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Industria y Comercio
SUPERINTENDENCIA

Radicación: P2013030 02/13/02
Fecha (H10) 2002-02-15 16:05:45 Folio 24
Trámite 000 PATENTES D 1 REGISTRADO/ 411 PRESENT
Dependencia: 2000 DIVISION DE NUEVAS CREACIONES

FORMULARIO UNICO DE SOLICITUD DE PATENTE
2000-3

1. ☒ Patente de Invención ☐ Patente de Modelo de Utilidad			
2. SOLICITANTE (71)	Nombre: AVENTIS PHARMACEUTICALS INC. Dirección: 300 Somerset Corporate Boulevard, Bridgewater, New Jersey 08807 Teléfono: (908) 231-3800 Fax: (908) 231-2626 E-mail: gander@aventis.com Domicilio: New Jersey, Estados Unidos de América	IDENTIFICACIÓN CC ☐ NIT ☐ CE ☐ Otro ☐ Número: _____	
3. REPRESENTANTE O APODERADO (74)	Nombre: GUSTAVO HOLLMANN RESTREPO Dirección: Cra. 11A No. 94-76 - Of. 308 Teléfono: 6161948 Fax: 6163976 E-mail: ghollmann@hotmail.com	IDENTIFICACIÓN CC ☒ NIT ☐ CE ☐ Otro ☒ Número: 2.878.023 TP 5903	
4. INVENTOR (ES) (72)	Nombre: James A. HENDRIX Dirección: 4 Aspen Drive Hillsborough Teléfono: _____ Fax: _____ E-mail: _____ Domicilio: New Jersey 08876, USA	IDENTIFICACIÓN CC ☐ Otro ☐ CE ☐ Número: _____	
5. Título (54) "NUEVOS DERIVADOS HETEROCICLICOS DE UREA Y SU USO COMO LIGANTES RECEPTORES D3 DE DOPAMINA".			
6. Clasificación Internacional (51) (C.07) A61K 31/16			
7. Prioridad SI ☒ NO ☐	(33) País de Origen Estados Unidos	(32) Fecha Febrero 16, 2001	(31) Número de Solicitud 60/269.654
8. Para publicar a partir de la fecha de la presente solicitud a los 6 meses ☐ 12 meses ☐ 18 meses ☒ Otro ☐			
9. Comprobante de pago No. 02711499 Fecha FEB 15 2002			
instrucciones para el diligenciamiento del presente formulario, ver reverso de esta página			

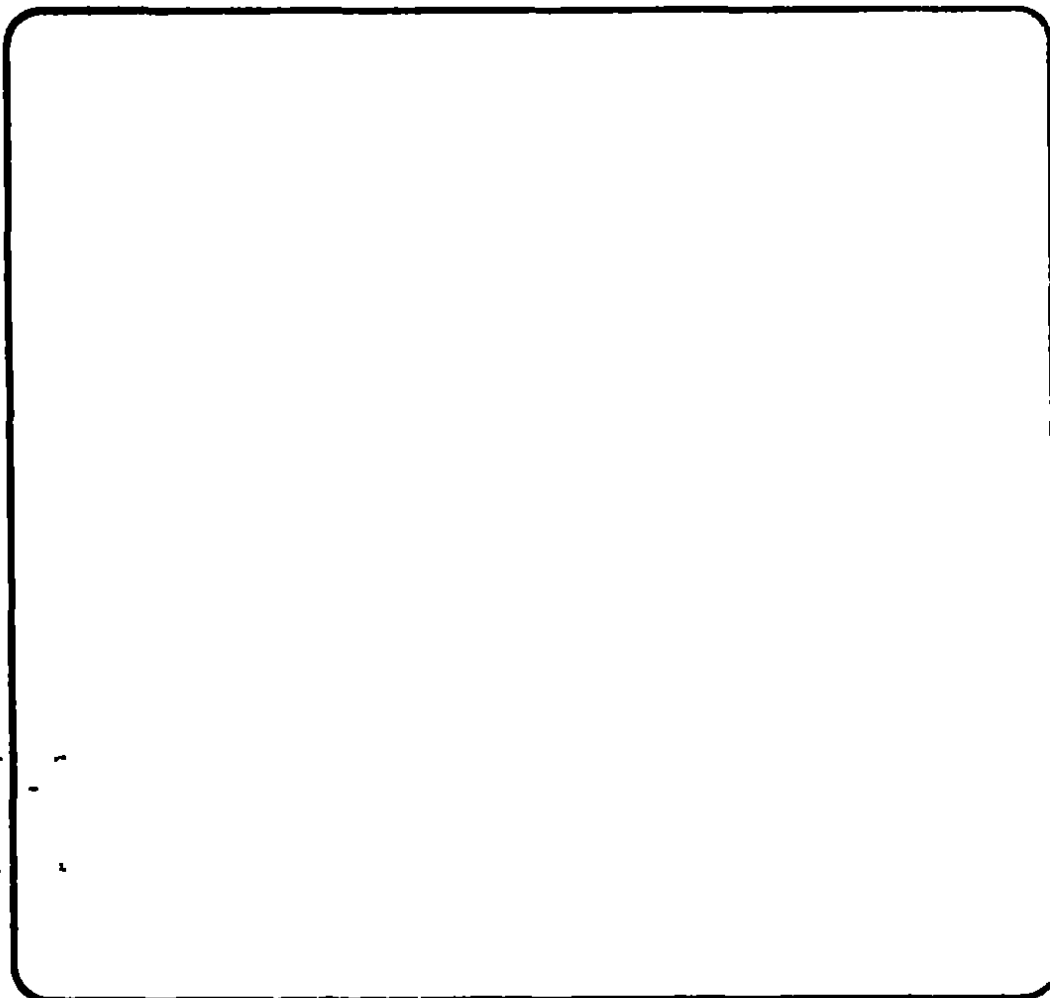
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ANEXOS

- ☒ Comprobante de pago de la tasa de presentación de la solicitud
- ☐ Comprobante de pago de la tasa por concepto de explotación de la invención pública
- ☒ Comprobante de pago por reivindicación de prioridad
- ☐ Certificado de existencia y representación legal cuando el solicitante sea persona jurídica
- ☐ Poderes si fuere el caso
- ☒ Copia certificada de la primera solicitud si se reivindica prioridad
- ☐ Traducción simple de la primera solicitud si se reivindica prioridad
- ☐ Documento de cesión del invento al solicitante si es necesario
- ☒ Descripción de la invención
- ☒ Una o más reivindicaciones
- ☒ Dibujos y/o planos necesarios
- ☐ De ser el caso copia del contrato de acceso
- ☐ De ser el caso documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas
- ☐ De ser el caso certificado de depósito del material biológico
- ☐ Arte final 12x12

11

FIGURA CARACTERÍSTICA



NOMBRE

GUSTAVO HOLLMANN RESTREPO

FIRMA

C.C. No. 2.878.023 Bogotá T.P. 5903

CONTINUACION NOMBRE DE LOS INVENTORES

<u>Inventor</u>	<u>Dirección</u>	<u>Domicilio</u>
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Matthias URMANN	Im Sylvaner 4b	Eschborn 65760 Alemania

Industria y Comercio
SUPERINTENDENCIA

NIT 800174084-2

Radicación: 02013028 000000000
Fecha (ASD): 2002-02-15 16:06:45
Trámite: 382 PATENTES D 1 REGISTRO/ ALL PRESENTA
Dependencia: 2829 DIVISION DE NUEVAS CREACIONES

RECIBO OFICIAL DE CAJA 02 - 11,499
FECHA: FEBRERO 15 DE 2002

CONSIGNACION

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	Nº PAGO	FECHA PAGO	Vr PAGO
JUSTAVO HOLLMAN	CONSIGNACION	BANCO POPULAR	050-00110-6	876727	15/02/2002	1 936 000 00

CONCEPTO

CANT	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01	SOLICITUDES	
		1 TRAMITES DE SOL DE PATENTE DE IN	378 000 00
		TOTAL	\$ 378 000 00

SON TRESCIENTOS SETENTA Y OCHO MIL PESOS

RESPONDE

RECIBO DE CAJA APLICADO AL EXPEDIENTE No

Carrera 13 No 27-00 Pisos 5 7 y 10
Conmutador 382 0840
Fax 350 5220
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Industria y Comercio
SUPERINTENDENCIA

NIT 900174089-2

SUPERINTENDENCIA Y COMERCIO
Radicación 820/3830 00000000 Folio 25
Fecha (AÑO) 2002-02-15 16:06:45
Tramite : 002 PATENTES D 1 REGISTRO/ 411 PRESENTI
Dependencia: 2820 DIVISION DE NUEVAS CREACIONES

RECIBO OFICIAL DE CAJA 02 - 11,495
FECHA FERRERO 1º DE 2002

CONSIGNACION

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JUSTAVO HOLLMAN	CONSIGNACION	BANCO POPULAR	050-00110-6	996727	15/02/2002	1,936,000 00

CONCEPTO

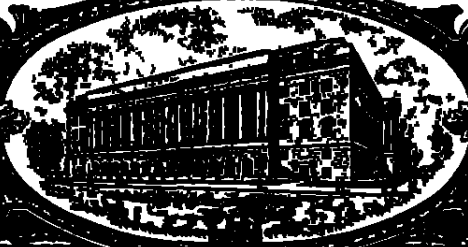
ANT	PERIÓDICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01	SOLICITUDES	
		02 INVOCACION DE UNA PRIORIDAD EN NU	106 000 00
		TOTAL	\$ 106,000 00

SON CIENTO SEIS MIL PESOS

RESPONSABLE

RECIBO DE CAJA APLICADO AL EXPEDIENTE No

Carrera 13 No 27-00 Pisos 5 7 y 10
Conmutador 382 0840
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Bogotá D C Colombia



THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

February 07, 2002

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OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT
APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A
FILING DATE UNDER 35 USC 111

APPLICATION NUMBER 60/269,654

FILING DATE. February 16, 2001

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS



P SWAIN

Certifying Officer

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PA 511221



THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

February 07, 2002

**THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM
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APPLICATION NUMBER 60/269,654

FILING DATE February 16, 2001



**By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS**

P. SWAIN

Certifying Officer

PROVISIONAL APPLICATION COVER SHEET

Certificate of Mailing 37 CFR 1.10

EL62018715545

"Express Mail" mailing number

16 February 2001

Date of Deposit

I hereby certify that this paper (along with any referred to as being attached or enclosed) is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed for Box Provisional Application, Commissioner of Patents and Trademarks, Washington D.C. 20231

Debra L. Wetherill

(Type or print name of person mailing paper)

Debra L. Wetherill

(Signature of person mailing paper)

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(b)(2).¹

Docket Number		A4016		Type a plus sign (+) inside this box	+
INVENTOR(S) / APPLICANT(S)					
LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)		
Hendrix	James	A.	Hillsborough New Jersey		
TITLE OF THE INVENTION (280 characters max) NOVEL HETEROCYCLIC UREA DERIVATIVES AND THEIR USE AS DOPAMINE D ₂ RECEPTOR LIGANDS					
CORRESPONDENCE ADDRESS					
Direct all correspondence to					
☒	Customer Number	005487		Place Customer Number Bar Code Label here	
	Type Customer Number here				
ENCLOSED APPLICATION PARTS (check all that apply)					
☒	Specification	Number of Pages	116		
☐	Drawings	Number of Sheets	Other (specify)		
METHOD OF PAYMENT					
PROVISIONAL FILING FEE AMOUNT(S) \$150.00					
☒	The Commissioner is hereby authorized to charge filing fees and credit L. post# Account No 18-1982 (Duplicate copies enclosed)				
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government					
☒	No				
☐	Yes, the name of the U.S. Government agency and the Government contract number are				

Respectfully submitted

Barbara E. Kurya, Reg. No. 34,650
Attorney/Agent for Applicant

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Patent Department
Route 202-206 / P.O. Box 6800 / Mail Code EMC G1
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X

Additional inventors are being named on separately numbered sheets attached hereto

PROVISIONAL APPLICATION FILING ONLY

Send to Box Provisional Application, Commissioner of Patents and Trademarks, Washington D.C. 20231

¹ If the invention was made by an agency of the United States Government or under a contract with an agency of the United States Government then acknowledge such

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PROVISIONAL APPLICATION COVER SHEET - Additional Page for Inventor Information

			Docket Number	A4018	Type a plus sign (+) inside this box	+
INVENTOR(S) / APPLICANT(S)						
LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)			
Strupczewski	Joseph	T	Flamington, New Jersey			

			Docket Number	A4018	Type a plus sign (+) inside this box	+
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			Docket Number	A4016	Type a plus sign (+) inside this box	+
INVENTOR(S) / APPLICANT(S)						
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			Docket Number	A4016	Type a plus sign (+) inside this box	+
INVENTOR(S) / APPLICANT(S)						
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Hemmerle	Horst		Bad Soden, Germany			

			Docket Number	A4018	Type a plus sign (+) inside this box	+
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LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)			
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			Docket Number		Type a plus sign (+) inside this box	
INVENTOR(S) / APPLICANT(S)						
LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)			

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Citizenship Country German

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Fax One 908-731 2626
Electronic Mail One Barbara Kurys@aventis.com

APPLICATION INFORMATION

Title Line One Novel Heterocyclic Urea Derivatives And
Title Line Two Their Use As Dopamine D3 Receptor Ligand
Title Line Three s
Total Drawing Sheets 0
Formal Drawings? No
Application Type Provisional
Docket Number A4016
Secrecy Order in Parent Appl ? No

Source PrintEPS Version 1 0 1

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 City Bad Soden

5

NOVEL HETEROCYCLIC UREA DERIVATIVES AND THEIR USE AS DOPAMINE D₃ RECEPTOR LIGANDS

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BACKGROUND OF THE INVENTION

The subject invention relates to novel heterocyclic derivatives that selectively bind to the dopamine D₃ receptor. The therapeutic effects of currently available antipsychotic agents (neuroleptics) are generally believed to be exerted via blockade of D₂ receptors, however this mechanism is also thought to be responsible for undesirable extrapyramidal side effects (eps) associated with many neuroleptic agents. Without wishing to be bound by theory, it has been suggested that blockade of the dopamine D₃ receptor may give rise to beneficial antipsychotic activity without significant eps (see for example Sokoloff et al *Nature* 1990 347 146-151 and Schwartz et al *Clinical Neuropharmacology* Vol 16 No 4, 295-314 1993). This receptor is found in high abundance in brain regions associated with emotional and cognitive functions. Compounds that selectively bind to the dopamine D₃ receptor are useful in treating certain central nervous system disorders. These central nervous system disorders include the following indications

25

1) Psychoses (including schizophrenia) -- See for example *Biochem Pharmacol* 1992, 3(4), 859-86, *Clin Neuropharmacol* 1993 16(4), 295-314 *Neuropsychopharmacology* 1997 16(6) 375-84 *Am J Psychiatry*, 1999 156(4) 610-616, *Psychopharmacology (Berl)*, 1995 120(1) 67-74

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2) Substance dependence and substance abuse -- See for example *Neuroreport*, 1997 8(9-10), 2373-2377, *J Pharmacol Exp Ther* 1996 278(3) 1128-37 *Brain Res Mol Brain Res*, 1997 45(2) 335-9

3) Mood Disorders (including mania, depressive disorders and bipolar disorders) – See for example *Clin Neuropharmacol*, 1998 21(3), 176-80, *Am J Med Genet*, 1998 81(2), 192-4 *J Clin Psychiatry*, 1995 56(11) 514-518 *J Clin Psychiatry*, 1995 56(9) 423-429, *Am J Med Genet* 1995 60(3), 234-237 *Pharmacopsychiatry*, 1999 32(4) 127-135, *J Affect Disord* 1999 52(1-3), 275-290, *Am J Psychiatry* 1999 156(4) 610-618

4) dyskinetic disorders (including Parkinson's Disease Parkinsonism Neuroleptic-Induced Tardive Dyskinesia and Gilles de la Tourette Syndrome) – See, for example *Clin Neuropharmacol*, 2000 23(1) 34-44 *Eur J Pharmacol* 1999 385(1), 39-46

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5) sleep disorders (including narcolepsy) - The D₃ agonist pramipexole causes narcolepsy. A D₃ antagonist would be useful for reversing this undesirable side effect. See *Aust Fam Physician* 1999 28(7) 737 *Neurology*, 1999, 52(9), 1906-1910

15 6) anxiety disorders (including obsessive compulsive disorders) – See, for example, *Physiol Behav*, 1997 63(1), 137-141, *J Clin Psychiatry*, 1995 56(9), 423-429 *J Psychiatry Neurosci* 2000 25(2) 185 *J Affect Disord* 1999 58(2-3) 219-226

20 7) nausea - Dopamine antagonists are used alone and in combination with 5HT₃ antagonists. See for example *Support Care Cancer* 1998 6(1) 8-12 *Support Care Cancer*, 2000, 8(3) 233-237 *Eur J Anaesthesiol*, 1999 16(5) 304-307

8) dementia – See for example, *Behav Brain Res*, 2000 109(1), 99-111 *Neuroscience* 1999 88(3) 743-749

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SUMMARY OF THE INVENTION

This invention relates to a class of compounds and pharmaceutically acceptable salts thereof which are selective modulators of dopamine D₃ receptors. The compounds may act as
30 agonists, partial agonists, antagonists or allosteric modulators of dopamine D₃ receptors and are useful for a variety of therapeutic applications.

In another aspect, the invention relates to a method for treating central nervous system disorders associated with the dopamine D₃ receptor activity in a patient in need of such
35 treatment comprising administering to the subject a therapeutically effective amount of a compound described herein for alleviation of such disorder. The central nervous system

conditions or disorders that may be treated with these compounds include Psychotic Disorders Substance Dependence, Substance Abuse, Dyskinetic Disorders (e.g. Parkinson's Disease, Parkinsonism, Neuroleptic-Induced Tardive Dyskinesia, Gilles de la Tourette Syndrome and Huntington's Disease), Nausea, Dementia, Anxiety Disorders, Sleep Disorders, Circadian Rhythm Disorders and Mood Disorders.

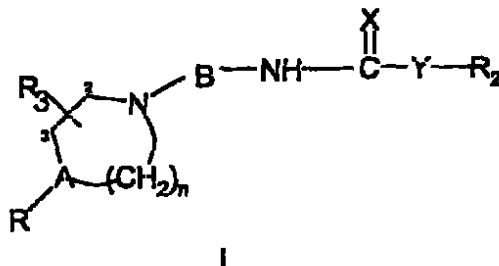
In yet another aspect, the subject invention is directed toward a pharmaceutical composition comprising an effective amount of a compound described herein with a pharmaceutically-acceptable carrier or diluent optionally in conjunction with one or more dopamine D₁, D₂, D₄, D₅ or 5HT receptor antagonists.

In yet another aspect, the subject invention is directed towards processes for the preparation of the class of compounds described herein.

Also within the scope of this invention are methods for using these novel compounds as imaging agents for dopamine D₂ receptors. Methods of using these compounds as imaging agents are presented, as are intermediates and methods for making the imaging agents.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided compounds of formula I:



wherein

A is CH or N

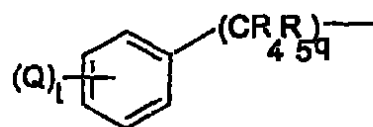
Y is O or NR₁

wherein R₁ is

a) hydrogen,

b) C₁-C₆alkyl, or

c)



wherein

each Q is independently hydrogen, C₁-C₈alkyl, halogen or trifluoromethyl

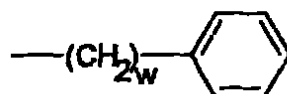
each R₄ and R₅ is independently hydrogen or C₁-C₈alkyl

t is 0 or 1, and

q is 0 or 1,

n is 2 or 3

R₆ is hydrogen, C₁-C₈alkyl or



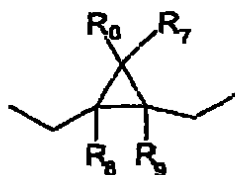
wherein w is 1, 2 or 3

X is O or S

-B- is a group selected from (a) - (e)

(a) -(CR₂₁R₂₂)_m-

(b)



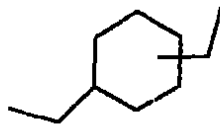
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(d)



(e)

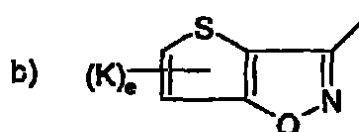
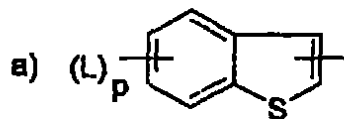


wherein

m is 3 4 5 or 6

each R_{21} and R_{22} is independently hydrogen, C_1 - C_6 alkyl or $(CH_2)_aOH$ wherein a is 0 1 or 2, R_6 , R_7 , R_e and R_g are each independently hydrogen, halogen or C_1 - C_3 alkyl with the proviso that when R_6 is C_1 - C_3 alkyl, R_g is not C_1 - C_3 alkyl

R is a group selected from (a) or (b)



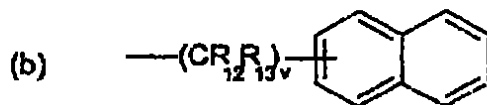
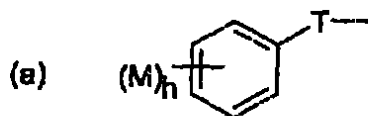
wherein

each L is independently hydrogen C_1 - C_6 alkyl, halogen or trifluoromethyl,K is hydrogen C_1 - C_6 alkyl CHO $(CH_2)_bOH$ $(CH_2)_bOC_1$ - C_6 alkyl or halogen

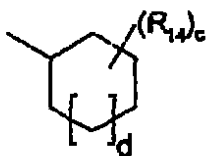
wherein b is 1 or 2

p is 0 1 or 2,

e is 0, 1 or 2

 R_2 is a group selected from (a) - (t)

(c)

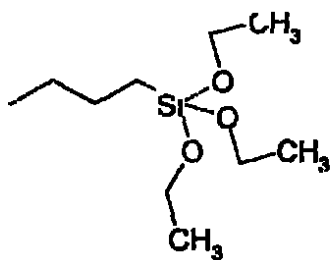


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(d)



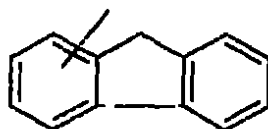
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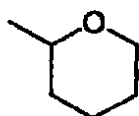
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(f) $-C_1-C_{12}alkyl$ (g) $-(CR_{15}R_{16})_n-CO_2C_1-C_8alkyl$

(h)

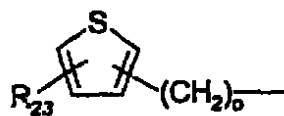


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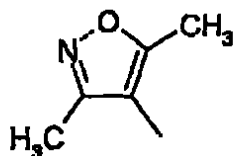


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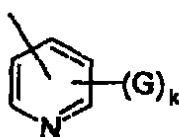
(j)



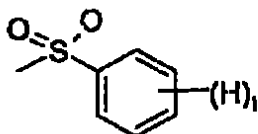
(k)

(l) $-\text{CH}_2\text{CH}_2\text{SO}_2\text{CH}_3$ (m) $-(\text{CR}_{17}\text{R}_{18})_r-\text{OC}_1-\text{C}_2\text{alkyl}$ (n) $-\text{CH}_2\text{CH}_2\text{OPh}$ (o) $-(\text{CR}_{19}\text{R}_{20})_q\text{CZ}_3$ (p) $-\text{CO}_2\text{PI}_1$ (q) $-\text{COCV}_3$

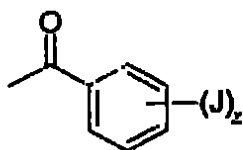
(r)



(s)



(t)



wherein

 $-\text{T}-$ is selected from (i) or (ii)(i) $-(\text{CR}_{10}\text{R}_{11})_u-$

wherein

 u is 0, 1 or 2 andeach R_{10} and R_{11} is independently hydrogen or $\text{C}_1-\text{C}_6\text{alkyl}$

(ii)



each M G H and J is independently selected from

- (1) hydrogen,
- (2) halogen,
- (3) C₁-C₆alkyl
- (4) C₁-C₆alkoxy
- (5) phenoxy
- (6) phenyl,
- (7) trifluoromethyl,
- (8) trifluoromethoxy,
- (9) -CO₂-R₁₀ wherein R₁₀ is hydrogen or C₁-C₆alkyl,
- (10) -COC₁-C₆alkyl,
- (11) hydroxy,
- (12) -(CH₂)₂-OR₂₀ wherein R₂₀ is hydrogen or C₁-C₆alkyl,
- (13) -C(CH₃)₂CH₃
- (14) -NO₂,
- (15) -SCH₃ and
- (16) benzyloxy,

each R₁₂ and R₁₃ is independently hydrogen or C₁-C₆alkyl,

v is 0 or 1

R₁₄ is hydrogen or C₁-C₄alkyl

c is 0 1 or 2

d is 0 or 1

each R₁₆ and R₁₈ is independently hydrogen or C₁-C₆alkyl,

s is 0 1 2 3 4 or 5

o is 1 or 2

each R₁₇ and R₁₉ is independently hydrogen C₁-C₆alkyl or

CO₂C₁-C₂alkyl

r is 2 or 3

each R₁₈ and R₂₀ is independently hydrogen or

C₁ C₂ alkyl,

R₂₃ is hydrogen or C₁-C₄alkyl,

Z is chlorine or fluorine

q is 0 or 1,

V is chlorine or fluorine, and

h, k, l and z are 0 1 2 or 3

The subject invention is directed toward compounds or pharmaceutically acceptable salts of
 5 Formula I as depicted above in either racemic or pure stereoisomeric forms

Terms used herein have the following meanings

a) "Pharmaceutically acceptable salts" means either an acid addition salt or a basic
 addition salt which is compatible with the treatment of patients for the intended use

10

"Pharmaceutically acceptable acid addition salt" is any non-toxic organic or inorganic acid
 addition salt of the base compounds represented by Formula I or any of its intermediates
 Illustrative inorganic acids which form suitable salts include hydrochloric hydrobromic
 sulfuric and phosphoric acid and acid metal salts such as sodium monohydrogen
 15 orthophosphate and potassium hydrogen sulfate Illustrative organic acids which form
 suitable salts include the mono-, di- and tri-carboxylic acids Illustrative of such acids are for
 example, acetic glycolic lactic pyruvic malonic, succinic, glutaric fumaric, malic tartaric
 citric, ascorbic maleic, hydroxymaleic, benzoic hydroxybenzoic, phenylacetic, cinnamic,
 salicylic, 2-phenoxybenzoic, p-toluenesulfonic acid and sulfonic acids such as
 20 methanesulfonic acid and 2-hydroxyethanesulfonic acid Either the mono- or di-acid salts
 can be formed and such salts can exist in either a hydrated solvated or substantially
 anhydrous form In general, the acid addition salts of these compounds are more soluble in
 water and various hydrophilic organic solvents and which in comparison to their free base
 forms generally demonstrate higher melting points

25

"Pharmaceutically acceptable basic addition salts" means non-toxic organic or inorganic
 basic addition salts of the compounds of Formula (I) or any of its intermediates Examples
 are alkali metal or alkaline-earth metal hydroxides such as sodium potassium calcium
 magnesium or barium hydroxides ammonia and aliphatic alicyclic, or aromatic organic
 30 amines such as methylamine, trimethylamine and picoline The selection criteria for the
 appropriate salt will be known to one skilled in the art

b) "Stereoisomers" is a general term for all isomers of the individual molecules that differ
 only in the orientation of their atoms in space It includes mirror image isomers

35 (enantiomers) geometric (cis/trans) isomers and isomers of compounds with more than one

chiral center that are not mirror images of one another (diastereoisomers)

c) "Alkyl" means a branched or straight chain alkyl or alkylene group, as is appropriate to the formula specified by the amount of carbons in the alkyl. e.g., C₁-C₆ alkyl means a one two three four five or six carbon branched or straight chain alkyl or alkylene, as the case may be or any ranges thereof, for example but not limited to C1-2 C1-3 C1-4 C1-5 C2-3, C2-4 C2-5 C2-6 C3-4 C3-5 C3-6 C4-5 C4-6 C5-6, etc

d) "Patient" means a warm blooded animal such as for example rat mice dogs cats guinea pigs, and primates such as humans

e) "Treat" or "treating" means to alleviate symptoms, eliminate the causation of the symptoms either on a temporary or permanent basis or to prevent or slow the appearance of symptoms of the named disorder or condition

f) "Therapeutically effective amount" means a quantity of the compound which is effective in treating the named disorder or condition

g) "Pharmaceutically acceptable carrier" is a non-toxic solvent, dispersant, excipient, adjuvant or other material which is mixed with the active ingredient in order to permit the formation of a pharmaceutical composition i.e. a dosage form capable of administration to the patient. One example of such a carrier is a pharmaceutically acceptable oil typically used for parenteral administration

h) "Psychoses" or "Psychotic Disorders" means conditions wherein the patient experiences a major mental disorder of organic and/or emotional origin characterized by derangement of the personality and loss of contact with reality, often with delusions hallucinations or illusions. Included under the term psychoses are the disorders schizophrenia schizophreniform disorder schizoaffective disorder delusional disorder brief psychotic disorder shared psychotic disorder, psychotic disorder not otherwise specified and substance-induced psychotic disorder as defined by the Diagnostic and Statistical Manual of Mental Disorders 4th ed., published 1994 by the American Psychiatric Association Washington D C USA, incorporated herein by reference

i) "Substance Dependence" means a condition wherein the patient exhibits a maladaptive pattern of substance use leading to clinically significant impairment or distress

There is a pattern of repeated self-administration that usually results in tolerance, withdrawal and compulsive drug-taking

5 j) "Substance Abuse" means a condition wherein the patient exhibits a maladaptive pattern of substance use manifested by recurrent and significant adverse consequences related to the repeated use of substances. There may be repeated failure to fulfill major role obligations, repeated use in situations in which it is physically hazardous, multiple legal problems, and recurrent social and interpersonal problems. Unlike the criteria for Substance Dependence, the criteria for Substance Abuse do not include tolerance, withdrawal, or a
10 pattern of compulsive use and instead only include the harmful consequences of repeated use.

15 k) "Parkinson's Disease" means a slowly progressive neurological condition characterized by tremor, rigidity, bradykinesia, and postural instability. Other manifestations include depression and dementia.

20 l) "Parkinsonism" means a condition where the patient exhibits Parkinsonian signs or symptoms (i.e., tremor, muscular rigidity, or akinesia) that develop in association with the use of neuroleptic medication.

25 m) "Neuroleptic-Induced Tardive Dyskinesia" means a disorder characterized by involuntary movements of the tongue, jaw, trunk, or extremities which have developed in association with the use of neuroleptic medication. The involuntary movements may be choreiform, athetoid, or rhythmic.

30 n) "Gilles de la Tourette Syndrome" means a condition manifested by motor and vocal tics. (A tic is a sudden, rapid, recurrent, nonrhythmic, stereotyped motor movement or vocalization.) The disturbance causes marked distress or significant impairment in social, occupational, or other important areas of functioning. The onset is before age eighteen years and the disturbance is not due to the physiological effects of a substance or general medical condition.

35 o) "Dementia" means disorders characterized by the development of multiple cognitive deficits that include memory impairment and are due to the direct physiological effects of a general medical condition, to the persisting effects of a substance, or to multiple etiologies (e.g., the combined effects of cerebrovascular disease and Alzheimer's disease). Memory

Impairment is required to make the diagnosis of a dementia and is a prominent early symptom. Dementia disorders share a common symptom presentation but are differentiated based on etiology. See Diagnostic and Statistical Manual of Mental Disorders, 4th ed American Psychiatric Association for diagnostic criteria.

p) "Anxiety Disorders" means disorders that include Seasonal Affective Disorder, Panic Disorder Without Agoraphobia, Panic Disorder with Agoraphobia, Agoraphobia Without History of Panic Disorder, Specific Phobia, Social Phobia, Obsessive-Compulsive Disorder, Post-traumatic Stress Disorder, Acute Stress Disorder, Generalized Anxiety Disorder, Anxiety Disorder Due to a General Medical Condition, Substance-Induced Anxiety Disorder, and Anxiety Disorder Not Otherwise Specified, as defined by the Diagnostic and Statistical Manual of Mental Disorders, 4th ed.

c) "Sleep Disorders" means disorders that include Primary Sleep Disorders, Sleep Disorder Related to Another Mental Disorder, Sleep Disorder Due to a General Medical Condition, and Substance-Induced Sleep Disorder as defined by the Diagnostic and Statistical Manual of Mental Disorders, 4th ed. Primary Sleep Disorders are those in which none of the etiologies listed below (i.e., another mental disorder, a general medical condition, or a substance) is responsible. Primary Sleep Disorders are presumed to arise from endogenous abnormalities in sleep-wake generating or timing mechanisms, often complicated by conditioning factors. Primary Sleep Disorders in turn are subdivided into Dyssomnias (characterized by abnormalities in the amount, quality, or timing of sleep) and Parasomnias (characterized by abnormal behavioral or physiological events occurring in association with sleep, specific sleep stages, or sleep-wake transitions). A representative example of a Primary Sleep Disorder is Narcolepsy. Narcolepsy is characterized by repeated irresistible attacks of refreshing sleep, cataplexy, and recurrent intrusions of elements of rapid eye movement (REM) sleep into the transition period between sleep and wakefulness.

r) "Mood Disorders" are disorders that have a disturbance in mood as the predominant feature. As defined by the Diagnostic and Statistical Manual of Mental Disorders, 4th ed, Mood Disorders are divided into the Depressive Disorders ("unipolar depression"), the Bipolar Disorders, and two disorders based on etiology – Mood Disorder Due to a General Medical Condition and Substance-Induced Mood Disorder. The Depressive Disorders (i.e., Major Depressive Disorder, Dysthymic Disorder, and Depressive Disorder Not Otherwise Specified) are distinguished from the Bipolar Disorders by the fact that there is no history of ever having had a Manic, Mixed, or Hypomanic Episode. The Bipolar Disorders (i.e., Bipolar I Disorder

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Bipolar II Disorder, Cyclothymic Disorder, and Bipolar Disorder Not Otherwise Specified) involve the presence (or history) of Manic Episodes, Mixed Episodes, or Hypomanic Episodes, usually accompanied by the presence (or history) of Major Depressive Episodes.

- 5 s) "Circadian Rhythm Disorder" means a persistent or recurrent pattern of sleep disruption leading to excessive sleepiness or insomnia that is due to a mismatch between the sleep-wake schedule required by a person's environment and his or her circadian sleep-wake pattern. The sleep disturbance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning. The disturbance does not occur
10 exclusively during the course of another Sleep Disorder or other mental disorder. The disturbance is not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition.

- Preferred compounds are those wherein X is O, Y is NH and -B- is group (a) or group (b).
15 When B is group (a), m is further preferred to be 4. When -B- is group (b), then R₆, R₇, R₈ or R₉ are further preferred to be hydrogen. R₂ is preferred to be group (a) or group (c). When R₂ is group (a), M is further preferred to be hydrogen, C₁-C₆alkyl, C₁-C₆alkoxy or phenyl and T is further preferred to be (I) wherein u is 0 or 1. When R₂ is group (c), d is further preferred to be 1 and R₁₄ is further preferred to be C₁-C₆alkyl.

- 20 Specific embodiments of the invention include the compounds set forth in Table III.

- Preferred embodiments of the invention are those compounds of Formula I set forth in Table III that exhibit enhanced D3 potency. Particularly preferred compounds include the following

- 25 1-(4-Methoxy-phenyl)-3-{4-[4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl}-urea

- 1-p-Tolyl-3-{4-[4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl}-urea

- 30 1-{4-[4-(2,6-Difluoro-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl}-3-p-tolyl-urea

- 1-{4-[4-(2-Chloro-6-fluoro-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl}-3-(4-chloro-phenyl)-urea

- 35 1-(4-Acetyl-phenyl)-3-[4-(4-thieno[2,3-d]sotazol-3-yl-piperidin-1-yl)-butyl]-urea

1-(4-Ethoxy-phenyl)-3-[4-(4-thieno[2,3-d]isoxazol-3-yl)-piperidin-1-yl]-butyl]-urea

1-(4-Ethoxy-phenyl)-3-[4-[4-(6-fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl]-urea

1-[4-[4-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl]-3-(2-fluoro-phenyl)-urea

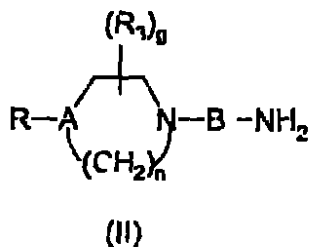
1-[4-[4-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl]-3-(3-trifluoromethyl-phenyl)-urea

1-[4-[4-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl]-3-(4-methoxy-phenyl)-urea

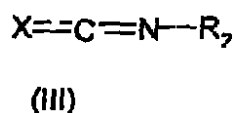
The compounds of the present invention may be prepared by various methods. Schemes I through VI show the different ways of preparing the compounds of Formula I.

