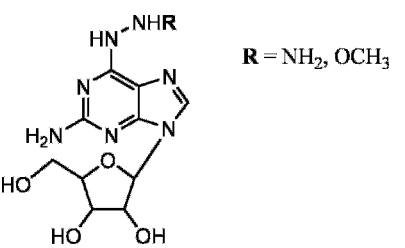
(III)

3. *N*-Aminoimidazole derivatives, (**IV**), prepared by De Clercq (5) were effective as retroviral enzyme inhibitors and used in treating HIV.



(IV)

4. D-Ribofuranosylpurine antiviral derivatives, (**V**), prepared by Loakes (6) were particularly effective as inhibitors of HIV reverse transcriptase-mediated DNA synthesis.



(V)

References

1. R.A. Vere Hodge *et al.*, US Patent 7,045,525 (May 16, 2006)
2. J. Du *et al.*, US Patent 6,855,821 (February 15, 2005)
3. K.A. Watanabe *et al.*, US Patent 6,908,924 (June 21, 2005)
4. G.R. Painter *et al.*, US Patent 6,939,965 (September 6, 2005)
5. E. De Clercq *et al.*, US Patent 7,049,332 (May 23, 2006)
6. D. Loakes *et al.*, US Patent 7,049,303 (May 23, 2006)

CHAPTER II

Addiction Disorders

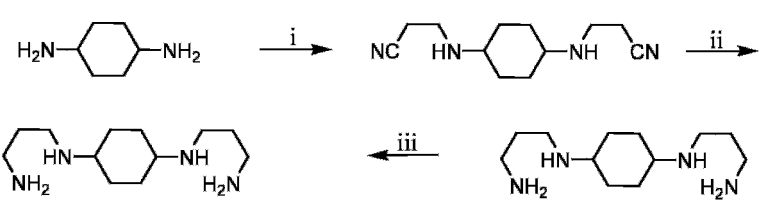
I. Alcohol Addiction

Title Compounds Used to Treat Alcoholism
B. Lojo *et al.*, US Patent 7,026,514 (April 11, 2006)
Assignee Garbil Pharma Investigacion Chile Ltda. (Santago de Chile, CL)
Utility Treatment of Alcohol Addiction

Invention Significance Current clinical methods for treating alcohol addiction are designed to impede acetaldehyde metabolism when alcohol is consumed to induce a variety of unpleasant and toxic syndromes. The current chemical agent behaves as an alcohol suppressant without the unpleasant side effects associated with the existing antialcoholism agents.

Reaction

Eq. 1



4 (p-$CH_3C_6H_4SO_2H \cdot H_2O$)

i- Acrylonitrile, ethyl alcohol
ii- Methyl alcohol, ammonia, hydrogen, Raney nickel
iii- Methanesulfonic acid, EtOAc, methyl alcohol

Experimental

1. **Preparation of *N,N′*-bis(2-cyanoethyl)-cyclohexane-1,4-diamine**

   A 5-l reactor was charged with 1,4-cyclohexanediamine (531.5 g), then warmed to 80°C, and treated with 675 ml acrylonitrile. The mixture was warmed 1 hour at 80°C and then 2 hours at 100°C. Thereafter, 960 ml ethyl alcohol was added and the mixture was cooled to ambient temperature and a solid was isolated. After washing with ethyl alcohol and drying, the product was isolated in 92.8% yield.

   Elemental analysis (%) Theory: C, 65.49; H, 9.09; N, 25.45. Found: C, 65.80; H, 9.20; N, 26.16.

2. **Preparation of *N,N′*-bis(3-aminopropyl)cyclohexane-1,4-diamine**

   The entire Step 1 product was dissolved in 21.21 methyl alcohol saturated with ammonia, then treated with Raney nickel catalyst (200 g), and hydrogenated at 50°C for 40 hours under 4 bar hydrogen. The catalyst was then removed by filtration, the mixture concentrated, and the product isolated in 93.7% yield as green oil.

   ^{1}H NMR spectrum (δ, ppm, $CDCl_3$) 1.05 (m, 4H), 1.48–1.66 (m, 10H), 1.92 (m, 4H), 2.35 (m, 2H), 2.65–2.79 (m, 8H)
   ^{13}C NMR spectrum (δ, ppm, $CDCl_3$) 31.8 (cyclohexane CH_2), 33.9 (CH_2), 40.1 (CH_2-NH_2), 44.7 (CH_2-NH), 56.6 (cyclohexane CH)
   IR spectrum (cm^{-1}, KBr) 3272 (NH_2, NH), 2940 (aliphatic CH).

3. **Preparation of *N,N″*-bis(3-aminopropyl)cyclohexane-1,4-diamine tetramethanesulfonate monohydrate**

   Methanesulfonic acid (350 ml) was dissolved in 1345 ml EtOAc and added to the Step 2 product (285.8 g), dissolved in 6880 ml methyl alcohol, and then stirred 2 hours. The mixture was then filtered, washed with ethyl alcohol, dried, and concentrated. The residue was stirred 2 hours in water (5 ml/g residue) containing activated charcoal, then filtered, and concentrated. The solid was dried at 40°C and product isolated in 67.2% yield, mp = 261–265°C.

   ^{1}H NMR spectrum (δ, ppm, DMSO-d_6) 1.4 (m, 4H), 1.91 (m, 4H), 2.14 (m, 4H), 2.3 (s, 12H), 2.90 and 3.20 (m 10H), 8 (10 H).
   ^{13}C NMR spectrum (δ, ppm, DMSO-d_6) 22.4 (cyclohexane CH_2), 24.6 (CH_2), 34.6 (CH_2-NH_2), 38.5 (acid CH_3), 38.6 (acid CH_3), 39.6 (CH_2-NH), 52.9 (cyclohexane CH)
   IR spectrum (cm^{-1}, KBr) 3440 (H_2O), 2944, 2860 (aliphatic CH), 1195 (SO_3)
   Elemental analysis (%) Theory: C, 30.48; H, 7.30; N, 8.88. Found: C, 30.79; H, 7.04; N, 8.88.

Derivatives

No additional derivatives were prepared.

Testing

I. *Antialcoholism Activity in Genetically Alcoholic Rats*: Initial Effects

Adult Wistar rats of both sexes having the UChB strain were used and considered to be genetically alcoholic.

A. *Reference period* consisted of 3 days prior to treatment. Measured consumption values were used as a reference for comparison purposes.
B. *Treatment period* consisted of the intragastrically administered experimental agent at 20 or 40 mg/kg for 6 or 3 consecutive days, respectively.
C. *Posttreatment period* consisted of 3 days immediately after treatment period where the duration of the effect and the reversibility of the changes were observed.
D. *After treatment period*. Using 20 mg/kg for 6 days after the treatment period, a significant ($p < 0.01$) reduction in alcohol consumption by 25% was observed following the first 6 hours after the agent was introduced. Treatment with 40 mg/kg for 3 days lowered alcohol consumption by 27% and remained at this level during the posttreatment period.

II. *Antialcoholism Activity*: Prolonged Treatment Effects

The effect of administrating 40 mg/kg of the experimental agent to Wistar rats for 30 consecutive days resulted in reduction in alcohol consumption by 71%, while water consumption gradually increased to 200%.

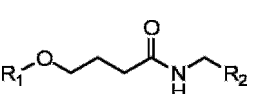
III. *Antialcoholism Activity*: Posttreatment Effects

The effect of withholding the experimental agent from Wistar rats for 27 consecutive days resulted in a gradual alcohol consumption increase, which reached an overall decrease of 24% at the end of the period.

Notes

1. Selected γ-hydroxybutyric acid amide derivatives, (**I**), prepared by Cacciaglia (1) were effective in the treatment of alcoholism and illustrated in Table 1.

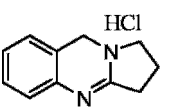
Table 1 γ-Hydroxybutyric acid amide derivatives effective in treating alcoholism prepared by Cacciaglia (1)



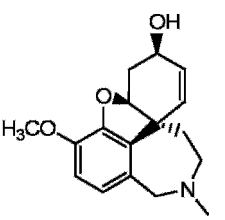
(I)

Entry	R_1	R_2
5	4-Methylphenyl	Hydrogen
7	4-methoxyphenyl	Hydrogen
14	1-Naphthyl	Methyl
15	Trifluoromethyl	Methyl

2. Desoxypeganine hydrochloride, (**II**), a reversibly acting cholinesterase and inhibitor monoamine oxidase, was determined by Asmussen (2) to be useful in the treatment of alcohol dependence. The cholinesterase inhibitor identified by Opitz (3), galathamine, (**III**), was also effective in treating chronic alcoholism.

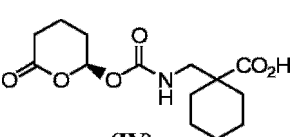


(II)



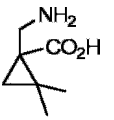
(III)

3. Wilkemeyer (4) determined that 3-pentanol, 2-pentanol, cyclopentanol, 4-methyl-1-pentanol, 2-methyl-2-pentanol, and 2,6-diisopropylphenol were effective in mitigating alcohol-induced cell adhesion disorders associated with alcohol addiction in adults, fetal alcohol syndrome, and neuropsychiatric behavioral disorders.
4. δ-Valerolacton derivatives, (**IV**), prepared by Gallop (5) were effective in treating psychiatric disorders, alcoholism, and manic behavior.



(IV)

5. Cyclopropyl-β-amino acids derivatives, (**V**), prepared by Schwarz (6) had an affinity for the α2δ subunit of a calcium channel and were used to treat pain caused by chronic alcoholism.



(V)

References

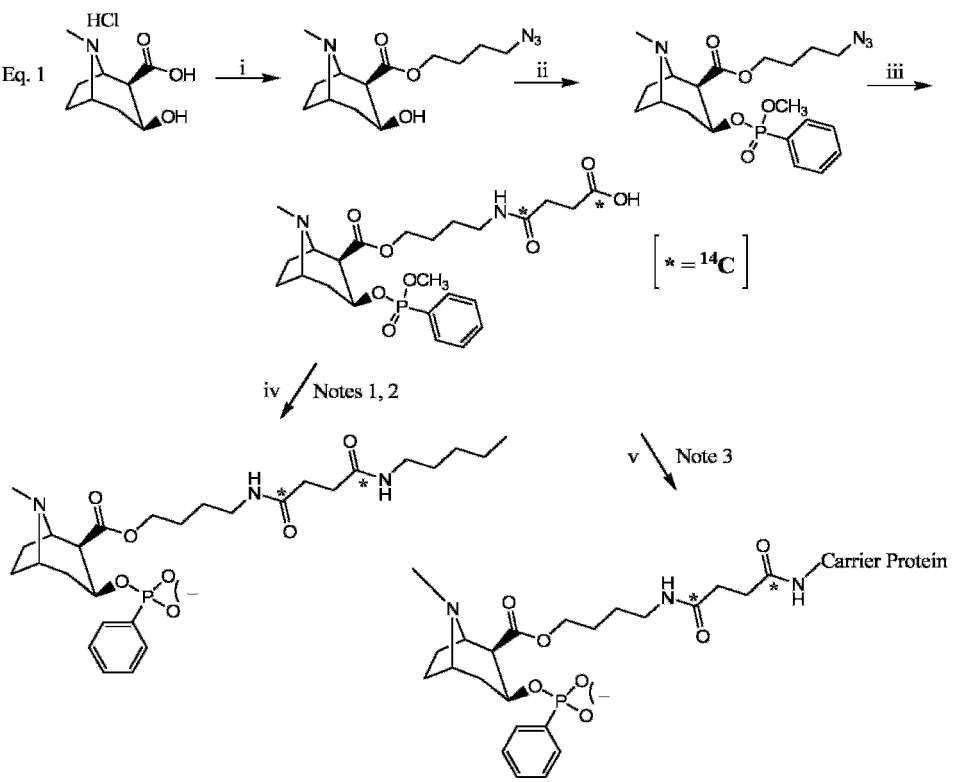
1. R. Cacciaglia *et al.*, US Patent 6,770,784 (August 3, 2004)
2. B. Asmussen *et al.*, US Patent 6,627,631 (September 30, 2003) and US Patent 6,599,511 (July 29, 2003)
3. K. Opitz, US Patent 5,932,238 (August 3, 1999)
4. M.F. Wilkemeyer *et al.*, US Patent 6,977,272 (December 20, 2005)
5. M.A. Gallop *et al.*, US Patent 7,026,351 (April 11, 2006)
6. J.B. Schwarz *et al.*, US Patent 7,030,267 (April 18, 2006)

II. Cocaine Addiction

Title Anticocaine Catalytic Therapy
D.W. Landry, US Patent 6,913,917 (July 5, 2005)
Assignee The Trustees of Columbia University of the City of New York
Utility Treatment of Cocaine Addiction Using Artificial Enzymes

Invention Significance Catalytic antibodies used to degrade cocaine, Mab3B9 and Mab612, were artificially activated using a modified TSA 1 immunogenic conjugate. The modification consists of incorporating phosphate methyl ester transition state intermediates into cocaine decomposition analogs. This discovery represents an entirely new treatment option for both cocaine addiction and drug overdose.

Reaction



i- DMF, tetramethylammonium hydroxide, 1-azido-4-iodobutane
ii- Benzene, phenylphosphonic dichloride, 1H-tetrazole, *N*, *N*-diiospropylamine
iii- Trimethylphosphine, THF, methyl alcohol, water, 1,4-^{14}C-succinic anhydride, benzyl alcohol, 1,3-dicyclohexylcarbodiimide, hydrogen, 10% palladium on carbon
iv- Acetonitrile, *N*-hydroxylphthalimide, 1,3-dicyclohexylcarbodiimide, CH_2Cl_2, trimethylsilyl bromide, amylamine, water
v- Acetonitrile, *N*-hydroxylphthalimide, 1,3-dicyclohexylcarbodiimide, CH_2Cl_2, trimethylsilyl bromide, ovalbumin

Experimental

1. **Preparation of 4-azido-butyl (−)-ecgoninate**

To (−)-ecgonine hydrogen chloride (1.6 mmol) dissolved in 4 ml methyl alcohol was added 40 ml DMF, tetramethylammonium hydroxide (6.4 mmol), and 1-azido-4-iodobutane (8 mmol) and the reaction stirred 12 hours at 50°C. The mixture was concentrated, purified by chromatography with silica gel using EtOAc/methyl alcohol/NH_4OH, 9:0.9:0.1, and the product isolated in 78% yield as oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.23 (m, 1H), 4.12 (m, 1H), 3.81 (m, 1H), 3.58 (m, 1H), 3.26 (t, 2H, $J = 7.0$ Hz), 3.18 (m, 1H), 2.74 (t, 1H, $J = 4.7$ Hz), 2.19 (s, 3H), 2.03 (m, 2H), 1.98–1.63 (m, 6H), 1.61–1.47 (m, 2H)
^{13}C NMR (500 MHz, $CDCl_3$) δ 173.73, 64.37, 64.29, 63.56, 61.58, 51.74, 50.94, 41.23, 40.26, 25.92, 25.61, 25.51, 24.82
MS (FAB) for $C_{13}H_{23}N_4O_3$ (M+1) Calc. 283.177 0; found 283.178 3.

2. **Preparation of 4-azido-butyl-(−)-ecgoninate methyl phosphate**

The Step 1 product (1.5 mmol) was dissolved in 10 ml benzene at 0°C and treated with phenylphosphonic dichloride (1.7 mmol), 1H-tetrazole (8 mg), and *N*,*N*-diisopropylethyl amine (3.4 mmol). The mixture was then stirred at ambient temperature and a precipitate isolated after 15 minutes. The mixture was stirred for an additional 12 hours, then treated with 0.1 ml methyl alcohol, and the mixture concentrated after an additional 4 hours of stirring. The residue was purified by chromatography with $CHCl_3$/methyl alcohol/NH_4OH, 9.5:0.5:0.02, and the product isolated in 89% yield as a mixture of diastereomers.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (m, 2H), 7.60 (m, 1H), 7.49 (m, 2H), 5.09 (m, 1/2H), 4.98 (m, 1/2H), 4.24 (m, 2H), 4.15–3.96 (m, 2H), 3.71 (d, 3/2H, $J = 14.6$ Hz),

3.68 (d, 2H, $J = 14.6$ Hz), 3.35–3.15 (m, 3H), 2.91 (s, 3/2H), 2.89 (s, 3/2H), 2.87 (t, 1/2H, $J = 7.5$ Hz), 2.59 (t, 1/2H, $J = 7.5$ Hz), 2.43–2.22 (m, 5/2H), 2.17–1.95 (m, 5/2H), 1.71–1.57 (m, 2H), 1.39 (m, 2H)
^{13}C NMR (500 MHz, $CDCl_3$) δ 161.55, 149.12, 134.32, 132.55, 129.80, 129.66, 66.72, 66.54, 66.45, 66.28, 64.80, 63.90, 63.81, 53.81, 51.60, 51.50, 49.58, 49.15, 40.30, 35.60, 35.27, 26.35, 26.06, 26.02, 25.82, 25.10, 23.98
MS (FAB) for $C_{20}H_{30}N_4O_5$ (M+1) Calc. 437.1954; found 437.1953.

3. **Preparation of butyl-(−)-ecgonine ^{14}C-1,4-succinamic acid methyl phosphate**

Trimethylphosphine (1.1 mmol) was added to the Step 2 product (0.5 mmol), dissolved in 6 ml THF/methyl alcohol/H_2O, 9:9:2, then stirred 5 hours at ambient temperature, and concentrated. The unstable amine (0.084 mmol) was dissolved in 5 ml dry CH_2Cl_2, then treated with 1,4-^{14}C-succinic anhydride (0.093 mmol), and stirred 12 hours. It was reconcentrated and the acid (0.087 mmol) esterified dissolved in 10 ml CH_2Cl_2 containing 1,3-dicyclohexylcarbodiimide (0.17 mmol), benzyl alcohol (0.35 mmol), and a catalytic amount of *N*,*N*-dimethylaminopyridine. After stirring 12 hours, the mixture was concentrated, purified by chromatography using methyl alcohol/$CHCl_3$, 0.5:99.5 to 2:98:0.5, and the diastereomeric intermediate mixture isolated in 59% yield as oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (m, 2H), 7.62 (m, 1H), 7.49 (m, 2H), 7.33 (m, 5H), 6.64 (br. S, 1/2H), 6.56 (br. s, 1/2H), 5.10 (s, 2H), 4.96 (m, 1/2H), 4.89 (m, 1/2H), 4.38–3.85 (m, 4H), 3.74 (d, 3/2H, $J = 15.2$ Hz), 3.68 (d, 3/2H, $J = 15.2$ Hz), 3.32–3.12 (m, 3H), 2.89 (s, 3/2H), 2.87 (s, 3/2H), 2.70–2.59 (m, 3H), 2.52–2.26 (m, 4H), 2.10–1.97 (m, 2H), 1.68 (m, 1H), 1.55 (m, 1H), 1.38 (m, 2H)
^{13}C NMR (500 MHz, $CDCl_3$) δ 173.55, 172.66, 171.37, 161.62, 161.28, 136.59, 134.17, 132.37, 129.56, 129.24, 128.88, 128.71, 67.04, 66.81, 66.64, 66.25, 64.66, 63.75, 53.74, 49.37, 49.00, 40.11, 39.42, 35.55, 35.26, 31.35, 30.31, 26.19, 26.06, 24.89, 23.91
MS (FAB) for $C_{31}H_{42}N_2O_8P$ (M+1)
Calc. 601.267 9; found 601.268 2

The intermediate (0.028 mmol) was dissolved in 10 ml methyl alcohol and stirred with a catalytic amount of 10% Pd on C under 1 atm hydrogen for 4 hours. The reaction mixture was filtered, concentrated, and the product quantitatively isolated.

^{1}H NMR (400 MHz, CD_3CD) δ 7.69 (m, 2H), 7.60 (m, 1H), 7.51 (m, 2H), 4.99 (m, 1H), 4.20–4.08 (m, 2H), 3.89 (m, 1H), 3.73 (d, 3/2H, $J = 21.5$ Hz), 3.66 (d, 3/2H, $J = 21.5$ Hz), 3.62 (m, 1H), 3.22 (m, 1H), 3.10 (m, 1H), 3.01 (m, 1H), 2.76 (s, 3/2H), 2.75 (s, 3/2H), 2.50 (m, 2H), 2.38–2.28 (m, SH), 2.04 (m, 2H), 1.61 (m, 1H), 1.50 (m, 1H), 1.34 (m, 3H)

^{13}C NMR (500 MHz, CD_3OD) δ 176.22, 174.52, 173.47, 162.22, 134.97, 132.79, 130.18, 67.66, 67.53, 66.99, 65.47, 64.44, 53.89, 39.63, 39.33, 35.99, 31.50, 30.23, 26.71, 24.65, 23.67
MS (EI) for $C_{24}H_{36}N_2O_8P$ Calc. 511.220 9 (M+1); found 511.221 8

4. **Preparation of butyl-(−)-ecgonine ^{14}C-1,4-*N*-butyl-succinamide methyl phosphate**

The Step 3 product (0.078 mmol) dissolved in 5 ml acetonitrile was treated with *N*-hydroxyphthalimide (0.086 mmol) and 1,3-dicyclohexylcarbodiimide (0.16 mmol), then stirred 1 hour at ambient temperature, and a white precipitate isolated. The filtrate was concentrated, then dissolved in 5 ml CH_2Cl_2, and treated with trimethylsilyl bromide (0.78 mmol). The mixture was stirred 1 hour and then concentrated. The residue was dissolved in 5 ml acetonitrile, then treated with amylamine (0.78 mmol), whereupon a bright orange color developed immediately that faded to light yellow within 1 hour. A second addition of amylamine (0.78 mmol) was introduced then stirred 12 hours at ambient temperature and concentrated. The mixture was then diluted with 3 ml water, then extracted twice with 5 ml $CHCl_3$, and the organic portion was re-extracted with 5 ml water. The combined aqueous fractions were concentrated, purified by high-pressure liquid chromatography on a Dynamax 300+, 12 μm, C-8 10×250 mm column using 4–40% CH_3CN/H_2O, 0.1% trifluoroacetic acid, and the product isolated in 36% yield.

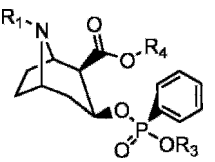
^{1}H NMR(400 MHz, CD_3OD) δ 7.72 (m, 2H), 7.56 (m, 1H), 7.47 (m, 2H), 4.12 (m, 3H), 3.87 (m, 1H), 3.23 (m, 2H), 3.14 (m, 3H), 2.77 (m, 4H) 2.58 (m, 4H), 2.34 (m, 3H), 2.16 (m, 1H), 1.97 (m, 2H), 1.55–1.48 (m, 6H), 1.26 (m, 4H), 0.846 (t, 3H, $J = 6.3$ Hz)
^{13}C NMR (500 MHz, CD_3OD) δ 175.76, 173.62, 133.83, 132.23, 131.01, 129.07, 66.56, 66.52, 65.26, 64.33, 41.13, 40.36, 39.33, 35.93, 31.13, 29.91, 29.48, 28.95, 26.57, 26.28, 24.73, 23.66, 23.22
Calc. 566.299 5 (M+1); found 566.299 7

5. **Preparation of butyl-(−)-ecgonine ^{14}C-1,4-*N*-TSA-succinamide methyl phosphate**

The Step 3 product (0.078 mmol) was dissolved in 5 ml acetonitrile and treated with *N*-hydroxyphthalimide (0.086 mmol) and 1,3-dicyclohexylcarbodiimide (0.16 mmol). After 1 hour at ambient temperature, a white precipitate was formed. The filtrate was concentrated, then dissolved in 5 ml CH_2Cl_2, and treated with trimethylsilyl bromide (0.78 mmol). The solution was then stirred 1 hour and concentrated. The residue was treated with ovalbumin (5 mg), dissolved in 5 ml 1 M $NaHCO_3$ (pH 8.0) at 0°C, then stirred vigorously 1 hour at ambient temperature, and purified by gel filtration chromatography using a Sephadex G-25 M column at pH 7.4 PBS. Protein-containing fractions were combined, then dialyzed overnight against PBS at 4°C at pH 7.4, and the coupling efficiency estimated to be 15:1 based on radiolabel incorporation.

Derivatives

Table 1 Selected (−)-ecgonine-derived experimental artificial enzymes designed to mimic the cocaine decomposition state intermediate. ^{1}H- and ^{13}C NMR for products and intermediates supplied by author



R_1	R_2	R_3	R_4
$(CH_2)_3NH^{14}CO(CH_2)_2{}^{14}CO$–NH–carrier protein	H	H	None
H	H	None	$(CH_2)_3NH^{14}CO(CH_2)_2{}^{14}CON$–H–carrier protein
H	$(CH_2)_3NH^{14}CO(CH_2)_2{}^{14}CO$–NH–carrier protein	CH_3	H

Testing

I. *Kinetic Measurements Using Selected Monoclonal Antibodies*

Catalytic antibodies were dissolved in 50 mM PBS at pH 8.0 (except 2A10 and 6A12 at pH 7.0), then incubated with ^{3}H-cocaine at five concentrations. At three time intervals, aliquots were acidified with cold HCl to pH 2 and partitioned using hexane/diethyl ether, 1:1, and the organic phase assayed by scintillation counting. Background hydrolysis was determined in identical reactions without antibody and observed rates corrected. Assays were performed in triplicate with standard error $< 10\%$. As a control, the release of benzoic acid was confirmed by HPLC using an analytical reverse-phase C18 column with an acetonitrile/water, 0.1% trifluoroacetic acid gradient, and the detector set at 220 nm.

The rate of hydrolysis of ^{3}H-phenyl-cocaine in the presence and absence of each monoclonal antibody as a function of substrate concentration was determined. Production of radiolabeled benzoic acid at time points corresponding to $< 5\%$ reaction extent provided initial rates. A saturation kinetics and a linear Lineweaver–Burk plot for each artificial enzyme were plotted. The first-order rate constants (k_{cat}) and Michaelis constants (K_m) of selected antibodies are provided in Table 2.

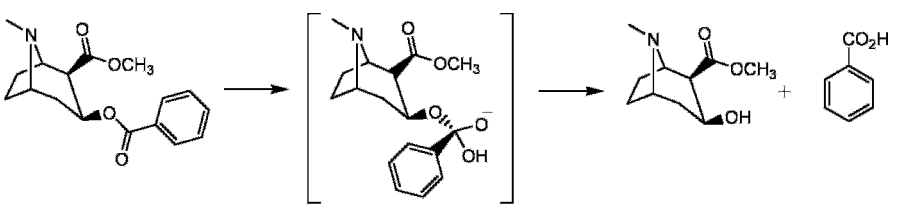
Table 2 The rate of hydrolysis of ^{3}H-phenyl-cocaine in the presence of selected modified monoclonal antibodies, Mab

Mab	K_m (μM)	k_{rate} (min^{-1})	k_{cat}/k_o
3B9	490	0.11	1 100
6A12	1 020	0.072	880
2A10	3 000	0.011	420
9A3	270	0.015	140
19G8	900	0.091	830
15A10	220	2.3	23 000
12H1	150	0.16	1 500
8G4G	530	0.60	5 500
8G4E	1 200	0.12	1 100

Notes

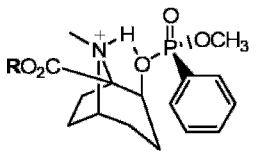
1. The cocaine decomposition mechanism and transition state, (**I**), is illustrated in Eq. 2. Thermodynamics and kinetics associated with this model are described by the author (1) in an earlier investigation.

Eq. 2

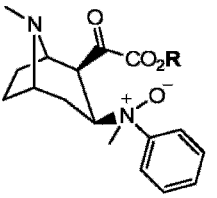


Decomposition Transition State (I)

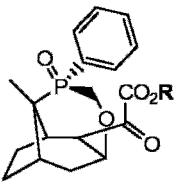
2. Other transition state analogs, (**II**), (**III**), and (**IV**), were previously prepared by the author (2) and were effective in activating Mab3B9 and Mab612 antibodies to degrade cocaine, where **R**=$CH_2CH_2CH_2NH$–$^{14}COCH_2CH_2{}^{14}CO$–NH–carrier protein.



(II)

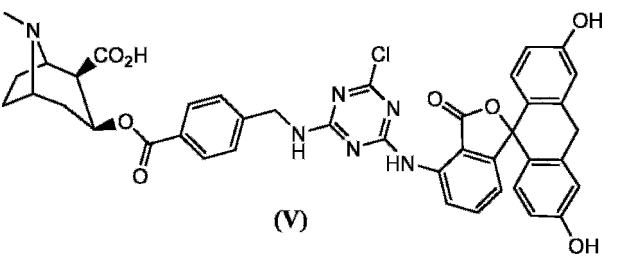


(III)



(IV)

3. Fluorescence polarization immunoassay using substituted benzoyl ecgonine derivative, (**V**), developed by Ungemach (3) was used to monitor/detect cocaine and cocaine metabolites.



(V)

References

1. D.W. Landry *et al.*, US Patent 5,977,314 (November 2, 1999)
2. D.W. Landry *et al.*, US Patent 6,566,084 (May 20, 2003) and US Patent 6,280,987 (August 28, 2001)
3. F.S. Ungemach *et al.*, US Patent 5,202,270 (May 13, 1993)

III. Opioid Addiction

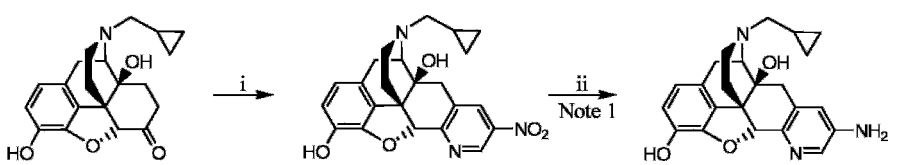
A. δ Receptor Antagonists

Title Pyridomorphinans, Thienomorphinans, and Use Thereof
S. Ananthan, US Patent 7,015,326 (March 21, 2006)
Assignee Southern Research Institute
Utility Opioid Antagonists and Analgesics

Invention Significance Naltrexone derivatives having significant selectivity toward the δ receptors and μ agonist characteristics have been prepared. These agents are useful as both opioid antagonists and as analgesics devoid of side effects such as addiction and respiratory depression.

Reaction

Eq. 1



i- 1-Methyl-3,5-dinitropyridin-2-one, methanolic ammonia, methyl alcohol
ii- Ethyl alcohol, 10% palladium on carbon, hydrogen

Experimental

1. **Preparation of 17-(cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-5′-nitropyrido[21,31:6,7]morphinan**

   A stirred solution of naltrexone (12.4 mmol) and 1-methyl-3,5-dinitropyridin-2-one (15.0 mmol) dissolved in 200 ml 2 M methanolic ammonia was refluxed 24 hours at 70°C and then concentrated. The residue was dissolved in minimum amount of methyl alcohol and then slurried with silica gel. The dried slurry was applied to the top of a chromatography column containing silica gel and eluted with $CHCl_3$ containing 0.1, 0.2, 0.3, 0.4, and 1.5% methyl alcohol. Fractions containing the product were pooled, concentrated, and 3.01 g product isolated, mp softens and foams

at 117–125°C, and decomposes at 142–156°C, TLC, $R_f = 0.44$ in $CHCl_3$/methyl alcohol/NH_4OH, 95:5:0.5.

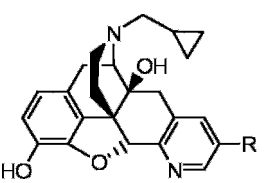
2. **Preparation of 5′-amino-17-(cyclopropylmethyl)-6,7-didehydro-3,14-dihydroxy-4,5α-epoxypyrido[2′,3′:6,7]morphinan**

   The Step 1 product (6.6 mmol) was dissolved in 300 ml warm ethyl alcohol and hydrogenated 24 hours at 50 psi hydrogen using 10% palladium on carbon (0.90 g) in a Paar shaker. The mixture was then filtered through a celite pad, concentrated, and 2.67 g product isolated, mp = 202–204° C (dec), TLC, $R_f = 0.34$ in $CHCl_3$/methyl alcohol, 9:1.

Derivatives

Selected pyridomorphinan and thienomorphinan derivatives are provided in Tables 1 and 2, respectively.

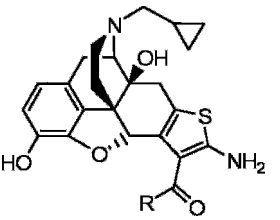
Table 1 The δ, μ, and κ opioid receptor binding profile using selected pyridomorphinans in rat or guinea pig brain membranes



Entry	R	K_i (nM) ± SEM			Selectivity Ratio	
		δ[a]	μ[b]	κ_1[c]	μ/δ	$\kappa_{1\delta}$
7a	H	0.78 ± 0.06	1.5 ± 0.09	8.8 ± 0.69	1.9	11
7b	Br	1.2 ± 0.13	15.5 ± 1.0	55.7 ± 7.0	13	46
7c	CN	4.5 ± 0.28	16.0 ± 1.8	33.9 ± 2.0	3.6	7.5
7d	$CO_2C_2H_5$	4.2 ± 0.27	37.0 ± 3.4	9.6 ± 0.93	8.8	2.3
7e	NO_2	5.5 ± 0.67	17.5 ± 2.0	92.0 ± 12.8	3.2	17
7f	NH_2	8.0 ± 0.3	12.8 ± 0.93	12.0 ± 1.2	1.6	1.5

[a] Displacement of [3H]DADLE (1.3–2.0 nM) in rat brain membranes using 100 nM DAMGO to block binding to μ sites.
[b] Displacement of [3H]DAMGO (1.4–2.0 nM) in rat brain membranes.
[c] Displacement of [3H]U69, 593 (1.2–2.2 nM) in guinea pig brain membranes.

Table 2 The δ, μ, and κ opioid receptor binding profile using selected thienomorphinans in rat or guinea pig brain membranes



Entry	R	K_i(nM) ± SEM			Selectivity Ratio	
		δ[a]	μ[b]	K_1[c]	μ/δ	$\kappa_{1\delta}$
8a	CN	2.6 ± 0.11	5.5 ± 0.2	1.5 ± 0.12	2.1	0.6
8b	CO_2CH3	6.6 ± 0.3	29.0 ± 4.0	8.7 ± 0.34	4.4	1.3
8c	$CO_2C_2H_5$	5.0 ± 0.2	20.0 ± 1.0	9.0 ± 0.81	4.0	1.8
8d	$CO_2CH_2C_6H_5$	7.0 ± 0.3	61.0 ± 3.0	48.0 ± 3.0	8.7	6.9
8e	$CONH_2$	3.7 ± 0.2	21.0 ± 1.3	2.0 ± 0.2	5.7	0.5
8f	COC_6H_5	14.0 ± 0.7	50.0 ± 3.0	14.0 ± 0.7	3.6	1.0

[a] Displacement of [3H]DADLE (1.3–2.0 nM) in rat brain membranes using 100 mM DAMGO to block binding to μ sites.

[b] Displacement of [3H]DAMGO (1.4–2.0 nM) in rat brain membranes.

[c] Displacement of [3H]U69, 593 (1.2–2.2 nM) in guinea pig brain membranes.

Testing

Biological Evaluations

I. *Radioligand Binding Assays*

Mu binding sites were labeled using ^{3}H-DAMGO. Rat membranes were prepared using partially thawed frozen rat brain, which was homogenized in 10 ml/brain of ice-cold 10 mM Tris–HCl, pH 7.0. Membranes were centrifuged twice, then resuspended with ice-cold buffer following each centrifugation. After the second centrifugation, the membranes were resuspended in 50 mM Tris–HCl, pH 7.4 (50 ml/brain) at 25°C. The materials were incubated, then placed in 50 mM Tris–HCl (pH 7.4) along with a protease inhibitor cocktail (PIC). The nonspecific binding was determined using 20 μM of levallorphan. Delta binding sites were labeled using ^{3}H-DAMGO and rat brain membranes.

Rat membranes were also incubated 2 hours at 25°C in 50 mM Tris–HCl (pH 7.4) containing 100 mM choline chloride, 3 mM $MnCl_2$, 100 nM DAMGO to block binding

to μ sites and PIC. Nonspecific binding was determined using 20 μM levallorphan. Kappa binding sites were labeled using ^{3}H-U69,593.

Guinea pig brain membranes were prepared as above incubated in 50 mM Tris–HCl, pH 7.4, containing 1 μg/ml captopril and PIC. Nonspecific binding was determined using 1 μM U69,593.

Testing results are provided in Tables 1–3 .

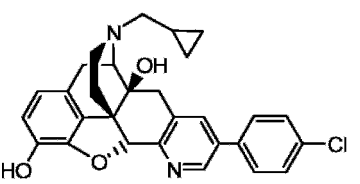
Table 3 Opioid antagonist and agonist potencies of selected pyrido- and thienomorphinans derivatives in the MVD and GPI smooth muscle preparations

Entry	Antagonist Activity					Agonist Activity	
	DPDPE (δ)[a] MVD		PL017 (μ)[b] GPI		K_c Ratio (μδ)	MVD IC_{50} (nm)	GPI IC_{50} (nm)
	IC_{50} ratio	K_e (nM[c])	IC_{50} ratio	K_e (nM[c])		Max resp[d] (%)	Max resp[d] (%)
7a[e]	27.9 ± 1.2	37	7.08 ± 3.44	164	4.4	0	0
7b	325 ± 127	3.1	43.9 ± 25.6	23	14	0	0
7c	23.6 ± 2.2	44	2.2 ± 1.1	–[f]	–	0	0
7d	50.1 ± 4.9	20	14.5 ± 5.2	74	3.7	0	0
7e	20.7 ± 4.2	51	4.7 ± 0.59	271	5.3	14	0
7f	43.3 ± 9.0	26	23.4 ± 4.8	49	1.9	6	0
8a	109.6 ± 12.1	9.6	160.3 ± 41.6	8.7	0.9	0	0
8b	53.5 ± 10.1	21	39.1 ± 11.6	35	1.7	0	0
8c	19.8 ± 6.6	68	46.0 ± 21.9	26	0.4	11	0
8d	289 ± 13	5.0	–[g]	–	–	11	40
8e	118.6 ± 38.4	10	47.8 ± 10.7	24	2.4	5	4
8f	24.4 ± 4.0	46	21.6 ± 7.7	62	1.3	15	25

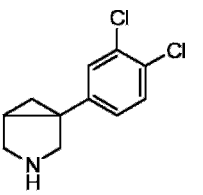
[a] DPDPE as the agonist.
[b] PL-017 as the agonist.
[c] K_c (nM) = [antagonist]/(IC_{50} ratio–1), where the IC_{50} ratio is the IC_{50} of the agonist in the presence of antagonist divided by the control IC_{50} in the same preparation ($n = 3$).
[d] Agonist activity, percentage inhibition of contraction at 1 μM.
[e] Data of 7a included for comparison. Data taken from Ananthan (1).
[f] The agonist effects precluded the determination of antagonist effects.
[g] IC_{50} ratio was not statistically different from 1.

Notes

1. Chlorophenylpyridomorphinan derivatives, (**I**), effective as κ opioid receptor antagonists were prepared by the author (1) in an earlier investigation and used in the treatment of heroin or cocaine addictions. 1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane, (**II**), prepared by Lippa (2) was effective as a dopamine-reuptake inhibitor and was used in the treatment of addiction disorders.

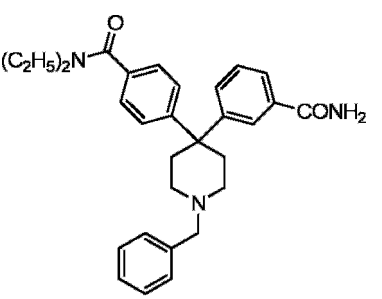


(I)



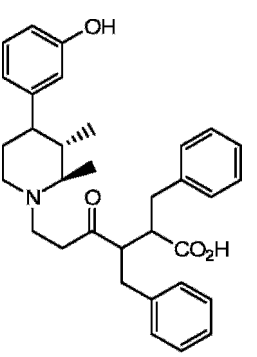
(II)

2. Delta opioid receptor antagonists consisting of 4,4-biaryl-piperidine derivatives, (**III**), prepared by Liras (3) were effective as a therapeutic heroin addiction treatment agent.



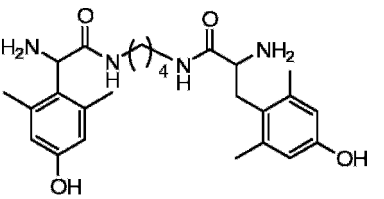
(III)

4. 3,4-*trans*-Dimethyl-4-aryl-piperidine derivatives, (**IV**), prepared by Le Bourdonnec (4) were effective as opioid receptor antagonists and used in treating morphine dependence.



(IV)

5. Peptide derivatives, (**V**), prepared by Okada (5) demonstrated a specific and very high μ opioid receptor binding affinity and were used as analgesics in severe pain management without devoid of side effects such as addiction and respiratory depression.



(V)

References

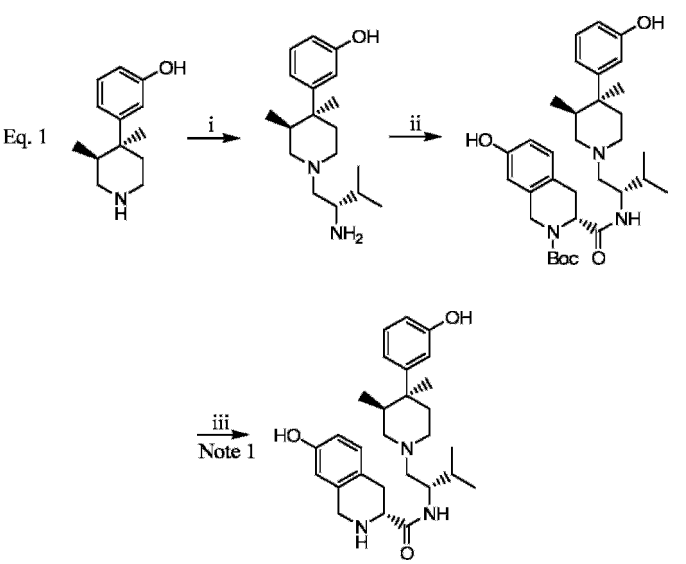
1. S. Ananthan, US Patent 6,465,479 (October 15, 2002)
2. A.S. Lippa *et al.*, US Patent 7,081,471 (June 25, 2006)
3. S. Liras, US Patent 6,720,336 (April 13, 2004)
4. B. Le Bourdonnec *et al.*, US Patent 6,992,090 (January 31, 2006)
5. Y. Okada *et al.*, US Patent 6,838,580 (January 4, 2005)

B. κ Opioid Receptor Antagonists

Title Kappa Opioid Receptor Ligands
F.I. Carroll *et al.*, US Patent 6,974,824 (December 13, 2005)
Assignee Research Triangle Institute
Utility Treatment of Heroin Addiction

Invention Significance N-Substituted *trans*-(3,4)-dimethyl-4-(3-hydroxyphenyl)piperidine derivatives have been prepared that bind with unusually high affinity and specificity to kappa opioid receptors relative to norbinaltorphimine. These selective kappa receptor antagonists are effective in ameliorating the effects of heroin addiction.

Reaction



i- Boc-L-valine, benzotriazol-1-yl-oxy-tris-(dimethylamino) phosphonium hexafluorophosphate, THF, triethylamine, CH_2Cl_2, trifluoroacetic acid, borane, dimethyl sulfide
ii- Boc-D-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, THF, benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate, triethylamine
iii- CH_2Cl_2, trifluoroacetic acid

Experimental

1. **Preparation of 3-[1-(2*S*-amino-3-methylbutyl)-3*S*,4*S*-dimethyl-4-piperidinyl]-phenol**

   (−)-(3*S*,4*S*)-Dimethyl-4-(3-hydroxyphenyl)piperidine was treated with Boc-L-valine and benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate in THF containing triethylamine. The intermediate was deprotected by dissolving in CH_2Cl_2 and treating with trifluoroacetic acid. Thereafter, the imine was reduced using borane dissolved in dimethyl sulfide and the product isolated.

2. **Preparation of *t*-butyl-(3*R*)-7-hydroxy-3-{[((1*S*)-1-{[(3*S*,4*S*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)amino]carbonyl}-3,4-dihydrohydro-2(1H)-isoquinolinecarboxylate**

   Boc-D-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid was dissolved in THF containing benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate and triethylamine and heated with the Step 1 product until amidation was completed. Thereafter, the coupled product was isolated.

3. **Preparation of (3*R*)-7-Hydroxy-*N*-((1*S*)-1-{[(3*S*,4*S*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide**

   The Step 2 product was deprotected by dissolving in CH_2Cl_2, then treating with trifluoroacetic acid, and the product isolated.

Derivatives

Selected derivatives are provided in Table 1.

Biological Testing

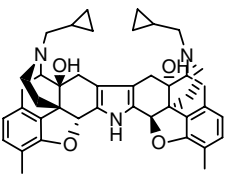
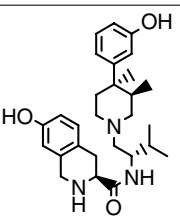
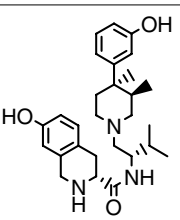
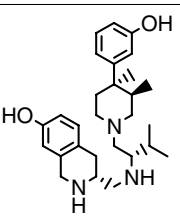
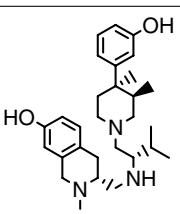
I. *Binding Effectiveness*

Binding affinities for experimental kappa antagonists and the standard kappa antagonist norbinaltorphimine at the μ, δ, and κ opioid and receptors using competitive binding assays were performed using the method previously described by the authors (1). Testing results are provided in Table 1.

II. *Antagonism Effectiveness*

Antagonism effectiveness was obtained by monitoring the ability of selected experimental agents to inhibit stimulation of [^{35}S]GTP-γ-S binding in guinea pig caudate and in cloned human receptors. Testing results are provided in Tables 2 and 3.

Table 1 Radioligand binding test results at the μ, δ, and κ opioid receptors for selected experimental agents. ^{1}H NMR data supplied by author

Entry	Structure	μ [^{3}H]DAMGO[a]	δ [^{3}H]DADLE[b]	κ [^{3}H]U69,593[c]	μ/κ	δ/κ
Reference	 Norbinaltorphimine	65.06	86	1.09	60	79
1		596	>4900	9.8	61	500
2		775	>4900	2.1	369	>2333
3		107	5572	0.63	170	8844
4		138	144	17.5	7.8	8.2

[a] [^{3}H] DAMGO, [(D-Ala^{2},MePhe^{4},Gly-ol^{5})enkephalin], is selective for the μ opioid receptor.
[b] [^{3}H]DADLE, [(D-Ala^{2},D-Leu^{5})enkephalin], is selective for δ opioid receptor.
[c] [^{3}H]U69,593 {[^{3}H](5α,7α,8β)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl] benzeneac-etamide} is selective for κ opioid receptor.

Table 2 Effectiveness of a selected experimental agent as an antagonist of [^{35}S]GTP-γ-S binding in guinea pig caudate stimulated by the opioid receptor subtype-selective agonists, DAMGO (μ), SNC80 (δ), and U69,593 (κ)

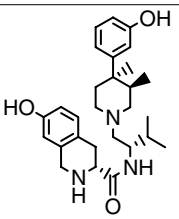
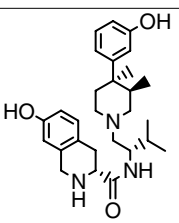
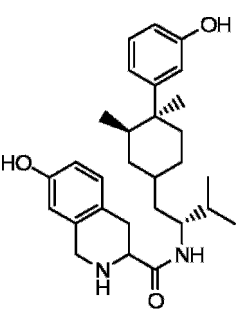
Entry	Structure	μ DAMGO	δ DADLE	κ U69,593	μ/κ	δ/κ
Reference	Norbinaltorphimine	16.7	10.2	0.038	439	268
2		2.16	>300	0.02	108	>15 000

Table 3 Effectiveness of a selected experimental agent as an antagonist of [^{35}S]GTP-γ-S binding in cloned human opioid receptors stimulated by DAMGO (μ)-, SNS-80 (δ)-, and U69,593 (κ)-selective opioid agonists

Entry	Structure	μ DAMGO	δ DADLE	κ U69,593	μ/κ	δ/κ
Reference	Norbinaltorphimine	15.8	12.1	0.07	225	172
2		3.42	> 100	0.006	570	>16 667

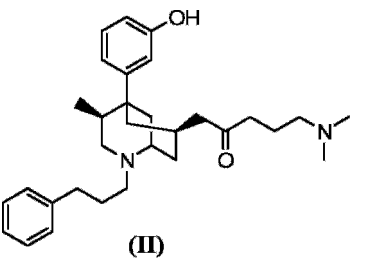
Notes

1. In another investigation by the authors (1), kappa opioid receptor antagonists consisting of 1,2,3,4-tetrahydro-3-isoquinolinecarboxamide derivatives, (**I**), were prepared and found effective in the treatment of heroin addiction.



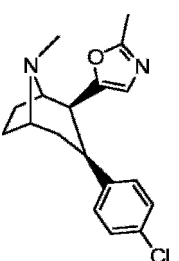
(I)

2. Azabicyclo[3.3.1]nonan-7-one derivatives, (**II**), effective as kappa opioid receptor antagonists, (**I**), were prepared by the authors (2) in an earlier investigation and used in the treatment of opioid addiction.

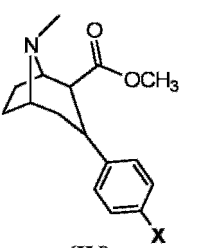


(II)

3. Tropane derivatives, (**III**) and (**IV**), previously prepared by the authors (3) have demonstrated high affinity for cocaine receptors in the brain, particularly dopamine and serotonin transporter sites.

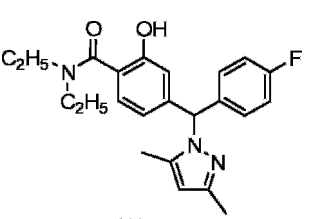


(III)



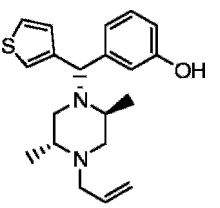
(IV) **X** = Cl, Br, I

4. 1-Diphenylmethyl-pyrazole derivatives, (**V**), prepared by McHardy (4) were effective as opioid receptor antagonists and used in treating addiction disorders.



(V)

5. The enantiomerically pure diarylmethylpiperzine derivative, (−)-3-((*S*)-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-(3-thienyl)-methyl)phenol, (**VI**), prepared by Chang (5) was effective as an opioid receptor antagonist and used as a therapeutic agent in treating drug and alcohol addictions.



(VI)

References

1. F.I Carroll *et al.*, US Patent 6,900,228 (May 31, 2005)
2. F.I. Carroll *et al.*, US Patent 6,559,159 (May 6, 2003)
3. F.I. Carroll *et al.*, US Patent 6,706,880 (March 16, 2004)
4. S.F. McHardy *et al.*, US Patent 6,960,609 (November 1, 2005)
5. K.-J. Chang, US Patent 6,924,288 (August 2, 2005)

CHAPTER III

Alzheimer's Disease

I. β-Amyloid Inhibitory Agents

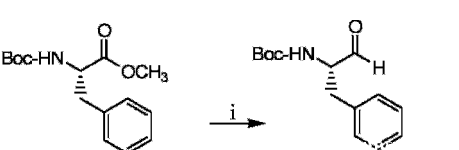
A. β-Secretase Enzyme Inhibitors

Title Substituted Amino Carboxamides for the Treatment of Alzheimer's Disease
M.A. Warpehoski *et al.*, US Patent 6,962,934 (November 8, 2005)
Assignee Elan Pharmaceuticals, Inc. and Pharmacia & Upjohn Company
Utility Treatment of Alzheimer's Disease

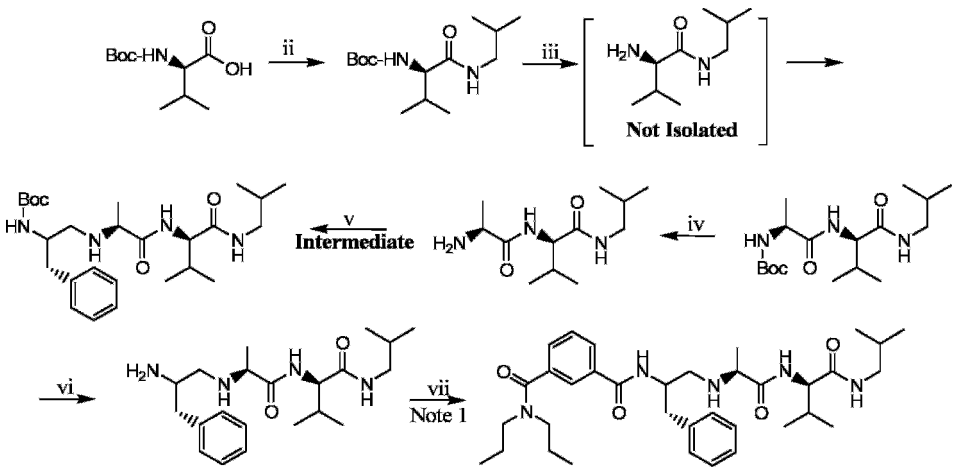
Invention Significance The presence of β-amyloid plaques is a defining feature of Alzheimer's disease. β-Amyloid plaques are composed of the amyloid β-peptide, βA_4, the latter of which is generated by proteolysis of the amyloid precursor protein which requires the β-secretase enzyme. To address this disorder, β-secretase enzyme inhibitors have been prepared to interrupt this cycle.

Reaction

Eq. 1



Intermediate



i- Toluene, diisobutylaluminum hydride, potassium sodium tartrate
ii- THF, carbonyldiimidazole, isobutylamine
iii- Trifluoroacetic acid, CH_2Cl_2, *N*-*t*-butoxycarbonylalanine, 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide, 1-hydroxybenzotriazole, DMF, *N*-methylmorpholine
iv- Trifluoroacetic acid, CH_2Cl_2
v- Sodium triacetoxyborohydride, 1,2-dichloroethane, THF, acetic acid
vi- Trifluoroacetic acid, CH_2Cl_2
vii- 3-[(Dipropylamino)carbonyl]benzoic acid,1-(3-dimethyl aminopropyl)-3-ethylcarbodiimide, 1-hydroxybenzotriazole, DMF

Experimental

1. **Preparation of *N*-*t*-butoxycarbonylphenylalanal**

N-*t*-Butoxycarbonylphenylalanine methyl ester (3.6 mmol) was dissolved in 15 ml toluene, then cooled to −78° C, and treated with the dropwise addition of 9.0 ml 1 M solution of diisobutylaluminum hydride in toluene over 5 minutes. After 1 hour, the reaction was slowly quenched with 1 ml methyl alcohol, and then poured into a cooled aqueous solution of potassium sodium tartrate. The mixture was stirred 2 hours, then extracted with diethyl ether, washed with water and brine, dried over Na_2SO_4, and concentrated to a colorless oil. The oil was purified by chromatography using silica gel with 20% EtOAc/heptane and the product isolated in 88% yield as a white solid.

^{1}H NMR ($CDCl_3$) δ 9.6 (s, 1H), 7.33–7.16 (m, 5H), 5.03 (b d, 1H), 4.43 (q, $J = 7$ Hz, 1H), 3.12 (d, $J = 7$ Hz, 2H), 1.43 (s, 9H)

2. **Preparation of *N*-2-(*t*-butoxycarbonyl)-*N*-1-isobutyl-L-valinamide**

N-*t*-Butoxycarbonylalanine (10 mmol) dissolved in THF was treated with carbonyldiimidazole (12 mmol), then the mixture stirred 50 minutes, and further treated with isobutylamine (20 mmol). After stirring 1 week, the mixture was concentrated and the residue dissolved in EtOAc. It was then washed with aqueous $KHSO_4$, water, and brine, dried using Na_2SO_4, and the product isolated as a white solid in 83% yield.

^{1}H NMR ($CDCl_3$) δ 6.0 (br, 1H), 5.07 (br d, 1H), 3.83 (m, 1H), 3.10 (m, 2H), 2.14 (m, 1H), 1.77 (m, 1H), 1.44 (s, 9H), 1.01–0.90 (overlapping d, 12H)
MS (ESI) Calc. for $C_{14}H_{28}N_2O_3 + H1$ 273.217 8; found 273.218 0

3. **Preparation of *N*-(*t*-butoxycarbonyl)-L-alanyl-*N*-1-isobutyl-L-valinamide**

In a separate vessel, the Step 2 product (5.95 mmol) was stirred 1 hour with 6 ml trifluoroacetic acid in 10 ml CH_2Cl_2 and then concentrated. The residue was dissolved in 5 ml DMF and 2.0 ml *N*-methylmorpholine added.

In another vessel, a mixture consisting of *N*-*t*-butoxycarbonylalanine (6.5 mmol), 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide (6.5 mmol), and 1-hydroxybenzotriazole (6.5 mmol) dissolved in 10 ml DMF was stirred 1 hour and then concentrated. This residue was then added to the first vessel and stirred 3 days. The reaction was quenched with aqueous $KHSO_4$, then diluted with EtOAc, washed with water, aqueous $NaHCO_3$, and brine, and dried over Na_2SO_4. The mixture was concentrated, triturated with diethyl ether to remove residual DMF, and the product isolated as a white solid in 73% yield.

^{1}H NMR ($CDCl_3$) δ 6.70 (d, $J = 9$ Hz, 1H), 6.32 (br, 1H), 5.0 (br d, 1H), 4.2 (m, 1H), 4.13 (m, 1H), 3.08 (m, 2H), 2.26 (m, 1H), 1.79 (m, 1H), 1.45 (s, 9H), 1.37 (d, $J = 7$ Hz, 3H), 0.96–0.89 (overlapping d, 12H)
MS (CI) m/z 344 (MH^+)

4. **Preparation of L-alanyl-*N*-1-isobutyl-L-valinamide**

The Step 3 product (0.72 mmol) was dissolved in 1 ml trifluoroacetic acid with 3 ml CH_2Cl_2, then stirred 30 minutes, concentrated, and 2 ml saturated $NaHCO_3$ solution added to the residue to raise the pH to 8. The residue was diluted with 100 ml diethyl ether, dried, concentrated, and 136 mg of product isolated as an off-white solid.

^{1}H NMR (MeOD)-d3 δ 4.66 (bs) 4.16 (d, $J = 8$ Hz, 1H), 4.00 (q, $J = 7$ Hz, 1H), 3.07 (dd, 1H), 2.98 (dd, 1H), 2.05 (m, 1H), 1.79 (m, 1H), 1.49 (d, $J = 7$ Hz, 3H), 0.99 (d, $J = 7$ Hz, 6H) 0.93 (d, $J = 7$ Hz, 6H).
HRMS (ESI) Calc. for $C_{12}H_{25}N_3O_2 + H1$ 244.202 5; found 244.202 3

5. **Preparation of *N*-[(2*S*)-2-[(*t*-butoxycarbonyl)amino]-3-phenylpropyl]-L-alanyl-*N*-1-isobutyl-L-valinamide**

The Step 1 product (0.42 mmol) and the Step 4 product (0.42 mmol) were mixed with sodium triacetoxyborohydride (0.66 mmol) in 4 ml apiece of 1,2-dichloroethane and

THF containing acetic acid (0.66 mmol) and a small amount of methyl alcohol and then stirred overnight. The reaction was quenched with aqueous $NaHCO_3$ and the mixture extracted with 70 ml EtOAc containing a small amount of methyl alcohol. The organic phase was washed with $NaHCO_3$ solution and brine, dried, and concentrated. The residue was purified by chromatography with silica gel using 2–5% methyl alcohol/CH_2Cl_2, and the product isolated as a white solid in 42% yield.

^{1}H NMR ($CDCl_3$) δ 7.76 (br d, 1H), 7.3–7.17 (m, 5H), 6.40 (br m, 1H), 4.8 (br d, 1H), 4.13 (dd, 1H), 3.87 (m, 1H), 3.1 (m, 2H), 3.0 (m, 1H), 2.9-2.7 (m, 3H), 2.62 (dd, 1H), 2.15 (m, 1H), 1.76 (m, 1H), 1.39 (s, 9H), 1.27 (d, $J = 7$ Hz, 3H), 0.96–0.89 (overlapping d, 12H)

6. **Preparation of *N*-[(2*S*)-2-amino-3-phenylpropyl]-L-alanyl-N-1-isobutyl-L-valinamide**

The Step 5 product (0.176 mmol) was stirred 30 minutes with 1 ml trifluoroacetic acid and 3 ml CH_2Cl_2, concentrated, and the residue treated with saturated aqueous $NaHCO_3$. The mixture was then extracted using 70 ml diethyl ether, washed with aqueous $NaHCO_3$, concentrated, and 75 mg product isolated as slightly yellow oil.

^{1}H NMR ($CDCl_3$) δ 7.96 (d, $J = 9$ Hz, 1H), 7.3–7.17 (m, 5H), 6.88 (br t, 1H), 4.13 (m, 1H), 3.27 (m, 1H), 3.13 (m, 1H), 3.05 (m, 1H), 2.94 (m, 1H), 3.83 (m, 2H), 2.7 (dd, 1H), 2.57 (dd, 1H), 2.08 (m, 1H), 1.72 (m, 1H), 1.31 (d, $J = 7$ Hz, 3H), 0.93–0.86 (overlapping d, 12H)

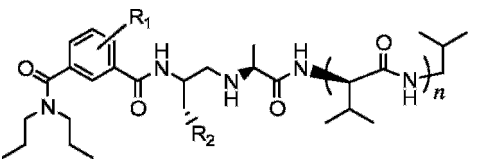
7. **Preparation of *N*-[(2*S*)-2-({3-[(dipropylamino)carbonyl]-benzoyl}amino)-3-phenylpropyl]-L-alanyl-*N*-1-isobutyl-L-valinamide**

A mixture consisting of 3-[(dipropylamino)carbonyl]benzoic acid (0.21 mmol), 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide (0.21 mmol), and 1-hydroxy-benzotriazole (0.22 mmol) was dissolved in 2.5 ml DMF, then stirred 1 hour, and treated with the Step 6 product (0.17 mmol) dissolved in 1 ml of DMF. The mixture was stirred 3 days at ambient temperature and was then quenched with 1 M KH_2PO_4. It was then diluted with EtOAc, washed with water, 1 M $NaHCO_3$, and brine, dried with Na_2SO_4, and concentrated. The residue was purified by chromatography using 5% methyl alcohol/CH_2Cl_2 and the product isolated in 51% yield.

^{1}H NMR ($CDCl_3$+MeOD) δ 7.79 (br d, 1H), 7.69 (s, 1H), 7.44 (m, 2H), 7.30 (m, 4H), 7.26 (m, obscured by $CHCl_3$), 7.19 (m, 2H), 4.33 (m, 1H), 4.03 (d, $J = 8$ Hz, 1H), 3.5–3.4 (m, 3H), 3.13–3.0 (m, 6H), 2.90 (m, 1H), 2.68 (dd, 1H), 2.0 (m, 1H), 1.8–1.7 (m, 3H), 1.5 (m, 2H), 1.31 (d, $J = 7$ Hz, 3H), 0.99 (t, $J = 7$ Hz, 3H), 0.93–0.86 (overlapping d, 12H), 0.72 (t, $J = 7$ Hz, 3H)
MS (FAB) m/z 608 (MH^+)

Derivatives

Table 1 Selected experimental agents and their corresponding mass spectra characterization data. Each agent was effective as a β-secretase enzyme inhibitor



Entry	R_1	R_2	*n*	HRMS (ESI)
3	Hydrogen	Isobutyl	1	574.43
4	5-Methyl	3,5-Difluorophenyl	0	559.35
6	Hydrogen	3,5-Difluorophenyl	1	644.40
8	Hydrogen	Cyclohexyl	1	614.41
10	Hydrogen	Phenyl	0	509.35

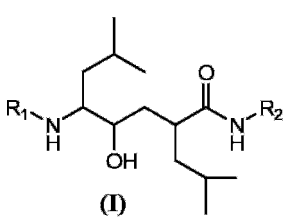
Testing

I. β-*Secretase Enzyme Assay*

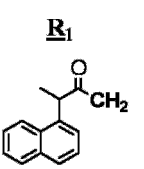
Enzyme activity and inhibition of enzyme activity were performed using the method of Chrysler (1). Although test results were not supplied by the author, experimental agents appearing in Table 1 were especially preferred.

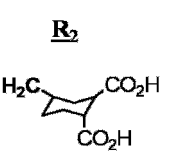
Notes

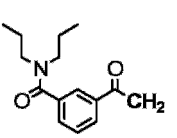
1. Hydroxyethylene analogs, (**I**), effective as β-secretase enzyme inhibitors prepared by Hom (2) were effective in treating amyloid plaques and slowing the progression of Alzheimer's disease.

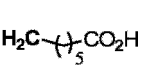


(I)

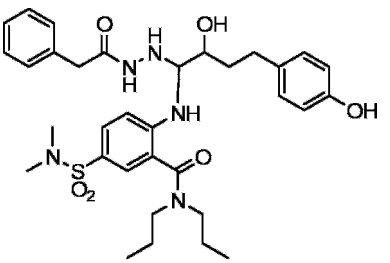






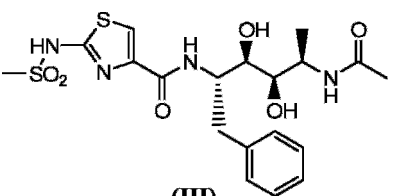


2. Schostarez (3) prepared aza-hydroxylethyl derivatives, (**II**), which were effective as β-secretase enzyme inhibitors in the treatment of amyloid-related diseases.



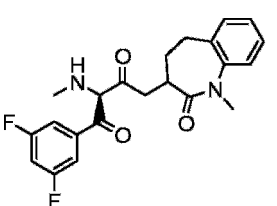
(II)

3. In subsequent investigations by Schostarez (4), diaminediol derivatives, (**III**), effective as β-secretase enzyme inhibitors were prepared, which were effective in reducing amyloid peptide formation and used in treating dementia and neurodegenerative disorders.

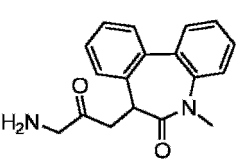


(III)

4. Lactones, (**IV**) and (**V**), prepared by Wu (5) and Audia (6), respectively, were effective as β-secretase enzyme inhibitors and useful in treating Alzheimer's disorder.

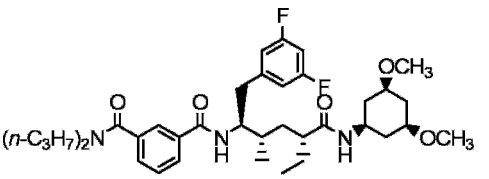


(IV)



(V)

5. Amide, (**VI**), derivatives of the current invention prepared by Hom (7) were also effective as β-secretase enzyme inhibitors and used in treating Alzheimer's disease.



(VI)

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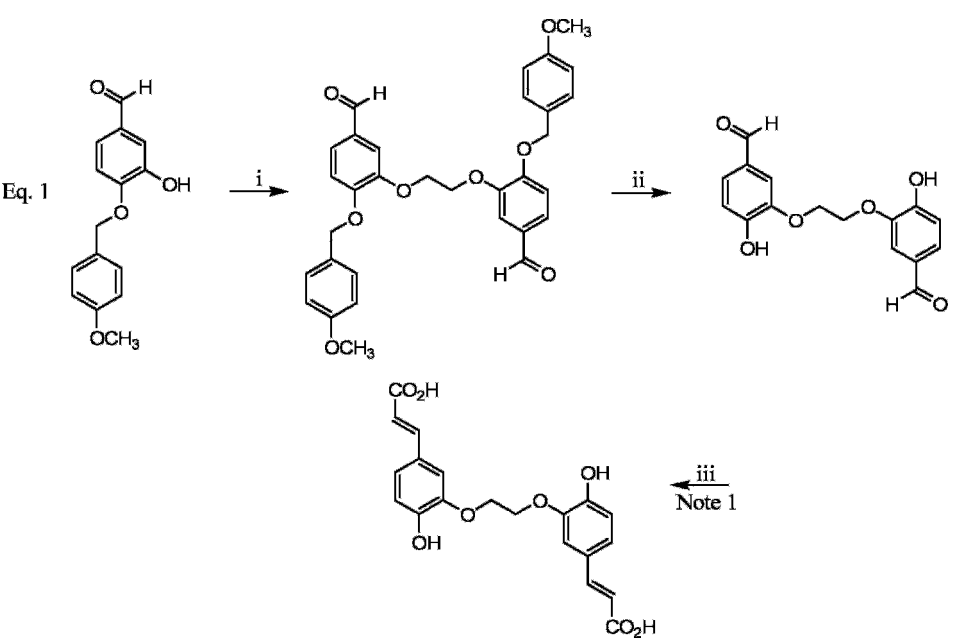
1. S.M.S. Chrysler *et al.*, US Patent 5,744,346 (April 28, 1998)
2. R. Hom *et al.*, US Patent 6,737,420 (May 18, 2004)
3. H. Schostarez *et al.*, US Patent 6,960,664 (November 1, 2005)
4. H. Schostarez *et al.*, US Patent 7,067,542 (June 27, 2006) and US Patent 7,053,109 (May 30, 2006)
5. J. Wu *et al.*, US Patent 6,951,854 (October 4, 2005)
6. J.E. Audia *et al.*, US Patent 6,958,330 (October 25, 2005)
7. R. Hom *et al.*, US Patent 6,992,081 (January 31, 2006)

B. Transthyretin Stabilizers

Title Ferulic Acid Dimers and Their Pharmaceutically Acceptable Salts, Their Preparation, and Use Thereof for Treating Dementia
J. Yu *et al.*, US Patent 7,005,539 (February 28, 2006)
Assignee Korea Institute of Science and Technology
Utility β-Amyloid Inhibitory Agent Useful in Treating Memory Loss Associated with Dementia

Invention Significance Although transthyretin is a stable tetrameric protein at physiological pH conditions, it is destabilized at low pH. Monomeric units from this disassociation subsequently aggregate to form β-amyloid plaque causing familial amyloid neuropathy. Ferulic acid dimers that stabilize the tetrameric integrity of transthyretin have been prepared to address this disorder.

Reaction



i- DMF, sodium hydride, ethylene glycol ditosylate
ii- Hydrochloric acid
iii- Malonic acid, pyridine, piperidine

Experimental

1. **Preparation of 1,2-[2-(*para*-methoxybenzyloxy)-5-formyl]phenoxyethane**

   4-(4-Methoxybenzyloxy)-3-hydroxybenzaldehyde (38.7 mmol) was dissolved in 200 ml DMF and 60% NaH (38.7 mmol) slowly added, and then stirred 30 minutes at ambient temperature. Ethylene glycol ditosylate (19.4 mmol) was added and the mixture stirred 48 hours at 30°C. It was then poured into 3000 ml water and a solid isolated. The solid was washed with 2000 ml water and 1000 ml hexane, dried, and the product isolated in 87.9% yield.

   ^{1}H NMR (300 MHz, DMSO) δ 9.79 (s, 2H), 7.52 (d, 2H, $J = 8.25$ Hz), 7.49 (s, 2H), 7.31 (d, 4H, $J = 8.52$ Hz), 7.24 (d, 2H, $J = 8.25$ Hz), 6.83 (d, 4H, $J = 8.52$ Hz), 5.11 (s, 4H), 4.20 (s, 4H)

2. **Preparation of 1,2-(2-hydroxy-5-formyl)phenoxyethane**

   The Step 1 product (12.6 mmol) dissolved in 250 ml ethyl alcohol was treated with 100 ml 1 M HCl, then refluxed 2 hours, and concentrated. The residue was added to 500 ml water and a solid formed, which was isolated by filtration. The solid was washed with 500 ml apiece water and hexane, dried, and the product isolated in 97% yield as a pale yellow solid.

   ^{1}H NMR (300 MHz, DMSO) δ 9.78 (s, 2H), 7.49 (d, 2H, $J = 1.74$ Hz), 7.44 (dd, 2H, $J = 1.74, 8.06$ Hz), 6.99 (d, 2H, $J = 8.06$ Hz), 4.42 (s, 4H)

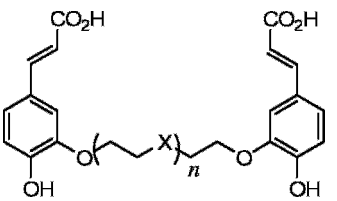
3. **Preparation of 1,2-[2-hydroxy-5-(2-carboxyvinyl)]phenoxyethane**

   The Step 2 product (3.31 mmol) and malonic acid (13.2 mmol) were dissolved in 27 ml pyridine, then treated with 1.01 ml piperidine, and the mixture heated 4 hours at 50 C. Once cooled to ambient temperature, crystals were formed, which were isolated by filtration. The crystals were dissolved in water and 2 M HCl added to obtain an acidic pH. The crystals were refiltered, washed with 200 ml water, dried, and the product isolated in 52.4% yield as white crystals.

   ^{1}H NMR (300 MHz, DMSO) δ 12.2 (bs, 2H), 9.55 (s, 2H), 7.49 (d, 2H, $J = 15.87$ Hz), 7.31 (s, 2H), 7.11 (d, 2H, $J = 8.19$ Hz), 6.83 (d, 2H, $J = 8.19$ Hz), 6.37 (d, 2H, $J = 15.87$ Hz), 4.39 (s, 4H)

Derivatives

Table 1 Selected ferulic acid derivatives evaluated as antineurodegenerative agents. Only Entry 1 was effective in improving learning and memory retention. ^{1}H NMR data for all derivatives supplied by author



Entry	X	*n*
1	None	0
2	CH_2	1
3	CH_2	2
4	O	1
5	O	2
6	O	3
7	NH	2
8	NH	2

Testing

I. *Effect of Administration, Learning, and Memory-Retention Ability on Mice*

Four groups of 10 mice aged 4–5 weeks, weighing 20–25 g, were administered one of the agents listed below:

(A) Reverse phase β-amyloid
(B) β-Amyloid
(C) Entry 1 and β-amyloid
(D) Ferulic acid and β-amyloid

After injection of 1.85 g sample to the cerebral ventricle on each of three consecutive days, passive avoidance testing on days 1 and 2 and Y-maze testing on days 3 and 4 were conducted. Learning and memory-retention ability of the mice were performed according to the method of Song (1), while spontaneous alternation behavior of mice in a Y-maze test was performed according to the method of Yamada (2).

Test results indicated that the step-through latency period was significantly higher for Group C mice administered with entry 1 and β-amyloid as compared with Group B mice administered β-amyloid alone.

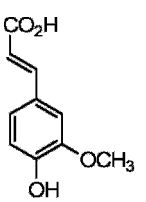
II. *Oral Toxicity Test of Korea Angelica Root Extract*

Twenty female and twenty male Sprague–Dawley rats aged 4 weeks were divided into four groups and orally administered experimental agents with solution dosages of 300, 1000, 3000, and 10 000 mg/kg one time.

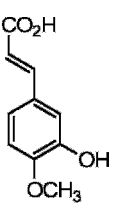
LD_{50} values of experimental agents were 3722 mg/kg for males and 2804 mg/kg for females.

Notes

1. Ferulic acid, (**I**), is obtained from the Korean angelica root by extraction. When administered to mice in the current investigation, a considerable improvement in memory retention was observed. This effect has also been reported by Castillo (3). Isoferulic acid, (**II**), was ineffective in treating dementia.

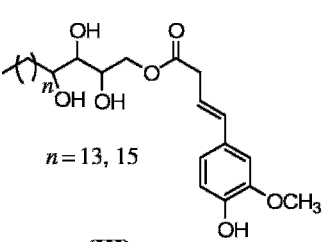


(I)

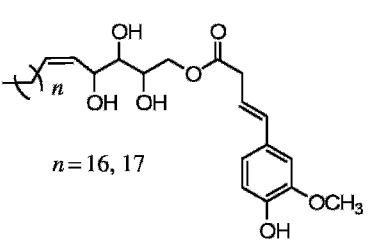


(II)

2. Snow (4) and Castillo (5) isolated an extract from the inner bark or root tissue within *Uncaria tomentosa*, which was effective in treating Alzheimer's disease and other amyloidoses. The structure of this agent remains undetermined.
3. Majeed (6) extracted ferulate esters, (**III**) and (**IV**), from *Commiphora wightii*, which were effective in the treatment of abnormal cell growth and neoplasia.



(III)



(IV)

References

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2. Yamada *et al.*, Eur. J. Pharmacol., 349, 15 (1998)
3. G. Castillo *et al.*, US Patent 6,607,758 (August 19, 2003)
4. A. Snow *et al.*, US Patent 6,939,570 (September 6, 2005)
5. G. Castillo *et al.*, US Patent 7,029,710 (April 18, 2006)
6. M. Majeed *et al.*, US Patent 6,436,991 (August 20, 2002)

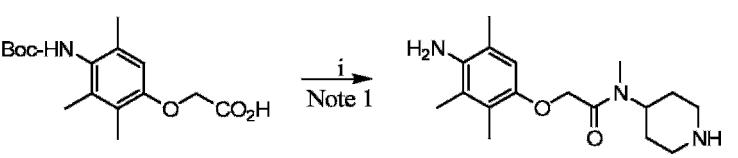
II. Calbindin D28Kd Inducing Agents

Title Aminophenoxyacetic Acid Derivatives and Pharmaceutical Composition Containing Thereof
H. Annoura *et al.*, US Patent 6,998,401 (February 14, 2006)
Assignee Suntory Limited
Utility Calbindin D28Kd Neuroprotective Activators in the Treatment of Alzheimer's Disease

Invention Significance Calbindin D28Kd is one of the Ca^{2+}-binding proteins. It is a macromolecular protein with 28 000 AMU and a neuroprotective agent but impractical to administer to the central nervous system. To address this problem, low molecular weight compounds have been prepared which can induce or increase calbindin D28Kd useful in treating ischemic brain disorders such as dementia, sequelae infarction, and cerebral arteriosclerosis.

Reaction

Eq. 1



i- 1-(*t*-Butoxycarbonyl)-4-methylaminopiperidine benzotriazol-1-yl-oxytris(dimethyl-amino) phosphonium hexafluorophosphate, triethylamine, DMF

Experimental

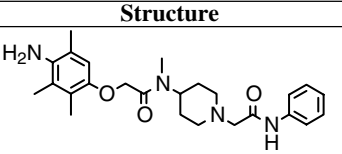
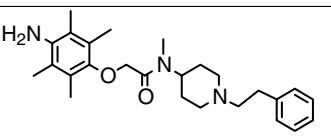
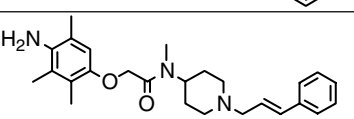
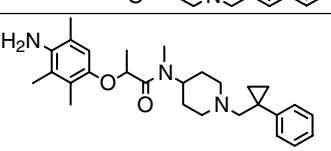
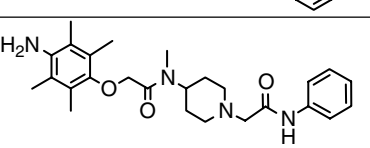
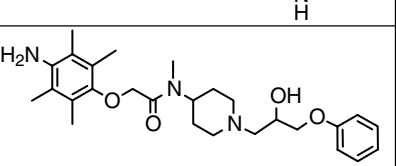
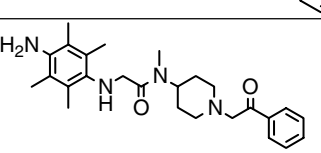
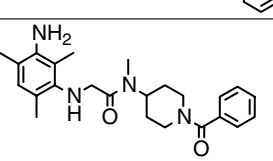
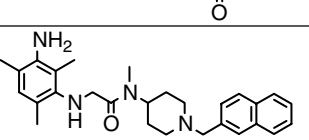
1. **Preparation of 2-(4-amino-2,3,5-trimethylphenoxy)-*N*-methyl-*N*-(4-piperidinyl)-acetamide**

A mixture consisting of 2-[4-(*t*-butoxycarbonylamino)-2,3,5-trimethylphenoxy]acetic acid (1.86 g), 1-(*t*-butoxycarbonyl)-4-methylaminopiperidine (2.94 g), and benzotriazol-1-yl-oxytris(dimethyl-amino)phosphonium hexafluorophosphate (1.43 sg) was dissolved in 30 ml DMF containing 1.26 ml triethylamine, then stirred overnight at ambient temperature. The mixture was then treated with 15 ml saturated $NaHCO_3$ solution and extracted with EtOAc. The extract was washed with brine, dried, and concentrated. The residue was dissolved in 30 ml CH_2Cl_2, then treated with 7.5 ml trifluoroacetic acid at 0°C, stirred 2 hours at ambient temperature, and concentrated.

The residue was purified by chromatography with silica gel using CH_2Cl_2/methyl alcohol, 12:1, and the product isolated in 81% yield.

Derivatives

Table 1 Selected aminophenoxyacetic acid derivatives and their corresponding melting points. FT-IR and ^1H NMR data also supplied by author

Entry	Structure	mp (°C) HCl salt
29		177–178
40		173–175
53		169–171
90		172–175
91		221–224
103		143–147
111		218–220
128		166–168
133		186–189

Testing

I. *Cytoprotective Effect Against Glutamate-Induced Cell Death*

Cytoprotective effect against glutamate-induced cell death was performed using the method of Mattoson (1). Testing results are provided in Table 2.

Table 2 Survival rate of living cells after treatment with 1 μM of selected experimental agents following incubation

Entry	Survival Rate[a] (%)
40	108
53	144
90	149
91	203
103	180
133	101
Untreated	100

[a] Testing results greater than 100% indicate that the untreated test group value was arbitrarily set at 100%.

II. *Calbindin D28Kd Inducing Effect*

Calbindin D28Kd inducing effect using selected experimental agents was determined according to the method of Mattoson (1). Testing results are provided in Table 3.

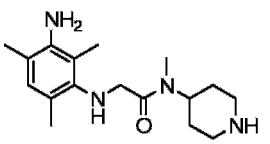
Table 3 Efficacy of selected experimental agents as calbindin D28Kd inducing agents

Entry	Amount of Induced Calbindin D28Kd[a](%)
29	122
40	150
111	167
128	171
Untreated	100

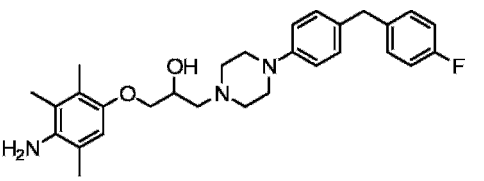
[a] Testing results greater than 100% indicate that the untreated test group value was arbitrarily set at 100%.

Notes

1. Additional aminophenoxyacetic acid derivatives, (**I**), and arylpiprazinopropanol derivatives, (**II**), having calbindin D28Kd inducing effects were prepared by the author (2,3), respectively, in previous investigations.

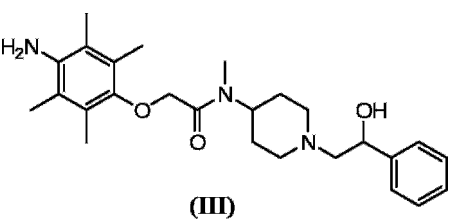


(I)



(II)

2. Aminophenoxyacetamide derivatives, (**III**), prepared by Takemoto (4) were effective in increasing calbindin D28k and used as neuroprotectants for treating cerebral functional and cerebral organic disorders.



(III)

References

1. M.P. Mattoson, Brain Res. Rev., 13, 179 (1988)
2. H. Annoura *et al.*, US Patent 6,559,146 (May 6, 2003)
3. H. Annoura *et al.*, US Patent 6,838,470 (January 4, 2005) and US Patent 6,525,199 (February 25, 2003)
4. N. Takemoto *et al.*, US Patent 7,067,533 (June 27, 2006)

III. Metabotropic Glutamate 5a Receptor Antagonists

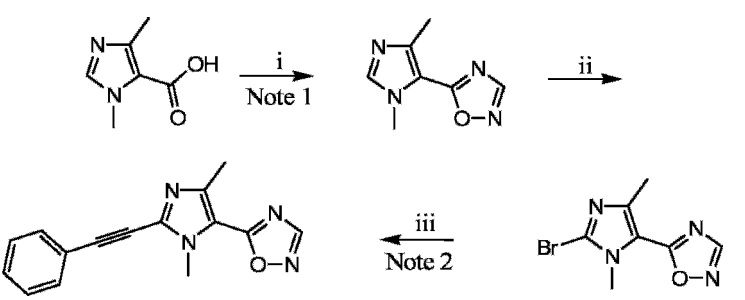
A. Phenylethynyl and Styryl Imidazole Antagonists

Title Phenylethynyl and Styryl Derivatives of Imidazole and Fused Ring Heterocycles
V. Mutel *et al.*, US Patent 6,927,232 (August 9, 2005)
Assignee Hoffman-La Roche Inc.
Utility Treatment of Alzheimer's Disorder

Invention Significance Glutamate is the major excitatory neurotransmitter in the brain and plays a unique role in a variety of central nervous system functions. The metabotropic glutamate receptors (mGluR) can be used in the treatment or in the prevention of acute or chronic neurological disorders such as Alzheimer's disease, cognitive, and psychiatric disorders. A method of treating Alzheimer's disease using phenylethynyl and styryl derivatives, which are effective as mGluR 5a antagonists, is described.

Reaction

Eq. 1



i- 1,1′-Carbonyldiimidazole, DMF, *N*-hydroxy-acetamidine
ii- $CHCl_3$, bromine
iii- Bis(triphenylphosphine)palladium(II) chloride, phenylacetylene, triethylamine, triphenylphosphine

Experimental

1. **Preparation of 5-(3,5-dimethyl-3H-imidazol-4-yl)-3-methyl-[1,2,4]oxadiazole**

   A solution of 3,5-dimethyl-3H-imidazole-4-carboxylic acid (7.14 mmol) and 1,1′-carbonyl-diimidazole (10.7 mmol) in 35 ml DMF was stirred 3 hours at ambient temperature and then treated with *N*-hydroxy-acetamidine (9.18 mmol). The mixture was stirred an additional 16 hours at 80°C, then concentrated, and the residue dissolved in 30 ml acetic acid. This mixture was stirred 2 hours at 100°C and was then reconcentrated. The residue was then treated with 50 ml saturated $NaHCO_3$ solution and extracted seven times with 30 ml CH_2Cl_2. The extracts were washed with 70 ml brine, dried using $MgSO_4$, concentrated, and the product isolated in 61% yield as a white solid, mp = 95°C.

   MS *m*/*z* 178.2 (M^+)

2. **Preparation of 5-(2-bromo-3,5-dimethyl-3H-imidazol-4-yl)-3-methyl-[1,2,4]-oxadiazole**

   The Step 1 product (3.93 mmol) was dissolved in 7 ml $CHCl_3$, then treated with the dropwise addition of bromine (5.89 mmol) dissolved in 7 ml $CHCl_3$, and stirred 26 hours at ambient temperature. The solution was concentrated and the residue poured into 40 ml saturated $NaHCO_3$ solution, then extracted twice with 30 ml CH_2Cl_2. The extracts were washed with 40 ml brine, dried using $MgSO_4$, concentrated, and yellow oil isolated. The residue was purified by chromatography using EtOAc/methyl alcohol, 98:2, and the product isolated as a white solid in 51% yield, mp = 89°C.

   MS *m*/*z* 256, 258 (M^+)

3. **Preparation of 5-(3,5-methyl-2-phenylethynyl-3H-imidazol-4-yl)-3-methyl-[1,2,4]oxadiazole**

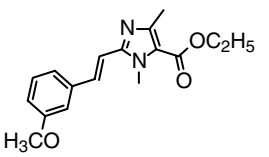
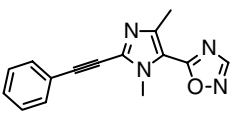
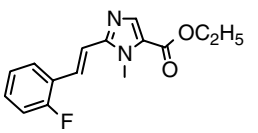
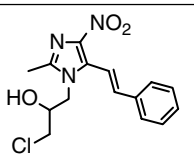
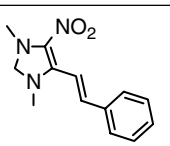
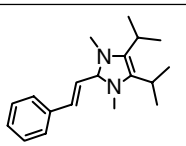
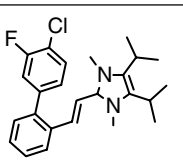
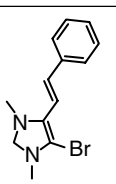
   A solution of the Step 2 product (2.02 mmol) dissolved in THF (10 mmol) was treated with bis(triphenylphosphine)palladium(II) chloride (0.1 mmol), phenylacetylene (3.03 mmol), triphenylphosphine (0.1 mmol), and triethylamine (6.07 mmol) at ambient temperature. Argon was bubbled through the mixture for 10 minutes and stirring was continued for 16 hours at 55°C. The mixture was then poured into 50 ml water and extracted twice with 50 ml EtOAc. Combined extracts were washed with 40 ml brine, dried, concentrated, and yellow oil isolated. The residue was purified by chromatography using EtOAc/toluene, 5:1, and the product isolated as a light yellow solid in 55% yield, mp = 137°C.

   MS *m*/*z* 278.1 (M^+)

Derivatives

Selected phenylethynyl and styryl imidazole derivatives are provided in Table 1.

Table 1 Selected phenylethynyl and styryl imidazole experimental derivatives and their associated mass spectra characterization data and inhibition concentrations (IC_{50}). Agents having mGluR5a receptor antagonist activity of 2 μM or less are preferred

Entry	Structure	MS (M + H^+)	IC_{50} (μM)
3		299.3	0.35
12		278.1	0.011
17		287.4	0.09
26		321.9	0.23
30		242	0.02
32		269.4	1.82
38		320.8	10
43		263.0	3.06

Testing

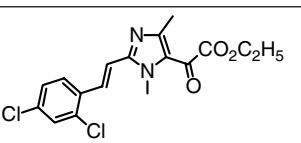
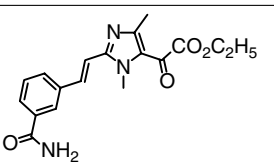
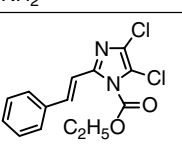
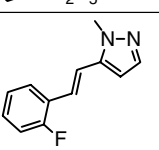
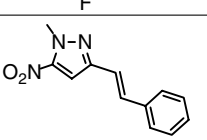
I. *mGluR5a Receptor Antagonist Testing*

cDNA encoding rat mGlu5a receptor was transfected into EBNA cells using the procedure of Schlaeger (1). $[Ca^{2+}]_i$ measurements were performed on mGlu5a-transfected EBNA cells after incubation of the cells with Fluo 3-AM 1 hour at 37°C followed by four washes with DMEM supplemented with Hank's salt and 20 mM HEPES. $[Ca^{2+}]_i$ measurements were done using a fluorometric imaging plate reader. Experimental agents evaluated as antagonists were tested against 10 μM glutamate as agonist. Inhibition curves were fitted with a four-parameter logistic equation giving IC_{50} and Hill coefficient using iterative nonlinear curve fitting software. Testing results are provided in Table 1.

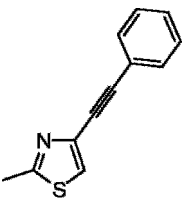
Notes

1. The Step 1 co-reagent, 3,5-dimethyl-3H-imidazole-4-carboxylic acid, was prepared according to the method of Leone-Bay (2).
2. Additional Step 3 derivatives effective as mGluR5a receptor antagonists were prepared by the author (3) in an earlier investigation and illustrated in Table 2.

Table 2 Selected styryl imidazole derivatives effective as mGluR5a receptor antagonists previously prepared by the author (3)

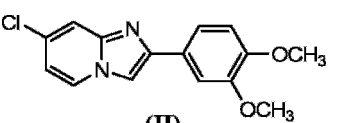
Structure	IC_{50} (μM)
	10
	2.12
	0.52
	0.07
	0.02

3. 4-(Phenylethynyl)-1,3-thiazole derivatives, (**I**), prepared by Cosford (4) were effective as GluR5a antagonists and used in treating neurodegenerative disorders such as Alzheimer's disease.



(I)

4. In earlier investigations by the author (5), mGluR5a receptor antagonists consisting of imidazo[1,2-a]pyridine derivatives, (**II**), were prepared and were effective in treating Alzheimer's disease.



(II)

References

1. E.-J. Schlaeger *et al.*, Cytotechnology, 30, 71 (1999)
2. A. Leone-Bay, US Patent 4,711,962 (December 8, 1987) and US Patent 4,571,257 (February 18, 1986)
3. V. Mutel *et al.*, US Patent 6,706,707 (March 16, 2004)
4. N.D.P. Cosford *et al.*, US Patent 6,956,049 (October 18, 2005)
5. V. Mutel *et al.*, US Patent 6,916,826 (July 12, 2005) and US Patent 6,861,437 (March 1, 2005)

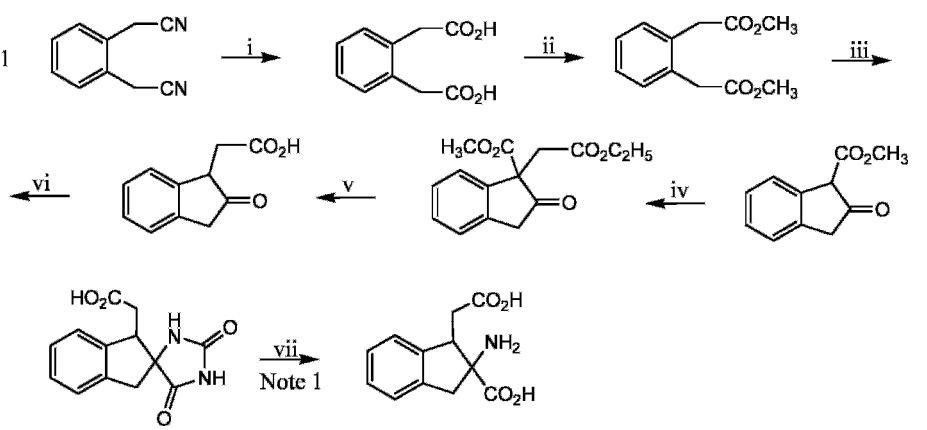
B. Metabotropic Glutamate Receptor Agonists/Antagonists

Title 2-Aminoindane Analogs
K. Curry, US Patent 7,034,055 (April 25, 2006)
Assignee Prescient Neuropharma Inc.
Utility Treatment of Acute and Chronic Neurological Disorders including Alzheimer's Disease

Invention Significance While current treatment options for neurological disorders reduce clinical symptoms, they also adversely impact the central nervous system because of the nonspecificity of the treatment agent. To address this concern, medicaments designed for treating central nervous system diseases associated to the metabotropic glutamate receptor system have been prepared which act as either agonist or antagonist.

Reaction

Eq. 1



i- Sulfuric acid
ii- Methyl alcohol, hydrogen chloride
iii- Benzene, sodium
iv- 1,8-Diazabicyclo-[5.4.0]undec-7-ene, ethylbromoacetate, DMF
v- Hydrochloric acid, acetic acid
vi- Ethyl alcohol, water, potassium cyanide, ammonium carbonate
vii- Sodium hydroxide, water, propylene oxide

Experimental

1. **Preparation of 1,2-phenylenediacetic acid**

   Phenylenediacetonitrile (10 g) and 50% sulfuric acid (180 g) were refluxed 2 hours, then poured onto ice, and 11.4 g product isolated. This compound was used without further purification.

2. **Preparation of dimethyl-1,2-phenylenediacetate**

   The Step 1 product was dissolved in 150 ml methyl alcohol saturated with hydrogen chloride gas, then refluxed 2 hours, cooled, and then concentrated. The residue was dissolved in 200 ml diethyl ether, then washed with saturated $NaHCO_3$ solution, dried with $MgSO_4$, and concentrated. The residue was purified by flash chromatography and 11.1 g product isolated.

3. **Preparation of methyl-2-indanone-1-carboxylate**

   The Step 2 product (10 g) dissolved in 50 ml benzene was treated with sodium (1.5 g) and stirred until hydrogen evolution stopped. The solution was then washed sequentially with 50 ml apiece 1 M HCl, water, and brine, then dried, and concentrated. The residue was recrystallized from water/methyl alcohol and the product isolated in 92% yield as a light yellow solid.

   ^{1}H NMR ($CDCl_3$) δ 3.18 (s, 2H, enolate), 3.59 (s, 2H), 3.79 (s, 3H, enolate), 3.97 (s, 3H), 7.05–7.62 (4H), 11.03 (s, 1H), 12.13 (s, 1H, enolate)
   The ratio of ketone:enolate 5.6:1

4. **Preparation of ethyl-1-methoxycarbonyl-2-oxo-indaneacetate**

   The Step 3 product (6.02 g) was treated with 9.44 ml 1,8-diazabicyclo-[5.4.0]undec-7-ene and 7.0 ml ethylbromoacetate dissolved in 25 ml DMF and stirred overnight. The mixture was extracted three times with 50 ml EtOAc then washed with water, dried, filtered, and concentrated. The residue was purified by flash chromatography using EtOAc/hexanes, 10:30–90:70, and the product isolated in 82% yield as oil.

   ^{1}H NMR ($CDCl_3$) δ 3.20–3.42 (AB Pattern, 2H), 3.59 (s, 3H), 3.70–3.95 (4H), 7.15–7.35 (4H)

5. **Preparation of 2-oxo-indaneacetic acid**

   The Step 4 product was refluxed 2 hours in 60 ml 6 M HCl/acetic acid, 1:1. The mixture was cooled then recrystallized using water/methyl alcohol and the product isolated in 71% yield.

   ^{1}H NMR ($CDCl_3$) δ 2.90–3.10 (m, 2H), 3.58 (s, 2H), 3.70 (m, 1H), 7.1–7.4 (4H)

6. **Preparation of 1-indaneacelic acid-2-(5,5′-hydantoin)**

   The Step 5 product (1.9 g) was dissolved in 20 ml ethyl alcohol/water, 1:1, was treated with potassium cyanide (2.0 mmol) and ammonium carbonate (4.0 mmol),

then placed into a sealed tube, and heated 16 hours at 90–100°C. It was cooled, then acidified with 6 M HCl, and concentrated. The residue was extracted with ethyl alcohol, filtered, and used without further purification.

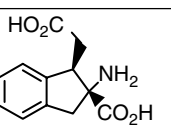
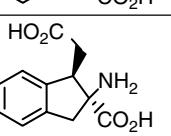
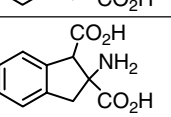
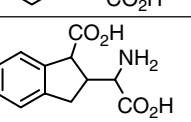
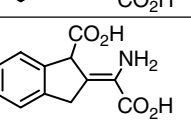
7. **Preparation of 2-amino-2-carboxyindaneacetic acid**

The Step 6 product was refluxed 16 hours with 45 ml 2 M NaOH, then acidified with 6 M HCl, and concentrated. The residue was dissolved in ethyl alcohol, then treated with propylene oxide, whereupon *cis* and *trans* isomers were precipitated. The isomers were separated by chromatography using a Spectrum 1X4 anion exchange resin with dilute acetic acid. Both *cis* and *trans* products were isolated as colorless crystals after recrystallization from methyl alcohol/water.

^{1}H NMR *Cis* isomer of compound (D_2O) δ 2.5–2.7 (m, 2H), 3.15 (d, 1H), 3.58 (d, 1H), 4.05 (m, 1H), 7.1–7.35 (m, 4H)
Trans isomer of compound ^{1}H NMR (D_2O) δ 2.55 (dd, 1H), 2.8 (dd, 1H), 3.23 (d, 1H), 3.55 (d, 1H), 3.85 (dd, 1H), 7.15–7.3 (m, 4H)
Analysis Calc. for compound: C, 61.27; H, 5.57; N, 5.95. Found: C, 57.05; H, 5.93; N, 5.45 contains 0.9 mol of H_2O

Derivatives

Table 1 Summary of experimental agents designed to modulate glutamate receptors by acting as either agonist or antagonists. ^{1}H NMR for all experimental agents provided by author

Entry	Structure
8 (*Cis*)	
8 (*Trans*)	
10	
14	
18	

Testing

I. *Ex Vivo Testing: Cyclic AMP Assay*

The Group II/III metabotropic glutamate receptors (mGluRs) testing protocol was performed using Sprague–Dawley rat tissue according to the method of Tovey (1). Cyclic adenosine 5′-monophosphate (AMP) assay testing results are provided in Table 2.

Table 2 Ex vivo testing results for cyclic AMP formation using Sprague–Dawley rat tissue. Experimental agents inhibiting forskolin-induced cyclic AMP accumulation are considered to be Group II/III agonists. Agents increasing forskolin-induced cyclic AMP accumulation caused by glutamate are considered to be Group II/III antagonists

Entry	Group II/III Agonist	EC_{50} (M)	Group II/III Antagonist	EC_{50} (M)
8 (*Cis*)	No	–	Yes	1.2×10^{-9}
8 (*Trans*)	Yes	1.1×10^{-7}	No	–

II. *In Vitro Testing: Phosphatidylinositol Assay*

Phosphatidylinositol hydrolysis testing was measured in clonal cell lines harboring a plasmid expressing the cloned metabotropic glutamate receptor in response to addition of glutamate agonists according to the method of Schoepp (2). Testing results are provided in Table 3.

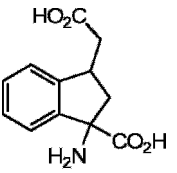
Table 3 Phosphatidylinositol assay from in vitro testing. Experimental agents causing an increase in intracellular free inositol phosphate accumulation are considered to be Group I agonists. Agents inhibiting inositol phosphate accumulation induced by ACPD[a] are considered to be Group I antagonists

Entry	Group I Agonist	EC_{50} (M)	Group I Antagonist	EC_{50} (M)
8 (*Cis*)	No	–	Yes	2.2×10^{-6}
8 (*Trans*)	No	–	Yes	1.6×10^{-3}

[a] *Trans*-(1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid.

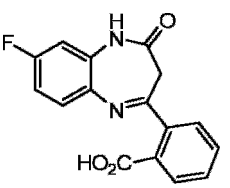
Notes

1. Additional 2-aminoindane derivatives, (**I**), effective as metabotropic glutamate receptor agonists or antagonists were prepared by the author (3) in an earlier investigation.

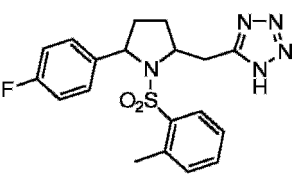


(I)

2. Diazepin-2-ones, (**II**), and sulfonyl pyrrolidine derivatives, (**III**), prepared by Adam (4) and Mutel (5), respectively, were effective as glutamate receptor antagonists and used in treating neurological diseases such as Alzheimer's disease and psychotic and schizophrenic disorders.

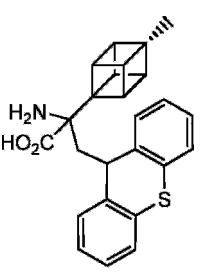


(II)



(III)

4. Cubane derivatives, (**IV**), prepared by Pajouhesh (6) were effective as metabotropic glutamate receptor agonists or antagonists and used in treating neurological disorders.



(IV)

References

1. S. Tovey *et al*., Clin. Chim. Acta, 56, 221 (1974)
2. D. Schoepp, Trends Pharmacol. Sci., 11, 508 (1990)
3. K. Curry, US Patent 6,699,909 (March 2, 2004)
4. G. Adam *et al*., US Patent 7,018,998 (March 28, 2006); US Patent 6,949,542 (September 27, 2005); and US Patent 6,960,578 (November 1, 2005)
5. V. Mutel *et al*., US Patent 6,995,182 (February 7, 2006)
6. H. Pajouhesh *et al*., US Patent 6,784,202 (August 31, 2004)

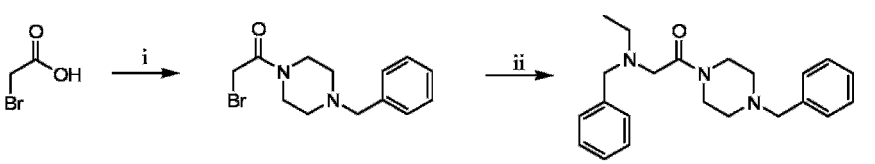
IV. Nerve Growth Factors/Neuroprotectants

Title Acyclic Piperidine Derivatives
D. Lauffer *et al.*, US Patent 6,949,655 (September 27, 2005)
Assignee Vertex Pharmaceuticals Incorporated
Utility Treatment of Alzheimer's disorder

Invention Significance Neurological diseases are associated with the death or injury to neuronal cells. Although some nerve growth factors currently exist, most cannot cross the blood–brain barrier; they are also unstable in plasma or have poor delivery properties. This art addresses these limitations by providing agents that cross the blood–brain barrier to stimulate nerve growth diminished by neurological diseases.

Reaction

Eq. 1



i- Diisopropylcarbodiimide,1-benzylpiperazine, CH_2Cl_2
ii- *N*-Benzyl-*N*-ethylamine, THF

Experimental

1. **Preparation of 1-(4-benzyl-piperiazin-1-yl)-2-bromo-ethanone**

Diisopropylcarbodiimide (6.78 mmol) was added to a solution of bromoacetic acid (3.99 mmol) dissolved in 30 ml CH_2Cl_2 and after 30 minutes a white precipitate was formed. The precipitate was filtered, then treated with 1-benzylpiperazine (5.82 mmol), and stirred 10 hours. The mixture was concentrated, the residue was purified by flash chromatography using CH_2Cl_2/EtOAc, and the product isolated in 89% yield.

MS (MH^+) m/z 297.88

2. **Preparation of 1-(4-benzyl-piperiazin-1-yl)-2-*N*-benzyl-*N*-ethylamine-ethanone**

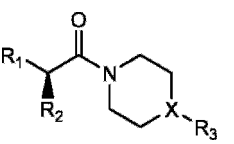
Library Synthesis: Combinatorial Synthesis of Compounds

The Step 1 product (0.25 mmol) was added to the well of a reaction block containing 5 ml THF and then *N*-benzyl-*N*-ethylamine (0.5 mmol) was added neat. The reaction block was shaken 24 hours then filtered, concentrated, and purified using reverse-phase HPLC with water/acetonitrile (0.1% TFA) and the product isolated as a trifluoroacetate salt.

Derivatives

Selected derivatives are provided in Table 1.

Table 1 Summary of physical constants of selected experimental agents and their effectiveness as neuroprotectants. "A" designates an EC_{50} of less than 100 nM; "B" designates an EC_{50} of between 100 and 500 nM; and "C" designates an EC_{50} of greater than 500 nM



Entry	R_1	R_2	X	R_3	MS (m/z)	EC_{50} (nM)
5	*N*-Benzyl-*N*-methylamine	CH_3	CH	Benzyl	351	A
6	*N*-Benzyl-*N*-ethylamine	H	CH	Benzyl	351	B
12	*N*,*N*-Dimethylamine	H	N	Di(4-fluorophenyl) methyl	374	C
13	*N*-Benzyl-*N*-methylamine	2-Butyl	CH	Benzyl	393	C
16	*N*-Benzyl-*N*-ethylamine	H	N	Di(4-fluorophenyl) methyl	464	C
20	*N*,*N*-Dibenzylamine	H	N	Di(4-fluorophenyl) methyl	526	C

Testing

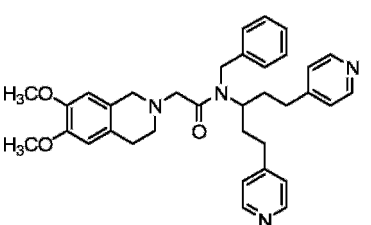
I. *Neuroprotection Assay*

The ventral mesencephalic region was dissected out of embryonic day 15 Sprague–Dawley rat embryos and dissociated into single-cell suspension by a combination of

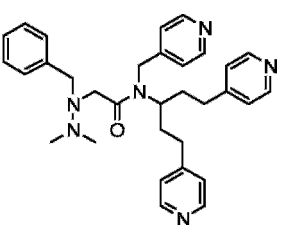
trypsinization and trituration according to the method of Costantini (1). Dissociated VM cells were plated into poly-L-ornithine-coated 96-well plates at a density of 85 000 cells/well in 100 μl DMEM supplemented with 18% heat-inactivated horse serum, 0.24% glucose, 2 mM glutamine and 50 U/ml penicillin/streptomycin, and incubated in a 5% CO_2 incubator. After 1 day in culture, DIV_1, the medium was replaced with 100 μl of a defined medium, DMEM supplemented with $1 \times N_2$ cocktail, 0.12% glucose, 2 mM glutamine, and 50 U/ml penicillin/streptomycin containing DMSO or various concentrations of the experimental agents. On DIV_5, neuroexcitotoxic injury was induced by the addition of using 100–400 μM of the glutamate receptor agonist NMDA. Cultures were incubated with neurotoxin for 20 hours and the effects of neurophilin compounds were assessed using high-affinity ^{3}H-dopamine uptake according to the method of Park (2). Testing results are summarized in Table 1.

Notes

1. In earlier investigations by the author, nerve growth agents consisting of 1,2,3,4-tetrahydro-isoquinoline, (**I**), (3), and azo-amino acid derivatives, (**II**), (4), were prepared and used in the treatment of neurological disorders.

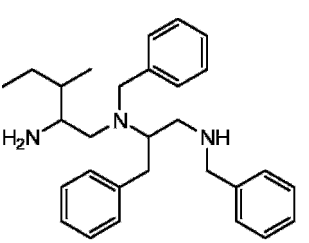


(I)

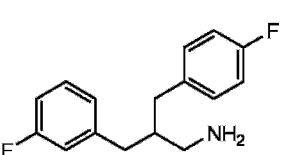


(II)

2. Polyaromatic amine derivatives, (**III**) and (**IV**), prepared by Tai (5) and Mueller (6), respectively, designed as NMDA selective receptor channel blockers to protect neuronal cell receptors from excitotoxic cell death were used in the treatment of Alzheimer's and Huntington's disease and amyotrophic lateral sclerosis.

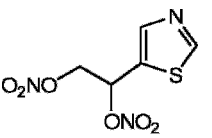


(III)



(IV)

3. Nitrate esters, (**V**), prepared by Thatcher (7) were effective as neuroprotectants for conditions associated with Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis.



(V)

References

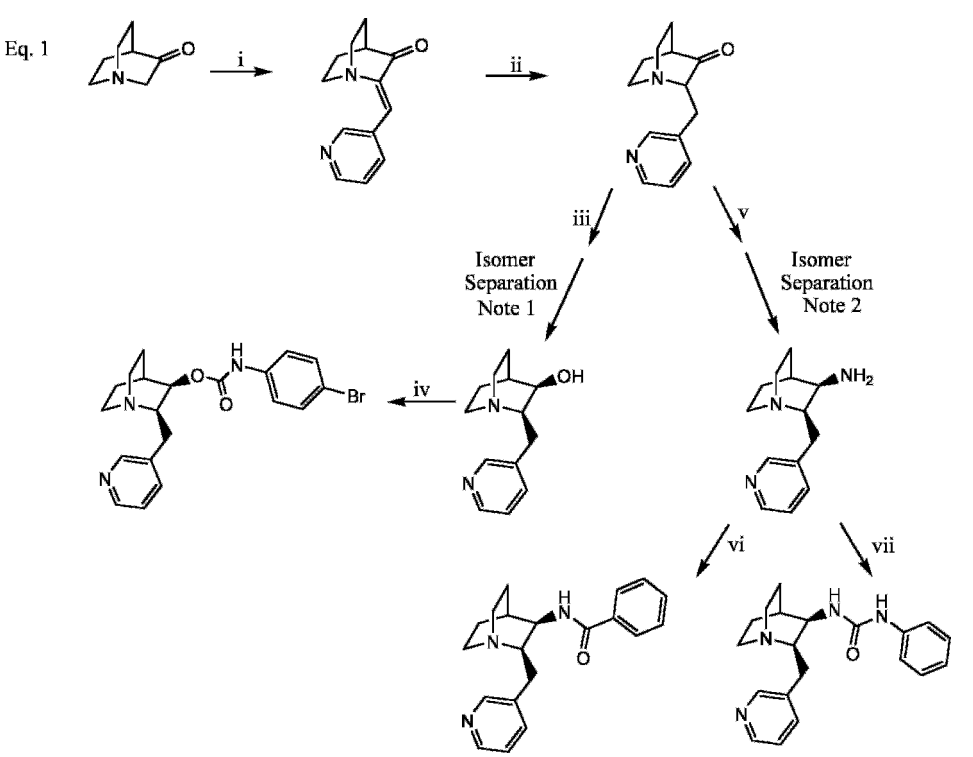
1. G.V. Costantini *et al.*, Neurobiol. Dis., 97 (1998)
2. Y. Park *et al.*, Brain Res., 599, 83 (1992)
3. D. Lauffer *et al.*, US Patent 6,747,042 (June 8, 2004)
4. D. Lauffer *et al.*, US Patent 6,716,860 (April 6, 2004)
5. K.-K. Tai *et al.*, US Patent 7,022,882 (April 4, 2006)
6. A.L. Mueller *et al.*, US Patent 6,750,244 (June 15, 2004)
7. G.R.J. Thatcher *et al.*, US Patent 6,916,835 (July 12, 2005)

V. Nicotinic Acetylcholinergic Receptor Subtype α7 Antagonists

Title 3-Substituted-2-(Arylalkyl)-1-Azabicycloalkanes and Methods of Use Thereof A.A. Mazurov *et al.*, US Patent 6,953,855 (October 11, 2005)
Assignee Targacept, Inc
Utility Treatment of Senile Dementia

Invention Significance Central nervous system disorders are characterized by an alteration in normal neurotransmission and are associated with presenile and senile dementia and microinfarct dementia. A method of modulating neurotransmission using medicaments which selectively interacts with the nicotinic acetylcholinergic receptor subtype, α7, *n*AChR, has been devised to address this disorder.

Reaction



i- 3-Pyridinecarboxaldehyde, potassium hydroxide, water
ii- Methyl alcohol, hydrochloric acid, 10% palladium on carbon
iii- Aluminum isopropoxide, isopropyl alcohol
iv- 4-Bromophenylisocyanate, toluene
v- Methyl alcohol, ammonium formate, zinc chloride, sodium cyanoborohydride
vi- Diphenylchlorophosphate, benzoic acid, triethylamine, CH_2Cl_2
vii- Phenyl isocyanate, $CHCl_3$

Experimental

1. **Preparation of 2-((3-pyridinyl)methylene)-1-azabicyclo[2.2.2]octan-3-one**

   3-Quinuclidinone hydrochloride (0.49 mol) was treated with potassium hydroxide (0.54 mol) dissolved in 420 ml methyl alcohol and then stirred 30 minutes at ambient temperature. 3-Pyridine-carboxaldehyde (0.54 mol) was then added and the mixture stirred for an additional 16 hours. Solid cakes, which formed on the flask walls, were broken up and dissolved with the addition of 390 ml rapidly stirring water. The mixture was kept cooled to 4°C overnight and a precipitate formed, which were collected by filtration. These crystals were then washed with water, air dried, and 80 g product isolated as yellow solid. A second product crop of 8 g was obtained by reducing the filtrate volume by 90%, so a total product yield of 82% was observed.

2. **Preparation of 2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]octan-3-one**

   The Step 1 product (93 mmol) was suspended in 200 ml methyl alcohol and treated with 46 ml 6 M HCl and 10% palladium on carbon (1.6 g), and then shaken 16 hours under 25 psi hydrogen. The mixture was filtered through celite, then concentrated, and a white gum isolated. The residue was treated with 50 ml apiece 2 M NaOH and $CHCl_3$ and then stirred 60 minutes. The aqueous phase was retreated with 5 ml 2 M NaOH to pH 10 and then washed with 25 ml brine, extracted three times with 10 ml $CHCl_3$, dried with $MgSO_4$, and concentrated. The residue was dissolved in 320 ml warm diethyl ether and cooled to 4°C. A white solid was isolated by filtration, which was washed with a small portion of cold diethyl ether, and then air dried. A second product crop of 8 g was obtained by reducing the filtrate volume by 90%, so a total product yield of 79% was obtained.

3. **Preparation of 2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]octan-3-ol**

   A mixture consisting of the Step 2 product (13.9 mmol), 165 ml isopropyl alcohol, and aluminum isopropoxide (50.9 mmol) was refluxed 3 hours and the mixture cooled to ambient temperature. The mixture was then concentrated and the gelatinous residue

diluted with 50 ml brine and 10 ml 50% aqueous NaOH, then extracted three times with 25 ml $CHCl_3$. Combined extracts were dried, then concentrated, and amber oil that became cream-colored solid was isolated. The product was isolated in 99.7% yield as a 93:7 mixture of diastereomers, the *cis* relative being the major component.

4. **Preparation of 2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl 4-bromophenyl carbamate**

   4-Bromophenylisocyanate (0.2 mmol) was combined with the Step 3 product in 1 ml toluene and heated to 100°C for 3 hours, then concentrated by centrifugal evaporation. The residue was dissolved in 5 ml DMF and purified by HPLC on a C18 silica gel column using acetonitrile/water gradients containing 0.05% trifluoroacetic acid as eluent.

5. **Preparation of 3-amino-2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]octane**

   The Step 2 product dissolved in 20 ml methyl alcohol was treated with 2.78 ml 1 M $ZnCl_2$ in diethyl ether, then stirred 30 minutes at ambient temperature, and treated with solid ammonium formate (167 mmol). The mixture was stirred an additional 60 minutes and solid sodium cyanoborohydride (27.8 mmol) was added in portions. After stirring overnight at ambient temperature, the mixture was quenched with 5 ml water and then partitioned between 10 ml 5 M NaOH and 20 ml $CHCl_3$. The aqueous layer was extracted with 20 ml $CHCl_3$, then dried with Na_2SO_4, filtered, and concentrated. The product consisted of yellow gum as 90:10 mixture of *cis* and *trans* amines, respectively.

6. **Preparation of *N*-(2-((3-pyridinyl)methyl)-1-azabicyclo[2.2.2]oct-3-yl)phenyl carboxamide**

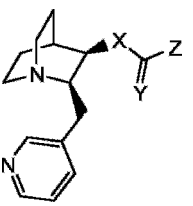
   Diphenylchlorophosphate (0.3 mmol) was added dropwise to solution of benzoic acid (0.3 mmol) and triethylamine (0.3 mmol) dissolved in 1 ml dry CH_2Cl_2, then stirred 60 minutes at ambient temperature. This mixture was treated with the Step 5 product (0.3 mmol) and triethylamine (0.6 mmol) dissolved in 0.5 ml in dry CH_2Cl_2 and stirred overnight at ambient temperature. The solution was diluted with 2 ml $CHCl_3$, then washed with 2 ml 5 M NaOH, and concentrated. The residue was dissolved in 5 ml methyl alcohol and purified by HPLC on a C18 silica gel column using acetonitrile/water gradients containing 0.05% trifluoroacetic acid as eluent and the product isolated.

7. **Preparation of *N*-phenyl-*N′*-(2-((3-pyridinyl) methyl)-1-azabicyclo[2.2.2]oct-3-yl)urea**

   A mixture consisting of phenyl isocyanate (0.3 mmol), the Step 5 product (0.3 mmol), and 1 ml $CHCl_3$ was stirred 48 hours at ambient temperature and concentrated. The residue was dissolved in 0.5 ml methyl alcohol methanol and purified as in Step 6 and the product isolated.

Derivatives

Table 1 Summary of selected 1-azabicycloalkanes derivatives and their corresponding mass spectral data



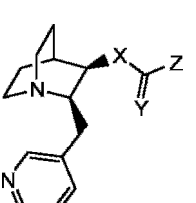
Entry	X	Y	Z	LCMS Mass (MH^+)
5	O	O	NH-4-C_6H_4−OCH_3	384.29
11	NH	S	C_6H_5	430.30
15	NH	O	4-C_6H_4−Br	402.24
20	NH	O	NH-4-C_6H_4−Br	417.22
27	NCH_3	O	NH-4-C_6H_4−Br	431.26

Testing

I. *Determination of Activity at the α7 nAChR Subtype*

Selective α7 agonists were determined using a functional assay FLIPR available in high throughput from Molecular Devices Corporation, Sunnyvale, CA. FLIPR was designed to read a fluorescent signal from each well of a 96- or 384-well plate as fast as twice a second for up to 30 minutes. This assay was used to accurately measure the functional pharmacology of α7*n*AChR and $5HT_3R$ subtypes. Testing results are provided in Table 2.

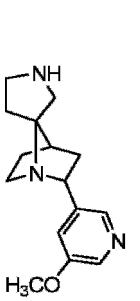
Table 2 Selectivity for α7 *n*AChR subtypes for selected 2-(3-pyridinyl)methyl derivatives



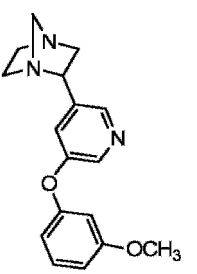
Entry	X	Y	X	K_i (nM)
3	NH	O	O-4-C_6H_4−Fl	120
4	NH	O	O-4-C_6H_4−OCH_3	7
5	NH	S	4-C_6H_5	40
6	NH	O	OC_6H_5	5
7	NH	O	OC_6H_5	53
8	NCH_3	O	NH-4-C_6H_4−Br	9

Notes

1. The Step 2 product mixture was resolved using *N*,*N*-dicyclohexylcarbodiimide and (*S*)-2-methoxy-2-phenylacetic acid.
2. The Step 5 product mixture was resolved using di-*p*-toluoyl-D-tartaric acid.
3. Spiro, (**I**), and diazo derivatives, (**II**), prepared by Bhatti (1) and Miller (2), respectively, were effective in treating central nervous disorders characterized by an alteration in normal neurotransmitter release present in presenile and senile dementia characteristic of Alzheimer's disease.

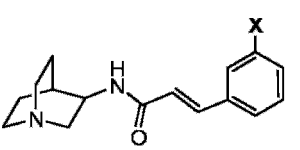


(I)



(II)

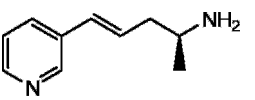
4. *Z*-Cinnamamide derivatives, (**III**), prepared by Bencherif (3) selectively bound to the α7 *n*AChR receptor were used in the treatment of Alzheimer's disease.



(III)

X = OCH_3
F, Cl, Br, I

5. Caldwell (4) prepared aryl substituted olefins, (**IV**), which were effective in activating nicotinic cholinergic receptors and used in the treatment of central nervous system disorders including Alzheimer's disease.



(IV)

References

1. B.S. Bhatti *et al.*, US Patent 6,956,042 (October 18, 2005)
2. C.H. Miller *et al.*, US Patent 6,852,721 (February 8, 2005)
3. M. Bencherif *et al.*, US Patent 7,067,261 (June 27, 2006)
4. W.S. Caldwell *et al.*, US Patent 7,045,538 (May 16, 2006)

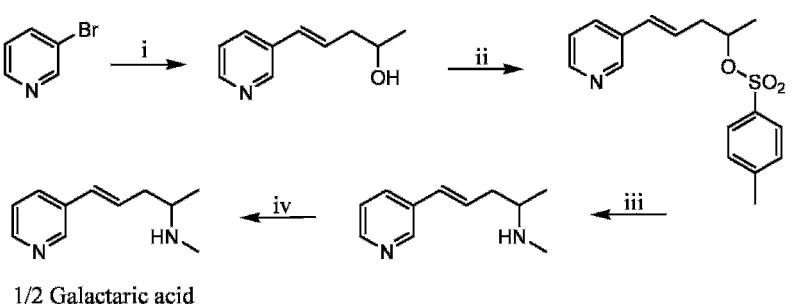
VI. Selective Nicotinic Acetylcholine Receptors Agonists

Title Compounds Capable of Activating Cholinergic Receptors
W.S. Caldwell *et al.*, US Patent 6,958,399 (October 25, 2005)
Assignee Targacept, Inc.
Utility Treatment of Alzheimer's Dementia and Parkinsonism

Invention Significance Current treatment methods related to cholinergic or dopaminergic deficiencies associated with Alzheimer's dementia and Parkinsonism induce skeletal muscle and ganglia sites side effects. This art addresses this concern using chemical agents that selectively activate nicotinic acetylcholine receptors to normalize neurotransmitter release.

Reaction

Eq. 1



i- Penten-2-ol, palladium(II) acetate, tri-*o*-tolylphosphine, triethylamine, acetonitrile
ii- Pyridine, *p*-toluenesulfonyl chloride
iii- Methylamine, ethyl alcohol
iv- Ethyl alcohol, galactaric acid, water

Experimental

1. **Preparation of (4*E*)-5-(3-pyridyl)-4-penten-2-ol**

A mixture consisting of 3-bromopyridine (47.46 mmol), 4-penten-2-ol (56.96 mmol), palladium(II) acetate (0.47 mmol), tri-*o*-tolylphosphine (1.89 mmol), 28.4 ml triethylamine, and 25 ml acetonitrile was heated 14 hours at 140°C in a sealed glass tube. The mixture was cooled to ambient temperature, then diluted with water, and

extracted three times with 200 ml $CHCl_3$. The combined extracts were dried over Na_2SO_2, filtered, concentrated, and the product isolated in 81.0% yield as pale yellow oil.

2. **Preparation of (4*E*)-5-(3-pyridyl)-4-penten-2-ol p-toluenesulfonate**

   The Step 1 product (30.67 mmol) dissolved in 30 ml dry pyridine at 0°C was treated with *p*-toluenesulfonyl chloride (46.01 mmol), then stirred 24 hours at ambient temperature. Pyridine was removed by rotary evaporation and 50 ml toluene added to the residue, which was subsequently removed by distillation. The residue was then stirred with100 ml saturated $NaHCO_3$ solution and extracted three times with 100 ml $CHCl_3$. The combined extracts were dried over Na_2SO_4 and then concentrated. The residue was purified by chromatography using aluminum oxide with EtOAc/hexane, 3:7, and the product isolated in 60.1% yield as viscous brown oil.

3. **Preparation of (4*E*)-*N*-methyl-5-(3-pyridyl)-4-penten-2-amine**

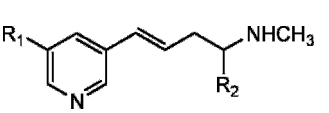
   A mixture consisting of the Step 2 product (17.66 mmol), 100 ml 40% methylamine solution in water, and 10 ml ethyl alcohol was stirred 18 hours at ambient temperature, then extracted three times with 100 ml $CHCl_3$. The extract was then dried, filtered, and concentrated. The residue was purified by chromatography using aluminum oxide with EtOAc/methyl alcohol, 7:3, and the product isolated in 51.6% yield as colorless oil, BP = 110–120°C at 0.1 mmHg.

4. **Preparation of (4*E*)-*N*-Methyl-5-(3-pyridyl)-4-penten-2-amine hemigalactarate**

   The Step 3 product (9.10 mmol) was dissolved in 20 ml ethyl alcohol by warming to 60°C, which was then treated with galactaric acid (4.54 mmol) followed by the dropwise addition of 0.5 ml water. Once cooled, ambient temperature crystals were formed and were isolated by filtration. The crystals were then washed with anhydrous diethyl ether, dried under vacuum at 40°C, and the product isolated in a 47.0% yield as a white solid, mp = 148–150°C.

Derivatives

Table 1 Selected (4*E*)-*N*-methyl-5-(3-pyridyl)-4-penten-2-amine hemigalactarate salt derivatives and their corresponding melting points. The corresponding D- and L-2,3-diacyl-tartaric acid salt derivatives were previously prepared by Dull (1)



Entry	R_1	R_2	mp (°C)
7	Hydrogen	Methyl	131–134
8	Hydrogen	(2*R*)-Methyl	131–133
9	Hydrogen	(2*S*)-Methyl	141–143
10	Isopropoxy	Methyl	210–213
11	Isopropoxy	(2*R*)-Methyl	150–153
12	Isopropoxy	(2*E*)-Methyl	140–143
13	Bromo	Methyl	143–145
14	Methoxy	Methyl	143–145

Testing

I. *Dopamine and Rubidium Ion Release*

The ability of experimental agents to pass across the blood–brain barrier, log *P*, was determined according to the method of Hansch (2), inhibition constants, K_i, calculated using the method of Chang (3), and dopamine and rubidium ion release obtained calculated according to the method of Dull (4). Inhibition constants, dopamine and rubidium ion release testing results are provided in Table 2.

Table 2 Test results of selected experimental agents indicating the ability of these materials to pass across the blood–brain barrier and impact on dopamine and rubidium ion release

Entry	Capacity to Pass Blood–Brain Barrier		Dopamine Release		Rubidium Ion Release	
	Log *P*	K_i (%)	EC_{50} (μm)	E_{max} (%)	EC_{50} (μM)	E_{max} (%)
7	1.924	83	6 600	113	3 100	35
8	1.924	520	27 400	76	4 390	32
9	1.924	34	2 600	162	2 600	162
10	2.957	10	100	57	100	60
11	2.957	62	624	38	88	14
12	2.957	11	106	85	220	58
13	2.026	284	202	18	–	0
14	2.026	22	5 000	110	–	–

II. *Determination of Interaction with Muscle Receptors and Ganglion Receptors*

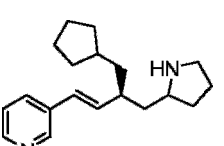
The effectiveness of experimental agents with muscle and ganglionic receptors was determined according to the method of Dull (4) and testing results are provided in Table 3.

Table 3 Effect of selected experimental agents in eliciting activation in muscle and rat ganglionic preparations

Entry	E_{max} (%)	
	Muscular Receptors	**Ganglionic Receptors**
7	13	62
8	0	36
9	0	18
10	15	36
11	0	4
12	0	0
13	6	8
14	1	2

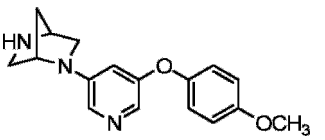
Notes

1. Olefinic azacyclic derivatives, (**I**), prepared by Dull (5) selectively activated nicotinic acetylcholine receptors and were used in the treatment of neurodegenerative diseases.

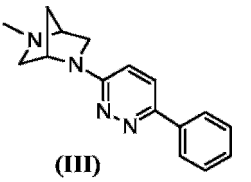


(I)

2. Diazabicyclo[2.2.1]heptane derivatives, (**II**) and (**III**), prepared by Miller (6) and Lochead (7), respectively, were effective as nicotinic receptor subtypes agonists and used in the treatment of central nervous systems disorders.

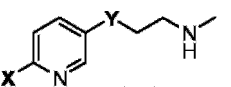


(II)



(III)

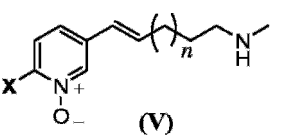
3. 5-Halo-pyridyloxyl- and thioalkylamines derivatives, (**IV**), prepared by Dull (8) were effective as nicotinic receptor agonists and used in the treatment of Alzheimer's disease.



(IV)

X	Y
Cl	O
Br	S

4. Pyridine *N*-oxide derivatives, (**V**), prepared by Dull (9) were effective as nicotinic receptor agonists and used in treating Alzheimer's disease and related neurodegenerative diseases.



(V)

X	n
i-C_3H_7	1
H	0
SC_2H_5	1

References

1. G.M. Dull, US Patent 6,743,812 (June 1, 2004)
2. P.M. Hansch *et al.*, J. Med. Chem., ii, 1 (1968)
3. E. Chang *et al.*, Biochem. Pharmacol., 22, 3099 (1973).
4. G.M. Dull *et al.*, US Patent 5,597,919 (January 28, 1997)
5. G.M. Dull *et al.*, US Patent 6,890,935 (May 10, 2005)
6. C.H. Miller *et al.*, US Patent No. 6,852,721 (February 8, 2005)
7. A. Lochead *et al.*, US Patent 6,635,645 (October 21, 2003)
8. G.M. Dull *et al.*, US Patent 6,627,648 (September 30, 2003)
9. G.M. Dull *et al.*, US Patent 6,455,554 (September 24, 2002)

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CHAPTER IV

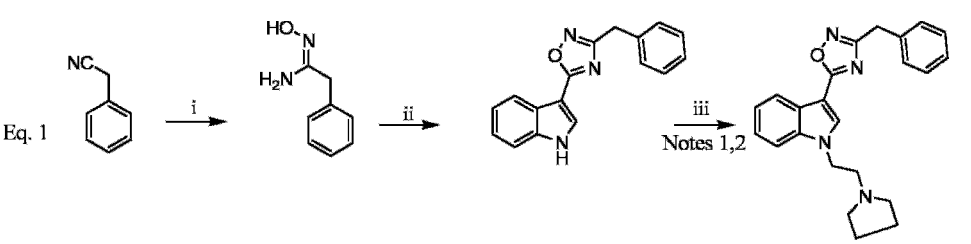
Analgesics

I. Cannabinoid CB_1 and CB_2 Receptor Modulators

Title 3-Oxadiazol-5-yl-1-Aminoalkyl-1H-Indole Derivatives
P.G. Moloney *et al.*, US Patent 6,930,118 (August 16, 2005)
Assignee Amrad Operations Pty. Ltd
Utility Treatment of Neuropathic Pain

Invention Significance Aminoalkylindole derivatives have been prepared that are effective as cannabinoid CB_1 and CB_2 receptor agonists or antagonists despite the absence of a carbonyl group at C-3 indole nucleus. These agents are particularly useful in pain management and in the treatment of inflammatory disorders.

Reaction



i- Hydroxylamine hydrogen chloride, ethyl alcohol, triethylamine
ii- Indole-3-carboxylic acid, carbonyldiimidazole, dimethyl ether
iii- Sodium hydride, DMF, (2-chloroethyl)pyrrolidine•HCl

Experimental

1. **Preparation of *N*-hydroxyl-2-phenyl-acetamidine**

   A mixture consisting of phenylacetonitrile (14 mmol), hydroxylamine hydrochloride (28 mmol), and 4.4 ml triethylamine dissolved in 25 ml ethyl alcohol was refluxed 16 hours and concentrated. The residue was washed with 100 ml aqueous 10% Na_2CO_3, then extracted with 150 ml EtOAc. The extract was dried, filtered, concentrated, and the product isolated in 57% yield, mp = 163–170°C.

   MS *m/z* 151 (M + 1)

2. **Preparation of 3-(3-Benzyl-[1,2,4]oxadiazol-5-yl)-1H-indole**

   In a separate vessel, indole-3-carboxylic acid (5.1 mmol) and carbonyldiimidazole (5.6 mmol) were stirred in 20 ml dimethyl ether for 16 hours.

   In another vessel, the Step 1 product (5.1 mmol) was dissolved in 20 ml dimethyl ether containing 3 Å crushed molecular sieves (900 mg), then stirred 30 minutes, and treated with sodium hydride (5.1 mmol). After stirring, an additional 30 minutes at ambient temperature, the contents of this vessel were added to the first vessel, then refluxed 24 hours, and concentrated. The residue was partitioned between 50 ml apiece of EtOAc and 5% aqueous $NaHCO_3$, then filtered, and phases separated. The aqueous phase was washed twice with 20 ml EtOAc, filtered, then concentrated, and an oily yellow solid isolated. The residue was purified by recrystallization using EtOAc/hexane and the product isolated in 51% as white crystals, mp = 198–199°C.

   ^{1}H NMR ($CDCl_3$) δ 4.16 (2H, s, CH_2), 7.21–7.48 (8H, m, ArH), 8.02 (1H, m, ArH), 8.28 (1H, m, ArH), 8.70 (1H, bs, NH)
   Analysis ($C_{17}H_{13}N_3O$) C, H, N
   MS *m/z* 276

3. **Preparation of 3-(3-Benzyl-[1,2,4]-oxadiazol-5-yl)-1-[2-(pyrrolidin-1-yl)ethyl]-1H-indole**

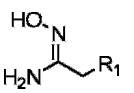
   The Step 2 product (0.43 mmol) dissolved in 2 ml DMF was treated with sodium hydride (0.95 mmol), then stirred 30 minutes at ambient temperature. The solution was further treated with 1-(2-chloroethyl)pyrrolidine•HCl (0.48 mmol), then stirred 30 minutes at ambient temperature and 24 hours at 100°C. The solution was concentrated and the residue was washed with 30 ml 10% Na_2CO_3 solution and then extracted with 20 ml EtOAc. The extract was then filtered and reconcentrated. The residue was purified by flash chromatography with $CHCl_3$/methyl alcohol, 19:1, and the product was isolated in 72% yield as a viscous yellow oil.

   ^{1}H NMR (methanol-d_4) δ 1.64 (4H, m, 2 × CH_2), 2.39 (4H, m, 2 × CH_2), 2.71 (2H, t, J = 6.9 Hz, CH_2), 4.01(2H, s, CH_2), 4.14 (2H, t, J = 6.9 Hz, CH_2), 7.10–7.38 (8H, m, ArH), 7.94 (1H, s, H2), 8.06 (1H, m, H4)

^{13}C NMR (methanol-d_3) δ 22.81, 31.53, 45.21, 53.59, 54.68, 100.0, 110.1, 120.5, 121.8, 123.0, 125.1, 126.5, 128.2, 128.6, 132.4, 135.9, 136.4, 169.1, 173.3
Analysis [Citrate salt hydrate and ethanol] ($C_{23}H_{24}N_4O \bullet C_6H_8O_7 \bullet H_2O \bullet C_2H_6O$) C, H, N
MS m/z 373 $(M+1)^+$

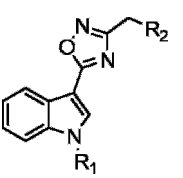
Derivatives

Table 1 Step 2 product intermediates and their corresponding melting points and/or mass spectral data



R_1	mp (°C)	MS, m/z $(M+1)$
4-Methoxyphenyl	108–109	181
2-Naphthylen-2-yl	117–119	–
Isonicotinyl	132–134	138
3-Phenylpropyl	–	213
Benzenecarboxy	–	137

Table 2 Selected 1H-indole derivatives and their corresponding mass spectral data. ^{1}H-NMR data supplied by author



Entry	R_1	R_2	MS (M+1)
2	Benzyl	2-(Morpholin-4-yl)ethyl	389
6	4-Methoxybenzyl	2-(Morpholin-4-yl)ethyl	419
8	Benzyl	Pentyl	–
11	Biphenyl	2-(Morpholin-4-yl)ethyl	451
12	3-Naphth-2-yl-methyl	Pyrolidin-1-yl ethyl	–
15	5-(Naphth-2-yl-methyl)	Pentanoic acid	426

Testing

I. CB_1 *and* CB_2 *Receptor Binding Assay*

Mouse vasa deferentia were dissected with capsular connective tissue intact and set up in 20 ml organ baths at 37°C in Mg^{2+}-free physiological salt solution. The epididymal end was attached to an isometric force transducer and the prostatic end tied to a fixed support between two parallel platinum field electrodes, 5 mm apart, 5 mm long. The tissues were stimulated to contract using trains of electrical field stimulation of three pulses (4 Hz), 0.5 ms duration, 100 V (80% maximal voltage) every 20 seconds for 10 minutes and was applied before and after both antagonist/agonist addition. Selected experimental agents were dissolved in DMSO and equilibrated with the tissue 30 minutes before being assayed to reflect the effect of predrug twitch force. Testing results are provided in Table 3.

Table 3 Receptor binding for selected chemical agents using anandamide and THC as references. Agents 11–15 are especially preferred

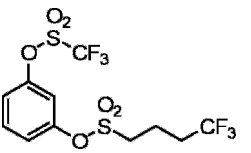
Entry	CB_1 (MVD), Percentage of Inhibition at 1.0 μM	IC_{50} (μM)	
		CB_1 (Binding)	CB_2 (Binding)
Anandamide	84	1	< 1
THC[a]	100	0.1	< 0.1
2	96	1	1
6	39	0.2	2.8
8	100	0.006	0.4
11	11	≫10	≫10
12	96	≫10	≫10
15	54	≫10	≫10

[a] Delta-9-tetrahydrocannabinol.

Notes

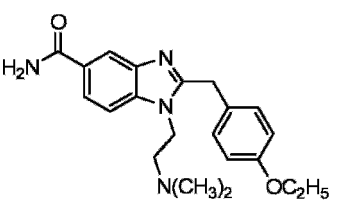
1. A common structural feature of previously reported CB_1 and CB_2 receptor modulators is the presence of a carbonyl or thiocarbonyl group substituted by an aryl group and attached to the 3- or 4-position of the indole nucleus as reported by Dutta (1). The current investigation indicates, however, that removal of the carbonyl group at C-3 on the indole template and substituting a five-membered heterocyclic group provides agents that exhibit very strong cannabinoid receptor activity.

2. Perfluoro heteroaryl sulfonates derivatives, (**I**), prepared by Heil (2) were effective CB_2 receptor antagonists and used in treating chronic pain and inflammatory disorders.

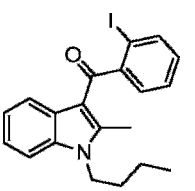


(I)

3. Selective CB_2 agonists consisting of imidazo[4,5-b]pyridine-6-carboxamide and benzimid-azoles, (**II**), prepared by Cheng (3) and indole derivatives, (**III**), prepared by Makriyannis (4), respectively, were effective as pain management agents without psychoactive side effects.

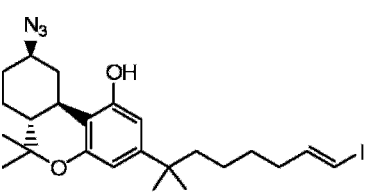


(II)

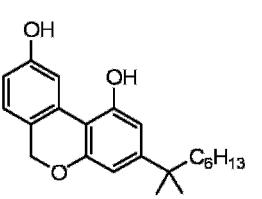


(III)

4. Bicyclic and tricyclic cannabinoids, (**IV**), prepared by Makriyannis (5) were effective as both CB_1 and CB_2 agonists/antagonists and used as an effective and safe treatment option in pain management. Dibenzo[b,d]pyran derivatives, (**V**), effective as selective CB_2 receptor agonists (**V**) also prepared by Makriyannis (6) were effective in treating pain.

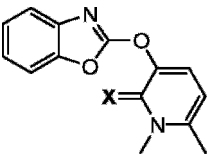


(IV)



(V)

5. CB_2 agonists/antagonists consisting of pyridone derivatives, (**VI**), were prepared by Tada (7) and were effective as analgesics and used in the treatment of moderate pain.



X = O, S

(VI)

References

1. T.E. Dutta *et al.*, Bioorg. Med. Chem., 5, 1591 (1997)
2. M. Heil *et al.*, US Patent 6,919,470 (June 19, 2005)
3. Y.-X. Cheng *et al.*, US Patent 7,030,139 (April 18, 2006)
4. A. Makriyannis, US Patent 6,900,236 (May 31, 2005)
5. A. Makriyannis *et al.*, US Patent 7,057,076 (June 6, 2006)
6. A. Makriyannis *et al.*, US Patent 6,995,187 (February 7, 2006)
7. Y. Tada *et al.*, US Patent 6,977,266 (December 20, 2005)

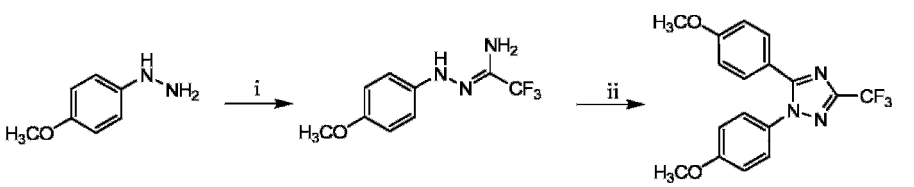
II. Cyclooxygenase-II Inhibitors

Title Triazole Derivatives
S. Aoki *et al.*, US Patent 6,927,230 (August 9, 2005)
Assignee Fujisawa Pharmaceutical Co., Ltd
Utility Analgesic and Blood Platelet Inhibitor

Invention Significance Nonsteroidal anti-inflammatory agents (NSAIDs) have inhibiting activities against both cyclooxygenase-I (COX-I) and cyclooxygenase-II (COX-II). Their therapeutic use, however, is limited because of side effects such as ulceration and renal toxicity associated with COX-I inhibition. To address this problem, COX-II inhibitors have been prepared that selectively impede prostaglandin biosynthesis while only marginally impacting COX-I levels.

Reaction

Eq. 1



i- Trifluoroacetoamidine, methyl alcohol, triethylamine
ii- 4-Methoxybenzoyl chloride, dioxane

Experimental

1. **Preparation of 2,2,2-trifluoro-*N*′-(4-methoxyphenyl)ethanehydrazonamide**

   A solution of trifluoroacetoamidine (37.8 mmol) in 20 ml methyl alcohol was treated with 4-methoxyphenylhydrazine hydrochloride (27 mmol) followed by 3.77 ml triethylamine, then stirred 6 hours at ambient temperature, and concentrated. The residue was treated with 20 ml water and 50 ml EtOAc/THF, 9:1, and the organic layer isolated. The aqueous layer was re-extracted with 50 ml EtOAc/THF, 9:1, and combined extracts were washed with water and brine. The solution was then dried using $MgSO_4$, concentrated, and 108.2% yield residue isolated and used without further purification.

2. **Preparation of 1,5-bis(4-methoxyphenyl)-3-(trifluoromethyl)-1H-1,2,4-triazole**

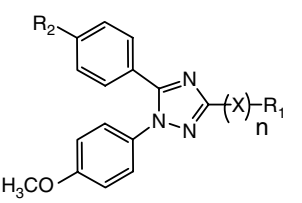
   The Step 1 product (3.95 mmol) dissolved in 10 ml dioxane was treated with pyridine (3.95 mmol) and 4-methoxybenzoyl chloride (3.95 mmol) dissolved in 3 ml dioxane,

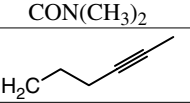
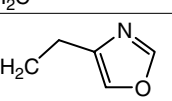
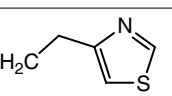
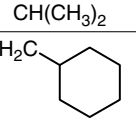
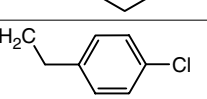
then refluxed 12 hours. The mixture was concentrated and the residue treated with 50 ml CH_2Cl_2 and 20 ml 0.1 M HCl. The organic layer was isolated and the aqueous component re-extracted with an additional 50 ml CH_2Cl_2. Combined extracts were then washed with 0.1 M HCl and brine, dried, and concentrated. The residue was purified by chromatography using silica gel with toluene/EtOAc, 9:1, then recrystallized using diisopropyl ether/hexane, and the product isolated in 48.6% yield as pale brown needles.

^{1}H NMR (DMSO-d_6, ppm) δ 7.45 (t, $J = 8.9$ Hz, 4H), 7.09 (d, $J = 8.9$ Hz, 2H), 6.98 (d, $J = 8.9$ Hz, 2H), 3.83 (s, 3H), 3.78 (s, 3H)
MS (ESI) m/z 350 (M + 1).

Derivatives

Table 1 Selected triazole derivatives and their corresponding mass spectral data. ^{1}H NMR data supplied by author



Entry	X_n	R_1	R_2	MS (M + H^+)
1	None	CF_3	OCH_3	350
4	None	CF_3	CH_3	334
5	O	CH_3	OCH_3	312
16	O	$CON(CH_3)_2$	OCH_3	369
17	O		OCH_3	336
19	None		OCH_3	393
20	O		OCH_3	395
23	O	$CH(CH_3)_2$	OCH_3	340
25	O		OCH_3	380
26	O		OCH_3	422

Testing

I. *Analgesic Activity Effect on Adjuvant Arthritis in Rats*

Arthritis was induced by injection of 0.5 mg of *Mycobacterium tuberculosis* in 50 μl of liquid paraffin into the right hind footpad of Lewis rats aged 7 weeks and the analgesic activity of a single dose of each experimental agent evaluated. Experimental agents were administered and the pain threshold measured 2 hours thereafter. The mechanical pain threshold of the left noninjected hind paw was determined by compressing the ankle joint with a balance pressure apparatus. The threshold pressure of rats squeaking or struggling was expressed in grams. The threshold pressure of rats treated with selected experimental agents was then compared with that of untreated rats. Chemical agents showing a ratio of 1.5 are considered to be effective analgesics and are provided in Table 2.

Table 2 Analgesic activity of selected experimental agents at a treatment dosage of 3.2 mg/kg effective as analgesics in the adjuvant arthritis tests using Lewis rats

Entry	Analgesic Coefficient
4	>1.5
5	>1.5
25	>1.5

II. *Inhibiting Activity Against COX-I and COX-II (Whole Blood Assay)*

A. *COX I Testing Protocol*

Fresh blood was collected by syringe without anticoagulants from volunteers with consent. The subjects had no apparent inflammatory conditions and had not taken any medication for at least 7 days prior to blood collection. Aliquots of human whole blood (500 μl) were immediately incubated with 2 μl of either DMSO vehicle or a selected experimental agent for 1 hour at 37°C to allow the blood to clot. At the end of incubation, 5 μl of 250 mM indomethacin was added to stop the reaction. The blood was centrifuged, a 100 μl aliquot of serum mixed with 400 μl methyl alcohol for protein precipitation, and the supernatant assayed for TXB_2 using the enzyme immunoassay. Experimental agent test results were expressed as percent inhibition of TXB_2 production relative to control incubations containing DMSO vehicle and IC_{50} determined. Testing results are summarized in Table 3.

B. *COX II Testing Protocol*

The aforementioned procedure was used with the exception that the supernatant was assayed for PGE_2 using a radioimmunoassay kit after conversion of PGE_2 to the methyl oximate derivative. Testing results are summarized in Table 3.

Table 3 Testing results summarizing the inhibiting activity against COX-I and COX-II using selected experimental agents. Experimental agents were particularly effective as selective COX-I inhibitors

Entry	COX-I IC_{50} (μM)	COX-II IC_{50} (μM)
4	<0.01	>0.1
16	<0.01	>0.1
17	<0.01	>0.1
20	<0.01	>0.1
23	<0.01	>0.1
25	<0.01	>0.1
26	<0.01	>0.1

III. *Inhibiting Activity on Aggregation of Platelet*

Testing Protocol

Blood from healthy human volunteers was collected into plastic vessels containing 3.8% sodium citrate (1/10 volume) and platelet-rich plasma obtained from the supernatant fraction of blood after centrifugation. Platelet-poor plasma was obtained by centrifugation of the remaining blood.

Platelet aggregation was measured according to the turbidimetric method with an aggregometer.

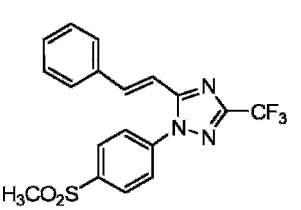
Platelet-rich plasma was preincubated 2 minutes at 37°C after the addition of a selected experimental agent or vehicle. Collagen was used as a platelet aggregation agonist with a final concentration of 0.5 μg/ml. The effect of each experimental agent was expressed as percentage inhibition agonist-induced platelet aggregation compared with the vehicle treatment. Each experimental agent was evaluated in six experiments and was required to produce a 50% inhibition agonist-induced platelet aggregation. Testing results are summarized in Table 4.

Table 4 Test results of selected experimental agents having inhibiting activity against platelet aggregation and useful in treating disorders induced by platelet aggregation

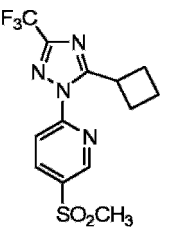
Entry	IC_{50} (μM)
1	<0.02
5	<0.02
19	<0.02
25	<0.02

Notes

1. 1H-1,2,4-Triazole derivatives, (**I**) and (**II**), prepared by Cho (1) and Sakya (2), respectively, were effective as selective COX-II inhibitors and used to reduce inflammation, pain, and fever.

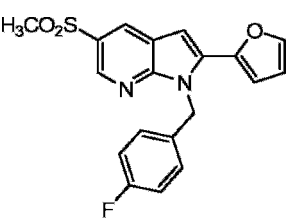


(I)



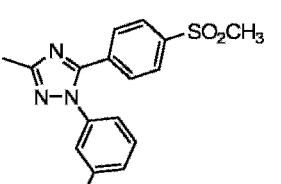
(II)

2. Heterocyclic-1H-pyrrolo[2,3-b]pyridine derivatives, (**III**), prepared by Matsuoka (3) were effective as selective COX-II inhibitors and used in treating pain and inflammation disorders.



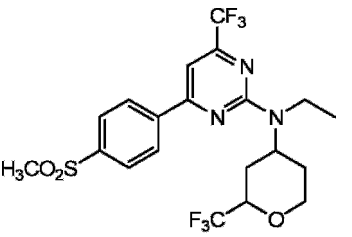
(III)

3. Pascal (4) prepared NSAIDs consisting of triazole derivatives, (**IV**), which were effective as COX-II inhibitors and used in treating pain and inflammation.



(IV)

4. Pyrimidine derivatives, (**V**), effective as selective COX-II inhibitors were prepared by Carter (5) and used in the treatment of pain, fever, and inflammation.



(V)

References

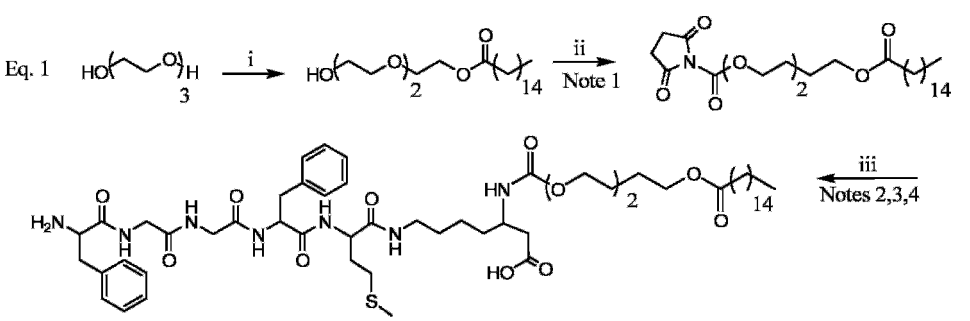
1. I.H. Cho *et al.*, US Patent 7,019,144 (March 28, 2006) and US Patent 6,849,652 (February 1, 2005)
2. S.M. Sakya *et al.*, US Patent 6,875,779 (April 5, 2005)
3. H. Matsuoka *et al.*, US Patent 6,875,770 (April 5, 2005)
4. J.-C. Pascal *et al.*, US Patent 6,803,380 (October 12, 2004)
5. M.C. Carter *et al.*, US Patent 7,084,148 (August 1, 2006) and US Patent 7,056,928 (June 6, 2006)

III. Enkephalin Delivery Agents

Title Enkephalin Conjugates
N.N. Ekwuribe *et al.*, US Patent 6,956,051 (October 18, 2005)
Assignee Nobex Corporation
Utility Blood–brain barrier Traversing Delivery Endogenous Opioid Peptides

Invention Significance A noninvasive method of traversing the blood–brain barrier using amphiphilic oligomeric conjugates for delivery of the neuroactive peptide, enkephalin, not requiring metabolic activation has been devised. This passive diffusion of endogenous and synthetic opioid peptides across the blood–brain barrier into the central nervous system is therapeutically useful in pain management.

Reaction



i- Palmitic anhydride, THF, pyridine
ii- Dimethylaminopyridine, THF, *N*,*N*′-disuccinimidyl carbonate
iii- Met-enkephalin, DMF, CH_2Cl_2, triethylamine

Experimental

1. **Preparation of triethylene glycol monohexadecyl ester**

Palmitic anhydride (10.104 mmol) was dissolved in 20 ml THF and 3 mol pyridine, then stirred at ambient temperature, and slowly treated with triethylene glycol (10.104 mmol). After stirring 1 hour, THF was removed and the mixture poured into ice-cold 10% sulfuric acid. The aqueous layer was extracted three times with 30 ml

EtOAc, then washed with water, brine, dried using $MgSO_4$, concentrated, and the product isolated.

2. **Preparation of succinimidyl triethylene glycol monohexadecyl ester**

   The Step 1 product (2.57 mmol) and dimethylaminopyridine (2.57 mmol) were dissolved in THF treated with *N*, *N*′-disuccinimidyl carbonate (0.691 g) in a single portion and then stirred overnight at ambient temperature. The mixture was concentrated, then diluted with EtOAc, and washed twice with 10 M HCl, water, and brine. The solution was dried, concentrated, and the product isolated as a white solid.

3. **General procedure for conjugation of met-enkephalin**

   Met-enkephalin (0.1854 mmol) dissolved in 5 ml DMF/CH_2Cl_2, 2:1, was treated with 25 μl triethylamine and cooled to 10°C. This solution was treated with the Step 2 product dissolved in 1 ml CH_2Cl_2, then stirred 2 hours at 10°C, and concentrated. The residue was dissolved in EtOAc, then reconcentrated, and 0.310 g product isolated having a mono/diconjugate ratio of 5.2:1, respectively.

Derivatives

Table 1 Oligomer molecular weight summary of selected enkephalin conjugates indicating the predominance of monoconjugates. Amphiphilic properties were only associated with singly conjugated derivatives

Enkephalin Conjugate	Expected MW	Observed MW
Cholesterol-PEG_2	1274	1275
Dihydroxyacetone-PEG_2	1144.4	1144.3
Linolenic-PEG_2	1093.4	1093.3
Cetyl-PEG_2	Avg. 1059	Avg. 1032
Palmitate-PEG_3	1116	1115.6
Cetyl-PEG_3	1101	1101.12

Testing

I. *Rat Paw-Hot Plate Test*

Adult, male Sprague–Dawley rats were assessed for analgesic activity using the rat paw-hot plate assay. The latency hot plate paw withdrawal was measured by a Hot Plate Analgesia Meter. The temperature of the hot plate was set and calibrated at 52°C and the rats were removed from the heat stimulus by 36 seconds after placement.

Thereafter, rats were given an injection of the opinoid inhibitor, naloxone, a μ-receptor antagonist, followed by an injection of cetyl-PEG_2-enkephalin to demonstrate that

the activity of cetyl-PEG_2-enkephalin is attributable to opioid μ-receptor binding. Testing results are provided in Tables 2 and 3.

Table 2 Summary of selected enkephalin conjugate experimental agents and their analgesic effects in rats during the rat paw-pot plate pest

Entry	Dosage (mg/kg)	Number of Rats	Analgesia Compared to Morphine After 5 minutes (%)	Analgesia Compared to Morphine After 30 minutes (%)
Morphine[a]	3	8	100	100
Enkephalin[a]	20	7	0	0
Cetyl-PEG-ENK	5	8	84	75
Dihydroxyacetone-PEG-ENK	20	8	130	67
Chelesterol-PEG-ENK	5	8	80	68
Linolenic-PEG-ENK	10	8	77	73

[a] Reference.

Table 3 Comparison of μ-receptor binding affinity of selected experimental enkephalin conjugates and their analgesic effect in rats

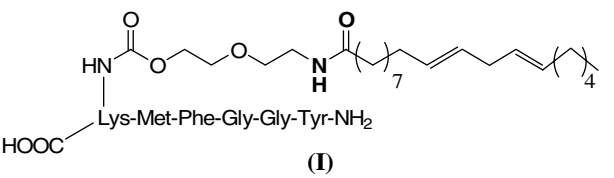
Drug or Conjugate	μ-Receptor Binding Affinity (%)
Naloxone[a]	100
Enkephalin[a]	67
Cetyl-ENK	100
Dihydroxyacetone-PEG-ENK	63
Cholesterol-PEG-ENK	95
Palm-TEG-ENK	76

[a] Reference.

Notes

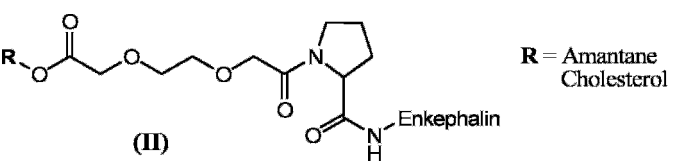
1. The Step 2 product activity was determined to be 67% after conjugating with insulin according to the method of Soltero (1).

2. The presence of the hexadecyl ester tail renders the Step 3 product a hydrolyzable amphiphilic oligomer. The linoleic amide tail in the amphiphilic oligomer linoleic met-enkephalinc-PEG-ENK, (**I**), previously prepared by the author (2) is nonhydrolyzable.



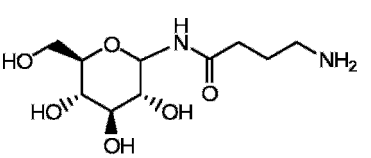
(I)

3. Pyrrolidine amide, (**II**), ENK conjugates prepared by the author (3) in a subsequent investigation were effective in traversing the blood–brain barrier and used as enkephalin delivery agents.



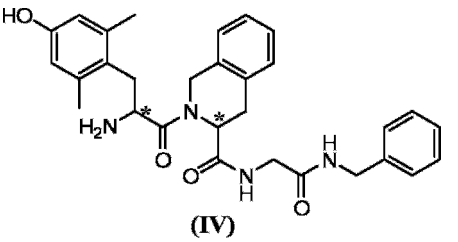
(II)

4. 4-Aminobutyramide of *N*-glucosyl amine, (**III**), prepared by Miller (4) was effective as a blood–brain barrier using conjugate for delivery of the neuroactive agents such as serotonin, dopamine, and enkephalin.



(III)

5. Dipeptidic, (**IV**), and tripepitidic conjugates effective in traversing the blood–brain barrier were prepared by Lazarus (5) and used as δ opioid receptor antagonists.



(IV)

References

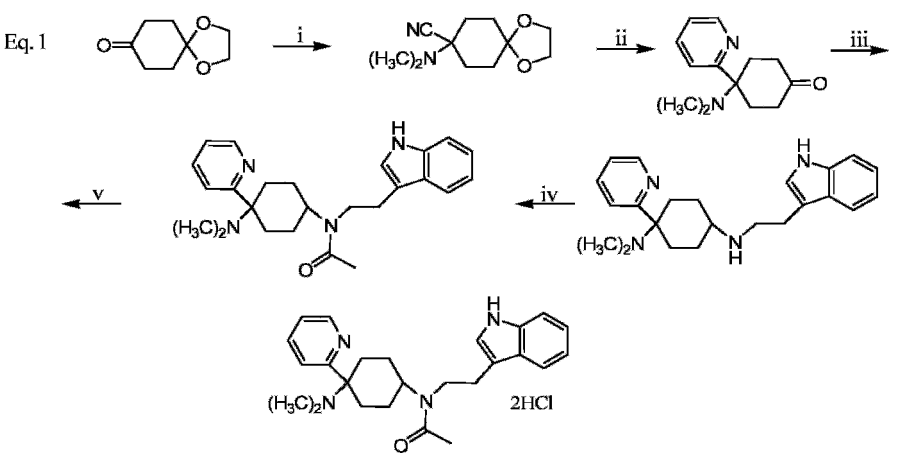
1. R. Soltero *et al.*, US Patent US 6,913,903 (July 5, 2005)
2. N. Ekwuribe *et al.*, and US Patent 6,930,090 (August 16, 2005)
3. N. Ekwuribe *et al.*, US Patent 7,030,084 (April 18, 2006) and US Patent 6,943,148 (September 13, 2005)
4. L.C.G. Miller *et al.*, US Patent 7,074,775 (July 11, 2006)
5. L.H. Lazarus *et al.*, US Patent 6,916,905 (July 12, 2005)

IV. ORL_1 Inhibitors

Title Substituted 2-Pyridine Cyclohexane-1,4-Diamine Compounds
B. Sundermann *et al.*, US Patent 6,998,409 (February 14, 2006)
Assignee Gruenenthal GmbH
Utility Treatment of Neuropathic Pain

Invention Significance The heptadecapeptide nociceptin is a ligand of the opioid receptor-like (ORL_1) that inhibits the activity of kainate- or glutamate-stimulated basal ganglia neurons resulting in acute, neuropathic, or chronic pain. To control this disorder, medicaments have been prepared that act on the nociceptin/ORL_1 receptor system or act as ORL_1 receptor inhibitors.

Reaction



i- Dimethylamine, dimethylamine•HCl, potassium cyanide
ii- Cyclopentadienyl cycloocta-1,5-diene cobalt(I), toluene, acetylene
iii- Acetic acid, tryptamine, THF, 1,2-dichloroethane, sodium triacetoxyboron hydride
iv- Pyridine, acetic anhydride
v- 2-Butanone, chlorotrimethylsilane

Experimental

1. **Preparation of 8-dimethylamino-1,4-dioxaspiro[4.5]decane-8-carbonitrile**

   A mixture consisting of 200 ml methyl alcohol, 1680 ml 40% aqueous dimethylamine solution, dimethylamine•HCl (303 g), and 200 g potassium cyanide (200 g) was added to 1,4-dioxaspiro[4.5]decan-8-one (200 g), then stirred 65 hours. A white suspension that formed was extracted four times with 800 ml diethyl ether, then concentrated, and the residue dissolved in 500 ml CH_2Cl_2. The organic phase was isolated, dried using Na_2SO_4, then concentrated, and 265 g product isolated as a white solid.

2. **Preparation of 4-dimethylamino-4-pyridin-2-yl-cyclohexanone**

   A stirred mixture consisting of the Step 1 product (4.4 g) and cyclopentadienyl cycloocta-1,5-diene cobalt(I) (50 mg) was dissolved in 100 ml toluene, then transferred into a reaction chamber, and saturated with acetylene, while the solution was irradiated 6 hours at 25°C. The mixture was concentrated. The residue (5.47 g) was then dissolved in 8.7 mfl water and 15 ml 12 M HCl, then stirred overnight at ambient temperature, and re-extracted three times with 100 ml diethyl ether. The aqueous phase was alkalified using 32% NaOH solution, then extracted three times with 100 ml CH_2Cl_2, dried, concentrated, and 3.72 g product isolated.

3. **Preparation of *N′*-[2-(1H-indol-3-yl)ethyl]-*N*, *N*-dimethyl-1-pyridin-2-yl-cyclohexane-1,4-diamine**

   Acetic acid (0.448 ml) was added to a solution of the Step 2 product (873 mg) and tryptamine (640 mg) dissolved in 40 ml THF containing 10 ml 1,2-dichloroethane and stirred 15 minutes. Sodium triacetoxyboron hydride (1.2 g) was introduced and the mixture stirred 3 days at ambient temperature and was then concentrated. The residue was dissolved in 40 ml apiece 1 M NaOH solution and diethyl ether, while the aqueous phase was extracted twice with 30 ml diethyl ether. Combined extracts were then dried and concentrated. The residue was purified by chromatography using silica gel with methyl alcohol/(methanol/ammonia), 100:1, and 617 mg product isolated as a white solid, mp = 150–152°C.

4. **Preparation of *N*-(4-dimethylamino-4-pyridin-2-yl-cyclohexyl)-*N*-[2-(1H-indol-3-yl)ethyl] acetamide**

   The Step 3 product (250 mg) dissolved in 5 ml pyridine was then treated with 0.64 ml acetic anhydride and the mixture stirred 22 hours at ambient temperature. Ice was then added and the mixture was then concentrated. The residue was dissolved in 20 ml apiece 1 M NaOH solution and EtOAc, then reconcentrated yielding 86 mg of a white solid. The aqueous phase was further extracted twice with 20 ml EtOAc, dried, concentrated, and 219 mg product isolated, mp = 209–210°C.

5. **Preparation of *N*-(4-dimethylamino-4-pyridin-2-yl-cyclohexyl)-*N*-[2-(1H-indol-3-yl)ethyl] acetamide•2HCl**

The Step 4 product (195 mg) dissolved in 25 ml 2-butanone was heated to 40°C and treated with 0.303 ml chlorotrimethyl silane. After workup, 219 mg product was isolated as a white solid, mp = 244–247°C.

Derivatives

Table 1 Selected diastereoisomers and their corresponding hydrogen chloride salt melting points for agents effective as ORL_1 receptor inhibitors. Optical activities for entries 5 and 6 provided by author

Entry	Agent	Diastereoisomer	Salt	mp (°C)
1	(*S*)-*N*-(4-Dimethylamino-4-pyridin-2-yl cyclohexyl)-*N*-[2-(1H-indol-3-yl)ethyl] acetamide	Nonpolar	2HCl	244–247
2	(*S*)-*N*-(4-Dimethylamino-4-pyridin-2-yl cyclohexyl)-*N*-[2-(1H-indol-3-yl)ethyl] acetamide	Nonpolar	3HCl	–
3	(*R*)-*N*-(4-Dimethylamino-4-pyridin-2-yl cyclohexyl)-*N*-[2-(1H-indol-3-yl)ethyl] acetamide	Polar	3HCl	225–230
4	(*S*)-*N′*-[2-(1H-Indol-3-yl)ethyl]-*N*,dimethyl-1-pyridin-2-yl cyclohexane-1,4-diamine	Nonpolar	3HCl	
5	(*S*)-2-(4-Dimethylamino-4-pyridin-2-yl cyclohexylamino)-3-(1H-indol-3-yl) methyl propionate	Nonpolar	3HCl	170–176
6	(*S*)-2-(4-Dimethylamino-4-pyridin-2-yl cyclohexylamino)-3-(1H-indol-3-yl) propionic acid	Nonpolar	2HCl	

Testing

I. *Measurement of* ORL_1 *Binding*

Experimental agents were evaluated in a receptor binding assay with ^{3}H-nociceptin/orphanin FQ with membranes of recombinant CHO–ORL_1 cells according to the method of Ardati (1). Testing results are provided in Table 2.

II. *Analgesia Testing in the Tail Flick Test in Mice*

Mice were placed into a test cage and the base of the tail exposed to a focused beam of heat from a tail flick type 50/08/1.bc electric lamp. The lamp intensity was

Table 2 Affinity values for ORL_1 binding assay using ^{3}H-nociceptin/orphanin FQ with membranes of recombinant CHO–ORL_1 cells and experimental derivatives

Entry	ORL_1 (K_i, μM)
1	0.18
2	0.013
3	0.34
4	0.093
5	0.47
6	0.28

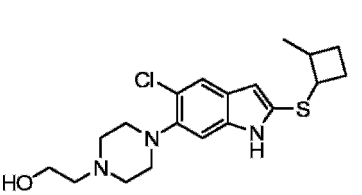
adjusted so that the time from switching on the lamp to the sudden flicking away of the tail for untreated mice was 3–5 seconds. The experimental agents were then administered intravenously, pain measured 10, 20, 40, and 60 minutes thereafter, and the analgesic activity determined. Maximum antinociceptive effect testing results are provided in Table 3.

Table 3 Maximum antinociceptive effect using the tail flick test in mice treated with intravenous dosage of 10 mg/kg using selected experimental agents

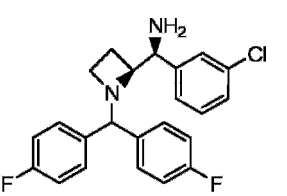
Entry	Maximum Antinociceptive Effect (%)
1	71
4	91

Notes

1. Benzimidazoles, (**I**), and azetidinyl amine derivatives, (**II**), prepared by Okamoto (2) and Burnett (3), respectively, were effective as ORL_1 receptor inhibitors and used in treating chronic pain.

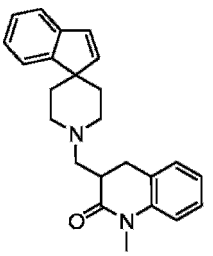


(I)

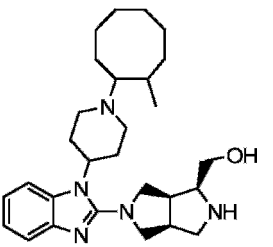


(II)

2. Spiro amines, (**III**), and benzimidazole derivatives, (**IV**), prepared by Ito (4,5), respectively, were effective as ORL_1 receptor agonists and useful as analgesics.

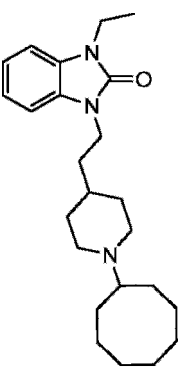


(III)



(IV)

3. Benzimidazolone derivatives, (**V**), prepared by Goehring (6) were effective as ORL_1 receptor agonists and used in the management of chronic pain.



(V)

References

1. J. Ardati *et al.*, Mol. Pharmacol., 51, 816, (1997)
2. O. Okamoto *et al.*, US Patent 6,969,712 (November 29, 2005)
3. D.A. Burnett *et al.*, US Patent 6,903,123 (January 7, 2005)
4. F. Ito *et al.*, US Patent 6,869,960 (March 22, 2005)
5. F. Ito *et al.*, US Patent 6,861,425 (March 1, 2005)
6. R.R. Goehring *et al.*, US Patent 6,872,733 (March 29, 2005)

CHAPTER V

Antibacterial Agents

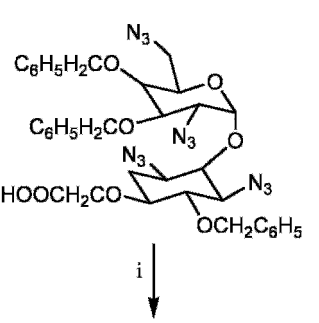
I. Aminoglycoside-modifying Enzyme Inhibitors Targeting Ribosomal RNA

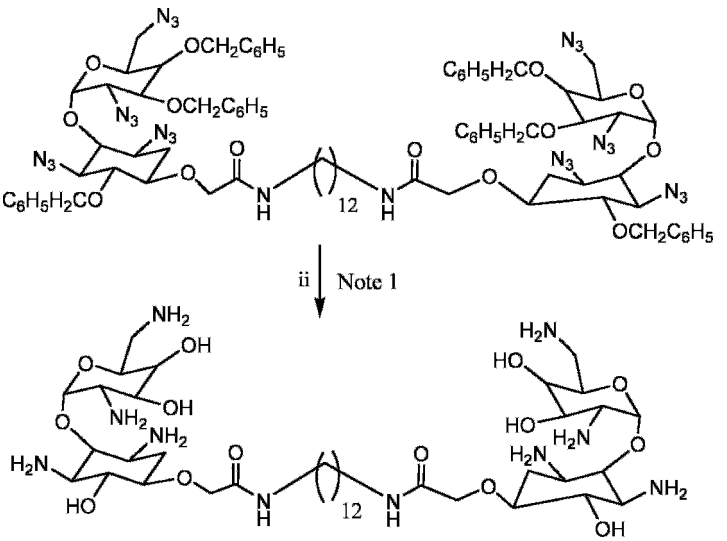
Title Bifunctional Antibiotics
C.-H. Wong *et al.*, US Patent 6,921,810 (July 26, 2005)
Assignee The Scripps Research Institute
Utility Treatment of Drug Resistant Bacterial Disorders

Invention Significance Deoxystreptamine-based aminoglycosides are a clinically important class of antibiotics that are effective against a broad range of microorganisms. Their effectiveness, however, is being compromised by the ongoing problem of antibiotic resistance. To address this concern, bifunctional aminoglycosides have been prepared that target both bacterial RNA and resistance-causing enzymes.

Reaction

Eq. 1





i- CH_2Cl_2, MP-carbodiimide resin, 1,12-dodecylamine
ii- Acetic acid, palladium hydroxide on carbon, hydrogen

Experimental

1. **Preparation of 5-ethylcarboxyl-1, 3, 2′, 6′-tetraazido-6, 3′, 4′-tri-*O*-benzylneamine diamine dimer**

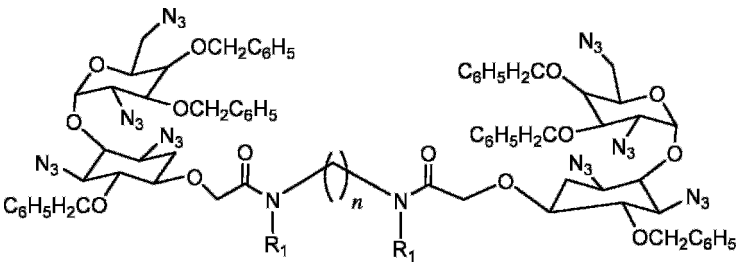
   Protected neamine dimers were prepared using a Quest 210 parallel synthesizer. 5-Ethylcarboxyl-1, 3, 2′, 6′-tetraazido-6, 3′, 4′-tri-*O*-benzylneamine (0.0826 mmol/tube was dissolved in 1.5 ml/tube containing CH_2Cl_2 distributed to each tube, which was then treated with MP-carbodiimide resin (1.15 mmol/g) followed by 1,12-dodecylamine (0.0413 mmol/tube). Solutions were agitated 16 hours, filtered, concentrated, and dimers isolated as colorless foams.

2. **General deprotection procedure**

   The Step 1 product dissolved in 1 ml/vial acetic acid was treated with 50 μg 20% $Pd(OH)_2$ on carbon, then hydrogenated 16 hours under 1 atm hydrogen, and concentrated. The residue was purified by flash chromatography on silica gel using $NH_4OH/CHCl_3/C_2C_5OH/C_4C_9OH$, 8:2:5:4, respectively. The isolated material was resuspended in water and applied to Dowex 50WX4-50 H^+, then washed with 5 ml water. The product was eluted with 3% NH_4OH and isolated as a colorless foam after lyophilization.

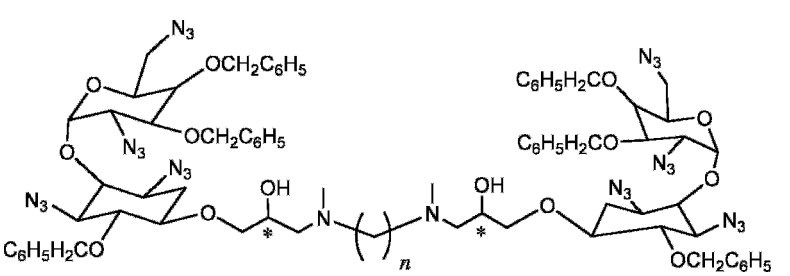
Derivatives

Table 1 Selected experimental neamine dimers prepared using the Quest 210 parallel synthesizer and their corresponding mass spectra characterization



Sample	*n*	R_1	Yield (%)	MALDI-TRMS, *m*/*z* $(M + Na)^+$
4	3	H	26	1569.645
8	7	H	60	1625.7021
9	8	H	47	1639.7137
10	9	H	44	1653.7423
11	10	H	54	1667.7483
12	11	H	50	1695.7802
13	12	H	47	1715.7344
14	12	$C_6H_5CHCONHCH_2CO_2H$	26	2215.8445 $(M+Cs)^+$

Table 2 Selected optically active experimental neamine dimers prepared using the Quest 210 parallel synthesizer and their corresponding mass spectra characterization



Sample	*n*	Yield (%)	MALDI-TRMS, m/z , $(M + H)^+$
21 (*S*, *S*-)	2	74	1593.7404
23 (*S*, *S*-)	4	54	1621.7658
25 (*R*, *R*-)	2	90	1593.7299
26 (*R*, *R*-)	3	77.9	1607.7531

Testing

I. *Antimicrobial Testing*

Antimicrobial testing was performed using the Kirby–Bauer Disk assay as described by Hendrix (1) and results summarized in Table 3.

Table 3 Antibiotic test results using selected experimental dimers. The numbers under each test strain are for diameters (mm) of zones of inhibition. All compounds except neomycin were spotted at 200 nmol/disk; neomycin was spotted at 33 nmol/disk

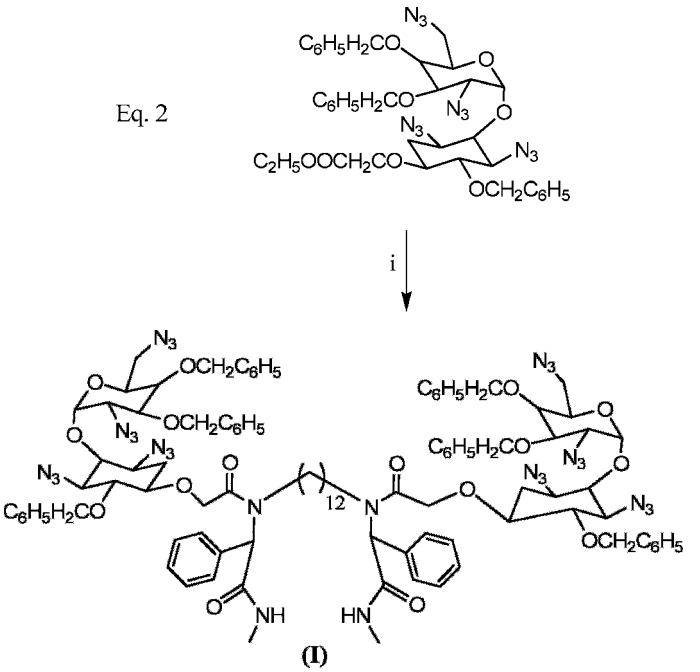
Sample	*Escherichia coli* ATCC 25922	*Staphylococcus aureus* ATCC 25923	K_d (μM)
Neamine[a]	17	17	10
Neomycin[a]	18.5	21	0.2
4	14.5	20	1.1
8	11	14.5	4.1
10	10.5	14.5	2.4
12	10	12.5	1.9
21	15	22.5	0.5
23	13	18	5.0
25	11	17.5	0.6
26	17	23	0.8

[a] Reference.

Notes

1. Step 2 dimer derivatives, (**I**), were also prepared by the author under Ugi conditions as illustrated in Eq. 2.

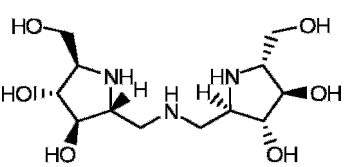
Eq. 2



(I)

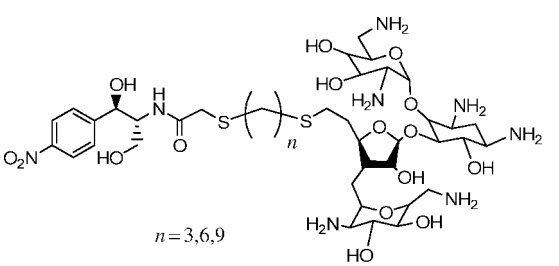
i-5-Ethylcarboxyl-1, 3, 2′, 6′-tetraazido-6, 3′, 4′-tri-*O*-benzylneamine, isocyanoacetate, benzaldehyde, diaminododecane, CH_2Cl_2, methyl alcohol

2. In an earlier investigation by the authors (2), iminocyclitol derivatives, (**II**), effective as hexoaminidases and glycosidase inhibitors were prepared.



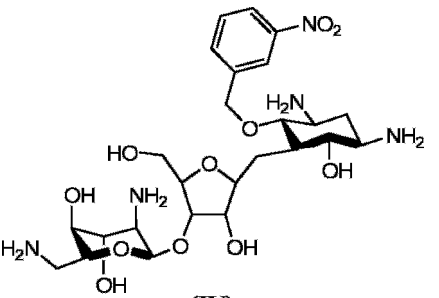
(II)

3. Yu (3) prepared heterodimeric conjugates of neomycin and chloramphenicol, (**III**), that recognized both stem and loop of RNA motif and showed an enhanced specificity against certain RNA targets.



(III)

4. Swayze (4) prepared 2-deoxystreptamine aromatic derivatives, (**IV**), which showed broad-spectrum activity against antibiotic-resistant pathogens.



(IV)

References

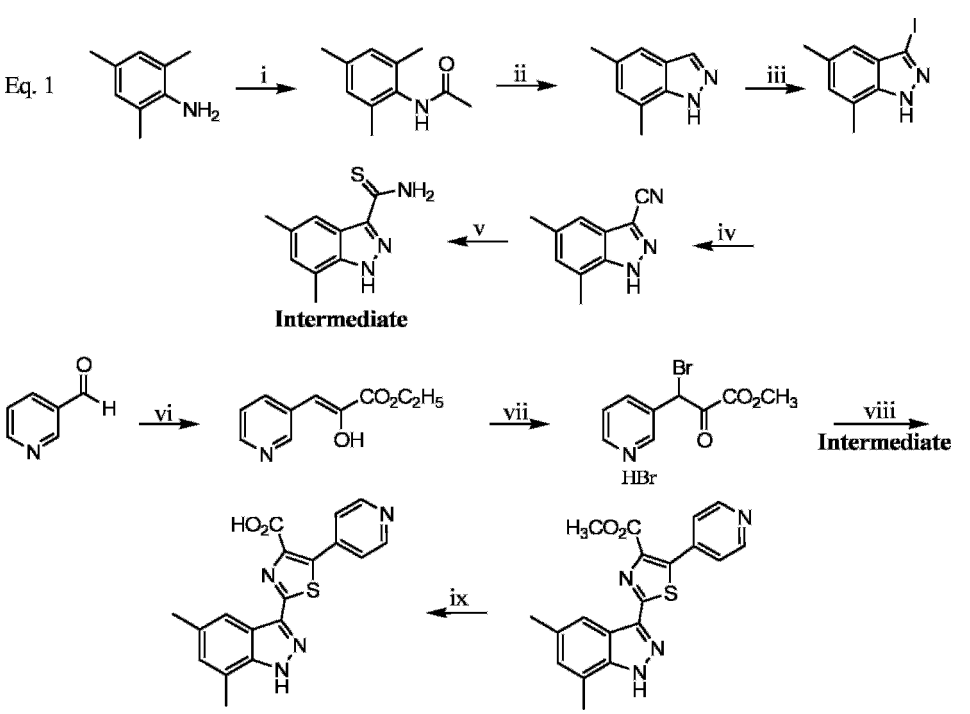
1. M. Hendrix *et al*., J. Am. Chem. Soc., 119, 3641 (1997)
2. C.-H. Wong *et al*., US Patent 6,774,140 (August 10, 2004)
3. J. Yu *et al*., US Patent 7,022,839 (April 4, 2006)
4. E. Swayze *et al*., US Patent 6,967,242 (November 22, 2005) and US Patent 6,541,456 (April 1, 2003)

II. Bacterial DNA Gyrase Inhibitors

Title Gyrase Inhibitors
K. Yager *et al.*, US Patent 6,984,652 (January 10, 2006)
Assignee Warner-Lambert Company LLC
Utility Treatment of Bacterial Infections

Invention Significance Gyrase is a topoisomerase found in many gram-positive and gram-negative bacteria which is required for the interconversion of topological isomers occurring during DNA replication. Although gyrase inhibitors currently exist, their use is impeded by high levels of antibiotic resistance and instances of drug toxicity. The current investigation addresses the need for new antibacterial agents that target the B subunit of bacterial DNA gyrase having favorable toxicity profiles

Reaction



i- Acetyl chloride, triethylamine, CH_2Cl_2
ii- Acetic acid, isoamyl acetate
iii- Iodine, potassium hydroxide
iv- Sodium cyanide, *N*-methyl-2-pyrrolidinone
v- Hydrogen sulfide, triethylamine, pyridine, triethylamine
vi- Diethyl ether, sodium hydride, ethyl alcohol, 3-pyridine-carboxaldehyde
vii- Bromine, methyl alcohol, THF
viii- Methyl alcohol, collidine, ethanesulfonic acid
ix- Methyl alcohol, sodium hydroxide

Experimental

1. **Preparation of *N*-(2,4,6-trimethyl-phenyl)-acetamide**

   A solution of 2,4,6-trimethylphenylamine (0.370 mol) dissolved in 500 ml CH_2Cl_2 was cooled to 0°C, then treated with acetyl chloride (380 mmol) over 5 minutes followed by the portion-wise addition of 53 ml triethylamine. The ice bath was removed and the mixture was stirred 2 hours at ambient temperature. An ivory-colored solid was collected by filtration, then suspended in 600 ml water, stirred 30 minutes, recollected by filtration, and dried. The original filtrate was washed three times with 100 ml apiece water and brine, dried with $MgSO_4$, concentrated to provide additional material, and the product isolated in 99% yield.

2. **Preparation of 5,7-dimethyl-1H-indazole**

   The Step 1 product (133 mmol) was dissolved in 300 ml toluene and glacial acetic acid (173 mmol), then slowly treated with isoamyl nitrite (173 mmol), and refluxed overnight. The mixture was poured into 1300 ml water and extracted twice with 300 ml EtOAc. Combined extracts were washed twice with 200 ml saturated $NaHCO_3$ solution, once with 100 ml brine, dried using $MgSO_4$, concentrated, and 19.5 g product isolated.

3. **Preparation of 3-iodo-5,7-dimethyl-1H-indazole**

   A mixture consisting of the Step 2 product (186 mmol) dissolved in 500 ml DMF was treated with iodine crystals (567 mmol) and KOH (945 mmol) and stirred 2 hours at ambient temperature. The mixture was concentrated to half volume, then poured into 250 ml 5% $NaHSO_3$ solution, and re-extracted three times with 250 ml diethyl ether. The extracts were washed twice with 200 ml water and once with 200 ml brine, dried using $MgSO_4$, and concentrated. The dark solid was suspended in 300 ml hot EtOAc and treated with 600 ml hexane, then cooled 2 hours, and 17.50 g crystals isolated. The filtrate was further concentrated and additional crystals were isolated using a silica gel plug with 20% EtOAc in hexanes. A total product yield of 27 g was obtained.

4. **Preparation of 5,7-dimethyl-1H-indazole-3-carbonitrile**

The Step 3 product (74.0 mmol) dissolved in 300 ml *N*-methyl-2-pyrrolidinone was treated with CuCN (222 mmol) and NaCN (148 mmol) and the mixture heated 18 hours at 130°C. The solution was poured into a mixture of 1500 ml 0.25 M KH_2PO_4 solution and 750 ml diethyl ether and celite (100 g) added. The suspension was stirred 30 minutes and was then filtered through sintered glass. The phases were separated and the aqueous layer extracted three times with 200 ml diethyl ether. Combined extracts were washed twice with 200 ml water and once with 200 ml brine, dried, concentrated, and the product isolated in 65% yield.

5. **Preparation of 5,7-dimethyl-1H-indazole-3-carboximidothioic acid**

A solution of the Step 4 product (22.0 mmol) dissolved in 50 ml 20% triethylamine/pyridine was cooled to 0°C and saturated with H_2S for 5 minutes. The vessel was then sealed and the mixture stirred 90 minutes at ambient temperature. Excess H_2S was removed under vacuum and the mixture was concentrated to a solid. The residue was suspended in 275 ml hexane and isolated by vacuum filtration. It was dried over P_2O_5 in vacuo, and the product isolated in 93% yield as an yellow solid.

6. **Preparation of 2-hydroxy-3-pyridin-3-yl-acrylic acid ethyl ester**

A reaction vessel was charged with 500 ml diethyl ether, then cooled to 0°C, and NaH (400 mmol) and 23.2 ml ethyl alcohol added followed by 3-pyridine carboxaldehyde (200 mmol) and 84.8 ml *N*,*N*-dimethyl glycine ethyl ester. The mixture stirred overnight at ambient temperature and was then heated 1 hour at 30°C. The mixture was transferred to a separation funnel and diluted with 500 ml apiece EtOAc and water. The organic phase was transferred to a beaker, then treated with 500 ml 1 M HCl, stirred 10 minutes at ambient temperature, and the phases separated. The aqueous layer was neutralized with solid $NaHCO_3$ and extracted three times with 300 ml EtOAc. The combined extracts were dried with Na_2SO_4, concentrated, and an yellow solid isolated. The residue was recrystallized using EtOAc/hexanes, 4:1, and the product isolated in 52% yield as a pale yellow solid.

7. **Preparation of 3-bromo-2-oxo-3-pyridin-3-yl-propionic acid methyl ester hydrobromide**

The Step 6 product (100 mmol) was dissolved in 400 ml THF and treated with the dropwise addition of bromine (100.0 mmol). The mixture was then treated with 10 ml methyl alcohol to maintain homogeneity and then stirred 30 minutes at ambient temperature. It was then concentrated and the solid residue triturated with 50 ml THF and three times with 50 ml EtOAc. The product was isolated as an yellow foam in quantitative yield after drying and used without further purification.

8. **Preparation of methyl- and ethyl 2-(5,7-dimethyl-1H-indazol-3-yl)-5-pyridin-3-yl-thiazole-4-carboxylate**

   A suspension of Step 5 product (22.0 mmol) in 100 ml methyl alcohol was heated at 50°C. In a separate vessel, the Step 7 product (44.0 mmol) dissolved in 88 ml methyl alcohol containing collidine (77.0 mmol) was cooled to 0°C, then added dropwise to the Step 5 product mixture, and heated 2 hours at 50°C. This mixture was then treated with ethanesulfonic acid (77.0 mmol), then heated to 50°C overnight. The solution was concentrated and the oily residue was treated with 100 ml saturated Na_2CO_3 solution. The resulting solid was rinsed twice with 30 ml water, dried under vacuum, purified by silica gel-plug filtration initial using 100% CH_2Cl_2, then 2–5% methyl alcohol/CH_2Cl_2, and the ester mixture isolated.

9. **Preparation of 2-(5,7-dimethyl-1H-indazol-3-yl)-5-pyridin-3-yl-thiazole-4-carboxylic acid**

   The Step 8 product mixture (22 mmol) was dissolved in 100 ml methyl alcohol, then treated with 50 ml 1 M NaOH, then refluxed 2 hours, and concentrated. The concentrate was acidified with 100 ml 1 M HCl and a solid residue isolated. The solid was rinsed three times with 20 ml water, dried, and 7.9 g product isolated. RP-HPLC Method: 10–95% B in 8 minutes.

Derivatives

Selected 5,7-dimethyl-1H-indazole derivatives are provided in Table 1.

Table 1 Selected experimental agents found to have a MIC values of 10 μM or less in the methicillin-resistant *S. aureus* assay. Only HPLC retention times supplied by author

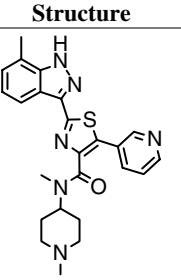
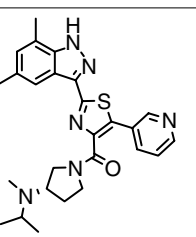
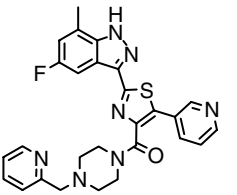
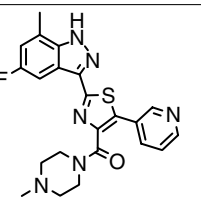
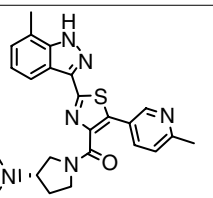
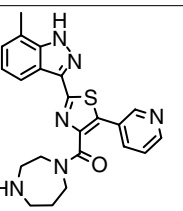
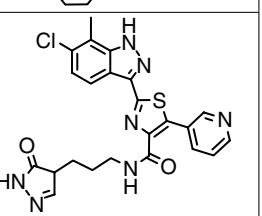
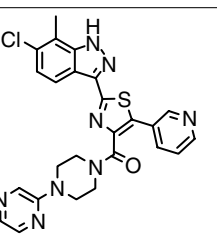
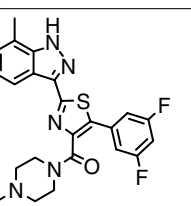
Entry	Name	Structure
2	2-(7-Methyl-1H-indazol-3-yl)-5-pyridin-3-yl-thiazole-4-carboxylic acid methyl-(1-methyl-piperidin-4-yl)-amide	
7	[2-(5,7-Dimethyl-1H-indazol-3-yl)-5-pyridin-3-yl-thiazol-4-yl]-[3-(isopropyl-methyl-amino)-pyrrolidin-l-yl]-methanone	

Table 1 Continued

20	[2-(5-Fluoro-7-methyl-1H-indazol-3-yl)-5-pyridin-3-yl-thiazol-4-yl]-(4-pyridin-2-ylmethyl-piperazin-1-yl)-methanone	
34	2-[(5-Fluoro-7-methyl-1H-indazol-3-yl)-5-pyridin-3-yl-thiazol-4-yl]-(4-methyl-piperazin-1-yl)-methanone	
42	(3-Dimethylamino-pyrrolidin-1-yl)-[2-(7-methyl-1H-indazol-3-yl)-5-(6-methyl-pyridin-3-yl)-thiazol-4-yl]-methanone	
51	[1,4]Diazepan-1-yl-[2-(7-methyl-1H-indazol-3-yl)-5-pyridin-3-yl-thiazol-4-yl]-methanone	
64	2-(6-Chloro-7-methyl-1H-indazol-3-yl)-5-pyridin-3-yl-thiazole-4-carboxylic acid [3-(5-oxo-4,5-dihydro-1H-pyrazol-4-yl)-propyl]-amide	
74	[2-(6-Chloro-7-methyl-1H-indazol-3-yl)-5-pyridin-3-yl-thiazol-4-yl]-(2,3,5,6-tetrahydro-[1,2′]bipyrazinyl-4-yl)-methanone	
95	[5-(3,5-Difluoro-phenyl)-2-(7-methyl-1H-indazol-3-yl)-thiazol-4-yl]-(4-methyl-piperazin-1-yl)-methanone	

Testing

I. *Bacteria Preparations*

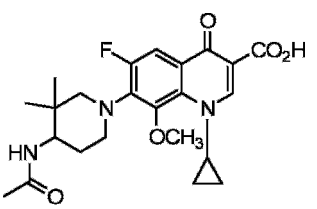
Bacterial colonies were suspended in 0.9% NaCl until a turbidity comparable to MacFarland's standard was achieved. Thereafter, the effectiveness of selected experimental agents was assayed at various concentrations using serial dilutions and absorbance methods at 600 nm with a bacterial stock of 1×10^8 bacteria/ml.

Tests Results

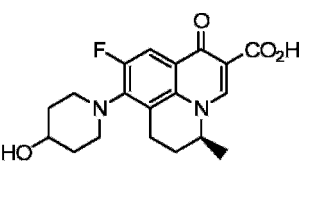
Selected experimental 5,7-dimethyl-1H-indazole agents provided in Table 1 had MIC values of 10 μM or less in the methicillin-resistant *Staphylococcus aureus* assay.

Notes

1. De Souza (1,2) prepared 7-substituted piperidino-quinolone carboxylic acid derivatives, (**I**) and (**II**), respectively, which were effective against multidrug-resistant pathogens in addition to providing broad-spectrum coverage to gram-positive and gram-negative microbes.

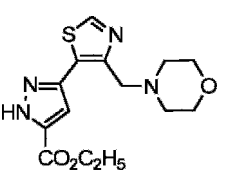


(I)



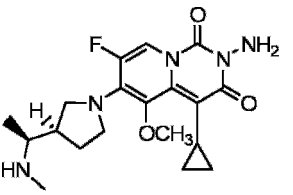
(II)

2. Thiazole, (**III**), oxazole, imidazole, and pyrazole gyrase inhibitors prepared by Charifson (3) were effective against the bacterial DNA gyrase two B subunits and used in treating antibiotic resistance.



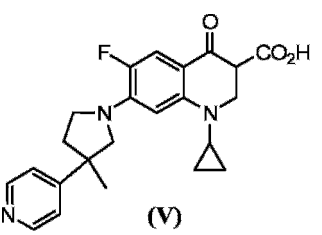
(III)

3. Pyrimidine derivatives, (**IV**), prepared by Ellsworth (4) showed antibacterial activities against ciprofloxacin-resistant strains in addition to being effective against *Streptococcus pyogenes* and *Escherichia coli* gyrase.

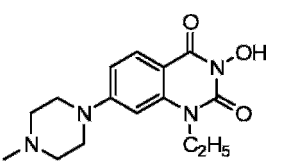


(IV)

4. 7-Pyridyl quinolone carboxylic acids, (**V**), and 7-piperazine quinazolin-2,4-dione derivatives, (**VI**), prepared by Park (5) and Domagala (6), respectively, exhibited broad-spectrum antibacterial activity with reduced cytotoxicity.



(V)



(VI)

References

1. N.J. De Souza *et al*., US Patent 6,964,966 (November 15, 2005) and US Patent 6,878,713 (April 12, 2005)
2. N.J. De Souza *et al*., US Patent 6,514,986 (February 4, 2003)
3. P. Charifson *et al*., US Patent 6,930,116 (August 16, 2005)
4. E.L. Ellsworth *et al*., US Patent 6,864,259 (March 8, 2005)
5. T.-H. Park *et al*., US Patent 6,825,199 (March 14, 2006)
6. J.M. Domagala *et al*., US Patent 6,825,199 (November 30, 2004)

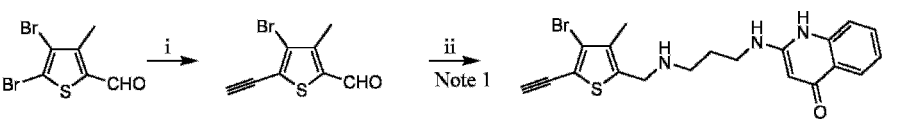
III. Methionyl t-RNA Synthetase Inhibitors

Title Substituted Thiophenes with Antibacterial Activity
J. Berge *et al*., US Patent 7,030,137 (April 18, 2006)
Assignee Replidyne, Inc.
Utility Treatment of Multidrug-resistant Methionyl t-RNA Synthetase

Invention Significance Thiophene-containing derivatives effective as bacterial methionyl t-RNA synthetase inhibitors and active against *Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus faecalis, Haemophilus influenzae*, and *Moraxella catarrhalis* organisms have been prepared. These agents are also effective against Staphylococci organisms such as *S. aureus* and coagulase-negative strains of Staphylococci such as *Staphylococcus epidermidis*.

Reaction

Eq. 1



i- Trimethylsilylacetylene, copper(I) iodide, bis(triphenylphosphine)palladium(II) chloride, triethylamine
ii- 2-(3-Aminoprop-1-ylamino)-1H-quinolin-4-one-diamine, DMF, acetic acid, sodium acetate, sodium triacetoxyborohydride

Experimental

1. **Preparation of 4-bromo-5-ethynyl-3-methylthiophene-2-carbaldehyde**

A mixture consisting of trimethylsilylacetylene (26.4 mmol), 4,5-dibromo-3-methylthiophene-2-carbaldehyde (52.8 mmol), copper(I) iodide (19 mg), and bis(triphenylphosphine)palladium(II) chloride (80 mg) dissolved in 35 ml triethylamine was stirred 2 hours at ambient temperature, then filtered, and concentrated. The residue was purified by chromatography using silica gel with petroleum ether containing up to 50% CH_2Cl_2. The product was then treated with methyl alcohol containing 10% concentrated NH_4OH, then stirred 15 minutes at ambient temperature, reconcentrated, and 0.73 g product isolated as a light brown foam.

^{1}H NMR δH (CDCl3) 9.99 (1H, s), 3.80 (1H, s), 2.55 (3H, s)

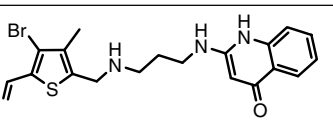
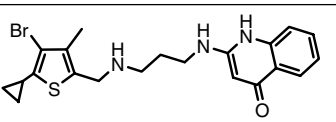
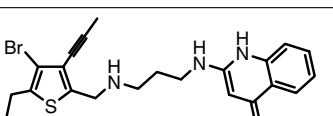
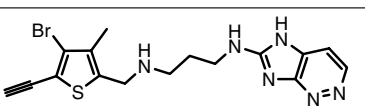
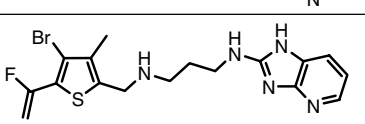
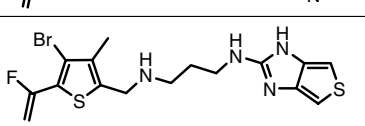
2. **Preparation of *N*-(4-bromo-5-ethynyl-3-methylthiophen-2-ylmethyl)-*N′*-(1H-quinolin-4-one)propane-1,3-diamine**

Reductive amination was performed by initially treating the Step 1 product (0.14 mmol) with 2-(3-aminoprop-1-ylamino)-1H-quinolin-4-one-diamine (0.135 mmol) dissolved in 0.2 ml DMF/acetic acid, 1:1, and sodium acetate (0.97 mmol), then stirring 75 minutes. This mixture was then treated with sodium triacetoxyborohydride (0.156 mmol), then stirred an additional 2 hours, and concentrated. The residue was partitioned between EtOAc and 1 M NaOH and the organic component dried using $MgSO_4$, concentrated, and the product isolated after chromatographic purification using silica gel.

Derivatives

Selected thiophene derivatives are provided in Table 1.

Table 1 Selective thiophene derivatives illustrating their effectiveness as methionyl t-RNA synthetase inhibitors against *Staphlococcus aureus, Streptococcus pneumoniae* and *Haemophilus influenzae*

Entry	Structure	MIC*[1] S. *aureus* (ug/ml)	MIC S. *pneumo* (ug/ml)	MIC H. *influenzae* (ug/ml)
3		0.06	0.06	16
6		0.06	0.13	32
9		0.06	0.06	64
14		0.25	0.25	0.13
17		0.06	0.25	64
21		0.06	0.25	8

[a] Minimum inhibitory concentration.

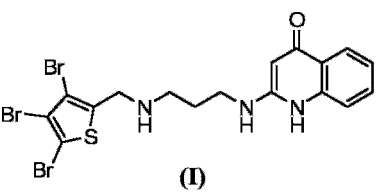
Testing

I. *Enzyme Inhibition: Aminoacylation Assay*

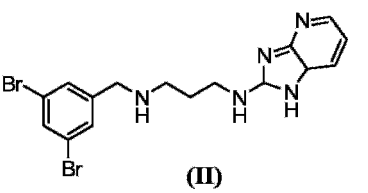
Experimental agents were assayed for their ability to inhibit the enzyme methionyl tRNA synthetase according to the method of Fleischmann (1). Aminoacylation assay test results are provided in Table 1.

Notes

1. Quinolones, (**I**), and 1H-imidazo[4,5-b]pyridine derivatives, (**II**), effective as methionyl t-RNA synthetase inhibitors were previously prepared by the author (2,3), respectively.

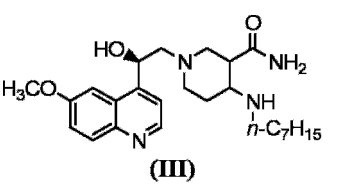


(I)

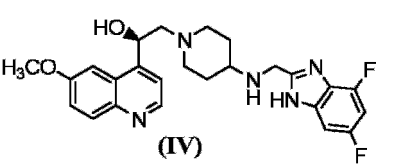


(II)

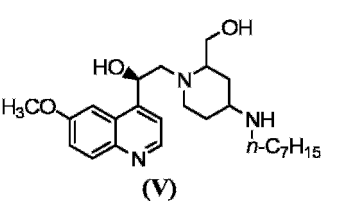
2. Aminopiperidine quinoline derivatives, (**III**), (**IV**), and (**V**), prepared by Davies (4,5) and Markwell (6), respectively, were effective as methionyl t-RNA synthetase inhibitors against *S. aureus* Oxford, *S. aureus* WCUH29, *S. pneumoniae* 1629, *S. pneumoniae* N1387, and *S. pneumoniae* ERY 2.



(III)

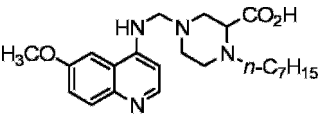


(IV)



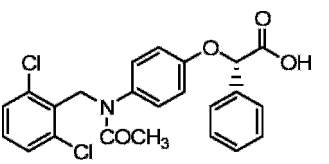
(V)

3. Piperazine quinoline analogues, (**VI**), prepared by Davies (7) were effective as methionyl t-RNA synthetase inhibitors associated with *S. aureus* Oxford, *S. aureus* WCUH29, *S. pneumoniae* 1629, *S. pneumoniae* N1387, *S. pneumoniae* ERY 2, and *E. faecalis* 1, *H. influenzae*.



(VI)

4. Christensen (8) prepared acetic acid derivatives, (**VII**), which were effective as fatty acid synthase inhibitors useful in the treatment of gram-positive and gram-negative bacterial infections such as *E. coli* and *S. aureus* and *S. epidermidis*.



(VII)

References

1. R.D. Fleischmann *et al*., Science, 269, 496, 512 (1995)
2. J.M. Berge *et al*., US Patent 6,320,051 (November 20, 2001)
3. J.M. Berge *et al*., US Patent 6,943,175 (September 13, 2005)
4. D.T. Davies *et al*., US Patent 7,001,913 (February 21, 2006) and US Patent 6,602,882 (August 5, 2003)
5. D.T. Davies *et al*., US Patent 6,962,917 (November 8, 2005)
6. R.E. Markwell *et al*., US Patent 6,989,447 (January 24, 2006)
7. D.T. Davies *et al*., US Patent 6,911,442 (June 28, 2005)
8. S.B. Christensen *et al*., US Patent 6,723,749 (April 20, 2004)

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CHAPTER VI

Anti-inflammatory Agents

I. Anti-inflammatory Cytokine Inhibitors

Title Anti-inflammatory Nitro- and Thio-fatty Acids
A. Ferrante *et al*., US Patent 6,924,309 (August 2, 2005)

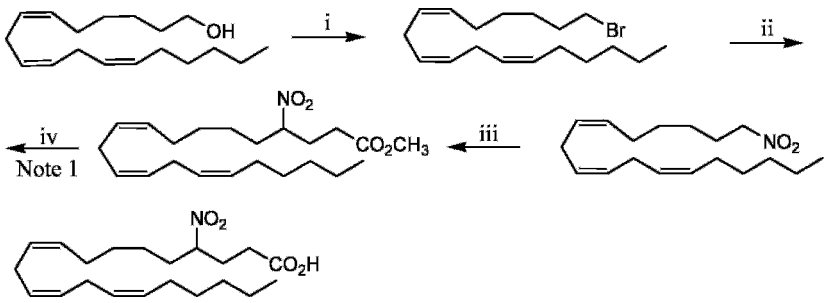
Assignee Children, Youth and Women's Health Service Incorporated (North Adelaide, AU) *and* Peptech Limited (AU)

Utility Anti-inflammatory Agents and Antioxidants

Invention Significance The lipoxygenase pathway of arachidonic acid is responsible for the generation of leukotrienes and lipoxins, both of which have been implicated in the pathology of inflammatory disorders including asthma. To enhance resistance to β-oxidation of polyunsaturated fatty acids (PUFAs), nitro- and thio-acid derivatives have been prepared, which retain biological activities of unmodified PUFAs. In addition to being effective as inflammatory cytokine inhibitors, they are also effective as antioxidants.

Reaction

Eq. 1



i- CH_2Cl_2, triphenylphosphine, carbon tetrabromide
ii- Acetone, sodium iodide, silver nitrate, diethyl ether
iii- Sodium hydroxide, water, methyl acrylate, tetrabutylammonium iodide
iv- Lithium hydroxide, 1,2-dimethoxyethane, hydrochloric acid

Experimental

1. **Preparation of 1-bromooctadecane**

Octadecan-1-ol (1.92 mmol) and triphenylphosphine (2.10 mmol) were dissolved in 25 ml CH_2Cl_2, then cooled in an ice bath, and treated with CBr_4 (1.90 mmol). The mixture was stirred overnight at ambient temperature and was then concentrated. The residue was purified by flash column chromatography using silica gel with hexane and the product was isolated in 96% yield as a waxy solid, mp = 26−28°C.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, 3H, $J = 6.7$ Hz, C18-H_3), 1.25–1.32 [m, 30H, (C3-17)-H_2)], 1.82–1.85 (m, 2H, C2-H_2), 3.40 (t, 2H, $J = 6.8$ Hz, C1-H_2)
^{13}C NMR (300 MHz, $CDCl_3$) δ 14.7, 23.2, 28.7, 29.3, 29.9, 30.0, 30.1, 30.1(6), 30.2(3), 32.5, 33.4, 34.6
IR (KBr) ν (cm^{-1}) 2920 (s), 2848 (s), 1468 (s), 1378 (w), 1254 (w), 1144 (m), 720 (m), 658 (s)
MS (EI) *m/z* (%) 334 (M^+, 8), 332 (M^+, 10), 253 (25), 151 (27), 149 (28), 137 (67), 135 (69), 113 (19), 97 (30), 85 (50), 71 (70), 57 (100)
HRMS *m/z* Calc. for $C_{13}H_{37}Br$ 334.2058 (M^+) and 332.2078 (M^+). Found 334.2070 and 332.2086

2. **Preparation of 1-nitrooctadecane**

The Step 1 product (1.44 mmol) dissolved in 25 ml acetone was treated with NaI (2.87 mmol), then stirred overnight at ambient temperature, and concentrated. The residue was mixed with 25 ml saturated $NaHSO_3$ solution and extracted three times with 25 ml diethyl ether, then dried using Na_2SO_4, and concentrated. The residue was then dissolved in diethyl ether containing $AgNO_3$ (2.64 mmol), then stirred 3 days, filtered through celite, and concentrated. The residue was purified by flash column chromatography using diethyl ether/hexane, 5:95, and the product isolated in 51% yield as a white solid, mp = 41−42°C.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, 3H, $J = 6.6$ Hz, C18-H_3), 1.25–1.34 [m, 30H, (C3-C17)-H_2], 1.96–2.05 (m, 2H, C2-H_2), 4.38 (t, 2H, $J = 7.1$ Hz, C1-H_2)
^{13}C NMR (300 MHz, $CDCl_3$) δ 14.7, 23.3, 26.7, 28.0, 29.4, 29.8, 29.9, 30.0, 30.1, 30.2, 30.3, 32.5, 76.3
IR (film) ν (cm^{-1}) 2954 (s), 2919 (s), 2850 (s), 1563 (s), 1470 (m), 1385 (w), 1147 (w), 742 (w), 720 (m), 630 (w)

MS (EI) *m/z* (%) 299 (M^+, < 1), 282 (4), 264 (20), 252 (7), 238 (7), 224 (7), 210 (5), 196 (4), 154 (5), 139 (7), 125 (20), 111 (40), 97(74), 83 (87), 69 (95), 57 (100), 55 (96)
Analysis Calc. for $C_{18}H_{37}NO_2$: C, 72.19; H, 12.45; N, 4.68. Found: C, 72.33; H, 12.77; N, 4.57
121 (35), 108 (63), 95 (84), 93 (75), 91 (69), 79 (100), 67 (95)

3. **Preparation of methyl 4-nitroheneicosanoate**

A solution of NaOH (3.4 mmol) and Bu_4NI (0.43 mmol) in 10 ml water was added to a solution of the Step 2 product (1.70 mmol) and methyl acrylate (5.13 mmol) dissolved in 10 ml CH_2Cl_2, then refluxed 24 hours. The solution was cooled and the layers separated. The organic phase was washed twice with 25 ml water, dried, and concentrated. The residue was purified by flash column chromatography using diethyl ether/hexane, 5:95, and the product isolated in 76% yield as a waxy solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, 3H, $J = 6.7$ Hz, C21-H_3), 1.19–1.25 (m, 30H, (C6-C20)-H_2), 1.69–1.78 (m, 1H), 1.92–2.30 (m,3H), 2.32–2.40 (m, 2H, C2-H_2), 3.69 (s, 3H, OCH_3), 4.50–4.59 (m, 1H, C4-H)
^{13}C NMR (300 MHz, $CDCl_3$) δ 14.7, 23.3, 26.2, 29.2, 29.5, 29.8, 29.9, 30.0, 30.1, 30.2, 30.3, 30.5, 32.5, 34.5, 52.5, 88.4, 173.0
IR (Nujol) ν (cm^{-1}) 2924 (s), 2853 (s), 1744 (s), 1554 (s), 1466 (m), 1439 (m), 1367 (m), 1201 (m), 1175 (m), 1120 (m), 829 (w), 722 (w)
MS (EI) *m/z* (%) 386 [$(M+1)^+$, 25], 368 (12), 354 (18), 339 (20), 305 (24), 287 (28), 263 (18), 221 (15), 193 (10), 179 (15), 165 (21), 151 (26), 137 (31), 123 (36), 111 (52), 97 (76), 83 (86), 69 (88), 55 (100)
HRMS *m/z* Calc. for $C_{22}H_{44}NO_4$ 386.3270 $(M+H)^+$. Found 386.3275
Analysis Calc. for $C_{22}H_{43}NO_4$: C, 68.53; H, 11.24; N, 3.63. Found: C, 68.39; H, 11.53; N, 3.50

4. **Preparation of 4-nitroheneicosanoic acid**

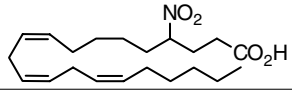
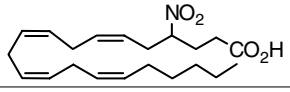
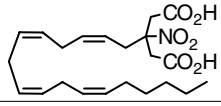
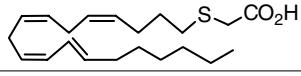
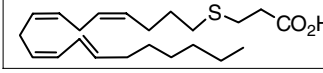
The Step 3 product (0.38 mmol) dissolved in 2 ml 1,2-dimethoxyethane was treated with 2 ml saturated LiOH solution and then stirred for 24 hours. The mixture was acidified with 10 ml 10% HCl, then extracted twice with 10 ml EtOAc, and concentrated. The residue was purified by flash chromatography using diethyl ether/hexane, 100:20, then diethyl ether/hexane/HOAc, 60:40:1, and the product isolated in 85% yield as a white solid, mp = 55−56°C.

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, 3H, $J = 7.1$ Hz, C21-H_3), 1.20–1.28 [m, 30H, (C6-C20)-H_2], 1.69–1.78 (m, 1), 1.98–2.30 (m, 3H), 2.39–2.48 (m, 2H, C2-H_2), 4.53–4.60 (m, 1H, C4-H)
^{13}C NMR (300 MHz, $CDCl_3$) δ 14.7, 23.3, 26.2, 28.8, 29.5, 29.8, 29.9, 30.0, 30.1, 30.2(6), 30.3(3), 32.5, 34.4, 88.2, 177.5
IR (KBr) ν (cm^{-1}) 3500–2600 (br), 2955 (m), 2919 (s), 2849 (s), 1698 (s), 1615 (w), 1543 (s), 1467 (m), 1445 (m), 1413 (w), 1360 (w), 1334 (w), 1266 (w), 923 (w), 827 (w), 723 (w), 612 (w)

MS (CI) *m/z* 389.3 $(M+NH_4)^+$
MS (EI) *m/z* (%) 354 [$(M-; OH)^+$, 2], 323 (19), 321 (19), 305 (17), 287 (14), 263 (12), 236 (5), 221 (9), 193 (10), 179 (15), 165 (15), 151 (17), 137 (20), 125 (25), 110 (73), 97 (100), 83 (64), 69 (64), 55 (73)
HRMS *m/z* Calc. for $C_{21}H_{40}NO_3$ 354.3008 $(M-; OH)^+$. Found 354.3006
Analysis Calc. for $C_{21}H_{41}NO_4$: C, 67.88; H, 11.12; N, 3.77. Found: C, 67.58; H, 11.08; N, 3.81

Derivatives

Table 1 Selected experimental nitro- and thio-arachidonic acid analogs and their corresponding overall yield and mass spectral data. ^{1}H- and ^{13}C- and NMR data supplied by author for products and intermediates

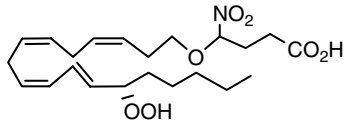
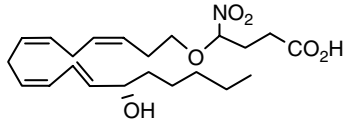
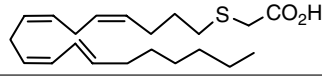
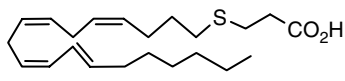
Entry	Yield (%)	MS
	51	293
	53	343
	88	463
	77	288
	–	301

Testing

I. *Antioxidative Properties Using the Thin Film Oxidation Protocol*

A stock solution of 2 ml CH_2Cl_2 containing arachidonic acid 1 (18 mg) and a selected experimental agent (18 mg) was prepared using lauric acid (18 mg) as an internal standard. Stock solution samples (100 μl) were placed in glass Petri dishes followed by 400 μl ethyl alcohol and the mixture evaporated leaving a thin film. The film was placed into a desiccator filled with oxygen and stored in darkness. Dishes were removed from the desiccator after 1, 2, 3, 5, and 7 days. The mixture on each dish was redissolved in diethyl ether and transferred to a 2 ml vial. After evaporation of the solvent, the residue was dissolved in 100 μl mobile phase of the HPLC and 10% of the solution analyzed by HPLC-RP containing octadecylsilane; 4.6 mm × 250 mm, 3 μm column. Testing results comparing HPLC retention times for arachidonic acid alone or when additized with selected experimental agents are provided in Table 2.

Table 2 Oxidative resistance of arachidonic acid when additized with experimental nitro- and thio-polyunsaturated fatty acids during thin film oxidation testing

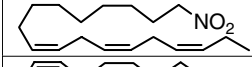
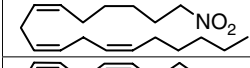
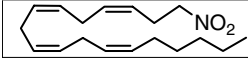
Film Oxidation Composition	Experimental Agent	Time (%)		Time [60 hours] after 10% AIBN*1
		70	7	
16		97	23	17
Arachidonic Acid and 16	–	88	27	11
17		92	41	44
Arachidonic Acid and 17	–	102	30	49
18		98	68	87
Arachidonic Acid and 18	–	99	28	57
19		101	102	100
Arachidonic Acid and 19	–	98	96	96

*1 AIBN = 2, 2′-Azobisisobutylonitrile

II. *Anti-inflammatory Testing*

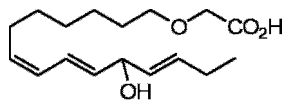
The effects of the experimental agents on lymphocyte activation and cytokine production were determined by ELISA using anticytokine antibodies. Testing results summarizing cytokine levels of tumor necrosis factor-α and interferon-γ using nitro-polyunsaturated fatty chemical agents at 20μM are provided in Table 3.

Table 3 The effect of selected experimental agents in suppressing mitogen-induced proliferation in response to PHA and *Streptococcus aureus* and to inhibit cytokine production

Agent	Stimulus (*S. aureus*), Tumor Necrosis Factor-α (% Reduction)	Stimulus (PHA), Interferon-γ (% Reduction)
	43.7	40.4
	48.2	37.6
	34	79.8

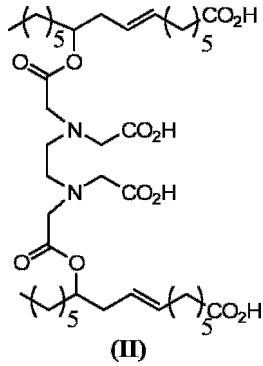
Notes

1. In an earlier investigation by the author (1), anti-inflammatory agents consisting of hydroxyl β-oxa-fatty acids, (**I**), were prepared and were effective in containing the formation of pro-inflammatory cytokines such as tumor necrosis factor-α.



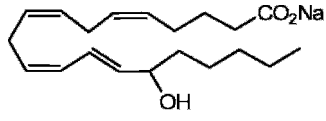
(I)

2. Fatty acid dimers, (**II**), trimers, and tetramers effective as PLA_2 inhibitors having antioxidant and anti-inflammatory properties were prepared by Franson (2) and used in treating nerve and tissue damage in central and peripheral neurological inflammatory conditions.



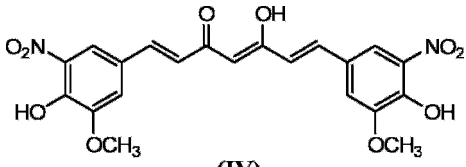
(II)

3. The topical ophthalmic application of hydroxyeicosatetraenoic acid derivatives, (**III**), prepared by Gamache (3) was effective in preventing ophthalmic inflammatory disorders involving cytokine secretion and used in treating conjunctivitis, iritis, uveitis, and episcleritis.



(III)

4. Reactive oxygen species inhibitors consisting of curcumin derivatives, (**IV**), were prepared by Pandol (4) and are used in treating inflammation disorders associated with pancreatitis.



(IV)

References

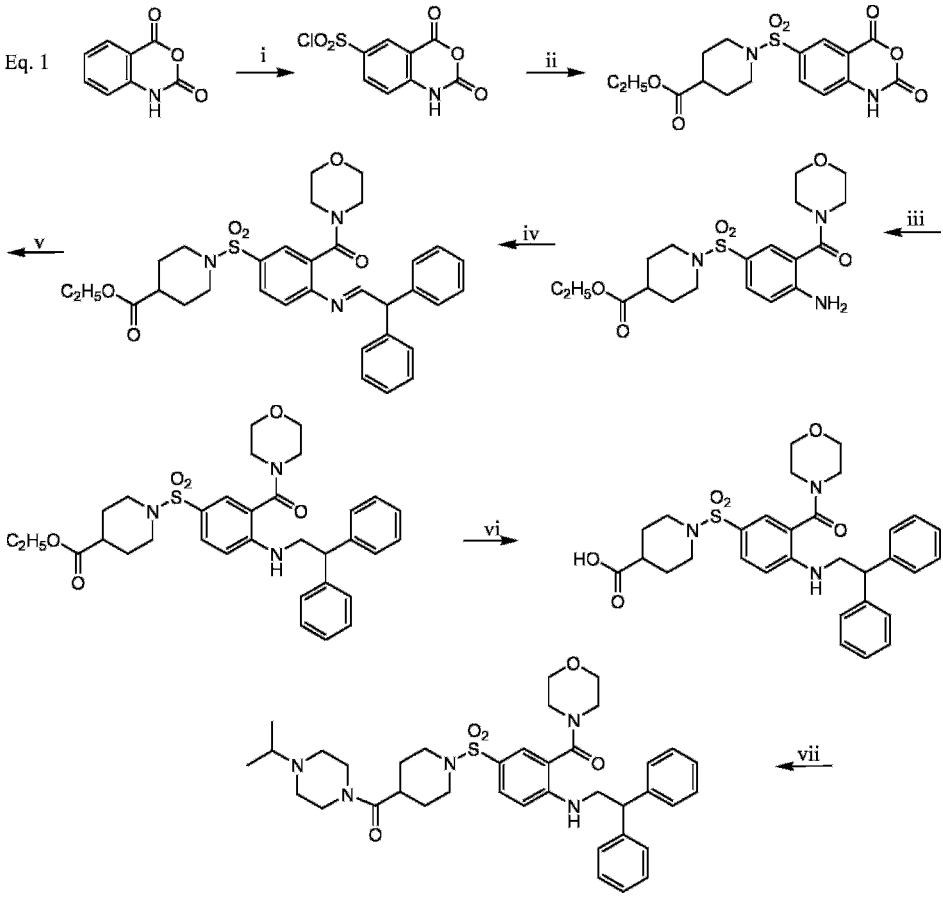
1. A. Ferrante *et al*., US Patent 6,376,688 (April 23, 2002) and US Patent 5,767,156 (June 16, 1998)
2. R.C. Franson *et al*., US Patent 6,600,059 (July 23, 2003) and US Patent 6,423,855 (June 23, 2002)
3. D.A. Gamache, US Patent 6,825,232 (November 30, 2004)
4. S.J. Pandol *et al*., US Patent 7,060,733 (June 13, 2006)

II. Bradykinin B_1 Receptor Antagonists

Title Bradykinin Receptor Antagonists
C.T. Brain *et al.*, US Patent 6,958,331 (October 25, 2005)
Assignee Novartis AG
Utility Treatment of Sepsis and Edema

Invention Significance Although peptide antagonists of bradykinin receptors are known, most have activity towards B_2 receptors. To augment the limited number of available treatment options, medicaments have been prepared that are active as bradykinin B_1 receptor antagonists useful for treating inflammatory diseases such as sepsis and edema.

Reaction



i- Chlorosulfonic acid, thionyl chloride
ii- Acetone, ethyl isonipecotate, triethylamine
iii- Morpholine, EtOAc
iv- EtOAc, 2,2-diphenylethanal, trimethylorthoacetate, trifluoroacetic acid, methyl-*t*-butyl ether
v- THF, 10% palladium on carbon, hydrogen, methyl-*t*-butyl ether
vi- THF, sodium hydroxide
vii- CH_2Cl_2, DMF, thionyl chloride, isopropylpiperazine, triethylamine

Experimental

1. **Preparation of 7-chlorosulfonyl isatoic anhydride**

   Isatoic anhydride (180.3 g) and 367 ml chlorosulfonic acid were mixed and stirred 21 hours at ambient temperature, then treated with 80.6 ml thionyl chloride over 2 hours. The mixture was stirred an additional 16 hours and then poured over ice (4.5 kg). The mixture was filtered, washed twice with 500 ml water, dried, and the product isolated.

2. **Preparation of 7-[(4-carboxyethoxy-piperidin-1-yl)sulfonyl] isatoic anhydride**

   The Step 1 product (270.2 g) was mixed with 2000 ml acetone, then cooled in an ice/methanol bath, and treated with a solution of ethyl isonipecotate (165.7 g) and triethylamine dissolved in 700 ml acetone over 1.5 hours. The cooling bath was removed and the mixture was stirred an additional 2 hours, then concentrated at 35°C. The residue was triturated with 1763 ml 0.5 M HCl, then filtered, washed with 1000 ml water, dried at 40°C for 3 days, and the product isolated.

3. **Preparation of 3-[(4-carboxyethoxy-piperidin-1-yl)sulfonyl]-2-*N*-morpholineamide aniline**

   The Step 2 product (342.4 g) dissolved in 3400 ml EtOAc was treated with morpholine (85.80 g) dissolved in 340 ml EtOAc added over 90 minutes. The mixture was stirred 30 minutes, then treated with charcoal (35.2 g), and filtered through celite. Solids were washed with EtOAc, the filtrate volume reduced to 2000 ml, and 1700 ml heptane added. The suspension was stirred overnight, filtered, washed with the mother liquor, dried overnight, and the product isolated.

4. **Preparation of ethyl {2-(2,2-diphenyl-ethylimino)-5-[4-(4-carboxy)-piperidine-1-sulfonyl]-phenyl}-morpholin-4-yl-methanone**

   The Step 3 product (324.7 g) and 4400 ml EtOAc were heated until dissolution then treated with 2,2-diphenyl ethanal (164.7 g), trimethyl orthoacetate (100.8 g), and trifluoroacetic acid (4.35 g). The mixture was stirred 3 days at ambient temperature

and then concentrated. The residue was triturated with 3200 ml MTBE, filtered, the filtrate washed with 320 ml MTBE, dried, and the product isolated.

5. **Preparation of ethyl {2-(2,2-diphenyl-ethylamino)-5-[4-(4-carboxy)-piperidine-1-sulfonyl]-phenyl}-morpholin-4-yl-methanone**

   A Parr reactor was charged with the Step 4 product (118.8 g) and 1200 ml THF, then heated until dissolution, and then treated with 10% palladium on carbon (0.47 g). The mixture was shaken under 50 psi hydrogen 24 hours at ambient temperature, then filtered through celite, and concentrated. The residue was triturated with 1100 ml MTBE, refiltered, washed with 110 ml MTBE, dried, and the product isolated.

6. **Preparation of {2-(2,2-diphenyl-ethylamino)-5-[4-(4-carboxy)-piperidine-1-sulfonyl]-phenyl}-morpholin-4-yl-methanone**

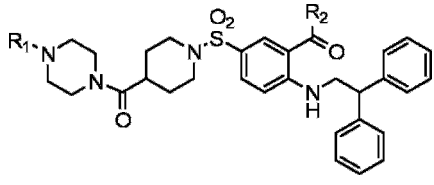
   A mixture of the Step 5 product (326.8 g), 3300 ml THF, and 540 ml 1.0 M NaOH was stirred 24 hours at ambient temperature and then concentrated. The residue was diluted with 1000 ml water and 600 ml 1.0 M HCl, then stirred 2 hours at ambient temperature. The suspension was filtered, washed with 1000 ml water, and the product isolated.

7. **Preparation of 2-(2,2-diphenyl-ethylamino)-5-[4-(4-isopropylpiperazine-1-carbonyl)-piperidine-1-sulfonyl]-phenyl}-morpholin-4-yl-methanone**

   A mixture consisting of the Step 6 product (130.2 g), 1300 ml CH_2Cl_2, DMF (0.82 g), and thionyl chloride (29.51 g) was stirred 2 hours at ambient temperature, then concentrated. The crude acid chloride was redissolved in 300 ml CH_2Cl_2, then treated with a mixture consisting of isopropylpiperazine (47.60 g), triethylamine (98.09 g), and 1000 ml CH_2Cl_2 at $-10°C$ over 60 minutes. The mixture was concentrated and the residue suspended in 2000 ml EtOAc, then washed twice with 500 ml apiece water and 500 ml brine, then dried using Na_2SO_4. The solution was concentrated to 500 ml, stirred 17 hours at ambient temperature, filtered, dried, and the product isolated as the free base.

Derivatives

Table 1 HPLC retention times of trifluoroacetate salts for selected experimental bradykinin B_1 receptor antagonists agents

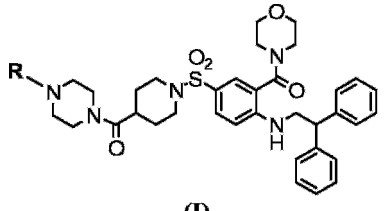


Entry	R_1	R_2	HPLC Retention Time (minutes)
2	CH_3	Morpholin-4-yl	5.68[a]
7	Benzyl	Morpholin-4-yl	25.93[b]
8	CH_3	Isopropylamine	5.09[c]
11	Isopropyl	3,6-Dihydro-2H-pyridin-1-yl	6.37[a]
13	CH_3	2,5-Dihydropyrrol-1-yl	5.10[c]
1.2	CH_3	Methyl	6.00[d]
1.7	CH_3	4-Difluoropiperidin-1-yl	5.7[e]
5.3	CH_3	2-Fluorobenzyl	6.100[a]
5.12	CH_3	2-Trifluoromethylbenzyl	6.998[a]
5.20	CH_3	Pyridin-4-ylmethyl	4.667[a]

[a] Hypersil 3 μm C 18 BDS column. Gradient elution 10–100% MeCN in water (+0.1% TFA) over 10 minutes.
[b] Nucleosil 5 μm C18 column, 25 cm × 4.6 mm. Gradient elution 10–100% MeCN in water (+0.1% TFA) over 40 minutes.
[c] Kingsorb 50 mm × 4.6 mm C18 column, 3 μm particle size; flow rate 3 ml/minute; 90% water (+10 mM NH_4OAc 0.3% HCOOH) 10% MeCN to 100% MeCN over 10 minutes.
[d] Waters Symmetry 3 μm C18 column; 5 × 0.46 cm. Gradient elution 10–100% MeCN in water (+0.1% TFA) over 10 minutes.
[e] Kingsorb 3 μm C18 column, 30 × 4.6 mm. Gradient elution 10% MeCN in water (+0.1% TFA) to 100% MeCN over 10 minutes.

Testing

Although bradykinin receptor binding testing data were not supplied by the author, 2-(2,2-diphenyl-ethylamino)-5-[4-(4-isopropylpiperazine-1-carbonyl)-piperidine-1-sulfonyl]-phenyl}-morpholin-4-yl-methanone, (**Ia**), and {2-(2,2-diphenyl-ethylamino)-5-[4-(4-methylpiperazine-1-carbonyl)-piperidine-1-sulfonyl]-phenyl}-morpholin-4-yl-methanone, (**Ib**), were especially preferred.

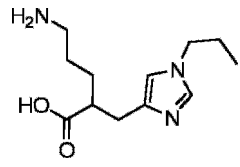


(I)

$R_{Ia} = CH_3$
$R_{IIa} = i\text{-}C_3H_7$

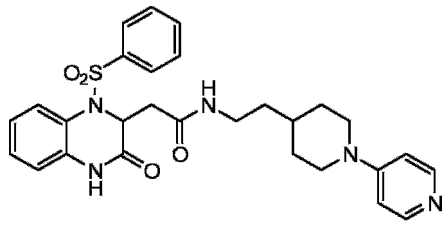
Notes

1. 1H-Imidazol-4-yl derivatives prepared by Allerton (1) were effective as agonists/antagonists and used in maintaining bradykinin levels in the body. The agent, (±)-5-amino-2-[(1-*n*-propyl-1H-imidazol-4-yl)methyl]pentanoic acid, (**II**), was especially preferred and used in treating inflammatory disorders.



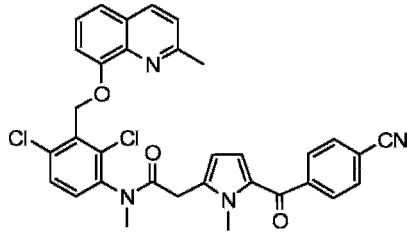
(II)

2. Grant (2) prepared sulfonylquinoxalone derivatives, (**III**), as bradykinin antagonists, which were used to relieve adverse symptoms, associated with bradykinin disorders including pain, inflammation, bronchoconstriction, and cerebral edema.



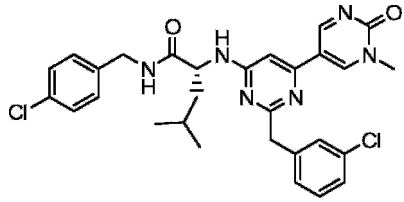
(III)

3. 2-Methyl-quinolin-8-yloxymethyl derivatives, (**IV**), prepared by Carson (3) were effective as bradykinin agonists/antagonists and used in the management of pain and inflammation.



(IV)

4. Pyrimidine, (**V**), triazines, and aniline-based bradykinin receptor antagonists, (**V**), prepared by Olmeyer (4) were effective in treating inflammation, pain, and multiple sclerosis.



(V)

References

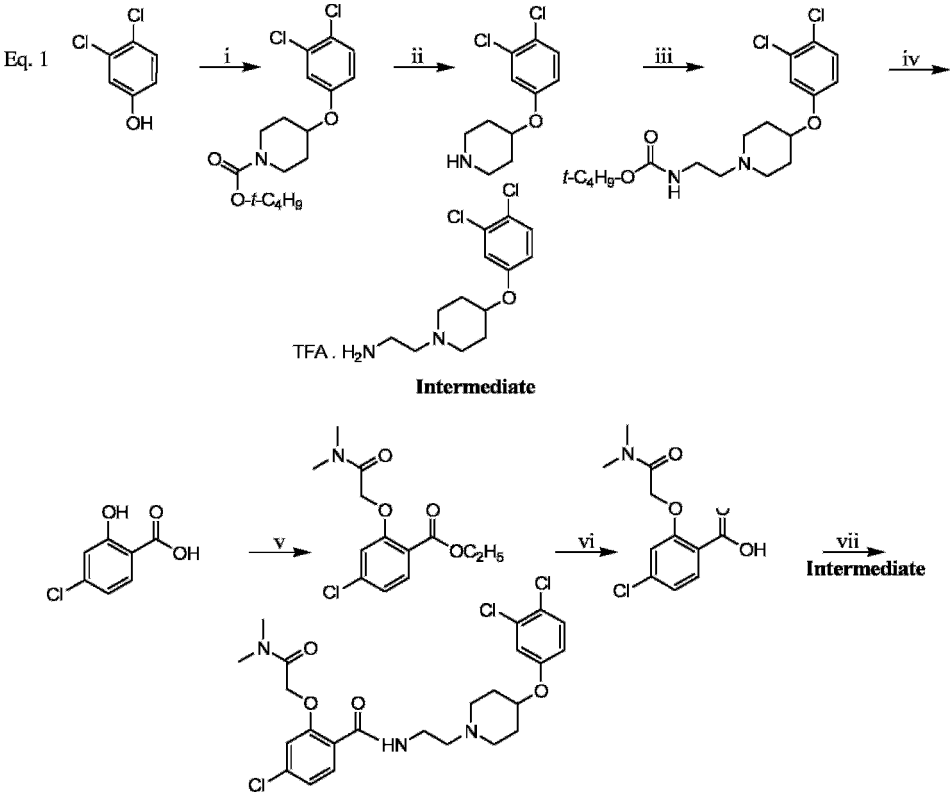
1. C.M.N. Allerton *et al*., US Patent 6,949,577 (September 27, 2005) and US Patent 6,919,457 (July 19, 2005)
2. F.S. Grant *et al*., US Patent 7,056,937 (June 6, 2006)
3. J.R. Carson *et al*., US Patent 6,958,349 (October 25, 2005)
4. M.H.J. Olmeyer *et al*., US Patent 6,919,347 (July 19, 2005)

III. CCR_1/CCR_3 Chemokine Antagonists

Title Compounds
A. Baxter *et al.*, US Patent 6,946,478 (September 20, 2005)
Assignee AstraZeneca AB
Utility Treatment of Respiratory Tract Obstructive Airway Disorders

Invention Significance Chemokines are mediated by subfamilies of G protein-coupled receptors including $CCR_{1–10}$, $CCR_{2A–B}$, and $CXCR_{1–4}$ receptors. These receptors are associated with immune and inflammatory disorders, particularly respiratory diseases. CCR_1 and/or CCR_3 chemokine receptor modulators have been prepared, which are effective in the treatment of respiratory tract obstructive airways diseases such as chronic or inveterate asthma and chronic obstructive pulmonary disease.

Reaction



i- Diethyl azodicarboxylate, triphenylphosphine, THF, *t*-butyl-4-hydroxy-1-piperidinecarboxylate
ii- CH_2Cl_2, trifluoroacetic acid
iii- DMF, triethylamine, *t*-butyl-2-bromoethylcarbamate
iv- CH_2Cl_2, trifluoroacetic acid
v- Cesium carbonate, 2-chloro-*N,N*-dimethylacetamide
vi- Water, methyl alcohol, lithium hydroxide hydrate
vii- 2-[4-(3,4-Dichlorophenoxy)-1-piperidinyl]ethylamine trifluoroacetate, *N,N*-carbonyldiimidazole, DMF, triethylamine

Experimental

1. **Preparation of *t*-butyl 4-(3,4-dichlorophenoxy)-1-piperidinecarboxylate**

   Diethyl azodicarboxylate (12.6 ml) was added to a solution of triphenylphosphine (20.8 g) dissolved in 300 ml THF at 0°C. After 15 minutes, 3,4-dichlorophenol (12.9 g) was added followed by the dropwise addition of *t*-butyl 4-hydroxy-1-piperidinecarboxylate (14.5 g) dissolved in 100 ml THF after 10 minutes. The solution was stirred 5 hours at ambient temperature and was then concentrated. The residue was partitioned between diethyl ether and brine and the organic phase was separated, dried, and evaporated to a gum. The residue was purified by chromatography using EtOAc/isohexane, 95:5, and 20 g product isolated.

   MS APCI (+ve): 246 (M−BOC + 2H)

2. **Preparation of 4-(3,4-dichlorophenoxy)piperidine**

   The Step 1 product was dissolved in 200 ml CH_2Cl_2, treated with 100 ml trifluoroacetic acid, and stirred 18 hours at ambient temperature. The solution was concentrated, the residual gum triturated with diethyl ether, and 16.2 g product isolated.

3. **Preparation of *t*-butyl 2-[4-(3,4-dichlorophenoxy)-1-piperidinyl]ethylcarbamate**

   The Step 2 product (6.55 g) was dissolved in 50 ml DMF, then treated with 7.9 ml triethylamine followed by *t*-butyl 2-bromoethylcarbamate (4.3 g) dissolved in 5 ml DMF. The mixture was stirred 3 days at ambient temperature and was then diluted with EtOAc and water. The organic phase was dried and concentrated to a gum. The gum was purified by chromatography using CH_2Cl_2/methyl alcohol, 95:5, and 5.7 g product isolated as a gum.

   MS APCI (+ve): 389 (M + H)

4. **Preparation of 2-[4-(3,4-dichlorophenoxy)-1-piperidinyl]ethylamine trifluoroacetate**

   The Step 3 product was dissolved in 200 ml CH_2Cl_2, treated with 100 ml trifluoroacetic acid, and then stirred 18 hours at ambient temperature. The mixture was concentrated, triturated with diethyl ether, and 5.7 g product isolated.

   MS APCI (+ve): 290 (M + H)

5. **Preparation of 2-(dimethylamino)-2-oxoethyl 4-chloro-2-[2-(dimethylamino)-2-oxoethoxy]benzoate**

   A mixture of 4-chloro-2-hydroxybenzoic acid (5 g), Cs_2CO_3 (17.5 g), and 2-chloro-*N, N*-dimethylacetamide (6.6 g) was stirred 3 hours at 70°C, then diluted with water and EtOAc. The organic phase was separated, dried, and concentrated to a gum. The gum was purified by chromatography using EtOAc/methyl alcohol, 9:1, and 8.0 g solid product isolated, mp = 140−141°C.

   MS APCI (+ve): 343 (M + H)

6. **Preparation of 4-chloro-2-[2-(dimethylamino)-2-oxoethoxy]benzoic acid**

   The Step 5 product (1.0 g) was dissolved in 15 ml water/methanol, 2:1, then treated with $LiOH \bullet H_2O$, and stirred 2 hours. The mixture was diluted with 2 M HCl and EtOAc and the organic phase separated. The solution was dried, concentrated, and 1.2 g product isolated, mp = 141−142°C.

   MS APCI (+ve): 258 (M + H)

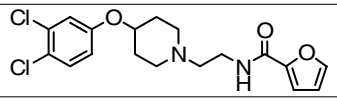
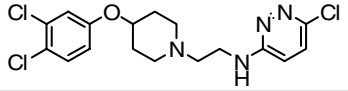
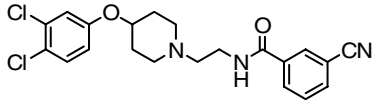
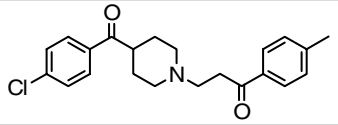
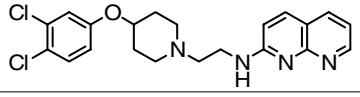
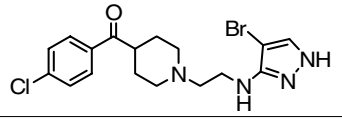
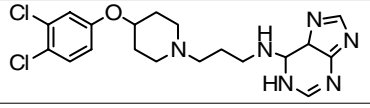
7. **Preparation of 4-chloro-*N*-{2-[4-(3,4-dichlorophenoxy)-1-piperidinyl]ethyl}-2-[2-(dimethylamino)-2-oxoethoxy]benzamide**

   The Step 6 product (0.3 g) and *N,N*-carbonyldiimidazole (0.19 g) were dissolved in 20 ml DMF and stirred 1 hour at ambient temperature. The mixture was then treated with the Step 4 product (0.42 g) and 0.32 ml triethylamine and stirred 20 hours. The solution was then diluted with water and diethyl ether, the organic phase separated, dried, and concentrated to a gum. The residue was purified by chromatography using CH_2Cl_2/methyl alcohol, 93:7, and 0.38 g product isolated, mp = 139−140°C.

   ^{1}H NMR δ (DMSO) 9.17 (t, 1H), 7.88 (d, 1H), 7.48 (d, 1H), 7.38 (d, 1H), 7.24 (d, 1H), 7.13 (dd, 1H), 6.99 (dd, 1H), 5.11 (s, 2H), 4.32 (m, 1H), 3.42 (m, 2H), 2.99 (s, 3H), 2.88 (s, 3H), 2.73 (m, 2H), 2.50 (m, 2H), 2.30 (m, 2H), 1.90 (m, 2H), 1.59 (m, 2H)
   MS (ESI) 528.12 (M + H)

Derivatives

Table 1 Selected 3,4-dichlorophenoxy-1-piperidinyl experimental agents and their corresponding mass spectra. All experimental agents were effective as CCR_1/R_3 chemokine antagonists using the calcium flux $[Ca^{2+}]_i$ assay. ^1H NMR for products and intermediates provided by the author

Entry	Structure	MS
8		383
25		403
59		418
87		385
109		417
164		427
242		421

Testing

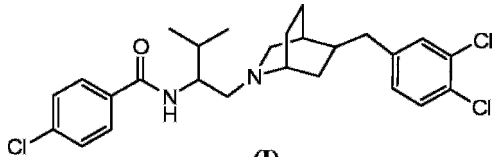
Testing consist of the calcium flux $[Ca^{2+}]_i$ assay using:

I. Human Eosinophils
II. Human Monocytes
III. Human Eosinophil Chemotaxis

Although testing results were not supplied by the author, 3,4-dichlorophenoxy-1-piperidinyl derivatives appearing in Table 1 were especially preferred.

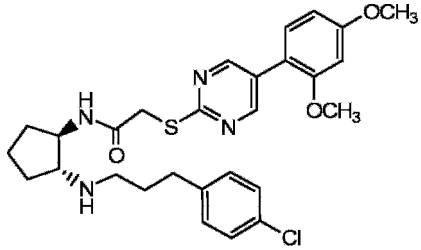
Notes

1. 2-Aza-bicyclo[2.2.2]octane derivatives prepared by Gong (1) were effective as CCR_3 receptor antagonists and used as a means of combating eosinophil-induced diseases including asthma.



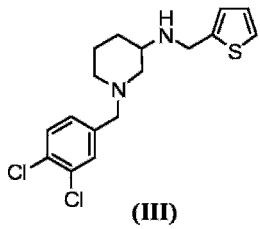
(I)

2. CCR_3 receptor antagonists consisting of *trans*-1,2-diaminocyclopentane derivatives, (**II**), were prepared by Du Bois (2) and used in treating pathological conditions such as asthma and rhinitis.

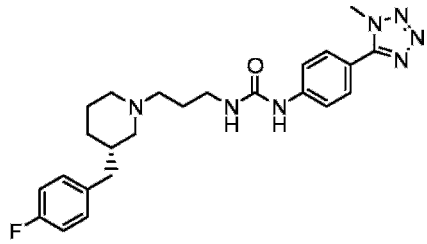


(II)

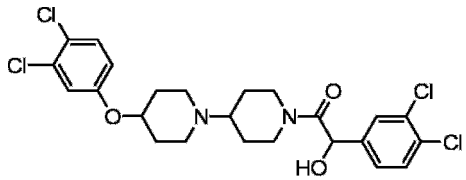
3. Piperidine amines, (**III**), prepared by Thom (3), *N*-ureidoalkyl-piperidines, (**IV**), prepared by Ko (4), and bipiperidine derivatives, (**V**), prepared by Rigby (5) were effective as CCR_1/CCR_3 chemokine antagonists and used in treating asthma and related allergic diseases as well as autoimmune pathologies such as rheumatoid arthritis.



(III)

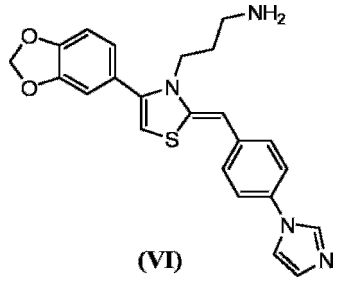


(IV)



(V)

4. Thiazol-2(3H)-ylidene derivatives, (**VI**), prepared by Fujiwara (6) were effective as CCR_1/CCR_3 chemokine antagonists and used to induce an immediate therapeutic response for patients suffering from atopic asthma and bronchial asthma.



(VI)

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1. L. Gong *et al*., US Patent 7,081,531 (July 25, 2006)
2. D.J. Du Bois *et al*., US Patent 7,049,317 (May 23, 2006)
3. S. Thom *et al*., US Patent 6,903,085 (June 7, 2005)
4. S.S. Ko *et al*., US Patent 6,919,368 (July 19, 2005)
5. A. Rigby *et al*., US Patent 6,903,115 (July 7, 2005)
6. N. Fujiwara *et al*., US Patent 6,919,361 (July 19, 2005)

IV. Cyclooxygenase-II Inhibitors

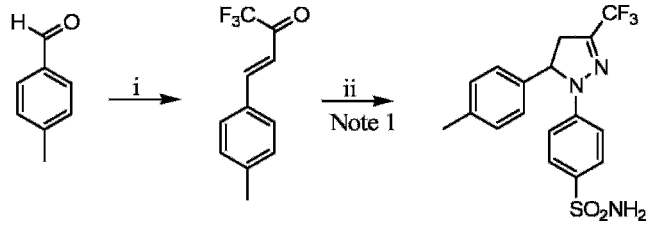
A. Trifluoromethyl Pyrazoline Medicaments

Title Pyrazoline Derivatives, Their Preparation, and Application as Medicaments
M.R. Cuberes-Altisent *et al*., RE38,963 (January 31, 2006)
Assignee Laboratorios del Dr. Esteve
Utility Nonsteroid Anti-inflammatory Agents with Reduced Gastrointestinal Toxicity

Invention Significance Although cyclooxygenase-I inhibitors (COX-I) are effective as nonsteroid anti-inflammatory drugs (NSAIDS), they are associated with varying degrees of gastric toxicity. The current invention addresses this concern by preparing COX-II-based NSAID agents with diminished gastrointestinal and renal toxicity and reduced bleeding times.

Reaction

Eq. 1



i- THF, lithium diisopropylamide, diethylmethyl phosphonate, *N*-phenyltrifluoroacetimidoyl chloride, 4-tolualdehyde
ii- 4-(Aminosulphonyl)phenylhydrazine chlorohydrate, acetic acid

Experimental

1. **Preparation of (*E*)-1,1,1-trifluoro-4-(4-methylphenyl)-3-butene-2-one**

THF (15 ml) was cooled to −70°C, then treated with 5 ml 2 M LDA in hexane and diethylmethyl phosphonate (5 mmol) dissolved in 5 ml THF, and shaken

30 minutes. The mixture was then treated with the dropwise addition of *N*-phenytrifluoroacetimidoyl chloride (5 mmol) over 1 hour followed by 4-tolualdehyde (5 mmol), then shaken 16 hours at ambient temperature. The solution was then treated with 10 ml of 2 M HCl, then shaken an additional 4 hours, and concentrated. The residue was extracted twice with 20 ml diethyl ether, then washed with 5% $NaHCO_3$ solution and brine until reaching pH 6. The solution was dried using Na_2CO_3, reconcentrated, the residue purified by column chromatography with silica gel under pressure using EtOAc/petroleum ether, 1:9, and the product isolated in 75% yield as clear oil.

IR (cm^{-1}) 1715, 1601, 1201, 1183, 1145, 1056, 811, 703
^{1}H NMR ($CDCl_3$) δ 2.4 (s, 3H), 6.97 (d, $J = 18$ Hz, 1H); 7.25 (d, $J = 9$ Hz, 2H); 7.54 (d, $J = 9$ Hz, 2H); 7.95 (d, $J = 18$ Hz, 1H)
TLC (petrol ether) $R_f = 0.16$

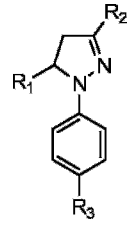
2. **Preparation of 1-(4-aminosulphonylphenyl)-4,5-dihydro-5-(4-methylphenyl)-3-trifluoromethyl-1H-pyrazole**

4-(Aminosulphonyl)phenylhydrazine chlorohydrate (3.69 mmol) and the Step 1 product (3.69 mmol) were dissolved in 15 ml of acetic acid, then refluxed 3 hours, and poured over water. The mixture was extracted with EtOAc, then washed with water, dried, and concentrated. The solid was recrystallized using ethyl alcohol/petroleum ether and the product isolated in 45% yield, mp = 140−143°C.

IR (KBr, cm^{-1}) 3356, 3268, 1594, 1326, 1170, 1139, 1120, 1097
^{1}H NMR ($CDCl_3$) δ 2.34 (s, 3H), 2.99–3.06 (dd, $J = 6.9$, 14 Hz, 1H); 3.66–3.73 (dd, $J = 12.6$, 14 Hz, 1H); 4.69 (broad s, 2H); 5.38–5.45 (dd, $J = 6.9$, 12.6 Hz, 1H); 7.04–7.11 (2d, $J = 8.1$, 9.3 Hz, 4H); 7.17 (d, $J = 8.1$ Hz, 2H); 7.70 (d, $J = 9.3$ Hz, 2H)
^{13}C NMR ($CDCl_3$) 20.9; 41.2; 64.5; 113.4; 120.5 (q, $J = 268$ Hz); 125.3, 127.6, 130.1; 133.2; 136.7; 138.3; 138.8 (q, $J = 38$ Hz); 146.0
TLC (EtOAc) $R_f = 0.89$

Derivatives

Table 1 Selected experimental pyrazoline derivatives and their corresponding melting points. IR and ^{1}H NMR data for products and intermediates supplied by author



Entry	R_1	R_2	R_3	mp (°C)
3	2,4-Difluorophenyl	Trifluoromethyl	Aminosulfonyl	160–162
7	4-Methylphenyl	Cyano	Methylsulfonyl	162–164
13	2,4-Dichlorophenyl	Trifluoromethyl	Aminosulfonyl	142–143
26	4-Fluoro	Methyl	Aminosulfonyl	206–208
30	4-Methylphenyl	Methyl	Aminosulfonyl	190–194
42	4-Methylpheny	Carboxamide	Methylsulfonyl	128–132
50	2,4-Dimethylphenyl	Difluoromethyl	Aminosulfonyl	181–183
59	4-Methylsulfonyl	Trifluoromethyl	Fluoro	105–106

Testing

I. *Inhibition of the Synthesis of Prostaglandins in Inflammatory Exudate and Mucus Membrane in Rats*

The selective inhibition of COX-II anti-inflammatory activity and the absence of gastric prostaglandin effects after oral administration was assayed according to the method of Tofanetti (1). Testing results are provided in Table 2.

Table 2 Comparison of in vivo COX-II/COX-I activity of Entry 3 and a reference NSAID at a dosage of 40 mg/kg, po

Entry	Inhibitor	Inflammatory exudates (%)	Gastric mucus (%)
3	COX-II	92	0
Meloxicam	COX-I	97	65

II. *Analgesic Activity Against "Hyperalgesia" by Thermal Stimulus of Preinflamed Rat Paw*

The analgesic activity of selected experimental agents was determined according to the method of Hargreaves (2). Testing results are provided in Table 3. The efficacy of entry 3 as a function of its ED_{50} is also provided in Table 4.

III. *Gastrointestinal Effects: Induction of Ulcers in Rats Submitted to Cold Stress*

Ulcerogenic effects at a gastrointestinal level of the experimental agents were determined after oral administration according to the method of Rainsford (3). Testing results are provided in Table 5.

Table 3 Comparison of analgesic activity against hyperalgesia by thermal stimulus of entry 3 and reference NSAIDs at 40 mg/kg, po

Entry	Inhibitor	Activity (%)
3	COX-2	100
Nimesulide	COX-1	97
Nabumetone	COX-1	95

Table 4 ED_{50}s of analgesic activity against hyperanalgesia by thermal stimulus of entry 3 and reference NSAIDs

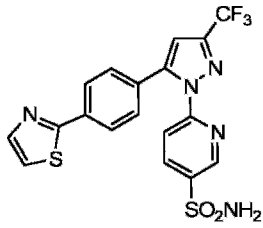
Entry	Inhibitor	ED_{50} (mg/kg)
3	COX-2	0.2
Nimesulide	COX-1	1.0
Nabumetone	COX-1	2.1

Table 5 Comparison of maximum nonulcerogenic dose induction of ulcers in rats submitted to cold stress

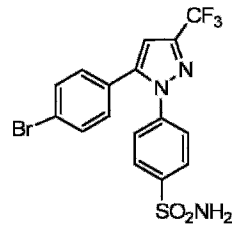
Entry	Inhibitor	Maximum Nonulcerogenic dose (mg/kg)
3	COX-2	> 80
Dichlophenac	COX-1	1.2
Piroxicam	COX-1	1.7

Notes

1. COX-II inhibiting agents consisting of sulfamoylheteroaryl-, (**I**), and arylpyrazole derivatives, (**II**), were prepared by Ando (4,5) and used in treating anti-inflammatory disorders without gastric side effects associated with COX-I inhibition.

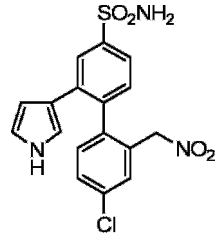


(I)



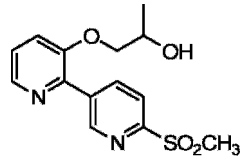
(II)

2. Nitrosated, (**III**), and nitrosylated benzenesulfonamides effective as COX-II inhibitors were prepared by Bandarage (6) and used for treating inflammation, pain, and fever.



(III)

3. 2, 3′-Bipyridine derivatives, (**IV**), effective as COX-II inhibitors having less severe side effects such as gastrointestinal and renal toxicity were prepared by Cho (7) and used in the treatment of inflammation and fever.



(IV)

References

1. O. Tofanetti *et al*., Med. Sci. Res., 17, 745 (1989)
2. K. Hargreaves *et al*., Pain, 32, 77 (1988)
3. K.D. Rainsford, Agents and Actions, 5, 553 (1975)
4. K. Ando *et al*., US Patent 6,951,876 (October 4, 2005) and US Patent 6,603,008 (August 5, 2003)
5. K. Ando *et al*., US Patent 6,949,536 (September 27, 2005)
6. U.K. Bandarage *et al*., US Patent 6,649,629 (November 18, 2003) and US Patent 6,593,347 (July 15, 2003)
7. I.-H. Cho *et al*., US Patent 6,946,558 (September 20, 2005)

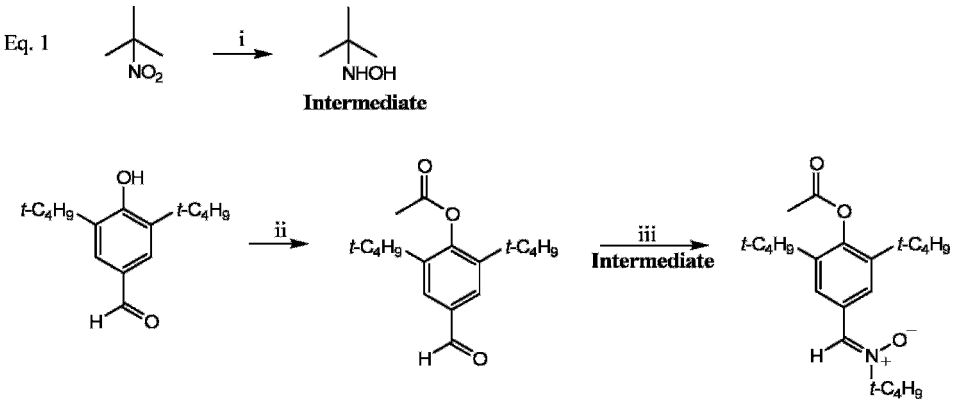
B. Aryl Nitrone Medicaments

Title 3,4,5-Trisubstituted Aryl Nitrone Compounds and Pharmaceutical Compositions Containing the Same
D.L. Waterbury *et al*., US Patent 6,998,419 (February 14, 2006)
Assignee Renovis, Inc.
Utility Selective COX-II Inhibitor with Reduced Gastrointestinal Toxicity

Invention Significance Nonsteroidal anti-inflammatory agents (NSAIDS) prevent the production of prostaglandins by nonselectively inhibiting both COX-I and COX-II enzymes in the arachidonic acid–prostaglandin pathway. This is particularly troublesome since the integrity of the gastrointestinal tract requires prostoglandins produced by the COX-I enzyme. This chapter addresses this concern by introducing agents that selectively inhibit COX-II.

Reaction

Eq. 1



i- Zinc, ammonium chloride, water
ii- Acetic anhydride, perchloric acid
iii- Benzene

Experimental

1. **Preparation of *N-t*-butylhydroxylamine**

A mixture of 2-methyl-2-nitropropane (503 g) and NH_4Cl (207 g) dissolved in 6000 ml water was treated with zinc dust (648 g) while maintaining the temperature

below 18°C, then stirred 15 hours. The mixture was then filtered, washed with 1750 ml hot water, saturated with K_2CO_2 (4.6 kg), and extracted twice with 1300 ml EtOAc. The solution was dried with Na_2SO_4 and the product isolated 75.7% yield as white crystals. It was subsequently used without further purification.

^{1}H NMR (270 MHz, $CDCl_3$) δ 1.090 (s, $3CH_3$)

2. **Preparation of 4-acetoxy-3,5-di-*t*-butylbenzaldehyde**

3,5-Di-*t*-butyl-4-hydroxybenzaldehyde (0.411 mol) and 300 ml acetic anhydride were mixed, then treated with 0.6 ml 70% perchloric acid, and stirred overnight at ambient temperature. The solution was then cooled in an ice bath and treated with the portionwise addition of 2000 ml ice water. An oil was formed, which solidified into brown lumps, and was washed with water and acetic acid, then dried under vacuum. The solid was boiled 10 minutes with 250 ml ethyl alcohol containing 25 ml 12 M HCl, then cooled, and resolidified by pouring into 2000 ml water. The solid was washed with 1000 ml water and the product isolated in 99.4% yield as a light brown solid, mp = 65.2−74.5°C

^{1}H NMR (270 MHz, $CDCl_3$) δ 9.96 (1H, s, carbonyl H), 7.86 (2H, s, phenyl H), 2.38 (3H, s, 3CH3), 1.39 (18H, s, 18CH3)

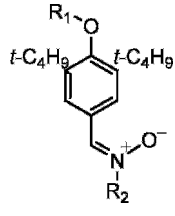
3. **Preparation of α-(4-acetoxy-3,5-di-*t*-butylphenyl)-*N*-*t*-butylnitrone**

The Step 2 product dissolved in 500 ml benzene was treated with Step 1 product (0.49 mol) and silica gel (20 g), then refluxed overnight, and concentrated. A grey solid was isolated, which was recrystallized in EtOAc and white crystals isolated. The solid was washed with hexanes, dried, and the product isolated in 74.4% yield as a crystalline white solid, mp = 227.0−248.9°C.

^{1}H NMR (270 MHz, $CDCl_3$) δ 8.32 (1H, s, phenyl H), 7.50 (1H, s, nitronyl H), 2.35 (3H, s, $1CH_3$), 1.61 (9H, s, $3CH_3$), 1.37 (s, $18CH_3$)
^{13}C NMR (270 MHz, $CDCl_3$) δ 170.7, 149.2, 142.6, 129.7, 128.4, 127.2, 70.7, 35.5, 31.4, 28.5, 22.6

Derivatives

Table 1 Melting points and Step 3 percent yields of selected nitrones. ^{1}H NMR and elemental analysis provided by author



Entry	R_1	R_2	Yield (%) (last step)	mp (°C)
1	CH_3CO	t-C_4H_9	74.4	227.0–248.9
4	CH_3CO	i-C_4H_9	52.3	176.8–180.4
5	CH_3CO	$CH_2(OH)C(CH_3)CH_3$	83.9	204.3–204.8
6	$CH_3CH_2CH_2CH_2CO$	t-C_4H_9	56.5	195.5–204.8
13	$CH_3CH_2CH_2CO$	t-C_4H_9	93.0	203.6–205.1
16	CH_3OCH_2	i-C_4H_9	69.0	202.2–205.9

Testing

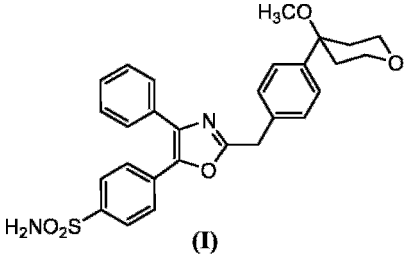
Biological testing was limited to the preferred experimental agent, Entry 1, provided in Table 2.

Table 2 Entry 1 testing results in four standard testing protocols. Only qualitative testing information were provided by author

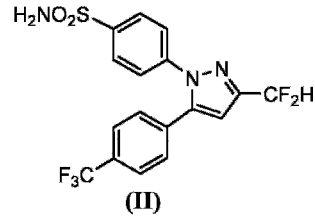
Test	Result
Inhibition of both COX-I and COX-II	Inactive
Inhibition of PGE_2	Active
Carrageenan Footpad Edema Assay	Active
Adjuvant Assay (Rat Adjuvant Arthritis)	Active
Collagen Arthritis Assay	Active

Notes

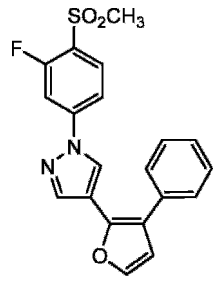
1. Selective inhibitors of COX-II or 5-lipoxygenase (5-LO) consisting of oxazolyl-, (**I**), and pyrazolylbenzene sulfonamide derivatives, (**II**), prepared by Talley (1,2), respectively, and sulfonyl derivatives, (**III**), prepared by Ando (3) were effective as anti-inflammatory agents with only marginal gastrointestinal side effects.



(I)

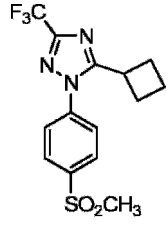


(II)



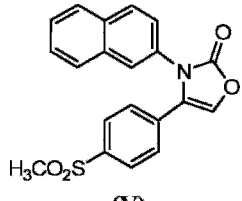
(III)

2. COX-II inhibitors consisting of methylsulfonylpyridinyl triazoles, (**IV**), prepared by Sakya (4) were effective anti-inflammatory agents and used in treating osteoarthritis and rheumatoid arthritis without concomitant gastrointestinal distress.



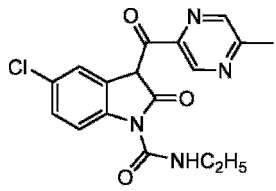
(IV)

3. 2-(3H)-Oxazolone derivatives, (**V**), prepared by Duran (5) were selective as COX-II inhibitors and used in treating inflammation, pain, fever, and asthma without ulcerogenic activity.



(V)

4. 2-Oxo-2,3-dihydro-indole derivatives, (**VII**), prepared by DeMello (6) had an especially favorable COX-II/COX-I selectivity ratio and used in treating inflammation disorders.



(VII)

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1. J.J. Talley *et al*., US Patent 6,998,415 (February 14, 2006)
2. J.J. Talley *et al*., US Patent 6,951,949 (October 4, 2005)
3. K. Ando *et al*., US Patent 6,949,536 (September 22, 2005)
4. S.M. Sakya *et al*., US Patent 6,875,779 (April 5, 2005)
5. C.P. Duran *et al*., US Patent 6,869,968 (March 22, 2005)
6. K.L. DeMello *et al*., US Patent 6,846,818 (January 25, 2005)

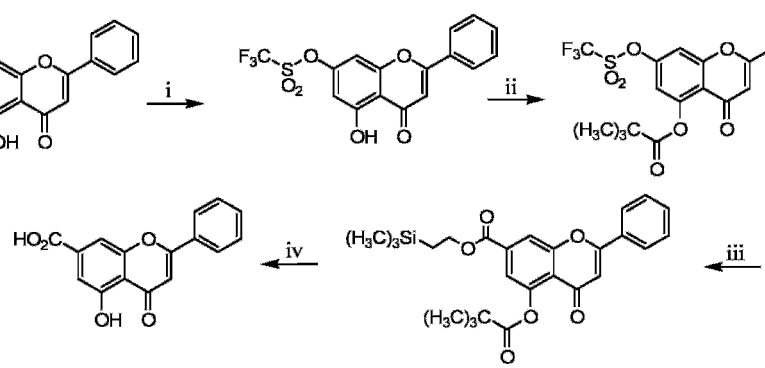
V. Interleukins-1 and -6 and Tumor Necrosis Factor Cytokine Inhibitors

Title 7-Carboxy-Flavone Derivatives Preparation Method and Therapeutic Use
J.-P. Gesson *et al.*, US Patent 6,965,039 (November 15, 2005)
Assignee Negma-Lerands
Utility Treatment of Rheumatoid Arthritis

Invention Significance A method of inhibiting interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor (TNF-α) cytokines using flavone and isoflavone derivatives containing a carboxylic acid function in the 7 position is described. Medicaments derived from these agents are useful in treating conditions such as rheumatoid arthritis or osteoarthritis without exhibiting the troublesome laxative side effect of similar materials.

Reaction

Eq. 1



i- Pyridine, trifluoromethanesulfonic anhydride, CH_2Cl_2
ii- Pivaloyl chloride, pyridine
iii- Bis(diphenylphosphino)propane, palladium acetate, carbon monoxide, 2-(trimethylsilyl)ethanol, triethylamine, DMSO
iv- Tetrabutylammonium fluoride, tetrahydrofuran, sodium hydroxide

Experimental

1. **Preparation of 7-trifluoromethanesulfonyloxy-5-hydroxy-2-phenyl-4-oxo-4H-chromene**

   A solution of 5,7-dihydroxyflavone (10 g) dissolved in 200 ml CH_2Cl_2 was treated with 12.7 ml pyridine followed by 6.6 ml of trifluoromethanesulfonic anhydride, then stirred 3 hours at 0°C. The mixture was neutralized with 1 M HCl and extracted with CH_2Cl_2. The extract was concentrated and the product isolated in 92% yield as a white powder.

   IR (cm^{-1}) 1655 (C=O), 1620 (C=C), 1436 (S=O)
   $\boldsymbol{R_f}$ = 0.58, EtOAc/petroleum ether, 30:70

2. **Preparation of 7-trifluoromethane-sulfonyloxy-2-phenyl-5-pivaloyloxy-4-oxo-4H-chromene**

   The Step 1 product (330 mg) dissolved in 4 ml pyridine at 0°C was treated with 0.16 ml pivaloyl chloride, then stirred 48 hours at 0°C, and concentrated. The residue was purified by flash chromatography using EtOAc/petroleum ether, 5:95 to 10:90, and the product isolated in 95% yield, mp = 126−128°C.

   IR (cm^{-1}) 1752 (C=O ester), 1657 (C=O), 1614 (C=C), 1427 (S=O)
   $\boldsymbol{R_f}$ = 0.65, EtOAc/petroleum ether, 10:90

3. **Preparation of 2-(trimethylsilyl)ethyl 2-phenyl-5-pivaloyloxy-4-oxo-4H-chromene-7-carboxylate**

   A mixture consisting of the Step 2 product (200 mg), 1,3-bis(diphenylphosphino) propane (8.7 mg), and $Pd(OAc)_2$ (4.7 mg) was placed under a carbon monoxide atmosphere, then treated with 0.2 ml 2-(trimethylsilyl)ethanol, 0.12 ml triethylamine, and 0.9 ml DMSO. The mixture was stirred 3 hours at 70°C and was then extracted with CH_2Cl_2. It was washed with 1 M HCl, then purified by flash chromatography using EtOAc/petroleum ether, 5:95 to 10:90, and the product isolated in 91% yield, mp = 205°C.

   $\boldsymbol{R_f}$ = 0.26, CH_2Cl_2/methyl alcohol, 97:3

4. **Preparation of 5-hydroxy-2-phenyl-4-oxo-4H-chromene-7-carboxylic acid**

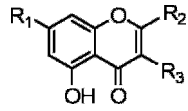
   The step 3 product (180 mg) was added to a solution of tetrabutylammonium fluoride (202 mg) dissolved in 2 ml THF at 0°C, then stirred 19 hours at ambient temperature. The mixture was then treated with 4.1 ml 1 M NaOH, then stirred an additional 72 hours. It was neutralized with 1 M HCl and extracted with EtOAc. The material

was purified by recrystallization using methyl alcohol/$CHCl_3$, 1:9, and the product isolated as a yellow powder, mp = 270°C (dec).

R_f = 0.24 CH_2Cl_2/methyl alcohol, 95:5
^{1}H NMR ($CDCl_3/CD_3OD = 9:1$) δ (ppm) 6.74 (s, 1H, H-3), 7.40 and 7.66 (2s, 2H, H-6 and H-8), 7.48–7.51 (m, 3H, H-3′ and H-4′), 7.88 (d, 2H, $J = 6.4$ Hz, H-2′)
^{13}C NMR (DMSO [sic]) δ (ppm) 106.4, 108.9, 111.3, and 112.8 (C-3, C-8, C-6, and C-4a), 127.1 (C-2′), 129.5 (C-3′), 130.6 (C-1′), 132.9 (C-4′), 137.6 (C-7), 156.0, 160.1, 165.1, and 166.1 (C-5, C-2, C-8a and COOH), 183.3 (C-4)
IR (cm^{-1}): 1724 (COOH), 1656 (C=O), 1614 (C=C)

Derivatives

Table 1 Selected 7-carboxy-flavone derivatives and their corresponding melting points. ^{1}H- and ^{13}C NMR data provided by author



Entry	R_1	R_2	R_3	mp (°C)
1	Carboxylic acid	Phenyl	Hydrogen	270
2	Dimethoxyphosphoryl	Phenyl	Hydrogen	219
3	Dihydrophosphoryl	Phenyl	Hydrogen	293–296
4	Carboxylic acid	4-Methoxyphenyl	Hydrogen	277–278
5	Carboxylic acid	Hydrogen	4-Methoxyphenyl	–
6	Carboxylic acid	4-Trifluoromethylphenyl	Hydrogen	278

Testing

I. *Interleukin-1, -6 and TNF-α Inhibiting Testing*

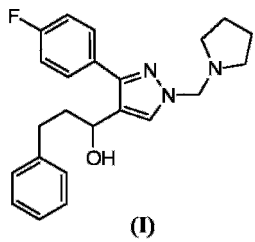
The inhibiting activity on the secretion of inflammatory cytokines was tested in vitro on the secretion of IL-1, IL-6, and TNF-α. Tests were performed on peripheral blood mononuclear cells according to the method of Schindler (1) using cycloheximide (IL-1) and dexamethasone (IL-6, TNF-α) as references. Testing results are provided in Table 2.

Table 2 Effectiveness of selected experimental agents in inhibiting the secretion of inflammatory cytokines

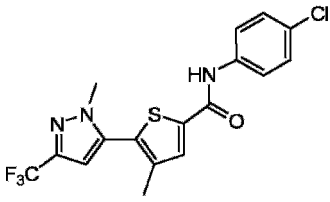
Entry	μM	IL-1β	IL-6	TNF-α
1	10	96	98	32
1	1	15	50	61
4	1	< 10	56	–
6	10	72	100	–

Notes

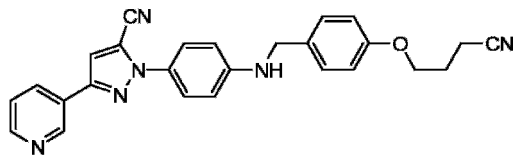
1. 4-Pyridylpyrazole derivatives, (**I**), prepared by Minami (2) were effective as IL-1, IL-6, TNF-α, and COX-II inhibitors and used in treating inflammation disorders. By contrast, pyrazole derivatives, (**II**) and (**III**), prepared by Kubota (3) and Betageri (4), respectively, impeded IL-2 production in T-lymphocytes and were also used in the treatment of inflammatory and autoimmune diseases.



(I)

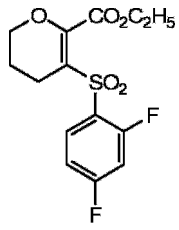


(II)



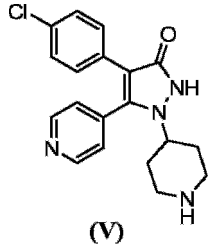
(III)

2. IL-1, IL-6, TNF-α inhibitors, and NO inhibitors prepared by Tamura (5) consisting of 3,4-dihydro-2H-pyran derivatives, (**IV**), were effective in the treatment of autoimmune and inflammatory diseases including septic shock.



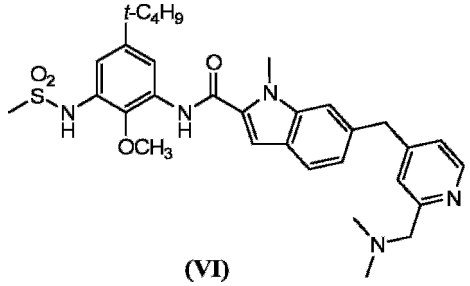
(IV)

3. 1,2-Dihydro-pyrazol-3-one derivatives, (**V**), prepared by Dominguez (6) were effective as IL-1 and TNF-α antagonists in response to inflammatory stimuli such as lipopolysaccharides and useful in treating rheumatoid arthritis.



(V)

4. TNF-α receptor inhibitors consisting of indole derivatives, (**VI**), were prepared by Cirillo (7) and used in the treatment of rheumatoid arthritis.



(VI)

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1. R. Schindler *et al*., Blood, 75, 40 (1990)
2. N. Minami *et al*., US Patent 7,087,624 (August 8, 2006)
3. H. Kubota *et al*., US Patent 6,958,339 (October 25, 2005)
4. R. Betageri *et al*., US Patent 6,506,747 (January 14, 2003)
5. N. Tamura *et al*., US Patent 7,078,540 (July 18, 2006)
6. C. Dominguez *et al*., US Patent 6,967,254 (November 22, 2005)
7. P.F. Cirillo *et al*., US Patent 7,078,419 (July 18, 2006)

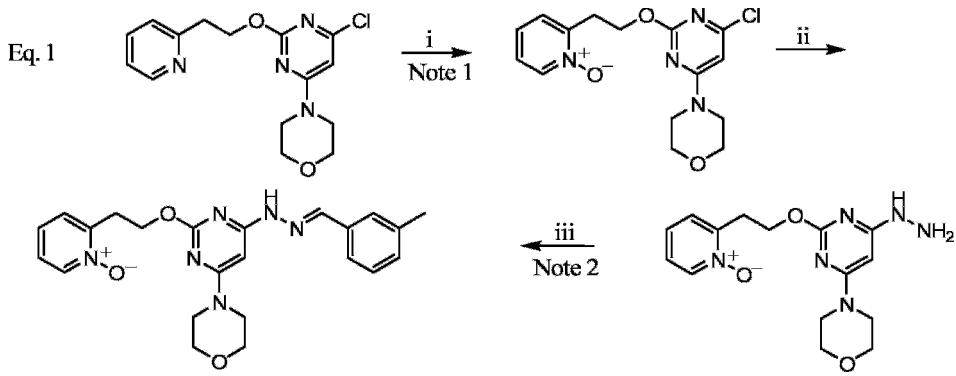
VI. Interleukin-12 Inhibitors

Title Pyrimidine Compounds
L. Sun *et al*., US Patent 6,958,332 (October 25, 2005)
Assignee Synta Pharmaceuticals Corp.
Utility Treatment of Crohn's Disease

Invention Significance Interleukin-12 (IL-12) promotes type 1 T helper (Th1) cell responses. Overproduction of IL-12, however, causes excessive Th1 responses, which results in inflammatory disorders. To address this disorder, compounds that downregulate IL-12 production have been prepared, which inhibit excessive Th1 production to control inflammatory-based disorders.

Reaction

Eq. 1



i- 3-Chloroperbenzoic acid, CH_2Cl_2, methyl alcohol
ii- Hydrazine, dioxane
iii- 3-Tolualdehyde, methyl alcohol, acetic acid

Experimental

1. **Preparation of 4-{6-chloro-2-[2-(1-oxy-pyridin-2-yl)-ethoxy]-pyrimidin-4-yl}-morpholine**

   A solution of 4-[6-chloro-2-(2-pyridin-2-yl-ethoxy)-pyrimidin-4-yl]-morpholine (5.0 mmol) in 40 ml CH_2Cl_2 was treated with 10 ml methyl alcohol followed by 70% 3-chloroperbenzoic acid (5.8 mmol), then stirred overnight at ambient temperature. The solution was poured into 35 ml saturated $NaHCO_3$ solution and the organic phase isolated. It was washed with 40 ml 10% Na_2SO_3 solution and 40 ml brine.

The solution was dried with Na_2SO_4, then concentrated, and the product isolated in 86.7% yield as a white solid.

^{1}H NMR ($CDCl_3$) (ppm), *J* (Hz) 8.25–8.23 (m, 1H), 7.41–7.7.38 (m, 1H), 7.20–7.16 (m, 2H), 6.14 (s, 1H), 4.71 (t, $J = 6.0$ Hz, 2H), 3.77–3.73 (m, 4H), 3.63–3.55 (m, 4H), 340 (t, $J = 6.0$ Hz, 2H)

2. **Preparation of 6-morpholin-4-yl-2-[2-(1-oxy-pyridin-2-yl)-ethoxy]-pyrimidin-4-yl]-hydrazine**

   Anhydrous hydrazine (20 mmol) was added to a solution of the Step 1 product (4.0 mmol) dissolved in 15 ml dioxane, then heated 2 hours to 95–100°C. The mixture was gradually formed, concentrated until a white solid began to precipitate, whereupon 15 ml water was added. The precipitate was isolated, washed with water to pH ~7, and the product isolated in 76.7% yield.

   ^{1}H NMR (DMSO-d_6) (ppm) 8.25 (s, 1H), 7.66 (s, 1H), 7.44–7.41 (m, 1H), 7.33–7.25 (m, 2H), 5.59 (s, 1H), 4.46 (t, $J = 6.0$ Hz, 2H), 3.64–3.61 (m, 4H), 3.41–3.38 (m, 4), 3.17 (t, $J = 6.0$ Hz, 2H)

3. **Preparation of *N*-(3-methyl-benzylidene)-*N*′-{6-morpholin-4-yl-2-[2-(1-oxy-pyridin-2-yl)-ethoxy]-pyrimidin-4-yl}-hydrazine**

   A mixture consisting of the Step 2 product (2.46 mmol) and *m*-tolualdehyde (2.58 mmol) dissolved in 7 ml methyl alcohol was treated with 2 drops acetic acid, then refluxed 15 minutes. Upon cooling, a solid was isolated, which was washed with a small amount of methyl alcohol and diethyl ether, then dried. The product was isolated in 89% yield as a white solid, mp = 187−188°C.

   ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm) 10.86 (s, 1H), 8.28–8.26 (m, 1H), 7.98 (s, 1H), 7.50–7.43 (m, 3H), 7.33–7.26 (m, 3H), 7.17 (d, $J = 7.8$ Hz, 1H), 6.05 (s, 1H), 4.53 (t, $J = 6.3$ Hz, 2H), 3.68–3.64 (m, 4H), 3.54–3.50 (m, 4H), 3.21 (t, $J = 6.3$ Hz, 2H), 2.33 (s, 3H)
   MS (ES) Calc. for $C_{23}H_{26}N_6O_3$ 434.21. Found 457.2 $(M + Na)^+$

Derivatives

No additional derivatives were prepared.

Testing

I. *Treatment of Crohn's Disease Using Dinitrobenzene Sulfonic Acid-induced Inflammatory Bowel Syndrome*

Wistar-derived male or female rats weighing 200 g, which fasted 24 hours, were used. Distal colitis was induced by intracolonic instillation of 2,4-dinitrobenzene sulfonic acid, which was injected to ensure that the solution remained in the colon. The Step 3

experimental agent was administered orally 2 and 24 hours before administering dinitrobenzene sulfonic acid with a vehicle and then administered daily for five additional days. One control group was similarly treated with the vehicle alone, while the other control group was treated with vehicle plus dinitrobenzene sulfonic acid. The animals were sacrificed 24 hours after the final treatment dose and each colon removed and weighed.

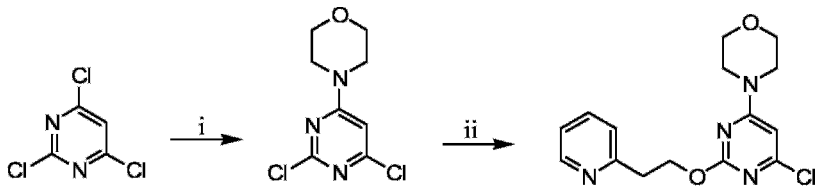
Results

The Step 3 experimental agent reduced inflammation by 63%.

Notes

1. The Step 1 co-reagent, (**I**), was prepared by Ono (1) as illustrated in Eq. 2.

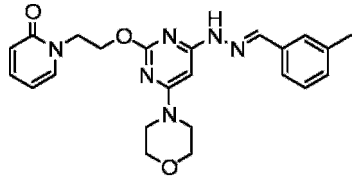
Eq. 2



(I)

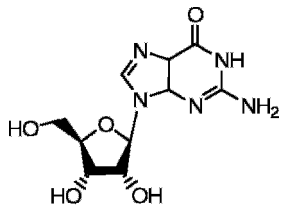
i- Morpholine, CH_2Cl_2, diisopropylethylamine
ii- 2-(2-Pyridin-2-yl)-1-hydroxyethane, DMF

2. In an earlier investigation by the author (2), pyrimidine derivatives, (**II**), were prepared, which were effective as IL-12 production regulators, and used in the treatment of Crohn's disease, sepsis, and rheumatoid arthritis.



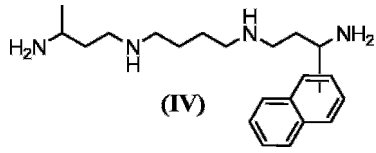
(II)

3. Salzman (3) determined that inosine derivatives, (**III**), were effective in regulating IL-12 levels and used in the treatment of chronic inflammatory disorders such as inflammatory bowel, ulcerative colitis, and Crohn's disease.



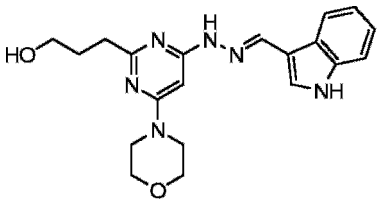
(III)

4. Polyamine derivatives, (**IV**), prepared by Burns (4) were effective in mitigating IL-12-initiated inflammation diseases associated with Crohn's disease.



(IV)

5. Pyrimidines, (**V**), and triazine derivatives, (**VI**), prepared by Ono (5,6), respectively, were effective in treating IL-12 overproduction-related disorders associated with Crohn's disease, sepsis, and rheumatoid arthritis.



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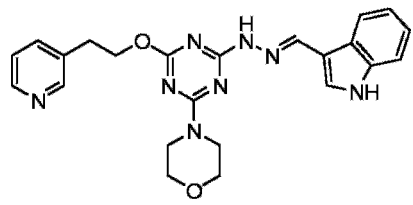


(VI)

References

1. M. Ono *et al*., US Patent 6,693,097 (February 17, 2004) and US Patent 6,384,032 (May 7, 2002)
2. L. Sun *et al*., US Patent 6,858,606 (February 22, 2005)
3. A.L. Salzman *et al*., US Patent 6,958,324 (October 25, 2005)
4. M.R. Burns *et al*., US Patent 6,919,483 (July 19, 2005)
5. M. Ono *et al*., US Patent 7,067,514 (June 27, 2006)
6. M. Ono *et al*., US Patent 7,045,517 (May 16, 2006)

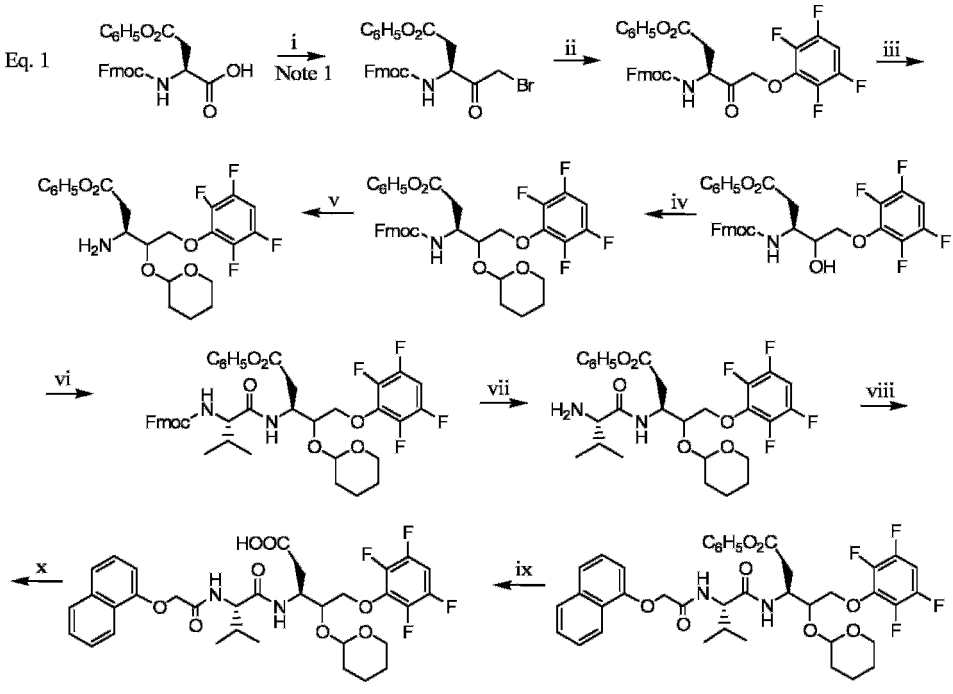
VII. Interleukin-1β-converting Enzyme Inhibitors

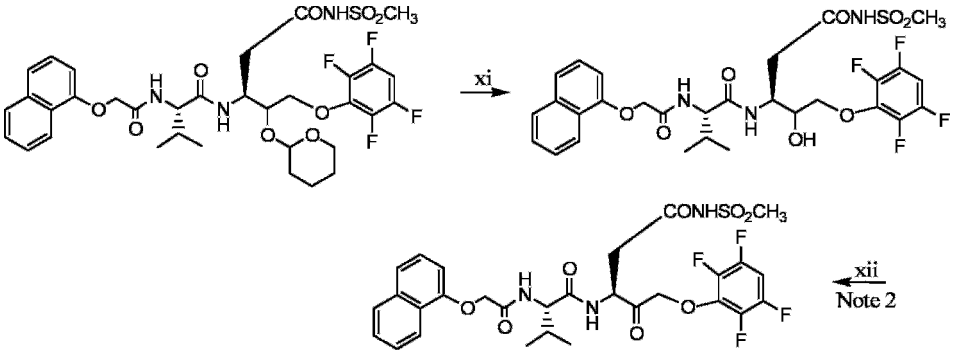
Title Inhibitors of the ICE/ced-3 Family of Cysteine Proteases
R.J. Ternansky *et al.*, US Patent 6,969,703 (November 29, 2005)
Assignee Idun Pharmaceuticals, Inc.
Utility Treatment of Inflammatory Diseases

Invention Significance Interleukin 1 (IL-1) is a major proinflammatory and immunoregulatory protein. Although inhibitors of interleukin-1β-converting enzyme (ICE) and related proteases are available, they exhibit limited cell penetration, adsorption, and poor metabolic stability. This investigation addresses these concerns by preparing dipeptide mimetics having improved cell penetration and metabolic stability resulting in enhanced bioavailability.

Reaction

Eq. 1





i- THF, isobutyl chloroformate, diethyl ether, potassium hydroxide, 1-methyl-3-nitro-1-nitrosoguanidine, hydrobromic acid, water
ii- Sodium iodide, acetone, potassium 2,3,5,6-tetrafluorophenoxide
iii- Sodium borohydride, methyl alcohol, THF
iv- 3,4-Dihydro-2H-pyran, pyridinium *p*-toluenesulfonate, CH_2Cl_2
v- Piperidine, DMF
vi- Fmoc-Val-OH, CH_2Cl_2, 1-hydroxybenzotriazole hydrate, 4-methylmorpholine 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
vii- Piperidine, DMF
viii- (1-Naphthoxy)acetic acid, CH_2Cl_2, 1-hydroxybenzotriazole hydrate, *N*-methyl-morpholine, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
ix- Palladium on carbon, methyl alcohol, hydrogen
x- 1,1′-Carbonyldiimidazole, THF, methanesulfonamide, 1,8-diazabicyclo[5.4.0]-undec-7-ene
xi- *p*-Toluenesulfonic acid, methyl alcohol
xii- Dess–Martin periodinane, CH_2Cl_2

Experimental

1. **Preparation of bromomethylketone**

   4-Methylmorpholine (6.8 mmol) was added to a solution of Fmoc-Asp(OBn)-OH (4.55 mmol) in 50 ml THF at −10°C followed by the addition of isobutyl chloroformate (6.0 mmol), then stirred 20 minutes, filtered, and the cake removed.

In a separate flask, 1-methyl-3-nitro-1-nitrosoguanidine (7.36 mmol) was added to a mixture of 14 ml diethyl ether and 8 ml 40% aqueous KOH, then stirred 10 minutes, and the phases separated.

The organic layer was transferred to the first vessel, then stirred 30 minutes, and treated with 2.10 ml 48% HBr, then stirred an additional 15 minutes at ambient temperature. The solution was diluted with EtOAc, then washed twice with saturated $NaHCO_3$ solution and once with brine, then dried, and concentrated. The residue was purified by flash chromatography with silica gel using 35% EtOAc/hexanes and the product isolated in 73% yield as a white solid.

^{1}**H NMR** (300 MHz, $CDCl_3$) δ 7.77 (d, $J = 8$ Hz, 2H), 7.58 (d, $J = 8$ Hz, 2H), 7.45–7.29 (m, 9H), 5.77 (d, $J = 9$ Hz, 1H), 5.12 (s, 2H), 4.79–4.71 (m, 1H), 4.63–4.42 (m, 2H), 4.21 (t, $J = 6$ Hz, 1H), 4.04 (s, 2H), 2.97 (ABXq, $J = 7, 5$ Hz)

2. **Preparation of Ketone**

Sodium iodide (0.720 mmol) was added to the Step 1 product (3.28 mmol) dissolved in 10 ml acetone followed by potassium of 2,3,5,6-tetrafluorophenol (3.45 mmol) and the mixture stirred 1 hour at ambient temperature. The reaction mixture was diluted with EtOAc, then washed twice with brine, dried, and concentrated. The residue was purified by flash chromatography using CH_2Cl_2/diethyl ether/hexanes, 1:1:3, and the product isolated in 80% as a white solid.

^{1}**H NMR** (300 MHz, $CDCl_3$) δ 7.76 (d, $J = 8$ Hz, 2H), 7.58 (d, $J = 8$ Hz, 2H), 7.44–7.27 (m, 9H), 6.85–6.73 (m, 1H), 5.73 (d, $J = 9$ Hz, 1H), 5.15–4.92 (m, 4H), 4.75–4.67 (m, 1H), 4.61–4.42 (m, 2H), 4.21 (t, $J = 6$ Hz, 1H), 3.00 (ABXq, $J = 18, 4$ Hz, 2H)

3. **Preparation of Alcohol**

Sodium borohydride (3.20 mmol) was added to the Step 2 product (2.63 mmol) dissolved in 7 ml apiece methyl alcohol and THF and the mixture stirred 30 minutes at 0°C. The mixture was quenched with saturated NH_4Cl solution and extracted three times with CH_2Cl_2. Extracts were washed with brine, then dried, and concentrated. The residue was purified by flash chromatography using EtOAc/hexanes, 1:1, and the product isolated in 87% yield as a white solid.

^{1}**H NMR** (300 MHz, $CDCl_3$) δ 7.78–7.74 (m, 2H), 7.57 (d, $J = 7$ Hz, 2H), 7.44–7.27 (m, 9H), 6.87–6.75 (m, 1H), 5.62 (d, $J = 9$ Hz, 0.3H), 5.44 (d, $J = 9$ Hz, 0.2H), 5.29–5.23 (m, 0.5H), 5.16–5.11 (m, 1H), 4.69 (d, $J = 6$ Hz, 1H), 4.59–4.37 (m, 4H), 4.30–4.04 (m, 3H), 3.35–3.09 (m, 1H), 2.94–2.41 (m, 2H)

4. **Preparation of THP Ether**

3,4-Dihydro-2H-pyran (3.4 mmol) and pyridinium *p*-toluenesulfonate (0.441 mmol) were added to the Step 3 product (2.28 mmol) dissolved in 12 ml CH_2Cl_2 and

stirred 16 hours at ambient temperature. The mixture was diluted with EtOAc and washed twice with saturated $NaHCO_3$ solution and once with brine, then dried, and concentrated. The residue was purified by flash chromatography first using 15% EtOAc/hexanes and then using EtOAc/hexanes, 1:1, and the product isolated in 69% yield as a colorless oil.

^{1}**H NMR** (300 MHz, $CDCl_3$) δ 7.76 (d, $J = 7$ Hz, 2H), 7.62–7.55 (m, 2H), 7.42–7.27 (m, 9H), 6.84–6.71 (m, 1H), 6.21 (d, $J = 9$ Hz, 0.3H), 5.65 (d, $J = 9$ Hz, 0.2H), 5.33–5.27 (m, 0.5H), 5.13 (t, $J = 3$ Hz, 2H), 4.72–4.04 (m, 8H), 3.91–3.73 (m, 1H), 3.51–3.36 (m, 1H), 2.98–2.57 (m, 2H), 1.86–1.61 (m, 2H), 1.57–1.43 (m, 4H)

5. **Preparation of Amine**

Piperidine (5.1 mmol) was added to the Step 4 product (1.57 mmol) dissolved in 10 ml DMF, then stirred 5 minutes at ambient temperature. The mixture was diluted with EtOAc, washed once with saturated NH_4Cl solution, twice with water and once with brine, then dried, and concentrated. The residue was purified by flash chromatography first using EtOAc/hexanes, 1:1, then using 80% EtOAc/hexanes, and the product isolated in 74% yield as a colorless oil.

^{1}**H NMR** (300 MHz, $CDCl_3$) δ 7.39–7.29 (m, 5H), 6.82–6.70 (m, 1H), 5.15 (s, 2H), 4.78–4.63 (m, 1H), 4.53–4.26 (m, 2H), 4.03–3.79 (m, 2H), 3.71–3.43 (m, 2H), 2.80–2.43 (m, 2H), 1.85–1.66 (m, 2H), 1.57–1.45 (m, 4H)

6. **Preparation of Dipeptide**

A mixture of the Step 5 product (1.15 mmol) and Fmoc-Val-OH (1.28 mmol) dissolved in 30 ml CH_2Cl_2 was treated with 1-hydroxybenzotriazole hydrate (1.76 mmol) followed by 4-methyl-morpholine (1.7 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide HCl (1.41 mmol), then stirred 16 hours at ambient temperature. The mixture was diluted with EtOAc and washed once with saturated NH_4Cl solution, saturated $NaHCO_3$, and once with brine, then dried, and concentrated. The residue was purified by flash chromatography using 35% EtOAc/hexanes and the product isolated in 91% yield as a white solid.

^{1}**H NMR** (300 MHz, $CDCl_3$) δ 7.76 (d, $J = 8$ Hz, 2H), 7.62–7.56 (m, 2H), 7.42–7.27 (m, 9H), 6.91–6.71 (m, 2H), 5.39–5.30 (m, 1H), 5.12–5.05 (m, 2H), 4.74–3.78 (m, 10H), 3.50–3.36 (m, 1H), 2.97–2.61 (m, 2H), 2.19–2.06 (m, 1H), 1.82–1.68 (m, 2H), 1.52–1.40 (m, 4H), 0.98–0.87 (m, 6H)

7. **Preparation of Amine**

Piperidine (3.6 mmol) was added to the Step 6 product (1.05 mmol) dissolved in 7 ml DMF and stirred 5 minutes at ambient temperature. The mixture was diluted with EtOAc, then washed once with saturated NH_4Cl solution, twice with water, once with brine, dried, and concentrated. The residue was purified by flash

chromatography first using EtOAc/hexanes, 1:1, then 20% methyl alcohol/CH_2Cl_2, and the product isolated in 100% yield as a yellow oil.

^{1}**H NMR** (300 MHz, $CDCl_3$) δ 8.07–7.63 (m, 1H), 7.40–7.27 (m, 5H), 6.83–6.71 (m, 1H), 5.17–5.07 (m, 2H), 4.76–4.62 (m, 1H), 4.60–4.26 (m, 2H), 4.24–4.09 (m, 2H), 3.92–3.80 (m, 1H), 3.52–3.39 (m, 1H), 3.22–3.16 (m, 1H), 2.97–2.60 (m, 2H), 2.31–2.16 (m, 1H), 1.84–1.64 (m, 2H), 1.59–1.44 (m, 4H), 0.98–0.94 (m, 3H), 0.81–0.76 (m, 3H)

8. **Preparation of Dipeptide**

The Step 7 product (1.01 mmol) and (1-naphthoxy)acetic acid (1.12 mmol) were dissolved in 25 ml CH_2Cl_2, then treated with 1-hydroxybenzotriazole hydrate (1.56 mmol) followed by *N*-methylmorpholine (1.5 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide HCl (1.25 mmol), and stirred 16 hours at ambient temperature. The mixture was diluted with EtOAc, washed once with saturated NH_4Cl solution, saturated $NaHCO_3$, and brine, then dried, and concentrated. The residue was purified by flash chromatography using 35% EtOAc/hexanes and the product isolated in 89% yield as a white foam.

^{1}**H NMR** (300 MHz, $CDCl_3$) δ 8.28–8.21 (m, 1H), 7.85–7.80 (m, 1H), 7.57–7.49 (m, 3H), 7.40–7.22 (m, 8H), 6.96–6.42 (m, 2H), 5.12–5.05 (m, 2H), 4.80–4.59 (m, 3H), 4.58–4.24 (m, 3H), 4.23–4.06 (m, 2H), 3.96–3.77 (m, 1H), 3.56–3.38 (m, 1H), 2.97–2.61 (m, 2H), 2.24–2.10 (m, 1H), 1.84–1.66 (m, 2H), 1.55–1.40 (m, 4H), 0.98–0.87 (m, 6H)

9. **Preparation of Acid**

Palladium (10%) on carbon (170 mg) was added to the Step 8 product (0.861 mmol) dissolved in 15 ml methyl alcohol and hydrogenated under a hydrogen balloon for 75 minutes. The mixture was filtered through celite and eluted with methyl alcohol. The solution was concentrated and the product isolated in 94% yield as a white solid.

10. **Preparation of Methyl Sulfonimide**

1,1′-Carbonyldiimidazole (0.900 mmol) was added to the Step 9 product (0.451 mmol) dissolved in 7 ml THF and stirred for 3 hours. The mixture was cooled to 0°C, then treated with methanesulfonamide (0.90 mmol) followed by 1,8-diazabicyclo[5.4.0]undec-7-ene (0.903 mmol), and stirred 4 hours at ambient temperature. The reaction mixture was diluted with EtOAc, washed once with 1 M HCl, twice with water, once with brine, then dried, and concentrated. The residue was reconcentrated from CH_2Cl_2 and the product isolated in 98% yield as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 10.22–9.99 (m, 1H), 8.24–8.17 (m, 1H), 7.85–7.69 (m, 1H), 7.59–7.47 (m, 3H), 7.40–7.27 (m, 2H), 6.94–6.66 (m, 2H), 4.85–4.62 (m, 4H), 4.55–4.02 (m, 6H), 3.59–3.45 (m, 1H), 3.22–3.19 (m, 3H), 2.89–2.55 (m, 2H), 2.21–2.09 (m, 1H), 1.88–1.67 (m, 2H), 1.63–1.43 (m, 4H), 1.00–0.86 (m, 6H)

11. **Preparation of Alcohol**

p-Toluenesulfonic acid (0.18 mmol) was added to the Step 10 product (0.426 mmol) dissolved in 5 ml methyl alcohol and the mixture stirred 30 minutes at ambient temperature. It was then diluted with EtOAc and washed twice with water and once with brine, then dried. The mixture was concentrated and the product isolated in 93% yield as a white solid.

^{1}H NMR (300 MHz, DMSO) δ 8.23–8.18 (m, 2H), 8.04–7.88 (m, 2H), 7.61–7.50 (m, 4H), 7.43–7.37 (m, 1H), 6.90 (d, $J = 8$ Hz, 1H), 5.61 (d, $J = 5$ Hz, 0.3H), 5.48 (d, $J = 6$ Hz, 0.7H), 4.83–4.72 (m, 2H), 4.58–3.75 (m, 5H), 3.17 (s, 1H), 3.13 (s, 2H), 2.74–2.37 (m, 2H), 2.03–1.91 (m, 1H), 0.84–0.76 (m, 6H)

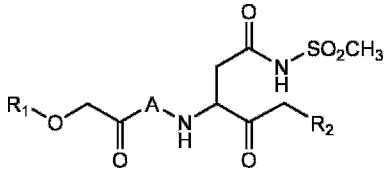
12. **Preparation of Methylsulfonimide**

Dess–Martin periodinane (0.479 mmol) was added to the Step 11 product (0.373 mmol) dissolved in 7 ml CH_2Cl_2, then stirred 30 minutes at ambient temperature. The mixture was diluted with EtOAc and washed twice with water and once with brine, then dried, and concentrated. The residue was purified by flash chromatography using 60% EtOAc/hexanes, then 80% EtOAc/hexanes, and the product isolated in 58% yield as a mixture of diastereomers as a white solid.

^{1}H NMR (300 MHz, DMSO) δ 8.84 (d, $J = 7$ Hz, 1H), 8.28–8.06 (m, 2H), 7.91–7.87 (m, 1H), 7.58–7.49 (m, 5H), 7.39 (t, $J = 8$ Hz, 1H), 6.89 (d, $J = 8$ Hz, 1H), 5.23 (s, 1H), 4.93–4.42 (m, 4H), 4.28–4.19 (m, 1H), 3.28 (s, 1.3H), 3.19 (s, 1.7H), 2.96–2.59 (m, 2H), 2.06–1.96 (m, 1H), 0.89–0.80 (m, 6H)
MS (ESI) *m/z* 654 $[(M^+)^{-1}]$

Derivatives

Table 1 Selected experimental dipeptide ICE/ced-3 family of cysteine proteases inhibitors having improved cell penetration and metabolic stability resulting in enhanced bioavailability. ^{1}H-, ^{13}C NMR, and mass spectral data supplied only for Entry 1 and its intermediates



Entry	R_1	R_2	A
1	1-Naphthyl	2,3,5,6-Tetrafluorophenyl	$-NHCH(CH(CH_3)_2)CO-$
3	1-Naphthyl	Fluoro	$-NHCH(CH(CH_3)_2)CO-$
19	1-Naphthyl	Methylphenylphosphate	$-NHCH(CH(CH_3)_2)CO-$
24	2,4,-Di-*t*-butyl phenyl	Diphenylphosphate	$-NHCH(CH_3)CO-$
41	2-Fluorophenyl	2,3,5,6-Tetrafluorophenyl	$-NHCH(CH(CH_3)_2)CO-$
77	2-Phenylphenyl	2,3,5,6-Tetrafluorophenyl	$-NHCH(CH_3)CO-$
91	5,6,7,8-Tetrahydronaphthyl	2,3,5,6-Tetrafluorophenyl	$-NHCH(CH_2(C_6H_{11}))CO-$
99	2-Phenylphenyl	Diphenylphosphate	$-NHCH(CH_2(C_6H_{11}))CO-$
125	5,6,7,8-Tetrahydronaphthyl	H	$-NHCH(CH(CH_3)_2)CO-$

Testing

I. *Assay for Inhibition of ICE/ced-3 Protease Family Activity*

A. Determination of IC_{50} Values

Fluorescence enzyme assays detecting the activity of selected experimental agents using recombinant ICE and CPP_{32} enzymes were performed using the methods of Thornberry (1) and Nicholson (2). The substrate used was Acetyl-Tyr-Val-Ala-Asp-amino-4-methylcoumarin (AMC) for the ICE assay and Acetyl-Asp-Glu-Val-Asp-amino-4-methylcoumarin for the CPP_{32}, Mch2, Mch3, and Mch5 assays. Enzyme reactions are run in an ICE buffer at ambient temperature in duplicate. The assays are performed by mixing the following components:

i- 50 μl ICE, Mch2, Mch5, CPP_{32} at 18.8, 38, 8.1, and 0.153 nM concentrations, respectively, or Mch3 enzyme in ICE buffer containing either 8.0 mM (ICE, Mch2, Mch3, CPP32) or 20 mM (Mch5) DTT
ii- 50 μl compound of Formula I or ICE buffer (control)
iii- 100 μl of 20 μM substrate

Fluorescent AMC product formation was monitored 1 hour at ambient temperature by measuring the fluorescence emission at 460 nm using an excitation wavelength of 360 nm and the IC_{50} determined where Cbz-ValAlaAsp-H was used as the reference. Testing results are summarized in Table 2.

B. Determination of the Dissociation Constant K_i and Irreversible Rate Constant k_3 for Irreversible Inhibitors

The irreversible inhibition, K_i, of an ICE/ced-3 family protease enzyme using selected experimental agents was determined according to the methods of Thornberry (1) and

Nicholson (2) using Cbz-ValAlaAsp-H was used as the reference. Testing results are provided in Table 3.

Table 2 IC_{50} testing results for Entry 1 and reference Cbz-ValAlaAsp-H indicating the effectiveness of the experimental agent as an ICE/ced-3 protease inhibitor

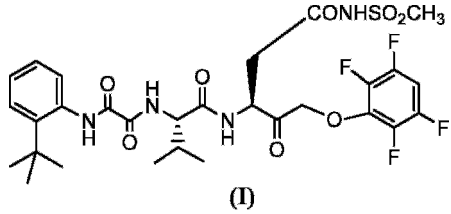
Entry	IC_{50} (μM)					
	Csp-1	**Csp-3**	**Csp-6**	**Csp-7**	**Csp-8**	**Csp-9**
1	0.004	0.002	0.002	0.004	0.006	0.005
Reference	0.064	47.0	> 10	> 10	2.96	0.87

Table 3 K_i activity of Entry 1 and reference Cbz-ValAlaAsp-H indicating the preferred irreversible inhibition properties of the experimental agent

Entry	K_i (μM)			
	Csp-1	**Csp-3**	**Csp-6**	**Csp-8**
1	0.20	0.08	0.40	0.60
Reference	0.015	0.820	0.594	0.018

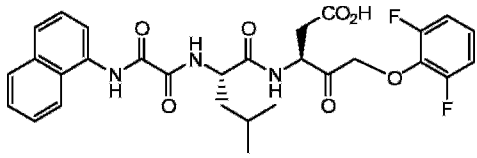
Notes

1. In earlier investigations by the author (3), oxamyl dipeptide derivatives, (**I**), were prepared, which were effective as ICE/ced-3 family inhibitors, and used in treating the patients suffering from inflammatory, autoimmune, and neurodegenerative diseases.



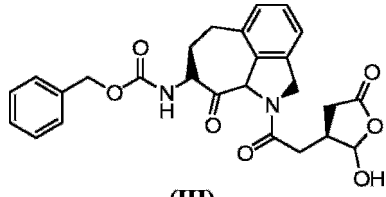
(I)

2. Oxamyl dipeptides, (**II**), prepared by Karanewsky (4) were effective as ICE inhibitors of cysteine proteases and used in the treatment of inflammatory, autoimmune, and neurodegenerative diseases and for the prevention of ischemic injury.



(II)

3. Tricyclics derivatives, (**III**), prepared by Fritz (5) were effective in treating inflammation-associated disorders with the IL-1β-converting enzyme family of proteases and used in treating inflammatory conditions such as arthritis, cholangitis, and colitis.



(III)

4. Dipeptide ICE inhibitors such as Cbz-Val-AspCH_2F were prepared by Keans (6) and used for reducing or treating apoptotic cell death.

References

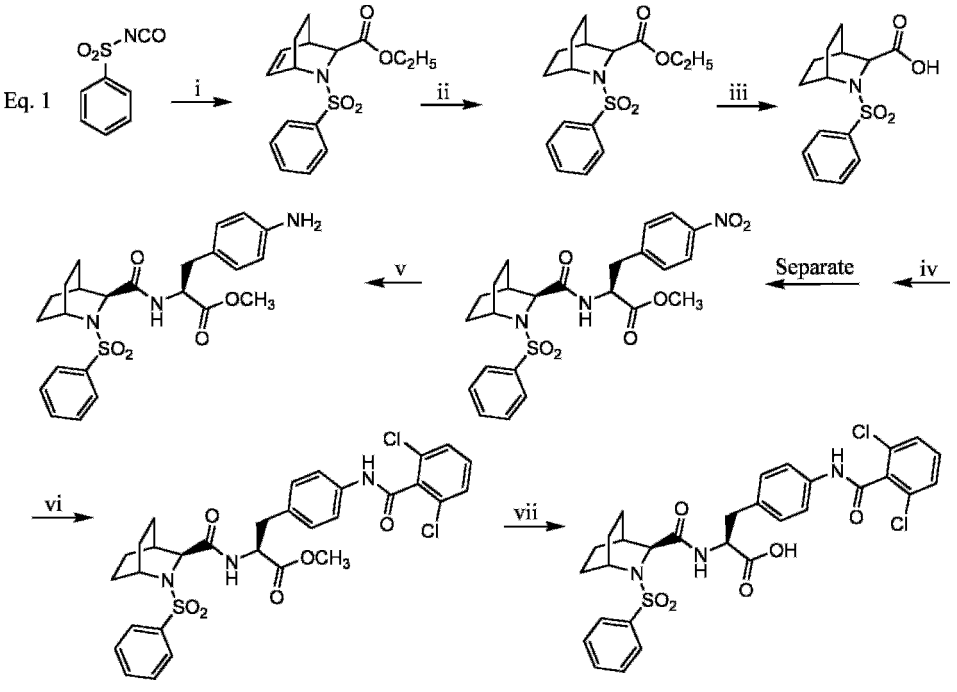
1. H.F. Thornberry *et al.*, Nature, 356, 774 (1992)
2. P.B. Nicholson *et al.*, Nature, 376, 37 (1995)
3. R.J. Ternansky *et al.*, US Patent 6,790,989 (September 14, 2004) and US Patent 6,515,173 (February 4, 2003)
4. D.S. Karanewsky *et al.*, US Patent 7,053,056 (May 20, 2006) and US Patent 6,544,951 (April 8, 2003)
5. L.C. Fritz *et al.*, US Patent 6,693,096 (February 17, 2004) and US Patent 6,200,969 (March 13, 2001)
6. J.F.W. Keans *et al.*, US Patent 6,949,516 (September 27, 2005)

VIII. α_4 Integrin Antagonists

Title Aza-bridged Bicyclic Amino Acid Derivatives as α_4 Integrin Antagonists
A. Dyatkin *et al.*, US Patent 6,960,597 (November 1, 2005)
Assignee Ortho-McNeil Pharmaceutical, Inc.
Utility Treatment of Inflammation

Invention Significance Intercellular adhesion is mediated by $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrin antagonists. At an injury site, for example, vascular endothelial cells release molecules that are adhesive for leukocytes, which subsequently release chemical mediators to combat infection. Excess intercellular adhesion, however, is associated with inflammatory conditions. To address this disorder, integrin antagonists have been developed for treating integrin-mediated diseases.

Reaction



i- Ethyl glyoxalate, toluene, 1,3-cyclohexadiene
ii- 10% Palladium on carbon, ethyl alcohol, hydrogen
iii- Ethyl alcohol, water, sodium hydroxide
iv- (*S*)-4-Nitrophenylalanine methyl ester hydrochloride, *N*-ethyl-*N*′-dimethylaminopropylcarbodiimide hydrochloride, diisopropylethylamine, CH_2Cl_2
v- Methyl alcohol, ammonium chloride, zinc
vi- Triethylamine, 2,6-dichlorobenzoylchloride
vii- Methyl alcohol, water, sodium hydroxide

Experimental

1. **Preparation of ethyl 2-[(phenyl sulfonyl)-2-azabicyclo[2.2.2]oct-3-yl]-3-ene-carboxylic acid**

   A 20 ml 50% solution of benzenesulfonyl isocyanate (0.10 mol) in toluene and 50 ml ethyl glyoxalate in toluene were refluxed 24 hours, then treated with 1,3-cyclohexadiene (0.21 mol), and refluxed an additional 10 hours. Upon cooling, a white precipitate was formed, which was recrystallized in EtOAc, and 19.1 g product isolated as a white solid.

2. **Preparation of ethyl 2-[(phenyl sulfonyl)-2-azabicyclo[2.2.2]oct-3-yl]carboxylic acid**

   A suspension consisting of the Step 1 product (19.0 g), 10% Pd/C (200 mg), and 200 ml ethyl alcohol was hydrogenated 24 hours. The mixture was filtered through celite and concentrated. The residue was recrystallized in EtOAc and 16.1 g product isolated as white crystals.

   ^{1}H NMR ($CDCl_3$) 7.98 (d, $J = 7$ Hz, 2H), 7.5 (m, 3H), 4.36 (m,1 H), 4.25 (q, $J = 8$ Hz, 2H), 3.65 (d, $J = 3$ Hz, 1H), 2.22 (m,1H), 2.0 (m, 1H), 1.9 (m, 1H), 1.5 (broad m, 6H), 1.28 (t, $J = 8$ Hz, 3H)

3. **Preparation of 2-[(phenyl sulfonyl)-2-azabicyclo[2.2.2]oct-3-yl]carboxylic acid**

   A solution of the Step 2 product in 200 ml ethyl alcohol and 50 ml 3 M NaOH solution was stirred 24 hours and then concentrated. The residue was dissolved in 300 ml water, then washed with EtOAc, and the organic layer discarded. The aqueous layer was acidified to pH 2 with 1 M HCl, then extracted with EtOAc, and dried using $MgSO_4$. The mixture was concentrated and 9.5 g product isolated as a white solid.

   ^{1}H NMR ($CDCl_3$) 7.98 (d, $J = 7$ Hz, 2H), 7.5 (m, 3H), 4.36 (m,1H), 3.70 (d, $J = 3$ Hz, 1H), 2.30 (m, 1H), 2.0 (m, 1H), 1.9–1.3 (broad m, 7H)

4. **Preparation of (3*S*,5*S*)-methyl 4-[benzyl)amino]-*N*-[[(3S)-2-(phenyl sulfonyl)-2-azabicyclo[2.2.2]oct-3-yl]carbonyl]-L-phenylalanine**

A mixture consisting of the Step 3 product, (*S*)-4-nitrophenylalanine methyl ester•HCl (1 equiv.), *N*-ethyl-*N*′-dimethylaminopropylcarbodiimide HCl (5.0 g), diisopropylethylamine (3 equiv.), and 150 ml CH_2Cl_2 was stirred 5 hours at ambient temperature, then washed with 50 ml apiece saturated $NaHCO_3$ solution and 1 M HCl. The solution was dried, concentrated, and a white solid foam isolated. The foam was recrystallized in hexane/ethyl acetate and 1.05 g product isolated as colorless crystals.

^{1}H NMR ($CDCl_3$) 8.14 (d, $J = 8$ Hz, 2H), 7.90 (d, $J = 7$ Hz, 2H), 7.62 (t, $J = 7$ Hz, 1H), 7.52 (t, $J = 7$ Hz, 2H), 7.38 (d, $J = 8$ Hz, 2H), 2.3 (m, 1H), 4.95 (q, $J = 8$ Hz, 1H)

When the mother liquor was concentrated and the residue purified by column chromatography with silica gel using hexane/ethyl acetate, 1:1, the (*R,S*) diastereomer was isolated.

5. **Preparation of (3*S*,5*S*)-methyl 4-[(benzyl)amino]-*N*-[[(3*S*)-2-(phenylsulfonyl)-2-azabicyclo[2.2.2]oct-3-yl]carbonyl]-L-phenylalanine**

   The Step 4 product (1.00 g) dissolved in methyl alcohol was treated with NH_4Cl (0.54 g) and zinc dust (4.5 g) and stirred 3 hours, then filtered through celite. The solution was concentrated and the residue was treated with 1 ml 10% acetic acid, then neutralized with $NaHCO_3$. The solution was then extracted three times with 50 ml EtOAc, washed with 15 ml water, dried with Na_2SO_4, reconcentrated, and 0.85 g product isolated.

   MS *m/z* 472.6 (MH^+)

6. **Preparation of methyl 4-[(2,6-dichlorobenzoyl)amino]-*N*-[[(3*S*)-2-(phenylsulfonyl)-2-azabicyclo[2.2.2]oct-3-yl]carbonyl]-L-phenylalanine**

   A mixture consisting of Step 5 product dissolved in 9 ml CH_2Cl_2, TEA (0.0024 mol), and 2,6-dichlorobenzoylchloride (0.453 g) was stirred 6 hours at ambient temperature, then diluted with 50 ml CH_2Cl_2. The solution was washed with 20 ml saturated $NaHCO_3$ solution and dried with Na_2SO_4. The mixture was concentrated, then purified by chromatography with silica gel using EtOAc/hexane, 1:1, and 1.05 g product isolated as a white solid.

7. **Preparation of 4-[(2,6-dichlorobenzoyl)amino]-*N*-[[(3*S*)-2-(phenylsulfonyl)-2-azabicyclo[2.2.2]oct-3-yl]carbonyl]-L-phenylalanine**

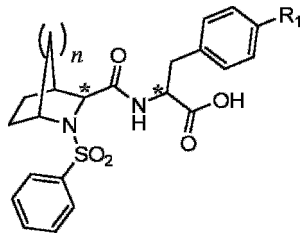
   The Step 6 product dissolved in 30 ml methyl alcohol was treated with 10 ml 3 M NaOH, then stirred 24 hours, and concentrated. The residue was dissolved in 100 ml water and washed with EtOAc. The aqueous layer was acidified to pH 2 using 1 M HCl, then extracted three times with EtOAc, and dried with $MgSO_4$. The mixture was concentrated and 0.76 g product isolated as a white crystalline solid.

   ^{1}H NMR ($CDCl_3$) 7.98 (s, 1H), 7.89 (d, $J = 8$ Hz, 2H), 7.65 (m, 2H), 7.54 (t, $J = 8$ Hz, 2H), 7.25 (m, 6H), 7.12 (d, $J = 7$ Hz, 1H), 4.93 (q, $J = 7$ Hz, 1H), 4.08

(d, $J = 3$ Hz, 1H), 3.83 (m, 1H), 3.33 (dd, $J = 13, 6$ Hz, 1H), 3.16 (dd, $J = 13, 7$ Hz, 1H), 2.22 (m, 1H), 1.8–1.0 (m, 8H)
(ESI) *m/z* 628 (free acid, $M-H^-$).

Derivatives

Table 1 Selected experimental integrin antagonists and their corresponding mass spectral data



Entry	Orientation	n	R_1	MS (*m/z*)
5a and 5B	*S,S* and *R,S*	1	*N*-Difluorophenyl amide	582
6, 6a, and 6b	Racemic, *S,S*, and *R,S*	1	*N*-6-Dichlorophenyl amide	628
16a and 16b	*S,S* and *R,S*	2	*N*-6-Dichlorophenyl amide	616
18a	*S,S*	2	2,6-Dimethoxyphenyl amine	592
19a and 19b	*S,S* and *R,S*	1	*N*-6-Dimethoxyphenyl amide	608
47a	*S,S*	2	4-(1,3-Dihydro-1,3-dioxo)-2H-isoindol-2-yl	588
52 and 52a	Racemic and *S,S*	2	4-(*N,N*-dimethyl)carbamate	530
53 and 53a	Racemic and *S,S*	2	(1-*t*-Butoxycarbonyl)-4-piperidinylamide	669
71 and 71a	Racemic and *S,S*	2	3,5-Dichloro-4-pyridinyl amide	540

Testing

I. *Ramos Cell Adhesion Assay ($\alpha_4\beta_1$-Mediated Adhesion/VCAM-1)*

Immulon 96-well plates were coated with 100 µl recombinant hVCAM-1 at 4.0 µg/ml in 0.05 M $NaCO_3$ buffer (pH 9.0) overnight at 4°C. Plates were washed three times in PBS with 1% BSA and blocked 1 hour at ambient temperature in

this buffer. PBS was removed and 50 μl selected experimental agents added. Ramos cells labeled with 5 μM calcein AM for 1 hour at 37°C were added to each well and allowed to adhere 1 hour at ambient temperature. Plates were washed three times in PBS + 1% BSA and cells were lyzed 15 minutes in 100 μl 1 M Tris (pH 8.0) with 1% SDS. The plate was read at 485 nm excitation and 530 nm emission. Testing results for experimental agent racemates, (*S,S*), and (*R,S*) diastereomers are provided in Tables 2–4, respectively.

II. *$\alpha_4\beta_7$–K562 Cell Adhesion Assay ($\alpha_4\beta_7$-Mediated Adhesion/VCAM-1)*

The aforementioned procedure was followed using a stable cell line of K562 cells expressing human $\alpha_4\beta_4$. Testing results for experimental agent racemates, (*S,S*), and (*R,S*) diastereomers are provided in Tables 2–4, respectively.

Table 2 Antagonist IC_{50} activity of Ramos $\alpha_4\beta_1$ and K562 $\alpha_4\beta_7$ cells to VCAM-1 using selected experimental agent racemates

Entry	$\alpha_4\beta_1$	$\alpha_4\beta_7$–K562
6	20	283
18	124	210
47	74	83
52	124	998
53	179	871
71	9	30

Table 3 Antagonist IC_{50} activity of Ramos $\alpha_4\beta_1$ and K562 $\alpha_4\beta_7$ cells to VCAM-1 using selected experimental agent (*S,S*) diastereomers

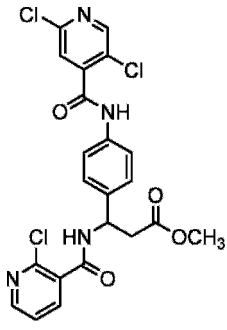
Entry	$\alpha_4\beta_1$	$\alpha_4\beta_7$-K562
5a	81	62
6a	23	217
16a	153	2090
18a	857	9000
19a	300	3822
47a	26	102
52a	45	436
53a	219	693
71a	6	33

Table 4 Antagonist IC_{50} activity of Ramos $\alpha_4\beta_1$ and K562 $\alpha_4\beta_7$ cells to VCAM-1 using selected experimental agent (*R,S*) diastereomers

Entry	$\alpha_4\beta_1$	$\alpha_4\beta_7$–K562
5b	394	3380
6b	222	421
16b	240	393
19b	1210	3220

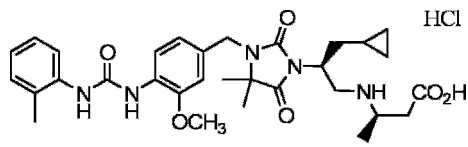
Notes

1. β-Alanine nicotinoyl derivatives, (**I**), prepared by Porter (1) were effective as $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrin agonists/antagonists and used in the treatment of immune or inflammatory disorders.



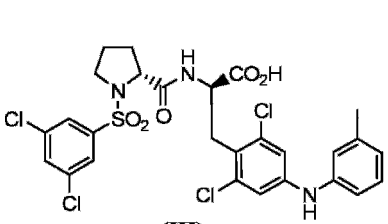
(I)

2. In an investigation by Wehner (2), imidazolidine derivatives, (**II**), were effective as integrin antagonists and used in treating rheumatoid arthritis or allergic diseases.

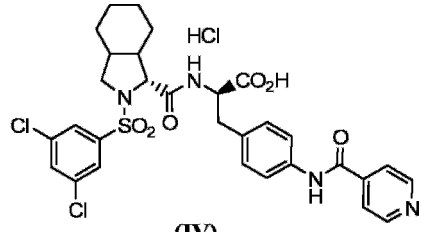


(II)

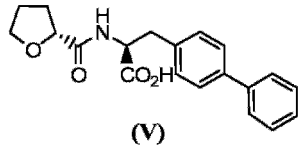
3. Doherty (3), Lin (4), and Hagmann (5) prepared $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrin antagonists consisting of *N*-arylsulfonyl-prolines, (**III**), *N*-arylsulfonyl aza-bicyclics, (**IV**), and furanic acid derivatives, (**V**), respectively, which were effective in treating inflammatory and adhesion pathologies.



(III)

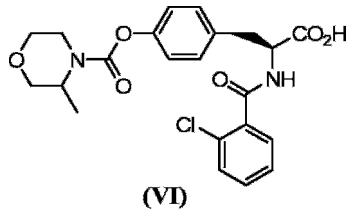


(IV)



(V)

4. Thiocarbamate derivatives, (**VI**), effective as α_4 integrin inhibitors having enhanced resistant to metabolism were prepared by Jackson (6) and used in treating diseases mediated by the binding interaction of α_4 integrin such as inflammatory disorders.



(VI)

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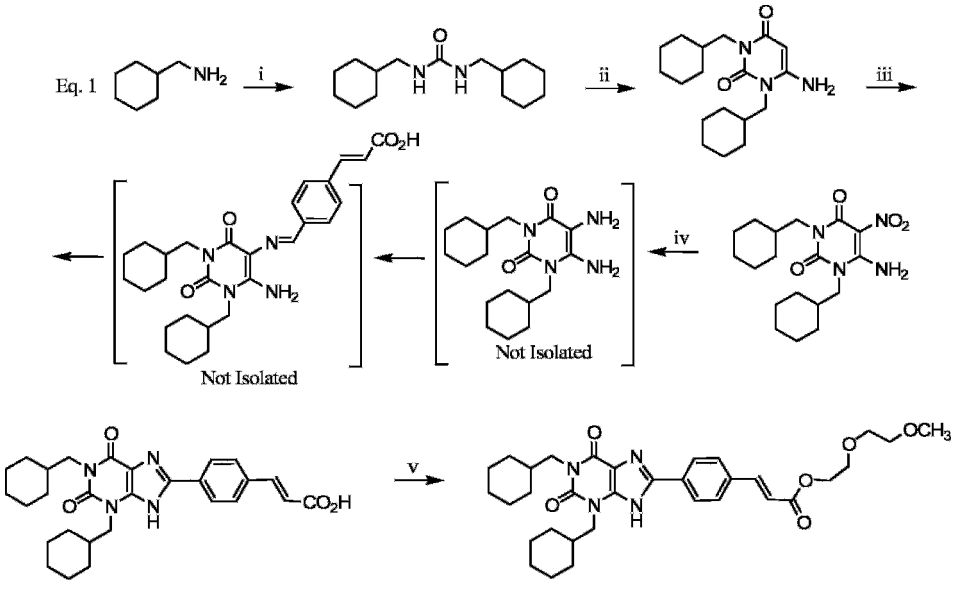
1. J.R. Porter *et al.*, US Patent 6,953,798 (October 11, 2005)
2. V. Wehner *et al.*, US Patent 6,962,937 (November 8, 2005)
3. G Doherty *et al.*, US Patent 6,943,180 (September 13, 2005)
4. L.S. Lin *et al.*, US Patent 6,855,708 (February 15, 2005)
5. W.K. Hagmann *et al.*, US Patent 6,953,798 (July 16, 2002)
6. D.Y. Jackson, US Patent 7,015,323 (March 21, 2006)

IX. Monocyte Chemoattractant Protein-1 Antagonists

Title Substituted (1,3-Bis(cyclohexylmethyl)-1,2,3,6-Tetrahydro-2,6-Dioxo-9H-Purin-8-yl-Phenyl Derivatives, Their Preparation, and Their Use in the Treatment of Inflammatory Conditions and Immune Disorders
S.M. Daluge *et al.*, US Patent 7,002,012 (February 21, 2006)
Assignee SmithKline Beecham Corporation
Utility Treatment of Respiratory Disorders

Invention Significance Adhesion of circulating leukocytes to the vascular endothelium is a crucial event in the pathogenesis of inflammatory responses. Monocyte chemoattractant protein-1 antagonists (MCP-1) have been prepared to diminish leukocyte infiltration into inflamed tissue. These oligomeric ether derivatives either block or inhibit these adhesive interactions thereby reducing or eliminating inflammatory disorders.

Reaction



i- Sodium hydroxide, water, phosgene, toluene
ii- Cyanoacetic acid, acetic anhydride
iii- Acetic acid, water, ethyl alcohol, sodium nitrite
iv- Ethyl alcohol, water, palladium on carbon, hydrogen, 4-formylcinnamic acid, methyl alcohol, dimethoxyethane, iodine, water, sodium thiosulfate
v- Triethylene glycol, monomethyl ether, xylenes

Experimental

1. **Preparation of 1,3-bis(cyclohexylmethyl)urea**

Cyclohexanemethylamine (68.66 g) and 200 ml 5 M NaOH were stirred at −10°C, then treated with phosgene (30.0 g) dissolved in 600 ml toluene, and stirred 20 minutes. A solid that formed was isolated, washed with 1500 ml water, dried, and the product isolated in 95% yield as a white powder, mp = 150−152°C.

^{1}H NMR (DMSO-d_6) δ 5.74 (br t, J = 5.8 Hz, 2, 2NH), 2.81 (t, J = 6.3 Hz, 4, 2NCH_2), 1.62, 1.25, and 0.85 (all m, 22, 2 cyclohexyl)
Analysis Calc. for $C_{15}H_{28}N_2O$: C, 71.38; H, 11.18; N, 11.10. Found: C, 71.22; H, 11.17; N, 11.15

2. **Preparation of 6-amino-1,3-bis(cyclohexylmethyl)uracil**

The Step 1 product (54.5 g) was treated with cyanoacetic acid dissolved in 260 ml acetic anhydride, then heated 2 hours to 80°C, and concentrated. The residual oil was dried by evaporation three times with 400 ml water/ethyl alcohol, 1:9, then dissolved in a mixture of 600 ml ethyl alcohol and 300 ml water at 80°C. The solution pH was raised to 10 using 10% aqueous $NaHCO_3$, then diluted with 75 ml water, and colorless crystals isolated. The crystals were washed three times with 500 ml, dried, and the product isolated in 94% yield, mp = 138−141°C.

^{1}H NMR (DMSO-d_6) δ 6.73 (br s, 2, NH_2), 4.63 (s, 1, H-5), 3.67 (d, J = 7.3 Hz, 2, NCH_2), 3.57 (d, J = 7.3 Hz, 2, NCH_2), 1.55 and 1.09 (both m, 22, 2 cyclohexyl)
Analysis Calc. for $C_{18}H_{29}N_3O_2 \bullet H_2O$: C, 64.07; H, 9.26; N, 12.45. Found: C, 63.98; H, 9.27; N, 12.48

3. **Preparation of 6-amino-1,3-bis(cyclohexylmethyl)-5-nitrosouracil**

The Step 2 product (25.0 g) was dissolved in 440 ml apiece glacial acetic acid, water, and ethyl alcohol, then heated to reflux and $NaNO_2$ (5.65 g) added. Upon slowly cooling to ambient temperature, a lavender precipitate was isolated, which was washed with water/ethyl alcohol, 1:1, dried, and the product isolated as light purple crystals in 86% yield, mp = 240−243°C.

^{1}H NMR (DMSO-d_6) δ 13.23 (br s, 1, = NOH), 9.00 (br s, 1, = NH), 3.73 (br t, J = 6.86 Hz, 4, 2 NCH_2), 2.0–1.6 and 1.7–1.1 (both m, total 22, 2 cyclohexyl)

Analysis Calc. for $C_{18}H_{28}N_4O_3$: C, 62.05; H, 8.10; N, 16.08. Found: C, 62.13; H, 8.12; N, 16.03

4. **Preparation of (*E*)-4-[1,3 bis(cyclohexylmethyl)-1,2,3,6-tetrahydro-2,6-dioxo-9H-purin-8-yl]cinnamic acid**

1,3-Bis(cyclohexylmethyl)-5,6-diaminouracil was initially prepared by shaking the Step 3 product (5.00 g) in a mixture of 250 ml methyl alcohol and 25 ml water with 10% palladium on carbon (0.50 g) under 50 psi hydrogen for 2 hours in a Parr shaker. The mixture was filtered, then concentrated to 25 ml. 4-Formylcinnamic acid (14.35 mmol) was then added and the yellow mixture concentrated and then dried by evaporation using 100% absolute ethyl alcohol.

The above intermediate was stirred in 115 ml dimethoxyethane and iodine (4.0 g) for 20 hours at 60°C, then treated with saturated $Na_2S_2O_3$ solution, and an yellow solid isolated. The solid was washed with water, dried, then purified by dissolving in 1 M NaOH and filtering through celite. It was acidified using HCl and the product isolated in 91% yield as a pale yellow powder, mp > 300°C.

^{1}H NMR (DMSO-d_6) δ 13.80 and 12.40 (both br m, 1 each, CO_2H and NH), 8.12 (d, $J = 8.3$ Hz, 2, 2 phenyl CH), 7.84 (d, $J = 8.4$ Hz, 2, 2 phenyl CH), 7.64 (d, $J = 16.0$ Hz, 1, CH=), 6.64 (d, $J = 16.0$ Hz, 1, CH=), 3.93 (d, $J = 7.0$ Hz, 2, CH_2N), 3.79 (d, $J = 6.8$ Hz, 2, CH_2N), 2.0–1.4 and 1.3–0.85 (both br m, 22 total, 2 cyclohexyl)
Analysis Calc. for $C_{28}H_{34}N_4O_4$: C, 68.55; H, 6.99; N, 11.42. Found: C, 68.45; H, 6.98; N, 11.48

5. **Preparation of (*E*)-4-(1,3-bis(cyclohexylmethyl)-1,2,3,6-tetrahydro-2,6-dioxo-9H-purin-8-yl)cinnamic acid triethylene glycol methyl ether ester**

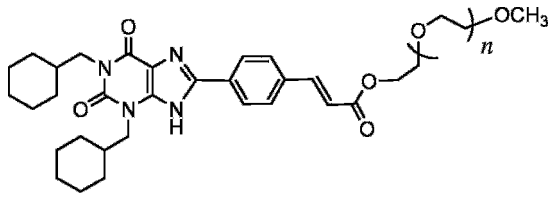
Triethylene glycol, monomethyl ether (80.0 g) was dried at 125°C three times using 50 ml xylenes, then treated with the Step 4 product (4.00 g), and the mixture further dried using 40 ml xylenes. Sulfuric acid (0.41 g) was added and the mixture was heated 2 hours to 190°C where 50 ml portions of xylenes were continuously added to remove water. Additional sulfuric acid (0.2 g) was added after 2 hours and the mixture further heated 3 hours at 140°C, while xylenes were continuously replaced. The reaction was then cooled to ambient temperature and filtered. The filtrate was diluted with 200 ml $CHCl_3$, washed four times with 50 ml water, dried using Na_2SO_4, and concentrated. The product was purified by chromatography with silica gel using 1–4% methyl alcohol/EtOAc, then recrystallized from EtOAc by the addition of hexanes, and the product isolated in 62% yield white powder, mp = 189−192°C.