The compounds of formula (I) of this invention can be synthesized by following or combining one or more of the steps described below, not necessarily in the order presented. Throughout the description of the synthetic steps, the definitions of R, R₂, R₃, n, X, Y, B and A are as given above unless otherwise stated or indicated, and other nomenclatures appearing below shall have the same meanings defined in their respective first appearances unless otherwise stated or indicated.

Compounds of formula I wherein Y is N may be prepared according to a process which comprises reacting a compound of formula (II)

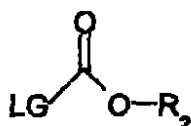


with a compound of formula (III)



Typically, this reaction is carried out in an organic solvent such as, for example, chloroform or tetrahydrofuran at a temperature of about 20 °C to about 25 °C for about 6 to 18 hours

- 5 Compounds of formula I wherein Y is O may be prepared according to a process which comprises reacting a compound of formula (II) with a compound of formula (IV) wherein "LG" is a suitable leaving group selected from bromine, chlorine, iodine or -OR₂ in the instance where formula (IV) is available as an anhydride



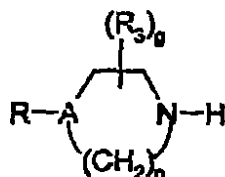
(IV)

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Typically this reaction is carried out in an organic solvent such as, for example, chloroform or tetrahydrofuran in the presence of a weak base such as, for example, amberlite-IRA-67 or triethylamine. The reaction is typically conducted at a temperature of about 20 °C to about 25 °C for about 6 to 18 hours

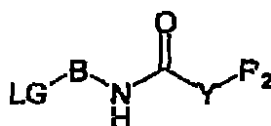
15

Alternatively compounds of formula I may be prepared according to a process which comprises reacting a compound of formula (V)



(V)

- 20 with a compound of formula (VI) wherein "LG" is a suitable leaving selected from chlorine, bromine, iodine, mesyl, tosyl, brosyl, triflyl, nosyl, nonafllyl or tresyl



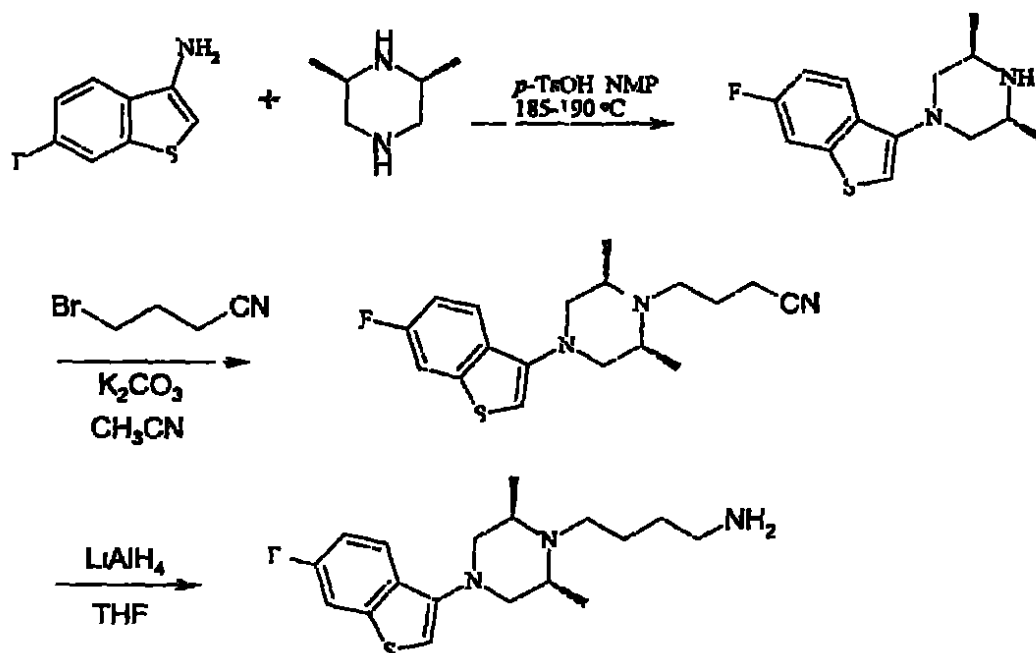
(VI)

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Typically, this reaction is carried out in an aqueous miscible solvent such as for example tetrahydrofuran or acetonitrile, in the presence of water and a base such as, for example, potassium carbonate cesium carbonate, or triethylamine at a temperature of about 50 C to about 75°C for about 12 to 24 hours

The compound of formula (II) may be prepared via synthetic methods well known in the art. The starting materials are either commercially available or readily synthesized via methods known from the literature. For example scheme I describes the coupling of an amino-substituted benzothiophene with a commercially-available substituted piperazine. The synthesis is analogous for the un-substituted piperazine analogs. The less sterically hindered piperazine nitrogen is more reactive and cleanly gives a single product in the benzo[b]thiophene coupling. The more sterically hindered nitrogen can then be alkylated with the appropriate alkylating agent

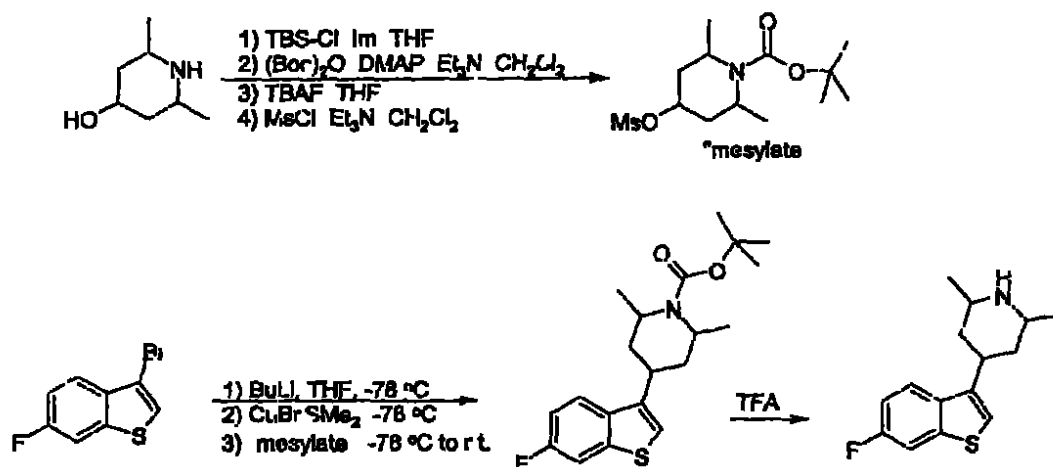
SCHEME I



Piperidine-substituted compounds may be prepared via syntheses analogous to those shown in the following reaction schemes II and III

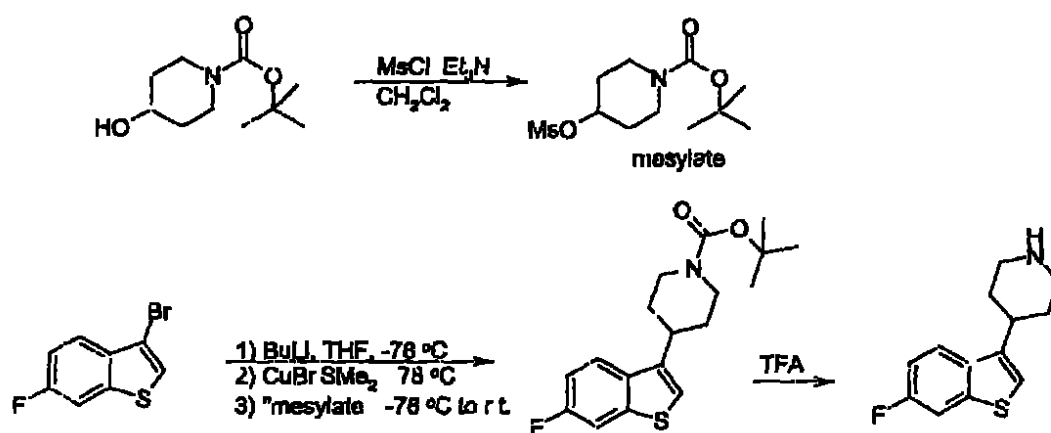
SCHEME II

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SCHEME III

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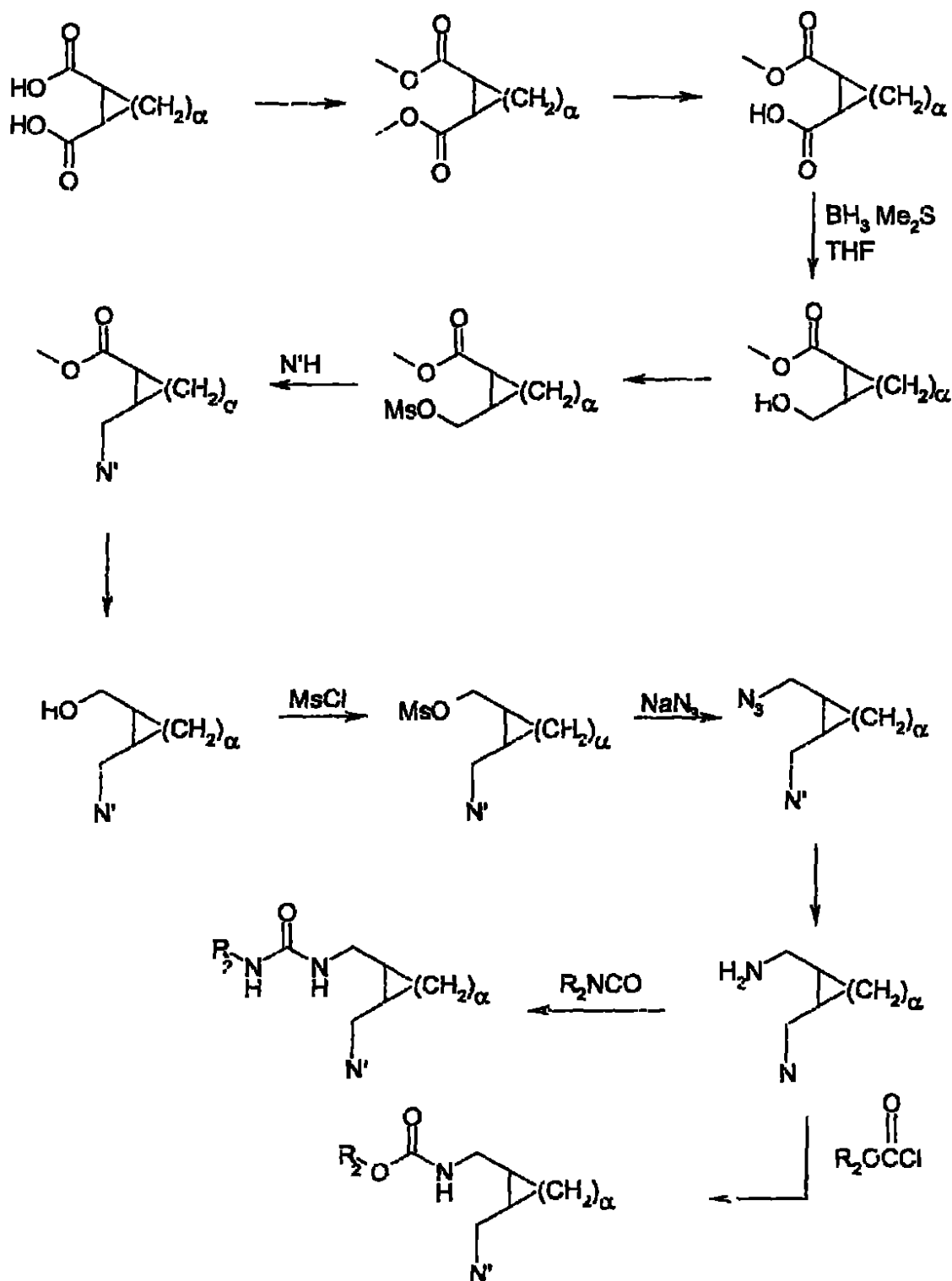
The preparation of various substituted aza- and diazacycloheptanes is described by Treiber et al in WO 9725324

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Compounds of formula I wherein B contains a carbocycle may be prepared as shown in Scheme IV

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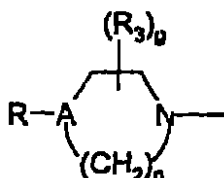
SCHEME IV



wherein R_2 is as hereinbefore defined

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α is 1, 2, 3 or 4, and N - is

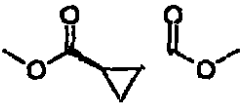
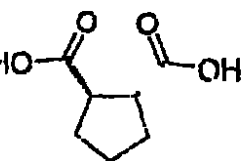
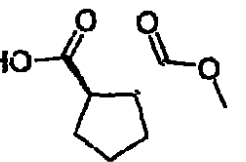
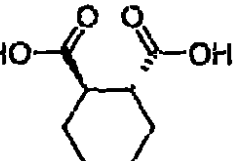
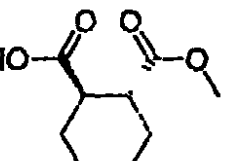


wherein R, A, R_3 , g and n are as hereinbefore defined

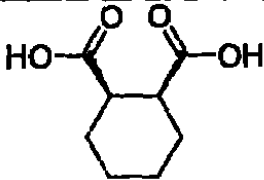
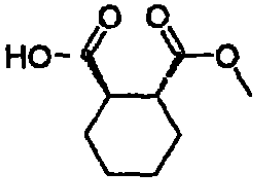
Many of the dicarboxylates or more advanced intermediates that are genencally described in Scheme IV are commercially available. Several of these are shown in Table 1. This table is used for illustrative purposes only and is not intended to limit the scope of the present invention in any way.

29 ²⁸

Table 1 - Starting Materials

Structure	Name	CAS #	Supplier
	Dimethyl cis-1,2-cyclopropane dicarboxylate	826-34-6	Acros
	Dimethyl trans-1,2-cyclopropane dicarboxylate	826-35-7	Acros
	Dimethyl 1-methyl-trans-1,2-cyclopropane dicarboxylate	702-92-1	Acros
	Dimethyl 3-methyl-trans-1,2-cyclopropane dicarboxylate	28363-79-3	Acros
	trans-Cyclobutane-1,2-dicarboxylic acid dimethylester		Syntec
	trans-D L-1,2-Cyclopentane-dicarboxylic acid	1461-97-8	Aldrich
	trans-2-Carbomethoxy cyclopentane-1-carboxylic acid		Reke
	trans-1,2 Cyclohexane dicarboxylic acid	2305-32-0	Aldrich Acros
	trans 2-Carbomethoxy cyclohexane-1-carboxylic acid		Rieke

29
30

Structure	Name	CAS #	Supplier
	cis-1,2-Cyclohexane dicarboxylic acid	610-09-3	Acros
	cis-2-Carbomethoxy cyclohexane-1-carboxylic acid		Rieke

5 When not commercially available, the appropriate starting material may be obtained via standard synthetic methods

When a compound of formula (I) is obtained as a mixture of enantiomers these may be separated by conventional methods such as crystallization in the presence of a resolving agent, or chromatography for example using a chiral HPLC column

10

Compounds of formula (I) have been found to exhibit affinity for dopamine receptors in particular D₂ receptors and are expected to be useful in the treatment of disease states which require modulation of such receptors such as psychotic conditions Preferred

15 compounds of the present invention are those which have higher affinity for dopamine D₂ than dopamine D₁ receptors

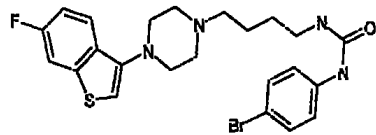
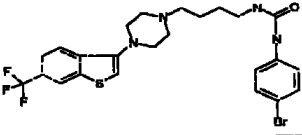
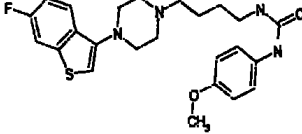
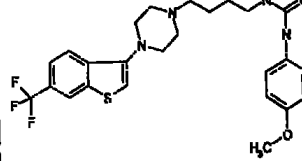
A major challenge in antipsychotic research is to produce agents with reduced side effects Orthostatic hypotension is a common side effect in antipsychotics that is associated with the

20 high potency that these agents have at the alpha-1 adrenergic receptor (hereinafter referred to as "α-1") A major goal of this work was to find agents with reduced α-1 potency

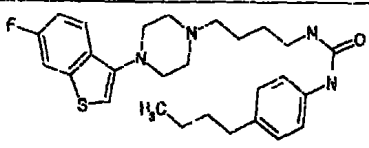
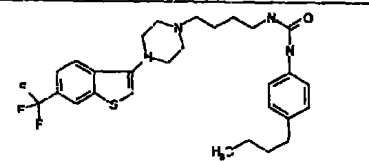
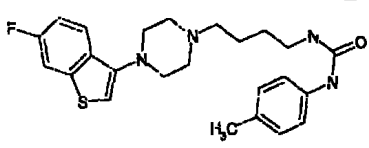
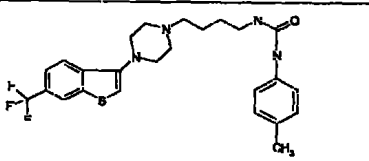
The 6-fluoromethyl benzo[b]thiophenes described herein have a clear and somewhat surprising advantage over the 6-fluoro benzo[b]thiophenes The 6-fluoro benzo[b]thiophenes

25 are clearly more potent at the alpha-1 adrenergic receptor than are the 6-trifluoromethyl benzo[b]thiophenes This is shown by comparing pairs of analogs that only differ in substitution at the 6-position of the benzo[b]thiophene (see Table II) In every case, the 6-fluoro benzo[b]thiophene is more potent than the corresponding 6-trifluoromethyl analog In

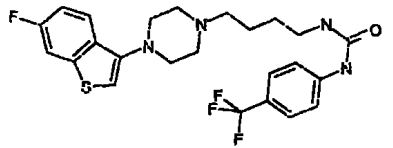
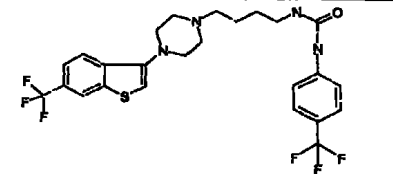
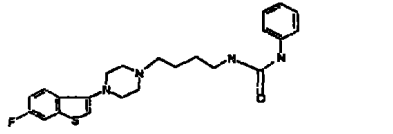
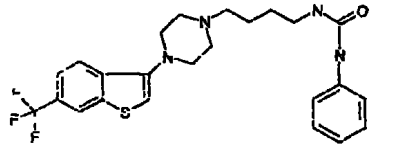
some cases this small structural difference in substitution at the 6-position produces a dramatic change in α -1 potency. There are several examples wherein 6-fluoro analogs exhibited nanomolar potency while the corresponding 6-trifluoromethyl analog exhibited micromolar potency.

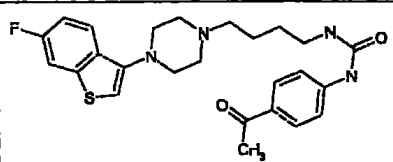
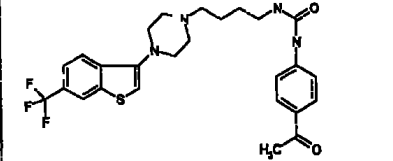
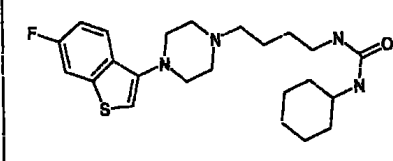
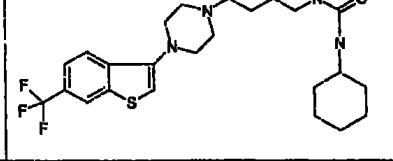
TABLE II			
MDL NUM	MOLSTRUCTURE	halpha1KI (nM)	halpha1 % I
814949		182	
8000		234	
814933		18	
815994		1220	11.9% Inh @ 1 uM

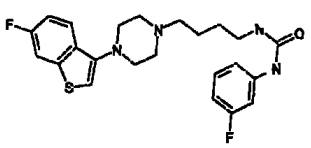
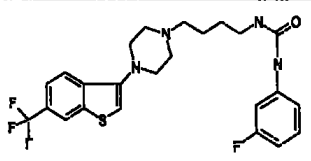
3

814837		532	
815995			42.4% inh @ 10 uM
814941		13.5	
815997		936	23.5% inh @ 1 uM

36

814952		44.6	
816002		435	30.2% inh @ 1 μ M
813581		15	
816005		56.8	

814932		22	
815993		74.9	
814945		45	
816001		83.8	

814953		35	
816003		58.7	

28

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Especially preferred compounds of the instant invention are those with a reduced liability for α -1 receptor binding while at the same time having a higher affinity for dopamine D_3 than dopamine D_2 receptors

- 5 Receptor affinity can be measured using standard methodology such as is described below

[N-Methyl- ^3H]Spiroperidol Binding to Cloned Human Dopamine D_3 Receptors

Purpose

10

This assay measures the *in vitro* activity of compounds on cloned human dopamine (D_3) receptors and predicts the direct dopamine-blocking properties of putative neuropsychiatric agents at human dopamine D_3 receptors

15

Methods

A Cloning

20

The D_3 gene was isolated from a human striatal cDNA library (Stratagene). The gene was sequenced and sub-cloned into the expression vector RC/RSV (Invitrogen). CHO (Chinese Hamster Ovary) cells were stably transfected with 10 μ g of the D_3 /RSV plasmid using the DOTAP method from Boehringer Mannheim and 72 clones that were G418 resistant were isolated. Using mRNA and binding displacement data a single high expressing clone was identified. This clone was then grown in large batches for the purpose of

25

developing a 96 well format assay

B Cell Culture

30

1 One plate (10 cm) with approximately 2-3 million D_3 cells per plate is incubated with 1 ml of Trypsin-EDTA at room temperature for ~2 min or until cells have lifted off plates. Four ml of Ham's F12 + 10% Fetal Bovine Serum + 1% Penicillin/Streptomycin + G418 (400 μ g/ml) medium are added to resuspend cells and 1 ml of this is added to each large plate (15 cm) containing 19 ml of the same medium as mentioned above

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2 The 5 large plates are incubated at 37°C + 5% CO₂ for ~ 3 days or until the cells are confluent.

3 After these plates are confluent, they are split into 10 large plates. Medium is
5 aspirated off. 2 ml of Trypsin-EDTA are added to each plate and plates are incubated at RT for 2 min or until cells have lifted off the plate. Eight ml of the F12 medium (same medium as #1 above) are added to each plate (10 ml total) to resuspend the cells and 5 ml are transferred to the 2 new plates containing 15 ml of the F12 media.

10 4 The 10 large plates are incubated at 37°C + 5% CO₂ for ~ 2 days or until the cells are confluent.

5 The 10 large plates are split into 60 large plates (using Trypsin-EDTA as #3 except 4
15 ml of F12 medium are added to resuspend cells and 1 ml is aliquoted to 6 new plates containing 19 ml of F12 medium each).

6 Plates are incubated at 37°C + 5% CO₂ for ~ 3 days or until cells are confluent.

7 The 60 large plates are then split into 60 roller bottles (100-150 million cells/bottle).
20 Medium is aspirated off. 2 ml of Trypsin-EDTA are added to each plate and incubated at RT for ~2 minutes or until cells have lifted off plates. Eight ml of F12 medium are added to each plate to resuspend cells and the entire 10 ml are added to 1 roller bottle containing 90 ml of the F12 medium.

25 8 The 60 roller bottles are immediately placed on their sides and transferred to the roller bottle incubator. They are incubated at 37°C + 5% CO₂ for ~ 3-5 days. Cells are spun at 30-40% motor speed in the Forma incubator.

9 Medium is poured off and cells are washed 2X in PBS.

30 10 Cells are then scraped off in 20 ml of PBS and the bottles are rinsed again with 5 ml of PBS to remove any remaining cells. Cells are stored on ice before membrane preparation.

11 The yield for 60 D₃ roller bottles has varied from ~ 250-500 mg.

Note All tissue culture reagents are from Gibco-BRL

C Membrane Preparation

The cells are harvested into 250 ml centrifuge tubes with 100 volumes of cold phosphate buffered saline (PBS) and spun down (1200xG for 10 min at 4°C). The medium is removed and 100 ml PBS are added to each centrifuge tube, cells are resuspended and spun down again. The PBS is removed and the final pellet is homogenized in an appropriate volume of 10% DMSO with a polytron on ice at a medium setting.

D Lowry Protein Assay

A 200 µl sample membrane homogenate is added to 200 µl of 1% SDS, vortexed and allowed to stand for 5 min. Aliquots (25, 50 and 100 µl) of this mixture are assayed in duplicate following the standard Bio-Rad DC protein assay protocol (kit catalog number 500-0112) and using reagent S. Absorbance readings are made at 750 nm (note the most accurate protein OD readings are between 0.1-0.5 units). The protein concentration is calculated using a standard curve generated concurrently with bovine serum albumin as standard.

E Storage/Freezing conditions

Following the determination of the protein concentration and Scatchard analysis, the protein is diluted into distilled water with 10% DMSO to the appropriate volume based on expression levels (Bmax). The concentrated protein is then aliquoted into 1.5 ml screw top cap Eppendorf tubes and placed into a -80°C freezer.

F Binding Assay Reagents

1 0.5M Tris Buffer pH 7.7

- a) 44.4 g Tris HCl
26.5 g Tris Base
q.s. to 1 Liter (0.5 M Tris buffer pH 7.7 at 37°C)

- b) make a 1:10 dilution in distilled H₂O (0.05 M Tris buffer pH 7.7)

2 Tns Buffer containing physiological salts

a) Stock buffer

5 NaCl 7.014 g
 KCl 0.372 g
 CaCl₂ 0.222 g
 MgCl₂ 0.204 g
 q.s. To 100 ml with 0.5 M Tns Buffer

b) Dilute 1:10 in distilled H₂O

This yields 0.05 M Tris HCl pH 7.7 containing NaCl (120 mM) KCl (5 mM) CaCl₂ (2 mM) and MgCl₂ (1 mM)

Optional: add 0.1% ascorbic acid and check pH (In assays with compounds that may oxidize)

3 a) 1.0 % polyethylenimine stock in 0.5M Tns (reagent 1 a)

b) Dilute 1:10 in distilled H₂O

20 4 [N-methyl-³H]-Spiropendol (60-80 Ci/mmol) is obtained from New England Nuclear catalog #NET-856

For K_i determinations [³H]NMSP is made up to a concentration of 2.7 nM in buffer 2b such that when 150 µl is added to each tube a final concentration of 0.4 nM is attained in the 1 ml assay. Samples of total CPM added are taken for each experiment to calculate the total ligand concentration.

5 S(-)-Etiopride is obtained from Research Biochemicals International (RBI catalog number E-101). A refrigerated stock (good for up to a month) solution of S(-)-etiopride is made at a concentration of 30 µM in buffer 2b. One hundred microliters are added to 3 wells for the determination of nonspecific binding (this yields a final concentration of 3 µM in the 1 ml assay).

6 Test Compounds

For most assays a 100 μ M stock solution of the test compound is made up in a suitable solvent (usually <0.1% acetic acid) and serially diluted with buffer 2b such that when 100 μ l of drug is combined with the total 1 ml assay final concentrations ranging from 10^{-5} to 10^{-8} M are attained. Characteristically eight concentrations are studied for each assay however higher or lower concentrations may be used depending on the potency of the drug.

G Binding Assay

750 μ l Tissue

150 μ l [^{3} H]NMSP

100 μ l vehicle (for total binding) or 30 μ M (-)-elidopride (for nonspecific binding) or appropriate drug concentration

The 96-Well Packard Unifilters GF/B are incubated for >1 h at 25°C in 0.1% polyethylamine (from 3 b). The cold tissue is added last and mixed on a orbital shaker for a few seconds and is then incubated at 37°C for 30 min in a shaking water bath. The assay is stopped by rapid filtration through Packard Unifilter plates. The filter membranes are then washed with 15 ml of ice-cold 0.05 M Tris buffer. The filters are then dried (~15 min under a heat lamp or incubated for 15 min in a 60°C oven) and a bottom seal is applied. Then 40 μ l of Packard Mirroscint 20 scintillation cocktail is added and a permanent topseal (Type P) is applied and heat sealed. The plates are then shaken on an orbital shaker for 1 h and placed in the Packard Topcount and counted for at least 5 minutes for each point.

Specific binding is defined as the difference between total binding and the binding in the presence of 3 μ M S-(-)-elidopride. Total binding is approximately 10% of the total added ligand. Cheng-Prusoff determination (K_i s) are performed using Pnsm software using a one-site competition curve analysis where the top and the bottom of the non-linear regression are held constant at 0% and 100% percent inhibition. The percent inhibition at each drug concentration is the mean of duplicate determinations.

[N-Methyl ³H]Spiroperidol Binding to Cloned Human Dopamine D₂Long Receptors

Purpose

- 5 This assay measures the *in vitro* activity of drugs on cloned human dopamine D₂Long (D₂L) receptors and predicts the direct dopamine-displacing properties of neuropsychiatric cardiovascular and renal agents at human dopamine D₂ receptors

Methods

10

A Cloning

The D₂L gene was isolated from a human striatal (caudate/putamen) cDNA library. The gene was sequenced and sub-cloned into the expression vector pRC/RSV (Invitrogen).
15 CHO (Chinese Hamster Ovary) cells were stably transfected and 72 clones that were geneticin (G418) resistant were isolated. Using mRNA and binding data a single high expressing cell line was identified (#44). This cell line was then grown in suspension culture for the purpose of developing a 96 well format assay.

20

B Cell Culture Conditions

1 Medium for adherent CHO cultures

Ham's F12 + 10% fetal bovine serum (FBS) + 400 µg/ml geneticin
(G418) + 10 ml/L penicillin-streptomycin (pen-strep)

25

2 Cells are transferred to suspension culture when at least 1.5 million cells are available (this allows for 300,000 cells/ml in a 50 ml spinner flask; this is the ideal suspension density). Cells are removed from flasks with trypsin, spun down (1000xG) and resuspended in fresh medium.

30

50% CHO-SFM II + 50% Ham's F12 w/ 10% FBS (final FBS conc. 5%)
+ 400 µg/ml G418 + pen-strep (10 ml/L)

35

3 After the transfer to suspension culture, growth is monitored and cell viability is assessed using trypan blue exclusion. Total and viable cell count on 5 sectors of the hemocytometer are recorded. When the viable cell density reaches 800,000 cells/ml, the volume is doubled.

4 After 1 week of growth in the 50/50 medium the cells are spun down and transferred to a new spinner flask and replaced with 75% CHO-SFM II / 25% Ham's F12 + 10% FBS plus the pen strep and G418. Thereafter every 3 days the medium is replaced with new medium

5 containing a decreasing amount of FBS as follows

ml of CHO SFM	ml of Ham's F12	Final % FBS conc
87.50	12.5	1.25
93.75	6.25	0.625
99.00	1.00	0.1

10

5 The final maintenance culturing medium is made up as follows

17 A stock mixture of 10 ml of pen strep, 0.5 ml of 400 mg/ml (active final concentration
18 200 mg/ml) G418 and 1 ml of FBS are mixed and filtered and refrigerated. A volume (11.5 ml)
19 of this mixture is added to a freshly opened 1 L bottle of CHO SFM II

15

C Membrane Preparation

21 The cells are harvested into 250 ml centrifuge tubes with 100 volumes of cold
22 phosphate buffered saline (PBS) and spun down (1200xG for 10 min at 4°C). The medium is
23 removed and 100 ml PBS are added to each centrifuge tube. Cells are resuspended and spun
24 down again. The PBS is removed and the final pellet is homogenized in an appropriate volume
25 of PBS with a polytron on ice at a medium setting

20

D Lowry Protein Assay

26 A 200 µl sample membrane homogenate is added to 200 µl of 1% SDS vortexed and
27 allowed to stand for 5 min. Aliquots (25, 50 and 100 µl) of this mixture are assayed in duplicate
28 following the standard Bio Rad DC protein assay protocol (kit catalog number 500-0112) and
29 using reagent S. Absorbance readings are made at 750 nm (note the most accurate protein
30 OD readings are between 0.1-0.5 units). The protein concentration is calculated using a
31 standard curve generated concurrently with bovine serum albumin as standard

30

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E Storage/Freezing conditions

Following the determination of the protein concentration the protein is diluted into distilled water with 10% DMSO to the appropriate volume based on expression levels (Bmax)

- 5 The concentrated protein is aliquoted into 1.5 ml screw top eppendorf tubes and placed into a -80°C freezer

F Binding Assay Reagents

- 10 1 0.5M Tris Buffer pH 7.7

- a) 44.4 g Tris HCl
26.5 g Tris Base
q.s. to 1 Liter (0.5 M Tris buffer pH 7.7 at 37°C)

- 15 b) make a 1:10 dilution in distilled H₂O (0.05 M Tris buffer pH 7.7)

- 2 Tris Buffer containing physiological salts

- 20 a) Stock buffer
NaCl 7.014 g
KCl 0.372 g
CaCl₂ 0.222 g
MgCl₂ 0.204 g
25 q.s. To 100 ml with 0.5 M Tris Buffer

- b) Dilute 1:10 in distilled H₂O

This yields 0.05 M Tris HCl pH 7.7 containing NaCl (120 mM) KCl (5 mM) CaCl₂ (2 mM) and MgCl₂ (1 mM)

- 30 Optional: add 0.1% ascorbic acid and check pH (in assays with compounds that may oxidize)

- 3 a) 1.0% polyethylenimine stock in 0.5M Tris (reagent 1 a)
b) Dilute 1:10 in distilled H₂O

4 [N methyl-³H]-Spiroperidol (60-90 Ci/mmol) is obtained from New England Nuclear catalog #NET 856

For K_i determinations [³H]NMSP is made up to a concentration of 2.7 nM in buffer 2b such that when 150 μl is added to each tube a final concentration of 0.4 nM is attained in the 1 ml assay. Samples of total CPM added are taken for each experiment to calculate the total ligand concentration.

5 S(-)-Etiopride is obtained from Research Biochemicals International (RBI catalog number E-101). A refrigerated stock (good for up to a month) solution of S(-)-etiopride is made at a concentration of 30 μM in buffer 2b. One hundred microliters are added to 3 wells for the determination of nonspecific binding (this yields a final concentration of 3 μM in the 1 ml assay).

15 6 Test Compounds

For most assays a 100 μM stock solution of the test compound is made up in a suitable solvent (usually <0.1% acetic acid) and serially diluted with buffer 2b such that when 100 μl of drug is combined with the total 1 ml assay final concentrations ranging from 10⁻⁵ to 10⁻⁸ M are attained. Characteristically eight concentrations are studied for each assay; however, higher or lower concentrations may be used depending on the potency of the drug.

G Binding Assay

75 750 μl Tissue
150 μl [³H]NMSP
100 μl vehicle (for total binding) or 30 μM (-)-etiopride (for nonspecific binding) or appropriate drug concentration

30 The 96-Well Packard Unifilters GF/B are incubated for >1 h at 25°C in 0.1% polyethylamine (from 3 b). The cold tissue is added last and mixed on a orbital shaker for a few seconds and is then incubated at 37°C for 30 min in a shaking water bath. The assay is stopped by rapid filtration through Packard Unifilter plates. The filter membranes are then washed with 15 ml of ice-cold 0.05 M Tris buffer. The filters are then dried (~15 min under a heat lamp or incubated for 15 min in a 60°C oven) and a bottom seal is applied. Then 40 μl of

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Packard Microscint 20 scintillation cocktail is added and a permanent topseal (Type P) is applied and heat sealed. The plates are then shaken on an orbital shaker for 1 h and placed in the Packard Topcount and counted for at least 5 minutes for each point.

5 Specific binding is defined as the difference between total binding and the binding in the presence of 3 μ M S-(-)-cilclopride. Total binding is approximately 10% of the total added ligand. Cheng-Prusoff determination (K_i s) are performed using Prism software using a one-site competition curve analysis where the top and the bottom of the non-linear regression are held constant at 0% and 100% percent inhibition. The percent inhibition at each drug
10 concentration is the mean of duplicate determinations.