^{1}H NMR (DMSO-d_6) δ 8.16 (d, $J = 8.0$ Hz, 2, 2 phenyl CH), 7.88 (d, $J = 8.3$ Hz, 2, 2 phenyl CH), 7.70 (d, $J = 16.1$ Hz, 1, CH =), 6.77 (d, $J = 16.1$ Hz, 1, CH =), 4.28 (m, 2, CO_2CH_2), 3.92 (d, $J = 6.8$ Hz, 2, CH_2N), 3.78 (d, $J = 6.8$ Hz, 2, CH_2N), 3.68 (m, 2, CH_2O), 3.6–3.5 (m, 6, 3 CH_2O), 3.40 (2, CH_2O), 3.23 (s, 3, CH_3), 2.0–1.5 and 1.3–0.9 (both br m, 22 total, 2 cyclohexyl)

Analysis Calc. for $C_{35}H_{48}N_4O_7$: C, 66.02; H, 7.60; N, 8.80. Found: C, 65.91; H, 7.58; N. 8.76

Derivatives

Table 1 Melting points of selected oligomeric methyl ether derivatives and Step 5 product yield. ^{1}H NMR and elemental analysis for products and intermediates supplied by author



Entry	*n* (ave.)	Yield (%)	mp (°C)
3	7.2	91	147–154
4	4	–	171–174
9	10	47	143–145
10	9	74	143–145
18	13	64	142–143

Testing

I. *Carrageenan Pleurisy Assay*

The anti-inflammatory activity of experimental agents was determined using the method of Vinegar (1) using a carrageenan dose of 0.075 mg/rat and harvesting pleural exudate 4 hours afterwards. Testing results are provided in Table 2.

II. *Acetic Acid Colitis Assay*

Anti-inflammatory testing was performed using the acetic acid colitis rat model described by Fretland (2). Experimental agents were administered 24, 16, and 4 hours prior to the 40 second instillation of 3% acetic acid solution in the proximal 6 cm of the colon under light anesthesia. The colon was immediately washed with 5 ml saline and neutrophil inflammation determined by measuring MPO levels in the scraped colonic mucosa. Testing results are provided in Table 2.

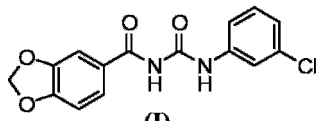
Table 2 Anti-inflammatory effectiveness of selected experimental agents determined using carrageenan pleurisy and acetic acid colitis assays

Entry	PEG Terminus	*n* (ave.)	Carrageenan Pleurisy (4 hours)		Acetic Acid Colitis (24 hours)	
			ED_{50} Cells (mg/rat)	ED_{50} Edema (mg/rat)	MPO	Tissue Weight
Reference[a]	n/a	n/a	0.02	0.015	0.03[b]	0.17[b]
9	CH_3	10	0.02	0.2	5.0	5.0
10	CH_3	9	0.5	0.5	50[c]	50[c]
14	CH_3	41.5	Inactive	0.5	NT	NT
15	CH_3	15, 16	0.4	0.2	50[c]	Inactive
16	OH	32.2	0.2	0.2	NT[d]	NT
17	OH	18.9	0.1	0.1	NT	NT
18	OH	13	0.1	0.1	NT	NT

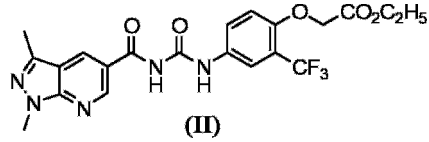
[a] Dexamethasone, 9-fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione.
[b] Intracolonic administration.
[c] Oral administration.
[d] Not tested.

Notes

1. Acyl urea derivatives, (**I**) and (**II**), prepared by Laborde (3,4), respectively, were effective as MCP-1 antagonists and used in the treatment of chronic or acute inflammatory or autoimmune diseases associated with aberrant lymphocyte or monocyte accumulation such as arthritis, asthma, and atherosclerosis.

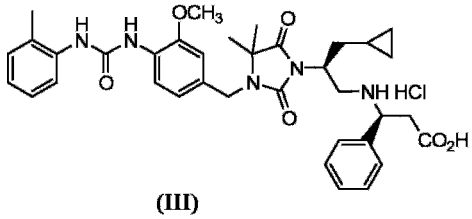


(I)



(II)

2. Urea- and L-aspartyl-L-glycine imidazolidine derivatives, (**III**) and (**IV**), prepared by Wehner (5,6), respectively, were effective as leukocytes inhibitors and used in treating rheumatoid arthritis and allergic diseases.



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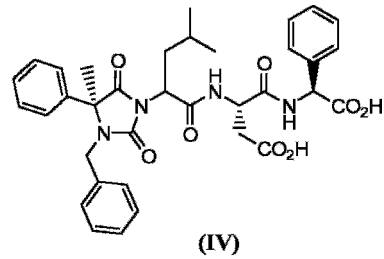
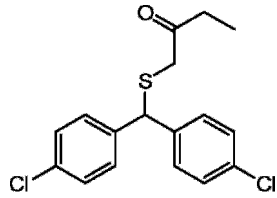


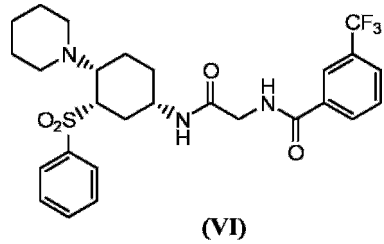
(IV)

3. MCP-1 receptor antagonists consisting of bis-(4-chloro-phenyl)-methanesulfonyl derivatives, (**V**), were prepared by Bratton (7) and used in the treatment of inflammatory bowel disease, rheumatoid arthritis, and atherosclerosis.



(V)

4. Trifluoromethyl benzamide derivatives, (**VI**), prepared by Cherney were effective as MCP-1 modulators and useful in treating inflammation disorders such as rheumatoid arthritis, asthma, and restinosis.



(VI)

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2. D. Fretland *et al.*, Proc. Soc. Exp. Biol. Med., 255, 572 (1990)
3. E. Laborde *et al.*, US Patent 6,998,407 (February 14, 2006)
4. E. Laborde *et al.*, US Patent 6,992,086 (January 31, 2006)
5. V. Wehner *et al.*, US Patent 6,962,937 (November 8, 2005)
6. V. Wehner *et al.*, US Patent 6,423,712 (July 23, 2002)
7. L.D. Bratton *et al.*, US Patent 7,067,538 (June 27, 2006)
8. R.J. Cherney, US Patent 7,087,604 (August 8, 2006)

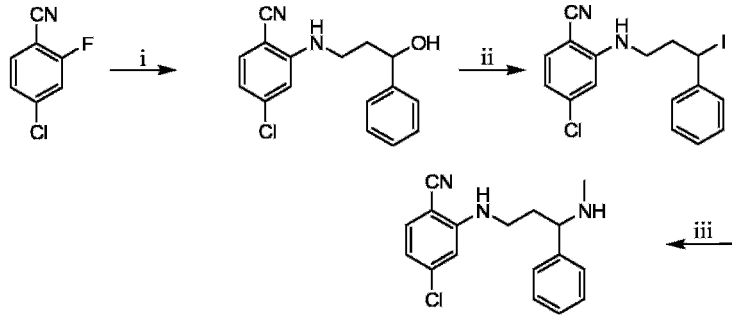
X. Nitric Oxide Synthase Inhibitors

Title Use of Phenylheteroalkylamine Derivatives
D. Cheshire *et al.*, US Patent 6,953,797 (October 11, 2005)
Assignee AstraZeneca AB
Utility Treatment of Rheumatoid Arthritis

Invention Significance The endothelial constitutive nitric oxide synthase (ecNOS) is involved with smooth muscle relaxation and the regulation of blood pressure and blood flow. Unregulated production of ecNOS, however, is implicated in the pathogenesis of inflammatory diseases including rheumatoid arthritis, osteoarthritis, and inflammatory bowel disease. Nitric oxide synthase inhibitors have been prepared that are particularly useful in the treatment or prophylaxis of these inflammatory diseases.

Reaction

Eq. 1



i- 3-Amino-3-phenyl-1-propanol, *N,N*-diisopropylethylamine
ii- Triphenylphosphine, THF, diethyl azodicarboxylate, lithium iodide
iii- Methylamine, methyl alcohol

Experimental

1. **Preparation of 4-chloro-2-[(3-hydroxy-1-phenylpropyl)amino]-benzonitrile**

A mixture consisting of 3-amino-3-phenyl-1-propanol (6.6 mmol), 4-chloro-2-fluorobenzonitrile (6.4 mmol) and 1.2 ml *N,N*-diisopropylethylamine was heated 5 hours at 140°C then cooled. The material was purified by chromatography with

silica gel using diethyl ether/isohexane, 1:4, and the product isolated in 58% yield as a colorless solid, mp = 88−90°C.

^{1}H NMR (300 MHz, d_6-DMSO) 7.5–7.2 (6H, m), 7.05 (1H, d), 6.63 (1H, dd), 6.51 (1H, d), 4.9 (1H, t), 4.73 (1H, q), 3.49 (2H, q), 2.1–1.88 (2H, m)
MS APCI = 287$[M+H]^+$

2. **Preparation of 4-chloro-2-[(3-iodo-1-phenylpropyl)amino]-benzonitrile**

A solution of triphenylphosphine (6.98 mmol) in 30 ml THF at 0°C was treated with diethyl azodicarboxylate (6.9 mmol) followed by treatment with lithium iodide and the Step 1 product (2.79 mmol) 20 minutes thereafter. The mixture was stirred 5 hours and then concentrated. The residue was purified by chromatography using diethyl ether/isohexane, 1:4, and the product isolated in 32% yield as a colorless solid.

^{1}H NMR (400 MHz, $CDCl_3$) 7.41–7.28 (6H, m), 6.65 (1H, d), 6.5 (1H, d), 4.94 (1H, br d), 4.6 (1H, q), 3.28–3.23 (1H, m), 3.1–3.04 (1H, m), 2.43–2.26 (2H, m)

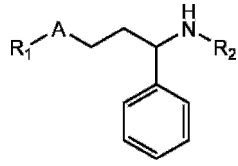
3. **Preparation of 4-chloro-2-[3-(methylamino)-1-(phenylpropyl)amino]-benzonitrile**

A solution of 3 ml methylamine in 20 ml methyl alcohol was treated with the Step 2 product (0.88 mmol), then stirred 20 hours at ambient temperature, and concentrated. The residue was purified by chromatography using 7 M methanolic ammonia/CH_2Cl_2 and the product was isolated in 64% yield as a pale pink-colored solid, mp = 119−120°C.

^{1}H NMR (300 MHz, $CDCl_3$) 8.22 (1H, d), 7.37–7.24 (6H, m), 6.52 (1H, dd), 6.27 (1H, d), 4.58 (1H, q), 2.8–2.66 (2H, m), 2.48 (3H, s), 2.14–2.05 (1H, m), 1.89–1.8 (1H, m)
MS APCI = 300/302 $[M+H]^+$

Derivatives

Table 1 Selected phenylheteroalkylamine derivatives and their corresponding mass spectral characterization data. All experimental agents were effective as ecNOS inhibitors



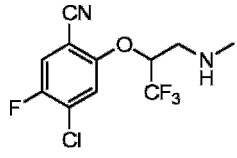
Entry	R_1	R_2	A	Salt	MS (M + 1)
1	3,5-Dichlorophenyl	Hydrogen	S	Fumarate	326
5	4-Bromo-1-cyanophenyl	Methyl	S	Oxalate	363
6	3,5-Dichlorophenyl	Methyl	SO_2	Trifluoroacetate	358
10	5-Chloro-2-nitrophenyl	Morpholn-1-yl	CH_2	Fumarate	376

Testing

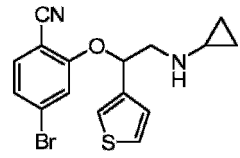
The activity of the chemical agents as nitric oxide synthase inhibiting agents was determined using the method of Förstermann (1). Although quantitative testing not supplied by author, all experimental agents appearing in Table 1 had IC_{50} values less than 15 μM.

Notes

1. In earlier investigations by the authors (2,3), alkoxy, (**I**), and heterocyclic derivatives, (**II**), respectively, were prepared, which were effective as nitric oxide synthase inhibitors.

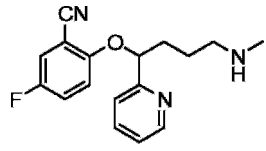


(I)



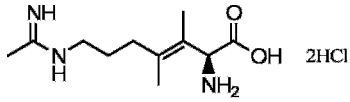
(II)

2. 2-Butoxy-benzonitrile derivatives prepared by Birkinshaw (4), (**III**), were effective as nitric oxide synthase inhibitors and used in the treatment of smooth muscle relaxation disorders and in the regulation of blood pressure and blood flow.



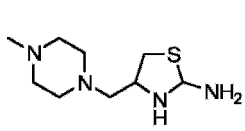
(III)

3. Amidine derivatives, (**IV**), prepared by Pitzele (5) were effective as nitric oxide synthase inhibitors and used in the treatment of hypertension and thrombosis.

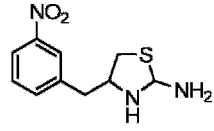


(IV)

4. 2-Aminothiazolines, (**V**), prepared by Bigot (6) and aminothiazoline aromatic derivatives, (**VI**), prepared by Carry (7) were effective as inhibitors of inducible nitric oxide synthase and used in the treatment of inflammatory pathologies such as arthritis, inflammatory bowel disorder, and asthma.

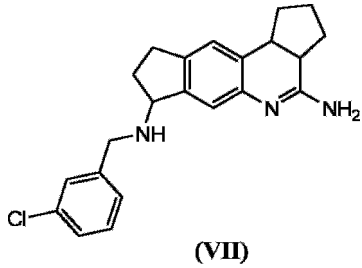


(V)



(VI)

5. Aminoalkyl-3, 4-dihydroquinoline derivatives, (**VII**), prepared by Jaroch (8) were effective as nitric oxide synthase inhibitors and used as anti-inflammatory agents.



(VII)

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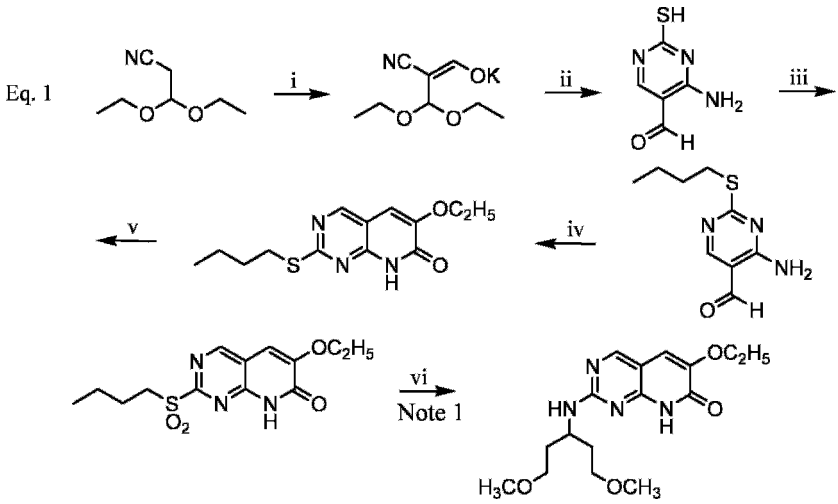
2. D. Cheshire *et al.*, US Patent 6,900,243 (May 31, 2005)
3. D. Cheshire *et al.*, US Patent 6,887,871 (May 3, 2005)
4. T. Birkinshaw *et al.*, US Patent 6,743,939 (June 1, 2004)
5. B.S. Pitzele *et al.*, US Patent 6,956,131 (October 18, 2005)
6. A. Bigot *et al.*, US Patent 6,953,796 (October 11, 2005)
7. J.-C. Carry *et al.*, US Patent 6,699,895 (March 2, 2004)
8. S. Jaroch *et al.*, US Patent 7,067,667 (June 27, 2006)

XI. P38 Protein Kinase Inhibitors

Title 6-Alkoxy-Pyrido-Pyridine
D.M. Goldstein *et al.*, US Patent 6,965,030 (November 15, 2005)
Assignee Roche Palo Alto LLC
Utility Treatment of Inflammation Diseases

Invention Significance Chemical agents, which are selective inhibitors against in vivo p38 kinase phosphorylation and able to mediate the production of inflammatory cytokines including TNF-α, IL-1, and cyclooxygenase-II, have been prepared. These agents address the need for additional treatment options for inflammation disorders while minimizing drug-induced side effects.

Reaction



i- Methyl formate, THF, potassium *t*-butoxide
ii- Thiourea, ethyl alcohol, sodium methoxide, methyl alcohol
iii- Potassium carbonate, acetone, 1-iodobutane
iv- Ethyl ethoxyacetate, toluene, potassium *t*-butoxide
v- CH_2Cl_2, 3-chloroperbenzoic acid
vi- 3-Methoxy-1-(2-methoxyethyl)propylamine, CH_2Cl_2

Experimental

1. **Preparation of 3,3-diethoxy-2-formylpropionitrile potassium**

   A mixture of 3,3-diethoxypropanenitrile (1.98 mol) and methyl formate (2.48 mol) in 1100 ml THF at 10°C was treated with 1.0 M potassium *t*-butoxide in THF (2.2 mol) over 45 minutes and the slurry stirred 2 hours at ambient temperature. The mixture was diluted with 400 ml hexane and stirred an additional 20 minutes and was then filtered. The filter cake was washed with hexanes/THF, 1:1, then dried, and the product isolated in 73.0% as a pale tan powder.

2. **Preparation of 4-amino-2-sulfanylpyrimidine-5-carbaldehyde**

   A slurry of thiourea (1.22 mol) in 90 ml ethyl alcohol was refluxed and then treated with a suspension of the Step 1 product (1.06 mol) in 85.5 ml 25% sodium methoxide in methyl alcohol and 285 ml ethyl alcohol in five aliquots over 10 minutes. An additional 150 ml ethyl alcohol was added to facilitate stirring and the mixture refluxed an additional hour. The mixture was cooled, concentrated, and the residue dissolved in 940 ml water. The crude product was precipitated by adding 280 ml 30% acetic acid and was then isolated. The filter cake was washed with 800 ml water and purified by trituration in1000 ml hot water for 30 minutes. The mixture was filtered and the product isolated in 72.3% yield as a bright yellow solid with an HPLC purity of 98.67%.

3. **Preparation of 4-amino-2-*n*-butyllthiopyrimidine-5-carbaldehyde**

   1-Iodobutane (902.2 mmol) was added dropwise over 20 minutes to a cooled solution of the Step 2 product (644.4 mmol) in 1500 ml acetone containing 325 mesh K_2CO_3 (1.29 mol) and then stirred 72 hours at ambient temperature. An additional 8 ml of 1-iodobutane was added and stirring continued 24 hours. The mixture was then concentrated and the residue diluted with 1000 ml water and then filtered. The solid was washed with 200 ml water and the product isolated in 94.8% yield with an HPLC purity of 95.8%.

4. **Preparation of 2-butylsulfanyl-6-ethoxy-8,8a-dihydro-4aHpyrido[2,3-d]pyrimidin-7-one**

   The Step 3 product (14.2 mmol) and ethyl ethoxyacetate (17.75 mmol) were stirred in 80 ml toluene at 0−5°C, then slowly treated with potassium *t*-butoxide (15.6 mmol), and then stirred 48 hours at 65°C. The mixture was further treated with 20 ml toluene and 2.4 ml ethyl ethoxy acetate and then stirred an additional 48 hours at 65°C. The solution was then concentrated and triturated with EtOAc to remove unreacted Step 3 reagent. The remaining solid was further triturated with $CHCl_3$ and 3.66 g product isolated with an HPLC purity of 80%.

5. **Preparation of 2-(butane-1-sulfonyl)-6-ethoxy-8H-pyrido[2,3-d]pyrimidin-7-one**

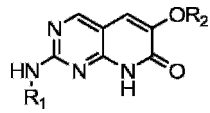
A solution of the Step 4 product (10.7 mmol) suspended in 40 ml CH_2Cl_2 was cooled to 0−5°C, then treated with the slow addition of 3-chloroperbenzoic acid (32.3 mmol), and stirred overnight at ambient temperature. The mixture was concentrated, the residue triturated with EtOAc, then purified by chromatography using CH_2Cl_2/methyl alcohol/acetone, 96:2:2, and 1 g product isolated.

6. **Preparation of 6-ethoxy-2[3-methoxy-1(2-methoxy-ethyl)-propylamino]-8,8a-dihydro-4aH-pyrido[2,3-d]pyrimidin-7-one**

A solution of the Step 5 product (0.16 mmol) and 3-methoxy-1-(2-methoxyethyl)propylamine (0.96 mmol) in 1 ml CH_2Cl_2 was heated 72 hours at 85°C then chromatographed directly using a Supelco™ 2 g/12 ml silica column with gradient solvent of CH_2Cl_2 to a final solvent mixture of CH_2Cl_2/methyl alcohol/acetone, 94:3:3. Two additional chromatographies were required to provide 24 mg product with an HPLC purity of 86%.

Derivatives

Table 1 Selected 6-alkoxy-pyrido[2,3-d]pyrimidin-7-one experimental derivatives and their corresponding salt product isolation. Entry 6 was especially preferred as a TNF-α, IL-1, and COX-II inhibitor



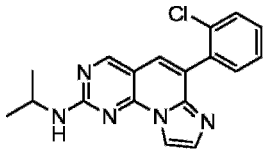
Entry	R_1	R_2	Isolated Product (mg) (HCl salt)
2	Tetrahydropyran-4-yl	Methyl	44
3	1-(Ethoxycarbonyl)-piperidin-4-yl	Ethyl	2.14
4	Piperidin-4-yl	Ethyl	4
5	1-(Ethoxycarbonyl)-piperidin-4-yl	2,6-Difluorophenyl	24
6	1-(Methanesulfonyl)-piperidin-4-yl	Ethyl	15

Testing

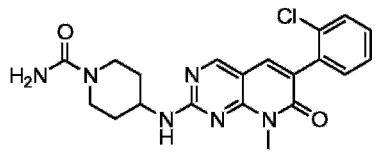
Although testing results were not supplied by author, the Step 6 product was especially preferred.

Notes

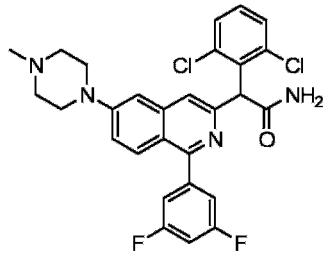
1. In investigations by the author (1), Chen (2), and Cochran (3), imidazo-, (**I**), pyrido[2,3-d]pyrimidin-7-ones, (**II**), and isoquinoline derivatives, (**III**), respectively, were prepared, which were effective in treating p38 kinase-mediated disorders by modulating IL-1, IL-6, and IL-8 and TNF-α. These agents were used in treating pathophysiological disorders such as rheumatoid arthritis, fever, and reduction of bone resorption.



(I)

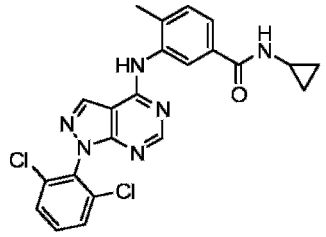


(II)

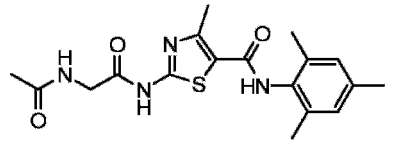


(III)

2. Pyrazolo-pyrimidine anilines, (**IV**), and thiazolecarboxamide derivatives, (**V**), prepared by Das (4,5), respectively, mediated the production of inflammatory cytokines including TNF, IL-1, IL-6, and COX-II and were used in treating inflammatory bowel disorder.

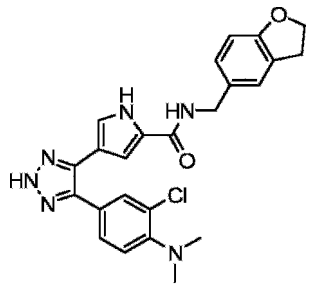


(IV)



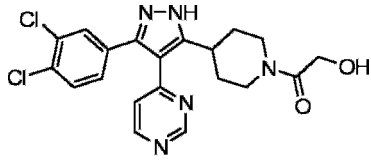
(V)

3. Hale (6) prepared triazole-containing kinase inhibitors, (**VI**), which were effective as p38 protein kinase inhibitors and used in treating immunomodulator diseases such as inflammatory disorders, restenosis, and cardiovascular disease.



(VI)

4. Pyrazole derivatives, (**VII**), prepared by Allen (7), were effective as p38 inhibitors and used in treating rheumatoid arthritis.



(VII)

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2. J.J. Chen *et al.*, US Patent 6,943,158 (September 13, 2005) and US Patent 6,861,423 (March 1, 2005)
3. J. Cochran *et al.*, US Patent 6,962,996 (November 8, 2005) and US Patent 6,759,535 (July 6, 2004)
4. J. Das *et al.*, US Patent 6,962,915 (November 8, 2005)
5. J. Das *et al.*, US Patent 6,979,694 (December 27, 2005)
6. M.R. Hale *et al.*, US Patent 6,962,936 (November 8, 2005)
7. K.K. Allen *et al.*, US Patent 6,897,318 (May 24, 2005)

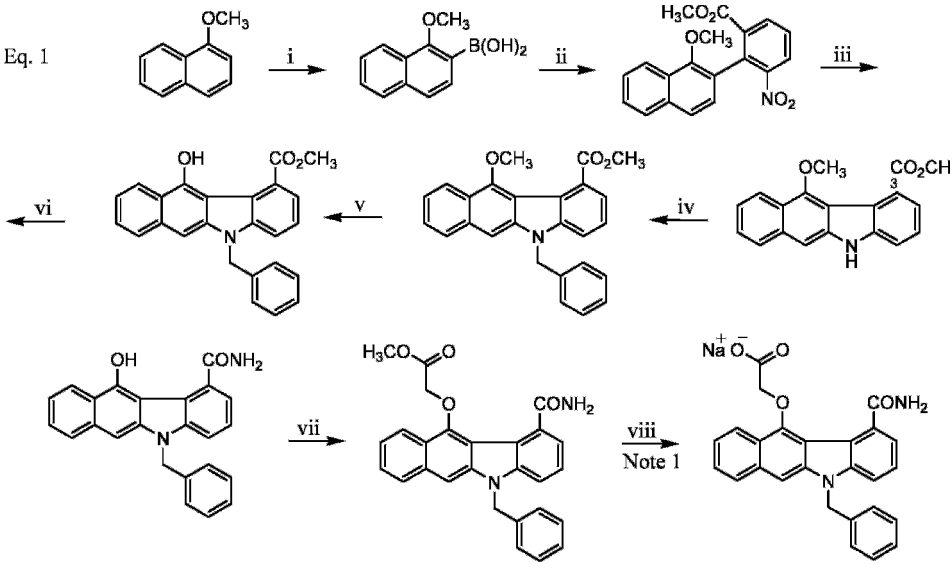
XII. Secretory Phospholipase A_2 Inhibitors

A. *N*-Benzyl Tetracyclic-based Medicaments

Title $sPLA_2$ Inhibitors
D.W. Beight *et al.*, US Patent 6,992,100 (January 31, 2006)
Assignee Eli Lilly and Company
Utility Treatment or Prevention of Sepsis

Invention Significance Human nonpancreatic secretory phospholipase-A_2 ($sPLA_2$) is a rate-limiting enzyme in the arachidonic acid cascade. Overproduction of $sPLA_2$, however, converts arachidonic acid into a variety of proinflammatory eicosanoids. *N*-Benzyl tetracyclic agents have been prepared for inhibiting $sPLA_2$-mediated fatty acid release for the treatment of septic shock and related inflammatory disorders.

Reaction



i- THF, *n*-butyllithium, triisopropyl borate
ii- 2-Chloro-3-nitrobenzoic acid methyl ester, tetrakis(triphenylphosphine) palladium, sodium carbonate, water, THF

iii- Triphenyl phosphite
iv- DMF, sodium hydride, benzyl bromide
v- Boron tribromide, CH_2Cl_2
vi- THF, ammonia (l)
vii- Benzyltrimethylammonium hydroxide, methyl alcohol, methyl bromoacetate, cesium carbonate
viii- Ethyl alcohol, sodium hydroxide

Experimental

1. **Preparation of 1-methoxynaphthalene-2-boronic acid**

A solution of 1-methoxynaphthalene (126.6 mmol) dissolved in 100 ml THF was treated with *n*-butyllithium (132.9 mmol) over 5 minutes at ambient temperature, then cooled to −50°C, and triisopropyl borate (190 mmol) added dropwise over 15 minutes. The resulting solid was treated with 100 ml THF, then warmed to 0°C, and poured into 100 ml 1 M HCl. The mixture was extracted with EtOAc, then dried using Na_2SO_4, concentrated, and the residue partially dissolved in diethyl ether. It was then triturated with hexane, washed with hexane, and the product isolated in 41% yield as an off-white crystalline solid.

^{1}H NMR (DMSO-d_6) δ 8.08–8.06 (m, 1H), 7.87–7.84 (m, 1H), 7.56 (d, 1H, $J = 8$ Hz), 7.50–7.46 (m, 3H), 6.51 (br s, 2H), and 3.91 (s, 3H)

2. **Preparation of 2-(1-methoxynaphthalen-2-yl)-3-nitrobenzoic acid, methyl ester**

A mixture consisting of the Step 1 product (11.0 mmol), 2-chloro-3-nitrobenzoic acid methyl ester (10.0 mmol), tetrakis(triphenylphosphine) palladium(0.5 mmol), and 2 M Na_2CO_3 solution (21.0 mmol) dissolved in 50 ml THF was refluxed 31 hours in the dark. The mixture was then cooled to ambient temperature and concentrated. The aqueous residue was dissolved in a mixture of EtOAc and brine, then separated, and extracted four times with 25 ml EtOAc. Combined extracts were washed with water, 1 M HCl, saturated $NaHCO_3$ solution and saturated brine, dried, and concentrated. The oily residue was purified by chromatography with silica gel using $CHCl_{3/toluene}$, 1:1, and the product isolated in 75% yield as an yellow solid, mp = 139−141°C, MW = 337.34.

^{1}H NMR ($CDCl_3$) δ 8.13–8.11 (m, 1H), 8.08 (d, 1H, $J = 9$ Hz), 8.00 (d, 1H, $J = 9$ Hz), 7.86–7.84 (m, 1H), 7.64 (d, 1H, $J = 9$ Hz), 7.60 (d, 1H, $J = 8$ Hz), 7.52–7.49 (m, 2H), 7.21 (d, 1H, $J = 8$ Hz), 3.61 (s, 3H), and 3.53 (s, 3H)
IR (KBr, cm^{-1}) 3020–2830 (multiple peak grouping), 1737, 1531, 1369, 1277, 1109, and 757
MS (ESI) *m/z* 306, 338, 360
Analysis Calc. for $C_{19}H_{15}NO_5$: C, 67.65; H, 4.48; N, 4.15. Found: C, 65.77; H, 4.31; N, 4.00

3. **Preparation of 11-methoxy-5H-benzo[b]carbazole-1-carboxylic acid, methyl ester**

   A solution of the Step 2 product (4.5 mmol) dissolved in triphenyl phosphite (22.5 mmol) was heated 19 hours at 160°C, then cooled to ambient temperature, and azeotropically dried with toluene. The material was purified by chromatography using a hexane/EtOAc elution gradient and the product isolated in 27% as an yellow solid, mp = 190−196°C.

   ^{1}H NMR ($CDCl_3$) δ 8.27 (d, 1H, $J = 9$ Hz), 8.08 (br s, 1H), 7.89 (d, 1H, $J = 9$ Hz), 7.54 (s, 1H), 7.50–7.45 (m, 3H), 7.41 (d, 1H, $J = 7$ Hz), 7.32 (d, 1H, $J = 8$ Hz), 4.03 (s, 3H), and 3.91 (s, 3H)
   IR ($CHCl_3$, cm^{-1}) 3469, 3030–2850 (multiple peak grouping), 1725, 1641, 1606, 1433, 1400, 1343, 1303, 1288, 1170, 1141, and 1090
   MS (ESI) *m/z* 274, 304, and 306
   Analysis Calc. for $C_{19}H_{15}NO_3$: C, 74.74; H, 4.95; N, 4.59. Found: C, 74.02; H, 5.02; N, 4.17

4. **Preparation of 5-benzyl-11-methoxy-5H-benzo[b]carbazole-1-carboxylic acid, methyl ester**

   A solution of the Step 3 product (1.5 mmol) dissolved in 5 ml DMF was treated with NaH (3.0 mmol) at ambient temperature followed by benzyl bromide (1.65 mmol) and the mixture stirred 4 hours. The mixture was diluted with EtOAc and water, solvent layers separated, and the aqueous layer extracted four times with 25 ml EtOAc. Combined extracts were washed with water, 1 M HCl, saturated $NaHCO_3$ solution and saturated brine, dried, concentrated, and triturated with hexanes. The yellow-orange gum was purified by chromatography using toluene/EtOAc, 9:1, and the product isolated in 86% yield as an yellow-orange foam.

   ^{1}H NMR ($CDCl_3$) δ 8.28 (d, 1H, $J = 9$ Hz), 7.88 (d, 1H, $J = 9$ Hz), 7.51–7.17 (m, 11H), 5.55 (s, 2H), 4.04 (s, 3H), and 3.94 (s, 3H)
   IR ($CHCl_3$, cm^{-1}) 3100–2850 (multiple peak grouping), 1725, 1634, 1596, 1453, 1346, 1310, 1287, 1171, and 1114
   MS (ESI) *m/z* 364, 396. **MS** (FD) *m/z* 395
   Analysis Calc. for $C_{26}H_{21}NO_3$: C, 78.97; H, 5.35; N, 3.54. Found: C, 78.38; H, 5.27; N, 3.53

5. **Preparation of 5-benzyl-11-hydroxy-5H-benzo[b]carbazole-1-carboxylic acid, methyl ester**

   Boron tribromide (1.56 mmol) was slowly added to a solution of the Step 4 product (1.2 mmol) dissolved in 8 ml CH_2Cl_2 at −10°C and the mixture stirred 90 minutes. It was quenched with 30 ml methyl alcohol, then warmed to ambient temperature, and stirred an additional 2 hours. The mixture was diluted with CH_2Cl_2 and washed with water, 1 M HCl, saturated $NaHCO_3$ solution, and saturated brine. The solution was dried, then concentrated, and a solid orange residue isolated. The residue was

purified by chromatography using a hexane/EtOAc elution gradient and the product was isolated in 47% yield as an orange foam.

^{1}H NMR ($CDCl_3$) δ 11.14 (br s, 1H), 8.60 (d, 1H, $J = 8$ Hz), 7.94 (br s, 1H), 7.75 (br s, 1H), 7.51–7.41 (m, 2H), 7.37–7.33 (t, 1H, $J = 7$ Hz), 7.26–7.21 (m, 5H), 7.12 (d, 2H, $J = 6$ Hz), 5.55 (s, 2H), and 4.15 (s, 3H)
IR (KBr, cm^{-1}) 3420 (br), 3050–2940 (multiple peak grouping), 1727, 1652, 1628, 1452, 1438, 1279, 1197, 1179, and 747
MS (ESI) *m/z* 350, 364, 380, and 382
Analysis Calc. for $C_{25}H_{19}NO_3$: C, 78.72; H, 5.02; N, 3.67. Found: C, 77.43; H, 4.78; N, 3.55

6. **Preparation of 5-benzyl-11-hydroxy-5H-benzo[b]carbazole-1-carboxylic acid amide**

A solution of the Step 5 product (0.60 mmol) dissolved in 8 ml THF was placed into a 203 mm × 38 mm pressure tube containing a small stirring bar, then cooled to −78°C, and treated with 8 ml NH_3 and sealed. The mixture was stirred 10 minutes at −78°C and 48 hours at ambient temperature. Excess NH_3 was released, the mixture concentrated, and the product isolated in 99% as an yellowish-green solid.

^{1}H NMR (DMSO-d_6) δ 11.52 (s, 1H), 9.08 (br s, 1H), 8.63 (br s, 1H), 8.37 (d, 1H, $J = 8$ Hz), 7.80 (d, 2H, $J = 8$ Hz), 7.73 (d, 1H, $J = 6$ Hz), 7.53–7.42 (m, 5H), 7.29–7.11 (m, 4H), and 5.69 (s, 2H)
IR (KBr, cm^{-1}) 3480–2920 (multiple peak grouping), 1725, 1658, 1645, 1631, 1594, 1578, 1440, 1295, and 749
MS (ESI) *m/z* 350, 365, and 367
Analysis Calc. for $C_{24}H_{18}N_2O_2$: C, 78.67; H, 4.95; N, 7.65. Found: C, 76.10; H, 5.17; N, 6.20

7. **Preparation of (5-benzyl-1-carbamoyl-5H-benzo[b]carbazol-11-yloxy)-acetic acid, methyl ester**

A solution of 40% aqueous benzyltrimethylammonium hydroxide (0.832 mmol) was added to a solution of the Step 6 product (0.64 mmol) dissolved in 5 ml DMF and treated with methyl bromoacetate (1.28 mmol), then stirred 2 hours at ambient temperature. Cesium carbonate (0.32 mmol) was then added as a solid and the mixture stirred an additional hour. The mixture was then diluted with EtOAc and washed with water, 1 M HCl, saturated $NaHCO_3$ solution, and saturated brine. It was dried using $MgSO_4$, concentrated, purified by chromatography using EtOAc, and the product isolated in 53% as a brown foam, mp = 120−125°C.

^{1}H NMR ($CDCl_3$) δ 8.30 (d, 1H, $J = 9$ Hz), 8.05 (d, 1H, $J = 9$ Hz), 7.51–7.46 (m, 3H), 7.36–7.26 (m, 7H), 7.18 (d, 1H, $J = 8$ Hz), 5.91 (br s, 2H), 5.81 (s, 2H), 4.72 (s, 2H), and 3.72 (s, 3H)
IR (KBr, cm^{-1}) 3430, 3330, 3150–2830 (multiple peak grouping), 1735, 1659, 1595, 1436, 1393, 1213, 1158, and 750

MS (ESI) *m/z* 422, 439. **MS** (FD) *m/z* 438
Analysis Calc. for $C_{27}H_{22}N_2O_4$: C, 73.96; H, 5.06; N, 6.39. Found: C, 70.52; H, 5.39; N, 5.42

8. **Preparation of (5-benzyl-1-carbamoyl-5H-benzo[b]carbazol-11-yloxy)-acetic acid, sodium salt**

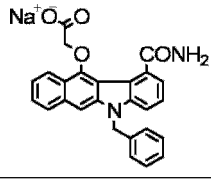
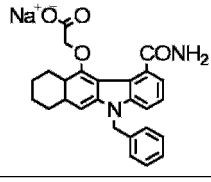
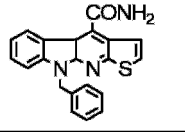
The Step 7 product (0.2 mmol) and 1 M NaOH (0.22 nM) dissolved in 5 ml ethyl alcohol was stirred 3.5 hours at 25°C and then diluted with diethyl ether/hexanes. The mixture was cooled and a precipitate isolated. The precipitate was washed with ethyl alcohol/diethyl ether/hexanes, dried, and the product isolated in 73% yield as a brown solid.

^{1}H NMR (DMSO-d_6) δ 8.44 (d, 1H, $J = 8$ Hz), 8.23 (br s, 1H), 7.97 (d, 1H, $J = 8$ Hz), 7.51–7.38 (m, 4H), 7.36–7.06 (m, 8H), 5.78(s, 2H), and 4.18 (s, 2H)
IR (KBr, cm^{-1}) 3426, 3061, 1728, 1664, 1594, 1495, 1419, 1380, 1347, 1321, 1305, 1264, 1216, 1157, 1013, 750, and 696.
MS (ESI) *m/z* 425. **MS** (FAB) *m/z* 447
Analysis Calc. for $C_{26}H_{19}N_2NaO_4$: C, 69.95; H, 4.29; N, 6.27. Found: C, 65.21; H, 4.42; N, 5.06

Derivatives

Selected tetracyclic derivatives are provided in Table 1.

Table 1 Mass spectra characterization and associated IC_{50} values for selected experimental agents determined using the chromogenic assay. ^{1}H NMR for products and intermediates supplied by author

Entry	Structure	MP °C	MS m/e	IC_{50} (μm)
1		120–125 (Methyl ester)	425	38.6
2		174–177 (Methyl ester)	429	410
3		242–245	356	–

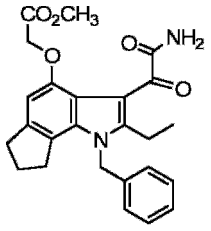
Testing

I. *Chromogenic Assay*

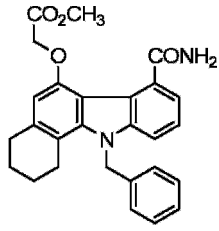
Experimental agents were evaluated as $sPLA_2$ inhibitors using the chromogenic recombinant human secreted phospholipase A_2 assay procedure described by Reynolds (1). Testing results are provided in Table 1.

Notes

1. Tri-, (**I**), and tetracyclic, (**II**), derivatives previously prepared by the authors (2,3), respectively, were effective as $sPLA_2$ inhibitors and used in the treatment of sepsis.

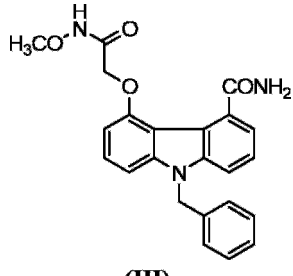


(I)



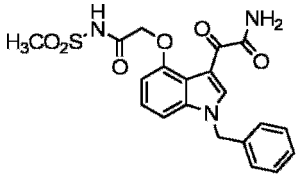
(II)

2. Substituted carbazoles, (**III**), prepared by Harper (4) were effective as $sPLA_2$ inhibitors and used in mediating release of fatty acids for treatment of septic shock.

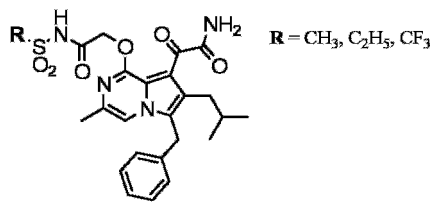


(III)

3. Indole derivatives, (**IV**), prepared by Mihelich (5) and pyrro[1,2-a]pyrazine derivatives, (**V**), prepared by Ogawa (6) were effective as $sPLA_2$ inhibitors and used in the treatment of sepsis.



(IV)



(V)

References

1. L.J. Reynolds *et al.*, Anal. Biochem., 204, 190 (1992)
2. D.W. Beight *et al.*, US Patent 6,974,831 (December 13, 2005) and US Patent 6,916,840 (July 12, 2005)
3. D.W. Beight *et al.*, US Patent 6,872,743 (March 29, 2005)
4. R.W. Harper *et al.*, US Patent 6,933,313 (August 23, 2005)
5. E.D. Mihelich *et al.*, US Patent 7,026,348 (April 11, 2006)
6. T. Ogawa *et al.*, US Patent 7,026,318 (April 11, 2006)

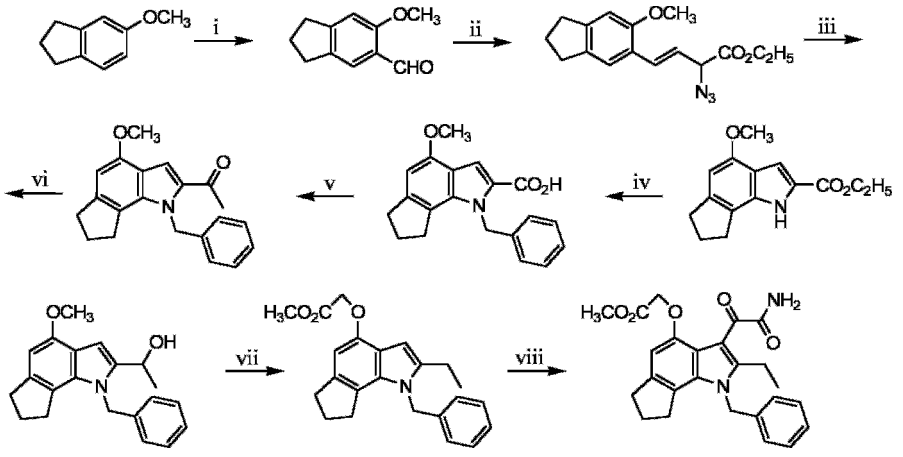
B. N-Benzyl Cyclopent[g]indole-based Medicaments

Title $sPLA_2$ Inhibitors
D.W. Beight *et al.*, US Patent 6,974,831 (December 13, 2005)
Assignee Eli Lilly and Company
Utility Treatment of Septic Shock Disorder

Invention Significance Human nonpancreatic secretory phospholipase A_2 ($sPLA_2$) is a rate-limiting enzyme in the arachidonic acid cascade which hydrolyzes membrane phospholipids. Medicaments that inhibit the $sPLA_2$-mediated release of arachidonic acid would be of general value in the treatment of conditions initiated by overproduction of $sPLA_2$. To address this need, $sPLA_2$ inhibitors have been prepared effective as anti-inflammatory agents useful in treating sepsis or rheumatoid arthritis.

Reaction

Eq. 1



i- Phosphorus oxychloride, DMF
ii- Sodium, ethyl alcohol, ethyl azidoacetate, diethyl ether
iii- Toluene
iv- Sodium hydride, DMF, benzyl bromide
v- THF, methyllithium
vi- Ethyl alcohol, sodium borohydride

vii- EtOAc, THF, ethyl alcohol, CH_2Cl_2, palladium on carbon, hydrogen, $CHCl_3$, boron tribromide, DMF, cesium carbonate, methyl bromoacetate

viii- CH_2Cl_2, oxalyl chloride, ammonium hydroxide, dioxane

Experimental

1. **Preparation of 2,3-dihydro-6-methoxy-1H-indene-5-carboxaldehyde**

   Phosphorus oxychloride (0.710 mol) was added to 60 ml DMF, then stirred 30 minutes at 0°C, and treated with 2,3-dihydro-6-methoxy-1H-indene (0.338 mol), then stirred 4 hours at 80°C. The mixture was cooled to ambient temperature, then poured over crushed ice, and stirred 18 hours. A precipitate that formed was recrystallized in 100% ethyl alcohol and the product isolated in 73% yield as yellow plates.

2. **Preparation of 3-(2,3-dihydro-6-methoxy-1H-inden-5-yl)-2-azido-2-propenoic acid ethyl ester**

   Sodium (0.600 mol) was dissolved in 400 ml 100% ethyl alcohol then cooled to −10°C. This was then treated with a mixture of the Step 1 product (0.150 mol) and the dropwise addition of ethyl azidoacetate (0.558 mol) dissolved in 100 ml diethyl ether, while the reaction remained −10°C. The mixture was warmed to 20°C over 3 hours, then poured into 700 ml water, and extracted twice with diethyl ether. The ethereal solution was washed with water, brine, then dried with Na_2SO_4, and concentrated. After cooling, the residue was cooled to −10°C for 24 hours and a solid was isolated. The solid was washed with hexanes, filtered, and the filtrate reconcentrated. The residue was purified by chromatography with silica gel using EtOAc/hexane, 5:95, and the product isolated in 53% yield as an yellow crystalline material, mp = 64−66°C.

   ^{1}H NMR ($CDCl_3$) δ 8.05 (s, 1H), 7.41 (s, 1H), 6.79 (s, 1H), 4.38 (q, $J = 7.3$ Hz, 2H), 3.84 (s, 3H), 2.89 (q, $J = 7.3$ Hz, 4H), 2.07 (quintet, $J = 7.3$ Hz, 2H), 1.38 (t, $J = 7.3$ Hz, 3H)
   ^{13}C NMR ($CDCl_3$) δ 163.97, 156.87, 147.90, 135.74, 125.81, 124.10, 120.36, 120.06, 61.99, 55.80, 33.92, 33.70, 32.16, 25.64, 14.26
   MS (FD+) *m/z* 287 (p)
   IR ($CHCl_3$, cm^{-1}) 2961, 2122, 1704, 1081
   Analysis Calc. for $C_{15}H_{17}N_3O_3$: C, 62.71; H, 5.96; N, 14.62. Found: C, 62.46; H, 5.99; N, 14.40

3. **Preparation of 2-carboethoxy-4-methoxy-1,6,7,8-tetrahydrocyclopent[g]indole**

   The Step 2 product (25.3 mmol) dissolved in 200 ml toluene was refluxed 6 hours, then cooled to ambient temperature, and a crystalline precipitate formed. The solid

was washed with hexanes and the product isolated in 52% yield as white needles, mp = 185−187°C.

^{1}H NMR ($CDCl_3$) δ 8.97 (bs, 1H, –NH), 7.36 (d, $J = 2.2$ Hz, 1H), 6.46 (s, 1H), 4.41 (q, $J = 7.0$ Hz, 2H), 3.93 (s, 3H), 3.03 (q, $J = 6.6$ Hz, 4H), 2.22 (quintet, $J = 7.3$ Hz, 2H), 1.42 (t, $J = 7.3$ Hz, 3H)
^{13}C NMR ($CDCl_3$) δ 162.31, 153.71, 143.03, 135.07, 125.55, 118.20, 117.80, 107.36, 97.40, 60.79, 55.39, 33.93, 29.32, 25.26, 14.42
MS (ES+) *m/z* 260 (p + 1)
IR ($CHCl_3$, cm^{-1}) 3400, 1698, 1258
Analysis Calc. for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.61; N, 5.40. Found: C, 69.71; H, 6.62; N, 5.45

4. **Preparation of 1-benzyl-2-carboxy-4-methoxy-1,6,7,8-tetrahydrocyclopent[g]-indole**

The Step 3 product (0.133 mol) was added to a slurry of sodium hydride (0.175 mol) in 350 ml DMF, then stirred 15 minutes, and treated with benzyl bromide (0.176 mol) and stirred an additional 48 hours at ambient temperature. The mixture was dissolved in 300 ml methyl alcohol/ THF, 1:1, then treated with 5 M NaOH at 50°C until the precipitate dissolved. The mixture was then treated with 12 M HCl until a pH 2 was obtained and the precipitate was isolated. The solid was washed with water, then dried 48 hours at 40°C, and the product isolated in 87% yield as a white solid, mp = 248−250°C.

^{1}H NMR (DMSO-d_6) δ 12.68 (bs, 1H), 7.29 (s, 1H), 7.22 (m, 3H), 6.80 (d, $J =$ 7.0 Hz, 2H), 6.52 (s, 1H), 5.94 (bs, 2H), 3.89 (s, 3H), 2.93 (m, 2H), 2.84 (t, $J =$ 7.3 Hz, 2H), 1.94 (quintet, $J = 7.3$ Hz, 2H)
^{13}C NMR (DMSO-d_6) δ 163.36, 162.59, 143.14, 140.25, 137.33, 128.47, 126.63, 126.11, 124.90, 117.19, 116.06, 108.63, 97.65, 55.09, 47.82, 32.99, 30.13, 24.48
MS (ES+) *m/z* 322 (p + 1)
IR (KBr, cm^{-1}) 3000, 1662, 1610, 1497
Analysis Calc. for $C_{20}H_{19}NO_3$: C, 74.75; H, 5.96; N, 4.36. Found: C, 74.59; H, 5.64; N, 4.38

5. **Preparation of 2-acetyl-1-benzyl-4-methoxy-1,6,7,8-tetrahydrocyclopent[g]-indole**

The Step 4 product (62.2 mmol) was dissolved in 350 ml THF, then treated with the dropwise addition of 1.4 M methyllithium in diethyl ether (189 mmol) at ambient temperature, and then stirred 2 hours. The mixture was quenched by pouring into saturated NH_4Cl solution, acidified with 12 M HCl acid, and extracted with diethyl ether. The ethereal layer was dried with Na_2SO_4, concentrated, and an amber oil obtained. The residue was purified by chromatography using EtOAc/hexanes, 1:9, and the product isolated in 52% yield as an yellow solid, mp = 147−149°C.

^{1}H NMR ($CDCl_3$) δ 7.52 (s, 1H), 7.23 (m, 3H), 6.90 (d, $J = 6.6$ Hz, 2H), 6.48 (s, 1H), 6.00 (bs, 2H), 3.97 (s, 3H), 3.07 (t, $J = 7.0$ Hz, 2H), 2.96 (t, $J = 7.7$ Hz, 2H), 2.55 (s, 3H), 2.09 (quintet, $J = 7.3$ Hz, 2H)
^{13}C NMR ($CDCl_3$) δ 190.43, 153.78, 145.14, 140.14, 138.65, 132.83, 128.49, 126.98, 125.49, 117.80, 116.77, 111.80, 97.49, 55.35, 49.02, 33.73, 30.87, 27.85, 25.12
MS (ES+) *m/z* 320 (p + 1)
IR ($CHCl_3$, cm^{-1}) 1665, 1606, 1490
Analysis Calc. for $C_{21}H_{21}NO_2$: C, 78.97; H, 6.63; N, 4.39. Found: C, 79.12; H, 6.80; N, 4.56

6. **Preparation of 1-benzyl-2-[2-(2-hydroxyethyl)]-4-methoxy-1,6,7,8-tetrahydrocyclo-pent[g]indole**

Sodium borohydride (25 mmol) was added to the Step 5 product (12.5 mmol) dissolved in 100 ml 100% ethyl alcohol and the mixture stirred 18 hours at ambient temperature. It was quenched with water, then concentrated. The aqueous residue was extracted twice with EtOAc, then washed with brine, and dried with Na_2SO_4. The mixture was concentrated and the product isolated in 90% yield as an off-white solid.

An analytical sample was prepared by recrystallization using EtOAc/hexane to provide white crystals, mp = 136−138°C.

^{1}H NMR ($CDCl_3$) δ 7.25 (m, 3H), 6.88 ($J = 6.2$ Hz, 2H), 6.69 (s, 1H), 6.51 (s, 1H), 5.70 (d, $J = 17.5$ Hz, 1H), 5.62 (d, $J = 17.9$ Hz, 1H), 4.83 (m, 1H), 3.97 (s, 3H), 3.05 (m, 1H), 2.97 (m, 3H), 2.08 (hextet, $J = 7.7$ Hz, 2H), 1.96 (d, $J = 6.2$ Hz, 3H)
^{13}C NMR ($CDCl_3$) δ 152.49, 140.56, 140.20, 139.91, 136.02, 128.79, 127.28, 125.40, 117.58, 116.86, 97.21, 97.06, 62.76, 55.35, 47.71, 33.30, 30.59, 25.38, 22.34
MS (ES+) *m/z* 322 (p + 1)
IR ($CHCl_3$, cm^{-1}) 1605, 1497, 1365
Analysis Calc. for $C_{21}H_{23}NO_2$: C, 78.47; H, 7.21; N, 4.36. Found: C, 78.73; H, 7.22; N, 4.51

7. **Preparation of 2-[(2-ethyl-1-benzyl-1,6,7,8-tetrahydrocyclopent[g]indol-4-yl)-oxy]acetic acid methyl ester**

A solution of the Step 6 product (10.9 mmol) dissolved in EtOAc/THF/ethyl alcohol, 1:1:1, was treated with 2 ml CH_2Cl_2 and 10% palladium on carbon (350 mg), then hydrogenated 2.5 hours at 40 psi hydrogen. The mixture was then filtered through celite and silica gel, then concentrated. The residue was dissolved in 20 ml $CHCl_3$ at 0°C, then treated with boron tribromide (9.5 mmol), and stirred 1 hour. The mixture was poured onto ice, then extracted with $CHCl_3$, and washed once with water and then brine, dried, and concentrated. The residue was dissolved in 10 ml DMF, then treated with cesium carbonate (4.14 mmol), and methyl bromoacetate (4.2 mmol). The mixture was stirred 24 hours at ambient temperature, then retreated with cesium carbonate and methyl bromoacetate, and stirred an additional 8 hours. The mixture

was then diluted with water and extracted twice with EtOAc. The extract was washed four times with water, once with brine, dried with Na_2SO_4, and concentrated. The residue was purified by chromatography using EtOAc/hexanes, 5:95, and the product isolated as a white solid in 21% yield. An analytical sample was prepared by recrystallization using hexanes to provide white crystals, mp = 117−119°C.

^{1}H NMR ($CDCl_3$) δ 7.25 (m, 3H), 6.88 (J = 6.6 Hz, 2H), 6.52 (s, 1H), 6.38 (s, 1H), 5.44 (s, 2H), 4.80 (s, 2H), 3.85 (s, 3H), 3.01 (t, J = 7.0 Hz, 2H), 2.93 (t, J = 7.7 Hz, 2H), 2.61 (q, J = 7.3 Hz, 2H), 2.06 (quintet, J = 7.3 Hz, 2H), 1.31 (t, J = 7.3 Hz, 3H)
MS (ES+) *m/z* 364 (p + 1)
IR ($CHCl_3$, cm^{-1}) 2955, 1760, 1738, 1496
Analysis Calc. for $C_{23}H_{25}NO_3$: C, 76.01; H, 6.93; N, 3.85. Found: C, 75.63; H, 7.27; N, 4.15

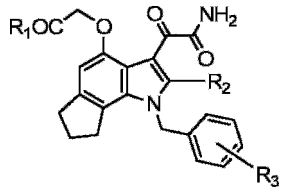
8. **Preparation of 2-[[3-(2-amino-1,2-dioxoethyl)-2-ethyl-1-benzyl-1,6,7,8-tetrahydrocyclo-pent[g]indol-4-yl]oxy]acetic acid methyl ester**

The Step 7 product (0.465 mmol) dissolved in 3 ml CH_2Cl_2 was cooled to 0°C, then treated with oxalyl chloride (1.86 mmol), and stirred 40 minutes. The mixture was concentrated and the residue was diluted with CH_2Cl_2 and reconcentrated. The residue was redissolved in 3 ml CH_2Cl_2, then treated with 6 ml 0.5 M ammonia in dioxane, and stirred 30 minutes. Thereafter, the solution was concentrated and the product isolated in 89% yield as an yellow solid, mp = 190−199°C.

^{1}H NMR ($CDCl_3$) δ 7.32 (m, 3H), 6.97 (d, J = 6.1 Hz, 2H), 6.62 (bs, 1H, –NH), 6.50 (s, 1H), 5.62 (bs, 1H, –NH), 5.51 (s, 2H), 4.74 (s, 2H), 3.82 (s, 3H), 2.90 (m, 6H), 2.04 (quintet, J = 7.3 Hz, 2H), 1.19 (t, J = 7.3 Hz, 3H)
MS (ES+) *m/z* 435 (p + 1)
IR (KBr, cm^{-1}) 3406, 1654, 1640, 1218
Analysis Calc. for $C_{25}H_{26}N_2O_5$: C, 69.11; H, 6.03; N, 6.45. Found: C, 67.97; H, 6.06; N, 6.60

Derivatives

Table 1 Selected experimental derivatives and their corresponding melting points and mass spectral properties $sPLA_2$ inhibitors. ^{1}H NMR and IR analytical data supplied by author



Entry	R_1	R_2	R_3	mp (°C)	MS FD + m/z
1	OCH_3	C_2H_5	Hydrogen	190–199	435
4	OH	CH_3	Hydrogen	255–257	407
6	OCH_3	CH_3	3-Fluoro	245–247	439
8	OCH_3	CH_3	2-Fluoro	228–230	439
10	OCH_3	C_2H_5	4-Fluoro	190–201	425
12	OCH_3	C_2H_5	Hydrogen	–	449
14	OCH_3	CH_3	Hydrogen	135–138	322
18	OCH_3	CH_3	2-Phenyl	–	496
20	OCH_3	CH_3	5-Bromothiophen-2-yl	–	581

Testing

I. *Chromogenic Assay*

The chromogenic assay procedure was performed according to the method of Reynolds (1). IC_{50} values were determined by plotting log concentration versus inhibition values in the range from 10 to 90% inhibition and are provided in Table 2.

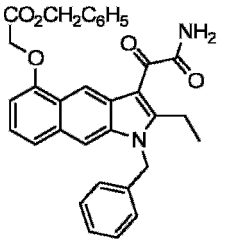
Table 2 Chromogenic assay test results of selected experimental indicating their effectiveness as $sPLA_2$ inhibitors

Entry	IC_{50} (μM)
1	0.108
4	0.010
6	0.132
12	0.100
14	0.109
18	0.190

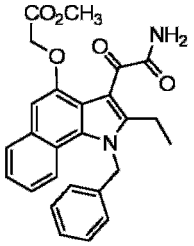
Notes

1. In earlier investigations by the authors (2–4) 1H-benz[f]indol-4-yl, (**I**), 1H-benz[g]-indol-4-yl, (**II**), and 6H-thieno[2,3-b]pyrrol-4-yl derivatives, (**III**), respectively, were prepared and were effective $sPLA_2$ inhibitors. The author (5) also prepared 2,3,4,11-tetraydro-1H-benzo[a]carbazol-6-yl derivatives, (**IV**), which were also effective as

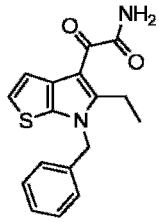
$sPLA_2$ inhibitors. All the forgoing agents were used in the treatment of inflammatory diseases, particularly sepsis.



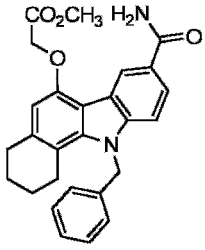
(I)



(II)

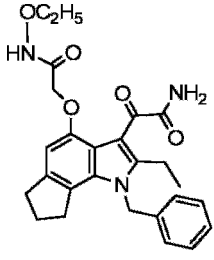


(III)

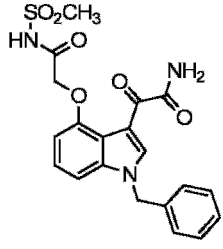


(IV)

2. Hydroxy- and alkoxyamide-1H-indole, (**V**), derivatives prepared by Harper (6) and acylsulfonamide derivatives, (**VI**), prepared by Mihelich (7) were effective as $sPLA_2$ inhibitors and used in the treatment of sepsis.

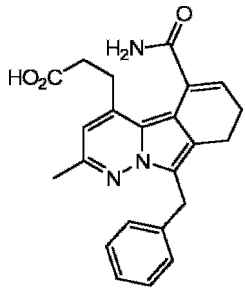


(V)



(VI)

3. Tricyclic azaindolizine derivatives, (**VII**), effective as $sPLA_2$ inhibitors were prepared by Fuji (8) and used in treating septic shock and adult respiratory distress syndrome.



(VII)

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1. L.J. Reynolds *et al.*, Analytical Biochemistry, 204, 190 (1992)
2. D.W. Beight *et al.*, US Patent 6,930,123 (August 16, 2005)
3. D.W. Beight *et al.*, US Patent 6,916,840 (July 12, 2005)
4. D.W. Beight *et al.*, US Patent 6,730,694 (May, 2004)
5. D.W. Beight *et al.*, US Patent 6,992,100 (January 31, 2006) and US Patent 6,872,743 (March 29, 2005)
6. R.W. Harper *et al.*, US Patent 6,831,095 (December 14, 2004)
7. E.D. Mihelich *et al.*, US Patent 7,026,348 (April 11, 2006)
8. M. Fuji *et al.*, US Patent 6,756,376 (June 29, 2004)

CHAPTER VII

Autoimmune Disorders

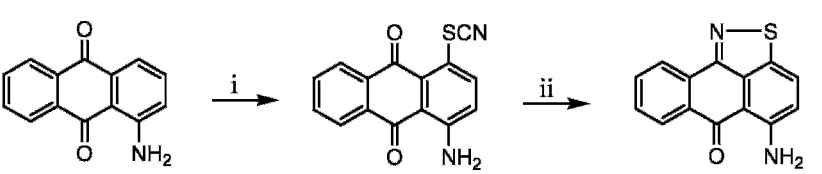
I. Jun N-terminal Kinase Inhibitors

Title Isothiazoloanthrones, Isoxazoloanthrones, Isoindolanthrones, and Derivatives Thereof as JNK Inhibitors and Compositions and Methods Related
S.T. Sakata *et al.*, US Patent 6,987,184 (January 17, 2006)
Assignee Signal Pharmaceuticals, LLC
Utility Treatment of Autoimmune Diseases

Invention Significance The Jun N-terminal kinase (JNK) pathway is activated by exposure of cells to environmental stress or by treatment of cells with proinflammatory cytokines such as TNF-α and IL-1. The JNK pathway has been implicated in the mediation of both autoimmune diseases and immunodeficiency disorders. To control this disorder, JNK signaling pathway inhibitors have been prepared that selectively impede the JNK cascade.

Reaction

Eq. 2



i- Ammonium thiocyanate, DMSO, sulfuric acid
ii- Ammonia (1)

Experimental

1. **Preparation of 4-thiocyano-1-aminoanthraquinone**

   A suspension of 1-aminoanthraquinone (3.0 g) in 45 ml of DMSO was treated with ammonium thiocyanate (9 g), then heated to 50°C, and 15 ml H_2SO_4 added dropwise. The mixture was stirred 16 hours at ambient temperature and was then diluted with 300 ml water. A suspension that formed was filtered and a solid isolated and dried. The procedure was repeated using the dried residue and the second crop was isolated. Combined solids were recrystallized in 250 ml *o*-dichlorobenzene and 1.9 g of product was isolated.

   ^{1}H NMR (DMSO-d_6): 8.19 (dd, 1H), 8.14 (dd, 1H), 7.93 (dt, 1H), 7.86 (dt, 1H), 7.81 (d, 1H), 7.43 (d, 1H)
   ES-MS (*m/z*) 281 $[M+1]^+$

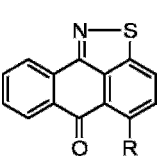
2. **Preparation of 3-aminoisothiazoloanthrone**

   A suspension of Step 1 product (300 mg) in 25 ml liquid ammonia was heated 5 hours at 140°C in a high pressure/temperature bomb apparatus, then diluted with 300 ml water, and filtered. The residue was purified using preparative HPLC using a 5 cm YMC C-18 column operated at a flow rate of 60 ml/min with a gradient elution from 40% aqueous acetonitrile with 0.1% trifluoroacetic acid to 100% acetonitrile with 0.1% trifluoroacetic acid over 20 minutes and 55 mg of product isolated.

   ^{1}H NMR (DMSO-d_6): 8.49 (d, 1H), 8.39 (d, 1H), 8.29 (d, 1H), 7.89 (t, 1H), 7.78 (t, 1H), 7.28 (d, 1H)
   ES-MS (*m/z*) 253 $[M+1]^+$

Derivatives

Table 1 Selected three-substituted experimental isothiazoloanthrone derivatives and their corresponding mass spectral data. Only entry 1 is effective as a JNK inhibitor. ^{1}H NMR data supplied by author



Entry	R	ES-MS (*m/z*) $[M+1]^+$
1	Amine	253
2	Hydroxyl	254
3	*N*-Acetyl	295
4	*N*-Benzamide	357
5	*N*-(4-Methoxycarbonyl)succinamide	367
6	*N*-(Pyridin-3-yl)amide	357

Testing

JNK testing was limited to 3-aminoisothiazoloanthrone.

I. *Selectivity for JNK*

Entry 1 was assayed for its inhibitory activity against selected protein kinases according to the method of Sefton (1). Testing results are provided in Table 2.

Table 2 Inhibitory activity of 3-amino isothiazoloanthrone against selected protein kinases

Enzyme	IC_{50} (nM)
JNK2	1
JNK3	400
p38-2	>30 000
MEK6	>30 000
LKK1	>30 000
IKK2	>30 000

II. *Jurkat T-cell IL-2 Production Assay*

Jurkat T cells (clone E6-1) were maintained in growth media consisting of RPMI 1640 medium containing 2 mM L-glutamine with 10% fetal bovine serum and penicillin/streptomycin. All cells were cultured at 37°C in 95% air and 5% CO_2. Cells were plated at a density of 0.2×10^6 cells/well in 200 µl of media. A selected experimental agent (20 mM) was diluted in growth media and added to each well as a 10× concentrated solution in a volume of 25 µl, then mixed, and preincubated with cells for 30 minutes. The compound vehicle, DMSO, was maintained at a final concentration of 0.5% in all samples. After 30 minutes, the cells were

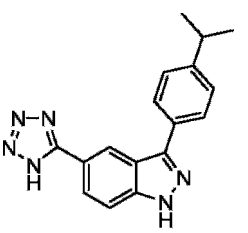
activated with phorbol myristate acetate (PMA) at final concentration 50 ng/ml and phytohemagglutinin (PHA) at a final concentration 2 μg/ml). PMA and PHA were added as a 10× concentrated solution made up in growth media and added in a volume of 25 μl per well. Cell plates were cultured 10 hours. Cells were pelleted by centrifugation and the media removed and stored at −20°C. Aliquots were analyzed by sandwich ELISA for the presence of IL-2.

Results

The IC_{50} value for entry 1 was 30 μM indicating its effectiveness as a JNK inhibitor.

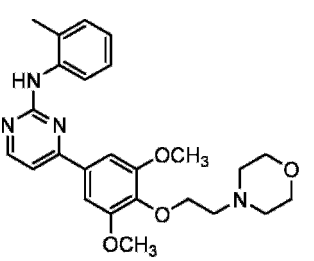
Notes

1. 1H-Indazole derivatives were prepared by Bhagwat (2) of which 5-{3-[4-(methylethyl)phenyl]-1H-indazol-5-yl}-2H-1,2,3,4-tetrazole, (**I**), was particularly effective as a JNK_2 inhibitor and used in the treatment of rheumatoid arthritis, spondylitis, osteoarthritis, and gout.

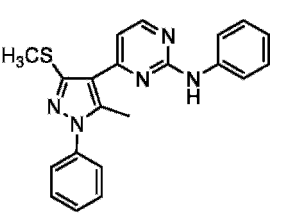


(I)

2. 1,3-Pyrimidine derivatives, (**II**), prepared by Bethiel (3) and thioalkyl 1,3, pyrimidine derivatives, (**III**), prepared by Choon-Moon (4) were effective as c-JNK inhibitors and kinases belonging to the Src family of protein kinases.

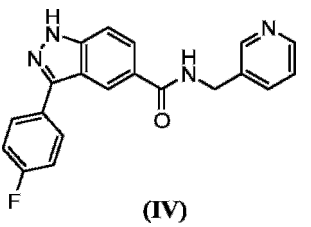


(II)



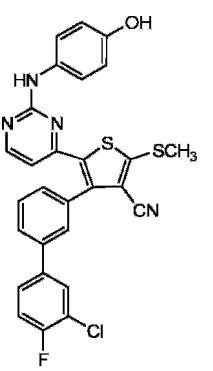
(III)

3. Oinuma (5) prepared 1H-indazole derivatives, (**IV**), which were effective as JNK inhibitory agents and as a medicament for the treatment of inflammatory disorders.



(IV)

4. c-JNK inhibitors prepared by Cao (6) consisting of pyrimidin-4-yl-4-thiophene derivatives, (**V**), were effective in treating autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus, scleroderma, and chronic thyroiditis.



(V)

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2. S.S. Bhagwat *et al.*, US Patent 6,897,231 (May 24, 2005)
3. R.S. Bethiel *et al.*, US Patent 6,949,544 (September 27, 2005)
4. Y. Choon-Moon, US Patent 6,884,804 (April 26, 2005)
5. H. Oinuma *et al.*, US Patent 6,982,274 (January 3, 2006)
6. J. Cao *et al.*, US Patent 7,084,159 (August 1, 2006)

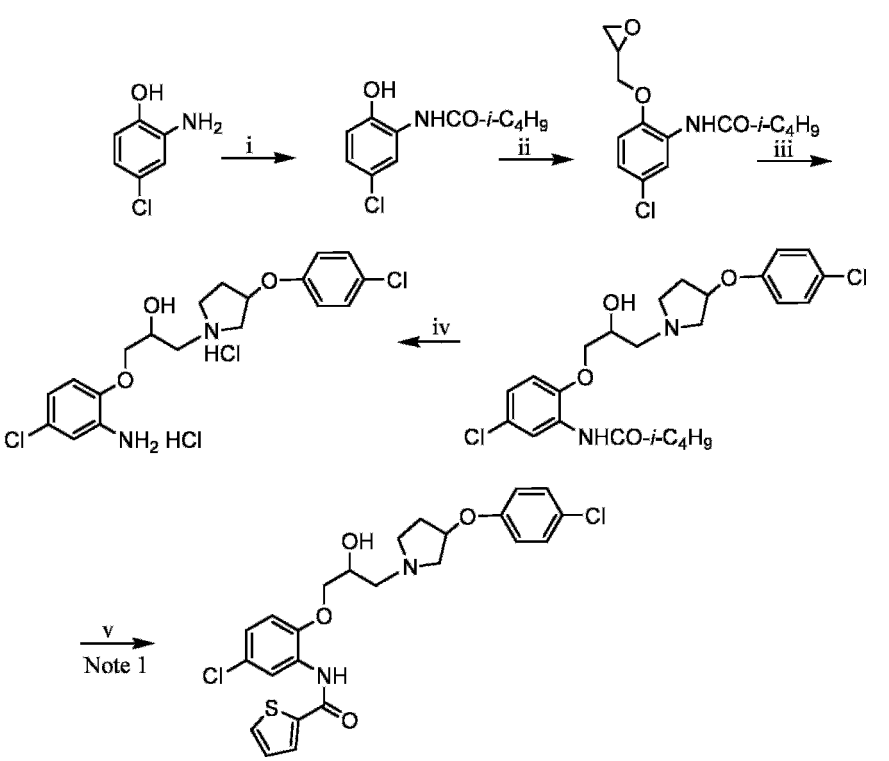
II. Macrophage Inflammatory Protein-1α Chemokine Receptor Antagonists

Title Compounds
T. Eriksson *et al.*, US Patent 7,005,439 (February 28, 2006)
Assignee AstraZeneca AB
Utility Treatment of Respiratory Tract Airway Disorders and Rheumatoid Arthritis Responses Autoimmune Pathologies

Invention Significance Neutrophils such as the macrophage inflammatory protein-1α (MIP-1α) are chemokines involved in immune and autoimmune inflammatory pathologies such as respiratory tract airway disorders and rheumatoid arthritis responses. MIP-1α receptor antagonists have been prepared to address these disorders.

Reaction

Eq. 1



i- Water, isobutyric anhydride
ii- Epibromohydrin, potassium carbonate, DMF
iii- 3-(4-Chlorophenoxy)-pyrrolidine, ethyl alcohol
iv- Hydrochloric acid
v- 2-Thiophenecarboxylic acid, 1-methyl-2-pyrrolidinone, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, 1-hydroxybenzotriazole, *N, N*-diisopropylethylamine

Experimental

1. **Preparation of *N*-(5-chloro-2-hydroxy-pheny)-isobutyramide**

   A suspension of 4-chloro-2-aminophenol (8.39 mmol) and 25 ml water was treated with 1.6 ml isobutyric anhydride, then stirred 30 minutes at 60°C, and cooled. A precipitate that formed was washed twice with water, dried, and the product isolated in 78% yield as a white solid.

   ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.11 (1H, s); 9.12 (1H, s); 7.94 (1H, d, $J =$ 2.5 Hz); 6.95 (1H, dd, $J = 8.7$, 2.6 Hz); 6.84 (1H, d, $J = 8.5$ Hz); 2.79 (1H, p, $J = 6.7$ Hz); 1.08 (6H, d, $J = 6.8$ Hz)

2. **Preparation of *N*-(5-chloro-2-oxiranylmethoxy-phenyl)-isobutyramide**

   A mixture consisting of the Step 1 product (1.87 mmol), epibromohydrin (2.06 mmol), K_2CO_3 (3.7 mmol), and 2 ml DMF was heated 2 hours at 60°C in a sealed vial. The mixture was then partitioned between EtOAc/water and the organic phase washed twice with water and once with brine. The solution was concentrated, the solid residue purified by chromatography on silica gel, and the product isolated in 44% yield as a white solid.

3. **Preparation of *N*-(5-chloro-{2-3-[3-(4-chloro-phenoxy)-pyrrolidin-1-yl]-2-hydroxy-propoxy}-phenyl)-isobutyramide**

   A mixture consisting of the Step 2 product (0.13 mmol), 3-(4-chlorophenoxy)-pyrrolidine (0.13 mmol), and 2 ml ethyl alcohol was heated 3 hours at 75°C in a sealed vial, then cooled, and concentrated. The residue was purified by chromatography and then lyophilized as the HCl salt. The product was isolated in 84% as a white solid and consisted of four stereoisomers.

   ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84–10.34 (1H, m); 9.12 (1H, s); 8.09 (1H, s); 7.36 (2H, dd, $J = 9.2$, 1.3 Hz); 7.11–7.00 (3H, m); 7.00 (2H, d, $J = 8.8$ Hz); 6.22–6.06 (1H, m); 5.22–5.10 (1H, m); 4.34 (1H, bs); 4.08–3.96 (1.5H, m); 3.95–3.87 (1H, m); 3.83–3.66 (1.5H, m); 3.61–3.23 (3H, m); 2.86 (1H, sept, $J = 6.6$ Hz);

2.64–2.51 (½H, m); 2.36–2.14 (1H, m); 2.14–2.00 (½H, m); 1.08 (6H, d, $J = 6.7$ Hz)
APCI-MS *m/z* 467.2 [MH^+]

4. **Preparation of 1-[(2-aminophenyl)oxyl]-3-{3-[(4-chlorophenyl)oxyl-] 1-pyrrolidinyl}-2-propanol dihydrochloride**

   The Step 3 product mixture was dissolved in 50 ml 12 M HCl and refluxed overnight. The precipitate was filtered, dried, and the product isolated in 65% yield.

   APCI-MS *m/z* 363, 365 [MH^+]

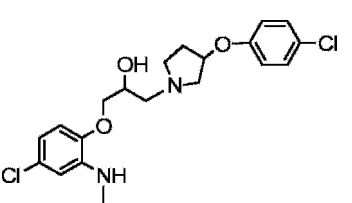
5. **Preparation of thiophene-2-carboxylic acid (2-{3-[3-(4-chloro-phenoxy)-pyrrolidin-1-yl]-2-hydroxy-propoxy}-phenyl)-amide**

   A solution of 80 µl apiece 0.2 M 1-methyl-2-pyrrolidinone 2-thiophenecarboxylic acid (*Step 5 Acid*), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, 1-hydroxybenzotriazole, 30 µl 0.2 M 1-methyl-2-pyrrolidinone, and 0.5 M apiece diisopropylethylamine and pyridine were stirred 30 minutes, then treated with 75 µl 0.2 M 1-methyl-2-pyrrolidinone containing the Step 4 product. The mixture was stirred overnight at ambient temperature, then concentrated, and diluted with 1000 µl CH_2Cl_2. The solution was washed with 800 µl apiece saturated $NaHCO_3$ solution and 1.81% HCl and then brine. The organic layer was reconcentrated and the product isolated in 51% yield.

   ^{1}H NMR (400 MHz, CD_3OD) δ 8 7.88–7.85 (d, 1H), 7.74–7.65 (m, 2H), 7.34–7.28 (m, 2H), 7.27–7.21(m, 1H), 7.20–7.15 (m, 1H), 7.14–7.09 (dd, 1H), 7.06–7.00 (m, 1H), 6.96–6.91 (m, 2H), 5.18–5.12 (m, 1H), 4.39–4.30 (m, 1H), 4.19–3.24 (m, 9H), 2.66–2.11 (m, 3H)
   APCI-MS *m/z* 473.2 [MH^+]

Derivatives

Table 1 Step 5 experimental pyrrolidine amide derivatives prepared by reacting the Step 4 product with a selected carboxylic acid and their corresponding mass spectral data



Step 5 Acid

Entry	Step 5 Acid	APCI-MS (*m/z*)
4	Pyrazine-2-carboxylic acid	469.2
7	3-Hydroxyl-butanoic acid	449.2
17	5-Methylthiophene-2-carboxylic acid	487.2
18	1-Acetylpyrrolidine-2-carboxylic acid	502.3
25	Pent-4-enoic acid	445.3
35	5-Oxy-hexanoic acid	475.3

Testing

I. *THP-1 Chemotaxis Assay*

Cells were washed by centrifugation in RPMI + 10% HIFCS + glutamax, then resuspended $2 \times 10^{+7}$ cells/ml in fresh medium, and calcein-AM added to give a final concentration of 5×10^{-6} M. The cells were diluted to 50 ml with medium and washed twice by centrifugation. Labeled cells were resuspended at a cell concentration of 1×10^{7} cells/ml and incubated with an equal volume of selected experimental agents for 30 minutes at 37°C in a humidified CO_2 incubator

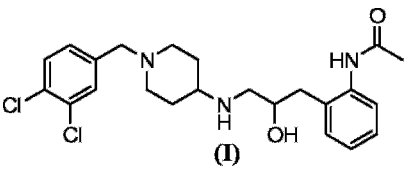
Chemotaxis was performed using 96-well chemotaxis plates employing 8 μm filters. Thirty microliters of chemoattractant supplemented with various concentrations of selected experimental agents or vehicle was added to the lower wells of the plate in triplicate. The filter was positioned on top and 25 μl of cells preincubated with a selected concentration of an experimental agent or vehicle added to the surface of the filter. The plate was incubated 2 hours at 37°C in a humidified CO_2 incubator, the remaining cells on the surface were removed by adsorption, and the entire plate centrifuged. The filter was then removed and the cells that had migrated to the lower wells were quantified by cell fluorescence of associated calcein-AM.

Results

Although quantitative data were not supplied by author, the experimental agent, *N*-(5-chloro-2-{3-[3-(4-chloro-phenoxy)-pyrrolidin-1-yl]-2-hydroxy-propoxy}-phenyl)-isobutyramide was especially preferred.

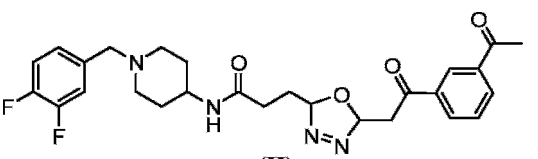
Notes

1. In an earlier investigation by the author (1), 4-piperidinyl[amino-(2-hydroxypropyl)oxy-phenyl]acetamide derivatives were prepared, which were effective as MIP-1α receptor antagonists. The derivative *N*-[2-(3-{[1-(3,4-dichlorobenzylpiperidinyl]aminohydroxy-propoxy)-phenyl]}acetamide, (**I**), was especially preferred.



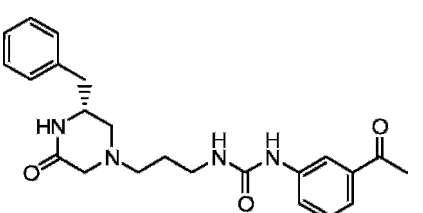
(I)

2. 3,4-Difluorobenzylpiperidinyl derivatives, (**II**), prepared by Brough (2) were effective as MIP-1α receptor antagonists and used in the treatment of autoimmune pathologies.



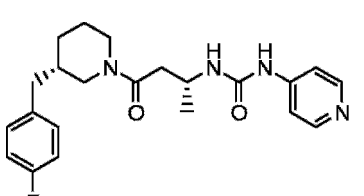
(II)

3. Piperizinone urea derivatives, (**IV**), prepared by DeLucca (3) were effective as MIP-1α receptor modulators and used in the treatment of asthma and allergic diseases.

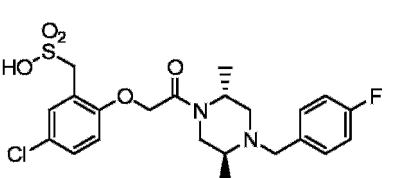


(III)

4. MIP-1α chemokine receptor activity modulators consisting of 4-fluorobenzyl-piperidinylamide ureas, (**IV**), prepared by Duncia (4) and sulfonic acid derivatives, (**V**), prepared by Hayward (5) were effective in treating inflammation and autoimmune disorders including asthma and allergic disorders.

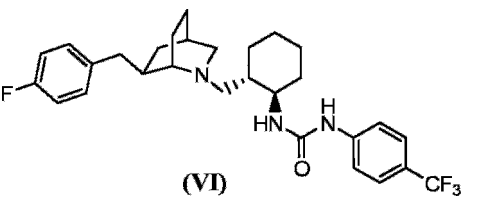


(IV)



(V)

5. 4-Fluorobenzyl bicyclic, (**VI**), and tricyclic amine derivatives prepared by Dunica (6) were effective as MIP-1α chemokine receptor antagonists and used in treating inflammatory and autoimmune diseases.



(VI)

References

1. T. Eriksson *et al.*, US Patent 6,911,458 (July 28, 2005)
2. S. Brough *et al.*, US Patent 6,958,350 (October 25, 2005)
3. G.V. DeLucca, US Patent 6,974,869 (December 13, 2005)
4. J.V. Duncia *et al.*, US Patent 6,984,651 (January 10, 2006)
5. M.M. Hayward, US Patent 6,974,817 (December 13, 2005)
6. J.V. Duncia *et al.*, US Patent 6,960,666 (November 1, 2005)

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CHAPTER VIII

Cardiovascular Disorders

I. Anticoagulants

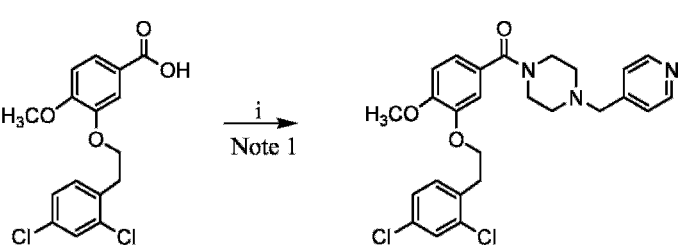
A. Blood Clotting Enzyme Factor Xa, FXa, or Factor VIIa, FVIIa Inhibitors

Title Oxybenzamide Derivatives Useful for Inhibiting Factor Xa or VIIa
M. Nazaré *et al*., US Patent 6,953,857 (October 11, 2005)
Assignee Aventis Pharma Deutschland GmbH
Utility Blood Coagulation Modulators

Invention Significance There continues to be a need for safe and effective therapeutic anticoagulants to limit or prevent thrombus formation. While current agents interrupt coagulation by interfering with steps associated with the coagulation cascade, thrombin is not directly inhibited. This investigation has addressed these limitations by providing chemical agents that are reversible inhibitors of blood clotting enzyme factors Xa (FXa) or factors VIIa (FVIIa) and are used in the treatment of thromboembolic disorders or restenoses.

Reaction

Eq. 1



i- DMF, *N*-ethylmorpholine,
1-pyridin-4-ylmethyl-piperazine,
O-((cyano-(ethoxycarbonyl)methylene)amino)-1,1,3,3-tetramethyluronium tetrafluoroborate

Experimental

1. **Preparation of {3-[2-(2,4-dichlorophenyl)-ethoxy]-4-methoxy-phenyl}-(4-pyridin-4-yl-methylpiperazin-1-yl)-methanone**

   3-[2-(2,4-Dichloro-phenyl)-ethoxy]-4-methoxy-benzoic acid (0.29 mmol) dissolved in 2 ml DMF was treated with *N*-ethylmorpholine (1.16 mmol), 1-pyridin-4-ylmethyl-piperazine (0.29 mmol), and O-((cyano(ethoxycarbonyl)methylene)amino)-1,1,3,3-tetramethyluronium tetrafluoroborate (0.3 mmol), then stirred 60 minutes at ambient temperature. The mixture was concentrated, then dissolved in CH_2Cl_2, washed three times with saturated $NaHCO_3$ solution, dried with Na_2SO_4, and reconcentrated. The residue was purified by chromatography with silica gel using *n*-heptane/EtOAc, 1:1, then EtOAc, and finally EtOAc/methyl alcohol, 10:1, and 102 mg product isolated.

   MS ES$^+$, $m/z = 500$ (M^+)

Derivatives

Selected derivatives are provided in Table 1.

Table 1 Selected experimental agents and corresponding mass spectra. Entries 79, 133, and 199 are especially preferred

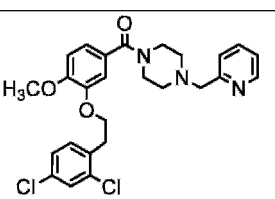
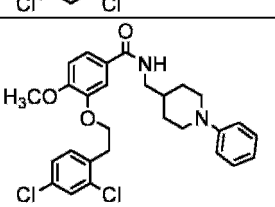
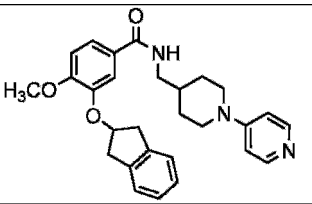
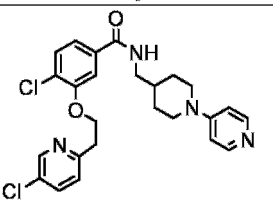
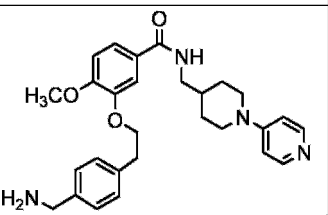
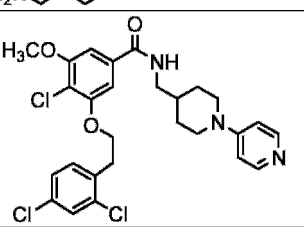
Entry	Structure	MS (ES$^+$)	Ki(FXa) (μm)
2		500	1.540
79		499	0.029

Table 1 Continued

95		588	29.734
133		485	0.020
143		460	38.79
199		550	0.01

Testing

I. *Factor Xa Assay*

In the assay for determining the inhibition of factor Xa activity TBS–PEG buffer consisting of 50 mM Tris–HCl, pH 7.8, 200 mM NaCl, 0.05% (w/v), PEG-8000, 0.02% (w/v), and NaN_3 was used. The IC_{50} was determined by combining in appropriate wells of half-area microtiter plate 25 μl human factor Xa in TBS–PEG, 40 μl 10% (v/v) DMSO in TBS–PEG as the uninhibited control or selected experimental agents diluted in 10% (v/v) DMSO in TBS–PEG with the substrate, *N*-α -benzyloxycarbonyl-D-Arg-Gly-L-Arg-*p*-nitroanilide in TBS–PEG.

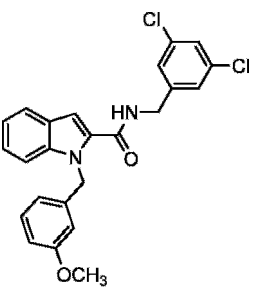
The assay was performed by first preincubating selected experimental agents and the enzyme for 10 minutes. Thereafter, the assay was initiated by adding the substrate to obtain a final volume of 100 μl. The initial velocity of chromogenic substrate hydrolysis was measured by the change in absorbance at 405 nm at 25°C during the linear portion of the time course. Inhibition constants, K_i, for factor Xa are summarized in Table 1.

Results

Entries 79, 133, and 199 appearing in Table 1 were especially preferred.

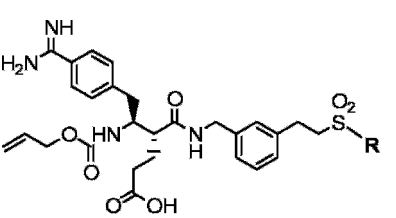
Notes

1. Indole derivatives, (**I**), prepared by the author (1) in an earlier investigation were also effective as factor Xa inhibitors.



(I)

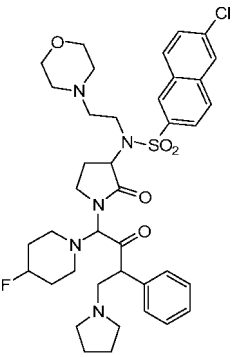
2. *N*-Guanidinoalkylamides analogs of the current invention, (**II**), prepared by Klingler (2) were effective as reversible inhibitors of the blood clotting enzymes factor Xa and/or factor VIIa and used in the treatment of thromboembolic diseases or restenosis.



(II)

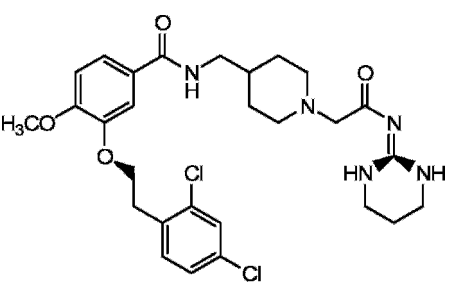
R
2-Acetylamino-1,3-thiazol-5-yl
3,5-Dichlorophenyl
4-Fluorophenyl
4-Methoxyphenyl
3-Trifluoromethylphenyl

3. Pyrrolidin-2-one derivatives, (**III**), prepared by Chan (3) were effective as factor Xa inhibitors and useful in treating diseases associated with coronary thrombosis.



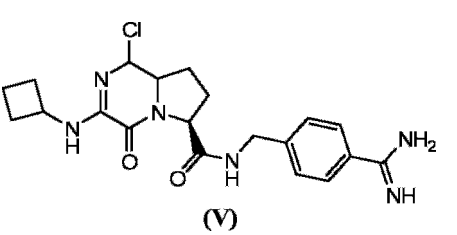
(III)

4. Nazare (4) prepared oxybenzamide derivatives, (**IV**) which were effective as factor Xa-specific blood clotting inhibitors with enhanced stability in plasma and liver.



(IV)

5. Amino-bicyclic pyrazinone, (**V**), and pyridinone derivatives prepared by Zhang (5) were effective as factor VIIa inhibitors and as selective inhibitors of serine protease enzymes associated with the coagulation cascade.



(V)

References

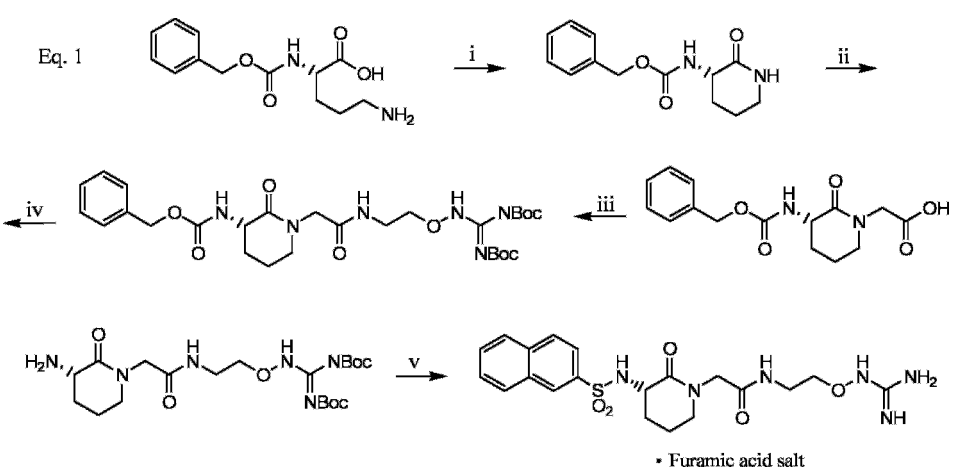
1. M. Nazaré *et al*., US Patent 6,906,804 (July 14, 2005)
2. O. Klingler *et al*., US Patent 6,664,393 (January 16, 2005)
3. C. Chan *et al*., US Patent 7,084,139 (August 1, 2006)
4. M. Nazare *et al*., US Patent 7,067,665 (June 27, 2006)
5. X. Zhang, US Patent 7,037,911 (May 2, 2006)

B. Factor Xa Inhibitions

Title Azacycloalkanone Serine Protease Inhibitors
S.C. Miller *et al.*, US Patent 6,962,942 (November 8, 2005)
Assignee 3-Dimensional Pharmaceuticals, Inc.
Utility Treatment of Thrombotic Occlusive Disorders

Invention Significance Factor Xa is a serine protease in the blood coagulation pathway forming a prothrombinase complex which ultimately converts prothrombin into thrombin. Although direct thrombin inhibitors are available, their use is associated with excessive bleeding, poor selectivity towards thrombin, and liver toxicity. This chapter addresses these limitations that inhibit thrombin production using a new chemical class of nonpeptidic factor Xa inhibitions.

Reaction



i- 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 4-methylmorpholine, 1-hydroxybenzotriazole, acetonitrile
ii- Lithium bis(trimethylsilyl)amide, THF, ethyl bromoacetate, ethylenediamine
iii- Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate, *t*-butyl-3-[(2-aminoethoxy)amino]-2-aza-3-[(*t*-butoxy)carbonylamino]prop-2-enoate, *N,N*-diisopropylethylamine, DMF

iv- Palladium on carbon, methyl alcohol, hydrogen
v- 2-Naphthalenesulfonyl chloride, polystyrene-*g*-dimethylaminopyridine, fumaric acid, CH_2Cl_2

Experimental

1. **Preparation of *N*-((3*S*)-2-oxo(3-piperidyl))(phenylmethoxy)carboxamide**

   1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (37.6 mmol) was added in a single portion to a solution of *N*-α-benzyloxycarbonyl-L-ornithine (37.6 mmol), 4-methylmorpholine (37.6 mmol), and 1-hydroxybenzotriazole (37.6 mmol) dissolved in 200 ml acetonitrile, then stirred overnight. The solution was filtered to remove white solids, then concentrated, and residue dissolved in CH_2Cl_2. The solution was washed with dilute HCl and $NaHCO_3$ solutions, then dried using $MgSO_4$. The mixture was reconcentrated and the product isolated in 75% yield as a white solid.

   ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (m, 5H), 6.20 (bs, 1H), 5.76 (bs, 1H), 5.11 (s, 2H), 4.10 (m, 1H), 3.32 (m, 1H), 2.50 (m, 1H), 1.92 (m, 2H), 1.61 (m, 1H)

2. **Preparation of 2-{(3*S*)-2-oxo-3-[(phenylmethoxy)carbonylamino]pipetidyl} acetic acid**

   A solution of 20.8 ml of 1.0 M Bis(trimethylsilyl)amide in THF lithium was added to an ice-cooled solution of the Step 1 product (18.9 mmol) dissolved in 20 ml THF followed by ethyl bromoacetate (94.5 mmol) and then stirred 30 minutes. The mixture was further treated with 10 ml ethylenediamine, then stirred an additional 30 minutes, and finally concentrated. The residue was dissolved in CH_2Cl_2, then washed with dilute HCl, dried with Na_2SO_4, and reconcentrated. The residue was redissolved in 25 ml methyl alcohol and treated with 50 ml 1.0 M NaOH. Methyl alcohol was then removed and the basic solution extracted three times with 100 ml with CH_2Cl_2. The aqueous layer was acidified with 1.0 M HCl to pH 1 and further extracted with CH_2Cl_2. Organic extracts were dried with Na_2SO_4, filtered, concentrated, and the product isolated in 98%. The product was used directly in the next step without additional purification.

3. **Preparation of *t*-butyl-3-{[2-(2-{(3*S*)-2-oxo-3-[(phenylmethoxy)carbonylamino]-piperidyl}acetylamino)ethoxy]-amino}-2-aza-3-[(t-butoxy)carbonylamino]prop-2-enoate**

   Benzotriazol-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate (12 mmol) was added in a single portion to a solution consisting of the Step 2 product (9.9 mmol), *t*-butyl 3-[(2-aminoethoxy)-amino]-2-aza-3-[(*t*-butoxy) carbonylamino]prop-2-enoate (9.9 mmol) and 3.45 ml *N,N*-diisopropylethylamine dissolved in 40 ml DMF, then stirred overnight. The mixture was diluted with

CH_2Cl_2, then washed with dilute solutions of HCl and $NaHCO_3$, dried using Na_2SO_4, concentrated, and 7 g crude product isolated. The product was used directly in the next step without additional purification.

4. **Preparation of *t*-butyl-3-({2-[2-((3*S*)-3-amino-2-oxopiperidyl)acetylamino] ethloxy}amino)-2-aza-3-[(*t*-butoxy)carbonylamino]prop-2-enoate**

A solution of the Step 3 product (7.0 g) was treated with 10% palladium on carbon (800 mg) and 100 ml methyl alcohol, then hydrogenated 2 hours under 1 atm hydrogen, then filtered through diatomaceous earth. The solution was concentrated and the residue partitioned between CH_2Cl_2 and dilute HCl. The aqueous phase was further extracted twice with CH_2Cl_2 and these extracts were discarded. The aqueous layer was basified with 1 M NaOH, then re-extracted twice with CH_2Cl_2, dried with Na_2SO_4, concentrated, and the residue isolated. The residue was used directly in the next step without additional purification.

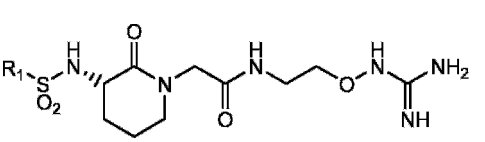
5. **Preparation of 2-{(3*S*)-3-[(2-naphthylsulfonyl)amino]-2-oxopiperidyl}-*N*-[2-(amidinoaminooxy)ethyl]acetamide•fumaric acid**

2-Naphthalenesulfonyl chloride (0.21 mmol) was added in a single portion to a solution of the Step 4 product (0.21 mmol) and polystyrene-*g*-dimethylaminopyridine (≈2 mmol) dissolved in 3 ml CH_2Cl_2, then stirred overnight. The mixture was then diluted with 3 ml acetonitrile and further treated with polystyrene-*g*-dimethylaminopyridine (1.1 mmol) and stirred 30 minutes. The resin was removed by filtration and the filtrate was concentrated. The residue was dissolved in 2 ml CH_2Cl_2, then stirred 30 minutes, and reconcentrated. The residue was purified by chromatography with silica gel using 10% methyl alcohol/CH_2Cl_2 saturated with ammonia. After isolation, the product was stirred in methyl alcohol containing fumaric acid (1 equiv.), reconcentrated, and the product isolated in 26% yield as a white solid.

MS $m/z = 463(M+1)$

Derivatives

Table 1 Selected 3-{[(2-arylsulfonyl)amino]-2-oxopiperidyl}-*N*-[2-(amidinoaminooxy)ethyl] acetamide•fumarate salts prepared and their corresponding mass spectral data



Fumaric Acid

Entry	R_1	MS, *m/z* (M + 1)
3	2-Nitrobenzyl	472
7	4-Propylphenyl	455
19	4-Nitrophenyl	458
24	3,4-Dimethoxyphenyl	473
27	4-(Phenyl)phenyl	489
34	4-(3-Methylphenyl)phenyl	503
42	4-(2-Naphthyl)phenyl	539

Testing

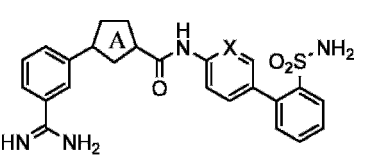
Although testing data were not supplied by author, the two experimental agents listed below were especially preferred:

i- 2-{(3*S*)-3-[(2-naphthylsulfonyl)amino]-2-oxopiperidyl}-*N*-[2-(amidinoaminooxy)ethyl]-acetamide

ii- 2-((3*S*)-3-{[(6-bromo(2-naphthyl))sulfonyl]amino}-2-oxopyrrolidinyl)-*N*-[2-(amidinoaminooxy)-ethyl]acetamide.

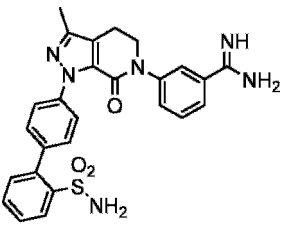
Notes

1. Heterocyclic aromatics, (**I**), prepared by Pruitt (1), where ring A consists of isoxazole, oxazole or thiazine and where X is either N or CH, were effective as factor Xa inhibitors. Additional ring A derivatives were prepared by Quan (2).



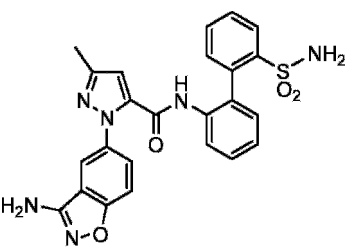
(**I**)

2. 4,5,6,7-Tetrahydro-1H-pyrazolo[3,4-c]pyridine derivatives, (**II**), and other 5–6 to 5–7 heterobicycles prepared by Pinto (3) were effective as trypsin-like serine protease inhibitors, especially for factor Xa.



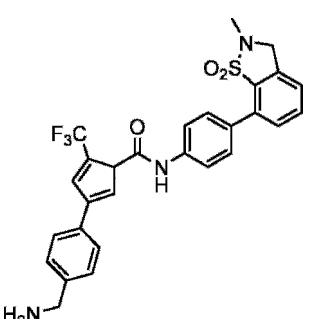
(II)

3. Lam (4) determined that the presence of either 3-amino- or 3-hydroxyl-benzisoxazol-5-yl in pyrazole derivatives such 1-(3′-aminobenzisoxazol-5′-yl)-3-methyl-5-[(2′-amino-sulfonyl-[1, 1′]-biphen-4-yl)aminocarbonyl]pyrazole, (**III**), mimicked the factor Xa-inhibiting properties of guanidine.



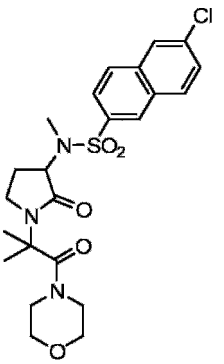
(III)

4. In another investigation, Wexler (5) determined that the presence of 1,1-dioxido-1,2-benzisothiazol-7-yl in pyrazole derivatives, (**IV**), generated factor Xa inhibitors, which were useful in the treatment of thromboembolic diseases.

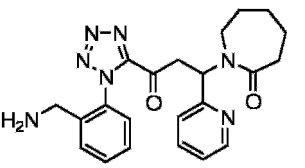


(IV)

5. Lactam-containing derivatives, (**V**) and (**VI**), prepared by Chan (6) and Pinto (7), respectively, were effective as factor Xa inhibitors and used in the amelioration of cardiovascular disorders.



(V)



(VI)

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3. D.J.P. Pinto *et al*., US Patent 6,960,595 (November 1, 2005) and US Patent 6,506,771 (January 14, 2003)
4. P.Y. Lam *et al*., US Patent 6,958,356 (October 25, 2005)
5. R.R. Wexler *et al*., US Patent 6,689,770 (February 10, 2004)
6. C. Chan *et al*., US Patent 7,084,139 (August 1, 2006)
7. D.J.P. Pinto *et al*., US Patent 6,967,208 (November 22, 2005)

C. Serine Protease Inhibitors

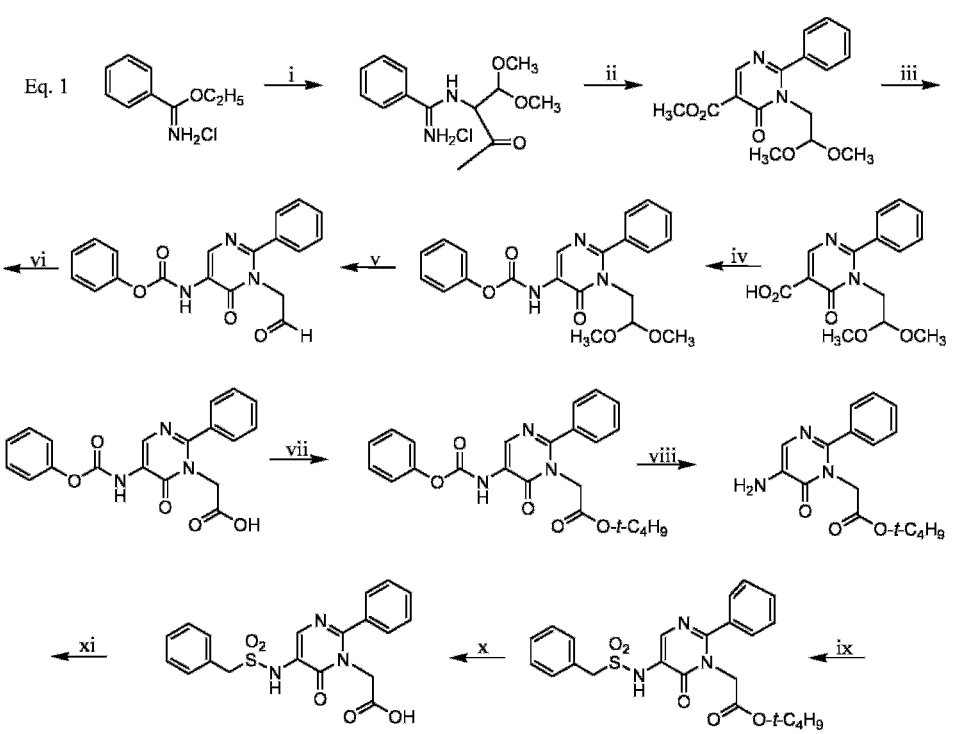
Title Substituted Polycyclic Aryl and Heteroaryl 1,2,4-Triazinones Useful for Selective Inhibition of the Coagulation Cascade
M.S. South *et al.*, US Patent 7,015,223 (March 21, 2006)

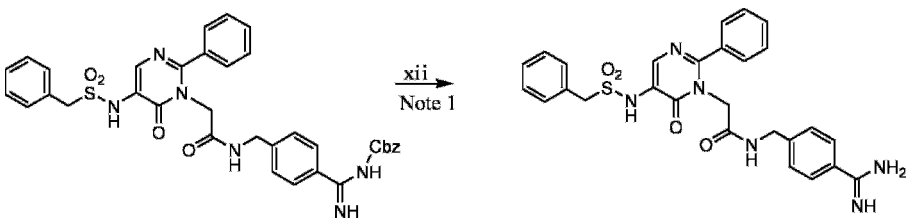
Assignee Pharmacia Corporation

Utility Anticoagulates in the Treatment of Thrombotic Disorders

Invention Significance Pyrimidinone derivatives have been found effective in impeding plasma coagulation by selectively inhibiting serine proteases or protease zymogens associated with either the extrinsic or intrinsic coagulation cascade pathways. These chemical agents are useful in the prevention or treatment of thrombotic conditions including coronary artery and cerebrovascular diseases.

Reaction





i- Methyl alcohol, aminoacetaldehyde dimethyl acetal
ii- Dimethyl methoxymethylenemalonate, methyl alcohol
iii- Pyridine, lithium iodide
iv- 1,4-Dioxane, triethylamine, diphenylphosphoryl azide
v- THF, hydrochloric acid
vi- THF, *t*-butyl alcohol, 2-methyl-2-butene, sodium chlorite, sodium dihydrogen phosphate monohydrate
vii- $CHCl_3$, oxalyl chloride, pyridine, 2-methyl-2-propanol
viii- Methyl alcohol, palladium on carbon, hydrogen
ix- THF, DMF, *N*-methylmorpholine, α-toluenesulfonyl chloride
x- Dioxane, hydrochloric acid
xi- DMF, *N,N*-diisopropylethylamine, *N*-hydroxybenzotriazole, 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride, (Cbz-amidino)benzylamine
xii- Methyl alcohol, hydrochloric acid, dioxane, palladium on carbon, hydrogen

Experimental

1. **Preparation of *N*-(2,2-dimethoxyethyl)benzamidine**

   A solution of ethyl benzimidate hydrochloride (496.9 mmol) in 300.0 ml methyl alcohol was cooled to 0°C, then treated with a solution of aminoacetaldehyde dimethyl acetal (670.9 mmol) in 75 ml methyl alcohol at such a rate that the temperature was kept below 5°C. The solution was stirred 3 days at or below 5°C, then concentrated, and an yellow oil isolated. The residue was dissolved in 750 ml 1 M NaOH, then extracted four times with 250 ml CH_2Cl_2, dried with $MgSO_4$, concentrated, and 108.13 g of crude *N*-(2,2-dimethoxyethyl)benzamidine was isolated as an yellow oil. It was used without further purification.

2. **Preparation of methyl 1-(2,2-dimethoxyethyl)-2-phenylpyrimidin-6(1H)-one-5-carboxylate**

   The Step 1 product (519.2 mmol) dissolved in 125 ml methyl alcohol was treated with dimethyl methoxymethylenemalonate (540.5 mmol), then heated 2 hours to

100°C to slowly distill off the solvent. A dark brown residue was isolated and dissolved in 1000 ml EtOAc, then washed twice with 500 ml saturated NH_4Cl solution, once with 500 ml brine, dried with $MgSO_4$, and concentrated. The residue was purified by MPLC using 25% EtOAc/hexane and the product isolated in 73% yield as a tan oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.73 (s, 1H), 7.59–7.49 (m, 5H), 4.86 (t, $J = 5.5$ Hz, 1H), 4.16 (d, $J = 5.4$ Hz, 2H), 3.95 (s, 3H), 3.32 (s, 6H)
^{13}C NMR (75 MHz, $CDCl_3$) δ 165.9, 164.6, 159.3, 158.2, 134.6, 130.9, 128.93, 128.78, 114.9, 101.4, 56.0, 55.1, 52.7, 49.1
HRMS (ES) Calc. for $C_{16}H_{19}N_2O_5$ 319.1294; found 319.1288

3. **Preparation of 1-(2,2-dimethoxyethyl)-2-phenylpyrimidin-6(1H)-one-5-carboxylate**

The Step 2 product (292.2 mmol) was dissolved in 420.0 ml dry pyridine, then treated with lithium iodide (732.2 mmol) at ambient temperature, then refluxed 2 hours. The mixture was concentrated and the residue was dissolved in 500 ml 1 M HCl. It was extracted four times with 250 ml CH_2Cl_2/methyl alcohol, 4:1, and extracts were washed twice with 250 ml 6 N HCl. The solution was then dried with $MgSO_4$ and concentrated. The residue was purified by recrystallization with EtOAc/hexane and the product isolated in 63% yield as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 12.99 (s, 1H), 8.97 (s, 1H), 7.63–7.51 (m, 5H), 4.78 (dd, $J = 4.3$, 5.5 Hz, 1H), 4.28 (d, $J = 5.4$ Hz, 2H), 3.30 (s, 6H)
^{13}C NMR (75 MHz, $CDCl_3$) δ 165.8, 165.1, 164.1, 159.1, 133.6, 131.5, 129.1, 129.0, 112.6, 101.0, 55.8, 49.2
HRMS (ES) Calc. for $C_{15}H_{17}N_2O_5$ 305.1137; found 305.1113

4. **Preparation of [5-[(benzyloxycarbonyl)amino]-2-phenyl-6-oxo-1,6-dihydro-1-pyrimidinyl] acetaldehyde dimethyl acetal**

A suspension of the Step 3 product (216.67 mmol) in 800 ml 1,4-dioxane was treated with triethylamine (358.7 mmol) followed by diphenylphosphoryl azide (238.5 mmol) at ambient temperature then refluxed 2 hours. Benzyl alcohol (434.8 mmol) was then added and the mixture refluxed an additional 14 hours and was then concentrated. The residue was dissolved in 1500 ml EtOAc then washed twice with 500 ml saturated NH_4Cl solution, once with 500 ml apiece 1 M NaOH and 500 ml brine, dried, and concentrated. The residue was purified by MPLC using 15–30% EtOAc/hexane and the product isolated in 46% yield as a light brown solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (br s, 1H), 7.53–7.32 (m, 11H), 5.20 (s, 2H), 4.68 (t, $J = 5.6$ Hz, 1H), 4.12 (d, $J = 5.6$ Hz, 2H), 3.22 (s, 6H)
^{13}C NMR (100 MHz, $CDCl_3$) δ 158.3, 153.7, 153.2, 135.9, 134.9, 134.7, 130.1, 129.1, 128.9, 128.8, 128.71, 128.66, 128.4, 125.1, 101.3, 67.7, 55.4, 48.6
HRMS (EI) Calc. for $C_{22}H_{24}N_3O_5$ 410.1716; found 410.1741

5. **Preparation of [5-[(benzyloxycarbonyl)amino]-2-phenyl-6-oxo-1,6-dihydro-1-pyrimidinyl] acetaldehyde**

   A solution of the Step 4 product (42.11 mmol) dissolved in 103 ml THF was treated with 35.0 ml 1 M HCl, then refluxed 12 hours, and concentrated. The residue was dissolved in 200 ml water, the pH was adjusted to 7 using solid $NaHCO_3$, and the emulsion was extracted four times with 150 ml CH_2Cl_2. The organic phase was washed with 200 ml water, dried with $MgSO_4$, concentrated, and 15.74 g product isolated. It was used without further purification.

6. **Preparation of [5[(benzyloxycarbonyl)amino]-2-phenyl-6-oxo-1,6-dihydro-1-pyrimidinyl] acetic acid**

   The Step 5 product (42.11 mmol) was added to 198 ml THF/*t*-butyl alcohol/2-methyl-2-butene, 1:1:1.3, cooled to 0°C, then treated with sodium chlorite (331.1 mmol), and sodium dihydrogen phosphate monohydrate (256.7 mmol) dissolved in 102 ml water. The biphasic solution was stirred for 10 minutes at 0°C and then 1 hour at ambient temperature and was then concentrated. The residue was diluted with 200 ml water and the pH adjusted to 3 using saturated $NaHCO_3$ solution and 1 M HCl. The aqueous solution was extracted four times with THF/CH_2Cl_2, 1:2, dried with $MgSO_4$, and concentrated. The residue was purified by trituration with diethyl ether and the product isolated in 88% yield as a white solid.

   ^{1}H NMR (300 MHz, d-DMSO) δ 13.34 (br s, 1H), 9.03 (s, 1H), 7.57–7.34 (m, 10H), 5.23 (s, 2H), 4.56 (s, 2H)
   ^{13}C NMR (75 MHz, d-DMSO) δ 169.4, 158.0, 154.6, 154.3, 137.1, 134.8, 130.9, 129.4, 129.1, 128.78, 128.72, 128.50, 125.5, 67.0, 48.8
   HRMS (EI) Calc. for $C_{20}H_{18}N_3O_5$ 380.1246; found 380.1246

7. **Preparation of [5-amino-2-phenyl-6-oxo-1,6-dihydro-1-pyrimidinyl]acetic acid *t*-butyl ester**

   The Step 6 product (13.84 mmol) in 70.0 ml $CHCl_3$ cooled to approximately 0°C was treated dropwise with 6.00 ml oxalyl chloride, then stirred 5 minutes at 0°C, and an additional 2 hours at ambient temperature. The mixture was concentrated and an yellow residue isolated. The residue was dissolved in 70.0 ml $CHCl_3$, then treated with 1.70 ml pyridine and 2-methyl-2-propanol (36.60 mmol). The solution

was stirred 1 hour at ambient temperature and was then refluxed 12 hours. Once cooled to ambient temperature, it was diluted with 300 ml $CHCl_3$, then washed with 100 ml apiece water, saturated $NaHCO_3$ solution, and brine, dried, and concentrated. The residue was purified by MPLC 25% EtOAc/hexanes and the product isolated in 49% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.81 (br s, 1H), 7.57-7.38 (m, 11H), 5.27 (s, 2H), 457 (s, 2H), 1.47 (s, 9H)
^{13}C NMR (75 MHz, $CDCl_3$) δ 166.4, 158.0, 153.2, 135.9, 135.0, 134.4, 130.6, 129.1, 128.9, 128.7, 128.5, 128.4, 125.4, 83.4, 67.7, 49.1, 28.2
HRMS (EI) Calc. for $C_{24}H_{26}N_3O_5$ 436.1872; found 436.1876

8. **Preparation of [5-amino-2-phenyl-6-oxo-1,6-dihydro-1-pyrimidinyl]acetic acid *t*-butyl ester**

A solution of the Step 7 product (4.282 mmol) in 21 ml methyl alcohol was treated with 211.3 mg 10% Pd/C, then hydrogenated 16 hours under 1 atm hydrogen at ambient temperature. The mixture was filtered through a pad of celite 545, concentrated, triturated from diethyl ether, and the product isolated in 99% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.39 (m, 6H), 4.48 (s, 2H), 4.06 (br s, 2H), 1.39 (s, 9H)
^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 158.6, 149.3, 134.9, 132.9, 130.0, 128.9, 128.5, 127.7, 82.9, 48.7, 28.1
HRMS (EI) Calc. for $C_{16}H_{20}N_3O_3$ 302.1505; found 302.1491

9. **Preparation of [5-α-toluenesulfonamide-2-phenyl-6-oxo-1,6-dihydro-1-pyrimidinyl]acetic acid *t*-butyl ester**

A solution of the Step 8 product (3.418 mmol) in 5.5 ml THF and 2.0 ml DMF was treated with 1.20 ml *N*-methylmorpholine, then stirred 10 minutes at 0°C and then treated over 5 minutes with α-toluenesulfonyl chloride (3.767 mmol) dissolved in 5.5 ml THF. The mixture was stirred 2 hours at 0°C and was then diluted with 150 ml EtOAc. The organic solution was washed twice with 25 ml 1 M HCl, twice with 25 ml saturated $NaHCO_3$ solution, once with 50 ml brine, dried, and concentrated. The yellow residue was triturated with diethyl ether, dried, and the product isolated in 74% yield as a white solid.

^{1}H NMR (400 MHz, d-DMSO) δ 9.34 (s, 1H), 7.76 (s, 1H), 7.55–7.28 (m, 10H), 4.59 (s, 2H), 4.49 (s, 2H), 1.32 (s, 9H)
^{13}C NMR (100 MHz, d-DMSO) δ 167.0, 158.8, 156.5, 142.1, 134.5, 131.7, 131.0, 130.1, 129.4, 129.0, 128.94, 128.58, 124.8, 83.0, 59.6, 49.2, 28.1
HRMS (EI) Calc. for $C_{23}H_{26}NO_3O_5S$ 456.1593; found 456.1597

10. **Preparation of [5-α-toluenesulfonamide-2-phenyl-6-oxo-1,6-dihydro-1-pyrimidinyl]-acetic acid**

The Step 9 product (2336 mmol) was dissolved in 9.0 ml 4 M HCl in dioxane, then stirred 12 hours at ambient temperature. The mixture was concentrated, the residue triturated with diethyl ether, and the product isolated in 87% yield as a white solid.

^{1}H NMR (400 MHz, d-DMSO) δ 9.32 (s, 1H), 7.74 (s, 1H), 7.51–7.30 (m, 10H), 4.58 (s, 2H), 4.48 (s, 2H)
^{13}C NMR (100 MHz, d-DMSO) δ 169.2, 158.7, 156.6, 141.9, 134.5, 131.7, 131.0, 130.1, 129.4, 129.0, 128.90, 128.56, 124.8, 59.6, 48.9
HRMS (EI) Calc. for $C_{19}H_{18}N_3O_5S$ 400.0967; found 400.0959

11. **Preparation of [5-α-toluenesulfonamide-2-phenyl-6-oxo-1,6-dihydro-1-pyrimidinyl]-acetic acid and 4-(Cbz-amidino)benzylamine**

A solution of the Step 10 product (1.018 mmol) in 10.0 ml DMF was treated with *N,N*-diisopropylethylamine (5.167 mmol), *N*-hydroxybenzotriazole (1.241 mmol), and 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (1.232 mmol). After stirring 30 minutes at ambient temperature, the mixture became homogeneous and was then treated with 4-(Cbz-amidino)-benzylamine (1.126 mmol) and stirred an additional 18 hours. The solution was then diluted with 50 ml EtOAc, washed once with 25 ml apiece 5% citric acid, saturated $NaHCO_3$ solution and brine, dried, and concentrated. The residue was purified by trituration with diethyl ether and the product isolated as a white solid.

^{1}H NMR (300 MHz, d-DMSO) δ 9.36–9.18 (br m, 3H), 8.82–8.78 (m, 1H), 7.98 (d, $J = 8.3$ Hz, 2H), 7.84, (s, 1H), 7.56–7.32 (m, 16H), 5.15 (s, 2H), 4.65 (s, 2H), 4.58 (s, 2H), 4.40 (d, $J = 5.4$ Hz, 2H)
HRMS (EI) Calc. for $C_{35}H_{33}N_6O_6S_2$ 665.2182; found 665.2177

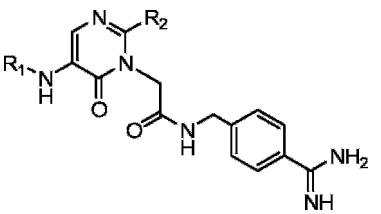
12. **Preparation of [5-α-toluenesulfonamide-2-phenyl-6-oxo-1,6-dihydro-1-pyrimidinyl]-acetic acid amidino benzylamine**

A solution of the Step 11 product (357.6 mmol) in 3.5 ml methyl alcohol/4 M HCl in dioxane, 4:1, was treated with 42.1 mg 10% Pd/C and hydrogenated 16 hours under 1 atm hydrogen at ambient temperature. The mixture was then filtered through a pad of Celite 545 and concentrated. The residue was purified by HPLC, gradient 5–95% acetonitrile/water with 0.1% trifluoroacetic acid, and the product isolated as a white solid.

^{1}H NMR (300 MHz, d-DMSO) δ 9.31–9.28 (m, 4H), 8.88 (br s, 1H), 7.81–7.77 (m, 3H), 7.60–7.54 (m, 5H), 7.43–7.37 (m, 7H), 4.65 (s, 2H), 4.58 (s, 2H), 4.42–4.41 (m, 2H)
HRMS (EI) Calc. for $C_{27}H_{27}O_4S$ 531.1815; found 531.1794

Derivatives

Table 1 Selected pyrimidinone derivatives and their corresponding mass spectral data. ^{1}H NMR product characterization supplied by author



Entry	R_1	R_2	Salt	(m/z+1)
1	Benzylsulfonyl	Phenyl	2TFA[a]	531.18
3	Benzyl	Phenyl	2HCl	481.23
6	*i*-Propyl	2-Aminophenyl	2TFA	598.24
7	Cyclobutyl	Pyridin-3-yl	–	554.25
8	Cyclobutyl	2-Aminophenyl	2TFA	446.23
13	Bethoxyacetyl	Phenyl	–	449.47
14	4-Methylbenzoyl	Phenyl	–	495.54
15	4-Nitrobenzoyl	Phenyl	–	526.52
17	2,4,6-Trimethylbenzoyl	Phenyl	–	523.60
18	Benzoyl	Phenyl	–	481.52
20	*i*-Propyl	3-Amino-5-methoxycarbonyl-phenyl	–	492
21	*i*-Propyl	3-Amino-5-hydroxycarboxy-phenyl	–	478

[a] Trifluoroacetic acid.

Testing

I. *TF-VIIa Assay*

In this assay, 100 nM recombinant soluble tissue factor and 2 nM recombinant human factor VIIa were added to a 96-well assay plate containing 0.4 mM of a selected experimental agent and *N*-methylsulfonyl-D-Phe-Gly-Arg-*p*-nitroaniline

containing either an inhibitor or a buffer. The reaction was immediately measured at 405 nm to determine background absorbance. Thereafter, the plate was incubated 60 minutes at ambient temperature and the rate of substrate hydrolysis measured by monitoring the reaction at 405 nm for the release of *p*-nitroaniline. Percent inhibition of TF-VIIa activity was determined using experimental and control samples. Testing results are provided in Table 2, column 2.

Table 2 Inhibitory activity of selected experimental pyrimidinone agents toward TF-VIIA, thrombin II, factor Xa, and trypsin II

Entry	IC_{50} or Inhibition (%) of TF-VIIa (30 μM)	IC_{50} or Inhibition (%) of Thrombin II TF-VIIa (μM)	IC_{50} or Inhibition (%) of Factor Xa (30 μM)	IC_{50} or Inhibition (%) of Trpysin II (30 μM)
1	15.4	22.4	–	0.5
2	> 30	> 30	–	> 30
3	1.0	1.0	–	0.6
6	0.05	43% at 30 μM	33% at 30 μM	< 0.04
7	0.7	11.3	33% at 30 μM	0.04
8	0.08	42% at 30 μM	15% at 30 μM	0.04
20	0.08	41% at 100 μM	85	0.03
21	0.07	26% at 100 μM	15% at 100 μM	0.05

II. *Thrombin Assay*

Human thrombin (0.28 nM) and 0.06 mM H-D-phenylalanyl-L-pipecolyl-L-arginine-*p*-nitroaniline dihydrochloride were added to a 96-well assay plate containing either an inhibitor or a buffer and a selected experimental agent. Thereafter, the evaluation proceeded using the aforementioned procedure. Testing results are provided in Table 2, column 3.

III. *Xa Assay*

Human factor Xa (0.3 nM) and 0.15 mM *N*-α-benzyloxycarbonyl-D-arginyl-L-glycyl-L-arginine-*p*-nitroanilne dihydrochloride were added to a 96-well assay plate containing either an inhibitor or a buffer and a selected experimental agent. Thereafter the evaluation proceeded using the aforementioned procedure. Testing results are provided in Table 2, column 4.

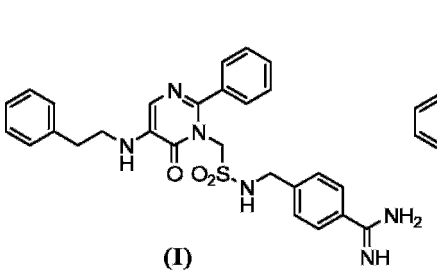
IV. *Trypsin Assay*

Trypsin (5 μg/ml), type IX from porcine pancreas, and 0.375 mM *N*-α-benzoyl-L-arginine-*p*-nitroanilide (L-BAPNA) were added to a 96-well assay plate containing

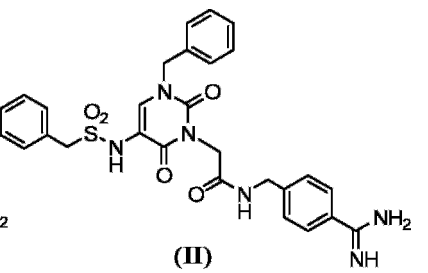
either an inhibitor or a buffer and a selected experimental agent. Thereafter, the evaluation proceeded using the aforementioned procedure. Testing results are provided in Table 2, column 5.

Notes

1. In earlier, (**I**), and subsequent investigations, (**II**), by the authors (1,2), respectively, additional pyrimidinone derivatives were prepared, which were effective as serine protease inhibitors, and used in the treatment of thrombotic disorders.

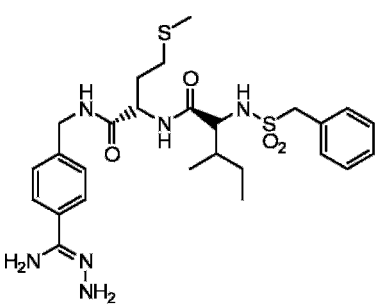


(**I**)

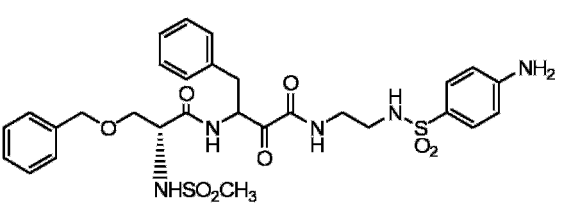


(**II**)

2. Peptide derivatives, (**III**), prepared by Shiraishi (3) selectively inhibited extrinsic blood coagulation by inhibiting factor VIIa. α-Ketoamide peptides, (**IV**), while those prepared by Chatterjee (4) were effective as cysteine and serine proteases inhibitors and used in treating coagulation disorders associated with thrombophlebitis and thrombosis.

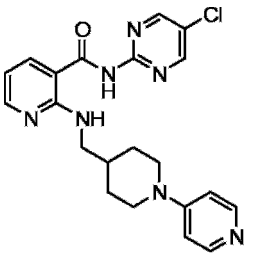


(**III**)



(**IV**)

3. Benzo-heterobicycles, (**IV**), prepared by Wade (5), (**V**), and Pinto (6), (**VI**), respectively, were effective as inhibitors of trypsin-based serine proteases, especially factor Xa, and used in in the treatment of thrombotic disorders.



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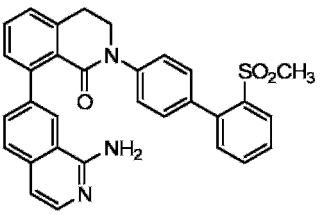
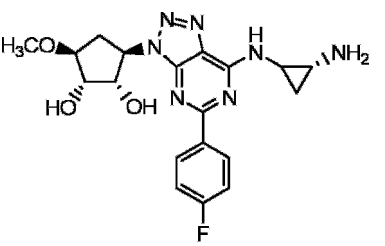


(VI)

4. Triazolo[4,5-d]pyrimidine compounds, (**VII**), prepared by Harden (7) were effective in controlling platelet-mediated occlusion and useful in treating thrombolysis compromised by reocclusion.



(VII)

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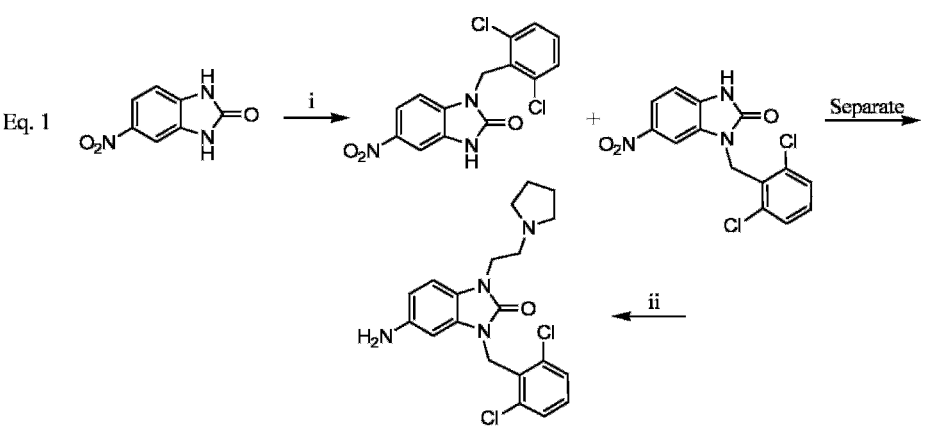
2. M.S. South *et al*., US Patent 7,015,230 (March 21, 2006)
3. T. Shiraishi *et al*., US Patent 7,001,887 (February 21, 2006)
4. S. Chatterjee *et al*., US Patent 7,001,907 (February 21, 2006) and US Patent 6,703,368 (March 9, 2004)
5. D.W. Wade *et al*., US Patent 6,689,780 (February 10, 2004)
6. D.J.P. Pinto, US Patent 6,998,408 (February 14, 2006) and US Patent 6,960,595 (November 1, 2005)
7. D. Harden *et al*., US Patent 6,974,868 (December 13, 2005)

D. Thrombin Receptor, PAR-1, Inhibitor

Title Benzimidazolone Peptidomimetics as Thrombin Receptor Antagonists
H.-C. Zhang *et al*., US Patent 6,943,149 (September 13, 2005)
Assignee Ortho McNeil Pharmaceutical, Inc.
Utility Treatment Platelet-mediated Thrombotic Disorders

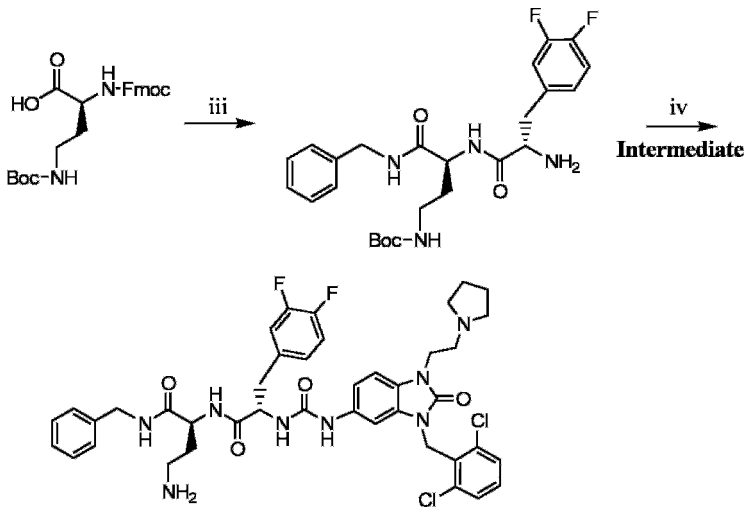
Invention Significance Activation of the human thrombin receptor, PAR-1, results in the formation of platelet aggregation causing thrombotic disorders such as myocardial infarction, stroke, angina, and atherosclerosis. Benzimidazolone peptidomimetic derivatives effective as thrombin receptor antagonists have been prepared to address this disorder.

Reaction



i- DMF, sodium hydride, 2,6-dichlorobenzylbromide, water
ii- DMF, sodium hydride, 2-chloroethylpyrrolidine hydrochloride, methyl alcohol, ferric chloride hexahydrate, charcoal, 1,1-dimethyl hydrazine

Eq. 2



iii- Acetonitrile, benzylamine, 1,2-dicyclohexylcarbodiimide, hydroxybenzotriazole, diisopropylcarbodiimide, Fmoc-3,4-difluorophenylalanine, diethylamine

iv- 4-Nitrophenyl chloroformate, CH_2Cl_2, diisopropylethylamine, trifluoroacetic acid

Experimental

1. **Preparation of 1-(2,6-dichlorobenzyl)-5-nitroimidazolone**

   5-Nitroimidazolone (8.04 mm) was dissolved in 20 ml DMF, then cooled to 0°C, and treated portionwise with an oil dispersion of 75% NaH (8.86 mmol). The mixture was stirred 5 minutes, then treated with 2,6-dichlorobenzylbromide (8.0 mmol) dissolved in 20 ml DMF, and the reaction stirred 30 minutes at 0°C and an additional 3 hours at ambient temperature. The solution was poured into 700 ml water and 2.38 g of a white solid consisting of a mixture of isomers, 1:1, was isolated. Isomers were separated by flash column chromatography using CH_2Cl_2/EtOAc, 2:1, and each product isolated.

2. **Preparation of 1-(2,6-dichlorobenzyl)-3-[2-(1-pyrrolidinyl)ethyl]-6-aminoimidazolone**

   The Step 1 product (105 mg) dissolved in 6 ml dry DMF was treated with 75% NaH (0.94 mmol), then stirred 5 minutes, and treated with 2-chloroethylpyrrolidine hydrochloride (0.60 mmol). The mixture stirred 2 hours at 50°C and was then partitioned between $CHCl_3$/2-propyl alcohol/water, 10:1:1, and the organic phase isolated. The solution was washed twice with water, once with brine, dried with K_2CO_3, then concentrated, and 130 mg of a light yellow solid isolated. The residue was dissolved in 8 ml methyl alcohol, then treated with ferric chloride hexahydrate (0.11 mmol), charcoal (5 mmol), and 1,1-dimethyl hydrazine (25 mmol), then refluxed 3 hours.

The solution was then cooled, filtered through dicalite, concentrated, and an oil isolated. The residue was dissolved in 25 ml 1 M HCl, then washed twice with diethyl ether, made alkaline with dilute NaOH, and extracted with CH_2Cl_2. The solution was rewashed twice with $NaHCO_3$ solution and brine, dried, concentrated, and 100 mg of product isolated.

3. **Preparation of benzenepropanamide, *N*-[(1*S*)-3-NH-Boc-1-[(phenylmethyl) amino-carbonyl]propyl]-2,3-dihydro-2-oxo-1H-benzimidazol-5-yl-amino carbonyl]amino-3,4-difluoro-**

Fmoc-α-*N*-Boc-γ-*N*-diaminobutyric acid (24.5 mmol) was stirred in 300 ml acetonitrile and treated sequentially with hydroxylbenzotriazole (24.4 mmol), benzylamine (24.3 mmol), and 1,2-dicyclohexylcarbodiimide (48.7 mmol), then stirred 3 hours at ambient temperature. A white solid was isolated, which was washed with 16 ml cold acetonitrile, then stirred 2 hours in 500 ml acetonitrile containing 25 ml diethylamine. The mixture was concentrated, then triturated three times with 400 ml hexane, and a white solid obtained. The solid was redissolved in 400 ml acetonitrile, then treated sequentially with hydroxybenzotriazole (19.1 mmol), Fmoc-3,4-difluorophenylalanine (19.1 mmol), and 1,2-dicyclohexylcarbodiimide (38.2 mmol) and stirred 16 hours at ambient temperature. The solution was then cooled in an ice bath and a white solid was isolated. The solid was washed with cold acetonitrile, then stirred 5 hours in 350 ml acetonitrile containing 35 ml diethylamine, and concentrated to a liquid. The liquid was triturated three times with hexane, dissolved in 250 ml $CHCl_3$, dried with Na_2SO_4, reconcentrated, and 8.0 g of product obtained.

4. **Preparation of benzenepropanamide, *N*-[(1*S*)-3-amino-1-[(phenylmethyl) amino]-carbonyl]propyl]-α-[[[[3-(2,6-dichlorophenyl)methyl]-2,3-dihydro-2-oxo-1-[2-(1-pyrrolidinyl)ethyl]-1H-benzimidazol-5-yl]amino]carbonyl]amino-3,4-difluoro-, (αS)-(1)**

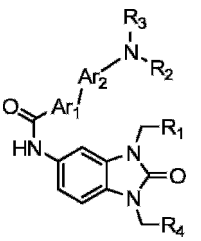
4-Nitrophenyl chloroformate (0.10 mm) was dissolved in 5 ml CH_2Cl_2, then cooled to −20°C, and treated with the Step 2 product (0.10 mmol) and diisopropylethylamine (0.10 mmol) dissolved in 1.5 ml CH_2Cl_2. The mixture was stirred for 30 minutes and then treated with the Step 3 product (0.10 mmol) and diisopropylethylamine (0.10 mmol) dissolved in 2 ml CH_2Cl_2, and stirred 30 minutes at −20°C and 16 hours at ambient temperature. The mixture was concentrated and 45 mg of a light yellow solid isolated. The solid was dissolved in 7 ml CH_2Cl_2 containing anisole (10 mg), then treated with 3 ml trifluoroacetic acid, and stirred 90 minutes at ambient temperature. The mixture was concentrated, then triturated three times with diethyl ether, and the product isolated as an oil.

^{1}H NMR (CD_3OD) δ 1.9–2.2 (m, 6H), 2.9–3.1 (m, 3H), 3.1–3.3 (m, 4H), 3.6 (dd, 2H), 3.8 (m, 2H), 4.25 (dd, 2H), 4.35 (s, 2H), 4.45 (dd, 2H), 5.32 (s, 2H), 7.0–7.5 (m, aromatics)
ES-MS *m/z* 821 (MH^+)
FAB-HRMS Calc. for $C_{41}H_{44}Cl_2F_2N_8O_4 + H^+$ 821.2909; found 821.2918

Derivatives

Selected five- and six-substituted benzimidazolone peptidomimetics derivatives are provided in Tables 1 and 2, respectively.

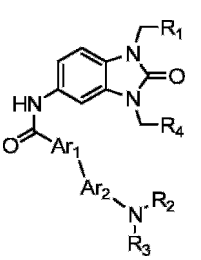
Table 1 IC_{50} values for selected five-substituted benzimidazolone peptidomimetics in a thrombin receptor binding assay versus IC_{50} values for platelet aggregation stimulated by thrombin. In all cases, amino acids have and L-absolute configuration. ^1H NMR data supplied by author



Entry	R_1	R_2	Ar_1	$Ar_2NR_2R_3$	IC_{50} (μM) Thr GFP Aggr[a]	IC_{50} (μM) Thr Receptor Bdg[b]
2	$CH_2N(CH_2)_4$	2-Me-Ph	3,4-DiF-Phe[c]	Dbu[d]-NHBn	7	3
3	CH_2NMe_2	2-Me-Ph	3,4-DiF-Phe	Dbu-NHBn	7.7	1.9
7	$CH_2N(CH_2)_4$	2,6-DiCl-Ph[e]	3,4-DiF-Phe	Dbu-NHBn	5.5	1.7

[a]Thrombin-induced gel-filtered platelet aggregation assay.
[b]Thrombin receptor (PAR-1) binding assay.
[c]3,4-Difluorophenylalanine.
[d]2,4-Diaminobutyric acid.
[e]2,6-Dichlorophenyl.

Table 2 IC_{50} values for selected six-substituted benzimidazolone peptidomimetics in a thrombin receptor binding assay versus IC_{50} values for platelet aggregation stimulated by thrombin. In all cases amino acids have an L absolute configuration. ^1H NMR data supplied by author



Entry	R_1	R_4	Ar_1	$Ar_2NR_2R_3$	IC_{50}(μM) Thr GFP Aggr[a]	IC_{50} (μM) Thr Receptor Bdg[b]
1	$CH_2N(CH_2)_4$	2,6-DiCl-Ph[c]	3,4-DiF-Phe[d]	Dbu[e]-NHBn	0.39	1.6
4	CH_2NMe_2	2-Me-Ph	3,4-DiF-Phe	Dbu-NHBn	0.23	0.7
5	$CH_2N(CH_2)_4$	2-Me-Ph	3,4-DiF-Phe	Dbu-NHBn	0.28	0.4
6	$CH_2N(CH_2)_4$	4-F-Ph	3,4-DiF-Phe	Dbu-NHBn	6	0.6
8	$CH_2N(CH_2)_4$	4-F-Ph	3,4-DiF-Phe	4-PyrAla[f]-$H(CH_2)_2NH_2$	5.8	0.5

[a] Thrombin-induced gel-filtered platelet aggregation assay.
[b] Thrombin receptor (PAR-1) binding assay.
[c] 2,6-Dichlorophenyl.
[d] 3,4-Difluorophenylalanine.
[e] 2,4-Diaminobutyric acid.
[f] 4-Pyridylalanine.

Testing

I. *In Vitro Thrombin Receptor (PAR-1) Binding Assay*

CHRF membranes described by Jones (1) were thawed, centrifuged, washed with binding buffer consisting of 50 mM HEPES containing 5 mM $MgCl_2$ and 0.1% BSA, and resuspended in 25 μg/100 ml binding buffer. Membranes (100 μl) were added to 24-Wallac plates and delivered to the Tomtech apparatus. In a typical experiment, a 6 μl experimental agent sample from a 125 μg/ml intermediary plate containing 20% DMSO and 44 μl buffer was delivered to the plate. Similarly, 6 μl 20% DMSO and 44 μl buffer were delivered to both column 1, NSB, and column 12, TB. Ten microliters Ser-pFPhe-Har-Leu-Har-Lys-Tyr-NH_2 and 500 μl SEQ ID NO 1 in deionized water were added to column 1. Fifty microliters tritiated SEQ ID NO 1, specific activity 46 Ci/mmol, was added to all the wells. The plates were mixed, incubated 30 minutes, and harvested with 10 mM HEPES/138 mM NaCl using the Skatron harvester. The filters were presoaked 3 hours in 0.5% polyethylenimine in HEPES/0.1 M *N*-acetylglucosamine, dried in a microwave, and placed in sample bags. Scintillation fluid (4.5 ml) was added, the bags sealed, placed in filter cassettes,

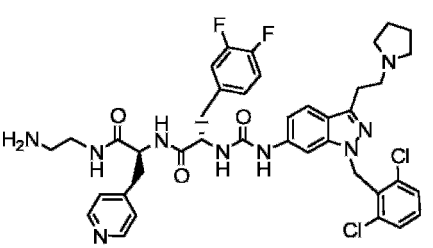
and analyzed with a microbeta counter. Testing results are provided in Tables 1 and 2.

II. *In Vitro Inhibition of Thrombin-induced Gel-filtered Platelet Aggregation Assay*

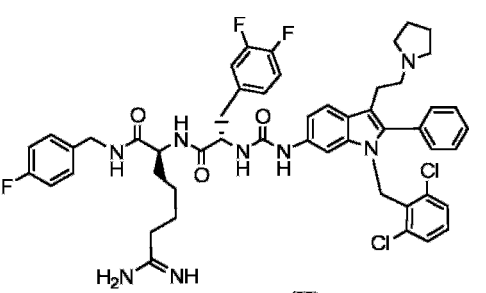
The percentage of platelet aggregation was calculated as the increase in light transmission of experimental agent-treated platelet concentrate versus control-treated platelet concentrate. Human blood was obtained from drug-free normal donors in tubes containing 0.13 M sodium citrate. Platelet-rich plasma was collected by centrifugation of whole blood, gel filtered through Sepharose 2B, and the platelet count adjusted to 2×10^7 platelets per sample. The following constituents were added to a siliconized cuvette: concentrated platelet filtrate and Tyrode's buffer consisting of 0.14 M NaCl, 0.0027 M KCl, 0.012 M $NaHCO_3$, 0.76 mM Na_2HPO_4, 0.0055 M glucose, 2 mg/ml BSA, and 5.0 mM HEPES at pH 7.4, in an amount equal to 350, 50 μl of 20 mM calcium and 50 μl of the experimental agent, respectively. Aggregation was monitored in a BIODATA aggregometer for 3 minutes following the addition of a selected experimental agent. Testing results are provided in Tables 1 and 2.

Notes

1. Additional PAR-1 inhibitors consisting of indazole, (**I**), and indole peptidomimetic derivatives, (**II**), were prepared by the author (2,3), respectively, and used in the treatment of platelet-mediated thrombotic disorders.

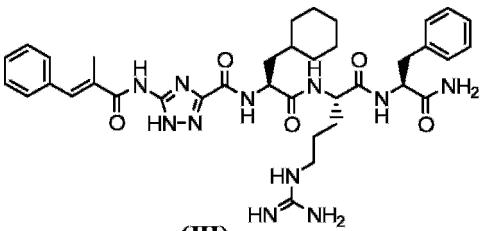


(I)

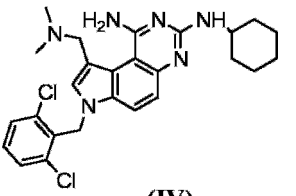


(II)

2. Triazole acyl-tripeptides, (**III**), prepared by McComsey (4) and aminomethyl-pyrroloquinazoline derivatives, (**IV**), prepared by Maryanoff (5) were effective as PAR-1 antagonists and used for treating PAR-1-mediated disorders.

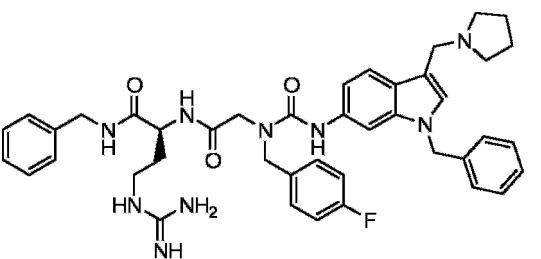


(III)



(IV)

3. Indazole and indole urea-peptoid derivatives, (**V**), prepared by McComsey (6) were effective as PAR-1 antagonists and used to mediate platelet thrombotic disorders.



(V)

References

1. E. Jones, Biochim. Biophys. Acta 1136, 272 (1992)
2. H.-C. Zhang *et al*., US Patent 7,049,297 (May 23, 2006)
3. H.-C. Zhang *et al*., US Patent 6,858,577 (February 22, 2005)
4. D.F. McComsey *et al*., US Patent 6,747,127 (June 8, 2004)
5. B.E. Maryanoff *et al*., US Patent 6,740,657 (May 25, 2004)
6. D.F. McComsey *et al*., US Patent 6,365,617 (June 8, 2002)

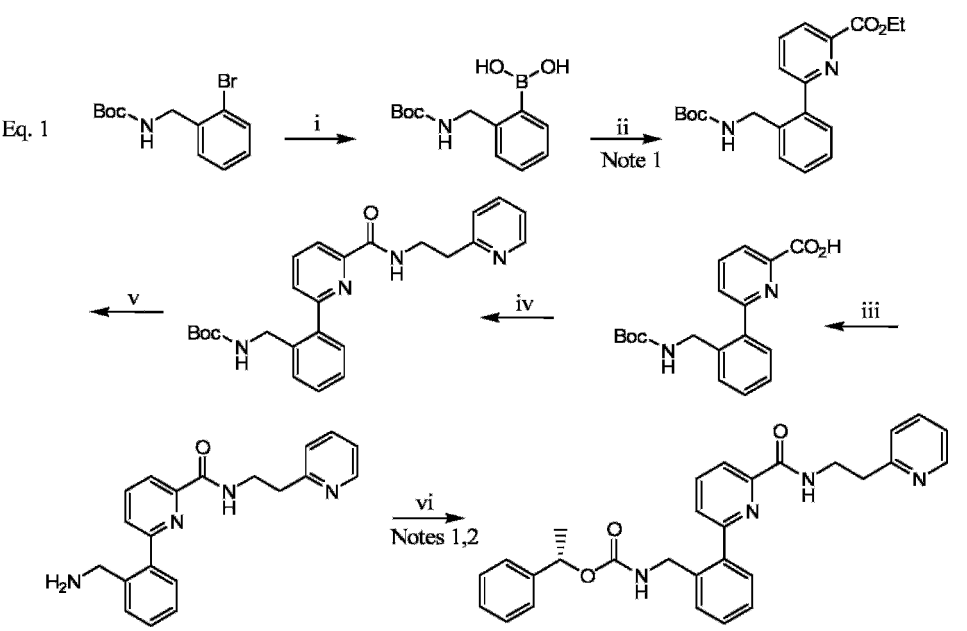
II. Arrhythmia

A. Potassium Channel Blockers of the Kv1.5 Channel

Title Ortho, Meta-substituted Bisaryl Compounds, Processes for Their Preparation, Their Use as Medicaments, and Pharmaceutical Preparations Comprising Them
S. Peukert *et al.*, US Patent 6,924,392 (August 2, 2005)
Assignee Aventis Pharma Deutschland GmbH
Utility Treatment of Arrhythmia

Invention Significance Atrial fibrillation (AF) and atrial flutters are the most persistent cardiac arrhythmias. While current potassium channel blockers of the Kv1.5 channel are effective as antiarrhythmics and reduce the reoccurrence rate of AF, their use is restricted because of potential proarrhythmic side effects. To address this problem, Kv1.5 channel blockers have been prepared, which are effective in terminating existing AF and atrium flutters while not inducing the formation of new fibrillation events.

Reaction



i- *N*-Boc-2-bromobenzylamine, THF, methyl lithium, *t*-butyllithium, trimethylborate
ii- 1,2-Dimethoxyethane, ethyl 6-bromopicolinate, tetrakistriphenylphosphine palladium
iii- Methyl alcohol, lithium hydroxide
iv- Triethylamine, *N,N*-dimethylaminopyridine, 2-pyridinyl-ethylamine, *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride
v- CH_2Cl_2, trifluoroacetic acid
vi- CH_2Cl_2, triethylamine, (*S*)-2-phenylethyloxy-carbonyloxysuccinimide

Experimental

1. **Preparation of 2-(*t*-butoxycarbonylaminomethyl)phenylboronic acid**

N-Boc-2-bromobenzylamine (20 mmol) dissolved in THF and cooled to −78°C was treated with 13.75 ml MeLi (22 mmol) followed *t*-BuLi (42 mmol) 60 minutes later, then further treated with trimethyl borate (80 mmol) after an additional 60 minutes. The mixture was warmed to ambient temperature and treated with HCl to pH 6, then extracted with CH_2Cl_2. The extract was washed with saturated brine, dried, and the product isolated in 100% yield as an yellow solid foam.

MS (FAB, sample treated with glycerol) $m/z = 308$ (M + 57), 252 (M + 1)

2. **Preparation of ethyl 6-[2-(*t*-butoxycarbonylaminomethyl)phenyl]pyridine-2-carboxylate**

Tetrakistriphenylphosphine palladium (0.41 mmol) and ethyl 6-bromopicolinate (8.3 mmol) were added to 83 ml 1,2-dimethoxyethane, then treated with the Step 1 product (12.5 mmol) followed by 8.3 ml 2 M Na_2CO_3 solution. The mixture was refluxed 18 hours, then cooled, and diluted with CH_2Cl_2. The solution was then washed with water, dried, and concentrated. The residue was purified by chromatography with silica gel and the product isolated in 72% yield as a viscous orange oil.

^{1}H NMR ($CDCl_3$) δ 8.13 (1H, dd, $J = 7.7$, 1.1 Hz); 7.96 (1H, t, $J = 7.7$ Hz); 7.7 (1H, dd, $J = 7.7$, 1.1 Hz); 7.74 (1H, d, $J = 7.7$ Hz); 7.52–7.38 (3H, m); 7.04 (1H, m); 4.54 (2H, q, $J = 7.0$ Hz); 4.22 (2H, m); 1.46 (9H, s); 1.44 (3H, t, $J = 7.0$ Hz)
MS (ES+) $m/z = 357$ (M + 1)

3. **Preparation of 6-[2-(*t*-butoxycarbonylaminomethyl)phenyl]pyridine-2-hydroxcarbonyl (general hydrolysis procedure)**

   The Step 2 product (1 equiv.) dissolved in 5 ml methyl alcohol/THF, 3:1, was treated with 1 M LiOH (2 equiv.) and the mixture stirred overnight at ambient temperature. The solution was diluted with water and the pH lowered to 3–4 using $KHSO_4$. The solution was extracted with CH_2Cl_2, dried, concentrated, and the product isolated.

4. **Preparation of 6-[2-(*t*-butoxycarbonylaminomethyl)phenyl]-2-(2-pyridin-2-ylethyl)-amide (general amidation procedure)**

   The Step 3 product (1 equiv.) dissolved in CH_2Cl_2 (20 ml/mmol) was treated with triethylamine (2 equiv.), *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (1.2 equiv.), *N,N*-dimethylaminopyridine (0.2 equiv.) and 2-pyridinyl-ethylamine (1.2 equiv.), then stirred overnight at ambient temperature. The solution was washed with water, then concentrated. The residue was purified by RP-HPLC and the product isolated.

5. **Preparation of 6-[(2-aminomethyl)phenyl]-2-(2-pyridin-2-ylethyl)amide (General Deprotection Procedure)**

   The product from Step 4 (1 equiv.) was dissolved in CH_2Cl_2/trifluoroacetic acid, 3:1 (10 ml/mmol), then stirred 3 hours at ambient temperature. The solution was concentrated, the residue co-evaporated with toluene, and the product used without further purification.

6. **Preparation of (*S*)-1-(phenylethyl){2-[6-(2-pyridin-2-ylethylcarbamoyl)pyridin-2-yl]benzyl}carbamate**

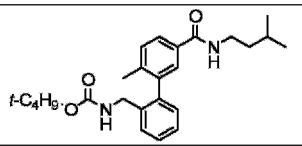
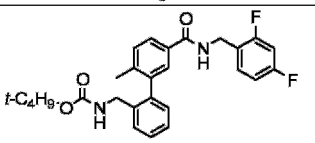
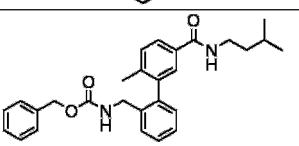
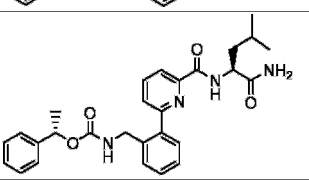
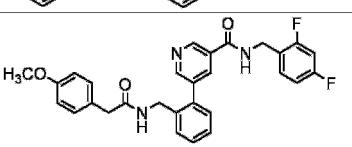
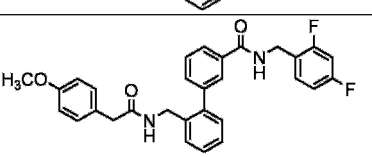
   The Step 5 product (0.06 mmol) dissolved in 3 ml CH_2Cl_2 was treated with triethylamine (0.07 mmol) and (*S*)-2-phenylethyloxy-carbonyloxysuccinimide (0.17 mmol), then stirred 18 hours. The solution was diluted with 15 ml CH_2Cl_2, washed with saturated $NaHCO_3$ solution, dried, and concentrated. The residue was purified by RP-HPLC and the product isolated in 28% yield as the bistrifluoroacetic acid salt.

   ^{1}H NMR ($CDCl_3$) δ 8.60 (1H, m), 8.05–7.86 (3H, m), 7.58–7.29 (13H, m), 5.75 (1H, q, $J = 6.6$ Hz), 5.65 (1H, br s), 4.22 (2H, m), 3.93 (2H, t, $J = 4.9$ Hz), 3.84 (2H, m), 1.48 (3H, d, $J = 6.6$ Hz)
   MS (ES+) $m/z = 481$ (M + 1)

Derivatives

Selected derivatives are provided in Table 1.

Table 1 Selected Step 6 experimental derivatives and accompanying mass spectra data and Kv1.5 channel inhibition concentration, IC_{50}. ^1H NMR for all entries supplied by the author. Entries 1, 13, 19, and 27 are especially preferred as potassium channel blockers of the Kv1.5 channel

Entry	Structure	MS (ES+): *m/z* (M+1)	IC_{50} (μm)
1		382	6.7
2		453	<100
13		487	7
19		489	2
25		501	<100
27		502	6

Testing

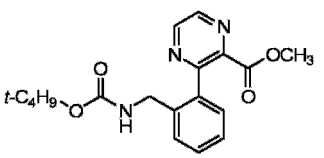
I. *Inhibition Concentration Determination for the Kv1.5 Channel*

Kv1.5 channels from humans were expressed in Xenopus oocytes. To this end, oocytes from *Xenopus laevis* were first isolated and defolliculated. RNA coding for Kv1.5 synthesized in vitro was then injected into these oocytes. After a Kv1.5 protein expression for 1–7 days, Kv1.5 currents were measured using the two-microelectrode voltage clamp technique. The Kv1.5 channels were activated using voltage jumps to 0 and 40 mV lasting 500 milliseconds. Selected experimental agents were evaluated at different concentrations and the percentage inhibition of Kv1.5 control current assayed

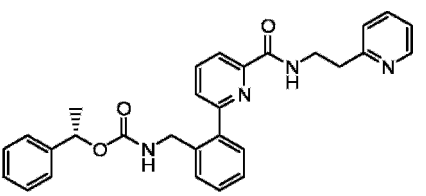
using the Hill equation to determine the inhibition concentration, IC_{50}. Testing results are provided in Table 1.

Notes

1. Pyrazine, (**I**), and pyridine derivatives, (**II**), prepared by the author (1,2), respectively, in earlier investigations were effective for the treatment and prophylaxis of atria arrhythmias.

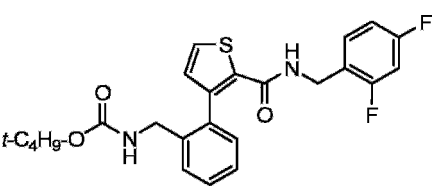


(I)



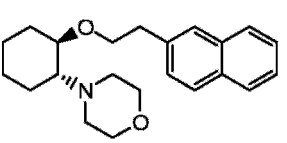
(II)

2. In a subsequent investigation by the author (3) arylated furan- and thiophenecarboxamides, (**III**), were prepared and were effective in the treatment of atrial fibrillation or atrial flutters.

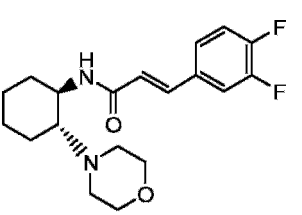


(III)

3. Morpholino derivatives, (**IV**) and (**V**), prepared by Beatch, (4,5), respectively, were effective as ion channel modulating agents and used in the treatment of arrhythmia.



(IV)



(V)

References

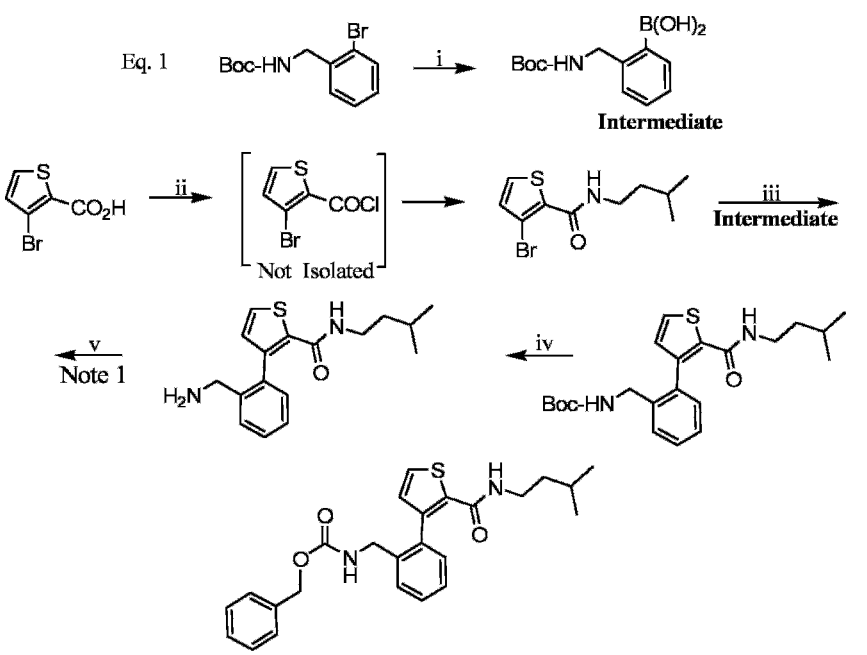
1. S. Peukert *et al*., US Patent 6,794,377 (September 21, 2004)
2. S. Peukert *et al*., US Patent 6,605,625 (August 12, 2003)
3. S. Peukert *et al*., US Patent 6,982,279 (January 3, 2006)
4. G.N. Beatch *et al*., US Patent 7,057,053 (June 6, 2006)
5. G.N. Beatch *et al*., US Patent 7,053,087 (May 30, 2006)

B. Ultrarapidly Activating Potassium Channel Blockers of the Kv1.5 Channel

Title Arylated Furan- and Thiophenecarboxamides, Processes for Their Preparation, Their use as Medicaments, and Pharmaceutical Preparations Comprising Them
S. Peukert *et al.*, US Patent 6,982,279 (January 3, 2006)
Assignee Aventis Pharm Deutschland GmbH
Utility Treatment of Arrhythmia

Invention Significance Class III antiarrhythmics mainly or exclusively block the rapidly activating potassium channel IK_r present in cells in both the human ventricle and atrium. Many of these agents, however, have an increased proarrhythmic risk thereby increasing proarrhythmic risk at low or normal heart rates. To address this concern, Kv1.5 potassium channel blockers have been prepared that ultrarapidly activate delayed rectifiers and inhibit a designated potassium current in the human atrium without increased susceptibility of new fibrillation events.

Reaction



i- THF, methyl lithium, *t*-butyl lithium, trimethyl borate
ii- Thionyl chloride, toluene, 3 methylbutylamine
iii- 1,2-Dimethoxyethane, tetrakis(triphenylphosphorus)palladium, sodium carbonate
iv- CH_2Cl_2, trifluoroacetic acid
v- CH_2Cl_2, triethylamine, benzyloxycarbonyloxysuccinimide

Experimental

1. **Preparation of 2-(*t*-butoxycarbonylaminomethyl)phenylboronic acid**

   N-Boc-2-bromobenzylamine (20 mmol) dissolved in THF previously cooled to −78°C was treated with methyl lithium (22 mmol) then 60 minutes and further treated with *t*-BuLi (42 mmol). The mixture was stirred an additional 60 minutes then treated with trimethyl borate (80 mmol) and the mixture warmed to ambient temperature. The mixture pH was raised to 6 using dilute HCl and the solution was then extracted with CH_2Cl_2. The extract was washed with brine and the product isolated in 100% yield as a pale yellow solid foam.

   MS (FAB, sample treated with glycerol) $m/z = 308$ (M + 57), 252 (M + 1)

2. **Preparation of 3-bromothiophene-2-carboxlic acid 3-methylbutylamide (General Procedure)**

   A mixture of 3-bromo-2-thienyl carboxylic acid (2.5 mmol) and 3 ml of thionyl chloride was refluxed 4 hours, then concentrated. The residue was co-evaporated twice with toluene, then extracted with 12.5 ml CH_2Cl_2, and treated with 3-methylbutylamine (3 mmol) and triethylamine (5.5 mmol). The mixture was stirred overnight and was then washed with $NaHCO_3$ solution, dried, concentrated, and around 1.5–2.5 mmol product isolated and used without further purification.

3. **Preparation of *t*-butyl {2-[2-(3-methylbutylcarbamoyl)thiophen-3-yl]benzyl} carbamate**

   Tetrakis(triphenylphosphorus)palladium (0.05 mmol) and the Step 2 (1 mmol) product were dissolved in 10 ml of 1,2-dimethoxyethane and after 10 minutes treated with the Step 1 product (1.5 mmol) and 1 ml 2 M Na_2CO_3 solution. The mixture was refluxed for 18 hours and was then diluted with CH_2Cl_2. The solution was washed with water, then purified by chromatography using RP-HPLC, and the product isolated in 13% as a viscous colorless oil.

^{1}H NMR ($CDCl_3$) δ 7.55–7.21 (5H, m), 6.90 (1H, d, $J = 4.8$ Hz), 5.58 (1H, brs), 4.82 (1H, brs), 4.10 (2H, d, $J = 6.2$ Hz), 3.18 (2H, m), 1.40 (9H, s), 1.16 (1H, m), 1.07 (2H, q, $J = 7.0$ Hz), 0.75 (6H, d, $J = 6.2$ Hz)
MS (ES+) *m/z* 403 (M + 1), 303 (M−99).

4. **Preparation of 3-(2-aminomethylphenyl)thiophene-2-carboxylic acid (3-methylbutyl)-amide (General Procedure)**

   The product from Step 4 (1 equiv.) was dissolved in CH_2Cl_2/trifluoroacetic acid, 3:1, then stirred 3 hours at ambient temperature and concentrated. The residue was co-evaporated with toluene and the product was isolated and used without further purification.

5. **Preparation of benzyl {2-[2-(3-methylbutylcarbamoyl)thiophen-3-yl]-benzyl} carbamate**

   The Step 4 product (0.09 mmol) was dissolved in 3 ml CH_2Cl_2, then treated with triethylamine (0.1 mmol) and benzyloxycarbonyloxysuccinimide (0.1 mmol), then stirred 18 hours at ambient temperature. The solution was diluted with 20 ml CH_2Cl_2, then washed with saturated $NaHCO_3$ solution, dried, and concentrated. The residue was purified by RP-HPLC and the product isolated in 70% yield.