**[³H]PRAZOSIN BINDING TO CLONED HUMAN ALPHA-1A ADRENERGIC RECEPTORS (α_{1A})
EXPRESSED IN CHINESE HAMSTER OVARY CELLS (CHO)**

Purpose

This *in vitro* assay is designed as a screen to identify compounds displaying an affinity for the human α_{1A} adrenoceptor subtype expressed in the membrane fragments of CHO cells. The assay measures the ability of the test compounds to displace [³H] prazosin from α_{1A} sites.

20 The identification of multiple vascular α_1 -adrenoceptors (α_{1A} , α_{1B} , α_{1D}) *in vitro* has provided impetus to define the role(s) of these subtypes in cardiovascular regulation *in vivo* (Vargas and Gorman, 1995). Hemodynamic studies in the unanesthetized rat suggest that vascular α_{1A} receptors are the major subtype involved in the sympathetic regulation of peripheral resistance and systemic arterial pressure (Pascik et al., 1989). Additional evidence for an involvement of peripheral α_{1A} receptors in the maintenance of arterial pressure was demonstrated by the findings that the selective α_{1A} antagonist 5-MU dose dependently lowered resting arterial pressure in awake conscious dogs (Pascik et al., 1989). A demonstrated inability of the irreversible antagonist chloroethylclonidine to reduce arterial pressure in rats when administered intravenously, is strong evidence against the role of α_{1B} and α_{1D} receptors in the acute regulation of arterial pressure (Vargas et al., 1993).

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Therefore the binding of compounds to α_{1A} adrenergic receptors is believed to be a good measure of a compound's potential to cause orthostatic hypotension and sedation as side effects. Prazosin is a potent antagonist of the human α_{1A} -adrenoceptor subtype which has been cloned and is expressed in the membrane fragments of CHO cells.

h α_{1A} receptor

The cloning of the human α_{1A} cDNA was accomplished first by screening a human prostate cDNA library (Clontech) from which a portion of the coding region was obtained. This DNA fragment was then used to screen a human leukocyte genomic library (Clontech) and the rest of the coding sequence was obtained. Later these two fragments were spliced together. The entire coding sequence was then fully sequenced including matching PCR sequence with original genomic coding sequence thus ensuring splice sites were joined correctly (Schwinn et al 1995). Once sequenced the gene was subcloned into the expression vector pcDNA3 (Invitrogen). Plasmid DNA was then used for transfection into CHO cells and G418 resistant clones were isolated. A clone expressing high levels of the h α_{1A} receptor (as determined by mRNA and receptor binding data) was chosen and pharmacologically characterized.

Culture Media

Media Ingredients for Adherent α_{1A} expressing CHO Culture

- A HAM's F-12 (Cellgro)
- B 10% 0.2 micron filtered Fetal Bovine Serum (FBS)(Cellgro)
- C 1% 0.2 micron filtered Penicillin-Streptomycin (Cellgro)
- D G418 0.2 micron filtered (Geneticin 400 μ g/ml)(Cellgro)

Cells are cultured using established methods and procedures in either 150x25mm culture plates (scale up to 100 plates) or a combination of these plates and 70 roller bottles. One culturing/harvest cycle typically requires 2 weeks and yields between 100-400mg protein. Plates or bottles are incubated at 37°C + 5% CO₂.

Storage

Cells are harvested by mechanical scraping, washed using PBS, collected in 250ml Corning polypropylene centrifuge tubes, spun down

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(1500RPM) and resuspended in dH₂O 10% DMSO (final volume per harvest is approximately 50ml) Protein determination is made using the Biorad DC Assay Kit Finally the appropriate volume is aliquoted into a 2ml Coming Cryovial (10mg/1-1.5 ml) which is stored at -80°C

5

Current Lot Data α_{1a} (clone # 7)
 Batch 1/14/98
 Receptor Concentration 2418 fmol/mg protein
 K_d 0.18nM
 Volume 1.5 ml/cryovial
 Protein Concentration approx 10mg/1.5ml

10

Assay Requirement: 0.5 cryovials per 96 well plate (assay volume = 200 μ l/well)

15

[³H]-Ligand [³H]-7-methoxy-Prazosin 1.0nM (NEN NBT-823)
 70-87 Ci/mmol

Materials Phentolamine mesylate (Research Biochemicals Int #P-131)
 96 well flat bottom plates (Beckman)
 Unifilter GF/B Plate (Packard)
 Polyethylenimine (Sigma #P-3134)
 TomTec or Packard Filtermate 196 Cell Harvesters
 Packard TopCount Scintillation Counter

20

Buffers A 50 mM Tris HCl 0.1% ascorbate pH 7.7 (incubation buffer)
 B 50 mM Tris HCl, pH 7.7 (wash buffer)

25

Procedure Assay additions are as follows (in the order indicated)
Total Binding = 50 μ l buffer A + 50 μ l [³H]prazosin + 100 μ l membrane
Nonsp. Bd = 50 μ l 10 μ M phentolamine + 50 μ l [³H]prazosin + 100 μ l membrane
Test Cpd = 50 μ l compound + 50 μ l [³H]prazosin + 100 μ l membrane

30

35

Compounds to be evaluated are weighed out to yield a 10 mM stock solution in DMSO in a 24 well polystyrene plate. This is diluted to a 0.5 mM stock in dH₂O. Serial dilutions in Buffer A are made from which 50

-40-

µl additions to the plate are made in duplicate in order to achieve the final concentrations desired. Typically one 96 well plate is used to evaluate 11 compounds at 4 concentrations (10^{-6} - 10^{-9} M) in duplicate. Total binding and nonspecific binding are determined in quadruplicate. Usually one standard is run with each assay.

[³H]Prazosin is made up in Buffer A such that when 50 µl are added per well the final concentration is 1.0 nM in a final assay volume of 200 µl. The final concentration should be verified by running a sample in a scintillation counter prior to adding the [³H]prazosin to the 96 well plate. Note: The radioactivity should be prepared just before the additions are made so that it is not allowed to sit on the bench for very long.

Packard GF/B Plate Pretreatment The filter plates are presoaked for at least 30 min in ice cold Buffer B containing 0.05% polyethyleneimine (200 µl/200 ml) to maximize filtration efficiency and minimize filter blank binding.

Incubation & Filtration Once buffer compounds [³H]prazosin and membrane have been added (and mixed) the 96 well plates are incubated for 40 min at 37°C and spaced 3-5 min apart. At 40 min the plates are filtered using a Tomtec Automated Cell Harvester. Filtration is immediately followed by washes of ice cold Buffer B (total vol ~7 ml).

Drying and Counting Each filter plate is dried under a heat lamp for 15 min. The back of the plate is sealed and 40 µl of Packard microscint fluid are added per well. Using Packard film each plate is heat sealed prior to being counted in a Packard Topcount Scintillation counter. A program has been written that counts each plate twice sending DPM, CPM and TSIS data to disk and printer.

Analysis of Results Raw DPM and CPM data are captured on disk and are imported into one of several software packages (Graphpad Prism Ver 2.0 Excel) residing on the LAN. Specific binding is defined as the difference between total binding and the binding in the presence

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of 10 μ M phentolamine. Total binding is less than 10% of the total added ligand. Software using one-site competition curve analysis is employed in the calculation of IC_{50} and K_i (Cheng-Prusoff equation 1973). The top and bottom of the non linear regression are held constant at 0% and 100% inhibition. The percent inhibition at each drug concentration is the mean of duplicate determinations.

References

- Vargas H M and A.J Gorman *Life Sciences* Vol 57 No 25 pp 2291-2308, 1995
- Morrow A L and I Creese *Mol Pharmacol* 29 321-330 1986
- Piascik M T J W Kusiak, and K W Barron *Eur J Pharmacol* 11 101-107 1989
- Vargas H M D Cunningham L Zhou H B Hartman and A J Gorman *J Pharmacol Exp Ther* 267 264 272 1993

The functional activity of compounds of the invention (i.e. whether they are antagonists, agonists or partial agonists) can readily be determined using the microphysiometer test method that follows.

Chinese Hamster Ovary (CHO) cells expressing the human dopamine D3 receptor were grown on the surface of a capsule cup. Cups are assembled and placed on the microphysiometer and buffer (Dulbecco's Modified Eagle's Medium without sodium bicarbonate and without serum) is perfused through the cup assembly until a stable baseline is achieved (4 hours). Buffer perfusion rate and solution changes were controlled by a computer. Intracellular acidification rate was measured in each of the 8 cup assemblies and recorded by the computer. Buffer containing test compound (10 nM, 100 nM and 1 μ M) was perfused through the cup assembly for 20 min, then buffer containing quinpirole (10 nM) and test compound (same concentrations) was perfused for an additional 1 min. This was followed by a recovery period of 10-60 min where buffer alone was perfused through the cups. Quinpirole increased acidification rate. A D₃ antagonist will inhibit this increase in a concentration dependent manner.

D₃ antagonists are of potential use as antipsychotic agents for example in the treatment of schizophrenia, schizo-affective disorders, psychotic depression and mania. Conditions which may be treated by D₃ agonists include include dyskinesic disorders such as

Parkinson's disease, neuroleptic-induced parkinsonism and tardive dyskinesias, depression, anxiety, dementia, circadian rhythm disorders, and drug (e.g., cocaine) dependency.

In accordance with yet another embodiment of the present invention, there is provided a method of modulating the activity of dopamine D₃ receptors, said method comprising contacting cell-associated dopamine D₃ receptors with a concentration of a compound of formula I or a physiologically acceptable salt thereof sufficient to modulate the activity of said dopamine D₃ receptor.

As employed herein, the phrase "modulating the activity of dopamine D₃ receptors" refers to a variety of therapeutic applications. Said therapeutic applications refer to the treatment of conditions or disorders which include dyskineic disorders, psychoses, anxiety disorders, mood disorders, dementia, sleep disorders, substance dependence, substance abuse and nausea.

The instant invention also provides a method of treating conditions or disorders of the central nervous system comprising administering to a patient in need thereof a therapeutically effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof.

The instant invention further provides a method of treating conditions or disorders of the central nervous system comprising administering to a patient in need thereof a therapeutically effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof in conjunction with one or more D₁, D₂, D₄, D₅ or 5HT receptor antagonists.

In treating a patient afflicted with a condition or disorder described above, a compound of formula I can be administered in any form or mode which makes the compound bioavailable in therapeutically effective amounts, including orally, sublingually, buccally, subcutaneously, intramuscularly, intravenously, transdermally, intranasally, rectally, topically, and the like. One skilled in the art of preparing formulations can determine the proper form and mode of administration depending upon the particular characteristics of the compound selected for the condition or disease to be treated, the stage of the disease, the condition of the patient and other relevant circumstances. For example, see Remington's Pharmaceutical Sciences, 18th Edition, Mack Publishing Co. (1990), incorporated herein by reference.

The compounds of Formula I can be administered alone or in the form of a pharmaceutical composition in combination with pharmaceutically acceptable carriers, the

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proportion and nature of which are determined by the solubility and chemical properties of the compound selected the chosen route of administration standard pharmaceutical practice and other relevant criteria

5 The compounds of formula I may be administered orally for example in the form of tablets troches capsules elixirs suspensions solutions syrups wafers chewing gums sprinkles and the like and may contain one or more of the following adjuvants binders such as microcrystalline cellulose gum tragacanth or gelatin excipients such as starch or lactose
10 disintegrating agents such as alginate acid Primogel corn starch and the like lubricants such as magnesium stearate or Sterotex glidants such as colloidal silicon dioxide and sweetening agents such as sucrose or saccharin may be added or a flavoring agent such as peppermint methyl salicylate or orange flavoring When the dosage unit form is a capsule it may contain in addition to materials of the above type a liquid carrier such as polyethylene glycol or a fatty oil Other dosage unit forms may contain other various materials which modify the physical
15 form of the dosage unit, for example as coatings Thus tablets or pills may be coated with sugar shellac or other enteric coating agents A syrup may contain in addition to the present compounds sucrose as a sweetening agent and certain preservatives dyes and colorings and flavors

20 The compounds of Formula I may also be administered topically and when done so the carrier may suitably comprise a solution ointment or gel base The base for example may comprise one or more of petrolatum lanolin polyethylene glycols, bee wax mineral oil diluents such as water and alcohol and emulsifiers and stabilizers

25 The solutions or suspensions may also include one or more of the following adjuvants sterile diluents such as water for injection saline solution fixative oils polyethylene glycols glycerine, propylene glycol or other synthetic solvents antibacterial agents such as benzyl alcohol or methyl paraben antioxidants such as ascorbic acid or sodium bisulfite
30 chelating agents such as ethylene diaminetetraacetic acid buffers such as acetates citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose The parenteral preparation can be enclosed in ampoules disposable syringes or multiple dose vials

35 The highly lipophilic esters amides and carbamates of compounds I are capable of sustained release in mammals for a period of several days or from about one to four weeks when formulated and administered as depot preparations as for example when injected in a

properly selected pharmaceutically acceptable oil. The preferred oils are of vegetable origin such as sesame oil, cottonseed oil, corn oil, coconut oil, soybean oil, olive oil and the like, or they are synthetic esters of fatty acids and polyfunctional alcohols such as glycerol or propyleneglycol.

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The depot compositions of formula I are prepared by dissolving a highly lipophilic ester, amide or carbamate of the instant invention in a pharmaceutically acceptable oil under sterile conditions. The oil is selected so as to obtain a release of the active ingredient over a desired period of time. The appropriate oil may easily be determined by consulting the prior art or without undue experimentation by one skilled in the art.

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The dosage range at which the compounds of formula I exhibit their ability to act therapeutically can vary depending upon the particular disease or condition being treated and its severity, the patient, the formulation, other underlying disease states that the patient is suffering from, and other medications that may be concurrently administered to the patient. Generally, the compounds of formula I will exhibit their therapeutic activities at dosages of between about 0.001 mg/kg of patient body weight/day to about 100 mg/kg of patient body weight/day.

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In a further aspect, the present invention provides novel radiolabeled imaging agents or formula I useful inter alia for imaging dopamine D₃ receptors in the CNS to diagnose CNS abnormalities.

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The radiolabeled (labeled and C-14 labeled) forms compounds of formula I are useful as radioligands to determine the binding of compounds to the dopamine D₃ receptor. They are also useful as labeled parent compounds to determine the metabolism of the compound in animals. Preferred for this purpose are compounds of formula I wherein R is group (a) with a radiolabeled ¹⁴C in the 3-position of the benzo[b]thiophene ring system, L is trifluoromethyl, p is 1, R₃ is hydrogen, n is 2 and A is N. Particularly preferred for this purpose are compounds of formula IA. As employed herein, a "compound of formula IA" shall refer to the compound of formula I wherein R is group (a) wherein L is trifluoromethyl substituted in the 6-position of the benzothiophene ring system and a radiolabeled ¹⁴C is in the 3 position of the benzo[b]thiophene ring system, p is 1, A is N, n is 2 and R₃ is hydrogen. Compounds of formula IA may be prepared in a manner analogous to that set forth in Example 21.

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Imbalances in dopamine production have been implicated in a variety of mental and

-45-

physical disorders such as Parkinson's disease (PD) It is thus desirable to diagnose and monitor such imbalances and to monitor the effectiveness of drugs and substances that affect brain chemistry New and powerful imaging methods that enable one to assess the living brain in vivo and thereby monitor brain chemistry and the effectiveness of drugs and substances that affect brain chemistry have been developed Methods such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) involve administering to a patient a radioactive tracer substance comprising a ligand that binds to the presynaptic or postsynaptic neuroreceptors in the patient's brain Emissions (primarily gamma rays are emitted from the positrons or photons from the radioactive tracer) are measured These emissions are indicative of the number and degree of occupancy of blocking of the neuroreceptors The number of neuroreceptors and the degree of occupancy or blocking is calculated utilizing a mathematical model and compared with an intra-person or inter-person control to determine the degree of drug response Further treatment of the patient with drugs is based on the comparisons made For these methods to be useful however, a ligand that has a high specificity and affinity for the desired receptor is required

It is believed that certain radioactive ligands may be selective for dopamine transporters and are thus potentially useful in evaluating changes in dopamine function in vivo and in vitro especially for patients with Parkinson's disease (PD) which is characterized by a selective loss of dopamine neurons in the basal ganglia and substantia nigra

Another aspect of this invention relates to methods for utilizing the compounds of the invention as CNS imaging agents Imaging techniques are non invasive diagnostic techniques that generally involve administering a compound with marker atoms that can be detected externally to the mammal Generally these methods comprise administering to a mammal a compound of the invention dissolved or dispersed in a suitable pharmaceutical carrier or diluent The compound of the invention selectively binds to dopamine D₂ thus permitting the imaging of CNS receptors and the ability to, inter alia evaluate brain chemistry the effectiveness of drugs and neuronal functions Imaging techniques suitable for practicing the present invention include but are not limited to single photon emission computed tomography (SPECT) and positron emission tomography (PET)

Radionuclides that are widely used in diagnostic nuclear medicine include technetium [^{99m}Tc] Iodine [¹²³I] carbon [¹¹C] and fluorine [¹⁸F] The radiolabeled imaging agent specifically exemplified herein contains the radionuclide ¹¹C It should be noted that the same or similar

radiochemistry reactions can be carried out using radionuclides other than ^{14}C

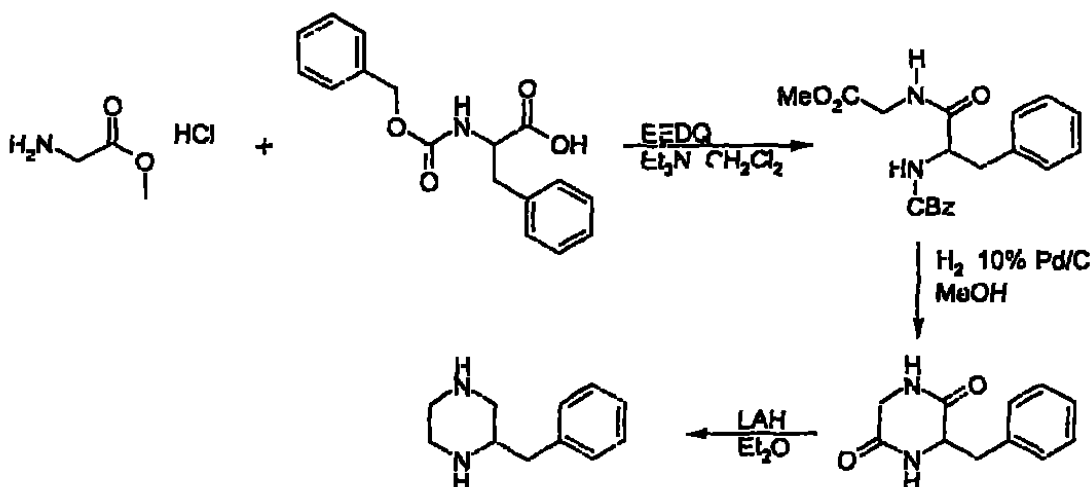
The invention is further illustrated by the following non-limiting examples and tabular information. These examples are understood to be illustrative only and are not intended to limit the scope of the present invention in any way. As used herein, the following terms have the indicated meanings: "g" refers to grams, "mmol" refers to millimoles, "ml" refers to milliliters, "°C" refers to degrees Celsius, "TLC" refers to thin layer chromatography, "LC/MS" refers to liquid chromatography mass spectrometry, "APCI" refers to atmospheric pressure chemical ionization, "mp" refers to melting point.

EXAMPLES

Example 1

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Synthesis of Intermediate Substituted Piperazines

10 Example 1(a), Preparation of intermediate 3-benzyl-piperazine

To a suspension of 3-benzyl-piperazine-2,5-dione (14.98 g, 73 mmol, prepared following generally the procedure of Halpern and Westley J Org Chem 1968 33 864) in dry diethyl ether (500 mL) is added dropwise to a solution of lithium aluminum hydride (400 mL of a 1 M solution in diethyl ether, 400 mmol, 5.4 eq). The suspension is heated at reflux for 23 hours and then cooled to 0°C . Water (70 mL) is then cautiously added and the resulting suspension is warmed to room temperature. After 3 hours the suspension is filtered and the solid washed with diethyl ether (1 L). The filtrate is concentrated under vacuum to provide crude title compound (11.40 g, 88%) as a yellow crystalline solid. A sample (2 g) is recrystallized from cyclohexane and then from toluene to provide the purified title compound (0.83 g) as a fine white crystals, mp $80-81^\circ\text{C}$.

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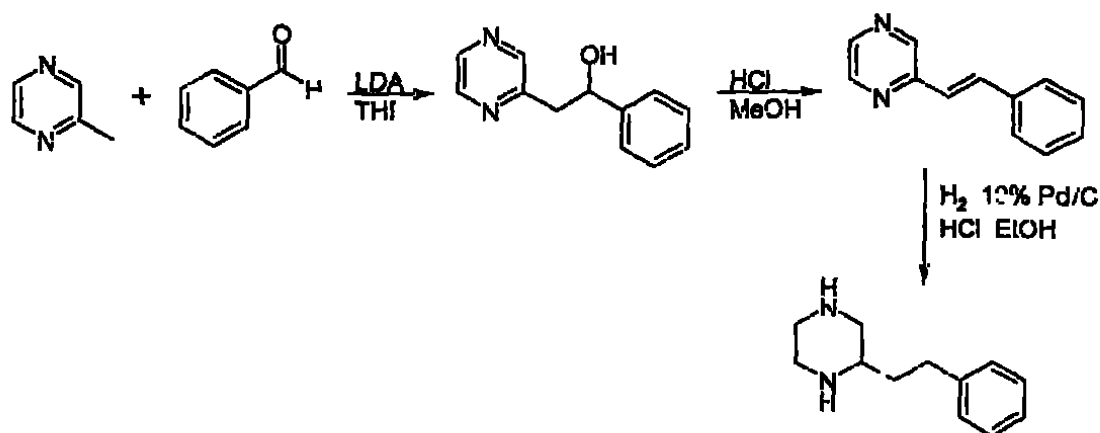
Anal. Calcd. For $\text{C}_{10}\text{H}_{18}\text{N}_2$

C 74.96, H 9.15, N 15.89,

Found

C 74.84, H 9.01, N 16.15

-48-



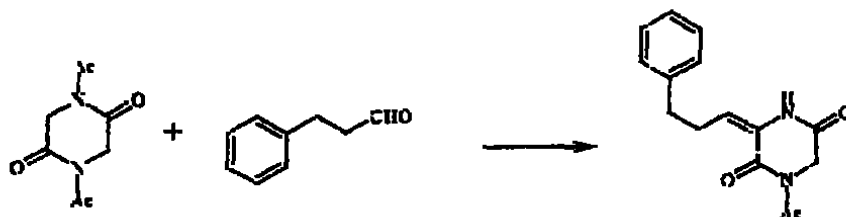
Example 1(b) To a solution of LDA (295 mL, 0.59 mol, 2 M in heptane/THF/ethylbenzene) in anhydrous THF (300 mL) cooled to -40°C was added 2-methylpyrazine (48.5 mL, 0.531 mol) dropwise via an addition funnel. The reaction was allowed to warm to 20°C and was stirred for 90 minutes when a solution of benzaldehyde (54 mL, 0.531 mol) in anhydrous THF (200 mL) was added dropwise via an addition funnel. After complete addition, the reaction was allowed to warm to room temperature and was stirred for 20 hours. The reaction was then cooled in an ice bath and saturated NH_4Cl (500 mL) was added. The resulting mixture was extracted with EtOAc (500 mL, 250 mL). The combined extracts were dried (Na_2SO_4), filtered and concentrated to a damp, beige solid. The product was triturated with Et₂O and collected then dried overnight to yield 56.0 g (53%) of a light brown solid, mp $81-84^{\circ}\text{C}$.

A solution of the above-obtained solid (56.0 g, 0.28 mol) in MeOH (1 L) and conc. HCl (290 mL) was stirred at reflux for 24 hours. The reaction was cooled to room temperature and concentrated to a dark liquid. The dark liquid was cooled in an ice bath and water (1 L) was added. The resulting solution was neutralized with a saturated solution of Na_2CO_3 and the product was extracted with EtOAc (1 L, 2 x 500 mL). The combined extracts were dried (Na_2SO_4), filtered and concentrated to yield 46 g of a dark brown solid. The solid was purified via flash column chromatography (40% EtOAc in heptane) yielding 22.7 g of the olefin as a brown foam.

A 1 L Parr shaker bottle was flushed with nitrogen and charged with 10% Pd/C (4.5 g, Degussa type) and the above-obtained olefin (20.0 g, 0.110 mol) in EtOH (450 mL). The reaction was hydrogenated at 50 psi for 3.5 hours when the reaction was filtered through a celite plug and rinsed with ethanol. The bottle was recharged with fresh 10% Pd/C (4.5 g, Degussa type), the filtrate and conc. HCl (15 mL). The reaction was hydrogenated at 50 psi for 18 hours when the reaction was diluted with warm MeOH and filtered through a plug of

celite. The solid was thoroughly washed with hot MeOH and the filtrate was concentrated to yield 11.2 g (39%) of the final product as the di-HCl salt mp 297-300

See Tetrahedron 30 1974 pp667-673 and Tet Lett 1979 pp4483-4486



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Example 1(c) DBU (14.0 g, 92 mmol) was added to a solution of the piperazine diacetate (18.2 g, 92 mmol) and aldehyde (12.3 g, 92 mmol) in 92 mL of DMF at ambient temperature. The resulting mixture was stirred at room temperature for 5h. The precipitated product was collected by filtration providing 17.1 g of product.

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The monoacetate (17.0 g, 62.8 mmol) and hydrazine hydrate (9.4 g, 188.6 mmol) in 125 mL of DMF were stirred at room temperature for 20 h. The precipitated solid was collected by filtration and washed with water and ethanol leaving 13.7 g of product.

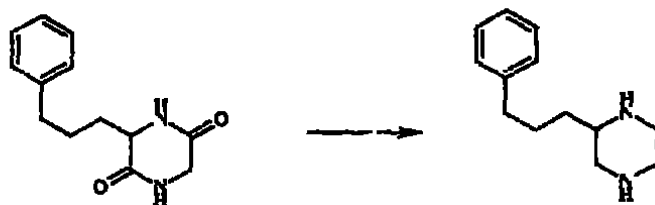
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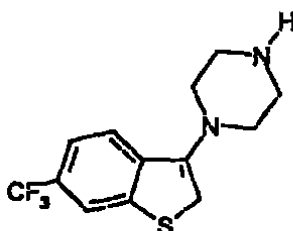
The olefin (13.6 g, 59.1 mmol) and palladium on carbon (2.7 g, 10% Pd/C, Degussa type 50% H₂O) in 1.2 l of methanol were shaken on a Parr hydrogenation apparatus at 40 psi of hydrogen until hydrogen uptake ceased. The mixture was diluted with dichloromethane and filtered through celite. Concentration of the filtrate provided 12.1 g of product.

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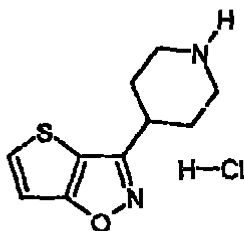
A solution of LAH (156 mL 158 mmol 1M in THF) was added dropwise to a 0°C solution of the piperazine dione (12.1 g 52.1 mmol) in 100 mL of THF. The mixture was heated to reflux and stirred overnight. The mixture was cooled to 0°C and 35 mL of water in 200 mL of THF was carefully added. The resulting mixture stirred for 1 h then it was filtered, the filter cake was washed with THF and the filtrate was concentrated in vacuo to give 7.4 g of product.

Example 2**1 (6-(trifluoromethyl)-1-benzothien-3-yl)-piperazine hydrochloride****2a 2-Carbomethoxy 3-amino-6-trifluoromethylbenzo[b]thiophene**

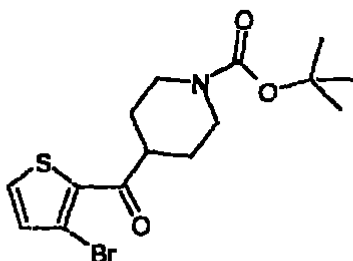
Equip a 22-L, 3-necked round-bottom flask with a mechanical stirrer, nitrogen bubbler and a thermocouple probe. Charge with 1.20 kg (5.55 mole) of 2-nitro-4-trifluoromethylbenzonitrile, 589.3 g (496 mL 5.55 mole) of methyl thioglycolate and 4.3 L of NMP. Cool the resulting yellow solution to 2 °C and add slowly over a period of 78 min a solution prepared from 466.0 g (11.11 mole 2.0 eq) of lithium hydroxide monohydrate in 3.36 L of water while maintaining the temperature between 2 - 20 °C. Allow the brown slurry to warm to 21 °C over a 2 h period and then dilute with 8.0 L of water (observe exotherm → 27 °C). Stir for 40 min and cool to 18 °C. Collect the product by filtration, rinsing with 10 L of water, then air-drying at ambient temperature to give 1.295 kg (84.7% yield) of 2-carbomethoxy-3-amino-6-trifluoromethylbenzo[b]thiophene as a light-yellow solid, 99.8% pure by HPLC assay.

2b 1-(6-(trifluoromethyl)-benzo[b]thien-3-yl)-piperazine hydrochloride

Equip a 12-L 3-necked round-bottom flask with a mechanical stirrer nitrogen bubbler and a thermocouple probe, and charge with 1.14 kg (4.14 mole) of 2-carbomethoxy-3-amino-6-trifluoromethylbenzo-[b]thiophene (Example 2a), 196.0 g (2.28 mole 0.55 eq) of piperazine, 4.0 L of NMP and 570 mL of xylene. Heat the solution and hold at 170 – 180 °C for 4 h at which time the reaction is ca. 98% complete as determined by HPLC assay. Cool the brown solution to 168 °C and then add 1.605 kg (18.63 mole 4.5 eq) of piperazine (temp -> 109 °C) following with 1.575 kg (28.28 mole 2.0 eq) of p-toluenesulfonic acid monohydrate (observe exotherm 109 -> 130 °C). Connect a Dean-Stark trap to the condenser and heat the reaction to collect an azeotrope. Remove a total of 410 mL of an aqueous distillate while allowing the pot temperature to increase from 145 to 165 °C. Monitor the progress of the reaction by GC/MS and HPLC assays. After 14 h at ca. 165 °C (>99% conversion by HPLC and GC/MS assay) cool the reaction to 30-35 °C and then quench into an extractor that contains 5 kg of ice, 12 L of water and 8.5 L of toluene. Separate the phases, wash the organic extract with 11 L of 0.5 N NaOH, 2 L of saturated aq. NaCl, and then extract with 6 L of 1 N HCl. Dilute the acidic aqueous extract with 1 kg of ice and basify to pH 11.2 by adding 624 g of 50% NaOH. Extract the resulting mixture with 9.5 L of toluene. Wash the toluene extract with 2 L of saturated aqueous NaCl, dry (Na₂SO₄) and filter. Charge the filtrate in a 22 L 3-necked round-bottomed flask (N₂, mechanical stirring, temperature control probe) and add a total of 3.7 L of 1N ethereal HCl at 20 – 27 °C so that the mixture is positive to Congo Red indicator paper. During the HCl addition add a total of 2.5 L of toluene to improve the stirring of the thick slurry that results. Stir at ambient temperature for 40 min, filter the slurry and wash with 4.5 L of toluene. After air drying obtain 1.165 kg (87% yield) of 3-piperazinyl-6-trifluoromethyl-benzo[b]thiophene hydrochloride as a light pink-beige solid, 99.1% pure by GC/MS assay.

Example 3**3-Piperidinyl-4-yl-thieno[2,3-d]isoxazole hydrochloride**

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3a 4-(3-Bromo-thiophene-2-carbonyl)-piperidine-1-carboxylic acid tert-butyl ester

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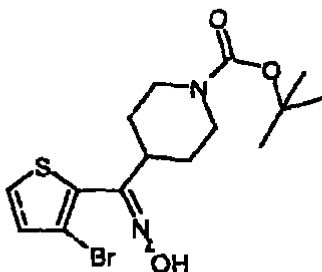
Stir a solution under nitrogen of 3-bromothiophene (21.0 mL, 0.224 mol) in tetrahydrofuran (1.0 L) at -78°C and add a 2.0M solution of lithium diisopropylamide in heptane/tetrahydrofuran/ethylbenzene (112 mL, 0.224 mol) for 45 min. Add dropwise over 2 h a solution of 4-(N-methoxy-N-methylcarboxamido)-1-piperidinecarboxylic acid 1,1-

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dimethylethyl ester (prepared according to US 5,134,139) (79.4 g, 0.291 mol) in tetrahydrofuran (800 mL). Stir for 2 h, add a saturated ammonium chloride solution and stir for an additional 0.5 h. Filter the resulting solid and pour the filtrate into water (800 mL). Extract the aqueous mixture with ether and concentrate to obtain a dark liquid. Pour the liquid into water (400 mL), add NaCl and extract the aqueous mixture with ether. Wash the

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extract with water, brine, and dry over Na₂SO₄. Filter and concentrate to obtain the crude product. Chromatograph the product over silica gel (pet ether/ether 4:1) to obtain 41.5 g (50%) of white solid.



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3b 4-[(3-Bromo-thiophen-2-yl)-hydroxyimino-methyl]-piperidine-1-carboxylic acid tert-butyl ester

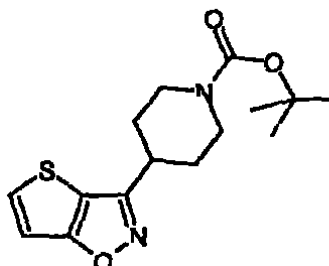
Stir a mixture of 4-(3-bromo-thiophene-2-carbonyl)-piperidine-1-carboxylic

acid tert-butyl ester (Example 3a) (41.5 g, 0.11 mol), hydroxylamine hydrochloride (15.4 g,

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0.23 mol) and pyridine (190 mL) at ambient temperature overnight. Pour the reaction into

water (500 mL) and extract with dichloromethane (3x). Wash the combined extracts with saturated CuSO_4 solution (2x), dry (MgSO_4) and concentrate to a green solid. Dissolve the solid in toluene (175 mL) and let stand at ambient temperature for 3 h. Collect the resulting crystals that form and wash with toluene (60 mL). Concentrate the filtrate and again dissolve the residue in toluene and proceed to collect additional crystals to obtain a total yield of 25 g (58%) of the title compound as a light green solid.



10 **3c** 4-Thieno[2,3-d]isoxazol-3-yl-piperidine-1-carboxylic acid tert-butyl ester

Add to a stirring solution of 4-[(3-bromo-thiophen-2-yl)-hydroxyimino-methyl]piperidine-1-carboxylic acid tert-butyl ester (Example 3b) (25 g, 64.2 mmol) in 2-methoxyethanol (200 mL) a solution of potassium hydroxide (7.2 g, 128.4 mmol) in water (20 mL). Heat the reaction to 80°C and then add copper powder (1.25 g). Stir at 80-70°C for 6 h and then at ambient temperature overnight. Pour the reaction mixture into water (500 mL) and extract with EtOAc (3x). Concentrate to a dark residue and purify by column chromatography over silica gel (heptane/EtOAc 4:1) to provide 9.8 g (50%) of a white solid.

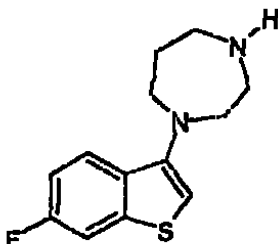
20 **3d** 3-Piperidinyl 4-yl thieno[2,3-d]isoxazole hydrochloride

Add ethereal HCl (10 mL) to 4-thieno[2,3-d]isoxazol-3-yl piperidine-1-carboxylic acid tert-butyl ester (Example 3c) (1.0 g, 3.2 mmol) and then methanol (1 mL) to effect solution. Permit to stand at ambient temperature for 1 h and then collect 0.34 g of white solid, mp 240-241°C. From the filtrate collect 0.25 g of additional white solid, mp 263-265°C. Both samples MS $m/z = 209$ ($M+H$)⁺.

Analysis (sample mp 263-265°C).