^{1}H NMR ($CDCl_3$) δ 7.54–7.24 (10H, m), 6.90 (1H, d, $J = 4.8$ Hz), 5.52 (1H, br s), 5.05 (3H, br s), 4.17 (2H, d, $J = 6.2$ Hz), 3.16 (2H, m), 1.14 (1H, m), 1.07 (2H, q, $J = 7.0$ Hz), 0.73 (6H, d, $J = 6.2$ Hz)
MS (ES+) *m/z* 437 (M + 1)

Derivatives

Table 1 Selected Step 2 intermediates prepared by condensing 3-bromo-2-thienylacetyl chloride with a primary amine

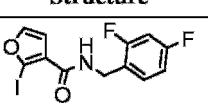
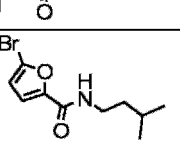
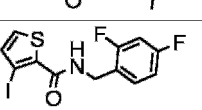
Step 2 Entry	Structure	MS (ES+): *m/z*
4		364
5		260
9		380

Table 2 Selected Step 3 intermediates prepared by palladium-catalyzed Suzuki coupling

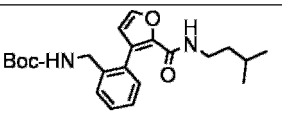
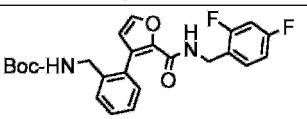
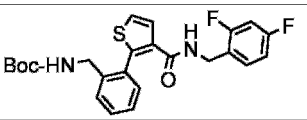
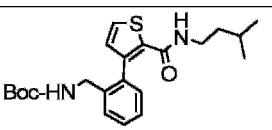
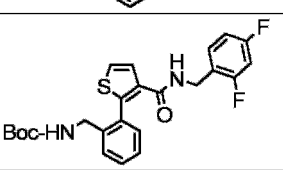
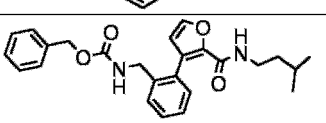
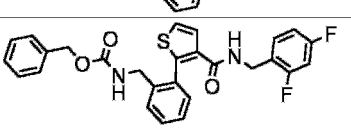
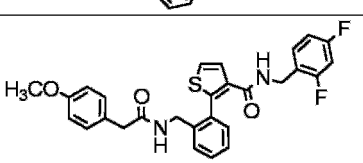
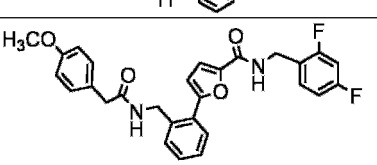
Step 3 Entry	Structure	MS (ES+), *m/z*
4		387
5		459
9		442

Table 3 Selected thiophen-3-yl-benzylcarbamate derivatives and their corresponding mass spectral characterization data. All derivatives were effective as antiarrhythmic potassium channel blocker agents

Product Entry	Structure	MS (ES+), *m/z*
1		437
5		459
13		421
16		493
23		507
29		491

Testing

I. *Inhibition Concentration Determination for Kv1.5 Channel*

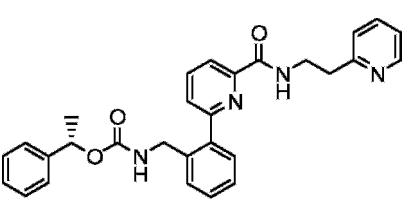
Kv1.5 channels from humans were expressed in Xenopus oocytes. To this end, oocytes from *Xenopus laevis* were first isolated and defolliculated. RNA coding for Kv1.5 synthesized in vitro was then injected into these oocytes. After a Kv1.5 protein expression for 1–7 days, Kv1.5 currents were measured using the two-microelectrode voltage clamp technique. The Kv1.5 channels were activated using voltage jumps to 0 and 40 mV lasting 500 milliseconds. Selected experimental materials of the current invention were evaluated at different concentrations and the percentage inhibition of Kv1.5 control current assayed using the Hill equation to determine the inhibition concentration, IC_{50}. Testing results are provided in Table 4.

Table 4 Selected experimental agents effective as antiarrhythmic potassium channel blockers and corresponding IC_{50} values

Entry	IC_{50} (μM)
1	4
5	3.1
13	5.6
16	1.9
23	3.9
29	4.2

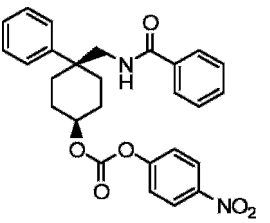
Notes

1. In an earlier investigation by the authors (1), pyridine derivatives, (**I**), were prepared, which were effective as antiarrhythmia agents.



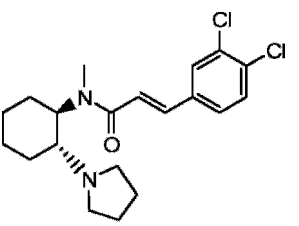
(I)

2. Ultrarapidly activating potassium Kv1.5 channel blockers consisting of benzamide derivatives, (**II**), prepared by Baker (4) were effective in treating atrial fibrillation disorders.



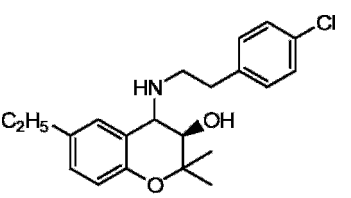
(II)

3. Aminocycloalkyl cinnamide derivatives, (**III**), prepared by Beatch (3) were effective in the treatment and termination of atrial fibrillation/flutter arrhythmia events.



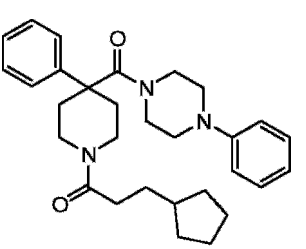
(III)

4. Substituted benzopyran derivatives, (**IV**), prepared by Ohara (4) were effective as potassium Kv1.5 channel blockers and used in the treatment of arrhythmic events.



(IV)

5. Piperidinyl derivatives, (**V**), prepared by Lloyd (5) were effective as ultrarapidly activating potassium Kv1.5 channel blockers and used in the treatment of arrhythmic events.



(V)

References

1. S. Peukert *et al*., US Patent 6,924,392 (August 2, 2005) and US Patent 6,794,377 (September 21, 2004)
2. R.K. Baker *et al*., US Patent 6,632,836 (October 14, 2003)
3. G.N. Beatch *et al*., US Patent 7,053,087 (May 30, 2006)
4. Y. Ohara *et al*., US Patent 7,041,700 (May 9, 2006)
5. J. Lloyd *et al*., US Patent 7,005,436 (February 28, 2006)

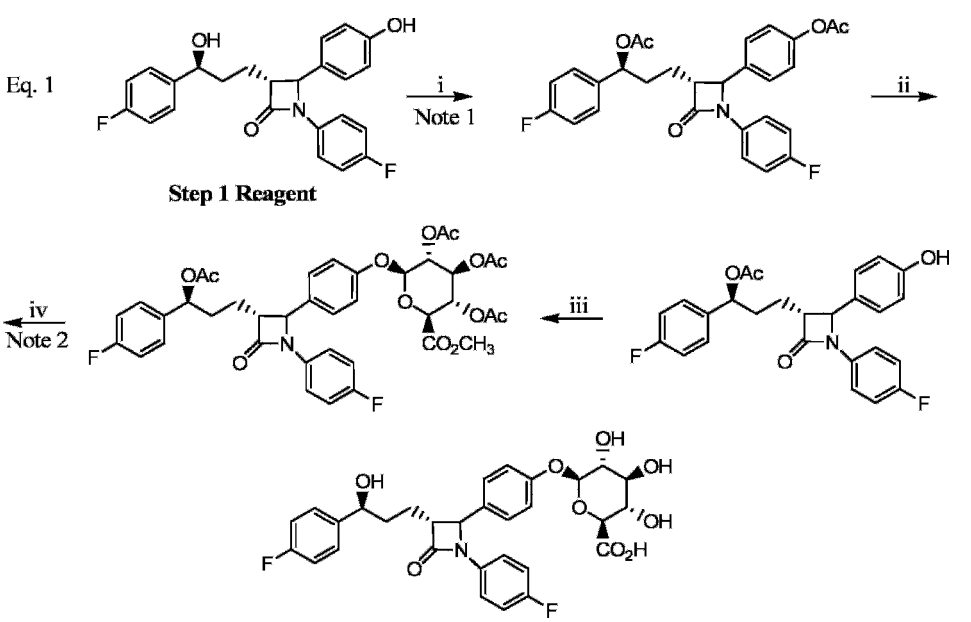
III. Atherosclerosis

A. Cholesterol Absorption Inhibitors

Title Substituted 2-Azetidinones Useful as Hypocholesterolemic Agents
A. Ghosal *et al*., US Patent 6,982,251 (January 3, 2006)
Assignee Schering Corporation (Kenilworth, NJ)
Utility Treatment and Prevention of Atherosclerosis

Invention Significance Atherosclerosis results from elevated plasma cholesterol and lipoprotein levels. However, when cholesterol absorption in the intestines is reduced, less cholesterol is delivered to the liver. A method for lowering cholesterol levels using hypocholesterolemic agents derived from 2-azetidinones and their glucose conjugates used individually or in combination with a cholesterol biosynthesis inhibitor addresses this problem.

Reaction



i- Acetic anhydride, dimethylaminopyridine, THF
ii- Sodium ethoxide, guanidine hydrochloride, methyl alcohol

iii- Boron trifluoride etherate, methyl (2,3,4-tri-*O*-acetyl-D-glucopyransyl)uronate-1-(2,2,2-trichloroacetimidate), CH_2Cl_2
iv- Methyl alcohol, triethylamine, water

Experimental

1. **Preparation of 1-(4-fluorophenyl-3(*R*)-(3(*S*)-acetyloxy)-3-(4-fluorophenyl)-propyl)]-4(*S*)-(4-acetyloxyphenyl)-2-azetidinone**

   Acetic anhydride (10.96 mmol) was added to 1-(4-fluorophenyl-3(*R*)-(3(*S*)-hydroxy-3-(4-fluorophenyl)propyl))-4(*S*)-(4-hydroxyphenyl)-2-azetidinone (4.98 mmol) containing dimethylaminopyridine (11.96 mmol) dissolved in 15 ml THF and the reaction monitored by TLC using 5% methyl alcohol/toluene. Thereafter, the mixture was then diluted with diethyl ether, washed with 1 M HCl and brine, and dried using Na_2SO_4. The solution was concentrated and the product isolated in 100% yield and used without additional purification.

   ^{1}H NMR 7.33 (2H, d, $J = 8.6$ Hz), 7.27(2H, m), 7.21 (2H, m), 7.11 (2H, d, $J = 8.5$ Hz), 7.02 (2H, t, $J = 8.6$ Hz), 6.94 (2H, d, $J = 8.5$ Hz), 5.70 (1H, t, $J = 7$ Hz), 4.60 (1H, d, $J = 2.4$ Hz), 3.06(1H, dt, $J = 7.9$, 2.4 Hz), 2.31 (3H, s), 2.06 (3H, s), 2.03 (1H, m), 1.86 (2H, m)
   HRMS (FAB) Calc. for M + H $C_{28}H_{25}NO_5F_2$ 493.1701; found 493.1695

2. **Preparation of 1-(4-fluorophenyl-3(*R*)-(3(*S*)-acetyloxy)-3-(4-fluorophenyl)-propyl)]-4(*S*)-(4-hydroxyphenyl)-2-azetidinone**

   The Step 1 product (4.97 mmol) dissolved in 15 ml methyl alcohol was treated with a mixture of sodium ethoxide (4.97 mmol) and guanidine hydrochloride (5.22 mmol) and the reaction was then monitored by TLC using 15% EtOAc/toluene. The mixture was then concentrated and the residue dissolved in EtOAc. The residue was purified by chromatography using 15% EtOAc/toluene and the product isolated in 95% yield.

   HRMS (FAB) Calc. for M + H $C_{26}H_{24}NO_4F_2$ 452.1673; found 452.1661

3. **Preparation of 2,3,4-tri-*O*-acetyl-1-*O*-[4-[*trans*-(3*R*,4*S*)-3-[3-(*S*)-acetyloxy-3-(4-fluorophenyl)propyl]-1-(4-fluorophenyl)-2-oxo-4-azetidinyl]phenyl]-β-D-glucopyranuronic acid methyl ester**

   Boron trifluoride etherate (0.74 mmol) was cooled to −25°C, then added to a solution of the Step 2 product (7.38 mmol), and the mixture treated with methyl (2,3,4-tri-*O*-acetyl-D-glucopyransyl)-uronate-1-(2,2,2-trichloroacetimidate) (8.86 mmol) dissolved in 74 ml in CH_2Cl_2. The solution was stirred 2 hours at −20°C, then 2 hours at 10°C, and finally quenched using NH_4Cl solution. It was diluted with EtOAc, then washed with saturated NH_4Cl solution, water and brine, and dried with Na_2SO_4. It was concentrated and the residue purified by chromatography with 40% EtOAc/hexanes, and the product isolated in 95% yield.

^{1}H NMR (400 MHz, CDCl.sub.3) 7.26 (4H, m), 7.21 (2H, m), 7.01 (4H, m), 6.93 (2H, t, $J = 8.4$ Hz), 5.69 (1H, t, $J = 6.7$ Hz), 5.34 (2H, m), 5.29 (1H, m), 5.15 (1H, d, $J = 7.2$ Hz), 4.56 (1H, d, $J = 2.1$ Hz), 4.17 (1H, m), 3.73 (3H, s), 3.02 (1H, dt, $J = 7.6$, 2.3 Hz), 2.07 (14H, m), 1.85 (2H, m)
HRMS (FAB) Calc. for M + H $C_{39}H_{40}NO_{13}F_2$ 768.2468; found 768.2460

4. **Preparation of 1-*O*-[4-[*trans*-(3*R*,4*S*)-1-(4-fluorophenyl)-2-oxo-3-[3-(*S*)-(hydroxy-4-fluorophenyl)propyl]]-4-azetidinyl]phenyl]-β-D-glucuronic acid**

The Step 3 product (6.98 mmol) was dissolved in 127 ml apiece of methyl alcohol and triethylamine, then treated with 445 ml water, and stirred overnight at ambient temperature. The mixture was quenched using 1 M HCl, diluted with EtOAc, and concentrated. The residue was acidified using 1 M HCl then diluted and extracted with EtOAc. The extract was washed with 1 M HCl, water and brine, then dried, and concentrated. The residue was dissolved in CH_2Cl_2 and purified by chromatography using a column charged with 15% methyl alcohol/CH_2Cl_2 and eluted with acetic acid/methyl alcohol/CH_2Cl_2, 5:15:80. Fractions containing the product were azeotroped three times with toluene and five times with CH_3OH, then concentrated, and the product isolated in 64% yield as a white solid.

^{1}H NMR (400 MHz, CD_3OD) 7.29 (6H, m), 7.09 (1H, d, $J = 8.6$ Hz), 6.70 (4H, m), 4.96 (1H, m), 4.80 (1H, d, $J = 2.0$ Hz), 4.59 (1H, m), 3.97 (1H, d, $J = 9.6$ Hz), 3.59 (1H, m), 3.49 (2H, m), 3.09 (1H, m), 1.86 (4H, m)
HRMS (FAB) Calc. for M + H $C_{30}H_{30}NO_9F_2$ 586.1889; found 586.1883

Derivatives

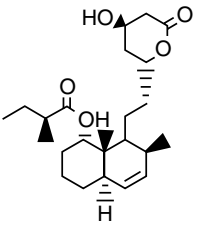
Only the Step 4 glucose conjugate was prepared.

Testing

I. *In Vivo Assay of Hypolipidemic Agents Using Hyperlipidemic Hamsters*

Hamsters were separated into groups of six and given a controlled cholesterol diet containing 0.5% cholesterol for seven days. Diet consumption was monitored to determine dietary cholesterol exposure in the presence of the experimental agent without the cholesterol biosynthesis inhibitor simvastatin. Treated animals received either the Step 4 product or the Step 1 reagent dissolved in 0.2 ml corn oil; untreated corn oil was given to the control group. Thereafter, animals were anesthetized and sacrificed by decapitation. Blood was evaluated for total cholesterol and lipids in plasma while the liver was assayed for free and esterified cholesterol and triglyceride content as summarized in Table 1.

Table 1 The effect of the Step 1 reagent and the Step 4 product in lowering cholesterol and cholesterol esters in conjunction with an unspecified amount of simvastatin[a]

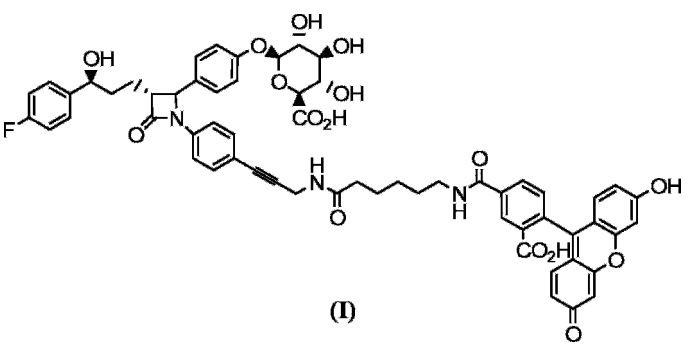


Entry	Reduction in Plasma Cholesterol (%)	Reduction in Cholesterol Esters (%)	Dose (mg/kg)
Step 1 Reagent	−59	−95	1
Step 4 Product	−58	−95	3

[a] 6(*R*)-[2-(8′-acyloxy-2′-methyl-6′-methyl)-polyhydronaphthyl-1′-ethyl]-4(*R*)-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one.

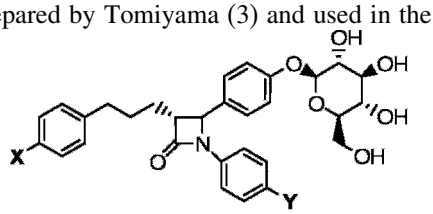
Notes

1. The preparation of the Step 1 reagent and other hydroxy-substituted azetidinone derivatives useful as hypocholesterolemic agents is described by Rosenblum (1).
2. In an earlier investigation by Altmann (2), the fluorescent 2-azetidinone cholesterol absorption inhibitor, (**I**), was prepared to monitor the dynamics of cholesterol absorption into a membrane.



(I)

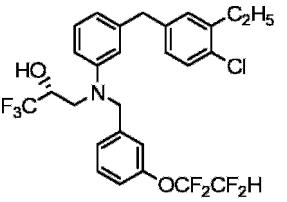
3. Additional 2-azetidinone derivatives, (**II**), effective as hypocholesterolemic agents were prepared by Tomiyama (3) and used in the treatment of atherosclerosis.



X = H, F
Y = H, Cl, F, OCH_3, CH_3

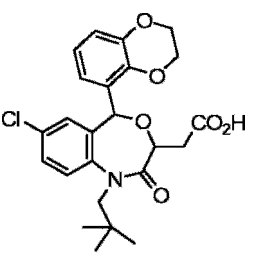
(II)

4. When used in conjunction with niacin or niceritol, Sikorski (4) determined that the benzyl amine cholesteryl ester transfer protein inhibiting agent, (**III**), was effective in treating atherosclerosis.



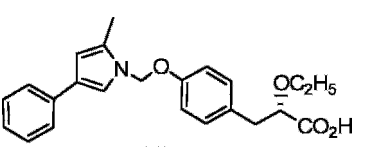
(III)

5. Squalene synthetase inhibitors, (**IV**), prepared by Hamanaka (5) were effective in the treatment of hypocholesterolemia, hypotriglyceridemia, and artherosclerosis.



(IV)

6. Heterocyclic β-aryl-α-substituted propanoic acids derivatives, (**V**), prepared by Lohray (6) were effective in treating hypolipidemic and hypocholesteremic disorders.



(V)

References

1. S.B. Rosenblum *et al*., US Patent 5,767,115 (June 16, 1998) and RE37,721 (March 28, 2002)
2. S.W. Altmann *et al*., US Patent 6,933,107 (August 23, 2005)
3. H. Tomiyama *et al*., US Patent 7,045,515 (May 16, 2006)
4. J.A. Sikorski *et al*., US Patent 6,890,958 (May 10, 2005)
5. E.S. Hamanaka *et al*., US Patent 6,537,987 (March 25, 2003)
6. B.B. Lohray *et al*., US Patent 7,041,837 (May 9, 2006) and US Patent 6,987,123 (January 17, 2006)

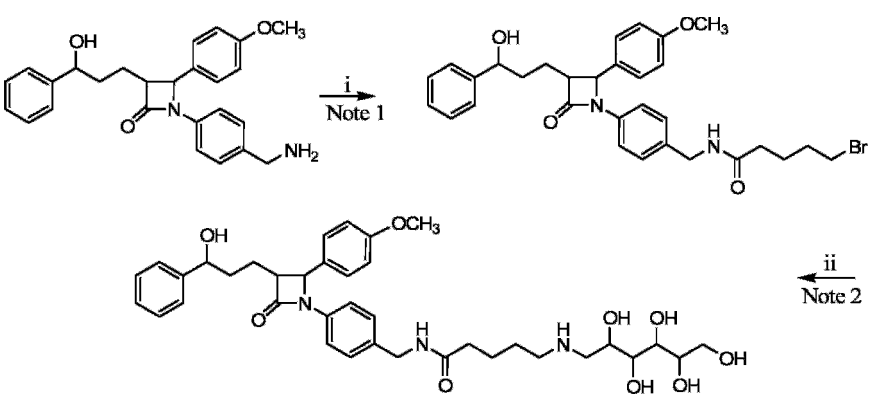
B. Hypolipidemia and Hypocholesterolemia Treatment Agents

Title Diphenylazetidinones, Process for Their Preparation, Medicaments Comprising These Compounds and Their Use
H. Glombik *et al*., US Patent 6,992,067 (January 31, 2006)
Assignee Aventis Pharma Deutschland GmbH
Utility Treatment of Arteriosclerosis

Invention Significance Diphenylazetidinone-based medicaments currently exist and are used for treating hypolipidemia and hypocholesterolemia. However, as a result of their high intestinal bioabsorption, liver toxicity has been observed. To minimize this side effect, diphenylazetidinone derivatives have been prepared having bioabsorption between 5 and 10% while continuing to remain effective in treating arteriosclerotic disorders.

Reaction

Eq. 1



i- CH_2Cl_2, triethylamine, 5-bromovalery l chloride
ii- DMF, 6-aminohexane-1,2,3,4,5-pentaol

Experimental

1. **Preparation of *N*-4-[3-(3-hydroxy-3-phenylpropyl)-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl]-benzyl-5-bromopentanamide**

   A solution of 1-(4-aminomethylphenyl)-3-(3-hydroxy-3-phenylpropyl)-4-(4-methoxyphenyl)-azetidin-2-one (416 mg) in 10 ml CH_2Cl_2 was treated with 0.2 ml

of triethylamine followed by ice-cold 5-bromovaleryl chloride (200 mg) dissolved in 2 ml CH_2Cl_2, then stirred 5 hours at ambient temperature. The mixture was diluted with 5 ml water and the solution pH lowered to 3 with 0.5 M HCl. The two phases were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic solutions were dried with Na_2SO_4 and then concentrated. The residue was purified by chromatography, and the product isolated, MW = 579.54.

MS (FAB) = 581/579 $(M+H^+)$

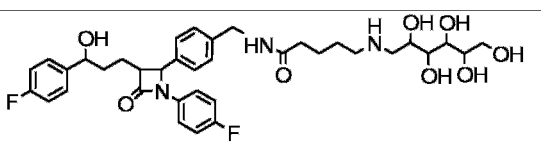
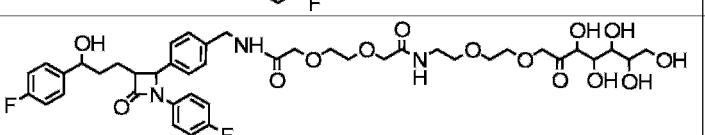
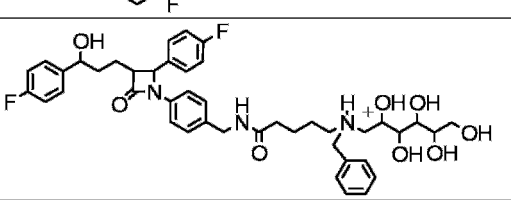
2. **Preparation of *N*-4-[3-(3-hydroxy-3-phenylpropyl)-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl]benzyl-5-(2,3,4,5,6-pentahydroxyhexylamino)pentanamide**

The Step 1 product (300 mg) dissolved in 10 ml DMF was treated with 6-aminohexane-1,2,3,4,5-pentaol (191 mg), then stirred 2 hours at 80°C, and concentrated. The residue was purified by chromatography with silica gel using CH_2Cl_2/methyl alcohol/12 M NH_4OH, 30:10:2, and the product isolated, MW = 679.82.

MS (FAB) = 680 $(M+H^+)$

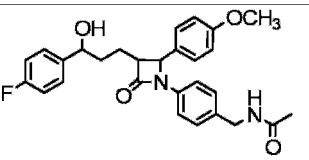
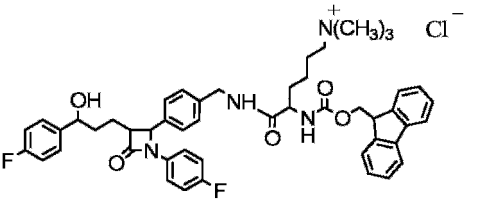
Derivatives

Table 1 Selected diphenylazetidinones derivatives and their corresponding mass spectral characterization data. Entry 20 is the *N*-acylated Step 1 co-reagent and was used as a reference in liver toxicity testing

Entry	Structure	MS (ESI), $(M+H^+)$
2		601
13		876
18		789

(*continued*)

Table 1 Continued

20		477
21		815

Testing

I. *Liver Toxicity Testing*

Mice were fed Intralipid® 20 containing 0.25 μCi of ^{14}C-cholesterol in 0.1 mg of cholesterol perorally by gavage just prior to when 0.5 ml/mouse of selected experimental agents were administered. Immediately thereafter, livers were removed, homogenized, aliquots incinerated to determine the amount of ^{14}C-cholesterol absorbed and ED_{50} values determined. Testing results are provided in Table 2.

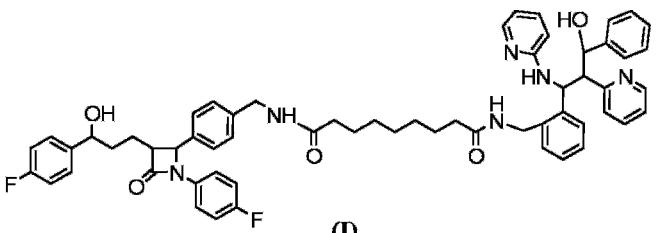
Table 2 The effect of experimental hypolipidemia agents on liver toxicity

Entry	Liver ED_{50} (mg/mouse)
2	0.1
13	0.3
18	1.0
20	0.03
21	1.0

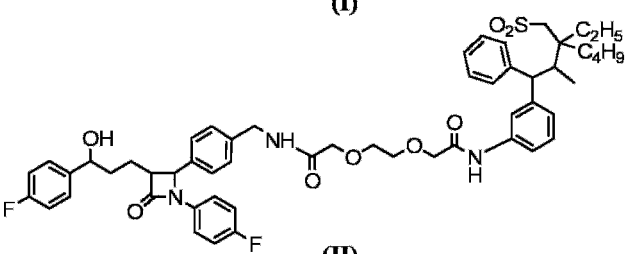
Notes

1. The preparation of the Step 1 co-reagent, 1-(4-aminomethylphenyl)-3-(3-hydroxy-3-phenylpropyl)-4-(4-methoxy-phenyl)azetidin-2-one and other hydroxy-substituted azetidinone derivatives useful in treating hypocholesterolemic diseases is described by Rosenblum (1).

2. In earlier investigations by the authors (2,3), pyridine-, (**I**), and 1,1-dioxo-2,3,4,5-tetrahydro-1H-benzo[b]thiepine derivatives, (**II**), respectively, were prepared having limited bioabsorption while remaining effective in the treatment of arteriosclerosis.

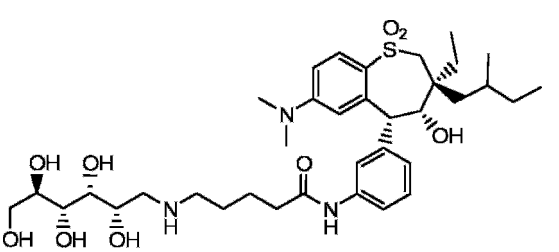


(I)



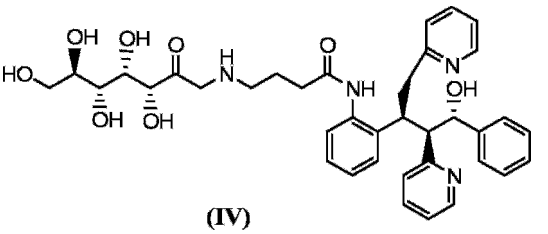
(II)

3. Sugar-functionalized benzothiepine 1,1-dioxide derivatives, (**III**), prepared by Frick (4) were effective at significantly lower treatment dosages in treating hypolipidemia and hypocholesterolemia.



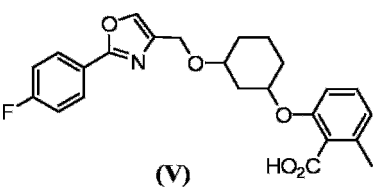
(III)

4. In conjunction with a statin such as simvastatin, 1,3-diaryl-2-pyridin-2-yl-3-(pyridin-2-ylamino)propanol derivatives, (**IV**), prepared by Kirsch (5) were effective as hypolipidemic agents in the treatment of arteriosclerosis.



(IV)

5. Diarylcycloalkyl derivatives, (**V**), prepared by the author (6) in earlier investigations were effective as lipid- and/or triglyceride-lowering chemical agents used in treating lipid metabolism disorders.



(V)

References

1. S.B. Rosenblum *et al*., US Patent 5,631,365 (May 20, 1997)
2. H. Glombik *et al*., US Patent 6,703,386 (March 9, 2004)
3. H. Glombik *et al*., US Patent 6,498,156 (December 24, 2002)
4. W. Frick *et al*., US Patent 6,642,269 (November 4, 2003)
5. R. Kirsch *et al*., US Patent 6,897,198 (March 24, 2005) and US Patent 6,596,728 (July 22, 2003)
6. H. Glombik *et al*., US Patent 6,884,812 (April 26, 2005) and US Patent 6,624,185 (September 23, 2003)

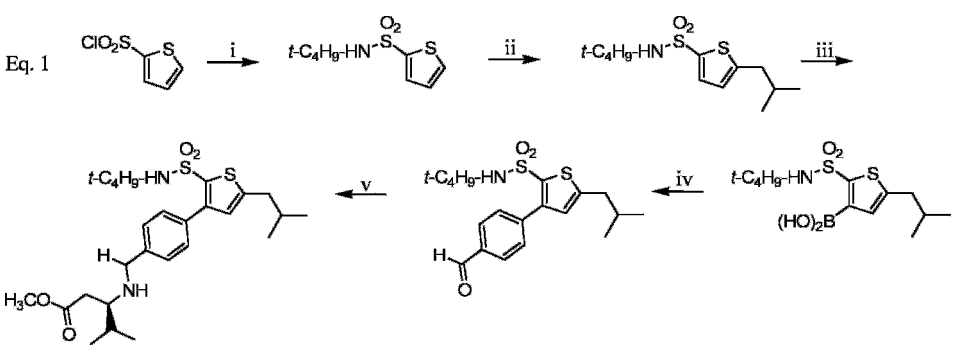
IV. Cardiac Hypertrophy: Angiotensin-(1–7) Receptor Agonists

Title *p*-Thienylbenzylamides as Agonists of Angiotensin-(1–7) Receptors, and Methods of Their Preparation and Use
H. Heitsch, US Patent 6,984,660 (January 10, 2006)
Assignee Aventis Pharma Deutschland GmbH
Utility Treatment of Cardiac Hypertrophy

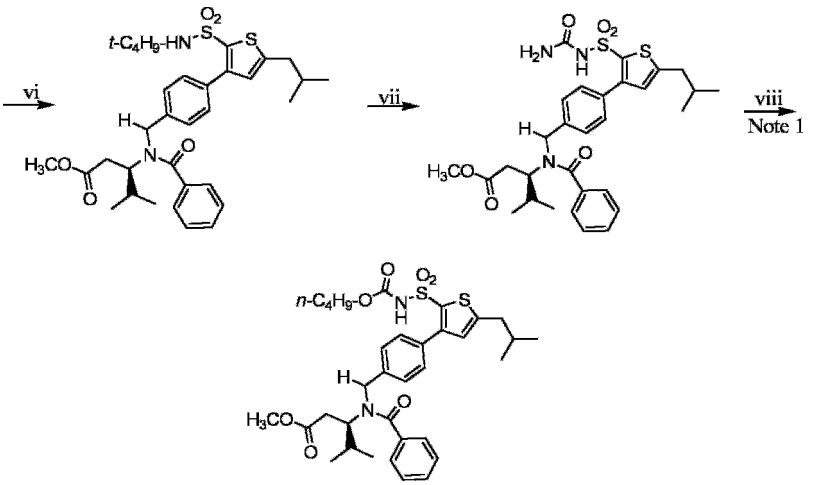
Invention Significance Angiotensin-(1–7) has an anti-proliferative effect on vascular smooth muscle cells and inhibits the proliferation of smooth muscle cells following vascular tissue damage. Although treatment medicaments exist, angiotensin-(1–7) receptor agonists of the current art exhibit improved activity and selectivity. Moreover, as a result of the stimulation of angiotensin-(1–7) receptors by these agonists, the production and release of the vasorelaxing and cardioprotective messengers such as cyclic guanosine monophosphate (cGMP) and nitrogen monoxide (NO) associated with endothelial cells is enhanced.

Reaction

Eq. 1



i- *N*-*t*-Butylamine, CH_2Cl_2
ii- *n*-Butyllithium, 1-iodo-2-methylpropane, THF
iii- *n*-Butyllithium, THF, trimethyl borate
iv- Ethyl alcohol, 4-bromobenzaldehyde, tetrakis (triphenylphosphine) palladium, toluene, cesium carbonate
v- THF, 5A molecular sieves, L-valine methyl ester hydrochloride, sodium cyanoborohydride, methyl alcohol



vi- Benzoyl chloride, triethylamine, CH_2Cl_2
vii- Anisole, trifluoroacetic acid
viii- 4-Pyrrolidinopyridine, *n*-butyl chloroformate, pyridine

Experimental

1. **Preparation of 2-(*N*-*t*-butyl)sulfonamidothiophene**

   While cooling with ice, *N*-*t*-butylamine (0.82 mol) was added dropwise to a solution of 2-thiophenesulfonyl chloride (0.27 mol) in 500 ml CH_2Cl_2 and the solution stirred 1 hour at ambient temperature. The mixture was then diluted with 500 ml 1 M HCl and the organic phase isolated. The solution was washed with water, dried over Na_2SO_4, concentrated, and 58.2 g product isolated as a pale yellow oil.

   R_f EtOAc/*n*-heptane 1:1, 0.39
   MS (ESI) *m/z*, 220 $[M+H]^+$

2. **Preparation of 5-isobutyl-2-[(*N*-*t*-butyl)sulfonamido]thiophene**

   n-Butyllithium (0.27 mol) in 15% hexane was added dropwise to the Step 1 product (0.11 mol) dissolved in 450 ml THF cooled to −78°C, then stirred 3 hours at −20°C and 2 hours at ambient temperature. The mixture was recooled to −20°C, then treated with 1-iodo-2-methylpropane (0.13 mol), then stirred 1 hour at 0°C and overnight at ambient temperature. The mixture was treated with 150 ml apiece NH_4Cl and water, extracted with EtOAc, dried over Na_2SO_4, and concentrated. The residue was purified by chromatography using EtOAc/*n*-heptane, 1:6, and 9.8 g of product isolated.

   R_f EtOAc/*n*-heptane 1:4, 0.28
   MS (ESI) *m/z*, 276 $[M+H]^+$

3. **Preparation of 5-isobutyl-2-[(*N*-*t*-butyl)sulfonamido]thiophene-3-boronic acid**

n-Butyllithium (54.4 ml) in 15% hexane was added dropwise to the Step 2 product (35.1 mmol) dissolved in 350 ml THF cooled to −78°C and solution warmed over 2 hours while stirring to ambient temperature. The mixture was recooled to 0°C, then treated with trimethyl borate (52.2 mmol), and stirred 1 hour at 0°C and an additional hour at ambient temperature. The organic phase was isolated, washed with water, dried with Na_2SO_4, and concentrated. The residue was purified by chromatography with silica gel using CH_2Cl_2/methyl alcohol, 30:1, and 10.3 g of product isolated as a pale brown oil.

$\boldsymbol{R_f}$ EtOAc/*n*-heptane 1:1, 0.22
MS (ESI) *m/z*, 320 $[M+H]^+$

4. **Preparation of 4-[5-isobutyl-2-[(*N*-*t*-butyl)sulfonamido]-3-thienyl]benzaldehyde**

The Step 3 product (14.5 mmol) dissolved in 75 ml of ethyl alcohol was added to a mixture of 4-bromobenzaldehyde (14.5 mmol) and tetrakis(triphenylphosphine) palladium (0.40 mmol) dissolved in 75 ml of toluene, then stirred 15 minutes at ambient temperature. The mixture was further treated with 16.9 ml 2 M Cs_2CO_3 solution, then refluxed 3 hours, and concentrated. The residue was dissolved in EtOAc, then washed with water, dried with Na_2SO_4, and reconcentrated. The residue was then purified by chromatography with silica gel using EtOAc/*n*-heptane, 1:4, and 5.46 g of product isolated as a slightly yellow-colored solid, mp = 140−143°C.

$\boldsymbol{R_f}$ EtOAc/*n*-heptane 1:2, 0.47
MS (FAB) *m/z*, 380 $[M+H]^+$

5. **Preparation of methyl 2-*N*-[4-[2-(*N*-*t*-butyl)sulfonamido-5-isobutyl-3-thienyl] benzyl]-amino-3-methyl-L-butyrate**

The Step 4 product (14.4 mmol) was dissolved in 160 ml THF and treated with 5 Å molecular sieves (16 g) and L-valine methyl ester hydrochloride (28.8 mmol), then stirred 30 minutes at ambient temperature. The solution was further treated with the dropwise addition of sodium cyanoborohydride (14.4 mmol) in 18 ml methyl alcohol at 0−5°C and the mixture stirred overnight at ambient temperature. The solution was concentrated, the residue purified by chromatography using EtOAc/*n*-heptane, 1:2, and 4.41 g of product isolated as an amorphous solid

$\boldsymbol{R_f}$ EtOAc/*n*-heptane, 1:4, 0.43
MS (ESI) $m/z = 495$ $[M+H]^+$

6. **Preparation of methyl 2-*N*-benzoyl-2-*N*-[4-[2-(*N*-*t*-butyl)sulfonamido-5-isobutyl-3-thienyl]benzyl]amino-3-methyl-L-butyrate**

A mixture consisting of the Step 5 product (2.02 mmol), 352 µl benzoyl chloride and 280 µl triethylamine dissolved in 20 ml CH_2Cl_2 was refluxed 1 hour, then washed

with water, dried, and concentrated. The residue was purified by chromatography using EtOAc/*n*-heptane, 1:4, and 1.20 g of product isolated as an amorphous solid.

R_f EtOAc/*n*-heptane, 1:4, 0.12
MS (ESI) $m/z = 599$ $[M+H]^+$

7. **Preparation of methyl 2-*N*-benzoyl-2-*N*-[4-[2-sulfonamido-5-isobutyl-3-thienyl] benzyl]-amino-3-methyl-L-butyrate**

A mixture consisting of the Step 6 product (1.92 mmol), anisole (21.5 mmol), and 12.2 ml trifluoroacetic acid was stirred 24 hours at ambient temperature, then concentrated. The residue was dissolved in EtOAc, washed with water and brine, dried using Na_2SO_4, then concentrated. The residue was purified by chromatography using EtOAc/*n*-heptane, 1:2, and 834 mg of product isolated as a white solid, mp = 50°C (softening)

R_f EtOAc/*n*-heptane, 1:2, 0.28
MS (ESI) $m/z = 543$ $[M+H]^+$

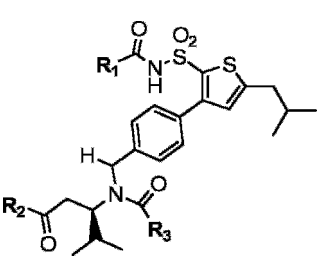
8. **Preparation of methyl 2-*N*-benzoyl-2-*N*-[4-[2-(*n*-butyloxycarbonylsulfonamido)-5-isobutyl-3-thienyl]benzyl]amino-3-methyl-L-butyrate**

A mixture consisting of the Step 7 product (0.74 mmol), 4-pyrrolidinopyridine (0.09 mmol), and 927 µl *n*-butyl chloroformate dissolved in 6 ml pyridine was stirred 2 days at ambient temperature, then concentrated. The residue was dissolved in CH_2Cl_2, washed with a 10% solution of citric acid and with water, dried with Na_2SO_4, and concentrated. The residue was purified by chromatography using CH_2Cl_2/methyl alcohol, 20:1, and 470 mg of product isolated as a slightly yellow amorphous foam.

R_f EtOAc/*n*-heptane, 1:1, 0.35
MS (ESI) $m/z = 643$ $[M+H]^+$

Derivatives

Table 1 Selected thienylbenzylamides derivatives and their corresponding mass spectral data and melting points. Retention factors were also supplied by author



Entry	R_1	R_2	R_3	MS (ESI) ($[M+H]^+$)	mp
3	*n*-Butyl	Hydroxide	Phenyl	629	–
4	2-Ethylaminocarbonyl	Methoxy	Phenyl	614	65
9	2-Ethylaminocarbonyl	Methoxy	Methyl	552	130
18	2-Methylaminocarbonyl	Methoxy	Methyl	610	158
19	2-Methylaminocarbonyl	Hydroxide	3-Methoxycarbonyl propinyl	582	128

Testing

I. *Binding Assay*

Binding affinities on endothelial cells using selected experimental agents were determined according to the method of Tallant (1). Testing results are provided in Table 2.

Table 2 Test results for selected experimental agents indicating optimum affinity for angiotensin-(1–7) receptors on endothelial cells. In both cases, negligible affinities for ANG II receptors of the AT_1 and AT_2 types were observed

Entry	IC_{50} (nM)
4	21
9	30

II. *cGMP Determinations*

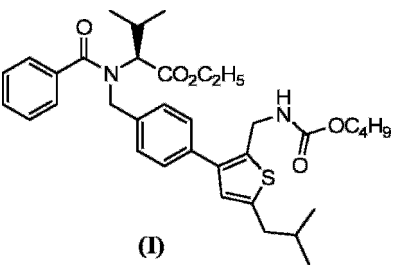
Agonistic effects on the production of cGMP by selected experimental agents were determined according to the modified method of Santos (2). Testing results are provided in Table 3.

Table 3 Test results indicating the agonistic effect on the production of cGMP using selected *p*-thienylbenzylamides

Entry	IC_{50} (1×10^{-7}M)
4	6.0
9	0.4

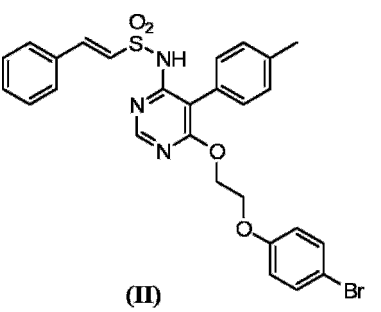
Notes

1. Additional *p*-thienylbenzyl amides, (**I**), effective as angiotensin-(1–7) receptors agonists were prepared by the author (3) in earlier investigations.



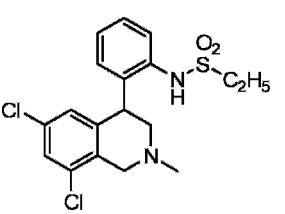
(I)

2. Arylethene-sulfonamides derivatives, (**II**), prepared by Boss (4) were effective as endothelin antagonists and used in the treatment of coronary diseases such as cardiac hypertrophy and cardiac insufficiency.



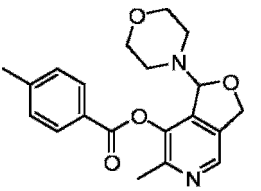
(II)

3. Hofmeister (5) prepared 4-phenyltetrahydroisoquinolinium derivatives, (**III**), for the treatment of left-ventricular hypertrophy and related thrombotic disorders.



(III)

4. 3-Acylated pyridoxal derivatives, (**IV**), prepared by Haque (6) were effective in the treatment of cardiovascular hypertrophy, hypertension, and congestive heart failure.



(IV)

References

1. V. Tallant *et al*., Hypertension, 29 [Part 2], 388 (1997)
2. A. Santos *et al*., Brain Res. Bull. 35, 293 (1994)
3. H. Heitsch, US Patent 6,538,144 (March 25, 2003), US Patent 6,429,222 (August 6, 2002), and US Patent 6,235,766 (May 22, 2001)
4. C. Boss *et al*., US Patent 6,951,856 (October 4, 2005)
5. A. Hofmeister *et al*., US Patent 6,911,453 (June 28, 2005)
6. W. Haque *et al*., US Patent 6,890,943 (May 10, 2005)

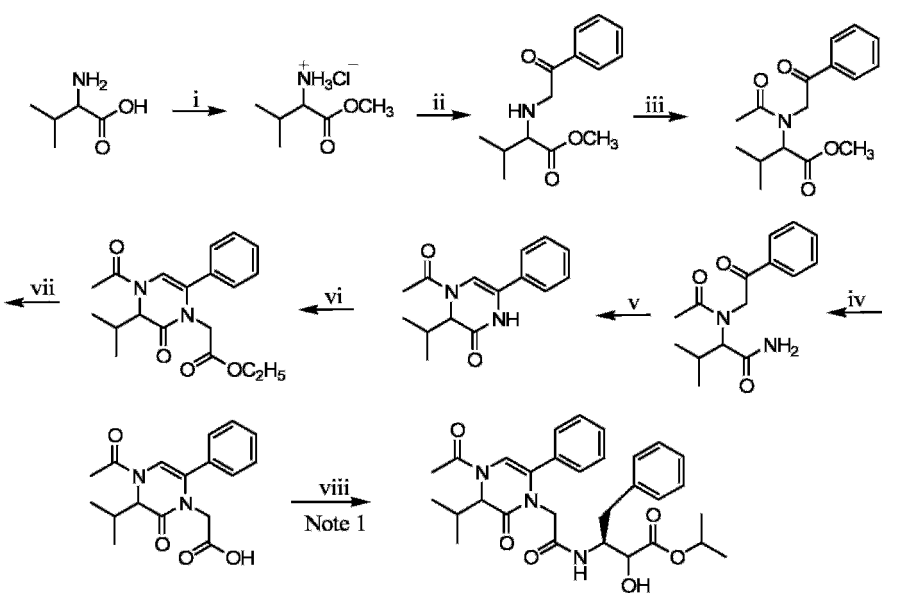
V. Cardiac Infarction: Chymase Inhibitors

Title Thiazine Derivatives
K. Nishimura *et al.*, US Patent 6,960,575 (November 1, 2005)
Assignee Santen Pharmaceutical Co., Ltd
Utility Treatment of Cardiac Infarction

Invention Significance Chymase enzyme causes angiotensin I to liberate the dipeptide, His-Leu, causing chymase-initiated disorders including cardiac infarction, heart failure, blood-vessel restenosis, or hypertension. To address this disorder, a new chemical class of chymase inhibitor containing 2-oxo-1,2,3,4-tetrahydropyrazine as the main skeleton has been prepared.

Reaction

Eq. 1



i- Methyl alcohol, thionyl chloride, D,L-valine
ii- Diisopropylethylamine, 2-bromoacetophenone, CH_2Cl_2
iii- Pyridine, CH_2Cl_2, acetyl chloride
iv- Methyl alcohol, ammonia
v- *p*-Toluenesulfonic acid monohydrate, water
vi- Sodium hydride, THF, ethyl bromoacetate

vii- Lithium hydroxide, water
viii- *N*-Methylmorpholine, 1-hydroxybenzotriazole, isopropyl (2*RS*,3*S*)-3-amino-2-hydroxy-4-phenylbutyrate, CH_2Cl_2, 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride

Experimental

1. **Preparation of D,L-valine methyl ester hydrochloride**

   Thionyl chloride (21.2 g) was added to 50 ml methyl alcohol cooled to −15°C, then stirred for 20 minutes, and treated with D,L-valine. The mixture was stirred overnight, then concentrated and the residue treated with diethyl ether. A precipitate that formed was filtered and 8.68 g of crystalline product isolated.

2. **Preparation of methyl (2*RS*)-2-(2-oxo-2-phenylethyl)aminoisovalerate**

   A suspension of the Step 1 product (3.36 g) in 100 ml CH_2Cl_2 was treated with diisopropylethylamine (7.66 ml) and 2-bromoacetophenone (4.2 g), then refluxed 4 days, and cooled. The solution was diluted with diethyl ether and extracted with 0.1 M HCl. The extract was neutralized with$NaHCO_3$ and the ethereal medium re-extracted with EtOAc. The organic layer was washed with saturated brine, dried, then concentrated, and 4.92 g product isolated, mp = 32.5−50.8°C.

   IR (KBr, cm^{-1}) 3334, 2964, 2614, 1730, 1687, 1598, 1448

3. **Preparation of methyl (2*RS*)-2-{*N*-acetyl-*N*-(2-oxo-2-phenylethyl)} aminoisovalerate**

   The Step 2 product (16.5 g) dissolved in 70 ml CH_2Cl_2 was treated with 10.7 ml pyridine, then cooled with ice, and 7.06 ml acetyl chloride added. The mixture stirred overnight at ambient temperature and was then concentrated. The residue was dissolved in EtOAc, then washed with 0.1 M HCl, saturated $NaHCO_3$ solution, brine, dried, and concentrated. The residue was purified by chromatography with silica gel and 18.6 g product isolated, mp = 85.5 − 86.0°C.

   IR (KBr, cm^{-1}) 1729, 1697, 1640

4. **Preparation of (2*RS*)-2-{*N*-acetyl-*N*-(2-oxo-2-phenylethyl)}aminoisovaleramide**

   The Step 3 product (1.0 g) dissolved in 5 ml methyl alcohol was saturated with ammonia gas, then placed into a sealed vessel, and stirred for 6 days. The solution was then concentrated, the residue purified by chromatography with silica gel column, and 926 mg product isolated.

   IR (Film, cm^{-1}) 3121, 1659, 1448, 1297

5. **Preparation of (3*RS*)-4-acetyl-3-isopropyl-2-oxo-6-phenyl-1,2,3,4-tetrahydro pyrazine**

   A catalytic amount of *p*-toluenesulfonic acid monohydrate was added to the Step 4 product (900 mg) dissolved in 1 ml toluene, then refluxed overnight. The solution was washed with saturated $NaHCO_3$ solution, brine, dried, and concentrated. The residue was purified by chromatography with silica gel and 757 mg product isolated, mp = 181.5−185.0°C

   IR (KBr, cm^{-1}) 3231, 1698, 1626, 1503

6. **Preparation of ethyl {(3*RS*)-4-acetyl-3-isopropyl-2-oxo-6-phenyl-1,2,3,4-tetrahydropyrazine-1-yl}acetate**

   Under ice cooling, NaH (27.9 mg) was added to a solution of the Step 5 product (150 mg) in 1 ml THF, then stirred for 30 minutes and treated with 77 μl ethyl bromoacetate. The mixture was stirred 1 hour at ambient temperature, then EtOAc and saturated NH_4Cl solution were added. The organic portion was washed with 0.1 M HCl, saturated $NaHCO_3$ solution and brine, then dried, and concentrated. The residue was purified by chromatography with silica gel and 195 mg product isolated, mp = 96.0−98.0°C.

   IR (KBr, cm^{-1}) 1751, 1686, 1666, 1645, 1576

7. **Preparation of {(3*RS*)-4-acetyl-3-isopropyl-2-oxo-6-phenyl-1,2,3,4-tetrahydropyrazin-1-yl}acetic acid**

   The Step 6 product (185 mg) dissolved in 5 ml ethyl alcohol was treated with 0.4 ml 4 M LiOH solution, then stirred for 35 minutes, and the mixture acidified with 1 M HCl. The solution was extracted with EtOAc and the extract was washed with brine. It was dried, concentrated, and 167 mg product isolated.

   IR (Film, cm^{-1}) 3400–2000, 1743, 1691, 1640, 1495, 1446

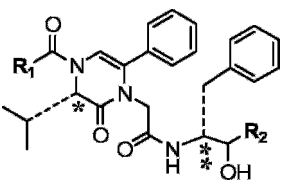
8. **Preparation of isopropyl (2*RS,3S*)-3-{(3*RS*)-4-acetyl-3-isopropyl-2-oxo-6 -phenyl-1,2,3,4-tetrahydropyrazin-1-yl}methylcarbonylamino-2-hydroxy-4-phenylbutyrate**

   The Step 7 product (160 mg) dissolved in 5 ml CH_2Cl_2 was treated with 67 μl methylmorpholine, isopropyl (2*RS*,3*S*)-3-amino-2-hydroxy-4-phenylbutyrate (144 mg) and 1-hydroxybenzotriazole (103 mg), then cooled, and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (116 mg) added. The mixture was stirred overnight and was then extracted using EtOAc. The extract was washed successively with 0.1 M NaOH solution, brine, 0.1 M HCl, brine, dried, and then concentrated. The residue was purified by chromatography with silica gel and 259 mg product isolated.

   IR (Film, cm^{-1}) 3328, 1731, 1680, 1531, 1447

Derivatives

Table 1 Selected experimental derivatives containing the 2-oxo-1,2,3,4-tetrahydropyrazine skeletal system. FTIR characterization and melting points provided by author



Entry	R_1	R_2	Orientation (*)	Orientation (**)
2-22	Benzoyl	Isopropoxycarbonyl	*R*	*S*
2-31	3-Pyridylcarbonyl	Isopropoxycarbonyl	*R,S*	*S*
2-32	4-Pyridylcarbonyl	Isopropoxycarbonyl	*R,S*	*S*
2-34	Benzenesulfonyl	Isopropoxycarbonyl	*R,S*	*S*
4.1	Acetyl	Trifluoromethylcarbonyl	*R,S*	*R,S*
5.1	Acetyl	1,3-Thiazol-2-yl	*R,S*	*S*
6.6	3-Pyridylcarbonyl	Hydrogen	*R,S*	*R*
8-6	3-Pyridylcarbonyl	Cyano	*R*	*R,S*
10.7	Benzoyl	1-(4,4-Dimethyl-4,5-dihydro-1,3-oxazol-2-yl)-carbonyl	*R,S*	*S*
21.2	1-Methoxycarbonyl-methylpyridinium bromide	1-(4,4-Dimethyl-4,5-dihydro-1,3-oxazol-2-yl)-carbonyl	*S*	*R*

Testing

I. *Chymase Inhibitory Effect*

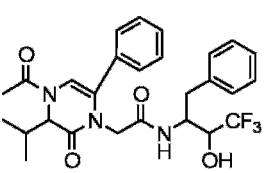
Chymase causes angiotensin I to liberate the dipeptide His-Leu, which was measured by the fluorescence intensity of the resulting dipeptide. A summary of experimental agent concentrations required to inhibit a chymase enzymatic activity by 50% is provided in Table 2.

Table 2 Summary of selected chemical agents containing the 2-oxo-1,2,3,4-tetrahydropyrazine skeleton and their corresponding IC_{50} values

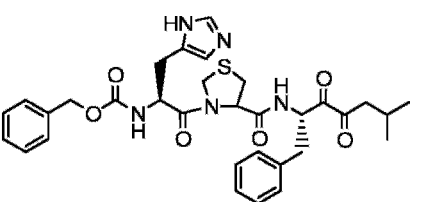
Entry	IC_{50} (10^{-6}M)
2-22	0.20
2-31	0.21
2-32	0.36
4.1	0.25
8.6	3.80
10.5	0.50

Notes

1. In earlier investigations by the authors (1,2), 2-oxo-1,2,3,4-tetrahydropyrazines, (**I**), and thiazolidine derivatives, (**II**), respectively, were prepared, which were effective as chymase inhibitors.

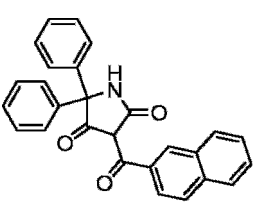


(I)

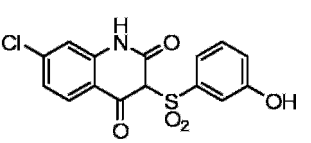


(II)

2. Imidazolidinediones, (**III**), prepared by Sakai (3) and 2,4(1H,3H)-quinazolinedione derivatives, (**IV**), prepared by Fukami (4) were effective as chymase inhibitors and used in the treatment of cardiovascular and inflammatory disorders.

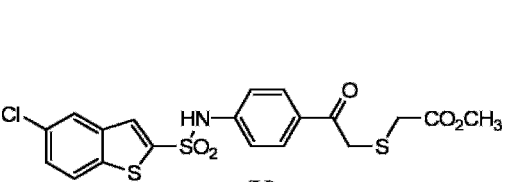


(III)

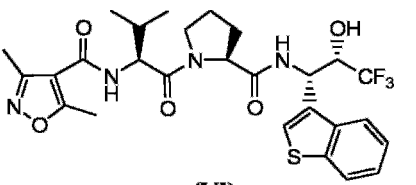


(IV)

3. Benzothiophenesulfonamide derivatives, (**V**) and (**VI**), prepared by Satoh (5) and Deguchi (6), respectively, were effective as chymase inhibitory agents and used for preventing or treating cardiac or circulatory diseases caused by abnormal increases of angiotensin II or endothelin I based on excess chymase activity.



(V)



(VI)

References

1. K. Nishimura *et al*., US Patent 6,713,472 (March 30, 2004)
2. K. Nishimura *et al*., US Patent 6,410,576 (June 25, 2002)
3. Y. Sakai *et al*., US Patent 6,919,365 (July 19, 2005)
4. H. Fukami *et al*., US Patent 6,921,766 (July 25, 2005)
5. S. Satoh *et al*., US Patent 7,071,220 (July 4, 2006)
6. T. Deguchi *et al*., US Patent 6,852,744 (February 8, 2005)

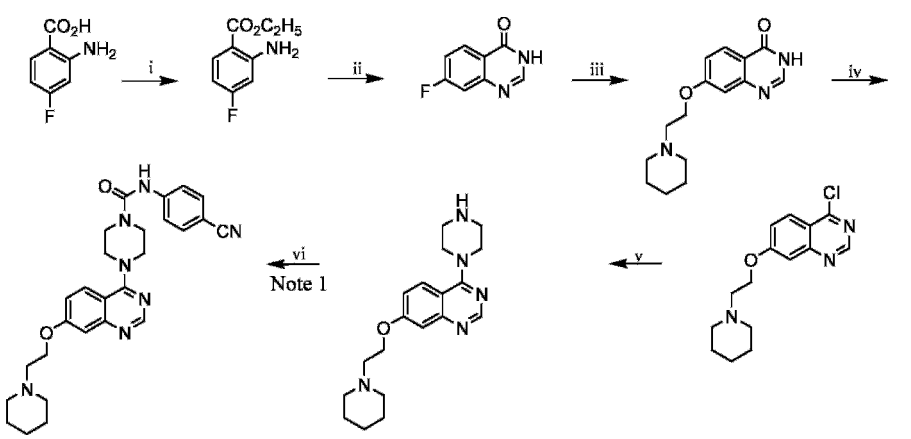
VI. Cell Proliferation Diseases: Phosphorylation Kinase Inhibitors

Title Nitrogenous Heterocyclic Compounds
A. Pandey *et al.*, US Patent 6,956,039 (October 18, 2005)
Assignee Millennium Pharmaceuticals, Inc.
Utility Treatment of Cell Proliferation Diseases in Cardiovascular Disorders

Invention Significance Platelet-derived growth factor (PDGF) is an aggravating factor for abnormal cell growth and cell wandering disorders characteristic of proliferative diseases such as arteriosclerosis, vascular reobstruction, and glomerulosclerosis. To treat these diseases, tyrosine receptor phosphorylation kinase inhibitors effective against PDGF Flt3 and CSF-1R, epidermal growth factor inhibitors (EGRF), fibroblast growth factor (FGF), and vascular endothelial growth factor receptor (VEGFR) have been prepared.

Reaction

Eq. 1



i- Ethyl alcohol, thionyl chloride
ii- Ammonium formate, DMF
iii- 1-Piperidineethanol, DMF, sodium hydride
iv- Phosphoryl chloride
v- Isopropyl alcohol, piperazine
vi- DMF, 4-cyanophenylisocyanate

Experimental

1. **Preparation of ethyl 2-amino-4-fluorobenzote**

   2-Amino-4-fluorobenzoic acid (5.41 mmol) dissolved in 15 ml ethyl alcohol was treated with 1.18 ml thionyl chloride, then refluxed overnight, and concentrated. The residue was dissolved in EtOAc, washed with 10% NaOH solution then dried, concentrated, and the product isolated in 82% as a solid.

   MS (ES) 184 (M + H)

2. **Preparation of 7-fluoro-quinazolinone**

   The Step 2 product (4.43 mmol) dissolved in 6 ml DMF was treated with ammonium formate (7.14 mmol) and the mixture heated to 140°C overnight. The mixture was cooled, then diluted with water and EtOAc. The organic layer was dried, concentrated, and the product quantitatively isolated.

   MS (ES) 165 (M + H)

3. **Preparation of 7-(2-piperidylethoxy)-quinazolinone**

   1-Piperidineethanol (5.18 mmol) dissolved in 3 ml DMF at 0°C was treated with sodium hydride (12.95 mmol), then stirred 30 minutes, and treated with the Step 2 product (1.73 mmol) dissolved in 3 ml DMF. The mixture was heated to 75°C overnight and then concentrated. The residue was purified by RP-HPLC and the product isolated in 97% yield as a creamy solid.

4. **Preparation of 4-chloro-7-(2-piperidylethoxy)quinazoline**

   A mixture consisting of the Step 3 product (1.68 mmol) and 5 ml $POCl_3$ was heated overnight at 75°C and then concentrated. The residue was azeotroped with toluene and the product isolated in 95% yield.

5. **Preparation of 4-piperazinyl-7-(2-piperidylethoxy)quinazoline**

   The Step 4 product (2.11 mmol) dissolved in 10 ml isopropyl alcohol was treated with piperazine (8.44 mmol), then heated 4 hours at 100°C, and concentrated. The residue was purified by RP-HPLC and the product isolated in 87% yield as a white solid.

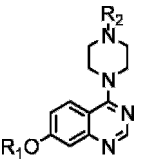
6. **Preparation of *N*-(4-cyanophenyl){4-[7-(2-piperidylethoxy)quinazolin-4-yl]piperazinyl}-carboxamide**

   4-Cyanophenylisocyanate (1.26 mmol) was added to a solution of the Step 5 product (0.84 mmol) in 2 ml DMF, then stirred overnight at ambient temperature, and concentrated. The residue was purified by RP-HPLC and the product isolated in 50% yield as a white solid.

   MS (ES) 487 (M + H)

Derivatives

Table 1 Selected quinazoline derivatives and their corresponding mass spectrum and product conversions



Entry	R_1	R_2	Yield (%)	MS (M + H)
1	1-Piperidineethanol	H	71 (five steps)	–
2	1-Piperidineethanol	N-(4-Cyanophenyl) carboxamide	50 (last step)	487

Testing

I. *HR5 Phosphorylation Assay*

The HR5 cell line is a cell line of CHO cells engineered to overexpress human β-PDGFR. The expression level of β-PDGFR in HR5 cells is around 5×10^4 receptor per cell. For the phosphorylation assay, HR5 cells were grown to confluency in 96-well microtiter plates followed by 16 hours serum starvation. Quiescent cells were incubated 30 minutes at 37°C, then treated with 8 nM PDGF BB. Cells were lyzed with 100 mM Tris, 750 mM NaCl, 0.5% Triton X-100, 10 mM sodium pyrophosphate, 50 mM NaF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, and 1 mM sodium vanadate followed by centrifugation. Clarified lysates were transferred into a second microtiter plate previously coated with 500 ng/well of 1B5B11anti-β-PDGFR mAb, incubated, washed three times with binding buffer, 250 ng/ml of either rabbit polyclonal anti-phosphotyrosine antibody or a selected experimental agent and then incubated. Thereafter, each well was rewashed three times and incubated with 1 μg/ml horseradish peroxidase-conjugated anti-rabbit antibody. Finally, wells were washed prior to adding ABTS and the rate of substrate formation monitored at 650 nm. HR5 IC_{50} testing results are provided in Table 2, column 2.

Table 2 Summary of IC_{50} MG63 and HR5 testing assays for selected quinazoline derivatives. Chemical agents having IC_{50} values less than 1 μM are especially preferred

Entry	IC_{50} (μM)	
	HR5	MG63 w/Human Plasma
1	0.150	0.103
2	2.27	3.56

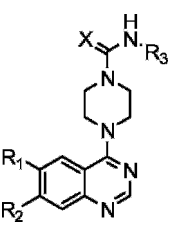
II. *MG63 Phosphorylation Assay*

The MG63 cell line is a human osteosarcoma tumor cell and is used for measuring endogenous β-PDGFR phosphorylation in these cells. The assay conditions are the same as those described for HR5 cell, except that PDGF-BB stimulation was provided in the presence or absence of 45% human plasma. MG63 w/human plasma IC_{50} testing results are summarized in Table 2, column 3.

Notes

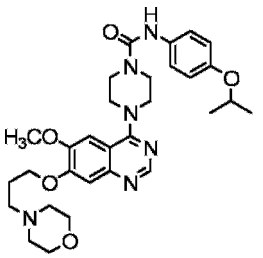
1. In an earlier investigation by co-author Matsuno (1), other quinazoline derivatives effective as phosphorylation kinase inhibitors were prepared and are provided in Table 3.

Table 3 Selected quinazoline derivatives previously prepared by the co-author Matsuno effective as PDGF kinase inhibitors and used in the treatment of cell proliferation diseases associated with cardiovascular disorders

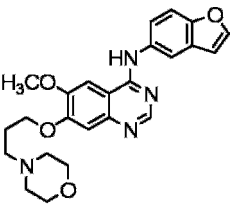


Entry	Z	R_1	R_2	R_3
16	S	OCH_3	OCH_3	2-Propyne
59	O	OCH_3	OCH_3	3,4-Trifluoromethoxyphenyl
115	O	OCH_3	OCH_3	4-Phenoxyphenyl
143	S	OCH_3	OCH_3	Cinnamide
207	S	OCH_3	OCH_3	4-(3-Cyclopentoxy)-4-methoxyphenyl)phenyl
329	O	F	OC_2H_5	3-(4-Cyanopyridine)

2. 7-(3-Morpholin-4-yl-piperizyl) quinazoline derivatives, (**I**), prepared by Kanter (2) were effective as kinase phosphorylation inhibitors and used in the treatment of proliferative diseases such as arteriosclerosis, vascular reobstruction after percutaneous coronary angioplasty, and bypass operation cell-proliferative. Quinazoline derivatives, (**II**), prepared by Lambert (3) were effective against the Src family of nonreceptor tyrosine kinases inhibitors and used in the treatment of uncontrolled cellular proliferation.

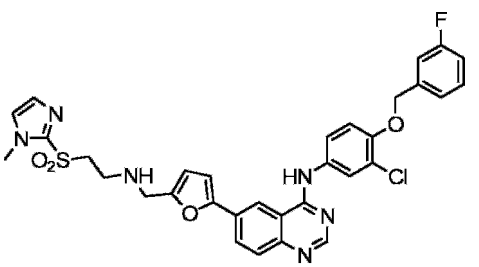


(I)



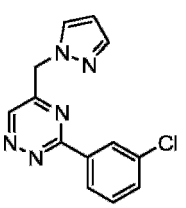
(II)

3. Anilinoquinazolines, (**III**), prepared by Cockerill (4) were effective as protein tyrosine kinase inhibitors and used in regulating cell growth associated with atherosclerosis and thrombosis.

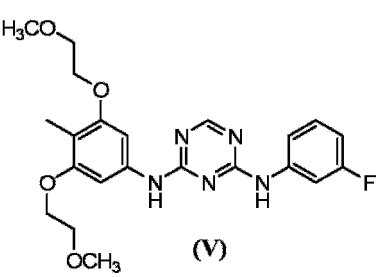


(III)

4. 1,2,4-, (**IV**), and 1,3,5-triazine derivatives, (**V**), prepared by Armistead (5) and Bebbington (6), respectively, were effective as phosphoryl transferase inhibitors and used in the treatment of cardiovascular diseases.

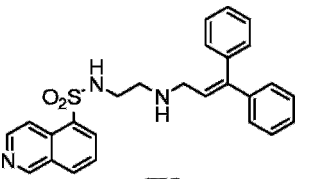


(IV)



(V)

5. Isoquinoline derivatives, (**VI**), effective as protein kinase inhibitors were prepared by Livnah (7) and used in treating cardiovascular pathologies.



(IV)

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2. J. Kanter *et al*., US Patent 6,951,937 (October 4, 2005)
3. C.M.P. Lambert *et al*., US Patent 6,939,866 (September 6, 2005)
4. G.S. Cockerill *et al*., US Patent 6,933,299 (August 23, 2005)
5. D.M. Armistead *et al*., US Patent 7,074,789 (July 11, 2006)
6. D. Bebbington *et al*., US Patent 7,091,343 (August 15, 2006)
7. N. Livnah *et al*., US Patent 6,949,565 (September 27, 2005)

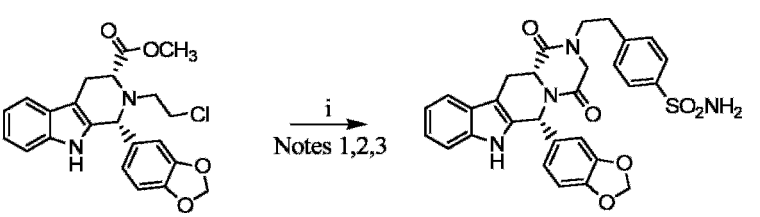
VII. Vasodilators: Cyclic Guanosine 3′,5′-Monophosphate-Specific Phosphodiesterase, PDE_5, Inhibitors

Title Pyrazino[1′,2′:1,6]pyrido[3,4-b]indole Derivatives
M.W. Orme *et al*., US Patent 6,911,542 (June 28, 2005)
Assignee Wyeth
Utility New Chemical Class of Vasodilators

Invention Significance Pyrazino[1′,2′:1,6]pyrido[3,4-b]indole-based medicaments are a new class of chemical agents effective as cGMP-specific PDE_5 inhibitors. These materials are effective as vasodilators, relaxants, and diuretics in the treatment of cardiovascular diseases such as angina and hypertension.

Reaction

Eq. 1



i- 4-(2-Aminoethyl)phenylsulfonamide, THF, methyl alcohol

Experimental

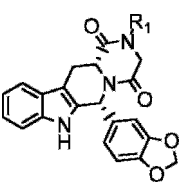
1. **Preparation of (6*R-trans*)-6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-[2-(4-phenylsulfamoyl)-ethyl]pyrazino[1′,2′:1,6]pyrido[3,4-b]indole-1,4-dione**

   A mixture consisting of (6*R-trans*)-6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-[2-(2-chloroethyl]pyrazino] [1′,2′:1,6]pyrido[3,4-b]indole (10.0 mmol) and 4-(2-aminoethyl)benzene-sulfonamide (20 mmol) dissolved in 50 ml methyl alcohol and 25 ml THF was heated 22 hours at 45°C, then concentrated. The residue was stirred 20 minutes in 40 ml methyl alcohol and a white solid was collected. The solid was washed five times with 20 ml methyl alcohol, three times with 20 ml hexanes, dried, and the product isolated in 89.9% yield as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 11.08 (s, 1H), 7.75 (d, $J = 8.2$ Hz, 2H), 7.54 (d, $J = 7.6$ Hz, 1H), 7.43 (d, $J = 8.2$ Hz, 2H), 7.31 (d, $J = 7.6$ Hz, 1H), 7.30–6.91 (m, 4H), 6.89 (s, 1H), 6.85–6.70 (m, 2H), 6.16 (s, 1H), 5.94 (s, 2H), 4.41 (dd, $J = 11.5$, 4.7 Hz, 1H), 4.17 (d, $J = 16.9$ Hz, 1H), 3.97 (d, $J = 16.9$ Hz, 1H), 3.73–3.50 (m, 2H), 3.48 (dd, $J = 15.8$, 4.4 Hz, 1H), 3.05–2.81 (m, 3H)
^{13}C-NMR (125 MHz, DMSO-d_6) δ (ppm) 167.1, 165.6, 147.1, 146.1, 143.0, 142.2, 137.0, 136.2, 133.9, 129.3, 125.8, 121.3, 119.0, 118.1, 111.3, 108.1, 106.9, 104.6, 101.0, 55.4, 55.1, 50.0, 48.5, 32.4, 22.1
API MS 559 $(C_{29}H_{26}N_4O_6S + H)^+$
TLC R_f (CH_2C_2/EtOAc, 3 : 1) = 0.11
Analysis Calc. for $C_{29}H_{26}N_4O_6S$: C, 62.35; H, 4.69; N, 10.03; S, 5.74. Found: C, 61.99; H, 4.76; N, 10.11; S, 5.81
$\boldsymbol{\alpha}_D^{27°C}$ +41.6° ($c = 1.0$, DMSO)

Derivatives

Table 1 Selected (6*R-trans*)-6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-pyrazino[1′, 2′,:1,6]pyrido[3,4-b]indole-1,4-dione derivatives their and corresponding physical properties.[1]H NMR data supplied by author



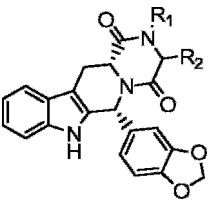
Entry	R_1	Yield (%)	mp (°C)	MS (M+1)	α_D^T (DMSO)
2	Hydroxyl	66	276–286	332	106.6 ($T = 27$°C, $c = 0.5$)
3	Methoxy	27	268–270	406	−91.7 ($T = 25$°C, $c = 0.5$)
4	Amine	82	272–278	391	75.7 ($T = 25$°C, $c = 1.0$)

Testing

I. *Inhibitory Effect on cGMP-PDE$_5$*

cGMP-PDE activity of selected experimental agents was determined according to the method of Wells (1). Testing results are provided in Table 2.

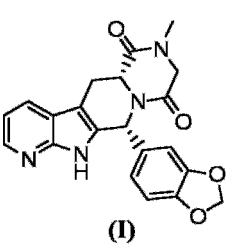
Table 2 Summary of in vitro testing for selected experimental agents exhibiting favorable IC_{50} PDE5 test results



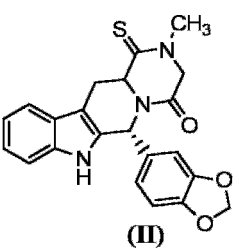
R_1	R_2	PDE_5 IC_{50} (μM)
Hydroxyl	Hydrogen	0.0075
4-(2-Aminoethyl)phenylsulfonamide	Hydrogen	0.0014
2-(*N,N*-Dimethy)ethyl-	Methyl	0.013
3-(Butyrolactame)propyl	Hydrogen	0.0054
1-Methyl-4-(3-propyl)-piperazine	Hydrogen	0.001
1-Acetic acid, *n*-octyl ester	Hydrogen	0.11

Notes

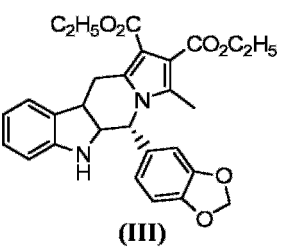
1. The preparation of the reagent of Step 1 co-reagent, (6*R-trans*)-6-(1,3-benzodioxol-5-yl)- 2,3,6,7,12,12a-hexahydro-2-[2-(2-chloroethyl]pyrazino][1′,2′:1,6]pyrido[3,4-b]indole, was previously prepared by the author (2).
2. Additional cGMP-specific PDF5 inhibitors, (**I**), (**II**), and (**III**), were prepared by the authors (3,4,5), respectively, in earlier investigations and used in the treatment of cardiovascular diseases.



(I)

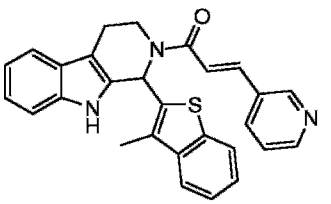


(II)



(III)

3. Carboline derivatives, (**IV**) and (**V**), prepared by Sawyer (6) and the author (7), respectively, were effective as PDE_5 inhibitors and used in the treatment of variant (Prinzmetal) angina and hypertension.



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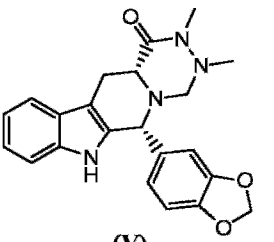

(V)

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2. M.W. Orme *et al*., US Patent 6,876,711 (April 15, 2005)
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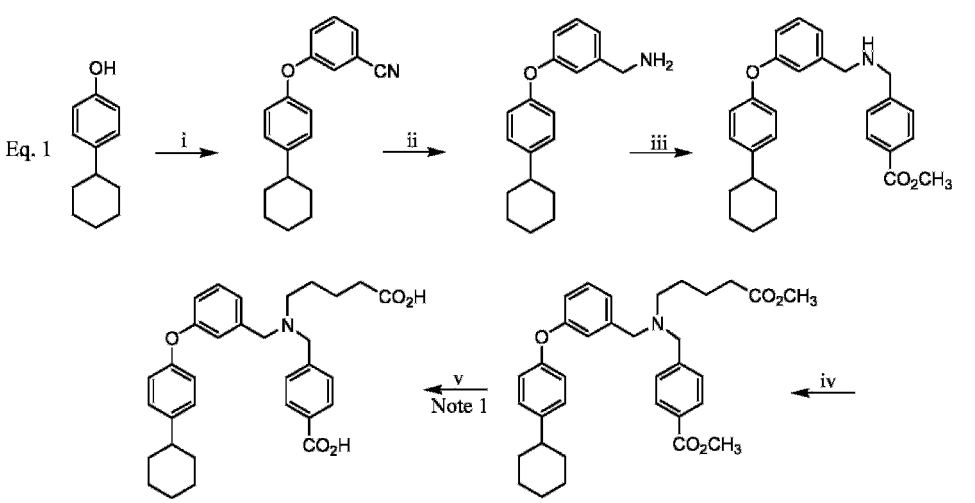
4. M.W. Orme *et al*., US Patent 6,858,620 (February 22, 2005)
5. M.W. Orme *et al*., US Patent 6,838,456 (January 4, 2005)
6. J.S. Sawyer *et al*., US Patent 6,992,192 (January 21, 2006)
7. M.W. Orme *et al*., US Patent 6,984,641 (January 10, 2006)

VIII. Vasorelaxants: Cyclic Guanosine Monophosphate Formation Stimulators

Title Derivatives of Dicarboxylic Acid Having Pharmaceutical Properties
M. Harter *et al*., US Patent 6,939,990 (September 6, 2005)
Assignee Bayer Aktiengesellschaft
Utility Treatment of Cardiovascular Disorders

Invention Significance Cyclic guanosine monophosphate (cGMP) regulation of phosphodiesterases, ion channels, and protein kinases, guanylate cyclase, plays a crucial role in the relaxation and proliferation of smooth muscle cells. Together with nitric oxide (NO) it forms the NO/cGMP system. If this system is interrupted, high blood pressure, angina pectoris, thromboses, and other cardiovascular symptoms result. A method of stimulating cGMP formation as a means of treating cardiovascular disorders is described.

Reaction



i- 3-Bromobenzonitrile, Cu(I) iodide, potassium carbonate, pyridine
ii- Diethyl ether, lithium aluminum hydride, THF

iii- Sodium triacetoxyborohydride, methyl 4-formylbenzoate, CH_2Cl_2
iv- Methyl 5-bromovalerate, acetonitrile, potassium carbonate
v- Sodium hydroxide, dioxane, water

Experimental

1. **Preparation of 3-(4-cyclohexylphenoxy)benzonitrile**

4-Cyclohexylphenol (6.81 mmol), 3-bromobenzonitrile (40.85 mmol), copper(I) iodide (6.81 mmol), and K_2CO_3 (13.62 mmol) were mixed in 24 ml of pyridine, then stirred 15 hours at 140°C, and cooled. After filtering the mixture through kieselguhr, the filter cake was washed with CH_2Cl_2 and the organic phase concentrated. The residue was diluted with EtOAc and water, then treated with 2 M HCl, and a precipitate that formed was removed. The filtrate was extracted twice with 2 M HCl, then washed with brine, dried using $MgSO_4$, and concentrated. The residue was purified by chromatography with silica gel using cyclohexane/EtOAc, 2:1, and the product isolated in 44% yield as a colorless oil.

R_f cyclohexane/EtOAc, 2:1, 0.71
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.42–7.32 (2H, m), 7.29–7.15 (4H, m), 6.95 (2H, d), 2.35 (1H, m), 1.96–1.60 (5H, m), 1.50–1.25 (5H, m)
MS (DCl, NH_3) 572 ($2M + NH_4^+$), 317 ($M + N_2H_7^+$), 295 ($M + NH_4^+$), 277 (M^+)

2. **Preparation of 3-(4-cyclohexylphenoxy)benzylamine**

The Step 1 product (2.16 mmol) dissolved in 6 ml diethyl ether at 0°C was added dropwise to 4.32 ml 1 M $LiAlH_4$ in THF and the mixture warmed to ambient temperature over 4 hours. The solution was then diluted with 10 ml saturated NH_4Cl solution and diethyl ether and the organic phase isolated. The ethereal solution was washed with water, dried over $MgSO_4$, concentrated, and the product isolated in 84% yield with 88.82% purity.

R_f CH_2Cl_2/methyl alcohol, 9/1,0.13
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.26 (2H, d), 7.16 (2H, d), 7.05–6.85 (4H, m), 3.86 (2H, s), 2.51 (1H, m), 1.93–1.79 (4H, m), 1.70–1.55 (2H, m), 1.48–1.31 (4H, m).
MS (EI) 280 (M^+)

3. **Preparation of methyl 4-({[3-(4-cyclohexylphenoxy)benzyl]amino}methyl) benzoate**

Sodium triacetoxyborohydride (3.80 mmol) was added to a solution of the Step 2 product (1.90 mmol) and methyl 4-formylbenzoate (1.90 mmol) in 5 ml CH_2Cl_2 and the mixture stirred overnight at ambient temperature. The mixture was then treated

with 10 ml saturated $NaHCO_3$ solution, then diluted with CH_2Cl_2. The organic phase was dried using $MgSO_4$, concentrated, and 429 mg of product isolated as a colorless oil.

R_f CH_2Cl_2/methyl alcohol, 10:1, 0.56
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.99 (2H, d), 7.40 (2H, d), 7.28 (1H, d), 7.18 (2H, d), 7.08–6.99 (2H, m), 6.97–6.87 (3H, m), 3.91 (3H, s), 3.85 (2H, s), 3.78 (2H, s), 2.51 (1H, m), 1.93–1.70 (5H, m), 1.50–1.32 (5H, m)
MS (ESI) 430 ($M + H^+$)

4. **Preparation of methyl 4-{[[3-(4-cyclohexylphenoxy)benzyl](5-methoxy-5-oxopentyl)-amino]methyl}benzoate**

The Step 3 product (0.89 mmol) and methyl 5-bromovalerate (0.98 mmol) dissolved in 3.3 ml acetonitrile were treated with K_2CO_3 (1.95 mmol), then refluxed 48 hours, and concentrated. The residue was dissolved in EtOAc, then washed with water, dried using Na_2SO_4, and concentrated. The residue was purified by chromatography using cyclohexane/EtOAc, 5:1, and the product isolated in 78% yield as a colorless oil.

R_f CH_2Cl_2, 0.09
^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 7.87 (2H, d), 7.39 (2H, d), 7.30 (1H, t), 7.23 (2H, d), 7.04 (1H, m), 6.95–6.83 (4H, m), 3.82 (3H, s), 3.60 (5H, s), 3.55 (4H, m), 2.51 (1H, m partially obscured by DMSO), 2.19 (2H, t), 1.90–1.73 (7H, m), 1.70–1.53 (2H, m), 1.45–1.32 (5H, m)
MS (ESI) 544 ($M + H^+$)

5. **Preparation of 4-({(4-Carboxybutyl)[3-(4-cyclohexylphenoxy)benzyl]amino}methyl)-benzoic acid**

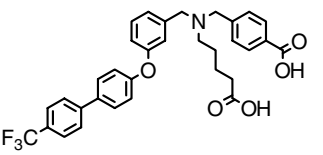
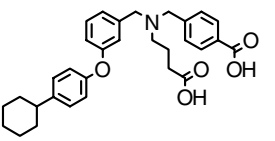
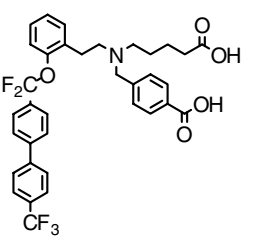
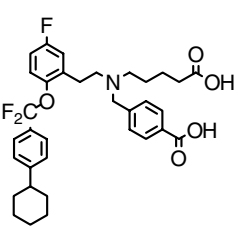
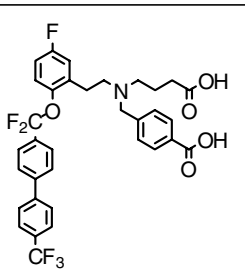
The Step 4 product (0.65 mmol) dissolved in 3.5 ml dioxane and 1.8 ml of water was treated with 195 µl 45% NaOH solution, then heated 2 hours at 90°C, and cooled. Dioxane was removed and the aqueous pH lowered to 4 with 1 M HCl. A precipitate that formed was washed with water, dried, and the product isolated in 83% yield as a white solid.

R_f EtOAc/methyl alcohol, 7:3, 0.38
^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 12.49 (2H, broad s), 7.89 (2H, d), 7.47 (2H, d), 7.29 (1H, t), 7.20 (2H, d), 7.05 (1H, d), 6.98–6.80 (4H, m), 3.55 (2H, s), 3.50 (2H, s), 2.51 (1H, m partially obscured by DMSO), 2.41 (2H, m), 2.08 (2H, m), 1.89–1.62 (6H, m), 1.49–1.13 (8H, m)
MS (ESI) 1030 ($2M + H^+$), 516 ($M + H^+$)

Derivatives

Selected derivatives are provided in Table 1.

Table 1 Selected experimental agents and results of vasorelaxant effect in vitro assay.[1]H NMR and R_f information for product and intermediates supplied by author

Entry	**Structure** (Note 3)	**IC_{50} (nM)**
2		55
3		36
6		0.041
12		0.4
17		0.26

Testing

I. *Vasorelaxant Effect In Vitro*

Rabbits were anesthetized by intravenous injection with thiopental sodium and exsanguinated. The arteria saphena was removed and divided into rings of 3 mm wide. The

individual rings were mounted on a pair of triangularly shaped hooks opened at the end and made of Remanium® wire having a diameter of 0.3 mm. Under pretension, each ring was introduced into a 5 ml organ bath containing Krebs–Henseleit solution at 37°C. The force of contraction was detected with Statham UC2 cells, amplified, and recorded in parallel on chart recorders. Contractions are generated by adding phenylephrine.

After four control cycles, a selected experimental agent was added and in each subsequent run, the concentration increased. Thereafter, the height of the contraction reached using the selected experimental agent was compared with the height of the contraction reached in preceding run and the concentration necessary to reduce the height of the control value by 50%, IC_{50}, calculated. Testing results are provided in Table 1.

II. *In Vivo Antifibrotic Action Assay*

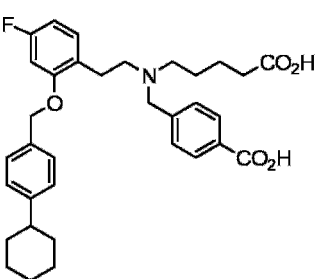
The antifibrotic action of the substances was investigated using the model of the porcine serum-induced rat liver fibrosis. Female Sprague–Dawley rats were treated twice per week with 0.5 ml/animal of sterile porcine serum, while control animals were also treated twice weekly with sterile physiological saline. Treatment with a selected experimental agent was once daily using 5 ml/kg of po solvent comprising 20% Cremophor, 10% Transcutol, and 70% H_2O and was carried out in parallel to the treatment with porcine serum. After 7 weeks of treatment, the animals were killed and the livers removed to quantify the collagen content. Hydroxyproline tissue concentration was determined according to the method of Prockop (1).

Results

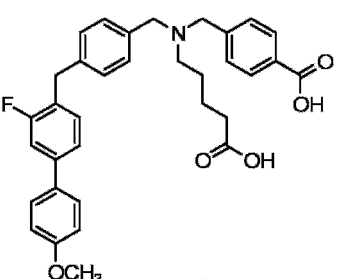
The hydroxyproline values agreed very well with the results of the morphometric fibrosis measurement. Without simultaneous administration of selected experimental agents, porcine serum treatment resulted in a pronounced accumulation of collagen in the liver. The formation of these collagen deposits was reduced in a dose-dependent manner using selected experimental agents.

Notes

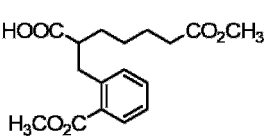
1. Halogenated dicarboxylic acids, (**I**) and (**II**), prepared by the author (2,3), respectively, and carboxylic ester diacids (**III**) prepared by Alonso-Alija (4) were also effective in stimulating cGMP formation and used in the treatment of cardiovascular diseases.



(I)

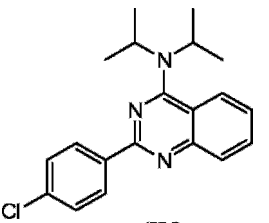


(II)



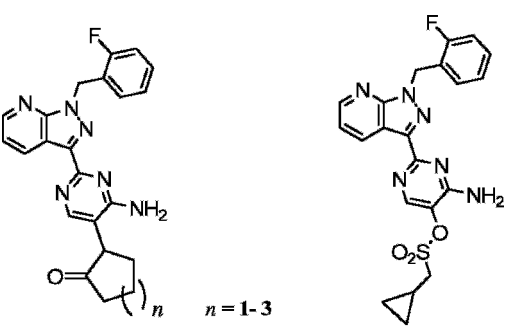
(III)

2. 4-Amino-2-aryl-tetrahydroquinazolines, (**IV**), prepared by Schindler (5) were effective in modulating the endogenous production of cGMP and were suitable for treatment of diseased states associated with a disturbed cGMP balance.



(IV)

3. Fluorobenzyl 1H-pyrazolo [3,4-b] lactams, (**V**), and sulfonates, (**VI**), prepared by Stasch (6,7), respectively, were effective in controlling cardiovascular disorders associated with disturbances of the NO/cGMP system.



(V) $n = 1\text{-}3$ **(VI)**

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5. U. Schindler *et al*., US Patent 7,045,526 (May 16, 2006)
6. J.-P. Stasch *et al*., US Patent 6,919,345 (July 19, 2005)
7. J.-P. Stasch *et al*., US Patent 6,903,089 (June 7, 2005)

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CHAPTER IX

Diabetes

Type I Diabetes

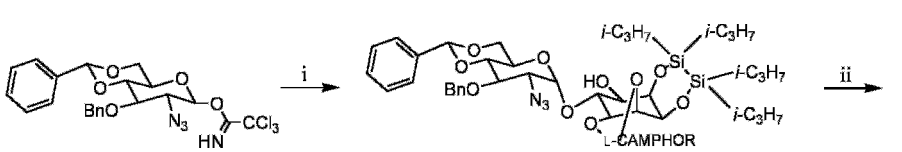
I. Insulin-dependent Enzyme Stimulants: Inositolphosphoglycan Mimics

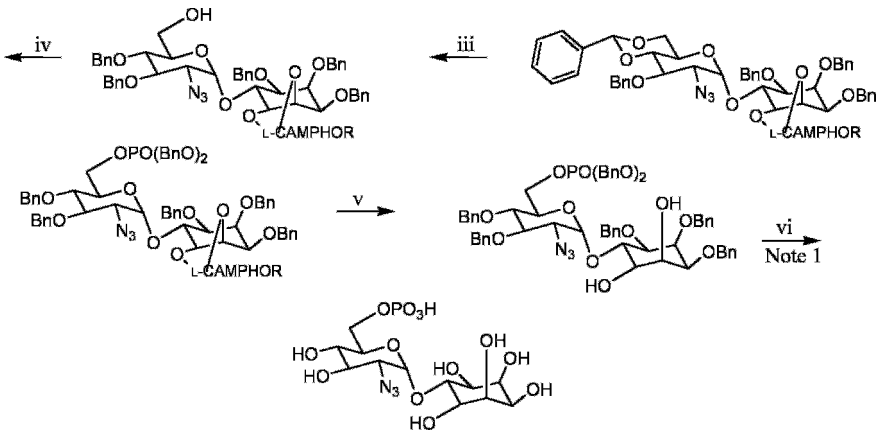
Title Compounds and Their Uses
M. Martin-Lomas *et al*., US Patent 6,953,781 (October 11, 2005)
Assignee Rodaris Pharmaceuticals Limited
Utility Treatment of Type I Diabetes

Invention Significance Inositolphosphoglycans (IPGs) have been postulated as mediators in the action of insulin and insulin-like growth factor (IGF-I). It remains, however, a significant problem to produce medicaments, which can mimic the activities of IPGs. The present invention describes methods for the preparation of IPG mimics suitable for the treatment of insulin dependent diabetes. The effectiveness of these agents is a result of the stimulation of insulin-dependent enzymes such as pyruvate dehydrogenase phosphatase and glycogen synthase phosphatase.

Reaction

Eq. 1





i- 1-D-1,2-*O*-(L-1,7,7-Trimethyl[2.2.1]-bicyclohept-2-ylidene)-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxanyl)-myo-inositol, trimethylsilyl trifluromethanesulfonate, diethyl ether, triethylamine

ii- THF, tetrabutyl ammonium fluoride, DMF, sodium hydride, benzyl bromide

iii- Borane–dimethylamine, CH_2Cl_2, boron trifluoride diethyl etherate

iv- 1-H-tetrazole, CH_2Cl_2, dibenzyl di-isopropylphosphoramidite, 3-chloroperbenzoic acid

v- CH_2Cl_2, trifluoroacetic acid

vi- Ethyl alcohol, 10% palladium on carbon, hydrogen

Experimental

1. **Preparation of 1′-D-6-*O*-(2′-azido-3′-*O*-benzyl-4′,6′-*O*-benzylidene-2′-deoxy-α-D-glucopyranosyl)-1,2-*O*-(L)-1,7,7-trimethyl[2.2.1]-bicyclohept-2-ylidene**

   A solution of trichloroacetimidate 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-D-glucopyranoside (3.217 mmol) and 1-D-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]-bicyclohept-2-ylidene)-3,4-*O*-(1,1,3,3-tetraisopropyldisiloxanyl)-myo-inositol (4.507 mmol) in 50 ml diethyl ether at −20°C was treated with 29 ml trimethylsilyl trifluromethanesulfonate, then gradually warmed over 5 hours to ambient temperature. The mixture was then quenched with 0.5 ml triethylamine and concentrated. The residue was purified by flash chromatography using a hexane/EtOAc gradient of 95:5–75:25 and the α(1–6) product isolated in 55% yield as a white solid.

2. **Preparation of 1′-D-6-*O*-(2′-azido-3′-*O*-benzyl-4′,6′-*O*-benzylidene-2′-deoxy-α-D-glucopyranosyl)-1,2-*O*-(L)-1,7,7-trimethyl[2.2.1]-bicyclohept-2-ylidene**

   The Step 1 product (1.735 mmol) dissolved in 20 ml THF at 0°C was treated with 3.81 ml 1 M tetrabutyl ammonium fluoride in THF, then stirred 15 minutes, and

concentrated. The residue was redissolved in 15 ml DMF, then recooled to 0°C, and treated with sodium hydride (7.801 mmol) and benzyl bromide (7.801 mmol). The mixture stirred 2 hours and was then quenched with methyl alcohol and diluted with 100 ml CH_2Cl_2. The solution was then washed twice with brine, dried, and concentrated. The residue was purified by flash chromatography using hexane/EtOAc, 95:5, and 1.410 g of product isolated.

3. **Preparation of 1′-D-6-*O*-(2′-azido-3′,4′-di-*O*-benzyl- 2′-deoxy-α-D-glucopyranosyl)-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]-bicyclohept-2-ylidene)-3,4,5-tri-*O*-benzyl-myo-inositol**

   The Step 2 product (0.484 mmol) was treated with borane–dimethylamine complex (1.952 mmol) in 40 ml CH_2Cl_2 at 0°C followed by the dropwise addition of 254 ml boron trifluoride diethyl etherate over 30 minutes. Stirring was continued for an additional hour at ambient temperature and the mixture was then quenched with using 15 ml saturated $NaHCO_3$ solution. The solution was diluted with 60 ml CH_2Cl_2, then washed three times with 100 ml brine, dried, and concentrated. The residue was purified by flash chromatography using hexane/EtOAc, 6:1, and the product isolated in 60% yield.

4. **Preparation of 1′-D-6-*O*-(2′-azido-3′, 4′-di-*O*-benzyl-2′-deoxy-6′-dibenzyl-phosphate-α-D-glucopyranosyl)-1,2-*O*-(L-1,7,7-trimethyl[2.2.1]-bicyclohept-2-ylidene)-3,4,5-tribenzyl-myo-inositol**

   A mixture of the Step 3 product (0.157 mmol) and 1-H-tetrazole (0.628 mmol) in 15 ml CH_2Cl_2 at 0°C was treated with the dropwise addition of 212 ml dibenzyl diisipropylphosphoramidite, then stirred 3 hours at ambient temperature. The mixture was then cooled to −40°C and further treated with 3-chloroperbenzoic acid (0.393 mmol) dissolved in 5 ml CH_2Cl_2. After stirring 45 minutes, it was diluted with 30 ml CH_2Cl_2 then washed twice with 50 ml apiece saturated $NaHCO_3$, $NaHCO_3$, and brine, dried, and concentrated. The residue was purified by flash chromatography using hexane/EtOAc, 4:1, and the product isolated in 80% yield.

5. **Preparation of 1′-D-6-*O*-(2′-azido-3′,4′-di-*O*-benzyl-2′-deoxy-6′-dibenzyl-phosphate-α-D-glucopyranosyl)-3,4,5-tri-*O*-benzyl-myo-inositol**

   The Step 4 product (0.091 mmol) dissolved in 10 ml CH_2Cl_2 was treated with 1 ml water and 0.42 ml trifluoroacetic acid, then stirred 4 hours at ambient temperature, and diluted with 40 ml CH_2Cl_2. The organic portion was washed twice with 50 ml saturated $NaHCO_3$ solution, three times with 50 ml brine, dried with $MgSO_4$, and concentrated. The residue was purified by flash chromatography using a hexane/EtOAc gradient of 1:1–1:2, then 100% EtOAc, and the product isolated in 86% yield as a white solid.

6. **Preparation of 1′-D-6-*O*-(2′-amino-2′-deoxy-4′-phosphate-α-D-glucopyranosyl)-myo-inositol (RGL1027)**

A suspension of the Step 5 product (14.840 mmol) in 0.6 ml ethyl alcohol was treated with 10% Pd/C (0.003 mmol), then stirred 36 hours under 1 atm hydrogen at ambient temperature. The mixture was concentrated and the residue suspended in water then filtered through celite. The filtrate was lyophilized and the product isolated in 74%.

^{1}H NMR (500 MHz, D_2O) δ 5.25 (broad s, 1H, $H_{1'}$), 3.96 (m, 1H, $H_{4'}$), 3.91 (m, 2H, $H_{3'} + H_2$), 3.84 (m, 1H, $H_{5'}$), 3.82 (m, 1H, $H_{6'b}$), 3.61 (m, 3H, $H_{6'a} + H_1 + H_6$), 3.52 (t, $J = 8.85$Hz, 1H, H_4), 3.41 (broad d, $J = 8.85$Hz, 1H, H_3), 3.27 (d, $J = 8.85$ Hz, 1H, H_5), 3.18 (m, 1H, $H_{2'}$)
^{13}C NMR (500 MHz, D_2O) d 97.40 ($C_{1'}$), 80.94 (C_6), 73.03 (C_5), 72.91 (C_4), 72.68 (C_2), 72.25 ($C_{5'}$), 72.08 (C_1), 71.99 ($C_{4'}$), 71.32 (C_3), 70.79 ($C_{3'}$), 60.36 ($C_{6'a} + C_{6'b}$), 55.07 ($C_{2'}$)

Derivatives

The effect of selected derivatives in stimulating PDH levels are provided in Tables 1 and 2.

Table 1 Effect of selected experimental agents at 100 μM in stimulating the insulin-dependent enzyme PDH levels. ^{1}H NMR for products provided by author

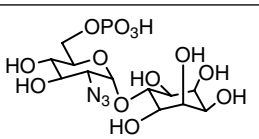
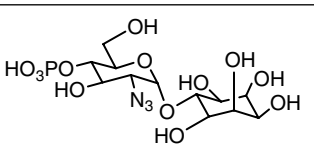
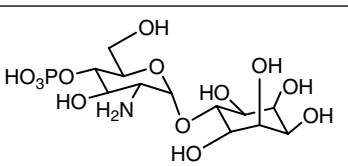
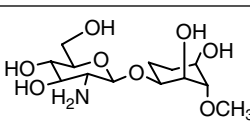
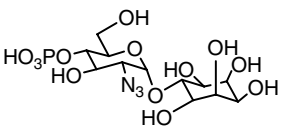
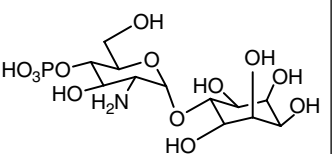
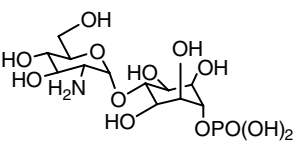
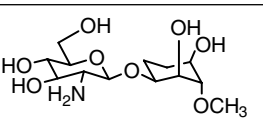
Entry	Structure	PDH Activation using 100 μM (%)
RGL1023		14
RGL1027		132
RGL1029		361
RGL1015		38

Table 2 Effect of selected experimental agents at 0.1, 1.0, and 10 μM in stimulating the insulin-dependent enzyme PDH levels

Entry	Structure	PDH Activation using 0.1 μM (%)	PDH Activation using 1.0 μM (%)	PDH Activation using 10.0 μM (%)
RGL1027		18	–	10
RGL1029		59	–	33
RGL1019		32	–	31
RGL1015		14	15	12

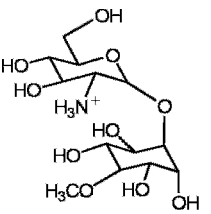
Testing

I. *Pyruvate Dehydrogenase Phosphatase Assay*

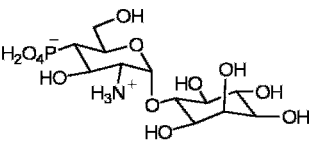
Pyruvate dehydrogenase phosphatase (PDH) assay was determined using the method of Caro (1). The effects of selected experimental agents in stimulating the insulin-dependent enzyme PDH at 100 and 0.1 μM, 1.0 and 10 μM are provided in Tables 1 and 2, respectively.

Notes

1. D-Pinitol, (**I**), and D-inositol, (**II**),-based PDH phosphatase mimics were prepared by the author (2,3), respectively, in earlier investigations and used in the treatment of insulin-dependent diabetes.

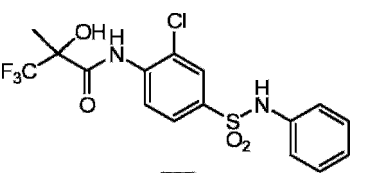


(I)



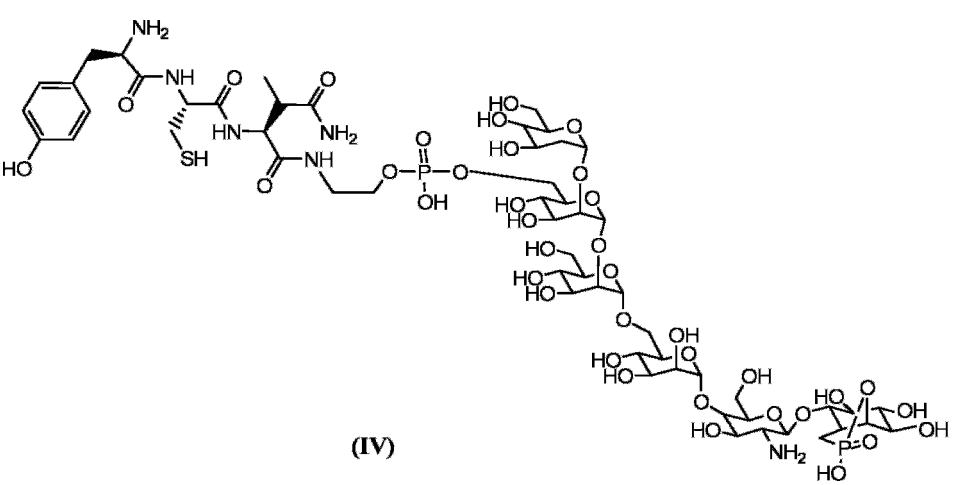
(II)

2. 3,3,3-Trifluoropropylphenyl amides, (**III**), prepared by Butlin (4) were effective in elevating insulin-dependent enzyme PDH levels and used in the treatment of Type I diabetes.



(III)

3. Phosphoinositoylglycans, (**IV**), prepared by Mueller (5) having a high binding affinity to proteins from plasma membrane of adipocytes were effective as glucose uptake agents by circumventing the insulin signaling cascade.



(IV)

References

1. W. Caro *et al*., Biochem. Mol. Med., 61, 214 (1997)
2. M. Martin-Lomas *et al*., US Patent 6,939,857 (September 6, 2005)
3. Martin-Lomas *et al*., US Patent 6,759,390 (July 6, 2004)
4. R.J. Butlin *et al*., US Patent 6,960,688 (November 1, 2005)
5. G. Mueller *et al*., US Patent 7,049,416 (May 23, 2006)

Type II Diabetes

I. Blood Glucose-Lowering Agents

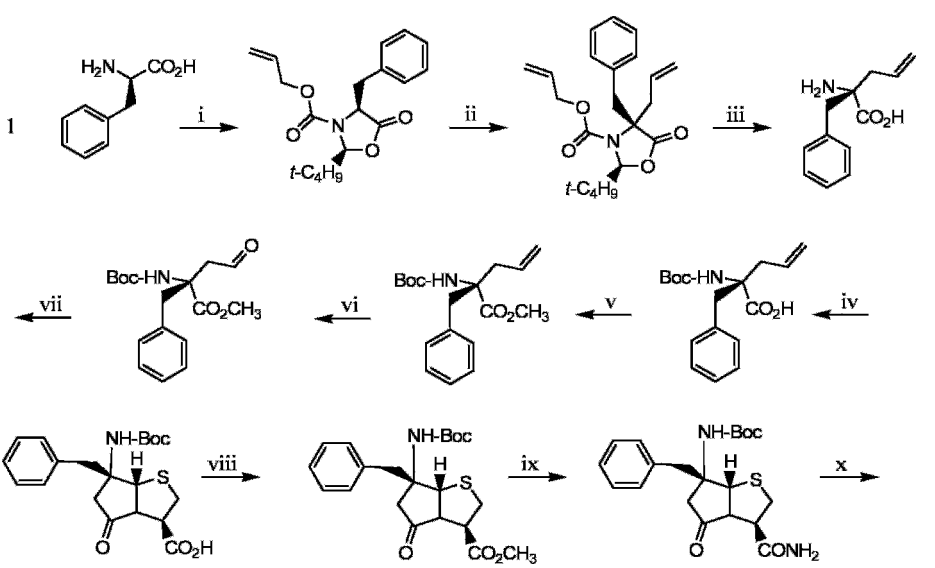
A. Dipeptidyl Peptidase-IV Inhibitors

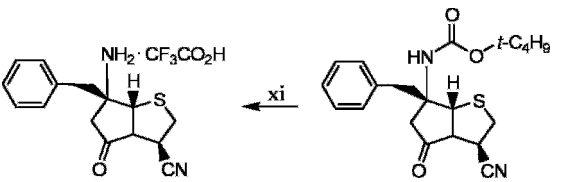
Title Bicyclic Cyanoheterocycles, Process for Their Preparation, and Their Use as Medicaments
H. Wagner *et al.*, US Patent 7,008,957 (March 7, 2006)
Assignee Sanofi-Aventis Deutschland GmbH
Utility Blood Glucose-lowering Agents for Treating Type II Diabetes

Invention Significance Resistance against the metabolic effects of insulin is one of the main features of noninsulin-dependent diabetes and is usually manifested by reduced inhibition of hepatic gluconeogenesis. To address this disorder, dipeptidyl peptidase-IV (DP-IV) inhibitors have been prepared which increase lipid and carbohydrate metabolism and thereby lower blood glucose levels.

Reaction

Eq. 1





i- Sodium hydroxide, toluene, *n*-pentane, trimethylacetaldehyde, CH_2Cl_2, allyl chloroformate
ii- Potassium bistrimethylsilylamide, toluene, allyl bromide
iii- THF, morpholine, tetrakistriphenylphosphinepalladium, acetic acid
iv- Sodium hydroxide, water, dioxane, di-*t*-butyl dicarbonate, potassium carbonate
v- Trimethylsilyldiazomethane, hexane, methyl alcohol
vi- Osmium tetroxide, *t*-butyl alcohol, sodium periodate, THF, water
vii- Pyridine, L-cysteine
viii- Trimethylsilyldiazomethane, hexane
ix- Ammonium hydroxide, methyl alcohol, methyl-*t*-butylether
x- DMF, cyanuric chloride
xi- Thioanisole, trifluoroacetic acid

Experimental

1. **Preparation of allyl (2*S*,4*S*)-4-benzyl-2-*t*-butyl-5-oxooxazolidine-3-carboxylate**

   (*S*)-Phenylalanine (5 g) and 30.5 ml 1 M NaOH were mixed and stirred 60 minutes at ambient temperature, then concentrated. The residue was treated with 50 ml toluene and reconcentrated. The residue was then suspended in 100 ml *n*-pentane containing 7.7 ml trimethylacetaldehyde and refluxed 15 hours, then concentrated. The residue was mixed with 40 ml toluene, then reconcentrated. The residue was suspended in 100 ml CH_2Cl_2 while stirring in an ice bath, then treated with 3.53 ml allyl chloroformate and stirred 4 days at ambient temperature. The mixture was partitioned between 100 ml CH_2Cl_2 and 75 ml saturated brine, then filtered, and the organic phase washed three times with 30 ml $NaHCO_3$, once brine, dried with Na_2SO_4, and concentrated. The oily residue was purified by column chromatography with silica gel using heptane/EtOAc, 45:5, and 3.4 g of product isolated as an oil.

2. **Preparation of allyl (2*S*,4*S*)-4-allyl-4-benzyl-2-*t*-butyl-5-oxooxazolidine-3-carboxylate**

   The Step 1 product (3.16 g) dissolved in 20 ml THF cooled to −65°C was treated dropwise with 20.9 ml 0.5 M potassium bistrimethylsilylamide in toluene, then

stirred 30 minutes, and further treated with 0.861 ml allyl bromide. The mixture was gradually warmed to ambient temperature, then stood overnight, and was then quenched with 15 ml saturated NH_4Cl solution. The mixture was extracted with EtOAc, washed with brine, concentrated, purified as in Step 1, and 2.0 g product isolated as an oil.

M + H 358

3. **Preparation of (*S*)-2-amino-2-benzylpent-4-enecarboxylic acid**

A mixture of the Step 2 product (2 g), 60 ml THF, 4.88 ml of morpholine, and tetrakistriphenylphosphinepalladium (300 mg) was stirred 20 minutes at ambient temperature, then concentrated. The residue was stirred 30 minutes in a mixture of 6 ml glacial acetic acid and 20 ml water, then reconcentrated. The residue was purified by chromatography using CH_2Cl_2/methyl alcohol, 9:1, and 680 mg of product isolated, mp = 221.2°C.

M + H 206

4. **Preparation of (*S*)-2-benzyl-2-*t*-butoxycarbonylaminopent-4-ene-carboxylic acid**

The Step 3 product (450 mg) dissolved in 8 ml dioxane and 6 ml water was treated with 2.2 ml 1 M NaOH followed by di-*t*-butyl dicarbonate (1.6 g) of and K_2CO_3 (350 mg), then stirred 7 hours at 40°C. The dioxane was stripped off in vacuo at ambient temperature, the aqueous phase pH lowered to 3–4 with 10% citric acid, and the mixture extracted with 10 ml EtOAc. The organic phase was dried, concentrated, and 490 mg product isolated. The product was used without further purification.

M + H 306

5. **Preparation of methyl (*S*)-2-benzyl-2-*t*-butoxycarbonylaminopent-4-ene-carboxylate**

The step 4 product (490 mg) dissolved in 8 ml methyl alcohol was treated portionwise with 5 ml 2 M trimethylsilyldiazomethane at ambient temperature. After the reaction was complete, it was quenched with glacial acetic acid and concentrated. The residue was purified by chromatography using CH_2Cl_2/methyl alcohol, 95:5, and 410 mg of product isolated as an oil.

M + H 320

6. **Preparation of methyl (*R*)-2-benzyl-2-*t*-butoxycarbonylamino-4-oxobutyrate**

The Step 5 product (410 mg) dissolved in 15 ml THF and 5 ml water was treated with 1.1 ml 2.5% solution osmium tetroxide in *t*-butyl alcohol followed by the portionwise addition of sodium periodate (686 mg), then stirred overnight at ambient temperature. The mixture was concentrated and the residue dissolved in 26 ml

1 M $NaHCO_3$ solution, then extracted with diethyl ether. The extract was dried, concentrated, and 400 mg of product isolated as an oil.

M + H 322

7. **Preparation of (3*R*,6*R*,7a*S*)-6-benzyl-6-*t*-butoxycarbonylamino-5-oxohexahydropyrrolo[2,1-b]thiazole-3-carboxylic acid**

   A mixture consisting of the Step 6 product (400 mg), 5 ml of pyridine, and L-cysteine (166 mg) was refluxed 4 hours and concentrated. The residue was purified by chromatography using CH_2Cl_2/methyl alcohol/acetic acid, 90:10:1, and 375 mg of product isolated as an oil.

   M + H 393

8. **Preparation of methyl (3*R*,6*R*,7a*S*)-6-benzyl-6-*t*-butoxycarbonylamino-5-oxohexahydropyrrolo[2,1-b]thiazole-3-carboxylate**

   The Step 7 product (250 mg) dissolved in 8 ml methyl alcohol was treated portionwise with 1.5 ml 2 M trimethylsilyldiazomethane in hexane at ambient temperature, then quenched with glacial acetic acid, and concentrated. The residue was purified by chromatography with silica gel using EtOAc/*n*-heptane, 1:1.5, and 250 mg product isolated as an oil.

   M + H 407

9. **Preparation of (3*R*,6*R*,7a*S*)-6-benzyl-6-*t*-butoxycarbonylamino-5oxohexahydropyrrolo[2,1-b]thiazole-3-carboxamide**

   A mixture of the Step 8 product (170 mg) and 10 ml 7 M NH_4OH in methyl alcohol was left to stand overnight at ambient temperature and then concentrated. The residue was stirred with MTBE and 130 mg of product isolated by filtration, mp = 76.5°C.

   M + H 392

10. **Preparation of *t*-butyl (3*R*,6*R*,7a*S*)-(6-benzyl-3-cyano-5-oxohexahydropyrrolo-[2,1-b]thiazol-6-yl)-carbamate**

    The Step 9 product (100 mg) dissolved in 3 ml DMF was treated with cyanuric chloride (28.25 mg) and stirred 2 hours at ambient temperature, then concentrated. The residue was purified by chromatography using diisopropyl ether/CH_2Cl_2, 100:10, and 50 mg of product isolated as an oil.

    M + H 374

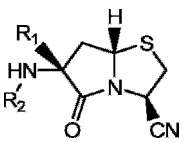
11. **Preparation of (3*R*,6*R*,7a*S*)-6-amino-6-benzyl-5-oxohexahydropyrrolo[2,1-b]-thiazole-3-carbonitrile trifluoroacetate**

    A mixture consisting of the Step 10 product (50 mg), 0.1 ml of thioanisole, and 1 ml trifluoroacetic acid was stirred in an ice bath for 15 minutes, then concentrated. The residue was stirred with diisopropyl ether and 35 mg product isolated, mp = 209°C.

    M + H 274

Derivatives

Table 1 Selected 5-oxohexahydropyrrolo[2,1-b]thiazole-3-carbonitrile derivatives and their corresponding mass spectra. Melting points for selected products provided by author



Entry	R_1	R_2	Salt[a]	[M + 1]
1	H	CH_3	TFA	274
2	H	*i*-C_3H_7	TFA	284
3	H	CH_3	TFA	284
4	3-(Phenyl)propyl	CH_3	TFA	302
5	Benzyl	H	TFA	274
6	Cyclopentyl	H	TFA	252
7	*sec*-Butyl	H	TFA	240
8	H	Hydroxylmethyl	TFA	214
9	H	Piperidin-1-ylmethyl	2TFA	281
10	H	Piperidin-4-yl	2TFA	267
11	H	Piperidin-1-ylmethyl	2TFA	281

[a] Trifluoroacetic acid.

Testing

I. *Measurement of Dipeptidyl Peptidase-IV Activity*

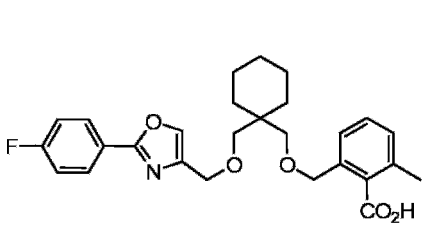
The assay was carried out using DPP-IV from porcine kidney at ambient temperature for approximately 10 minutes and quenched using 20 μl 1 M $ZnCl_2$. H-Ala-Pro-AFC conversion was determined fluorimetrically by measuring the emission at 535 nm after excitation at 405 nm. When selected experimental agents were evaluated, the added buffer volume was adapted so that a total volume of 200 μl was maintained for the assay mixture. IC_{50}s of experimental agents were determined by varying the inhibitor concentrations with the substrate concentration according to the method of Dixon (1). Testing results are provided in Table 2.

Table 2 DPP-IV activities for selected experimental agents. K_i and K_m values were also determined but were not supplied by author

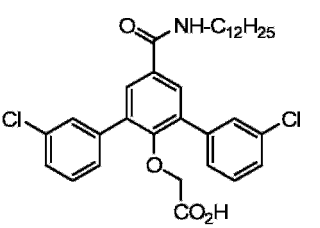
Entry	IC_{50}	Salt
1	48 nM	Trifluoroacetic acid
2	47 nM	Trifluoroacetic acid
6	2 μM	Trifluoroacetic acid
7	18 nM	Trifluoroacetic acid
8	400 nM	Trifluoroacetic acid
9	110 nM	Trifluoroacetic acid
10	300 nM	Bistrifluoroacetic acid
11	500 nM	Bistrifluoroacetic acid

Notes

1. 2-Phenyl oxazoles, (**I**), and 2,3,5-trisubstituted biphenyls, (**II**), prepared by Glombik (2) and Butera (3), respectively, were effective in treating metabolic disorders associated with insulin resistance.

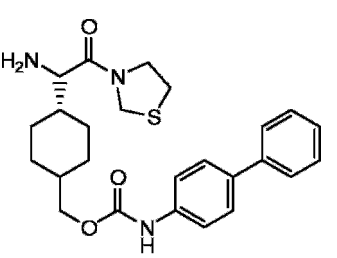


(I)



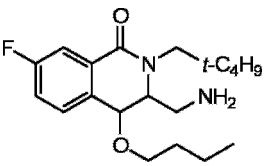
(II)

2. 1,3-Thiazolidin-3-yl derivatives (**III**) prepared by Ashton (4) were effective as DPP-IV inhibitors and used in treating elevated levels of plasma glucose associated with noninsulin-dependent diabetes mellitus.



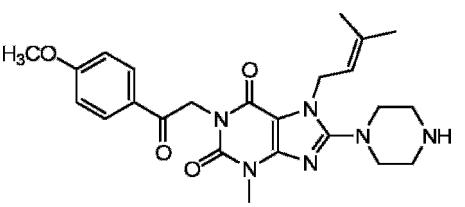
(III)

3. Isoquinolinone derivatives, (**IV**), prepared by Oi (5) were extremely effective as DPP-IV inhibitors and used in treating metabolic disorders associated with insulin resistance disorders.



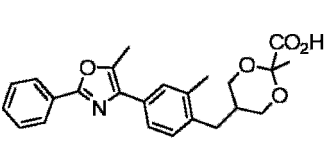
(IV)

4. Yoshikawa (6) prepared xanthine derivatives, (**V**), which were effective as DPP-IV inhibitors and useful for treating, preventing, or improving diabetic diseases.

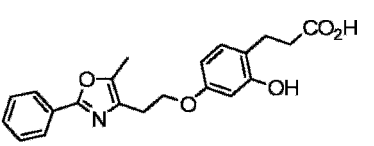


(V)

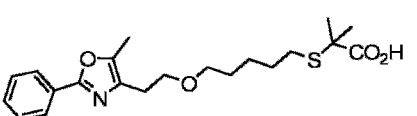
5. 5-Methyl-2-phenyl-oxazole derivatives, (**VI**), (**VII**), and (**VIII**), prepared by Kuwabara (7), Fakhoury (8), and Tajima (9), respectively, were effective in the treatment of hyperlipidemia associated with Type II diabetes.



(VI)



(VII)



(VIII)

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1. M. Dixon *et al*., Enzymes, 3rd Edition (1979), Academic Press, pp. 47–206.
2. H. Glombik *et al*., US Patent 6,884,812 (April 26, 2005)
3. J.A. Butera *et al*., US Patent 7,008,636(March 7, 2006)

4. W.T. Ashton *et al*., US Patent 7,026,316 (April 11, 2006)
5. S. Oi *et al*., US Patent 7,034,039 (April 25, 2006)
6. S. Yoshikawa *et al*., US Patent 7,074,798 (July 11, 2006)
7. K. Kuwabara *et al*., US Patent 6,998,412 (February 14, 2006)
8. S.A. Fakhoury *et al*., US Patent 6,716,842 (April 6, 2004)
9. H. Tajima *et al*., US Patent 6,664,281 (December 16, 2003)

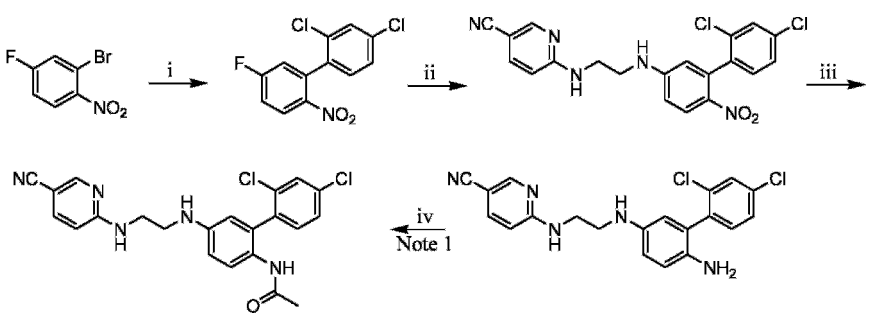
B. Glycogen Synthase Kinase 3 Inhibitors

Title Carbocycle-based Inhibitors of Glycogen Synthase Kinase 3
A.S. Wagman *et al.*, US Patent 6,989,382 (January 24, 2006)
Assignee Chiron Corporation
Utility Treatment of Type II Diabetes

Invention Significance Glycogen synthase kinase 3 (GSK_3) is a serine/threonine kinase existing in α- and β-isoforms and constitutively active in resting cells. Upon insulin activation, GSK_3 is inactivated thereby allowing the activation of glycogen synthase and other insulin-dependent events such as glucose transport. These events result in increased hepatic glucose production and an inadequate insulin response. To address this concern, GSK_3 inhibitors have been prepared, which are useful in the treatment of diabetes and other disorders associated with GSK_3 activity.

Reaction

Eq. 1



i- 2,4-Dichlorobenzene boronic acid, sodium carbonate, benzene, water, tetrakis(triphenylphosphine)-palladium(0)
ii- Acetonitrile, 2-(2-aminoethylamino)-5-cyanopyridine, *N,N*-diisopropylethylamine
iii- Palladium on carbon, hydrazine
iv- CH_2Cl_2, trifluoroacetic anhydride, tin(II) chloride, hydrochloric acid, dioxane, water, EtOAc, acetyl chloride, *N,N*-diisopropylethylamine

Experimental

1. **Preparation of 2-(2,4-dichlorophenyl)-4-fluoro-1-nitrobenzene**

   A mixture consisting of 1-bromo-5-fluoro 2-nitrobenzene (4.5 mmol), 2,4-dichlorobenzene boronic acid (4.7 mmol), Na_2CO_3 (13.5 mmol) in benzene, and 3 ml water was treated with tetrakis(triphenylphosphine)-palladium(0) (0.2 mmol) and heated at 75°C overnight. The mixture was partitioned between EtOAc and water and the organic layer separated, then washed with brine. It was dried with $MgSO_4$ and then concentrated. A brown solid residue was recrystallized and the product isolated in 80% yield as a white solid.

   HPLC 13.2 min (100%)
   MS (M + H/Z), 266

2. **Preparation of 6-({2-[(2′,4′,-dichloro-6-nitro-1,1′,-biphenyl-3-yl)amino]ethyl}-amino)-nicotinonitrile**

   The Step 1 product (0.1 mmol) dissolved in acetonitrile was treated with 2-(2-aminoethylamino)-5-cyanopyridine (0.1 mmol) and *N,N*-diisopropylethylamine (0.1 mmol) and heated to 85°C overnight. The mixture was partitioned between EtOAc and water and the organic layer was dried and concentrated. The residue was purified by chromatography with silica gel using 5% methyl alcohol/CH_2Cl_2 and 25 mg of product isolated.

   HPLC 13.1 min (90%)
   MS (M + H/Z), 428

3. **Preparation of 6-({2-[(6-amino-2′,4′-dichloro-1,1′-biphenyl-3-yl)amino]ethyl}-amino)-nicotinonitrile**

   The Step 2 product (40 mg) was dissolved in ethyl alcohol containing a catalytic amount of 10% Pd/C then treated with 500 μl hydrazine, and refluxed 2 hours. The catalyst was removed by filtration, the mixture concentrated, and 22 mg of product isolated.

   HPLC 6.7 minutes (80%)
   MS (M + H/Z), 398

4. **Preparation of *N*-[2′,4′-dichloro-5-({2-[(5-cyanopyridin-2-yl)amino]ethyl}-amino)-1,1′-biphenyl-2-yl]acetamide**

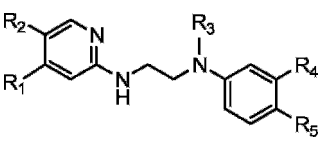
   The Step 3 product (0.1 mmol) dissolved in CH_2Cl_2 was treated with 50 μl trifluoroacetic anhydride, then stirred 10 minutes, and concentrated. The mixture was then treated with $SnCl_2$ (0.5 mmol) and 2 ml of 100 μl HCl in dioxane/water, 1:4, and then stirred 60 minutes. The solution was partitioned between EtOAc and water and 44 mg of *N*-[4-amino-3-(2,4-dichlorophenyl)phenyl]-*N*-{2-[(5-cyano(2-pyridyl))amino]ethyl}-2,2,2-trifluroacetamide isolated.

The amide intermediate was dissolved in 10 μl CH_2Cl_2, then treated with acetyl chloride and 13 μl *N,N*-diisopropylethylamine. It was then partitioned between EtOAc and water and the organic layer isolated, then dried, and concentrated. The residue was then dissolved in water/dioxane, 1:1, followed by 10 mg K_2CO_3 and then stirred 2 hours. The mixture was partitioned between EtOAc and the organic layer isolated. This organic layer was dried, triturated with diethyl ether, and 20 mg of product isolated.

HPLC 7.2 minutes (99%)
MS (M + H/Z), 440

Derivatives

Table 1 Selected experimental agents and their corresponding mass spectral data. ^{1}H NMR for all products and intermediates supplied by author. Entries 3, 8, 9, and 11 had IC_{50}s of 1 μM or less and were active as GSK3 inhibitors



Entry	R_1	R_2	R_3	R_4	R_5	MS
3	H	NO_2	H	2,4-Dichlorophenyl	NO_2	448
8	NO_2	NH_2	H	2,4-Dichlorophenyl	*N*-2-Methylglycinamide	469
9	H	NO_2	CH_3	2,4-Dichlorophenyl	NO_2	462
11	NO_2	NH_2	H	4-Methyl-piperazin-2-one	2,4-Dichlorophenyl	531
12	NO_2	NH_2	CH_3	Pyrrolidin-2-one	2,4-Dichlorophenyl	515
15	NO_2	NH_2	CH_3	Pyrrolidin-2-one	2,4-Dichlorophenyl	515
17	H	CN	H	2-Phenyl-1H-imidazol-1-yl	2,4-Dichlorophenyl	–

Testing

I. *Screening for GSK_3 Inhibitory Activity Using a Cell-Free Assay* (Note 1)

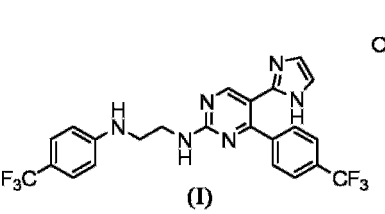
Experimental agents were tested for inhibition of human $GSK_3\beta$, the nucleotide sequence for human $GSK_3\beta$, according to the method of Hughes (1).

Results

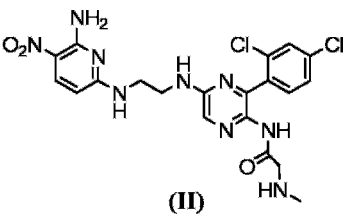
Entries 3, 8, 9, and 11 appearing in Table 1 exhibited IC_{50}s of 1 μM and were especially preferred.

Notes

1. Pyrimidines, (**I**), and pyrazine derivative, (**II**), effective as GSK_3 inhibitors were prepared by co-author Nuss (2,3), respectively, in earlier investigations.

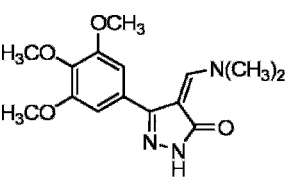


(I)

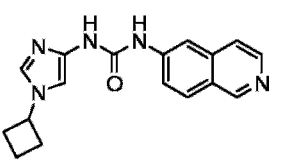


(II)

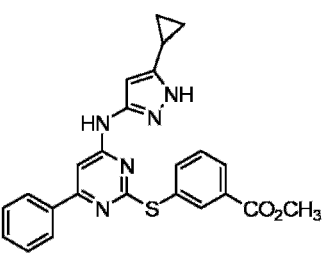
2. 2,4-Dihydro-pyrazol-3-ones, (**III**), imidazoles, (**IV**) and pyrazole derivatives, (**V**), prepared by Green (4), Sanner (5), and Bebbington (6), respectively, were effective as GSK_3 inhibitors and used in treating Type II diabetes.



(III)

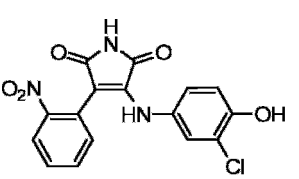


(IV)

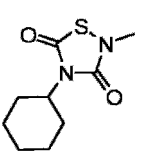


(V)

3. 1H-pyrrole-2,5-diones, (**VI**), prepared by Coghlan (7) and 1,2,4-thiadiazolidin-3,5-dione derivatives, (**VII**), prepared by Martinez Gil (8) were effective as a GSK_3 inhibitor and used in the treatment of Type II diabetes.



(VI)



(VII)

References

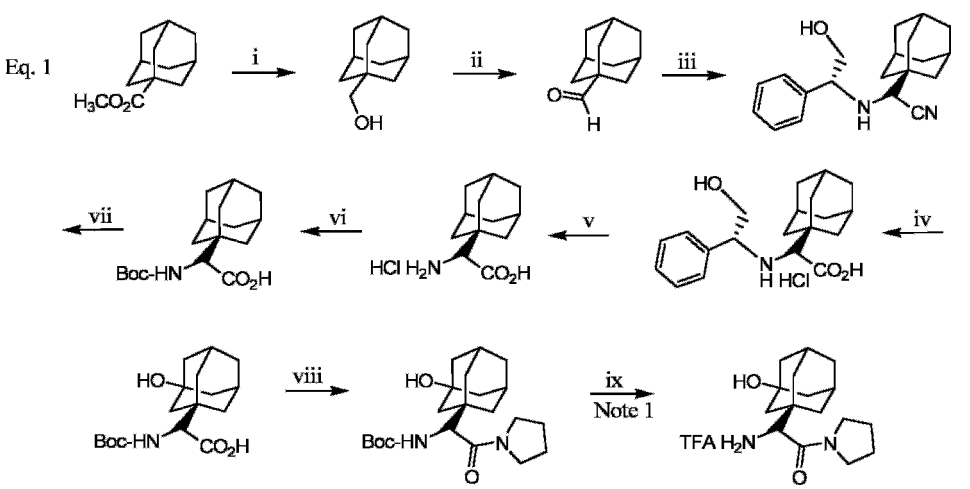
1. Hughes *et al*., Eur. J. Biochem., 203, 11 (1992)
2. J.M. Nuss *et al*., US Patent 6,489,344 (December 3, 2002)
3. J.M. Nuss *et al*., US Patent 6,949,547 (September 27, 2005)
4. J. Green *et al*., US Patent 6,916,798 (July 12, 2005)
5. M.A. Sanner *et al*., US Patent 7,078,529 (July 18, 2006) and US Patent 6,756,385 (June 29, 2004)
6. D. Bebbington *et al*., US Patent 6,989,385 (July 24, 2006)
7. M.P. Coghlan *et al*., US Patent 6,719,520 (April 13, 2004)
8. A. Martinez Gil *et al*., US Patent 6,872,737 (March 29, 2005)

C. Dipeptidyl Peptidase IV Inhibitors

Title Adamantylglycine-based Inhibitors of Dipeptidyl Peptidase IV and Methods
L.G. Hamann *et al*., US Patent 6,995,183 (February 7, 2006)
Assignee Bristol Myers Squibb Company
Utility Treatment of Hyperglycemia in Type II Diabetics

Invention Significance Dipeptidyl peptidase IV (DPP-IV) is an aminodipeptidase that is responsible for the metabolic cleavage of peptide GLP-1(7–36) into peptide GLP-1(9–36). GLP-1(7–36) stimulates insulin secretion and inhibits glucagon secretion. To potentiate higher levels of GLP-1(7–36) and thereby ameliorate the diabetic condition, DPP-IV inhibitors have been prepared.

Reaction



i- THF, lithium aluminum hydride
ii- CH_2Cl_2, DMSO, oxalyl chloride
iii- Water, sodium bisulfite, potassium cyanide, (*R*)-(−)-phenylglycinol, methyl alcohol
iv- Hydrochloric acid, acetic acid
v- Methyl alcohol, acetic acid, hydrogen, palladium(II) hydroxide
vi- DMF, potassium carbonate, di-*t*-butyldicarbonate
vii- Potassium permanganate, potassium hydroxide, water

viii- 1-Hydroxybenzotriazole hydrate, CH_2Cl_2, pyrrolidine, 3-ethyl-3′-(dimethylamino)propyl-carbodiimide hydrochloride, triethylamine

ix- Trifluoroacetic acid, CH_2Cl_2

Experimental

1. **Preparation of adamantane-1-hydroxymethyl**

   Adamantane-1-methoxycarbonyl (0.055 mmol) was dissolved in 150 ml THF, then treated with 1 M $LiAlH_4$ (69 mmol), and stirred 90 minutes at ambient temperature. The mixture was cooled to 0°C, then quenched sequentially with 5.1 ml water, 5.1 ml 15% NaOH, and 10.2 ml water. The mixture was stirred 15 minutes at ambient temperature and then filtered. The solids were washed twice with 100 ml EtOAc and then combined with the filtrate and concentrated. The residue was purified by flash column chromatography with silica gel using 10% EtOAc/CH_2Cl_2, and the product isolated in 96% yield as a white solid.

2. **Preparation of adamantane-1-aldehyde**

   A mixture of 150 ml CH_2Cl_2 and 10.3 ml DMSO was cooled to −78°C, then treated with 6.7 ml oxalyl chloride, and stirred 15 minutes. The Step 1 product (58.2 mmol) dissolved in 75 ml CH_2Cl_2 was added and the reaction stirred 60 minutes, then diluted with 400 ml diethyl ether, and the layers separated. The organic layer was washed three times with 150 ml cold 10% KH_2PO_4 aqueous solution and 100 ml brine, then dried with Na_2SO_4, and concentrated. The residue was purified by flash column chromatography using CH_2Cl_2 and the product isolated in 98% yield as a white solid.

3. **Preparation of (*S*-(−)adamantane-1-cyano-1-phenylglycinol**

   The Step 2 product (57 mmol) was suspended in 145 ml water and cooled to 0°C, then treated with a mixture consisting of $NaHSO_3$ (57 mmol), KCN (59 mmol), and (*R*)-(−)-phenylglycinol (57 mmol) dissolved in 55 ml methyl alcohol. The mixture was stirred 2 hours at ambient temperature, then refluxed 16 hours, and then diluted with 200 ml EtOAc. The layers separated and the aqueous fraction was re-extracted with EtOAc. The combined extracts were washed with 50 ml brine, dried with Na_2SO_4, and concentrated. The residue was purified by flash column chromatography using 20% EtOAc/hexanes and the product isolated in 65% yield as a white solid.

   MS *m/z* 311 $(M+H)^+$

4. **Preparation of (*S*-(−)adamantane-1-phenylglycine hydrogen chloride salt**

   The Step 3 product (18 mmol) was heated in 120 ml 12 M HCl and 30 ml HOAc for 18 hours at 80°C, filtered after cooling, and the product isolated in 78% yield as a white solid.

   MS *m/z* 330 $(M+H)^+$

5. **Preparation of (*S*)-adamantylglycine hydrogen chloride salt**

   The Step 4 product (14 mmol) was dissolved in 50 ml methyl alcohol and 10 ml HOAc, then treated with Pearlman's catalyst (20% $Pd(OH)_2$, 1.04 g, 20% (w/w)) and hydrogenated 18 hours under 50 psi hydrogen. The reaction was filtered and the catalyst washed three times with 25 ml methyl alcohol. The filtrate was concentrated and the product isolated as a white solid and used without further purification.

6. **Preparation of (*S*)-*N*-*t*-butoxycarbonyl-adamantylglycine**

   The Step 5 product (14 mmol) dissolved in 50 ml DMF was treated with K_2CO_3 (42 mmol) and di-*t*-butyldicarbonate (14 mmol), then stirred 19 hours at ambient temperature, and concentrated. The residue was mixed with 100 ml apiece water and diethyl ether and the layers were then separated. The aqueous phase was extracted twice with 100 ml diethyl ether and extracts saved. The aqueous phase was then cooled to 0°C and re-extracted with 200 ml EtOAc. The aqueous phase pH was lowered to 3 with 1 M HCl and the solution further extracted with 100 ml EtOAc. The combined extracts were washed with 50 ml brine, dried with Na_2SO_2, and concentrated. The residue was purified by flash chromatography using 5% methyl alcohol/CH_2Cl_2/0.5% HOAc and the product isolated in 92% yield as a white foam.

   MS *m/z* 310 $(M+H)^+$

7. **Preparation of (*S*)-*N*-*t*-butoxycarbonyl-3-hydroxyadamantylglycine**

   The Step 6 product (1.94 mmol) was slowly added to a solution of $KMnO_4$ (2.13 mmol) in 6 ml 2% aqueous KOH at 60°C and the temperature gradually raised to 90°C over 90 minutes. The mixture was then cooled to 0°C and treated with 50 ml EtOAc and 1 M HCl to lower the pH to 3. Layers were separated and the aqueous portion further extracted with 50 ml EtOAc. Extracts were washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash chromatography using 2% (200 ml), 3% (200 ml), 4% (200 ml), and 5% (500 ml) methyl alcohol/CH_2Cl_2 and 0.5% HOAc and the product isolated in 51% yield as a white solid.

   MS *m/z* 326 $(M+H)^+$

8. **Preparation of (*S*)-[1-(3-hydroxyadamantan-1-yl)-2-oxo-2-pyrrolidin-1-ylethyl]-carbamic acid *t*-butyl ester**

   A solution of the Step 7 product (0.16 mmol) and 1-hydroxybenzotriazole hydrate (0.16 mmol) in CH_2Cl_2 was cooled to 0°C, then stirred 30 minutes, and treated sequentially with pyrrolidine (0.16 mmol), 3-ethyl-3′-(dimethylamino)propyl-carbodiimide hydrochloride (0.16 mmol), and 60 µl triethylamine. Stirring was continued for additional 30 minutes at 0°C and 2 days at ambient temperature. The mixture was then partitioned between 1.5 ml water and 20 ml EtOAc and combined organic extracts washed with 1.5 ml brine, dried with Na_2SO_4, and concentrated.

The residue was purified by flash chromatography with using EtOAc/hexane (0–100% gradient) and the product isolated in 85.2% yield as a white foam.

MS *m/z* 380 $(M+H)^+$

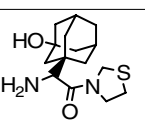
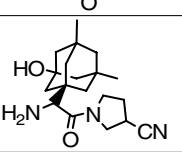
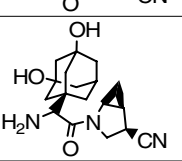
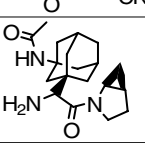
9. **Preparation of (*S*)-[1-(3-hydroxyadamantan-1-yl)-2-oxo-2-pyrrolidin-1-ylethyl]-amine, trifluoroacetic acid salt**

The Step 8 product (0.13 mmol) was dissolved in 0.44 ml trifluoroacetic acid/CH_2Cl_2, 1:1, then stirred 60 minutes at ambient temperature, and concentrated. The residue was purified by trituration with diethyl ether and preparative HPLC and the product isolated in 68.4% yield as a white solid.

MS *m/z* 279 $(M+H)^+$

Derivatives

Table 1 Selected adamantanyl derivatives and their corresponding mass spectral data. HPLC data supplied by the author. Cyclopropyl-fused dehydro pyrrolidines were prepared using $(C_2H_5)_2Zn$, $ClCH_2I$, and *N*-Boc-4,5-L-proline ester derivatives in CH_2Cl_2 according to the method of co-author Robl (1)

Entry	Structure	Salt	MS *m/z*
2		Trifluoroacetic acid	297
5		None	307
8		None	303
13		None	331

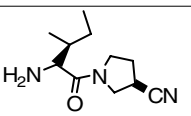
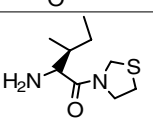
Testing

I. *In Vivo Evaluation of DPP-IV Inhibitors*

Experimental agents were evaluated in vitro as DPP-IV inhibitors using Zucker rats according to the method of Truett (2). Fasted male diabetic fatty Zucker rats were orally dosed with water or with selected experimental agents at 3 μmol/kg. Thereafter, an oral glucose tolerance testing was conducted 4 hours after dosing and

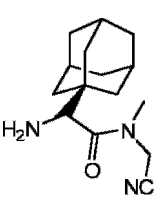
plasma glucose levels monitored over a 2 hour period. Two reference agents were also evaluated. Testing results are provided in Table 2.

Table 2 DPP-IV inhibitor effectiveness measured by plasma glucose lowering after dosing fasted male diabetic fatty Zucker rats with 3 μmol/kg with either an experimental or reference agent

Entry	Structure	K_i (nM)	Glucose Reduction (%)	
			After 0.5 hours	After 4 hours
5	–	133	70	66
8	–	17	80	67
Reference 1		2	30	5
Reference 2		110	50 (at 110 mol/kg)	–

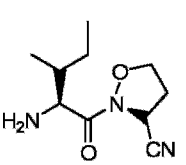
Notes

1. Acyclic cyanomethyl adamantanyl derivatives, (**I**), prepared by Magnin (3) were effective as DPP-IV inhibitors and used in treating Type II diabetes, hyperglycemia, and hypoglycemia.

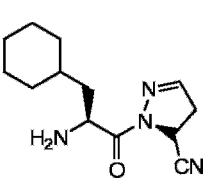


(I)

2. [(2*S*, 3(2*S*)]-2,1-Oxazoline-, (**II**), and 1,2-pyrazoline derivatives, (**III**), prepared by Sulsky (4) were useful as DPP-IV inhibitors and were used in the treatment of diabetes and associated disorders

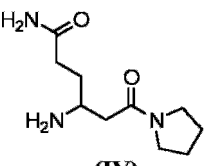


(II)

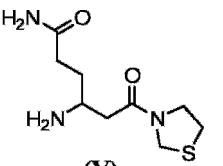


(III)

3. Demuth (5) determined that both glutaminyl pyrrolidines, (**IV**), and glutaminyl thiazolidine derivatives, (**V**), were effective in treating conditions mediated by DPP-IV or DPP-IV-like enzymes such as impaired glucose tolerance and diabetes mellitus.

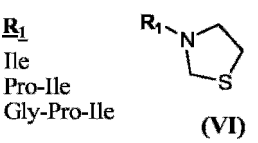


(IV)

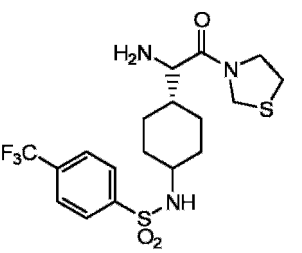


(V)

4. Peptidyl thiazolidines, (**VI**), DPP-IV prodrugs prepared by Demuth (6) and dipeptidyl thiazolidine derivatives, (**VII**), prepared by Ashton (7) were effective as DPP-IV inhibitors and used in the treatment of diabetes mellitus and impaired glucose tolerance disorders.



(VI)



(VII)

References

1. J.A. Robl *et al*., US Patent 6,395,767 (May 28, 2002)
2. G. Truett *et al*., Proc. Natl Acad. Sci., 88, 7806 (1991)
3. D.R. Magnin *et al*., US Patent 6,995,180 (February 7, 2006)
4. R.B. Sulsky *et al*., US Patent 6,573,287 (June 3, 2003)
5. H.-U. Demuth *et al*., US Patent 7,060,719 (June 13, 2006); US Patent 6,946,480 (September 20, 2005); and US Patent 6,548,481 (April 15, 2003)
6. H.-U. Demuth *et al*., US Patent 7,084,120 (August 1, 2006)
7. W.T. Ashton *et al*., US Patent 7,026,316 (August 11, 2006)

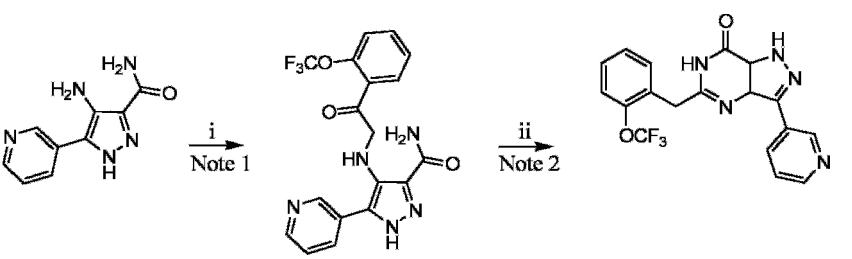
II. Insulin Resistance Syndrome: Cyclic Nucleotide Phosphodiesterase-9 Inhibitors

Title Treatment of Insulin Resistance Syndrome and Type II Diabetes with PDE9 Inhibitors
D.A. Fryburg *et al.*, US Patent 6,967,204 (November 22, 2005)
Assignee Pfizer, Inc.
Utility Treatment of Insulin Resistance Syndrome in Type II Diabetes

Invention Significance Diabetes mellitus is characterized by metabolic defects in production and utilization of carbohydrates resulting in elevated blood glucose. Current treatments include administration of exogenous insulin, oral administration of drugs, and dietary therapies and exercise regimens. A more effective method for treating this disorder utilizes cyclic guanosine 3′,5′-monophosphate-specific phosphodiesterase (cGMP–PDE9) inhibitors to amplify the cyclic nucleotide signal, which enhances insulin uptake, and is described.

Reaction

Eq. 1



i- Carbonyldiimidazole, 2-triflouoromethyoxyphenyl acetic acid, THF
ii- Potassium *t*-butoxide, isopropyl alcohol

Experimental

1. **Preparation of 5-pyridine-3-yl-4-[2-(2-trifluoromethoxy-phenyl)-acetylamino]-1H-pyrazole-3-carboxylic acid amide**

   Carbonyldiimidazole (0.886 mmol) was added to a solution of 2-triflouoromethy-oxyphenylacetic acid (0.886 mmol) in 5 ml THF at room temperature, then treated

with 4-amino-5-pyridine-3-yl-1H-pyrazol-3-carboxylic acid amide (0.886 mmol) and stirred additional 18 hours. The solution was diluted with 20 ml brine, then extracted twice with 20 ml EtOAc. The solution was dried using $MgSO_4$, then concentrated, and 345 mg of product isolated as an off-white solid.

LRMS (electrospray) *m/z* $[M+Na]^+$ 428, $[M-H]^+$ 404

2. **Preparation of 3-pyridin-3-yl-5-(2-trifluoromethoxy-benxyl)-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one**

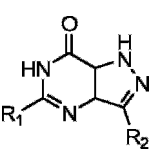
The Step 1 product (0.85 mmol) and potassium *t*-butoxide (2.55 mmol) were suspended in 5 ml isopropyl alcohol, then heated 18 hours at 55°C, and concentrated. The residue was partitioned between 20 ml apiece EtOAc and water and the aqueous phase acidified to pH 2 with 2 M HCl. The aqueous component was extracted twice with 15 ml apiece EtOAC and CH_2Cl_2, dried with $MgSO_4$, and reconcentrated. The residue was initially purified by flash column chromatography with silica gel using CH_2Cl_2/methyl alcohol, 99:1 to 95:5, then triturated using 3 ml apiece methyl alcohol, CH_2Cl_2, and diethyl ether and 13 mg product isolated as an off-white solid.

^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 9.34 (1H, brs), 8.57–8.61 (1H, d), 8.43–8.48 (1H, m), 7.32–7.47 (5H, m), 4.18 (2H, s)

LRMS (electrospray) *m/z* $[M-H]^+$ 386

Derivatives

Table 1 Selected experimental agents and their corresponding preparative HPLC retention times



Entry	R_1	R_2	Retention Time[a] (minutes)
19	4-Methoxybenzyl	Isopropyl	1.77
29	2,6-Dichlorobenzyl	Isopropyl	1.97
44	4-Chlorophenylmethyl	Isopropyl	1.97
52	2-Phenoxybenzyl	Isopropyl	2.03
61	4-Methylpentyl	Isopropyl	1.94
76	2-Fluorobenzyl	Isopropyl	1.80
93	Cyclopropylmethyl	*n*-Butyl	1.14

Table 1 Continued

Entry	R_1	R_2	Retention Time[a] (minutes)
127	2-Phenoxybenzyl	Isobutyl	1.65
148	2,6-Difluorobenzyl	Cyclopentyl	1.44

[a]Preparative HPLC conditions listed below:

Column: Phenomenex Luna C18, 5 μm, 150 cm × 10 mm id
Temperature: Ambient
Eluent A: 0.05% Diethylamine (aqueous)
Eluent B: Acetonitrile
Sample solvent: 90% dimethyl sulfoxide in water
Initial pump conditions: A (%) 90, B (%) 10, flow 6 ml/minute
Detection: Gilston 119 UV detector, 225 nm
Injection volume: 600 μl

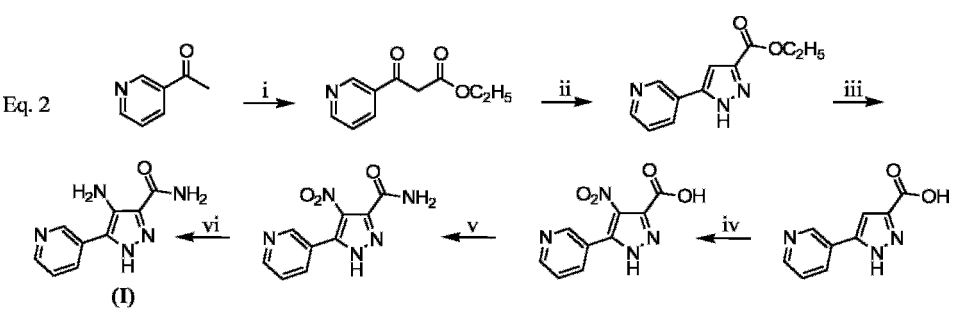
Testing

Although testing data were not supplied by author, the cGMP–PDE9 inhibitor, 5-(3-chloro-benzyl)-3-isopropyl-1,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one, was particularly preferred.

Notes

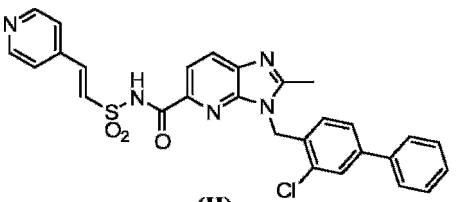
1. The preparation of Step 1 co-reagent, 4-amino-5-pyridine-3-yl-1H-pyrazol-3-carboxylic acid amide, (**I**), was provided by the author and illustrated in Eq. 2.

Eq. 2



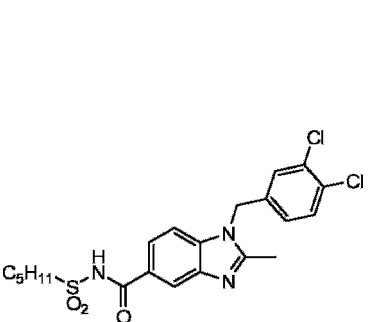
i- Sodium, ethyl alcohol, diethyloxalate
ii- Hydrazine hydrate, ethyl alcohol
iii- Sodium hydroxide, water, 1,4-dioxan, hydrochloric acid
iv- Sulfuric acid, nitric acid
v- Oxalyl chloride, CH_2Cl_2, DMF, ammonia
vi- Palladium on carbon, ethyl alcohol, hydrogen

2. cGMP–PDE inhibitors consisting of 3H-Imidazo[4,5-b]pyridine sulfonamide derivatives, (**II**), were prepared by Oku (1) and were effective in treating insulin resistance syndrome, impaired glucose tolerance disorder, and Type II diabetes.

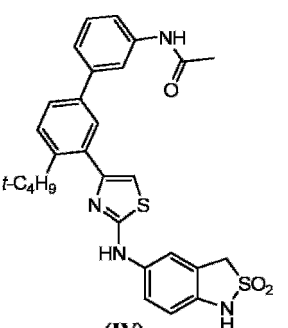


(II)

3. Benzothiophenes, (**III**), prepared by Yamasaki (2) and thiazole–benzoisothiazole dioxide derivatives, (**IV**), prepared by Petry (3) were effective as cGMP–PDE inhibitors and used as blood sugar level-depressing agents in the treatment of insulin resistance syndrome associated with Type II diabetes.

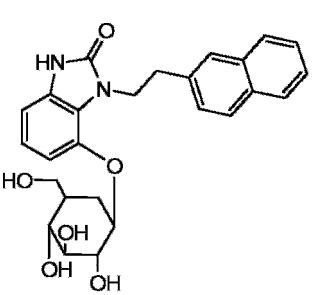


(III)

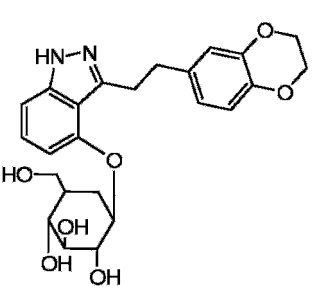


(IV)

4. β-D-Glucopyranoside derivatives containing 1,3-dihydro-benzoimidazol-2-one, (**V**), and 1H-indazole, (**VI**), prepared by Urbanski (4) and Patel (5), respectively, were effective in treating insulin resistance syndrome and reducing related risk factors for the development of Type II diabetes.

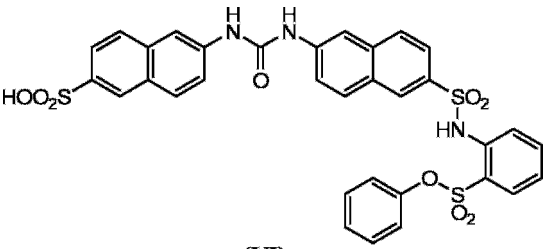


(V)



(V)

5. Naphthalene ureas, (**VI**), prepared by Spevak (6) were effective in treating hyperglycemia prevalent in Type II diabetes by stimulating the kinase activity of the insulin receptor to enhance the uptake of glucose.



(VI)

References

1. N. Oku *et al*., US Patent 7,060,721 (June 13, 2006) and US Patent 6,890,934 (May 10, 2005)
2. N. Yamasaki *et al*., US Patent 6,911,469 (June 28, 2005) and US Patent 6,869,950 (March 22, 2005)
3. S. Petry *et al*., US Patent 7,094,794 (August 22, 2006)
4. M. Urbanski, US Patent 7,094,764 (August 22, 2006)
5. M. Patel *et al*., US Patent 7,084,124 (August 1, 2006)
6. W.R. Spevak *et al*., US Patent 7,071,231 (July 4, 2006)

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CHAPTER X

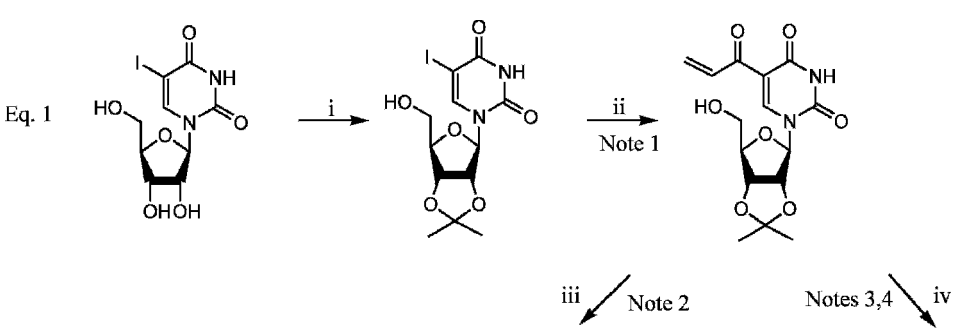
Diagnostics

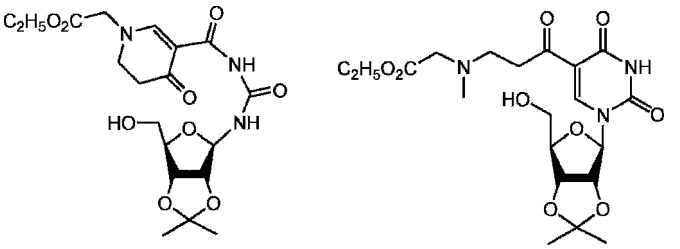
I. Urea Nucleosides as Diagnostic and Therapeutic Agents

Title Urea Nucleosides as Therapeutic and Diagnostic Agents
G. Kirschenheuter, US Patent 6,914,138 (July 5, 2005)
Assignee Gilead Sciences, Inc.
Utility Diagnostic and Therapeutic Agents

Invention Significance Only a limited number of nucleosides exist that can be used as diagnostic agents or in antitumor and antiviral therapeutics as strand cleavage agents for sequencing oligonucleotides. To address this need, modified urea oligonucleotides have been prepared which exhibit different binding affinities and can used diagnostically and therapeutically as antitumor, antibacterial, or antiviral drugs.

Reaction





i- Acetone, *p*-toluenesulfonic acid
ii- Palladium acetate, copper(I) iodide, triphenylphosphine, THF, carbon monoxide, vinyltributyl tin
iii- Glycine ethyl ester hydrochloride, calcium hydride, DMF
iv- Sarcosine ethyl ester hydrochloride, DMF, triethylamine

Experimental

1. **Preparation of 5-Iodouridine-2′, 3′-isopropylidene**

   A suspension of 5-iodouridine (27 mmol) and 0.5 g of toluenesulfonic acid in 600 ml acetone were refluxed 4 hours and then passed through an addition funnel packed with 4 Å molecular sieves. The reaction volume was then reduced to approximately 150 ml and passed through a 125 ml pad of flash silica gel eluting with 500 ml acetone. The solution was concentrated, then recrystallized using 225 ml ethyl alcohol, and the product isolated in 87% yield as a white crystalline solid.

   ^{1}H NMR (d_6-DMSO) δ 1.28 (s, 3H), 1.48 (s, 3H), 3.56 (ddd, 1H, $J = 4.0$, 4.9, 11.8 Hz), 3.61 (ddd, 1H, $J = 4.0$, 4.5, 11.8 Hz), 4.09 (dt, 1H, $J = 3.7$, 4.0 Hz), 4.75 (dd, 1H, $J = 3.5$, 6.3 Hz), 4.92 (dd, 1H, $J = 2.5$, 6.3 Hz), 5.20 (t, 1H, $J = 5.1$ Hz), 5.82 (dd, 1H, $J = 2.4$ Hz), 8.33 (s, 1H), 11.76 (br s, 1H)
   ^{13}C NMR (d_6-DMSO) δ 25.09, 26.93, 61.04, 69.49, 80.22, 83.84, 86.87, 91.29, 112.83, 146.09, 150.01, 160.55
   IR (KBr, diffuse reflectance accessory) ν_{max} (cm^{-1}) 3428, 3232, 1707, 1662, 1605, 1454, 1270, 1114, 854, 786, 629

2. **Preparation of 5-(1′-propenoyl)uridine-2′, 3′-isopropylidene**

   A mixture consisting of the Step 1 product (10 mmol), palladium(II) acetate (2.0 mmol), copper(I) iodide (6.0 mmol), triphenylphosphine (6.0 mmol) and 150 ml THF was mixed in a pressure apparatus that was then charged with 55 psi carbon monoxide and heated to 70°C. The mixture was then treated with a solution of vinyltributyl tin (15 mmol) in 200 ml THF over 6 hours, then stirred an additional 4 hours, filtered, and concentrated. The residue was dissolved in 50 ml CH_2Cl_2, then refiltered through Hyflo. The mixture was reconcentrated to approximately 25 ml, then added dropwise to 300 ml hexane and the crude product isolated in 85% yield by filtration. The residue was purified by flash chromatography using 0.5–5.0% methyl alcohol/$CHCl_3$, and the product isolated as an yellow solid.

^{1}H NMR (d_6-DMSO) δ 1.28 (s, 3H), 1.48 (s, 3H), 3.52–3.64 (m, 2H), 4.23 (dt, 1H, $J = 3.1, 3.9$ Hz), 4.75 (dd, 1H, $J = 2.9, 6.2$ Hz), 4.94 (dd, 1H, $J = 2.1, 6.2$ Hz), 5.15 (t, 1H, $J = 4.7$ Hz), 5.78 (dd, 1H, $J = 2.2, 10.4$ Hz), 5.85 (d, 1H, $J = 2.1$ Hz), 6.25 (dd, 1H, $J = 2.1, 17.2$ Hz), 7.44 (dd, 1H, $J = 10.3, 17.2$ Hz), 8.64 (s, 1H), 11.76 (br s, 1H)
^{13}C NMR (d_6-DMSO) δ 25.01, 26.87. 61.14, 80.60, 84.58, 87.66, 93.07, 111.23, 112.49, 128.13, 134.60, 148.84, 149.53, 161.37, 184.98
IR (KBr, film) ν_{max} (cm^{-1}) 1694

3. **Preparation of the urea nucleoside**

Glycine ethyl ester hydrochloride (1.2 mmol) dissolved in 1 ml DMF was treated with calcium hydride (0.65 mmol), then left standing overnight. The mixture was filtered through a plug of glass wool, then added to the Step 2 product (1.0 mmol) dissolved in 1 ml DMF, and stirred 48 hours. The mixture was concentrated and coevaporated three times with ethyl alcohol. The residue was purified by flash chromatography using 1–5% methyl alcohol/$CHCl_3$, and the product isolated in 19% yield.

^{1}H NMR ($CDCl_3$) δ 1.32 (t, 3 H, $J = 7.4$ Hz), 1.35 (s, 3 H), 1.56 (s, 3 H), 2.61–2.67 (m, 2 H), 3.62–3.81 (m, 3H), 3.90 (dd, 1 H, $J = 1.8, 12.0$ Hz), 4.19 (d, 1 H, $J = 18$ Hz), 4.25 (q, 1 H, $J = 7.1$ Hz), 4.26 (q, 1 H, $J = 7.1$ Hz), 4.32 (br s, 1 H), 4.26–4.60 (br, –OH), 4.66 (d, 1 H, $J = 18$ Hz), 4.70 (dd, 1 H, $J = 1.7, 6.0$ Hz), 4.91 (d, 1 H, $J = 6.0$ Hz), 5.88 (dd, 1 H, $J = 1.7, 10.1$ Hz), 8.15 (s, 1 H), 9.23 (d, 1 H, $J = 10.1$ Hz), 11.09 (br s, 1 H)
^{13}C NMR ($CDCl_3$) δ 14.11, 25.04, 26.81, 34.62, 47.78, 57.51, 62.10, 63.51, 82.63, 86.17, 88.17, 99.99, 112.42, 153.30, 160.47, 165.45, 168.00, 189.58
IR (KBr, film) ν_{max} (cm^{-1}) 3424(br), 3264(br), 2985, 2938, 1744, 1665(s), 1593(s), 1206(s), 1096(s)

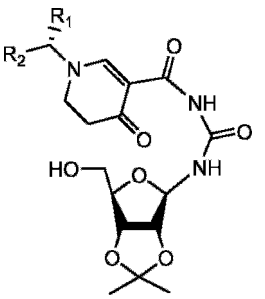
4. **Synthesis of uridine nucleoside**

Sarcosine ethyl ester hydrochloride (1.0 mmol) was suspended in 1 ml DMF, treated with triethylamine (1.1 equiv.) and the Step 2 product (1.0 mmol), then stirred overnight. The solution was quenched with 10 ml water, then extracted three times with 10 ml chloroform, dried over Na_2SO_4, and concentrated. The residue was coevaporated four times with ethyl alcohol and the product isolated in 95% yield as a white foam.

^{1}H NMR ($CDCl_3$) δ 1.27 (t, 3H, $J = 7.1$ Hz), 1.36 (s, 3H), 1.58 (s, 3H), 2.37 (s, 3H), 2.82–2.90 (m, 2H), 3.07 (dt, 1H, $J = 6.9, 16.8$ Hz), 3.21–3.34 (m, 3H), 3.84 (dd, 1H, $J = 3.0, 11.9$ Hz), 3.96 (dd, 1H, $J = 2.2, 6.2$ Hz), 4.17 (q, 2H, $J = 7.1$ Hz), 4.46–4.47 (m, 1H), 4.91 (dd, 1H, $J = 2.0, 6.2$ Hz), 4.95 (dd, 1H, $J = 2.3, 6.2$ Hz), 5.83 (d, 1H, $J = 2.3$ Hz), 8.66 (s, 1H)
^{13}C NMR ($CDCl_3$) 514.18, 25.14, 27.07, 39.96, 42.25, 51.65, 58.11, 60.63, 62.47, 81.03, 85.27, 87.99, 95.57, 111.35, 113.77, 149.03, 149.67, 161.16, 170.89, 195.37
IR (KBr, film) ν_{max} (cm^{-1}) 3493, 3225, 3068, 2986, 2934, 2834, 1727(s), 1698(s), 1592(m), 1456(m), 1384, 1282, 1212(m), 1104(m), 1069(m), 853, 798, 733, 580

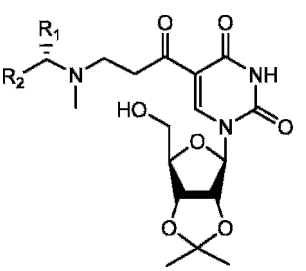
Derivatives

Table 1 Selected urea nucleosides derivatives and overall product conversions. ^{1}H NMR for products and intermediates provided by author



R_1	R_2	Yield (%)
i-C_3H_7	$CO_2C_2H_5$	32
C_6H_5	$CO_2C_2H_5$	32
CH_2-4-imidazole	H	
CH_2-4-imidazole	$CO_2C_2H_5$	–

Table 2 Selected uridine nucleosides derivatives and overall product conversions. ^{1}H NMR for products and intermediates provided by author



R_1	R_2	Yield (%)
i-C_3H_7	$CO_2C_2H_5$	90
C_6H_5	$CO_2C_2H_5$	93
CH_2-4-imidazole	H	–

Testing

Although testing and results were not provided by the author, the utility of experimental agents is provided below:

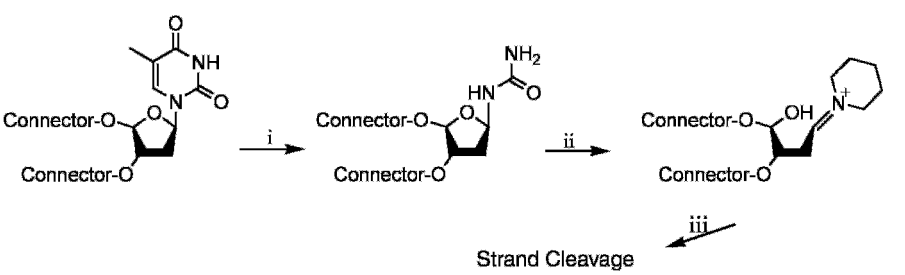
1. Strand cleavage agents for sequencing oligonucleotides
2. Diagnostic tools after labeling nucleosides with a radiolabel or fluorescent tag including rhodamine or fluorescein
3. Pre- and post-systematic evolution of ligands for exponential (SELEX) process useful for preparing oligonucleotides alone or in combination with other nucleosides
4. Antiviral agents, especially those that result in increased specificity to viral kinases
5. Antineoplastic agents

Notes

1. The pathway for strand cleavage using the Maxam–Gilbert sequencing method (1) with a thymidine residue and the cleavage method of the current invention are illustrated in Eqs 2 and 3, respectively.