Calc For	$\text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_5 \cdot \text{HCl}$	49.08% C	5.35% H	11.45% N
Found		49.03% C	5.29% H	11.25% N

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Example 4**1 (6 Fluoro benzo[b]thiophen-3-yl)-[1,4]diazepane**

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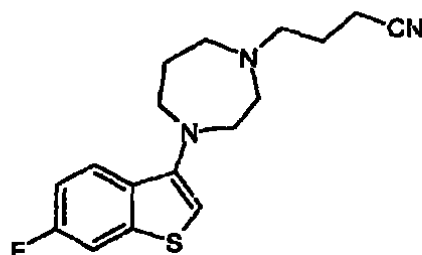
4a 3-Amino-6-fluoro-benzo[b]thiophene-2-carboxylic acid

At 50°C add to a stirring solution of 2-carbomethoxy-3-amino-6-fluoro-benzo[b]thiophene (prepared according to US 5,143,923) (90.1 g, 0.4 mol) in H₂O (450 mL) a 50% aqueous solution of NaOH (64 g, 0.8 mol) over 2-3 min. Heat the reaction to 70-73°C and continue to stir for 3 h. Add 10% aqueous isopropanol (45 mL) and bring to reflux. Remove the isopropanol under N₂ and add H₂O (300 mL). Cool the reaction mixture to between 7-10°C and add concentrated HCl (80 mL). Add H₂O (650 mL), cool to 5-7°C, filter the resulting solid and wash the filter cake with H₂O (2x150 mL). Dry the solid under vacuum at 35°C to obtain 80.6 g (94.7%) of solid mp 160-163°C. TLC on silica gel (dichloromethane/methanol 3:1) R_f = 0.69.

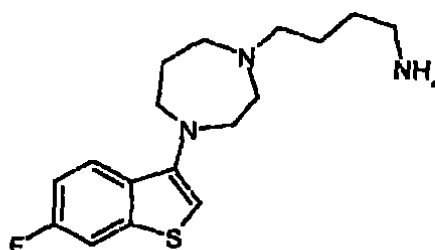
4b 1-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepane

Heat a solution of 3-amino-6-fluoro-benzo[b]thiophene-2-carboxylic acid (5.0 g, 24 mmol) in 1-methyl-2-pyrrolidinone (5 mL) to 100°C for 2 h and then introduce a stream of nitrogen to cool the solution to room temperature. Add homopiperazine (9.5 g, 95 mmol) and p-toluene sulfonic acid monohydrate (9.0 g, 47 mmol) and heat the mixture to 145°C for 4 h. After that time, cool the reaction mixture to room temperature, dilute with ethyl acetate (30 mL) and wash with brine (3x15 mL). Separate the organic layer and dry over MgSO₄. Evaporate the solvent and purify the crude product by column chromatography (SiO₂, 100 g CH₂Cl₂/MeOH 9:2 then CH₂Cl₂/MeOH/NH₄OH 9:2:0.15) to give 3.9 g (65%) of yellow solid. LC/MS (LiChrospher 5 µ, RP-18, 250 mm) CH₃CN/ Water gradient 20% → 100% (25 min) Flow 1.5 mL/min. t_R = 10.74 min, m/z = 250.3.

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Example 55 **4-[4-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazapan-1-yl]butyronitrile**

Add potassium carbonate (39.3 g, 284 mmol) to a solution of 1-(6-fluoro benzo[b]thiophen-3-yl)-[1,4]diazepane (Example 4) (23.7 g, 95 mmol) and 4-bromobutyronitrile (21.0 g, 142 mmol) in acetonitrile (400 mL) and stir the mixture under reflux for 10 h. Filter the mixture, evaporate the solvent and dissolve the residue in ethyl acetate (EtOAc). Wash with water and saturated sodium chloride solution and dry the organic phase over $MgSO_4$. Evaporate the solvent under vacuum and purify the crude product by column chromatography (EtOAc/MeOH 9:1) to give 12.9 g of a yellow oil. LC/MS (LiChrospher 5 μ , RP-18, 250 mm CH_3CN /Water(0.05% TFA)-gradient 2% $\rightarrow$ 98% (20 min) Flow: 0.75 mL/min)

15 $t_R = 9.46$ min, $m/z = 317.3$ Example 6

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4-[4-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazapan-1-yl]butylamine

Add over 30 min at room temperature a solution of $LiAlH_4$ in diethyl ether (1M, 72.5 mL) to a solution of 4-[4-(6-fluoro-benzo[b]thiophen-3-yl)-[1,4]diazapan-1-yl]butyronitrile (Example 5)

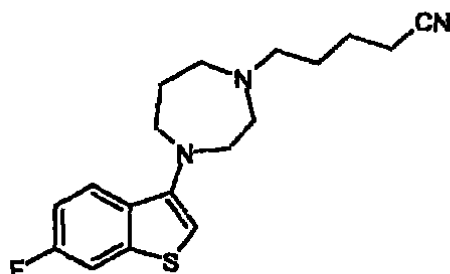
25 (11.5 g, 38.2 mmol) in dry diethyl ether (200 mL). Heat the solution to reflux for 5 h. After that,

time, allow the solution to cool to room temperature and carefully quench the reaction with water and aqueous sodium hydroxide solution. Separate the phases and re-extract the aqueous phase with EtOAc. Dry the combined organic phases over MgSO_4 and remove the solvent under vacuum. Purify the crude product by column chromatography

- 5 (CH₂Cl₂/MeOH/NH₄OH 9:2:0.25) to obtain 8.9 g of a colorless oil. LC/MS (LiChrospher 5 μ RP-18, 250 mm CH₃CN/ Water (0.05% TFA)- gradient 2% $\rightarrow$ 68% (20 min) Flow 0.75 mL/min), t_R = 7.79 min m/z = 321.3

Example 7

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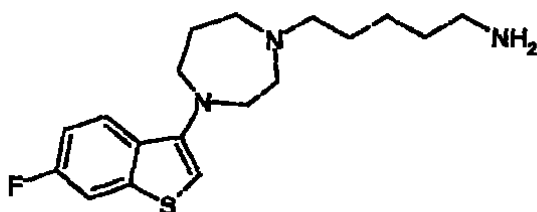


4-[4-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]pentanenitrile

- 15 Follow the procedure of Example 5 and substitute pentanenitrile for butyronitrile therein to obtain the title compound. (LiChrospher 5 μ RP-18, 250 mm CH₃CN/ Water (0.05% TFA)- gradient 2% $\rightarrow$ 98% (20 min) Flow 0.75 mL/min) t_R = 10.4 min m/z = 331.5

Example 8

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4-[4-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]pentylamine

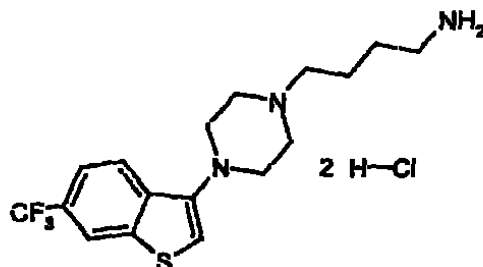
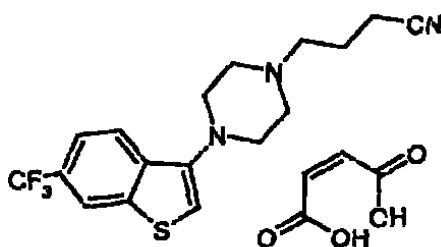
- 25 Follow the procedure of Example 6, and substitute 4-[4-(6-fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]pentanenitrile (Example 7) therein to obtain the title compound. LC/MS (

-57-

LiChrospher 5 μ RP-18, 250 mm CH₃CN/ Water(0.05% TFA)- gradient 2% $\rightarrow$ 98 % (20 min)
Flow 0.75 mL/min) t_R = 8.31 min m/z = 335.5

Example 8

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4-[6-(Trifluoromethyl)-benzo[b]thien-1-yl]-1-piperazinebutan-1-amine dihydrochloride

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9a 4-[6-(Trifluoromethyl)-benzo[b]thien-3-yl]-1-piperazinebutyl-nitrile (Z)-2-butenedioate

Reflux a mixture of 1-[6-(trifluoromethyl)-benzo[b]thien-3-yl]-piperazine (Example 2) (10.1 g, 35.3 mmol), 4-bromobutyronitrile (8.25 g, 42.3 mmol), anhydrous potassium carbonate (8.00 g, 57.9 mmol) and anhydrous acetonitrile (80 mL) for 18 h. Filter the slurry, wash the insolubles with dichloromethane (2x150 mL), and concentrate the filtrate under vacuum. Take up the residue in dichloromethane (125 mL), wash with 5% aqueous NaOH (75 mL), water (75 mL), and dry (K₂CO₃). Concentrate under vacuum and chromatograph the crude product over silica gel (EtOAc) to obtain 10.3 g (82%) of amber oil. Add to an ethanolic solution of the oil (1.2 g, 3.40 mmol), maleic acid (400 mg, 3.45 mmol), and concentrate the solution under vacuum to receive a gum. Triturate the gum with EtOAc to afford a solid. Recrystallize the solid from methanol/EtOAc to obtain 1.01 g of white crystals, mp 158-159°C.

25

Analysis

Calc for $C_{21}H_{22}F_3N_3O_4S$	53.73%C	4.72%H	8.95%N
Found	53.57%C	4.65%H	8.86%N

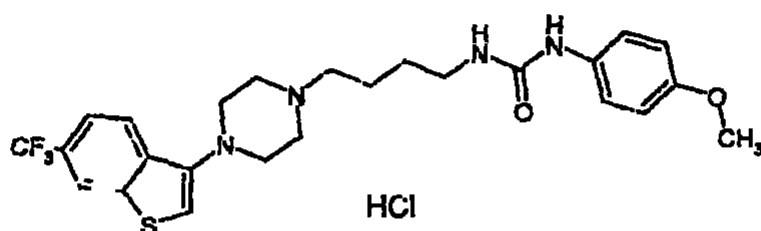
5 **9b 4-[6-(Trifluoromethyl)-benzo[b]thien-1-piperazinebutanamine dihydrochloride**

Under N_2 add dropwise a solution of 4-(6-trifluoromethyl)-benzo[b]thien-3-yl)-1-piperazinebutyl-nitrile (free base of Example 9a) (9.00 g, 25.5 mmol) in anhydrous tetrahydrofuran (THF, 70 mL) to a stirred cooled ($3^\circ C$) suspension of $LiAlH_4$ (1.06 g, 27.9 mmol) in anhydrous THF (120 mL). Maintain the temperature at $3^\circ C$ for 5 min and then stir at ambient temperature for 21 h. Cool the mixture to $0^\circ C$ and treat sequentially with H_2O (1 mL), 15% aqueous NaOH (1 mL) and H_2O (3 mL). After 20 min at room temperature, filter the mixture, wash the insolubles with dichloromethane (2x50 mL), and concentrate the filtrate under vacuum. Take the residue up in dichloromethane (150 mL), wash sequentially with 5% aqueous NaOH (75 mL), H_2O (75 mL) and then dry (K_2CO_3). Remove the solvent under vacuum and purify the residue by chromatography over silica gel (ethanol/ NH_4OH 95/5) to obtain 5.32 g (58%) of the free base of the title compound. To a solution of the free base (639 mg) in ethanol, add ethanolic HCl until the solution is acidic (pH 2-3). Concentrate under vacuum to a gum and triturate the gum with ethanol to obtain an off-white solid. Recrystallize the solid from MeOH/ $CHCl_3$ to obtain 485 mg of white powder, mp $256-258^\circ C$.

Analysis

Calculated for $C_{17}H_{22}F_3N_3S \cdot 2HCl$	47.45%C	5.62%H	9.76%N
Found	47.10%	5.67%H	9.62%N

Example 10

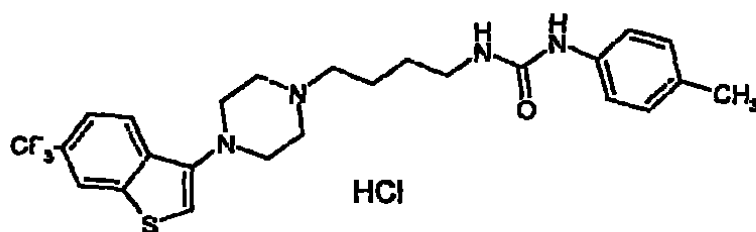
30 **1-(4-Methoxy-phenyl)-3-{4-[4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl}-urea hydrochloride**

Under nitrogen add 4-methoxyphenyl isocyanate (0.42 g, 2.8 mmol) in CHCl_3 (18 mL) to a stirring solution of 4-[6-(trifluoromethyl)-benzo[b]thien-1-yl]piperazinebutanamine (free base of Example 9b) (1.0 g, 2.8 mmol) in CHCl_3 (10 mL). Allow the reaction to stir at ambient temperature for 18 h, filter the resulting solid, wash with CHCl_3 and obtain 635 mg of product as a white solid, mp 202-204°C. Concentrate the filtrate and obtain 1.0 g of a sticky residue. Chromatograph the residue over 40 g of silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 24:1) and obtain an additional 110 mg of product. Combine the samples, dissolve in hot EtOAc-MeOH (100 mL-2 mL), filter and after cooling add ethereal HCl to precipitate the hydrochloride salt. After 2 h collect 787 mg of the salt as a white solid, mp 228-231°C. Stir the solid in refluxing acetonitrile for 45 min, allow the mixture to cool and filter the solid to obtain 570 mg of the title compound as a white solid, mp 240-242°C.

Analysis

Calculated for $\text{C}_{28}\text{H}_{28}\text{F}_3\text{N}_4\text{O}_2\text{S}\cdot\text{HCl}$	55.29%C	5.57%H	10.32%N
Found	55.21%C	5.71%H	10.22%N

Example 11



1-(4-Methyl-phenyl)-3-{4-[4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl}-urea hydrochloride

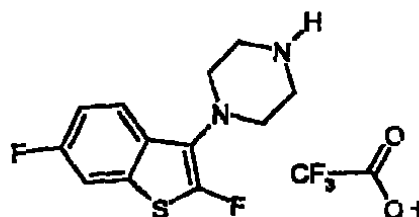
Add p-tolyl isocyanate (0.41 g, 3.1 mmol) in CHCl_3 (8 mL) to a stirring solution of 4-[6-(trifluoromethyl)-benzo[b]thien-1-yl]piperazinebutanamine (free base of Example 9b) (1.1 g, 3.1 mmol) in CHCl_3 (8 mL). Allow the reaction to stir at ambient temperature for 63 h, filter the resulting solid, wash with CHCl_3 and obtain 677 mg of product as a beige solid. Dissolve the solid in hot EtOAc-MeOH (100 mL-2 mL), filter and after cooling add ethereal HCl to precipitate a hydrochloride salt. Collect 669 mg of the salt as a white solid, mp 231-234°C.

-60-

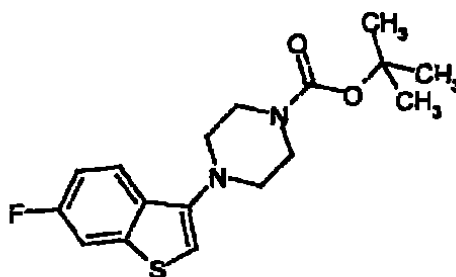
Recrystallize the solid from absolute ethanol to obtain 595 mg (37%) of the title compound as a white solid mp 240-242°C

Analysis

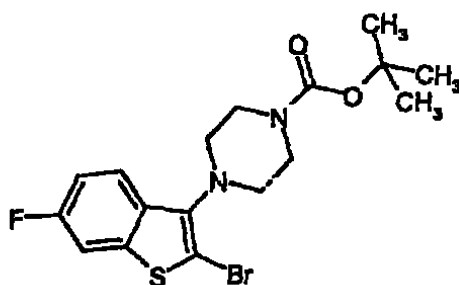
5	Calculated for $C_{25}H_{20}F_3N_4OS \cdot HCl$	56.97%C	5.74%H	10.63%N
	Found	57.02%C	5.83%H	10.68%N

Example 12

1-(2,6-Difluoro-benzo[b]thien-3-yl)-piperazine trifluoroacetate

12a 4-(6-Fluoro-benzo[b]thiophen-3-yl)-piperazine-1-carboxylic acid tert-butyl ester

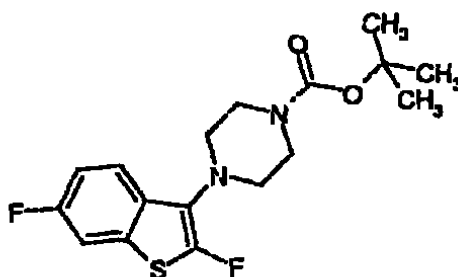
Add a solution of di-*tert*-butyl dicarbonate (5.15 g, 23.6 mmol) in $CHCl_3$ (15 mL), dropwise over 45 min to a solution at -85°C of 1-(6-fluorobenzo[b]thiophen-3-yl)-piperazine (prepared according to US 5 143 923) (2.8 g, 11.8 mmol), 4-(dimethyl-amino)pyridine (0.16, 1.3 mmol) and diisopropylethylamine (4.3 mL, 3.2 g, 24.8 mmol) in $CHCl_3$ (50 mL). Following complete addition, stir the reaction at ambient temperature for 20 h and then pour the reaction into a mixture of cold (5°C) 5% aqueous NaOH/EtOAc (150/150 mL). Extract the product into EtOAc, wash the extract with H_2O brine and concentrate to a red oil. Purify the crude oil over silica gel (EtOAc) to obtain 3.6 g of red oil. LC/MS m/z = 337 (M+H)⁺



12b 4-(2-Bromo-6-fluoro-benzo[b]thiophen-3-yl)-piperazine-1-carboxylic acid tert-butyl ester

Add N-bromosuccinimide (0.59 g, 3.3 mmol) to a stirring solution of 4-(6-fluoro-benzo[b]thiophen-3-yl)-piperazine-1-carboxylic acid tert-butyl ester (Example 12a) (1.00 g, 2.97 mmol) in CHCl_3 (32.8 mL) and reflux for 30 min. Allow cooling to room temperature and filter. Evaporate the solvent and purify the residue by chromatography over silica gel (EtOAc/heptane 9:1) to obtain 0.53 g (43%) of oil. MS $m/z = 416$ ($M+H$)⁺.

In an alternative procedure, add N-bromosuccinimide (1.319 g, 6.62 mmol) to a stirring solution of 4-(6-fluoro-benzo[b]thiophen-3-yl)-piperazine-1-carboxylic acid tert-butyl ester (Example 12a) (2.226 g, 6.62 mmol) in CCl_4 and reflux for 2 h. Allow cooling to room temperature and filter. Evaporate the solvent and purify the residue by chromatography over silica gel (EtOAc/heptane 9:1) to obtain 2.34 g (94%) of oil.



12c 4-(2-Fluoro-6-fluoro-benzo[b]thiophen-3-yl)-piperazine-1-carboxylic acid tert-butyl ester

At a temperature of -65°C stir under nitrogen, a solution of the 4-(2-bromo-6-fluoro-benzo[b]thiophen-3-yl)-piperazine-1-carboxylic acid tert-butyl ester (Example 12b) (15.59 g, 37.55 mmol) in anhydrous THF (247 mL) and add, dropwise, n-butyllithium in hexane (2.5M, 19.53 mL, 48.82 mmol). Stir for 30 min and then add, dropwise, N-fluorobenzenesulfonimide

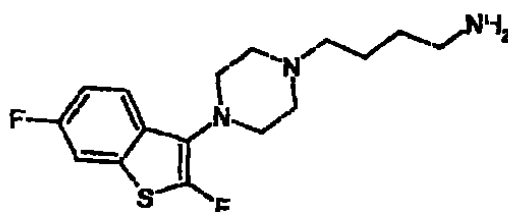
-62-

(17.76 g, 56.33 mmol) dissolved in anhydrous THF. Stir overnight at ambient temperature, cool the reaction to 0°C, add saturated NaCl solution and then water. Extract the mixture with EtOAc (3x's), combine the extracts and wash with water and brine. Dry the extract (MgSO₄) and concentrate to obtain 11.0 g of oil. Chromatograph the oil over silica gel (ether/pet ether 9:1) and obtain 6.28 g (52%) of red oil. MS m/z 354 (M+H)⁺.

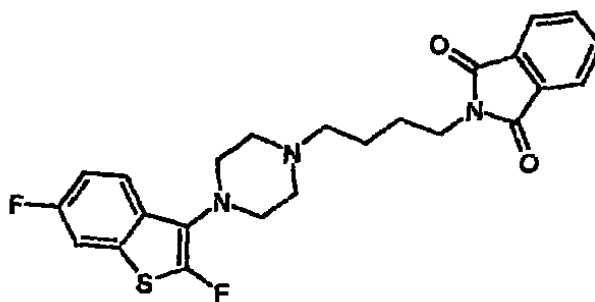
12d 1-(2,6-Difluoro-benzo[b]thien-3-yl)-piperazine trifluoroacetate

Stir a solution of 4-(2-fluoro-6-fluoro-benzo[b]thiophen-3-yl)-piperazine-1-carboxylic acid tert-butyl ester (Example 12c) (250 mg, 0.70 mmol) in trifluoroacetic acid (2.2 mL) at ambient temperature for 30 min. Evaporate the trifluoroacetic acid and treat the residue with ether. Stir the suspension at ambient temperature for 2 h, and filter the resulting white solid to obtain 191 mg (58%) of the trifluoroacetate salt. MS m/z = 255 (M+H)⁺.

Example 13



4-[6-(2,6-Difluoro-benzo[b]thien-1-yl)-piperazine]butanamine



13a 2-[4-[6-(2,6-Difluorobenzo[b]thiophen-3-yl)piperazin-1-yl]butyl]isobutylisindole-1,3-dione

Stir and reflux under argon a mixture of 1-(2,6-difluoro-benzo[b]thien-3-yl)-piperazine (free base of Example 12d) (1.48 g, 5.8 mmol), bromobutylphthalimide (1.65 g, 5.8 mmol), triethylamine (1.2 mL) and acetonitrile (25 mL) for 4 h. Allow the reaction to cool and then

dilute with dichloromethane. Wash the organic solution with water saturated K_2CO_3 solution and dry (K_2CO_3). Concentrate the solvent and obtain 2.55 g of solid. Chromatograph the solid over silica gel ($CH_2Cl_2/MeOH$ 49/1) to obtain 2.1 g of solid. mp 123-125°C, MS, $m/z = 456 (M+H)^+$

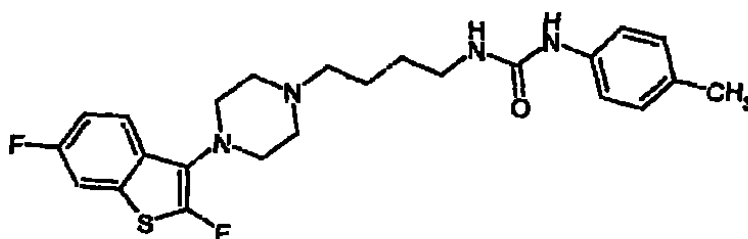
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13b 4-[6-(2,6-Difluoro-benzo[b]thien-1-yl)-piperazin-1-yl]butanamine

Stir a suspension under argon of 2-[4-[4-(6-fluorobenzo[b]thiophen-3-yl)piperazin-1-yl]butyl]isoindole-1,3-dione (Example 13a) (2.05 g, 4.5 mmol) in anhydrous MeOH (30 mL) and add hydrazine (0.5 mL, 15.9 mmol). Reflux for 2.5 h and allow cooling to ambient temperature. Cool the reaction in an ice bath and add 1M HCl to a pH ~1. Filter the mixture, cool the filtrate in an ice bath and add 50% aqueous NaOH to basify. Extract the aqueous mixture with dichloromethane, wash the extract with H_2O , dry with K_2CO_3 and concentrate to obtain 1.4 g of oil which crystallizes upon standing. LC/MS $m/z = 326 (M+H)^+$

15

Example 14



20 1-(4-Methyl-phenyl)-3-[4-[4-(2,6-difluoro-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl]-urea

Add a solution of 4-methylphenyl isocyanate (43.3 mg, 0.32 mmol) in $CHCl_3$ (1-2 mL) to a solution of 4-[6-(trifluoromethyl)-benzo[b]thien-1-yl]-piperazinebutanamine (Example 13b) (3.5 mL of a 0.086M solution in $CHCl_3$, 0.31 mmol) and shake the reaction for 20 h. Filter the resulting solid or concentrate the reaction and collect the solid after trituration with EtOAc to obtain 102.7 mg of the title compound. LC/MS (Ymc005-AQ 4x50 mm water/ CH_3CN /acetic acid, 94.5/5.0/0.5 100% for 0.1 min then water/ CH_3CN /acetic acid, 5.0/94.5/0.5 linear gradient $\rightarrow$ 100% (2 min, hold 4 min) Flow 1.0 mL/min) $t_R = 3.22$ min $m/z = 459 (M+H)^+$

30

Examples 15-18

The following HPLC conditions are referred to in Examples 15-18

5 HPLC Condition I

A) 95/5/0 1% Water/Acetonitrile/Formic Acid,

B) 5/95/0 1% Water/Acetonitrile/Formic Acid

Column YMC ODS-A 4x50 mm Flow rate 2 mL/minute

10 The initial HPLC conditions consisted of 100% (A) flowing at 2 mL/minute After the initial injection a linear gradient was performed so that at 2 minutes the HPLC conditions were 100% B These conditions were then held for 3 4 minutes at which time the system switched back to initial conditions and equilibrated for the next analysis

HPLC Condition II

15 A) 95/5/0 1% Water/Acetonitrile/Formic Acid

B) 5/95/0 1% Water/Acetonitrile/Formic Acid

Column YMC ODS-A 2x50 mm, Flow rate=1 mL/minute

20 The initial HPLC conditions consisted of 100% (A) flowing at 0.1 mL/minute After the initial injection a linear gradient was performed so that at 2 minutes the HPLC conditions were 100% B These conditions were then held for 3 5 minutes at which time the system switched back to initial conditions and equilibrated for the next analysis

HPLC Condition III

A) 95/5/0 5% Water/Acetonitrile/Formic Acid

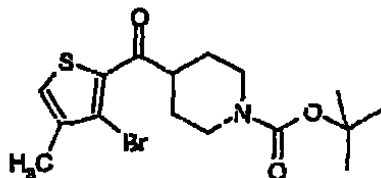
25 B) 5/95/0 5% Water/Acetonitrile/Formic Acid

Column YMC ODS-A 4x50 mm Flow rate=2 mL/minute

The initial HPLC conditions consisted of 100% (A) flowing at 2 mL/minute After the initial injection a linear gradient was performed so that at 2 minutes the HPLC conditions were 100% B These conditions were then held for 3 4 minutes at which time the system switched
30 back to initial conditions and equilibrated for the next analysis

Example 15**1-{4-[4-(6-Methyl-thieno[2,3-d]isoxazol-3-yl)-piperidin-1-yl]-butyl}-3 (substituted)phenyl-ureas and bis-ureas**

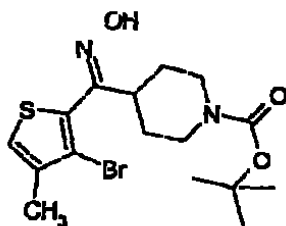
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15a Preparation of 4-[1-(3-bromo-4-methyl-thiophen-2-yl)-methanoyl]-piperidine-1-carboxylic acid tert-butyl ester

- 10 Under inert conditions, add a 2.0 M solution (in tetrahydrofuran/n-heptane) of lithium diisopropylamide (29.65 mmol, 14.83 mL, 1.05 equivalents) to a cold (-78° C) solution of 3-bromo-4-methylthiophene (28.24 mmol, 5.00 g, 1.00 equivalents) in dry tetrahydrofuran (27.33 mL). Stir at -78° C for 1 hour and add a solution of 4-(methoxy-methyl-carbamoyl)-piperidine-1-carboxylic acid tert-butyl ester (28.24 mmol, 7.69 g, 1.00 equivalents) dropwise.
- 15 Continue stirring at -78° C for 3 hours. Quench the reaction mixture with saturated ammonium chloride (aqueous, 55 mL) and allow to warm to room temperature. Extract the reaction mixture with a mixture of ethyl acetate/diethyl ether (1:1, 3 x 40 mL). Combine the extracts and dry over magnesium sulfate, filter and evaporate. Purify the residue via flash column chromatography using a mixture of n-heptane/ethyl acetate (4:1) to yield a yellow, crystalline solid (9.84 g).
- 20 MS (CI, methane) m/z 388 (MH⁺), LC/MS (APCI) m/e 288 (M-100) retention time 2 min. 43 sec. Condition I.

15b Preparation of 4-[1-(3-bromo-4-methyl-thiophen-2-yl)-1-hydroxyimino-methyl]-piperidine-1-carboxylic acid tert-butyl ester

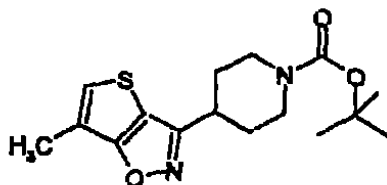
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Add ammonium hydroxide hydrochloride (50.68 mmol, 3.52 g, 2.00 equivalents) to a stirred solution of 4-[1-(3-bromo-4-methyl-thiophen-2-yl)-methanoyl]-piperidine-1-carboxylic acid tert-butyl ester (25.54 mmol, 9.84 g, 1.00 equivalents) in pyridine (47.5 mL). Stir at room temperature overnight and at 70° C for 4 hours. Cool the reaction mixture and add

- 5 hydrochloric acid (3 M solution, 115 mL). Extract the reaction mixture with dichloromethane (115 mL), filter the organic layer, wash with water (100 mL), dry over magnesium sulfate, filter and evaporate. Recrystallize the resulting residue from toluene to yield a white solid (4.84 g). LC/MS (APCI), m/e 403 (MH⁺), retention time 2 min. 32 sec. Condition I.

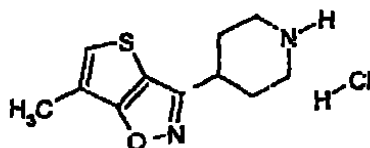
10 **15c Preparation of 4-(6-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidine-1-carboxylic acid tert-butyl ester**



- 15 Add cesium carbonate (3.72 mmol, 1.21 g, 1.50 equivalents) and copper iodide (0.25 mmol, 47 mg, 0.10 equivalents) to a stirred solution of 4-[1-(3-bromo-4-methyl-thiophen-2-yl)-1-hydroxyimino-methyl]-piperidine-1-carboxylic acid tert-butyl ester (2.48 mmol, 1.00 g, 1.00 equivalents) in 2-methoxy ethanol (25 mL). Stir the resulting mixture at room temperature overnight and filter to remove the inorganic material. Concentrate the filtrate and partition the resulting oil between ethyl acetate (75 mL) and water (25 mL). Extract the aqueous layer
- 20 with ethyl acetate (2 x 75 mL) and wash the combined organic layers with saturated sodium chloride (aqueous, 25 mL), dry over magnesium sulfate, filter and evaporate. Purify the residue via flash column chromatography eluting with n-heptane/ethyl acetate (4:1) to yield a white solid (588 mg). MS (CI, methane) m/e 323 (MH⁺). LC/MS (ESI) m/e 345 (MNa⁺). retention time 2.05 minutes. Condition II.

25

15d Preparation of 6-methyl-3-piperidin-4-yl-thieno[2,3-d]isoxazole hydrochloride



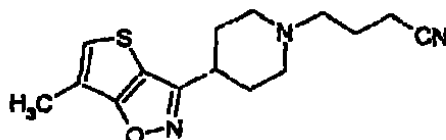
Stir a solution of 4-(6-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidine-1-carboxylic acid tert-butyl ester (6.84 mmol, 2.85 g, 1.00 equivalents) in hydrochloric acid (48.75 mL, 1 M solution in

-67-

diethyl ether) and methanol (2.00 mL) at room temperature for 3.5 hours. Filter the suspension, collect the white solid and dry to yield the desired product (659 mg). Allow the mother liquor to age overnight, filter, collect the white solid and dry to yield additional desired product (1.252 g). LC/MS (ESI) m/e 223 (MH^+) retention time 1.14 minutes. Condition II

5

15e Preparation of 4-[4-(6-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidin-1-yl]-butyronitrile



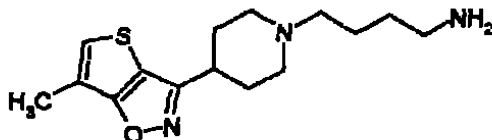
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Add potassium carbonate (17.72 mmol, 2.45 g, 2.40 equivalents), potassium iodide (0.73 mmol, 123 mg, 0.10 equivalents), and 4-bromobutyronitrile (8.88 mmol, 0.88 mL, 1.20 equivalents) to a stirred solution of 6-methyl-3-piperidin-4-yl-thieno[2,3-d]isoxazole hydrochloride (7.38 mmol, 1.91 g, 1.00 equivalents) in acetonitrile (10.84 mL) and water (3.60 mL). Stir the resulting mixture at reflux overnight. Cool to room temperature, filter the reaction mixture and wash the solid material collected with dichloromethane and evaporate the filtrate. Take the residue up in dichloromethane (45 mL), wash with sodium hydroxide (aqueous, 18 mL, 2 M), water (18 mL), saturated sodium hydroxide (aqueous, 18 mL), dry over magnesium sulfate, filter and evaporate. Purify the residue via flash column chromatography using a gradient and eluting with a mixture of n-heptane/ethyl acetate (0.5/9.5) to ethyl acetate (100%) to yield the desired product as a brown oil (663 mg). LC/MS (ESI) m/e 290 (MH^+) retention time 1.19 minutes. Condition II

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15f Preparation of 4-[4-(6-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidin-1-yl]-butylamine



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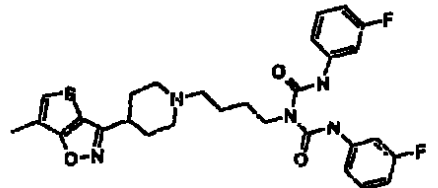
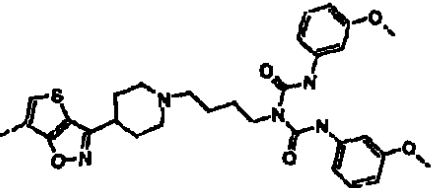
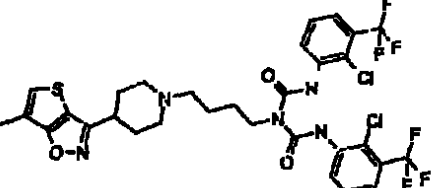
Under inert conditions, add lithium aluminum hydride (3.42 mmol, 3.42 mL, 1.50 equivalents, 1.0 M solution in tetrahydrofuran) to a stirred solution of 4-[4-(6-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidin-1-yl]-butyronitrile (2.28 mmol, 660 mg, 1.00 equivalents) in tetrahydrofuran (dry, 12.86 mL). Stir the resulting solution at room temperature for 2.5 hours. Quench the reaction mixture by adding water (0.16 mL), then sodium hydroxide (aqueous, 0.16 mL, 2 M solution) and then water (0.5 mL). Dilute the resulting suspension with dichloromethane (16 mL) and vigorously stir for 30 minutes. Filter the resulting mixture through a bed of celite®

dry over magnesium sulfate filter and evaporate to yield the desired product (457 mg) as a brown oil LC/MS (ESI) m/e 294 (MH⁺) retention time 0.58 minutes Condition II

The following 1-[4-[4-(6-Methyl-thieno[2,3-d]isoxazol-3-yl) piperidin-1-yl]-butyl]-3-(substituted)phenyl-ureas and bis-ureas were prepared using 4-[4-(6-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidin-1-yl]-butylamine (15f) and carrying out the procedures found subsequent to the table

Compound Number	Product	LC/MS (ESI) Condition II
15-1		m/e 457 (MH ⁺), retention time 1.40 minutes
15-2		m/e 427 (MH ⁺), retention time 1.40 minutes
15-3		m/e 431 (MH ⁺) retention time 1.38 minutes
15-4		m/e 431 (MH ⁺) retention time 1.39 minutes
15-5		m/e 443 (MH ⁺) retention time 1.38 minutes
15-6		m/e 515 (MH ⁺) retention time 1.52 minutes
15-7		m/e 620 (MH ⁺) retention time 1.65 minutes

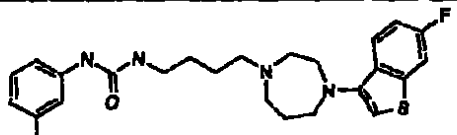
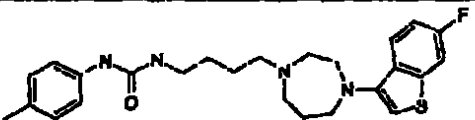
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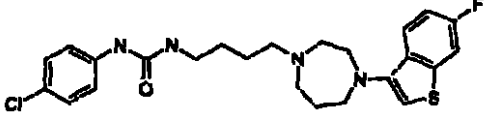
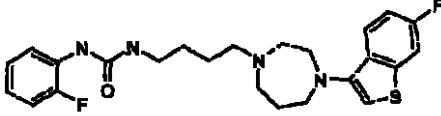
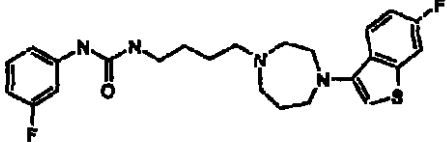
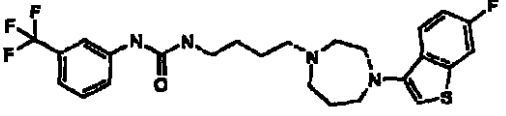
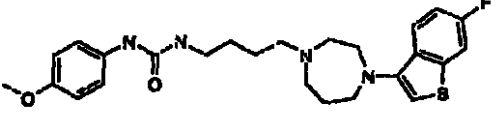
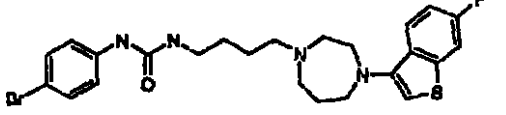
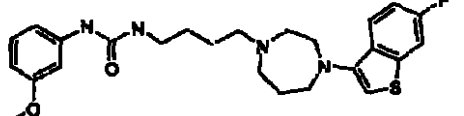
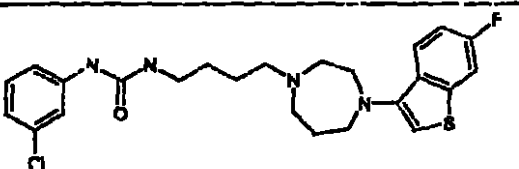
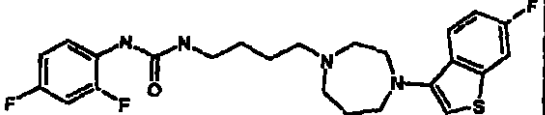
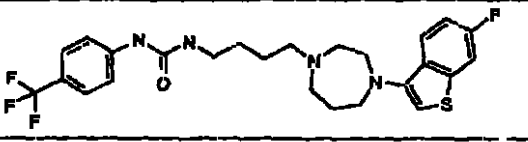
15-8		m/e 587 (MH ⁺), retention time 1.62 minutes
15-9		m/e 592 (MH ⁺), retention time 1.59 minutes
15-10		m/e 736 (MH ⁺), retention time 1.80 minutes

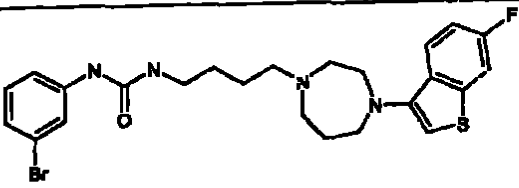
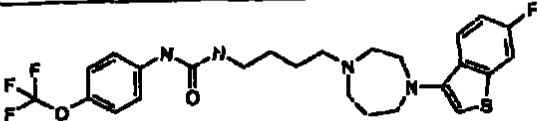
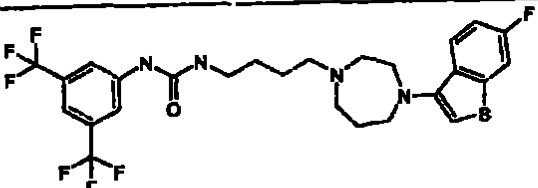
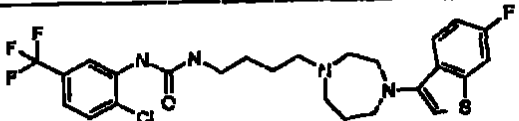
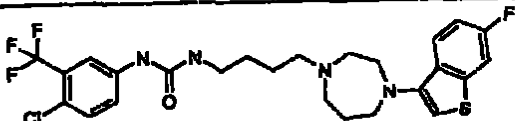
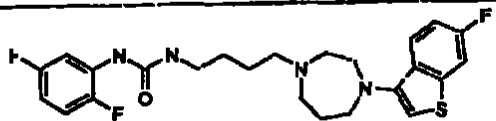
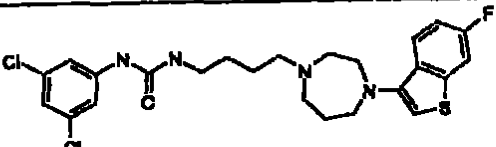
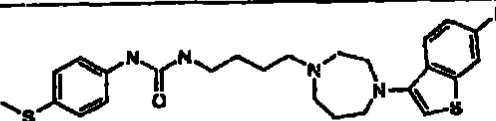
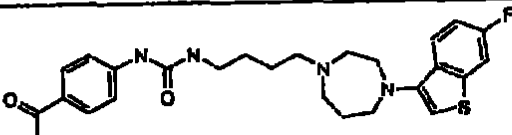
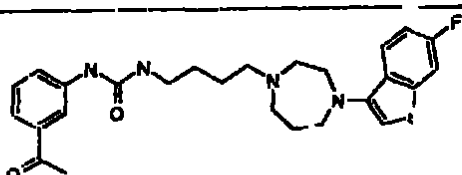
Add a solution of 4-[4-(6-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidin-1-yl]-butylamine (0.26 mmol, 75 mg, 1.00 equivalents) in dichloromethane (1.00 mL) to the desired isocyanate (0.50 mmol, 1.95 equivalents). Shake each reaction mixture overnight at room temperature, evaporate and purify the reaction mixtures via flash column chromatography (ISCO Combi Flash) using a gradient and eluting with ethyl acetate (100%) to a mixture of ethanol in ethyl acetate (1:5 - 8:5). Concentrate to give each desired product.

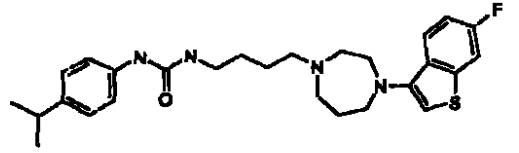
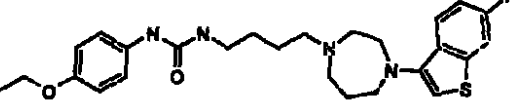
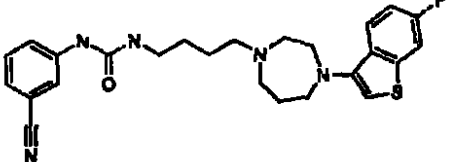
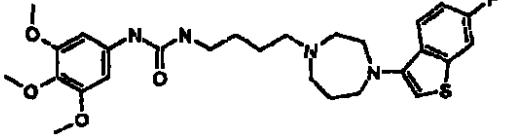
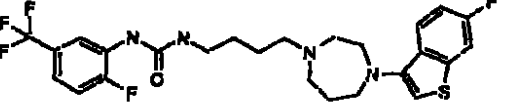
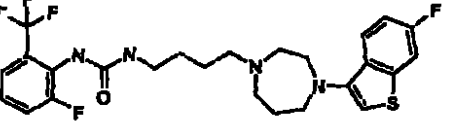
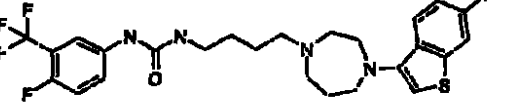
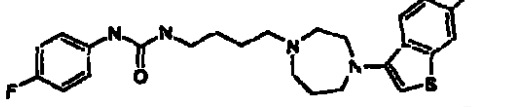
Example 16

Preparation of 1-[4-[4-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl]-3-(substituted)phenyl-ureas

Compound Number	Product	LC/MS (APCI) Condition III
16-1		m/e 455 (MH ⁺), retention time 1 min 44 sec
16-2		m/e 455 (MH ⁺), retention time 1 min 45 sec

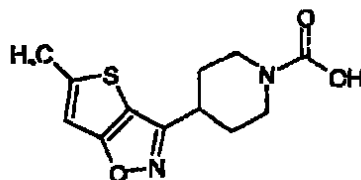
16-3		m/e 475 (MH ⁺) retention time 1 min 46 sec
16-4		m/e 459 (MH ⁺), retention time 1 min 42 sec
16-5		m/e 459 (MH ⁺) retention time 1 min 43 sec
16-6		m/e 509 (MH ⁺) retention time 1 min 47 sec.
16-7		m/e 471 (MH ⁺), retention time 1 min 41 sec
16-8		m/e 519 (MH ⁺) retention time 1 min 47 sec.
16-9		m/e 471 (MH ⁺) retention time 1 min 42 sec.
16-10		m/e 475 (MH ⁺), retention time 1 min 46 sec
16-11		m/e 477 (MH ⁺), retention time 1 min 43 sec
16-12		m/e 509 (MH ⁺), retention time 1 min 48 sec.

16-13		m/e 519 (MH ⁺), retention time 1 min 47 sec
16-14		m/e 525 (MH ⁺), retention time 1 min 49 sec
16-15		m/e 577 (MH ⁺) retention time 1 min 55 sec.
16-16		m/e 543 (MH ⁺) retention time 1 min 51 sec
16-17		m/e 543 (MH ⁺) retention time 1 min 51 sec.
16-18		m/e 477 (MH ⁺) retention time 1 min 48 sec
16-19		m/e 509 (MH ⁺) retention time 1 min 51 sec.
16-20		m/e 487 (MH ⁺), retention time 1 min 48 sec.
16-21		m/e 483 (MH ⁺) retention time 1 min 39 sec
16-22		m/e 483 (MH ⁺) retention time 1 min 40 sec

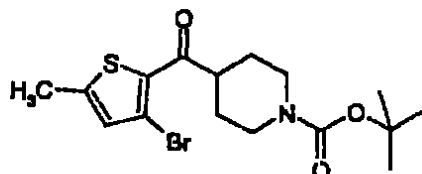
16-23		m/e 483 (MH ⁺) retention time 1 min 50 sec.
16-24		m/e 485 (MH ⁺), retention time 1 min 44 sec
16-25		m/e 466 (MH ⁺) retention time 1min 43 sec.
16-26		m/e 531 (MH ⁺), retention time 1 min 39 sec
16-27		m/e 527 (MH ⁺) retention time 1 min 49 sec.
16-28		m/e 527 (MH ⁺), retention time 1 min 43 sec.
16-29		m/e 527 (MH ⁺), retention time 1 min 50 sec
16-30		m/e 459 (MH ⁺) retention time 1 min 42 sec.

The above compounds were prepared in a manner analogous to Example 15. Evaporate the pure fractions from a purification performed via flash column chromatograph (ISCO Combi Flash) to yield each compound as an oil. Dry each oil under the vacuum pump to give the final desired compounds.

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Example 174-(5-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidine-1-carboxylic acid

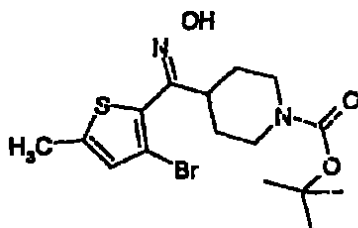
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17a Preparation of 4-[1-(3-bromo-5-methyl-thiophen-2-yl)-methanoyl]-piperidine-1-carboxylic acid tert-butyl ester

- 10 Prepared essentially as in Example 15a except that 2-bromo-5-methyl thiophene is used as the starting material. In addition, 1.20 equivalents of lithium diisopropylamide and 1.24 equivalents of 4-(methoxy-methyl-carbamoyl)-piperidine-1-carboxylic acid tert-butyl ester are used for the reaction. Accordingly, stirring time of the reaction mixture may vary. Purification of the residue via flash column chromatography uses a gradient with a mixture of ethyl acetate/n-heptane (1/9) to ethyl acetate/n-heptane (2/8) to yield a yellow oil. LC/MS (ESI), m/e 332 (M-56) and 389 (MH⁺); retention time 2.15 minutes. Condition II.
- 15

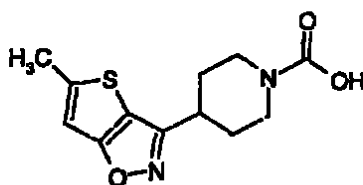
17b Preparation of 4-[1-(3-bromo-5-methyl-thiophen-2-yl)-1-hydroxyimino-methyl]-piperidine-1-carboxylic acid tert-butyl ester

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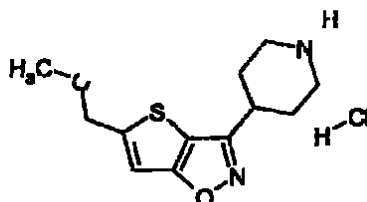
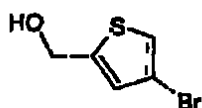


- Prepared essentially as Example 15b except that 4-[1-(3-bromo-5-methyl-thiophen-2-yl)-methanoyl]-piperidine-1-carboxylic acid tert-butyl ester is used as the starting material and the reaction mixture was stirred at 70° C for 6 hours. LC/MS (ESI) m/e 347 (M-56) and 403 (MH⁺); retention time 2.03 minutes. Condition II.
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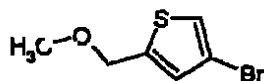
17c Preparation of 4-(5-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidine-1-carboxylic acid

Prepared essentially as Example 15c except that 4-[1-(3-bromo-5-methyl-thiophen-2-yl)-1-hydroxyimino-methyl]-piperidine-1-carboxylic acid tert butyl ester is used as the starting material. Two other differences are 1) 0.05 equivalents of copper iodide is used and 2) no partition between ethyl acetate and water accompanied by subsequent extraction with ethyl acetate is required. Purification of the residue via flash column chromatography uses a mixture of ethyl acetate/n-heptane (1/4) to yield a white solid. LC/MS (ESI) m/e 345 (MNa⁺) retention time 2.12 minutes. Condition II

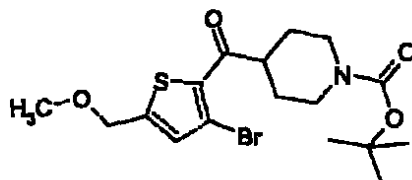
Example 185-methoxymethyl-3-piperidin-4-yl-thieno[2,3-d]isoxazole hydrochloride18a Preparation of (4-bromo-thiophen-2-yl)-methanol

Under inert conditions add sodium borohydride (13.82 mmol, 0.523 g, 2.08 equivalents) in absolute ethanol (16 mL) dropwise over a period of 15 minutes to a stirred mixture of 4-bromothiophene-2-carboxaldehyde (26.58 mmol, 5.08 g, 1.00 equivalents) in cold (0° C) absolute ethanol (32 mL). Stir the resulting mixture at room temperature for 2.5 hours and add glacial acetic acid dropwise until the effervescence ceases. Evaporate the resulting solution, take the residue up in diethyl ether (75 mL), wash with water (15 mL) and brine (15 mL) and dry over magnesium sulfate. Filter and evaporate to yield the product as a colorless oil (5.13 g).

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18b Preparation of 4-bromo-2-methoxymethyl-thiophene

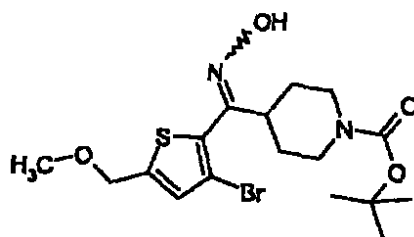
- 5 Add sodium hydride (737 mg, 29.23 mmol, 1.10 equivalents, 95%) to a solution containing methyl iodide (1.65 mL, 26.57 mmol, 1.00 equivalents) and (4-bromo-thiophen-2-yl)-methanol (5.13 g, 26.57 mmol, 1.00 equivalents) in tetrahydrofuran (dry, 25 mL). Stir the resulting mixture at room temperature overnight and evaporate. Partition the residue between water (100 mL) and dichloromethane (100 mL). Extract the aqueous layer with dichloromethane (100 mL), combine the organic layers, dry over magnesium sulfate, filter and evaporate to yield the desired product as a yellow oil.

18c. Preparation of 4-[1-(3-bromo-5-methoxymethyl-thiophen-2-yl)-methanoyl]-piperidine-1-carboxylic acid tert-butyl ester

- 15 Add lithium diisopropyl amide (13.20 mL, 26.37 mmol, 1.05 equivalents) to a stirred, cold (-78° C) solution of 4-bromo-2-methoxymethyl-thiophene (5.20 g, 25.11 mmol, 1.00 equivalents) in tetrahydrofuran (dry, 24.30 mL). Stir at -78° C for 1 hour and add a solution of 4-(methoxy-methyl-carbamoyl)-piperidine-1-carboxylic acid tert-butyl ester (6.84 g, 25.11 mmol, 1.00 equivalents) in tetrahydrofuran (dry, 19.40 mL) dropwise. Stir the resulting solution at -78° C for 3 hours. Quench the reaction mixture with saturated sodium chloride (aqueous, 50 mL). Allow the resulting mixture to warm to room temperature and extract with a mixture of ethyl acetate/diethyl ether (1:1, 3 x 35 mL). Combine the extracts, dry over magnesium sulfate, filter and evaporate. Purify the residue via flash column chromatography eluting with a mixture of n-heptane/ethyl acetate (4:1) to yield the desired product as a yellow oil (9.47 g). LC/MS (ESI), m/e 362 (M-56) and 418 (MH⁺). Retention time 2.08 minutes. Condition II.
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- 25

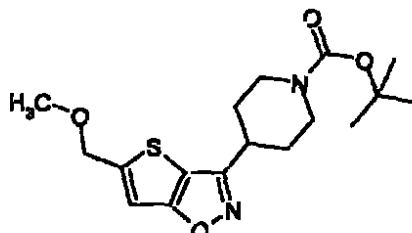
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18d Preparation of 4-[1-(3-bromo-5-methoxymethyl-thiophen-2-yl)-1-hydroxyimino-methyl]-piperidine-1-carboxylic acid tert-butyl ester



5 Add hydroxylamine hydrochloride (2.29 g, 45.27 mmol, 2.00 equivalents) to a stirred solution of 4-[1-(3-bromo-5-methoxymethyl-thiophen-2-yl)-methanoyl]-piperidine-1-carboxylic acid tert-butyl ester (9.47 g, 22.64 mmol, 1.00 equivalents) in pyridine (42.40 mL). Stir the resulting solution at room temperature overnight and then at 70° C for 4 hours. Cool the reaction mixture slightly, add hydrochloric acid (3N, 100 mL) and extract the resulting mixture with dichloromethane (100 mL). Wash the extract with water (100 mL), dry over magnesium sulfate, filter and evaporate to yield the desired product as a yellow oil (9.48 g).

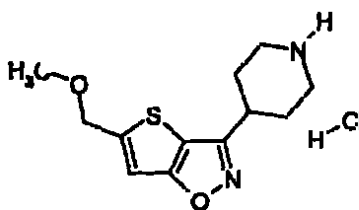
18e Preparation of 4-(5-methoxymethyl-thieno[2,3-d]isoxazol-3-yl)-piperidine-1-carboxylic acid tert-butyl ester



15 Add cesium carbonate (1.13 g, 3.46 mmol, 1.50 equivalents) and copper iodide (44 mg, 0.23 mmol, 0.10 equivalents) to a stirred solution of 4-[1-(3-bromo-5-methoxymethyl-thiophen-2-yl)-1-hydroxyimino-methyl]-piperidine-1-carboxylic acid tert-butyl ester (1.00 g, 2.31 mmol, 1.00 equivalents) in 2-methoxy ethanol (23.30 mL). Stir the resulting mixture at room temperature overnight or up to 3 days and filter through celite. Evaporate the filtrate, partition the residue between ethyl acetate (70 mL) and water (23 mL) and separate. Extract the aqueous layer with ethyl acetate (3 x 70 mL), combine the organic layers, dry over magnesium sulfate, filter and evaporate. Purify the residue via flash column chromatography eluting with a mixture of hexane:ethyl acetate (4:1) to yield the desired product as a yellow oil. LC/MS (ESI) m/e 375 (MNa⁺) retention time 1.98 minutes. Condition II

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18f Preparation of 5-methoxymethyl-3-piperidin-4-yl-thieno[2,3-d]isoxazole hydrochloride

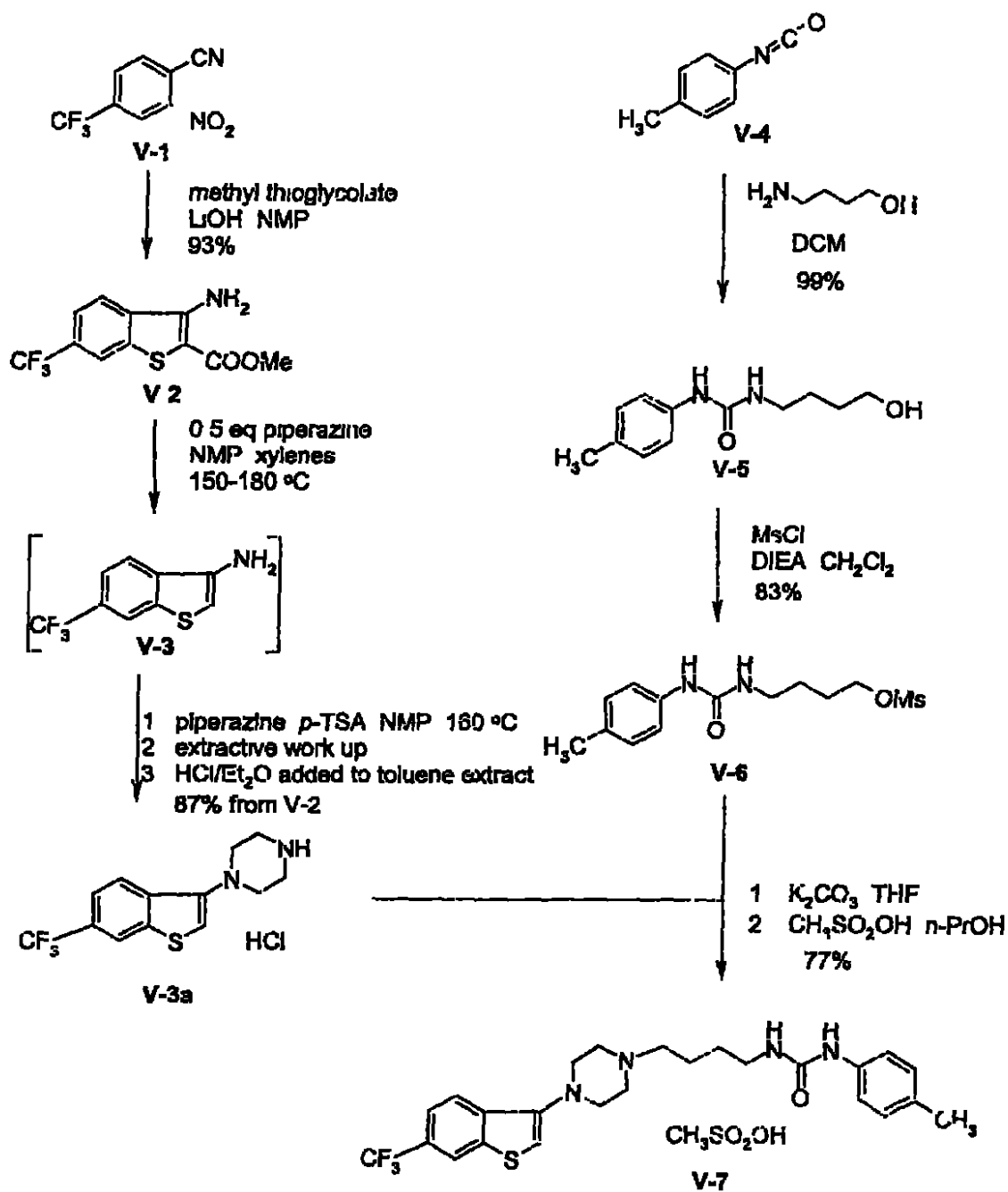
Stir a solution of 4-(5-methoxymethyl-thieno[2,3-d]isoxazol-3-yl)-piperidine-1-carboxylic acid
 tert-butyl ester (2.21 g, 6.68 mmol, 1.00 equivalents) and hydrochloric acid (1.0 M in diethyl
 ether, 35 mL) overnight to form a suspension. Add additional hydrochloric acid (1.0 M in
 diethyl ether, 10 mL). Stir the suspension overnight, filter and wash the solid with ether.
 Collect the solid and dry to yield the desired product as a dark blue solid. LC/MS (ESI) m/e
 253 (MH^+) retention time 1.17 minutes. Condition II.

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Example 19

Scheme V



General Gas chromatography/mass spectroscopy was accomplished using a HP Model 5972 system with the following conditions 0.25 mm x 30 m HP 5MS column cross-linked 5% Ph Me silicone, 0.25 μ film thickness injector at 250 °C, detector at 280 °C, 50 °C for 1 min ramp at 20 °C/min to 300 °C, 300 °C for 5 min to 10 min

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2-Carbomethoxy-3-amino-6-trifluoromethylbenzo[b]thiophene (V-2)

A 22-L, 3-necked, round-bottom flask equipped with a mechanical stirrer nitrogen bubbler, and a thermocouple probe was charged with 1.20 kg (5.55 mole) of 2-nitro-4-trifluoromethylbenzonitrile, 496 mL (589.3 g 5.55 mole) of methyl thioglycolate, and 4.3 L of NMP. The resulting yellow solution was cooled (-10 °C bath) to 1 °C, and a solution prepared from 486.0 g (11.11 mole, 2.0 eq) of lithium hydroxide monohydrate in 3.36 L of water was added over a period of 2 h while maintaining a temperature of 2-16 °C. The tan-orange slurry was stirred at 0-8 °C for 45 min, then was diluted with 8.0 L of water (T_{max} → 21 °C). After stirring for 30 min and cooling to 14 °C the product was collected by filtration rinsing with 15 L of water then air-drying at ambient temperature to give 1.43 kg (93.5% yield) of 2-carbomethoxy-3-amino-6-trifluoromethylbenzo[b]thiophene as a light-yellow solid.

3 Piperazinyl-6-trifluoromethylbenzo[b]thiophene hydrochloride (V-3a)

A 22-L, 3-necked, round-bottom flask equipped with a mechanical stirrer nitrogen bubbler and a thermocouple probe was charged with 1.42 kg (5.17 mole) of 2-carbomethoxy-3-amino-6-trifluoromethylbenzo[b]thiophene (V-2) 245.0 g (2.84 mole 0.55 eq) of piperazine, 5.0 L of NMP and 720 mL of xylene. The solution was heated to and held at 165-176 °C for 4.5 h, at which time the reaction was ca. 97% complete as determined by hplc assay. A total of 2.00 kg (23.22 mole 4.5 eq) of piperazine ($T > 120$ °C) and 1.97 kg (10.36 mole 2.0 eq) of *p*-toluenesulfonic acid monohydrate (exotherm observed 120 → 140 °C) were added to the brown solution. A Dean-Stark trap was connected to the condenser, and the reaction was heated to collect an azeotrope. A total of 350 mL of an aqueous distillate was removed allowing the pot temperature to increase from 140 to 158 °C. The Dean-Stark trap was disconnected. The progress of the reaction was monitored by GC/MS assays. After 12 h at ca. 158-162 °C (>99% conversion by GC/MS assay) the reaction was cooled to 40 °C then quenched into an extractor that contained 6.2 kg of ice, 15 L of water, and 10.6 L of toluene. The phases were separated, and the aqueous layer was extracted with 2 L of toluene. The combined organic extract was washed with 13.7 L of 0.5 N NaOH.

followed by 2.5 L of saturated aq. NaCl, then was extracted with 10 L of 1 N HCl. The acidic aqueous extract was diluted with 1.2 kg of ice, then was basified to pH 10.7 by adding 770 g of 50% NaOH. The resulting mixture was extracted with 11 L of toluene. The toluene extract was washed with 2.5 L of saturated aq. NaCl, dried (Na_2SO_4) and filtered. The filtrate was charged into a 22 L, 3-necked, round-bottomed flask (N_2 , mechanical stirring, TC probe). A total of 4.5 L of 1 N ethereal HCl was added at 20 – 27 °C until the mixture was positive to Congo Red indicator paper. After stirring at ambient temperature for 35 min, the slurry was filtered and washed with 5 L of toluene. After air drying, 1.44 kg (86.5% yield) of 3-piperazinyl-6-fluoromethyl-
benzo[b]thiophene hydrochloride (V-3a) was obtained as a light pink-beige solid.

***N*-(4-Hydroxybutyl)-*N'*-(4-tolyl) urea (V-5)**

A 12-L, 3-necked, round-bottom flask equipped with a mechanical stirrer, nitrogen bubbler, and a thermocouple probe, was charged with 8 L of methylene chloride and 367.7 g (4.13 moles, 1.10 eq.) of 4-amino-1-butanol, and the resulting solution was cooled to 0 – 5 °C. A solution of 499.3 g (3.75 moles, 1.00 eq.) of 4-tolyl isocyanate in 0.5 L of methylene chloride was added over a period of 2.5 h while maintaining a temperature of 0 – 12 °C. The white slurry was stirred for 1 h at 0 – 10 °C, then was filtered, washed with 3 L of methylene chloride, and air dried to afford 0.83 kg (99.9%) of *N*-(4-hydroxybutyl)-*N'*-(4-tolyl) urea (V-5) as a white solid.

***N*-(4-Butylmesylate)-*N'*-(4-tolyl) urea (V-6)**

A 22-L, 3-necked, round-bottom flask equipped with a mechanical stirrer, nitrogen bubbler and a thermocouple probe, was charged with 2.057 kg (9.25 moles) of *N*-(4-hydroxybutyl)-*N'*-(4-tolyl) urea (V-5), 13 L of DCM, and 2.02 L (1.5 kg, 11.57 mole, 1.25 eq.) of diisopropylethylamine. The white suspension was cooled to 6 °C and a solution of 1.22 kg (10.64 mole, 1.15 eq.) of methanesulfonyl chloride in 0.25 L of DCM was added over a period of ca. 2 h while maintaining a pot temperature of 5 – 12 °C. After stirring for 10 min at 5 – 10 °C (100% conversion by hplc assay) the light-yellow solution was quenched into an extractor that contained 9 L of 1 N HCl. The phases were separated. The organic phase was washed with 9 L of 1 N HCl, washed with 9 L of 5% aq. NaHCO_3 , dried (MgSO_4), filtered and then was partially concentrated (30 °C, 150 mbar) to a mass of 6.41 kg. After stirring in an ice bath for 30 min, the resulting slurry was filtered, washed with 2 L of cold DCM, and air dried to give 2.32 kg (83.4%) of *N*-(4-butylmesylate)-*N'*-(4-tolyl) urea (V-6) as a white solid.

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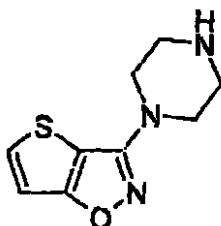
1-*p*-Tolyl-3-[4-[4-(6-trifluoromethylbenzo[*b*]thieny-3-yl) piperazin-1-yl]butyl]-urea (V-7, free base)

A 22-L 3-necked round-bottom flask equipped with a mechanical stirrer, nitrogen bubbler, and a thermocouple probe was charged with 1.42 kg (4.40 mole) of V-3a, 1.757 kg (5.85 mole, 1.33 eq) of *N*-(4-butylmesylate)-*N'*-(4-tolyl) urea (V-6), 4.26 L of THF, 1.23 L of water, and 1.52 kg (11.00 mole, 2.50 eq) of K₂CO₃. Using a temperature controller, the biphasic mixture was heated at 48 - 51 °C for 22 h. The resulting thick slurry was charged to a 20-gal glass-lined steel reactor along with rinses of 9.8 L of THF and 1.05 L water, and heated to reflux (65 °C). A total of 13.1 L of water was added to the biphasic mixture over a period of ca. 10 min during which the temperature dropped to ca. 60 °C. The batch was then slowly cooled (crystallization occurred at 43 °C) to 0 - 5 °C and aged at this temperature for 30 min, then was filtered, slurry-rinsed in several portions using a cold mixture of 5 L THF/0.42 L water, and given a final rinse with 3 x 12 L of water. After air drying, 1.72 kg (79.7%) of 1-*p*-tolyl-3-[4-[4-(6-trifluoromethylbenzo[*b*]thieny-3-yl) piperazin-1-yl]butyl]-urea (V-7, free base) as a white fluffy solid was obtained.

1-*p*-Tolyl-3-[4-[4-(6-trifluoromethylbenzo[*b*]thieny-3-yl) piperazin-1-yl]butyl]-urea methanesulfonate (V-7)

A 22-L 3-necked round-bottom flask equipped with a mechanical stirrer, nitrogen bubbler, and a thermocouple probe, was charged with 4.045 kg (8.245 mole) of V-7 (free base) and 11 L of *n*-propanol. A solution of 792.4 g (8.245, 1.00 eq) of methanesulfonic acid in 1 L of *n*-propanol was added in one portion. An exotherm was observed (T → 39 °C), and the mixture became nearly homogeneous at the end of the addition. The mixture was heated to 92 °C, at which temperature all solids had dissolved. The solution was filtered through a coarse frit to remove a trace amount of extraneous matter. The clear, red-brown filtrate was charged into a 20-gal glass-lined steel reactor along with 3.3 L of *n*-PrOH rinse. After slowly cooling and seeding at 51 °C, crystallization commenced at 47 °C. Upon further crystallization and cooling to 37 °C, the slurry became very thick and was diluted with 12.1 L of *n*-PrOH. After cooling to 0 - 10 °C and stirring for 60 min, product was collected by filtering, rinsing with 5 L of cold *n*-PrOH, and drying (tray oven, 105-115 °C, 100 mm Hg, N₂ bleed) to give 4.67 kg (96.5%) of 1-*p*-tolyl-3-[4-[4-(6-trifluoromethylbenzo[*b*]thieny-3-yl) piperazin-1-yl]butyl] urea methanesulfonate (V-7) as a white to off-white solid.

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Example 20

Synthesis of BOC protected piperazine-thienylisoxazole

5 **3-Bromothiophene-2-carbaldehyde oxime**

3-Bromothiophene-2-carbaldehyde (Maybridge) (28.7 gm 0.15 mol) in ethanol (50 ml) was added in one portion to a solution of hydroxylamine hydrochloride (13.8 gm 0.2 mole) sodium hydroxide (8 gm 0.2 mol) in water (30 ml) and ethanol (100 ml). The mixture was stirred at 0°C for 2 hours and was kept at 0°C overnight. The reaction mixture was diluted with cold water (600 ml) and the precipitated solids were collected by filtration to provide 20.5 gm (67%) of product. The aqueous layer was further extracted with ethyl acetate and the combined organic layers were washed with brine, dried with magnesium sulfate, filtered and concentrated in vacuo to leave an additional 8.9 g of product.

15 **3-Bromothiophene-2-hydroximidoyl chloride**

To a solution of 3-bromothiophene-2-carbaldehyde oxime (10.8 gm 52.4 mmol) hydrogen chloride (14.5 ml, 4M in dioxane) in DMF (100 ml) was added oxone (16.9 gm 1.05 equiv) in one portion at room temperature. The mixture was stirred at ambient temperature overnight. At the end of the reaction, DMF solution was poured into water and product was extracted into ethyl acetate. The organic solution was washed with brine, dried over magnesium sulfate, filtered and concentrated in vacuo to 12.68 gm of product, which was used in the next reaction without further purification.