Maxam–Gilbert Sequencing Method

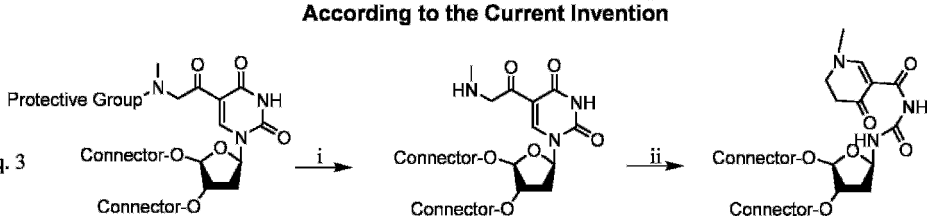
Eq. 2



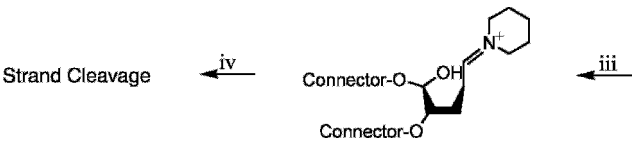
i- Hydrazine
ii- Cyclohexyl amine
iii- β-Elimination

Modified Maxam–Gilbert Sequencing Method According to the Current Invention

Eq. 3

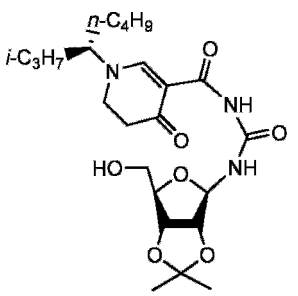


i- Deprotect
ii- Ring opening reaction

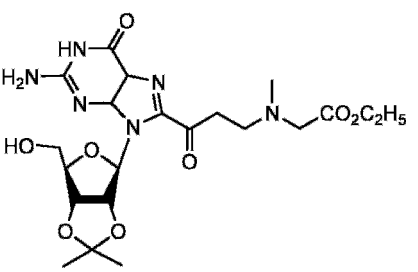


iii- Cyclohexyl amine
iv- β-Elimination

2. Additional urea derivatives, (**I**), and purine nucleosides, (**II**), were previously prepared by the authors (2,3) and used as diagnostic or therapeutic agents.

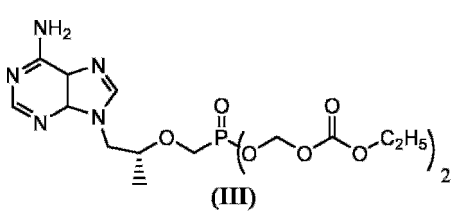


(I)

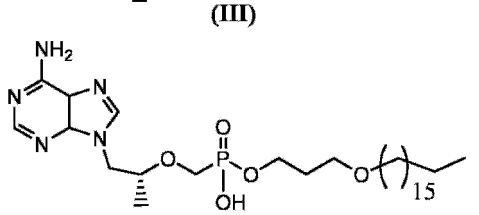


(II)

3. Antiviral phosphonoalkoxy nucleotides, (**III**) and (**IV**), having increased oral bioavailability were prepared by Arimilli (4) and Hostetler (5), respectively, and used in the treatment of DNA viruses or RNA viruses vital infections in humans.



(III)



(IV)

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1. A.M. Maxam *et al*., Proc. Natl. Acad. Sci. USA, 74, 560 (1977)
2. G. Kirschenheuter, US Patent 6,441,161 (August 27, 2002)
3. G. Kirschenheuter, US Patent 6,143,882 (November 7, 2000)
4. M.N. Arimilli *et al*., RE38,333 (November 25, 2003)
5. K.Y. Hostetler *et al*., US Patent 7,094,772 (August 22, 2006)

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CHAPTER XI

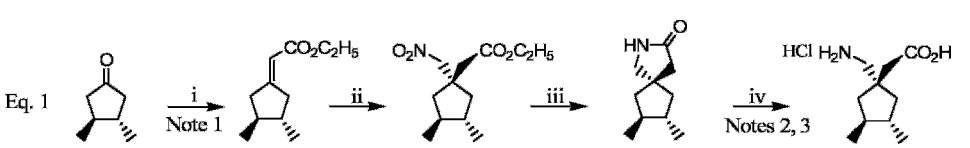
Epilepsy

I. Calcium Channel $\alpha_2\delta$ Subunit Antagonists

Title Cyclic Amino Acids and Derivatives Thereof Useful as Pharmaceutical Agents
J.S. Bryans *et al*., US Patent 6,921,835 (July 26, 2005)
Assignee Warner Lambert Company
Utility Treatment of Epilepsy and Faintness Attacks

Invention Significance A method for treating seizure disorders using cyclic amino acids having significantly higher binding affinities to the $\alpha_2\delta$ subunit of a calcium channel compared to that of the existing anticonvulsant agents is described. Their effectiveness in treating convulsion disorders represents the next generation of antiseizure medication.

Reaction



i- THF, sodium hydride, triethylphosphonoacetate, tetrabutylammonium fluoride
ii- Methyl nitrate, THF, tetrabutylammonium fluoride
iii- Methyl alcohol, Raney nickel, hydrogen
iv- 1,4-Dioxane, hydrochloric acid

Experimental

1. **Preparation of *trans*-(3,4-dimethyl-cyclopentylidene)-acetic acid ethyl ester**

   Sodium hydride (18.42 mmol) was suspended in 50 ml THF cooled to 0°C, then treated with triethylphosphonoacetate (19.30 mmol), and stirred 15 minutes. The mixture was further treated with *trans*-3,4-dimethylcyclopentanone (17.54 mmol) dissolved in 10 ml 1 M tetrabutylammonium fluoride in THF, then stirred 2 hours at ambient temperature. The solution was then partitioned between 200 ml diethyl ether and 150 ml water and the organic phase separated and washed with brine. The solution was then dried with $MgSO_4$ and concentrated. The residue was purified by flash chromatography with silica gel using EtOAc/heptane, 1:9, and the product isolated in 94% yield as a colorless oil.

   ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (3H, d, $J = 6$ Hz), 1.03 (3H, d, $J = 6$ Hz), 1.26 (3H, t, $J = 7$ Hz), 1.49 (2H, m), 2.07 (1H, m), 2.24 (1H, m), 2.61 (1H, m), 4.13 (2H, q, $J = 7$ Hz), 5.72 (1H, s)
   MS (CI+) *m/z*: 183 ([MH^+], 18%)

2. **Preparation of *trans*-(3,4-dimethyl-1-nitromethyl-cyclopentyl)-acetic acid ethyl ester**

   The Step 1 product (16.2 mmol) dissolved in 10 ml THF was treated with 1.9 ml nitromethane and 22 ml 1.0 M tetrabutylammonium fluoride in THF then stirred 6 hours at 70°C. The mixture was then diluted with 50 ml EtOAc and washed with 30 ml 2 M HCl and 50 ml brine. The organic phase was collected, then dried, and concentrated. The residue was purified by flash chromatography using EtOAc/heptane, 1:9, and the product isolated in 29% yield as a clear oil.

   ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (6H, d, $J = 6$ Hz), 1.10–1.39 (5H, m), 1.47 (2H, m), 1.87 (1H, m), 2.03 (1H, m), 2.57 (2H, ABq, $J = 16, 38$ Hz), 4.14 (2H, q, $J = 7$ Hz), 4.61 (2H, ABq, $J = 12, 60$ Hz)
   MS (ES+) *m/z* 244 ([MH^+], 8%)
   IR (film) ν(cm^{-1}) 1186, 1376, 1549, 1732, 2956

3. **Preparation of (±)-(*trans*)-7,8-dimethyl-spiro[4.4]nonan-2-one**

   The Step 2 product (4.7 mmol) was dissolved in 50 ml ethyl alcohol and shaken over Raney nickel catalyst 5 hours at 30°C under 40 psi hydrogen, then filtered through celite. The filtrate was concentrated and the product isolated in 95% as a pale yellow oil, which solidified on standing.

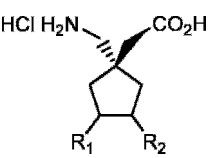
   ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.98 (6H, d, $J = 6$ Hz), 1.32 (2H, m), 1.46 (2H, m), 1.97 (2H, m), 2.27 (2H, ABq, $J = 16, 27$ Hz), 3.23 (2H, s), 5.62 (1H, br s)
   MS (ES+) *m/z* 168 ([MH^+], 100%)
   IR (film) ν (cm^{-1}) 1451, 1681, 1715, 2948, 3196

4. **Preparation of (±)-(*trans*)-(1-aminomethyl-3,4-dimethyl-cyclopentyl)-acetic acid hydrochloride**

The Step 3 product (4.40 mmol) was refluxed 4 hours in a mixture of 5 ml 1,4-dioxane and 15 ml 6 M HCl, then diluted with 20 ml water, and washed three times with 20 ml CH_2Cl_2. The aqueous solution was concentrated, the residue triturated with EtOAc, and the product isolated in 69% yield as a white solid.

^{1}H NMR (400 MHz, d_6-DMSO) δ 0.91 (6H, d, $J = 6$ Hz), 1.18 (2H, m), 1.42 (2H, m), 1.72 (1H, m), 1.87 (1H, m), 2.42 (2H, ABq, $J = 16, 24$ Hz), 2.90 (2H, ABq, $J = 12, 34$ Hz), 8.00 (3H, br s), 12.34 (1H, br s)
MS (ES+) *m/z*: 186 ($[MH - HCl]^+$, 100%)

Derivatives



Entry	R_1	R_2	*n*	Yield (%) (Step 4)	MS $[MH - HCl]^+$
1	CH_3	CH_3	1	96	186
2	CH_3	CH_3	1	48	186
3	CH_3	CH_3	1	59	186
4	H	CH_3	1	65	230
5	H	H	0	72	144
6	$CH_2C_6H_5$	H	0	62	234

Testing

I. *Prevention of Audiogenic Seizures in DBA/2 Mice*

Mice were placed into an enclosed chamber with a high-frequency speaker in the center top lid. An audio signal generator was used to produce a continuous sinusoidal tone that was swept linearly in frequency between 8 and 16 kHz once every 10 milliseconds. The average sound pressure level during stimulation was approximately 100 dB at the chamber floor. DBA/2 mice in the vehicle-treated group

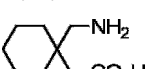
responded to the sound stimulus with a characteristic seizure sequence consisting of wild running followed by clonic seizures, tonic extension, and finally by respiratory arrest and death in 80% of the mice. In this group, the entire sequence of seizures-to-respiratory arrest lasted approximately 15–20 seconds.

The incidence of all the seizure phases in the mice receiving selected experimental agents and vehicle-treated mice was recorded and the occurrence of tonic seizures was used for calculating anticonvulsant ED_{50} values. All experimental agents were dissolved in distilled water and were given by oral gavage in a volume of 10 ml/kg of body weight. Insoluble experimental agents were suspended in 1% carboxymethocellulose.

Table 1 Test results for dose-dependent suppression of sound-induced tonic seizures in mice. Experimental agents used show a comparable/superior binding affinity to the $\alpha_2\delta$ subunit similar to that of the anticonvulsant drug, Neurontin®[a]

Entry	IC_{50} (μM) at $\alpha_2\delta$ binding site	Percentage of MPE[b] 2 hours postdose at 30 mg/kg PO	Percentage of Protected 1 hour postdose 30 mg/kg PO
1	0.034	72	100
2	0.022	118	100
3	1.0	–	–
4	0.088	53	100
5	0.598	4	20
6	>10	0	Not tested

[a] 1-(Aminomethylcyclohexane)acetic acid, (**I**)



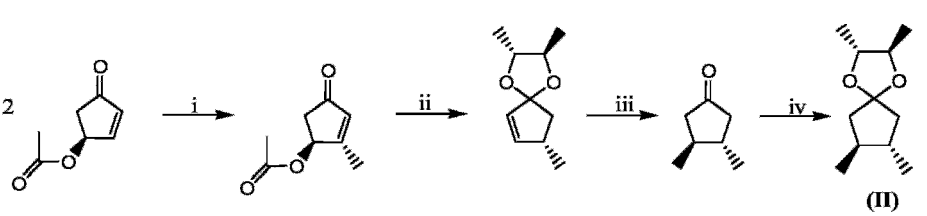
(**I**)

[b] Maximum possible effect.

Notes

1. The Step 1 co-reagent, *trans*-3,4-dimethylcyclopentanone, was prepared by Blakemore (1) by hydrogenation of the corresponding ketal, (**II**), as illustrated in Eq. 2.

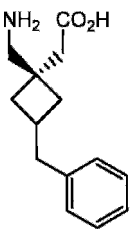
Eq. 2



(**II**)

i- THF, methyl magnesium chloride, dimethyl zinc
ii- DBU, CH_2Cl_2
iii- THF, methyl magnesium chloride, dimethyl zinc
iv- (2*R*,3*R*)-(–)-2,3butanediol, *p*-toluenesulfonic acid, benzene

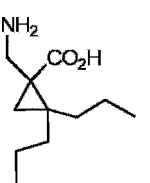
2. In earlier investigations by the authors (2,3), benzylcyclobutyl-, (**III**), and bicyclic amino acid derivatives, (**IV**), respectively, were prepared, which were effective as high binding $\alpha_2\delta$ subunit to a calcium channel, and used as anticonvulsants.



(III)

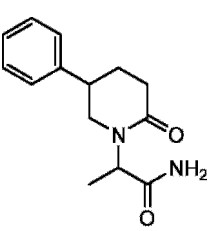
(IV)

3. Cyclopropyl-β-amino acid derivatives, (**V**), having high binding affinities to the $\alpha_2\delta$ subunit of a calcium channel were prepared by Schwarz (4) and used in the treatment of epilepsy.



(V)

4. 2-Oxo-piperidinyl-, (**VI**), and 2-oxo-azepanyl alkanoic acid derivatives prepared by Michel (5) were effective in the treatment of epilepsy and related seizure disorders.



(VI)

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1. D.C. Blakemore *et al*., US Patent 6,872,856 (May 29, 2005)
2. J.S. Bryan *et al*., US Patent 6,635,673 (October 21, 2003)
3. J.S. Bryan *et al*., US Patent 6,689,906 (February 10, 2004)
4. J.B. Schwarz *et al*., US Patent 7,030,267 (April 18, 2006)
5. P. Michel *et al*., US Patent 7,087,596 (August 8, 2006)

II. *N*-Methyl-D-Aspartic Acid Receptor Antagonists

Title Derivatives and Pharmaceutical Compositions of *n*-Hydroxyalkyl Tetramethylcyclopropane Carboxamide, Having Antiepiletic, Neurological and CNS Activity, and Method for Their Preparation
M. Bialer *et al.*, US Patent 6,960,687 (November 1, 2005)

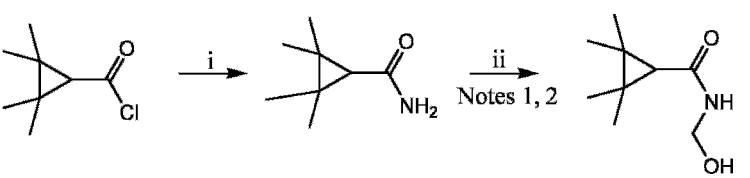
Assignee Yissum Research Development Company of the Hebrew University of Jerusalem

Utility Anticonvulsive for the Treatment of Epilepsy

Invention Significance Approximately 25% of epileptic patients do not respond favorably to the treatment by four major antiepileptic drugs including phenytoin, carbamazepine, phenobarbital, and valproic acid. This chapter addresses this problem by introducing an amide derivative of tetramethylcyclopropane carboxylic acid as a fifth treatment option.

Reaction

Eq. 1



i- Ammonium hydroxide
ii- Trimethylamine, formaldehyde, THF

Experimental

1. **Preparation of tetramethylcyclopropylcarboxamide**

Tetramethylcyclopropylcarbonylchloride (0.093 mol) was added dropwise to 100 ml 25% ammonium hydroxide at ambient temperature and stirred overnight. The mixture was then treated with 100 ml water and extracted twice with 200 ml CH_2Cl_2. The extract was washed with a carbonate buffer solution, dried, and concentrated. The residue was treated with 100 ml $CHCl_{3/hexane}$ and crystals collected by filtration. The crystals were washed with hexane and the product isolated in 76% yield as a white solid, mp = 90°C

2. **Preparation of *N*-hydroxylmethyl tetramethylcyclopropylcarboxamide**

   The Step 1 product (0.028 mol) was added to 100 ml THF containing 20 ml apiece trimethylamine and formaldehyde, then stirred 12 hours at 60°C, and concentrated. The residue was treated with $CHCl_3$ and crystals collected by filtration. The crystals were washed hexane and the product isolated in 63% yield as a white solid, mp = 135°C.

Derivatives

Only the Step 2 derivative was prepared.

Testing

I. Model 1 *Maximal Electroshock Seizure Test*

   The maximal electroshock seizure (MES) test was used to show efficacy of antiepileptic agents against partial and generalized seizure type epilepsy among therapy-resistant epileptic patients.

II. Model 2 *Subcutaneous Metrazole Test*

   The second model, subcutaneous metrazole test (sc Met), measured seizure threshold and is a standard screening procedure to show efficacy of agents against seizure threshold and absence seizures.

III. Model 3 *6 Hz Psychomotor Seizure Model*

   The third model is the 6 Hz psychomotor seizure model, which screens for focal seizures and is used to find new antiepileptic agents with novel mechanisms of action. In these studies, convulsions were inhibited or prevented in mice following intraperitoneal administration of the experimental agent.

   Results

   The test agent showed anticonvulsant activity in mice in the MES test. The ED_{50} in the MES model was between 150 and 300 mg/kg in mice (Table 1). This value is slightly lower than that of valproic acid in mice. The results are indicative of the experimental agent having an efficacy against generalized seizures and complex partial seizures that evolve into generalized motor seizures.

IV. *Neurotoxicity*

   Neurotoxicity was assessed in mice following ip administration in the rotorod ataxia test. Testing results are provided in Table 1.

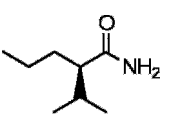
Table 1 Anticonvulsant activity, ED_{50}, and neurotoxicity, TD_{50}, were obtained 15 minutes after ip administration of the experimental agent to mice in comparison to valproic acid

Biological Measurement	Experimental Agent	Valproic Acid[a]
MES ED_{50} (mg/kg)	150–300	271
sc Met ED_{50} (mg/kg)	120	149
6 Hz 22 mA (mg/kg)	75	42
6 Hz 32 mA (mg/kg)	91	126
6 Hz 44 mA (mg/kg)	134	310
Neurotoxicity TD_{50} (mg/kg)	146	283
PI (6 Hz 44 mA)	1.1	0.91

[a] 2-Propyl-pentanoic acid.

Notes

1. (2*R*)-, (**I**), (*S*)-, and racemic propylisopropyl acetamides effective as NMDA receptor antagonists were prepared by the authors (1) in a subsequent investigation and were effective in treating epileptic disorders as summarized in Table 2.

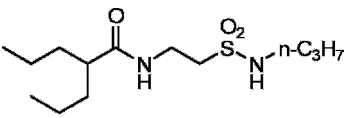


(I)

Table 2 Anticonvulsant activity of (*S*)-, (*R*)-, and racemates of propylisopropyl acetamides when ip administered to mice

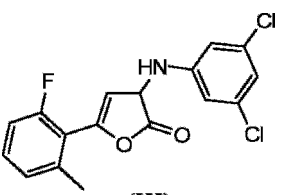
Biological Measurement	(*S*)-Propylisopropyl acetamide	(*R*)-Propylisopropyl acetamide	Racemate	*S/R*
MES	122	145	110	1.32
sc Met	77	80	67	1.19
Neurotox	< 120	118	< 145	> 0.81
PI-MES	< 0.98	0.81	< 1.3	–
PI-sc Met	< 1.56	1.46	< 2.2	–
ED_{50} (mg/kg)	26	16	22	–

2. Valproyltaurinamide derivatives, (**II**), prepared by amidation of 2-propyl-pentanoic acid (valproic acid) using 2-aminoethanesulfonic acid (taurine) previously prepared by the authors (2) was effective as an antiseizure medicament.



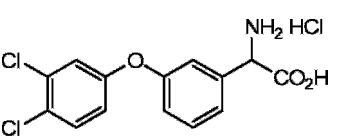
(II)

3. Substituted γ-lactone derivatives, (**III**), prepared by Sundermann (3) were effective as NMDA antagonists and used in treating chronic pain, neuropathic pain, and epilepsy.



(III)

4. Diaryl ether aminoacid derivatives, (**IV**), prepared by Weaver (4) were effective in inhibiting or preventing ictogenesis and epileptogenesis and used in the treatment of seizure disorders.



(IV)

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2. M. Bialer *et al*., US Patent 6,958,416 (October 25, 2005)
3. C. Sundermann *et al*., US Patent 6,956,055 (October 18, 2005)
4. D.F. Weaver *et al*., US Patent 6,930,112 (August 16, 2005)

CHAPTER XII

Gastrointestinal Disorders

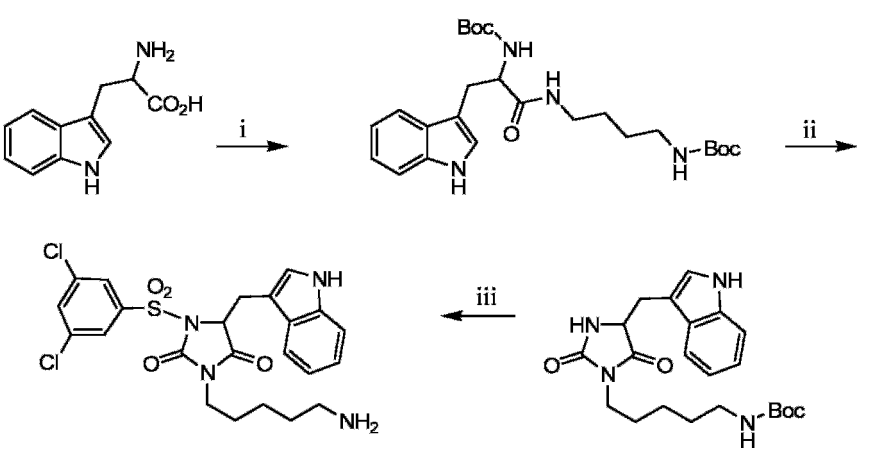
I. Somatostatin Receptor Agonists: Treatment of Excess Gastrointestinal Secretion

Title Hydantoin Derivatives with Affinity for Somatostatin Receptors
D. Berney *et al.*, US Patent 7,019,004 (March 28, 2006)
Assignee Novartis AG
Utility Treatment of Excess Gastrointestinal Secretion

Invention Significance Somatostatin directly impacts the inhibition of gastrin release from the gastrointestinal tract. Disorders with an etiology associated with excess gastrointestinal secretion may include peptic ulcers, disturbances of gastrointestinal motility, and irritable bowel syndrome. To address these concerns associated with excess gastrin release, somatostatin receptor agonists have been prepared.

Reaction

Eq. 1



i- *N*-Boc-1,5-pentanediamine, THF,
dicyclohexylcarbodiimide
ii- THF, tetrabutylammonium fluoride trihydrate
iii- Sodium hexamethyldisilazide, THF,
2,5-dichlorobenzenesulfonyl chloride

Experimental

1. **Preparation of *N*-α-*t*-butyloxycarbonyl-D,L-tryptophan-[5-amino-(*N*-*t*-butyloxy carbonyl)-*n*-pentanyl] amide**

   A stirred solution of mono-*N*-Boc-1,5-pentanediamine (6.3 mmol) and D,L-tryptophan (7.0 mmol) in 30 ml THF was treated with dicyclohexylcarbodiimide (7.5 mmol) at ambient temperature and stirred 1 hour. The mixture was filtered, concentrated, and warm diethyl ether added to the residue. Upon cooling, crystallization occurred and the product isolated as a light brown powder, mp = 97−98°C.

2. **Preparation of 3-[5′-amino-(*N*-*t*-butyloxycarbonyl)-*n*-pentanyl]-5-[(indol-3-yl) methyl]-imidazolidine 2,4-dione**

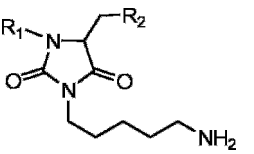
   The Step 1 product (6.0 mmol) dissolved in 50 ml THF was treated with tetrabutylammonium fluoride trihydrate (18 mmol), then refluxed 24 hours, and concentrated. The residue was dissolved in EtOAc, then washed with brine, dried with Na_2SO_4, reconcentrated, and a viscous brown oil isolated. The residue was purified by MPLC chromatography with silica gel using EtOAc/hexane, 2:1, and the product isolated as a light yellow oil, which crystallized upon standing, mp = 136−137°C.

3. **Preparation of (±)-1-(2′, 5′-dichloro-1′-benzenesulfonyl)-3-(5′-amino-*n*-pen tanyl)-5-[(indol-3-yl)-methyl]-imidazolidine-2,4-dione**

   The Step 2 product (1.0 mmol) dissolved in 5 ml THF at −40°C was treated with 1.1 ml 1 M sodium hexamethyldisilazide (1.1 mmol) in THF and after 30 minutes the mixture was further treated with 2,5-dichlorobenzenesulfonyl chloride (1.1 mmol). The solution was stirred overnight at ambient temperature and then treated with saturated NH_4Cl solution and concentrated. The residue was dissolved in EtOAc, then washed with brine, dried with Na_2SO_4, concentrated, and an oil isolated. The residue was purified by MPLC using EtOAc/hexane, 2:1, and the product isolated as a colorless viscous oil.

Derivatives

Table 1 Selected 3-(5-amino-*n*-pentan-1-yl)-5-[(indol-3-yl)-methyl]-imidazolidine-2,4-dione derivatives and their corresponding mass spectral characterization data. ^{1}H NMR data supplied by author



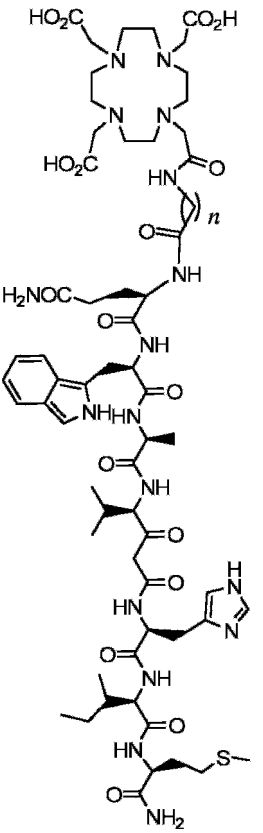
Entry	R_1	R_2	FAB-MS (MH^+)
2	$-SO_2$-4-C_6H_4-CH_3	Indol-3-yl	469
4	$-SO_2$-3-C_6H_4-CH_3	Indol-3-yl	523
12	$-SO_2$-3-CN-C_6H_4-4′-C_6H_4-4″-C_6H_4OH	7-Methyl-indol-3-yl	602
16	$-SO_2$-3-CN-C_6H_4-4′-C_6H_4-4″-C_6H_4OH	Indol-3-yl	563
30	$-SO_2$-3-CON$(CH_3)_2$-C_6H_4-4′-(3-Cl-C_6H_4)-4″-C_6H_4OH	5-Methyl-indol-3-yl	682

Testing

Testing data were not supplied by author.

Notes

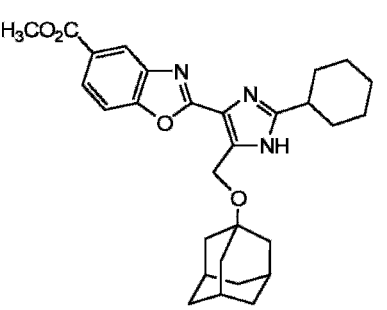
1. The somatostatin analogue, D-Phe-c(Cys-Tyr(I)-D-Trp-Lys-Val-Cys)-Thr-NH_2 containing a Tyr phenyl ring iodinated in the 3- or 5-position was prepared by Gordon (1) and was effective in inhibitory excess pituitary, pancreas, and gastrointestinal tract secretions.
2. Cyclen peptide derivatives, (**I**), prepared by Hoffman (2) were capable of binding to the gastrin releasing peptide receptor. When radiolabeled with either ^{99}Tc or ^{188}Re, they were used as therapeutic or diagnostic agents.



$n = 0,3,4,7,9$

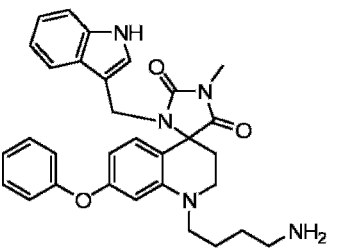
(I)

3. Gastrin and cholecystokinin receptor ligands consisting of 1H-imidazole-4-yl-benzooxazole derivatives, (**II**), prepared by Kalindjian (3) were effective in controlling excess gastric acid secretion.

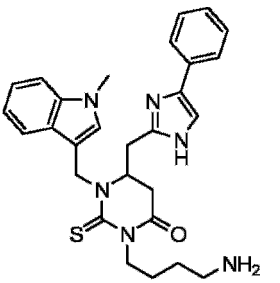


(II)

4. Spiro hydantoins, (**III**) prepared by Webb (4) and hydantoin and thiohydantoin, (**IV**), derivatives prepared by Poitout (5) were effective as somatostatin receptor agonists and were useful in treating hypersecretion disorders.



(III)



(IV)

References

1. T.D. Gordon *et al*., US Patent 7,094,753 (August 22, 2006)
2. T.J. Hoffman *et al*., US Patent 7,060,247 (June 13, 2006)
3. S.B. Kalindjian *et al*., US Patent 7,034,048 (April 25, 2006)
4. T. Webb *et al*., US Patent 6,903,074 (March 29, 2005)
5. L. Poitout *et al*., US Patent 6,759,415 (July 6, 2004)

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CHAPTER XIII

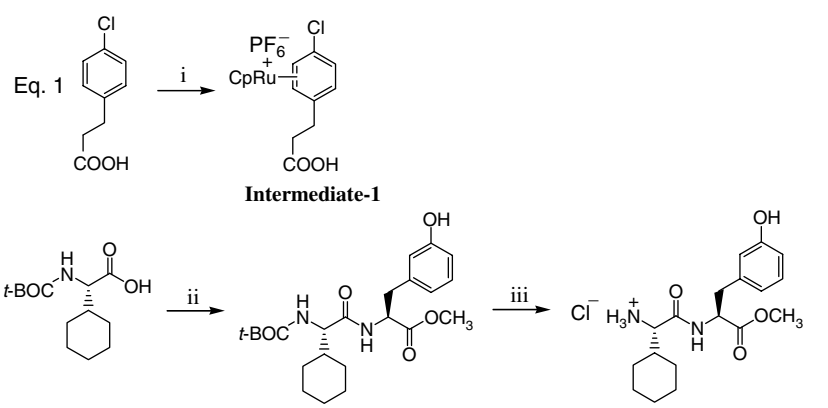
Hepatitis C

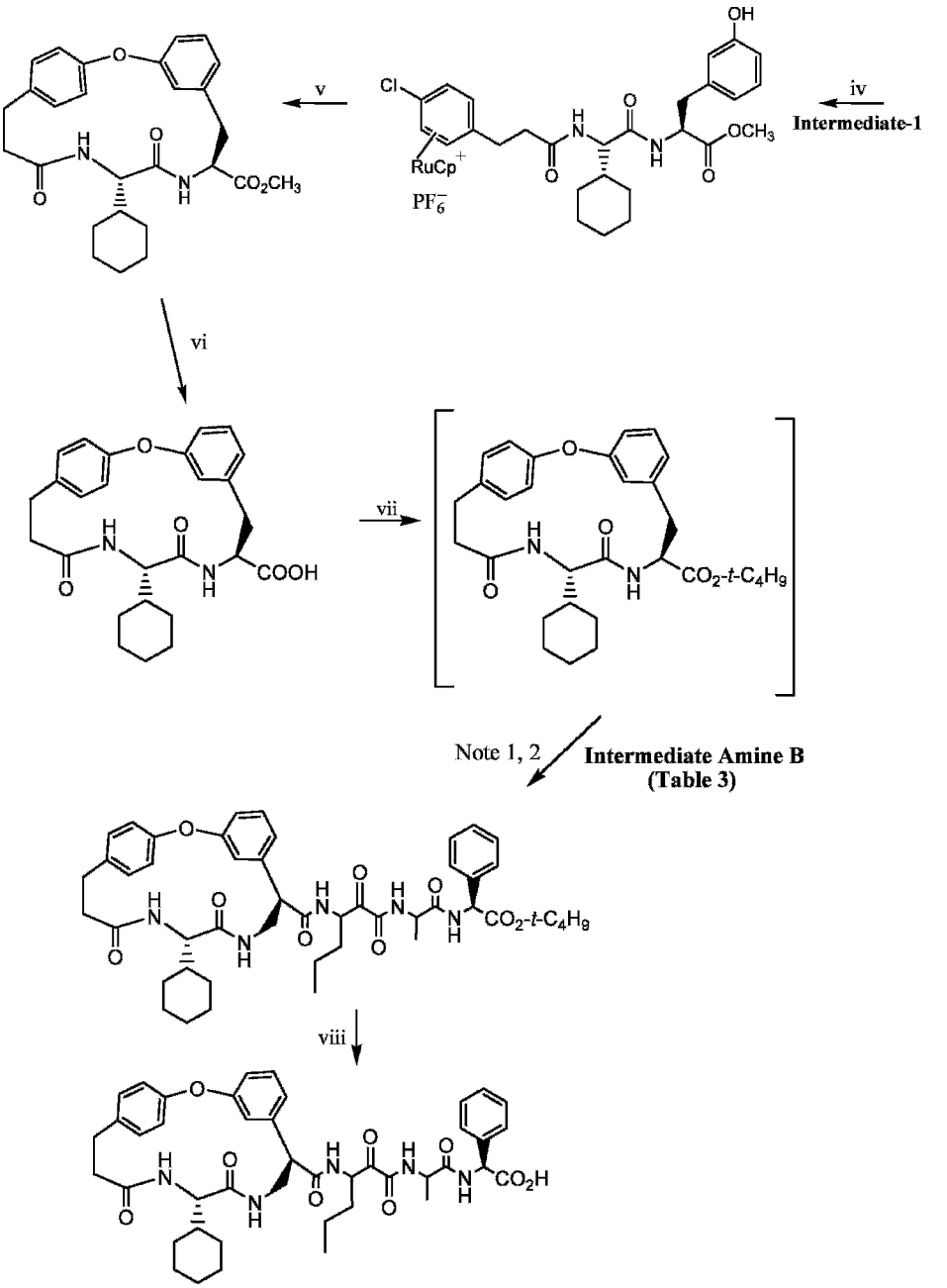
I. Hepatitis C Virus Protease Inhibitors: Macrocyclic NS3 Serine Protease Inhibitors

Title Macrocyclic NS3 Serine Protease Inhibitors of Hepatitis C Virus Compromising Alkylated and Aryl Alanine P2 Moities
S. Venkatraman *et al.*, US Patent 6,914,122 (July 5, 2005)
Assignee Schering Corporation
Utility Treatment of Hepatitis C

Invention Significance Hepatitis C virus (HCV) is a (+)-sense single-stranded RNA virus implicated as the major causative agent in non-A, non-B hepatitis, NANBH, particularly in blood-associated NANBH. The current invention discloses novel HCV NS3 serine protease macrocyclic derivatives useful as antiviral chemical agents in the treatment of hepatitis C virus.

Reaction





i- Cyclopentadiene-trisacetonitrilo-ruthenium hexafluorophosphate, CH_2Cl_2

ii- CH_2Cl_2, 4-methylmorpholine, 3-hydroxyl-tyrosine methyl ester hydrogen chloride, isobutyl chloroformate

iii- Hydrochloric acid, Dioxane, diethyl ether
iv- Cyclopentadiene-n^6-4-chlorophenylpropionic acid-ruthenium hexafluorophosphate, DMF, 3-hydroxy-1,2,3-benzotriazin-4(3H)-one, diisopropylethyl amine, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
v- Cesium carbonate, DMF
vi- Methyl alcohol, CH_2Cl_2, lithium hydroxide hydrate
vii- 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 3-hydroxy-1,2,3-benzotriazin-4(3H)-one, diisopropylethyl amine, *Intermediate Amine B*, DMF, Dess–Martin reagent
viii- Trifluoroacetic acid, CH_2Cl_2

Experimental

1. **Preparation of cyclopentadiene-n^6-4-chlorophenylpropionic acid-ruthenium hexafluorophosphate**

   A solution of 4-chlorophenylpropionic acid (10.8 mmol) in 200 ml CH_2Cl_2 was treated with $CpRu(CH_3CN)_3$ PF_6 (10.8 mmol), then refluxed 2 hours. The mixture was cooled to ambient temperature and colorless crystals formed. The crystals were isolated, washed with diethyl ether/CH_2Cl_2, 1:1, and 3.3 g analytically pure product isolated.

   ^{1}H NMR (400 MHz, $CD_3C(O)CD_3$, δ (ppm), J) 6.77 (d, 2H, $J = 7.0$ Hz), 6.53 (d, 2H, $J = 7$ Hz), 5.64 (s, 5H), 2.87 (t, 2H, $J = 7.0$ Hz), 2.74 (t, 2H, $J = 7.0$ Hz)
   MS (Electron spray, *m/z* relative intensity) 350.9 ($C_{14}H_{14}ClRu^+$, M^+, 100)
   Analytical $C_{14}H_{14}ClF_6O_2PRu$. Calc.: C, 33.92%; H, 2.85%; Cl, 7.15%; P, 6.25%. Found: C, 34.04%; H, 3.04%; Cl, 7.09%; P, 5.71%

2. **Preparation of Boc-cyclohexylglycine-3-hydroxyl-tyrosine methyl ester**

   A solution of Boc-cyclohexylgylcine monohydrate (24.00 mmol) in 50 ml CH_2Cl_2 was treated with 4-methylmorpholine (26.0 mmol), then cooled to -10°C and treated with 3.5 ml isobutyl chloroformate. A white suspension was stirred until the temperature rose to 5°C. In a separate beaker 3-hydroxyl-tyrosine methyl ester hydrochloride salt (26.5 mmol) dissolved in 30 ml DMF was treated with 4-methylmorpholine (26.0 mmol) and stirred 15 minutes at ambient temperature. This mixture was added to the aforementioned mixture, then stirred 1 hour at ambient temperature, and then diluted with 100 ml 1 M HCl. The aqueous layer was extracted three times with 200 ml EtOAc, then washed with 100 ml apiece 1 M HCl, 1 M NaOH, and brine, dried with Na_2SO_4, and concentrated. The residue was purified by chromatography with silica gel using EtOAc/hexanes, 3:7, and the product isolated in 53% yield as a colorless foam.

3. **Preparation of Boc-cyclohexylglycine-3-hydroxyl-tyrosine, methyl ester, hydrogen chloride**

   A solution of the Step 2 product (23.04 mmol) in 100 ml 4 M HCl in dioxane was stirred 24 hours at ambient temperature, then concentrated, and the solid resuspended in diethyl ether. The mixture was filtered, washed with diethyl ether, and 8.2 g product isolated as a colorless solid.

   ^{1}H NMR (400 MHz, d_4-CD_3OD, δ, ppm) 7.09 (t, 1H, $J = 8.0$ Hz), 6.71–6.36 (m, 3H), 4.69 (dd, 1H, $J = 6.0$s, 3.2 Hz), 3.69 (s, 3H), 3.66 (d, 1H, $J = 5.2$ Hz), 3.15–3.10 (dd, 1H, $J = 5.6$, 4.0 Hz), 1.87–1.69 (m, 6H), 1.32–1.10 (m, 5H)

4. **Preparation of cyclopentadiene-n^6-ruthenium-4-chlorophenylpropionic acid-cyclohexyglycine-*meta*-tyrosine, methyl ester**

   The Step 1 product (4.0 mmol) dissolved in 20 ml DMF was treated with 3-hydroxy-1,2,3-benzotriazin-4(3H)-one (6.0 mmol) and diisopropylethyl amine (16.0 mmol), then cooled to 0°C. The mixture was then treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (5.0 mmol), then stirred 30 minutes, and treated with the Step 3 product (4.0 mmol). This mixture was stirred 12 hours at ambient temperature, then concentrated in vacuo. The residue was diluted with 200 ml water, then extracted three times with 100 ml into CH_2Cl_2, and re-extracted with 100 ml HCl. The combined extracts were washed once with 100 ml apiece $NaHCO_3$ and brine solutions and used without further purification.

5. **Preparation of the cyclization product**

   The Step 5 product (1.47 g crude) dissolved in 150 ml DMF was treated with Cs_2CO_3 (7.37 mmol), then stirred 16 hours at ambient temperature, and concentrated. The residue was dissolved in 200 ml water, then extracted three times with 100 ml CH_2Cl_2, and re-extracted with 100 ml brine. The extracts were dried with Na_2SO_4 and then concentrated in vacuo. The residue was photolytically decomplexed by dissolving in 35 ml acetonitrile and photolyzed in a Raynot ($\lambda = 350$ nm) for 48 hours. The decomplexed mixture was concentrated, the residue purified by chromatography using EtOAc/hexanes, 7:3, and the product isolated in 52% yield as a colorless solid.

6. **Preparation of cyclization acid**

   The Step 5 product (0.65 mmol) was dissolved in a mixture consisting of 10 ml methyl alcohol, 20 ml CH_2Cl_2, and 5 ml water, then treated with $LiOH \bullet H_2O$ (2.2 mmol) and stirred 2 hours at ambient temperature. The mixture was acidified with 6 M HCl, then extracted three times with 100 ml CH_2Cl_2. The organic layers was dried with Na_2SO_4, concentrated, and the product isolated in 66% yield as a colorless solid.

7. **Preparation of cyclization amide-*E*-alcohol (General Procedure)**

   A solution of the Step 6 product (0.22 mmol) in 2.5 ml DMF was treated with 3-hydroxy-1,2,3-benzotriazin-4(3H)-one (0.33 mmol) and diisopropylethyl amine

(1.1 mmol), then cooled to 0°C, and treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.33 mmol). The solution was stirred 20 minutes, then treated with *Intermediate Amine B* (0.27 mmol), and stirred 12 hours at ambient temperature. The reaction mixture was concentrated and the residue dissolved in 30 ml water. The solution was extracted three times with 50 ml CH_2Cl_2, then re-extracted with 30 ml apiece using 1 M HCl and 1 M NaOH. The extracts were concentrated and 58 mg product isolated as a colorless solid.

MS (Electron spray, *m/z* relative intensity) 693 [$(M+1)^+$, 100], 637 (41), 494 (55), 394 (51), 338 (13)

This intermediate (0.14 mmol) was dissolved in 2.0 ml CH_2Cl_2, then treated with the Dess–Martin reagent (0.28 mmol), and stirred 2 hours at ambient temperature. The mixture was concentrated, the residue was purified by chromatography using methyl alcohol/CH_2Cl_2, 1:32, and 47 mg of product isolated as a colorless solid.

MS (Electron spray, *m/z* relative intensity) 691 $(M+1)$

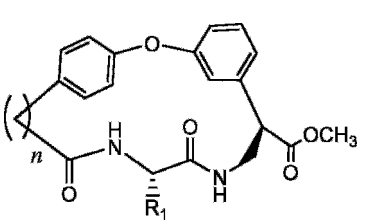
8. **Preparation of cyclization amide-*E*-triketo**

The Step 7 product (60.0 μmol) was treated with 4 ml CF_3COOH/CH_2Cl_2, then stirred 2 hours at ambient temperature, and concentrated. The residue was treated with 4 ml heptanes, then reconcentrated, and the product quantitatively isolated as a white solid.

MS (Electron spray, *m/z* relative intensity) 768 [$(M+1)^+$, 100]

Derivatives

Table 1 Selected macrocyclic esters and their corresponding mass spectral data. Esters were converted into hepatitis C antiviral agents after amidation with selected *Intermediate Amines* illustrated in Table 3



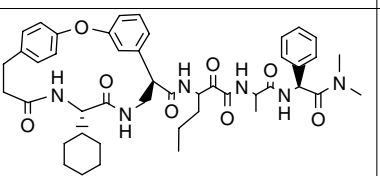
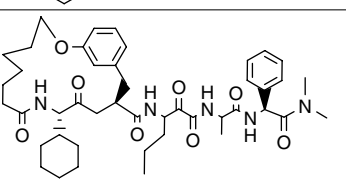
n	R_1	MS FAB (Relative Intensity)
2	Hydrogen	383
3	Cyclohexyl	493
4	Cyclohexyl	493

Testing

I. *Assay for HCV Protease Inhibitory Activity*

Spectrophotometric assays for the HCV serine protease were performed on selected experimental agents using the method of Zhang (1). This assay is based on the proteolysis of chromogenic ester substrates and is suitable for continuous monitoring of HCV NS3 protease activity. Testing results are provided in Table 2.

Table 2 Testing results for selected experimental agents indicating their utility as NS3 serine protease inhibitors

Entry	Structure	EC_{50} (μm)
35		20
36		9

Notes

1. Selected *Intermediate Amine* co-reagents prepared by the author and used in amidation of the Step 7 product are illustrated in Table 3.

Table 3 *Intermediate Amines* used in coupling with selected Step 6 macrocyclic ester intermediate

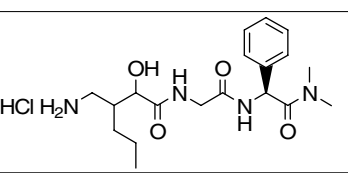
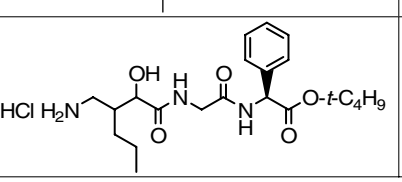
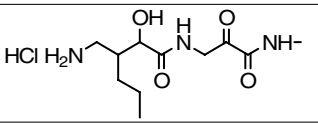
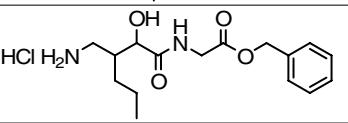
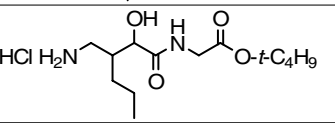
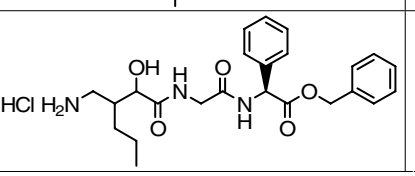
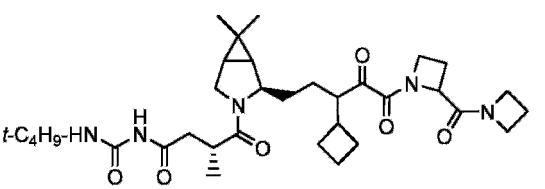
Intermediate Amine	Structure	LRMS, *m/z*, MH^+
A		365.0
B		Not purified

Table 3 Continued

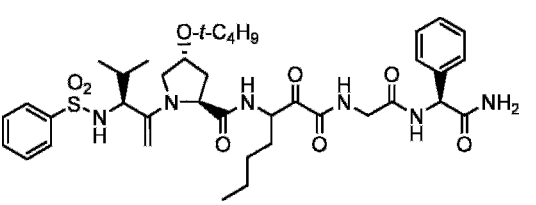
Intermediate Amine	Structure	LRMS, *m/z*, MH^+
C		351.1
D		Not purified
E		Not purified
F		Not purified

2. An additional *Intermediate Amine*, (**I**), used in coupling with selected Step 7 derivatives was prepared by Arasappan (2), which was effective as an NS3 serine protease inhibitor of hepatitis C.



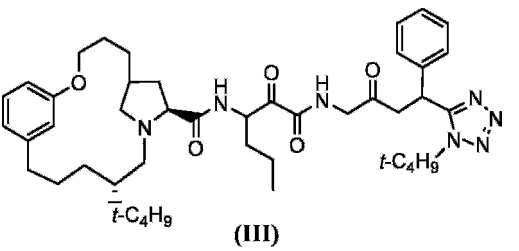
(I)

3. Peptide derivatives, (**II**), prepared by Saksena (3) were effective as NS3 serine protease inhibitors of hepatitis C when used in pharmaceutical compositions containing α-interferon or pegylated interferon.



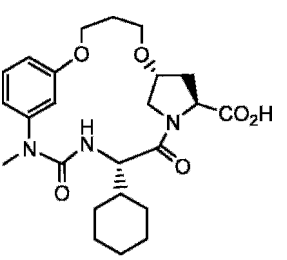
(II)

4. Zhu (4) prepared diaryl peptide derivatives, (**III**), containing a 2- or 5-tetrazolyl terminus, which were effective as NS3 serine protease inhibitors of the hepatitis C virus.



(III)

5. Macrocyclic diether derivatives, (**IV**), prepared by Chen (5) were also effective as NS3 serine protease inhibitors of the hepatitis C virus after conversion into amide derivatives using *Intermediate Amines* described in Table 3.



(IV)

References

1. R. Zhang *et al.*, Anal. Biochem., 270, 268 (1999)
2. A. Arasappan *et al.*, US Patent 6,894,072 (May 17, 2005)
3. A.K. Saksena *et al.*, US Patent 7,012,066 (March 14, 2006)
4. Z. Zhu *et al.*, US Patent 6,911,428 (June 28, 2005)
5. K.X. Chen *et al.*, US Patent 6,846,802 (January 25, 2005)

CHAPTER XIV

Hormonal Disorders

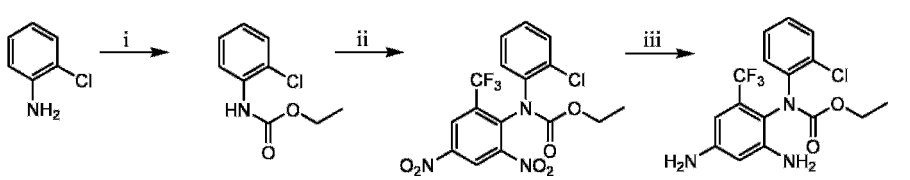
I. Growth Hormone Releaser: Benzimidazolidinones

Title Benzimidazolidinone Derivatives
K. Okazaki *et al.*, US Patent 6,939,887 (September 6, 2005)
Assignee Sumitomo Pharmaceuticals Co., Ltd
Utility Treatment of Growth Hormone Deficiency

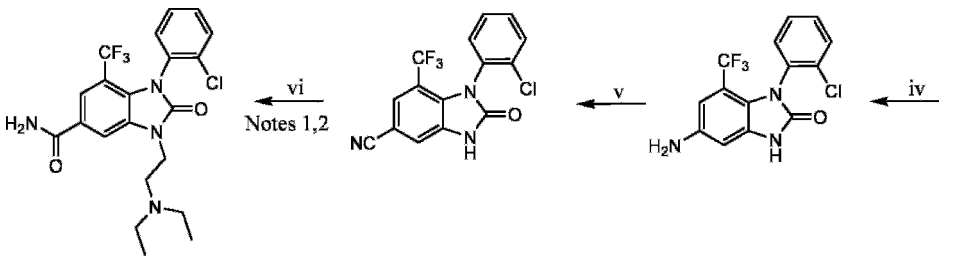
Invention Significance Growth hormone deficiency results in symptoms of dwarfism and negatively impacts body metabolism. Agents that increase growth hormone levels generate growth hormone releasing factors. This investigation describes the preparation of orally administrable nonpeptidic benzimidazolidinone derivatives which promote secretion of growth hormone releasing factors.

Reaction

Eq. 1



i- Pyridine, ethyl chloroformate, hydrochloric acid
ii- Diglyme, sodium hydride,
2-chloro-3,5-dinitrobenzotrifluoride
iii- EtOAc, 10% palladium on carbon, hydrogen



iv- Ethyl alcohol, sodium hydride
v- DMSO, copper(I) cyanide, water, *t*-butyl nitrite
vi- DMF, sodium hydride, 2-chlorotriethylamine hydrochloride, triethylamine, 2-methyl-2-propanol, potassium hydroxide

Experimental

1. **Preparation of ethyl 2-chlorophenylcarbamate**

   2-Chloroaniline (1.4463 g) dissolved in 10 ml pyridine was treated with1.3 ml ethyl chloroformate under ice-cooling, then stirred 1 hour. The solution was then treated with 1 M HCl and extracted with EtOAc. The organic layer was washed with 1 M HCl, brine, dried with $MgSO_4$, concentrated, and the product isolated in 98% yield.

   ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (3H, t, $J = 7.1$ Hz), 4.24 (2H, q, $J = 7.0$ Hz), 6.97 (1H, dt, $J = 7.7$, 1.4 Hz), 7.14 (1H, brs), 7.25 (1H, dt, $J = 7.9$, 1.5 Hz), 7.33 (H, dd, $J = 8.1$, 1.5 Hz), 8.16 (1H, brd, $J = 8.1$ Hz)

2. **Preparation of ethyl 2-chlorophenyl[2,4-dinitro-6-(trifluoromethyl) phenyl] carbamate**

   The Step 1 product (62.6 mg) dissolved in 1 ml diglyme was treated with sodium hydride (12.6 mg) and 2-chloro-3,5-dinitrobenzotrifluoride (77.1 mg), then stirred 1 hour at ambient temperature, and treated with 1 M HCl. The mixture was extracted with EtOAc, then washed with 1 M HCl and saturated $NaHCO_3$ solutions, dried, and concentrated. The residue was purified by preparative TLC using hexane/EtOAc, 3:1, and the product isolated in 93% yield.

   ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29–1.21 (3H, m), 4.32–4.23 (2H, m), 7.28–7.03 (3H, m), 7.51–7.35 (1H, m), 8.80 (1H, d, $J = 2.8$ Hz), 8.96 (1H, brd, $J = 14.1$ Hz)

3. **Preparation of ethyl 2-chlorophenyl[2,4-diamino-6-(trifluoromethyl)phenyl] carbamate**

   The Step 2 product (0.645 g) dissolved in 100 ml EtOAc was treated with 10% palladium on carbon (0.58 g) and the mixture stirred 11 hours under a hydrogen

atmosphere. The mixture was filtered, washed with EtOAc, concentrated, and the product isolated in 98% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (3H, t, $J = 7.0$ Hz), 3.98 (4H, brs), 4.21 (2H, q, $J = 7.0$ Hz), 6.13 (1H, d, $J = 2.4$ Hz), 6.39 (1H, d, $J = 2.5$ Hz), 7.23–7.10 (3H, m), 7.42 (1H, d, $J = 7.7$ Hz)

4. **Preparation of 5-amino-1-(2-chlorophenyl)-7-(trifluoromethyl)-1,3-dihydro-2H-benzimidazol-2-one**

   The Step 3 product (68.8 mg) dissolved in 6 ml ethyl alcohol was treated with sodium hydride (14.7 mg) and refluxed 2 hours. After cooling, it was treated with saturated $NaHCO_3$ solution, then extracted three times with $CHCl_3$. The solution was washed with water, dried, and concentrated. The residue was purified by preparative TLC using $CHCl_{3/methylalcohol}$, 5:1, and 46.2 mg product isolated.

5. **Preparation of 1-(2-chlorophenyl)-2-oxo-7-(trifluoromethyl)-2,3-dihydro-1H-benzimidazole-5-carbonitrile**

   The Step 4 product (46.2 mg) dissolved in 1 ml DMSO was treated with CuCN (16.4 mg), then heated at 55°C. At the same temperature, *t*-butyl nitrite (43.6 mg) dissolved in 0.5 ml DMSO was added dropwise over 90 minutes and the mixture stirred an additional hour at 60°C. After cooling, water was added and the mixture was extracted twice with EtOAc, then washed with brine, dried, and concentrated. The residue was purified by chromatography using $CHCl_{3/methylalcohol}$, 20:1, and 17.6 mg product isolated.

6. **Preparation of 1-(2-chlorophenyl)-3-[2-(diethylamino)ethyl]-2-oxo-7-(trifluoro methyl) -2,3-dihydro-1H-benzimidazole-5-carboxamide**

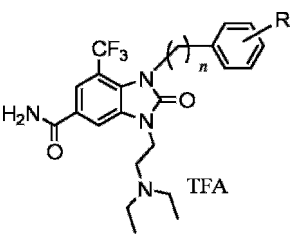
   The Step 5 product (17.6 mg) dissolved in 1.5 ml DMF was treated with 2-chlorotriethylamine hydrochloride (11.7 mg) dissolved in 2 ml DMF containing 0.011 ml triethylamine, then stirred 1 hour at 60 – 70°C. After the mixture cooled, it was treated with saturated $NaHCO_3$ solution, then extracted with EtOAc. The extract was washed with water, dried, and concentrated. The residue was dissolved in 1 ml 2-methyl-2-propanol and heated to 50°C. This solution was then treated with KOH (73.1 mg) and stirred 1 hour. After cooling, water was added and the mixture was re-extracted four times with EtOAc and the combined extracts washed with brine, dried, and concentrated. The product was purified by HPLC and isolated in 6% yield.

   ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20 (6H, t, $J = 7.1$ Hz), 3.65–3.15 (6H, m), 4.37 (2H, t, $J = 6.6$ Hz), 7.72–7.51 (5H, m), 7.98 (1H, s), 8.19 (1H, brs), 8.21 (1H, s), 9.64 (1H, brs)
   HPLC Retention time 13.8 minutes

Derivatives

Selected benzimidazolidinone derivatives are provided in Table 1.

Table 1 Growth hormone release promoting activity of selected experimental agents. Growth hormone releasing activity is the ratio of growth hormone level of the supernatant in the presence of a selected experimental agent in 10,000 nM to that in its absence. ^{1}H NMR data for products and intermediates provided by author



Entry	R	*n*	Growth Hormone Activity
3	3-Chloro	1	1.2
5	2-Chloro	1	1.3
7	4-Chloro	0	3.0
8	2,4-Dichlorochloro	0	2.1
9	3-Trifluoromethyl	1	1.3
16	2-Methyl	1	1.0

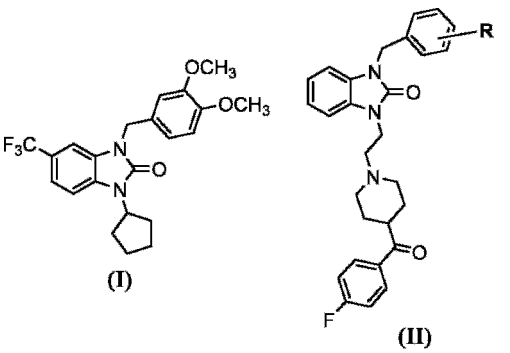
Testing

I. *Growth Hormone Release Promoting Activity*

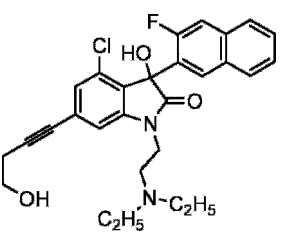
Experimental agents were evaluated for their growth hormone release promoting activity according to the method of Smith (1).

Notes

1. The preparation of additional 1H-benzimidazol-2-one derivatives, (**I**) and (**II**), effective as growth hormone releasing is described by Sawada (3) and Gallagher (4), respectively.

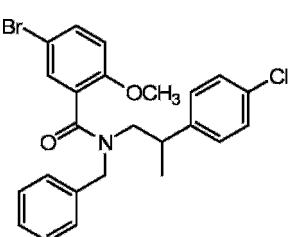


2. Orally administrable nonpeptide oxindole derivatives, (**III**), prepared by Tokunago (4) were effective in stimulating the release of growth hormone factor.



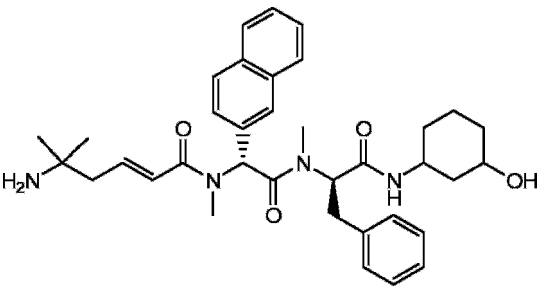
(III)

3. Phenyl-alkylene benzamide derivatives, (**IV**), effective as orexin antagonists were useful in the treatment of growth hormone deficiency associated with hypothalamic disorders.



(IV)

4. (2*E*)-5-Amino-5-methylhex-2-enoic acid derivatives, (**V**), effective as growth hormone releasing agents were prepared by Peschke (6) and used in treating medical disorders resulting from a deficiency in growth hormone.



(V)

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1. R.G. Smith *et al.*, Science, 260, 1640 (1993)
2. K. Sawada *et al.*, US Patent 6,582,351 (June 24, 2003)
3. P.T. Gallagher *et al.*, US Patent 6,075,039 (June 13, 2000)
4. T. Tokunago *et al.*, US Patent 6,576,656 (June 10, 2003)
5. W.N. Chan *et al.*, US Patent 7,078,565 (July 18, 2006)
6. B. Peschke *et al.*, US Patent 6,919,315 (July 19, 2005)

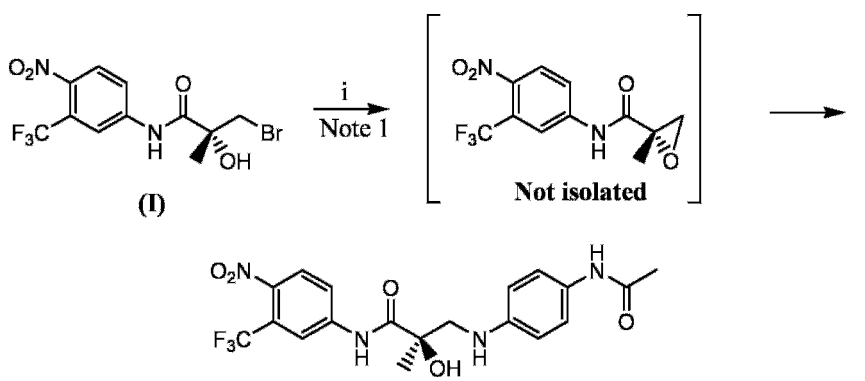
II. Nonsteroidal Selective Androgen Receptor Modulators: 4-Nitro-3-(Trifluoromethyl)phenyl Propionamides

Title N-Bridged Selective Androgen Receptor Modulators and Methods of Use Thereof
J.T. Dalton *et al.*, US Patent 7,022,870 (April 4, 2006)
Assignee University of Tennessee Research Foundation
Utility Nonsteroidal Selective Androgen Receptor Modulators Useful for Treating Androgen-related Disorders

Invention Significance Androgen decline in aging males is characterized by osteoporosis, hair loss, anemia, and benign prostate hyperplasia while in females it is characterized by higher incidences of endometriosis, breast cancer, uterine cancer, and ovarian cancer. A method of addressing these conditions using nonsteroidal selective androgen receptor modulators (SARMs) is described.

Reaction

Eq. 1



i- Hexafluoro-2-propanol, 4-aminophenylacetamide

Experimental

1. **Preparation of *S*-3-(4-acetylamino-phenylamino)-2-hydroxy-2-methyl-*N*-(4-nitro-3-trifluoromethylphenyl)propionamide**

   N-[4-Nitro-3-(trifluoromethyl)phenyl]-(2*R*)-3-bromo-2-hydroxy-2-methylpropanamide (0.26 mmol) and (4-aminophenyl)acetamide (0.26 mmol) were dissolved in

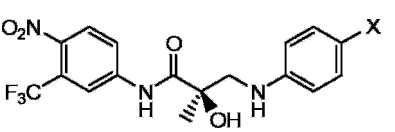
1.5 ml of hexafluoro-2-propanol and stirred at ambient temperature until TLC indicated the reaction was complete. The mixture was concentrated and the residue dissolved in 30 ml water, then re-extracted three times with 30 ml EtOAc. The extracts were dried with $MgSO_4$, concentrated to an oil, purified with preparative TLC using 10% methyl alcohol/$CHCl_3$, and the product isolated in 27% yield as a tan solid, mp = 143 − 145°C.

^{1}H NMR (DMSO-d_6) δ 10.48 (s, 1H, NH), 9.48 (s, 1H, NH), 8.48 (s, 1H, ArH), 9.28 (d, $J = 9$ Hz, $J - 2$ Hz, 1H, ArH), 8.16 (d, $J = 9$ Hz, 1H, ArH), 7.21 (d, $J = 8$ Hz, 2H, ArH), 6.57 (d, $J = 8.0$ Hz, 2H, ArH), 6.06 (s, 1H, OH), 5.1 (bs, 1H, NH), 3.41 (dd, J–12, 3 Hz, 1H, (CH_2(1)), 3.11 (dd, $J = 12$, 3 Hz, 1H, (CH_2(2)), 1.93(s, 3H, Me), 1.41 (s, 3H, Me)
Analysis ($C_{19}H_{19}F_3N_4O_5$) Calc.: C, 52.74%; H, 4.89%; N, 11.58;. Found: C, 52.4%; H, 4.9%; N, 11.2%
Calculated Mass 440.13, [M−H] 438.8

Derivatives

Selected derivatives are provided in Table 1.

Table 1 Evaluation of selected experimental agents as selective androgen receptor modulators. Entry 16 had a moderate androgen receptor affinity while entry 17 displayed a very high propensity as an androgen selective receptor binding agent. ^{1}H NMR data supplied by author



Entry	X	K_i(nm)
16	*N*-acetyl	135 ± 12
17	Fluoro	10 ± 1

Testing

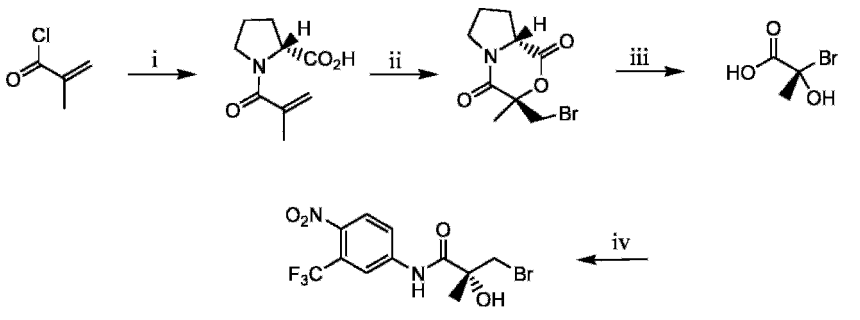
I. *Androgen Receptor Binding Affinities*

Androgen receptor binding affinities were determined using competitive binding assays described by Kirkovsky (1) and summarized in Table 1.

Notes

1. The preparation of the Step 1 co-reagent, *N*-[4-nitro-3-(trifluoromethyl)phenyl]-(2*R*)-3-bromo-2-hydroxy-2-methylpropan amide, (**I**), was previously prepared by the authors (2) and illustrated in Eq. 2.

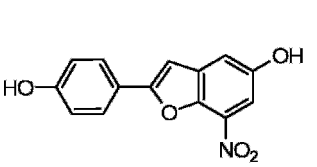
Eq. 2



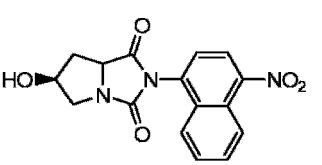
(I)

i- (*R*)-Pyrrolidine-2-carboxylic acid, sodium hydroxide, acetone
ii- *N*-Bromosuccinimide, DMF
iii- Hydrobromic acid
iv- Thionyl chloride, 4-nitro-3-trifluoromethylaniline

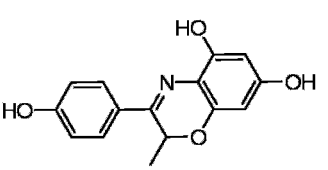
2. 2-Phenyl benzofuran derivatives, (**II**), prepared by Miller (3), naphthalene derivatives, (**III**), prepared by Hamann (4), and 3,4-dihydro-3-aryl-hydroxybenzoxazines, (**IV**), prepared by Dickson (5) were effective as nonsteroidal androgen receptor modulators and used in the treatment of age-related disorders.



(II)

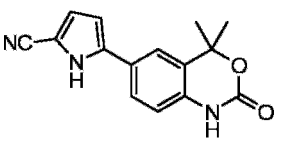


(III)

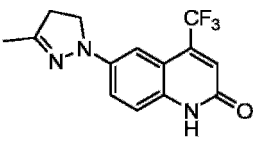


(IV)

3. Progesterone receptor agonists consisting of 1H-pyrrole-2-carbonitrile derivatives, (**V**), prepared by Collins (6) and 4,5-dihydro-3-metshyl-pyrazole derivatives, (**VI**), prepared by Zhi (7) were effective in lowering the incidence of breast, uterine, and ovarian cancers in older women.



(V)



(VI)

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1. L. Kirkovsky *et al.*, J. Med. Chem. 43, 581 (2000)
2. J.T. Dalton *et al.*, US Patent 6,569,896 (May 27, 2003)
3. C.P. Miller *et al.*, US Patent 7,022,733 (April 4, 2006)
4. L. Hamann *et al.*, US Patent 6,992,102 (January 31, 2006)
5. J.K. Dickson *et al.*, US Patent 7,015,219 (March 21, 2006)
6. M.A. Collins *et al.*, US Patent 6,982,261 (January 3, 2006)
7. L. Zhi *et al.*, US Patent 6,818,779 (November 15, 2005)

CHAPTER XV

Immunosuppressants

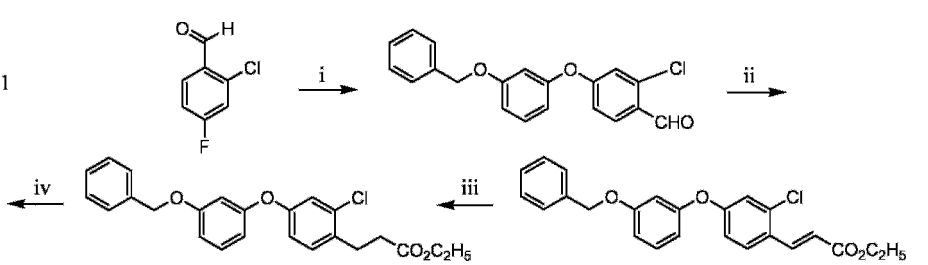
I. Immunosuppressants Mediated by Lymphocyte Interactions with EDG Receptor-Mediated Signal Transduction

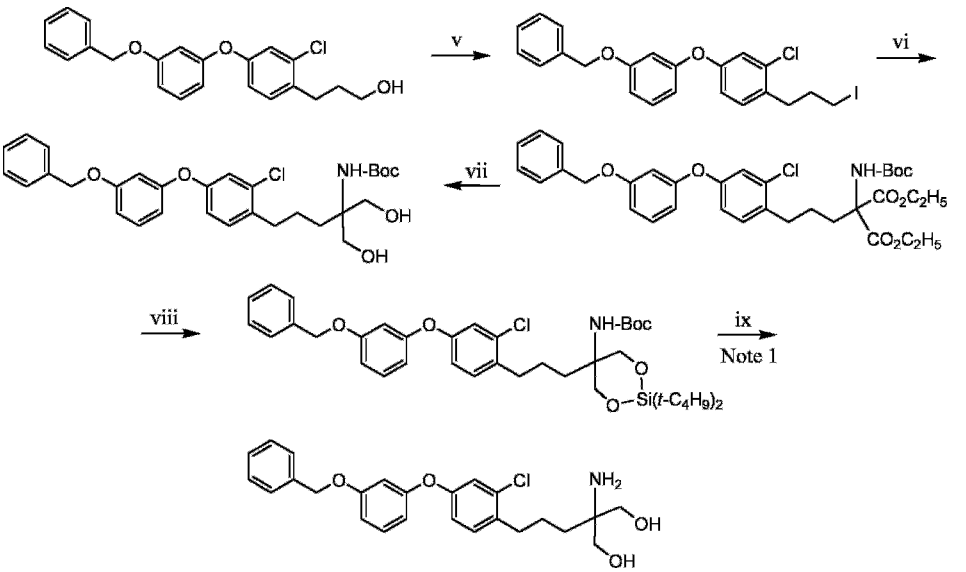
Title Diaryl Ether Derivative, Addition Salt Thereof, and Immunosuppressant
Y. Kohno *et al*., US Patent 6,963,012 (November 8, 2005)
Assignee Kyorin Pharmaceutical Co., Ltd
Utility Immunosuppressants Following Organ Transplant Procedures

Invention Significance The use of immunosuppressive agents following organ transplantation is a necessary medical protocol to avoid organ rejection. Existing immunosuppressive agents, however, are associated with renal failure and toxic side effects requiring multiple drug combined therapy. Experimental agents exhibiting significant immunosuppressive effects with nominal side effects have been prepared to address this problem.

Reaction

Eq. 1





i- DMF, 3-benzyloxyphenol, potassium carbonate
ii- Sodium hydride, THF, ethyl (diethylphosphono)acetate
iii- Ethyl alcohol, bismuth chloride, sodium borohydride
iv- THF, lithium aluminum hydride
v- THF, imidazole, triphenylphosphine, iodine
vi- Sodium *t*-butoxide, diethyl 2-*t*-butoxycarbonylaminomalonate, THF, DMF
vii- THF, lithium borohydride, ethyl alcohol
viii- 3-Isopropylphenylboric acid, copper(II) acetate, CH_2Cl_2, triethylamine
ix- THF, tetrabutylammonium fluoride

Experimental

1. **Preparation of 4-(3-benzyloxyphenoxy)-2-chlorobenzaldehyde**

   Potassium carbonate (5.53 g) was added to 2-chloro-4-fluorobenzaldehyde (3.35 g) and 3-benzyloxyphenol (4.23 g) dissolved in 70 ml DMF and the solution and stirred for 3 hours at 150°C. The mixture was decanted into water, then extracted with EtOAc, washed with water and brine, dried with Na_2SO_4, and concentrated. The residue was purified by chromatography with silica gel using hexane/EtOAc, 6:1, and 6.73 g product isolated as a colorless powder.

2. **Preparation of ethyl 4′-(3-benzyloxyphenoxy)-2′-chlorocinnamate**

   Sodium hydride (960 mg) was added to 4.8 ml ethyl (diethylphosphono)acetate dissolved in 150 ml THF solution at 0°C, then stirred for 30 minutes. The Step 1

product (6.73 g) dissolved in 20 ml THF was added dropwise and the mixture stirred an additional 60 minutes. The mixture was treated with water, then worked up as in Step 1, and 7.36 g of product isolated.

3. **Preparation of ethyl 4′-[(3-benzyloxy)phenoxy]-2′-chlorodihydrocinnamate**

The Step 2 product (7.36 g) dissolved in 100 ml ethyl alcohol at 0°C was treated with bismuth chloride (2.84 g) followed by three 0.9 g additions of sodium borohydride and the mixture stirred 3 hours at ambient temperature. Ice water was added to the mixture and the crystallized inorganic deposits filtered out through celite. The filtrate was extracted with EtOAc, washed with water and brine, purified as in Step 1, and 7.40 g product isolated as a colorless oil.

4. **Preparation of 4′-[(3-benzyloxy)phenoxy]-2′-chlorodihydrocinnamyl alcohol**

The Step 3 product (7.40 g) dissolved in 100 ml THF at 0°C was treated with lithium aluminum hydride (500 mg) followed by 20% aqueous NaOH after 10 minutes. Insoluble inorganic deposits were then filtered out through celite. The reaction mixture was worked up as described in Step 1 and 6.37 g product isolated as a colorless oil.

5. **Preparation of 4′-[(3-benzyloxy)phenoxy]-2′-chlorodihydrocinnamyl iodide**

The Step 4 product (6.37 g) dissolved in 150 ml THF at 0°C was treated sequentially with imidazole (2.45 g), triphenylphosphine (9.44 g), and iodine (9.14 g), then stirred 60 minutes. The product was worked up as in Step 1 using hexane/EtOAc, 20:1, and 7.90 g of product isolated as a colorless oil.

6. **Preparation of ethyl 5-[4-(3-benzyloxyphenoxy)-2-chlorophenyl]-2-*t*-butoxy carbonylamino-2-ethoxycarbonylpentanoate**

Sodium *t*-butoxide (1.40 g) was added to a solution of 3.60 ml diethyl 2-*t*-butoxycarbonylaminomalonate dissolved in 130 ml THF containing 20 ml and DMF at ambient temperature and heated 30 minutes at 80°C. The mixture was cooled to ambient temperature and the Step 5 product (6.22 g) dissolved in 20 ml THF solution was added dropwise and then refluxed 5 hours. The mixture was then decanted into ice water and extracted with EtOAc, worked up as described in Step 1 using hexane/EtOAc, 4:1, and 6.84 g product isolated as a colorless oil.

FAB-MS 626 ($[M+H]^+$)
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22–1.30 (6H, m), 1.42 (9H, s), 1.57 (2H, br s), 2.37 (2H, br), 2.70 (2H, t, $J = 7.8$ Hz), 4.19–4.29 (4 H, m), 5.03 (2 H, s), 5.95 (1 H, bs), 6.57–6.62 (2 H, m), 6.74 (1 H, dd, $J = 8.3$, 2.4 Hz), 6.83 (1 H, dd, $J = 8.3$,

2.4 Hz), 6.98 (1H, d, $J = 2.4$ Hz), 7.13 (1 H, d, $J = 8.3$ Hz), 7.23(1 H, t, $J = 8.3$ Hz), 7.33–7.43(5 H, m)

7. **Preparation of 2-[4-(3-benzyloxyphenoxy)-2-chlorophenyl]propyl-2-*t*-butoxy carbonylamino-1,3-propanediol**

The Step 6 product (6.84 g) dissolved in 150 ml THF at 0°C was treated with lithium borohydride (960 mg) and 10 ml ethyl alcohol, then stirred 8 hours. At ambient temperature, the mixture was added to ice water and the organic solvent removed by distillation. A 10% aqueous solution of citric acid was added to the residue to adjust the pH to 3 followed by extraction with EtOAc. The mixture was worked up as described in Step 1 using hexane/EtOAc, 1:1, and the product isolated in 84% yield as a colorless viscous oil.

FAB-MS 542($[M + H]^+$)
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (9H, s), 1.66 (4H, br s), 2.69 (2H, t, $J = 6.8$ Hz), 3.40 (2H, br), 3.60 (2H, dd, $J = 11.3$, 5.9 Hz), 3.84 (2H, dd, $J = 11.3$, 3.8 Hz), 4.92 (1H,br s), 5.03 (2H, s), 6.59–6.62 (2H, m), 6.75 (1H,dd, $J = 8.3$, 2.5 Hz), 6.84 (1H, dd, $J = 8.3$, 2.5 Hz), 7.00 (1H,d, $J = 2.5$ Hz), 7.14 (1H, d, $J = 8.3$ Hz), 7.24 (1H, t, $J = 8.3$ Hz), 7.31–7.43 (5H, m)

8. **Preparation of 2-[(4-(3-benzyloxy)phenoxy)-2-chlorophenyl]propyl-5-*t*-butoxy carbonylamino-2,2-di-*t*-butyl-1,3,2-dioxasilane**

The Step 7 product (1.50 g) dissolved in 30 ml THF containing 0.84 ml 2,6-lutidine was treated with 1.67 ml di-*t*-butylsilyl bistrifluoromethanesulfonate dissolved in 5 ml CH_2Cl_2 at 0°C and stirred 60 minutes. Thereafter, the mixture was worked up as described in Step 1 and 1.67 g of product isolated as a colorless powder.

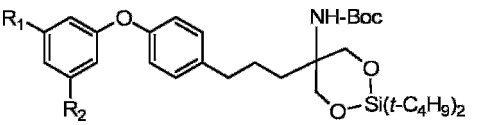
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (9H, s), 1.07 (9H, s), 1.23 (6H, d, $J = 6.8$ Hz), 1.43 (9H, s), 1.55 (4H, br s), 2.55 (2H, t, $J = 7.1$ Hz), 2.84–2.91 (1H, m), 3.89 (2H, d, $J = 11.7$ Hz), 4.23 (2H, d, $J = 11.7$ Hz), 4.91 (1H, br s), 6.75–6.79 (1H, m), 6.89–6.91 (1H, m), 6.91 (2H, d, $J = 8.8$ Hz), 6.95 (1H, d, $J = 7.8$ Hz), 7.09 (2H, d, $J = 8.8$ Hz), 7.22 (1H, t, $J = 7.8$ Hz)

9. **Preparation of 2-amino-2-[(4-(benzoxy)phenoxy)2-chlorophenyl]propyl-1,3-pro panediol hydrochloride**

The Step 8 product (188 mg) dissolved in 3 ml THF was treated with 1.61 ml 1 M tetrabutylammonium fluoride in THF, then stirred 2 hours at ambient temperature. The mixture was worked up as described in Step 1 using hexane/EtOAc, 3:2, and 107 mg of product isolated.

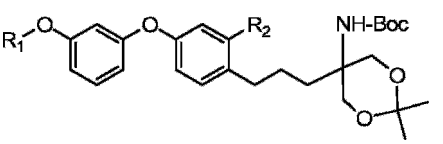
Derivatives

Table 1 Selected Step 8 dioxasilane intermediates and corresponding Step 9 conversions



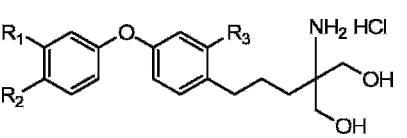
Entry	R_1	R_2	Yield (%) (last step)
110	Fluoro	Hydrogen	60
115	Methoxy	Hydrogen	69
120	Hydroxylmethyl	Hydrogen	41
122	Fluoro	Fluoro	79
124	Trifluoromethyl	Trifluoromethyl	83

Table 2 Selected Step 8 dimethyl ketal intermediates and corresponding Step 9 conversions



Entry	R_1	R_2	Yield (%) (last step)
131	3-Chlorobenzyl	Hydrogen	100
134	3-Methoxybenzyl	Chloro	94
137	2-Phenylethyl	Hydrogen	86
139	2-Phenylethyl	2-Phenylethyl	100
142	3-Chlorobenzyl	Hydrogen	100

Table 3 Selected experimental agents and corresponding melting points. ^{1}H NMR for products supplied by author



Entry	R_1	R_2	R_3	mp (°C)
148	3-Phenylpropoxy	Hydrogen	Hydrogen	95–98
173	Benzoxy	Hydrogen	Chloro	Amorphous
186	Hydrogen	Benzyl	Hydrogen	Amorphous
202	Hydrogen	Heptyl	Hydrogen	Amorphous
224	3-(3-Chlorophenyl)propoxy	Hydrogen	Chloro	94–96
225	3-(3,5-Dichlorophenyl)propoxy	Hydrogen	Hydrogen	92–95

Testing

I. *Suppress Host versus Graft Rejection in Mice Assay*

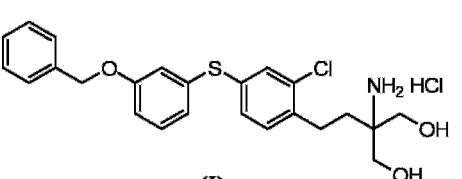
Selected experimental agents were evaluated for their effectiveness as immunosuppressants according to the method of Levenson (1) using 9–11-week-old male BALB/c mice. Testing results are provided in Table 4.

Table 4 The immunoactivity of selected experimental dosages as determined in the suppress host versus graft rejection in mice assay

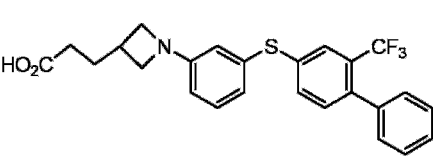
Entry	Dosage (mg/kg)	Immunosuppressant Activity (%)
148	3	78
173	3	100
186	1	87
202	10	92
224	0.3	71
225	0.03	70

Notes

1. Diaryl sulfide, (**I**) and (**II**), prepared by the authors (2) and Marsilje (3), respectively, were effective in mediating by lymphocyte interactions with EDG receptors and used as immunosuppressants following organ transplant procedures.

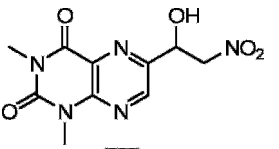


(I)



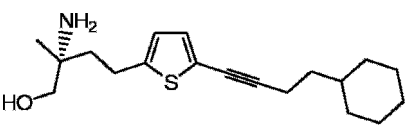
(II)

2. Polysubstituted pteridinediones (lumazines), (**III**), prepared by Waer (4) were effective in inhibiting transplant rejection as evaluated by the in vitro lymphocyte activation test and were synergistic when used in conjunction with other immunosuppressant drugs such as pentoxyfylline, tacrolimus, and rapamycin.



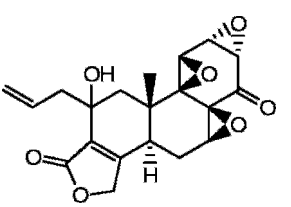
(III)

3. α, β-Amino alcohol derivatives, (**IV**), prepared by Nishi (5) were effective in mediating by lymphocyte interactions with EDG receptors as indicated in the suppress host versus graft rejection assay in mice.



(IV)

4. Dai (6) prepared triptolide derivatives, (**V**), which were effective in treating or preventing transplantation rejection including graft-versus-host disease.



(V)

References

1. A. Levenson *et al*., Transplantation, 55, 3, 578 (1993)
2. Y. Kohno *et al*., US Patent 6,960,692 (November 1, 2005)
3. T.H. Marsilje *et al*., US Patent 7,060,697 (June 13, 2006)
4. M.J.A. Waer *et al*., US Patent 6,946,465 (September 20, 2005)
5. T. Nishi *et al*., US Patent 6,964,976 (November 15, 2005) and US Patent 6,723,745 (April 20, 2004)
6. D. Dai *et al*., US Patent 7,098,348 (August 29, 2006)

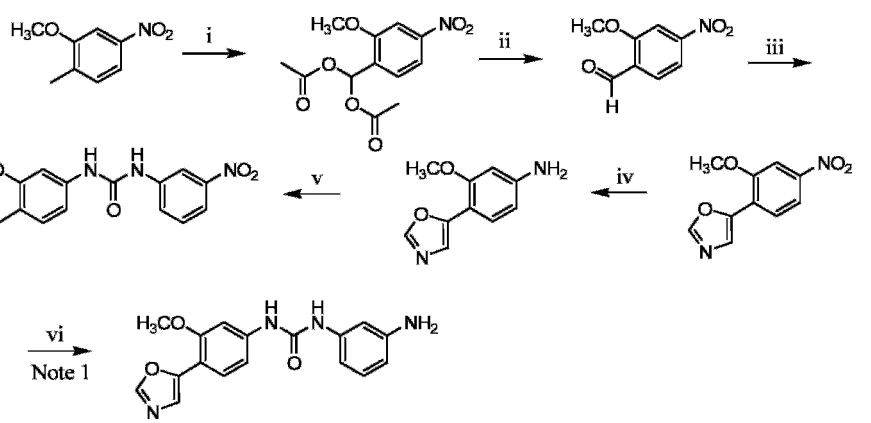
II. Inosine-5′-monophosphate Dehydrogenase Inhibitors

Title Inhibitors of IMPDH Enzymes
D.M. Armistead *et al*., US Patent 6,967,214 (November 22, 2005)
Assignee Vertex Pharmaceuticals Incorporated
Utility Immunosuppressants Following Organ Transplant Procedures

Invention Significance Inosine-5′-monophosphate dehydrogenase (IMPDH) is an enzyme involved in guanosine nucleotide synthesis required for organisms to divide and replicate. Although tiazofurin, ribavirin, and mizoribine inhibit IMPDH, they exhibit broad cellular toxicity, lack of IMPDH enzyme specificity, and sustained response in monotherapy. Immunosuppressive agents with high IMPDH enzyme specificity have been prepared to address these concerns.

Reaction

Eq. 1



i- Acetic acid, acetic anhydride, sulfuric acid, chromium trioxide
ii- Dioxane, hydrochloric acid
iii- Tosylmethyl isocyanide, potassium carbonate, methyl alcohol
iv- EtOAc, palladium on carbon, hydrogen
v- CH_2ClCH_2Cl, 3-nitrophenyl isocyanate
vi- Ethyl alcohol, tin chloride dihydrate

Experimental

1. **Preparation of 2-(dioxyacyl)methyl-5-nitro-anisole**

   2-Methyl-4-nitroanisole (29.1 mmol) dissolved in 46 ml apiece glacial acetic acid and acetic anhydride at 0°C was treated dropwise with 6.9 ml 18 M sulfuric acid and CrO_3 (80.8 mmol) portionwise over 60 minutes. The mixture was stirred an additional 15 minutes, then poured over ice, and a precipitate isolated. The solid was washed with cool water, then purified by flash chromatography with a solvent gradient of 15–50% EtOAc/hexanes, and the product isolated in 24% yield as a white solid.

2. **Preparation of 2-methoxy-4-nitro-benzaldehyde**

   A stirred suspension of the Step 1 product (307 mmol) in 100 ml dioxane was treated with 20 ml 12 M HCl and refluxed overnight. Upon cooling to ambient temperature, 40.65 g of precipitate was isolated as a light yellow crystalline solid. When the filtrate was reduced to c. 80 ml, an additional 8.91 g precipitate was obtained upon addition of hexanes. After drying, the product was isolated in 89.1% yield.

3. **Preparation of 5-nitro-2-(oxazol-5-yl)-anisole**

   A mixture of the Step 2 product (2.51 mmol), tosylmethyl isocyanide (2.51 mmol), and K_2CO_3 (251 mmol) dissolved in methyl alcohol was refluxed 90 minutes, then concentrated, and the residue dissolved in CH_2Cl_2. The solution was washed with water and brine, then dried over Na_2SO_4, and reconcentrated. After recrystallization in diethyl ether/hexanes, the product was isolated in 68% yield.

4. **Preparation of 5-amino-2-(oxazol-5-yl)-anisole**

   A mixture of the Step 3 product (19.1 mmol) in 150 ml EtOAc containing 10% Pd/C (1.05 g) was hydrogenated at 40 psi hydrogen overnight. The mixture was filtered, concentrated, the residue purified by flash chromatography using 30–40% EtOAc/hexanes, and the product isolated in 93% yield.

5. **Preparation of *N*-(3-methoxy-4-(oxazol-4-yl)phenyl-*N′*-4-nitrophenyl urea**

   The Step 4 product (0.394 mmol) dissolved in 5 ml CH_2Cl_2 was treated with 3-nitrophenyl isocyanate (0.591 mmol) at ambient temperature, then stirred overnight. The mixture was filtered, then washed with CH_2Cl_2, and the product isolated in 79% yield.

6. **Preparation of *N*-4-aminophenyl *N′*-(3-methoxy-4-(oxazol-4-yl)phenyl) urea**

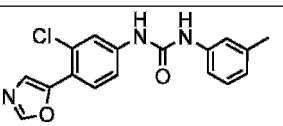
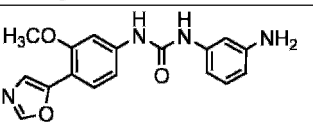
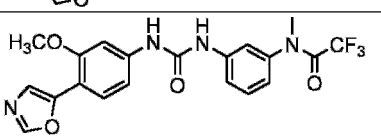
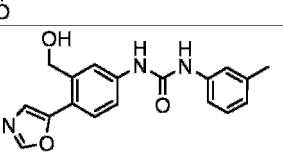
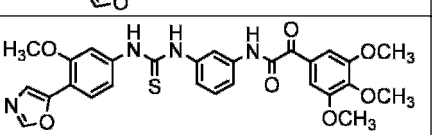
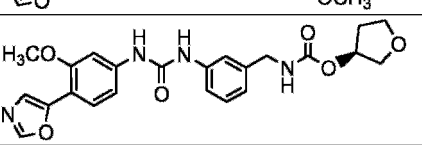
   A stirred suspension of the Step 5 product (0.268 mmol) in 20 ml ethyl alcohol was treated with $SnCl_2 \bullet 2H_2O$ (1.34 mmol), then refluxed 90 minutes and a precipitate that formed was removed by filtration. The filtrate was diluted with EtOAc, then washed with 2 M NaOH and brine, dried with Na_2SO_4, and concentrated. The residue

was purified byflash chromatography using 2.5–5% methyl alcohol/CH_2Cl_2, recrystallized, and the product isolated in 18% yield.

R_f 0.20 (5% MeOH/CH_2Cl_2)
^{1}H NMR (500 MHz, d_6-DMSO) δ 8.83 (s), 8.44 (s), 8.35 (s), 7.59 (d), 7.48 (d), 7.40 (s), 6.97–7.04 (dd), 6.86–6.92 (t), 6.83 (d), 6.54 (dd), 6.20 (dd), 5.05 (br s), 3.92 (s)

Derivatives

Table 1 Selected experimental ureas and corresponding last step conversions. ^{1}H NMR product data supplied by author

Entry	Structure	Yield (%) [last step]
43		74
59		18
97		7
102		68
108		35
120		85

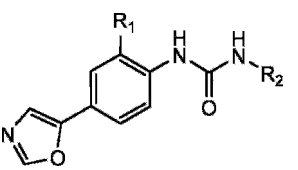
Testing

I. *IMPDH Activity Inhibition Assay*

IMPDH activity was assayed following an adaptation of the method of Magasanik (1). Enzyme activity was measured spectrophotometrically by monitoring the increase in

absorbance at 340 nm due to the formation of NADH ($\varepsilon^{340} = 6220 M^{-1}cm^{-1}$). The assay mixture consisted of 0.1 M Tris at pH 8.0, 0.1 M KCl, 3 mM EDTA, 2 mM DTT, 0.1 M IMP, and enzyme (IMPDH human type II) at 15–50 nM. This solution was incubated 10 minutes at 37°C, NAD added to a final concentration of 0.1 M, and the rate measured by following the linear increase in absorbance at 340 nm for 10 minutes using a 1 cm path length in a 1 ml cuvette. Selected experimental agents were evaluated by dissolution in DMSO to 20 mM and added to the assay mixture at a final volume of 2–5% (v/v). K_i test results are provided in Table 2.

Table 2 IMPDH activity inhibition assay testing results using selected experimental urea agents

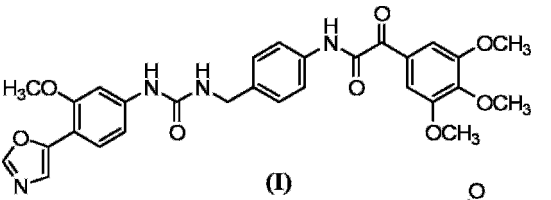


Entry	R_1	R_2	K_i
55	Methoxide	3-Methylphenyl	A
106	Methoxide	5-(*N*-Acetoxy)-indolinyl	A
24	Hydrogen	3-Aminophenyl	B
73	Chloro	3-Iodophenyl	B
1	Hydrogen	Benzyl	C
9	Hydrogen	3-Trifluoromethyl-4-chlorophenyl	C
4	Hydrogen	3,4-Difluorophenyl	D
53	Hydrogen	3-(3-Furanyl)-phenyl	D

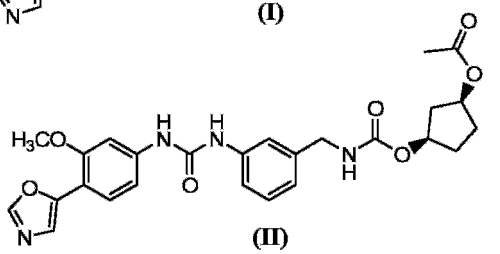
A, 0.01–50 nm activity; B, 51–1000 nm activity; C, 1000–10,000 nm activity; D, exceeds 10 000 nm activity.

Notes

1. Additional urea-based IMPDH inhibitors, (**I**) and (**II**), were prepared by the authors (2) and Stamos (3), respectively, and used as immunosuppressive agents following transplant procedures.

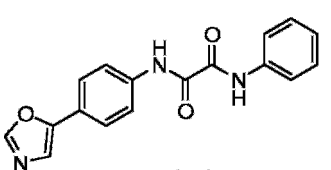


(I)

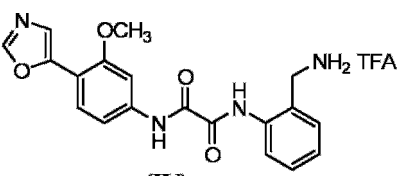


(II)

2. Oxazolyl derivatives, (**III**) and (**IV**), prepared by Gu (4) and Broadhurst (5), respectively, were useful in treating or preventing IMPDH-associated disorders such as transplant rejection and autoimmune diseases.

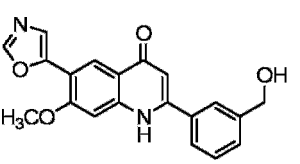


(III)

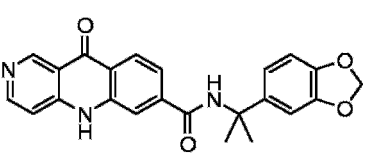


(IV)

3. 4(1H)-Quinolinones, (**V**), and heterocyclic acridone derivatives, (**VI**), prepared by Iwanowicz (6) and Chen (7), respectively, were effective in treating IMPDH-associated disorders, particularly allogenic rejection.



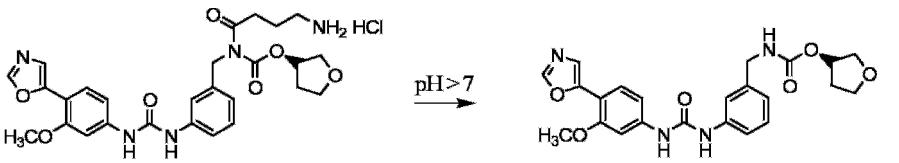
(V)



(VI)

4. Immunosuppressant IMPDH carbamate prodrug, (**VII**), illustrated in Eq. 2, prepared by Stamos (8) were used in the treatment or prophylaxis of transplant rejection and autoimmune disorders.

Eq. 2



(VII)

References

1. B. Magasanik *et al*., J. Biol. Chem., 226, 339 (1957)
2. D.M. Armistead *et al*., US Patent 6,541,496 (April 1, 2003)
3. D. Stamos *et al*., US Patent 7,087,642 (August 8, 2006)
4. H.H. Gu *et al*., US Patent 7,060,720 (July 12, 2006) and US Patent 7,053,111 (May 30, 2006)
5. M.J. Broadhurst *et al*., US Patent 6,867,299 (March 15, 2005)
6. E.J. Iwanowicz *et al*., US Patent 6,919,335 (July 19, 2005)
7. P. Chen *et al*., US Patent 6,916,809 (July 12, 2005)
8. D. Stamos *et al*., US Patent 6,825,224 (November 30, 2004)

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CHAPTER XVI

Improved Synthetic Methods

I. Antineoplastic Agents

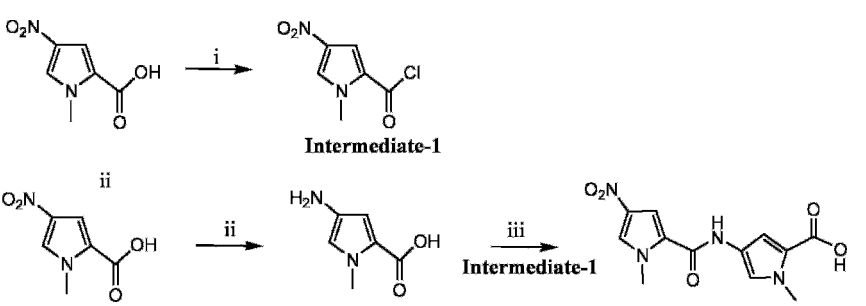
A. Distamycin Derivative

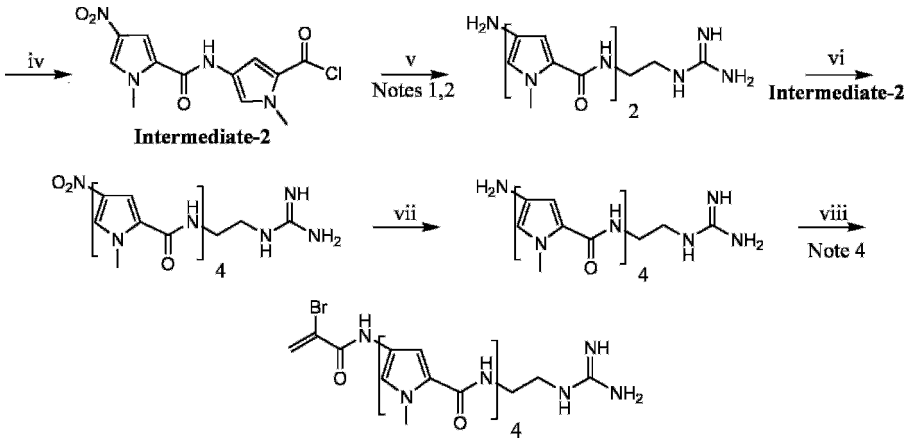
Title Process for Preparing Distamycin Derivatives
F. Caldarelli *et al.*, US Patent 6,906,199 (June 14, 2005)
Assignee Plantaceutica Italia S.p.a.
Utility Antineoplastic DNA Sequence Binding Agent

Invention Significance Acryloyl-distamycin-guanidino derivatives are cytotoxic agents, which selectively bond to a site of a DNA abundant with AT base pairs. Although they possess remarkable antitumor properties, their preparation is multistep, low yielding, and requires extensive product purification. This chapter addresses these logistical concerns using a synthetically improved route for preparing a distamycin derivative, which requires limited intermediate purifications.

Reaction

Eq. 1





i- Thionyl chloride, toluene
ii- Hydrochloric acid, palladium on carbon, dioxane, water, hydrogen
iii- Sodium bicarbonate, dioxane, water
iv- Thionyl chloride, DMF, CH_2Cl_2
v- 2-Aminoethylguanidine•2HCl, sodium bicarbonate, water, palladium on carbon, hydrochloric acid
vi- Dioxane, water, sodium bicarbonate, palladium on carbon, hydrochloric acid
vii- Palladium on carbon, hydrochloric acid, water, dioxane, hydrogen, acetone
viii- 2-Bromoacrylic acid, *N′*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrogen chloride, sodium bicarbonate, palladium on carbon, hydrochloric acid

Experimental

1. **Preparation of *N*-methyl-4-nitropyrrole-2-carboxyxlic acid chloride**

   Thionyl chloride (3.16 mol) was added to a solution of *N*-methyl-4-nitropyrrole-2-carboxylic acid (1.17 mol) dissolved in 1500 ml toluene, then stirred 3 hours at 100°C, and concentrated. A suspension formed which was stirred 2 hours with cyclohexane at ambient temperature. The mixture was then filtered, dried, and the product isolated in 96% yield. The product was used without further purification.

2. **Preparation of *N*-methyl-4-aminopyrrole-2-carboxylic acid**

   N-Methyl-4-nitropyrrole-2-carboxylic acid (0.58 mol) dissolved in 350 ml was treated with 2 M HCl, Pd/C (5 g) and 1200 ml dioxane/water, 2:1, then hydrogenated 4 hours at ambient temperature. The catalyst was then removed by filtration and the mixture

concentrated. The suspension was filtered and the crude product isolated in 92% yield. The product was used directly without further purification.

3. **Preparation of *N*-methyl-4-[(*N′*-methyl-4-nitro-pyrrolyl-2yl)carbonylamino]-pyrrole-2-carboxylic acid**

   The Step 1 product dissolved in 350 ml dioxane was treated with a mixture of the Step 2 product (0.56 mol) and $NaHCO_3$ dissolved in 300 ml dioxane/water, 1:1, and the mixture stirred 1 hour at ambient temperature. The solution was diluted with 100 ml water and the pH raised to 3 using 2 M HCl. The solution was concentrated and the product isolated in 90% yield. The material was used directly without further purification.

4. **Preparation of *N*-methyl-4-[(*N′*-methyl-4-nitro-pyrrolyl-2-yl)carbonylamino]-pyrrole-carboxylic acid chloride**

   The Step 3 product (0.45 mol) dissolved in 3 ml DMF was treated with thionyl chloride (2.3 mol) dissolved in 2500 ml CH_2Cl_2, then refluxed 6 hours, cooled, and filtered. The crude product was washed with 1000 ml hexane, filtered, dried, the product isolated in 90% yield. The material was used directly without further purification.

5. **Preparation of *N*-methyl-4-[(*N′*-methyl-4-nitro-pyrrolyl-2-yl)carbonylamino]-pyrrole-2-carboxylic acid chloride ethylguanidine hydrochloride**

   The Step 4 product (0.057 mol) was added to a solution of 300 ml dioxane containing 2-aminoethylguanidine•2HCl (0.057 mol) and $NaHCO_3$ (0.17 mol) dissolved in 100 ml water, then stirred 2 hours at ambient temperature. It was then treated with 60 ml 2 M HCl and Pd/C (3 g), then hydrogenated 3 hours at ambient temperature. The mixture was filtered and then treated with the Step 3 product (0.057 mol) dissolved in 120 ml dioxane containing $NaHCO_3$ (0.17 mol) and stirred 4 hours at room temperature. It was then concentrated, filtered, the residue washed with acetone, refiltered, dried, and the product isolated in 85% yield.

6. **Preparation of 2-[1-methyl-4-[1-methyl-4-[1-methyl-4-(1-methyl-aminopyrrole-2-carboxamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine hydrochloride**

   A mixture consisting of the entire Step 5 product and Step 4 product (0.057 mol) was dissolved in 120 ml dioxane containing $NaHCO_3$ (0.17 mol) and the suspension stirred 4 hours at ambient temperature. The mixture was then concentrated until a suspension was obtained and then filtered, then dried, and the product isolated in 85% yield.

7. **Preparation of 2-[1-methyl-4-[1-methyl-4-[1-methyl-4-(1-methyl-aminopyrrole-2-carboxamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine hydrochloride**

   A mixture consisting of the Step 6 product (0.067 mol), 550 ml dioxane, 450 ml water, 120 ml 2 M HCl, and Pd/C (13 g) was hydrogenated 3 hours at ambient temperature, then filtered. The solution was then treated with 1300 ml acetone cooled overnight at 4°C, then filtered, and the product isolated in 90% yield.

8. **Preparation of *N*-(5-{[(5-{[(5-{[(2-{[amino(imino)methyl]amino}ethyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-carbonyl-1-methyl-1H-pyrrole-2-carboxamide hydrochloride**

   A vessel containing bromoacrylic acid (7.56 mmol), *N′*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide HCl (7.56 mmol), $NaHCO_3$ (14.3 mmol), 60 ml dioxane, and the Step 7 product (2 mmol) dissolved in 30 ml dioxane/water, 2:1, was stirred 1 hour at ambient temperature. The solution pH was then lowered to 4.5 using 2 M HCl and the mixture concentrated. The suspension was filtered, the crude material dried, and product isolated in 85% yield, HPLC area > 98%.

Derivatives

Only the Step 8 product was prepared.

Steps 1–8 product conversions

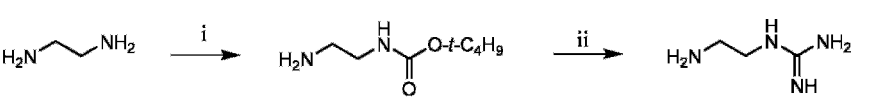
Table 1 Reaction conversions for Steps 1–8 for products isolated prior to purification

Reaction Step	Yield (%)
1	96
2	92
3	90
4	90
5	85
6	85
7	90
8	85

Notes

1. The Step 5 co-reagent, 2-aminoethylguanidine•2HCl, (**I**), was prepared by the author as illustrated in Eq. 2.

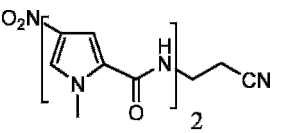
Eq. 2



(**I**)

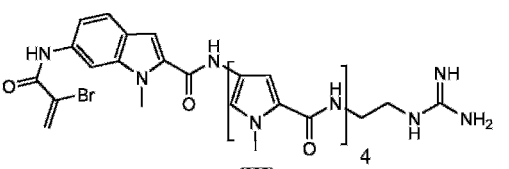
i- Dioxane, di-*t*-butyl-dicarbonate
ii- *O*-Methylisourea hydrogensulfate, triethylamine

2. An alternative method for preparing the Step 5 product is described by Cozzi (1), which uses Pinner reaction conditions with *N*-methyl-4-[(*N′*-methyl-4-nitro-pyrrolyl-2-yl)carbonylamino]-pyrrole-2-carboxylic acid-2-cyanoethylene, (**II**).

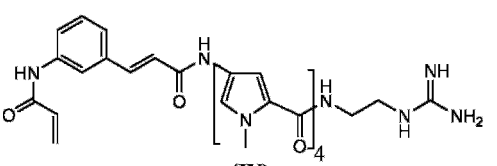


(II)

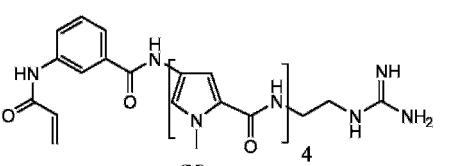
3. Distamycin derivatives containing benzoheterocyclic, (**III**), and cinnamoyl components, (**IV**), prepared by Cozzi (2,3), respectively, and amidophenyl derivatives, (**V**), prepared by Beria (4) were isolated in only moderate yields.



(III)



(IV)



(V)

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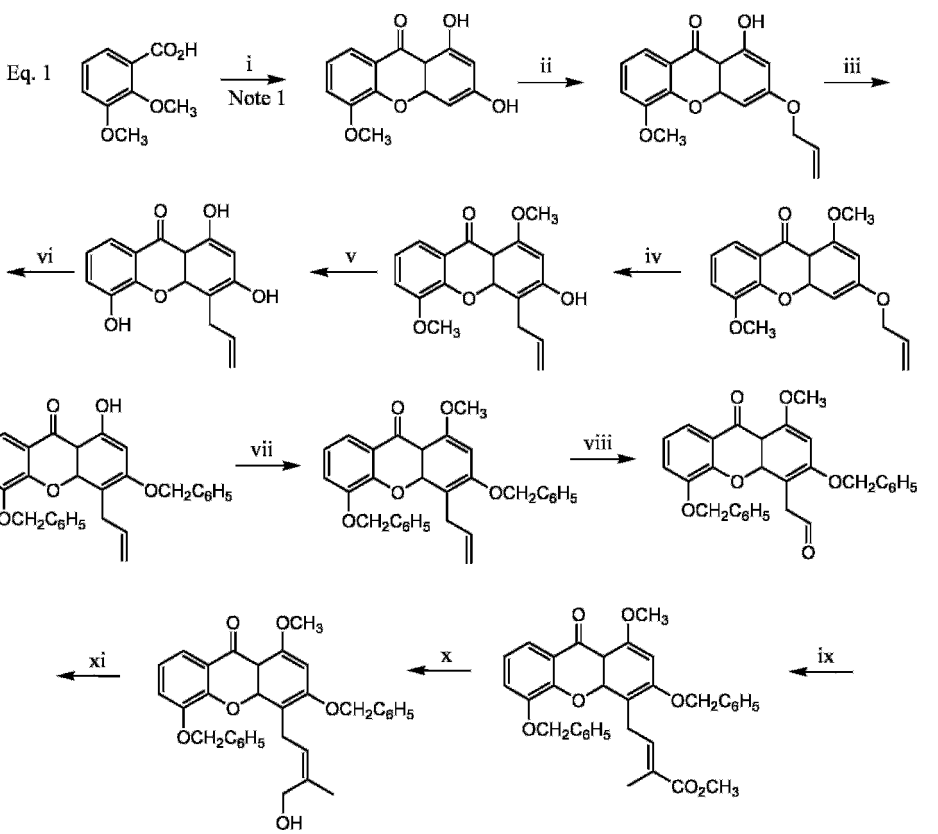
1. P. Cozzi *et al.*, US Patent 6,482,920 (November 19, 2002)
2. P. Cozzi *et al.*, US Patent 6,153,642 (November 28, 2000)
3. P. Cozzi *et al.*, US Patent 6,596,845 (July 22, 2003)
4. I. Beria *et al.*, US Patent 5,753,629 (May 19, 1998)

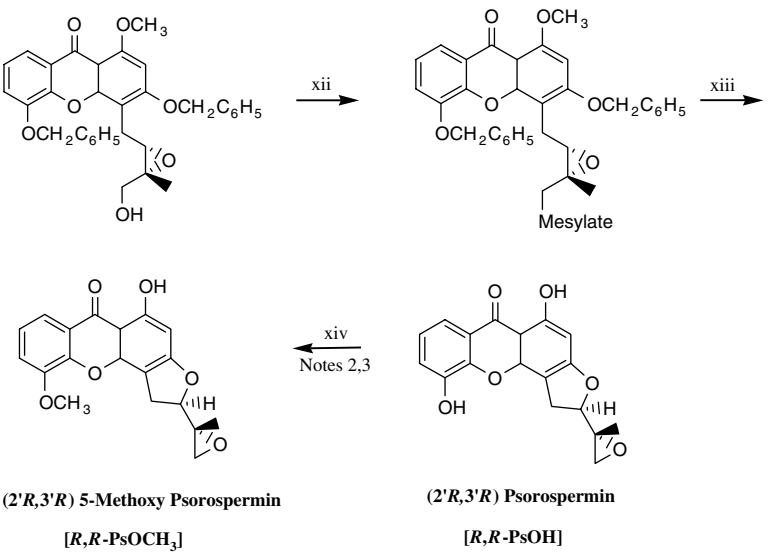
B. Psorospermin Derivative

Title Process to Prepare Psorospermin
J.P. Whitten *et al.*, US Patent 6,933,396 (August 23, 2005)
Assignee Cylene Pharmaceuticals
Utility Antiprolification Agents

Invention Significance Methods for synthesizing the cytotoxic agent psorospermin, enantiomers, and derivatives without stereoisomer separation are described. From this process it was determined that (2′*R*,3′*R*) 5-methoxy psorospermin is especially effective for the treatment of hepatoma, lymphomas, and other proliferative disorders.

Reaction





i- Zinc chloride, phosphorus oxychloride, phlorglucinol
ii- Potassium carbonate, allyl bromide
iii- Potassium carbonate, methyl iodide
iv- Mesytlene
v- Boron tribromide, CH_2Cl_2
vi- Potassium carbonate, DMF, benzyl bromide
vii- Sodium hydride, DMF, methyl iodide
viii- Dioxane, osmium tetroxide, sodium periodate
ix- Methyl methacrylate phosphonate, 18-crown-6, THF, potassium hexamethyldisilazide
x- CH_2Cl_2, diisobutyl aluminum hydride
xi- CH_2Cl_2, (–) diisopropyl tartrate, titanium isopropoxide, *t*-butyl hydroperoxide, 3 Å molecular sieves
xii- CH_2Cl_2, methanesulfonyl chloride, triethylamine
xiii- EtOAc, ethyl alcohol, potassium carbonate, Raney nickel
xiv- Methyl iodide, potassium carbonate

Experimental

1. **Preparation of 5-methoxy-1,3-dihydroxy-xanthone**

Zinc chloride (1.47 mol) and phosphorus oxychloride (7.71 mol) were mixed and heated 30 minutes at 50°C, then treated with 2,3-dimethoxybenzoic acid (1.37 mol). The mixture was stirred 1 hour, then treated with phlorglucinol (1.58 mol), stirred an additional hour, and cooled to ambient temperature. The reaction contents were then poured into 10 l ice water and stirred 20 minutes. The aqueous layer

was decanted from the red solid and replaced with 3 l water, then stirred 5 minutes, and the solid isolated. It was dissolved in 1 M NaOH at 50°C, then neutralized with 1 M HCl. The solid was finally dried and the product isolated in 76% yield.

2. **Preparation of 5-methoxy-3-allyloxy-1-hydroxy-xanthone**

The Step 1 product (0.52 mol) and K_2CO_3 (1.05 mol) dissolved in 1 l acetone were treated with allyl bromide (0.59 mol), then refluxed 60 hours, and concentrated. The residue was extracted with 1.5 l EtOAc and residual K_2CO_3 removed using dilute sulfuric acid. The organic layer was dried over $MgSO_4$, then filtered, and concentrated in vacuo. The residue was redissolved in 1 l acetone, filtered through silica gel, concentrated, and 150 g of product isolated as a pale yellow solid.

3. **Preparation of 5-methoxy-3-allyloxy-1-methoxy-xanthone**

The Step 2 product (0.5 mol) dissolved in 500 ml DMF was treated with K_2CO_3 (0.72 mol) and methyl iodide (1.28 mol), then heated 1 hour at 80°C, and then cooled. The solution was diluted with 2 l EtOAc and residual K_2CO_3 removed using dilute sulfuric acid. The mixture was dried with $MgSO_4$, then concentrated, and the product isolated in 77% yield as a pale yellow solid.

4. **Preparation of 1,5-dimethoxy-4-allyl-xanthone**

The Step 3 product (0.38 mol) was refluxed in 300 ml mesytlene 36 hours, cooled, filtered, washed with 300 ml diethyl ether, and the product isolated in 42% yield as a white solid.

5. **Preparation of 1,3,5-trihydroxy-4-allyl-xanthone**

The Step 4 produ‘ct (186 mmol) suspended in 200 ml CH_2Cl_2 at −20°C was treated with BBr_3 (650 mol), then stirred 1.5 hours at ambient temperature, then poured over ice. The solid was isolated by filtration, then dried, and the product obtained in 91% yield as a white solid.

6. **Preparation of 3,5-dibenzyloxy-1-hydroxy-4-allyl-xanthone**

The Step 5 product (68 mmol) dissolved in DMF was treated with K_2CO_3 (336 mmol) and benzyl bromide (526 mmol), then heated to 110°C for 8 hours, and then cooled. The mixture was diluted with 1.4 l EtOAc and residual K_2CO_3 removed using 2 M sulfuric acid. The organic layer was washed twice with 500 ml brine, dried, concentrated, and the product isolated in 81% yield as a solid.

7. **Preparation of 3,5-dibenzyloxy-1-methoxy-4-allyl-xanthone**

The Step 6 product (135 mmol) dissolved in 300 ml DMF was treated portionwise with NaH (60% in oil, 225 mmol) at ambient temperature followed by CH_3I (1.28 mol), then heated to 50°C for 90 minutes. The mixture was chilled in an ice bath and treated with 20 ml methyl alcohol to remove excess NaH. The mixture was diluted with 1.2 l EtOAc, then washed three times with 500 ml brine, dried,

filtered through silica gel, concentrated, and the product isolated in 85% yield as a solid.

8. **Preparation of 3,5-dibenzyloxy-1-methoxy-xanthone-4-aldehyde**

The Step 7 product (113 mmol) dissolved in 600 ml dioxane was treated with 200 ml water and 4 ml 4% aqueous solution of osmium tetroxide, then stirred 5 minutes, and treated with sodium periodate (469 mmol). The mixture was heated 3 hours at 35°C, then cooled to ambient temperature, and diluted with 1 l EtOAc. The solution was then washed with water, dried, and concentrated. The residue was recrystallized using THF and 15 g of product was isolated. The remaining filtrate was purified by chromatography with silica gel and EtOAc/hexanes, 1:1, so that a total product yield of 44% was observed.

9. **Preparation of *cis*-3,5-dibenzoxy-1-methoxy-4-[4-(2-methoxycarbonyl-2-butene]-xanthone**

Methyl methacrylate phosphonate (24 mmol) and 18-crown-6 (57 mmol) dissolved in 300 ml THF were cooled to −78°C, then treated with 50 ml 0.5 M in toluene potassium hexamethyldisilazide, and stirred 30 minutes. The Step 8 product was added as powder to this mixture and stirred 6 hours at −78°C and then overnight at ambient temperature. The mixture was then diluted with 1.2l EtOAc, washed four times with 500 ml water, dried, and concentrated. The residue consisted of a mixture of 10 g of *E/Z*, 1:10, respectively, isomer mixture, and the *Z*-isomer isolated after recrystallization in EtOAc.

10. **Preparation of allylic alcohol**

The Step 9 product (9.1 mmol) dissolved in 200 ml CH_2Cl_2 at −78°C was treated dropwise with 18.2 ml 1.0 M in CH_2Cl_2 DiBALH, then stirred 30 minutes, quenched with 1 M HCl, and then warmed to ambient temperature. The solution was extracted using CH_2Cl_2, then, dried and concentrated. The residue was purified by chromatography with silica gel using EtOAc/hexanes, 1:1, and 3.86 g product isolated as a white solid.

11. **Preparation of epoxy alcohol**

A mixture consisting of 3 Å molecular sieves (2.0 g) in 60 ml CH_2Cl_2 and (−) diisopropyl tartrate (8.87 mmol) was cooled to 0°C, then treated with titanium isopropoxide (8.87 mmol), and stirred 15 minutes. The mixture was further cooled to −78°C and treated with the Step 10 product (7.39 mmol) dissolved in 10 ml CH_2Cl_2 followed by 7.4 ml 5 M *t*-butyl hydroperoxide in decane, then stirred overnight at −25°C. The solution was filtered and then stirred 1 hour with $Na_2S_2O_4$ solution. It was refiltered, then extracted three times with 100 ml CH_2Cl_2, dried using Na_2SO_4, and concentrated. The residue was purified by chromatography using EtOAc/hexanes, 1:1, and the product isolated in 78% as a white solid.

12. **Preparation of mesylate**

The Step 11 product (5.76 mmol) dissolved in 60 ml CH_2Cl_2 was treated with methanesulfonyl chloride (6.34 mmol) at 0°C followed by the dropwise addition of triethylamine (6.9 mmol) and then stirred for 30 minutes. It was then quenched with 1 M HCl and the organic layer isolated. The organic phase was washed with brine, dried, filtered through silica gel, and concentrated. The residue was recrystallized using EtOAc/ethyl alcohol, 1:1, and the product isolated in 95% yield as a white solid.

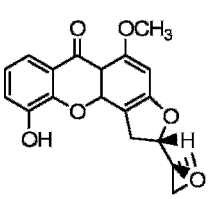
13. **Preparation of (2′*R*,3′*R*) Psorospermin**

The Step 12 product (1.6 mmol) was dissolved in 60 ml apiece of EtOAc and ethyl alcohol containing K_2CO_3 (2.17 mol), then treated with 0.5 ml slurry Raney nickel, and heated to 60°C while monitoring the extent of the reaction using TLC. The reaction catalyst was removed by filtration and the solution concentrated. The residue was purified by chromatography using methyl alcohol/CH_2Cl_2, 5:95, and the product isolated in 70% yield as a white solid.

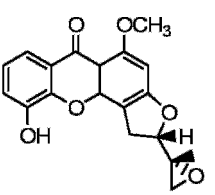
14. **Preparation of (2′*R*,3′*R*) 5-Methoxy Psorospermin**

The Step 13 product (10 mg) was dissolved in 10 ml acetone and refluxed with 0.05 ml methyl iodide and K_2CO_3 for 30 minutes. The mixture was concentrated and the product isolated.

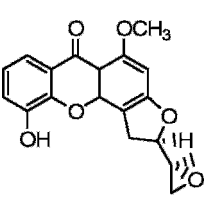
Derivatives



(2'*S*,3'*S*)



(2'*S*,3'*R*)



(2'*R*,3'*S*)

Testing

I. *Methionine Synthase Testing*

MTS cytotoxicity data were collected for a comparison of (*R,R*)-psorospermin with (*R,R*)-5-methoxy-psorospermin, (*R,S*)-5-methoxy-psorospermin, (*S,R*)-5-methoxy-psorospermin, and (*S,S*)-5-methoxy-psorospermin in cell viability assays with various tumor cell lines. Test results are provided in Table 1.

Table 1 IC_{50} MTS cytotoxicity data with experimental agents in cell viability assays with various tumor cell lines

Tumor Cell Line	*R,R*-PsOH (μM)	*R,R*-PsOMethyl (μM)	*R,S*-PsOMethyl (μM)	*S,R*-PsOMethyl (μM)	*S,S*-PsOMethyl (μM)
8226		0.072	0.345	0.221	0.494
MIA PaCa-2		0.1	0.36	0.38	1.18
HT-29	0.5	0.178	1.21	0.319	1.79
MDA-MB-468	0.4	0.181	1.29	0.405	1.73
DU 145		0.23	0.67	0.57	2.51
H522	0.5	0.38	1.35	0.77	2.77
HeLa		0.39	2.5	3.2	4.45

II. *Blood Plasma Level Determination*

Blood plasma levels of psorospermin were measured over 24 hours after a 5 mg/kg iv injection in Sprague–Dawley rats and are summarized in Table 2.

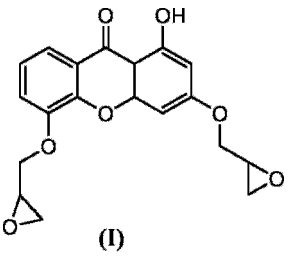
Table 2 Primary pharmacokinetics testing of psorospermin using Sprague–Dawley rats

Time (hours)	Plasma Concentration Study 1 (ng/ml)	Plasma Concentration Study 2 (ng/ml)	Plasma Concentration Study 3 (ng/ml)
0.5	6.91	8.02	23.1
1.0	BQL[a]	2.30	BQL
2.0	BQL[a]	BQL	BQL
4.0	BQL[a]	BQL	BQL
24.0	BQL[a]	BQL	BQL

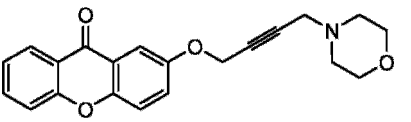
[a]BQL, below the quantifiable limit.

Notes

1. Xanthone epoxides, (**I**), and 4-morpholinobut-2-yn derivatives, (**II**), prepared by Lin (1) and Bombardelli (2), respectively, were effective as antiproliferative agents and in containing the spread of abnormal cell growth.

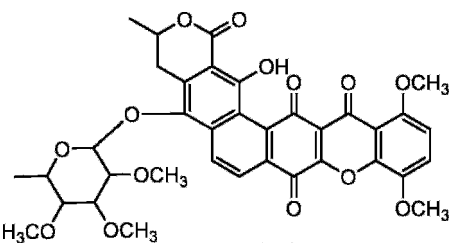


(I)



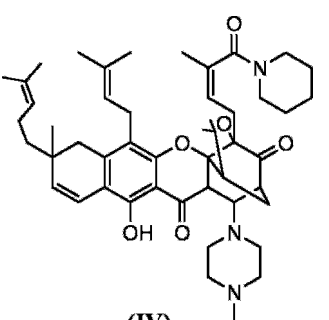
(II)

2. Canedo (3) identified polycyclic xanthone derivatives, (**III**), a novel class of antitumor compounds isolated from a new marine microbe strain PO13-046 belonging to the genus *Actinomadura* sp., which has activity against several cancel cell lines.



(III)

3. Gambogic derivatives, (**IV**), prepared by Cai (4) were also effective as antiproliferatives and used in the treatment of Hodgkin's disease and non-Hodgkin's lymphomas.



(IV)

References

1. C.-N. Lin *et al.*, US Patent 5,981,774 (November 4, 1999) and US Patent 5,741,813 (April 11, 1998)
2. E. Bombardelli *et al.*, US Patent 6,608,089 (August 19, 2003)
3. L.M. Canedo *et al.*, US Patent 6,812,245 (November 2, 2004)
4. S.X. Cai *et al.*, US Patent 6,613,762 (September 2, 2003)

II. Macular Degeneration Treatment Agent

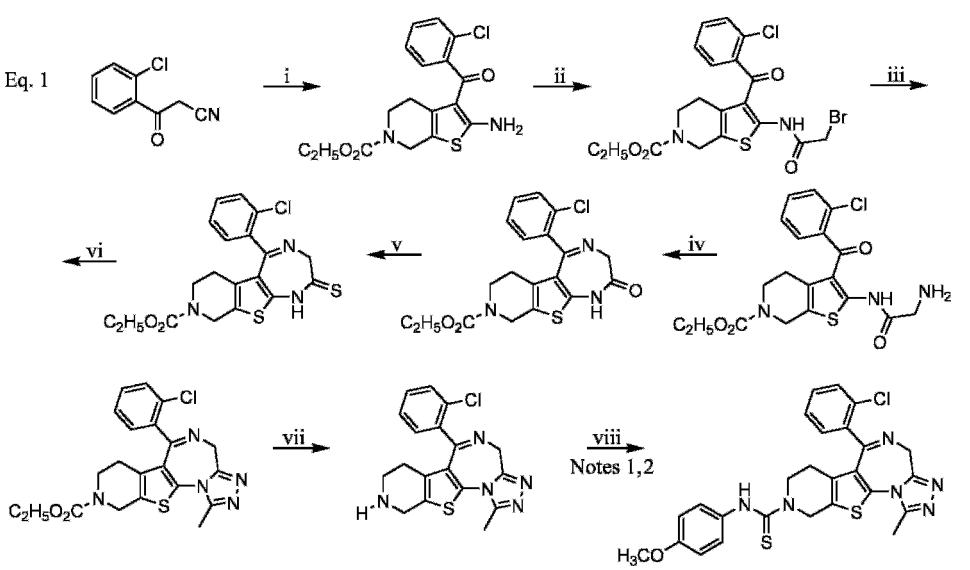
Title Synthesis and Use of Thienotriazolodiazepines
N.G. Bazan *et al.*, US Patent 6,987,105 (January 17, 2006)

Assignee Board of Supervisors of Louisiana State University And Agricultural and Mechanical College (Baton Rouge, LA)

Utility Treatment of Age-related Macular Degeneration

Invention Significance Age-related macular degeneration involves a complex pathophysiology characterized by photoreceptor cell death and in some cases also pathological neovascularization. Although a thienotriazolodiazpene derivative exists effective as a platelet-activating factor antagonist for treating this disorder, its synthesis is multistep and low yielding. This chapter addresses this logistical concern by providing a more direct method for its preparation and isolation.

Reaction



i- *N*-Ethoxycarbonyl-4-piperidone, sulfur, methyl alcohol
ii- CH_2Cl_2, bromoacetyl bromide
iii- THF, ammonia
iv- Pyridine, acetic acid, toluene

v- Dimethoxyethane, Lawesson reagent
vi- Acetyl hydrazine, dioxane
vii- Methyl alcohol, sodium hydroxide
viii- Toluene, 4-methoxyphenyl isothiocyanate

Experimental

1. **Preparation of 2-amino-3-(2-chlorobenzoyl)-6-ethoxycarbonyl-4,5,6,7-tetrahydropyrido-[3,4-b]thiophene**

   A mixture consisting of 2-chlorobenzoylacetonitrile (0.178 mol), *N*-ethoxycarbonyl-4-piperidone (0.178 mol), sulfur (0.21 mol), and 15.65 ml morpholine in 190 ml methyl alcohol was refluxed 2 hours, then cooled. The precipitate that formed was filtered, then washed with diethyl ether, and 43 g product isolated as a yellow solid. The filtrate was then concentrated and the residue purified by chromatography with silica gel using hexane/EtOAc, 4:1. The solid was recrystallized in methyl alcohol and the product isolated in 81% yield as pale yellow prisms, mp = 194−195°C.

   IR (KBr) (cm^{-1}) 3259, 2983, 1679, 1578, 1432, 1298, 1270, 1233, 1117
   ^{1}H NMR (300 MHz, $CDCl_3$) δ (pp.) 7.42–7.20 (m, 4H), 4.37 (bt, 2H, $J = 2.0$ Hz), 4.13 (q, 2H, $J = 7.1$ Hz); 3.41 (bt, 2H, $J = 5.8$ Hz), 1.80–1.74 (m, 2H), 1.25 (t, 3H, $J = 7.1$ Hz)
   ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) 188.9, 174.2, 167.0, 155.3, 141.2, 130.3, 130.1, 129.7, 127.7, 126.9, 114.3, 112.9, 61.6, 42.6, 40.8, 25.9, 14.6
   Analysis Calc. for $C_{17}H_{17}ClN_2O_3S$ (364.85 g/mol): C (%), 55.96; H (%), 4.70; N (%), 7.68. Found: C (%), 55.6; H (%), 4.64; N (%), 7.56

2. **Preparation of 2-bromoacetamido-3-(2-chlorobenzoyl)-6-ethoxycarbonyl-4,5,6,7-tetrahydropyrido[3,4-b]thiophene**

   The Step 1 product (0.137 mol) dissolved in 800 ml CH_2Cl_2 at 0°C was treated with the dropwise addition of 13.25 ml bromoacetyl bromide and the mixture stirred overnight at ambient temperature. The mixture was poured over 500 ml ice water, then extracted with CH_2Cl_2, dried over Na_2SO_4, and concentrated. The residue was poured into cold ethyl alcohol/diethyl ether and a yellow solid precipitate was isolated. The solid was washed with a mixture of ethyl alcohol/diethyl ether and 48 g product was isolated. The filtrate was further concentrated and the residual oil purified by chromatography in a silica gel using CH_2Cl_2 where an additional 12 g product was isolated. After recrystallization in ethyl alcohol, the product yield was isolated in 90% yield as a pale yellow solid, mp = 84−86°C.

   IR (KBr) (cm^{-1}): 2927, 1707, 1693, 1680, 1622, 1520, 1431, 1231
   ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 12.88 (bs, 1H, NH), 7.45–7.28 (m, 4H), 4.56–4.53 (m, 2H), 4.15 (q, 2H, $J = 7.0$ Hz), 4.13 (s, 2H), 3.46 (bt, 2H, $J = 5.7$ Hz), 1.91–1.87 (m, 2H), 1.26 (t, 3H, $J = 7.0$ Hz)

Analysis Calc. for $C_{19}H_{18}BrClN_2O_4S$ (485.79 g/mol): C (%), 46.98; H (%), 3.73; N (%), 5.77. Found: C (%), 46.92; H (%), 3.84; N (%), 5.64

3. **Preparation of 3-aminoacetamido-3-(2-chlorobenzoyl)-6-ethoxycarbonyl-4,5,6,7-tetrahydropyrido[3,4-b]thiophene**

The Step 2 product (0.103 mol) dissolved in 800 ml THF at 0°C was treated with NH_3 gas for 12 hours, then concentrated, and diluted with 500 ml EtOAc. The organic layer was washed once with water, three times with 100 ml brine, dried, and concentrated. The residue was purified by chromatography using EtOAc and the product isolated in 83% yield after recrystallization in ethyl alcohol as a white-yellow solid, mp = 95−97°C.

IR (KBr) (cm^{-1}) 3330, 2935, 1692, 1618, 1501, 1434, 1232, 1082
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 13.07 (bs, 1H),7.42–7.31 (m, 4H), 4.54–4.51 (m, 2H), 4.14 (q, 2H, $J = 6.9$ Hz), 3.66 (s, 2H), 3.44 (bt, 2H, $J = 5.8$ Hz), 1.90–1.87 (m, 2H), 1.25 (t, 3H, $J = 6.9$ Hz)
^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) 191.2, 178.5, 171.9, 155.3, 150.6, 140.5, 131.0, 130.2, 130.0, 127.7, 127.1, 120.3, 112.9, 61.6, 44.8, 42.6, 40.8, 25.5, 14.6
Analysis alc. for $C_{19}H_{20}ClN_3O_4S$ (421.91 g/mol): C (%), 54.09; H (%), 4.78; N (%), 9.96. Found: C (%), 54.38; H (%), 4.91; N (%), 9.22

4. **Preparation of 5-(2-chlorophenyl)-8-ethtoxycarbonyl-6,7,8,9-tetrahydro-3H-pyrido[4′,3′:4,5] thieno[3,2-f]-1,4-diazepine-2-one**

A solution of the Step 3 product (0.071 mol) in a mixture of 93 ml pyridine, 30 ml acetic acid, and 560 ml toluene was refluxed with a Dean–Stark apparatus for 5 hours, then concentrated. The residue was purified by chromatography using hexane/EtOAc, 1:1, and the product isolated in 78% after recrystallization in acetonitrile as a white solid, mp = 209−210°C.

^{1}H NMR (300 MHz, $CDCI_3$) δ (ppm) 7.50–7.34 (m, 4H), 4.53 (s, 2H), 4.41–4.38 (m, 2H), 4.14 (q, 2H, $J = 7.2$ Hz), 3.48–3.44 (m, 2H), 1.83–1.78 (m, 2H), 1.24 (t, 3H, $J = 7.2$ Hz)
MS (*m/z*, relative intensity) 405 (8), 403 (M^+, 19), 376 (36), 374 (100), 348 (9), 346 (23), 302 (9), 239 (14)
Analysis Calc. for $C_{19}H_{18}ClN_3O_3S$ (403.89 g/mol): C (%), 56.50; H (%), 4.49; 10.40. Found: C (%), 56.23; H (%), 4.55; N (%), 10.17

5. **Preparation of 5-(2-chlorophenyl)-8-ethoxycarbonyl-6,7,8,9-tetrahydro-3H-pyrido[4′,3′:4,5]-thieno[3,2-f]-1,4-diazepine-2-thione**

A solution of the Step 4 product (0.052 mol) in 175 ml dimethoxyethane at 90°C was treated with the Lawesson reagent (0.031 mol), then stirred 2 hours, and cooled. The solution was concentrated and the residue purified by chromatography using CH_2Cl_2/acetone, 9.5:0.5 and then 9:1. The product was isolated in 78% yield as a white yellowish solid after recrystallization using acetonitrile, mp = 236−237°C.

IR (KBr) (cm^{-1}) 3155, 2982, 1692, 1593, 1564, 1481, 1433, 1352, 1233
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 11.00 (bs., 1H), 7.42–7.32 (m, 4H), 4.80–4.76 (m, 2H), 4.54 (s, 2H), 4.15 (q, 2H, $J = 7.1$ Hz), 3.47–3.44 (m, 2H), 1.83–1.80 (m, 2H), 1.26 (t, 3H, $J = 7.1$ Hz)
^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) 196.2, 165.4, 155.3, 144.7, 137.1, 133.0, 131.2, 131.1, 130.0, 128.0, 127.1, 126.0, 64.4, 61.9, 42.6, 40.6, 25.3, 14.6
MS (*m/z*, relative intensity) 419 (M^+, 29), 392 (42), 391 (22), 390 (100), 346 (15), 283 (13), 237 (5), 149 (8)
Analysis Calc. for $C_{19}H_{18}ClN_3O_2S_2$ (419.96 g/mol): C (%), 54.34; H (%), 4.32; N (%), 10.01. Found: C (%), 54.57; H (%), 4.33; N (%), 9.84

6. **Preparation of 6-(2-chlorophenyl)-9-ethoxycarbonyl-1-methyl-7,8,9,10-tetrahydro-4H-pyrido[4′,3′:4,5]thieno[3,2-f]-1,2,4-triazolo[4,3-a]-1,4-diazepine**

A mixture of the Step 5 product (0.0429 mol) and acetyl hydrazine (0.051 mol) dissolved in 220 ml of dioxane was heated 8 hours at 130°C, then concentrated. The residue was purified by chromatography using CH_2Cl_2/methyl alcohol, 9.5:0.5, and the product isolated in 74% yield as yellowish prisms after recrystallization from acetonitrile, mp = 234−235°C.

IR (KBr) (cm^{-1}) 2982, 1698, 1604, 1468, 1414, 1233, 1117
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.42–7.31 (m, 4H), 5.59 (d, 1H, $J = 11.0$ Hz), 4.85 (d, 1H, $J = 16.6$ Hz), 4.44 (d, 1H, $J = 16.6$ Hz), 4.22–4.19 (m, 1H), 4.14 (q, 2H, $J = 7.0$ Hz), 3.90–3.87 (m, 1H), 3.19–3.14 (m, 1H), 2.67 (s, 3H), 2.04–2.01 (m, 1H), 1.79–1.73 (m, 1H), 1.24 (t, 3H, $J = 7.0$ Hz)
^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) 164.5, 155.1, 153.5, 149.5, 137.4, 134.3, 132.5, 131.3, 131.1, 130.9, 130.1, 128.9, 127.2, 112.8, 61.8, 46.9, 42.6, 40.5, 25.3, 14.5, 11.9
MS (*m/z*, relative intensity) 443 (3), 441 (M^+,10), 414 (39), 412 (100), 329 (9), 327 (21), 302 (5), 300 (15), 264 (17), 237 (19).
Analysis Calc. for $C_{21}H_{20}ClN_5O_2S$ (441.94 g/mol): C (%), 57.07; H (%), 4.56; N (%), 15.85. Found: C (%), 56.96; H (%), 4.61; N (%), 15.63

7. **Preparation of 6-(2-chlorophenyl)-1-methyl-7,8,9,10-terahydro-4H-pyrido[4′3′:4,5]thieno-[3,2-f]-1,2,4-triazolo[4,3-a]-1,4-diazepine**

A solution the Step 6 product (0.0317 mol) dissolved in 100 ml methyl alcohol was treated with 90 ml 4 M NaOH, then heated at 90°C overnight. The mixture was diluted with brine, then extracted three times with 100 ml EtOAc, dried, and concentrated. The residue was recrystallized in acetone and the product isolated in 92% yield as a white solid, mp = 196−198°C.

IR (KBr) (cm^{-1}) 2932, 1603, 1542, 1496, 1415, 1379, 1324, 1084, 1034
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.44–7.29 (m, 4H), 5.57 (d, 1H, $J = 12.3$ Hz), 4.19 (d, 1H, $J = 12.3$ Hz), 4.01 (s, 2H), 3.00–2.97 (m, 1H), 2.82–2.80 (m, 1H), 2.67 (s, 3H), 2.02–1.99 (m, 1H), 1.65–1.62 (m, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) 164.8, 153.6, 149.5, 137.7, 132.5, 131.7, 131.5, 130.9, 130.8, 129.4, 127.1, 112.8, 46.9, 44.3, 42.6, 26.3, 12.0
MS (*m/z*, relative intensity) 371 (24), 369 (M^+, 66), 340 (12), 306 (20), 305 (100), 265 (25), 264 (85), 237 (41), 235 (33), 137 (58)
Analysis Calc. for $C_{18}H_{16}ClN_5S$ (369.88 g/mol): C (%), 58.45; H (%), 4.36; N (%), 18.93. Found: C (%), 58.55; H (%), 4.04; N (%), 18.64

8. **Preparation of (6-(2-chlorophenyl)-9-[(4-methoxyphenyl)thiocarbamoyl]-1-methyl-7,8,9,10-tetrahydro-4H-pyrido[4′,3′:4,5]thieno[3,2-f]-1,2,4-triazolo[4,3-a]-1,4-diazepine**

A suspension of the Step 7 product (0.0135 mol) in 60 ml toluene was treated with the dropwise addition of 4-methoxyphenyl isothiocyanate (13.5 mmol) and the mixture stirred 3 hours at ambient temperature. A white precipitate that formed was removed by filtration and washed with toluene and diethyl ether. The solid was recrystallized from acetone and the product isolated in 79% yield as a white solid, mp = 184–187°C.

IR (KBr) (cm^{-1}) 3232, 1603, 1511, 1415, 1241, 1206, 1033
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.53 (bs, 1H, NH), 7.44–7.28 (m, 4H), 7.07 (d, 2H, $J = 8.8$ Hz), 6.84 (d, 2H, $J = 8.8$ Hz), 5.57 (d, 1H, $J = 13.3$ Hz), 5.24 (d, 1H, $J = 15.3$ Hz), 4.78 (d, 1H, $J = 15.3$ Hz), 4.28–4.18 (m, 2H), 3.80 (s, 3H), 3.60–3.49 (m, 1H), 2.64 (s, 3H), 2.20–2.12 (m, 1H), 1.80–1.70 (m, 1H)
^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) 183.9, 164.4, 157.7, 153.5, 149.6, 137.2, 134.5, 132.6, 132.4, 131.7, 131.3, 130.9, 130.2, 128.8, 128.6, 127.3, 126.5, 114.2, 55.4, 47.8, 46.8, 46.0, 25.1, 11.9
MS (*m/z*, relative intensity) 534 (M^+, 0.3), 409 (6), 397 (20), 369 (100), 193 (24), 165 (88)
Analysis Calc. for $C_{26}H_{23}ClN_6OS_2$ (535.0938 g/mol): C (%), 58.36; H (%),4.33; N (%), 15.71. Found: C (%), 58.45; H (%), 4.40; N (%), 15.69

Derivatives

Only the Step 8 derivative was prepared.

Testing

I. *Neuroprotective Effect on Bright Light-Treated Photoreceptors*

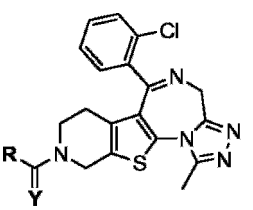
Sprague–Dawley rats (150–175 g males) were dark adapted for 3 days. Two hours before light treatment, rats were injected with either 30 mg/kg of the experimental agent, the vehicle consisting of cyclodextrin alone or with the experimental agent, or received no treatment. Rats were then placed in 5 in. diameter cylindrical tubes and placed within an eight-light array of 10 in. diameter fluorescent lights, 350 $\mu E/m^2$ s, up to 5 hours daily, then sequestered 10 days in darkness, and eyes collected. Testing results are provided in Table 1.

Table 1 Neuroprotective effectiveness using the synthetically improved Step 8 product on rats after exposure to fluorescent lights

Treatment	Photoreceptor Layer
Light exposure	30% Nuclei lost
Light + vehicle	30%
Light + vehicle + Step 8 product	5% Loss

Notes

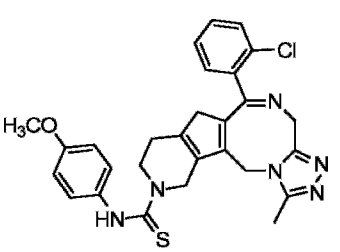
1. Thienotriazolodiazepines derivatives, (**I**), were initially prepared by Braquet (1) in a 11-step synthetic process and were effective as platelet-activating factor inhibitors and used as antiasthmatic, antiallergic, and gastrointestinal protects.



(I)

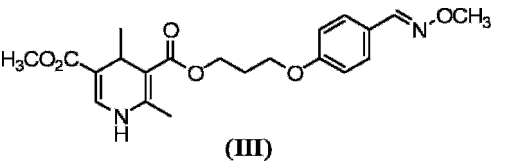
Y	R
S	*i*-Propyl
O	2,3,4-Trimethylphenyl
O,S	4-*t*-Butylphenyl
S	4-*t*-Butoxyphenyl
S	Hexadecyl

2. In an earlier investigation, co-author Hunt (2) prepared the Step 8 1,4-diazepin analog, (**II**), which was effective as an intracellular binding platelet-activating factor antagonist and used in treating in vivo and in vitro tumor growth and angiogenesis.



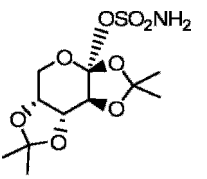
(II)

3. Other platelet-activating factor antagonists consisting of 1,4-dihydropyridine derivatives, (**III**), were prepared by the authors (3) and used in treating neurological diseases.



(III)

4. Chu (4) demonstrated that the sustained use of the antiepileptic drug, Topiramate®, (**IV**), was effective in treating age-related macular degeneration by inducing growth of new optic nerve.



(IV)

References

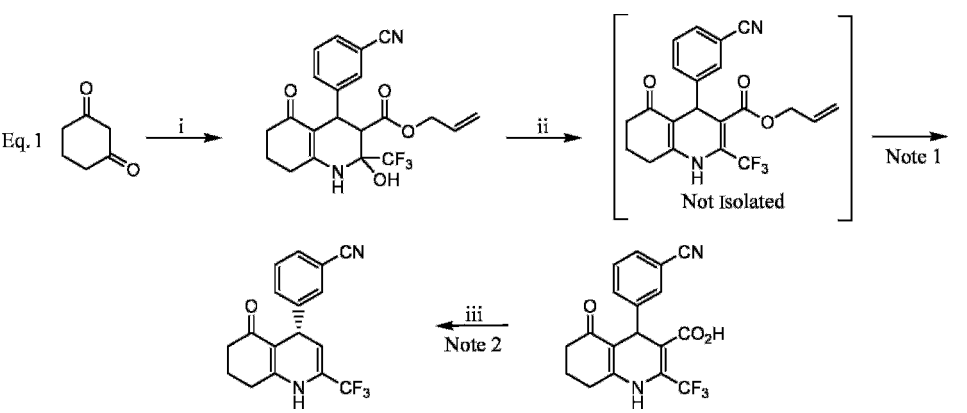
1. P. Braquet *et al.*, US Patent 5,492,906 (February 20, 1996)
2. J. D. Hunt *et al.*, US Patent 6,979,682 (December 27, 2005)
3. N.G. Bazan *et al.*, US Patent 6,566,359 (May 20, 2003)
4. P.-H. Chu *et al.*, US Patent 6,949,518 (September 27, 2005)

III. Urinary Incontinence Treatment Agent

Title Method
A. Jones *et al.*, US Patent 7,012,145 (March 14, 2006)
Assignee Astrazeneca AB
Utility Treatment of Overactive Bladder Disorder Using a K-ATP Channels Opener

Invention Significance Inappropriate smooth muscle activation is associated with urinary incontinence or overactive bladder disorder. A potent K-ATP channel agent effective on the smooth muscles found in the human bladder has been prepared which requires no intermediate purifications to address this disorder.

Reaction



i- 3-Cyanobenzaldehyde, ammonium acetate, ethyl alcohol, allyl 4,4,4-trifluoro-3-oxobutanoate, MTBE
ii- *n*-Butyl acetate, *p*-toluenesulfonic acid, water, ethyl alcohol, acetic acid, chlorotris(triphenylphosphine) rhodium (Wilkinson's catalyst)
iii- (1*S*)-1-Phenylethyl-1-amine, ethyl alcohol, 1-methylpyrrolidone, acetonitrile

Experimental

1. **Preparation of allyl 4-(3-cyanophenyl)-2-hydroxy-5-oxo-2-trifluoromethyl)-1,2,3,4,5,6,7-3-octahydroquinoline-3-carboxylate**

   A slurry of 3-cyanobenzaldehyde and ammonium acetate in ethyl alcohol at ambient temperature was treated sequentially with 1,3-cyclohexanedione dissolved in ethyl

alcohol, allyl 4,4,4-trifluoro-3-oxobutanoate, and ammonium acetate in ethyl alcohol. The mixture was refluxed, then concentrated, and the residue treated with water. At ambient temperature, the mixture was washed with water and MTBE, then concentrated, and the product isolated. The material was used directly without further purification.

2. **Preparation of 4-(3-cyanophenyl)-5-oxo-2-(trifluoromethyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxylic acid**

   The Step 1 product was stirred at 125°C in *n*-butyl acetate with *p*-toluenesulfonic acid, then cooled, filtered, and washed with water. The mixture was then treated with water, ethyl alcohol, chlorotris(triphenylphosphine)rhodium, degassed, and heated for 3–5 hours at 75°C. The racemic acid was extracted with 2 M NaOH, then washed with butyl acetate. It was then treated with methyl alcohol followed by the slow addition of 2 M HCl and the product isolated by filtration. The material was used directly without further purification.

3. **Preparation of 3-[(4*S*)-5-oxo-2-(trifluoromethyl)-1,4,5,6,7,8-hexahydroquinolin-4-yl]-benzonitrile**

   The Step 2 product was co-crystallized with (1*S*)-1-phenylethyl-1-amine from ethyl alcohol, decarboxylated using 1-methylpyrrolidone at 95°C, and the product isolated after being recrystallized from acetonitrile.

Derivatives

No additional derivatives were prepared.

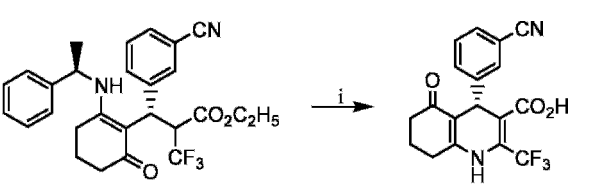
Testing

The effectiveness of the experimental agent in the treatment of incontinence was previously demonstrated by Ohnmacht (1).

Notes

1. The Step 2 product was also prepared by Patel (2) through an α,β-unsaturated ketone intermediate, (**I**), illustrated in Eq. 2.

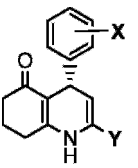
Eq. 2



(**I**)

i- Ammonium hydroxide, acetonitrile, hydrochloric acid, acetonitrile

2. 5-Oxo-1,4,5,6,7,8-hexahydroquinolin derivatives, (**II**), originally prepared by Ohnmacht (2) required extensive chromatographic separations and purification before use.



X = 3-CN, 3-NO_2, 3-CF_3
4-Cl
Y = CH_3, CF_3

(II)

References

1. I. Patel *et al.*, US Patent 6,995,289 (February 7, 2006)
2. C.J. Ohnmacht, Jr., *et al.*, US Patent 5,455,253 (October 3, 1995)

CHAPTER XVII

Incontinence

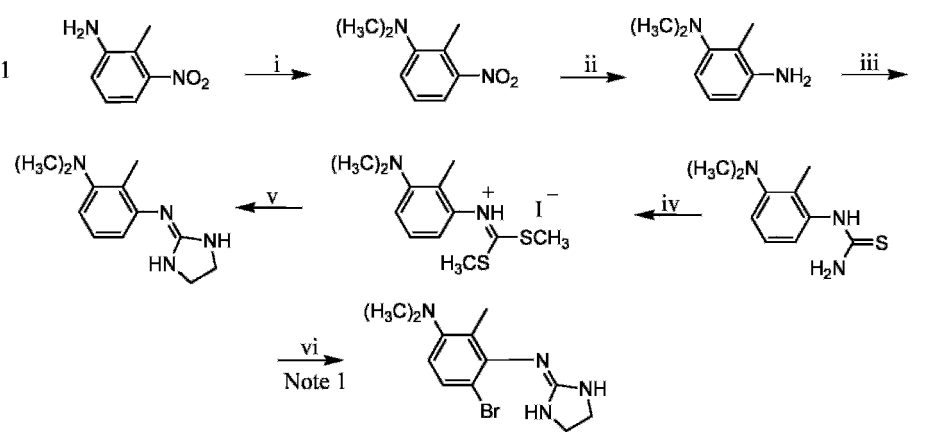
I. α_{1L}-Adrenoreceptor Agonists

Title Compounds and Methods for Treating Urinary Incontinence
F. Esser *et al.*, US Patent 7,019,021 (March 28, 2006)
Assignee Boehringer Ingelheim Pharma GmbH & Co. KG
Utility Treatment of Stress Incontinence in Women

Invention Significance Stress incontinence in women may be caused by pelvic floor weakness, a congenitally short urethra, or reduction in estrogen levels following menopause. α_{1L}-Adrenoreceptor agonists have been prepared that restrict urethra contractions for extended periods with only marginal concomitant blood pressure increase to address this disorder.

Reaction

Eq. 1



i- Water, dimethylsulphate, potassium carbonate
ii- Methyl alcohol, hydrogen, Raney nickel
iii- Acetone, potassium thiocyanate, benzoyl chloride, potassium hydroxide, ethyl alcohol
iv- Methyl alcohol, methyliodide
v- Methyl alcohol, 1,2-diaminoethane
vi- $CHCl_3$, bromine

Experimental

1. **Preparation of *N,N*-dimethyl-2-methyl-3-nitroaniline**

   A mixture of 2-methyl-3-nitroaniline (83.6 g), K_2CO_3 (190 g), and 260 ml water was refluxed and 27 ml dimethylsulphate added dropwise over 1 hour. The mixture was refluxed an additional hour, then cooled, and the top layer removed. The aqueous phase was extracted four times with diethyl ether, then dried with $MgSO_4$, concentrated, and 73 g product isolated.

2. **Preparation of 3-dimethylamino-2-methylaniline**

   The Step 1 product (73 g) was dissolved in 800 ml methyl alcohol, then hydrogenated at 20°C under 5 bar of hydrogen using Raney nickel, and the product isolated.

3. **Preparation of *N*-(3-dimethylamino-2-methylphenyl)-thiourea**

   A mixture consisting of the Step 2 product (57 g), 1150 ml acetone, KSCN (36.6 g), and 43.8 ml benzoyl chloride was refluxed 3 hours, then poured onto 2.4 kg crushed ice. The precipitate was isolated, then treated with KOH (85 g), 85 ml of water, and 255 ml ethyl alcohol, then heated 2 hours at 60°C. The mixture was cooled, then diluted with 850 ml of water, ethyl alcohol distilled off, and 72 g of product isolated.

4. **Preparation of *N*-(3-dimethylamino-2-methylphenyl)-*S*-methyl-isothiourea hydroiodide**

   The Step 3 product (72 g) dissolved in 345 ml methyl alcohol was treated with 22.6 ml of methyliodide, then refluxed 2 hours. The mixture was cooled, concentrated, and 120 g product isolated.

5. **Preparation of 2-(3-dimethylamino-2-methylphenyl-imino)-imidazolidine**

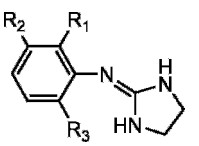
   The Step 4 product (120 g) dissolved in 350 ml methyl alcohol was treated with 34.4 ml of 1,2-diaminoethane, then refluxed 17 hours, and concentrated. The residue was dissolved in water and the pH adjusted to 7 using dilute HCl. The solution was initially extracted three times with EtOAc, then the aqueous phase made alkaline with 5 M NaOH, and the solution re-extracted three times with EtOAc. Extracts were combined, dried with $MgSO_4$, concentrated, and an oil was isolated. The residue was purified by chromatography with silica gel using toluene/dioxane/ethyl alcohol/NH_4OH, 10:8:3:1, and 17.9 g of product isolated, mp = 116−118°C.

6. **Preparation of 2-(6-bromo-3-dimethylamino-2-methylphenylimino)-imidazolidine**

The Step 5 product (6.55 g) dissolved in 75 ml $CHCl_3$ was treated with 1.53 ml bromine, then stirred 2 hours at 0°C, and concentrated. The residue was mixed with dilute HCl and then extracted twice with diethyl ether. The aqueous phase was made alkaline with dilute NaOH and re-extracted three times with diethyl ether. The combined ether extracts were concentrated, the residue purified as described in Step 5, and 3.4 g product isolated as a white powder, mp = 157–158°C.

Derivatives

Table 1 Selected imidazolidine derivatives and their corresponding melting points



Entry	R_1	R_2	R_3	Salt	mp (°C)
1	Methyl	Dimethylamino	Bromo	–	157–158
4	H	N-Acetyl	Chloro	–	236–238
5	Methyl	Phthalimido	H	–	189–190
6	H	Phthalimido	Chloro	–	239–241
10	H	Amino	H	HCl	194–196
13	Methyl	Amino	H	HCl	204–206

Testing

I. *α_{1L}-Adrenoreceptor Agonists Testing*

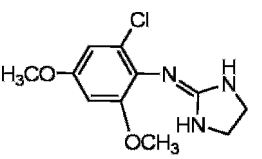
Phenylephrine and entry 1, 30 and 10 μg/kg, respectively, were introduced into the vena femoralis through a polyethylene cannula. Compared with phenylephrine, the experimental agent exhibited a potency factor 2.73 times greater with regard to the contraction of the urethra and with a duration longer by a factor of 4.3. Moreover, the increase in blood pressure associated with entry 1 was only 1.39 times that of phenylephrine. Testing results are provided in Table 2.

Table 2 Effect of entry 1 and reference phenylephrine as α_{1L}-adrenoreceptor agonists in the treatment of stress incontinence in women

Agent	Urethra Contraction	Duration	Blood Pressure Increase	Duration
Phenylephrine	100	100	100	100
Entry 1	273	430	139	117

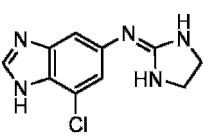
Notes

1. Additional 2-imidazolidine derivatives, (**I**), effective as α_{1L}-adrenoreceptor agonists were prepared by the authors (1) in earlier investigations and used in the treatment of stress incontinence in women.

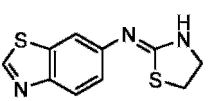


(I)

2. Benzimidazoles, (**II**), and benzothiazole derivatives, (**III**), effective as α_{1L}-adrenoreceptor agonists were prepared by Jeon (2) and used in treating incontinence in women.

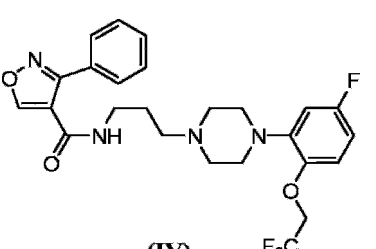


(II)



(III)

3. Isoxazolecarboxamide derivatives, (**III**), effective as α_{1L}-adrenoreceptor agonists were prepared by Leonardi (3) and were effective in treating incontinence as well as voiding problems such as weak stream and incomplete bladder emptying.



(IV)

References

1. F. Esser *et al.*, US Patent 6,858,594 (February 22, 2005); US Patent 6,747,051 (June 8, 2004); and US Patent 6,602,897 (August 5, 2003)
2. Y. Jeon *et al.*, US Patent 6,723,741 (April 20, 2004) and US Patent 6,498,177 (December 24, 2002)
3. A. Leonardi *et al.*, US Patent 6,680,319 (January 20, 2004)

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CHAPTER XVIII

Irritable Bowel Syndrome

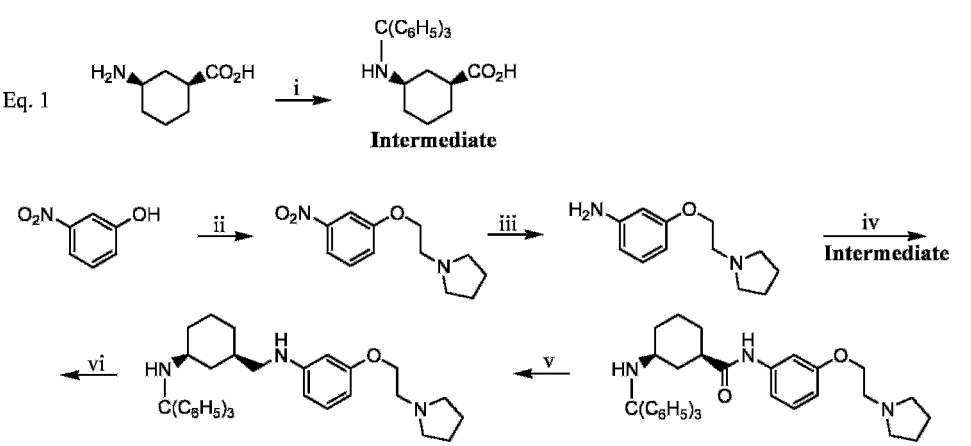
I. Nonpeptidyl Motilin Antagonists

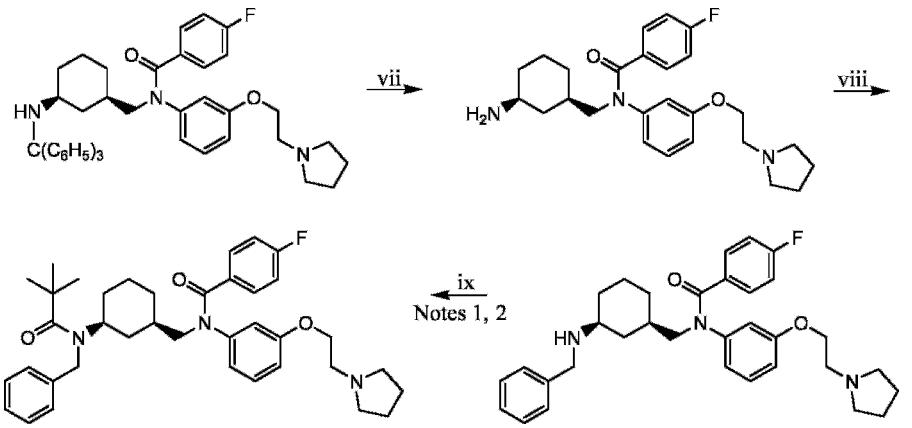
Title Substituted Diamines Derivatives Useful for Motilin Antagonists
S.G Johnson *et al.*, US Patent 6,967,199 (November 22, 2005)
Assignee Ortho-McNeil Pharmaceutical, Inc.
Utility Treatment of Irritable Bowel Syndrome

Invention Significance Motilin is a 22 amino acid peptide which is a component of the gastrointestinal system. Excess motilin, however, is associated with abdominal cramping and diarrhea characteristic of irritable bowel syndrome. Nonpeptidyl motilin antagonists that suppress smooth muscle contractions with limited side effects have been prepared to address this problem.

Reaction

Eq. 1





i- CH_2Cl_2, acetonitrile, triethylamine, chlorotrimethylsilane, triphenylmethyl chloride
ii- DMF, sodium hydride, 1-(2-chloroethyl)pyrrolidine•HCl
iii- Palladium on carbon, EtOAc, hydrogen
iv- Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate, *N*-trityl-*cis*-3-aminocyclohexanecarboxylic acid, diisopropylethylamine
v- Lithium aluminum hydride, THF, potassium sodium tartrate
vi- 4-Fluorobenzoyl chloride, CH_2Cl_2, triethylamine
vii- Trifluoroacetic acid, triethylsilane, CH_2Cl_2
viii- Benzaldehyde, toluene, titanium(IV) isopropoxide
ix- CH_2Cl_2, trimethylacetyl chloride

Experimental

1. **Preparation of *N*-trityl-*cis*-3-aminocyclohexanecarboxylic acid**

A mixture of chlorotrimethylsilane (0.205 mmol) was added to a suspension of *cis*-3-aminocyclohexanecarboxylic acid (0.205 mmol) suspended in 500 ml CH_2Cl_2/acetonitrile, 5:1, and refluxed for 2 hours. Once cooled, triethylamine (0.410 mmol) was added dropwise to the mixture followed immediately by the portionwise addition of triphenylmethyl chloride (0.205 mmol). The mixture was stirred 18 hours and sufficient methyl alcohol was added to dissolve the vessel contents. The solution was concentrated and the residue was partitioned between 800 ml diethyl ether/10% citric acid, 1:1. The ether layer was collected and combined with a 150 ml diethyl ether extraction from the citric acid layer. Combined fractions were extracted three times with 250 ml 2 M NaOH and once with 100 ml water. These layers were washed twice with 150 ml diethyl ether, cooled to 0°C, acidified to pH 7 with 12 M HCl, and re-extracted three times with 200 ml EtOAc. The extract was dried over $MgSO_4$, then concentrated, and the product isolated in 85% yield as a white foam.

MS 384 (M^-)
^{1}H NMR ($CDCl_3$) δ 0.44–0.95 (br m, 3H), 0.97–1.22 (br m, 2H), 1.30–1.48 (br m, 1H), 1.53–1.79 (br m, 2H), 1.8–2.04 (br m, 1H), 2.10–2.29 (br m, 1H), 6.95–7.24 (m, 9H), 7.36–7.59 (m, 6H)

2. **Preparation of 1-(2-(3-nitrophenoxy)ethyl)pyrrolidine**

3-Nitrophenol (23.7 mmol) dissolved in 20 ml DMF was added dropwise to 60% NaH (66.2 mmol) in 30 ml DMF at 0°C and the mixture stirred until hydrogen evolution ceased. This mixture was then treated portionwise with 1-(2-chloroethyl)pyrrolidine•HCl (33.1 mmol), stirred 18 hours at ambient temperature, and quenched with 50 ml 2 M NaOH. The mixture was extracted three times with 50 ml diethyl ether and the extract washed twice with 50 ml water, dried over $MgSO_4$, and concentrated. The residue was purified with a silica gel plug using 10% EtOAc/hexane followed by chromatographic purification with silica gel using 40% EtOAc/hexane containing 2% triethylamine and the product isolated as a pale yellow oil.

MS 237 (MH^+)
^{1}H NMR ($CDCl_3$) δ 1.78–1.88 (m, 4H), 2.55–2.66 (m, 4H), 2.94 (t, $J = 5.8$ Hz, 2H), 4.18 (t, $J = 5.8$ Hz, 2H), 7.23–7.28 (m, 1H), 7.42 (virtual t, $J = 8.2$ Hz, 1H), 7.75–7.76 (m, 1H), 7.80–7.83 (m, 1H)

3. **Preparation of 1-(2-(3-aminophenoxy)ethyl)pyrrolidine**

The Step 2 product (14.8 mmol) dissolved in 20 ml EtOAc containing 10% palladium on carbon (400 mg) was hydrogenated 10 hours under 50 psi hydrogen, then filtered through Celite 545. The mixture was extracted three times with 20 ml 1 M HCl, washed twice with 20 ml diethyl ether, and the pH adjusted to > 10 with 2 M NaOH. The aqueous layer was re-extracted three times with 20 ml diethyl ether, dried, and concentrated. The residue was purified by chromatography with a silica gel pad using 75% EtOAc/hexane/1% triethylamine, and the product isolated as a pale yellow oil.

MS 207 (MH^+)
^{1}H NMR ($CDCl_3$) δ 1.72–1.80 (m, 2H), 2.54–2.71 (m, 2H), 2.88 (t, $J = 8.2$ Hz, 2H), 3.48–3.79 (br s, 2H), 4.07 (t, $J = 8.2$ Hz, 2H), 6.22–6.39 (m, 3H), 7.05 (virtual t, $J = 9.1$ Hz, 1H)

4. **Preparation of *N*-(3-(2-(1-pyrrolidino)ethyloxy)phenyl)-*cis*-3-(triphenylmethylamino)-cyclohexylcarboxamide**

Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (9.3 mmol) was added to a solution consisting of the Step 1 product (3.3 g, 8.4 mmol), the Step 3 product (7.0 mmol), and diisopropylethylamine (9.3 mmol) dissolved in 30 ml CH_2Cl_2 and stirred overnight. The solution was concentrated onto silica gel and purified by flash chromatography using 20% EtOAc/2% triethylamine/hexane,

then 60% EtOAc/2% triethylamine/hexane and the product isolated in 78% yield as a white foam.

MS 596 (MNa^+), 574 (MH^+), 332 (MH^+–trt), 243 (trt^+)

5. **Preparation of *N*-(3-(2-(1-pyrrolidino)ethyloxy)phenyl)-*N*-*cis*-3-(triphenylmethylamino)-cyclohexylmethylamine**

   The Step 4 product (3.7 mmol) dissolved in 10 ml THF was treated with lithium aluminum hydride(5.8 mmol), then refluxed 8 hours. The mixture was cooled, quenched with saturated potassium sodium tartrate solution, filtered through Celite 545, and the filtrate concentrated. The residue was dissolved in 20 ml EtOAc, washed twice with 20 ml water, dried, reconcentrated, and the product isolated as a white foam.

   MS 582 (MNa^+), 560 (MH^+), 318 (MH^+–trt), 243 (trt^+)

6. **Preparation of *N*-(3-(2-(1-pyrrolidino)ethyloxy)phenyl)-*N*-[*cis*-3-(triphenylmethylamino)-cyclohexylmethyl]-4-fluorophenylcarboxamide**

   The Step 5 product (2.6 mmol) and triethylamine (2.9 mmol) dissolved in 10 ml CH_2Cl_2 were treated with 4-fluorobenzoyl chloride (2.9 mmol) dissolved in 5 ml CH_2Cl_2 and the reaction quenched after 3 hours with 3 ml 2 M NaOH. The mixture was extracted three times with 20 ml CH_2Cl_2, dried, and concentrated onto silica gel. The residue was purified by chromatography using a silica gel column preconditioned with triethylamine using 50% EtOAc/2% triethylamine/hexane and the product isolated as a white foam.

   MS 682 (MH^+), 440 ($MH^+ \frac{1}{N}$trt), 243 (trt^+)

7. **Preparation of *N*-(3-(2-(1-pyrrolidino)ethyloxy)phenyl)-*N*-[*cis*-(3-aminocyclohexyl)-methyl]-4-fluorophenylcarboxamide**

   The Step 6 product (2.57 mmol) was treated with 35 ml of a mixture consisting of 10% trifluoroacetic acid/1% triethylsilane/CH_2Cl_2, then stirred 3 hours. The solution was extracted three times with 20 ml 1 M HCl, washed twice with 20 ml CH_2Cl_2, cooled to 0°C, and made basic with NaOH. The mixture was re-extracted three times with 20 ml EtOAc, dried, reconcentrated, and the product isolated as a pale yellow oil.