(4-(t-Butoxycarbonyl)piperazinyl)-3-bromo-2-thienyl methanone oxime

25 3-bromothiophene-2-hydroximidoyl chloride (16.4 gm, 68 mmol) in tetrahydrofuran (THF, 70 ml) was added dropwise to a solution of N-(t-butoxycarbonyl)piperazine (14 gm 1.1 equiv) DABCO (9.5 gm 1.25 equiv) in DMF (100 ml) at 0°C over 25 minutes. The mixture was stirred for 3.5 hrs. At the end, the mixture was poured into water and was extracted with ethyl acetate. The organic was washed with brine and dried over magnesium sulfate. The solvent was removed on a rotary evaporator. The crude product (30.5 gm) was purified by chromatography on a Blotage cartridge (400 gm of silica gel), eluting

with methanol in dichloromethane (0-5 % of MeOH) The product thus obtained weighed 24.6 gm (85%)

(t-BOC-piperazine)-3-thienylbenzoxazole

- 5 A mixture of (4-t-Butoxycarbonylpiperazinyl)-3-bromo-2-thienyl methanone oxime (10.3 gm 26.4 mmol), cesium carbonate (10.7 gm 32.7 mmol), and copper iodide (500 mg) in methoxyethanol (200 ml) was stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate, then washed with water. The aqueous solution was extracted three times with ethyl acetate. The organic solution (total 600 ml) was washed with brine and was
- 10 dried over magnesium sulfate then concentrated to an oil (~10 gm). This material was purified by chromatography using a Biotage cartridge (120 gm of silica gel) eluting with 0-8% Methanol in dichloromethane. The product thus obtained as light oil (5.1 gm, 62%)

Example 21

15

The following scheme exemplifies the synthesis of a radiolabeled C14 intermediate useful for the preparation of certain compounds within the scope of the present invention

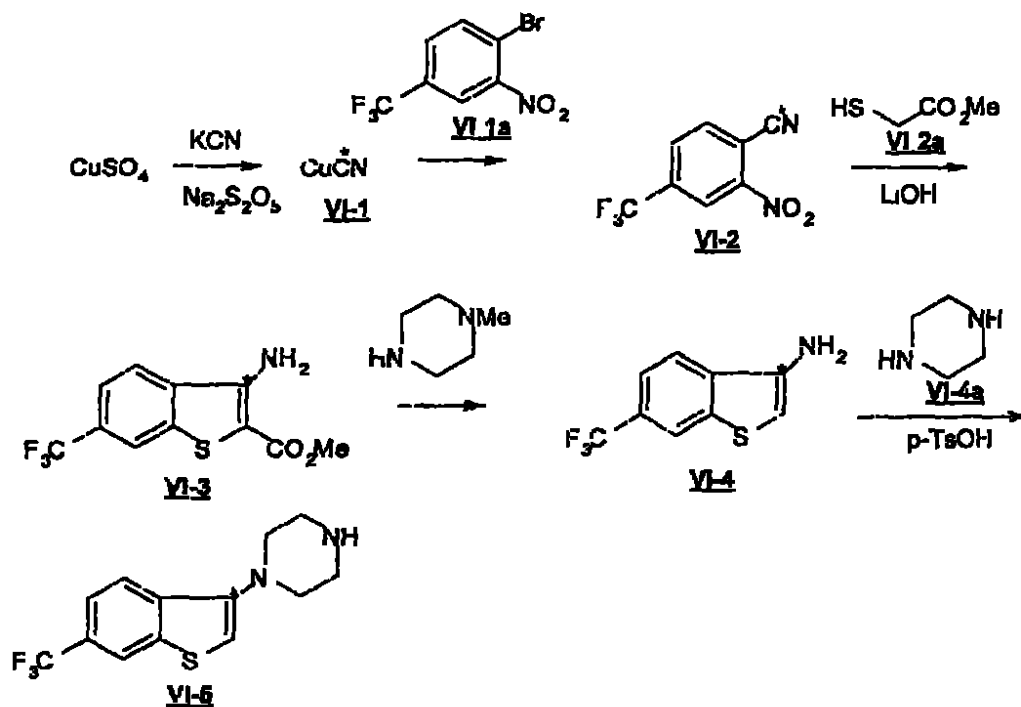
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Scheme VI

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General Analytical thin layer chromatography (TLC) was performed on E Merck TLC plates with silica gel 60 F₂₅₄ (0.25 mm). TLC plates used in the analysis of radioactive samples were scanned on a BIOSCAN system 2000 Imaging Scanner using P-10 gas (10% methane 90% argon). Identity of the intermediates was established by co-migration in radio-TLC and/or radio-HPLC with the standard samples of unlabeled analogues. Flash chromatography was performed using silica gel with a particle size of 40-63 μm . Specific activity was determined on a Packard Minaxi Tri-Carb Liquid Scintillation Analyzer (Model 1600 TR) using Bio-Safe II as scintillation cocktail.

Purification of compounds VI-2, VI-3, VI-4, VI-5 and VI-6 was monitored by HPLC (conditions A) which was carried out on Waters 500 Controller, Waters 996 Photodiode Array Detector, Millennium Chromatography Manager and Beta-Ram Radioactive Flow Through Monitor System, Model 2 (IN/US Systems Inc.). Final purity determination of VI-7 by HPLC (conditions B) was performed on Waters Model 510 Pumps, Waters 680 Gradient Controller, Waters 715 Ultra Wisp Autosampler, Waters 484 Tunable Absorbance Detector and Beta-Ram Radioactive Flow-Through Monitor System, Model 2 (IN/US Systems Inc.).

Conditions A YMC Basic 5 μ m C18 4.6x250 mm mobile phase A (v/v) 50/50 acetonitrile/0.1N ammonium formate mobile phase B (v/v) 75/25 acetonitrile/0.1N ammonium formate flow rate 1.0 mL/min uv detection at 254 nm

5	Gradient:	Time (minutes)	%MP A	%MP B
		0	100	0
		15	100	0
		25	0	100
		30	0	100
10		35	100	0

Conditions B Ultramex 5 μ m C8 4.6 x 150 mm mobile phase (v/v/v) 50/50/0.25 acetonitrile/0.05 M potassium phosphate buffer pH 3.0/triethylamine flow rate 1.0 mL/min uv detection at 210 nm.

15

[^{14} C] Copper (I) Cyanide (VI-1)

A solution of copper (II) sulfate pentahydrate

(4.10 g 16.67 mmol) in water (13.3 mL) was heated to 70 °C and a solution of sodium metabisulfite (1.94 g 6.28 mmol) in water (3.3 mL) at 70 °C was added in one minute

20 Immediately a solution of [^{14} C] potassium cyanide (245.5 mg 200 mCi 3.77 mmol S.A. 53.0 mCi/mmol) and unlabeled potassium cyanide (0.84g, 12.9 mmol) in water (3.3 mL) at 70 °C was added in one minute. A white solid precipitated out of solution and blue color of the solution was discharged. After stirring for 10 min at 70 °C the mixture was filtered hot and the solid was washed with hot water (15mL) and ethanol (15mL). The white solid was dried

25 under vacuum (0.1 mm Hg) for 27 h 45 min to give VI-1 (1.393 g 186.6 mCi) in 93.3% yield

2-Nitro-4-(trifluoromethyl)-[7- ^{14} C]benzonitrile (VI-2)

To a suspension of [^{14} C]copper (I) cyanide (VI-1) (1.393 g 15.55 mmol 186.6 mCi) in 1-methyl-2-pyrrolidinone (NMP 10mL) was added 4-bromo-3-nitrobenzotrifluoride (6.33 g 23.45 mmol) and the mixture was heated at 190-195 °C for 1 h. Ethyl acetate (25mL) and water (20 mL) were added at room temperature and the mixture was filtered through celite. To the filtrate more water (20mL) and ethyl acetate (25mL) were added and the aqueous layer was extracted with ethyl acetate (90mL). The organic extract was washed with iron (III) chloride solution (50mL) prepared by dissolving iron (III) chloride (7.468 g 46.04 mmol) in

water (50 mL) The organic extract was further washed with water (30mL) sat. sodium chloride (15mL), dried (Na_2SO_4) and the solvent was removed *in vacuo*

The residue was purified by flash chromatography on silica gel (hexane/ethyl acetate 9/1-7/3) to provide an oil which was dissolved in hexane (70 mL) The solvent was removed

5 under reduced pressure and residue was dried under vacuum for 15 h 40 min to provide VI-2 (3.01 g, 167.13 mCi, 89.6% yield) as a yellow solid Radio-TLC (hexane/ethyl acetate 9/1), $R_f = 0.21$, HPLC (System A), RCP 99.86% (ret. time, 9.2 min)

[3- ^{14}C]-3-Amino-2-carbomethoxy-6-trifluoromethylbenzo[b]thiophene (VI-3)

10 Nitrile (VI-2) (3.01g, 13.9 mmol, 167.13mCi) was dissolved in DMF (14mL) and methyl thioglycolate (1.78g, 15.94 mmol, 95%) was added in one minute The mixture was cooled to 0-5 °C and a solution of lithium hydroxide (0.689 g, 28.77 mmol) in water (9.2mL) was added dropwise in 12 minutes After the addition cooling bath was removed and the mixture was stirred at room temperature for 4 hours Water (70mL) was added at 0-5 °C and the mixture

15 was stirred for 15 min at 0-5 °C the solid was collected on a filter washed with water (20 mL) and dried under vacuum (0.1 mm Hg) for 40 h 15 min to provide VI-3 (3.469 g, 151.24 mCi, 90.49% yield) Radio-TLC (CH_2Cl_2) $R_f = 0.372$ HPLC (system A) RCP 99.92% (ret time 16.722 min)

[3- ^{14}C]-3-Amino-6-trifluoromethylbenzo[b]thiophene (VI-4)

20 To a solution of benzo[b] thiophene (VI-3) (3.469 g, 12.6 mmol, 151.2 mCi) in NMP (14mL) was added 1-methylpiperazine (6.69g, 66.79 μmol) and the mixture was heated at 140-145 °C for 5 h The mixture was allowed to cool to room temperature, poured into water (60mL) and extracted with ethyl acetate (140 mL) The organic extract was washed with water (30

25 mL) sat. sodium chloride (10mL) dried (Na_2SO_4) and the solvent was removed *in vacuo* The residue was purified by flash chromatography on silica gel (hexane/ethyl acetate 1/1) to yield a greenish solid which was dried under vacuum (0.1 mm Hg) for 14 h to provide VI-4 (w 66g, 146.95 mCi, 97.16% yield) Radio-TLC (hexane/ethyl acetate 1/5), $R_f = 0.407$, HPLC (system A) RCP 99.44 % (ret time 10.552 min)

30

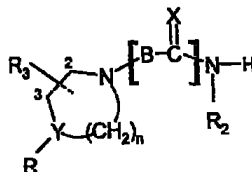
1-[6-(trifluoromethyl)benzo[b]thien-3-yl]-[3- ^{14}C]piperazine (VI-5)

To a solution of benzo[b]thiophene (VI-4) (2.66 g, 12.24 mmol, 146.95 mCi) in NMP (17mL) was added piperazine (4.309g, 50.02 mmol) and p-toluenesulfonic acid (4.76g, 25.02 mmol) at room temperature The mixture was heated at 170 °C for 20 h 24

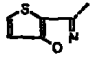
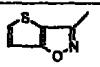
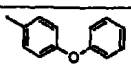
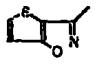
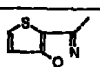
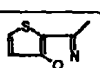
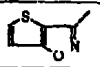
35 min allowed to cool to room temperature and poured into a solution of sodium carbonate

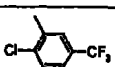
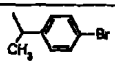
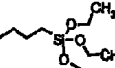
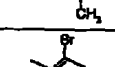
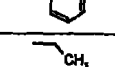
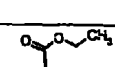
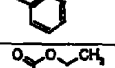
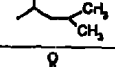
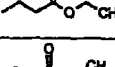
(4.70 g, 44.3 mmol) in water (60 mL). The mixture was extracted with ethyl acetate (20 mL), dried (Na_2SO_4) and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$, 9/1/0.2) and product was dried under vacuum (0.1 mm Hg) for 11 h 50 min. Ethanol (absolute, 30 mL) was added to the product and solvent was removed under reduced pressure. The residue was dried under vacuum (0.1 mm Hg) for 24 h 55 min to provide VI-5 (3.44 g, 144.18 mCi, 98.1% yield) as an oil. Radio-TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$, 9/1/0.2) $R_f=0.46$. HPLC (system A) RCP 99.86% (retention time 5.807 min).

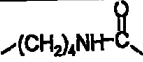
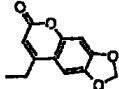
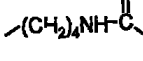
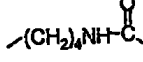
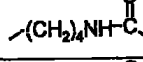
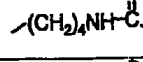
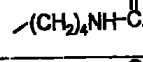
TABLE III



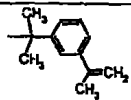
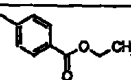
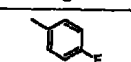
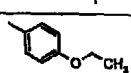
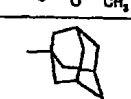
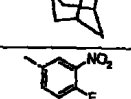
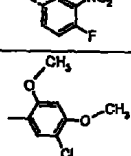
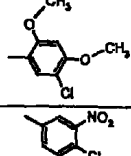
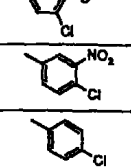
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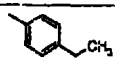
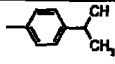
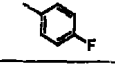
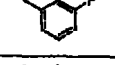
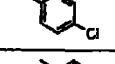
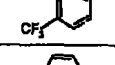
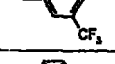
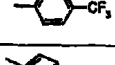
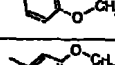
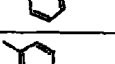
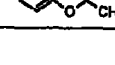
815945		2			II	CH	116
815946		2			H	CH	28 3
815947		2			H	CH	26
815948		2			H	CH	6 04
815949		2			H	CH	13 6
815950		2			H	CH	4 59
815951		2			H	CH	2 23
815952		2			H	CH	27

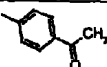
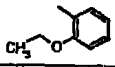
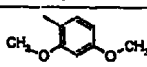
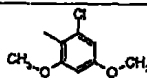
44

815953		2	$(CH_2)_4NH-C(=O)-$		H	CH	67.9
815954		2	$(CH_2)_4NH-C(=O)-$		H	CH	14.9
81595		2	$(CH_2)_4NH-C(=O)-$		H	CH	23.5
81595		2	$(CH_2)_4NH-C(=O)-$		H	CH	5.24
816210		2	$(CH_2)_3NH-C(=O)-$		H	CH	187
816557		2	$(CH_2)_4NH-C(=O)-$		H	CH	20.7
816558		2	$(CH_2)_4NH-C(=O)-$		H	CH	33.3
816559		2	$(CH_2)_4NH-C(=O)-$		H	CH	13.1
816560		2	$(CH_2)_4NH-C(=O)-$		H	CH	10.9

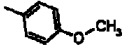
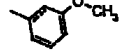
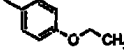
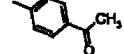
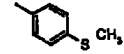
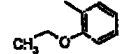
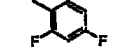
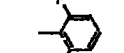
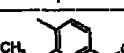
10/

816561		2	$-(CH_2)_4NH-C(=O)-$		H	CH	24.2
816562		2	$-(CH_2)_4NH-C(=O)-$		H	CH	6.99
816563		2	$-(CH_2)_4NH-C(=O)-$		H	CH	21.5
816564		2	$-(CH_2)_4NH-C(=O)-$		H	CH	55.9
816565		2	$-(CH_2)_4NH-C(=O)-$		H	CH	55.5
816566		2	$-(CH_2)_4NH-C(=O)-$		H	CH	95.1
816567		2	$-(CH_2)_4NH-C(=O)-$		H	CH	73.8
816568		2	$-(CH_2)_4NH-C(=O)-$		H	CH	11.2
816748		2	$-(CH_2)_4NH-C(=O)-$		H	CH	97.2
816749		2	$-(CH_2)_4NH-C(=O)-$		H	CH	11.5
816750		2	$-(CH_2)_4NH-C(=O)-$		H	CH	92.7

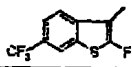
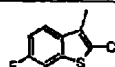
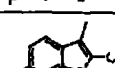
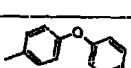
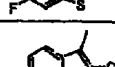
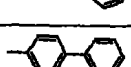
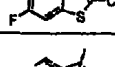
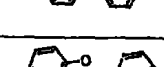
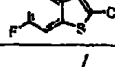
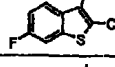
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816752		2	$(CH_2)_5NH-C(=O)-$		H	CH	157
816753		2	$(CH_2)_5NH-C(=O)-$		H	CH	115
816754		2	$(CH_2)_5NH-C(=O)-$		H	CH	108
816755		2	$(CH_2)_5NH-C(=O)-$		H	CH	80.8
816756		2	$(CH_2)_5NH-C(=O)-$		H	CH	96.1
816757		2	$(CH_2)_5NH-C(=O)-$		H	CH	92.6
816758		2	$(CH_2)_5NH-C(=O)-$		H	CH	116
816759		2	$(CH_2)_5NH-C(=O)-$		H	CH	107
816760		2	$(CH_2)_5NH-C(=O)-$		H	CH	121
816761		2	$(CH_2)_5NH-C(=O)-$		H	CH	82.9

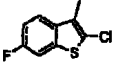
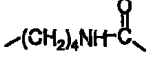
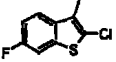
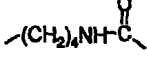
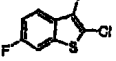
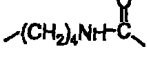
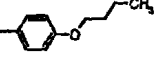
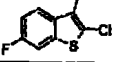
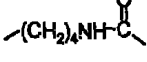
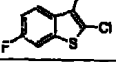
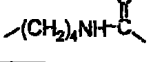
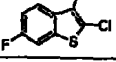
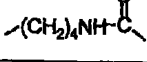
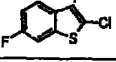
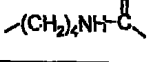
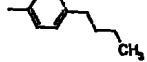
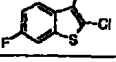
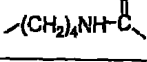
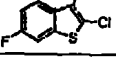
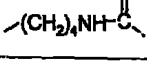
816762		2	$-(CH_2)_5NH-C(=O)-$		H	CH	57.1
816763		2	$-(CH_2)_5NH-C(=O)-$		H	CH	78.9
816764		2	$-(CH_2)_5NH-C(=O)-$		H	CH	85.4
816765		2	$-(CH_2)_5NH-C(=O)-$		H	CH	104
816766		2	$-(CH_2)_5NH-C(=O)-$		H	CH	42.9
816767		2	$-(CH_2)_5NH-C(=O)-$		H	CH	60.1
816768		2	$-(CH_2)_5NH-C(=O)-$		H	CH	23.4
816769		2	$-(CH_2)_5NH-C(=O)-$		H	CH	57.8
816770		2	$-(CH_2)_5NH-C(=O)-$		H	CH	28.2
816771		2	$-(CH_2)_5NH-C(=O)-$		H	CH	74.1

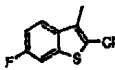
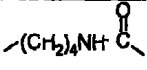
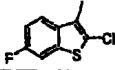
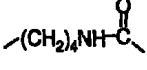
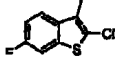
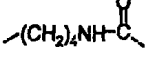
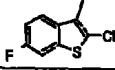
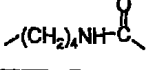
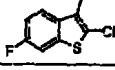
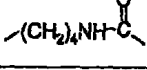
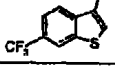
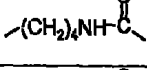
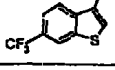
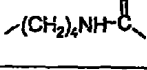
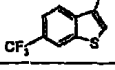
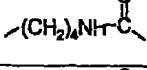
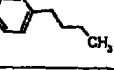
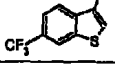
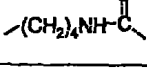
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816861		2	$-(CH_2)_6NH-C(=O)-$		H	CH	14.8
816862		2	$-(CH_2)_6NH-C(=O)-$		H	CH	19
816863		2	$-(CH_2)_6NH-C(=O)-$		H	CH	20
816864		2	$-(CH_2)_6NH-C(=O)-$		H	CH	21.5
816865		2	$-(CH_2)_6NH-C(=O)-$		H	CH	11.9
816866		2	$-(CH_2)_6NH-C(=O)-$		H	CH	8.25
816867		2	$-(CH_2)_6NH-C(=O)-$		H	CH	7.38
816868		2	$-(CH_2)_6NH-C(=O)-$		H	CH	58.6
816869		2	$-(CH_2)_6NH-C(=O)-$		H	CH	23.5
816870		2	$-(CH_2)_6NH-C(=O)-$		H	CH	17.6

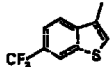
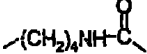
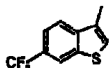
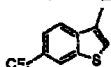
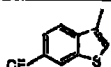
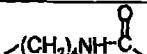
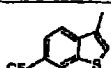
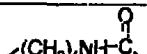
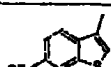
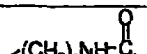
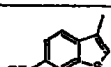
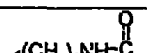
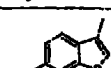
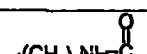
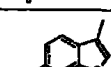
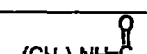
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816872		2	$(CH_2)_8NH-C(=O)-$		H	CH	19
816873		2	$(CH_2)_5NH-C(=O)-$		H	CH	22
816874		2	$(CH_2)_8NH-C(=O)-$		H	CH	6.84
816875		2	$(CH_2)_8NH-C(=O)-$		H	CH	35.4
816876		2	$(CH_2)_8NH-C(=O)-$		H	CH	46.9
816877		2	$(CH_2)_8NH-C(=O)-$		H	CH	19.1
816878		2	$(CH_2)_5NH-C(=O)-$		H	CH	33.8
816879		2	$(CH_2)_8NH-C(=O)-$		H	CH	47
816880		2	$(CH_2)_8NH-C(=O)-$		H	CH	14.7

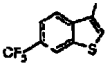
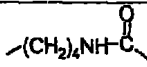
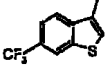
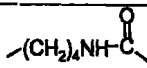
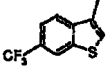
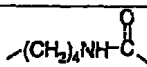
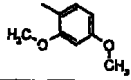
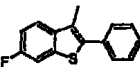
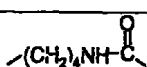
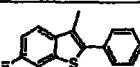
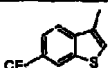
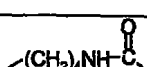
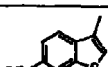
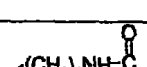
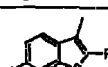
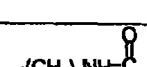
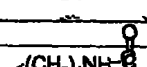
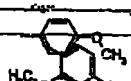
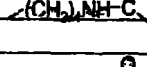
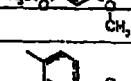
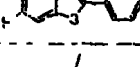
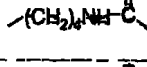
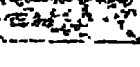
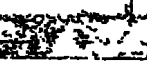
816881		2	$(CH_2)_8NH-C(=O)-$		H	CH	23.8
816882		2	$(CH_2)_8NH-C(=O)-$		H	CH	58.6
816883		2	$(CH_2)_8NH-C(=O)-$		H	CH	35.5
816968		2	$(CH_2)_8NH-C(=O)-$		H	CH	24.2
817075		2	$(CH_2)_4NH-C(=O)-$		H	CH	12.7
818280		2	$(CH_2)_8NH-C(=O)-$		H	CH	21.7
818283		2	$(CH_2)_8NH-C(=O)-$		H	CH	13.1
818308		2	$(CH_2)_8NH-C(=O)-$		H	CH	35.1
818309		2	$(CH_2)_8NH-C(=O)-$		H	CH	32.0
818310		2	$(CH_2)_8NH-C(=O)-$		H	CH	13.3

818316		2	$-(CH_2)_3NH-C(=O)-$		H	CH	426
818318		2	$-(CH_2)_3NH-C(=O)-$		H	Cl	384
818426		2	$-(CH_2)_3NH-C(=O)-$		H	CH	158
817510		2	$-(CH_2)_4NH-C(=O)-$		H	N	0 665
815158		2	$-(CH_2)_4NH-C(=O)-$		H	N	7 4
815159		2	$-(CH_2)_4NH-C(=O)-$		H	N	85 4
815160		2	$-(CH_2)_4NH-C(=O)-$		H	N	192
815161		2	$-(CH_2)_4NH-C(=O)-$		H	N	75 9
815162		2	$-(CH_2)_4NH-C(=O)-$		H	N	38
815163		2	$-(CH_2)_4NH-C(=O)-$		H	N	25 2

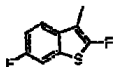
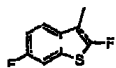
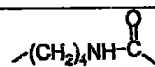
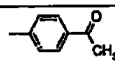
815164		2			H	N	319
815165		2			H	N	214
815166		2			H	N	37
815167		2			H	N	48
815168		2			H	N	334
815169		2			H	N	112
815170		2			H	N	177
815171		2			H	N	38
815172		2			H	N	56

815173		2			H	N	46.5
815174		2			H	N	6
815175		2			H	N	11.8
815176		2			H	N	17.1
815177		2			H	N	18
815993		2			H	N	2.26
815994		2			H	N	8.87
815995		2			H	N	17.5
815996		2			H	N	5.85

815997		2			H	N	4.5
815998		2			H	N	22.5
815999		2			H	N	19
816000		2			H	N	6.31
816001		2			H	N	4.68
816002		2			H	N	12.5
816003		2			H	N	1.58
816004		2			H	N	6.11
816005		2			F	N	2.54

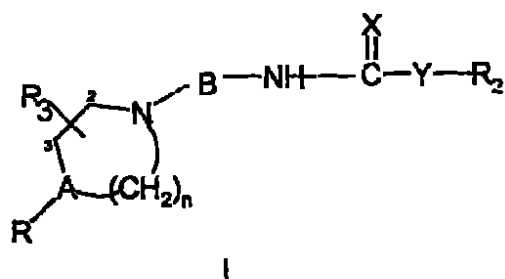
816006		2			H	N	7.42
816097		2			H	N	17.5
816008		2			H	N	17
816226		2			H	N	184
816232		2			H	N	651
815944A HCl Salt		2			H	N	5.94
815997A FCl Salt		2			H	N	4.08
817510		2			H	N	0.665
817511		2			H	N	1
816008		2			H	N	17
816226		2			H	N	184
816226		2			H	N	184

A 4016

817512		2			H	N	1.09
817513		2			H	N	0.546

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1 A compound of the formula (I)



wherein

A is CH or N

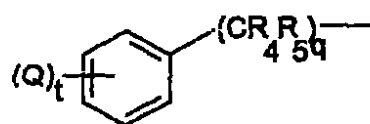
Y is O or NR₁

wherein R₁ is

a) hydrogen,

b) C₁-C₈alkyl, or

c)



wherein

each Q is independently hydrogen, C₁-C₈alkyl, halogen or trifluoromethyl,

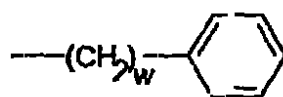
each R₄ and R₅ is independently hydrogen or C₁-C₈alkyl,

t is 0 or 1, and

q is 0 or 1

n is 2 or 3

R₃ is hydrogen, C₁-C₈alkyl or



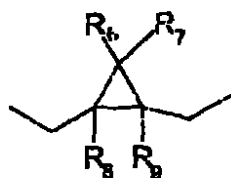
wherein w is 1, 2 or 3,

X is O or S

-B- is a group selected from (a) - (e)

(a) -(CR₂₁R₂₂)_m-

(b)



5

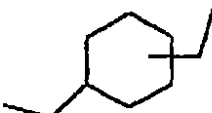
(c)



(d)



(e)



10

wherein

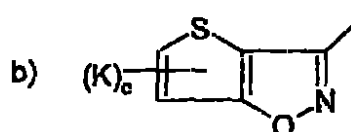
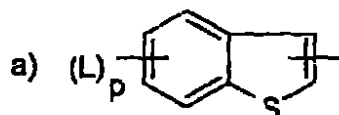
m is 3 4 5 or 6

each R_{21} and R_{22} is independently hydrogen C_1 - C_8 alkyl or $(CH_2)_aOH$ wherein a is 0, 1 or 2,

15

R_6 R_7 R_8 and R_9 are each independently hydrogen halogen or C_1 - C_8 alkyl with the proviso that when R_6 is C_1 - C_8 alkyl R_9 is not C_1 - C_8 alkyl,

R is a group selected from (a) or (b)



20

wherein

each L is independently hydrogen C_1 - C_8 alkyl, halogen or trifluoromethyl

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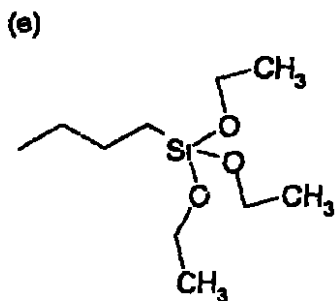
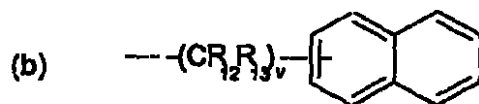
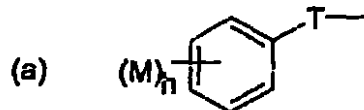
K is hydrogen, C₁-C₈alkyl CHO (CH₂)_bOH (CH₂)_bOC₁-C₈alkyl or halogen

wherein b is 1 or 2,

p is 0 1 or 2,

e is 0 1 or 2

R₂ is a group selected from (a) - (i)

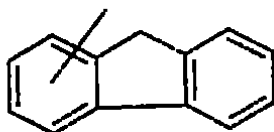


(f) C₁-C₁₂alkyl

(g) -(CR₁₆R₁₅)_e-CO₂C₁-C₈alkyl

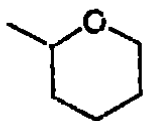
-108-

(h)

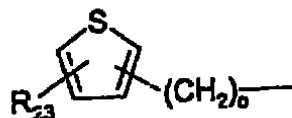


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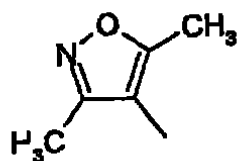
(i)



(j)



(k)



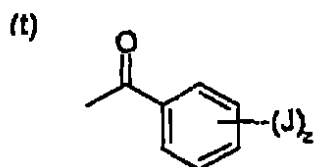
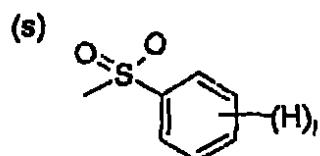
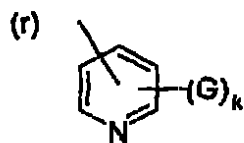
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(l) $-\text{CH}_2\text{CH}_2\text{SO}_2\text{CH}_3$ (m) $-(\text{CR}_{17}\text{R}_{18})_r-\text{OC}_1-\text{C}_2\text{alkyl}$ (n) $-\text{CH}_2\text{CH}_2\text{OPh}$ (o) $-(\text{CR}_{19}\text{R}_{20})_q\text{CZ}_3$

15

(p) $-\text{CO}_2\text{Ph}$ (q) $-\text{COCV}_3$

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wherein

-T- is selected from (i) or (ii)

(i) $-(CR_{10}R_{11})_u-$

wherein

u is 0, 1 or 2 and

each R_{10} and R_{11} is independently hydrogen or C_1-C_8 alkyl,

(ii)



each M, G, H and J is independently selected from

(1) hydrogen

(2) halogen

(3) C_1-C_8 alkyl,(4) C_1-C_8 alkoxy

(5) phenoxy

(6) phenyl

(7) trifluoromethyl

(8) trifluoromethoxy,

(9) $-CO_2 R_{19}$ wherein R_{19} is hydrogen or C_1-C_8 alkyl,(10) $-COC_1-C_8$ alkyl,

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- (11) hydroxy
 (12) $-(CH_2)_n-OR_{20}$ wherein R_{20} is hydrogen or C_1-C_6 alkyl
 (13) $-C(CH_3)_2CH_3$
 (14) $-NO_2$
 (15) $-SCH_3$, and
 (16) benzyloxy

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each R_{12} and R_{13} is independently hydrogen or C_1-C_6 alkyl,
 v is 0 or 1,
 R_{14} is hydrogen or C_1-C_4 alkyl
 c is 0, 1 or 2
 d is 0 or 1
 each R_{15} and R_{16} is independently hydrogen or C_1-C_6 alkyl,
 s is 0, 1, 2, 3, 4 or 5,
 o is 1 or 2
 each R_{17} and R_{18} is independently hydrogen, C_1-C_6 alkyl or
 $CO_2C_1-C_2$ alkyl,
 r is 2 or 3,
 each R_{19} and R_{20} is independently hydrogen or
 C_1-C_2 alkyl
 R_{23} is hydrogen or C_1-C_4 alkyl
 Z is chlorine or fluorine
 q is 0 or 1,
 V is chlorine or fluorine, and
 h, k, l and z are 0, 1, 2 or 3

2 A compound according to claim 1 wherein X is O, Y is NH and $-B-$ is group (a)

30 3 A compound according to claim 2 wherein R_2 is group (a)

4 A compound according to claim 3 wherein m is 4, M is hydrogen, C_1-C_6 alkyl, C_1
 C_6 alkoxy, or phenyl and T is (i) wherein u is 0 or 1

35 5 A compound according to claim 2 wherein R_2 is group (c)

- 6 A compound according to claim 5 wherein wherein m is 4 d is 1 and R₁₄ is C₁-C₈alkyl
- 5 7 A compound according to claim 1 wherein X is O Y is NH and -B- is group (b)
- 8 A compound according to claim 6 wherein R₇ is group (a)
- 9 A compound according to claim 7 wherein R₆, R₇, R₈ or R₉ are hydrogen, M is
10 hydrogen C₁-C₈alkyl C₁-C₈alkoxy or phenyl and T is 0 or 1
- 10 A compound according to claim 6 wherein R₂ is group (c)
- 11 A compound according to claim 9 wherein R₆, R₇, R₈ or R₉ are hydrogen d is 1 and
15 R₁₄ is C₁-C₈alkyl
- 12 The compound of claim 1 which is 1-(4-methoxy-phenyl)-3-[4-[4-(6-trifluoromethyl-
benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl]-urea
- 20 13 The compound of claim 1 which is 1-p-tolyl-3-[4-[4-(6-trifluoromethyl-
benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl]-urea
- 14 The compound of claim 1 which is 1-[4-[4-(2,6-difluoro-benzo[b]thiophen-3-yl)-
piperazin-1-yl]-butyl]-3-p-tolyl-urea
- 25 15 The compound of claim 1 which is 1-[4-[4-(2-chloro-6-fluoro-benzo[b]thiophen-3-yl)-
piperazin-1-yl]-butyl]-3-(4-chloro-phenyl)-urea
- 16 The compound of claim 1 which is 1-(4-acetyl-phenyl)-3-[4-(4-thieno[2,3-d]isoxazol-3-
30 yl-pipendin-1-yl)-butyl]-urea
- 17 The compound of claim 1 which is 1-(4-ethoxy-phenyl)-3-[4-(4-thieno[2,3-d]isoxazol-3-
yl-pipendin-1-yl)-butyl]-urea

18 The compound of claim 1 which is 1-(4-ethoxy-phenyl)-3-{4-[4-(6-fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl}-urea

19 The compound of claim 1 which is 1-{4-[4-(6-fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl}-3-(2-fluoro-phenyl)-urea

20 The compound of claim 1 which is 1-{4-[4-(6-fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl}-3-(3-trifluoromethyl-phenyl)-urea

21 The compound of claim 1 which is 1-{4-[4-(6-fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl}-3-(4-methoxy-phenyl)-urea

22 A method of modulating the activity of dopamine D₃ receptors said method comprising contacting cell-associated dopamine D₃ receptors with a concentration of a compound of claim 1

23 A method of treating conditions or disorders of the central nervous system comprising administering to a patient in need thereof a therapeutically effective amount of a compound of claim 1

24 The method of claim 23 wherein the central nervous system disorder is selected from Psychotic Disorders, Substance Dependence, Substance Abuse, Dyskinetic Disorders, Dementia, Anxiety Disorders, Sleep Disorders, Circadian Rhythm Disorders, Mood Disorders and Nausea

25 The method of claim 24 wherein the Psychotic Disorder is Schizophrenia

26 The method of claim 24 wherein the compound of claim 1 is administered in conjunction with one or more dopamine D₁, D₂, D₄, D₅, or 5HT receptor antagonists

27 A pharmaceutical composition comprising an effective amount of a compound of claim 1 with a pharmaceutically-acceptable carrier or diluent

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28 A pharmaceutical composition comprising an effective amount of a compound of claim 1 with a pharmaceutically-acceptable carrier or diluent in conjunction with one or more dopamine D₁, D₂, D₄, D₅ or 5HT receptor antagonists

5 29 A depot pharmaceutical composition which comprises a pharmaceutically acceptable carrier and a therapeutically effective amount of the compound of claim 1 wherein the compound contains an acylated hydroxy group or an acylated amino group

10 30 The depot pharmaceutical composition of claim 29 wherein the hydroxy group is acylated or the amino group is acylated with (C₁-C₁₈)alkanoyl group or a (C₄-C₁₂)alkoxycarbonyl group

31 The composition of claim 29 which contains a pharmaceutically acceptable oil

15 32 The composition of claim 31 wherein the oil is selected from the group consisting of coconut oil, peanut oil, sesame oil, cotton seed oil, corn oil, soybean oil, olive oil and synthetic esters of fatty acids and polyfunctional alcohols

20 33 A method for providing a long acting antipsychotic effect which comprises injecting into a mammal an amount of the composition of claim 29 sufficient to produce a long acting antipsychotic effect.

25 34 A method for providing a long acting antipsychotic effect which comprises injecting into a mammal an amount of the composition of claim 30 sufficient to produce a long acting antipsychotic effect.

30 35 A method for providing a long acting antipsychotic effect, which comprises injecting into a mammal an amount of the composition of claim 31 sufficient to produce a long acting antipsychotic effect.

36 A compound of claim 1 wherein one or more of the atoms contained therein is a radionuclide

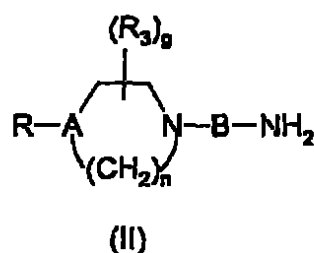
37 A compound of claim 1 wherein R is group (a) with a radiolabeled ¹⁴C in the 3-position of the benzo[b]thiophene ring system L is trifluoromethyl p is 1, R₃ is hydrogen n is 2 and A is N

5 38 A diagnostic method for monitoring neuronal functions in a mammal comprising
introducing into a mammal a radiolabeled compound according to claim 36

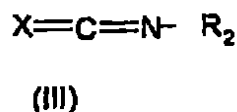
39 The method of claim 38 wherein said diagnostic method is performed using single
 40 photon emission computed tomography (SPECT) or positron emission tomography (PET)

10

40 A process for preparing a compound of claim 1 wherein Y is N which comprises reacting a compound of formula (II)

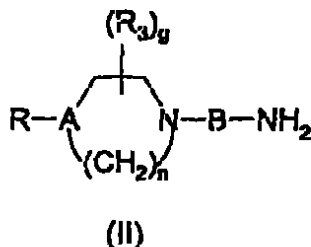


15 wherein R, R₃, g, n, B and A are as defined in formula I of claim 1
with a compound of formula (III)



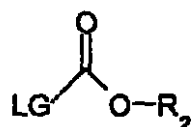
wherein X and R₂ are as defined in formula I of claim 1

20 41 A process for preparing a compound of formula I wherein Y is O which comprises
reacting a compound of formula (II)



wherein R, R₃, g, n, B and A are as defined in formula I of claim 1 with a compound of formula IV

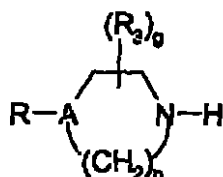
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(IV)

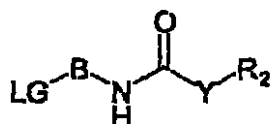
wherein "LG" is a suitable leaving group selected from bromine chlorine or iodine and R_2 is as defined in formula I of claim 1

- 5 42 A process for preparing compounds of formula I which comprises reacting a compound of formula (V)



(V)

wherein R_3 g R A and n are as defined in formula I of claim 1
with a compound of formula (VI)



(VI)

wherein "LG" is a suitable leaving selected from chlorine bromine iodine or mesyl
and B Y and R_2 are defined in formula I of claim 1

ABSTRACT

5 The invention relates to heterocyclic substituted urea derivatives that display selective binding to dopamine D₃ receptors. In another aspect, the invention relates to a method for treating central nervous system disorders associated with the dopamine D₃ receptor activity in a patient in need of such treatment comprising administering to the subject a therapeutically effective amount of said compounds for alleviation of
10 such disorder. The central nervous system disorders that may be treated with these compounds include Psychotic Disorders, Substance Dependence, Substance Abuse, Dyskinetic Disorders (e.g. Parkinson's Disease, Parkinsonism, Neuroleptic-Induced Tardive Dyskinesia, Gilles de la Tourette Syndrome and Huntington's Disease), Dementia, Anxiety Disorders, Sleep Disorders, Circadian Rhythm Disorders and
15 Mood Disorders. The subject invention is also directed towards processes for the preparation of the compounds described herein as well as methods for making and using the compounds as imaging agents for dopamine D₃ receptors.

5

NOVEL HETEROCYCLIC UREA DERIVATIVES AND THEIR USE AS DOPAMINE D₃ RECEPTOR LIGANDS

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BACKGROUND OF THE INVENTION

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The subject invention relates to novel heterocyclic derivatives that selectively bind to the dopamine D₃ receptor. The therapeutic effects of currently available antipsychotic agents (neuroleptics) are generally believed to be exerted via blockade of D₂ receptors, however this mechanism is also thought to be responsible for undesirable extrapyramidal side effects (eps) associated with many neuroleptic agents. Without wishing to be bound by theory, it has been suggested that blockade of the dopamine D₃ receptor may give rise to beneficial antipsychotic activity without significant eps (see for example Sokoloff et al, *Nature*, 1990, 347: 146-151, and Schwartz et al, *Clinical Neuropharmacology*, Vol 16, No 4, 295-314, 1993). This receptor is found in high abundance in brain regions associated with emotional and cognitive functions. Compounds that selectively bind to the dopamine D₃ receptor are useful in treating certain central nervous system disorders. These central nervous system disorders include the following indications:

1) Psychoses (including schizophrenia) – See, for example, *Biochem Pharmacol*, 1992, 3(4), 659-66, *Clin Neuropharmacol*, 1993, 16(4), 295-314, *Neuropsychopharmacology*, 1997, 16(6), 375-84, *Am J Psychiatry*, 1999, 156(4), 610-616, *Psychopharmacology (Berl)*, 1995, 120(1), 67-74

2) Substance dependence and substance abuse – See, for example, *Neuroreport*, 1997, 8(9-10), 2373-2377, *J Pharmacol Exp Ther*, 1996, 278(3), 1128-37, *Brain Res Mol Brain Res*, 1997, 45(2), 335-9

3) Mood Disorders (including mania, depressive disorders and bipolar disorders) – See, for example, *Clin Neuropharmacol*, 1998, 21(3), 176-80, *Am J Med Genet*, 1998, 81(2), 192-4, *J Clin Psychiatry*, 1995, 56(11), 514-518, *J Clin Psychiatry*, 1995, 56(9), 423-429, *Am J Med Genet*, 1995, 60(3), 234-237, *Pharmacopsychiatry*, 1999, 32(4), 127-135, *J Affect Disord*, 1999, 52(1-3), 275-290, *Am J Psychiatry*, 1999, 156(4), 610-616

4) dyskinetic disorders (including Parkinson's Disease, Parkinsonism, Neuroleptic-Induced Tardive Dyskinesia and Gilles de la Tourette Syndrome) – See, for example, *Clin Neuropharmacol*, 2000, 23(1), 34-44, *Eur J Pharmacol*, 1999, 385(1), 39-46

5) sleep disorders (including narcolepsy) - The D₃ agonist pramipexole causes narcolepsy. A D₃ antagonist would be useful for reversing this undesirable side effect. See *Aust Fam Physician*, 1999, 28(7), 737, *Neurology*, 1999, 52(9), 1908-1910

6) anxiety disorders (including obsessive compulsive disorders) – See, for example, *Physiol Behav*, 1997, 63(1), 137-141, *J Clin Psychiatry*, 1995, 56(9), 423-429, *J Psychiatry Neurosci*, 2000, 25(2), 185, *J Affect Disord*, 1999, 56(2-3), 219-226

7) nausea - Dopamine antagonists are used alone and in combination with 5HT₃ antagonists. See, for example, *Support Care Cancer*, 1998, 6(1), 8-12, *Support Care Cancer*, 2000, 8(3), 233-237, *Eur J Anaesthesiol*, 1999, 16(5), 304-307

8) dementia – See, for example, *Behav Brain Res*, 2000, 109(1), 99-111, *Neuroscience*, 1999, 89(3), 743-749

Dopamine D₃ receptor ligands are also useful for the treatment of renal dysfunction (See WO 200067847)

SUMMARY OF THE INVENTION

This invention relates to a class of compounds and pharmaceutically acceptable salts thereof which are selective modulators of dopamine D₃ receptors. The compounds may act as agonists, partial agonists, antagonists or allosteric modulators of dopamine D₃ receptors, and are useful for a variety of therapeutic applications.

In another aspect, the invention relates to a method for treating central nervous system

disorders associated with the dopamine D₃ receptor activity in a patient in need of such treatment comprising administering to the subject a therapeutically effective amount of a compound described herein for alleviation of such disorder. The central nervous system conditions or disorders that may be treated with these compounds include Psychotic Disorders, Substance Dependence, Substance Abuse, Dyskinetic Disorders (e.g. Parkinson's Disease, Parkinsonism, Neuroleptic-Induced Tardive Dyskinesia, Gilles de la Tourette Syndrome and Huntington's Disease), Nausea, Dementia, Anxiety Disorders, Sleep Disorders, Circadian Rhythm Disorders and Mood Disorders.

In yet another aspect, the subject invention is directed toward a pharmaceutical composition comprising an effective amount of a compound described herein with a pharmaceutically-acceptable carrier or diluent optionally in conjunction with one or more dopamine D₁, D₂, D₄, D₅ or 5HT receptor antagonists

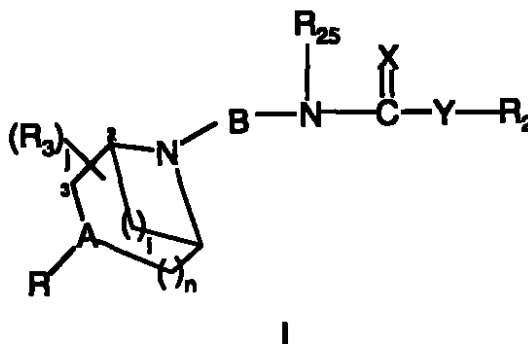
In yet another aspect, the subject invention is directed towards processes for the preparation of the class of compounds described herein

Also within the scope of this invention are methods for using these novel compounds as imaging agents for dopamine D₃ receptors. Methods of using these compounds as imaging agents are presented, as are intermediates and methods for making the imaging agents

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided compounds of formula I

A compound of the formula (I)



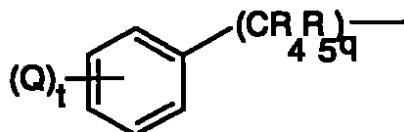
wherein

A is CH or N.

Y is O or NR₁

wherein R_1 is

- a) hydrogen,
- b) C_1 - C_8 alkyl, or
- c)



wherein

each Q is independently hydrogen, C_1 - C_8 alkyl, halogen or trifluoromethyl,

each R_4 and R_5 is independently hydrogen or C_1 - C_8 alkyl,

t is 0 or 1, and

q is 0 or 1,

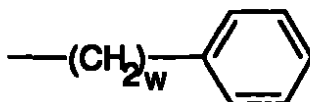
j is 0, 1 or 2,

n is 1 or 2,

when n is 1, i is 0 or 2,

when n is 2, i is 0,

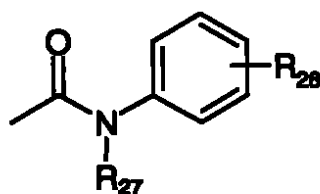
R_3 is hydrogen, C_1 - C_8 alkyl or



wherein w is 1, 2 or 3,

X is O or S,

R_{25} is hydrogen or



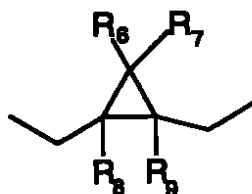
wherein R_{27} is hydrogen or C_1 - C_8 alkyl and R_{28} is hydrogen,

C_1 - C_8 alkoxy, halogen or C_1 - C_8 alkyl that is unsaturated or saturated,

-B- is a group selected from (a) - (e)

(a) $-(CR_{21}R_{22})_m-$

(b)

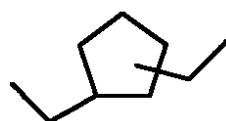


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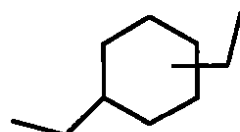
(c)



(d)



(e)



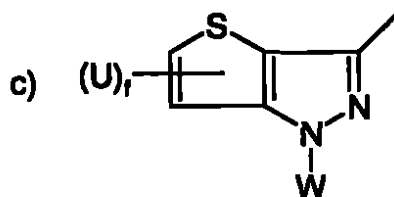
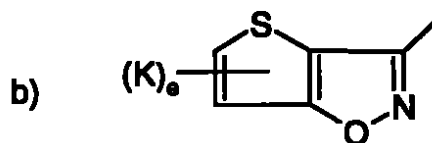
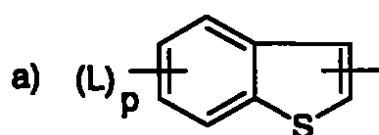
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wherein

 m is 3, 4, 5, or 6,each R_{21} and R_{22} is independently hydrogen, C_1 - C_8 alkyl or $-(CH_2)_aOH$ wherein a is 0, 1 or 2,

15

 R_6 , R_7 , R_8 and R_9 are each independently hydrogen, halogen or C_1 - C_3 alkyl with the proviso that when R_8 is C_1 - C_3 alkyl, R_9 is not C_1 - C_3 alkyl, R is a group selected from (a) - (c)

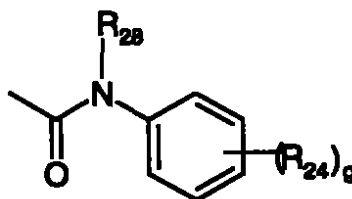


wherein

each L is independently hydrogen, C₁-C₆alkyl, halogen or trifluoromethyl,

5 each U is independently hydrogen, C₁-C₆alkyl, CHO, halogen, -(CH₂)_bOH, or -(CH₂)_bOC₁-C₆alkyl wherein b is 1 or 2,

each K is independently hydrogen, C₁-C₆alkyl, CHO, halogen, -(CH₂)_bOH, or -(CH₂)_bOC₁-C₆alkyl wherein b is as hereinbefore defined, W is hydrogen or



10

wherein R₂₈ is hydrogen or C₁-C₆alkyl,

each R₂₄ is independently trifluoromethyl, trifluoromethoxy, C₁-C₆alkoxy or halogen, and g is 0, 1 or 2,

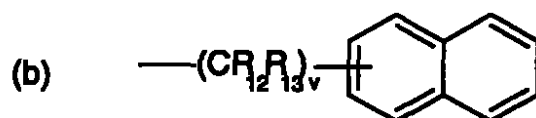
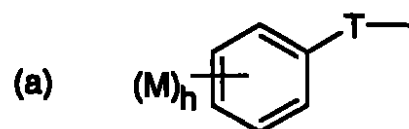
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p is 0, 1 or 2,

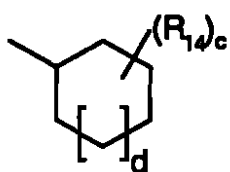
e is 0, 1 or 2,

f is 0, 1, or 2,

R₂ is a group selected from (a) - (t)



(c)

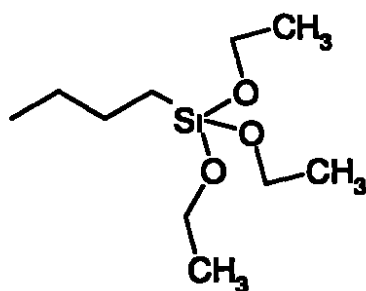


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(d)



(e)

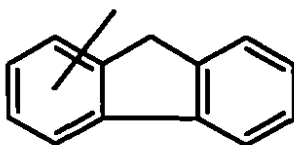


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(f) $-C_1-C_{12}$ alkyl

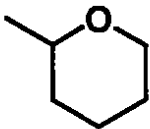
(g) $-(CR_{18}R_{19})_8-CO_2C_1-C_8$ alkyl

(h)

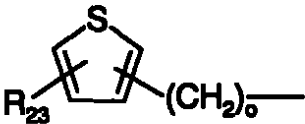


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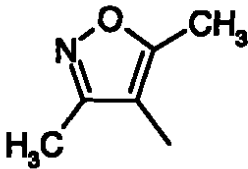
(i)



(i)



(k)

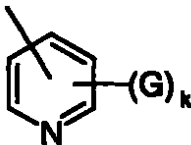


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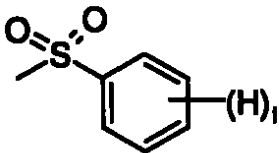
- (l) $-\text{CH}_2\text{CH}_2\text{SO}_2\text{CH}_3$
- (m) $-(\text{CR}_{17}\text{R}_{18})_r-\text{OC}_1-\text{C}_2\text{alkyl}$
- (n) $-\text{CH}_2\text{CH}_2\text{OPh}$
- (o) $-(\text{CR}_{19}\text{R}_{20})_q\text{CZ}_3$
- (p) $-\text{CO}_2\text{Ph}$
- (q) $-\text{COCV}_3$

10

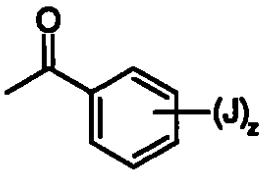
(r)



(s)



(t)



wherein

15

- T- is selected from (i) or (ii)
- (i) $-(\text{CR}_{10}\text{R}_{11})_u-$
wherein
u is 0, 1 or 2, and

each R_{10} and R_{11} is independently hydrogen or C_1 - C_8 alkyl,

(ii)



each M, G, H and J is independently selected from

- (1) hydrogen,
- (2) halogen,
- (3) C_1 - C_8 alkyl,
- (4) C_1 - C_8 alkoxy,
- (5) phenoxy,
- (6) phenyl,
- (7) trifluoromethyl,
- (8) trifluoromethoxy,
- (9) $-CO_2-R_{19}$ wherein R_{19} is hydrogen or C_1 - C_8 alkyl,
- (10) $-COC_1$ - C_8 alkyl,
- (11) hydroxy,
- (12) $-(CH_2)-OR_{20}$ wherein R_{20} is hydrogen or C_1 - C_8 alkyl,
- (13) $-C(CH_2)CH_3$,
- (14) $-NO_2$,
- (15) $-SCH_3$,
- (16) benzyloxy, and
- (17) $-CN$,

each R_{12} and R_{13} is independently hydrogen or C_1 - C_8 alkyl,
 v is 0 or 1,

R_{14} is hydrogen or C_1 - C_4 alkyl,

c is 0, 1 or 2,

d is 0 or 1,

each R_{15} and R_{16} is independently hydrogen or C_1 - C_8 alkyl,

s is 0, 1, 2, 3, 4 or 5,

o is 1 or 2,

each R_{17} and R_{18} is independently hydrogen, C_1 - C_8 alkyl or CO_2C_1 - C_2 alkyl,

r is 2 or 3,

each R_{19} and R_{20} is independently hydrogen or

C_1 - C_2 alkyl,

R_{23} is hydrogen or C_1 - C_4 alkyl,

Z is chlorine or fluorine,

q is 0 or 1,

V is chlorine or fluorine, and

h , k , l , and z are 0, 1, 2, or 3

The subject invention is directed toward compounds or pharmaceutically acceptable salts of Formula I as depicted above in either racemic or pure stereoisomeric forms

Terms used herein have the following meanings

a) "Pharmaceutically acceptable salts" means either an acid addition salt or a basic addition salt which is compatible with the treatment of patients for the intended use

"Pharmaceutically acceptable acid addition salt" is any non-toxic organic or inorganic acid addition salt of the base compounds represented by Formula I or any of its intermediates. Illustrative inorganic acids which form suitable salts include hydrochloric, hydrobromic, sulfuric and phosphoric acid and acid metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids which form suitable salts include the mono-, di- and tri-carboxylic acids. Illustrative of such acids are, for example, acetic, glycolic, lactic, pyruvic, malonic, succinic, glutamic, fumaric, malic, tartaric, citric, ascorbic, maleic, hydroxymaleic, benzoic, hydroxybenzoic, phenylacetic, cinnamic, salicylic, 2-phenoxybenzoic, p-toluenesulfonic acid and sulfonic acids such as methanesulfonic acid and 2-hydroxyethanesulfonic acid. Either the mono- or di-acid salts can be formed, and such salts can exist in either a hydrated, solvated or substantially anhydrous form. In general, the acid addition salts of these compounds are more soluble in water and various hydrophilic organic solvents and which in comparison to their free base forms, generally demonstrate higher melting points.

"Pharmaceutically acceptable basic addition salts" means non-toxic organic or inorganic basic addition salts of the compounds of Formula (I) or any of its intermediates. Examples

are alkali metal or alkaline-earth metal hydroxides such as sodium, potassium, calcium, magnesium or barium hydroxides, ammonia, and aliphatic, alicyclic, or aromatic organic amines such as methylamine, trimethylamine and picoline. The selection criteria for the appropriate salt will be known to one skilled in the art.

5

b) "Stereoisomers" is a general term for all isomers of the individual molecules that differ only in the orientation of their atoms in space. It includes mirror image isomers (enantiomers), geometric (cis/trans) isomers, and isomers of compounds with more than one chiral center that are not mirror images of one another (diastereoisomers).

10

c) "Alkyl" means a branched or straight chain alkyl or alkylene group, as is appropriate to the formula, specified by the amount of carbons in the alkyl, e.g., C₁-C₆ alkyl means a one, two, three, four, five or six carbon branched or straight chain alkyl or alkylene, as the case may be, or any ranges thereof, for example, but not limited to, C₁-2, C₁-3, C₁-4, C₁-5, C₂-3, C₂-4, C₂-5, C₂-C₆, C₃-C₄, C₃-5, C₃-6, C₄-5, C₄-6, C₅-6, etc.

15

d) "Patient" means a warm blooded animal, such as for example rat, mice, dogs, cats, guinea pigs, and primates such as humans.

20

e) "Treat" or "treating" means to alleviate symptoms, eliminate the causation of the symptoms either on a temporary or permanent basis, or to prevent or slow the appearance of symptoms of the named disorder or condition.

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f) "Therapeutically effective amount" means a quantity of the compound which is effective in treating the named disorder or condition.

30

g) "Pharmaceutically acceptable carrier" is a non-toxic solvent, dispersant, excipient, adjuvant or other material which is mixed with the active ingredient in order to permit the formation of a pharmaceutical composition, i.e., a dosage form capable of administration to the patient. One example of such a carrier is a pharmaceutically acceptable oil typically used for parenteral administration.

35

h) "Psychoses" or "Psychotic Disorders" means conditions wherein the patient experiences a major mental disorder of organic and/or emotional origin characterized by derangement of the personality and loss of contact with reality, often with delusions, hallucinations or illusions. Included under the term psychoses are the disorders

schizophrenia, schizophreniform disorder, schizoaffective disorder, delusional disorder, brief psychotic disorder, shared psychotic disorder, psychotic disorder not otherwise specified, and substance-induced psychotic disorder, as defined by the Diagnostic and Statistical Manual of Mental Disorders, 4th ed , published 1994 by the American Psychiatric Association,

5 Washington D C USA, incorporated herein by reference

i) "Substance Dependence" means a condition wherein the patient exhibits a maladaptive pattern of substance use, leading to clinically significant impairment or distress. There is a pattern of repeated self-administration that usually results in tolerance, withdrawal,
10 and compulsive drug-taking

j) "Substance Abuse" means a condition wherein the patient exhibits a maladaptive pattern of substance use manifested by recurrent and significant adverse consequences related to the repeated use of substances. There may be repeated failure to fulfill major role obligations, repeated use in situations in which it is physically hazardous, multiple legal
15 problems, and recurrent social and interpersonal problems. Unlike the criteria for Substance Dependence, the criteria for Substance Abuse do not include tolerance, withdrawal, or a pattern of compulsive use and instead only include the harmful consequences of repeated use

20

k) "Parkinson's Disease" means a slowly progressive neurological condition, characterized by tremor, rigidity, bradykinesia, and postural instability. Other manifestations include depression and dementia

25 l) "Parkinsonism" means a condition where the patient exhibits Parkinsonian signs or symptoms (i.e. tremor, muscular rigidity, or akinesia) that develop in association with the use of neuroleptic medication

m) "Neuroleptic-Induced Tardive Dyskinesia" means a disorder characterized by
30 involuntary movements of the tongue, jaw, trunk, or extremities which have developed in association with the use of neuroleptic medication. The involuntary movements may be choreiform, athetoid or rhythmic

n) "Gilles de la Tourette Syndrome" means a condition manifested by motor and vocal
35 tics. (A tic is a sudden, rapid, recurrent, nonrhythmic, stereotyped motor movement or vocalization.) The disturbance causes marked distress or significant impairment in social,

occupational, or other important areas of functioning The onset is before age eighteen years and the disturbance is not due to the physiological effects of a substance or general medical condition

- 5 o) "Dementia" means disorders characterized by the development of multiple cognitive deficits that include memory impairment and are due to the direct physiological effects of a general medical condition, to the persisting effects of a substance, or to multiple etiologies (e g , the combined effects of cerebrovascular disease and Alzheimer's disease) Memory impairment is required to make the diagnosis of a dementia and is a prominent early
- 10 symptom Dementia disorders share a common symptom presentation but are differentiated based on etiology See Diagnostic and Statistical Manual of Mental Disorders, 4th ed , American Psychiatric Association, for diagnostic criteria
- p) "Anxiety Disorders" means disorders that include Seasonal Affective Disorder, Panic
- 15 Disorder Without Agoraphobia, Panic Disorder with Agoraphobia, Agoraphobia Without History of Panic Disorder, Specific Phobia, Social Phobia, Obsessive-Compulsive Disorder, Post-traumatic Stress Disorder, Acute Stress Disorder, Generalized Anxiety Disorder, Anxiety Disorder Due to a General Medical Condition, Substance-Induced Anxiety Disorder, and Anxiety Disorder Not Otherwise Specified, as defined by the Diagnostic and Statistical
- 20 Manual of Mental Disorders, 4th ed
- q) "Sleep Disorders" means disorders that include Primary Sleep Disorders, Sleep Disorder Related to Another Mental Disorder, Sleep Disorder Due to a General Medical Condition, and Substance-Induced Sleep Disorder as defined by the Diagnostic and
- 25 Statistical Manual of Mental Disorders, 4th ed Primary Sleep Disorders are those in which none of the etiologies listed below (i e , another mental disorder, a general medical condition, or a substance) is responsible Primary Sleep Disorders are presumed to arise from endogenous abnormalities in sleep-wake generating or timing mechanisms, often complicated by conditioning factors Primary Sleep Disorders in turn are subdivided into
- 30 Dyssomnias (characterized by abnormalities in the amount, quality, or timing of sleep) and Parasomnias (characterized by abnormal behavioral or physiological events occurring in association with sleep, specific sleep stages, or sleep-wake transitions) A representative example of a Primary Sleep Disorder is Narcolepsy Narcolepsy is characterized by repeated irresistible attacks of refreshing sleep, cataplexy, and recurrent intrusions of elements of rapid
- 35 eye movement (REM) sleep into the transition period between sleep and wakefulness

r) "Mood Disorders" are disorders that have a disturbance in mood as the predominant feature. As defined by the Diagnostic and Statistical Manual of Mental Disorders, 4th ed., Mood Disorders are divided into the Depressive Disorders ("unipolar depression"), the Bipolar Disorders, and two disorders based on etiology – Mood Disorder Due to a General Medical Condition and Substance-Induced Mood Disorder. The Depressive Disorders (i.e., Major Depressive Disorder, Dysthymic Disorder, and Depressive Disorder Not Otherwise Specified) are distinguished from the Bipolar Disorders by the fact that there is no history of ever having had a Manic, Mixed, or Hypomanic Episode. The Bipolar Disorders (i.e., Bipolar I Disorder, Bipolar II Disorder, Cyclothymic Disorder, and Bipolar Disorder Not Otherwise Specified) involve the presence (or history) of Manic Episodes, Mixed Episodes, or Hypomanic Episodes, usually accompanied by the presence (or history) of Major Depressive Episodes.

s) "Circadian Rhythm Disorder" means a persistent or recurrent pattern of sleep disruption leading to excessive sleepiness or insomnia that is due to a mismatch between the sleep-wake schedule required by a person's environment and his or her circadian sleep-wake pattern. The sleep disturbance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning. The disturbance does not occur exclusively during the course of another Sleep Disorder or other mental disorder. The disturbance is not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition.

Preferred compounds are those wherein X is O, Y is NH and -B- is group (a) or group (b). When B is group (a), m is further preferred to be 4. When -B- is group (b), then R₆, R₇, R₈ or R₉ are further preferred to be hydrogen. R₂ is preferred to be group (a) or group (c). When R₂ is group (a), M is further preferred to be hydrogen, C₁-C₆alkyl, C₁-C₆alkoxy or phenyl and T is further preferred to be (i) wherein u is 0 or 1. When R₂ is group (c), d is further preferred to be 1 and R₁₄ is further preferred to be C₁-C₆alkyl.

Specific embodiments of the invention include the compounds set forth in Table III.

Preferred embodiments of the invention are those compounds of Formula I set forth in Table III that exhibit enhanced D3 potency. Particularly preferred compounds include the following

1-(4-Methoxy-phenyl)-3-{4-[4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl}-urea

1-p-Tolyl-3-{4-[4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl}-urea

1-{4-[4-(2,6-Difluoro-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl}-3-p-tolyl-urea

5 1-{4-[4-(2-Chloro-6-fluoro-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl}-3-(4-chloro-phenyl)-urea

1-(4-Acetyl-phenyl)-3-[4-(4-thieno[2,3-d]isoxazol-3-yl-piperidin-1-yl)-butyl]-urea

10 1-(4-Ethoxy-phenyl)-3-[4-(4-thieno[2,3-d]isoxazol-3-yl-piperidin-1-yl)-butyl]-urea

1-(4-Ethoxy-phenyl)-3-[4-[4-(6-fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl]-urea

1-{4-[4-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl}-3-(2-fluoro-phenyl)-urea

15 1-{4-[4-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl}-3-(3-trifluoromethyl-phenyl)-urea

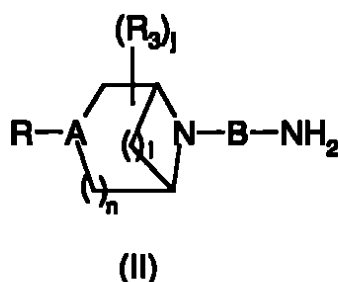
20 1-{4-[4-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl}-3-(4-methoxy-phenyl)-urea

1-(3-Imidazol-1-yl-propyl)-3-[(1R, 2R)-2-[4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-ylmethyl]-cyclopropylmethyl]-urea (MDL 833306)

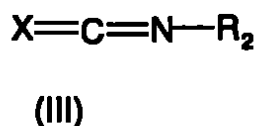
25 The compounds of the present invention may be prepared by various methods. Schemes I through VI show the different ways of preparing the compounds of Formula I

30 The compounds of formula (I) of this invention can be synthesized by following or combining one or more of the steps described below, not necessarily in the order presented. Throughout the description of the synthetic steps, the definitions of R, R₂, R₃, n, j, i, X, Y, B and A are as given above unless otherwise stated or indicated, and other nomenclatures appearing below shall have the same meanings defined in their respective first appearances unless otherwise stated or indicated.

Compounds of formula I wherein Y is N may be prepared according to a process which comprises reacting a compound of formula (II)

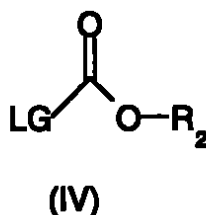


wherein R_3 , R, n, i, B, j, A and R as defined in formula I, with a compound of formula (III)



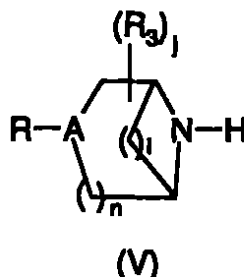
Typically, this reaction is carried out in an organic solvent such as, for example, chloroform or tetrahydrofuran at a temperature of about 20 °C to about 25 °C for about 6 to 18 hours

Compounds of formula I wherein Y is O may be prepared according to a process which comprises reacting a compound of formula (II) with a compound of formula (IV) wherein "LG" is a suitable leaving group selected from bromine, chlorine, iodine or $-\text{OR}_2$ in the instance where formula (IV) is available as an anhydride



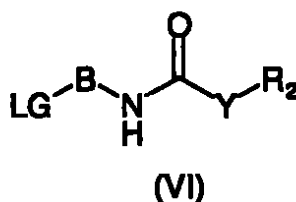
Typically, this reaction is carried out in an organic solvent such as, for example, chloroform or tetrahydrofuran in the presence of a weak base such as, for example, amberlyte-IRA-67 or triethylamine. The reaction is typically conducted at a temperature of about 20 °C to about 25 °C for about 6 to 18 hours

Alternatively, compounds of formula I may be prepared according to a process which comprises reacting a compound of formula (V)



wherein R, A, n, i, R₃ and j are as hereinbefore defined,

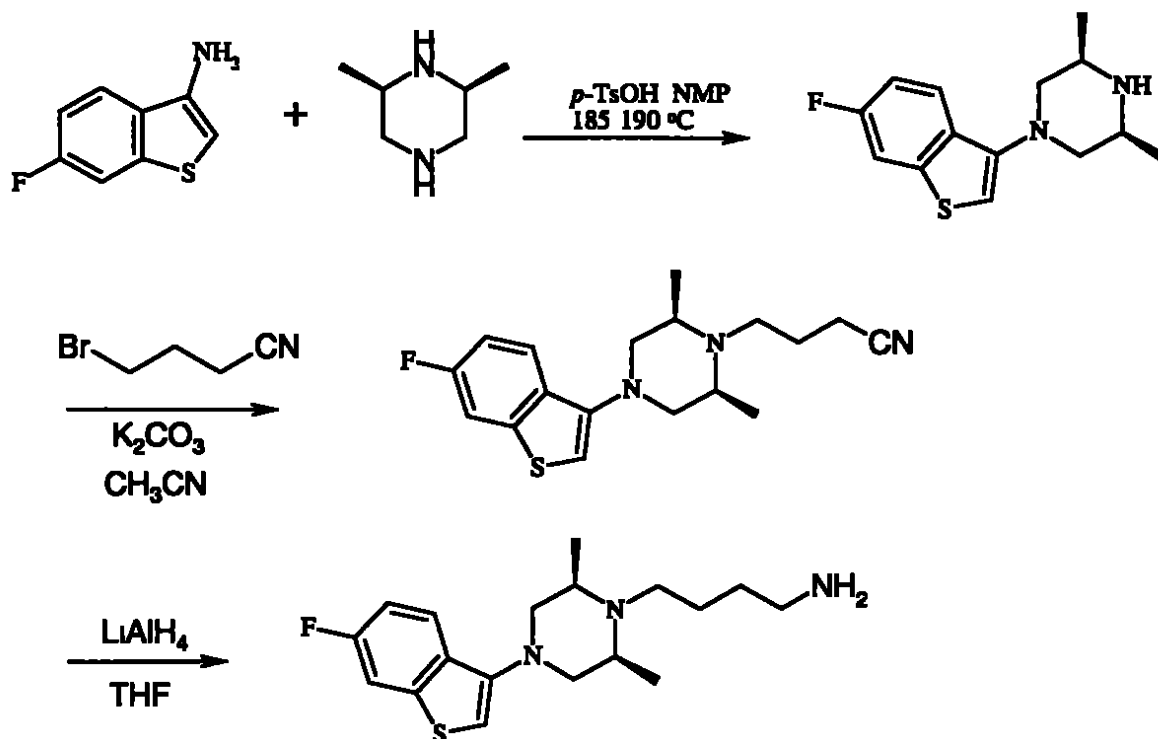
with a compound of formula (VI) wherein R₂, B and Y are as hereinbefore defined and "LG" is a suitable leaving selected from chlorine, bromine, iodine, mesyl, tosyl, brosyl, triflyl, nosyl, nonafllyl or tresyl



Typically, this reaction is carried out in an aqueous miscible solvent such as, for example, tetrahydrofuran or acetonitrile, in the presence of water and a base such as, for example, potassium carbonate, cesium carbonate, or triethylamine, at a temperature of about 50°C to about 75°C for about 12 to 24 hours

The compound of formula (II) may be prepared via synthetic methods well known in the art. The starting materials are either commercially available or readily synthesized via methods known from the literature. For example, scheme 1 describes the coupling of an amino-substituted benzthiophene with a commercially-available substituted piperazine. The synthesis is analogous for the un-substituted piperazine analogs. The less sterically hindered piperazine nitrogen is more reactive and cleanly gives a single product in the benzo[b]thiophene coupling. The more sterically hindered nitrogen can then be alkylated with the appropriate alkylating agent.