   MS 462 (MNa^+), 440 (MH^+)

8. **Preparation of *N*-(3-(2-(1-pyrrolidino)ethyloxy)phenyl)-*N*-(*cis*-3-(benzylamino)-cyclohexyl)methyl-4-fluorophenylcarboxamide**

   A mixture of the Step 7 product (2.3 mmol) and benzaldehyde (2.5 mmol) dissolved in 4 ml toluene was treated with titanium(**IV**) isopropoxide (2.8 mmol) and stirred 18 hours. The mixture was then treated with 0.80 ml ethyl alcohol followed by the portionwise addition of sodium triacetoxyborohydride (2.8 mmol) and stirred an

additional 4 hours. The mixture was quenched with 2 M NaOH, the precipitate filtered off through Celite 545, the filtrate dried, concentrated, and the product isolated. The product was used without additional purification.

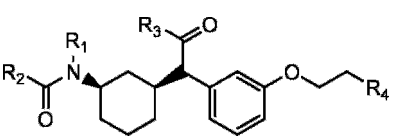
9. **Preparation of *N*-(3-(2-(1-pyrrolidino)ethyloxy)phenyl)-*N*-[(*cis*-3-(benzylamino)-(*cis*-trimethylacetal)]-cyclohexyl) methyl-4-fluorophenylcarboxamide**

The Step 9 product was dissolved in 4 ml CH_2Cl_2 and treated with trimethylacetyl chloride (2.5 mmol), then stirred 2 hours. The mixture was neutralized with saturated $NaHCO_3$ solution, extracted three times with 10 ml CH_2Cl_2, dried, and concentrated onto silica gel. The product was purified by flash chromatography using 50% EtOAc/1% triethylamine/hexane and 690 mg product isolated as a white foam. The addition of 1.2 ml 1 M HCl dissolved in 5 ml diethyl ether provided the final product.

MS 614 (MH^+); HPLC (RT 4.11 minutes)

Derivatives

Table 1 Selected experimental diamine derivatives and their corresponding mass spectral data. ^{1}H NMR characterization supplied by author



Entry	R_1	R_2	R_3	R_4	MS (MH^+)
43	Benzyl	2-(Phenylethyl)	Phenylamino	*N*-Morpholinyl	675
65	3-Nitrobenzyl	Trichloromethyl	Phenylamino	*N*-Morpholinyl	732
90	2-Pyridyl-methyl	Trichloromethyl	4-Fluorophenyl	*N*-Morpholinyl	691
232	2-Chlorobenzyl	*t*-Butyl	4-Fluorophenyl	*N*-Methyl-*N*-benzylamino	650
238	2-Chlorobenzyl	Trichloromethyl	4-Fluorophenyl	1-Piperidinyl	–
265	Hydrogen	3-Carboxy-1,2,2-trimethylcyclopentyl	4-Fluorophenyl	1-Piperidinyl	–

Testing

I. *Rabbit Tissue Bath In Vivo Testing*

A Covance white rabbit was euthanized and the duodenum excised. The lumen was rinsed with saline and the tissue placed in cold, aerated Tyrode's buffer, then cut into 3 cm segments starting at the proximal end. These segments were tied on both ends with 3-0 silk suture, one end of which was attached to an S-hook on a custom-made glass support rod placed in a 15 ml isolated tissue bath and the other end attached to a Grass Force Displacement Transducer FT03. The tissue was maintained in room temperature Tyrode's buffer (pH 7.4) and continually gassed with 95% O_2–5% CO_2. The tissues were adjusted to 1.0 g resting tension and maintained at that tension throughout the equilibration period.

Tissues were washed twice during a 30-minute equilibration period, then readjusted to 1 g resting tension, and after equilibration treated with 3 μM carbamoyicholine chloride. After maximal contraction, tissues were rewashed three times with Tyrode's buffer, left undisturbed 20 minutes, rewashed, and readjusted to 1 g resting tension. The tissues were retreated with carbamoyicholine chloride and this contraction was considered as the maximum contraction value. Thereafter, selected experimental agents dissolved in 30% DMSO–50 mM HEPES were evaluated by adding to the tissue bath followed by a 20 minutes incubation period. Tissues were then treated with porcine motilin, and when maximum contraction was attained, tissues we retreated with another aliquot of carbamoyicholine chloride to ascertain the contraction inhibiting effect for each experimental agent. Testing results are provided in Table 2.

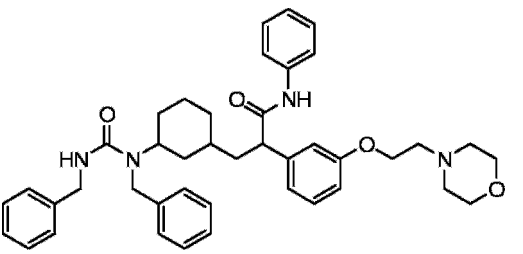
Table 2 Effect of selected experimental agents on colon contraction inhibition using the rabbit tissue bath in vivo testing protocol

Entry	Colon Contraction Inhibition at 1 mM (%)	IC_{50} (μM)
43	74	0.73
65	80	0.035
90	95	0.49
232	89	0.17
238	98	0.097
265	67	–

Notes

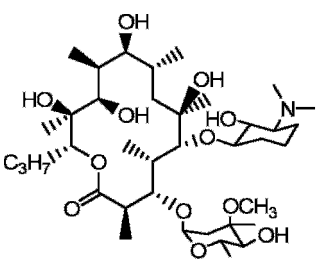
1. Although erythromycin is also effective in treating irritable bowel syndrome, its use is limited because of gastrointestinal side effects.

2. Additional substituted diamine derivatives, (**I**), effective as motilin antagonists were prepared by the authors (1) in an earlier investigation.

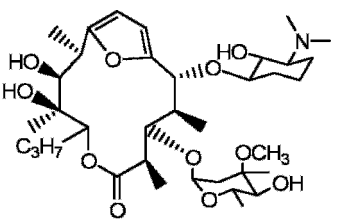


(I)

3. Erythromycin motilide, (**II**), and furan erythromycin motilide derivatives, (III), prepared by Santi (2) and Ashley (3), respectively, were effective as motilin receptor agonists and used in treating conditions associated with impaired gastric motility.

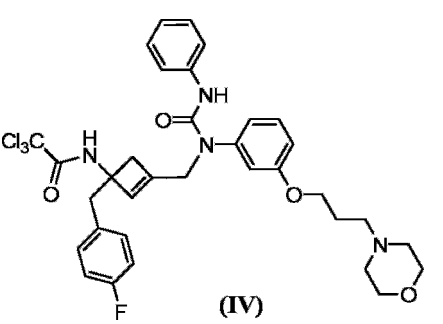


(II)



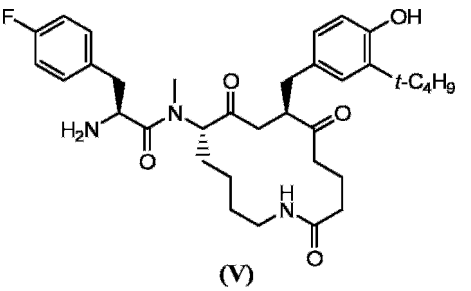
(III)

4. Cyclobutene, (**IV**), and cyclopentene derivatives prepared by Chen (4,5), respectively, were effective as both motilin receptor antagonists and contractile smooth muscle antagonists and used in treating irritable bowel syndrome disorder.



(IV)

5. Cyclic motilin receptor antagonists, (**V**), prepared by Matsuoka (6) were effective in treating irritable bowel syndrome in patients showing diarrhea or hypermotilinemia conditions.



(**V**)

References

1. S.G. Johnson *et al*., US Patent 6,511,980 (January 28, 2003)
2. D.V. Santi *et al*., US Patent 6,946,482 (September 20, 2005)
3. G. Ashley *et al*., US Patent 6,946,482 (September 13, 2003) and US Patent 6,750,205 (June 15, 2004)
4. R.H. Chen *et al*., US Patent 6,667,309 (December 22, 2003)
5. R.H. Chen *et al*., US Patent 6,624,165 (September 23, 2003) and US Patent 5,972,939 (October 26, 1999)
6. H. Matsuoka *et al*., US Patent 7,018,981 (March 28, 2006)

CHAPTER XIX

Malaria

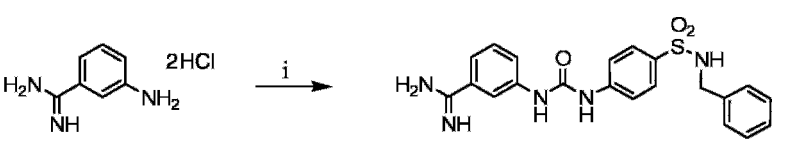
I. Intracellular Protein-Degradation Inhibitors

Title Compounds for the Treatment of Protozoal Diseases
A. Aschenbrenner *et al.*, US Patent 6,949,567 (September 27, 2005)
Assignee 4SC AG
Utility Treatment of Protozoal Diseases

Invention Significance Protozoal infections are characterized by hyperproliferation of the infectious agent independent of the parasites living intracellularly or extracellularly of their respective host cells. A method of controlling malaria and related disorders using asymmetrical amidinyl diphenylurea derivatives as modulators of cellular pathways in eukaryotes is described.

Reaction

Eq. 1



i- 1,1-Thiocarbonyldiimidazole, nitromethane, methyltriflate, DMA, diisopropylethyl amine

Experimental

1. **Preparation of 3-[3-(4-benzylsulfamoyl-phenyl)-thioureido]-benzamidine**

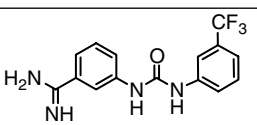
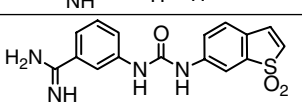
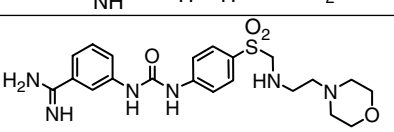
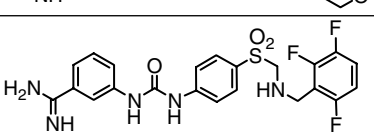
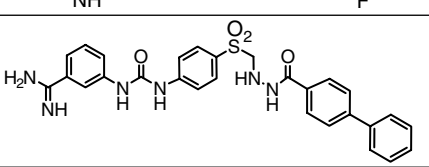
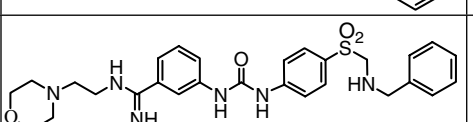
   1,1-Thiocarbonyldiimidazole (0.8 mmol) dissolved in 5 ml nitromethane was cooled to 4°C, then treated dropwise with methyl triflate (1.6 mmol). The reaction was

stirred 30 minutes, then treated dropwise with 4-amino-*N*-benzylbenzenesulfonamide (0.8 mmol) dissolved in 2 ml DMA, and stirred an additional 2.5 hours at ambient temperature. 3-Aminobenzamidine•2HCl (0.8 mmol) and diisopropylethyl amine (0.8 mmol) dissolved in 1 ml DMA were then added and the mixture stirred 16 hours at ambient temperature. The solution was concentrated and the residue purified by flash chromatography using 95–45% EtOAc/methyl alcohol and the product isolated in 15% yield.

Derivatives

Selected experimental derivatives are provided in Table 1.

Table 1 Biological activity of selected experimental agents. ^{1}H NMR and MS data for intermediates and products supplied by author

Entry	Structure	Anti-plasmodial activity[a] (3D7)	Anti-plasmodial activity[b] (Dd2)	Human proteasome inhibition[c]
2		–	B[b]	C (5 M)
18		A	A	–
22		–	–	B (5 M)
54		–	–	A (50 M)
71		B	–	A (50 M)
103		C	A	B (5 M)

[a] A, IC_{50} <1 μM; B, IC_{50} 1−10 μM; C, IC_{50} 10−100 μM.
[b] A, 90–100% inhibition; B, 75–90%; C, 50–75% inhibition.
[c] Inhibitor concentration of 5 or 50 μM.

Testing

I. *Antiplasmodial Activity*

For the determination of the antiplasmodial activity of the compounds, the multi-resistant Dd2 strain of *Plasmodium falciparum* was used and [8-^{3}H]-hypoxanthine incorporated into the parasitic nucleic acids measured. The plasmodia were incubated with 0.3% parasitemia and an erythrocyte hematocrit of 2.5% in the presence of different concentrations of selected experimental agents in a final volume of 200 µl. The medium employed was RPMI 1640 containing 10% of heat-treated human serum and 3 mg/l of gentamycin. In the incubations, the concentrations of the compounds varied from 0.3 to 100 µM. After 48 hours, each batch was treated with 50 µl [8-^{3}H]hypoxanthine and incubated additional 18 hours. Testing results are provided in Table 1.

II. *Antiplasmodial Activity Against the 3D7 Chloroquine-sensitive Strain*

Antiplasmodial activity against the 3D7 chloroquine-sensitive strain of *P. falciparum* was determined using the method of Wright (1). Testing results are provided in Table 1.

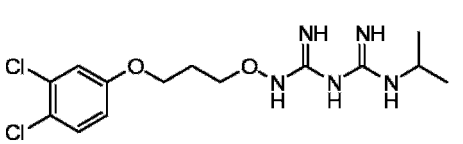
III. *Inhibition of Human 20S Proteasome*

The activity of human 20S proteasome was measured by monitoring the release of 7-amino-4-methylcoumarin (AMC) from the fluorogenic peptide Suc-Leu-Leu-Val-Tyr-AMC.

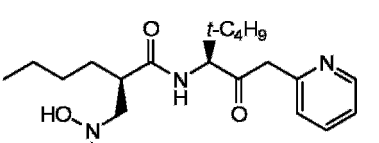
Selected experimental agents were incubated with 0.25 µg human 20S proteasome in the assay buffer (25 mM Hepes, 500 µM EDTA, 3% SDS, pH 7.6) for 10 minutes in a final volume of 200 µl. The rate of AMC released was measured by monitoring the increase of fluorescence having an excitation of 390 nm and an emission of 460 nm. For IC_{50} determination, a selected experimental agent was added so that a final concentration between 5 and 0.005 µM was achieved. Testing results are provided in Table 1.

Notes

1. Hydroxylamine derivatives, (**I**) and (**II**), prepared by Jacobus (2) and Todd (3), respectively, were effective in reducing Plasmodium sp., Mycobacterium sp., *P. falciparum*, and *Pneumocystis carinii* infection levels in subjects.

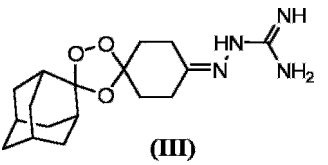


(I)



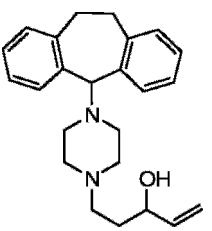
(II)

2. Adamantyl spiro- and dispiro-1,2,4-trixolane derivatives, (**III**), prepared by Vennerstrom (4) were effective against *P. falciparum*.



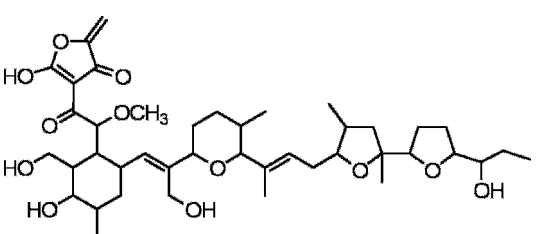
(III)

3. Dibenzosuberanyl piperazine derivatives, (**IV**), prepared by Takeuchi (5) were effective in restoring medicament sensitivity of antimalarial agents and used in the treatment of plasmodium infections.



(IV)

4. Takeuchi (6) demonstrated that the tetronic acid-containing antibiotic, (**V**), was effective in the treatment of antiprotozoal infections including *Plasmodium berghei*.



(V)

References

1. P. Wright *et al*., J. Med. Chem., 44, 19, 3187 (2001)
2. D.P. Jacobus *et al*., US Patent 6,693,217 (February 17, 2004) and US Patent 6,551,614 (April 22, 2003)
3. R.S. Todd *et al*., US Patent 6,716,878 (April 6, 2004)
4. J.L. Vennerstrom *et al*., US Patent 6,906,205 (June 14, 2005) and US Patent 6,825,230 (November 30, 2004)
5. T. Takeuchi *et al*., US Patent 6,881,841 (April 19, 2005)
6. T. Takeuchi *et al*., US Patent 6,939,892 (September 6, 2005)

II. Treatment of Protozoal Infections Using Gem-Dihydroperoxides

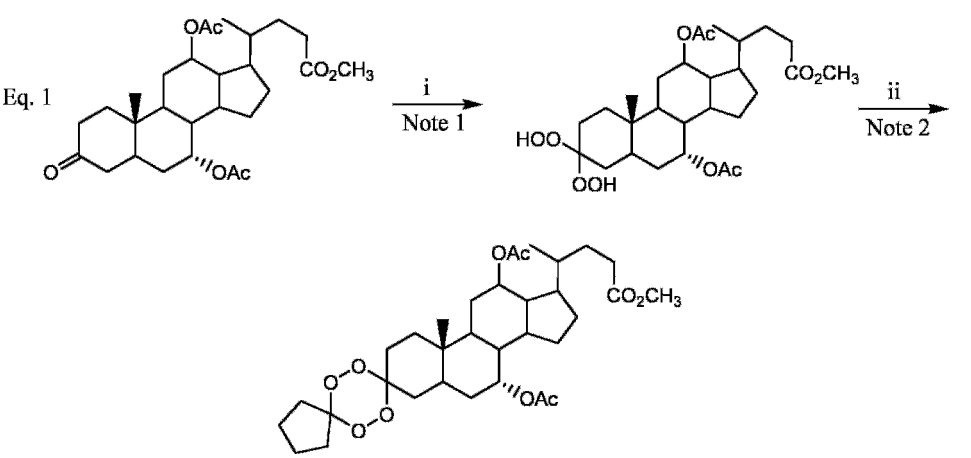
Title Mixed Steroidal 1,2,4,5-Tetraoxane Compounds and Methods of Making and Using Thereof
B. Solaja *et al*., US Patent 6,906,098 (June 14, 2005)

Assignee The United States of America as Represented by the Secretary of the Army

Utility Treatment of Drug-Resistant Malaria

Invention Significance The endoperoxide bridge undergoes reductive decomposition forming free radicals and electrophilic intermediates, which have antimalarial activity. Although antimalarial agents such as artesunate and arteether are effective in treating malaria, drug-induced and dose-related neurotoxicity has been observed. This investigation addresses these drug resistance and toxicity concerns.

Reaction



i- Hydrogen peroxide, hydrochloric acid, CH_2Cl_2, acetonitrile

ii- Cyclopentanone, sulfuric acid, CH_2Cl_2, acetonitrile

Experimental

1. **Preparation of methyl 3,3-dihydroperoxy-7α,12α-diacetoxy-5β-cholan-24-oate**

   Methyl 3,3-dihydroperoxy-7α,12α-diacetoxy-5β-cholan-24-oate (5.94 mmol) dissolved in 120 ml acetonitrile/CH_2Cl_2, 3:1, was treated with 6.18 ml 30% H_2O_2 and five drops 12 M HCl, then stirred 2 hours at 22°C. The mixture was treated with 80 ml water and 120 ml CH_2Cl_2 and the organic layer washed twice with 10 ml $NaHCO_3$ solution and three times with 20 ml brine. After drying with Na_2SO_4, the crude material was isolated as a colorless solid in 97% yield. The solid was purified by chromatography using silica gel with heptane/EtOAc, 1:1, recrystallized, and the product isolated as colorless prisms, mp = 197−199°C.

   IR (KBr) (cm^{-1}) 3429, 2954, 1736, 1636, 1439, 1382, 1254, 1124, 1077
   IR (CCl_4) (cm^{-1}) 3442, 2952, 1736, 1636, 1559, 1541, 1508, 1439, 1381, 1253, 1127, 1103, 1077
   ^{1}H NMR 9.06 (bs, 2H, HOO–C(3), exchangeable with D_2O), 5.08 (bs, H–C(12)), 4.90 (bs, H–C(7)), 3.67 (s, CH_3O_2C(24)), 2.16 (s, CH_3COO–), 2.13 (s, CH_3COO–), 0.95 (s, H_3C-C(10)), 0.08 (d, J = 6.0 Hz, H_3C-C(20)), 0.73 (s, H_3C-C(13))
   ^{13}C NMR (50 MHz, $CDCl_3$) 175.06, 171.22, 110.65, 75.62, 71.10, 51.66, 47.32, 45.07, 43.30, 38.29, 37.62, 34.58, 32.16, 30.90, 30.66, 28.57, 27.10, 25.69, 24.31, 22.73, 22.12, 21.63, 21.50, 17.43, 12.17
   ESI-MS (*m/z*) 1031.63 ($[2M+Na]^+$, 15), 577.32 ($[M+Na]^+$, 4), 572.36 (4), 559.29 (4), 543.28 ($[M+Na-H_2O_2]^+$, 19), 527.30 ($[M+Na-H_2O_2-H_2O]^+$, 100), 522.36 (33), 385.28 (75)

2. **Preparation of methyl 7α,12α-diacetoxy-5β-cholan-24-oate-3- spiro-6′-(1′,2′,4′,5′-tetraoxacyclohexane)- 3′-spirocyclopentane**

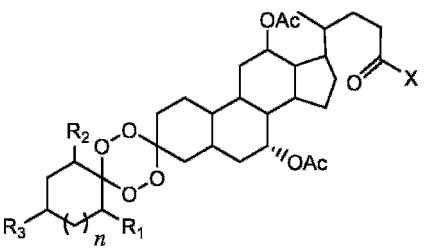
   The Step 1 product (0.90 mmol) dissolved in 14 ml CH_2Cl_2 was treated with cyclopentanone (1.80 mmol) and cooled to 0°C. After 30 minutes, 599 µl of 1:10 mixture of cold H_2SO_4/CH_3CN was added dropwise and the mixture stirred 15 minutes at 0°C. The mixture was worked up as in Step 1 using heptane/EtOAc, 85:15, and the product isolated in 26%, mp = 180−182°C.

   $[\alpha]_D^{20}$ 49.56 (c = 1.02, $CHCl_3$)
   IR (KBr) (cm^{-1}) 2954, 2875, 1736, 1440, 1379, 1242, 1076, 1032
   ^{1}H NMR 5.09 (bs, H–C(12)), 4.92 (bs, H–C(7)), 3.66 (s, CH_3O_2C(24)), 2.13 (s, CH_3COO–), 2.08 (s, CH_3COO–), 0.94 (s, H_3C-C(10)), 0.81 (d, J = 6.0 Hz, H_3C-C(20)), 0.73 (s, H_3C-C(13))
   ^{13}C NMR 174.42, 170.49, 119.71, 108.35, 75.12, 70.52, 53.35, 51.38, 47.14, 44.88, 43.17, 37.47, 35.12, 34.52, 34.42, 34.18, 32.08, 30.66, 30.56, 28.28, 26.28, 25.57, 24.88, 24.40, 24.15, 23.75, 22.62, 21.98, 21.45, 21.29, 17.32, 12.06

ESI-MS (***m/z***)1263.75 ($[2M+Na]^+$, 25), 659.35 ($[M+K]^+$, 18), 643.37 ($[M+Na]^+$, 100), 638.41 ($[M+NH_4]^+$, 41)

Derivatives

Table 1 Summary of selected 1,2,4,5-tetraoxane derivatives and their corresponding melting points



Entry	*n*	R_1	R_2	R_3	X	mp (°C)
1	0	H	H	H	OCH_3	182–184
2	1	CH_3	CH_3	H	OCH_3	213–215
3	1	H	H	(4″R)-CH_3	OCH_3	82–84
4	1	H	H	H	$NH(CH_2)_2CH_3$	208–210
5	1	H	H	H	OH	213–216
6	1	H	H	(4″R)-CH_3CH_2	$NH(CH_2)_2CH_3$	112–116
7	1	H	CH_3	H	NH_2	139–143
8	1	H	H	(4″R)-C_6H_5	$NHCH_3$	133–136
9	3	H	H	H	OCH_3	181–183

Testing

I. *In Vitro Evaluation of Antimalarial Activity*

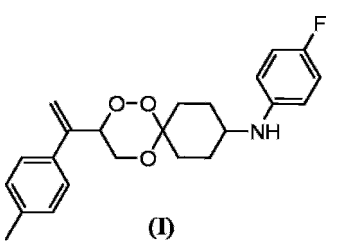
The activity of selected experimental agents against parasite clones of chloroquine-susceptible malaria strains, Sierra Leone, D6, and chloroquine-resistant malaria strain, Indochina, W2, was determined using the method of Desjardins (1). Testing results are provided in Table 2.

Table 2 Effectiveness of selected 1,2,4,5-tetraoxane derivatives against D6 and W2 malaria strains

Entry	IC_{50} (ng/ml)		Resistance Index
	Strain D6	Strain W2	
1	24.30	17.18	0.71
2	20.67	236.70	11.45
3	6.48	3.27	0.50
4	9.77	4.50	0.46
5	18.98	11.93	0.63

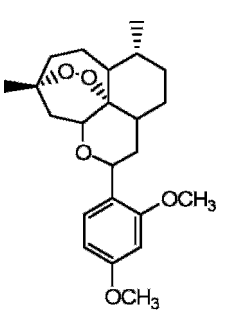
Notes

1. Vinyl-1,2,4-Trioxyspiro antimalarial agents, (**I**), prepared by Singh (2) were effective in treating multidrug-resistant malaria strains.



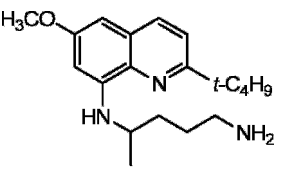
(I)

2. Haynes (3) prepared C-10 substituted artemisinin endoperoxide derivatives, (**II**), which were particularly effective in the treatment of malaria, neosporosis, and coccidiosis.



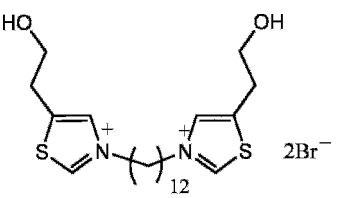
(II)

3. 8-Aminoquinoline derivatives, (**III**), prepared by Jain (4) have a broad spectrum of activity against blood and tissue stages of human malaria parasites as well as being useful in treating drug-sensitive and multidrug-resistant malaria.



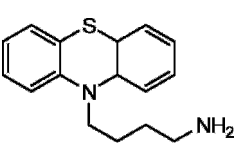
(III)

4. Quaternary dithiazolium derivatives, (**IV**), prepared by Vial (5) were effective as antiparasitics and used as antimalarial and antibabesiosis agents.



(IV)

5. Chemosensitizing agents prepared by Lin (6) consisting of phenothiazine derivatives, (**V**), were effective against chloroquine-resistant *Plasmodium falciparum*.



(V)

References

1. R.E. Desjardins *et al*., Antimicrob. Agents Chemother. 16, 710 (1979)
2. C. Singh *et al*., US Patent 7,071,226 (July 4, 2006)
3. R.K. Haynes *et al*., US Patent 6,984,640 (January 10, 2006)
4. R. Jain *et al*., US Patent 6,979,740 (December 27, 2005)
5. H. Vial *et al*., US Patent 6,972,343 (December 6, 2005)
6. A.J. Lin *et al*., US Patent 6,800,618 (October 5, 2004)

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CHAPTER XX

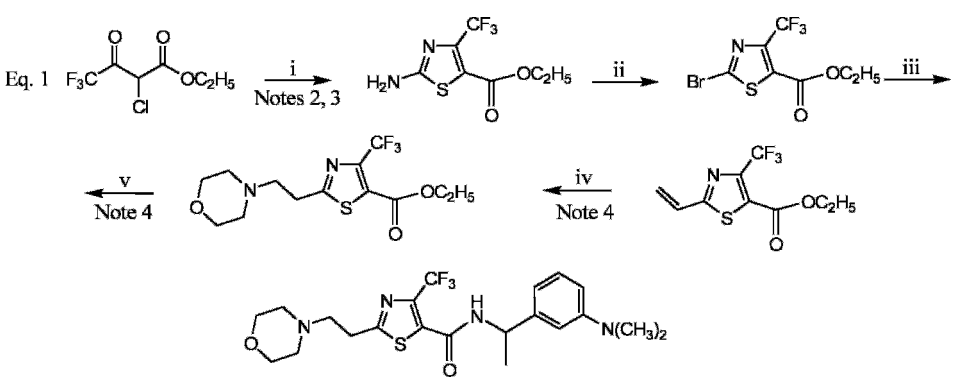
Migraine Headaches

I. KCNQ Potassium Channels Modulators

Title Aminoalkyl Thiazole Derivatives as KCNQ Modulators
K.M. Boy *et al.*, US Patent 6,933,308 (August 23, 2005)
Assignee Bristol-Myers Squibb Company
Utility Treatment of Migraine Headaches

Invention Significance Although the symptom pattern and severity vary among migraine sufferers, abnormal synchronous neuron firing is associated with migraine headaches. A method for treating this disorder using KCNQ potassium channel openers to increase neuron hyperpolarization and diminish abnormal synchronous neuron firing is described.

Reaction



i- Ethyl alcohol, thiourea
ii- Hydrobromic acid, sodium nitrite, copper(I) bromide
iii- Toluene, tetrakis(triphenylphosphine)palladium(0), tributylvinyltin, 2,6-di-*t*-butyl-4-methyl phenol

iv- Ethyl alcohol, morpholine
v- Lithium hydroxide,
[3-(1-aminoethyl)phenyl]-dimethylamine,
3-(dimethoxyphosphoryloxy)-1,2,3-benzotriazine, DMF

Experimental

1. **Preparation 2-amino-4-trifluoromethyl-thiazole-5-carboxylic acid ethyl ester**

   Ethyl 2-chloro-4,4,4-trifluoroacetoacetate (230 mmol) dissolved in 250 ml ethyl alcohol was treated with thiourea (230 mmol), then refluxed 3 hours, and concentrated. The residue was dissolved in diethyl ether and washed with aqueous $NaHCO_3$ and brine. The solution was dried with $MgSO_4$, then recrystallized with EtOAc and hexanes, and the product isolated in 75% yield as white crystals.

   ^{1}H NMR (300 MHz, MeOD-d_4) δ 4.26 (q, $J = 7.1$ Hz, 2H), 1.31 (t, $J = 7.1$ Hz, 3H)

2. **Preparation of 2-bromo-4-trifluoromethyl-thiazole-5-carboxylic acid ethyl ester**

   The Step 1 product (167 mmol) dissolved in 300 ml 48% HBr at 0°C was treated dropwise with sodium nitrite (250 mmol) dissolved in 200 ml water over 1 hour, then stirred additional 30 minutes. The mixture was then treated with CuBr (167 mmol) dissolved in 200 ml 48% HBr for over 30 minutes and the mixture stirred 30 minutes at 0°C and 2 hours at ambient temperature. The solution was extracted three times with CH_2Cl_2, then concentrated. The residue was purified by chromatography using silica gel with hexane and then 2.5% EtOAc/hexane and the product isolated in 93% yield.

   ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.40 (q, $J = 7.1$ Hz, 2H), 1.38 (t, $J = 7.1$ Hz, 3H)

3. **Preparation of 4-trifluoromethyl-2-vinyl-thiazole-5-carboxylic acid ethyl ester**

   The Step 2 product (57.3 mmol) dissolved in 150 ml toluene was treated with a mixture consisting of tetrakis(triphenylphosphine)palladium(0) (1.14 mmol), tributylvinyltin (63.0 mmol), and a catalytic amount of 2,6-di-*t*-butyl-4-methyl phenol, then stirred in a sealed tube at 120°C for 25 minutes. The mixture was then cooled and extracted with 250 ml EtOAc. The extract was washed twice with 150 ml 1 M NaOH and once with 150 ml brine, dried, concentrated, and a brown oil isolated. This residue was purified by flash chromatography using 0–10% EtOAc/hexane and the product isolated in 76% yield as an yellow oil.

   ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.88 (dd, $J = 11.0$, 17.6 Hz, 1H), 6.20 (d, $J = 17.6$ Hz, 1H), 5.74 (d, $J = 11.0$ Hz, 1H), 4.38 (q, $J = 7.2$ Hz, 2H), 1.37 (t, $J = 7.1$ Hz, 3H)

4. **Preparation of 2-(2-morpholin-4-yl-ethyl)-4-trifluoromethyl-thiazole-5 -carboxylic acid ethyl ester (general procedure)**

   A solution of the Step 3 product (1.59 mmol) dissolved in 4 ml methyl alcohol was treated with morpholine (1.91 mmol), then stirred 1 hour at 25°C, and concentrated. The residue was purified by flash chromatography using 0–10% methyl alcohol/$CHCl_3$ and the product isolated in 50% yield as a brown oil.

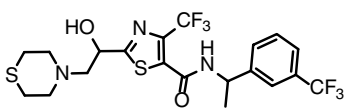
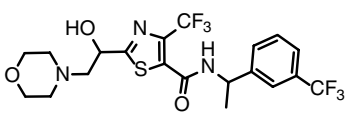
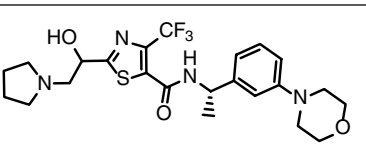
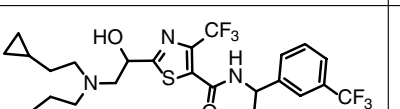
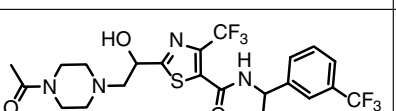
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.38 (q, $J = 7.2$ Hz, 2H), 3.80 (t, $J = 4.8$ Hz, 4H), 3.18 (t, $J = 6.4$ Hz, 2H), 2.72 (t, $J = 6.2$ Hz, 2H), 2.55 (t, $J = 4.4$ Hz, 4H), 1.37 (t,$J = 7.0$ Hz, 3H)

5. **Preparation of 2-(2-morpholin-4-yl-ethyl)-4-trifluoromethyl-thiazole-5-[1-(3-dimethylamine)phenyl]-ethyl-1-carboxamide (general procedure)**

The Step 4 product (0.8 mmol) dissolved in 2 ml THF was treated with 1 ml 1 M LiOH, then refluxed 1 hour, cooled, and treated with 1 ml 1 M HCl. The mixture was concentrated and the residue (0.145 mmol) dissolved in 1 ml DMF. The solution was treated with 3-(dimethoxyphosphoryloxy)-1,2,3-benzotriazine (0.18 mmol) followed by [3-(1-aminoethyl)-phenyl]-dimethylamine (0.145 mmol) dissolved in 0.5 ml DMF containing 81 µl triethylamine. The solution was stirred 8 hours at 25°C, then filtered, and applied directly to a preparatory HPLC column (C18, 10–100% methyl alcohol/water/0.1% trifluoroacetic acid) for purification. The residue was then dissolved in 3 ml methyl alcohol and applied to a SAX column having hydroxide as the counter ion. The product was eluted with 10 ml methyl alcohol and isolated.

Derivatives

Table 1 Summary of minimum concentration responses and EC_{50} for selected hydroxyl thiazole derivatives

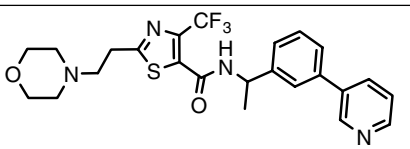
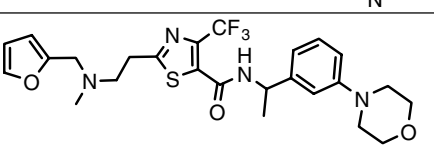
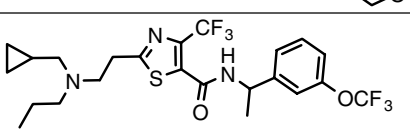
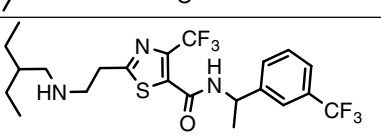
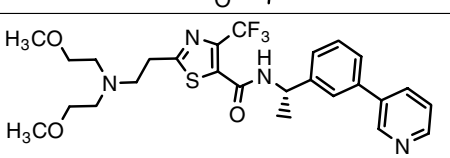
Entry	Structure	Retention Time (minutes)	MS (M^+)	Minimum Concentration Response (µM)
46		0.637	472	3
52		1.363	497	10
59		0.953	498	30
60		1.477	523	3
61		1.363	538	10

Testing

I. *Thallium Flux Assay for KCNQ Channel Openers*

The thallium assay was performed using the method of Weaver (1). EC_{50} values were calculated by fitting the resulting amplitudes to a single-site logistic equation. EC_{50} was defined as the concentration of test compound required to yield 50% of the maximal response. Maximal response, i.e., maximal opening, was the largest signal amplitude divided by the negative control amplitude generated by any concentration of a test compound. Testing results are provided in Table 2.

Table 2 Summary of minimum concentration responses and EC_{50} for selected hydroxyl thiazole derivatives

Entry	Structure	Minimum Concentration Response (μM)	EC_{50} (μM)
8		10	+[a]
11		30	+
28		0.3	+++[b]
34		3	+
40		30	++[c]

[a] 1000–20 000.

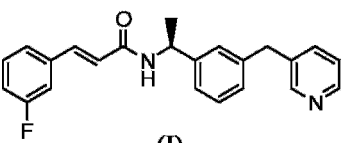
[b] <50 nM.

[c] 50–1000 nM.

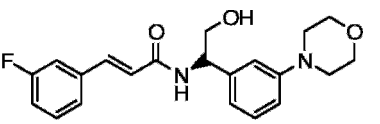
Notes

1. In previous and subsequent investigations by co-authors Wu (2,3), acrylamide, (**I**), and hydroxyethyl acrylamide derivatives, (**II**), respectively, were prepared that were

effective in treating migraine attacks and other disorders sensitive to KCNQ potassium channel opening activity.

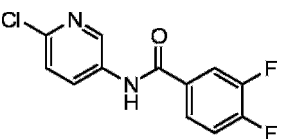


(I)



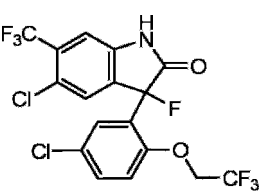
(II)

2. Voltage-dependent potassium channel openers KCNQ consisting of benzanilides, (**III**), prepared by McNaughton-Smith (4) were effective in treating migraines and related diseases modulated by potassium channel opening agents.



(III)

3. 3-Fluoro-2-oxindole derivatives, (**IV**), prepared by Dworetzky (5) were effective modulators of KCNQ potassium channels and used in treating migraine and mechanistically related disease.



(IV)

References

1. C.D. Weaver, WO 02/31508 (2002)
2. Y.-J. Wu *et al.*, US Patent 6,900,210 (May 31, 2005) and US Patent 6,831,080 (December 14, 2004)
3. Y.-J. Wu *et al.*, US Patent 7,045,551 (May 16, 2006)
4. G.A. McNaughton-Smith *et al.*, US Patent 6,989,398 (January 24, 2006) and US Patent 6,737,422 (May 18, 2004)
5. S.I. Dworetzky *et al.*, US Patent 6,855,829 (February 15, 2005)

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CHAPTER XXI

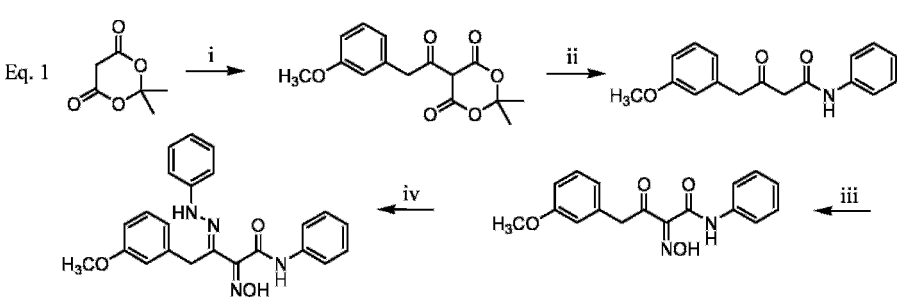
Obesity

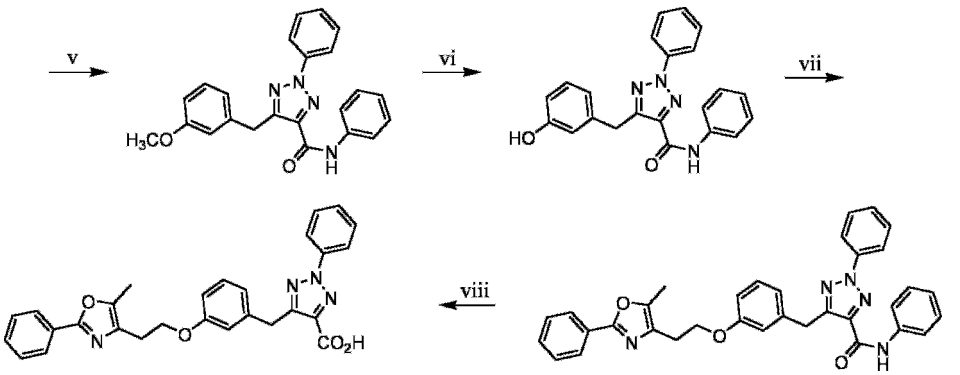
I. Peroxisome Proliferator Receptor-γ Inhibitor and Peroxisome Proliferator Receptor-α Stimulator

Title Substituted Azole Acid Derivatives Useful as Antidiabetic and Antiobesity Agents and Method
P.T. Cheng *et al.*, US Patent 6,967,212 (November 22, 2005)
Assignee Bristol-Myers Squibb Company
Utility Treatment of Obesity

Invention Significance Azole acid derivatives have been prepared for treating obesity and dyslipidemia which simultaneously inhibit the peroxisome proliferator-activated receptor-γ (PPAR-γ) and stimulate the peroxisome proliferator-activated receptor-α (PPAR-α). These agents are also effective in modulating blood glucose, triglyceride, and insulin levels.

Reaction





i- Pyridine, CH_2Cl_2, 3-methoxyphenylacetyl chloride
ii- Toluene, aniline
iii- Sulfuric acid, sodium nitrite, sodium hydroxide
iv- Phenylhydrazine, magnesium sulfate, ethyl alcohol
v- Trifluoroacetic anhydride, trifluoroacetic acid
vi- CH_2Cl_2, borontribromide
vii- 5-Phenyl-2-methyl-oxazole-4-ethanol mesylate, potassium carbonate, DMF
viii- Potassium hydroxide, ethyl alcohol

Experimental

1. **Preparation of 2,2-dimethyl-1,3-dioxane-4,6-dione-5-(3-methoxybenzyl) ketone**

   A solution of 2,2-dimethyl-1,3-dioxane-4,6-dione (65 mmol) and pyridine (100 mmol) dissolved in CH_2Cl_2 at 0°C was treated with the dropwise addition of 3-methoxyphenylacetyl chloride (10.0 g) over 2 hours, then stirred an additional 2 hours at ambient temperature. The mixture was partitioned between 2 M HCl and CH_2Cl_2, the organic layer isolated, dried, and concentrated. The residue was isolated as an oil and used without further purification.

2. **Preparation of 3-keto-4-(3-methoxyphenyl)-*N*-phenylbutamide**

   The Step 1 product and aniline (54 mmol) were dissolved in 20 ml toluene and refluxed 3 hours. The solution was washed with 1 M HCl, concentrated into a small volume, where upon a precipitate was formed, and the product isolated in 59% yield as an yellow solid.

3. **Preparation of 2-oxime-3-keto-4-(3-methoxyphenyl)-*N*-phenylbutamide**

   A mixture consisting of the Step 2 product (14 mmol) and $NaNO_2$ (20 mmol) dissolved in 14 ml 1 M NaOH at 0°C was treated with 5 ml 18 M H_2SO_4 then stirred 30 minutes. A precipitate was formed, which was washed with water, then purified by chromatography with silica gel using hexane/EtOAc from 5:1 to 3:1 and the product isolated 68% yield as yellow crystals.

4. **Preparation of 4-(3-methoxyphenyl)-2-oxime-3-*N*-phenylhydrazide-*N*-phenyl-butamide**

   A mixture consisting of the Step 3 product (0.32 mmol), phenylhydrazine (0.55 mmol), and $MgSO_4$ (200 mg) was refluxed in 10 ml ethyl alcohol for 2 hours and then concentrated. The residue was recrystallized using hexane/CH_2Cl_2, 1:1, and the product isolated in 70% yield as yellow crystals.

5. **Preparation of 5-(3-methoxybenzyl)-2-phenyl-4-*N*-phenylamido-1,2,3-triazole**

   A mixture consisting of the Step 4 product (0.22 mmol) and 1 ml apiece trifluoroacetic anhydride and trifluoracetic acid were heated in a sealed tube for 10 hours at 45°C, then concentrated. The residue was partitioned between EtOAc and $NaHCO_3$ and the organic phase isolated. It was dried with Na_2SO_4 and then reconcentrated. The residue was purified by chromatography using hexane/EtOAc, 3:1, and the product isolated in 35%yield as an yellow solid.

6. **Preparation of 5-(3-hydroxylbenzyl)-2-phenyl-4-*N*-phenylamido-1,2,3-triazole**

   The Step 5 product (0.078 mmol) dissolved in 2.0 ml CH_2Cl_2 was cooled to −70°C, then treated dropwise with 1 ml 1 M BBr_3 in CH_2Cl_2. The mixture was warmed to 0°C and then stirred 3 hours. The solution was recooled to −20°C and quenched with NH_4Cl solution. This mixture was rewarmed to ambient temperature, then stirred additional 30 minutes, and extracted with EtOAc. The organic phase was washed with 1 M HCl and water, dried, concentrated, and the product isolated in 99% yield. The oil was used without further purification.

7. **Preparation of 5-(3-(2-(5-methyl-2-phenyl-oxazol-4-yl)ethoxy)benzyl)-2-phenyl-4-*N*-phenylamido-1,2,3-triazole**

   A mixture consisting of the Step 6 product (0.081 mmol), 5-phenyl-2-methyl-oxazole-4-ethanol mesylate (0.11 mmol), and K_2CO_3 (3.61 mmol) dissolved in 3 ml DMF was stirred 12 hours at 80°C, filtered, and then concentrated. The residue was purified by chromatography using hexane/EtOAc, 3:1, and the product isolated in 36% yield as a light brown solid.

8. **Preparation of 4-hydroxycarboxy-5-(3-(2-(5-methyl-2-phenyl-oxazol-4-yl) ethoxy)benzyl)-2-phenyl-1,2,3-triazole**

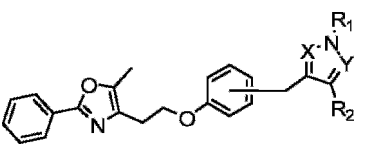
   A solution of the Step 7 product (0.054 mmol) and KOH (3.6 mmol) in 3.0 ml ethyl alcohol was heated to 90°C for 24 hours in a sealed tube, then partitioned between EtOAc and 1 M HCl, and the organic phase isolated. It was washed with water, dried, and concentrated. The residue oil was purified by preparative HPLC and the product isolated in 31% yield as a solid.

[M+H]$^+$ 481.1

^{13}C NMR (100 MHz, $CDCl_3$) δ 10.2, 24.5, 31.7, 65.6, 113.6, 114.7, 119.4, 121.6, 124.5, 126.7, 128.4, 129.2, 129.3, 129.5, 130.1, 131.9, 137.5, 139.1, 139.6, 146.8, 151.4, 157.9, 160.2, 163.5

Derivatives

Table 1 Selected pyrrole and triazole experimental agents and their corresponding mass spectral data. ^{1}H NMR for experimental materials supplied by author



Entry	R_1	R_2	Orientation	X	Y	$[M+H]^+$
1	Phenyl	Carboxylic acid	*meta*	N	N	481
2	4-Methylphenyl	Carboxylic acid	*meta*	N	N	495
5	Carboxylic acid	4-Methylphenyl	*para*	N	N	495
21	Phenyl	Carboxylic acid	*para*	CH	CH	479
45	4-Fluorophenyl	Carboxylic acid	*para*	CH	CH	597
51	3-Methylphenyl	Carboxylic acid	*meta*	N	N	495

Testing

A description of testing protocols was not supplied by author.

I. *Body Mass Assay in Obese Mice Assay*

Table 2 Effect on body fat mass and lean body mass for diet-induced obese mice using Entry 1 for 3 weeks at 10 mg/kg per day

Treatment	Fat Body Mass (%)	Lean Body Mass (%)
Vehicle	47.2 ± 1.5	50.5 ± 1.4
Entry 1	41.5 ± 1.8	56.0 ± 1.8
Net effect	-12	11

II. *Plasma glucose, Triglyceride, and Free Fatty Acids Assay*

Table 3 Effect of plasma glucose, triglyceride, and free fatty acids in obese diabetic mice treated with Entry 1 at 10 mg/kg per day

Treatment	Glucose (mg/dl)	Triglyceride (mg/dl)	Free Fatty Acids (mequiv./l)
Vehicle	780.9 ± 43.8	265.2 ± 34.3	1.18 ± 0.06
Entry 1	683.0 ± 25.2	145.3 ± 12.5	0.76 ± 0.12
Net effect	−13	−45	−36

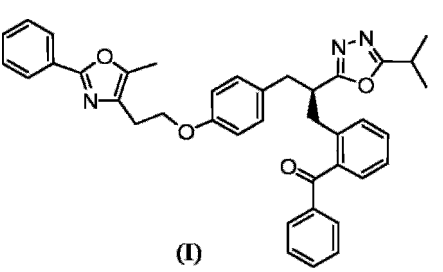
III. *Liver triglycerides and Alanine Aminotransferase Assay*

Table 4 Effect of liver triglycerides and alanine aminotransferase blood levels on diet-induced obesity in mice using Entry 1 at 10 mg/kg per day

Treatment	Liver Triglycerides (mg/g)	Alanine Aminotransferase (IU/l)
Vehicle	72.5 ± 4.8	158.8 ± 20.2
Entry 1	55.4 ± 7.0	98.0 ± 12.6
Net effect	−24	−38

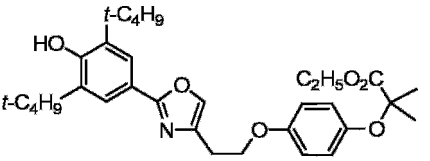
Notes

1. 1,3-Oxazolyl derivatives containing 1,2,4-oxadiazolyl-, 1,3,4-thiadiazolyl-, and 1,3,4-oxadiazolyl substituents, (**I**), were prepared by Cobb (1) and were effective as PPAR-γ receptor antagonist and used in the treatment of diabetes, obesity, and metabolic syndrome disorders.



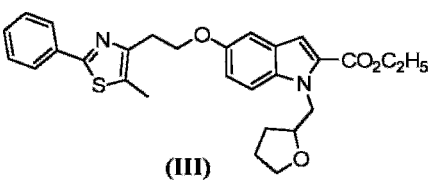
(I)

2. Oxazolyl-aryloxyacetic acid derivatives, (**II**), prepared by Brooks (2) were effective as PPAR agonists and used in treating diabetes, obesity, hyperglycemia, and hyperlipidemia.

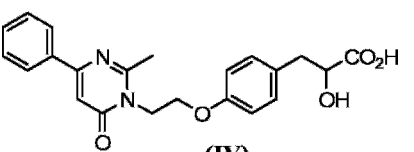


(II)

3. PPAR-γ inhibitors consisting of 1H-indole-2-carboxylate derivatives, (**III**), and β-aryl-α-hydroxy propionic acid derivatives, (**IV**), prepared by Stolle (3) and Gurram (4), respectively, were effective in the treatment of diseases caused by increased fat accumulation or lipid storage, such as osteoporosis, obesity, and acne.

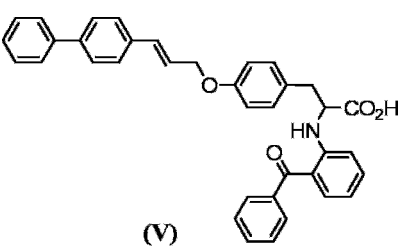


(III)



(IV)

4. Vinyl *N*-(2-benzoylphenyl)-L tyrosine derivatives, (**V**), prepared by Jeppesen (5) were partial PPAR-α, PPAR-γ, and PPAR-δ agonists and used in treating obesity, hyperglycemia, hyperlipidemia, and hypercholesterolemia.



(V)

References

1. J.E. Cobb *et al.*, US Patent 6,506,781 (January 14, 2003)
2. D.A. Brooks *et al.*, US Patent 6,982,278 (January 3, 2006)
3. A. Stolle *et al.*, US Patent 6,787,651 (September 7, 2004)
4. R.M. Gurram *et al.*, US Patent 7,053,217 (May 30, 2006)
5. L. Jeppesen *et al.*, US Patent 7,067,530 (June 27, 2006)

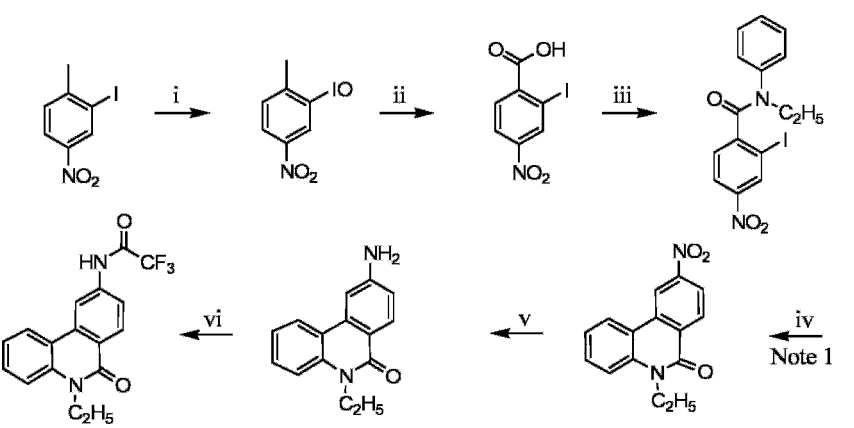
II. Subtype NPY-5 Receptor Antagonists

Title Aminophenanthridinone and Aminophenanthridine as NPY-5 Antagonists
M. Hammond *et al.*, US Patent 6,958,347 (October 25, 2005)
Assignee Pfizer Inc.
Utility Treatment of Obesity and Obesity-related Disorders

Invention Significance Neuropeptide Y (NPY) is a 36 amino acid peptide neurotransmitter implicated in the pathophysiology of feeding disorders. Its activation is related to stimulation of consummatory behavior and obesity. A method of modulating food intake using subtype NPY-5 receptor antagonists has been prepared to address this health problem.

Reaction

Eq. 1



i- Acetic acid, acetic anhydride, sulfuric acid, chromium trioxide
ii- Sodium iodide, water, acetic acid, sulfur dioxide
iii- Oxalyl chloride, CH_2Cl_2, DMF, dimethylaminopyridine, triethylamine, *N*-ethylaniline
iv- Palladium acetate, triphenylphosphine, acetonitrile, silver carbonate
v- 10% Palladium-on-carbon, ethyl alcohol, hydrogen
vi- Trifluoroacetic anhydride, pyridine, CH_2Cl_2

Experimental

1. **Preparation of 2-iodosyl-4-nitro-benzoic acid**

   2-Iodo-4-nitrotoluene (0.19 mol) was dissolved in a mixture of 380 ml acetic acid, 190 ml acetic anhydride, and 112 ml 18 M sulfuric acid previously cooled in an ice bath. Chromium trioxide (0.57 mol) was then added portionwise for over 30 minutes, which was accompanied by a temperature increase to 90°C. The thick suspension was cooled 30 minutes in an ice bath, poured over ice (2 kg), and a precipitate isolated by filtration. The solid was washed with 800 ml methyl alcohol, then 200 ml diethyl ether, dried, and the product isolated in 94% yield.

2. **Preparation of 2-iodo-4-nitrobenzoic acid**

   A suspension of the Step 1 product (0.18 mol) in 250 ml water was treated with a single portion of solid potassium iodide (0.33 mol) and 50 ml acetic acid, then stirred 1 hour at ambient temperature. Gaseous sulfur dioxide was bubbled through the mixture until a pale green suspension was formed. The solid was isolated, co-evaporated three times with 250 ml toluene, dried, and the product isolated in 64% yield.

3. **Preparation of *N*-ethyl-2-iodo-4-nitro-*N*-phenylbenzamide**

   The Step 2 product dissolved in 137 ml CH_2Cl_2 was treated dropwise with oxalyl chloride (0.14 mol) followed by 0.1 ml DMF and the mixture stirred 1 hour at ambient temperature. A second addition of 0.1 ml DMF was added to the mixture, which was then followed by additional 2 hours of stirring. The solution was concentrated and the residue dissolved in 137 ml CH_2Cl_2, then treated with dimethylaminopyridine (50 mg) and triethylamine (0.102 mol). The solution was then cooled to 0°C and *N*-ethylaniline (82 mmol) added over 10 minutes, then stirred 16 hours at 23°C. The mixture was diluted with 150 ml water and the layers separated. The organic layer was washed twice with 100 ml 1 M HCl, dried using Na_2CO_3, and concentrated. The residue was purified by flash column chromatography using CH_2Cl_2 and the product isolated in 93% yield.

4. **Preparation of 5-ethyl-9-nitro-5H-phenanthridin-6-one**

   Palladium acetate (3.68 mmol) was added to a suspension of silver carbonate (36.8 mmol), triphenylphosphine (7.36 mmol), and the Step 3 product (18.4 mmol) in 92 ml acetonitrile, then refluxed 30 minutes. The mixture was cooled, filtered through a plug of celite, and the filter cake washed with 100 ml apiece EtOAc and diethyl ether. The filtrates were washed with 100 ml brine, dried, and concentrated. The residue was purified by flash column chromatography using 150 ml diethyl ether followed by 150 ml EtOAc and the product isolated in 58% yield as an yellow solid.

5. **Preparation of 9-amino-5-ethyl-5H-phenanthridin-6-one**

   The Step 4 product (20 mmol) dissolved in 400 ml ethyl alcohol was treated with 10% palladium on activated carbon (500 mg) and hydrogenated 18 hours at 50 psi hydrogen. The mixture was filtered, concentrated, and the product quantitatively isolated.

MS *m/z* 239 (M^+)
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.1 (d, $J = 7.5$ Hz, 1H), 8.0 (d, $J = 8.5$ Hz, 1H), 7.5 (m, 2H), 7.4 (s, 1H), 7.3 (m, 1H), 6.8 (dd, $J = 8.7$, 2.1 Hz, 1H), 4.3 (q, $J = 6.9$ Hz, 2H), 1.2 (t, $J = 7.1$ Hz, 3H)

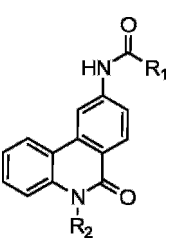
6. **Preparation of *N*-(5-ethyl-6-oxo-5,6-dihydro-phenanthridin-9-yl)-2,2,2-trifluoro-acetamide**

Trifluoroacetic anhydride (0.24 mmol) was added to a solution of the Step 5 product (0.16 mmol) and pyridine (0.49 mmol) in 3 ml CH_2Cl_2, then stirred 16 hours at ambient temperature. The mixture was diluted with 10 ml EtOAc/hexanes, 1:1, and then washed successively with 10 ml solutions of water, brine, NH_4Cl, and rewashed with brine. The organic layer was dried, concentrated, the residue purified by flash chromatography using 50% diethyl ether in hexanes, and the product isolated in 61% yield.

MS *m/z* 335 ($M^+ + 1$)
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.8 (d, $J = 2.1$ Hz, 1H), 8.6 (d, $J = 8.5$ Hz, 1H), 8.3 (d, $J = 8.1$ Hz, 1H), 8.2 (s, 1H), 7.6 (m, 2H), 7.4 (d, $J = 8.5$ Hz, 1H), 7.3 (t, $J = 7.9$ Hz, 1H), 4.5 (q, $J = 7.1$ Hz, 2H), 1.4 (t, $J = 7.3$ Hz, 3H)

Derivatives

Table 1 Selected 6-oxo-5,6-dihydro-phenanthridine derivatives and their corresponding mass spectral data. ^{1}H NMR characterization provided by author



Entry	R_1	R_2	MS (M + 1)
4	Trifluoroacetamide	Ethyl	335
5	2-Pyridin-4-yl-acetamide	Ethyl	358
6	2-Dimethylaminoacetamide	Ethyl	324
7	3-Piperidin-1-yl-propanamide	Ethyl	378
8	2-Dimethylaminoacetamide	Isopropyl	338
9	2-Pyridin-4-yl-acetamide	Isopropyl	372
10	3-Piperidin-1-yl-propanamide	Isopropyl	392
12	2-Hydroxy-isobutyamide	Ethyl	325

Testing

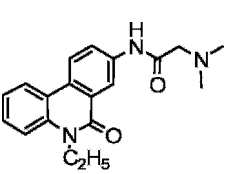
I. [^{125}I]*Peptide YY (PYY) Binding at Human NPY Receptors Expressed in Sf9 Cells*

Baculovirus-infected Sf9 cells expressing NPY-5 receptors were harvested after 48 hours and NPY-Y5 receptor cDNA using the method of Sambrook (1). Harvested cells were homogenized, centrifuged, and the nuclei pelletized. They were then recentrifuged, washed, suspended in phosphate-buffered saline, and stored in aliquots at −80°C. Purified membranes were washed, then suspended in a binding buffer, and added to polypropylene tubes containing 0.035 nM [^{125}I]PYY(porcine). Selected experimental agents were added in concentrations ranging from 10^{-12} to 10^{-5} M followed by a buffer solution to yield a final volume of 0.5 ml. Nonspecific binding was determined in the presence of 1 mM NPY(human) and accounted for approximately 10% of total binding. After a 2 hour incubation period at ambient temperature, the reaction was terminated. Samples were then filtered and a gamma counter was used to count filters having 85% efficiency or higher. Testing results are provided in Table 2.

Table 2 The effectiveness of selected 6-oxo-5,6-dihydro-phenanthridine derivatives as NPY-5 antagonists

Entry	NPY-5, K_i(nM)	Treatment Trials
4	43	2
5	12	4
6	41	4
8	38	4
9	16	4
Reference[a]	2165	2

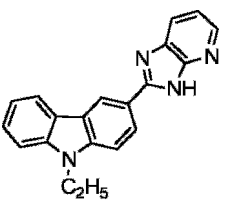
[a] The reference agent was 2-dimethylamino-*N*-(5-methyl-6-oxo-5,6-dihydro-phenanthridin-8-yl)-acetamide, (**I**).



(I)

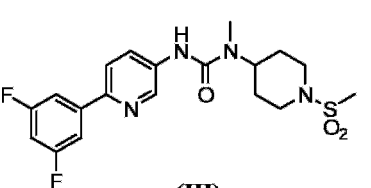
Notes

1. 9H-Carbazole derivatives, (**II**), prepared by Elliott (2) were effective as NPY-5 antagonists and used in the treatment of feeding disorders including obesity and obesity-related diseases.

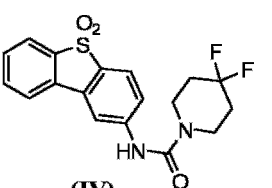


(II)

2. Pyridin-3-yl-, (**III**), and dibenzothiophene urea derivatives, (**IV**), prepared by Stamford (3) and Block (4), respectively, were effective NPY-5 antagonists and used in the treatment of obesity.

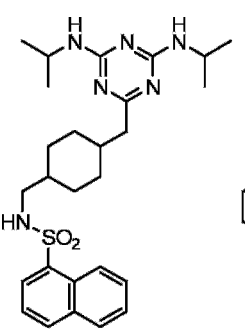


(III)

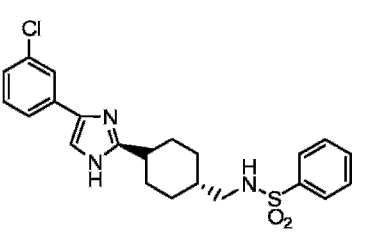


(IV)

3. 1H-indole, (**IV**), and 1H-imidazole sulfonamide derivatives, (**V**), prepared by Marzabadi (5) and Blum (6), respectively, were effective as NPY-5 antagonists and used to control aberrant eating behavior.



(IV)



(V)

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2. R.L. Elliott, US Patent 6,982,267 (January 3, 2006)
3. A. Stamford *et al.*, US Patent 6,946,476 (September 20, 2005) and US Patent 6,946,476 (September 20, 2005)
4. M.H. Block *et al.*, US Patent 6,967,216 (November 22, 2005)
5. M.R. Marzabadi *et al.*, US Patent 6,989,379 (January 24, 2006)
6. C.A. Blum *et al.*, US Patent 7,034,034 (April 25, 2006)

CHAPTER XXII

Ocular Disorders

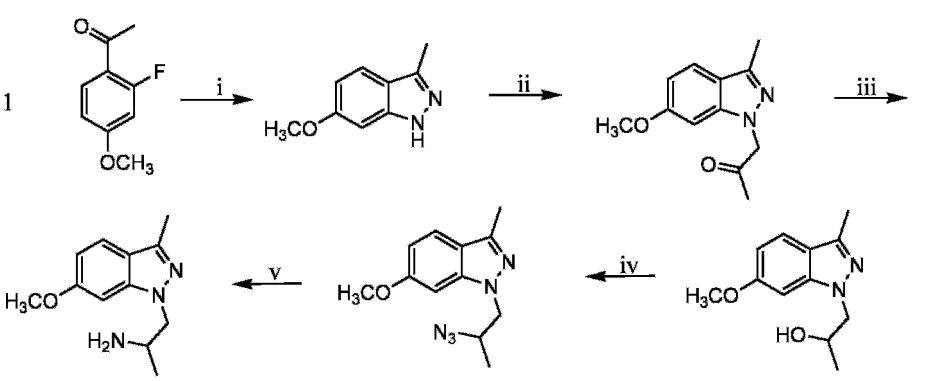
I. Glaucoma: Serotonergic 5-HT_2 Receptor Agonists

Title 6-Hydroxy-indazole Derivatives for Treating Glaucoma
J.A. May *et al.*, US Patent 6,956,036 (October 18, 2005)
Assignee Alcon, Inc.
Utility Treatment of Drug-Resistant Glaucoma

Invention Significance The early detection and prompt treatment of ocular hypertension can prevent loss of visual function or impede its progressive deterioration. There are, however, some individuals who do not respond well when treated with certain existing topical glaucoma medications. This chapter is designed to address that concern.

Reaction

Eq. 1



i- Ethyl alcohol, hydrazine hydrate, ethylene glycol, CH_2Cl_2
ii- DMF, sodium hydride, chloroacetone

iii- Sodium borohydride, methyl alcohol
iv- CH_2Cl_2, methanesulfonyl chloride, DMF, sodium azide
v- Methyl alcohol, 10% palladium-on-carbon, hydrogen

Experimental

1. **Preparation of 6-methoxy-3-methyl-1H-indazole**

   A solution of 2-fluoro-4-methoxyacetophenone (11.3 mmol) dissolved in 20 ml ethyl alcohol was treated with 1.4 ml hydrazine hydrate and then refluxed 6 hours. This mixture was concentrated and the residue treated with 10 ml ethylene glycol. This mixture was heated 18 hours at 150°C, then cooled, and diluted with 50 ml water. It was then extracted three times with 60 ml CH_2Cl_2, washed with 10 ml brine, dried with $MgSO_4$, and concentrated. The residue was recrystallized from EtOAc and the product isolated in 59% yield.

   MS (ES) *m/z* 163 (M^+)

2. **Preparation of 1-(6-methoxy-3-methyl-indazol-1-yl)-propan-2-one**

   The Step 1 product (6.7 mmol) dissolved in 10 ml DMF was treated with sodium hydride (10.2 mmol), then stirred 30 minutes at ambient temperature. The mixture was then treated with 0.79 ml chloroacetone and heated 6 hours at 60°C. After cooling, it was diluted with 10 ml saturated NH_4Cl solution and extracted three times with 65 ml EtOAc. The combined extracts were washed with 10 ml brine, dried, and concentrated. The residue was purified by chromatography using 20–30% EtOAc/hexane and the product isolated in 88% yield as an oil.

   MS (ES) *m/z* 219 (M^+)

3. **Preparation of 1-(6-methoxy-3-methyl-indazol-1-yl)-propan-2-ol**

   Sodium borohydride (5.5 mmol) was added to a solution of the Step 2 product (5.5 mmol) dissolved in 10 ml methyl alcohol, then stirred 2 hours at ambient temperature. The mixture was concentrated, the residue treated with 10 ml saturated NH_4Cl solution, and then extracted three times with 50 ml EtOAc. The combined extracts were washed with 10 ml brine, dried, concentrated, the residue purified as in Step 2, and the product isolated in 56% yield as an oil.

   MS (ES) *m/z* 221 (M^+)

4. **Preparation of 1-(2-azido-propyl)-6-methoxy-3-methyl-1H-indazole**

   A solution of the Step 3 product (3.0 mmol) in 10 ml CH_2Cl_2 at 0°C was treated with triethylamine (3.9 mmol) and methanesulfonyl chloride (3.9 mmol), then stirred 30 minutes. The mixture was then diluted with 50 ml apiece diethyl ether and water and the organic layer was separated. The aqueous component was re-extracted twice with 50 ml diethyl ether. Combined ethereal extracts were washed with 30 ml brine,

dried, evaporated, and the residue dissolved in 6 ml DMF containing sodium azide (3.9 mmol). This mixture was heated 12 hours at 70°C, then poured into water, and re-extracted three times with 50 ml diethyl ether. The combined extracts were washed with brine, dried, and concentrated. The residue was purified by chromatography using 10% EtOAc/hexane and the product isolated in 69% yield as an oil.

MS (ES) *m/z* 246 (M^+)

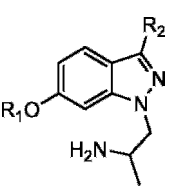
5. **Preparation of 2-(6-methoxy-3-methyl-indazol-1-yl)-1-methylethylamine fumarate**

Palladium on carbon (10%, 0.10 g) was added to a solution of the Step 4 product (2.0 mmol) in methyl alcohol and stirred 18 hours at room temperature under a hydrogen atmosphere. The mixture was filtered through a filter aid, concentrated, and the residue isolated in 96% yield. The residue was converted into the fumaric acid salt and crystallized from methyl alcohol/diethyl ether and the product isolated as a colorless solid, mp = 150−152°C.

MS (ES) *m/z* 191 (M^+)
Analysis Calc. for $C_{12}H_{17}N_3O$: C, 55.58; H, 6.86; N, 11.44.
Found: C, 55.41; H, 6.85; N, 11.37

Derivatives

Table 1 Selected experimental indazole derivatives and their corresponding melting points and mass spectra characterization



Entry	R_1	R_2	Salt	mp (°C)	MS (M^+)
1	Methyl	Methyl	Fumarate	150–152	191
2	Hydrogen	Methyl	Fumarate	166–168	206
3	2,2-Dimethylcarbonyl	Hydrogen	Fumarate	180–182	276
4	Methyl	Hydrogen	–	151–152	206
5	Methyl	Chloride	–	–	240
6	Hydrogen	Chloride	–	–	226

Testing

I. *5-HT$_2$ Receptor Binding Assay*

The relative affinities of the experimental at the 5-HT$_2$ receptors were determined according to the method of Johnson (1). IE$_{50}$ testing results are provided in Table 2.

II. *5-HT$_2$ Functional Assay: Phosphoinositide (PI) Turnover Assay*

The agonist activity of serotonergic compounds at the 5-HT$_2$ receptor was determined in vitro using the ability of the compounds to stimulate the production of [^{3}H]inositol phosphates in [^{3}H]myo-inositol-labeled A7r5 rat vascular smooth muscle cells by their ability to activate the enzyme phospholipase C.

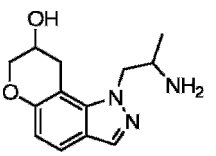
These cells are grown in culture plates maintained in a humidified atmosphere of 5% CO_2 and 95% air and fed semiweekly with Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/l glucose and supplemented with 2 mM glutamine, 10 μg/ml gentamicin, and 10% fetal bovine serum. For the purpose of conducting the phosphoinositide (PI) turnover experiments, the A7r5 cells were cultured in 24-well plates. Confluent cells were exposed for 24–30 hours to 1.5 μCi [^{3}H]-myo-inositol (18.3 Ci/mmol) in 0.5 ml serum-free medium. Cells were rinsed once with DMEM/F-12 containing 10 mM LiCl prior to incubation with a selected experimental agent in 1.0 ml of the same medium for 1 hour at 37°C after which the medium was aspirated and quenched with 1 ml 0.1 M formic acid. The chromatographic separation of [^{3}H]-inositol phosphates, [^{3}H]-IPs, on an AG-1-X8 column was performed by sequential washes with water and 50 mM ammonium formate followed by elution of the total [^{3}H]-IPs fraction with 1.2 M ammonium formate containing 0.1 M formic acid. The eluate was collected and [^{3}H]-IPs determined by scintillation counting on a beta counter. Concentration–response data were analyzed to determine agonist potency, EC$_{50}$, and efficacy, E_{max}. Testing data are supplied in Table 2.

Table 2 Effects of selected experimental indazole derivatives on the 5-HT$_2$ receptor binding and functional assays. IC$_{50}$ values less than 50 nM reflect high affinity for the 5-HT$_2$ receptor. Agents are considered full agonists if their EC$_{50}$ values have an efficacy of $>80\%$ to α-methylserotonin reference

Entry	IC$_{50}$ (nM)	EC$_{50}$ (nM)	Efficacy (%) (E_{max})
α-Methylserotonin (Reference)	3.5	189	104
1	3.1	578	71
2	3.0	483	87
4	–	243	73
5	2.0	541	64
6	2.2	1050	84

Notes

1. Fused pyranoindazolyl, (**I**), prepared by Chen (2) and -indolyl derivatives, (**II**), and naphthyl-azoles, (**III**), prepared by the authors (3,4), respectively, were effective as 5-HT$_2$ agonists and used in the treatment of glaucoma.



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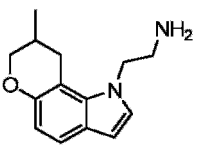
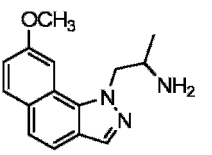
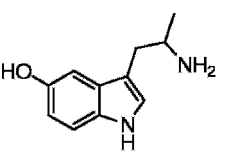


(II)

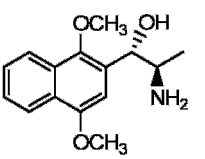


(III)

2. 5-HT$_2$ receptor agonists consisting of 2-aminopropyl-indoles, (**IV**), prepared by the author (5) and naphthylaminopropane derivatives, (**V**), prepared by Hellberg (6) were used in lowering and controlling normal or elevated intraocular pressure associated with glaucoma.

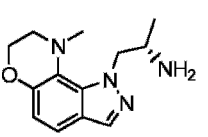


(IV)

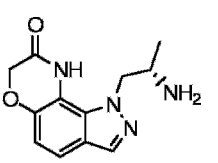


(V)

3. A new class of 5-HT$_2$ receptor agonists were prepared by Dantanarayana (7) consisting of [1,4]oxazino[2,3-g]indazole derivatives, (**VI**) and (**VII**), and used in treating drug-resistant glaucoma.



(VI)



(VII)

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3. J.A. May *et al*., US Patent 7,012,090 (March 14, 2006)
4. J.A. May *et al*., US Patent 6,933,392 (August 23, 2005) and US Patent 6,884,816 (April 26, 2005)
5. J.A. May *et al*., US Patent 6,956,036 (October 18, 2005)
6. M.R. Hellberg *et al*., US Patent 7,071,225 (July 4, 2006)
7. A.P. Dantanarayana *et al*., U.S. Patent 6,989,445 (January 24, 2006)

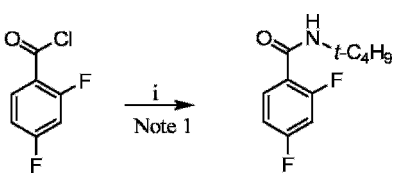
II. Retinopathy: Platelet-activating Factor Antagonists

Title Amide Derivative
K. Ikeda *et al.*, US Patent 6,936,736 (August 30, 2005)
Assignee Sumitomo Manufacturing Company
Utility Topical Mydriatica for the Treatment of Retinal Neurodegenerative Disorders

Invention Significance Amide derivatives effective in treating neurodegenerative disorders such as retinitis pigmentosa, senile macular degeneration, and glaucoma have been prepared. When these agents were administered by eye drops, only modest improvement was observed. Application of these identical agents by topical mydriatica administration, however, dramatically improved their effectiveness.

Reaction

Eq. 1



i- *t*-Butylamine, triethylamine, CH_2Cl_2

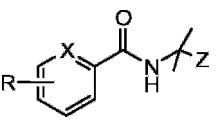
Experimental

1. **Preparation of *N-t*-Butyl-2,4-difluorobenzamide**

   A solution of *t*-butylamine (4.05 mmol) and triethylamine (5.02 mmol) in 2.0 ml CH_2Cl_2 was cooled to 0°C, then treated with the dropwise addition of 2,4-difluorobenzoyl chloride (2.07 mmol) dissolved in 3 ml CH_2Cl_2, and stirred 2.5 hours. The solution was then washed with saturated $NaHCO_3$ solution, extracted three times with EtOAc, rewashed with saturated $NaHCO_3$ solution, dried with $MgSO_4$, and the product isolated in 93% yield.

Derivatives

Table 1 Selected *N*-alkyl-halobenzamide derivatives and their corresponding reaction conversions. ^{1}H-NMR characterization data supplied by author



Entry	R	X	Z	Yield (%)
1	2,4-Difluoro	CH	Methyl	93
6	4-Fluoro	CH	Methyl	96
27	2,5-Difluoro	CH	Hydroxylmethyl	68
40	3-Chloro	N	Methyl	–
47	2,5-Difluoro	CH	Hydroxylmethyl	–
48	2-Fluoro	CH	Methyl	97
49	4-Bromo-2-fluoro	CH	Methyl	96
50	Hydrogen	$N^{+}-O^{-}$	Methyl	83

Testing

I. *Pharmacological Effect Against Constant White Light-induced Retinal Damage*

In the initial phase, male Wistar rats were maintained in cyclic light/dark for 1 week, then placed within an apparatus, and irradiated 24 hours using a white fluorescent lamp. Following this, the rats were moved to a darkroom and dark-adapted 1–4 hours.

In the second phase, the rats were anesthetized, then placed in a stereotaxic frame, and topical mydriatica was administrated. Electrodes were then put on corneal surface, the center of the forehead, and the lower part of the lobe. The response in active potential of the retina to the flash stimulation with fixed energy was determined from the recorded electroretinogram (ERG). Retina damage was evaluated by the decrease in the amplitude of ERG α-wave originating from retinal photoreceptors. Experimental agents were intraperitoneally injected just prior to irradiating rats to a 24-hour white fluorescent lamp to assess the protective effect of the agent. Protection by selected experimental against retinal damage is represented as percent recovery value. Testing results are provided in Table 2.

Table 2 Percent recovery against white light-induced retinal damage for male Wistar rats administered experimental agents by topical mydriatica

Entry	Percent Recovery (Mean ± SEM)
1	95.5 ± 12.3
6	80.1 ± 8.9
27	45.7 ± 10.1
40	82.3 ± 25.6
47	58.2 ± 2.7

II. *Pharmacological Effect Against Constant White Light-induced Retinal Damage Using Eye Drops*

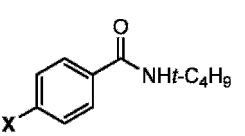
The aforementioned experimental protocol was used but selected experimental agents were administered by eye drops. Tests results are summarized in Table 3.

Table 3 Percent recovery against white light-induced retinal damage for male Wistar rats administered experimental agents as eye drops

Entry	Concentration (mg/ml)	Percent Recovery (Mean ± SEM)
1	2	11.6 ± 2.6
25	1.2	16.2 ± 3.2
48	3.2	15.0 ± 3.7
49	0.4	5.1 ± 1.8
50	10	19.1 ± 7.5

Notes

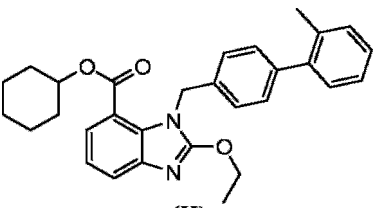
1. Additional amide derivatives, (**I**) were prepared by the authors (1) in an earlier investigation and used in the treatment of retinal neurodegenerative disorders.



X = Br, Cl, F
CH_3, OCH_3
NH_2

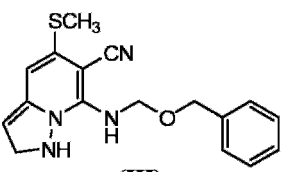
(I)

2. Benzimidazole derivatives, (**II**), prepared by Nakagawa (2) were effective in the treatment of simple or preproliferative retinopathy.

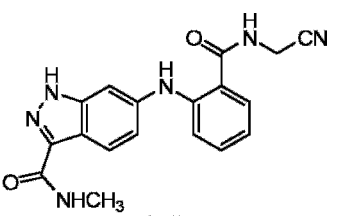


(II)

3. Tyrosine kinase inhibitors consisting of fused heterocyclic derivatives, (**III**) and (**IV**), were prepared by Kato (3) and Borchardt (4), respectively, and used in treating diabetic retinopathy.

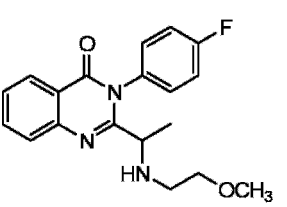


(III)



(IV)

4. Substituted quinazolin-4(3H)-ones, (**V**), effective as $CXCR_3$ chemokine receptor modulators and anthranilic acid amides, (**VI**), effective as VEGF-receptor tyrosine kinase inhibitors were prepared by Medina (5) and Bold (6), respectively, and used in the treatment of diabetic retinopathy and age-related macular degeneration.



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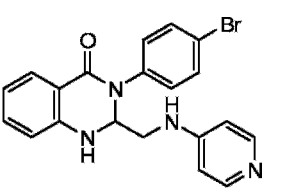

(VI)

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2. S. Nakagawa *et al*., US Patent 7,064,141 (June 20, 2006)
3. F. Kato *et al*., US Patent 7,067,520 (June 27, 2006)
4. A.J. Borchardt *et al*., US Patent 7,053,107 (May 30, 2006)
5. J.C. Medina *et al*., US Patent 7,053,215 (May 30, 2006)
6. G. Bold *et al*., US Patent 7,067,543 (June 27, 2006)

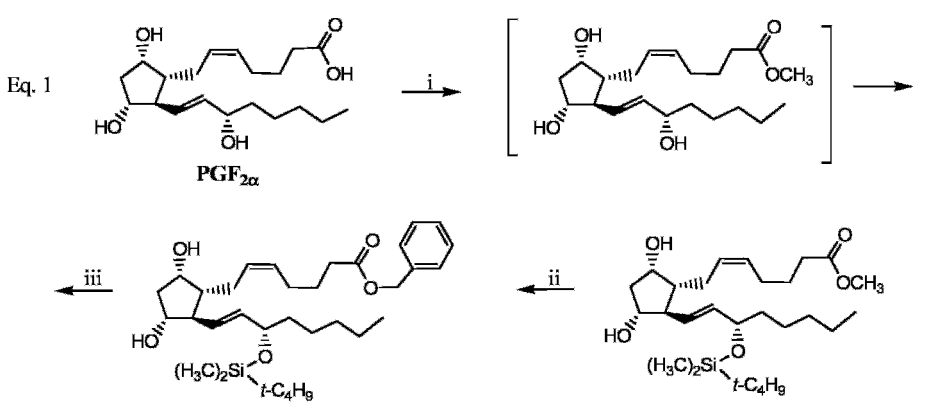
III. Short-Term Treatment of Ocular Hypertension

A. Thromboxane A_2 Receptor Agonists

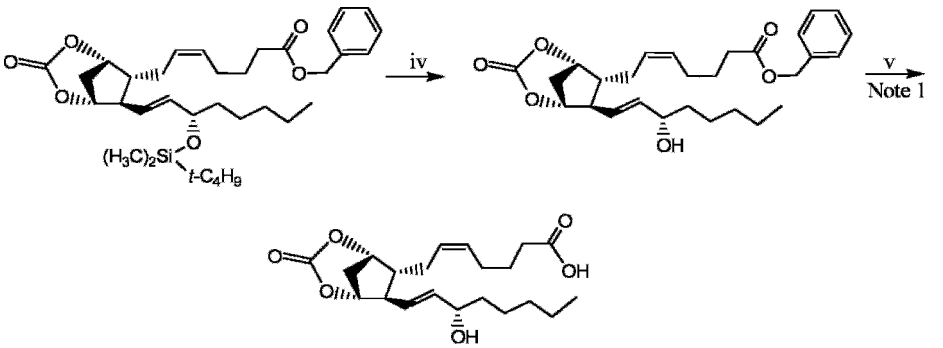
Title Thromboxane Ligands Without Blood Clotting Side Effects
R.M. Burk *et al.*, US Patent 7,019,149 (March 28, 2006)
Assignee Allergan, Inc.
Utility Short-term Treatment of Intraocular Pressure

Invention Significance Thromboxane is an intermediate in the metabolic pathway of arachidonic acid. Thromboxane A_2 mediates potent vasoconstriction and irreversible platelet aggregation. Thromboxane A_2 receptor agonists have been prepared which are effective as vasoconstrictors that do not cause blood clotting. These agents are useful in treating ocular hypotension resulting from postsurgical and postlaser trabeculectomy ocular hypertensive episodes and glaucoma.

Reaction



i- Diazomethane, diethyl ether, *n*-butyl boronic acid, CH_2Cl_2, 2,6-lutidine, *t*-butyldimethyl-silyltrifluoromethane sulfonate
ii- Lithium hydroxide, water, THF, citric acid
iii- CH_2Cl_2, pyridine, triphosgene, ethyl alcohol



iv- THF, tetrabutylammonium fluoride
v- Palladium on carbon, 1-methyl-1,4-cyclohexadiene, methyl alcohol

Experimental

1. **Preparation of cyclopentane heptenoic acid, 5-*cis*-2-(3α-*t*-butyldimethylsilyloxy-1-*trans*-octenyl)-3,5-dihydroxy, [1α, 2β, 3α, 5α]methyl ester**

   A suspension of $PGF_{2\alpha}$ (1.53 mmol) in 20 ml diethyl ether was cooled to 0°C, then treated dropwise with solution of diazomethane in diethyl ether until yellow color persisted. The solution was warmed to 25°C for 30 minutes, concentrated, and the ester used directly without any purification.

   The crude $PGF_{2\alpha}$ ester was treated with *n*-butyl boronic acid (1.84 mmol) dissolved in 3.1 ml CH_2Cl_2, then refluxed 2 hours, and concentrated. It was immediately diluted with 3 ml CH_2Cl_2 at 0°C, then treated with 2,6-lutidine (3.7 mmol), and *t*-butyldimethylsilyltrifluoromethane sulfonate (2.9 mmol) and stirred 16 hours at 23°C. The mixture was concentrated, diluted with 40 ml methyl alcohol, stirred 24 hours, and reconcentrated. The residue was purified by flash chromatography with silica using hexane/EtOAc, 2:1, and the product isolated in 92% yield as an oil.

2. **Preparation of cyclopentane heptenoic acid, 5-*cis*-2-(3-*t*-butyldimethylsilyloxy-1-*trans*-octenyl)-3,5-dihydroxy, [1α, 2β, 3α, 5α]benzyl ester**

   A solution of the Step 1 product (1.17 mmol) dissolved in 3.5 ml 0.5 M LiOH and 7.0 ml THF was stirred 24 hours at 23°C, then acidified with 10% citric acid. The mixture was extracted with EtOAc, dried with $MgSO_4$, filtered, and concentrated. The residue was treated with *O*-benzyl-*N*, *N*′-diisopropylisourea (1.76 mmol) in 7.0 ml benzene, then heated 24 hours at 65°C, and concentrated. The residue was purified as in Step 1 and the product isolated in 85% yield.

3. **Preparation of 7-[6-carbobenzoxy-2-*cis*-hexenyl]-6-[3α-*t*-butyldimethylsilyloxy-1-*t*-octenyl]-3-oxo-2,4-dioxobicyclo[3.2.1]octane**

   The Step 2 product (0.591 mmol) was dissolved in 1.6 ml of CH_2Cl_2 and cooled to −78°C. The mixture was treated with 0.18 ml pyridine (0.6 mmol) followed by triphosgene (0.5 mmol) dissolved in 1 ml CH_2Cl_2, and stirred 60 minutes before warming to ambient temperature. After standing overnight, the reaction was quenched with saturated NH_4Cl solution, then diluted with EtOAc, and washed with 1 M HCl, $NaHCO_3$, and brine. The organic layer was dried, concentrated, and the product isolated in 68% yield.

4. **Preparation of 7-[6-carbobenzoxy-2-*cis*-hexenyl]-6-[3α-hydroxy-1-*trans*-octenyl]-3-oxo-2,4-dioxobicyclo[3.2.1]octane**

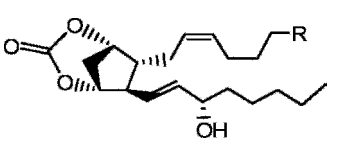
   The Step 3 product (0.1037 mmol) dissolved in 1.0 ml THF was treated with 0.20 ml 1.0 M solution of Bu_4NF and stirred 16 hours at 23°C, then diluted with EtOAc. It was washed with water, then brine, dried, then concentrated. The residue was purified by chromatography using hexane/EtOAc, 1:1, and the product isolated in 62% yield.

5. **Preparation of 7-[6-carboxy-2-*cis*-hexenyl]-6-[3α-hydroxy-1-*trans*-octenyl]-3-oxo-2,4-dioxobicyclo[3.2.1]octane**

   A suspension of the Step 4 product (0.0531 mmol) and 10% palladium on carbon (8 mg) in a mixture of 1.25 ml 1-methyl-1,4-cyclohexadiene and methyl alcohol, 1:4, was heated 20 minutes at 35°C, then diluted with CH_2Cl_2 and filtered. The filtrate was concentrated, the residue purified as in Step 4 using EtOAc, and the product isolated in 99% yield.

Derivatives

Table 1 Selected $PGF_{2\alpha}$ derivatives evaluated as thromboxane A_2 agonists. Very limited characterization data were supplied by author



Entry	R
4	CH_2OH
7	CO_2H
15	$CONH_2$
17	$CONHCH(CH_3)_2$

Testing

I. *Platelet Aggregation Study*

The efficacy of entry 4, columns 3 and 4, in resisting agonist-induced platelet aggregation initiated by arachidonic acid and adenosine diphosphate (ADP) is provided in Table 2.

Table 2
Effect of entry 4 shown in columns 3 and 4 in resisting agonist-induced platelet aggregation induced by arachidonic acid and ADP

Agonist	Agonist Response: Max Response (%)	Entry 4, Pretreatment	
		1×10^{-7} M	1×10^{-6} M
Arachidonic Acid (800 μM)	101.6 + 1.3	98.0 + 1.8	98.8 + 1.1
ADP (20 μM)	100 + 0 (standard)	99.9 + 1.4	97.6 + 1.8
ADP (2 μM)	73.8 + 11.17	68.3 + 12.9	73.1 + 14.3

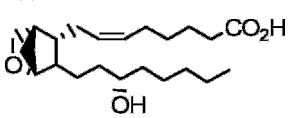
II. *Effects on Intraocular Pressure*

The effect of selected experimental agents on intraocular pressure was measured using an applanation pneumatonometry. In this testing phase, dogs and monkeys were administered 25 μl of a selected experimental agent dissolved in a vehicle to the ocular surface, while the contralateral eye received the vehicle alone as a control. Dog and monkey intraocular pressure studies are provided in Tables 3 and 4, respectively.

Table 3 The effect of experimental agents, Entries 4 and 7, and reference agent, 9,11-dideoxy-9α,11α, methanoepoxy prostaglandin $F_{2\alpha}$, on canine intraocular pressure at predetermined times after dosing

Entry	Dose(%)	2 HR	4 HR	6 HR
Example 7	0.01	−9.7[a]	−11.4[a]	−11.25[a]
Example 4	0.1	−6.7[a]	−7.7[a]	−8.5[a]
Reference[b]	0.1	+0.86	+1.75	+2.7

[a] $p < 0.01$, Student's paired t test.
[b] Reference consisted of 9,11-dideoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$, (**I**).



(I)

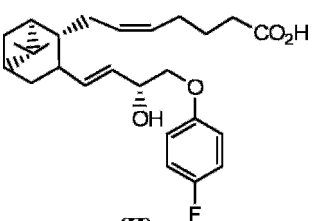
Table 4 The effect of experimental agent, Entry 7, and reference agent, 9,11-dideoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$, on monkey intraocular pressure at predetermined times after dosing

Entry	Dose (%)	2 HR	4 HR	6 HR	24 HR	26 HR	28 HR	30 HR	50 HR
Reference		2.0[a]	0.3	1.0[b]					
7	0.01	−0.4	0	0	−1.0	3.2[b]	−4.6[a]	−3.2	−3.8

[a] $p < 0.01$.
[b] $p < 0.05$ Student's paired t test.

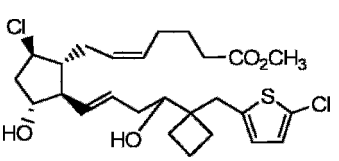
Notes

1. Additional noncoagulating thromboxane agonist derivatives were prepared by the authors (1) in an earlier investigation. [1*S*-[1α,2α-(*Z*),3β-(1*E*, 3*S**)-4α]]-7-[3-(3-hydroxy)-4-(4-iodophenoxy)-1-butenyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-hepten-1-ol, (**II**), is especially preferred for treating acute angle-closure glaucoma.



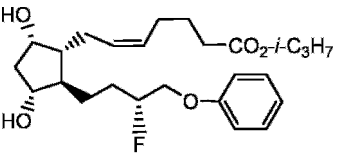
(II)

2. In a subsequent investigation by the authors (2), ω-cycloalkyl 17-heteroaryl PGE_2 derivatives, (**III**), were prepared as EP_2-receptor agonists and used in the treatment of interocular pressure associated with glaucoma.



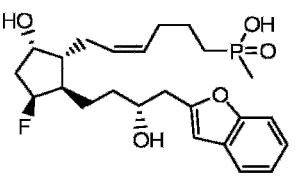
(III)

3. 15-Fluoro prostaglandin, (**IV**), $PGF_{2\alpha}$ analogs effective as thromboxane A_2 receptor agonists were prepared by Klimko (3) and used in the treatment of glaucoma and ocular hypertension.

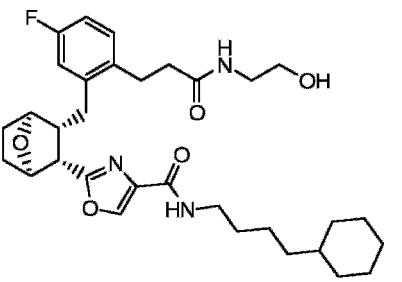


(IV)

4. Thromboxane A_2 receptor antagonists consisting of 2-decarboxy-2-phosphinico prostaglandins (**V**), and prostamide derivatives, (**VI**), prepared by deLong (4) and Krauss (5), respectively, and were used in glaucoma therapy.



(V)



(VI)

References

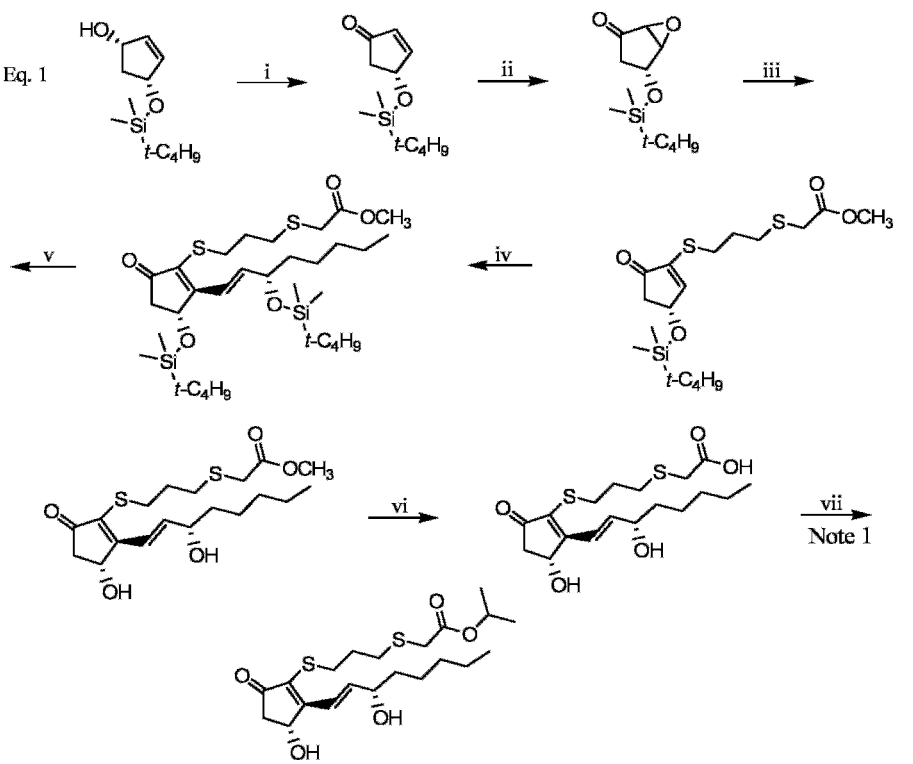
1. R.M. Burk *et al*., US Patent 6,818,779 (November 16, 2004)
2. R.M. Burk *et al*., US Patent 7,022,726 (April 4, 2006)
3. P.G. Klimko *et al*., US Patent 6,680,339 (January 20, 2004) and US Patent 6,649,653 (November 18, 2003)
4. M.A. deLong *et al*., US Patent 7,074,942 (July 11, 2006)
5. A.H. Krauss *et al*., US Patent 7,045,634 (May 16, 2006)

B. Prostanoic Acid Derivatives as EP4 Agonists

Title EP_4 Agonists as Agents for Lowering Intraocular Pressure
D.F. Woodward *et al.*, US Patent 6,956,057 (October 18, 2005)
Assignee Allergan, Inc.
Utility Short-term Treatment of Ocular Hypertension

Invention Significance 3,7-Thiaprostanoic acid derivatives have been prepared which are potent ocular hypotensives suitable for both long- and short-term management of glaucoma. These agents are particularly useful in pre- and postsurgical adjuncts especially as postlaser trabeculectomy ocular hypertensive episodes.

Reaction



i- Tetrapropylammonium perruthenate, 4-methylmorpholine *N*-oxide, molecular sieves, CH_2Cl_2
ii- Hydrogen peroxide, sodium hydroxide, water

iii- CH_2Cl_2, (3-mercaptopropylsulfanyl)acetic acid methyl ester, alumina
iv- *t*-Butyllithium, *t*-butyl[(*S*)-1-((*E*)-2-iodovinyl) hexyloxy]dimethylsilane, diethyl ether, lithium 2-thienylcyanocuprate
v- Hydrogen fluoride–pyridine, acetonitrile
vi- Acetonitrile, cationic exchange resin
vii- Isopropyl-*p*-tolyltriazene, acetone

Experimental

1. **Preparation of (*R*)-4-(*t*-butyldimethylsilanyloxy)cyclopent-2-enone**

   Tetrapropylammonium perruthenate (0.027 mmol) was added to a mixture of (1*S*,4*R*)-4-(*t*-butyldimethylsilanyloxy)cyclopent-2-enol (0.54 mmol), 4-methylmorpholine *N*-oxide (0.81 mmol), and 270 mg crushed 4 Å molecular sieves dissolved in 10 ml CH_2Cl_2. The mixture was stirred 30 minutes at ambient temperature, then filtered through a plug of silica gel with CH_2Cl_2, concentrated, and the product isolated in 86% yield.

2. **Preparation of (*R*)-4-(*t*-butyldimethylsilanyloxy)-6-oxabicyclo[3.1.0]hexan-2-one**

   The Step 1 product (11.5 mmol) dissolved in 30 ml methyl alcohol was treated with 4.5 ml 30% hydrogen peroxide containing 46 µl 1 M NaOH, then stirred 90 minutes at 0°C. The mixture was concentrated, washed with brine, and extracted three times with CH_2Cl_2. The combined organics were rewashed with brine, dried with Na_2SO_4, reconcentrated, and the product isolated.

3. **Preparation of {3-[(R)-3-(t-butyldimethylsilanyloxy)-5-oxocyclopent-1-enylsulfanyl]-propylsulfanyl}acetic acid methyl ester**

   The Step 2 product dissolved in 30 ml CH_2Cl_2 was treated with (3-mercaptopropyl-sulfanyl)acetic acid methyl ester (10.7 mmol), then cooled to 0°C, and basic alumina (11.9 g) added. The mixture was then stirred 18 hours at ambient temperature, filtered through celite, and concentrated. The residue was purified by flash chromatography with silica gel using hexane/EtOAc, 6:1, and the product isolated in 80% yield.

4. **Preparation of (3-{(1*R*,2*S*,3*R*)-3-(*t*-butyldimethylsilanyloxy)-2-[(*S*)-(*E*)-3-(*t*-butyldimethylsilanoxy)oct-1-enyl]-5-oxocyclopentylsulfanyl} propylsulfanyl) acetic acid methyl ester**

   t-Butyl[(*S*)-1-((*E*)-2-iodovinyl) hexyloxy]dimethylsilane (1.25 mmol) dissolved in 6.0 ml diethyl ether was cooled to −78°C, then treated dropwise with 1.47 ml 1.7 M *t*-butyllithium in pentane. The mixture was stirred for 30 minutes, then further treated with 6.0 ml 0.25 M lithium 2-thienylcyanocuprate in THF, and stirred an additional 30 minutes. This solution was then treated with the Step 3 product (1.1 mmol) dissolved in 1 ml diethyl ether, then stirred for additional 60 minutes, and quickly poured

into saturated NH_4Cl solution cooled to 0°C. The mixture was extracted with EtOAc, then washed with brine, dried using Na_2SO_4, and concentrated. The residue was purified by flash chromatography using 100% hexane followed by hexane/EtOAc, 8:1, and the product isolated in 39% yield.

5. **Preparation of {3-[(1*R*,2*S*,3*R*)-3-hydroxy-2-((*S*)-(*E*)-3-hydroxyoct-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid methyl ester**

   Hydrogen fluoride–pyridine (220 μl) was added to a solution of the Step 4 product (0.11 mmol) in 2.0 ml CH_3CN at 0°C, then stirred 60 minutes at ambient temperature. The mixture was recooled to 0°C, then quenched with saturated $NaHCO_3$ solution, and extracted four times with CH_2Cl_2. The combined organics were washed with brine, dried, and concentrated. The residue was purified by flash chromatography using 100% CH_2Cl_2 followed by CH_2Cl_2/methyl alcohol, 30:1, and the product isolated in 90% yield.

6. **Preparation of {3-[(1*R*,2*S*,3*R*)-3-hydroxy-2-((*S*)-(*E*)-3-hydroxyoct-1-enyl)-5-oxocyclopentylsulfanyl]propylsulfanyl}acetic acid**

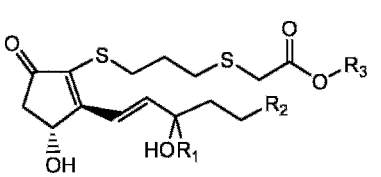
   The Step 5 product (0.124 mmol) dissolved in 10 ml CH_3CN was treated with 3.0 ml phosphate buffer (pH 7.2) followed by 400 μl cationic exchange resin (1.34 mol/l), then stirred 16 hours at 23°C, and extracted three times with EtOAc. The combined extracts were washed with brine, dried, and concentrated. The residue was purified by flash chromatography using EtOAc and the product isolated in 11% yield.

7. **Preparation of {3-[(1*R*,2*S*,3*R*)-3-hydroxy-2-((*S*)-(*E*)-3-hydroxyoct-1-enyl)-5-oxocyclopentylulfanyl]propylsulfanyl}acetic acid isopropyl ester**

   Isopropyl-*p*-tolyltriazene (200 μl) was added dropwise to a solution of the Step 6 product (0.026 mmol) in 5.0 ml acetone, then stirred 60 minutes at ambient temperature, and quenched with 1 M HCl. The mixture was concentrated and the residue extracted twice with CH_2Cl_2, dried, filtered, and then reconcentrated. The residue was purified by flash chromatography using hexane/EtOAc, 4:1, and the product isolated in 38% yield.

Derivatives

Table 1 Selected thiaprostanoic acid derivatives and their corresponding testing data indicating varying effectiveness as EP_4 agonists. Only limited product characterization data supplied by author



Entry	R_1	R_2	R_3	EP_4
9L	Hydrogen	2-Naphthyl	Methyl	40
14H	Hydrogen	2-Benzothienyl	Hydrogen	27
21L	Hydrogen	2-Naphthyl	Ethyl	13
24L	Hydrogen	3-Benzothienyl	Hydrogen	30
25L	Hydrogen	3-Benzothienyl	Hydrogen	5
34H	Methyl	2-Benzothienyl	Hydrogen	32

Testing

I. *EP_4 Agonist Assay*

Human Recombinant EP_4 Receptor: Stable Transfectants

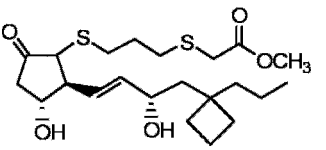
Plasmids encoding the human EP_4 receptor were prepared by cloning the respective coding sequences into the eukaryotic expression vector pCEP4. The cells were human embryonic kidney cells (HEK-293) transfected with the EBNA-1 protein. These HEK-293–EBNA cells were grown in a medium containing Geneticin, G418, to maintain expression of the EBNA-1 protein. HEK-293 cells were grown in DMEM with 10% fetal bovine serum, 250 μg/ml G418, and 200 μg/ml gentamicin or penicillin/streptomycin. Selection of stable transfectants was achieved with 200 μg/ml hygromycin.

For transfection, the cells were grown to 50–60% confluency, then plasmid pCEP4 incorporating cDNA inserts for the respective human prostanoid receptor and HEPES-buffered saline added dropwise. After 30 minutes, 9 ml DMEM and DNA/DMEM/calcium phosphate mixture were added to the cells. Thereafter, the cells were incubated for 5 hours at 37°C, calcium phosphate solution removed, and the cells treated with 10% glycerol in DMEM. The glycerol solution was then replaced by DMEM with 10% FBS, the cells incubated overnight, and the medium was replaced by DMEM/10% FBS containing G418 and penicillin/streptomycin. The following day, hygromycin B was added.

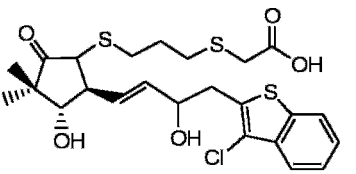
Ten days after transfection, hygromycin B-resistant clones were individually evaluated with selected experimental agents. Testing results are provided in Table 1.

Notes

1. 3,7-Thiaprostanoic acid derivatives, (**I**) and (**II**), prepared by co-author Burke (1) and Donde (2), respectively, were effective as EP_4 agonists and used in lowering intraocular pressure.

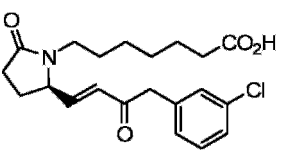


(I)

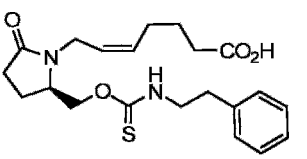


(II)

2. 8-Azaprostaglandin, (**III**), and -thiocarbonate analogs, (**IV**), prepared by Old (3,4), respectively, were as effective as EP_4 agonists and used in treating ocular hypertensive conditions resulting from postsurgical and postlaser trabeculectomy ocular hypertensive episodes.

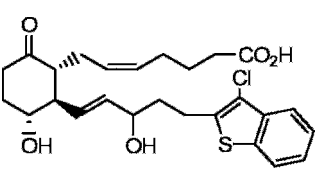


(III)



(IV)

3. Cyclohexyl prostaglandin analogs derivatized with 3-chloro-benzo[b]thiophen-2-yl, (**V**), prepared by Old (5) were effective as EP_4 agonists and used in the treatment and management of glaucoma.



(V)

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2. Y. Donde, US Patent 6,875,787 (April 5, 2005)
3. D.W. Old *et al*., US Patent 6,906,097 (June 14, 2005) and US Patent 6,573,294 (June 3, 2003)
4. D.W. Old *et al*., US Patent 7,005,442 (February 28, 2006) and US Patent 6,734,201 (May 11, 2004)
5. D.W. Old *et al*., US Patent 7,015,243 (March 21, 2006)

CHAPTER XXIII

Osteoporosis

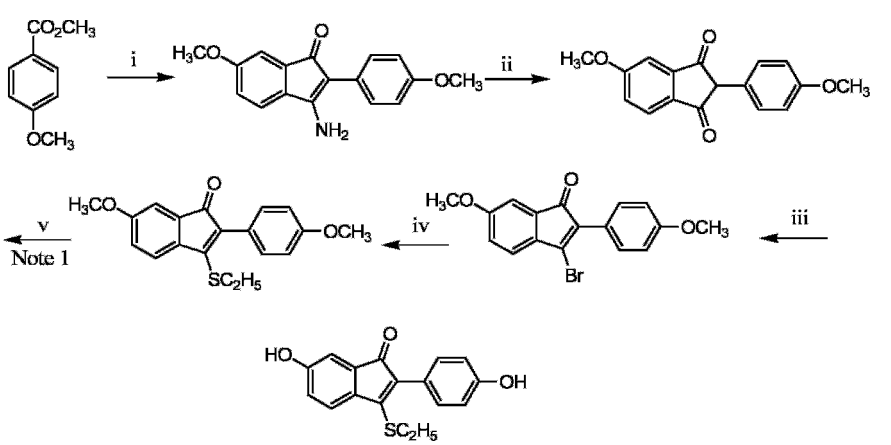
I. Estrogen Receptor, ER-β, Agonists

Title Substituted Indenones as Estrogenic Agents
R.E. McDevitt *et al.*, US Patent 6,903,238 (June 7, 2005)
Assignee Wyeth
Utility Treatment of Osteoporosis

Invention Significance Hormone replacement therapy for osteoporosis by postmenopausal women using estrone, estriol, or ethynyl estradiol not containing progestins is associated with higher risks of endometriosis and/or endometrical cancer. This invention addresses the need for a bone-sparing treatment option utilizing estrogenic agents that minimize proliferative effects in the uterus and breast.

Reaction

Eq. 1



i- 4-Methoxyphenylacetonitrile, THF, lithium diisopropylamide
ii- Sulfuric acid, water
iii- Triphenylphosphine, carbon tetrabromide, $CHCl_3$
iv- Sodium ethanthiolate, DMF, triethylamine
v- CH_2Cl_2, borontrifluoride, CH_2Cl_2

Experimental

1. **Preparation of 3-amino-5-methoxy-2-(4-methoxyphenyl)-1H-inden-1-one**

   A mixture consisting of methyl 4-methoxybenzoate (7.47 mmol) and 4-methoxyphenylacetonitrile (6.79 mmol) dissolved in 15 ml THF was added to 20 ml 2.0 M lithium diisopropylamide at −10°C, then gradually warmed to ambient temperature, and stirred overnight. It was then quenched with water and concentrated. The residue was recrystallized using isopropyl alcohol and the product isolated in 65% yield as a red-orange solid, mp = 213°C.

   MS *m/z* 281 $(M+)^+$
   Analysis Calc. for $C_{17}H_{15}NO_3$: C, 72.58; H, 5.37; N, 4.98. Found: C, 71.7; H, 5.21; N, 4.87

2. **Preparation of 5-methoxy-2-(4-methoxyphenyl)-1H-inden-1,3(2H)-dione**

   The Step 1 product (1.78 mmol) was suspended in a solution of 20% sulfuric acid, then heated to 120°C for 60 minutes. The reaction was cooled, the residual washed with water, and the product isolated as a light white solid in 99% yield, mp = 163°C.

   MS *m/z* 281 $(M-H)^+$
   Analysis Calc. for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00

3. **Preparation of 3-bromo-6-methoxy-2-(4-methoxyphenyl)-1H-inden-1-one and 3-bromo-5-methoxy-2-(4-methoxyphenyl)-1H-inden-1-one**

   Step 2 product (1.77 mmol) and triphenylphosphine (1.95 mmol) were suspended in 10 ml anhydrous $CHCl_3$, then heated to 45°C, and treated with CBr_4 (1.95 mmol) dissolved in 2 ml $CHCl_3$ over 15 minutes, and stirred 4 hours. The solution was then washed with EtOAc and concentrated. The residue was purified by flash chromatography using 10–20% EtOAc/petroleum ether, providing a partially separated mixture of the following:

   3-bromo-6-methoxy-2-(4-methoxyphenyl)-1H-inden-1-one as a red solid, mp = 152°C

   MS *m/z* 344 $(M)^+$

and 3-bromo-5-methoxy-2-(4-methoxyphenyl)-1H-inden-1-one as an orange solid, mp = 142°C

MS *m/z* 344 $(M)^+$
Analysis Calc. for $C_{17}H_{13}BrO_3$: C, 59.15; H, 3.8; N, 0.00. Found: C, 58.94; H, 3.58; N, 0.06

4. **Preparation of 3-(thioethyl)-6-methoxy-2-(4-methoxyphenyl)-1H-inden-1-one**

A mixture consisting of 3-bromo-6-methoxy-2-(4-methoxyphenyl)-1H-inden-1-one (0.4345 mmol), sodium ethanethiolate (1.09 mmol) and 0.5 ml triethylamine dissolved in 3 ml DMF was heated 24 hours at 80°C and then cooled. The mixture was then poured into water and extracted with EtOAc. Extracts were washed with brine, dried, purified by flash chromatography using 10% EtOAc/petroleum ether, and the product isolated in 89% yield as a dark red solid, mp = 88−90°C.

MS *m/z* 327 $(M+H)^+$
Analysis Calc. for $C_{19}H_{18}O_3S$: C, 69.91; H, 5.56; N, 0.00. Found: C, 69.95H, 5.66; N, 0.04

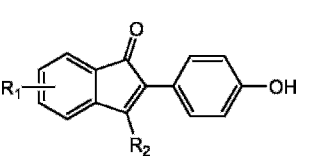
5. **Preparation of 3-(ethylthio)-6-hydroxy-2-(4-hydroxyphenyl)-1H-inden-1-one**

The Step 4 product (0.89 mmol) dissolved in 2.0 ml CH_2Cl_2 cooled to −78°C was treated with BF_3 (5.3 mmol), then gradually warmed to ambient temperature, and stirred 60 minutes. The mixture was then poured into an ice slurry containing NH_4OH and extracted with EtOAc. Combined extracts were washed with brine, dried, and concentrated. The residue was purified by flash chromatography using 5–10% ethyl alcohol/CH_2Cl_2 and the product isolated as a dark red solid, mp = 78−81°C.

MS *m/z* 299 $(M+H)^+$
Analysis Calc. for $C_{17}H_{14}O_3S \bullet 1.0H_2O$: C, 64.54; H, 5.10; N, 0.00. Found: C, 64.92; H, 5.10; N, 0.43

Derivatives

Table 1 Selected 2-(4-hydroxyphenyl)-1H-inden-1-one derivatives and their corresponding melting points and mass spectral characterization data



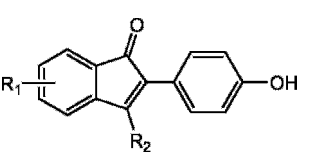
R_1	R_2	mp (°C)	MS $(M+H)^+$
5-Hydroxyl	Methyl	138–146	299
5-Hydroxyl	Phenyl	222–225	315
5-Hydroxyl	Thioethyl	100–102	326
5,7-Dihydroxyl	Phenyl	128–130	331
6-Hydroxyl	Thioethyl	88–90	327
6-Hydroxyl	Methyl	240–245	253
4,6-Dihydroxy	Bromine	350	333
4,6-Dihydroxy	Ethylthio	75–77	357

Testing

I. *Selective Estrogen Receptor Modulator Assay*

Experimental agents were evaluated for their ability to compete with the reference 17-β-estradiol using the radioligand binding assay described by Miller (1) and to determine the relative binding affinities of selected experimental agent for both ERα and ERβ receptors. Testing results are provided in Table 2.

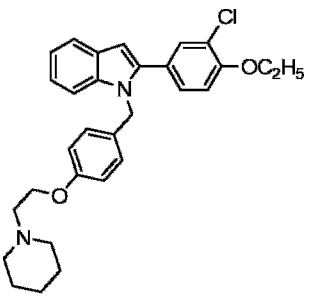
Table 2 ERα and ERβ binding affinities for selected 2-(4-hydroxyphenyl)-1H-inden-1-one derivatives. Although derivatives exhibited a range of activity, the higher receptor affinity selectivity profile of ERβ over ERα indicates their usefulness in treating disorders modulated by ERβ



R_1	R_2	Erβ IC_{50}(μM)	ER αIC_{50}(μM)
6-Hydroxyl	Thioethyl	0.008	0.01
6-Hydroxyl	Hydrogen	0.008	0.01
5-Hydroxyl	Hydroxyl	2.70	>5.0
5,7-Dihydroxyl	Methyl	0.002	0.031
4,6-Dihydroxyl	Thiomethyl	0.150	0.50
4,6-Dihydroxyl	Thioethyl	0.002	0.003

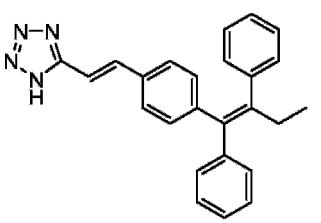
Notes

1. Estrogenic replacement agents consisting of 2-phenyl-1-[4-(2-aminoethoxy)-benzyl]-indole derivatives, (**I**), were prepared by Miller (2) and used in the treatment of osteoarthritis while showing little or no uterine stimulation.



(I)

2. Selective ERα and ERβ receptor modulators consisting of triphenylethylenes, (**II**), and naphthyl benzoxazole derivatives, (**III**), were prepared by Kaltenbach (3) and Malamas (4), respectively, and used in treating estrogen-stimulated diseases such as breast, uterine, and ovarian cancers.



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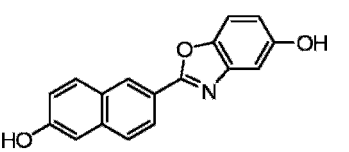
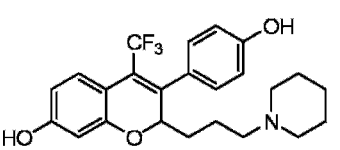


(III)

3. Estrogen antagonists consisting of 4-fluoroalkyl-2H-benzopyrans, (**IV**), and 3,4-dihydroquinoline derivatives were prepared by Kuenzer (5) and Wallace (6), respectively, for use as antiuterus growth agents.



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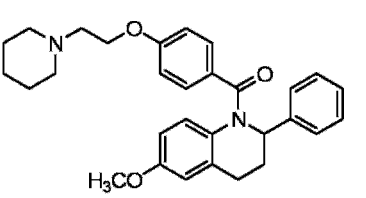

(V)

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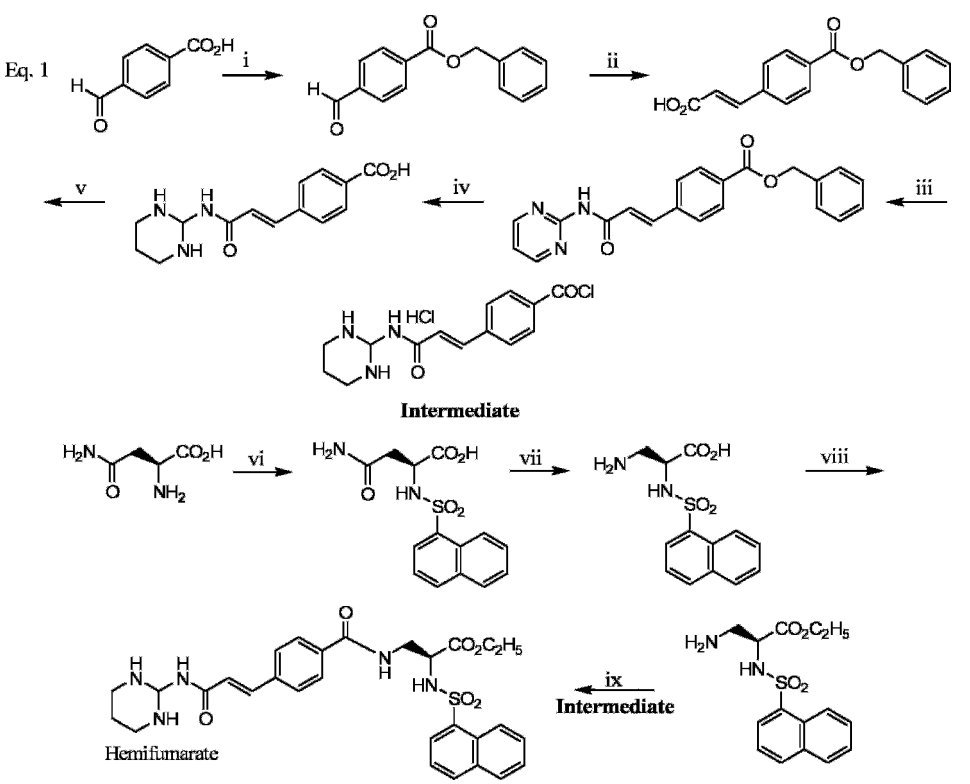
1. C.P. Miller *et al*., US Patent 5,998,402 (December 7, 1999)
2. C.P. Miller *et al*., US Patent 6,951,852 (October 4, 2005) and US Patent 6,787,538 (September 7, 2004)
3. R.F. Kaltenbach *et al*., US Patents 7,045,540 (May 16, 2006) and US Patents 6,927,224 (August 9, 2005)
4. M.S. Malamas *et al*., US Patents 6,960,607 (November 1, 2005)
5. H. Kuenzer *et al*., US Patents 6,844,336 (January 18, 2005)
6. O.B. Wallace, US Patents 7,056,931 (June 6, 2006)

II. VITRONECTIN $\alpha_v\beta_3$ RECEPTOR ANTAGONISTS

Title 1,4,5,6-Tetrahydropyrimidine Derivative as a Vitronectin Inhibitor
G. Breipohl *et al.*, US Patent 6,900,318 (May 31, 2005)
Assignee Aventis Pharma Deutscland GmbH
Utility Treatment of Osteoporosis

Invention Significance Bone resorption is caused by osteoclasts attached to the surface which secrete proteolytic enzymes and acids causing bone destruction. The attachment of osteoclasts to the bones is controlled by vitronectin receptors $\alpha_v\beta_3$. A method of controlling bone resorption disorders, including osteoporosis, using selected vitronectin inhibitors is described.

Reaction



i- DMF, potassium carbonate, benzyl bromide
ii- Malonic acid, pyridine
iii- Toluene, thionyl chloride, CH_2Cl_2, 2-aminopyrimidine
iv- Hydrogen, acetic acid, 10% palladium on charcoal
v- Toluene, thionyl chloride
vi- Sodium hydroxide, THF, naphthalene-1-sulfonyl chloride
vii- Sodium hydroxide, water, bromine, sodium hypobromite
viii- Ethyl alcohol, hydrogen chloride
ix- CH_2Cl_2, sodium bicarbonate, fumaric acid

Experimental

1. **Preparation of benzyl 4-formylbenzoate**

   4-Formylbenzoic acid (2 mol) dissolved in 1000 ml DMF was treated with K_2CO_3 (2.2 mol) over 30 minutes followed by benzyl bromide (2.2 mol), then stirred 4 hours at 45°C. The mixture was poured into 3000 ml ice water, extracted three times with 1000 ml EtOAc, dried with Na_2SO_4, concentrated, and 503 g crude oil product isolated. The material was used without further purification.

2. **Preparation of 4-benzyloxycarbonylcinnamic acid**

   Malonic acid (2.4 mol) dissolved in 360 ml pyridine was treated with the entire Step 1 product and 20 ml of piperidine, then refluxed 7 hours. The mixture was cooled to ambient temperature, then treated with 2000 ml water, and the pH lowered to 1.8 using 600 ml 12 M HCl. A precipitate that formed was isolated then washed with water, dried at 50°C under reduced pressure, and the product isolated.

   MS (CI) 283.2 $(M+H)^+$
   ^{1}H-NMR (DMSO-d_6) δ (ppm) 12.58 (s, broad, 1H); 8.02 (d, 2H); 7.85 (d, 2H); 7.65 (d, 1H); 7.55–7.30 (m, 5H); 6.7.0 (d, 1H); 5.38 (s, 2H)

3. **Preparation of benzyl 4-(2-(pyrimidin-2-ylcarbamoyl)vinyl)benzoate**

   The Step 2 product (1 mol) suspended in 2000 ml toluene was treated with 108 ml thionyl chloride, then stirred 7 hours until a clear solution of the acid chloride was obtained, and then concentrated. The residue was dissolved in 1000 ml of CH_2Cl_2, then treated dropwise over 1 hour with 2-amino-pyrimidine (1 mol) dissolved in 2000 ml CH_2Cl_2 containing 81 ml of pyridine at 0°C. The mixture was stirred 1 hour at ambient temperature, then concentrated, and the residue dissolved in 2500 ml hot ethyl alcohol. This solution was diluted with 1500 ml water, then slowly cooled to 0°C, and a precipitate formed. The solid was isolated, washed with water, dried at 60°C, and the product isolated in 88% yield.

   MS (ES) 360.2 $(M+H)^+$
   ^{1}H-NMR (DMSO-d_6) δ (ppm) 10.85 (s, broad, H); 8.72 (s, 1H); 8.68 (s, 1H); 8.02 (d, 2H); 7.85 (d, 2H); 7.65 (d, 1H); 7.55–7.18 (m, 7H): 5.38 (s, 2H)

4. **Preparation of 4-(2-(1,4,5,6-tetrahydropyrimidin-2-ylcarbamoyl)ethyl)benzoic acid**

A mixture consisting of the Step 3 product (0.85 mol), 10% palladium on charcoal (25 g) and 8000 ml 20% acetic acid was hydrogenated 6 hours at 40°C under 2 bar hydrogen pressure. After standing overnight, the mixture was warmed to 70°C, then filtered. The catalyst was washed with 1500 ml 20% acetic acid and the combined filtrates concentrated. The residue was recrystallized in 1000 ml water, then washed with cold water, dried under reduced pressure at 50°C, and the product isolated in 90.6% yield.

MS (ES) 276.1 $(M+H)^+$
^{1}H-NMR (trifluoroacetic acid) δ (ppm) 11.55 (s); 8.15 (d, 2H); 7.40 (d, 2H); 3.62 (dd, 4H); 3.18 (dd, 2H); 2.95 (dd, 2H); 2.1.5 (m, 2H)

5. **Preparation of 4-(2-(1,4,5,6-tetrahydropyrimidin-2-ylcarbamoyl)ethyl)benzoyl chloride hydrochloride**

The Step 4 product (0.4 mol) suspended in 1500 ml of toluene was treated with 35 ml thionyl chloride, then stirred 2 hours at 70°C, and further treated an additional 16 ml thionyl chloride until sulfur dioxide evolution stopped. The solution was concentrated and 139.5 g crude product isolated. The material was used without further purification.

MS (FAB) 294.1 ($(M+H)^+$ of the free base)
^{1}H-NMR ($CDCl_3$) δ (ppm) 13.05 (s, broad, 1H); 9.35 (s, broad, 2H); 8.05 (d, 2H); 7.40 (d, 2H); 3.50 (m, 4H); 3.10 (dd, 2H); 2.95 (dd, 2H); 2.05 (m, 2H)

6. **Preparation of (2*S*)-3-carbamoyl-2-(naphthalene-1-sulfonylamino)propionic acid**

(*S*)-Asparagine (1 mol) was dissolved in a mixture of 800 ml water and 500 ml 2 M NaOH and 500 ml THF added. This mixture was then cooled to 0°C while keeping the pH between 12.0 and 12.5 with 2 M NaOH. The solution was then treated with naphthalene-1-sulfonyl chloride (1 mol) dissolved in 500 ml THF over 1 hour and stirred 1 hour at 0°C while keeping the pH 12.5. The mixture was stirred for additional 2 hours at ambient temperature and then left overnight. The solution pH was then lowered to 7 using 12 M HCl and THF removed under vacuum. The solution was then cooled to 0°C and the aqueous residue acidified to pH 0.8 using 12 M HCl. After stirring 30 minutes, a precipitate was filtered, washed with water, dried, and the product isolated in 75% yield.

MS (ES) 323.1 $(M+H)^+$
^{1}H-NMR (DMSO-d_6) δ (ppm) 12:50 (s, 1H); 8.65 (d, 1H); 8.35 (d, 1H); 8.20 (d, 1H); 8.17 (d, 1H); 8.09 (d, 1H); 7.80-7.60 (m, 3H); 7.28 (s, 1H); 6.82 (s, 1H); 4.15 (m, 1H); 2.45 (dd, 1H); 2.23 (dd, 1H)

7. **Preparation of (2*S*)-3-amino-2-(naphthalene-1-sulfonylamino)propionic acid**

In the first vessel, a solution of NaOH (4.1 mol) dissolved in 940 ml water was treated with bromine (0.5 mol) over 45 minutes at 0°C. In a second reaction vessel, the Step 6 product (0.4 mol) dissolved in 400 ml 2 M NaOH containing 16 g NaOH was cooled to 5°C, then treated with the sodium hypobromite solution from the first vessel while keeping reaction below 10°C. The mixture was stirred for 15 minutes at 10°C, then warmed to 45°C within 30 minutes. The heating was removed and an exothermic reaction proceeded for about 1 hour with a peak temperature of about 50°C. The mixture was gradually heated to 70°C over 20 minutes and maintained at that temperature for 10 minutes, then cooled to 40°C. The solution pH was then lowered to 6.8 using 330 ml 12 M HCl and a precipitate formed. After standing overnight at ambient temperature, the mixture was cooled to 10°C, filtered, washed with water, and the product isolated in 83% yield.

MS (FAB) 295.0 $(M+H)^+$
^{1}H-NMR (DMSO-d_6) δ (ppm) 8.58 (d, 1H); 8.25 (d, 2H); 8.09 (d, 1H); 7.90 (s, very broad, 3H); 7.80–7.60 (m, 3H); 3.35 (s, very broad, 2H); 3.18 (m, 1H); 3.05 (dd, 1H); 2.82 (dd, 1H)

8. **Preparation of ethyl (2*S*)-3-amino-2-(naphthalene-1-sulfonylamino)propionate hydrochloride**

The Step 7 product (0.5 mol) suspended in 1000 ml ethyl alcohol was treated with hydrogen chloride gas for 2 hours and a clear solution obtained. The solution was concentrated and the residue dissolved in hot ethyl alcohol. Diisopropyl ether was added until precipitation began and the product crystallized overnight at ambient temperature. The mixture was filtered, washed with diisopropyl ether, dried, and the product isolated in 83% yield.

MS (ES) 323.2 ($(M+H)^+$ of the free base)
^{1}H-NMR (DMSO-d_6) δ (ppm) 8.95 (s, broad, 1H); 8.63 (d, 1H); 8.40-8.20 (m, 3H); 8.13 (d, 1H); 8.09 (d, 1H); 7.80–7.60 (m, 3H); 4.12 (m, 1H); 3.50–3.36 (m, 2H); 3.08 (dd, 1H); 2.92 (dd, 1H); 0.55 (t, 3H)

9. **Preparation of ethyl (2*S*)-2-naphthalene-1-sulfonylamino-3-(4-(2-(1,4,5, 6-tetrahydropyrimidin-2-ylcarbamoyl)ethyl)benzoylamino)propionate hemifumarate**

The Step 8 product (0.07 mol) was dissolved in 100 ml CH_2Cl_2 by the addition of 30 ml aqueous $NaHCO_3$ (0.21 mol), then treated with the Step 5 product (0.07 mol) over 30 minutes. The mixture was stirred for additional 1 hour and the layers were separated. The organic layer was extracted with $NaHCO_3$ solution and dried using Na_2SO_4. The sodium sulfate was filtered and washed with CH_2Cl_2. The filtrate was concentrated to about 100 ml, then treated with fumaric acid (0.035 mol), and the mixture refluxed until a clear solution was obtained. Upon cooling a precipitate

formed and was completed by addition of 300 ml EtOAc. The solid was isolated, washed with EtOAc, dried, and the product isolated in 79.5% yield, mp = 201°C.

MS (FAB) 580.3 ($(M+H)^+$ of the free base)
^{1}H-NMR (DMSO-d_6) δ (ppm) 9.73 (s, broad, 1H); 8.8 (s, very broad, 1H); 8.63 (d, 1H); 8.33 (t, 1H); 8.16 (d, 1H); 8.09 (d, 1H); 8.01 (d, 1H); 7.72–7.50 (m, 5H); 7.24 (d, 2H); 6.50 (s, 1H); 4.08 (t, 1H); 3.66–3.45 (m, 4H); 3.34 (q, 2H); 3.27 (t, 4H); 2.88 (dd, 2H); 2.59 (dd, 2H); 1.79 (m, 2H); 0.79 (t, 3H)

Derivatives

Only the Step 9 derivative was prepared.

Testing

I. *Parathyroid Hormone-induced Hypercalcemia in the Thyroparathyroidectomized Rat Model of Bone Resorption*

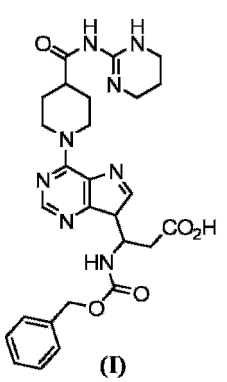
In this in vivo model, stimulation of bone resorption was induced in thyroparathyroidectomized rats by the infusion of parathyroid hormone. The changes in bone resorption were monitored by measuring the serum calcium concentration, which is directly related to the extent of bone resorption.

Results

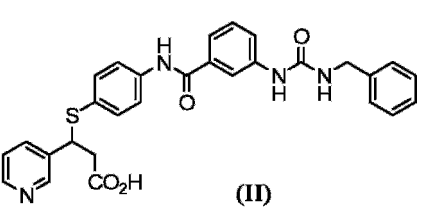
When the experimental agent was administered po twice at 10 mg/kg, bone resorption was diminished by approximately 45%.

Notes

1. Propanoic acid derivatives containing purine, (**I**), prepared by co-author Peyman (1) and 3-sulfanyl-3-pyridinyl, (**II**), prepared by Bandiera (2) were effective as vitronectin receptor antagonists and used in the treatment of bone resorption disorders.

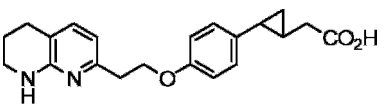


(I)



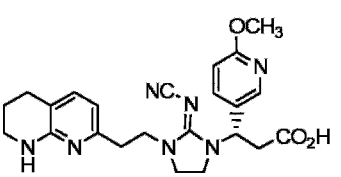
(II)

2. Cycloalkyl alkanoic acids, (**III**), prepared by Srinivasan (3) were effective as vitronectin $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ receptor integrin antagonists and used to decrease bone resorption and to restore the normal balance of bone forming/resorbing period.



(III)

3. Cyclic urea derivatives, (**IV**), prepared by Hutchinson (4) and amine salt derivatives, (**V**), prepared by Wells (5) were effective as $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ receptor integrin antagonists and used for inhibiting bone resorption disorders including osteoporosis and periodontal disease.



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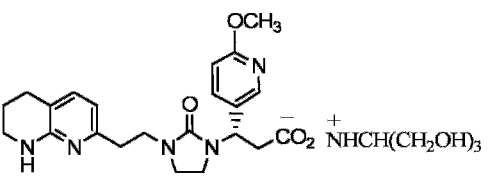
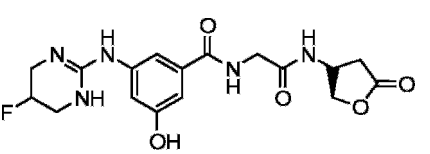


(V)

5. Lactone derivatives, (**VII**), prepared by Ruminiski (6) were selective $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ receptor integrin antagonists and used in treating osteoporosis.



(VI)

References

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2. T. Bandiera *et al*., US Patent 6,974,828 (December 13, 2005)
3. N. Srinivasan *et al*., US Patent 6,921,767 (July 26, 2005)
4. J.H. Hutchinson *et al*., US Patent 6,916,810 (July 12, 2005)
5. K.M. Wells *et al*., US Patent 7,074,930 (July 11, 2006)
6. P. Ruminiski *et al*., US Patent 6,906,051(June 14, 2005)

CHAPTER XXIV

Parkinson's Disease

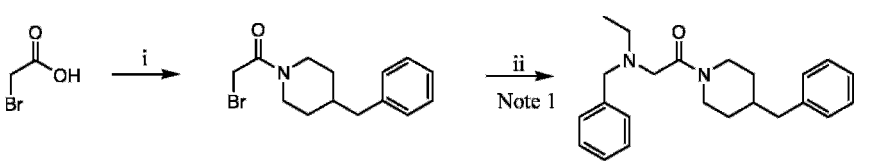
I. Blood–Brain Barrier Crossing Nerve Growth Factor Stimulators

Title Acyclic Piperidine Derivatives
D. Lauffer *et al.*, US Patent 6,949,655 (September 27, 2005)
Assignee Vertex Pharmaceuticals Incorporated
Utility Stimulation of Neural Axons in Nerve Cells in the Treatment of Parkinson's disease

Invention Significance Neurological diseases are associated with the death of or injury to neuronal cells. Although nerve growth factors currently exist, they do not readily cross the blood–brain barrier, are unstable in plasma, and have poor drug delivery properties. This chapter addresses these limitations and is designed for treating neuronal damage associated with Parkinson's disease.

Reaction

Eq. 1



i- CH_2Cl_2, diisopropylcarbodiimide, 1-benzylpiperazine
ii- *N*-Benzyl-*N*-ethylamine, THF

Experimental

1. **Preparation of 1-(4-benzyl-piperidin-1-yl)-2-bromo-ethanone**

   Bromoacetic acid (3.99 mmol) dissolved in 30 ml CH_2Cl_2 was treated with diisopropylcarbodiimide (6.78 mmol) and after 30 minutes a white precipitate formed, which was removed by filtration. The filtrate was treated with 1-benzylpiperazine (5.82 mmol), then stirred 10 hours, and concentrated. The residue was purified by flash chromatography using CH_2Cl_2/EtOAc and the product isolated in 89% yield.

   MS (MH^+) *m/z* 297.88

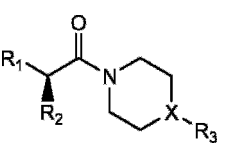
2. **Preparation of 1-(4-benzyl-piperidin-1-yl)-2-*N*-benzyl-*N*-ethylamine-ethanone** (***combinatorial synthesis of compounds***)

   The Step 1 product (0.25 mmol) was added to the wells of a reaction block containing 5 ml THF and treated with *N*-benzyl-*N*-ethylamine (0.5 mmol) or other selected amines neat. The reaction block was shaken 24 hours, filtered, and concentrated. The residue was purified by reverse-phase HPLC with water/acetonitrile (0.1% TFA) and the product isolated as the trifluoroacetate salt.

Derivatives

Selected piperidin-1-yl derivatives are provided in Table 1.

Table 1 Summary of physical constants of selected experimental agents and their corresponding EC_{50} data derived from the Neuroprotection Assay



Entry	R_1	R_2	X	R_3	MS (m/z)	EC_{50} (nM)
5	*N*-Methyl-*N*-benzylamine	Methyl	CH	Benzyl	351.21	A[a]
12	*N,N*-Dimethylamine	H	N	Di(4-fluorophenyl)methyl	374.49	C[b]
13	*N*-Methyl-*N*-benzylamine	2-Butyl	CH	Benzyl	393.61	C
16	*N*-Ethyl-*N*-benzylamine	H	N	Di(4-fluorophenyl)methyl	464.1	C
20	*N,N*-Dibenzylamine	H	N	Di(4-fluorophenyl)methyl	526.1	C

[a] $EC_{50} < 100$ nM.
[b] $EC_{50} > 500$ nM.

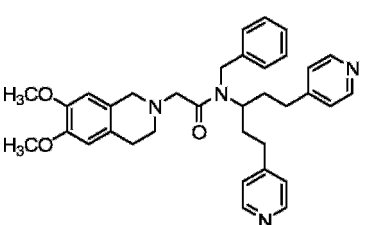
Testing

I. *Neuroprotection Assay*

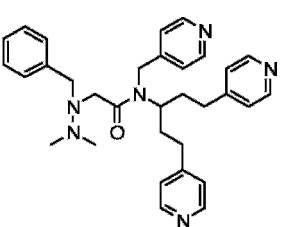
The ventral mesencephalic (VM) region was dissected out of embryonic day Sprague–Dawley rat embryos and dissociated into a single cell suspension by a combination of trypsinization and trituration according to the method of Costantini (1). Dissociated VM cells were plated into poly-L-ornithine-coated 96-well plates at a density of 85 000 cells/well in 100 µl of DMEM supplemented with 18% heat-inactivated horse serum, 0.24% glucose, 2 mM glutamine, and 50 U/ml penicillin/streptomycin, then incubated in a 5% CO_2 incubator. After 1 day in culture (DIV_1) the medium was replaced with 100 µl of a medium consisting of DMEM supplemented with 1 × N2 cocktail (Gibco-BRL), 0.12% glucose, 2 mM glutamine, and 50 U/ml penicillin/streptomycin containing DMSO or various concentrations of selected experimental agents. On DIV_5, neuroexcitotoxic injury was induced by the addition of 100–400 µM of the glutamate receptor agonist NMDA. Cultures were incubated with the neurotoxin for 20 hours and the effects assessed using high-affinity ^{3}H-dopamine uptake according to the method of Park (2). Testing results are provided in Table 1.

Notes

1. In earlier investigations by the authors (3,4), neurotrophic agents consisting of 1,2,3,4-tetrahydoisoquinoline, (**I**), and azo amino acid derivatives, (**II**), respectively, were prepared and used in the treatment of Parkinson's disorder and related neurological diseases.

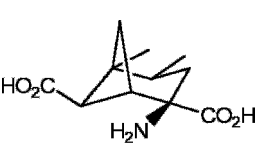


(I)



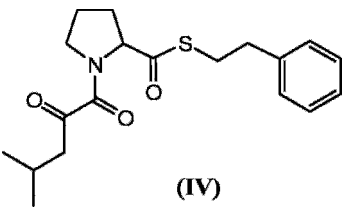
(II)

2. Bicyclic amino acid derivatives, (**III**), prepared by Kozikowski (5) were effective as metabotropic glutamate receptor ligands and used in the treatment of neuronal cell damage associated with Parkinson's disease.



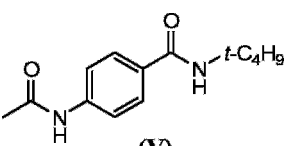
(III)

3. Neurotrophic agents prepared by Hamilton (6) consisting of thioester derivatives, (**IV**), were used in treating neurological disorders including physically damaged nerves and related neurodegenerative diseases.



(IV)

4. Acetamidobenzamide derivatives, (**V**), prepared by Flitter (7) were effective in treating dopamine-associated neurodegenerative disorders including Parkinson's disorder.



(V)

References

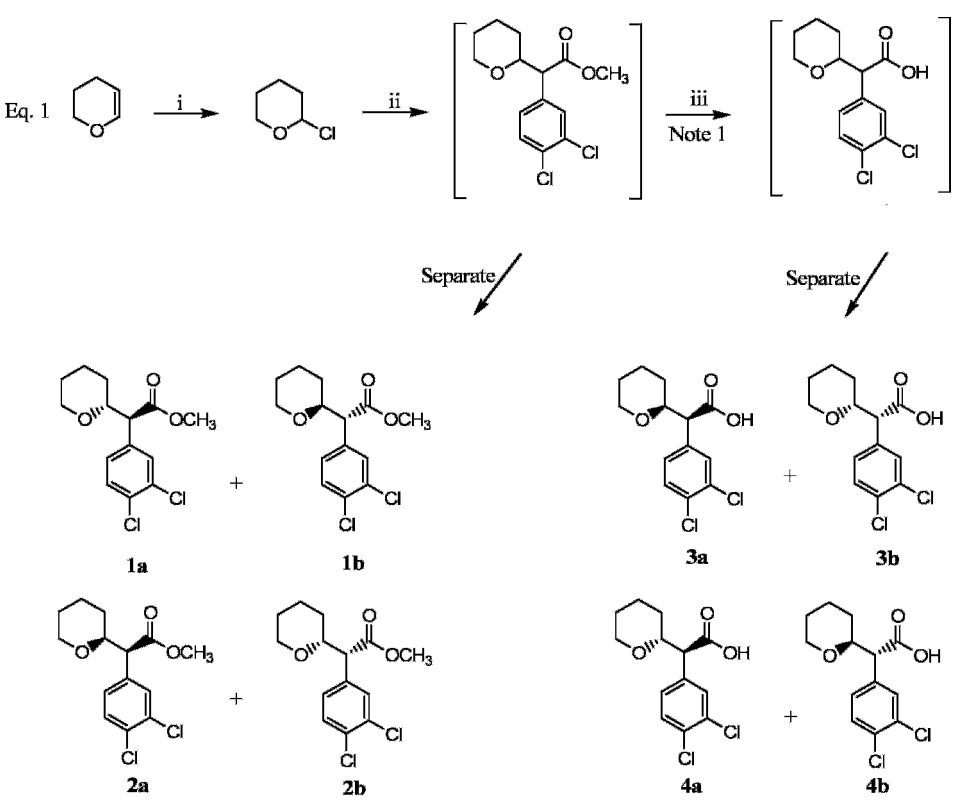
1. G.V. Costantini *et al.*, Neurobiol. Dis., 97–106 (1998)
2. Y. Park *et al.*, Brain Res., 599, 83 (1992)
3. D. Lauffer *et al.*, US Patent 6,747,042 (June 8, 2004)
4. D. Lauffer *et al.*, US Patent 6,716,860 (April 6, 2004)
5. A.P. Kozikowski *et al.*, US Patent 6,825,211 (November 30, 2004)
6. G.S. Hamilton *et al.*, US Patent 6,984,639 (January 10, 2006)
7. W. Flitter *et al.*, US Patent 7,005,546 (February 28, 2006)

II. Selective Serotonin-to-Dopamine Monoamine Transporters

Title Compounds with High Monoamine Transporter Affinity
P.C. Meltzer *et al.*, US Patent 7,026,516 (April 11, 2006)
Assignee President and Fellows of Harvard College and Organix, Inc.
Utility Treatment of Parkinson's Disease

Invention Significance Undesirable side effects associated with the use of monoamine transporters in treating psychiatric and neurological diseases are a result of limited transporter selectivity. To address this problem, medicaments having a selective serotonin-to-dopamine monoamine transport ratio of at least 10 or higher have been prepared.

Reaction



i- Hydrogen chloride, diethyl ether
ii- *n*-Butyllithium, diisopropylamine, diethyl ether, methyl 3,4-dichlorophenylacetate, THF, hydrochloric acid
iii- $CHCl_3$, trimethylsilyl iodide

Experimental

1. **Preparation of 2-chlorotetrahydropyran**

   Dry hydrogen chloride gas was bubbled through a solution of 3,4-dihydro-2H-pyran (0.41 mol) in 150 ml diethyl ether cooled in a dry ice/acetone bath for 2 hours. The mixture was concentrated, the residue fractionally distilled under reduced pressure, and the product isolated in 75% yield as a colorless oil. The material was used without further purification.

   ^{1}H NMR ($CDCl_3$) δ 1.4–1.8 (m, 3H), 1.9–2.2 (m, 3H), 3.7–3.8(m, 1H), 3.9–4.1 (m, 1H), 6.27 (t, $J = 0.54$Hz, 1)

2. **Preparation of 2-(3,4-dichlorophenyl)tetrahydropyran-2-yl acetic acid methyl ester diastereomer mixture**

   n-Butyllithium (20.8 ml, 2.5 M) in hexane was added dropwise to a solution of diisopropylamine (48 mmol) dissolved in 100 ml diethyl ether and stirred 90 minutes at 0°C. The mixture was then treated dropwise with methyl 3,4-dichlorophenylacetate (44 mmol) dissolved in 20 ml THF over 30 minutes and stirred additional 2 hours. It was further cooled to −78°C and stirred 20 minutes, then treated dropwise with the Step 1 product (44 mmol) dissolved in 40 ml of THF. This solution was stirred overnight at ambient temperature and was then treated with 104 ml 0.5 M cold HCl. The mixture was extracted with 400 ml EtOAc and the organic layer washed with brine, dried with Na_2SO_4, and concentrated. The residue was purified by chromatography using 5–15% EtOAc/hexane and a mixture consisting of 2.97 g of (2*S*,2′*R*) Product #1 isolated as an oil and 4.27 g of (2*R*,2′*S*) Product #2 isolated as a solid, mp = 65°C.

   (2*S*,2′*R*) Product #1 (oil):
   $R_f = 0.59$ (20% ethyl acetate in hexane)
   ^{1}H NMR ($CDCl_3$) δ 7.47 (d, $J = 2.2$Hz, 1H), 7.38 (d, 8.5 Hz, 1H), 7.21 (dd, $J = 2.2$, 8.5 Hz, 1H), 3.93–3.80 (m,2H), 3.67 (s, 3H), 3.55 (d, $J = 11.5$Hz, 1H), 3.40–3.26 (m, 1H), 1.90–1.20 (m, 6H)
   ^{13}C NMR 171.74, 136.61, 132.37, 131.56, 130.97, 130.26, 128.49, 78.22, 68.95, 56.94, 52.33, 31.67, 29.97, 25.72, 23.24

   (2*R*,2′*S*) Product #2 (solid):
   $R_f = 0.50$ (20% ethyl acetate in hexane)
   ^{1}H NMR ($CDCl_3$) δ 7.47 (d, $J = 1.9$Hz, 1H), 7.38 (d, $J = 8.3$Hz, 1H), 7.19 (dd, $J = 1.9$, 8.3 Hz, 1H), 3.99 (dt)

^{13}C NMR 172.69, 135.45, 132.83, 132.07, 130.63, 128.18, 79.24, 68.91, 57.41, 52.36, 29.16, 25.72, 23.13

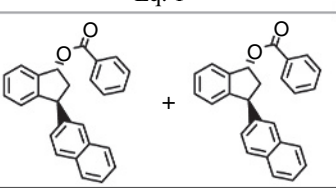
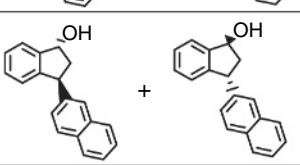
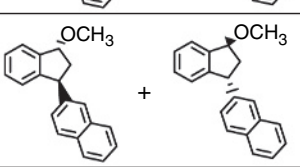
3. **Preparation of 2-(3,4-dichlorophenyl)tetrahydropyran-2-yl acetic acids**

The combined Step 2 product mixture (28 mmol) was dissolved in anhydrous 10 ml $CHCl_3$ and treated with trimethylsilyl iodide (70 mmol), then stirred at 80°C overnight, and concentrated. The residue was then treated with 1% sodium thiosulfate solution and 25 ml of diethyl ether, and the two layers were separated. The organic phase was dried, filtered, and concentrated. The residue was purified by chromatography using 30–50% EtOAc/hexane and acids having a combined weight of 5.2 g were isolated.

^{1}H NMR ($CDCl_3$) δ 7.43 (d, $J = 2.2$Hz, 1H), 7.40 (d, $J = 8.5$Hz, 1H), 7.21 (dd, $J = 8.3$, 2.2 Hz, 1H), 3.98 (dd, $J = 10.8$, 2.3 Hz, 1H), 3.87 (m, 1H), 3.61 (d, $J = 7.4$Hz, 1H), 3.4 (m, 1H), 1.95–1.20 (m, 6H)

Derivatives

Table 1 Selected tetrahydropyran and 3-aryl-oxaindanes derivatives and their corresponding melting points and optical rotations. ^{1}H NMR structural characterization provided by author

Entry	Structure	Melting Point (°C)	$[\alpha]_D^{20}$ ($c = 1$, $CHCl_3$)
3a	Eq. 1	138.9–139.9	+18.8
3b	Eq. 1	139.1–140	–19.1
4a	Eq. 1	124.2–125.2	+14.0
4b	Eq. 1	124.1–125.1	–13.9
8		–	–
9		Clear oils	–
10		Yellowish oils	–

Testing

I. *In Vitro Binding Assays*

The affinities and transporter selectivities of experimental agents were assessed in brain tissue of adult cynomolgus or rhesus monkeys (*Macaca fasicularis* or *Macaca mulatta*). Caudate–putamen was the source of the dopamine and serotonin transporters. The dopamine transporter affinity was measured with [^{3}H]WIN 35428 ([^{3}H]CFT), while the serotonin transporter was measured with [^{3}H]citalopram. Testing data are provided in Table 2.

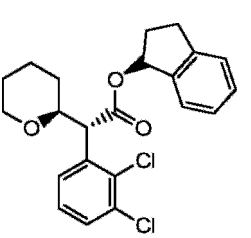
Table 2 Binding affinities data of selected experimental agents as inhibitors of [^{3}H]WIN 35428 binding to the dopamine transporter and [^{3}H]citalopram binding to the serotonin transporter in adult cynomolgus or rhesus monkeys

Entry	DAT IC_{50} (nM)[a]	SERT IC_{50} (nM)[b]	DAT/SERT Ratio
1a	736 ± 59	>10 000	>10
1b	193 ± 3.5	>10 000	>50
2a	34 ± 8.6	1655 ± 317	49
2b	17 ± 1.3	>10 000	>588
8 (mixture)	127	8000	63
9 (mixture)	146	12 000	82.2
10 (mixture)	128	10 000	78

[a] Inhibition of WIN 35428 binding to the dopamine transporter.
[b] Inhibition of citalopram binding to the serotonin transporter.

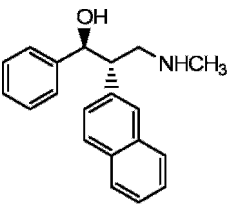
Notes

1. Tetrahydropyranyl esters, (**I**), having favorable DAT-to-SERT monoamine transport ratios were previously prepared by the author (1) and used in the treatment of neurodegenerative diseases.



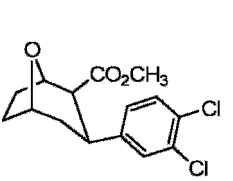
(I)

2. A related structural analog, (**II**), prepared by Richelson (2) was effective as a selective norepinephrine or epinephrine neurotransmitter reuptake inhibitor.

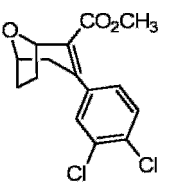


(II)

3. Nonamine tropane derivatives, (**III**), prepared by co-author Madras (3) were selective as serotonin transporters and used in the treatment of neuropsychiatric disorders. Other nonamine tropane derivatives, (**IV**), prepared by the author (4) having a high DAT-to-SERT selectivity were used in treating cocaine addiction.

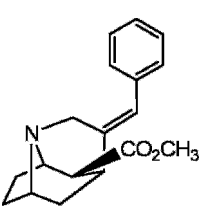


(III)



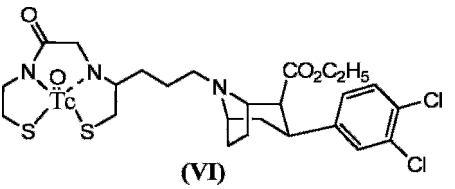
(IV)

4. Tropane derivatives, (**V**), prepared by Kozikowski (5) were highly selective as dopamine transporters as well as for selectively inhibiting serotonin reuptake and/or norepinephrine and used in the treatment of psychiatric and neurodegenerative disorders.



(V)

5. Radiopharmaceutical dopamine transporter imaging agents, (**VI**), having a selective dopamine/serotonin transport ratio of 30 or higher containing a chelating ligand complex to ^{99}Te were previously prepared by the author (6).



(VI)

References

1. P.C. Meltzer *et al.*, US Patent 6,525,206 (February 25, 2003)
2. E. Richelson *et al.*, US Patent 6,914.080 (July 5, 2005)
3. B.K. Madras *et al.*, US Patent 6,677,338 (January 13, 2004)
4. P.C. Meltzer *et al.*, US Patent 6,670,375 (December 30, 2003)
5. A.P. Kozikowski *et al.*, US Patent 6,982,271 (January 3, 2006)
6. P.C. Meltzer *et al.*, US Patent 6,548,041 (April 15, 2003)

CHAPTER XXV

Proliferative Disorders

I. Antiangiogenic Agents

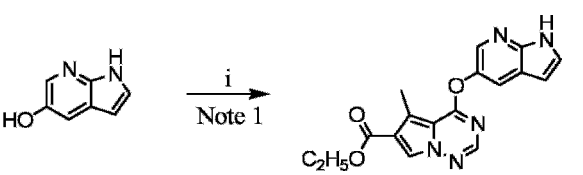
A. Kinase Growth Factor VEGFR-2 and FGFR-1 Inhibitors

Title Azaindole Kinase Inhibitors
R.S. Bhide *et al.*, US Patent 6,969,717 (November 29, 2005)
Assignee Bristol-Myers Squibb Company
Utility Antiangiogenic Agents in the Treatment of Cancer

Invention Significance The overexpression and activation of VEGFR-2 and FGFR-1 in tumor-associated vasculature suggest their role in tumor angiogenesis. Angiogenesis and subsequent tumor growth is inhibited by antibodies directed against VEGF ligand and VEGF receptors. To address this disorder, selectively active kinase growth factor VEGFR-2 and FGFR-1 inhibitors have been prepared that are useful in treating pathological angiogenesis associated with cancer and other metastatic diseases. The current agents are further distinguished by their ease of preparation and high purity.

Reaction

Eq. 1



i- Sodium hydride, DMF, 4-chloro-5-methylpyrrolo[2,1-f]
[1,2,4]triazine-6-carboxylic acid ethyl ester

Experimental

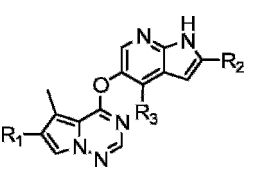
1. **Preparation of 5-methyl-4-(1H-pyrrolo[2,3-b]pyridin-5-yloxy)-pyrrolo [2,1-f][1,2,4]triazine-6-carboxylic acid ethyl ester**

   Sodium hydride (0.36 mmol) was added to 5-hydroxy-7-azaindole (0.36 mmol) dissolved in 1.5 ml DMF at 0°C, then treated with 4-chloro-5-methylpyrrolo[2,1-f] [1,2,4]triazine-6-carboxylic acid ethyl ester (0.32 mmol), and stirred 16 hours at ambient temperature. The reaction was quenched with 20 ml saturated NH_4Cl solution, then extracted three times with 25 ml EtOAc, and the extract washed with 50 ml brine. The solution was dried, then concentrated, and the residue purified by preparative HPLC, $RT = 7.12$ minute.

   ^{1}H-NMR δ 8.10 (1H, s), 7.91 (1H, br. s), 7.82 (1H, s), 7.31 (1H, s), 6.85 (1H, br. s) 4.31 (2H, q, $J = 7.3$ Hz), 2.79 (3H, s), and 1.33 (3H, t, $J = 7.3$ Hz)
   MS *m/z* 338 $(M+H)^+$ and 379 $(M+AcCN)^+$

Derivatives

Table 1 Selected 1,2,4-triazine derivatives and their corresponding mass spectral data. ^{1}H-NMR characterization data supplied by author



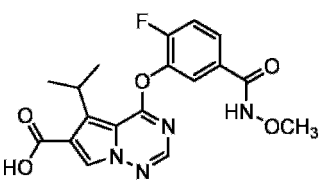
Entry	R_1	R_2	R_3	LCMS $(M+H)^+$
3	Benzoxy	Methyl	Hydrogen	386
5	Hydroxyl	Hydrogen	Fluoro	300
7	(2*S*)-Hydroxyl-1-propoxy	Hydrogen	Fluoro	358
12	3-Methoxy-(2*S*)-hydroxyl-1-propoxy	Hydrogen	Fluoro	388
14	3-Methoxy-(2*S*)-hydroxyl-1-propoxy	Methyl	Fluoro	402
15	2-(*N*-Aminosulfamide)-1-ethoxy	Hydrogen	Fluoro	422
20	2-Amino-1-ethoxy	Hydrogen	Fluoro	385

Testing

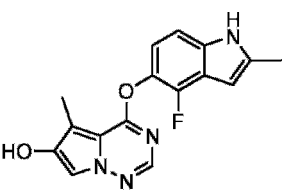
Although testing results were not supplied by author, 4-(4-fluoro-1H-pyrrolo[2,3-b] pyridin-5-yloxy)-5-methyl-pyrrolo[2,1-f][1,2,4]triazin-6-ol was especially preferred.

Notes

1. The preparation of the Step 1 co-reagent, 4-chloro-5-methylpyrrolo[2,1-f] [1,2,4]triazine-6-carboxylic acid ethyl ester, is described by author.
2. In earlier investigations by the authors (1,2), kinase growth factor VEGFR-2 and FGFR-1 inhibitors consisting of triazine-4-phenoxy-, (**I**), and -4-indole derivatives, (**II**), respectively, were prepared and used as antiangiogenic agents.

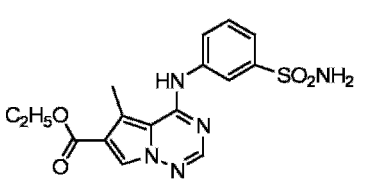


(I)

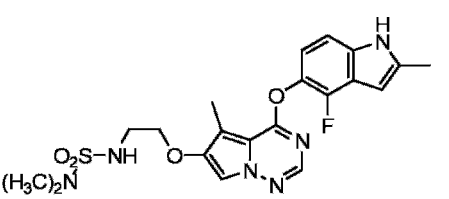


(II)

3. Aminosulfonyl triazine derivatives, (**III**) and (**IV**), prepared by Leftheris (3) and the authors (4), respectively, were effective as kinase growth factor VEGFR-2 and FGFR-1 and useful as antiangiogenic agents. In addition, they were also useful for the treatment of other diseases associated with signal transduction pathways operating through growth factor receptors.

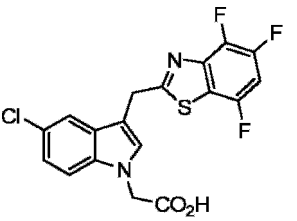


(III)

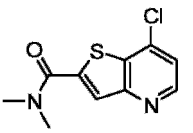


(IV)

4. Trifluorobenzothiazol-2-yl-indolealkanoic acids, (**V**), and thieno[3.2-b]pyridine-2-carboxylic amide derivatives, (**VI**), prepared by Sredy (5) and Luzzio (6), respectively, were effective as antiangiogenic agents and used in treating hyperproliferative disorders such as cancer.

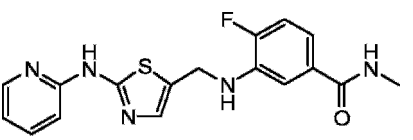


(V)



(VI)

5. 2-(Pyridin-2-yl-amino)-thiazol-5-yl-aminomethyl derivatives, (**VII**), prepared by Borzilleri (7) inhibited the tyrosine kinase activity of growth factor receptors of VEGFR-2 and FGFR-1 and were useful as antiangiogenic agents.



(VII)

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2. R.S. Bhide *et al*., US Patent 6,933,386 (August 23, 2005)
3. K. Leftheris *et al*., US Patent 6,670,357 (December 30, 2003)
4. R.S. Bhide *et al*., US Patent 6,869,952 (March 22, 2005)
5. J. Sredy *et al*., US Patent 6,964,980 (November 15, 2005)
6. M.J. Luzzio *et al*., US Patent 6,964,961 (November 15, 2005)
7. R.M. Borzilleri *et al*., US Patent 7,084,160 (August 1, 2006)

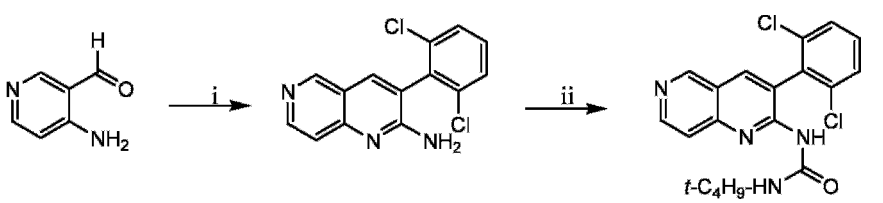
B. Tyrosine Kinase-2 Receptor Inhibitors

Title Compounds and Uses Thereof
M.A. Semones, US Patent 7,005,434 (February 28, 2006)
Assignee SB Corporation
Utility Antiangiogenic Agents

Invention Significance The angiogenesis cascade is a neovascularization process resulting from the activation of endothelial cells by an angiogenic signal. The tyrosine kinase receptor (Tie-2) plays a pivotal role in this process since it is largely restricted to endothelial cells in its expression. Excessive Tie-2 levels, however, are associated with proliferative diseases. To address this concern, Tie-2 inhibitors have been prepared to block the angiogenesis cascade.

Reaction

Eq. 1



i- 2,6-Dichlorophenylacetonitrile, sodium hydroxide, ethyl alcohol
ii- *t*-Butyl isocyanate, CH_2Cl_2, sodium hydride

Experimental

1. **Preparation of 3-[2,6-dichlorophenyl]-1,6-naphthyridin-2-amine**

   A mixture consisting of 4-aminonicotinaldehyde (0.78 mmol), 2,6-dichloro phenylacetonitrile (1.2 mmol), 10% aqueous NaOH (0.25 mmol) and 700 μl ethyl alcohol was heated 24 hours at 65°C, then cooled, and concentrated. The residue was diluted with 700 μl DMSO, then filtered. The solution was purified with preparative reverse-phase chromatography and the product isolated in 84% yield as an yellow solid.

   MS (ES+) $m/z = 290$

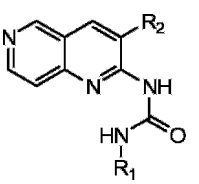
2. **Preparation of 3-[2,6-dichlorophenyl]-1,6-naphthyridin-2′-2-[N′-(1,1-dimethylethyl)-urea]**

The Step 1 product (0.176 mmol) and *t*-butyl isocyanate (0.176 mmol) were dissolved in 1 ml CH_2Cl_2, then treated with NaH (0.176 mmol), and stirred 24 hours. The mixture was filtered, washed with 1 ml DMF, and concentrated. The residue was purified by chromatography using silica gel with CH_2Cl_2/methyl alcohol, 25:1, and the product isolated in 36% yield as a white solid.

^{1}H NMR ($CDCl_3$) 9.32 (s, 1H), 8.72 (m, 1H), 8.40 (m, 1H), 7.95 (m, 1H), 7.55 (m, 3H), 1.50 (s, 9H)
MS (ES+) $m/z = 390$

Derivatives

Table 1 Selected experimental 1,6-naphthyridin derivatives and their corresponding mass spectra characterization data. All derivatives were effective as Tie-2 inhibitors



Entry	R_1	R_2	LC/MS (ES^+) (M + H), *m/z*
3	3-Thioph-3-yl	*t*-Butyl	327
10	3-Methylphenyl	*t*-Butyl	335
18	5-Acetyl-thioph-2-yl	Cyclohexyl	395
25	3-Pyridin-2-yl	3-Acetylphenyl	384
35	3,5-Dimethylphenyl	3-Chloro-4-fluorophenyl	421
45	Tetrahydropyran-2-yl	3-Chloro-4-fluorophenyl	377
55	2-Methylphenyl	Ethyl	323
67	4-Fluorophenyl	3,4-Difluorophenyl	395

Testing

I. Tests were conducted using the follow assays:

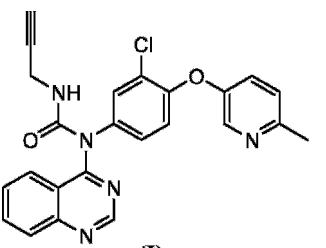
i- Tie2 receptor signal transduction
ii- Murine airpouch granuloma model of chronic inflammation (1)
iii- Angiogenesis in vivo—Matrigel model (2)

Results

Although quantitative testing data were not supplied by the author, 1,6-naphthyridin derivatives provided in Table 1 were especially preferred.

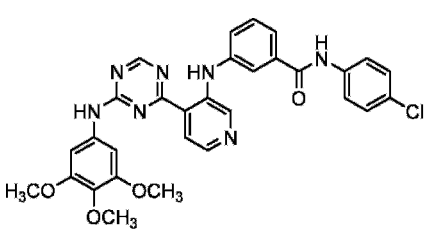
Notes

1. Quinazoline urea derivatives, (**I**), prepared by Kath (3) were effective as tyrosine kinase inhibitors such as c-erbB-2, c-met, tie-2, PDGFr, FGFr, and VEGFR and used in the treatment of proliferative disorders.

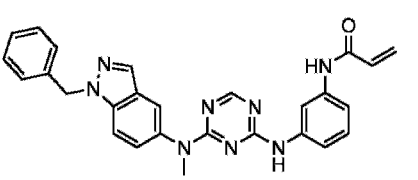


(I)

2. 1,3,5-s-Triazine derivatives, (**II**) and (**III**), prepared by Geuns-Meyer (4) and Buchanan (5), respectively, were effective in treating deregulated angiogenesis associated with ocular neovascularization.

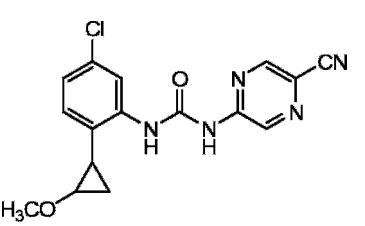


(II)



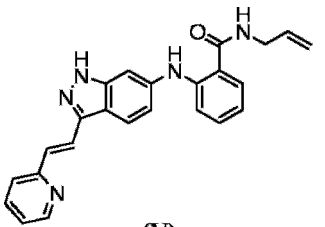
(III)

3. Li (6) prepared (5-cyano-pyrazin-2-yl)urea derivatives, (**IV**), that were effective as kinase inhibitors to treat hyperproliferative states such as cancer.



(IV)

4. Indazole derivatives, (**V**) and (**VI**), prepared by Borchardt (7) and Kania (8), respectively, were designed to modulate tyrosine kinase signal transduction and thereby inhibit unwanted angiogenesis and cellular proliferation.



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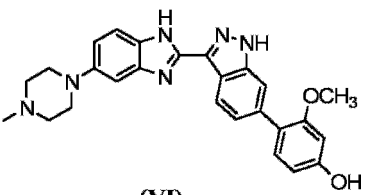

(VI)

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2. L. Biancone *et al*., J. Exp. Med., 186, 147 (1997)
3. J.C. Kath *et al*., US Patent 6,890,924 (May 10, 2005)
4. S.D. Geuns-Meyer *et al*., US Patent 6,864,255 (March 8, 2005)
5. J.L. Buchanan *et al*., US Patent 6,881,737 (April 19, 2005)
6. G. Li *et al*., US Patent 7,056,925 (June 6, 2006)
7. A.J. Borchardt *et al*., US Patent 7,053,107 (May 30, 2006)
8. R.S. Kania *et al*., US Patent 6,891,044 (May 10, 2005)

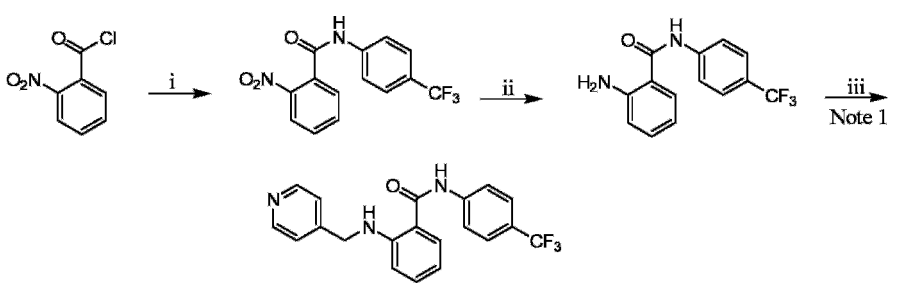
C. Vascular Endothelial Growth Factor Receptor Tyrosine Kinase Inhibitors

Title *N*-Aryl (thio) Anthranilic Acid Amide Derivatives, Their Preparation, and Their Use as VEGF
K.-H. Altmann *et al.*, US Patent 7,002,022 (February 21, 2006)
Assignee Novartis AG
Utility Antiangiogenic Agents

Invention Significance Vascular endothelial growth factor (VEGF) is a dimeric disulfide-linked 46-kDa glycoprotein, which is released by tumor cells to stimulate growth of blood capillaries to accelerate tumor proliferation. To impede this process, VEGF receptor tyrosine kinase inhibitors have been prepared that curtail tumor angiogenesis factors associated with VEGF-dependent cell proliferation.

Reaction

Eq. 1



i- CH_2Cl_2, 4-aminobenzotrifluoride, triethylamine
ii- Raney nickel, methyl alcohol, hydrogen
iii- Sodium cyanoborohydride, acetic acid, 4-pyridinecarboxaldehyde, methyl alcohol

Experimental

1. **Preparation of 2-nitro-*N*-(4-trifluoromethylphenyl)benzamide**

2-Nitrobenzoyl chloride (15 mmol) and 4-dimethylaminopyridine (10 mg) dissolved in 10 ml CH_2Cl_2 were added to 4-aminobenzotrifluoride (16.5 mmol) and triethylamine (18.8 mmol) dissolved in 100 ml CH_2Cl_2, then stirred 16 hours at ambient temperature. The mixture was treated with 50 ml saturated $NaHCO_3$, then extracted twice

with 50 ml CH_2Cl_2, and concentrated. Extracts were dried using Na_2SO_4 and then reconcentrated. The residue was purified by chromatography with silica gel using 10–50% EtOAc in hexane and the product isolated as a colorless crystalline solid.

2. **Preparation of 2-amino-*N*-(4-trifluoromethylphenyl)benzamide**

   The Step 1 product (6.19 mmol) dissolved in 200 ml methyl alcohol was hydrogenated 60 minutes at ambient temperature using Raney nickel (400 mg), then filtered, and concentrated. The residue was purified by recrystallization using CH_2Cl_2/hexane and the product isolated as a colorless crystalline solid, mp = 160−161°C.

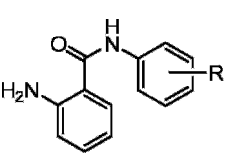
3. **Preparation of 2-[(4-pyridyl)methyl]amino-*N*-[4-(trifluoromethyl)phenyl] benzamide**

   A mixture of the Step 2 product (3.57 mmol), 0.15 ml acetic acid, and 4-pyridinecarboxaldehyde (3.57 mmol) dissolved in 15 ml methyl alcohol was treated portionwise with $NaBH_4$ (11.5 mmol) over 30 minutes, then stirred 16 hours at ambient temperature. The mixture was diluted with 100 ml CH_2Cl_2, then treated with 50 ml saturated $NaHCO_3$ solution, and extracted three times with 50 ml CH_2Cl_2. Extracts were then concentrated and the residue was purified by chromatography using 33% EtOAc/hexane. The solid was recrystallized using 2-propanol/hexane and the product isolated as a colorless crystalline solid, mp = 171–175°C.

^{1}H-NMR (DMSO-d_6) δ 4.49 (d, J = 6.1 Hz, 2H), 6.56 (d, J = 8.4 Hz, 1H), 6.66 (t, J = 85 Hz, 1H), 7.26 (t, J = 8.4 Hz, 1H), 7.33 (d, J = 5.9 Hz, 2H), 7.71 (d, J = 8.5 Hz, 2H), 7.72 (m, 1H), 7.90 (t, J = 6.1 Hz, 1H), 7.96 (d, J = 8.5 Hz, 2H), 8.49 (d, J = 5.9 Hz, 2H) and 10.46 (s, 1H)

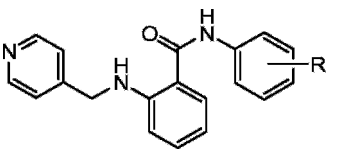
Derivatives

Table 1 Melting points of Step 2 amine intermediates prepared by hydrogenation of the 2-nitro-*N*-(4-trifluoromethylphenyl)benzamide precursor



Entry	R	mp (°C)
2c	4-Chloro	156–173 (HCl)
2e	3-Fluoro-2-methyl	149–151
2f	3-Chloro-5-trifluoromethyl	174–175
2j	3,5-Bistrifluoromethyl	192–193
2o	3-Cyano	161–163
2r	3-Aminocarbonyl	187–189

Table 2 Selected anthranilic acid derivatives prepared by reductive alkylation of the Step 2 amine intermediate using 4-pyridinecarboxaldehyde and their corresponding melting points. ^{1}H-NMR data supplied by author



Entry	R	mp (°C)
4	4-Chloro	134–139
5	4-Methyl	–
6	3-Fluoro-4-methyl	116–124
7	3-Trifluoromethyl	162–172
8	3-Fluoro-5-trifluoromethyl	190–194
15	3-(*i*-Propyl)	144–147
20	3-Dimethylamino	152–154

Testing

I. *VEGF Receptor Tyrosine Kinase Antagonist Assay*

VEGF receptor tyrosine kinase antagonist assay was performed according to the method of Shibuya (1). Testing results are provided in Table 3.

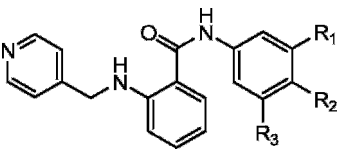
Table 3 Effectiveness of selected experimental agents as Flt-1 VEGF receptor tyrosine kinase antagonists. IC_{50} values above 0.01 μM are preferred

Entry	IC_{50} (μM)
4	0.18
5	0.26
7	0.56

Notes

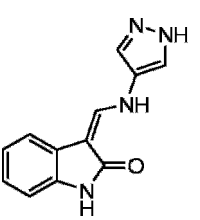
1. Additional benzamide VEGF receptor tyrosine kinase antagonists prepared by the authors (2) in an earlier investigation are summarized in Table 4.

Table 4 2-[(4-Pyridyl)methyl]amino-*N*-phenyl]benzamide derivatives effective as VEGF receptor tyrosine kinase antagonists previously prepared by the authors (2)

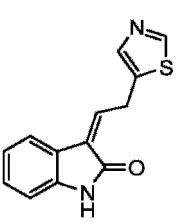


Entry	R_1	R_2	R_3
2	F	CF_3	H
7	CF_3	Cl	H
8	Cl	H	CF_3
10	F	H	CF_3
11	CF_3	H	CF_3

2. Andrews (3) and Tang (4) prepared VEGF receptor tyrosine kinase antagonists consisting of 1,3-dihydro-2H-indol-2-one derivatives, (**I**) and (**II**), respectively, which were effective as abnormal cell proliferation inhibitors.

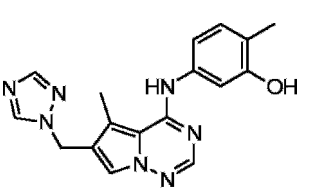


(I)



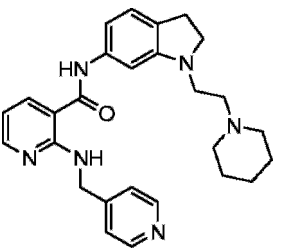
(II)

3. Pyrrolotriazine inhibitors, (**III**), prepared by Hunt (5) inhibited tyrosine kinase activity in VEGFR-2, FGFR-1, PDGFR, HER-1, and HER-2 growth factor receptors.



(III)

4. *N*-[1-(-Piperidylethyl)indolin-6-yl](2-[(4-pyridinylmethyl)amino](3-pyridyl)]carboxamide, (**IV**), and other alkyl amine derivatives prepared by Chen (6) were effective as VEGF inhibitors and were used in the treatment of angiogenesis-mediated diseases.



(IV)

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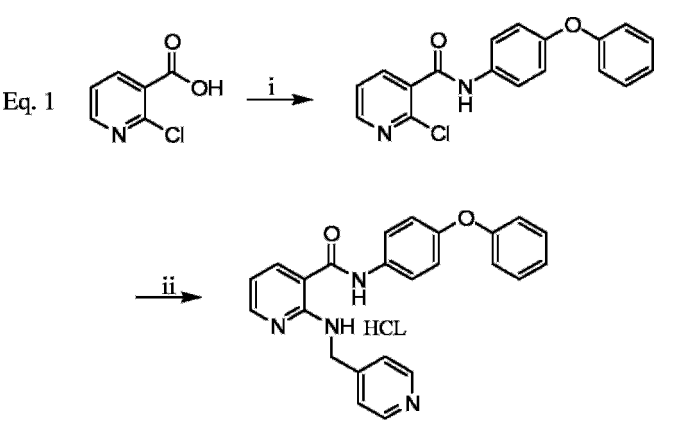
2. K.-H. Altmann *et al*., US Patent 6,878,720 (April 12, 2005)
3. S.W. Andrews *et al*., US Patent 7,005,444 (February 28, 2006)
4. P.C. Tang *et al*., US Patent 6,987,113 (January 17, 2006)
5. J.T. Hunt *et al*., US Patent 6,982,265 (January 3, 2006)
6. G. Chen *et al*., US Patent 6,995,162 (February 7, 2006)

D. Vascular Endothelial Growth Factor Tyrosine Kinase Receptor Inhibitors

Title Substituted Alkylamine Derivatives and Methods of Use
G. Chen, US Patent 6,995,162 (February 7, 2006)
Assignee Amgen Inc.
Utility Antiangiogenic Agents

Invention Significance Neovascularization occurs when angiogenesis becomes deregulated. In diseased states, high levels of angiogenic vascular endothelial growth factors (VEGF) are released by tumor cells to stimulate blood capillary growth and proliferation of tumor endothelium. To address this proliferative disorder, heterocyclic agents have been discovered that impede angiogenesis and associated disorders by inhibiting VEGF receptor tyrosine kinase activity.

Reaction



i- Triethylamine, THF, ethyl chloroformate, 4-phenoxyaniline
ii- 4-Aminomethylpyridine, hydrochloric acid, methyl alcohol

Experimental

1. **Preparation of (2-chloro(3-pyridyl))-*N*-(4-phenoxy-phenyl)carboxamide**

   2-Chloronicotinic acid (5.0 mmol) and 1.6 ml triethylamine were dissolved in 50 ml THF at 0°C, then treated with ethyl chloroformate (5.0 mmol), and stirred 60 minutes while the mixture warmed to ambient temperature. The mixture was further treated with 4-phenoxyaniline (5.0 mmol), then stirred 14 hours, and partitioned between water and EtOAc. The aqueous layer was extracted twice with 50 ml EtOAc and combined organic layers were washed with brine, dried over Na_2SO_4, then concentrated. The residue consisted of a brown oil and was used without further purification.

   MS *m/z* 325 (M + 1)
   Calc. for $C_{18}H_{13}ClN_2O_2$ 324.07

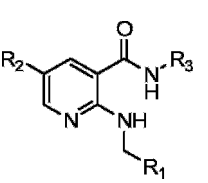
2. **Preparation of *N*-(4-phenoxyphenyl){2-[(4-pyridylmethyl)amino](3-pyridyl)}-carboxamide hydrochloride**

   A mixture of the Step 1 product (1.5 mmol) and 4-aminomethylpyridine (4.5 mmol) was heated for 48 hours at 90°C, then cooled and poured into saturated $NaHCO_3$ solution. The mixture was extracted with EtOAc and extracts washed with brine, dried, then concentrated. The residual brown oil was purified by chromatography using EtOAc/hexanes, 2:1, and the free amine isolated as clear oil. The free amine was converted to hydrogen chloride salt by dissolving in 5 ml methyl alcohol and treating with 3 equiv. of an HCl ethereal solution. The mixture was then concentrated and the product isolated as a light yellow solid.

   MS (ES+) 397 $(M+H)^+$; (ES−) 395 (M − H)
   Calc. for $C_{24}H_{20}N_4O_2$ 396.16

Derivatives

Table 1 Selected experimental agents and their corresponding mass spectra characterization data prepared and evaluated as VEGF inhibiting agents. Entries 7, 67, 99, 165, 840, 862, and 1000 had VEGF-stimulated human umbilical vein endothelial cells proliferation levels below 50 nm



Entry	R_1	R_2	R_3	MS (M + H)
7	Quinolin-6-yl	H	4-Chlorophenyl	389
8	Quinolin-6-yl	H	3,4-Dichlorophenyl	423
67	Pyridin-4-yl	Fluoro	4-*i*-Propylphenyl	456
99	Pyridin-4-yl	H	4-*n*-Butylphenyl	361
165	Pyridin-4-yl	H	4-Trifluoromethylphenyl	373
184	Pyridin-4-yl	Pyridin-3-yl	4-Chlorophenyl	–
840	Pyridin-4-yl	H	3-(2-(Piperidin-1-yl))ethoxy-5-trifluoromethylphenyl	564
862	Pyridin-4-yl	H	4,4-Dimethyl-1-tetralon-7-yl	416
1000	2-Methoxypyridin-4-yl	H	3,3-Dimethyl-2,3-dihydro-1H-indol-6-yl	403
1011	2-(Azetid-3-yloxy)-4-pyridin-4-yl	H	4-*t*-Butylphenyl	431

Testing

I. *Human Umbilical Vein Endothelial Cells Proliferation Assay*

Human umbilical vein endothelial cells (HUVEC) were trypsinized, then washed in DMEM, 10% FBS plus antibiotics, and centrifuged. The cells were then resuspended in DMEM and 10% FBS and antibiotics to achieve a concentration of 3×10^5 cells/ml. Thereafter the cells were diluted to 3×10^4 cells/ml in DMEM and 10% FBS plus antibiotics and incubated 22 hours at 37°C.

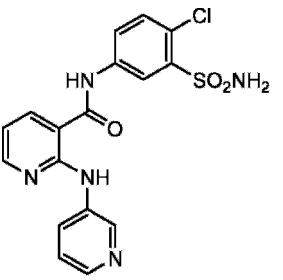
Growth factor solutions DMEM, 10% FBS plus antibiotics containing either VEGF or bFGF were prepared at 50, 10, 2, 0.4, 0.08, and 0 ng/ml. Selected experimental agents were evaluated in solutions of VEGF at 550 ng/ml or bFGF at 220, 50, or 20 ng/ml. For growth factor control curves, the media on wells B4-G6 of plates 1 and 2 were replaced with media containing VEGF or bFGF at concentrations ranging from 50 to 0 ng/ml. Thereafter cells were incubated at 37°C for additional 72 hours.

Results

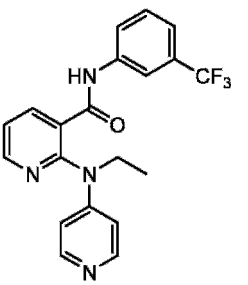
Experimental agents 7, 67, 99, 165, 840, 862, and 1000 appearing in Table 1 had VEGF-stimulated HUVEC proliferation levels below 50 nm.

Notes

1. Additional 2-amino-nicotinamide derivatives, (**I**) and (**II**), prepared by Askew (1) and Manley (2), respectively, were effective as VEGF receptor tyrosine kinase inhibitors and used to treat diseases associated with deregulated angiogenesis.

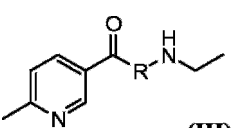


(I)



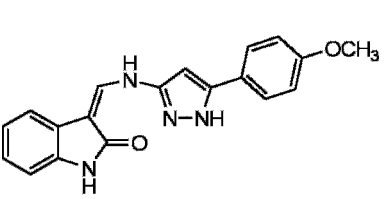
(II)

2. 6-Methyl nicotinyl-*N*-ethyl di- and tripeptide derivatives, (**III**) and (**IV**), respectively, prepared by Haviv (3) were effective in treating conditions that arose from or were exacerbated by angiogenesis.

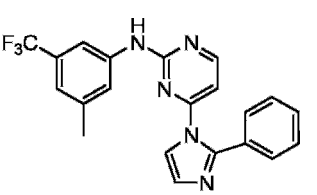


(III) R = Ile-Arg
(IV) R = Ile-Arg-Pro

3. 3-(Heteroarylamino)methylene-1,3-dihydro-2H-indol-2-ones, (**V**), prepared by Andrews (4) and pyrimidin-2-amine derivatives, (**VI**), prepared by Bilodeau (5) were effective as tyrosine kinase signal transduction regulating agents when evaluated using the VEGF-stimulated Ca^{2+} signal in vitro assay.

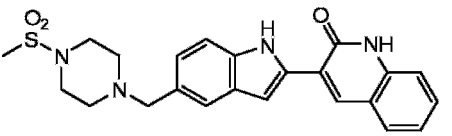


(V)

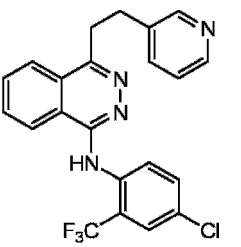


(VI)

4. 1H-Indol-2-yl-1H-quinolin-2-ones, (**VII**), and phthalazine derivatives, (**VIII**), prepared by Fraley (6) and Bold (7), respectively, were effective in inhibiting or modulating tyrosine kinase signal transduction and were used to treat tyrosine kinase-dependent diseases and conditions, such as angiogenesis, cancer, and tumor growth.



(VII)



(VIII)

References

1. B. Askew *et al*., US Patent 6,878,714 (April 12, 2005)
2. P.W. Manley *et al*., US Patent 6,624,174 (September 23, 2003)
3. F. Haviv *et al*., US Patent 7,001,984 (February 21, 2006)
4. S.W. Andrews *et al*., US Patent 7,005,444 (February 28, 2006)
5. M.T. Bilodeau *et al*., US Patent 6,958,340 (October 25, 2005)
6. M.E. Fraley *et al*., US Patent 6,960,590 (November 1, 2005)
7. G. Bold *et al*., US Patent 6,911,440 (June 28, 2005)

II. Antineoplastic Agents

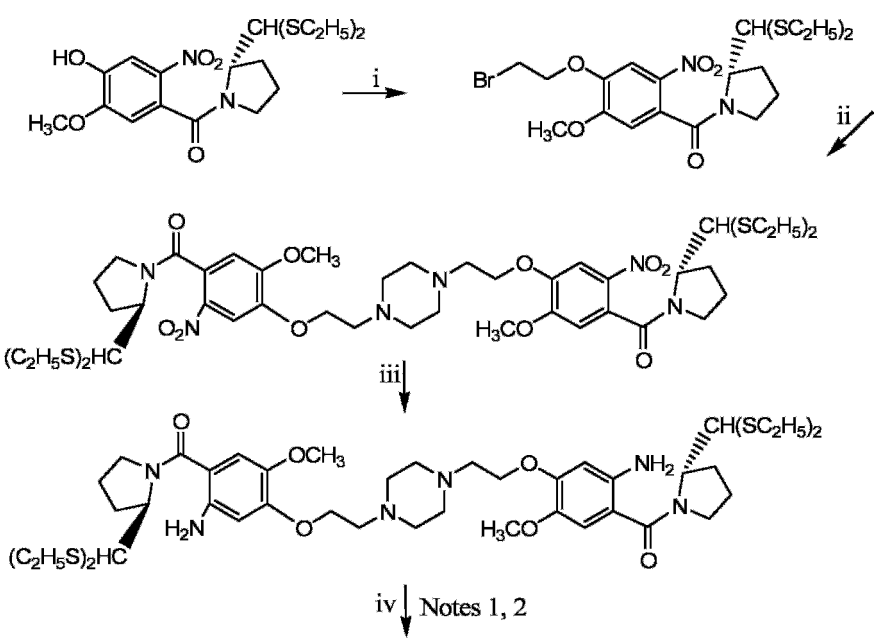
A. Bifunctional Cytotoxic DNA Crosslinking Agents

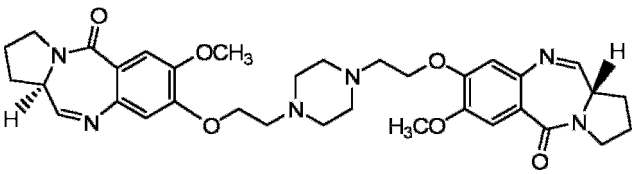
Title Pyrrolo[2,1-c][1,4]benzodiazepines Compounds and Process Thereof
A. Kamal *et al.*, US Patent 7,015,215 (March 21, 2006)
Assignee Council of Scientific and Industrial Research (Hyderabad, India)
Utility Versatile Antineoplastic Agents

Invention Significance Pyrrolo[2,1-c][1,4]benzodiazepines are antitumor antibiotics that react covalently with DNA to form an N_2–guanine adduct. Their clinical use, however, is hindered by their limited water solubility, drug resistance, and metabolic inactivation. In order to enhance their antitumor effectiveness, bifunctional analogs of varying separation lengths have been prepared that are capable of crosslinking DNA at specific sequence sites.

Reaction

Eq. 1





i- 1,2-Dibromoethane, potassium carbonate, acetone
ii- Piperazine, potassium carbonate, acetone
iii- Methyl alcohol, tin chloride dihydrate
iv- Mercury chloride, mercury oxide, acetonitrile, water

Experimental

1. **Preparation of (2*S*)-*N*-[4-(2-bromoethoxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal**

   A mixture consisting of (2*S*)-*N*-(4-hydroxy-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxaldehyde diethylthioacetal (2 mmol), 1,2-dibromoethane (2.5 mmol), and K_2CO_3 (3 mmol) dissolved in 40 ml acetone was refluxed 48 hours, then poured into water, and extracted with EtOAc. The solution was concentrated, the residue purified using chromatography with silica gel using EtOAc/hexane, 1:1, and the product isolated.

   ^{1}H-NMR ($CDCl_3$) Γ 1.20–1.4 (m, 6H), 1.75–2.2 (m, 4H), 2.6–2.9 (m, 4H), 3.20–3.33 (m, 2H), 3.67 (t, 2H), 3.95 (s, 3H), 4.37 (t, 2H), 4.62–4.78 (m, 1H), 4.85 (d, 1H), 6.82 (s, 1H), 7.67 (s, 1H)

2. **Preparation of 1,1′-{[(bisethane-1,*N*-diyl)piperazine]dioxy}bis[(11a*S*)-7-methoxy-2-nitro-benzoylpyrrolidin-2-carboxaldehyde diethylthioacetal]**

   A mixture consisting of the Step 1 product (1 mmol), piperazine (0.5 mmol), and K_2CO_3 (3 mmol) dissolved in 20 ml acetone was refluxed 48 hours, then poured into water, and extracted with EtOAc. The solution was concentrated, the residue purified by chromatography using EtOAc/hexane, 9:1, and the product isolated.

   ^{1}H-NMR ($CDCl_3$) Γ 1.29–1.41 (m, 12H), 1.7–2.39 (m, 8H), 2.60–2.90 (m, 20H), 3.17–3.3 (m, 4H), 3.92 (s, 6H), 4.2 (t, 4H), 4.60–4.70 (m, 2H), 4.81 (d, 2H), 6.8 (s, 2H), 7.7 (s, 2H)
   FAB MS 939 $(M + H)^+$

3. **Preparation of 1,1′-{[(bisethane-1,*N*-diyl)piperazine]dioxy}bis[(11a*S*)-7-methoxy-2-aminobenzoylpyrrolidin-2-carboxaldehyde diethylthioacetal]**

   The Step 2 product (1.0 mmol) dissolved in 10 ml methyl alcohol was treated with $SnCl_2 \cdot 2H_2O$ (5.0 mmol), then refluxed 90 minutes, and pH of the medium raised

to 8 using saturated $NaHCO_3$ solution. The product was extracted three times with 20 ml EtOAc, dried, and concentrated. The residue was isolated and used without further purification.

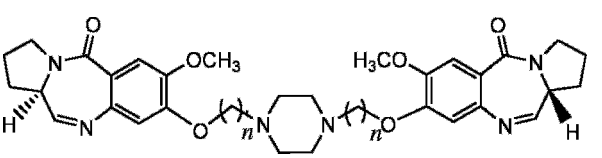
4. **Preparation of crude 1,1′-{[(bisethane-1,*N*-diyl)piperazine]dioxy}bis[(11a*S*)-7-methoxy-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one]**

A mixture consisting of the Step 3 product (1 mmol), $HgCl_2$ (2.93 mmol), and HgO (3.18 mmol) dissolved in 15 ml CH_3CN/water, 3:1, was stirred 12 hours at ambient temperature, then concentrated. The residue was diluted with EtOAc and saturated $NaHCO_3$ solution, then filtered through celite. The solution was then washed with EtOAc and reconcentrated. The residue was purified by chromatography eluting first with EtOAc to remove mercuric salts and then using with $CHCl_3$/methylalcohol, 9:1.

^{1}H-NMR ($CDCl_3$) Γ 1.92–2.42 (m, 8H), 2.60–2.95 (m, 12H), 3.2–3.88 (m, 6H), 3.92 (s, 6H), 4.14–4.28 (m, 4H), 6.76 (s, 2H), 7.5 (s, 2H), 7.66 (d, 2H)
FAB MS 631 $(M+H)^+$

Derivatives

Table 1 Selected difunctional pyrrolo[2,1-c][1,4] benzodiazepines derivatives of various separation lengths and their corresponding mass spectral data



Entry	*n*	FAB MS, $(M+H)^+$
1	2	631
2	3	659
3	4	687
4	5	–

Testing

I. *Cytotoxicity*

Experimental agents were evaluated in vitro against 60 human tumor cells derived from nine cancer types (leukemia, nonsmall cell lung, colon, melanoma, ovarian, prostate, and breast cancer). For each experimental agent, dose–response curves for

each cell line were measured at five concentrations at 10-fold dilutions. A protocol of 48 hours continuous drug exposure was used and a sulforhodamine B protein assay was used to estimate cell viability or growth. The concentration causing 50% cell growth inhibition (GI_{50}) total cell growth inhibition (TGI) and 50% cell death (LC_{50}) compared with the control was calculated. Testing data are provided in Table 2. Mean graph midpoint values of $\log_{10}$ TGI, $\log_{10}$ LC_{50}, and $\log_{10}$ GI_{50} are provided in Table 3.

Table 2 In vitro anticancer activity of selected difunctional pyrrolo[2,1-c][1,4]benzodiazepine derivatives against a variety of tumors underscoring relevance of alkane spacers

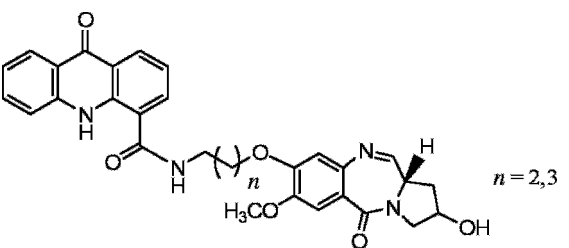
Cancer	Entry 1		Entry 2		Entry 3		Entry 4	
	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}
Leukemia								
HL-60(TB)	– 5.49	– 4.00	– 6.66	– 4.00	– 8.00	– 4.00	– 5.38	– 4.00
K-62	– 4.58	– 4.00	– 5.76	– 4.00	– 7.63	– 4.00	– 5.46	– 4.20
Nonsmall cell lung								
A549/ATCC	– 4.10	– 4.00	– 4.37	– 4.00	– 7.23	– 4.00	– 4.66	– 4.00
EKVX	– 4.00	– 4.00	– 4.47	– 4.00	– 6.36	– 4.00	– 4.33	– 4.00
Colon								
HCT-116	– 4.47	– 4.00	– 4.92	– 4.00	– 6.42	– 4.00	– 4.00	– 4.00
HCT-15	– 4.39	– 4.00	– 4.25	– 4.00	– 7.92	– 6.37	– 4.25	– 4.00
Melanoma								
M14	– 4.61	4.00	– 5.55	– 4.00	– 7.60	– 4.17	– 4.76	– 4.00
SK-MEL-2	– 4.57	– 4.00	– 4.82	– 4.00	– 7.34	– 4.00	–	–
Ovarian								
IGROVI	– 4.21	– 4.00	– 5.26	– 4.00	– 6.79	– 4.00	– 5.13	– 4.00
OVCAR-3	– 4.68	– 4.00	– 5.47	– 4.00	– 7.91	– 4.00	– 5.32	– 4.00
Renal								
786-0	– 4.84	– 4.00	– 5.30	– 4.00	– 8.00	– 4.00	– 5.61	– 4.00
A498	– 4.29	– 4.00	– 5.73	– 4.00	– 6.89	–	– 5.00	4.00
Prostate								
PC-3	– 44	– 4.00	– 5.53	– 4.00	– 7.02	– 4.00	– 5.41	– 4.00
DU-145	– 4.36	– 4.00	– 5.62	– 4.00	– 7.60	– 4.00	– 5.37	– 4.00
Breast								
MCF7	– 4.88	– 4.00	– 6.01	– 4.00	– 8.00	– 4.00	– 5.69	– 4.00
NCI/ADR-RES	– 4.00	– 4.00	– 4.00	– 4.00	– 6.47	– 4.00	– 4.00	– 4.00

Table 3 Log_{10} GI_{50}, log_{10} TGI, and log10 LC_{50} mean graphs midpoints for in vitro cytotoxicity testing using selected difunctional pyrrolo[2,1-c][1,4] benzodiazepine derivatives

Entry	Log_{10} GI_{50}	Log_{10} TGI	Log_{10} LC_{50}
1	−4.69	−4.16	−4.03
2	−5.19	−4.22	−4.01
3	−7.70	−5.95	−4.47
4	−5.14	−4.26	−4.04

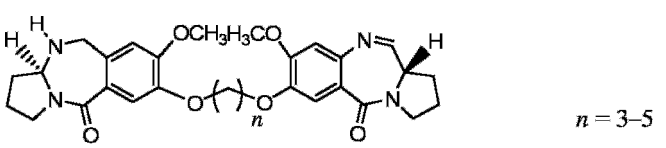
Notes

1. In a subsequent investigation by the author (1) antineoplastic agents consisting of pyrrolo[2,1-c][1,4]benzodiazepine acridone, (**I**), and acridine hybrids were prepared which were effective against leukemia, nonsmall cell, lung, and colon cancers.

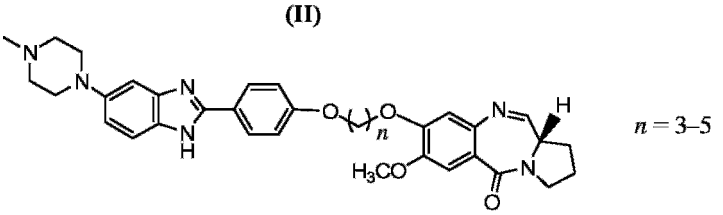


(I)

2. In earlier investigations by the authors (2,3), noncrosslinking pyrrolo[2,1-c][1,4] benzodiazepine hybrid derivatives, (**II**) and (**III**), respectively, were prepared having significant DNA binding ability and antitumor activity and used in treating proliferative disorders.

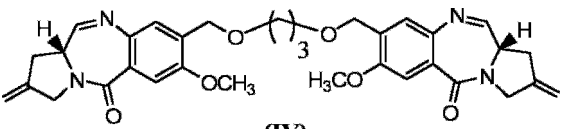


(II)



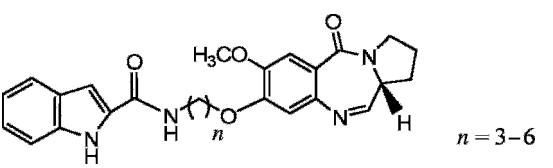
(III)

3. Pyrrolo[2,1-c][1,4]benzodiazepin-5-one dimer derivatives, (**IV**), prepared by Thurston (4) were effective as cytotoxic agents against human lung, colon, CNS, melanoma, renal, and breast cancer cell lines.



(IV)

4. Antineoplastic agents consisting of pyrrolo[2,1-c][1,4]benzodiazepine–indole derivatives, (**V**), containing varying aliphatic spacer lengths were prepared by Wang (5) and were effective in the treatment of nonsmall cell lung, CNS, colon, and renal cancers.



$n = 3-6$

References

1. A. Kamal *et al*., US Patent 7,056,913 (June 6, 2006)
2. A. Kamal *et al*., US Patent 6,884,799 (April 26, 2005)
3. A. Kamal *et al*., US Patent 6,951,853 (October 4, 2005)
4. D.E. Thurston *et al*., US Patent 7,067,511 (June 27, 2006) and US Patent 7,049,311 (May 23, 2006)
5. J.-J. Wang, US Patent 6,939,869 (September 6, 2005)

B. Cyclin-dependent Kinase-2/Cyclin Kinase Inhibitors

Title 3(5)-Amino-pyrazole Derivatives, Process for Their Preparation, and Their Use as Antitumor agents
P. Pevarello *et al*., US Patent 7,034,049 (April 25, 2006)

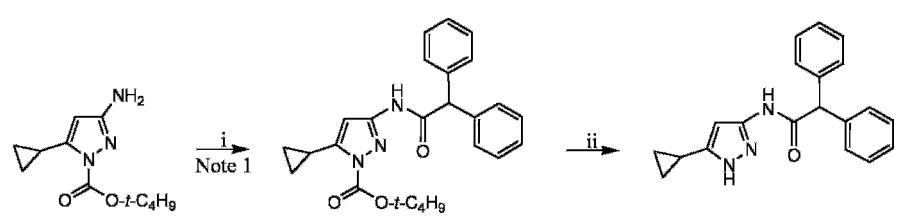
Assignee Pharmacia Italia S.p.A. (Milan, IT) and Pharmacia & Upjohn Company LLC

Utility Cytotoxic Agents with Diminished Cellular Metabolic Toxicity

Invention Significance Significant toxicity and side effects are often associated with cytotoxic agents because of their indiscriminate effect on cellular metabolic pathways in both normal and tumor cells. To address this concern, cyclin-dependent kinase-2 (cdk2), cdk2/cyclin kinase inhibitors with comparable cytotoxic efficacy but reduced toxicity have been prepared for treating proliferative disorders associated with altered cell-dependent kinase activity.

Reaction

Eq. 1



i- Diphenylacetic acid, CH_2Cl_2, *N*-(3-dimethyl aminopropyl)-*N′*-ethylcarbodiimide•HCl
ii- Trifluoroacetic acid, CH_2Cl_2

Experimental

1. **Preparation of *N*-(5-cyclopropyl-1-terbutoxycarbonyl-pyrazol-3-yl)-2,2-diphenyl acetamide**

   Diphenylacetic acid (0.215 mmol) dissolved in 3 ml CH_2Cl_2 at 0°C was treated with *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (0.215 mmol) and after 1 hour further treated with *t*-butyl-3-amino-5-cyclopropyl-1H-pyrazole-1-carboxylate (0.179 mmol). The mixture was stirred 16 hours at ambient temperature,

then diluted with CH_2Cl_2, and washed with saturated $NaHCO_3$ solution. It was dried with Na_2SO_4, then purified by chromatography using hexane/EtOAc, and the product isolated in 80% yield.

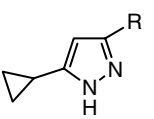
2. **Preparation of *N*-(5-cycloproyl-1H-pyrazol-3-yl)-2,2-diphenyl acetamide**

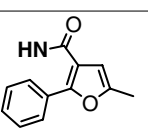
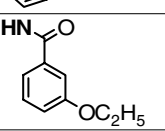
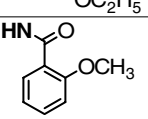
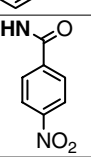
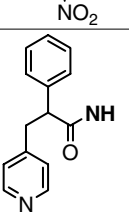
The Step 1 product was treated with 15 ml 10% trifluoroacetic acid in CH_2Cl_2, then concentrated, and redissolved in CH_2Cl_2. It was washed with saturated $NaHCO_3$ solution, then dried using Na_2SO_4, and the product isolated in 92% yield, mp = 218−220°C.

1H NMR (400 MHz, DMSO-d_6) (ppm) 0.62 (m, 2H, cyclopropyl CHH + CHH); 0.88 (m, 2H, cyclopropyl CHH + CHH); 1.81 (dddd, 1H, J = 5.2, 5.2, 8.4, 8.4 Hz, cyclopropyl CH); 5.17 (s, 1H, $CHPh_2$); 6.17 (s, 1H, pyrazole CH); 7.30 (m, 10H, phenyl CH); 10.6 (s, 1H, amidic NH); 12.04 (s, 1H, pyrazole NH)
ESI (+) MS *m/z* 318 (100, MH^+)

Derivatives

Table 1 Selected cdk/cyclin kinase inhibitory agent derivatives and their corresponding analytical characterization data. ^{1}H NMR data supplied by author



Entry	R	Analytical
2		MS, *m/z* = 308
4		MS, *m/z* = 285
6		mp = 118–120°C
7		183–185°C
10		–

Testing

I. *Cdk2/cyclin Inhibitors Testing*

The inhibiting activity of selected experimental agents was determined through a method of assay based on the use of the MultiScreen-PH 96-well plate in which phosphocellulose filter paper was placed at the bottom of each well allowing binding of positive charged substrate after a washing/filtration step.

When a radioactivity-labeled phosphate was transferred by the ser/threo kinase to the filter-bound histone, light emitted was measured in a scintillation counter.

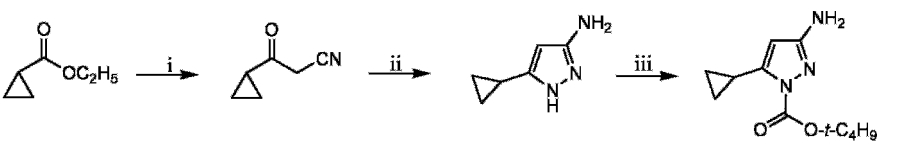
Results

Although testing results were not provided by the author, *N*-(5-cyclopropyl-1H-pyrazol-3-yl)-2,2-diphenyl acetamide was especially preferred.

Notes

1. The Step 1 co-reagent, *t*-butyl-3-amino-5-cyclopropyl-1H-pyrazole-1-carboxylate, (**I**), was previously prepared by the author (1) as illustrated in Eq. 2.

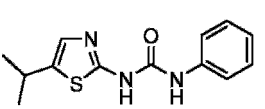
Eq. 2



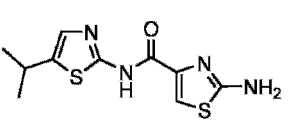
(I)

i- Sodium hydride, dioxane, acetonitrile
ii- Ethyl alcohol, hydrazine hydrate
iii- CH_2Cl_2, $NaHCO_3$, *t*-butoxycarbonyl anhydride

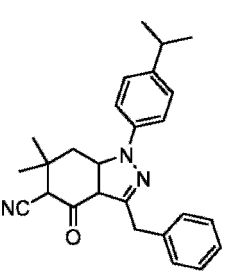
2. Cdk2/cyclin inhibitors consisting of 2-ureido-thiazoles, (**II**), 2-carbonylamino-thiazoles (**III**), and 4,5,6,7-tetrahydro indazole derivatives, (**IV**), were previously prepared by the authors (2–4), respectively, and were effective in treating cell proliferative disorders associated with altered cell-dependent kinase activity.



(II)

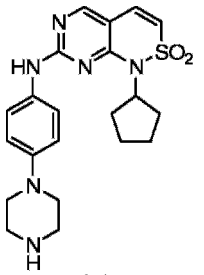


(III)

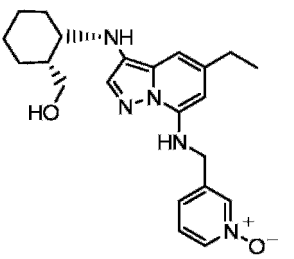


(IV)

3. 2-Thia-1,6,8-triaza-naphthalene-2,2-dioxides derivatives, (**V**), prepared by Repine (5) and pyrazolo[1,5-a]triazine derivatives, (**VI**), prepared by Guzi (6) were effective as cyclin-dependent kinase inhibitors and used in treating cell proliferative disorders.

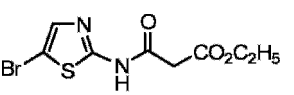


(V)



(VI)

4. In a subsequent investigation by the author (7), cdk2/cyclin kinase inhibitors were prepared consisting of (5-bromo-1,3-thiazol-2-yl)amino derivatives, (**VII**), and were effective as cytotoxic agents without producing indiscriminate damage to both normal and tumor cells.



(VII)

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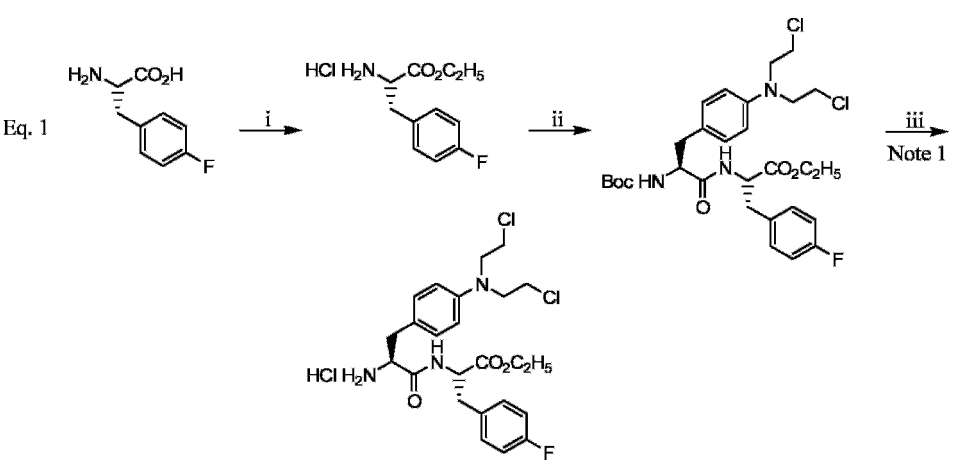
1. P. Pevarello *et al*., US Patent 6,218,418 (April 17, 2001)
2. P. Pevarello *et al*., US Patent 6,863,647 (March 8, 2005)
3. P. Pevarello *et al*., US Patent 6,784,198 (August 31, 2004)
4. P. Pevarello *et al*., US Patent 6,716,856 (April 6, 2004)
5. J.T. Repine, US Patent 7,026,313 (April 11, 2006)
6. T.J. Guzi *et al*., US Patent 7,038,045 (May 2, 2006)
7. P. Pevarello *et al*., US Patent 7,037,929 (May 2, 2006)

C. Mustard-based Alkylating Chemotherapeutic Agents

Title Melphalan Derivatives and Their Use as Cancer Chemotherapeutic Drugs
R. Lewensohn *et al*., US Patent 6,992,207 (January 31, 2006)
Assignee Oncopeptides AB
Utility Treatment of Drug-resistant Tumors

Invention Significance More effective alkylating agents consisting of di- and tripeptide melphalan derivatives have been prepared that are useful in the treatment of solid malignant tumors. Their enhanced cytotoxic activity addresses an urgent need for the development of agents useful in treating tumors that have developed primary resistance to conventional therapy or secondary resistance.

Reaction



i- Ethyl alcohol, hydrogen chloride
ii- *N-t*-Butoxycarbonyl-L-melphalan, CH_2Cl_2, triethylamine, (benzotriazol-1-yloxy)-tripyrrolidino-phosphonium hexafluorophosphate
iii- EtOAc, hydrogen chloride

Experimental

1. **Preparation of L-*p*-fluorophenylalanine ethyl ester hydrochloride**

   L-*p*-Fluorophenylalanine (1.18 mmol) was dissolved in 5 ml ethyl alcohol previously bubbled with hydrogen chloride, then heated to 100°C, and refluxed 18 hours. The mixture was cooled, then concentrated, and the product isolated in 98% yield as white crystals.

   ^{1}H NMR (CD_3OD) δ 7.30–7.26 (m, 2H, Ph–H), 7.13–7.06 (m, 2H, Ph–H), 4.29–4.22 (m, 2H, CH_2–Ph), 3.31–3.10 (m, 3H, CH_2CH_3, α-H), 1.24 (t, 3H, CH_2CH_3)

2. **Preparation of *N-t*-butoxycarbonyl-L-melphalanyl-L-*p*-fluorophenylalanine ethyl ester**

   N-t-Butoxycarbonyl-L-melphalan (0.387 mmol) was dissolved in 4 ml CH_2Cl_2, then treated with (benzotriazol-1-yloxy)-tripyrrolidino-phosphonium hexafluorophosphate (0.387 mmol) and 54 µl triethylamine (0.387 mmol), then stirred 60 minutes at ambient temperature. The mixture was then treated with the Step 1 product (0.387 mmol) and 54 µl triethylamine dissolved in 4 ml CH_2Cl_2, then stirred overnight. The solution was extracted with saturated $NaHCO_3$ solution followed by 10% citric acid, then dried using $MgSO_4$, and concentrated providing 200 mg of yellow oil. The residue was purified by gradient chromatography using diethyl ether/pentane, 1:2, 1:1, 1:0, and the product isolated in 43% yield and used without further purification.

3. **Preparation of L-melphalanyl-L-*p*-fluorophenylalanine ethyl ester hydrochloride**

   The Step 2 product (0.11 mmol) was dissolved in 5 ml EtOAc that was previously saturated with hydrogen chloride gas, then stirred 30 minutes at ambient temperature, and concentrated. The residue was recrystallized using ethyl alcohol/diethyl ether and the product isolated as white crystals.

   ^{1}H NMR (CD_3OD) δ 7.23 (d, 2H, Ph–H, Phe), 7.13 (d, 2H, Ph–H, Phe), 7.01 (d, 2H, Ph–H, Mel), 6.72 (d, 2H, Ph–H, Mel), 4.68 (dd, 1H, α-CH, Phe), 4.61 (br s, 1H, α-CH, Mel), 4.12 (q, 2H, CH_2CH_3), 3.80–3.62 (m, 8H, CH_2-mustard), 3.22–2.86 (m, 4H, CH_2–Ph), 1.21 (t, 3H, CH_2CH_3)
   Elemental analysis C, 53.7; H, 5.8; N, 7.9 (calc. C, 54.1; H, 6.1; N, 7.9)

Derivatives

Testing

I. *Fluorometric microculture cytotoxicity assay (FMCA)*

Cytostatic activity of selected experimental agents was performed, then compared with the activity of melphalan according to the method of Larsson (1). Cytotoxic drug resistance against a line panel of malignant cells using selected experimental agents and existing antineoplastic agents are provided in Tables 2 and 3,

Table 1 Preferred melphalan-based mustard agents having enhanced cytotoxicity in the treatment of selected human tumor specimens. ^{1}H NMR and elemental analysis supplied for all compounds; ^{13}C NMR supplied for JV-3, -28, and P2

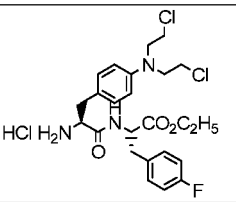
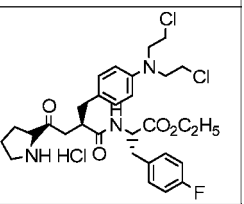
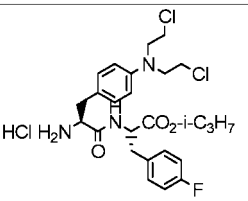
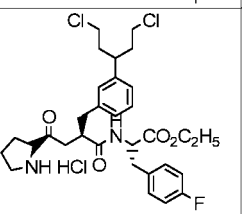
Entry	Structure	Entry	Structure
J1		JV-3	
JV-28		P2	

Table 2 IC_{50} (μM) of preferred experimental agents in a line panel representing defined forms of cytotoxic drug resistance

Cell line[a]	J1	JV-3	JV-28
CEM/S	0.03	0.06	0.06
CEM/R	0.01	0.02	0.04
ACHN	0.4	1.72	0.05
H69	0.04	0.2	0.05
H69AR	0.9	1.36	0.37
8226S	0.81	1.51	0.51
8226Dox40	1.76	5.66	0.89
8226LR5	2.47	3.52	1.88
U937gtb	0.07	0.23	0.1
U937vcr	0.11	0.42	0.15
MEAN	0.66	1.47	0.41

[a]U-937 GBT, lymphoma; RPMI 8226, myeloma; NCI-H69, small cell lung cancer; CCRF-CEM/R and CEM/S, leukemia; ACHN, renal carcinoma; U-937-vcr subline 8226Dox40, vincristine resistance; 8226LR5, melphalan resistance; H69AR, doxorubicin resistance.

respectively. Tumor cell survival rates of primary cultures of human tumor cells using selected experimental agents and existing antineoplastic agents are provided in Table 4.

Table 3 IC_{50} (μM) for Entry P2 and existing antineoplastic agents in a line panel representing defined forms of cytotoxic drug resistance

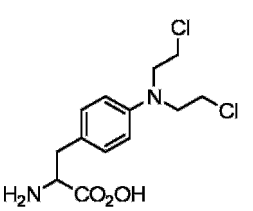
Cell Line	Melphalan	P2	Sarcolysine	Doxorubicin	Vincristine	Topotecan
CEM/S	1.48	0.94	2.54	0.18	0.08	0.02
CEM/R	0.94	0.63	1.70	1.31	0.01	0.02
ACHN	133.3	4.59	390	14.2	109	16.3
H69	13.44	4.59	41.15	1.66	69.8	1.72
H69AR	48.74	4.27	61.4	11.6	121	180.5
8226S	10.82	5.11	30.4	0.13	0.01	0.87
8226Dox40	35.18	5.43	41.38	4.97	0.9	0.59
8226LR5	30.61	7.4	31.88	0.55	0.08	0.21
U937gtb	1.8	1.78	3.78	0.11	0.01	0.02
U937vcr	2.95	1.77	6.64	0.42	0.08	0.02
Mean	27.93	3.35	61.09	3.51	30.1	20.0

Table 4 Tumor cell survival index percent for Entry J1 and P2, melphalan and other existing antineoplastic agents using primary cultures of human tumor cells

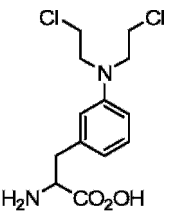
Agent	Concentration (μM)	Hematological ($n = 42$)	Solid ($n = 22$)
J1	0.66	13.7	46.9
Melphalan	3.3	41	88.8
P2	0.66	34.7	86.0
AraC	10.3	–	102
Vincristine	3.0	39	93
Vinorelbine	3.1	50	73
Docetaxel	6.2	–	67
Cisplatin	6.7	63	79
Doxorubicin	0.92	29	97
Etoposide	8.5	47	81

Notes

1. Structures of melphalan, (**I**), and sarcolysine, (**II**), are provided.

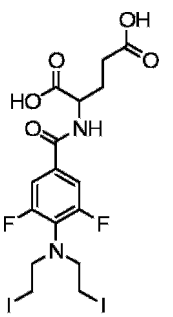


(I)



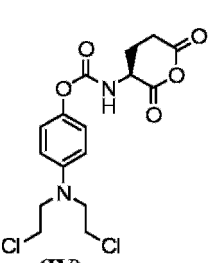
(II)

2. 3,5-Difluoro-4-[bis(2-iodoethyl)amino]benzoyl-L-glutamic acid, (**III**), prepared by Springer (2) was effective as an alkylating agent and used in treating cells lines associated with breast cancer.



(III)

3. Cytotoxic carbamate mustard prodrugs, (**IV**) and (**V**), prepared by Siedlecki (3) were delivered to patients as an antibody/enzyme complex to minimize general toxicity during antibody-directed enzyme prodrug therapy (ADEPT), a targeted cytotoxic cancer therapy. Other carbamate mustard prodrug derivatives, (**VI**), were prepared by Springer (4).



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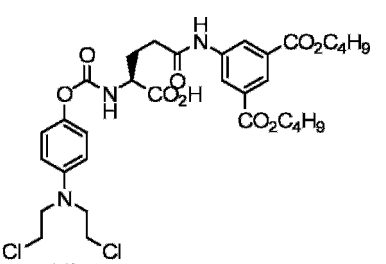

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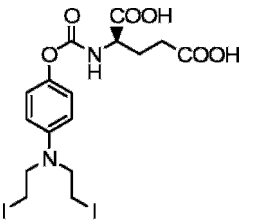
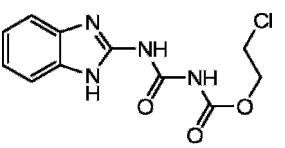


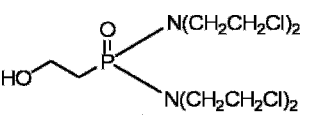
(VI)

4. Cytotoxic alkylating agents consisting of benzimidazolecarbmate derivatives, (**VII**), prepared by Quada (5) were effective against colon and melanoma tumor cells.



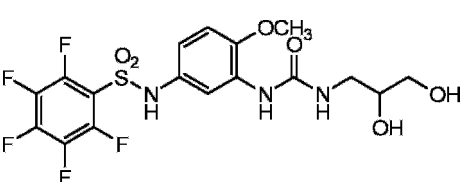
(VII)

5. (2-Chloroethyl)phosphorodiamidate mustards, (**VII**), prepared by Herr (7) were effective as antineoplastic agents especially in the treatment of solid tumors.



(VII)

6. Houze (8) prepared pentafluorophenylsulfonanilide urea derivatives, (**VIII**), that were effective in arresting the growth of HeLa cells derived from a human cervical adenocarcinoma tumor cells.



(VIII)

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2. C.J. Springer *et al*., US Patent 6,852,755 (February 8, 2005)
3. P.S. Siedlecki *et al*., US Patent 6,737,541 (May 18, 2004)
4. C.J. Springer *et al*., US Patent 6,916,949 (July 12, 2005)
5. J.C. Quada, Jr. *et al*., US Patent 6,720,349 (April 13, 2004)
6. R.J. Herr *et al*., US Patent 6,506,739 (January 14, 2003)
7. J.B. Houze, US Patent 7,060,718 (June 13, 2006)

D. Yujungamycin-based Alkylating Chemotherapeutic Agents

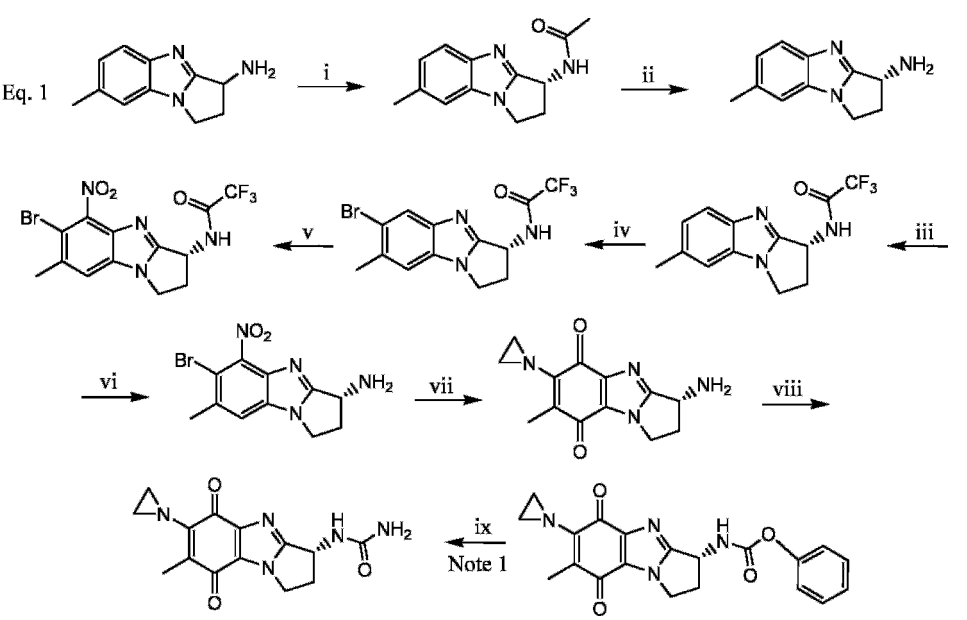
Title Treatment of Neoplasms with Yujungamycins
E.B. Skibo, US Patent 6,998,413 (February 14, 2006)

Assignee Arizona Board of Regents acting for and on behalf of Arizona State University

Utility Treatment of Central Nervous System, Ovarian, and Lung Cancers

Invention Significance Yujungamycin, pyrrolo[1,2-a]benzimidazole-based aziridinyl quinine, is a new class of .DNA-cleaving agent designed to alkylate the phosphate backbone and upon reduction and create a hydrolytically labile phosphotriester. Thereafter the reducing enzyme DT-diaphorase activates antitumor agents by two-electron reduction in cancerous tissues. To further exploit these agents additional yujungamycin derivatives have been prepared.

Reaction



i- ALTUS 20 CLEC enzyme, EtOAc
ii- Hydrochloric acid
iii- Trifluoroacetic acid, trifluoroacetic anhydride
iv- Bromine, acetic acid
v- Nitric acid, acetic anhydride
vi- Methyl alcohol, ammonia
vii- 5% Pd, hydrogen, methyl alcohol, monopotassium phosphate, water, potassium acid fluoride (Fremy's salt), azirindine
viii- Pyridine, phenylchloroformate
ix- Ammonia, CH_2Cl_2

Experimental

1. **Preparation of *R*-(+)-3-*N*-acetyl-2,3-dihydro-7-methyl-1H-pyrrolo[1,2-a] benzimidazole**

   3-Amino-2,3-dihydro-7-methyl-1H-pyrrolo[1,2-a]benz-imidazole (2.9 mmol) was added to ALTUS 20 CLEC (310 mg) in 104 ml EtOAc, then stirred 30 minutes, and filtered. The solution was washed three times with 10 ml $CHCl_3$, then concentrated. The residue was purified by chromatography using silica gel with 3% methyl alcohol in $CHCl_3$, then recrystallized from EtOAc/hexane, and the product isolated in 37% yield, mp = 219−220°C.

   ^{1}H NMR ($CDCl_3$) δ 7.75 (1H, s, acetamido proton) 7.47 (1H, d, $J = 8.4$ Hz, 5-aromatic proton), 7.02 (1H, d, $J = 8.4$ Hz, 6-aromatic proton), 6.88 (1H, s, 8-aromatic proton), 5.41 (1H, m, 3-methine proton), 4.0–3.8 (2H, m, 1-methylene protons), 3.25–3.15 (1H, m, 2-methylene proton), 2.55–2.39 (1H, m, 2-methylene proton) 2.43 (3H, s, methyl), 2.09 (3H, s, methyl)
   IR (KBr pellet) (cm^{-1}) 3245, 3068, 2990, 1635, 1560, 1420
   MS (EI) *m/z* 229 (M^+), 186 ($M^+ - COCH3$), 171,158, 145, 133, 116, 104
   TLC (chloroform/methanol [90:10]) $R_f = 0.38$
   Rotations (*R*)-(+) $[\alpha]_D^{25} = +104.4°$ ($c = 0.41$, methyl alcohol)

2. **Preparation of *R*-(+)-3-amino-2,3-dihydro-7-methyl-1H-pyrrolo[1,2-a] benzimidazole**

   The Step 1 product (0.877 mmol) was dissolved in 12 ml 1.2 M HCl, then refluxed 9 hours, and concentrated. The white residue was dissolved in water buffered to pH 7 with saturated $NaHCO_3$ solution, then extracted five times with 30 ml $CHCl_3$, dried with Na_2SO_4, and concentrated. The residue was recrystallized using $CHCl_3$/hexane and the product isolated in 95% yield as a white solid.

   Rotations *R*-(+) $[\alpha]_D^{25} = +20.9°$ ($c = 1.1$, methyl alcohol) and *S*-(−) $[\alpha]_D^{25} = -20.7°$ ($c = 0.2$, methyl alcohol)

3. **Preparation of *R*-(+)-3-*N*-trifluoroacetyl-2,3-dihydro-7-methyl-1H-pyrrolo-[1,2- a]-benzimidazole**

   The Step 2 product (0.83 mmol) was dissolved in 3 ml of trifluoroacetic and 3 ml of trifluoroacetic anhydride and stirred 20 minutes at ambient temperature, then poured into 150 ml of 0.1 M phosphate buffer (pH 7.0). The mixture was extracted three times using 20 ml EtOAc, then washed twice with 10 ml saturated $NaHCO_3$ solution, dried with Na_2SO_4, and concentrated. The residue was recrystallized from EtOAc/hexane and the product isolated in 65% yield.

   Rotation (*R*)-(+) $[\alpha]_D^{25} = +101.2°$ ($c = 0.55$, methyl alcohol)
   Analysis $C_{13}H_{12}F_3N_3O$

4. **Preparation of *R*-(+)-3-*N*-trifluoroacetyl-6-bromo-2,3-dihydro-7-methyl-5-nitro-1H-pyrrolo-[1,2-a]benzimidazole**

   The Step 3 product (1.76 mmol) dissolved in 25 ml of acetic acid was treated with 0.25 ml 0.8 M bromine dissolved in acetic acid, then stirred 20 minutes at ambient temperature, and quenched with 500 ml 0.1 M phosphate buffer (pH 7.0). The mixture was extracted three times with 80 ml EtOAc, then washed twice with 80 ml saturated $NaHCO_3$ solution, dried, and concentrated. The residue was recrystallized from EtOAc/hexane and the product isolated in 85% yield, mp = 215−217°C.

   ^{1}H NMR ($CDCl_3$) δ 9.9 (1H, bs, amide NH), 7.61 (1H, s, 5-aromatic proton), 6.83 (1H, s, 8-aromatic proton), 5.48–5.40 (1H, m, 3-methine proton), 4.17–3.95 (2H, m, 1-methylene protons), 3.33–3.26 (1H, m, 2-methylene proton), 2.77–2.65 (1H m, 2-methylene proton), 2.42 (3H, s, methyl)
   IR (KBr pellet) (cm^{-1}) 3178, 2984, 2859, 1730, 1570, 1522, 1445, 1221, 1186, 1148
   TLC (chloroform/methanol [90:10]) $R_f = 0.50$
   MS (EI) *m/z* 363 & 361 (M^+, 79 Br & 81 Br), 264 & 266 ($M^+ - COCF3$), 223, 169, 130, 90
   Rotations (*R*)-(+) $[\alpha]_D^{25} = +92.6°$ ($c = 0.47$, methyl alcohol); (*S*)-(−) $[\alpha]_D^{25} = -97.8°$ ($c = 0.18$, MeOH)
   Analysis $C_{13}H_{11}BrF_3N_3O$

5. **Preparation of *R*-(+)-3-*N*-trifluoroacetyl-6-bromo-2,3-dihydro-7-methyl-5-nitro-1H-pyrrolo-[1,2-a]benzimidazole**

   The Step 4 product (1.4 mmol) was slowly added to 12 ml fuming nitric acid, cooled to 0°C followed by 1.14 ml acetic anhydride with stirring while cooling for 5 minutes. Cooling was removed and the mixture stirred 90 minutes at ambient temperature and then poured into 450 ml ice water. The solution was buffered to pH 7 with $NaHCO_3$, then extracted three times with 50 ml EtOAc, dried, and concentrated. The yellow residue was recrystallized using a minimum amount of EtOAc facilitated by the

addition of hexane and the product isolated in 89% yield as light yellow crystals, mp = 245−247°C.

^{1}H NMR ($CDCl_3$) δ 10.21 (1H, s, amide NH), 7.38 (1H, s, 8-aromatic proton), 4.75–4.70 (1H, m, 3-methine proton), 4.52–4.42 (1H, m, 1-methylene proton), 4.20–4.12 (1H, m, 1-methylene proton), 3.50–3.40 (1H, m, 2-methylene proton), 2.95–2.85 (1H, m, 2-methylene proton), 2.58 (3H, s, methyl)
IR (KBr pellet) (cm^{-1}) 3246, 3044, 1741, 1657, 1570, 1538, 1370, 1229, 1159
TLC (chloroform/methanol [90:10]) $R_f = 0.60$
MS (EI Mode) m/z 406 (M^+), 361 (M^+−NO2), 309, 293, 280, 263, 189
Rotations *R*-(+) $[\alpha]_D^{25} = +70.12°$ ($c = 0.42$, methyl alcohol); *S*-(−) $[\alpha]_D^{25} = 64.0°$ ($c = 0.39$, methyl alcohol)
Analysis $C_{13}H_{10}BrF_3N_4O_3$

6. **Preparation of *R*-(−)-3-amino-6-bromo-2,3-dihydro-7-methyl-5-nitro-1H-pyrrolo[1,2-a]benzimidazole**

Gaseous ammonia was bubbled into 45 ml methyl alcohol, cooled to −70°C until the volume doubled, then treated with the Step 5 product (0.737 mmol), then stirred 48 hours at ambient temperature. The mixture was concentrated, the residue recrystallized from $CHCl_3$/*hexane*, and the product isolated in 66% yield as light brown crystals, mp = 145−148°C.

^{1}H NMR ($CDCl_3$) δ 7.36 (2H, s, aromatic proton), 4.58 (1H, dd, $J = 7.9$, 6.6 Hz, 3-methine proton), 4.29–4.21 and 4.09–4.01 (2H, 2m, 1-methylene protons), 3.15–3.03 and 2.51–2.39 (2H, 2m, 2-methylene), 2.58 (3H, s, 7-methyl)
IR (KBr pellet) (cm^{-1}) 3389, 2924, 1532, 1458, 1381, 1294, 878
TLC (chloroform/methanol [80:20]) $R_f = 0.22$
MS (EI mode) m/z 310 and 312 (M^+, 79 Br & 81 Br), 293 and 295 (M^+, −NH3), 263 and 265 (M^+-NH_3-NO)
Rotations *R*-(−) $[\alpha]_D^{25} = -8.1°$ ($c = 0.42$, methyl alcohol); *S*-(+) $[\alpha]_D^{25} = 10.1°$ ($c = 0.16$, methyl alcohol)
Analysis $C_{11}H_{11}N_4O_2$

7. **Preparation of *R*-(−)-3-amino-6-azindinyl-2,3-dihydro-7-methyl-1H-pyrrolo[1,2-a]benzimidazole 5,8-dione**

A mixture consisting of the Step 6 product (0.225 mmol), 70 ml methyl alcohol, and 5% Pd on charcoal (70 mg) was hydrogenated 24 hours at 50 psi hydrogen. The mixture was then filtered through celite, concentrated, and combined with 30 ml water containing KH_2PO_4 (0.333 g). This solution was combined with 50 ml apiece water containing KH_2PO_4 (1.0 g) and Fremy salt (0.700 g), then stirred 6 hours at ambient temperature, and concentrated. The residue was purified using a 25 ml Baker Bond Phenyl reverse-phase column prepared with 100% water and combined fractions concentrated. The residue was dissolved in 10 ml methyl alcohol containing 0.3 ml azirindine, then stirred 5 hours at ambient temperature, and reconcentrated.

The residue was purified by flash chromatography using $CHCl_3$/methyl alcohol, 95:5, then recrystallized using $CHCl_3$/*hexane*, and the product isolated in 10.3% yield as a red solid, mp > 240°C (dec).

^{1}H NMR (DMSO-d_6) δ 4.23–4.15 and 4.06–3.97 (3H, 2m, 3-methine and 1-methylene), 2.89–2.78 and 2.27–2.16 (2H, 2m, 2-methylene), 2.29 (4H, s, aziridinyl protons), 1.94 (3H, s, 7-methyl)
IR (KBr pellet) (cm^{-1}) 3387, 2996, 2924, 1674, 1632, 1576, 1518, 1377, 1341, 1312, 1140, 988
TLC (chloroform/methanol [80:20]) $R_f = 0.17$
MS (EI mode) *m/z* 258 (M^+), 240, 214
Analysis $C_{13}H_{14}N_4O_2$

8. **Preparation of *R*-(−)-3-phenoxyamido-6-azindinyl-2,3-dihydro-7-methyl-1H-pyrrolo[1,2-a]benz-imidazole 5,8-dione**

The Step 7 product (0.482 mmol) dissolved in 9 ml of dry pyridine was cooled to 0°C, then treated with 0.3 ml phenylchloroformate, then stirred 15 minutes at 0°C and 60 minutes at ambient temperature. The solution was diluted with 50 ml EtOAc, then washed three times with 15 ml 20% aqueous acetic acid, twice with 10 ml 0.12 M HCl, twice with 15 ml of water, and dried. The solution was concentrated, the residue recrystallized using $CHCl_3$/hexane, and the product isolated in 78% yield as a light brown solid, mp = 175−178°C.

^{1}H NMR (DMSO-d_6) δ 8.55 (1H, d, $J = 9$ Hz, amide proton), 7.87 (1H, s, C(8) proton), 7.4–6.7 (5H, 3m, aromatic protons), 5.28 (1H, q, $J = 6.6$ Hz, 3-methine), 4.36–4.26 and 4.18–4.09 (2H, 2m, 1-methylene), 3.13–3.01 and 2.61–2.49 (2H, 2m, 2-methylene), 2.54 (3H, s, 7-methyl)
IR (KBr pellet) (cm^{-1}) 3306, 1736, 1537, 1491, 1208
TLC (chloroform/methanol [80:20]) $R_f = 0.66$
MS (EI mode) *m/z* 430 and 432 (M^+, 79 Br & 81 Br)
Rotations *R*-(−) $[\alpha]_D^{25} = +13.6°$ ($c = 0.26$, methyl alcohol); *S*-(+) $[\alpha]_D^{25} = -14.0°$ ($c = 0.33$, methyl alcohol)
Analysis $C_{18}H_{15}BrN_4O_4$

9. **Preparation of *R*-(−)-3-urea-6-azindinyl-2,3-dihydro-7-methyl-1H-pyrrolo[1,2-a]benzimidazole 5,8-dione**

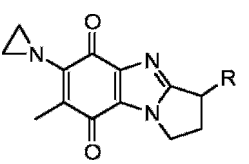
A solution of the Step 8 product (0.348 mmol) dissolved in 10 ml CH_2Cl_2 was added to 20 ml liquid ammonia and stirred for 30 minutes at −70°C, then 3 hours at ambient temperature. The mixture was concentrated, the residue recrystallized using $CHCl_3$/*hexane*, and the product isolated in 84.5% yield as an off-white solid, mp = 240−241°C.

^{1}H NMR (DMSO-d_6) δ 7.83 (1H, d, $J = 1.2$ Hz, aromatic proton), 6.69 (1H, d, $J = 8.4$ Hz, amide protons), 5.69 (2H, s, amide protons), 5.22 (1H, m, 3-methine),

4.30–4.21 and 4.11–4.03 (2H, 2m, 1-methylene), 2.99–2.88 and 2.41–2.30 (2H, 2m, 2-methylene), 2.54 (3H, s, 7-methyl)
IR (KBr pellet) (cm^{-1}) 3410, 3293, 2922, 1657, 1588, 1532, 1371
TLC (chloroform/methanol [80:20]) $R_f = 0.34$
MS (EI mode) *m/z* 353 and 355 (M^+, 79 Br & 81 Br)
Analysis $C_{12}H_{12}N_5O_3$

Derivatives

Table 1 Selected yujungamycin derivatives having significant antineoplastic activity. Although physical data were only supplied for yujungamycin C, additional data were supplied by the authors (1) in an earlier investigation



Agent	R
Yujungamycin A	$-NHCOCH_3$
Yujungamycin B	NH_2
Yujungamycin C	$-NHCONH_2$

Testing

I. *Cytotoxic Activity*

The cytotoxic activity of the experimental agents was evaluated according to the method of Boyd (2). Testing results are provided in Table 2.

II. *DT-Diaphorase Reduction Kinetics Study*

Kinetic studies were carried out under anaerobic conditions in 0.05 M Tris–HCl buffer (pH 7.4) with Thunberg cuvettes and a 2 mM stock solution of selected experimental agents dissolved in DMSO. The experimental agent was added to the top port and DT-diaphorase and NADH in the Tris buffer were added to the bottom. Thereafter both ports were purged with argon for 20 minutes and then equilibrated to 30°C. Ports were then mixed and the reaction monitored 25 minutes at

296 nm to obtain initial rates. The concentrations upon mixing were 0.3 mM NADH, $1 - 20 \times 10^{-5}$ M quinone, and 14.5 nM of enzyme active sites. The value of $\Delta\varepsilon$ was calculated from the initial and final absorbance values for complete quinone reduction and were used to calculate V_{max} (M/s) using the Lineweaver–Burk plot. Kinetic data are provided in Table 3.

Table 2 Summary of antineoplastic activity of selected experimental agents. The 3-unsubstituted and the 3-hydroxy derivatives were devoid of activity, while the 3-propanoate derivative showed 100% animal toxicity. A subcutaneous score of 14 indicates that the experimental agent can kill cancer cells at locations distant from the site of injection

Agent	Intraperitonal Score	Subcutaneous Score	Antitumor Activity
Yujungamycin A	40	8	Melanoma, nonsmall cell, lung cancer, breast cancer, ovarian cancer
Yujungamycin B	24	6	Nonsmall cell lung cancer, CNS cancer, ovarian cancer
Yujungamycin A (−)	38	14	–

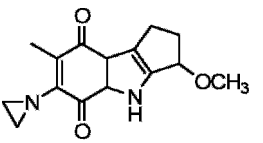
Table 3 Kinetic parameters associated with the DT-diaphorase reduction of the quinone portion using selected yujungamycin derivatives. Reduction of derivatives at the DT-diaphorase substrate was influenced by both enantiomer orientation and hydrogen bonding

Agent	$K_m \times 10^5$	V_{max} (nM/s)	$K_{cat}/K_m \times 10^{-4}$
S-(−)-Yujungamycin A	12.7	47.6	3.74
R-(+)-Yujungamycin A	3.8	28	7.5
R-(+)-Yujungamycin B	2.4	14.6	6.1
S-(−)-Yujungamycin C	5.15	21.8	4.23
R-(+)Yujungamycin C	4.6	39.3	8.59

Notes

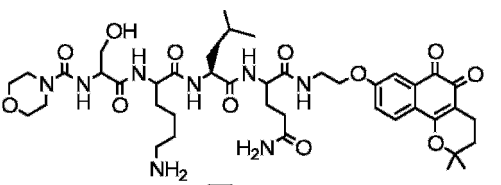
1. Cyclopent[b]indolequinones, (**I**), another new class of reductive alkylating agent having high in vitro cytotoxicity and antitumor activity, were prepared by the authors

(1) in an earlier investigation. Biomolecular labeling studies by Turnbull (3) indicated that cyclopent[b]indole–quinine alkylates the phosphate residue of nucleic acids in the binding region containing the adenine–thymine base pair.

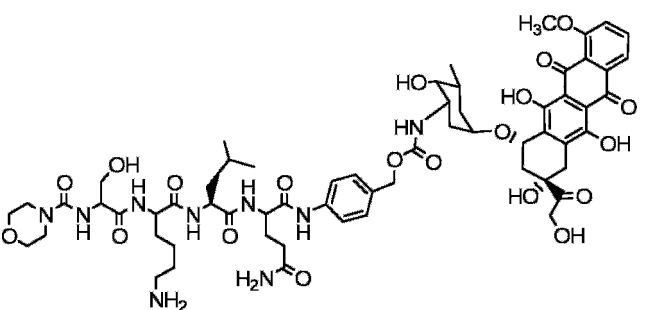


(I)

2. Tetrapeptide quinones derived from β-lapachone, (**II**), and doxorubicin, (**III**), prepared by Blokhin (4) were effective against human lung adenocarcinoma cell line, A549, and human prostatic cancer cell line, DUPRO.

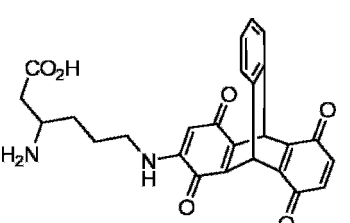


(II)



(III)

3. Triptycene diquinone derivatives, (**IV**), prepared by Hua (5) were effective in inhibiting nucleic acid or protein syntheses and used in the treatment of multidrug-resistant tumor cells.



(IV)

References

1. E.B. Skibo *et al*., US Patent 6,846,840 (January 25, 2005)
2. M.R. Boyd, Oncology 3, 1 (1989)
3. K.D. Turnbull, US Patent 6,657,052 (December 2, 2003)
4. A.V. Blokhin *et al*., US Patent 6,809,176 (October 26, 2004)
5. D. Hua *et al*., US Patent 6,828,450 (December 7, 2004)

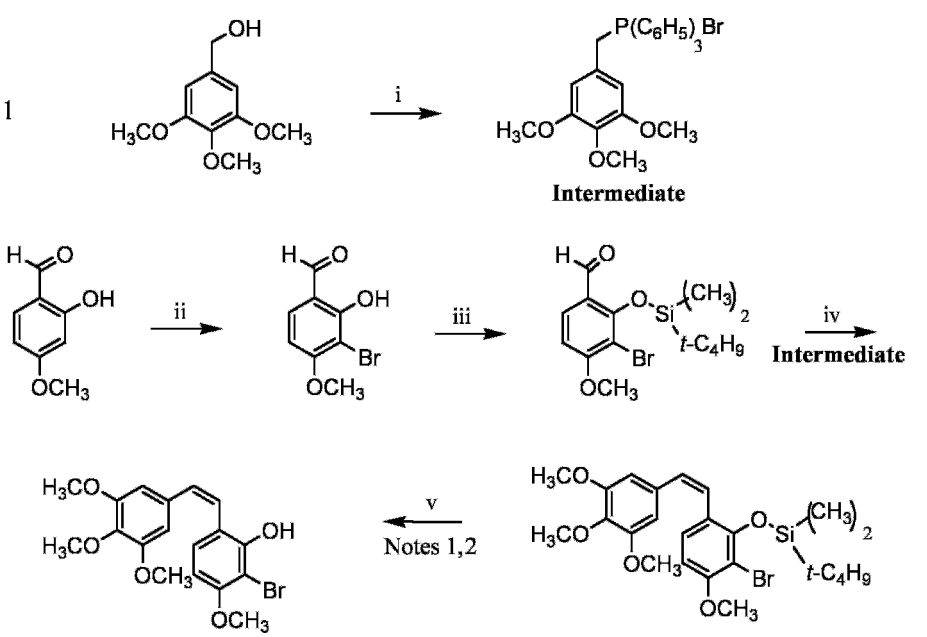
III. Antineovascularization Agents: Combretastatin A-1 and A-4 Inhibitors

Title Functionalized Stilbene Derivatives as Improved Vascular Targeting Agents
D.J. Chaplin *et al.*, US Patent 6,919,324 (July 19, 2005)
Assignee Oxigene, Inc. and Baylor University
Utility Antineovascularization Retinal Agents

Invention Significance Stilbenoid tubulin-binding agents structurally related to combretastatin A-1 (CA-1) and combretastatin A-4 (CA-4) have been prepared that are effective as CA-1 and CA-2 inhibitors. The prodrug forms, CA-1P and CA-4P, are also effective as vascular targeting agents (VTAs) and useful in the treatment of solid tumor cancers and diseases associated with unwanted retinal neovascularization.

Reaction

Eq. 1



i- Carbontetrabromide, triphenylphosphine, CH_2Cl_2
ii- Mercuric acetate, ethyl alcohol, acetic acid, sodium bromide, bromine, $CHCl_3$
iii- Diisopropylethylamine, DMF, *t*-butyldimethylsilyl chloride
iv- Butyllithium, 3,4,5-trimethoxybenzyltriphenyl-phosphonium bromide, THF
v- DMF, potassium fluoride, hydrobromic acid

Experimental

1. **Preparation of 3,4,5-trimethoxybenzyltriphenylphosphonium bromide**

   CBr_4 (15.4 mmol) dissolved in 80 ml acetone at 0°C was treated with 3,4,5-trimethoxybenzyl alcohol (11.3 mmol) and triphenylphosphine (15.3 mmol), then stirred 12 hours, and filtered through celite. The mixture was concentrated and the residue dissolved in 50 ml CH_2Cl_2 and treated with PPh_3 (12.4 mmol), then heated overnight. The solution was treated with ice-cold water, then extracted with CH_2Cl_2, washed with brine, dried using Na_2SO_4, and concentrated. The residue was recrystallized from ethyl alcohol/hexane and the product isolated in 85% yield.

2. **Preparation of 2-hydroxy-3-bromo-4-methoxybenzaldehyde**

   2-Hydroxy-4-methoxybenzaldehyde (20 mmol) was treated with mercuric acetate (20 mmol), then refluxed in 100 ml ethyl alcohol containing 1% acetic acid, and further treated with sodium bromide solution. Bromine (1 equiv.) dissolved in $CHCl_3$ containing acetic acid was then added and the mixture concentrated. The residue was purified by chromatography with silica gel using EtOAc in hexane and the product isolated in 47.2% yield.

3. **Preparation of 2-(*t*-butyldimethylsilyl)-3-bromo-4-methoxy benzaldehyde**

   The Step 2 product dissolved in 15 ml DMF was treated with 3 ml diiospropy-lethylamine and *t*-butyldimethlsilyl chloride (12.8 mmol), then stirred 30 minutes at ambient temperature, and treated with ice (20 g). The mixture was extracted three times with 25 ml diethyl ether, and washed once with 25 ml water and twice with 15 ml saturated $NaHCO_3$ solution. The solution was concentrated and the product isolated in 83.1% yield.

4. **Preparation of 2′-oxy-(*t*-butyldimethylsilyl)-3′-bromo-3,4,4′,5-tetramethoxy-(*Z*/*E*)-stilbene**

   Butyllithium (2 M) in hexane (3 mmol) was added to a suspension of the Step 1 product (3 mmol) in THF (50 ml) at −15°C, then stirred 30 minutes at ambient temperature. The solution was then treated with Step 3 product (2.8 mmol), then stirred 3 hours, and diluted with cold water. The mixture was extracted three times with

25 ml diethyl ether, then washed with water, and concentrated. Both *Z*- and *E*-2′-oxy-(*t*-butyldimethylsilyl)-3′-bromo-3, 4, 4′,5-tetramethoxystilbene isomers were isolated in a combined yield of 78.7%.

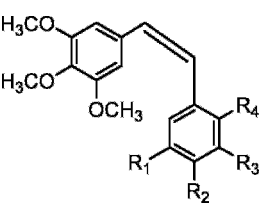
5. **Preparation of 2′-hydroxy-3′-bromo-3,4,4′,5-tetramethoxy-(*Z*)-stilbene**

The Step 4 product mixture (1.46 MMOL) dissolved in 7 ml DMF was treated with KF (1.46 mmol) and HBr (2.92 mmol), then stirred 2 days, and diluted with 15 ml water. The mixture was extracted three times using 15 ml EtOAc, then washed with water, dried, and concentrated. The residue was purified by chromatography using hexane/EtOAc, 7:3, and the product isolated in 43.8% yield.

^{1}H NMR (δ, ppm) 6.95 (1H, d, $J = 11.5$ Hz), 6.56 (1H, d, $J = 8.5$ Hz), 6.52 (1H, d, $J = 11.5$ Hz), 6.44 (2H, s), 3.80 (3H, s), 3.61 (3H, s), 3.54 (3H, s)
^{13}C NMR (δ, ppm) 155.00, 153.38, 150.72, 128.40, 126.07, 122.48, 118.59, 133.00, 103.93, 103.49, 60.98, 56.44, 56.15

Derivatives

Table 1 Selected *Z*-stilbene derivatives and last-step conversions. ^{1}H NMR data supplied by author



Entry	R_1	R_2	R_3	R_4	Yield (%)
1B	OH	OCH_3	OH	H	80
16	H	OCH_3	OH	Br	51.5
28A	H	OCH_3	OH	NO	65
36A	H	OH	OH	OH	43
38	H	OCH_3	H	$NHCOCH(NH_2)CH_2OH$	38
40B	H	H	$OPO_3^{-2}Na^{+2}$	H	30

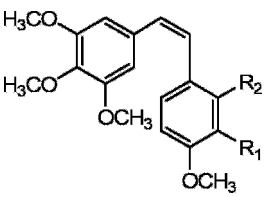
Testing

I. *MTT Cytotoxicity Assay*

Exponentially growing cells were treated with selected experimental agents for 1 hour and 5 days. Insoluble agents were dissolved in 0.3% DMSO before

administering. Cell viability was determined by the calorimetric MTT assay using 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide according to the method of Berridge (1). Testing results are provided in Table 2.

Table 2 Effectiveness of functionalized *Z*-stilbenoid derivatives as CA-1 and CA-2 inhibitors using the MTT cytotoxicity assay after 1-hour and 5-day treatment periods

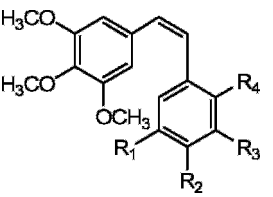


Entry	R_1	R_2	IC_{50} (μM)	
			1 hour	5 days
Combretastin A-1 **(reference)**	OH	OCH_3	10	0.05
Combretastin A-4 **(reference)**	OH	OH	0.1	0.05
16	OH	Br	2	0.0067
45	H	$NHCOCH(NH_2)CH_2OH$	10	0.05
46A	H	OH	43	0.068
191	$OPO_2(OC_6H_{11})O^- NH_4^+$	H	35	0.13
210	$OPO_2(OCH_2CF_3)O^- NH_4^+$	H	25	0.07

II. *Vascular Shutdown Assay*

The vascular effects of experimental agents were assessed in tumor-bearing mice using a fluorescent-bead assay. A MHEC-5T hemangioendothelioma tumor model was established by subcutaneous injection of 0.5×10^6 cultured MHEC5-T cells into the right flank of Fox Chase CB-17 SCID mice and allowed to grow to a size of 300 mm^3 before ip injection with a single dose of saline control or a selected experimental agent. At 24 hours post-treatment, mice were iv injected with 0.25 ml of diluted FluoSphere beads in physiological saline in the tail vein and sacrificed after 3 minutes. Tumor cryosections at a thickness of 8 μm were examined directly using quantitative fluorescent microscopy. Testing results are provided in Table 3.

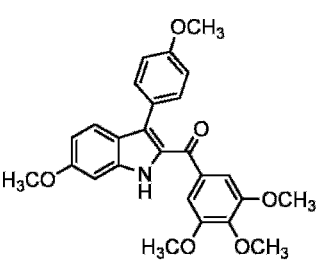
Table 3 Percent vascular blood flow shutdown using functionalized *Z*-stilbenoid derivatives according to the MHEC-5T hemangioendothelioma test method



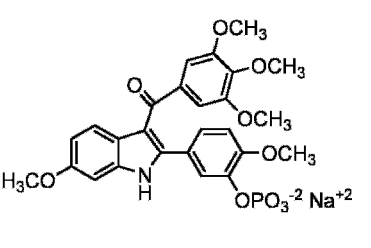
Entry	R_1	R_2	R_3	R_4	Blood Flow Shutdown (%)
2B	OPO_3^{-2} Na^{+2}	OCH_3	OPO_3^{-2} Na^{+2}	H	65
27B	H	OCH_3	OPO_3^{-2} Na^{+2}	OCH_3	41
29B	OCH3	OPO_3^{-2} Na^{+2}	H	I	43
33B	OPO_3^{-2} Na^{+2}	OCH_3	H	OPO_3^{-2} Na^{+2}	51
45	H	OCH_3	H	$NHCOCH(NH_2)CH_2OH$	43
210	H	OCH_3	$OPO_2(OC_8H_{17})O^-NH_4^+$	H	89

Notes

1. 6-Methoxy indole-containing combretastatin derivatives, (**I**), and prodrugs, (**II**), prepared by Pinney (2) were found effective as antimitotic and antitubulin polymerization agents and used in the selective destruction of tumor cell vasculature.

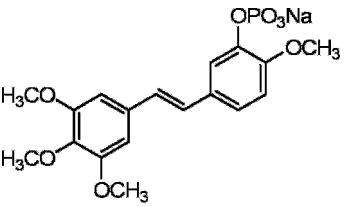


(I)



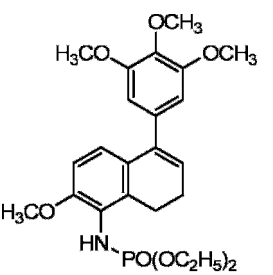
(II)

2. Pettit (3) prepared water-soluble, *trans*-isomer derivatives, (**III**), of combretastatin A-4, which were used in the treatment of neoplastic diseases.



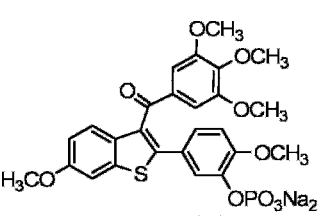
(III)

3. Tubulin-binding agents prepared by Pinney (4) consisting of dihydronaphthalene derivatives, (**IV**), exhibited potent tumor cell cytotoxicity by inhibiting the polymerization of α,β-tubulin heterodimers into the microtubule structures and were used in the treatment of proliferation diseases.



(IV)

5. Pero (5) prepared noncytotoxic stilbene phosphate prodrug derivatives, (**V**), which were converted into the dephosphorylated cytotoxic drug equivalents, (**VI**), by action of endothelial enzymes which then acted selectively at the sites of vascular proliferation.



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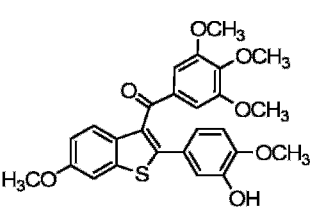
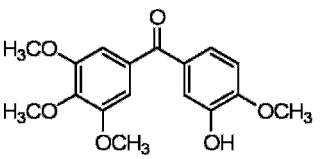
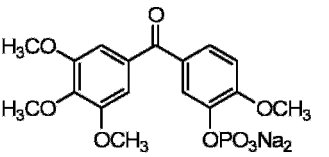


(VI)

6. Pettit (6) prepared phenstatin, (**VII**), and prodrug derivatives, (**VIII**), which were more effective tubulin polymerization inhibitors than colchicine and more effective as tubulin binder inhibitors than combretastatin.



(VII)



(VIII)

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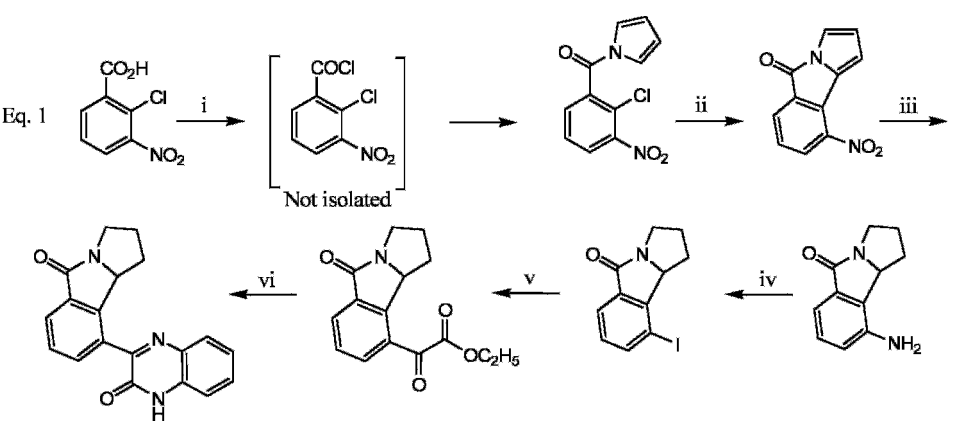
1. M.V. Berridge *et al*., Biochemica, 4, 15 (1996)
2. H. Pinney *et al*., US Patent 6,849,656 (February 1, 2005) and US Patent 6,593,374 (July 15, 2003)
3. G.R. Pettit *et al*., US Patent 7,018,987 (March 28, 2006)
4. H. Pinney *et al*., US Patent 7,001,926 (February 21, 2006)
5. R.W. Pero *et al*., US Patent 6,956,054 (October 18, 2005) and US Patent 6,538,038 (March 25, 2003)
6. G.R. Pettit *et al*., US Patent 6,943,194 (September 13, 2005)

IV. Brain, Head, and Neck Cancer: Cyclin-dependent Kinase-4 or -6 Kinase Inhibitors

Title Pyrazinone Derivatives
T. Hayama *et al.*, US Patent 6,914,062 (July 5, 2005)
Assignee Banyu Pharmaceutical Co., Ltd
Utility Treatment of Brain Cancer

Invention Significance Cell cycle regulation is through the cyclin-dependent kinase (CDK) family. CDK controls cell cycle progression by phosphorylation of target proteins and retinoblastoma (RB). Abnormalities that impede the formation of the CDK–RB complex are found in human cancer and are characterized by high levels of cyclin D1, CDK4, and depletion of functionalized RB protein. This investigation provides a method for impeding cancer cell growth by using CDK4 or CDK6 kinase inhibitors that are useful in treating human solid cancers in the brain, head, and neck.

Reaction



i- Thionyl chloride, 4-dimethylaminopyridine, triethylamine, pyrrole, CH_2Cl_2
ii- Potassium acetate, DMAc, tetrakistriphenylphosphine palladium

iii- 10% Palladium on carbon, methyl alcohol, THF, hydrogen
iv- Acetic acid, hydrochloric acid, sodium nitrite, sodium iodide, sodium thiosulfite
v- THF, *n*-butyllithium, ethyloxalyl chloride
vi- Ethyl alcohol, 1,2-phenylenediamine

Experimental

1. **Preparation of 1-(2-chloro-3-nitrobenzoyl)pyrrole**

   2-Chloro-3-nitrobenzoic acid (10.0 mmol) and 30 ml thionyl chloride were mixed, then treated with 4-dimethylaminopyridine (1.00 mmol), then refluxed 12 hours. The mixture was concentrated, then treated with 3.5 ml pyrrole, and 7.0 ml triethylamine dissolved in 80 ml CH_2Cl_2 and stirred 6 hours at ambient temperature. The solution was diluted with EtOAc and the organic phase was washed with brine and dried using $MgSO_4$. The solution was concentrated, the residue purified by chromatography with silica gel using hexane/EtOAc, 1:0–7:3, and 2.43 g product isolated as yellow oil.

2. **Preparation of 9-nitro-5H-pyrrolo[2,1-a]isoindol-5-one**

   The Step 1 product (9.60 mmol) dissolved in 180 ml in DMAc was treated with potassium acetate (19.2 mmol) and tetrakistriphenylphosphine palladium (0.960 mmol), then stirred overnight at 130°C. The mixture was diluted with EtOAc/diethyl ether, 1:2, washed with water and brine, dried, and concentrated. The residue was purified by chromatography using hexane/$CHCl_3$, 1:0–1:1, and 2.24 g product isolated as a brown solid.

   ^{1}H NMR ($CDCl_3$) δ 6.34 (1H, t, $J = 3.2$ Hz), 7.10 (1H, dd, $J = 3.3, 0.85$ Hz), 7.21(1H, m), 7.35 (1H, dd, $J = 8.3, 7.3$ Hz), 7.94 (1H, dd, $J = 7.3, 1.0$ Hz), 8.28 (1H, dd, $J = 8.5, 1.0$ Hz)

3. **Preparation of 9-amino-1,2,3,9b-tetrahydro-5H-pyrrolo[2,1-a]isoindol-5-one**

   The Step 2 product (2.24 g) dissolved in 80 ml methyl alcohol/THF, 1:1, was treated with 10% palladium on carbon catalyst (0.200 g), then stirred 12 hours at ambient temperature under hydrogen. The mixture was then filtered through a celite pad and concentrated. The residue was purified by chromatography using $CHCl_3$/methyl alcohol, 1:0–98:2–95:5, and 1.03 g product isolated as a brown solid.

   ^{1}H NMR (DMSO-d_6) δ 0.80–0.93 (1H, m), 2.10–2.30 (2H, m), 2.43–2.51(1H, m), 3.18–3.24 (1H, m), 3.38–3.47 (1H, m), 4.50 (1H, dd, $J = 10, 5.5$ Hz), 5.34 (2H, s), 6.72 (1H, d, $J = 7.9$ Hz), 6.76 (1H, d, $J = 7.4$ Hz), 7.11 (1H, t, $J = 7.6$ Hz)

4. **Preparation of 9-iodo-1,2,3,9b-tetrahydro-5H-pyrrolo[2,1-a]isoindol-5-one**

   The Step 3 product (38.7 mmol) dissolved in 19.3 ml acetic acid containing 7.7 ml 12 M HCl at 0°C was treated with the slow addition of 13.5 ml aqueous $NaNO_2$ (42.6 mmol) followed by the dropwise addition of 40 ml aqueous KI (7.71 g), then stirred 1 hour at 0°C. The mixture was then quenched with saturated $Na_2S_2O_3$ solution, then stirred additional 30 minutes at ambient temperature, and extracted with EtOAc. The extract was washed with saturated $NaHCO_3$ solution and brine, dried, and then concentrated. The residue was purified by chromatography using hexane/EtOAc, 5:1–1:1, and 5.95 g product isolated as a colorless solid.

5. **Preparation of ethyl (5-oxo-1,2,3,9b-tetrahydro-5H-pyrrolo[2,1-a]isoindol-9-yl)oxoacetate**

   The Step 4 product (3.88 mmol) was dissolved in 100 ml THF, then treated with 3.10 ml 1.5 M butyllithium in hexanes, then cooled to −78°C, and further treated with 867 μl cooled ethyloxalyl chloride dissolved in 10 ml THF. The mixture was stirred 5 minutes and then returned to ambient temperature where it was quenched with saturated NH_4Cl solution. It was extracted with EtOAc, washed with saturated brine solution, dried, and concentrated. The residue was purified by chromatography using hexane/EtOAc, 1:2, and 212 mg product isolated as a colorless oil.

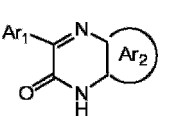
6. **Preparation of 9-(3-oxo-3,4-dihydroquinoxaline-2-yl)-1,2,3,9b-tetrahydro-5H-pyrrolo[2,1-a]isoindol-5-one**

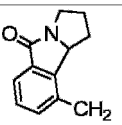
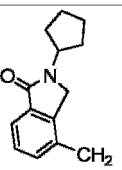
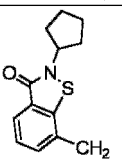
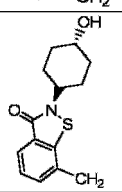
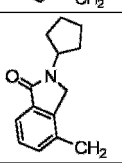
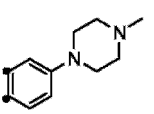
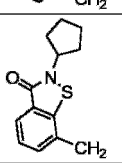
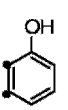
   A mixture of the Step 5 product (0.366 mmol) and 1,2-phenylenediamine (0.449 mmol) was dissolved in 1 ml ethyl alcohol and heated 12 hours at 100°C in a sealed tube. The solution was then concentrated, the residue purified by TLC using $CHCl_3$/methyl alcohol, 19:1, and 35 mg product isolated as an yellow solid.

   ^{1}H NMR (DMSO-d_6) δ 1.00–1.20 (1H, m), 2.12–2.31 (3H, m), 3.22–3.64 (2H, m), 5.25–5.32 (1H, m), 6.35–8.51 (7H, m), 12.7 (1H, s)
   MS 318 $(M+1)^+$

Derivatives

Table 1 Selected pyrazinone derivatives and their corresponding mass spectra characterization. Approximately 115 derivatives were prepared by the author



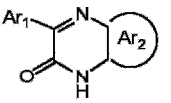
Entry	Ar_1	Ar_2	MS $(M+1)^+$
3			346
22			360
28			364
58			394
83			505
91			380

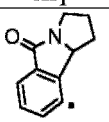
Testing

I. *D2–CDK4 Inhibition*

The method of Kitagawa (1) was used to determine cyclin D2–CDK4 activity. The synthetic peptide Arg-Pro-Pro-Thr-Leu-Ser-Pro-Ile-Pro-His-Ile-Pro-Arg that corresponds to the amino acid sequence No. 775–787 of RB protein was used as a substrate. IC_{50} testing results are provided in Table 2.

Table 2 Inhibition of CDK4 cell growth using selected pyrazinone derivatives using the synthetic peptide Arg-Pro-Pro-Thr-Leu-Ser-Pro-Ile-Pro-His-Ile-Pro-Arg as the substrate



Entry	Ar_1	Ar_2	IC_{50} (μM)
1			0.12
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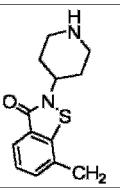
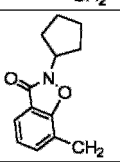
28	Table 1	Table 1	0.051
36			0.091
40			0.10
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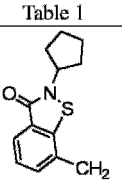
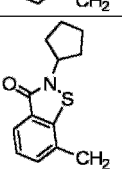
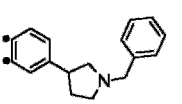
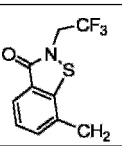
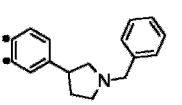
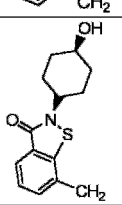
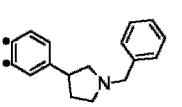
97			0.012
98			0.003
103			0.035
105			0.004

II. *Inhibition of Cell Growth*

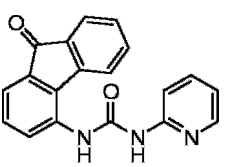
Inhibition of cell growth was determined by measuring the amount of formazan generated by UV–vis at 450 nm using live cells of T98G or U-2OS. Testing results appear in Table 3.

Table 3 Test results using selected experimental pyrazinone agents on 50% inhibition for cell growths of T98G, U-2 OS, and CDK6

Entry	IC_{50} (μM) (T98G Cell)	IC_{50} (μM) (U-2 OS Cell)	IC_{50} (μM) (Cyclin D2-CDK6)
1	0.30	10.33	–
3	0.18	10.21	0.088

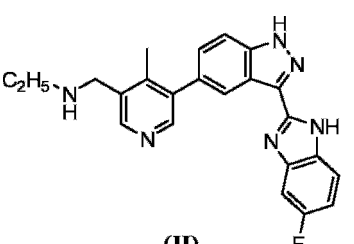
Notes

1. In a subsequent investigation, CDK4 and/or CDK6 inhibitors consisting of biarylurea derivatives, (**I**), were prepared by authors (2) and used for treating solid malignant tumors.

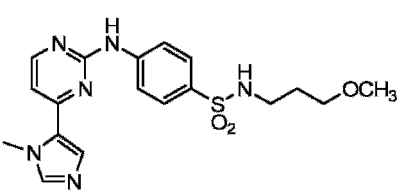


(I)

2. 3,5 Disubstituted indazoles, (**II**), and 2,4-disubstituted pyrimidines, (III), prepared by Kephart (3), were effective as CDK1, CDK2, CDK4, and CDK6 inhibitors and used in treating cancer and other disease states associated with unwanted angiogenesis and/or cellular proliferation.

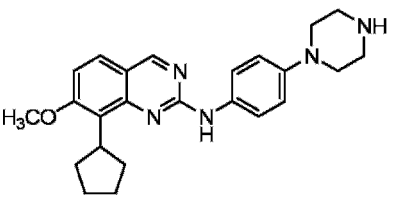


(II)



(III)

3. Aminoquinazolines, (IV) and pyrido[2,3-d]pyrimidine-2,7-diamine derivatives, (V), prepared by Barvian (4) and Booth (5), respectively, were effective as CDK2 and CDK4 inhibitors and used as cell regulators in the treatment of proliferative disorders.



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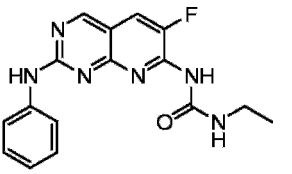
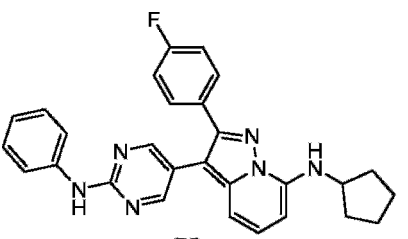


(V)

4. Pyrazolopyridinyl pyrimidines, (V), prepared by Chamberlain (6) and imidazolo-5-yl-2-anilino-pyrimidine derivatives, (VI), prepared by Breault (8) were effective as CDK2, CDK4 and CDK6 cell cycle kinase inhibitors.



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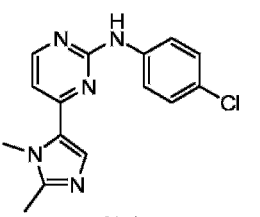

(VI)

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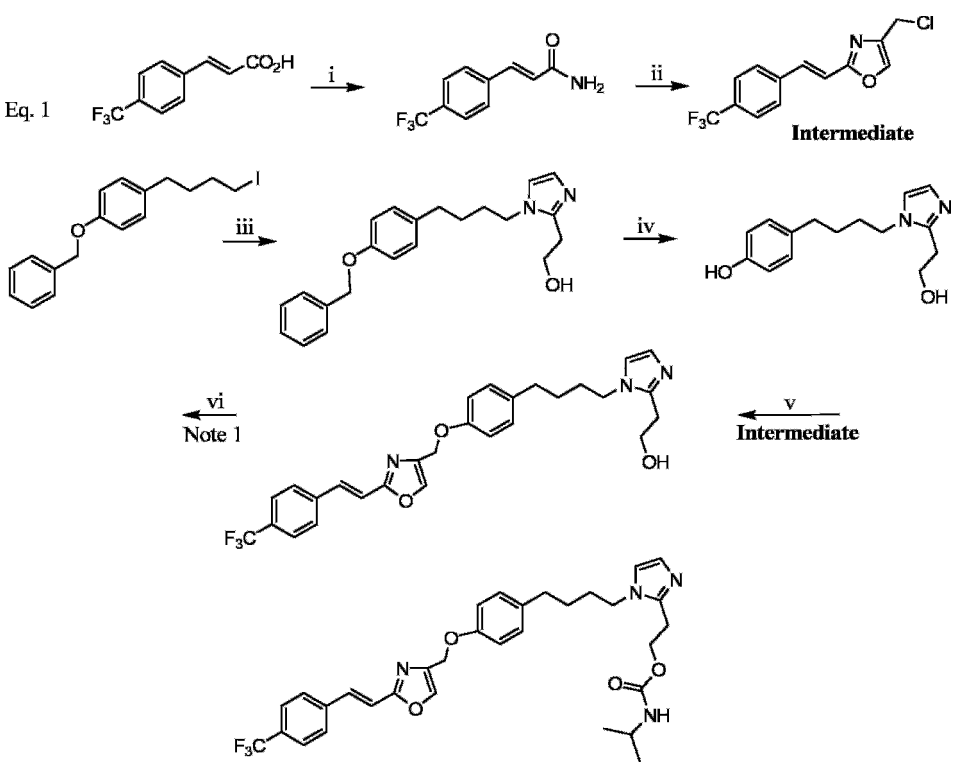
3. S.E. Kephart *et al*., US Patent 7,008,953 (March 7, 2006)
4. M.K. Barvian *et al*., US Patent 6,982,260 (January 3, 2006)
5. J.R. Booth *et al*., US Patent 7,053,070 (May 30, 2006)
6. S.D. Chamberlain *et al*., US Patent 7,087,618 (August 8, 2006)
7. G.A. Breault *et al*., US Patent 6,969,714 (November 29, 2005)

V. Breast Cancer: Human Epidermal Growth Factor Tyrosine Kinase Inhibitors

Title Heterocyclic Compounds, Oxazole Derivatives, Process for Preparation of the Same, and Use Thereof
A. Tasaka *et al.*, US Patent 6,984,653 (January 10, 2006)
Assignee Takeda Pharmaceutical Company Limited
Utility Treatment of Breast Cancer

Invention Significance The human epidermal growth factor receptor-2 gene (HER2) is found in both breast and ovarian cancers. The HER2 encodes transmembrane-type glycoprotein which subsequently increases tyrosine kinase activity. Very high-activity and low-toxicity oxazole derivatives effective as HER2 tyrosine kinase inhibiting agents have been prepared to address this concern.

Reaction



i- DMF, THF, oxalyl chloride, EtOAc, ammonia
ii- 1,3-Dichloroacetone, toluene
iii- 2-(2-Hydroxyethyl)imidazole, potassium carbonate, DMF
iv- Palladium on carbon, hydrogen, methyl alcohol
v- DMF, sodium hydride
vi- Sodium hydride, isopropyl isocyanate

Experimental

1. **Preparation of (*E*)-3-(4-trifluoromethylphenyl)-2-propenamide**

   A suspension of 4-trifluoromethylcinnamic acid (19.4 g) and six drops of DMF in 100 ml THF was treated with 11.7 ml oxalyl chloride at 0°C, then stirred 2 hours at ambient temperature, and concentrated. The residue was dissolved in 60 ml EtOAc, then poured into 120 ml mixture of 25% NH_4OH/EtOAc, 5:1, and the water layer salted out. The organic layer was extracted once with 650 ml EtOAc/THF, 12:1, and twice with 100 ml EtOAc, then dried with $MgSO_4$, and concentrated. The residue was recrystallized using EtOAc/hexane and 18.0 g product isolated as colorless plate crystals.

   ^{1}H-NMR ($CDCl_3$) δ 5.58 (2H, br s), 6.53 (1H, d, $J = 15.8$ Hz), 7.63–7.72 (5H, m)
   IR (KBr) (cm^{-1}) 3326, 3167, 1686, 1636, 1617, 1404, 1190

2. **Preparation of 4-chloromethyl-2-[(E)-2-(4-trifluoromethylphenyl)ethenyl]-1,3-oxazole**

   A mixture of the Step 1 product (17.9 g) and 1,3-dichloroacetone (14.8 g) dissolved in 83 ml toluene was refluxed 9 hours using the Dean–Stark apparatus. After cooling, the mixture was diluted with water, then extracted with EtOAc, washed with brine, dried, and concentrated. The residue was purified by chromatography using silica gel with hexane/EtOAc, 6:1–5:1, and 15.1 g product isolated as colorless needle crystals.

   ^{1}H-NMR ($CDCl_3$) δ 4.55 (2H, d, $J = 0.8$ Hz), 7.00 (1H, d, $J = 16.2$ Hz), 7.56 (1H, d, $J = 16.2$ Hz), 7.64–7.68 (5H, m)
   IR (KBr) (cm^{-1}) 1350, 1325, 1170, 1136, 1113, 1071, 959, 826, 727, 708

3. **Preparation of 2-(1-{4-[4-(benzyloxy)phenyl]butyl}-1H-imidazol-2-yl)-1-ethanol**

   A mixture consisting of benzyl 4-(4-iodobutyl)phenyl ether (14.29 g), 2-(2-hydroxyethyl)imidazole (13.1 g), and K_2CO_3 was stirred in 390 ml DMF 16 hours at 60°C, then cooled, filtered, and concentrated. The residue was dissolved in EtOAc, then washed with water, brine, and reconcentrated. The residue was purified by chromatography using EtOAc/methyl alcohol, 9:1–1:9, then recrystallized using EtOAc/methyl alcohol, and 10.99 g product isolated as colorless crystals, mp = 75−77°C.

^{1}H-NMR ($CDCl_3$) δ 1.53–1.82 (4H, m), 2.58 (2H, t, $J = 7.1$ Hz), 2.78 (2H, t, $J = 5.5$ Hz), 3.81 (2H, t, $J = 6.9$ Hz), 4.03 (2H, t, $J = 5.5$ Hz), 5.04 (2H, s), 6.80 (1H, d, $J = 1.2$ Hz), 6.90 (2H, d, $J = 8.6$ Hz), 6.93 (1H, d, $J = 1.2$ Hz), 7.05 (2H, d, $J = 8.6$ Hz), 7.34–7.47 (5H, m)
IR (KBr) (cm^{-1}) 3144, 3032, 2934, 2859, 1611, 1582, 1514, 1495, 1456, 1431, 1381, 1298, 1273, 1244, 1175, 1150, 1121, 1109, 1051, 1026

4. **Preparation of 4-{4-[2-(2-hydroxyethyl)-1H-imidazol-1-yl]butyl}phenol**

The Step 3 product (10.67 g) was dissolved in methyl alcohol containing 10% palladium carbon (1.6 g) and hydrogenated 8 hours at ambient temperature, then filtered. The mixture was concentrated and 5.3 g of product isolated, mp = 118−119°C.

^{1}H-NMR ($CDCl_3$) δ 1.50–1.80 (4H, m), 2.55 (2H, t, $J = 7.0$ Hz), 2.79 (2H, t, $J = 5.8$ Hz), 3.82 (2H, t, $J = 7.0$ Hz), 3.97 (2H, t, $J = 5.8$ Hz), 3.85–4.40 (1H, br), 6.77 (2H, d, $J = 8.4$ Hz), 6.80 (1H, s), 6.94 (1H, s), 6.96 (2H, d, $J = 8.4$ Hz)
IR (KBr) (cm^{-1}) 3600–2400, 1615, 1593, 1516, 1489, 1456, 1373, 1252, 1171, 1150, 1125, 1103, 1055

5. **Preparation of 2-[1-[4-[4-[2-(E)-2-(4-trifluoromethylphenyl)ethenyl]oxazol-4-yl]methoxy-phenyl]butyl-1H-imidazol-2-yl]-1-ethanol**

The Step 4 product (260 mg) was treated with NaH (40.6 mg) and 4 ml DMF at 0°C, then stirred 30 minutes at ambient temperature, and further treated with the Step 2 product (316 mg). The mixture was stirred 15 hours at ambient temperature and then diluted with water. Crystals that formed were isolated, washed with water and isopropyl ether, recrystallized using acetone/hexane, and 393 mg product isolated as pale yellow needle crystals.

^{1}H-NMR ($CDCl_3$) δ 1.56–1.74 (4H, m), 2.59 (2H, t, $J = 6.6$ Hz), 2.78 (2H, t, $J = 5.4$ Hz), 3.82 (2H, t, $J = 6.8$ Hz), 4.03 (2H, t, $J = 5.4$ Hz), 5.02 (2H, d, $J = 1.2$ Hz), 6.81 (1H, d, $J = 1.6$ Hz), 6.90–6.95 (4H, m), 7.02 (2H, d, $J = 16.2$ Hz), 7.52–7.69 (6H, m)
IR (KBr) (cm^{-1}) 1512, 1323, 1244. 1175, 1132, 1113, 1067, 1055

6. **Preparation of 2-[1-[4-[4-[[2-[(E)-2-[4-(trifluoromethyl)phenyl]ethenyl]-1,3-oxazol-4-yl]methoxy]phenyl]butyl]-1H-imidazol-2-yl]ethyl isopropylcarbamate**

The Step 5 product (300 mg) dissolved in 3 ml CH_2Cl_2 at 0°C was treated with NaH (21.6 mg), then stirred 30 minutes at ambient temperature, then further treated with 0.288 ml isopropyl isocyanate, and stirred additional 5 hours at ambient temperature. The mixture was diluted with water, extracted with EtOAc, washed with 1 M NaOH solution, brine, dried, and concentrated. The residue was recrystallized from EtOAc/hexane and 315 mg product isolated as a colorless crystal powder.

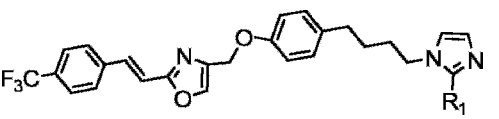
^{1}H-NMR ($CDCl_3$) δ 1.12–1.16 (6H, m), 1.61–1.66 (2H, m) 1.71–1.80 (2H, m), 2.59 (2H, t, $J = 7.5$ Hz), 2.97 (2H, t, $J = 7.2$ Hz), 3.81–3.89 (3H, m), 4.41 (2H, t,

$J = 7.2$ Hz), 4.53 (1H, br s), 5.02 (2H, d, $J = 0.6$ Hz), 6.81 (1H, d, $J = 1.2$ Hz), 6.91–7.09 (6H, m), 7.53–7.69 (6H, m)
IR (KBr) (cm^{-1}) 1711, 1512, 1325, 1246, 1069

Derivatives

Selected oxazole derivatives are provided in Table 1.

Table 1 Effectiveness of selected oxazole derivatives in suppressing receptor tyrosine phosphorylation in human breast cancer cells. ^{1}H-NMR data for experimental agents supplied by author



Entry	R_1	Phosphorylation Inhibition of HER-2, IC_{50} (μM)
1	Ethyl isopropylcarbamate	<4
3	Ethyl 1-pyrroridecarboxylate	<4
5	Propanamide	<4
6	Propanoyl pyrrolidine	<4
11	2-(Methylsulfonyl)ethyl	<4
16	Ethyl methanesulfonamide	<4

Testing

I. *Suppression of Receptor Tyrosine Phosphorylation in Human Breast Cancer Cells*

A suspension of human breast cancer cell BT-474 (500 μl, ~300000 cells) was sown into a 24-well plate and cultured at 37°C in the presence of 5% carbon dioxide. After 24 hours, 250 μl of a solution of a selected experimental agent was added, then reacted 2 hours and the protein removed and reacted with an antiphosphotyrosine antibody. IC_{50} testing results are provided in Table 1.

II. *Inhibitory Action on Breast Cancer Cell BT-474 Proliferation in Vitro*

A suspension of human breast cancer cell BT-474 (100 μl, ~6000 cells) was sown into a 96-well microplate and cultured at 37°C in the presence of 5% carbon dioxide. On the following day, 100 μl of a selected experimental agent was added and cultured 5 days. After the culture medium containing the chemical agent was removed, cells

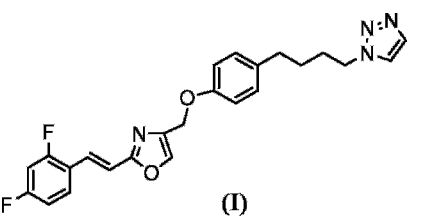
were prepared and dyed according to the method of Skehan (1) and absorbance measured at 550 nm to quantify protein content and IC_{50} values were determined. Testing results are provided in Table 2.

Table 2 Effectiveness of selected experimental agents in suppressing BT-474 cell proliferation in vitro

Entry	Cell Growth Inhibition BT-474, IC_{50} (μM)
1	<0.05
3	<0.05
5	<0.05
6	<0.05
16	0.15

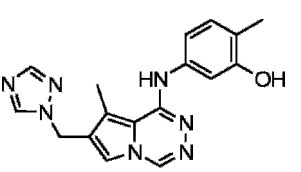
Notes

1. 1,2,3-Triazine derivatives, (**I**), previously prepared by the author (2) were effective as HER2 inhibitors and used in the treatment of breast cancer.



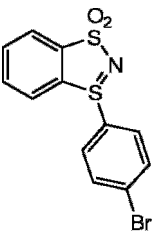
(I)

2. Hunt (3) prepared 1,2,4-triazine derivatives (**III**), which were effective as tyrosine kinase-inhibiting agents for VEGFR-2, FGFR-1, PDGFR, HER-1, and HER-2 growth factors and effective in treating breast and other cancers.



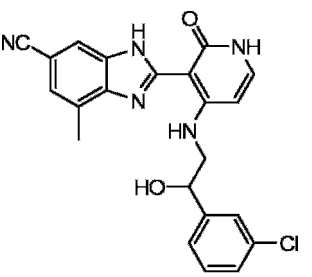
(III)

3. Benzo-, (**IV**), and azabenzodithiazole derivatives prepared by Sang (4) were effective as HER-1, HER-2, and HER-4 inhibitors and used in the treatment of proliferative diseases.



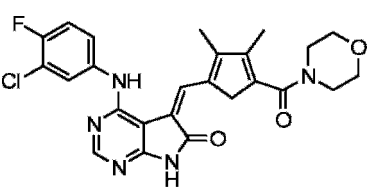
(IV)

4. HER-1 and HER-2 inhibitors consisting of 4-methyl-1H-benzimidazole derivatives, (**V**), prepared by Wittman (5) were effective against the MCF-7 human breast tumor cell line.



(V)

5. 7-Aza-indolin-2-one derivatives, (**VI**) prepared by Liang (6) were effective as HER-2 growth factor receptor tyrosine inhibitors and used in the treatment of uncontrolled cell proliferation associated with breast and ovarian cancers.



(VI)

References

1. M.L. Skehan *et al*., J. Natl Cancer Inst., 82, 1107 (1990)
2. A. Tasaka *et al*., US Patent 6,716,863 (April 6, 2004)
3. J.T. Hunt *et al*., US Patent 6,982,265 (January 3, 2006)
4. X. Sang *et al*., US Patent 7,087,621 (August 8, 2006)
5. M.D. Wittman *et al*., US Patent 7,081,454 (July 25, 2006)
6. C. Liang *et al*., US Patent 6,908,930 (June 21, 2005)

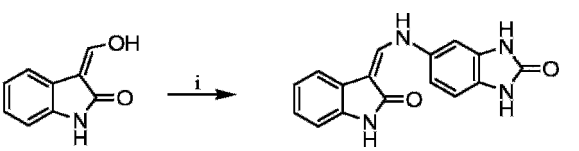
VI. Breast and Prostate Cancer: TrkA, TrkB, and TrkC Protein Tyrosine Kinase Inhibitors

Title Oxindole Derivatives
P.A. Harris *et al.*, US Patent 6,964,977 (November 15, 2005)
Assignee SmithKline Beecham Corporation
Utility Treatment of Breast and Prostate Cancer

Invention Significance Loss of regulation in the growth factor-signaling pathway is a frequent occurrence in cancer. A method of treating aberrant cellular proliferation disorders by the selective inhibition of the tyrosine family of protein kinases (TrkA, TrkB, and TrkC) using oxindole derivatives is described. This novel chemical art is an improvement over the existing treatment agents in that the materials are selective tyrosine kinase inhibitors and are prepared in one step.

Reaction

Eq. 1



i- 5-Aminobenzimidazolone, ethyl alcohol

Experimental

1. **Preparation of 5-{[(Z)-(2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]amino}-1,3-dihydro-2H-benzimidazol-2-one**

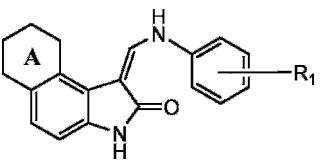
   A mixture of 3-(hydroxymethylene)-1,3-dihydro-2H-indol-2-one (1.00 mmol) and 5-aminobenzimidazolone (1.00 mmol) dissolved in 5 ml of ethyl alcohol was heated 90 minutes at 55°C, then concentrated. The residue was recrystallized using DMSO/methyl alcohol and the product isolated in 51% yield as an yellow solid.

   ^{1}H NMR (DMSO-d_6) δ 6.80 (d, $J = 7.7$ Hz, 1H), 6.85–6.98 (m, 4H), 7.00 (s, 1H), 7.54 (d, $J = 7.4$ Hz, 1H), 8.50 (d, $J = 12.7$ Hz, 1H), 10.39 (s, 1H), 10.56 (s, 1H), 10.72 (d, $J = 12.7$ Hz, 1H), 10.74 (s, 1H)
   APCI-Ms *m/z* 291 $(M\text{-}H)^-$

Derivatives

Selected derivatives are provided in Table 1.

Table 1 Effectiveness of selected experimental agents as TrkA tyrosine kinase inhibitors. ^{1}H-NMR supplied by the author



Entry	Ring A	R_1	Substrate Phosphorylation TrkA
1	None	4-(1,3-Dihydro-2H-benzimidazol-2-one)	+++[a]
2	None	4-(1H-1,2,4-Triazol-1-yl)	+++
3	None	4-[3-(3-Ethyl-2,6-piperidinone)]	+++
4	[1,2,3]Triazolo	3-(5-Amino-1,3,4-oxadiazol-2-yl)	+++
5	None	4-Acetamide	++[b]
6	None	4-(1H-1,2,4-Triazol-3-yl)	++

[a] $IC_{50} < 0.010\,\mu M$.
[b] $IC_{50} = 0.01 - 0.10\,\mu M$.

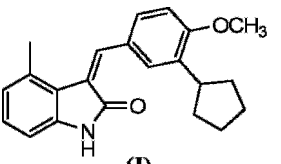
Testing

I. *Tyrosine Kinase Inhibition Assay*

Tyrosine kinase activity was measured using the synthetic Src peptide substrate NH_2-RRRAAAEEIYGEI-NH_2, while the enzyme was a GST fusion of the intracellular domain expressed in SF9 cells. Testing results are provided in Table 1.

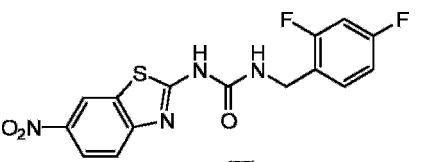
Notes

1. 3-Methylidenyl-2-indolinone derivatives, (**I**), effective as modulators of protein kinase effective in the treatment of protein kinase-related cellular disorders including cancer, were previously prepared by Tang (1) and used in treating cellular disorders such as cancer.



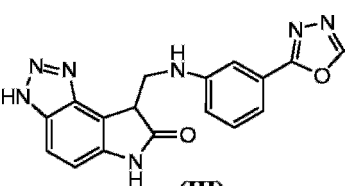
(I)

2. Benzothiazole derivatives, (**II**), prepared by Scott (2) were useful as tyrosine inhibitor kinases and used in the treatment of hyperproliferative diseases, especially cancer.



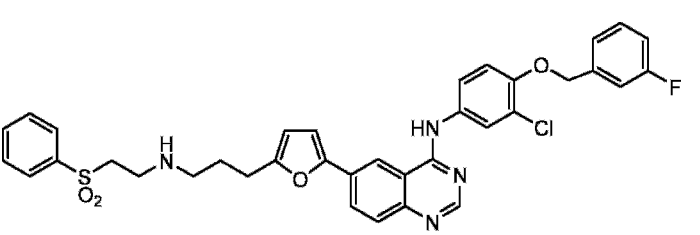
(II)

3. Oxindole derivatives, (**III**), prepared by Harris (3) were effective as Trk family protein tyrosine kinase inhibitors and were useful in cancer therapy.



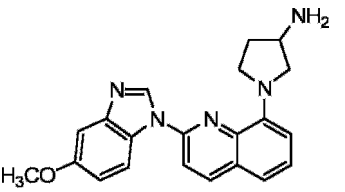
(III)

4. Cockerill (4) prepared protein tyrosine kinase inhibitors consisting of anilinoquinazoline derivatives, (**IV**), to regulate aberrant protein tyrosine kinase activity characterized by overexpression or mutation resulting in uncontrolled cell growth.



(IV)

5. Protein tyrosine kinase inhibitors consisting of benzimidazole derivatives, (**V**), prepared by Barth (5) were useful in the treatment of abnormal cell growth in mammals.



(V)

References

1. P.C. Tang, US Patent 6,855,730 (February 15, 2005)
2. B. Scott *et al*., US Patent 7,091,227 (August 15, 2006)
3. P.A. Harris *et al*., US Patent 6,964,977 (November 15, 2005)
4. G.S. Cockerill *et al*., US Patent 7,084,147 (August 1, 2006) and US Patent 6,933,299 (August 23, 2005)
5. W.E. Barth *et al*., US Patent 7,019,147 (May 28, 2006)

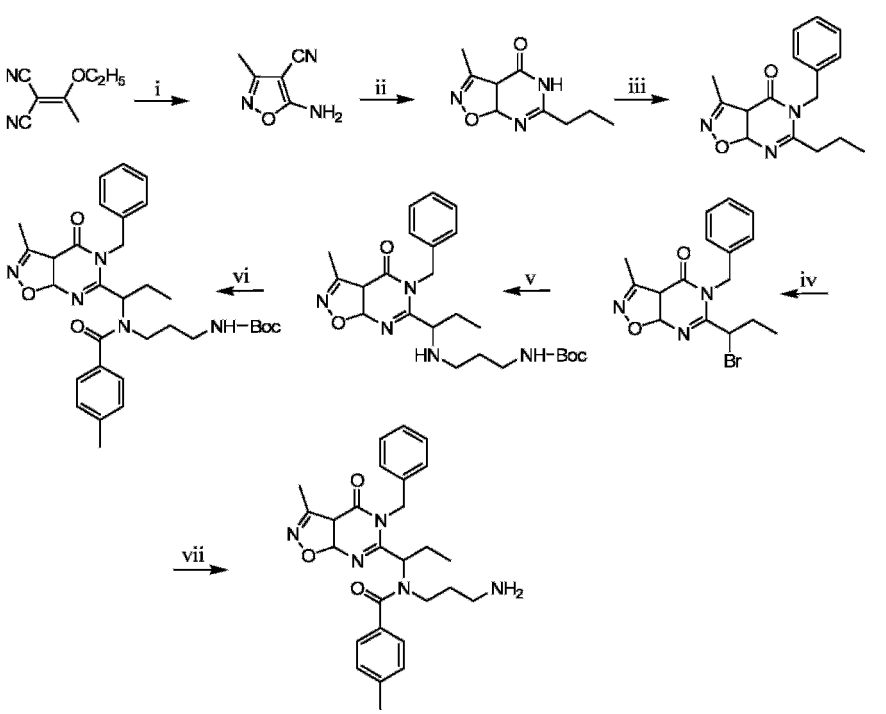
VII. Cell Growth Regulators: Eg5 Motor Protein Inhibitors

Title Bicyclicpyrimidones and Their Use to Treat Diseases
K.S. Kim *et al.*, US Patent 7,022,850 (April 4, 2006)
Assignee Bristol-Myers Squibb Co.
Utility Treatment of Ovarian Cancer

Invention Significance Cancer is a symptom of unregulated cell mitosis from overexpression of positive or negative cell cycle regulators. Eg5 is one of the several proteins localized to the mitotic spindle and is required for the formation of the bipolar mitotic spindle. A method of restoring cell life cycle order and inducing mitotic arrest using agents that specifically target Eg5 proteins has been discovered that is useful in the treatment of proliferative diseases.

Reaction

Eq. 1



i- Hydroxylamine hydrochloride, sodium hydroxide, water
ii- Butyric anhydride, sulfuric acid
iii- THF, lithium hexamethyldisilazide, benzylbromide, sodium iodide
iv- Acetic acid, sodium acetate, bromine
v- Ethyl alcohol, *N*-Boc-1,3-diaminopropane
vi- $CHCl_3$, 4-toluoyl chloride
vii- CH_2Cl_2, trifluoroacetic acid, triethylsilane

Experimental

1. **Preparation of 5-amino-3-methyl-isoxazole-4-carbonitrile**

   A mixture of hydroxylamine hydrochloride (0.4 mol) and NaOH (0.4 mol) dissolved in 1000 ml water was treated with 2-(1-ethoxy-ethylidene)-malononitrile (0.4 mol) over 60 minutes, then stirred 18 hours at ambient temperature. The suspension was filtered, then washed with 1000 ml cold EtOAc, air dried, and the product isolated in 72% yield as a white solid.

   ^{1}H NMR (DMSO-d_6) δ 8.32 (s, 2 H), 2.1 (s, 3H)

2. **Preparation of 3-methyl-6-propyl-5-H-isoxazolo[5,4-d]pyrimidin-4-one**

   A suspension of the Step 1 product (0.2 mol) in 15 ml butyric anhydride at 0°C was treated with 1.5 ml 18 M H_2SO_4, then stirred 60 minutes at 100°C, cooled, and then poured into ice water. A white precipitate was collected, washed with water, air dried, and the product isolated in 52% yield as a white solid.

   ^{1}H NMR (DMSO-d_6) δ 2.61 (t, $J = 7.5$ Hz, 2H), 2.42 (s, 3H), 1.70 (q, $J = 7.5$ Hz, 2H), 0.89 (t, $J = 7.5$ Hz, 3H)
   LC/MS (ESI) 194 $(M+H)^+$

3. **Preparation of 5-benzyl-3-methyl-6-propyl-5H-isoxazolo[5,4-d]pyrimidin-4-one**

   A suspension of the Step 2 product (9.3 mmol) in 40 ml THF was treated with 13.9 ml 1 M lithium hexamethyldisilazide, then stirred 60 minutes. It was further treated with benzylbromide (13.9 mmol) and NaI (1.0 mmol), then stirred 40 hours at 50°C, and cooled to ambient temperature. The mixture was partitioned between diethyl ether and brine and the organic phase was washed with water, then concentrated. The residue was purified by flash chromatography with silica gel using 15% EtOAc/hexane and the product isolated in 20% yield as a white solid.

   ^{1}H NMR (DMSO-d_6) δ 7.35 (m, 5H), 5.41 (s, 2H), 2.79 (t, $J = 7.5$ Hz, 2H), 2.53 (s, 3H), 1.72 (q, $J = 7.5$ Hz, 2H), 0.90 (t, $J = 7.5$ Hz, 3H)
   LC/MS (ESI) 284 $(M+H)^+$

4. **Preparation of (±)-5-benzyl-6-(1-bromo-propyl)-3-methyl-5H-isoxazolo[5,4-d]-pyrimidin-4-one**

   The Step 3 product (1.85 mmol) dissolved in 20 ml acetic acid was treated with sodium acetate (2.22 mmol), then bromine (1.85 mmol), and stirred 72 hours at 55°C. The cooled mixture was poured into water and a precipitate was formed. The solid was collected, air dried, and the product isolated in 30% yield as a white solid.

   ^{1}H NMR (DMSO-d_6) δ 7.35 (m, 5H), 5.71 (d, $J = 16.5$ Hz, 1H), 5.21 (d, $J = 16.5$ Hz, 1H), 5.16 (t, $J = 7.5$ Hz, 1H), 2.51 (s, 3H), 2.49 (m, 1H), 2.28 (m, 1H), 0.78 (t, $J = 7.5$ Hz, 3H)
   LC/MS (ESI) 284 $(M+H)^+$

5. **Preparation of (±)-{3-[1-(5-benzyl-3-methyl-4-Oxo-4,5-dihydro-isoxazolo-[5,4-d]pyrimidin-6-yl)-propylamino]-propyl}-carbamic acid t-butyl ester**

   A solution of the Step 4 product (0.55 mmol) in 25 ml ethyl alcohol was treated with *N*-Boc-1,3-diaminopropane (1.38 mmol), then refluxed 6 hours, and concentrated. The residue was purified by flash chromatography with silica gel using EtOAc/hexane, 1:1, and the product isolated in 66% yield as yellow oil.

   ^{1}H NMR (DMSO-d_6) δ 7.35 (m, 5H), 5.47 (m, 2H), 4.67 (s, 1H), 2.84 (m, 2H), 2.54 (m, 2H), 2.50 (s, 3H), 1.83 (m, 2H), 1.60 (m, 2H), 1.37 (s, 9H), 0.71 (m, 3H)
   LC/MS (ESI) 456 $(M+H)^+$

6. **Preparation of (±)-{3-[[1-(5-benzyl-3-methyl-4-oxo-4,5-dihydro-isoxazolo-[5,4-d]pyrimidin-6-yl)-propyl]-(4-methyl-benzoyl)-amino]-propyl}-carbamic acid *t*-butyl ester**

   A solution of the Step 5 product (0.11 mmol) in 10 ml $CHCl_3$ was treated with 4-toluoyl chloride (0.11 mmol) and triethylamine (0.11 mmol), then stirred 18 hours at ambient temperature, and concentrated. The residue was purified by preparative reverse-phase HPLC on a YMC S5 ODS 30×100 mm column and the product isolated in 95% yield as an yellow glass.

   LC/MS 100% at 4.32 minutes
   (ESI) 574 $(M+H)^+$

7. **Preparation of (±)-*N*-(3-amino-propyl)-*N*-[1-(5-benzyl-3-methyl-4-oxo-4,5-dihydroisoxazolo[5,4-d]pyrimidin-6-yl)-propyl]-4-methyl-benzamide**

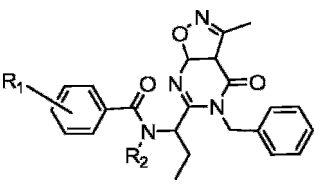
   A solution of the Step 6 product (0.10 mmol) in 2.5 ml CH_2Cl_2 was treated with 150 ml triethylsilane and 1 ml trifluoroacetic acid, then stirred 30 minutes at ambient temperature. The mixture was concentrated and the product isolated in 65% yield as a colorless film.

   ^{1}H NMR (DMSO-d_6) δ 7.62 (s, 1H), 7.25 (m, 7H), 7.22 (s, 1H), 5.77 (d, $J = 16.5$ Hz, 1H), 5.39 (s, 1H), 4.90 (d, $J = 15.9$ Hz, 1H), 3.40 (t, $J = 7.0$ Hz, 2H), 2.92 (s, 3H),

2.54 (s, 3H), 2.09 (m, 1H), 1.92 (m, 1H), 1.91 (m, 1H), 1.89 (t, $J = 7.0$ Hz, 3H), 0.68 (m, 1H)
LC/MS (ESI) 474 $(M+H)^+$

Derivatives

Table 1 Selected bicyclicpyrimidone derivatives and their corresponding mass spectra characterization. All experimental derivatives were effective as antiproliferative agents



Entry	R_1	R_2	$(M+H)^+$
4	4-Methyl	*n*-Butyl	473
3	4-Methyl	3-Morpholin-4-yl-propyl	544
8	4-Methyl	2-Carbamoylethyl	488
12	2,3-Dichloro	2-Cyanoethyl	524
17	4-*t*-Butyl	2-Cyanoethyl	481
22	4-Chloro	2-Cyanoethyl	504
41	2,3-Dichloro	2-Carbamoylethyl	542
47	3-Trifluoromethyl	2-Carbamoylethyl	542

Testing

I. *Cell Culture*

Cell lines were maintained in RPMI-1640 plus 10% fetal bovine serum and plated at a density of 3000–6000 cells/well and were grown overnight. Cells were then treated in triplicate with a seven concentration dose–response curve. The maximum concentration of DMSO never exceeded 0.5%. Cells were exposed to selected experimental agents for 72 hours and proliferation measured using XTT or MTS from Promega. Ovarian, breast, prostate, lung, leukemia, and colorectal human cancer cell lines used included A2780S, SKBR3, MDA-MB-231, PC3, LX-1, K562, HT-29, WiDr, HCT-15, and HCT116, respectively.

Results

All experimental agents exhibited activity in the 72-hour cell proliferation assay and inhibited cell proliferation in one or more of the cell lines listed with an IC_{50} less than or equal to about 10 μM.

II. *Clonogenic Growth Assay*

Colony growth inhibition was measured for A2780 ovarian carcinoma cells using a standard clonogenic assay. In these experiments, 200 cells/well were seeded into six-well tissue culture plates for 18 hours. The assay medium consisted of RPMI-1640 plus 10% fetal bovine serum. Cells were then treated in duplicate with a six concentration dose–response curve. The maximum concentration of DMSO never exceeded 0.25%. Cells were exposed to selected experimental agents for 4, 8, or 24 hours. Thereafter, the experimental agent was removed, cells washed with 2 volumes of PBS, the normal growth medium replaced, and colonies were fed with fresh media every third day. Colony number was scored on days 10–14 using a Optimax imaging station and the concentration of the experimental agent required to inhibit 50 or 90% of colony formation was determined by nonlinear regression analysis.

Results

When exposed to cells for 24 hours, all experimental agents exhibited activity in the clonogenecity assay.

III. *Cell Cycle Analysis*

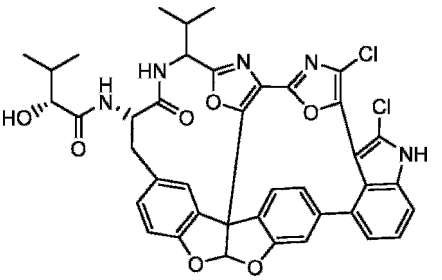
The cell cycle profile of cells treated with selected experimental agents was monitored by flow cytometry using A2780 ovarian carcinoma cells seeded at a density of 2×10^5 cells/well in standard six-well culture plates and grown 17 hours. Thereafter, cells were exposed to selected experimental agents at varying concentrations for 2–24 hours and cell populations harvested. Cells were stained with propidium iodide to determine DNA content and with other immunological reagents such as protein biomarkers for mitosis and apoptosis including antiphospho-ThreonineProline, anti-M Phase Phospoprotein 2 (MMP2), and anti-p85 PARP.

Results

All experimental agents exhibited activity in the cell cycle profile analysis assay producing significant increases in mitotic and apoptotic fractions of the cell population.

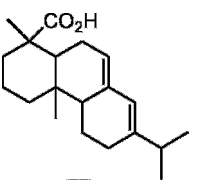
Notes

1. Diazonamide derivatives, (**I**), prepared by Harran (1) were modeled from toxins extracted from the marine invertebrate *Diazona angulata* and were effective in treating disorders associated with abnormal cell mitosis.



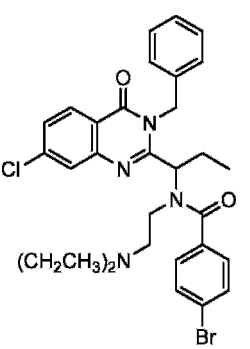
(I)

2. Lin (2) determined that the toxic compound, abietic acid, (**II**), a major ingredient in pine resin, was effective as an antimitosis agent and in reducing the tumor size in malignant growths.



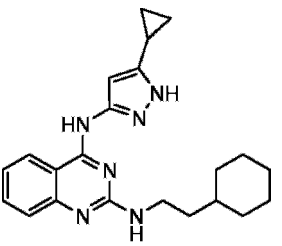
(II)

3. Bergnes (3) prepared quinazolinone derivatives, (**III**), that were effective in the treatment of cellular proliferative diseases by causing mitotic arrest and monopolar spindle formation.



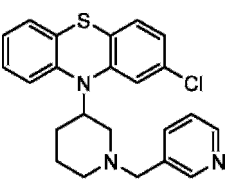
(III)

4. Pyrazole derivatives, (**IV**), prepared by Bebbington (4) were effective as Aurora-2 protein kinase inhibitors and impeded the accurate segregation of chromosomes during mitosis and used in treating colon and breast cancers in humans.



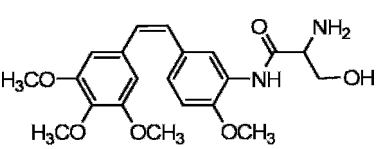
(IV)

5. Phenothiazine kinesin inhibitors such as 2-chloro-10-(1-pyridin-3-yl-methylpiperidin-3-yl)-10H-phenothiazine, (**V**), prepared by Finer (5) were effective in causing malformation of the mitotic spindle during mitosis and used in treating proliferative disorders.



(V)

6. Morinaga (6) observed that the stilbene derivative (*Z*)-1-(3-(2-amino-3-hydroxypropamide)-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethane, (**VI**), in conjunction with the neoplasm inhibitor Cisplatin®, $(NH_3)_2Cl_2Pt(II)$, strongly inhibited mitosis without patient weight loss associated with other antitumor agents such as Vindesine®.



(VI)

References

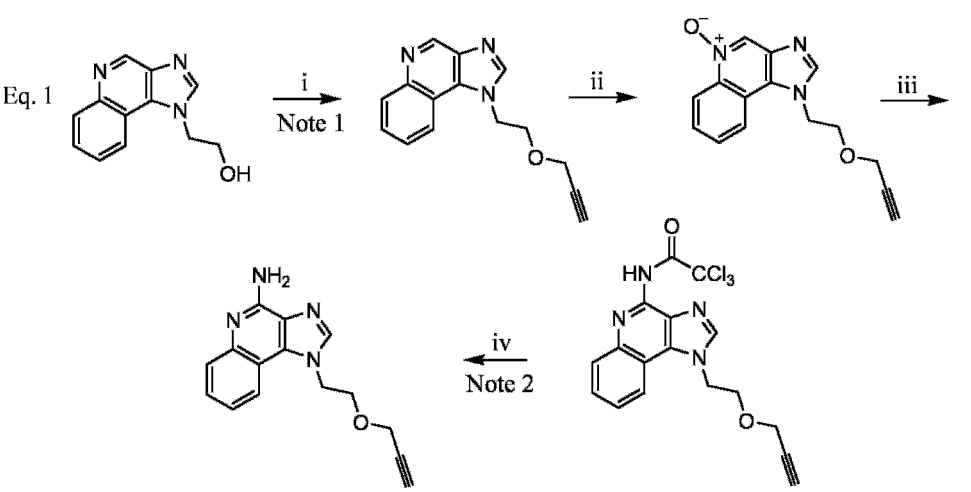
1. P.G. Harran *et al*., US Patent 7,022,720 (April 4, 2006)
2. C.-H. Lin *et al*., US Patent 7,015,248 (March 21, 2006)
3. G. Bergnes *et al*., US Patent 7,009,049 (March 7, 2006) and US Patent 6,831,085 (December 14 2004)
4. D. Bebbington *et al*., US Patent 6,989,385 (January 24, 2006)
5. J.T. Finer *et al*., US Patent 6,992,082 (January 31, 2006)
6. Y. Morinaga *et al*., US Patent 6,992,106 (January 31, 2006)

VIII. Cervical Cancer: Immune Response Modifiers as Antineoplastic Agents

Title Aryl Ether-Substituted Imidazoquinolines
P.D. Heppner *et al.*, US Patent 6,989,389 (January 24, 2006)
Assignee 3M Innovative Properties Co.
Utility Treatment of Cervical Intraepithelial Neoplasias

Invention Significance A new class of antineoplastic agents has been prepared that behave as immune response modifiers by inducing the biosynthesis of cytokines tumor necrosis factor-α (TNF-α) and interferon-α (IFN-α) without the concomitant production of significant levels of inflammatory cytokines. These agents are useful in the treatment of neoplastic diseases such as cervical intraepithelial neoplasias.

Reaction



i- Sodium hydroxide, CH_2Cl_2, propargyl bromide, benzyltrimethylammonium chloride
ii- $CHCl_3$, 3-chloroperoxybenzoic acid
iii- Trichloroacetyl isocyanate, CH_2Cl_2
iv- CH_2Cl_2, methyl alcohol, sodium methoxide

Experimental

1. **Preparation of 2-(1H-imidazo[4,5-c]quinolin-1-yl)ethyl (2-propynyl) ether**

   2-(1H-Imidazo[4,5-c]quinolin-1-yl)-1-ethanol (0.133 mol) was slowly added to a mixture of 240 ml of 50% NaOH solution and 240 ml CH_2Cl_2, then treated with propargyl bromide (0.266 mol), and benzyltrimethylammonium chloride (0.013 mmol), and stirred 16 hours at ambient temperature. The layers were separated and the aqueous fraction further extracted with CH_2Cl_2. Fractions were combined, then washed with water, dried using $MgSO_4$, and concentrated. The residue was mixed with diethyl ether and an orange solid was isolated. The solid was recrystallized using EtOAc and the product isolated as an yellow crystalline solid, mp = 124−126°C.

   ^{1}H NMR (300 MHz, DMSO) δ 9.21 (s, 1H), 8.44 (m, 1H), 8.36 (s, 1H), 8.18 (m, 1 H), 7.71 (m, 2H), 4.93 (t, $J = 5.1$ Hz, 2H), 4.14 (d, $J = 2.4$ Hz, 2H), 3.98 (t, $J = 5.1$ Hz, 2 H), 3.35 (t, $J = 2.2$ Hz, 1H)
   HRMS (ESI) Calc. for $C_{15}H_{14}N_3O$ (MH^+) 252.1137; found 252.1141
   Analysis Calc. for $C_{15}H_{13}N_3O$: C (%), 71.70; H (%), 5.21; N (%), 16.72. Found: C (%), 71.85; H (%), 5.25; N (%), 16.90

2. **Preparation of 1-[2-(2-propynyloxy)ethyl]-1H-imidazo[4,5-c]quinoline-5*N*-oxide**

   The Step 1 product (78.4 mmol) dissolved in $CHCl_3$ at 0°C was treated with 3-chloroperoxy benzoic acid (15.7 g, 57–86%), then stirred 30 minutes and warmed to ambient temperature. TLC monitoring indicated the presence of Step 1 product, so two separate additions of chloroperoxy benzoic acid (4 g) were added. The mixture was then treated with 10% NaOH, extracted multiple times with CH_2Cl_2, and dried. The solution was concentrated and 18.5 g product isolated.

   HRMS (ESI) Calc. for $C_{15}H_{14}N_3O_2$ (MH^+) 268.1086; found 268.1098

3. **Preparation of *N*-{1-[2-(2-propynyloxy)ethyl]-1H-imidazo[4,5-c]quinolin-4-yl}-2,2,2-trichloroacetamide**

   Trichloroacetyl isocyanate (82.2 mmol) was added dropwise to a mixture of the Step 2 product (68.5 mmol) dissolved in 300 ml CH_2Cl_2, then stirred for 30 minutes. TLC monitoring indicated the presence of Step 2 product, so additional trichloroacetyl isocyanate (4.5 g) was added. After 1 hour, TLC analysis indicated that the reaction was complete and the product was isolated as a pale yellow solid using the Step 2 workup.

4. **Preparation of 1-[2-(2-propynyloxy)ethyl]-1H-imidazo[4,5-c]quinolin-4-amine**

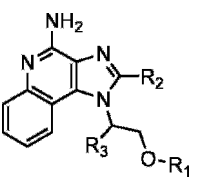
   The Step 3 product dissolved in 200 ml methyl alcohol and 150 ml CH_2Cl_2 was treated with sodium methoxide (50 g of 25% in methanol), then stirred overnight at ambient temperature. A precipitate that formed was isolated by filtration and a

second crop was obtained by reducing the solvent volume to 100 ml. The solids were dried and 16.4 g of product isolated as an off-white solid, mp = 225 – 227°C.

^{1}H NMR (300 MHz, DMSO) δ 8.13 (s, 1H), 8.08 (br d, $J = 7.8$ Hz, 1H), 7.62 (br d, $J = 8.3$ Hz, 1H), 7.44 (br t, $J = 7.6$ Hz, 1H), 7.24 (br t, $J = 7.5$ Hz, 1H), 6.54 (s, 2H), 4.81 (t, $J = 5.4$ Hz, 2H), 4.14 (d, $J = 2.4$ Hz, 2H), 3.93 (t, $J = 5.1$ Hz, 2H), 3.38 (t, $J = 2.4$ Hz, 1H)
HRMS (ESI) Calc. for $C_{15}H_{15}N_4O$ (MH^+) 267.1246; found 267.1253
Analysis Calc. for $C_{15}H_{14}N_4O$ (H_2O)1/4: C (%), 66.53; H (%), 5.40; N (%), 20.69. Found: C (%), 66.33; H (%), 5.18; N (%), 21.12

Derivatives

Table 1 Selected 1H-imidazo[4,5-c]quinoline derivatives and their corresponding mass spectral data. ^{1}H-NMR and IR for products supplied by author



Entry	R_1	R_2	R_3	MS (*m/z*)
1	1-Propynyl	Hydrogen	Hydrogen	267
16	3-Phenylpropyl	2-Ethoxymethyl	Hydrogen	404
22	3-Phenylpropyl	Hydrogen	Hydrogen	347
46	4-Cyanobenzyl	Hydrogen	Ethyl	371
108	3-Phenylprop-2-ynyl	Hydrogen	(R)-Ethyl	370
120	3-Phenylprop-2-ynyl	Methoxyethyl	Hydrogen	401
126	Benzoyl	Methyl	Hydrogen	375
141	4-Cyanobenzoyl	Methyl	Hydrogen	371

Testing

I. *Cytokine Induction in Human Cells*

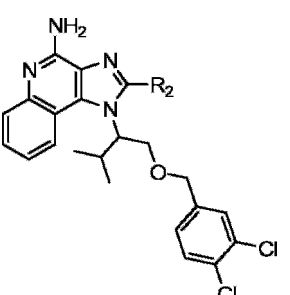
An in vitro human blood cell system was used to assess IFN-α and TNF-α levels using the method of Testerman (1). Testing results are provided in Table 2.

Table 2 Minimum concentration required to induce IFN-α and TNF-α cytokine in human cells using selected experimental agents

Entry	Minimum Amount of Experimental Agent to Induce IFN-α (μM)	Minimum Amount of Experimental Agent to induce TNF-α (μM)
1	0.12	1.11
16	0.01	0.37
22	0.12	0.37
46	0.01	3.33
108	0.00017	0.04
120	0.01	0.01
126	0.04	0.04
141	0.12	0.12

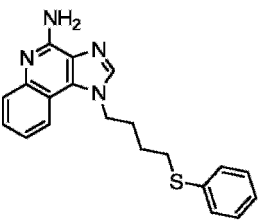
Notes

1. The preparation of the Step 1 co-reagent, 2-(1H-imidazo[4,5-c]quinolin-1-yl)-1-ethanol, was described by Gerster (2).
2. 1H-imidazo[4,5-c]quinoline benzoxy ethers, (**I**), prepared by Dellaria (3) were effective in inducing IFN-α and TNF-α cytokine in human cells and used in the treatment of neoplastic disorders.

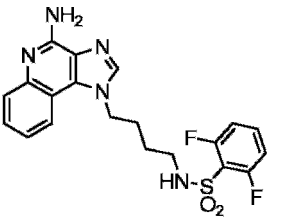


(I)

3. 1H-imidazo[4,5-c]quinoline thioethers, (**II**), and sulfonamide derivatives, (**III**), prepared by Bonk (4) and Lindstrom (5), respectively, were effective as antineoplastic agents by inducing IFN-α and TNF-α cytokine in human cells and used in treating cervical cancer.

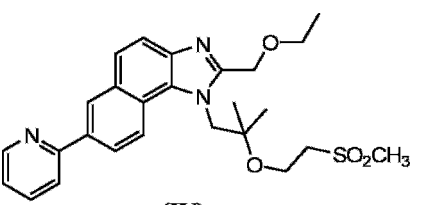


(II)



(III)

4. Aryl 1H-imidazo[4,5-c]quinoline derivatives, (**IV**), prepared by Hays (6) were effective for inducing cytokine biosynthesis in animals and used in the treatment of neoplastic diseases.



(IV)

References

1. R.B. Testerman *et al*., J. Leukocyte Biol., 58, 365 (1995)
2. J.F. Gerster *et al*., US Patent 5,605,899 (February 25, 1997)
3. J.F. Dellaria *et al*., US Patent 6,953,804 (October 11, 2005)
4. J.D. Bonk *et al*., US Patent 6,949,649 (September 27, 2005)
5. K.J. Lindstrom, US Patent 6,924,293 (August 2, 2005)
6. D.S. Hays *et al*., US Patent 7,091,214 (August 15, 2006)

IX. Colon Cancer

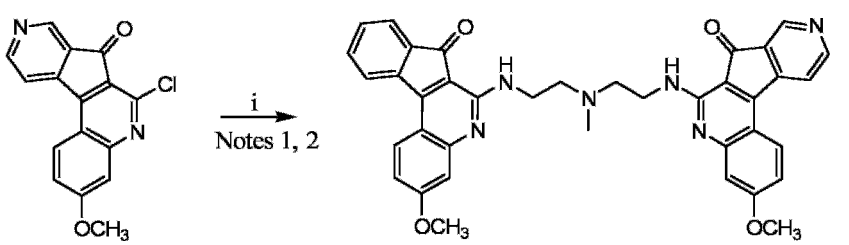
A. Immune Response Modifiers as Antineoplastic Agents

Title Polycyclic Compounds Exhibiting Antitumor Activities
K. Kawasaki *et al.*, US Patent 6,946,472 (September 20, 2005)
Assignee Hoffman-La Roche Inc.
Utility Treatment of Colon Cancer

Invention Significance Although farnesyl and geranylgeranyl transferase inhibitors, indeno[2,1-c]quinolin-7-one derivatives, are effective as antitumor agents, their acute toxicity is low and they are only marginally effective in treating solid tumors. To address these concerns, indeno[2,1-c]quinolin-7-one dimer derivatives have been prepared having very high toxicities effective in reducing solid tumor growth.

Reaction

Eq. 1



i- *N*-Methyl-2,2′-diaminodiethylamine, potassium carbonate, DMF

Experimental

1. **Preparation of 3-methoxy-6-(2-{methyl-[2-(3-methoxy-7-oxo-7H-5,9-diaza-benzo[c]fluoren-6-ylamino)-ethyl]-amino}-ethylamino)-5,9-diaza-benzo[c]fluoren-7-one**

   A mixture consisting of 6-chloro-3-methoxy-5,9-diaza-benzo[c]fluoren-7-one (21 mg), *N*-methyl-2,2′-diaminodiethylamine (3.3 mg), and K_2CO_3 (10 mg) was suspended in 0.5 ml DMF and stirred 13 hours at 90°C, then concentrated. The residue was purified by chromatography on silica gel using CH_2Cl_2/methyl alcohol,

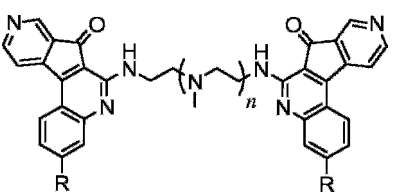
30:1, and CH_2Cl_2/methyl alcohol/25% NH_4OH, 30:1:0.4, and the product isolated as a reddish powder.

^{1}H-NMR ($CDCl_3$) δ 2.57 (3H, s), 2.88 (4H, brt, J = ca. 6 Hz), 3.69 (4H, q-like, J = ca. 6 Hz), 3.87 (6H, s), 6.78 (2H, dd, J = 9.2 Hz, 2.7 Hz), 6.85 (2H, d, J = 2.7Hz), 7.19 (2H, brt, J = ca. 5 Hz), 7.50 (2H, dd, J = 4.9 Hz, 1 Hz), 7.66 (2H, d, J = 9.2 Hz), 8.41 (2H, d, J = 1Hz), 8.69 (2H, d, J = 4.9 Hz)
ESI-MS *m/z* 638 (MH^+)

Derivatives

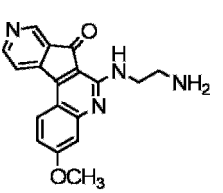
Selected dimer derivatives are provided in Table 1.

Table 1 The antitumor activity of selected chemical agents against in vitro growth of colorectal cancer using the HCT116 cell line. The acute toxicity, LD_{50}, of these experimental compounds was more than 90 mg/kg as determined by intravenous administration in mice



Entry	R	*n*	HCT116, IC_{50} (nM)
Reference[a]	OCH_3	–	12
1	OCH_3	1	0.64
6	Cl	1	0.02
11	H	1	0.3
13	H	2	0.13

[a] The reference agent was 6-(2-dimethylaminoethylamino)-3-hydroxy-7H-indeno[2,1-c]quinolin-7-one, (**I**).



(I)

Testing

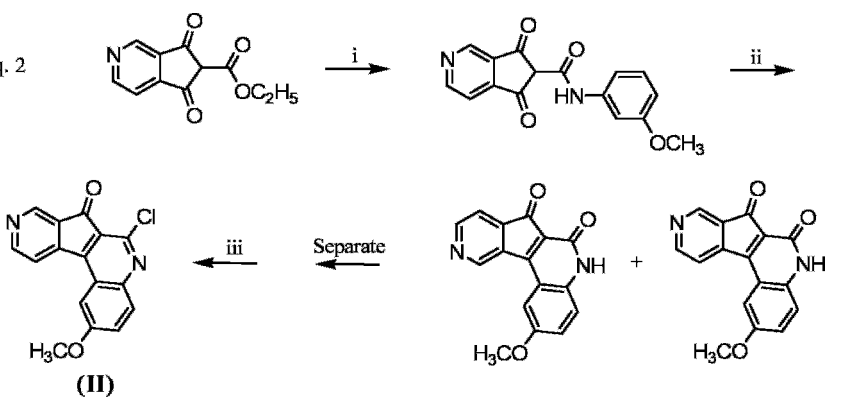
I. *Antiproliferative Activity Assay*

A suspension of tumor cells was inoculated to serially diluted 96-well microtest plate, incubated 4 days at 37°C, and monolayer cell growth measured by using WST-8. IC_{50} values of selected experimental agents were calculated as the concentration of the agent yielding 50% OD of the control growth. Testing results are provided in Table 1.

Notes

1. The preparation of the Step 1 co-reagent, 6-chloro-3-methoxy-5,9-diaza-benzo[c]fluoren-7-one, (**II**), by Aoyama (1) is illustrated in Eq. 2. The effectiveness of these derivatives, (**III**), against HCT116 cells is provided in Table 2.

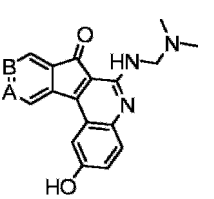
Eq. 2



(II)

i- Acetic acid, 3-anisidine, toluene
ii- Polyphosphoric acid
iii- Phosphorous oxychloride

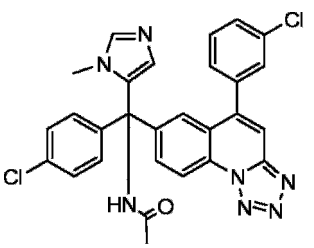
Table 2 Antitumor activity of tricyclic 6-(2-dimethylamino-ethylamino) derivatives against in vitro growth of colorectal cancer. The reference agent, (**I**), is provided Table 1



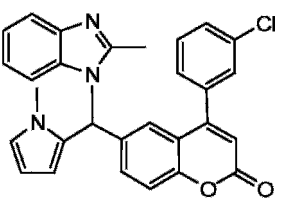
(III)

Entry	A	B	HCT116, IC_{50} (50 ng/ml)+
Reference	CH	N	12
2	CH	N	2.2
7	N	CH	3.9

2. Polycyclic 1,2-annelated quinoline derivatives, (**IV**) and (**V**), prepared by Angibaud (2,3) respectively, were effective as farnesyl and geranylgeranyl transferase inhibitors and used in treating mutations or overexpression of oncogenes associated with human colon and pancreatic carcinomas.

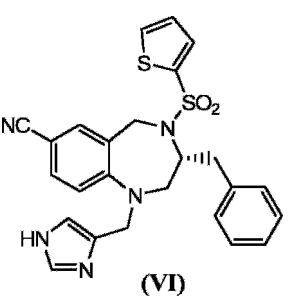


(IV)



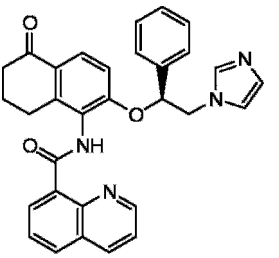
(V)

3. 3,7-Disubstituted-2,3,4,5-tetrahydro-1H-1,4-benzodiazepine derivatives, (**VI**), prepared by Kronenthal (4) were effective as farnesyl protein transferase inhibitors and used in the treatment of colon cancer and other proliferative diseases.



(VI)

4. Denny (5) prepared 5-substituted tetralone derivatives, (**VII**), which were effective as farnesyl transferase inhibitors, and used for treating uncontrolled or abnormal tissue proliferation such as in cancer.



(VII)

References

1. T. Aoyama *et al*., US Patent 6,680,326 (January 20, 2004)
2. P.R. Angibaud *et al*., US Patent 6,914,066 (July 5, 2005)
3. P.R. Angibaud *et al*., US Patent 7,067,531 (June 27, 2006)
4. D.R. Kronenthal *et al*., US Patent 7,074,921 (July 11, 2006)
5. W.A. Denny *et al*., US Patent 6,943,183 (September 13, 2005)

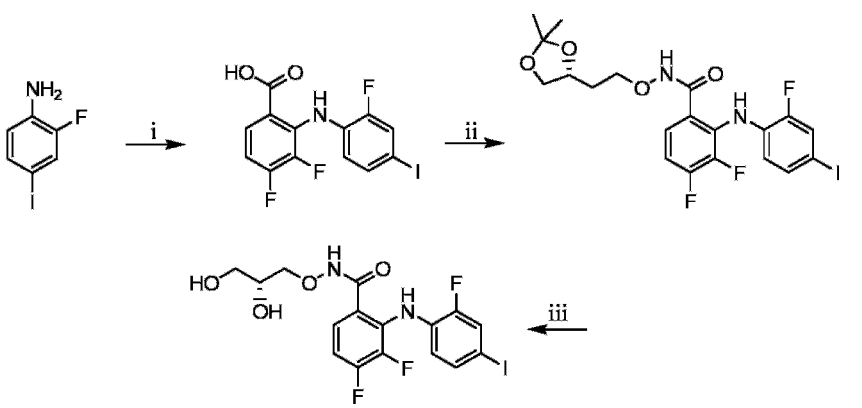
B. MAPK/ERK Kinase Inhibitors

Title Oxygenated Esters of 4-Iodo Phenylamino Benzhydroxamic Acids
S.D. Barrett *et al.*, US Patent 6,960,614 (November 1, 2005)
Assignee Warner-Lambert Company
Utility Treatment of Colon Tumors

Invention Significance MAPK/ERK Kinase (MEK) enzymes are dual specificity kinases involved in proliferative, immunomodulation, and inflammation diseases. Proliferative disorders are caused by intracellular signaling defects when MAP kinases MEK1 and MEK2 become activated. To impede this activation process, selective MEK1 and MEK2 inhibitors have been prepared while remaining inactive against other enzymes including MKK3, PKC, Cdk2A, phosphorylase kinase, EGF, and PDGF receptor kinases.

Reaction

Eq. 1



i- THF, lithium diisopropylamide, heptane, ethylbenzene, 5-bromo-2,3,4-trifluorobenzoic acid
ii- THF, diphenylphosphinic chloride, *N*-methyl morpholine, (*R*)-*O*-(2,2-dimethyl-[1,3]dioxolan-4-ylmethyl)-hydroxylamine
iii- Methyl alcohol, water, *p*-toluenesulfonic acid hydrate

Experimental

1. **Preparation of 3,4-difluoro-2-(2-fluoro-4-iodo-phenylamino)-benzoic acid**

   2-Fluoro-4-iodoaniline (0.00791 mol) dissolved in 10 ml THF was cooled to −78°C, then treated with 6 ml 2.0 M lithium diisopropylamide in THF/heptane/ethylbenzene, and the suspension stirred 10 minutes. The mixture was further treated with 5-bromo-2,3,4-trifluorobenzoic acid (0.00392 mol) dissolved in 15 ml THF, then stirred 18 hours at ambient temperature, and concentrated. The residue was treated with 100 ml 10% HCl and the suspension was extracted twice with 150 ml diethyl ether, then dried using $MgSO_4$. The solution was reconcentrated and an orange solid was isolated. The solid was triturated with boiling CH_2Cl_2, then cooled. The solid was isolated by filtration, then rinsed with CH_2Cl_2, and the product isolated, mp = 200−201°C.

2. **Preparation of *N*-((*R*)-2,2-dimethyl-[1,3]dioxolan-4-ylmethoxy)-3,4-difluoro-2-(2-fluoro-4-iodo-phenylamino)-benzamide**

   The Step 1 product (5.10 mmol) dissolved in THF cooled to −15°C was treated dropwise with diphenylphosphinic chloride (6.63 mol), then stirred 20 minutes and further treated with *N*-methyl morpholine (6.375 mmol), and stirred additional 20 minutes. (*R*)-*O*-(2,2-dimethyl-[1,3]dioxolan-4-ylmethyl)-hydroxylamine (5.1 mmol) was added and the mixture stirred for 1 hour. Additional *N*-methyl morpholine (6.37 mmol) was added and the mixture stirred 12 hours at ambient temperature and was then concentrated. The residue was diluted with EtOAc, washed twice with saturated $NaHCO_3$ solution, once with brine, dried using Na_2SO_4, and reconcentrated. The residue was purified by chromatography with silica gel using hexane/EtOAc, 4:1, and the product isolated in 68% yield.

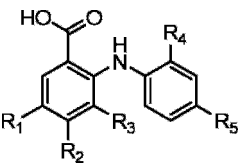
3. **Preparation of *N*-[(*R*)-2,3-dihydroxy-propoxy]-3,4-difluoro-2-(2-fluoro-4-iodo-phenylamino)-benzamide**

   The Step 2 product (0.40 mmol) suspended in methyl alcohol/water, 10:1, was treated with *p*-toluenesulfonic acid hydrate (0.04 mmol), then stirred 18 hours at ambient temperature, and then diluted with EtOAc. The organic solution was washed twice with saturated $NaHCO_3$ solution, once with brine, dried using Na_2SO_4, filtered, and concentrated. The residue was recrystallized from EtOAc and heptane and the solid washed with heptane/CH_2Cl_2, 1:1. The solid was dried in vacuo at 60°C and the product isolated in 70% yield as a white solid. The product shrinks at 90.8°C, mp = 115−117°C.

   Analysis C, 40.92; H, 3.16; N, 5.41; F, 11.30; I, 23.92 (6.75% EtOAc, 0.96% heptane)

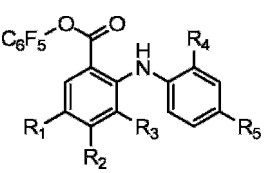
Derivatives

Table 1 Selected Step 1 phenylamino-benzoic acid intermediates and their corresponding melting points. ^{1}H-NMR data supplied by author



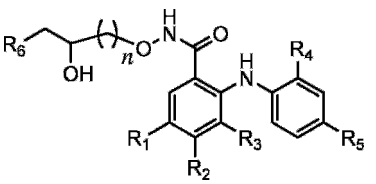
Entry	R_1	R_2	R_3	R_4	R_5	mp (°C)
3	Cl	F	F	CH_3	I	246–250
7	Br	F	F	I	Cl	302–304
10	H	F	H	CH_3	I	224–229
15	Br	F	F	I	F	258–259
21	F	F	H	CH_3	I	238–239

Table 2 Selected pentafluorobenzene phenylamino-benzoate intermediates and their corresponding mass spectral characterization data. ^{1}H-NMR data supplied by author



Entry	R_1	R_2	R_3	R_4	R_5	MS (APCI–)
34	Br	F	H	CH_3	I	576
38	Cl	Cl	F	F	I	592
41	H	F	F	CH_3	I	574
42	H	F	F	CH_3	Br	554 (*m/z*)
45	H	F	F	F	F	450

Table 3 Selected phenylamino benzhydroxamic esters and their corresponding mass spectral characterization data. ^{1}H-NMR data supplied by author



Entry	R_1	R_2	R_3	R_4	R_5	R_6	*n*	MS (M + 1)
91	Br	F	F	CH_3	I	OH	1	557/559 (APCI–)
97	Br	F	F	I	Cl	OH	2	571/573 (APCI–)
141	Cl	F	F	CH_3	I	CF_3	1	496.9
184	F	F	F	Cl	I	OC_6H_5	1	592.2
196	H	F	F	F	Cl	OC_6H_5	1	467.0
212	F	F	F	F	F	CF_3	1	413.4
218	H	H	H	Br	F	OCH_3	1	493.3

Testing

I. *Cellular Assay for Measuring MEK Inhibition*

The evaluation of selected experimental agents as MEK inhibitors was performed by measuring their ability to inhibit phosphorylation of MAP kinase IRK in murine colon 26 (C26) carcinoma cells. Since ERK1 and ERK2 represent the only known substrates for MEK, measurement of ERK phosphorylation inhibition in cells provided direct readout of cellular MEK inhibition by these agents. IC_{50} testing results associated with cellular inhibition of ERK phosphorylation are provided for Steps 1 and 2 intermediates and phenylamino benzhydroxamic derivatives in Table 5.

Table 4 Cellular inhibition of ERK phosphorylation using selected Steps 1 and 2 intermediates

Entry	IC_{50} (μM)
3	0.004
7	0.005

(*continued*)

Table 4 Continued

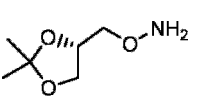
Entry	IC_{50} (μM)
10	0.0003
15	0.001
21	0.0018
34	0.001
38	>1.00
42	0.0027
45	0.019

Table 5 Cellular inhibition of ERK phosphorylation using selected phenylamino benzhydroxamic derivatives

Entry	Inhibition at 0.1 μM (%)	Inhibition at 1.0 μM (%)	Inhibition at 10 μM (%)
91	90.4	99.3	–
97	30.1	91.9	–
141	–	26.7	82.6
184	48.6	92.4	–
196	10.2	82.8	–
212	6.2	78.7	–

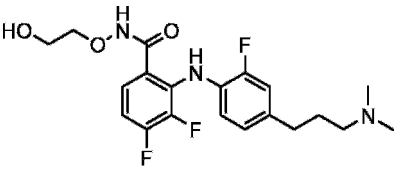
Notes

1. The preparation of the Step 2 co-reagent, (*R*)-*O*-(2,2-dimethyl-[1,3]dioxolan-4-ylmethyl)-hydroxylamine, (**I**), is described by the author.



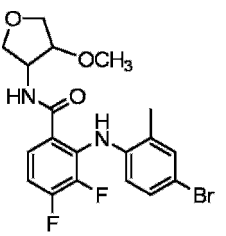
(I)

2. *N*-(4-Halophenyl)-anthranilic acid hydroxamate esters derivatives, (**II**), prepared by Rewcastle (1), were effective as MEK inhibitors and used in the treatment of proliferative diseases such as colon cancer.

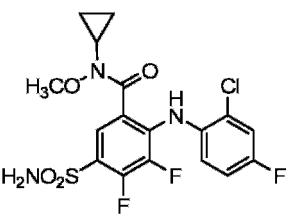


(II)

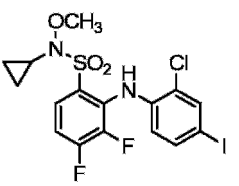
3. In earlier investigations by the authors (2,3), MEK inhibitors consisting of amides, (**III**), and benzenesulfonamide derivatives, (**IV**), respectively, and sulfohydroxamate derivatives, (**V**), prepared by Tecle (4) were effective in the treatment of proliferative disorders.



(III)

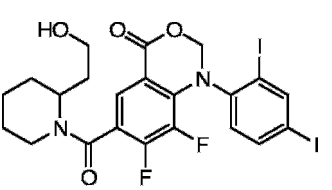


(IV)



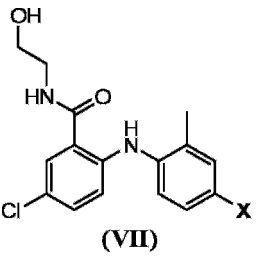
(V)

4. 3,1-Benzoxazine-3-one derivatives, (**VI**), prepared by Biwersi (5) were effective against diseases modulated by the MEK cascade such as colon cancer.



(VI)

5. In a subsequent investigation by the authors (6), 2-(4-bromo or 4-iodo phenylamino) benzoic acid derivatives, (**VII**), were prepared that were effective as inhibitors of MEK and used in treating cancer and other proliferative diseases.



X = Br, I

(VII)

References

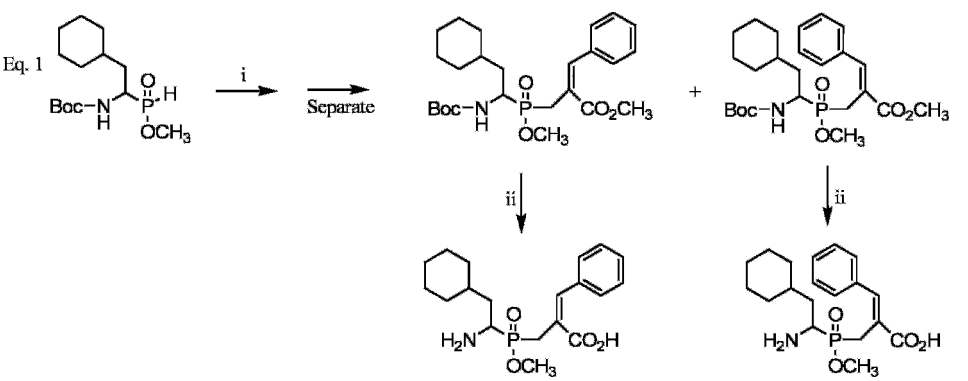
1. G.W. Rewcastle *et al*., US Patent 7,078,438 (July 18, 2006) and US Patent 6,891,066 (May 10, 2005)
2. S.D. Barrett *et al*., US Patent 6,821,963 (November 23, 2004)
3. S.D. Barrett *et al*., US Patent 6,770,778 (August 3, 2004) and US Patent 6,750,217 (June 15, 2004)
4. H. Tecle, US Patent 6,835,749 (December 28, 2004)
5. C. Biwersi *et al*., US Patent 7,001,905 (February 21, 2006)
6. S.D. Barrett *et al*., US Patent 7,019,033 (March 28, 2006)

C. Renal Dipeptidase Inhibitors

Title Design and Synthesis of Renal Dipeptidase Inhibitors
S.R. Khan *et al.*, US Patent 6,927,212 (August 9, 2005)
Assignee The Johns Hopkins University
Utility Treatment of Colon Tumors

Invention Significance Renal dipeptidase (RDP) is a zinc-containing hydrolytic enzyme that is overexpressed in both benign and malignant colon tumors. To limit enzyme overexpression, α-aminophosphinic acid derivatives have been prepared that are effective as RDP inhibitors. These agents are diagnostically and therapeutically effective in the detection and treatment of colon tumors.

Reaction



i- Trimethyl-2-phosphonoacrylate, methyl alcohol, sodium methoxide, benzaldehyde
ii- Trifluoroacetic acid, hydrochloric acid

Experimental

1. **Preparation of *cis/trans* methyl-1-amino-2-cyclohexylethyl-1-methyl-(3-phenyl acrylic acid)phosphinate** (generic procedure)

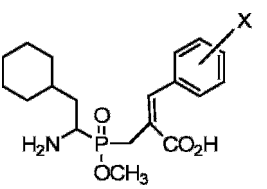
   Methyl-*N*-Boc-1-amino-2-cyclohexylethyl phosphinate (1.64 mmol) dissolved in 2.5 ml methyl alcohol at 0°C was treated dropwise with 0.90 ml 2.0 M sodium methoxide (1.8 mmol) over 10 minutes followed by the dropwise addition of 2-trimethylphosphono-acrylate (2.44 mmol) over 2 minutes. The mixture was stirred

30 minutes at ambient temperature, then recooled to 0°C, and further treated with benzaldehyde (2.0 equiv.) over 2 minutes. The mixture was then stirred 1 hour at ambient temperature and diluted with EtOAc. The solution was washed with phosphate buffer, dried using Na_2SO_4, and purified by medium-pressure liquid chromatography.

The protecting groups were removed by treatment with trifluoroacetic acid and hydrochloric acid treatment.

Derivatives

Table 1 Selected *Z*-α-aminophosphinic acid derivatives and their corresponding mass spectral data. The equivalent *E*-α-analogs were also isolated. ^{1}H-NMR for both *E,Z*-isomers supplied by author



Entry	X	LC-MS, *m/z*, $[M]^+$
2	4-F	369
4	4-Br	430
6	3-I	369
8	4-CF_3	419
10	$N(C_2H_5)_2$	422
12	3-Cl–4-F	403
14	3,4-Cl	420
16	3-Br–4-F	448

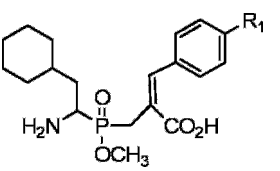
Testing

I. *RDP Inhibitor Assay*

The assay buffer to determine the activity of experimental agents consisted of 20 mM Tris (pH 8) containing 10 μM $ZnCl_2$. A 1 mM substrate of ε-DNP-L-Lys-D-Amp stock solution in DMSO was prepared and selected experimental agents drawn from a reservoir of methyl alcohol diluted with water.

The RDP inhibition activity of experimental agents was determined using crude lysates prepared from human colon cancers. A mixture of 20 μl of each experimental agent and 20 μl lysate in 150 μl assay buffer was incubated 30 minutes, cooled 30 minutes to the plate temperature, and immersed in a 2 μl/well and 1 mM of substrate ε-DNP-L-Lys-D-Amp in DMSO. IC_{50} values were determined using small intestine lysate and K_i values determined using the small intestine colon cancer lysate. IC_{50} and K_i testing data are provided in Table 2.

Table 2 Testing results of selected experimental agents as RDP inhibitors where IC_{50} were values were determined using small intestine lysate and K_i values determined using the small intestine colon cancer lysate. Entries 8, 10, and 12 were especially preferred



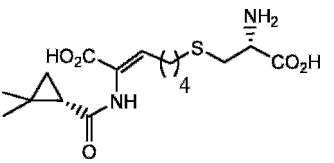
Entry	R_1	Olefin Geometry	K_i (nM)	IC_{50} (nM)
6	H	*Z*	1.12	28
7	H	*E*	1.6	40
8	F	*Z*	0.6	5
9	F	*E*	0.72	15
10	Br	*Z*	0.72	6
11	Br	*E*	2.4	30
12	I	*Z*	0.6	8
13	4-I	*E*	2.0	25

Results

From the data supplied in Table 1, entries 8, 10, and 12 are potent RDP inhibitors while 15 and 16 were only marginally active. In general, compounds with the *Z* configuration, such as experimental agents 8, 10, and 12, were significantly more active from their *E*-experimental agent counterparts, 9, 11, and 13.

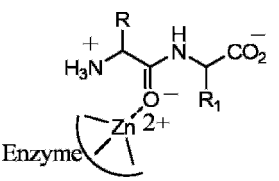
Notes

1. Experimental agents were modeled on the amino acid cilastatin, (**I**), prepared by Smith (1), which was determined by Nitanai (2) to be effective in complexing with the RDP enzyme.

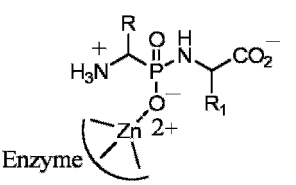


(I)

2. The effectiveness of the experimental agents resides in their ability to mimic the tetrahedral intermediate of the overexpressed RDP enzyme reaction, (**II**), by forming the inactive phosphorus analog, (**III**).



(II)



(III)

References

1. A.B. Smith *et al*., J. Am. Chem. Soc., 117, 10,879 (1995)
2. Y. Nitanai *et al*., J. Mol. Biol., 321, 177 (2002)

X. Leukemia

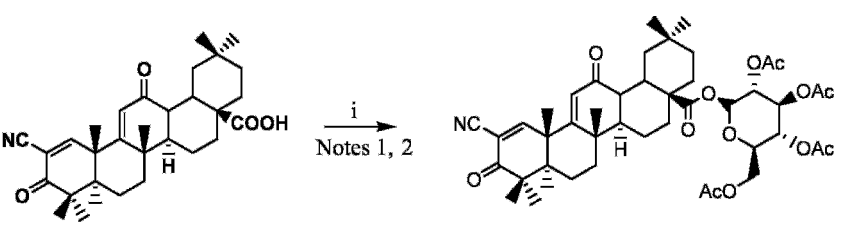
A. Inducible Nitric Oxide Synthase Inhibitors

Title Inhibitors and Methods of Use Thereof
T. Honda *et al.*, US Patent 6,974,801 (December 13, 2005)
Assignee The Trustees of Dartmouth College
Utility Treatment of Leukemia

Invention Significance New C-17 substituted 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid triterpenoid (CDDO) derivatives have been prepared that suppress formation of inducible nitric oxide synthase (iNOS) in macrophages stimulated by interferon-γ in mouse macrophages. All the new triterpenoid derivatives are more potent than previously known CDDOs in treating conditions associated with inflammatory enzyme disorders.

Reaction

Eq. 1



i- Tetra-*O*-acetyl-β-D-glucopyranoside bromide, CH_2Cl_2, Aliquat 336, water, potassium carbonate

Experimental

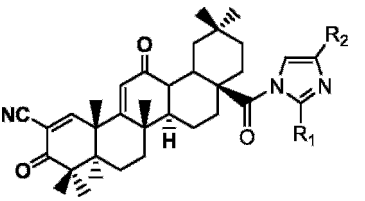
1. **Preparation of tetra-*O*-acetyl-*β*-D-glucopyranyl-2-cyano-3,12-dioxooleana-1,9(11)-dien-28-ate** (generic procedure for preparing oleananetriterpenoids esters)

   2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid and tetra-*O*-acetyl-β-D-glucopyranoside bromide were dissolved in CH_2Cl_2, then treated with K_2CO_3 dissolved in water containing Aliquat 336 added, and the reaction extent monitored by ^{1}H-NMR. A β-product configuration was assigned on the appearance of the two anomeric protons at δ 5.70 ppm (1H, d, $J = 7.8$ Hz).

Derivatives

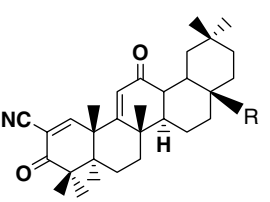
Selected triterpenoid ester derivatives are provided in Tables 1 and 2.

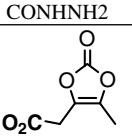
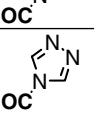
Table 1 IC_{50} values of selected imidazole 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid derivatives. IC_{50} values between 0.01 and 1 pM level are preferred. Very limited characterization data supplied by author



Entry	R_1	R_2	Yield (%)	IC_{50}
9	CH_3	Hydrogen	71	0.00001
10	Hydrogen	CH_3	70	0.00001
12	*n*-C_3H_7	Hydrogen	61	0.02
14	C_6H_5	Hydrogen	66	0.05
15	Hydrogen	Bromine	55	0.03

Table 2 IC_{50} values of selected 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic derivatives. IC_{50} values between 0.01 and 1 pM level are preferred. Very limited characterization data supplied by author



Entry	R	Yield (%)	IC_{50}
Reference	COOH	–	0.44
1	CN	81	0.0035
2	CO-D-Glu(OAc)4	75	0.070
4	CONHNH2	55	0.26
5		62	0.3
6		83	0.00003
8		55	0.05

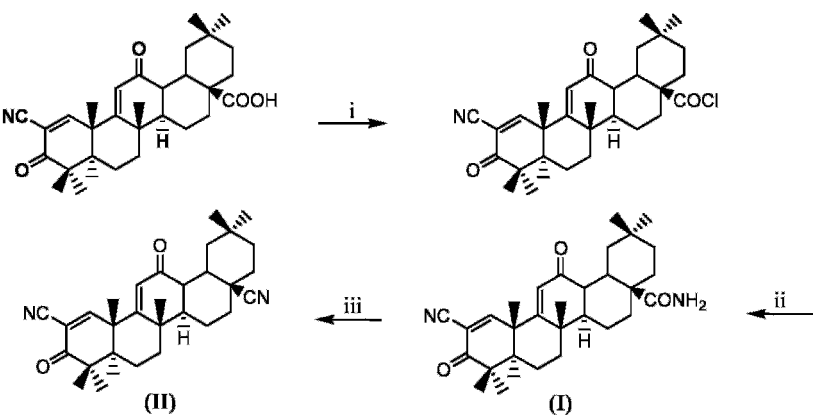
Testing

The inhibitory activities of selected experimental agents on iNO production induced by INF-γ in mouse macrophages are provided in Tables 1 and 2.

Notes

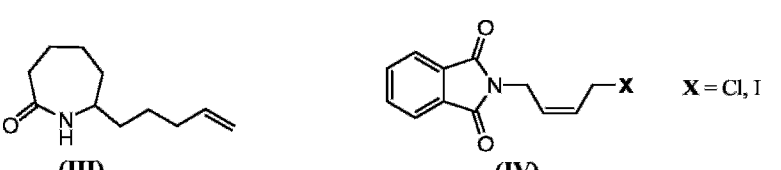
1. The preparation of the co-reagent, 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid, is described by Gribble (1).
2. CDDO C-17 amide, (**I**), and dicyano derivatives, (**II**), were also prepared by the author as illustrated in Eq. 2.

Eq. 2

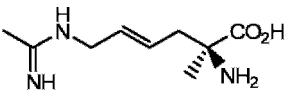


i- Oxalyl chloride, CH_2Cl_2
ii- Benzene, ammonia
iii- Thionyl chloride

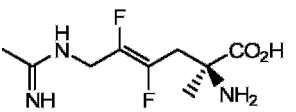
3. iNOS inhibitors consisting of unsaturated lactams, (**III**), imides, (**IV**), and hexanoic amine acid derivatives, (**V**), were prepared by Manning (2) for use as treatment agents for cancers affecting epithelial cells throughout the body.



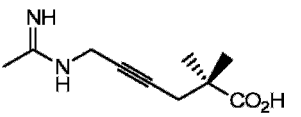
4. Fluorinated amino acids, (**VI**), and hexynoic derivatives, (**VII**), prepared by Durley (3) were effective as iNOS inhibitors and used in the treatment of epithelial cell-derived neoplasia.



(V)



(VI)



(VII)

References

1. G.W. Gribble *et al.*, US Patent 6,552,075 (April 22, 2003) and US Patent 6,326,507 (December 4, 2001)
2. P.T. Manning *et al.*, US Patent 7,012,098 (March 14, 2006)
3. R.C. Durley *et al.*, US Patent 7,005,450 (February 28, 2006)

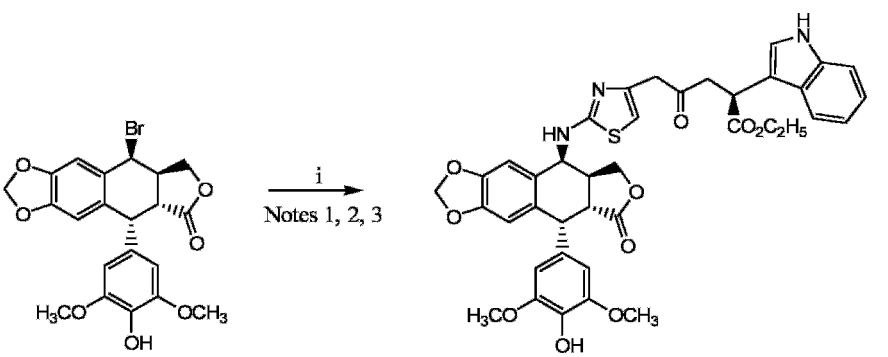
B. Topoisomerase II Inhibitors

Title Anticancer Compounds
Q. Shi *et al.*, US Patent 6,903,133 (June 7, 2005)
Assignee Plantaceutica, Inc.
Utility Treatment of Leukemia

Invention Significance Podophyllotoxin is extracted from the mandrake root. Only two derivatives of the extract, etoposide and teniposide, are effective as chemotherapeutic agents by inhibiting topisomerase II and are used in treating leukemia and sarcomas. The current invention substantially expands podophyllotoxin derivatives by providing a single step and high yielding method for their preparation.

Reaction

Eq. 1



i- CH_2ClCH_2Cl, THF, barium carbonate,
2-amino-[4″-(ethyl-L-tryptophan-*N*-acetyl)-2″-thiazine

Experimental

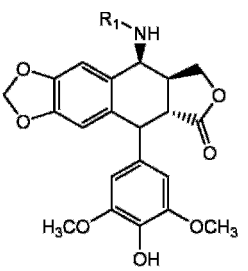
1. **Preparation of 4′-*O*-demethyl-4β-[4″-(ethyl-L-tryptophan-*N*-acetyl)-2″-thiazolyl amino]-4-desoxypodophyllotoxin (general procedure)**

   4′-*O*-Demethyl-4β-bromo-4-desoxypodophylotoxin (1 equiv.) dissolved in CH_2ClCH_2Cl/THF, 1:1, was treated with 2-amino-[4″-(ethyl-L-tryptophan-*N*-acetyl)]-2″-thiazine (1.2 equiv.) and $BaCO_3$ (1.5 equiv.), then refluxed while monitoring by TLC. The mixture was cooled to ambient temperature, then filtered, and concentrated. The residue was purified by chromatography with silica gel using CH_2Cl_2/EtOAc/acetone and the product isolated.

^{1}H NMR δ 8.35 (br s, 1H, NH), 7.96 (d, $J = 8$ Hz, 1H, 7‴-H), 7.42 (d, $J = 8$ Hz, 1H, 4‴-H), 7.05 (s, 1H, 3‴-H), 6.97 (m, 2H, 5‴, 6‴-H), 6.82 (s, 1H, H-5), 6.49 (s, 1H, 8-H), 6.30 (s, 2H, 2′, 6′-H), 6.22 (s, 1H, 5″-H), 5.95 (2H, d, $J = 12$ Hz, OCH_2O), 4.98 (m, 2H, 4, 9‴-H), 4.51 (d, $J = 5$ Hz, 1H, 1-H), 4.12 (t, $J = 7$ Hz, 2H, $OC\underline{H}_2CH_3$), 3.82 (s, 6H, 3′, 5′-OCH_3), 3.52 (d, $J = 7$ Hz, 2H, 6″-H), 3.42 (m, 2H, 11, 8‴-H), 3.28 (dd, $J = 4$, 15 Hz, 1H, 8‴-H), 3.16 (t, $J = 10$ Hz, 1H, 11-H), 2.78 (dd, $J = 5$, 14 Hz, 1H, 2-H), 2.58 (m, 1H, 3H)
ESI MS 754 [M + H], 753 [M − H]

Derivatives

Table 1 Selected podophyllotoxin derivatives prepared by reacting 4′-*O*-demethyl-4β-4-desoxypodphylotoxin and heterocyclic amines and their corresponding physical data. ^{1}H-NMR for products supplied by author.



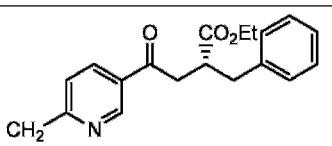
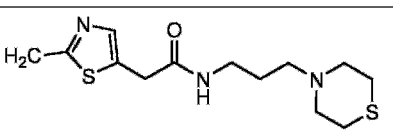
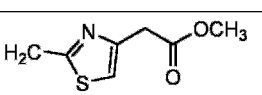
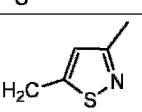
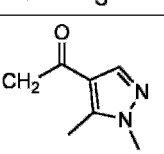
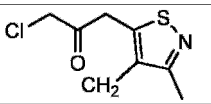
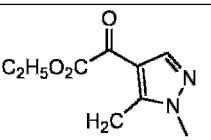
Entry	R	MP (°C)	ESI MS
15		233–236	694.4 [M − H]
28		105–109 (dec)	667 [M + 1]
36		116–120 (dec)	553 [M − H]
45		–	495.2 [M − 1]
85		140–143	550 [M − H]

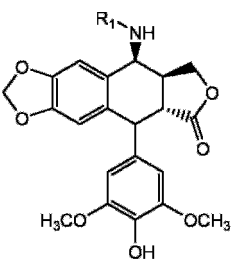
Table 1 (Continued)

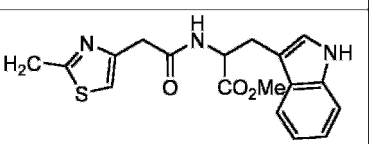
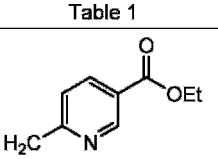
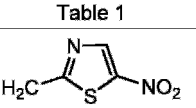
Entry	R	MP (°C)	ESI MS
190		178–180 (dec)	586.2 [M – H]
201		185–189	511.2 [M – 1]

Testing

Selected experimental agents were evaluated for cytotoxicity against KB cells using nasopharyngeal carcinoma cells and tested for stimulation of cellular protein-linked DNA breaks using etoposide as a positive control. Testing results are provided in Table 2.

Table 2 Cytotoxity of selected podophyllotoxin derivatives against KB cells and cellular protein-linked DNA breaks

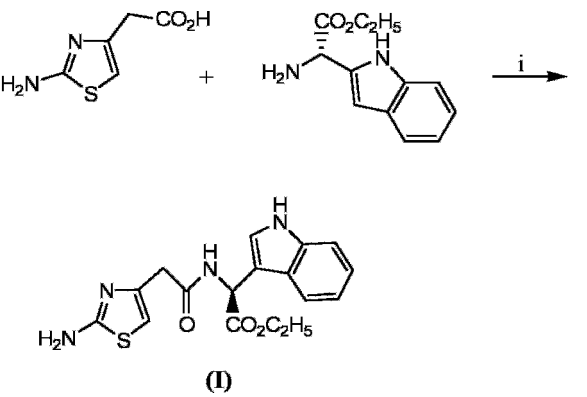


Entry	R	IC_{50} Against KB Cells	PLDB in KB Cells
1	 (R,S-)	Low	High
15	Table 1	Low	High
36	Table 1	Low	High
39		Low	High
45	Table 1	Low	High
49		Low	Low
201	Table 1	Low	Low

Notes

1. The preparation of the Step 1 co-reagent, 2-amino-[4″-(ethyl-L-tryptophan-*N*-acetyl)-2″-thiazine, (**I**), is illustrated in Eq. 2 and described by the author.

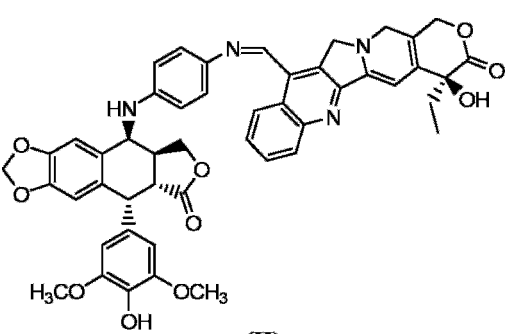
Eq. 2



(I)

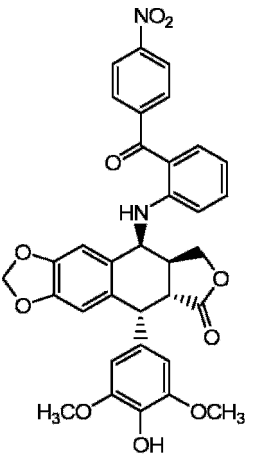
i- 1,3-Dicyclohexylcarbodiimide, toluene

2. Covalently linked conjugates, (**II**), consisting of a topoisomerase I inhibitor such as a camptothecin group and a topoisomerase II inhibitor such as epipodophyllotoxin, were prepared by Lee (1) and used in treating small cell lung cancer.

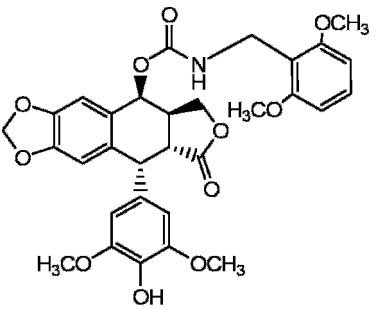


(II)

3. Benzoyl-anilino podophyllotoxin derivatives, (**III**), prepared by Kamal (2) and carbamate and thiocarbamate derivatives, (**IV**), prepared by Monneret (3), respectively, were effective against murine leukemia from L1210 cells.

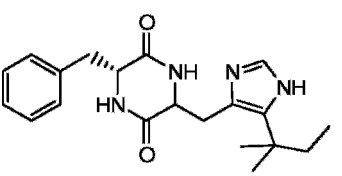


(III)

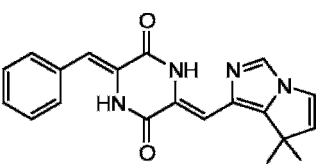


(IV)

4. Topoisomerase II inhibitors consisting of phenylahistins, (**V**), and dehydrophenylahistin derivatives, (**VI**), were prepared by Hayashi (4,5), respectively, and used in treating A-549 tumor cells derived from human lung cancer.



(V)



(VI)

References

1. K.-H. Lee *et al*., US Patent 6,207,673 (March 27, 2001)
2. A. Kamal *et al*., US Patent 7,087,641 (August 8, 2006)
3. C. Monneret *et al*., US Patent 6,878,746 (April 12, 2005)
4. Y. Hayashi *et al*., US Patent 7,026,322 (April 11, 2006)
5. Y. Hayashi *et al*., US Patent 7,064,201 (June 20, 2006)

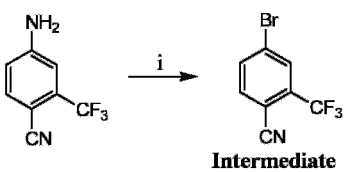
XI. Prostate Cancer: Nonsteroidal Antiandrogenic Antagonists

Title Antiandrogenic Biphenyls
F. Labrie *et al.*, US Patent 6,933,321 (August 23, 2005)
Assignee Endorecherche, Inc Pty. Ltd
Utility Treatment of Prostate Cancer

Invention Significance Antiandrogenic nonsteroidal biphenyl antagonists that substantially lack agonistic properties have been prepared. In addition to being therapeutically effective in slowing or stopping androgen-dependent diseases such as prostate cancer and benign prostatic hyperplasia, these agents are readily and inexpensively prepared.

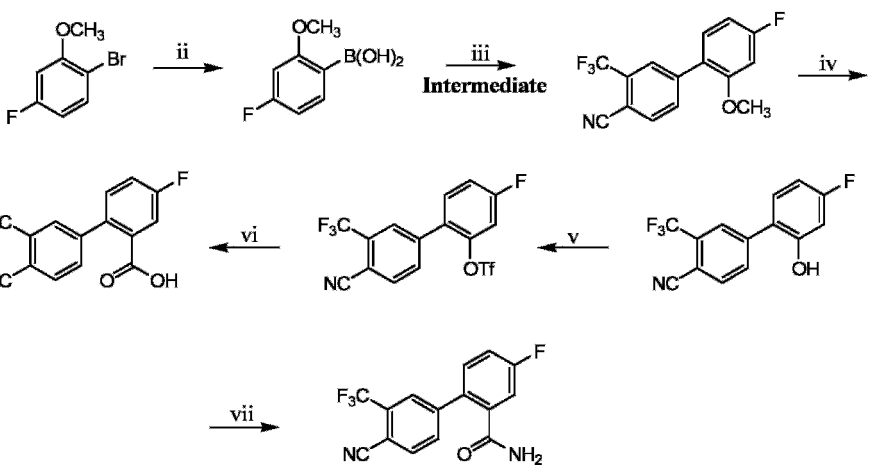
Reaction

Eq. 1



Intermediate

Eq. 2



Intermediate

i- Hydrobromic acid, sodium nitrite, copper(I) bromide
ii- THF, butyllithium, trimethyl borate
iii- Ethyl alcohol, sodium bicarbonate, 4-bromo-2-trifluoromethylbenzonitrile, tetrakis(triphenylphosphine)palladium

iv- CH_2Cl_2, boron tribromide
v- CH_2Cl_2, triethylamine, dimethylaminopyridine, triflic anhydride
vi- DMF, methyl alcohol, trimethylamine, diphenyl-1-pyrenylphosphine, palladium(II) acetate, carbon monoxide
vii- Ammonia, sodium cyanide, methyl alcohol

Experimental

1. **Preparation of 4-bromo-2-trifluoromethylbenzonitrile**

   4-Cyano-3-trifluoromethylaniline (107.5 mmol) dissolved in 300 ml 47% hydrobromic acid cooled to 0°C was treated with $NaNO_2$ (322 mmol) followed by the slow addition of CuBr (322.5 mmol), then stirred 3 hours at 20°C, recooled to 0°C, and treated with 500 ml water. An yellow precipitate was isolated, which was washed with 50 ml 10% HCl, 100 ml water, dried, and the product isolated in 87% yield.

   ^{1}H NMR (300 MHz, acetone-d_6) δ 8.19 (s, 1H, C_3-H), 8.16 (d, 1H, $J = 8$ Hz, C_6-H), 8.05 (d, 1H, $J = 8$ Hz, C_5-H)

2. **Preparation of 4-fluoro-2-methoxyphenylboronic acid**

   The Step 1 product (29 mmol) dissolved in 50 ml THF was cooled to −78°C, then treated with 17.4 ml BuLi. After 30 minutes 4.9 ml trimethyl borate was added and the mixture warmed to ambient temperature over 75 minutes. The solution was recooled to 0°C, then treated with 20 ml 10% HCl, concentrated, and 50 ml brine added to the residue. The solution was extracted twice with diethyl ether, then washed with 10% HCl and then brine. The solution was dried with $MgSO_4$, concentrated, and the product isolated in 82% yield as an yellow solid.

   ^{1}H NMR (400 MHz, acetone-d_6) δ 7.84 (t, 1H, $J = 8$ Hz, C_6-H), 6.85 (dd, $J = 12$, 2 Hz, C_3-H), 6.76 (td, $J = 8$, 2 Hz, C_5-H)

3. **Preparation of 4′-fluoro-2′-methoxy-3-trifluoromethyl-biphenyl-4-carbonitrile** (general Suzuki coupling method)

   The Step 1 product (4 mmol) dissolved in a mixture of 55 ml toluene and 20 ml ethyl alcohol was treated with 20 ml 0.8 M $NaHCO_3$ and the Step 2 product (4.8 mmol), then degassed 10 minutes and treated with $Pd(PPh_3)_4$ (0.4 mmol). The mixture was heated 2 hours at 95°C, then poured in brine, and extracted with CH_2Cl_2. The extract was dried with $MgSO_4$ and concentrated. The residue was purified by chromatography with silica gel using 250 ml EtOAc/hexanes, 1:9, and the product isolated in 81% yield as a white solid.

   ^{1}H NM (300 MHz, acetone-d_6) δ 8.02–8.14 (m, 3H, $C_{2,5,6}$-H), 7.53 (dd, 1H, $J = 8$, 7 Hz, C_6'-H), 7.03 (dd, 1H, $J = 11$, 2 Hz, C_3'-H), 6.89 (td, 1H, $J = 8$, 2 Hz, C_5'-H)

4. **Preparation of 4′-fluoro-2′-hydroxy-3-trifluoromethyl-biphenyl-4-carbonitrile**

   A solution of the Step 3 product (3.58 mmol) in 3 ml CH_2Cl_2 was treated with 7.5 ml 1 M BBr_3 in CH_2Cl_2 and the solvent slowly removed to dryness by heating 60 minutes to 60°C. The dry mixture was heated an additional 30 minutes, then redissolved in CH_2Cl_2. The solution was washed with water and brine, dried, then concentrated, and the product isolated in 99% yield as a white solid.

   ^{1}H NMR (300 MHz, acetone-d_6) δ 9.50 (bs, 1H, OH), 8.08–8.20 (m, 3H, $C_{2,5,6}$-H), 7.53 (t, 1H, $J = 7$ Hz, C_6'-H), 6.78–6.86 9 (m, 2H, $C_{3',5}$-H)

5. **Preparation of trifluoromethanesulfonic acid-4′-cyano-4-fluoro-3′-trifluoromethyl-biphenyl-2-yl ester**

   A solution of the Step 4 product (3.56 mmol) in 100 ml CH_2Cl_2 was treated with 992 μl triethylamine and dimethylaminopyridine (0.356 mmol) at ambient temperature, then cooled to 0°C and treated with 900 μl triflic anhydride. The mixture was stirred 45 minutes at 1°C, then quenched with 50 ml saturated NH_4Cl solution. It was extracted with CH_2Cl_2, dried, and then concentrated. The residue was purified by chromatography with silica gel using EtOAc/hexanes, 1:2.3, and the product isolated in 99% yield as an oil.

   ^{1}H NMR (400 MHz, acetone-d_6) δ 7.98 (d, 1H, $J = 8$ Hz, C_5'-H), 7.90 (s, 1H, C_2'-H), 7.79 (d, 1H, $J = 8$ Hz, C_6'-H), 7.52 (dd, 1H, $J = 6$, 8 Hz, C_6-H), 7.22–7.34 (m, 2H, $C_{3',5'}$-H)

6. **Preparation of 4′-cyano-4-fluoro-3-trifluoromethyl-biphenyl-2-carboxylic acid methyl ester**

   The Step 5 product dissolved in 80 ml DMF was initially treated with 25 ml methyl alcohol, 1.5 ml triethylamine, 1.5 ml Et_3N and diphenyl-1-pyrenylphosphine (0.212 mmol). The mixture was subsequently treated with $Pd(OAc)_2$ (0.247 mmol), then heated 90 minutes at 95°C, and saturated with carbon monoxide. The mixture was heated for additional 30 minutes under a carbon monoxide atmosphere, then quenched with 100 ml ice. The mixture was extracted twice with CH_2Cl_2, dried, and concentrated. The residue was purified by chromatography with silica gel using EtOAc/hexanes, 1:4, and the product isolated in 99% yield as a crystalline solid.

   ^{1}H NMR (300 MHz, acetone-d_6) δ 8.14 (d, 1H, $J = 8$ Hz, C_5'-H), 7.93 (s, 1H, C_2'-H), 7.85 (d, 1H, $J = 8$ Hz, C_6'-H), 7.71–7.75 (dd, 1H, $J = 2.6$, 8 Hz, C_3-H), 7.50–7.53 (m, 2H, $C_{5,6}$-H), 3.70 (s, 3H, $COOCH_3$)

7. **Preparation of 4′-cyano-4-fluoro-3′-trifluoromethyl-biphenyl-2-carboxylic acid amide**

   A Schlenk tube equipped with a magnetic stirrer and 60 ml ammonia at −78°C was treated with NaCN (176 mmol) and the Step 6 product (4.64 mmol) dissolved in 80 ml dry methyl alcohol. The mixture was heated 16 hours at 70°C, then excess ammonia

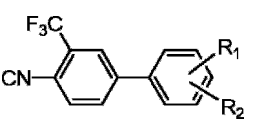
was removed, and the contents were poured in brine. The solution was extracted with CH_2Cl_2 and then concentrated. The residue was purified by chromatography using EtOAc/hexanes, 1:1.5, and the product isolated in 76% yield as a white crystalline solid.

^{1}H NMR (400 MHz, acetone-d_6) δ 8.14 (d, 1H, $J = 8$ Hz, C_5'-H), 8.00 (s, 1H, C_2'-H), 7.93 (d, 1H, $J = 8$ Hz, C_6'-H), 7.62 (d, 1H, $J = 8$ Hz, C_6-H), 7.44 (t, 1H, $J = 8$ Hz, C_5-H), 7.39 (d, 1H, $J = 8$ Hz, C_3-H)
^{13}C NMR (acetone-d_6) δ 108.93, 115.75, 116.06, 116.17 (CN), 117.51, 117.79, 121.86, 125.47, 127.97, 128.03, 129.09, 131.64, 132.07, 132.49, 132.91, 133.35, 133.47, 133.94, 134.18, 135.86, 139.997, 146.35, 161.65, 164.95, 169.57 (CO)
IR (KBr, cm^{-1}) 3431.3, 3299.7, 3181.6, 2237.0, 1668.9, 1326.7, 1179.7, 1130.6, 823.0

Derivatives

Selected derivatives are provided in Table 1.

Table 1 Selected 4′-cyano-3′-trifluoromethyl biphenyl derivatives evaluated as nonsteroidal antiandrogen inhibitors. ^{1}H-NMR for products supplied by author.



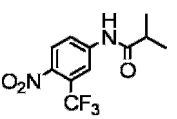
Entry	R_1	R_2	In Vitro Shionogi		In Vivo Rats	
			K_i	Antiandrogenic Activity, IC_{50} (nM)	Prostate efficacy versus FLU[a] per os DHT (%)	SV efficacy versus FLU[a] per os DHT (%)
4004	2-Fluoro	5-Fluoro	<0.2	>500	14	32
4046	2-Fluoro	4-Fluoro	<0.2	>500	0	13
4057	3-Fluoro	4-Methoxy	1.2	84	0	15
4068	3-Methoxy	4-Fluoro	0.9	96	0	0
4080	3-Bromo	4-Methoxy	0.6	102	4	0
4174	2-Fluoro	4-Methoxy	2.1	77	38	53

(*continued*)

Table 1 Continued

Entry	R_1	R_2	In Vitro Shionogi		In Vivo Rats	
			K_i	Antiandrogenic Activity, IC_{50} (nM)	Prostate efficacy versus FLU[a] per os DHT (%)	SV efficacy versus FLU[a] per os DHT (%)
4186	Hydrogen	4-Cyano	<0.2	>500	10	34
4265	Hydrogen	3-(2-Trifluoromethyl-1-hydroxylethyl)	0.7	116	0	24

[a] Throughout testing, flutamide, 2-methyl-*N*-[4-nitro-3-(trifluoromethyl) phenyl] propanamide, (**I**), a nonsteroidal antiandrogen inhibitor, was used as a reference.



(I)

Testing

I. *Shionogi assay*

Testing consisted of in vitro determination of androgenic/antiandrogenic activity on mouse mammary carcinoma Shionogi cells and in vivo determination of systemic antiandrogenic activity of immature male rats. A summary of these testing results is provided in Table 1 where:

Column 4: Represents the ratio of inhibition constant, K_i, for the inhibition of dihydrotestosterone (DHT)-stimulated Shionogi mouse mammary carcinoma cell number for the antiandrogen hydroxyflutamide versus a selected experimental agent. Higher values are preferable.
Column 5: Represents the dose (nM) that inhibits 50% (IC_{50}) of the DHT-stimulated Shionogi mouse mammary carcinoma cell number. Lower values are preferable.
Column 6: Represents a comparison of antiandrogenic efficacy (%) of selected experimental agents in rat prostate to that of flutamide (FLU).
Column 7: Represents a comparison of antiandrogenic efficacy (%) of experimental agents in rat seminal vesicle to that of FLU.

II. *Antiandrogenic Assay*

Testing for antiandrogenic activity in a hamster ear area was also performed and testing results are provided in Table 2 where:

Column 2: Represents the daily dose for 14 days of a selected experimental agent applied onto a region between the two cartilage ridges of the ventral surface of left pinna.
Column 3: Represents the percentage antiandrogenic inhibition of the area of the sebaceous glands of the left ear of animals treated with selected experimental agents versus the area of the sebaceous glands of the left ear of control animals.

Table 2 Antiandrogenic activity in hamster ears using selected 4′-cyano-3′-trifluoromethyl biphenyl derivatives

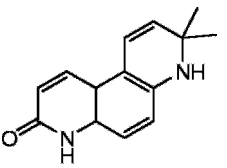
Entry	Dose (μg)	Inhibition Versus Control (%)
Step 7 Product	3	4
4004	10	60
4046	30	5
4057	10	64
4068	30	15
4080	30	5
4174	10	86
4186	30	0
4265	30	0
4977	10	73
4977	1	26
4977	3	48
4977	10	63

Results

For systemic use, 3-chloro-2′, 4′-difluoro-biphenyl-4-carbonitrile, is especially preferred. For topical application, 3-chloro-2′,6′-difluoro-biphenyl-4-carbonitrile is preferred.

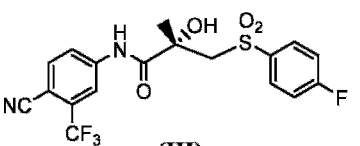
Notes

1. In a subsequent investigation by Zhi (1), tricyclic derivatives, (**II**), were prepared, which were effective as nonsteroidal partial agonists and antagonists as indicated by cotransfection assays.

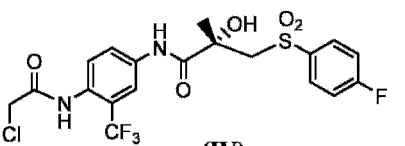


(II)

2. An irreversible nonsteroidal antiandrogen agent useful in the treatment of prostate cancer was prepared by Miller (2) by modifying the androgen antagonist, Casodex, (**III**), through *N*-haloacylation, (**IV**).

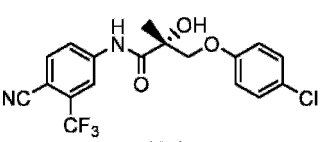


(III)

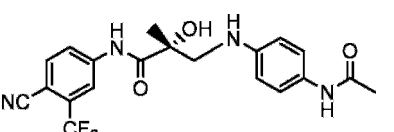


(IV)

3. Selective nonsteroidal androgen receptor modulators consisting of phenoxy, (**IV**), and anilino, (**V**), derivatives, were prepared by Dalton (3,4), respectively, and used in the treatment of androgen-dependent diseases including prostate cancer.



(IV)



(V)

References

1. L. Zhi *et al*., US Patent 7,026,484 (April 11, 2006)
2. D.D. Miller *et al*., US Patent 7,041,844 (May 9, 2006)
3. J.T. Dalton *et al*., US Patent 7,026,500 (April 11, 2006); US Patent 6,569,896 (May 27, 2003); and US Patent 6,492,554 (December 10, 2002)
4. J.T. Dalton *et al*., US Patent 7,022,870 (April 4, 2006)

CHAPTER XXVI

Psychiatric

I. Major Depression

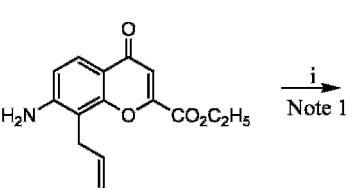
A. 5-HT_{1A} Agonists

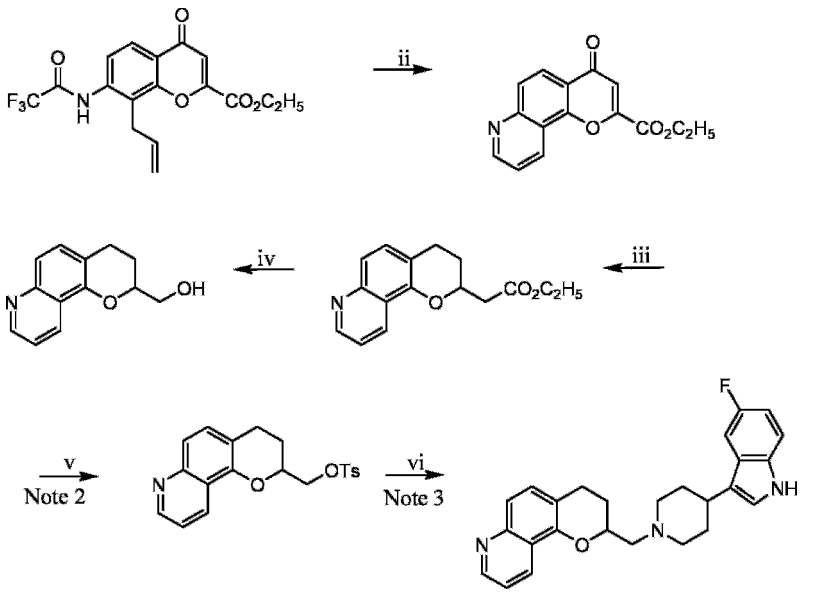
Title Antidepressant Azahetorocyclylmethyl Derivatives of 7,8-Dihydro-6H-5-oxa-1-aza-phenathrene
R. Zhao *et al.*, US Patent 7,030,245 (April 18, 2006)
Assignee Wyeth
Utility Treatment of Major Depression in Unresponsive Patients

Invention Significance Although existing selective serotonin reuptake inhibitors are successful in treating major depression, they have a slow onset of action and are therapeutically effective in fewer than two-thirds of patients. To address these concerns, 5-HT_{1A} agonists have been prepared that act directly on postsynaptic serotonin receptors to increase serotonergic neurotransmission during the latency period as well as stimulating the somatodendritic autoreceptors to produce clinical improvement in unresponsive patients.

Reaction

Eq. 1





i- CH_2Cl_2, *N,N*-diisopropylethylamine, trifluoroacetic anhydride
ii- Dioxane, selenium dioxide
iii- Ethyl alcohol, palladium hydroxide on carbon, hydrogen
iv- THF, lithium borohydride
v- CH_2Cl_2, *p*-toluenesulfonyl chloride, *N,N*-diisopropylethylamine, *N,N*-dimethylaminopyridine
vi- DMSO, 5-fluoro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole

Experimental

1. **Preparation of 8-allyl-4-oxo-7-(2,2,2-trifluoro-acetylamino)-4H-chromene-2-carboxylic acid ethyl ester**

   8-Allyl-7-amino-4-oxo-4H-chromene-2-carboxylic acid ethyl ester (32 mmol) dissolved in 400 ml of CH_2Cl_2 was treated first with *N,N*-diisopropylethylamine (50 mmol), then trifluoroacetic anhydride (50 mmol) while cooling the mixture in an ice bath. The mixture was stirred 1 hour at ambient temperature, then washed with 200 ml apiece 2 M HCl, saturated $NaHCO_3$ solution, and brine, dried with Na_2SO_4, and filtered. The material was concentrated and 12.2 g product isolated as a pale yellow solid, mp = 136–137°C.

   Elemental Analysis Calc. for $C_{17}H_{14}F_3NO_5$: C, 55.29; H, 3.82; N, 3.79. Found: C, 55.28; H, 3.79; N, 3.76

2. **Preparation of 8-oxo-8H-5-oxa-1-aza-phenanthrene-6-carboxylic acid ethyl ester**

The Step 1 product (10 mmol) dissolved in 100 ml dioxane was treated with selenium dioxide (40 mmol), then refluxed 5 hours. Additional selenium dioxide (1.0 g) was added and the mixture was further refluxed 8 hours. The reaction was then cooled, diluted with 500 ml water, and extracted with 300 and 200 ml portions of EtOAc. The extracts were washed with water and brine, dried, concentrated, and 2.0 g product isolated as an yellow solid. An analytically pure sample was obtained by chromatography with silica gel using 0–5% EtOAc/CH_2Cl_2, mp = 146−147°C.

Elemental Analysis Calc. for $C_{15}H_{11}NO_4 \bullet 1.1\ H_2O$: C, 62.33; H, 4.60; N, 4.85. Found: C, 62.09; H, 4.15; N, 4.79

3. **Preparation of (1,3,4,6,7,8-hexahydro-2H-5-oxa-1-aza-phenanthren-6-yl)-6-carboxylic acid ethyl ester**

The Step 2 product (7.4 mmol) was dissolved in 200 ml ethyl alcohol containing 20% palladium hydroxide on carbon (0.50 g) and hydrogenated 48 hours under 60 psi hydrogen. The mixture was filtered through celite, concentrated, where TLC on silica gel indicated that the reaction was incomplete. The residue was then dissolved in 150 ml acetic acid, then treated with 20% palladium hydroxide on carbon (0.50 g), and hydrogenated 48 hours at 60 psi hydrogen. The mixture was worked up as above, the residue purified by chromatography using 2% ethyl alcohol/$CHCl_3$, and 0.80 g of product isolated.

^{1}H NMR ($CDCl_3$) doublet 6.65 δ (1H); doublet 6.1 δ (1H); multiplet 4.7 δ (1H); quartet 4.25 δ (2H); broad singlet 3.65 δ (1H); multiplet 3.2 δ (2H); multiplet 2.65 δ (4H); multiplet 2.2 δ (2H); multiplet 1.95 δ (2H); triplet 1.25 δ (3 H)

4. **Preparation of (1,3,4,6,7,8-hexahydro-2H-5-oxa-1-aza-phenanthren-6-yl)-methanol**

The Step 3 product (3.1 mmol) dissolved in 25 ml THF was treated with lithium borohydride (10 mmol), then stirred 48 hours at ambient temperature. The mixture was quenched with 5 ml methyl alcohol, then stirred an additional hour, and finally diluted with 300 ml EtOAc. The solution was washed with 100 ml apiece water and brine, dried, concentrated, and 0.83 g product isolated as a white solid.

^{1}H NMR ($CDCl_3$) doublet 6.65 δ (1H); doublet 6.1 δ (1H); multiplet 4.1 δ (1H); multiplet 3.8 δ (2H); multiplet 3.2 δ (2H); broad singlet 2.8 δ (1H); multiplet 2.55 δ (4H); multiplet 1.9 δ (2H); multiplet 1.2 δ (2H)

5. **Preparation of toluene-4-sulfonic acid 7,8-dihydro-6H-5-oxa-1-aza-phenanthren-6-yl-methyl ester**

The Step 4 product (1.3 mmol) was dissolved in 75 ml CH_2Cl_2 and *p*-toluenesulfonyl chloride (3.1 mmol) added. The mixture was placed in an ice/isopropyl

alcohol bath and treated with *N,N*-diisopropylethylamine (3.1 mmol) and *N,N*-dimethylaminopyridine, then stirred 5 days. Afterwards it was washed with 150 ml apiece 2 M HCl, saturated $NaHCO_3$ solution, and brine, dried with $MgSO_4$, and concentrated to an orange oil. The residue was purified by chromatography using 0–5% methyl alcohol/CH_2Cl_2 and 0.22 g product isolated.

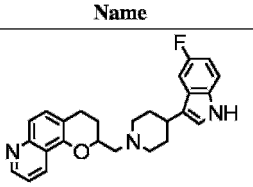
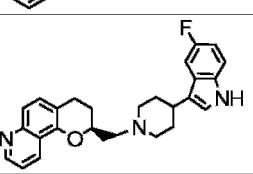
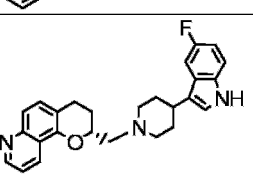
^{1}H NMR ($CDCl_3$) doublet 8.8 δ (1H); doublet 8.25 δ (1H); doublet 7.8 δ (2H); doublet 7.6 δ (1H); multiplet 7.3 δ (4H); multiplet 4.4 δ (1H); doublet 4.3 δ (2H); multiplet 2.9 δ (2H); singlet 2.4 δ (3H); multiplet 2.0 δ (2H)

6. **Preparation of (*RS*)-6-[4-(5-fluoro-1H-indol-3-yl)-3,6-dihydro-2H-pyridin-1-ylmethyl]-7,8-dihydro-6H-5-oxa-1-aza-phenanthrene**

The Step 5 product (1.67 mmol) and 5-fluoro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole (1.8 mmol) were dissolved in 200 ml DMSO, then heated 13 hours at 75–80°C, cooled, and partitioned between 400 ml apiece EtOAc and saturated $NaHCO_3$ solution. The organic phase was washed with brine, dried, and concentrated to an oil. The oil was purified by chromatography using CH_2Cl_2 to elute impurities and then using 5% methyl alcohol/CH_2Cl_2 and 0.19 g product isolated as an yellow oil. The oil was recrystallized from isopropyl alcohol containing 0.05 g of oxalic acid and 0.043 g oxalate salt isolated as a yellow solid, mp = 148°C.

Derivatives

Table 1 Selected 1,3,4,6,7,8-hexahydro-2H-5-oxa-1-aza-phenanthren-6-yl derivatives and their corresponding physical properties. R- and S-enantiomers were prepared using either the (*R*)- or (*S*)-[2-(*t*-butyl-(dimethyl-silanyloxy-methyl)-8-methyl-chroman-7-yl]-carbamic acid *t*-butyl ester in Step 1

Entry	Name	Analytical
1		mp = 148°C
2		Oil, $[\alpha]_D^{25}$ = +40.66° (*c* = 7.7, DMSO)
3		mp = 210°C $[\alpha]_D^{25}$ = –60.03° (*c* = 4.5, DMSO)

Testing

I. *High Serotonin 5-HT$_{1A}$ Receptor Affinity Test*

The high affinity for the serotonin 5-HT$_{1A}$ receptor affinity, K_i, test was performed by determining the ability of experimental agents to displace ^{3}H-dipropylaminotetralin from the 5-HT$_{1A}$ serotonin receptor using the modified procedure of Hall (1). Testing results are provided in Table 2, column 2.

II. *Agonist Activity at 5-HT$_{1A}$ Testing*

Agonist activity at the 5-HT$_{1A}$ receptors was established by using a ^{35}S-GTPγS binding assay similar to the procedure of Lazareno (2). Testing results for agonistic receptor affinity (K_i), column 3, maximum stimulatory effect (E_{max}), and potency (EC_{50}), are provided in Table 2.

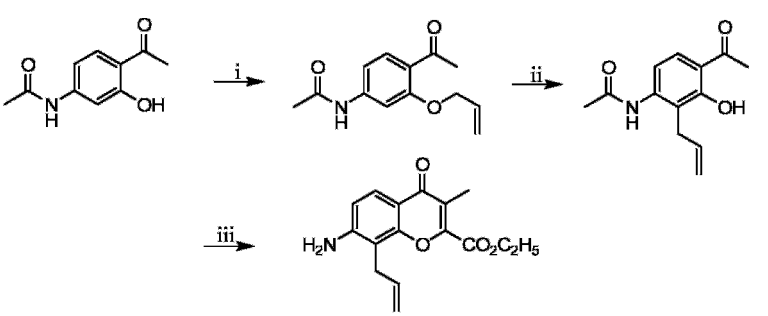
Table 2 5-HT$_{1A}$ receptor affinity and agonist activity of selected azahetorocyclylmethyl derivatives

Entry	K_i (nM)		EC_{50} (nM)	E_{max} (nM)
	5-HT$_{1A}$ Receptor Affinity	Agonistic Receptor Activity		
1	8.00	60.92	103.0	(49.0)
2	5.96	88.60	–	(5.00)
3	6.00	82.32	78.9	(52.0)

Notes

1. The preparation of the Step 1 co-reagent, 8-allyl-7-amino-4-oxo-4H-chromene-2-carboxylic acid ethyl ester, (**I**), was provided by the author and illustrated in Eq. 2.

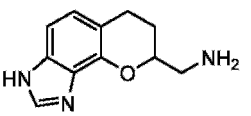
Eq. 2



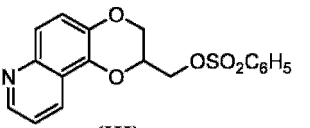
(**I**)

i- Sodium hydride, allyl bromide, DMF
ii- *N,N*-Dimethyl aniline
iii- Sodium, diethyl oxalate, hydrochloric acid, ethyl alcohol

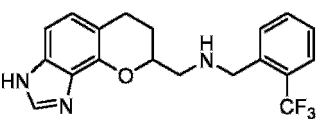
2. Step 5 analogs, (**II**) and (**III**), were prepared by Mewshaw (3) and Stack (4), respectively, and used to prepare dopamine agonists, (**IV**), and 5-HT_{1A} antagonists, (**V**).



(II)



(III)



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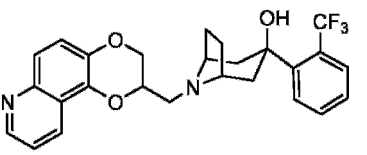
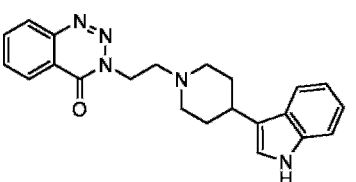
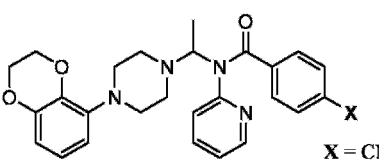


(V)

3. 5-HT_{1A} receptor antagonists consisting of 3-indolyl-4-piperidino heterocycles, (**VI**), and piperazine derivatives, (**VII**), with improved onset of action were prepared by Venkatesan (5) and Childers (6), respectively, and used in the treatment of depression and anxiety disorders.



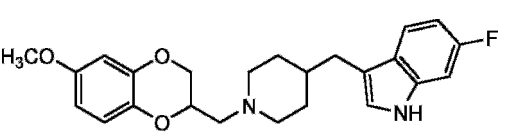
(VI)



X = CN, NO_2, CF_3

(VII)

4. 2,3-Dihydro-1,4-benzodiozan derivatives, (**VIII**), prepared by Husbands (7) were effective as both serotonin reuptake inhibitors and 5-HT_{1A} receptor antagonists and used in treating depression and addiction disorders.



(VIII)

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2. T. Lazareno *et al*., Br. J. Pharmacol., 109, 1120 (1993)
3. R.E. Mewshaw *et al*., US Patent 6,541,502 (April 1, 2003) and US Patent 6,541,501 (April 1, 2003)
4. G.P. Stack *et al*., US Patent 6,861,427 (March 1, 2005)
5. A.M. Venkatesan *et al*., US Patent 6,939,870 (September 6, 2005)
6. W.E. Childers *et al*., US Patent 7,026,320 (April 11, 2006) and US Patent 7,049,330 (May 23, 2006)
7. G.E.M. Husbands *et al*., US Patent 7,041,683 (May 9, 2006)

B. Noradrenaline Reuptake Inhibitors

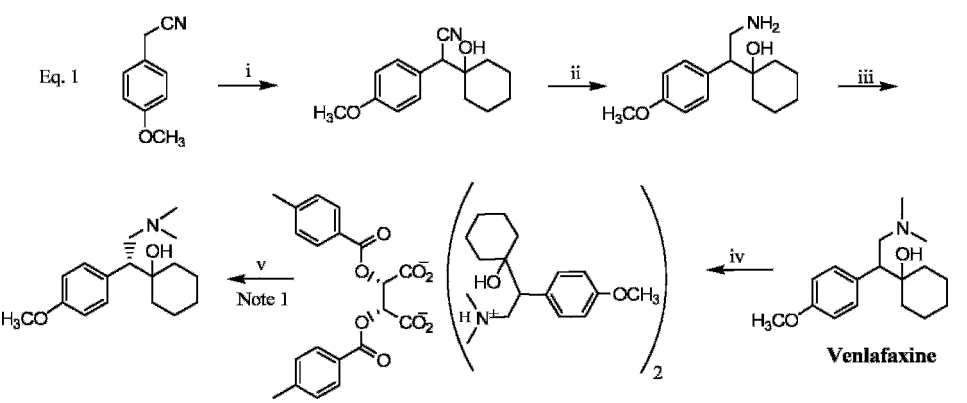
Title Methods of Treating and Preventing Pain, Anxiety, and Incontinence Using Derivatives of (−)-Venlafaxine
T.J. Jerussi *et al.*, US Patent 6,911,479 (June 28, 2005)

Assignee Wyeth

Utility Treatment of Psychiatric Disorders Using Optically Active (−)-Venlafaxine Derivatives as Noradrenaline Reuptake Inhibitors

Invention Significance Venlafaxine is an antidepressant containing an unresolved asymmetric benzylic carbon. Reports indicate that the (−)-enantiomer is a potent inhibitor of norepinephrine synaptosomal uptake while the (+)-enantiomer is a more selective serotonin uptake inhibitor. Methods of preparing (−)-venlafaxine derivatives and their use as noradrenaline reuptake inhibitors are described.

Reaction



i- THF, lithium diisopropylamide, cyclohexane
ii- Methyl alcohol, cobalt(II) chloride, sodium borohydride
iii- Formic acid, formaldehyde, water
iv- Di-*p*-toluoyl-D-tartaric acid, ethylacetate venlafaxine
v- Water, sodium hydroxide

Experimental

1. **Preparation of 1-[cyano-(4-methoxyphenyl)methyl]cyclohexanol**

   A solution of 4-methoxybenzylnitrile (0.36 mol) in 400 ml THF was cooled to −78°C and treated dropwise with 200 ml 2.0 M lithium diisopropylamide in THF while

maintaining the reaction temperature below −65°C, then stirred 30 minutes at −78°C. The mixture was then treated with cyclohexanone (0.40 mol) at a rate such that the reaction temperature did not rise above −65°C, then stirred 2 hours at −78°C, and finally quenched by pouring into 1000 ml saturated NH_4Cl solution containing ice. The mixture was extracted four times with 200 ml EtOAc and combined extracts washed three times with 100 ml water and once with 100 ml brine. The solution was then dried with Na_2SO_4 and concentrated. The residue consisted of a colorless solid that was triturated with hexane, filtered, washed with hexane, and the product isolated in 80.7% yield as a colorless solid.

^{1}H NMR ($CDCl_3$) 7.30 and 6.90 (q, 4H), 3.80 (s, 3H), 3.75 (s, 1H), 1.55 (m, 10H)
^{13}C NMR ($CDCl_3$) 159.8, 130.8, 123.8, 120.0, 114.1, 72.9, 55.5, 49.5, 34.9, 25.3, 21.6

2. **Preparation of 1-[2-amino-1-(4-methoxyphenyl)ethyl]cyclohexanol**

The Step 1 product (0.16 mol) dissolved in 1000 ml methyl alcohol was treated with cobalt chloride (0.32 mol) and the reaction stirred until a clear dark blue solution was obtained. Sodium borohydride (1.63 mol) was added portionwise while maintaining the temperature below 35°C, then stirred 2 hours at ambient temperature. The reaction was cooled in an ice/water bath, then slowly quenched with 1000 ml 3 M HCl to keep the temperature below 25°C, and stirred an additional 30 minutes. Methyl alcohol was removed in vacuo and the aqueous layer extracted three times with 300 ml EtOAc. The aqueous layer was cooled in ice/water bath, then basified with 600 ml 12 M NH_4OH, and re-extracted four times with 200 ml EtOAc. Combined extracts were dried with Na_2SO_4, concentrated, and the product isolated in 83.6% yield as an yellow gum.

^{1}H NMR ($CDCl_3$) 7.20 and 6.85 (q, 4H), 3.80 (s, 3H), 3.20 (m, 2H), 2.70 (t, 3H), 2.35 (br s, 3H), 1.40 (m, 10H)
^{13}C NMR ($CDCl_3$) 158.4, 132.6, 130.6, 113.7, 73.7, 56.7, 55.3, 42.4, 37.3, 34.5, 26.0, 21.9

3. **Preparation of (±)-venlafaxine**

The Step 2 product (0.13 mol) was dissolved in 55 ml 88% formic acid and 330 ml water, then treated with 41 ml 37% formaldehyde, and refluxed 20 hours. The mixture was cooled to ambient temperature, then concentrated to 150 ml, and acidified to pH 2.0 using 3 M HCl. The mixture was then extracted six times with 50 ml EtOAc until a pink impurity was removed. The aqueous layer was then cooled in ice/water bath and basified with 50% NaOH. This mixture was then extracted three times with 75 ml EtOAc and combined extracts washed three times with 25 ml water, once with brine, and dried. The solution was concentrated and the product isolated in 92.6% yield as an yellow gum that slowly turned into a pale yellow solid.

^{1}H NMR ($CDCl_3$) 7.05 and 6.80 (q, 4H), 3.80 (s, 3H), 3.30 (t, 1H), 2.95 (dd, 1H), 2.35 (s, 6H), 2.30 (dd, 1H), 1.30 (m, 10H)

^{13}C NMR ($CDCl_3$) 158.4, 132.9, 130.3, 113.5, 74.4, 61.4, 55.3, 51.8, 45.6, 38.2, 31.3, 26.2, 21.8, 21.5
MS 277, M^+

4. **Preparation of (*R*)-venlafaxine di-*p*-toluoyl-D-tartrate**

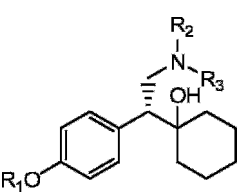
   To a stirred solution of the Step 4 product (0.072 mol) in 150 ml EtOAc was added a solution of di-*p*-toluoyl-D-tartaric acid (0.041 mol) dissolved in 120 ml EtOAc and within 15 minutes, a colorless solid began precipitating out. The suspension was stirred 4 hours at ambient temperature and the solid isolated by filtration. It was washed with EtOAc, then dried in vacuo, and the product isolated in 37.6% yield as a colorless solid.

5. **Preparation of (–)-venlafaxine**

   The Step 4 product (8.0 mmol) was treated with 50 ml cold 2 M NaOH and the aqueous layer extracted three times with 100 ml EtOAc. Combined extracts were washed once with 25 ml cold 2 M NaOH and then with a sufficient amount of water to obtain pH of 7. The solution was dried using Na_2SO_4, concentrated, and the product isolated in 100% yield as a colorless solid, HPLC > 99.95, ee ^{1}H, ^{13}C and MS data as in (±)-venlafaxine.

Derivatives

Table 1 Optically active (−)-venlafaxine derivatives and corresponding optical activities



Name	R_1	R_2	R_3	$[\alpha]_D^{25}$ ($c = 0.25$, EthOH)
O-Desmethylvenlafaxine	Hydrogen	Methyl	Methyl	−35.2
N-Desmethylvenlafaxine	Methyl	Hydrogen	Methyl	–
Venlafaxine	Methyl	Methyl	Methyl	−2.4

Testing

The method of Muth (1) was used to determine receptor binder specificity and synaptosomal uptake determined according to the method of Wood (2).

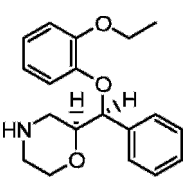
Results

Although testing results were not supplied by the author, the experimental agents are listed below were effective as noradrenaline reuptake inhibitors:

i- (−)-*O*-Desmethylvenlafaxine
ii- (−)-*N*-Didesmethylvenlafaxine
iii- (−)-*N,O*-Didesmethylvenlafaxine
iv- (−)-*N,N*-Didesmethylvenlafaxine

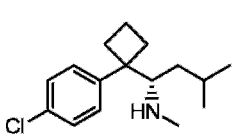
Notes

1. Wong (3) determined that optically pure *S,S*-2-[(2-ethoxyphenoxy)(phenyl)methyl] morpholine, (**I**), was about 5–8.5 times more effective as norepinephrine reuptake inhibitors than those containing the racemic mixture of Reboxetine®.



(I)

2. Jerussi (4) prepared amine metabilites *R*-(−)-desmethylsibutramine, (**II**), and *R*-(−)-didesmethylsibutramine, (**III**), from [*N*-1-[1-(4-chlorophenyl)-cyclobutyl]-3-methylbutyl]-*N,N*-dimethylamine, Sibutramine®, (**IV**), which were more effective as noradrenaline and serotonin reuptake than the unresolved antidepressant.



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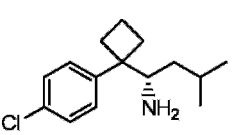

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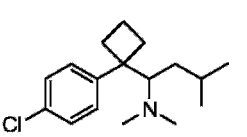
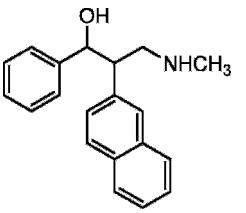


(IV)

Sibutramine

3. Richelson (5) prepared four stereoisomers of *N*-methyl-3-hydroxy-2-(2′-naphthyl)-3-phenylpropylamine, (**V**), each of which was more effective as norepinephrine or epinephrine reuptake inhibitor than the unresolved antidepressant.



(V)

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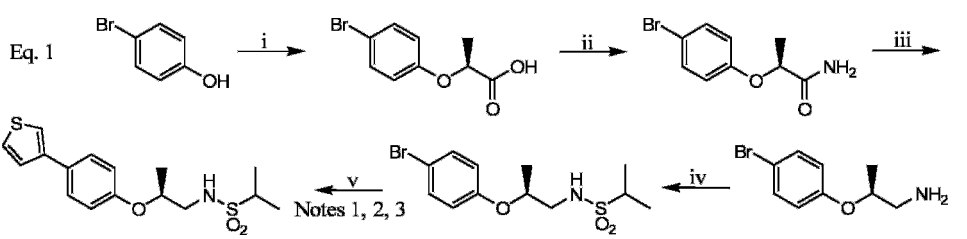
2. M.D. Wood *et al*., J. Neurochem. 37, 795 (1981)
3. K.H.F. Wong *et al*., US Patent 6,987,107 (January 17, 2006)
4. T.P. Jerussi *et al*., US Patent 7,071,234 (July 4, 2006)
5. E. Richelson *et al*., US Patent 6,914,080 (July 5, 2005)

C. Repotentiating α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid and Kainic Acid Glutamate Receptors

Title Sulfonamide Derivatives
J.Z. Davison *et al.*, US Patent 6,911,476 (June 28, 2005)
Assignee Eli Lilly and Company
Utility Treatment of Depression and Other Psychiatric Disorders

Invention Significance L-Glutamate mediates the excitatory pathway of nerve impulses between a neurotransmitter and a surface receptor. For reasons that are unclear, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainic acid receptors became deactivated and desensitized to glutamate. To address this problem, sulfonamide treatment agents have been prepared to repotentiate these receptors.

Reaction



i- THF, sodium hydride, (*S*)-α-chloropropionic acid
ii- *N*-Methyl morpholine, chlorodimethoxytriazine, ammonium chloride
iii- Borane dimethylsufide, THF
iv- CH_2Cl_2, sodium hydroxide, triethylamine, *N,N*-dimethylaminopyridine, isopropylsulfonyl chloride
v- Thiophene-3-boronic acid, tetrakis(triphenylphosphine)palladium, sodium carbonate, 1,4-dioxane

Experimental

1. **Preparation of (2*S*)-2-(4-bromophenoxy)propanoic acid**

A refluxing mixture consisting of sodium hydride (1.37 mol) and 500 ml THF was treated dropwise with 4-bromophenol (0.275 mol) dissolved in 200 ml THF over

60 minutes. Refluxing was continued until hydrogen evolution gas stopped and the mixture was then treated with (*S*)-α-chloropropionic acid (0.550 mol) over 60 minutes. The reaction was refluxed 2 hours, then quenched with 1000 ml water at reflux temperature, cooled, and extracted with 1400 ml CH_2Cl_2. The aqueous layer was extracted with 700 ml CH_2Cl_2, then acidified with 180 ml 6 M HCl, and dried with $MgSO_4$. The extraction solvent was exchanged with hexane and the product isolated in 96.4% yield as a tan precipitate.

^{1}H NMR ($CDCl_3$) δ 1.65–1.66 (d, 3H, $J = 6.83$Hz), 4.72–4.76 (m, 1H), 6.76–6.78 (d, 2H, $J = 9.03$Hz), 7.37–7.39 (d, 2H, $J = 9.03$Hz), 11.0
^{13}C NMR ($CDCl_3$) δ 18.61, 72.40, 114.43, 117.15, 132.75, 156.57, 178.31

2. **Preparation of (2*S*)-2-(4-bromophenoxy)propanamide**

The Step 1 product dissolved in 300 ml THF at ambient temperature was treated with 11.02 ml *N*-methyl morpholine followed immediately by chlorodimethoxytriazine (17.6 g) and the mixture stirred 45 minutes. An additional 17.64 ml *N*-methyl morpholine and NH_4Cl (8.04 g) were added and the reaction capped and stirred an additional 24 hours. A precipitate that formed was removed by filtration and the filtrate concentrated yielding an oil. The oil was dissolved in 150 ml CH_2Cl_2, then treated with 200 ml 1 M HCl, and stirred vigorously. The organic phase was then dried, the solvent exchanged with hexane, and the product isolated in 77.4% yield as a tan precipitate.

^{1}H NMR ($CDCl_3$) δ 1.56–1.58 (d, 3H, $J = 6.83$Hz), 4.6 (m, 1H), 5.58 (b, 1H), 6.47 (b, 1H), 6.78–6.81 (d, 2H, $J = 9.03$Hz), 7.40–7.42 (d, 2H, $J = 9.03$Hz)

3. **Preparation of (2*S*)-2-(4-bromophenoxy)propylamine**

The Step 2 product (77.93 mmol) dissolved in 300 ml THF ambient temperature was treated with 23.42 ml 10 M solution of borane dimethylsufide over 20 minutes, then refluxed 4.5 hours. The mixture was quenched with 4 M HCl in dioxane solution over 30 minutes and 105 ml ethyl alcohol added over the same time period. The mixture was concentrated to a white slurry, then azeotroped using 200 ml toluene, and the residue dissolved in 100 ml apiece EtOAc and diethyl ether. The mixture was stirred, filtered, and dried under a house vacuum for 18 hours at 45°C and the product isolated in 63% yield as a white precipitate.

^{1}H NMR (DMSO) δ 1.21–1.22 (d, 3H, $J = 6.1$Hz), 2.90–2.98 (m, 1H), 3.03–3.05 (m, 1H), 4.67–4.70 (m, 1H), 6.96–6.98 (d, 2H, $J = 9.03$Hz), 7.43–7.45 (d, 2H, $J = 9.03$Hz)
^{13}C NMR (DMSO) δ 17.48, 43.75, 71.63, 113.37, 119.26, 132.88, 156.82

4. **Preparation of [(2*S*)-2-(4-bromophenoxy)propyl][(methylethyl)sulfonyl]amine**

The Step 3 product (71.2 mmol) dissolved in 200 ml CH_2Cl_2 at ambient temperature was treated with 150 ml 2 M NaOH, then stirred 45 minutes. The organic layer was separated, dried, and the filtrate concentrated to an oil. The oil was dissolved in 225 ml

CH_2Cl_2 and treated with 23.6 ml triethylamine and *N,N*-dimethylaminopyridine (0.43 g), then cooled to −20°C. A solution of 9.5 ml isopropylsulfonyl chloride dissolved in 25 ml CH_2Cl_2 was then added over a period of 30 minutes while maintaining the temperature at −20°C and the mixture stirred overnight at ambient temperature. The reaction was quenched with 100 ml 3 M HCl solution, the organic layer separated, dried, concentrated, and the product isolated in 100% yield as an oil.

^{1}H NMR ($CDCl_3$) δ 1.25–1.26 (d, 3H, $J = 6.35$Hz), 1.33–1.35 (d, 6H, $J = 6.83$Hz), 3.14–3.19 (m, 1H), 3.22–3.26 (m, 1H), 3.34–3.36 (m, 1H), 4.43–4.46 (m, 1H), 4.74–4.76 (m, NH), 6.77–6.79 (d, 2H, $J = 9.03$Hz), 7.35–7.37 (d, 2H, $J = 9.03$Hz)
^{13}C NMR ($CDCl_3$) δ 16.91, 48.59, 53.94, 74.13, 113.84, 118.09, 132.72, 156.51

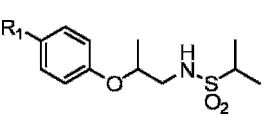
5. **Preparation of [(methylethyl)sulfonyl]{2-[4-(4-(3-thienyl)phenoxy)]propyl}amine**

A mixture consisting of the Step 4 product (0.773 mmol), thiophene-3-boronic acid (0.938 mmol), tetrakis(triphenylphosphine)palladium(0) (0.004 mmol), 276 mg sodium carbonate dissolved in 1.3 ml water, and 5 ml 1,4-dioxane was refluxed overnight under a nitrogen atmosphere. The reaction was quenched with water, extracted three times with 25 ml CH_2Cl_2, dried with Na_2SO_4, filtered, and concentrated. The brown oily residue was purified by chromatography with silica gel using hexane/EtOAc, 1:1, and the product isolated in 48.4% yield.

MS 340.0 ($M^* + 1$)
Analysis Theory: C, 56.61; H, 6.24; N 4.13. Found: C, 56.41; H, 6.12; N, 4.11

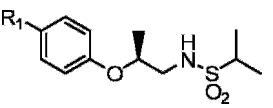
Derivatives

Table 1 Selected racemic sulfonamide derivatives and their corresponding mass spectral characterization data. Limited testing data supplied by author



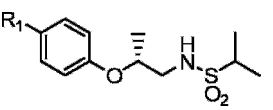
R_1	Electrospray-MS($M^* + 1$)
4-(2H-Benzo[3,4-d]1,3-dioxane-5-yl	378
2-Benzaldehyde	362
4-Trifluoromethylphenyl	402
2-Methoxyphenyl	364
3-Chlorophenyl	369
4-Methylphenyl	348
4-Cyanomethylphenyl	373

Table 2 Selected (2*S*)-sulfonamide derivatives and their corresponding mass spectral characterization data



R_1	Electrospray-MS ($M^* + 1$)
3-(Methylsulfonylamino)phenyl	427
4-Cyanophenyl	359
4-Bromo	336

Table 3 Selected (2*R*)-sulfonamide derivatives and their corresponding mass spectral characterization data



R_1	Electrospray-MS ($M^* + 1$)
3-(Methylsulfonylamino)phenyl	427
4-Cyanophenyl	359

Testing

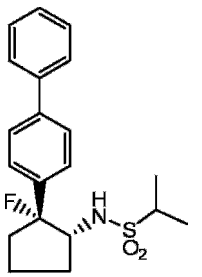
I. *Glutamate Receptor-mediated Potentiate Assay*

The ability of selected experimental agents to potentiate glutamate receptor-mediated response was determined using fluorescent calcium indicator dyes and by measuring glutamate-evoked efflux of calcium into GluR4-transfected HEK293 cells.

Testing results were not supplied by author.

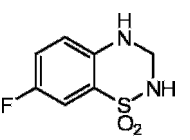
Notes

1. Cyclopentyl sulfonamide derivatives, (**I**), prepared by Cantrell (1) were effective as AMPA and kainic acid receptor repotentiators and used in treating depression and related psychiatric disorders.



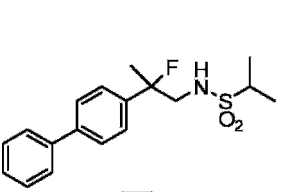
(I)

2. Pirotte (2) prepared benzothiadiazine derivatives, (**II**), which were effective as selective AMPA receptor activating agents and used in the treatment of depression-related illnesses.

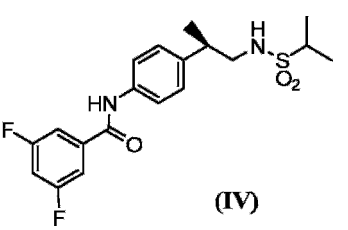


(II)

3. Mono-, (**III**), and difluoro-sulfonamide derivatives, (**IV**), prepared by Bender (3) and Aikins (4), respectively, were effective in reactivating and resensitizing AMPA and kainic acid receptors to glutamate.

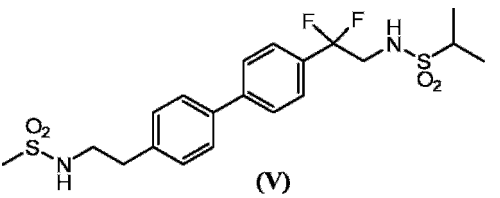


(III)



(IV)

4. Biphenyl-disulfonamide derivatives, (**V**), prepared by Arnold (5) were effective as AMPA and kainic acid receptor repotentiating and used in treating depression.



(V)

References

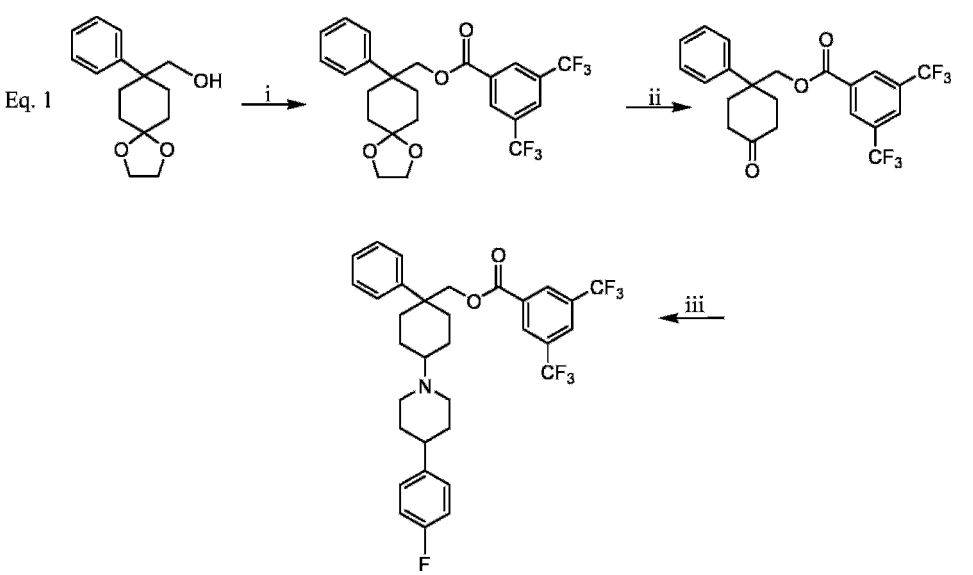
1. B.E. Cantrell *et al*., US Patent 6,900,353 (May 31, 2005)
2. B. Pirotte *et al*., US Patent 6,894,043 (May 17, 2005)
3. D.M. Bender *et al*., US Patent 7,034,045 (April 25, 2006)
4. J.A. Aikins *et al*., US Patent 6,803,484 (October 12, 2004)
5. M.B. Arnold *et al*., US Patent 6,713,516 (March 30, 2004)

D. Tachykinin Neurokinin-1 Receptor Antagonists

Title Cyclohexyl Derivatives and Their Use as Therapeutic Agents
J.L.Castro Pineiro *et al*., US Patent 6,953,792
(October 11, 2005)
Assignee Merck Sharp & Dohme Limited
Utility Treatment of Depression and Mood Disorders

Invention Significance Cyclohexyl derivatives have been prepared that have both a strong affinity and high selectivity for the tachykinin neurokinin-1 (NK_1) receptors. These agents are useful in the treatment of depression and dysthymic mood or other disorders mediated by NK_1 antagonists.

Reaction



i- 3,5-Bis(trifluoromethyl)benzoyl chloride, CH_2Cl_2, triethylamine, 4-dimethylamino pyridine
ii- Hydrochloric acid, water, acetone
ii- Sodium cyanoborohydride, zinc chloride, methyl alcohol, 4-(4-fluorophenyl)-piperidine

Experimental

1. **Preparation of (1,4-dioxa-8-phenylspiro[4.5]decan-8-yl)methyl 3,5-bis(trifluoromethyl)-benzoate**

   3,5-Bis(trifluoromethyl)benzoyl chloride (0.64 mmol) was added to 8-phenyl-1,4-dioxaspiro[4.5]-decane-8-methanol (0.6 mmol) dissolved in 3 ml CH_2Cl_2 and the mixture stirred 16 hours at ambient temperature. The solution was then treated with triethylamine (0.6 mmol) and 4-dimethylamino pyridine (1 crystal), then stirred 1 hour at ambient temperature. The mixture was then retreated with 3,5-bis(trifluoromethyl)benzoyl chloride (0.32 mmol), then stirred additional 1 hour, and diluted with 10 ml water. The solution was extracted three times with 10 ml CH_2Cl_2 and combined fractions washed with brine, dried with $MgSO_4$, and concentrated. The residue was purified by flash chromatography with silica gel using isohexane/EtOAc, 5:1, and the product isolated in 78% yield as a colorless oil contaminated with about 10% (4-oxo-1-phenylcyclohexyl)-methyl 3,5-bis(trifluoromethyl)benzoate.

   ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.55–1.73 (5H, m), 1.97 (2H, dt, $J = 3.7$, 13.7 Hz), 2.39 (2H, m), 3.89–3.98 (4H, m), 7.23–7.27 (1H, m), 7.35–7.39 (2H, m), 7.45–7.47 (2H, m), 8.02 (1H, s), 8.31 (2H, s)

2. **Preparation of (4-oxo-1-phenylcyclohexyl)methyl 3,5-Bis(trifluoromethyl) benzoate**

   The Step 1 product (0.45 mmol) dissolved in acetone was treated with 2 ml 2 M HCl, then refluxed 30 minutes, and cooled. The solution was then concentrated and the residue treated with saturated Na_2CO_3 solution, then re-extracted three times with 20 ml EtOAc. The combined extractions were washed with brine, dried, concentrated, and the product isolated in 95% yield as a colorless solid.

   ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.04–2.13 (2H, m), 2.37–2.42 (4H, m), 2.66–2.76 (2H, m), 4.37 (2H, s), 7.34 (1H, t, $J = 7.3$Hz), 7.46 (2H, t, $J = 7.7$Hz), 7.53–7.56 (2H, m), 8.04 (1H, s), 8.32 (2H, s)

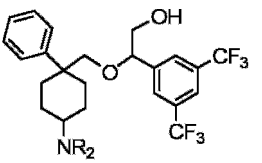
3. **Preparation of 1-([4-[4-fluorophenyl]piperidin-1-yl]-1-phenylcyclohexane)-methyl 3,5-bis(trifluoromethyl)benzoate**

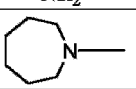
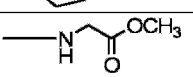
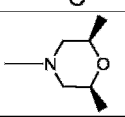
   A mixture of sodium cyanoborohydride (0.11 mmol) and zinc chloride (0.05 mmol) in 2 ml methyl alcohol was added to a solution containing the Step 2 product (0.05 mmol) and 4-(4-fluorophenyl)piperidine (0.05 mmol) dissolved in 2 ml methyl alcohol, then stirred overnight at ambient temperature. The mixture was concentrated and the residue treated with 3 ml saturated $NaHCO_3$ solution and 5 ml CH_2Cl_2. The layers were separated and the organic component poured into an SCX cartridge, then washed four times with 2 ml methyl alcohol. It was then eluted twice with 2 ml 2 M methyl alcohol/ammonia, concentrated, and the product was isolated in 70% yield as a colorless solid.

   (ES$^+$) *m/z* 530 (M + 1)

Derivatives

Table 1 Selected racemic cyclohexyl derivatives and their corresponding mass spectral data for each product. ^{1}H NMR for products provided by author

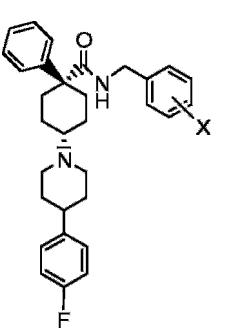


Entry	NR_2	MS (ES+) (M + 1)
69		544
74		534
78		560

Testing

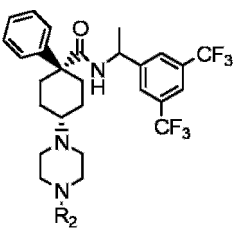
Although testing methods were not described by the author all experimental agents provided in Tables 2 and 3 were found to be active with an IC_{50} at the NK_1 receptor of less than 100 nM.

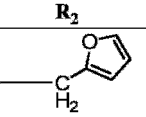
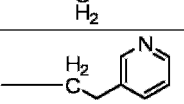
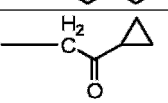
Table 2 Selected 4-(4-fluorophenyl)piperidine-1-yl cyclohexyl derivatives and their corresponding mass spectral data for each product. ^{1}H NMR for products provided by author



Entry	X	MS (ES+) (M + 1)
159	2-Chloro	505
170	4-Trifluoromethyl	534
173	2,4,6-Trimethoxy	561

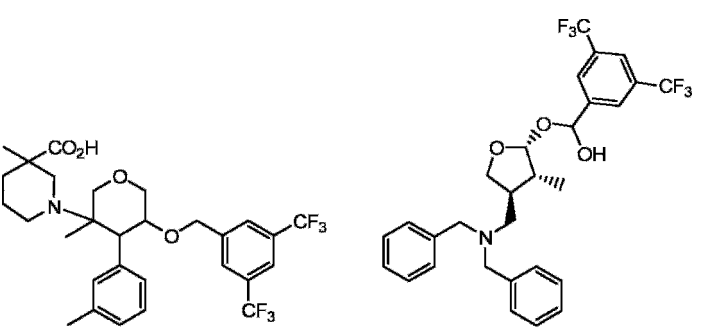
Table 3 Selected 4-(-fluorophenyi)pipeline-1-cyclohexyl derivatives and associated mass spectra for each product. ^{1}H NMR for products provided by author



Entry	R_2	MS (ES+) (M + 1)
230		608
245		619
252		596

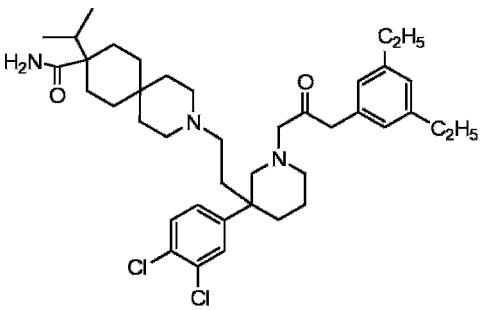
Notes

1. Tetrahydropyran, (**I**), and tetrahydrofuran derivatives, (**II**), prepared by the author (1) and Williams (2), respectively, were effective as NK_1 receptor antagonists and used in treating depression and mood disorders.

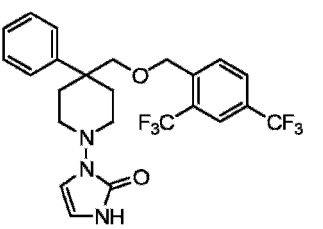


(I) (II)

2. Piperidine derivatives, (**III**) and (**IV**), prepared by Dasseux (3) and Shih (4), respectively, were effective as NK_1 antagonists and were used in treating physiological disorders and symptoms associated with depression and anxiety.

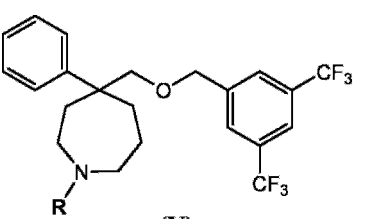


(III)



(IV)

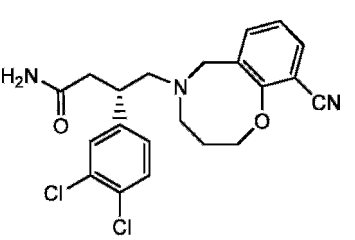
3. 4-Phenylazepan derivatives, (**V**), prepared by Wu (5) were effective as both NK_1 antagonists and selective serotonin reuptake inhibitors and used in treating major depression disorders.



(V)

R = CH_3, C_2H_5, *i*-C_3H_7
$CH_2C_6H_5$

4. 3,4,5,6-Tetrahydro-2H-benzo[b][1,5]oxazocine derivatives, (**VI**), prepared by Bernstein (6) were effective as either NK_1 or NK_2 receptor antagonists and used in treating anxiety disorders.



(VI)

References

1. C. Pineiro *et al*., US Patent 6,906,085 (July 14, 2005) and US Patent 6,489,343 (December 3, 2002)
2. B.J. Williams *et al*., US Patent 6,964,981 (November 15, 2005)
3. J.-L.H. Dasseux *et al*., US Patent 6,951,940 (October 4, 2005)
4. N.-Y. Shih *et al*., US Patent 7,041,682 (May 9, 2006)
5. Y.-J. Wu *et al*., US Patent 7,098,203 (August 29, 2006)
6. P. Bernstein, US Patent 6,924,277 (August 2, 2005)

CHAPTER XXVII

Skin Disorders

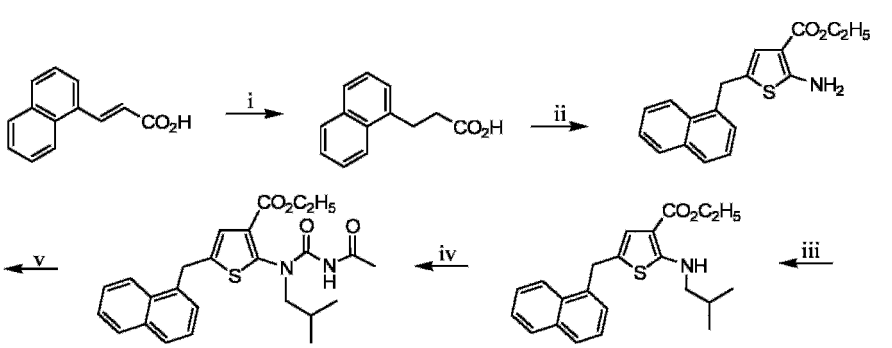
I. Inflammatory Skin Diseases: Interleukin-2 Inhibitors

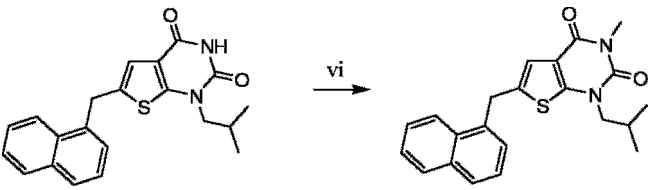
Title Treatment of Skin Disorders Using Thieno[2,3-d]pyrimidinediones
D. Cheshire *et al*., US Patent 6,984,644 (January 10, 2006)
Assignee AstraZeneca AB
Utility Treatment of Inflammatory Skin Diseases

Invention Significance T-cells play an important role in the immune response. In autoimmune diseases, however, T-cells are activated against particular tissues. Interleukin-2 (IL-2) is an essential autocrine growth factor for T-cells and the inhibition of IL-2 transcription can be used to modulate this and related disorders. A method for inhibiting IL-2 transcription using thieno[2,3d]pyrimidine-2,4(1H,3H)-dione derivatives for the treatment of skin-related disorders is described.

Reaction

Eq. 1





i- 10% Palladium on carbon, THF, hydrogen
ii- Oxalyl chloride, CH_2Cl_2, DMF, THF, 10% palladium on carbon, 2,6-lutidine, hydrogen, ethyl cyanoacetate, sulfur
iii- Sodium borohydride, 2-methylpropanoic acid
iv- Acetyl chloride, silver cyanate, toluene
v- Sodium ethoxide, ethyl alcohol
vi- Sodium ethoxide, iodomethane

Experimental

1. **Preparation of 3-(1-naphthyl)propanoic acid**

   Palladium on carbon (10%, 1.00 g) was added to a suspension of 3-(1-naphthyl)acrylic acid (50.0 g) in 500 ml THF and hydrogenated 18 hours at 6 atm of hydrogen. The mixture was then filtered through a kieselguhr pad and the pad washed three times with 10 ml EtOAc. The filtrate was concentrated and 50.0 g product isolated as a solid.

   ^{1}H NMR (DMSO-d_6) δ 2.65 (2H, t); 3.30 (2H, t); 7.37–7.46 (2H, m); 7.49–7.60 (2H, m); 7.79 (1H, d); 7.93 (1H, d); 8.07 (1H, d); 12.10 (1H, s, br)

2. **Preparation of ethyl 2-amino-5-(1-naphthalenylmethyl)-3-thiophenecarboxylate**

   A solution of 7.40 ml oxalyl chloride dissolved in 50 ml CH_2Cl_2 was added dropwise to a suspension of the Step 1 product (8.50 g) in 100 ml CH_2Cl_2 containing 0.1 ml DMF and stirred 2 hours at ambient temperature. The mixture was concentrated and the residual oil dried 4 hours in vacuo at 50°C.

   The oil was dissolved in 45 ml THF and added to a mixture of 10% palladium on carbon (0.50 g) and 5.82 ml 2,6-lutidine in 30 ml THF, then hydrogenated 4 days at 2 atm hydrogen. The mixture was then filtered through a kieselguhr pad, the filtrate concentrated, and a solid isolated. The solid was then dissolved in 20 ml DMF and treated with 4.53 ml ethyl cyanoacetate and sulfur (1.36 g), then stirred 2 hours at 50°C. The mixture was then treated with 300 ml water and 50 ml brine and extracted three times with 300 ml diethyl ether. The extracts were dried with $MgSO_4$ and concentrated. The residue was purified by chromatography with silica using diethyl ether/hexane, 2:3, and 11.00 g product isolated.

   MS (APCI) 312.1 ($(M+H)^+$)
   ^{1}H NMR (DMSO-d_6) δ 1.20 (3H, t); 4.12 (2H, q); 4.35 (2H, s); 6.56 (1H, s); 7.08 (2H, s, br); 7.41–7.56 (4H, m); 7.84 (1H, d); 7.90–7.96 (1H, m); 8.09-8.13 (1H, m)

3. **Preparation of ethyl 2-(2-methylpropyl)amino-5-(1-naphthalenylmethyl)-3-thiophenecarboxylate**

   Sodium borohydride (1.3 g) was added in 10 portions for over 5 hours to the Step 2 product (5.50 g) dissolved in 40 ml 2-methylpropanoic acid at 0°C and the mixture stirred at ambient temperature for 16 hours. Sodium borohydride (1.8 g) was again added in 10 portions over 8 hours and stirring continued for additional 16 hours. The mixture was then poured into 1000 ml water and neutralized with $NaHCO_3$. The mixture was extracted twice with 500 ml EtOAc and extracts were dried with $MgSO_4$, then concentrated. The residue was purified by chromatography using diethyl ether/hexane, 1:3, and 6.20 g product isolated, mp = 57 − 59°C.

   ^{1}H NMR (DMSO-d_6) δ 0.86 (6H, d); 1.22 (3H, t); 1.66–1.92 (1H, m); 2.91 (2H, dd); 4.14 (2H, q); 4.40 (2H, s); 6.70 (1H, s); 7.43–7.57 (4H, m); 7.84 (1H, dd); 7.92–7.95 (1H, m); 8.11–8.14 (1H, m)

4. **Preparation of *N′*-acetyl-*N*-(2-methylpropyl)-*N*-[3-ethoxycarbonyl-5-(1-naphthalenylmethyl)-2-thienyl]urea**

   A stirred suspension of silver cyanate (2.37 g) in 50 ml toluene was treated with 1.08 ml acetyl chloride, then stirred 60 minutes. The mixture was then treated with the Step 3 product (4.646 g) and stirring continued an additional 16 hours. The solution was filtered, the solid residue washed with 50 ml diethyl ether, and extracts concentrated. The residue was purified by chromatography using diethyl ether/hexane, 1:1, and 5.05 g product isolated as an oil.

   MS (APCI) 453.1 ($(M + H)^+$)
   ^{1}H NMR ($CDCl_3$) δ 0.87 (6H, d); 1.29 (3H, t); 1.78–1.92 (1H, m); 2.44 (3H, s); 3.06–3.80 (2H, br); 4.24 (2H, q); 4.53 (2H, s); 7.09 (1H, s); 7.30 (1H, s, br); 7.41–7.58 (4H, m); 7.84 (1H, d); 7.90 (1H, dd); 7.99 (1H, dd)

5. **Preparation of 1-(2-methylpropyl)-6-(1-naphthalenylmethyl)thieno[2,3-d]pyrimidine-2,4(1H,3H)-dione**

   A solution of the Step 4 product (0.20 g) in 4 ml ethyl alcohol was treated with sodium ethoxide (0.036 g), then stirred 3 hours. Additional sodium ethoxide (0.036 g) was added and the mixture stirred an additional 3 hours and was then poured into 20 ml 2 M HCl. The solution was extracted twice with 20 ml EtOAc and extracts dried with $MgSO_4$, then concentrated. The residue was recrystallized using EtOAc/hexane, 1:1, and 0.105 g product isolated, mp = 189 − 190°C.

   MS (APCI) 365.1 ($(M + H)^+$)
   ^{1}H NMR (DMSO-d_6) δ 0.84 (6H, d); 2.02–2.18 (1H, m); 3.57 (2H, d); 4.60 (2H, s); 7.01 (1H, s); 7.48–7.59 (4H, m); 7.87 (1H, dd); 7.95 (1H, dd); 8.16 (1H, dd); 11.34 (1H, s, br)

6. **Preparation of 3-methyl-1-(2-methylpropyl)-6-(1-naphthalenylmethyl) thieno[2,3-d]-pyrimidine-2,4(1H,3H)-dione**

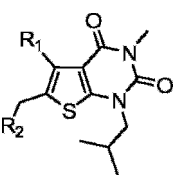
   A solution of the Step 6 product (0.30 g) in 6 ml ethyl alcohol was treated with sodium ethoxide (0.18 g), then stirred 6 hours. The solution was then treated with 0.165 g

iodomethane and stirred an additional 16 hours, then retreated with iodomethane (0.165 g), and stirred an additional 24 hours. The solution was then poured into 20 ml 2 M HCl where it was extracted twice with EtOAc, dried, and concentrated. The residue was purified by chromatography using diethyl ether/hexane, 1:1, triturated with diethyl ether, and 0.24 g product isolated, mp = 137−138°C.

MS (APCI) 379.1 ($(M+H)^+$)
^{1}H NMR ($CDCl_3$) δ 0.93 (6H, d); 2.18–2.32 (1H, m); 3.38 (3H, s); 3.68 (2H, d); 4.52 (2H, s); 7.04 (1H, t); 7.40–7.52 (4H, m); 7.82 (1H, d); 7.86–7.90 (1H, m); 7.95–8.02 (1H, m)

Derivatives

Table 1 Selected thieno[2,3-d]pyrimidine-2,4(1H,3H)-dione derivatives and their corresponding physical data. When evaluated for their effectiveness as IL-2 inhibitors using the human mixed lymphocyte reaction assay, all experimental agents had IC_{50} values less than 1×10^{-6}M



Entry	R_1	R_2	mp (°C)	MS (APCI) (M + H)
6	3-Hydroxylthiopropyl	1-Naphthyl	130–131	469
13	Hydrogen	Phenyl	91–93	315
24	N-(Carboxamido) acetamide	1-Naphthyl	235–236	479
37	(5-Nitropyridine-2-yl)thio	1-Naphthyl	200–201	533
48	N-(2-Hydroxyethyl)urea	1-Naphthyl	227–228	481
51	Hydrogen	1-Hydroxy-1-(3-fluorophenyl) methyl	54	363
59	Hydrogen	1-Hydroxy-1-(6-quinolinyl) methyl	Foam	396
77	Hydrogen	2-Chloro-6-fluorophenyl	122	381
89	*N,N*-Dimethylamide	Phenyl	238	373

Testing

I. *Inhibition of Human Mixed Lymphocyte Reaction*

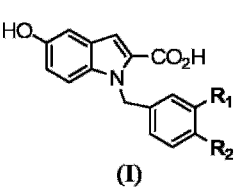
The mixed lymphocyte reaction (MLR) test was performed in 96-well flat-bottomed microtiter plates. Selected experimental agents were prepared as 10 mM stock solution in DMSO and a 50-fold dilution of this was prepared in RPMI. Serial dilutions were prepared from this subsequent solution and 10μl of the diluted stock was added to the well to give concentrations in the assay starting at 9.5μm. In each well was placed 1.5×10^5 donor cells to give a final volume of 0.2 ml RPMI 1640 medium supplemented with 10% human serum, 2 mM L-glutamine, and penicillin/streptomycin. Cells were incubated at 37°C in a humidified atmosphere containing 5% carbon dioxide for 120 hours. ^{3}H-Thymidine (0.5 μCi) was added in the final 6 hours of incubation and cell radioactivity levels determined, which were indicative of T-cell proliferation.

Results

When evaluated for their effectiveness using MLR testing, all experimental agents had IC_{50} values less than 1×10^{-6}M.

Notes

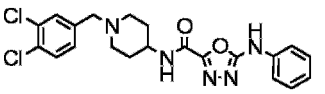
1. Indole derivatives, (**I**), prepared by Faull (1) were effective as immunosuppressant inhibitors and used in treating psoriasis and delayed-type skin hypersensitivity.



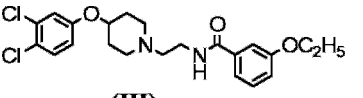
(I)

R_1	R_2
OCH_3	Cl
Cl	OCH_3
Cl	CH_3
NO_2	CH_3
Cl	OCF_3
NO_2	Cl
F	CH_3
HCC-	Cl

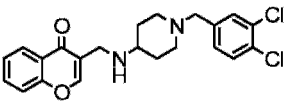
2. Piperidinyl derivatives, (**II**), (**III**), and (**IV**), prepared by Brough (2), Baxter (3), and Thom (4), respectively, were effective in modulating immunologically mediated disorders and used in the treatment of psoriasis, atopical dermatitis, contact dermatitis, and other eczmatous dermatitides.



(II)

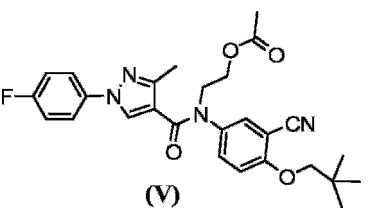


(III)



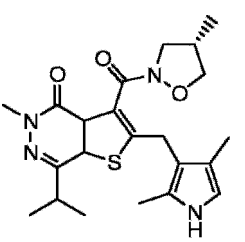
(IV)

3. Methylpyrazole derivatives, (**V**), prepared by Ushio (5) were effective as IL-2, IL-4, IL-7, IL-9, IL-13, or IL-15 inhibitors and used in treating psoriasis and related immune disorders.



(V)

4. Thienopyridazinones derivatives, (**VI**), prepared by Cooper (6) were effective as T-cell proliferation inhibitors and used in treating psoriasis, atopical dermatitis, contact dermatitis, and related disorders.



(VI)

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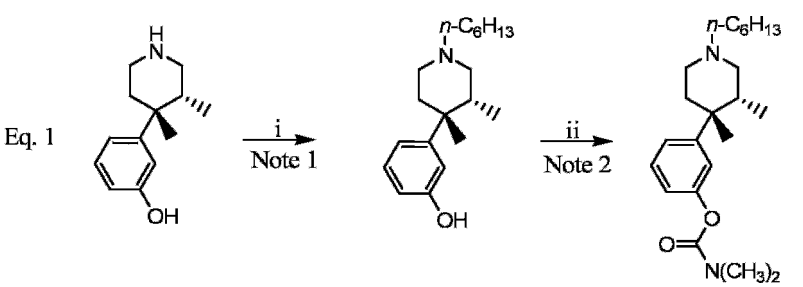
1. A.W. Faull *et al*., US Patent 6,984,657 (January 10, 2006) and US Patent 6,911,465 (June 28, 2006)
2. S. Brough *et al*., US Patent 6,958,350 (October 25, 2005)
3. A. Baxter *et al*., US Patent 6,946,478 (September 20, 2005)
4. S. Thom *et al*., US Patent 6,903,085 (June 7, 2005)
5. H. Ushio *et al*., US Patent 7,015,218 (March 21, 2006)
6. M.E. Cooper *et al*., US Patent 7,064,126 (June 20, 2006)

II. Treatment of Pruritus: Opioid Receptor Antagonists

Title Compounds Useful in Therapy
S.P. Gibson *et al.*, US Patent 7,012,083 (March 14, 2006)
Assignee Pfizer Inc.
Utility Treatment of Pruritus

Invention Significance Itching or pruritus is a dermatological symptom associated with inflammatory skin disease and is usually caused by hypersensitivity reactions induced by insect bites, environmental allergens, or by bacterial and fungal infections. Although symptoms are alleviated by using either corticosteroids or antihistamines, both agents have undesired side effects. The current art addresses the limited treatment options by introducing potent and effective antipruritic agents.

Reaction



i- Sodium bicarbonate, DMF, 1-bromohexane
ii- Dimethylcarbamyl chloride, THF, pyridine, triethylamine

Experimental

1. **Preparation of (±)-1-hexyl-*trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine**

   A solution of (±)-3-(*trans*-3,4-dimethylpiperidinyl)phenol (9.8 mmol) in 50 ml DMF was treated with $NaHCO_3$ (20.95 mmol) and 1-bromohexane (9.9 mmol) then reflux 3 hours. The mixture was diluted with 100 ml water, extracted four times with 50 ml CH_2Cl_2, washed with 100 ml brine, dried with $MgSO_4$, and concentrated. The residue

was purified by chromatography with silica gel using EtOAc/hexane/0.880 ammonia, 30:70:1, and the product isolated in 91% yield as a light brown oil.

^{1}H NMR ($CDCl_3$, selected data from the free base) 0.75 (d, 3H), 0.85 (t, 3H), 1.15–1.25 (m, 6H), 1.3 (s, 3H), 2.0 (m, 1H), 2.35 (m, 4H), 2.6 (m, 2H), 6.55–7.2 (m, 4H)
MS (TSI+) *m/z* [MH^+] 290.2; $C_{19}H_{31}NO + H$ requires 290.3

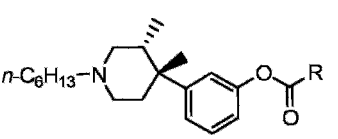
2. **Preparation of (±)-3-(1-hexyl-*trans*-3,4-dimethyl-4-piperidinyl)phenyl dimethylcarbamate**

A mixture of the Step 1 product (1.00 mmol) and dimethylcarbamyl chloride (1.1 mmol) dissolved in 2 ml apiece THF and pyridine was treated with triethylamine (2 mmol) then stirred 24 hours. The mixture was diluted with 20 ml water then extracted three times with 20 ml CH_2Cl_2, washed with 30 ml brine, dried with Na_2SO_4, and concentrated. The residue was purified by chromatography using methyl alcohol/CH_2Cl_2/0.880 ammonia with a gradient of 10:989:1 to 30:967:3 and the product isolated in 91% yield as a yellow oil.

^{1}H NMR (C_6D_6, selected data from the free base) 0.85 (t, 3H), 0.90 (d, 3H), 1.17 (s, 3H), 1.19–1.29 (m, 6H), 1.33–1.43 (m, 3H), 1.78 (m, 1H), 2.05–2.40 (m, 6H), 2.51 (s, 3H), 2.55 (s, 3H), 2.63 (m, 1H), 6.93 (m, 1H), 7.04–7.12 (m, 2H), 7.24 (m, 1H)
MS (APCI+) *m/z* [MH+] 361.3; $C_{22}H_{36}N_2O_2 + H$ requires 361.3

Derivatives

Table 1 Selected (±)-3-(1-hexyl-*trans*-3,4-dimethyl-4-piperidinyl)phenyl derivatives and accompanying mass spectra characterization. ^{1}H NMR data supplied by author



Entry	R	*m/z* [MH^+]
2	$NHCH_3$	347.2
3	$N(C_2H_5)_2$	389.3
4	$C(CH_3)_3$	374.3
6	2-Hydroxylphenyl	410.7
9	2-Aminophenyl	408.7
11	2,2-Diphenylpropyl	498.4

Testing

1. Antipruritic Assay

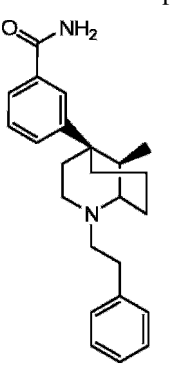
   The compounds of the invention were evaluated as antipruritic agents by measuring their ability to inhibit hind leg scratching induced in rats using a known pruritogenic agent according to the method of Berendsen (1).

   Results

   Although test data were not supplied by the author (±)-3-(1-hexyl-*trans*-3,4-dimethyl-4-piperidinyl)phenyl dimethylcarbamate and -methylcarbamate were especially preferred.

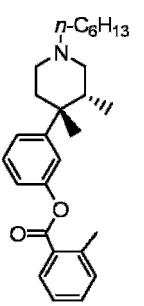
Notes

1. The Step 1 co-reagent, (±)-3-(*trans*-3,4-dimethylpiperidinyl)phenol, is an opioid receptor antagonist which was prepared according to the method Le Bourdonnec (2).
2. Piperidine derivatives, (**I**), effective as opioid receptor antagonists were prepared by Dolle (3) and were effective in the treatment of pruritus.



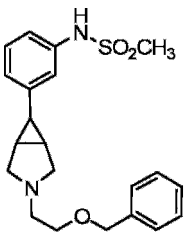
(I)

3. In a separate investigation by the author (4) Step 2 benzoate derivatives, (**II**), were prepared and were also effective as opioid receptor antagonists and used as antipruritic agents.

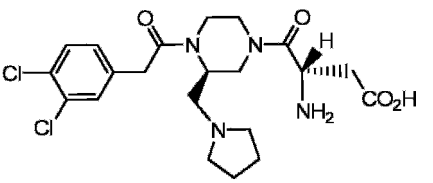


(II)

4. Kappa opiate receptor agonists including 3-azabicyclo[3.1.0]hexane derivatives, (**III**), and piperazine derivatives, (**IV**), prepared by Banks (5) and Kruse (6), respectively, were effective in treating pruritic disorders.

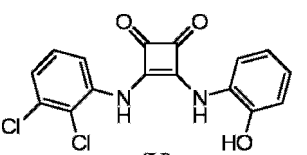


(III)



(IV)

5. Dianilino squarates, (**V**), prepared by Palovich (6) were effective in the treatment of diseases mediated by the chemokine, interleukin-8, and were used in the treating atopic dermatitis resulting in pruritus.



(V)

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1. E. Berendsen *et al*., Eur. J. Pharmacol., 194, 201 (1991)
2. B. Le Bourdonnec *et al*., US Patents 6,992,193 (January 31, 2006) and US Patent 6,992,090 (January 31, 2006)
3. R.E. Dolle *et al*., US Patent 7,087,749 (August 8, 2006)
4. S.P. Gibson *et al*., US Patent 7,012,083 (March 14, 2006)
5. B.J. Banks *et al*., US Patent 7,049,444 (May 23, 2006)
6. L.I. Kruse *et al*., US Patent 6,960,612 (November 1, 2005)
7. M.R. Palovich *et al*., US Patent 7,008,962 (March 7, 2006)

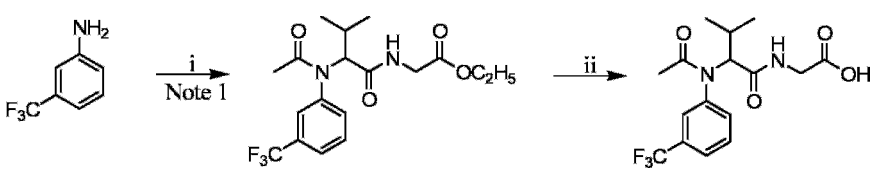
III. Treatment of Skin Aging: Leukocyte Elastase Inhibitor

Title Compounds of the *N*-Acylamino Amide Family, Compositions Comprising Same, and Uses
M. Dalko *et al*., US Patent 6,987,128 (January 17, 2006)
Assignee L'Oreal S.A.
Utility Treatment of Skin Aging

Invention Significance Cutaneous microstresses generated by prolonged exposure to UV or irritants results in accelerated loss of the skin's natural elasticity which causes accelerated skin aging. These environmental stresses also cause collagen fiber degradation resulting in the appearance of flaccid and wrinkled skin. To address these problems chemical agents have been prepared that are effective in controlling enzyme activity of the leukocyte elastase inhibitor associated with chronobiological and photo-induced skin aging.

Reaction

Eq. 1



i- Isobutyraldehyde, methyl alcohol, acetic acid, ethyl isocyanoacetate
ii- Acetone, sodium hydroxide

Experimental

1. **Preparation of ethyl {2-[acetyl(3-trifluoromethyl-phenyl)amino]-3-methylbutyrylamino}-acetate**

   A mixture consisting of 3-trifluoromethylaniline (1.15 equiv.) and 0.63 ml isobutyraldehyde dissolved in 15 ml methyl alcohol was stirred 15 minutes at 20°C, then treated with 0.46 ml of acetic acid, and stirred an additional 10 minutes at 20°C. The mixture was further treated with 0.8 ml 95% ethyl isocyanoacetate (1 equiv.), then

stirred 48 hours at 20°C, and concentrated. The residue was purified by chromatography with silica gel using heptane/ethyl acetate, $R_f = 0.5$, and the product isolated in 91% yield.

^{1}H NMR (200 MHz; $CDCl_3$) δ (ppm) 0.9 (6H, q), 1.3 (3H, t), 1.8 (3H, s), 2.3 (1H, m), 4.0 (2H, q), 4.2 (2H, q), 4.4 (2H, d), 7.3 (1H, t), 7.5 (4H, m)

2. **Preparation of {2-[acetyl(3-trifluoromethylphenyl)amino]-3-methylbutyrylamino}acetic acid**

The Step 1 product (2 g) was dissolved in 30 ml of acetone and treated with 30 ml 2 M NaOH, then stirred 6 hours at 20°C, and concentrated. The residue was acidified to pH 2 with 12 M HCl, then extracted with CH_2Cl_2, dried, and reconcentrated. The residue was then redissolved in aqueous 10% basic ethyl alcohol, reacidified to pH 2 with 12 M HCl, dried with Na_2SO_4, and the product isolated in 70% yield.

^{1}H NMR (200 MHz; DMSO) δ (ppm) 0.9 (6H, q), 3.7 (2H, m), 1.8 (4H, m), 4.8 (2H, d), 7.6 (4H, m), 8.4 (1H, t), 12.5 (1H, s)

Derivatives

Only the Step 2 derivative was prepared.

Testing

I. *In Vitro Antielastase Activity Determination*

Human leukocyte elastase (40 mU/ml) and 0.1% of the experimental agent were applied to the substrate methyl-*O*-succinate alanine alanine proline valine-*p*-nitroanilide, then incubated at 37°C for 60 minutes. Elastase activity was then evaluated by spectrophotometry. Testing results are provided in Tables 1 and 2.

Table 1 The effectiveness of 0.1% experimental agent as a leukocyte elastase inhibitor in comparison to selected elastase-inhibiting agents

Entry	Elastase Activity Inhibition (%)
Experimental agent-1	67
Reference 1[a]	17
Reference 2[b]	20
Reference 3[c]	13

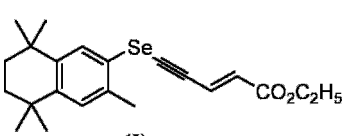
[a] Ethyl 2-{benzyl[(diethoxyphosphoryl)-acetyl]amino}-3-methylbutyrylamino)acetate.
[b] [2-(Acetylbenzylamino)-3-methylbutyrylamino]acetic acid.
[c] Ethyl [2-(acetylbenzylamino)-3-methylbutyrylamino]acetate.

Table 2 The effectiveness of the experimental agent as a leukocyte elastase inhibitor at various concentrations

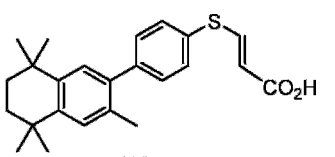
Concentration (%)	Elastase Activity Inhibition (%)
0.01	53
0.05	50
0.1	68
0.2	68

Notes

1. Breton (1) observed that the effectiveness of the experimental agent was enhanced when used in conjunction with metalloproteinase transcriptional inhibitors, Batimastat®, [4-(*N*-hydroxyamino)-2*R*-isobutyl-3*S*-(thiophen-2-yl-thiomethyl)-succinyl]-L-(phenylalanine-*N*-methylamide), or Marimastat®, [2*S*-[N4(*R**),2*R**,3*S*]]-N4[2,2-1-[(methylamino)carbonyl]-propyl]-*N*-1,2-dihydroxy-3-(2-methyl-propyl)butanediamide).
2. Tetrahydronaphthyl derivatives, (**I**) and (**II**), prepared by Diaz (2) and Bernardon (3), respectively, were effective in reducing photo-induced and chronobiological skin aging.

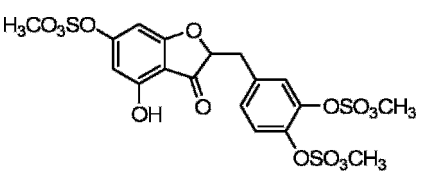


(I)



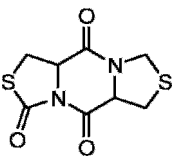
(II)

3. Benzofuranone derivatives, (**III**), prepared by Pflücker (4) were effective in protecting skin against oxidative stress and chronobiological aging.



(III)

4. Hexahydropyrrolo[1,2-a]thiazolo[3,4-d]pyrazine-3,5,10-trione derivatives, (**IV**), prepared by Erdelmeier (5) stimulated the intracellular synthesis of glutathione and were effective as photoprotective skin agents.



(IV)

References

1. L. Breton *et al*., US Patent 6,884,425 (April 26, 2005)
2. P. Diaz *et al*., US Patent 6,921,777 (July 26, 2005)
3. J.-M. Bernardon *et al*., US Patent 6,825,360 (November 30, 2004) and US Patent 6,818,666 (November 14, 2004)
4. F. Pflücker *et al*., US Patent 6,903,134 (June 7, 2005)
5. I. Erdelmeier *et al*., US Patent 7,022,317 (April 4, 2006)

CHAPTER XXVIII

Sleep Disorders

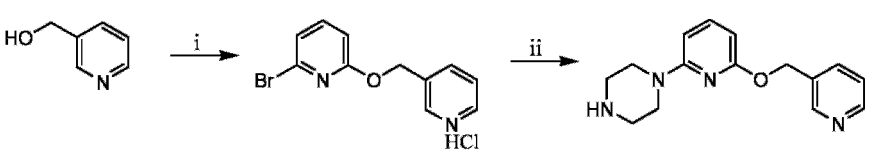
I. Treatment of Circadian Rhythm Disorders Using $5HT_7$ Receptor Agonists

Title 4-(2-Pyridyl) Piperazines Having $5HT_7$ Receptor Agonist Activity
W.M. Welch *et al.*, US Patent 7,009,051 (March 7, 2006)
Assignee Pfizer Inc.
Utility Treatment of Circadian Rhythm-based Sleep Disorders

Invention Significance Seasonal affective disorder, bipolar disorder, jet lag, and sleep deprivation are associated with circadian rhythm sleep disorders. Selective partial agonists at serotonin 7 receptors ($5HT_7$) capable of modulating circadian rhythms have been prepared to treat this disorder.

Reaction

Eq. 1



i- Sodium hydride, THF, 2,6-dibromopyridine, hydrochloric acid
ii- Dioxane, piperazine, sodium iodide, hydrogen chloride

Experimental

1. **Preparation of 2-bromo-6-(pyridin-3-ylmethoxy)-pyridine hydrogen chloride**

   3-Pyridycarbinol (100 mmol) dissolved in 250 ml THF was cooled to $0-5°C$ and oil-free NaH (110 mmol) added portionwise over 30 minutes forming a white suspension. After stirring an additional 15 minutes, 2,6-dibromopyridine (100 mmol) was added and the mixture refluxed overnight. The mixture was cooled to ambient temperature, then poured into ice water, and extracted three times with EtOAc. Combined extracts were washed with brine, dried with $MgSO_4$, and concentrated. A residual yellow oil was isolated, which was dissolved in 500 ml diethyl ether, then treated portionwise with HCl gas dissolved in diethyl ether forming a crystalline solid. The solid was isolated, washed with diethyl ether, air dried, and 12.44 g product isolated. A second crop was obtained by retreating the filtrate with HCl dissolved in diethyl ether solution so that a total of 25.07 g product was isolated, $mp = 171-173°C$.

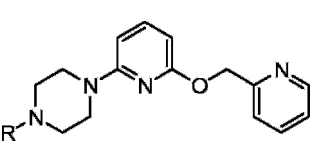
   ^{1}H NMR ($CDC1_3$) δ 5.56 (s, 2H), 6.80 (d, $J = 7$ Hz, 1H), 7.13 (d, $J = 7$ Hz, 1H), 7.50 (t, 1H), 7.94 (m, 1H), 8.48 (d, $J = 8$ Hz, 1H), 8.71 (m, 1H), 8.88 (s, 1H)

2. **Preparation of 1-[6-(pyridin-3-ylmethoxy)-pyridin-2-yl]-piperazine**

   The Step 1 product (94.6 mmol) suspended in 275 ml dry dioxane was treated with piperazine (756.5 mmol) and NaI (94.6 mmol), then refluxed 48 hours. The mixture was cooled, dissolved in cold water, extracted four times with EtOAc, and extracts washed once with water and then with brine. The solution was dried, concentrated, and a pale yellow oily residue was dissolved in 500 ml diethyl ether. The ethereal solution was treated with 75 ml 1 M HCl in diethyl ether and seeded with previously prepared Step 2 product. Product crystals that formed overnight were isolated, washed with diethyl ether, dried in a stream of dry nitrogen, and the product isolated in 75% yield as an off-white solid, $mp = 141-143°C$.

Derivatives

Table 1 Selected 4-(2-pyridyl)-piperazine prepared according to the aforementioned method. Entry 1 is especially preferred. Very limited physical data supplied by author



Entry	R
1	Hydrogen
3	Methyl
5	Benzyl
7	2-Methoxyethyl
10	2-Benzoxyethyl
13	3-Cyanopropyl
25	4-Fluorobenzyl

Testing

$5HT_7$ Receptor agonist activity of selected experimental agents was determined using standardized testing assays including:

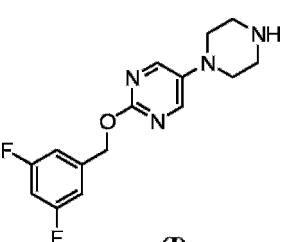
i- radioligand binding assay using HEK-293 cells
ii- $5HT_7$ receptor-mediated adenylate cyclase activity

Results

Although quantitative testing information was not provided by the author, Entry 1 is especially preferred.

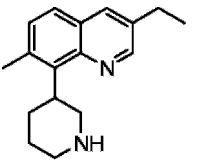
Notes

1. 2-(2,5-Difluoro-benzyloxy)-4-piperazin-1-yl-pyrimidine, (**I**), and related pyrimidine isomers prepared by Chiang (1) were effective as $5\text{-}HT_{2c}$ agonists and used in treating biological sleep rhythm disorders and neurodegenerative regulation diseases.



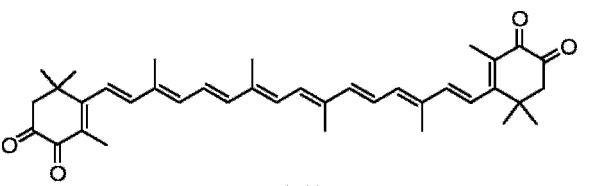
(I)

2. 8-Piperidin-quinoline derivatives, (**II**), prepared by Wlodecki (2) were effective as $5HT_7$ agonists and used in treating circadian rhythm disorders.



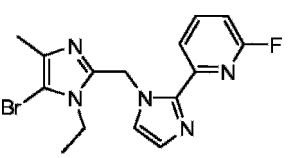
(II)

3. Kiso (3) observed that the liposoluble antioxidant astaxanthin, (**III**), significantly enhanced the circadian rhythm normalizing action of melatonin.



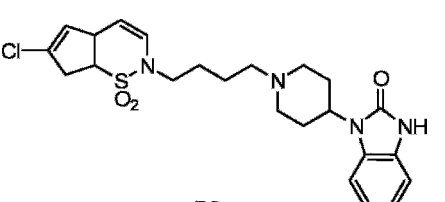
(III)

4. Imidazole derivatives, (**IV**), effective as $GABA_A$ receptor agonists were prepared by Maynard (4) and used in neuropathologies associated with the pattern of wakefulness and sleep.



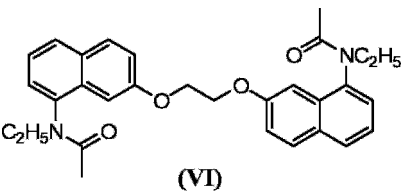
(IV)

5. 6-Chloro-2-[4-[4-(2H-benzimidazo-2-oxo-1-yl)piperidin-1-yl]butyl]-2H-thieno[3,2-e]-1,2-thiazine 1,1-dioxide hydrochloride, (**V**), and related $5HT_7$ receptor agonists prepared by May (5) were effective in the treatment of circadian rhythm disorders.



(V)

6. Dimeric naphthyl derivatives, (**VI**), prepared by Lesieur (6) were effective in treating or in preventing melatoninergic disorders associated with circadian rhythm disorders and sleep disorders.



(VI)

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1. P. Chiang *et al*., US Patent 6,995,159 (February 7, 2006)
2. B. Wlodecki *et al*., US Patent 6,894,062 (May 17, 2005)
3. Y. Kiso *et al*., US Patent 7,001,611 (February 21, 2006)
4. G. Maynard *et al*., US Patent 7,030,144 (April 18, 2006)
5. J.A. May *et al*., US Patent 7,060,704 (June 13, 2006) and US Patent 6,960,579 (November 1, 2005)
6. D. Lesieur *et al*., US Patent 6,635,650 (October 21, 2003)

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CHAPTER XXIX

Thyroid Disorders

I. Nongenomic Thyroid Disorders: T_3 Thyroid Hormone Receptor Agonists

Title Thyronamine Derivatives and Analogs and Methods of use Thereof
T.S. Scanlan *et al*., US Patent 6,979,750 (December 27, 2005)

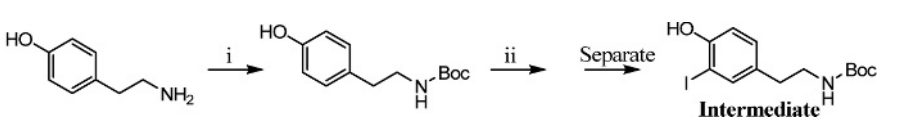
Assignee The Regents of the University of California (Oakland, CA) and Oregon Health & Science University (Portland, OR)

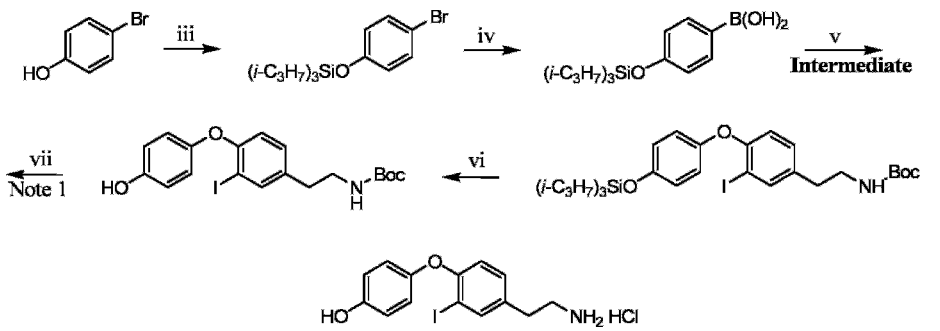
Utility Treatment of Nongenomic Thyroid Disorders

Invention Significance The majority of known biological activities of thyroid hormone are mediated by the binding of 3,5,3′-triiodothyronine (T_3) to thyroid hormone receptors. The physiological effects of thyroid hormone not explainable using this route are referred to as nongenomic. Although the second route is not well understood, it is characterized by a rapid onset in response to hormone and/or insensitivity to the translation inhibitor cyclohexamide. To better understand, modulate, and regulate these nongenomic effects, thyronamine derivatives and thyroid hormone analogs have been prepared.

Reaction

Eq. 1





i- Di-*t*-butyl dicarbonate, THF, water, sodium bicarbonate
ii- CH_2Cl_2, DMF, sodium methoxide, iodine monochloride
iii- *p*-Bromophenol, CH_2Cl_2, triisopropylsilyl chloride, imidazole
iv- *n*-Butyllithium, triisopropyl borate, THF
v- CH_2Cl_2, 4 Å molecular sieves, copper(II) acetate, triethylamine, pyridine
vi- Tetrabutylammonium fluoride, THF
vii- Hydrochloric acid, EtOAc

Experimental

1. **Preparation of *N-t*-Boc-tyramine**

A solution of $NaHCO_3$ (127 mmol) in 250 ml water was added to tyramine (115 mmol) suspended in 500 ml THF containing excess di-*t*-butyl dicarbonate, then stirred 24 hours. The mixture was diluted with diethyl ether and the aqueous phase extracted twice with diethyl ether. Combined extracts were washed with 0.5 M HCl, water, and brine, dried over $MgSO_4$, and concentrated. The yellow oily residue was purified by flash chromatography with silica using hexanes/EtOAc, 3:1, and the product isolated in 91% yield.

^{1}H NMR (400 MHz, chloroform-d) δ 6.99 (d, $J = 8.4$ Hz, 2H), 6.78 (d, $J = 8.4$ Hz, 2H), 4.67 (brd s, 1H), 4.47 (brd s, 1H), 3.32 (brd q, $J = 6.4$ Hz, 2H), 2.69 (t, $J = 6.8$ Hz, 2H), 1.44 (s, 9H)
^{13}C NMR (100 MHz, chloroform-d) δ 156.3, 154.8, 130.2, 129.7, 115.5, 79.7, 42.0, 35.2, 28.4
HRMS (EI+) for $C_{13}H_{19}NO_3$ calc. 237.1365; found 237.1367

2. **Preparation of *N-t*-Boc-3-iodotyramine**

The Step 1 product (63.3 mmol) was dissolved in a mixture of 250 ml CH_2Cl_2 and 60 ml DMF, then cooled to −40°C. The solution was treated first with sodium methoxide (127 mmol) followed by the dropwise addition of iodine monochloride (100 mmol) while maintaining the temperature below −30°C for 30 minutes.

The mixture was then diluted with diethyl ether, washed with 0.5 M HCl, and extracted with diethyl ether. The combined organic layers were washed twice with 0.1 M $Na_2S_2O_3$, water, and brine, and dried. The mixture was concentrated, the residue purified by flash chromatography using CH_2Cl_2/EtOAc, 100:1 to 50:1 to 10:1, and the product isolated in 19% yield.

^{1}H NMR (400 MHz, chloroform-d) δ 7.49 (s, 1H), 7.05 (d, $J = 7.6$ Hz, 1H), 6.91 (d, $J = 8.0$ Hz, 1H), 5.62 (brd s, 1H), 4.56 (brd s, 1H), 3.31 (brd q, $J = 5.6$ Hz, 2H), 2.69 (brd t, $J = 6.8$ Hz, 2H), 1.44 (s, 9H)
^{13}C NMR (100 MHz, chloroform-d) δ 155.9, 153.6, 138.3, 133.0, 130.0, 115.0, 85.5, 79.4, 41.8, 34.8, 28.4
HRMS (EI+) for $C_{13}H_{18}1NO_3$ calc. 363.0331; found 363.0336

3. **Preparation of 1-bromo-4-(tri-isopropyl)silyloxy-benzene**

A solution of *p*-bromophenol (23.1 mmol) dissolved in 40 ml CH_2Cl_2 was treated with triisopropylsilyl chloride (23.4 mmol), then cooled to 0°C, and further treated with imidazole (57.9 mmol) and stirred 30 minutes. The mixture was then warmed to ambient temperature and stirred an additional 12 hours. It was then diluted with diethyl ether, washed twice with 5 M HCl, $NaHCO_3$ solution, water, brine, and dried. The crude product was purified by bulb-to-bulb distillation and the product isolated in 82% as a clear oil, BP = 149−150°C at 2.0 mmHg.

^{1}H NMR (400 MHz, chloroform-d) δ 7.30 (d, $J = 8.8$ Hz, 2H), 6.75 (d, $J = 8.8$ Hz, 2H), 1.24 (septet, $J = 7.2$ Hz, 3H), 1.09 (d, $J = 7.2$ Hz, 18H)
^{13}C NMR (100 MHz, chloroform-d) δ 155.2, 132.2, 121.7, 113.2, 17.8, 12.6
FTIR (thin film) (cm^{-1}) 2945, 2892, 2867, 1586, 1487, 1274, 909, 883, 828, 732
HRMS (EI+) for $C_{15}H_{25}BrOSi$ calc. 328.0858; found 328.0844

4. **Preparation of 4-(triisopropyl)silyloxyphenyl boronic acid**

A solution of the Step 3 product (1.64 mmol) in 15 ml THF at −78°C was treated dropwise with *n*-butyllithium (1.96 mmol), then stirred 30 minutes, and further treated with triisopropyl borate (2.17 mmol) in one portion. The mixture was stirred 1 hour at −78°C and then 4 hours at ambient temperature. It was quenched with 5 ml 3 M HCl, then stirred an additional 30 minutes at 0°C, and the aqueous layer extracted three times with EtOAc. Extracts were combined, dried, and concentrated. The residue was purified using a chromatography column loaded with CH_2Cl_2, then eluted with hexanes/EtOAc, 10:1 to 3:1 to 1:1, and the product isolated in 68% yield.

^{1}H NMR (400 MHz, chloroform-d) δ 8.10 (d, $J = 8.4$ Hz, 2H), 6.98 (d, $J = 8.4$ Hz, 2H), 1.30 (septet, $J = 7.6$ Hz, 3H), 1.13 (d, $J = 7.6$ Hz, 18H)
^{13}C NMR (100 MHz, chloroform-d) δ 160.1, 137.4, 119.6, 17.9, 12.7

5. **Preparation of *N-t*-Boc-4′-triisopopylsilyloxy-3-iodothyronamine**

The Step 4 product (11.3 mmol) and the Step 2 product (5.52 mmol) dissolved in 60 ml CH_2Cl_2 were treated with 4 Å powdered molecular sieves, then stirred 10 minutes, and treated with copper(II) acetate (5.60 mmol), triethylamine (27.3 mmol), and pyridine (27.2 mmol). The mixture was stirred overnight at ambient temperature, then diluted with diethyl ether, and filtered through celite. The filtrate was washed with 0.5 M HCl, water, brine, dried, and concentrated. The residue was purified by chromatography using hexanes/EtOAc, 10:1, and the product isolated in 36% as a slightly yellow oil.

^{1}H NMR (400 MHz, chlorofom-d) δ 7.65 (d, $J = 1.6$ Hz, 1H), 7.05 (app d, $J = 8.0$ Hz, 1H), 6.86 (s, 4H), 6.68 (d, $J = 8.8$ Hz, 1H), 4.57 (brd s, 1H), 3.33 (brd q, $J = 6.5$ Hz, 2H), 2.72 (t, $J = 6.8$ Hz, 2H), 1.44 (s, 9H), 1.25 (septet, $J = 7.2$ Hz, 3H), 1.10 (d, $J = 7.2$ Hz, 18H)
^{13}C NMR (100 MHz, chlorofom-d) δ 156.1, 155.7, 152.3, 150.1, 139.7, 135.3, 129.8, 120.7, 120.1, 117.5, 87.6, 79.2, 41.6, 34.8, 28.3, 17.8, 12.5
FTIR (cm^{-1}) 3360, 2944, 2867, 1704, 1502, 1479, 1366, 1232, 1194, 1171, 910, 883, 734
HRMS (EI+) for $C_{28}H_{421}NO_4Si$ calc. 611.1928; found 611.1917

6. **Preparation of *N-t*-Boc-3-iodothyronamine**

The Step 5 product (1.0 mmol) dissolved in 10 ml THF was treated with 1.5 ml 1 M tetrabutylammonium fluoride, then stirred 30 minutes, and diluted with EtOAc. The mixture was washed with 0.5 M HCl, then extracted with EtOAc, and washed with water, brine, dried, and concentrated. The residue was purified by flash chromatography loaded with CH_2Cl_2, then eluted with hexanes/EtOAc, 5:1 to 3:1, and the product isolated in 85% yield.

^{1}H NMR (400 MHz, chloroform-d) δ 7.64 (d, $J = 2.0$ Hz, 1H), 7.01 (app dd, $J = 8.4$, 2.0 Hz, 1H), 6.87 (app dt, $J = 9.2$, 2.8 Hz, 2H), 6.82 (app dt, $J = 9.2$, 2.8 Hz, 2H), 6.67 (d, $J = 8.4$ Hz, 1H), 6.20 (brd s, 1H), 4.65 (brd s, 1H), 3.33 (brd q, $J = 6.5$ Hz, 2H), 2.71 (brd t, $J = 7.0$ Hz, 2H), 1.45 (s, 9H)
^{13}C NMR (100 MHz, chloroform-d) δ 156.2, 156.1, 152.5, 149.8, 139.7, 135.2, 129.8, 120.4, 117.6, 116.3, 87.8, 79.7, 41.8, 34.9, 28.4
HRMS (EI+) for $C_{19}H_{221}NO_4$ $[M + H - C_4H_9]$ calc. 398.9968; found 398.9950

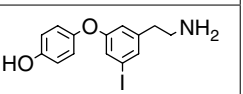
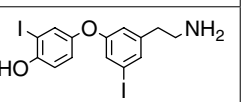
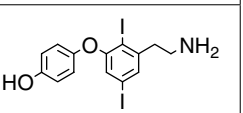
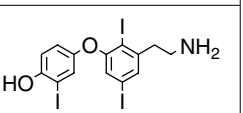
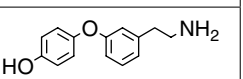
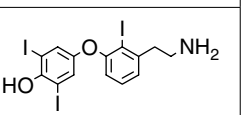
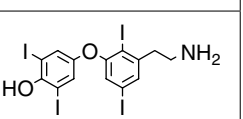
7. **Preparation of 3-iodothyronamine hydrochloride**

The Step 6 product (0.054 mmol) was dissolved in 2 ml 3 M HCl solution, then stirred 15 hours at ambient temperature, and a white precipitate isolated. An additional 2 ml HCl was added, the reaction mixture stirred overnight, and a total product yield of 93% isolated by filtration.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.44 (s, 1H), 8.12 (brd s, 3H), 7.76 (s, 1H), 7.20 (d, $J = 8.0$ Hz, 1H), 6.79 (s, 4H), 6.68 (d, $J = 8.4$ Hz, 1H), 2.98 (app brd q, $J = 7.2$ Hz, 2H), 2.84 (t, $J = 7.4$ Hz, 2H)
HRMS (EI+) for $C_{14}H_{14}INO_2$ [M−NH_3] calc. 337.9812

Derivatives

Table 1 Selected iodothyronamines derivatives prepared according to the current method. ^{1}H- and ^{13}C NMR data for products and intermediates supplied by author

Symbol	Experimental Agent	Structure
T_1AM	3-Iodothyronamine	
3,3′-T_2AM	3,3′-Diodothyronamine	
T_2AM	3,5-Diodothyronamine	
T_3AM	3,5,3′-Triiodothyronamine	
T_0AM	Thyronamine	
rT_3AM	3,3′,5′-Triodothyronamine	
T_4AM	Thyroxamine	

Testing

I. *Functional Role for Thyronamine Derivatives and Analogs as Signaling Molecules in an In Vitro Rat Trace Amine Receptor Assay*

Selected synthetic iodothyronamines were assayed for their ability to stimulate cAMP accumulation in human embryonic kidney (HEK) cells stably expressing trace amine receptor (rTAR-1) as well as cells transfected with an empty vector. None of

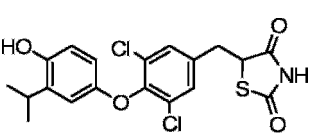
the experimental agents tested had any effect on cAMP accumulation in the cells that received an empty vector. However, several experimental iodothyronamines were found to stimulate cAMP accumulation in the rTAR-1 expressing cells in a dose-dependent fashion suggesting that the iodothyronamines are not promiscuous agonists of catecholamine receptors. The potency index of effective concentration for half-maximal stimulation, EC_{50}, of rTAR-1 was calculated from the dose–response curve for each compound. Testing results are provided in Table 2.

Table 2 Rank order potencies of selected iodothyronamines on the activation of rTAR-1. EC_{50} values between 1 and 45 nM are especially preferred

Entry	Rat TAR EC_{50} (nM)
3-Iodothyronamine (T_1AM)	14
3,3′-Diodothyronamine (3,3′-T_2AM)	41
3,5-Diodothyronamine (T_2AM)	56
3,5,3′-Triiodothyronamine (T_3AM)	87
Thyronamine (T_0AM)	131
3,3′,5′-Triodothyronamine (rT_3AM)	> 1000
Thyroxamine (T_4AM)	> 1000

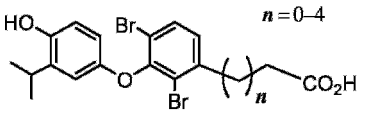
Notes

1. Thyroid receptor agonists consisting of thiazolidine-2,4-dione derivatives, (**I**), were prepared by Chiang (1) and used in the treatment of thyroid diseases including hypothyroidism and thyroid cancer.

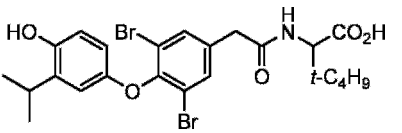


(I)

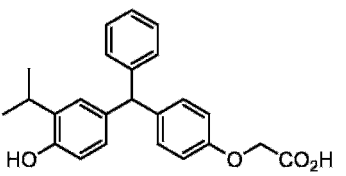
2. Bromine-containing synthetic thyroid agonists, (**II**) and (**III**), prepared by Li (2) and Hangeland (3), respectively, and phenolic carboxylic acid derivatives, (**IV**), prepared by the author (3) were effective for diseases dependent on the expression of T_3-regulated gene and used in treating hypothyroidism.



(II)

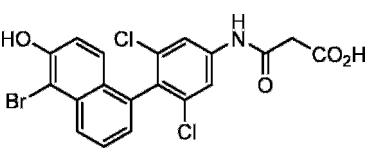


(III)



(IV)

3. Phenyl naphthol derivatives, (**V**), prepared by Hangeland (5) were also effective as thyroid receptor agonists and used in treating hypothyroidism.



(V)

References

1. Y.-C.P. Chiang, US Patent 6,960,604 (November 1, 2005) and US Patent 6,620,830 (September 16, 2003)
2. Y.-N. Li *et al.*, US Patent 6,465,687 (October 15, 2002)
3. J. Hangeland *et al.*, US Patent 6,989,402 (January 24, 2006)
4. T.S. Scanlan *et al.*, US Patent 6,107,517 (April 27, 2000)
5. J. Hangeland *et al.*, US Patent 6,951,844 (October 4, 2005)

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CHAPTER XXX

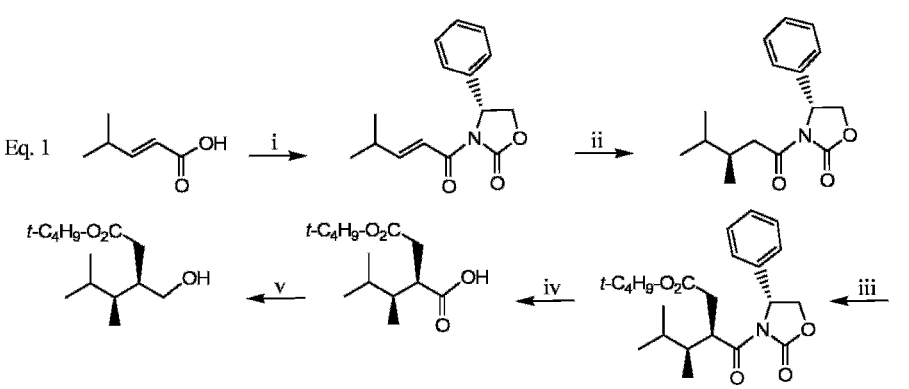
Tinnitus

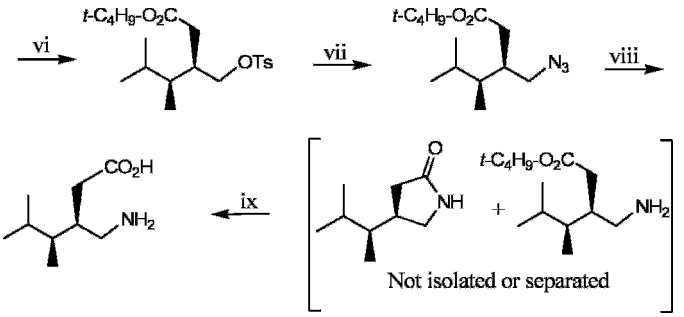
I. Gabapentin Analogs as Antagonists of the α2β Subunit of a Calcium Channel

Title Method of Treating Tinnitus
D.J. Dooley *et al.*, US Patent 7,026,505 (April 11, 2006)
Assignee Warner-Lambert Company
Utility Treatment of Tinnitus

Invention Significance Tinnitus is a medical disorder characterized by the sensation of perceived sound without an external stimulus. Although α2β ligands have been successful in treating this disorder, only a limited number of medicaments are commercially available. The current invention addresses the limited number of treatment options by providing new amino acid treatment agents.

Reaction





i- (*R*)-(–)-4-Phenyl-2-oxazolidinone, trimethylacetyl chloride, triethylamine, lithium chloride, THF
ii- Methyl magnesium chloride, copper(I) bromide–dimethyl sulphide, THF
iii- Sodium bis(trimethylsilyl)amide, *t*-butyl bromoacetate, THF
iv- Lithium hydroxide, hydrogen peroxide, THF, water
v- Borane–methyl sulfide, THF
vi- *p*-Toluenesulfonyl chloride, triethylamine, dimethylaminopyridine, CH_2Cl_2
vii- Sodium azide, DMSO
viii- Raney nickel, methyl alcohol, hydrogen
ix- Hydrochloric acid, Dowex 50WX8 ion exchange resin

Experimental

1. **Preparation of [*R*-(*E*)]-3-(4-methyl-pent-2-enoyl)-4-phenyl-oxazolidin-2-one**

Trimethylacetyl chloride (0.065 mol) was added to 4-methyl-2-pentenoic acid (0.06 mol) and triethylamine (0.187 mol) dissolved in 200 ml THF at −20°C. After 1 hour the mixture was treated with LiCl (0.55 mol) and (*R*)-(–)-4-phenyl-2-oxazolidinone (0.05 mol) and a thick suspension formed, which was stirred 20 hours at ambient temperature. The suspension was filtered and the filtrate concentrated. The residue was recrystallized using hexane/EtOAc, 5:1, and the product isolated in 68% yield as a white solid.

^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5H), 7.18 (dd, 1H, $J = 15.4$, 1.2 Hz), 7.02 (dd, 1H, $J = 15.4$, 6.8 Hz), 5.45 (dd, 1H, $J = 8.8$, 3.9 Hz), 4.68 (t, 1H, $J = 8.8$ Hz), 4.22 (dd, 1H, $J = 8.8$, 3.9 Hz), 2.50 (m, 1H), 1.04 (d, 1H, $J = 1.4$ Hz), 1.02 (d, 1H, $J = 1.4$ Hz)
MS *m/z* (relative intensity) 260 [M + H, 100%]

2. **Preparation of (3*R*,3*R**)-3-(3,4-dimethyl-pentanoyl)-4-phenyl-oxazolidin-2-one**

Methyl magnesium chloride (3 M) in THF was added to copper(I) bromide–dimethyl sulphide dissolved in 45 ml THF at −20°C and after 20 minutes treated dropwise with the Step 1 product (0.014 mol) dissolved in 20 ml THF over 10 minutes. The mixture was stirred 2.5 hours and then quenched with saturated NH_4Cl solution. The layers were separated and the aqueous portion extracted with diethyl ether. The combined organic phases were washed with 1 M HCl, 5% aqueous NH_4OH, dried with $MgSO_4$, concentrated, and the product isolated in 88% yield as a white solid.

^{1}H NMR ($CDCl_3$) δ 7.30 (m, 1H), 5.40 (dd, 1H, $J = 8.8$, 3.7 Hz), 4.63 (t, 1H, $J = 8.8$ Hz), 4.21 (dd, 1H, $J = 8.8$, 3.7 Hz), 2.85 (dd, 1H, $J = 16.1$, 5.6 Hz), 2.8 (dd, 1H, $J = 16.1$, 8.5 Hz), 1.90 (m, 1H), 1.56 (m, 2H), 0.83 (d, 3H, $J = 6.8$ Hz), 0.78 (d, 3H, $J = 6.8$ Hz), 0.75 (d, 3H, $J = 6.8$ Hz)
MS *m/z* (relative intensity) 276 [M + H, 100%]

3. **Preparation of [3*R*-(3*R**(*R**),4*S**)]-4,5-dimethyl-3-(2-oxo-4-phenyl-oxazolidine-3-carbonyl)-hexanoic acid *t*-butyl ester**

Sodium bis(trimethylsilyl)amide (14.4 ml, 1 M) in THF was added to a solution of the Step 2 product dissolved in 35 ml THF at −78°C. After 35 minutes, the mixture was treated with *t*-butyl bromoacetate (0.018 mol) and the solution immediately warmed to −40°C then stirred 3 hours. The mixture was quenched with saturated NH_4Cl solution and the layers were separated. The aqueous phase was extracted with diethyl ether, then dried, and concentrated. The residue was purified by flash chromatography using hexane/EtOAc, 9:1 to 5:1, and the product isolated in 82% yield as a white solid.

^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5H), 5.37 (dd, 1H, $J = 8.4$, 3.1 Hz), 4.67 (t, 1H, $J = 8.7$ Hz), 4.41 (dt, 1H, $J = 12.0$, 3.5 Hz), 4.25 (dd, 1H, $J = 8.68$, 3.1 Hz), 2.65 (dd, 1H, $J = 16.9$, 12.0 Hz), 2.25 (dd, 1H, $J = 16.9$, 3.5 Hz), 1.6 (m, 1H), 1.45 (m, 1H), 1.23 (s, 9H), 1.02 (d, 1H, $J = 6.5$ Hz), 0.93 (d, 1H, $J = 6.7$ Hz), 0.80 (d, 1H, $J = 7.0$ Hz)
MS *m/z* (relative intensity) 429 [M − H + CH_3CN, 100%], 388 [M−H, 20%]

4. **Preparation of (3*R*,4*S*)-2-(1,2-dimethyl-propyl)-succinic acid 4-*t*-butyl ester**

The Step 3 product (9.3 mmol) was dissolved in 54 ml THF and 15 ml water, then treated with 20 ml 0.8 M lithium hydroxide and 5.76 ml 30% aqueous H_2O_2 solution. After stirring 7 hours, the mixture was diluted with water containing sodium bisulfite (10 g) and stirred an additional 30 minutes. The two layers were then separated and the aqueous phase extracted with diethyl ether. The aqueous solution pH was then lowered to 2 with 1 M HCl, then re-extracted with diethyl ether, and extracts combined. They were then dried, concentrated, the residue purified by flash chromatography using hexane/EtOAc, 5:1, and the product isolated in 95% yield as a colorless oil.

^{1}H NMR ($CDCl_3$) δ 3.0 (m, 1H), 2.55 (dd, 1H, $J = 16.6$, 11.2 Hz), 2.27 (dd, 1H, $J = 16.6$, 3.4 Hz), 1.70 (m, 1H), 1.53 (m, 1H), 1.45 (m, 1H), 1.43 (s, 9H), 0.95 (d, 1H, $J = 6.8$ Hz), 0.90 (d, 1H, $J = 6.6$ Hz), 0.83 (d, 1H, $J = 6.8$ Hz)
MS *m/z* (relative intensity) 243 [M−H, 100%]

5. **Preparation of (3*R*,4*S*)-3-hydroxymethyl-4,5-dimethyl-hexanoic acid *t*-butyl ester**

The Step 4 product (8 mmol) dissolved in 20 ml THF at 0°C was treated with 16 ml 2 M borane–methyl sulfide complex in THF, then stirred 20 hours, and quenched with methyl alcohol. The mixture was concentrated, the residue purified as in Step 4, and the product isolated in 70% yield as a colorless oil.

^{1}H-NMR ($CDCl_3$) δ 3.62 (m, 1H), 2.32 (m, 1H), 2.14 (m, 1H), 1.6 (m, 1H), 1.45 (s, 9H), 1.35 (m, 1H), 0.93 (d, 1H, $J = 6.8$ Hz), 0.86 (d, 1H, $J = 6.8$ Hz), 0.77 (d, 1H, $J = 6.9$ Hz)
MS *m/z* (relative intensity) 175 [M−*t*Bu, 100%]

6. **Preparation of (3*R*,4*S*)-4,5-dimethyl-3-(toluene-4-sulfonyloxymethyl)-hexanoic acid *t*-butyl ester**

p-Toluenesulfonyl chloride (4.4 mmol) was added to a solution consisting of the Step 5 product (3.7 mmol), dimethylaminopyridine (0.08 mmol), and triethylamine (8.88 mmol) in 20 ml CH_2Cl_2 at 0°C and the mixture stirred 15 hours at ambient temperature. The solution was washed with 1 M HCl, brine, dried, and concentrated. The residue was purified by flash chromatography using 100–92% hexane/EtOAc, and the product isolated in 86% yield as a thick gum.

^{1}H NMR ($CDCl_3$) δ 7.80 (d, 2H, $J = 8.2$ Hz), 7.25 (d, 2H, $J = 8.2$ Hz), 3.92 (m, 1H), 2.38 (s, 3H), 2.20 (m, 2H), 1.95 (m, 1H), 1.40 (m, 1H), 1.32 (s, 9H), 1.27 (m, 1H), 0.78 (d, 1H, $J = 6.6$ Hz), 0.73(d, 1H, $J = 6.6$ Hz), 0.63(d, 1H, $J = 7.1$ Hz)
MS *m/z* (relative intensity) 311 [85%], 198 [100%], 157 [95%]

7. **Preparation of (3*R*,4*S*)-3-azidomethyl-4,5-dimethyl-hexanoic acid *t*-butyl ester**

A solution of the Step 6 product (3.1 mmol) and sodium azide (6.2 mmol) in 15 ml DMSO was warmed to 60°C for 2.5 hours. The reaction was then diluted with 100 ml water, extracted with diethyl ether, dried, and concentrated. The residue was purified by flash chromatography using hexane/EtOAc, 9:1, and the product isolated in 80% yield as a colorless oil.

^{1}H NMR ($CDCl_3$) δ 3.4 (dd, 1H, $J = 12.21$, 6.11 Hz), 3.3 (dd, 1H, $J = 21.11$, 6.59 Hz), 2.30 (dd, 1H, $J = 15.14$, 3.66 Hz), 2.25 (m, 1H), 2.05 (dd, 1H, $J = 15.14$, 9.04 Hz), 1.55 (m, 1H), 1.45 (s, 9H), 1.35 (m, 1H), 0.95 (d, 1H, $J = 6.59$ Hz), 0.90 (d, 1H, $J = 6.83$ Hz), 0.80 (d, 1H, $J = 7.08$ Hz)
MS *m/z* (relative intensity) 228 [M − N_2, 35%], 172 [M − N_2−*t*Bu, 100%]

8. **Preparation of (3*R*,4*S*)-3-aminomethyl-4,5-dimethyl-hexanoic acid *t*-butyl ester and [4*R*-[4*R**(*S**)]]-4-(1,2-dimethyl-propyl)-pyrrolidin-2-one**

The Step 7 product (2.5 mmol) and Raney nickel (1 g) in 50 ml methanol were shaken 4 hours under an atmosphere of hydrogen, filtered, and concentrated. The residue provided a mixture consisting of the corresponding amine and lactam, which was used without purification.

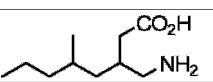
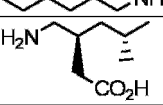
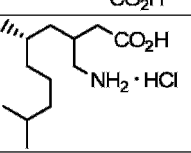
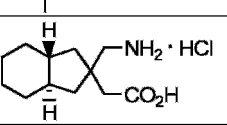
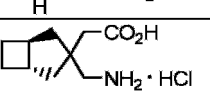
9. **Preparation of (3*R*,4*S*)-3-aminomethyl-4,5-dimethyl-hexanoic acid**

A solution of the Step 8 product mixture (500 mg) dissolved in 3 M HCl was refluxed 9 hours and then stirred 15 hours at ambient temperature. The solution was concentrated and the residue purified by ion exchange chromatography using Dowex 50WX8 strongly acidic resin, oxalate salt formation, then using ion exchange chromatography Dowex 50WX8 strongly acidic resin, and 343 mg product isolated as a white solid.

^{1}H NMR (D_2O) δ 2.87 (m, 2H), 2.22 (dd, 1H, $J = 15.4$, 3.4 Hz), 2.12 (m, 1H), 1.93 (dd, 1H, $J = 15.4$, 9.5 Hz), 1.38 (m, 1H), 1.12 (m, 1H), 0.77 (d, 1H, $J = 6.6$ Hz), 0.74 (d, 1H, $J = 6.6$ Hz), 0.70 (d, 1H, $J = 6.8$ Hz)
MS *m/z* (relative intensity) 174 [M + H, 100%]

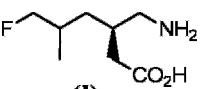
Derivatives

Table 1 Selected experimental amino acids and their corresponding mass spectral characterization data. ^{1}H NMR for products and intermediates supplied by author

Entry	Structure	MS
11		188 [M+H]
20		174 [M+H]
29		230 [Cl^+] (m/z)
35		198 [M+H]
40		184 [M+H]

Testing

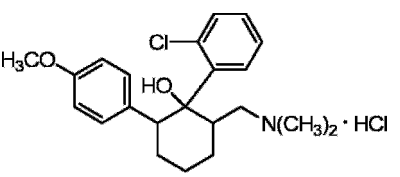
Although testing information was not supplied by the author, (*S*)-3-aminomethyl-6-fluoro-5-methyl-hexanoic acid, (**I**), was especially preferred.



(**I**)

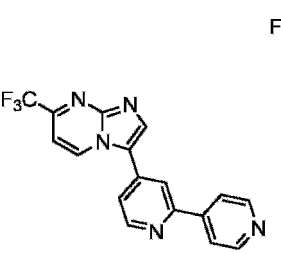
Notes

1. 1-Aminobutan-3-ol derivatives, (**I**), prepared by Buschmann (1), were effective as gabapentin antagonists and used in treating tinnitus.

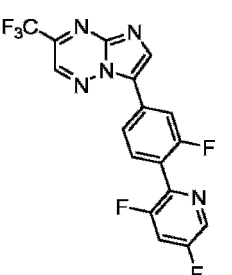


(**I**)

2. $GABA_A\alpha2$, $\alpha3$, or $\alpha5$ receptor antagonists consisting of imidazo[1,2-a]pyrimidine, (**II**), and imidazo[1,2-b]-[1,2,4]triazine, (**III**), prepared by Blackaby (2) and Bettati (3), respectively, were effective in treating tinnitus and other pain-inducing disorders.

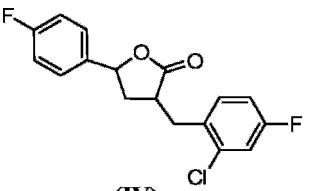


(**II**)

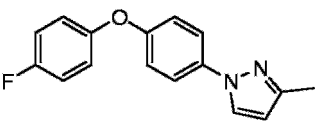


(**III**)

3. γ-Lactones, (**IV**), effective as NMDA antagonists prepared by Sundermann (4) and aryl pyrazole sodium channel modulators, (**V**), prepared by Hogenkamp (5) were effective in treating chronic pain sensations perceived by patients afflicted with tinnitus and related disorders.

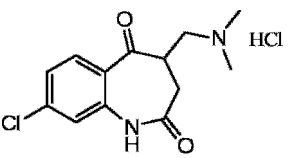


(IV)



(V)

4. Benzo[b]azepin-2-one derivatives, (**VI**), prepared by Sattlegger (6) were effective in treating chronic or neuropathic pain associated with tinnitus and related conditions.



(VI)

References

1. H. Buschmann *et al*., US Patent 7,022,739 (April 4, 2006)
2. W.P. Blackaby *et al*., US Patent 7,030,128 (April 18, 2006)
3. M. Bettati *et al*., US Patent 6,936,608 (August 30, 2005)
4. B. Sundermann *et al*., US Patent 6,956,055 (October 18, 2005)
5. D.J. Hogenkamp *et al*., US Patent 7,078,426 (July 18, 2006)
6. M. Sattlegger *et al*., US Patent 7,041,662 (May 9, 2006)

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Appendix: Patent Assignees

I. Academic

Arizona Board of Regents acting for and on behalf of Arizona State University
Centre National de la Recherche Scientifique
Children, Youth and Women's Health Service Incorporated (North Adelaide, AU)
Commissariat a l'Engergie Atomique
Council of Scientific and Industrial Research (Hyderabad, India)
Emory University
Korea Institute of Science and Technology
Oregon Health & Science University (Portland, OR)
President and Fellows of Harvard College
Southern Research Institute
The Johns Hopkins University
The Regents of the University of California (Oakland, CA)
The University of Georgia Research Foundation, Inc.
The Scripps Research Institute
The Trustees of Columbia University of the City of New York
The Trustees of Dartmounth College
The United States of America as Represented by the Secretary of the Army
University of Tennessee Research Foundation
Yissum Research Development Company of the Hebrew University of Jerusalem

II. Industrial

Alcon, Inc.
Allergan, Inc.
Amgen Inc.
Amrad Operations Pty. Ltd
AstraZeneca AB

Aventis Pharma Deutschland GmbH
Aventis Pharma S.A.
Banyu Pharmaceutical Co., Ltd
Boehringer Ingelheim Pharma GmbH & Co. KG
Bristol Myers Squibb Company
Chiron Corporation
Cylene Pharmaceuticals
3-Dimensional Pharmaceuticals, Inc.
Elan Pharmaceuticals, Inc. and Pharmacia & Upjohn Company
Eli Lilly and Company
Endorecherche, Inc. Pty. Ltd
Fujisawa Pharmaceutical Co., Ltd
Garbil Pharma Investigacion Chile Ltda. (Santago de Chile, CL)
Gilead Sciences, Inc.
Gruenenthal GmbH
Idun Pharmaceuticals, Inc. Hoffman-La Roche Inc.
Kyorin Pharmaceutical Co., Ltd
Laboratorios del Dr. Esteve
L'Oreal S.A.
3M Innovative Properties Co.
Merck Sharp & Dohme Limited
Negma-Lerands
Nobex Corporation
Novartis AG
Oncopeptides AB
Organix, Inc.
Ortho-McNeil Pharmaceutical, Inc.
Oxigene, Inc. and Baylor University
Peptech Limited (AU)
Pfizer, Inc.
Pharmacia Corporation
Pharmasset, Inc.
Pharmacia Italia S.p.A. (Milan, IT) and Pharmacia & Upjohn Company LLC
Plantaceutica, Inc.
Plantaceutica Italia S.p.a.
Prescient Neuropharma Inc.
Renovis, Inc.
Replidyne, Inc.
Research Triangle Institute
Rodaris Pharmaceuticals Limited
Roche Palo Alto LLC
Sanofi-Aventis Deutschland GmbH
SB Corporation

4SC AG
Santen Pharmaceutical Co., Ltd
Schering Corporation
Signal Pharmaceuticals, LLC
SmithKline Beecham Corporation
Sumitomo Manufacturing Company
Sumitomo Pharmaceuticals Co., Ltd
Suntory Limited
Synta Pharmaceuticals Corp.
Targacept, Inc.
Takeda Pharmaceutical Company Limited
Vertex Pharmaceuticals Incorporated
Warner Lambert Company
Wyeth

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