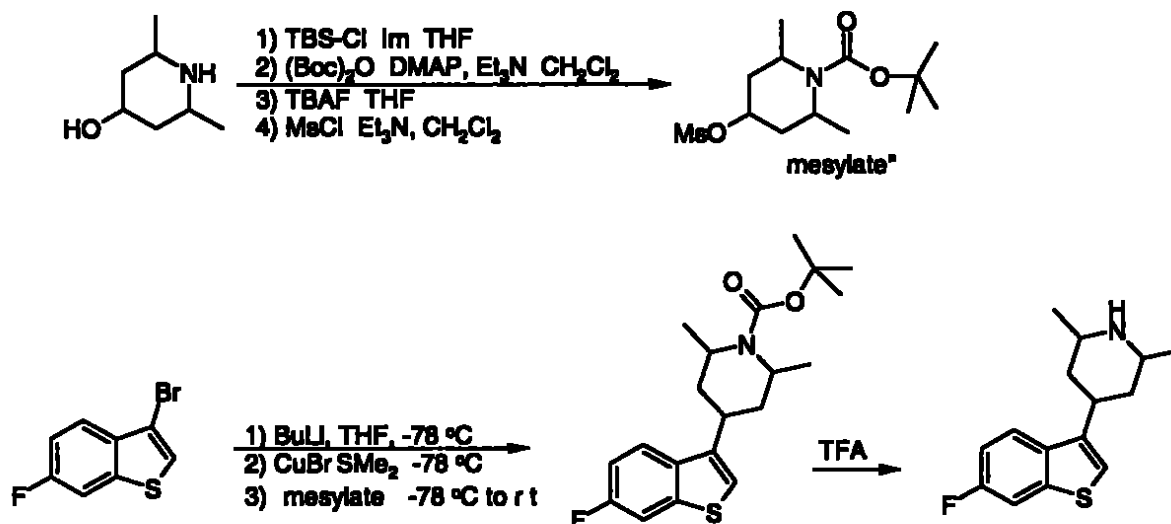
SCHEME I



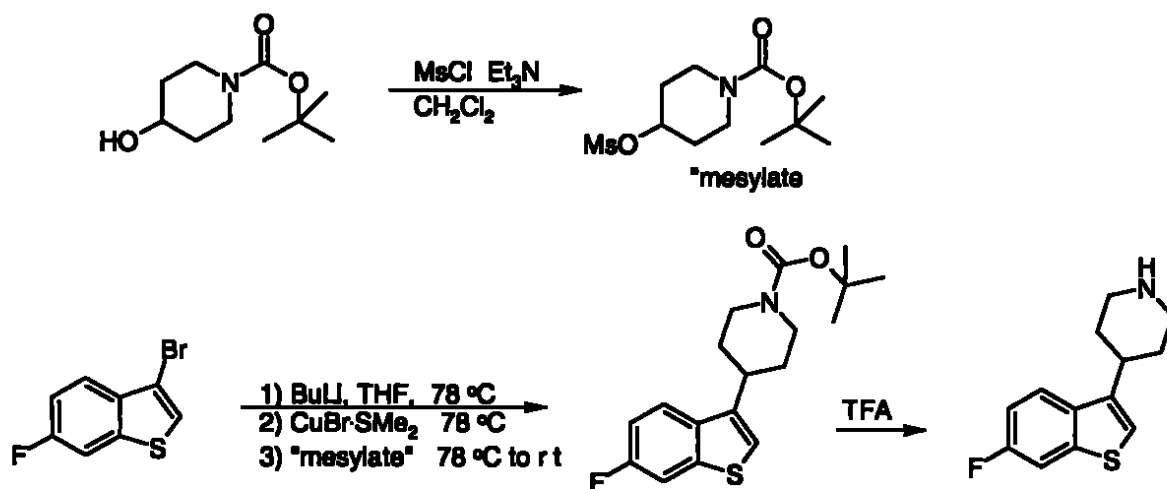
Piperidine-substituted compounds may be prepared via syntheses analogous to those shown in the following reaction schemes II and III

SCHEME II

10



SCHEME III



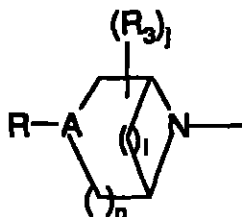
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The preparation of various substituted aza- and diazacycloheptanes is described by Treiber et al in WO 9725324

Compounds of formula I wherein B contains a carbocycle may be prepared as shown in Scheme IV

10

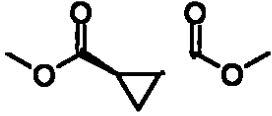
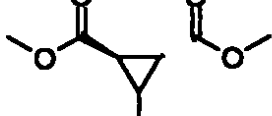
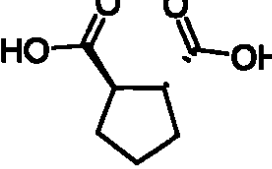
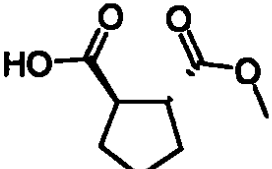
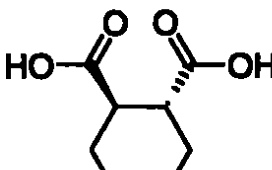
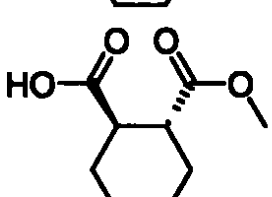
α is 1, 2, 3 or 4, and N'- is



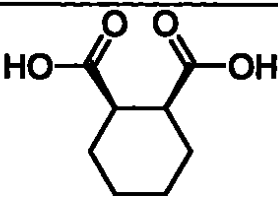
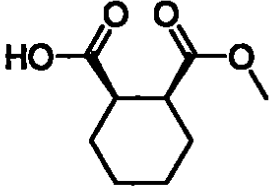
wherein R, A, R₃, j, i and n are as hereinbefore defined

Many of the dicarboxylates or more advanced intermediates that are genencally described in Scheme IV are commercially available. Several of these are shown in Table 1. This table is used for illustrative purposes only and is not intended to limit the scope of the present invention in any way.

Table 1 - Starting Materials

Structure	Name	CAS #	Supplier
	Dimethyl cis-1,2-cyclopropane dicarboxylate	826-34-6	Acros
	Dimethyl trans-1,2-cyclopropane dicarboxylate	826-35-7	Acros
	Dimethyl 1-methyl-trans-1,2-cyclopropane dicarboxylate	702-92-1	Acros
	Dimethyl 3-methyl-trans-1,2-cyclopropane dicarboxylate	28363-79-3	Acros
	trans-Cyclobutane-1,2-dicarboxylic acid dimethylester		Syntec
	trans-D, L-1,2-Cyclopentane-dicarboxylic acid	1461-97-8	Aldrich
	trans-2-Carbomethoxy cyclopentane-1-carboxylic acid		Rieke
	trans-1,2-Cyclohexane dicarboxylic acid	2305-32-0	Aldrich Acros
	trans-2-Carbomethoxy cyclohexane-1-carboxylic acid		Rieke

147
148

Structure	Name	CAS #	Supplier
	cis-1,2-Cyclohexane dicarboxylic acid	610-09-3	Acros
	cis-2-Carbomethoxy cyclohexane-1-carboxylic acid		Rieke

5 When not commercially available, the appropriate starting material may be obtained via standard synthetic methods

When a compound of formula (I) is obtained as a mixture of enantiomers these may be separated by conventional methods such as crystallization in the presence of a resolving agent, or chromatography, for example using a chiral HPLC column

10

Compounds of formula (I) have been found to exhibit affinity for dopamine receptors, in particular D₃ receptors, and are expected to be useful in the treatment of disease states which require modulation of such receptors, such as psychotic conditions Preferred compounds of the present invention are those which have higher affinity for dopamine D₃ than dopamine D₂ receptors

15

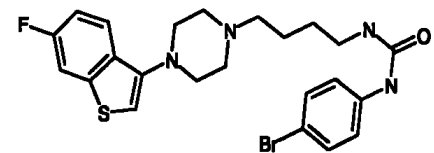
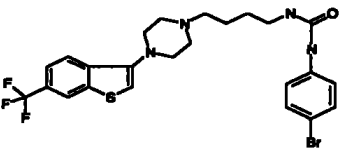
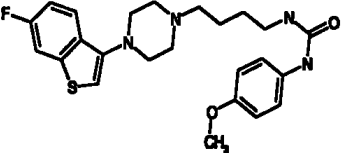
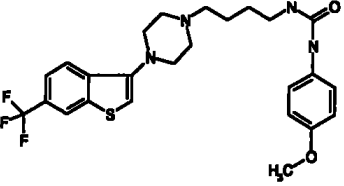
A major challenge in antipsychotic research is to produce agents with reduced side effects Orthostatic hypotension is a common side effect in antipsychotics that is associated with the high potency that these agents have at the alpha-1 adrenergic receptor (hereinafter referred to as "α-1") A major goal of this work was to find agents with reduced α-1 potency

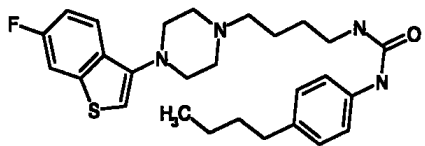
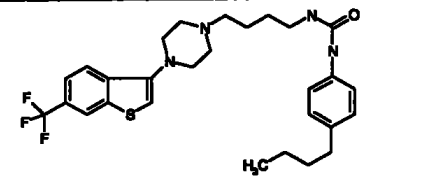
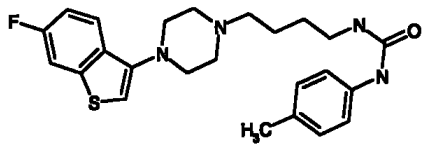
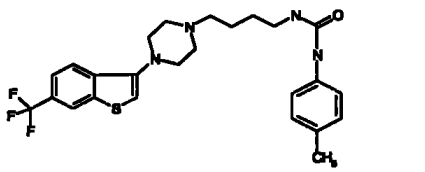
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The 6-trifluoromethyl benzo[b]thiophenes described herein have a clear and somewhat surprising advantage over the 6-fluoro benzo[b]thiophenes The 6-fluoro benzo[b]thiophenes are clearly more potent at the alpha-1 adrenergic receptor than are the 6-trifluoromethyl benzo[b]thiophenes This is shown by comparing pairs of analogs that only differ in substitution at the 6-position of the benzo[b]thiophene (see Table*II) In every case, the 6-fluoro benzo[b]thiophene is more potent than the corresponding 6-trifluoromethyl analog In

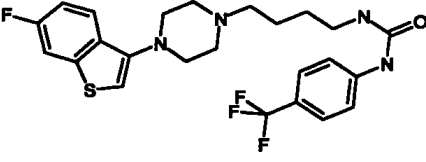
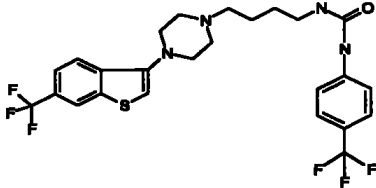
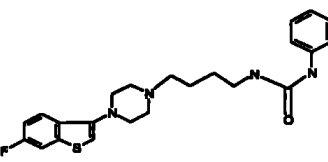
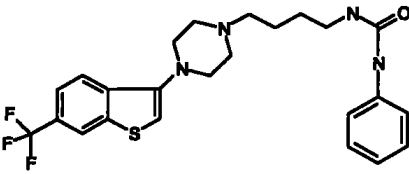
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some cases this small structural difference in substitution at the 6-position produces a dramatic change in α -1 potency. There are several examples wherein 6-fluoro analogs exhibited nanomolar potency while the corresponding 6-trifluoromethyl analog exhibited micromolar potency.

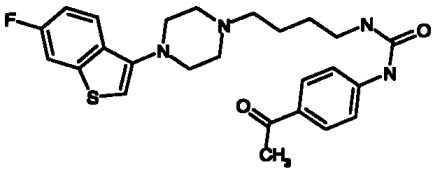
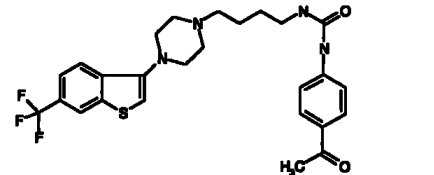
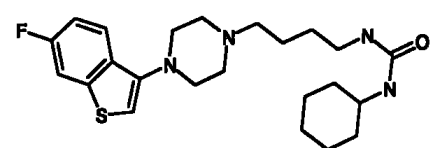
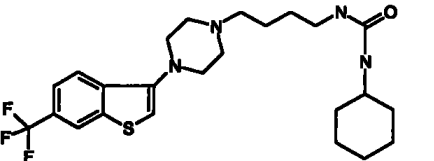
MDL NUM	MOLSTRUCTURE	halpha1KI (nM)	halpha1 % I
814949		18 2	
816000		234	
814933		1 8	
815894		1220	11 8% inh @ 1 uM

814937		532	
815995			42.4% Inh @ 10 uM
814941		13.5	
815997		936	23.5% Inh @ 1 uM

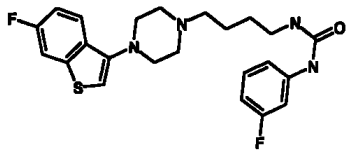
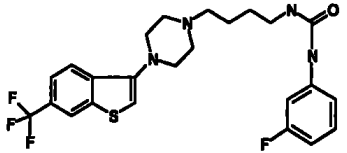
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814952		44.8	
816002		435	30.2% Inh @ 1 uM
813581		15	
816005		56.8	

11

814932		22	
815993		74.9	
814945		45	
816001		83.6	

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814953		35	
816003		567	

Especially preferred compounds of the instant invention are those with a reduced liability for α -1 receptor binding while at the same time having a higher affinity for dopamine D₃ than dopamine D₂ receptors

5 Receptor affinity can be measured using standard methodology such as is described below

[N-Methyl-³H]Spiroperidol Binding to Cloned Human Dopamine D₃ Receptors

Purpose

10

This assay measures the *in vitro* activity of compounds on cloned human dopamine (D₃) receptors and predicts the direct dopamine-blocking properties of putative neuropsychiatric agents at human dopamine D₃ receptors

15

Methods

A Cloning

20

The D₃ gene was isolated from a human striatal cDNA library (Stratagene). The gene was sequenced and sub-cloned into the expression vector RC/RSV (Invitrogen). CHO (Chinese Hamster Ovary) cells were stably transfected with 10 μ g of the D₃/RSV plasmid using the DOTAP method from Boehringer Mannheim and 72 clones that were G418 resistant were isolated. Using mRNA and binding displacement data a single high expressing clone was identified. This clone was then grown in large batches for the purpose of developing a 96 well format assay.

25

B Cell Culture

30

1 One plate (10 cm) with approximately 2-3 million D₃ cells per plate is incubated with 1 ml of Trypsin-EDTA at room temperature for ~2 min or until cells have lifted off plates. Four ml of Ham's F12 + 10% Fetal Bovine Serum + 1% Penicillin/Streptomycin + G418 (400 μ g/ml) medium are added to resuspend cells and 1 ml of this is added to each large plate (15 cm) containing 19 ml of the same medium as mentioned above.

-31-

2 The 5 large plates are incubated at 37°C + 5% CO₂ for ~ 3 days or until the cells are confluent

3 After these plates are confluent, they are split into 10 large plates Medium is
5 aspirated off, 2 ml of Trypsin-EDTA are added to each plate and plates are incubated at RT for 2 min or until cells have lifted off the plate Eight ml of the F12 medium (same medium as #1 above) are added to each plate (10 ml total) to resuspend the cells and 5 ml are transferred to the 2 new plates containing 15 ml of the F12 media

10 4 The 10 large plates are incubated at 37°C + 5% CO₂ for ~ 2 days or until the cells are confluent

5 The 10 large plates are split into 60 large plates (using Trypsin-EDTA as #3 except 4 ml of F12 medium are added to resuspend cells and 1 ml is aliquoted to 6 new plates
15 containing 19 ml of F12 medium each)

6 Plates are incubated at 37°C + 5% CO₂ for ~ 3 days or until cell are confluent

7 The 60 large plates are then split into 60 roller bottles (100-150 million cells/bottle)
20 Medium is aspirated off , 2 ml of Trypsin-EDTA are added to each plate and incubated at RT for ~2 minutes or until cells have lifted off plates Eight ml of F12 medium are added to each plate to resuspend cells and the entire 10 ml are added to 1 roller bottle containing 90 ml of the F12 medium

25 8 The 60 roller bottles are immediately placed on their sides and transferred to the roller bottle incubator They are incubated at 37°C + 5% CO₂ for ~ 3-5 days Cells are spun at 30-40% motor speed in the Forma Incubator

9 Medium is poured off and cells are washed 2X in PBS

30 10 Cells are then scraped off in 20 ml of PBS and the bottles are rinsed again with 5 ml of PBS to remove any remaining cells Cells are stored on ice before membrane preparation

11 The yield for 60 D₃ roller bottles has varied from ~ 260-500 mg

Note All tissue culture reagents are from Gibco-BRL

C Membrane Preparation

5 The cells are harvested into 250 ml centrifuge tubes with 100 volumes of cold phosphate buffered saline (PBS) and spun down (1200xG for 10 min at 4°C) The medium is removed and 100 ml PBS are added to each centrifuge tube, cells are resuspended and spun down again The PBS is removed and the final pellet is homogenized in an appropriate volume of 10% DMSO with a polytron on ice at a medium setting

D Lowry Protein Assay

10 A 200 µl sample membrane homogenate is added to 200 µl of 1% SDS, vortexed and allowed to stand for 5 min Aliquots (25, 50 and 100 µl) of this mixture are assayed in duplicate following the standard Bio-Rad DC protein assay protocol (kit catalog number 500-0112) and using reagent S Absorbance readings are made at 750 nm (note the most accurate protein OD readings are between 0.1-0.5 units) The protein concentration is calculated using a standard curve generated concurrently with bovine serum albumin as standard

E Storage/Freezing conditions

20 Following the determination of the protein concentration and Scatchard analysis, the protein is diluted into distilled water with 10% DMSO to the appropriate volume based on expression levels (Bmax) The concentrated protein is then aliquoted into 1.5 ml screw top cap Eppendorf tubes and placed into a -80°C freezer

F Binding Assay Reagents

1 0.5M Tris Buffer, pH 7.7

30 a) 44.4 g Tris HCl
26.5 g Tris Base
q.s. to 1 Liter (0.5 M Tris buffer, pH 7.7 at 37°C)

35 b) make a 1:10 dilution in distilled H₂O (0.05 M Tris buffer, pH 7.7)

2 Tris Buffer containing physiological salts**a) Stock buffer**

5 NaCl 7 014 g
 KCl 0 372 g
 CaCl₂ 0 222 g
 MgCl₂ 0 204 g
 q s To 100 ml with 0 5 M Tris Buffer

10

b) Dilute 1 10 in distilled H₂O

This yields 0 05 M Tris HCl, pH 7 7, containing NaCl (120 mM), KCl (5 mM), CaCl₂ (2 mM)
and MgCl₂ (1 mM)

15

Optional add 0 1% ascorbic acid and check pH (in assays with compounds that may
oxidize)

3 a) 1 0 % polyethylenimine stock in 0 5M Tris (reagent 1 a)**b) Dilute 1 10 in distilled H₂O**

20

**4 [N-methyl-³H]-Spiropendol (60-90 Ci/mmol) is obtained from New England Nuclear;
catalog #NET-856**

25

For K_d determinations [³H]NMSP is made up to a concentration of 2 7 nM in buffer 2b,
such that when 150 µl is added to each tube a final concentration of 0 4 nM is attained in the 1
ml assay Samples of total CPM added are taken for each experiment to calculate the total
ligand concentration

30

**5 S(-)-Eticlopride is obtained from Research Biochemicals International (RBI
catalog number E-101) A refrigerated stock (good for up to a month) solution of S(-)-
eticlopride is made at a concentration of 30 µM in buffer 2b One hundred microliters are
added to 3 wells for the determination of nonspecific binding (this yields a final concentration of
3 µM in the 1 ml assay)**

6 Test Compounds

For most assays, a 100 μ M stock solution of the test compound is made up in a suitable solvent (usually <0.1% acetic acid) and serially diluted with buffer 2b, such that when 100 μ l of drug is combined with the total 1 ml assay, final concentrations ranging from 10^{-5} - 10^{-8} M are attained. Characteristically eight concentrations are studied for each assay, however, higher or lower concentrations may be used, depending on the potency of the drug.

G Binding Assay

750 μ l Tissue

150 μ l [^{3} H]NMSP

100 μ l vehicle (for total binding) or 30 μ M (-)-eticlopride (for nonspecific binding) or appropriate drug concentration

The 96-Well Packard Unifilters GF/B are incubated for >1 h at 25°C in 0.1% polyethylamine (from 3,b). The cold tissue is added last and mixed on an orbital shaker for a few seconds and is then incubated at 37°C for 30 min in a shaking water bath. The assay is stopped by rapid filtration through Packard Unifilter plates. The filter membranes are then washed with 15 ml of ice-cold 0.05 M Tris buffer. The filters are then dried (~15 min under a heat lamp or incubated for 15 min in a 60°C oven) and a bottom seal is applied. Then 40 μ l of Packard Microscint 20 scintillation cocktail is added and a permanent topseal (Type P) is applied and heat sealed. The plates are then shaken on an orbital shaker for 1 h and placed in the Packard Topcount and counted for at least 5 minutes for each point.

Specific binding is defined as the difference between total binding and the binding in the presence of 3 μ M S-(-)-eticlopride. Total binding is approximately 10% of the total added ligand. Cheng-Prusoff determinations (K_i 's) are performed using Prism software using a one-site competition curve analysis where the top and the bottom of the non-linear regression are held constant at 0% and 100% percent inhibition. The percent inhibition at each drug concentration is the mean of duplicate determinations.

[N-Methyl-³H]Spiroperidol Binding to Cloned Human Dopamine D₂Long Receptors**Purpose**

- 5 This assay measures the *in vitro* activity of drugs on cloned human dopamine D₂Long (D₂L) receptors and predicts the direct dopamine-displacing properties of neuropsychiatric, cardiovascular and renal agents at human dopamine D₂ receptors

Methods

10

A Cloning

- The D₂L gene was isolated from a human striatal (caudate/putamen) cDNA library
The gene was sequenced and sub-cloned into the expression vector pRC/RSV (Invitrogen)
15 CHO (Chinese Hamster Ovary) cells were stably transfected and 72 clones that were geneticin (G418) resistant were isolated Using mRNA and binding data a single high expressing cell line was identified (#44) This cell line was then grown in suspension culture for the purpose of developing a 96 well format assay

20

B Cell Culture Conditions**1 Medium for adherent CHO cultures**

Ham's F12 + 10% fetal bovine serum (FBS) + 400 µg/ml geneticin
(G418) + 10 ml/L penicillin-streptomycin (pen-strep)

25

- 2 Cells are transferred to suspension culture when at least 1.5 million cells are available (this allows for 300,000 cells/ml in a 50 ml spinner flask, this is the ideal suspension density)
Cells are removed from flasks with trypsin, spun down (1000xG) and resuspended in fresh medium

30

50% CHO-SFM II + 50% Ham's F12 w/ 10% FBS (final FBS conc 5%)
+ 400 µg/ml G418 + pen-strep (10 ml/L)

- 3 After the transfer to suspension culture, growth is monitored and cell viability is assessed using trypan blue exclusion Total and viable cell count on 5 sectors of the hemocytometer are recorded When the viable cell density reaches 600,000 cell/ml, the volume is doubled
35

4 After 1 week of growth in the 50/50 medium, the cells are spun down and transferred to a new spinner flask and replaced with 75% CHO-SFM II / 25% Ham's F12 + 10% FBS plus the pen-strep and G418. Thereafter every 3 days, the medium is replaced with new medium
5 containing a decreasing amount of FBS as follows

ml of CHO SFM	ml of Ham'S F12	Final % FBS conc
87.50	12.5	1.25
93.75	6.25	0.625
99.00	1.00	0.1

10
5 The final maintenance culturing medium is made up as follows

A stock mixture of 10 ml of pen-strep, 0.5 ml of 400 mg/ml (active, final concentration 200 mg/ml) G418 and 1 ml of FBS are mixed and filtered and refrigerated. A volume (11.5 ml)
15 of this mixture is added to a freshly opened 1 L bottle of CHO-SFM II

C Membrane Preparation

The cells are harvested into 250 ml centrifuge tubes with 100 volumes of cold
20 phosphate buffered saline (PBS) and spun down (1200xG for 10 min at 4°C). The medium is removed and 100 ml PBS are added to each centrifuge tube, cells are resuspended and spun down again. The PBS is removed and the final pellet is homogenized in an appropriate volume of PBS with a polytron on ice at a medium setting.

25 D Lowry Protein Assay

A 200 µl sample membrane homogenate is added to 200 µl of 1% SDS, vortexed and allowed to stand for 5 min. Aliquots (25, 50 and 100 µl) of this mixture are assayed in duplicate following the standard Bio-Rad DC protein assay protocol (kit catalog number 500-0112) and
30 using reagent S. Absorbance readings are made at 750 nm (note: the most accurate protein OD readings are between 0.1-0.5 units). The protein concentration is calculated using a standard curve generated concurrently with bovine serum albumin as standard.

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E Storage/Freezing conditions

Following the determination of the protein concentration, the protein is diluted into distilled water with 10% DMSO to the appropriate volume based on expression levels (Bmax)

- 5 The concentrated protein is aliquoted into 1.5 ml screw top eppendorf tubes and placed into a -80°C freezer

F Binding Assay Reagents

- 10 1 0.5 M Tris Buffer, pH 7.7

- a) 44.4 g Tris HCl
 26.5 g Tris Base
 q.s. to 1 Ltr (0.5 M Tris buffer, pH 7.7 at 37°C)

15

- b) make a 1:10 dilution in distilled H₂O (0.05 M Tris buffer, pH 7.7)

- 2 Tris Buffer containing physiological salts

- 20 a) Stock buffer

 NaCl 7.014 g

 KCl 0.372 g

 CaCl₂ 0.222 g

 MgCl₂ 0.204 g

25

 q.s. To 100 ml with 0.5 M Tris Buffer

- b) Dilute 1:10 in distilled H₂O

This yields 0.05 M Tris HCl, pH 7.7, containing NaCl (120 mM), KCl (5 mM), CaCl₂ (2 mM) and MgCl₂ (1 mM)

- 30 Optional add 0.1% ascorbic acid and check pH (in assays with compounds that may oxidize)

- 3 a) 1.0 % polyethyleneimine stock in 0.5 M Tris (reagent 1 a)

- b) Dilute 1:10 in distilled H₂O

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4 [N-methyl-³H]-Spiroperidol (60-90 Ci/mmol) is obtained from New England Nuclear; catalog #NET-856

5 For K_i determinations [³H]NMSP is made up to a concentration of 2.7 nM in buffer 2b, such that when 150 µl is added to each tube a final concentration of 0.4 nM is attained in the 1 ml assay. Samples of total CPM added are taken for each experiment to calculate the total ligand concentration.

10 5 S(-)-Eticlopride is obtained from Research Biochemicals International (RBI catalog number E-101). A refrigerated stock (good for up to a month) solution of S(-)-eticlopride is made at a concentration of 30 µM in buffer 2b. One hundred microliters are added to 3 wells for the determination of nonspecific binding (this yields a final concentration of 3 µM in the 1 ml assay).

15 6 Test Compounds

For most assays, a 100 µM stock solution of the test compound is made up in a suitable solvent (usually <0.1% acetic acid) and serially diluted with buffer 2b, such that when 100 µl of drug is combined with the total 1 ml assay, final concentrations ranging from 10⁻⁵ - 10⁻⁸ M are attained. Characteristically eight concentrations are studied for each assay, however, higher or lower concentrations may be used, depending on the potency of the drug.

20

G Binding Assay

750 µl Tissue

25 150 µl [³H]NMSP

100 µl vehicle (for total binding) or 30 µM (-)-eticlopride (for nonspecific binding) or appropriate drug concentration

30 The 96-Well Packard Unifilters GF/B are incubated for >1 h at 25°C in 0.1% polyethylamine (from 3,b). The cold tissue is added last and mixed on a orbital shaker for a few seconds and is then incubated at 37°C for 30 min in a shaking water bath. The assay is stopped by rapid filtration through Packard Unifilter plates. The filter membranes are then washed with 15 ml of ice-cold 0.05 M Tris buffer. The filters are then dried (~15 min under a heat lamp or incubated for 15 min in a 60°C oven) and a bottom seal is applied. Then 40 µl of

Packard Microscint 20 scintillation cocktail is added and a permanent topseal (Type P) is applied and heat sealed. The plates are then shaken on an orbital shaker for 1 h and placed in the Packard Topcount and counted for at least 5 minutes for each point.

- 5 Specific binding is defined as the difference between total binding and the binding in the presence of 3 μ M S-(-)-eticlopride. Total binding is approximately 10% of the total added ligand. Cheng-Prusoff determination (K_i 's) are performed using Prism software using a one-site competition curve analysis where the top and the bottom of the non-linear regression are held constant at 0% and 100% percent inhibition. The percent inhibition at each drug
10 concentration is the mean of duplicate determinations.

**[³H]PRAZOSIN BINDING TO CLONED HUMAN ALPHA-1A ADRENERGIC RECEPTORS (α_{1a})
EXPRESSED IN CHINESE HAMSTER OVARY CELLS (CHO)**

15

Purpose

This *in vitro* assay is designed as a screen to identify compounds displaying a affinity for the human α_{1a} adrenoceptor subtype expressed in the membrane fragments of CHO cells. The assay measures the ability of the test compounds to displace [³H] prazosin from α_{1a} sites.

20

The identification of multiple vascular α_1 -adrenoceptors (α_{1a} , α_{1b} , α_{1d}) *in vitro* has provided impetus to define the role(s) of these subtypes in cardiovascular regulation *in vivo* (Vargas and Gorman, 1995).

25

Hemodynamic studies in the unanesthetized rat suggest that vascular α_{1a} receptors are the major subtype involved in the sympathetic regulation of peripheral resistance and systemic arterial pressure (Piascik et al, 1989). Additional evidence for an involvement of peripheral α_{1a} receptors in the maintenance of arterial pressure was demonstrated by the findings that the selective α_{1a} antagonist 5-MU dose dependently lowered resting arterial pressure in awake conscious dogs (Piascik et al, 1989). A demonstrated inability of the irreversible antagonist, chloroethylclonidine, to reduce arterial pressure in rats when administered intravenously, is strong evidence against the role of α_{1b} and α_{1d} receptors in the acute regulation of arterial pressure (Vargas et al, 1993).

30

-40-

Therefore, the binding of compounds to α_{1A} adrenergic receptors is believed to be a good measure of a compound's potential to cause orthostatic hypotension and sedation as side effects. Prazosin is a potent antagonist of the human α_{1A} -adrenoceptor subtype, which has been cloned and is expressed in the membrane fragments of CHO cells

h α_{1A} receptor

The cloning of the human α_{1A} cDNA was accomplished first by screening a human prostate cDNA library (Clontech), from which a portion of the coding region was obtained. This DNA fragment was then used to screen a human leukocyte genomic library (Clontech), and the rest of the coding sequence was obtained. Later these two fragments were spliced together. The entire coding sequence was then fully sequenced including matching PCR sequence with original genomic coding sequence, thus ensuring splice sites were joined correctly (Schwinn et al, 1995). Once sequenced, the gene was subcloned into the expression vector pcDNA3 (Invitrogen). Plasmid DNA was then used for transfection into CHO cells and G418 resistant clones were isolated. A clone expressing high levels of the h α_{1A} receptor (as determined by mRNA and receptor binding data) was chosen and pharmacologically characterized.

Culture Media

Media Ingredients for Adherent α_{1A} expressing CHO Culture

- A HAM's F-12 (Cellgro)
- B 10% 0.2 micron filtered Fetal Bovine Serum (FBS)(Cellgro)
- C 1% 0.2 micron filtered Penicillin-Streptomycin (Cellgro)
- D G418 0.2 micron filtered (Geneticin 400 μ g/ml)(Cellgro)

Cells are cultured using established methods and procedures in either 150x25mm culture plates (scale up to 100 plates) or a combination of these plates and 70 roller bottles. One culturing/harvest cycle typically requires 2 weeks and yields between 100-400mg protein. Plates or bottles are incubated at 37°C + 5% CO₂.

Storage

Cells are harvested by mechanical scraping, washed using PBS, collected in 250ml Corning polypropylene centrifuge tubes, spun down

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(1500RPM) and resuspended in dH₂O 10% DMSO (final volume per harvest is approximately 50ml) Protein determination is made using the Biorad DC Assay Kit Finally, the appropriate volume is aliquoted into a 2ml Corning Cryovial (10mg/1-1.5 ml) which is stored at -80°C

5

Current Lot Data α_1 (clone # 7)

Batch 1/14/98

Receptor Concentration

2418 fmoles/mg protein

 K_d

0.18nM

10

Volume

1.5 ml/cryovial

Protein Concentration

approx 10mg/1.5ml

Assay Requirement 0.5 cryovials per 96 well plate (assay volume = 200 μ l/well)

15

[³H]-Ligand[7-methoxy-³H]-Prazosin 1.0nM (NEN, NET-823)

70-87 Ci/mmol

Materials

Phentolamine mesylate (Research Biochemicals Int #P-131)

96 well flat bottom plates (Beckman)

20

Unifilter GF/B Plate (Packard)

Polyethylenimine (Sigma #P-3134)

TomTec or Packard Filtermate 196 Cell Harvesters

Packard TopCount Scintillation Counter

25

Buffers**A** 50 mM Tris HCl, 0.1% ascorbate, pH 7.7 (incubation buffer)**B** 50 mM Tris HCl, pH 7.7 (wash buffer)**Procedure**

Assay additions are as follows (in the order indicated)

Total Binding = 50 μ l buffer A + 50 μ l [³H]prazosin + 100 μ l membrane

30

Nonsp. Bd = 50 μ l 10 μ M phentolamine + 50 μ l [³H]prazosin + 100 μ l membrane**Test Cpd** = 50 μ l compound + 50 μ l [³H]prazosin + 100 μ l membrane

35

Compounds to be evaluated are weighed out to yield a 10 mM stock solution in DMSO in a 24 well polystyrene plate. This is diluted to a 0.5 mM stock in dH₂O. Serial dilutions in Buffer A are made from which 50

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µl additions to the plate are made in duplicate in order to achieve the final concentrations desired. Typically, one 96 well plate is used to evaluate 11 compounds at 4 concentrations (10^{-6} - 10^{-9} M) in duplicate. Total binding and nonspecific binding are determined in quadruplicate. Usually one standard is run with each assay.

[³H]Prazosin is made up in Buffer A such that when 50 µl are added per well the final concentration is 1.0 nM in a final assay volume of 200 µl. The final concentration should be verified by running a sample in a scintillation counter prior to adding the [³H]prazosin to the 96 well plate. Note: The radioactivity should be prepared just before the additions are made so that it is not allowed to sit on the bench for very long.

Packard GF/B Plate Pretreatment The filter plates are presoaked for at least 30 min in ice cold Buffer B containing 0.05% polyethyleneimine (200 µl/200 ml) to maximize filtration efficiency and minimize filter blank binding.

Incubation & Filtration Once buffer, compounds, [³H]prazosin and membrane have been added (and mixed), the 96 well plates are incubated for 40 min at 37°C and spaced 3-5 min apart. At 40 min, the plates are filtered using a Tomtec Automated Cell Harvester. Filtration is immediately followed by washes of ice cold Buffer B (total vol ~7 ml).

Drying and Counting Each filter plate is dried under a heat lamp for 15 min. The back of the plate is sealed and 40 µl of Packard microscint fluid are added per well. Using Packard film, each plate is heat sealed prior to being counted in a Packard Topcount Scintillation counter. A program has been written that counts each plate twice sending DPM, CPM and TSIS data to disk and printer.

Analysis of Results Raw DPM and CPM data are captured on disk and are imported into one of several software packages (Graphpad Prism Ver 2.0, Excel) residing on the LAN. Specific binding is defined as the difference between total binding and the binding in the presence

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of 10 μ M phentolamine. Total binding is less than 10% of the total added ligand. Software using one-site competition curve analysis is employed in the calculation of IC_{50} and K_i (Cheng-Prusoff equation, 1973). The top and bottom of the non-linear regression are held constant at 0% and 100% inhibition. The percent inhibition at each drug concentration is the mean of duplicate determinations.

References

- Vargas, H M and A J Gorman *Life Sciences* Vol 57, No 25, pp 2291-2308, 1995
- Morrow, A L and I Creese *Mol Pharmacol* 29 321-330, 1986
- Piascik, M T, J W Kusiak, and K W Barron *Eur J Pharmacol* 11 101-107, 1989
- Vargas, H M, D Cunningham, L Zhou, H B Hartman and A J Gorman *J Pharmacol Exp Ther* 267 264-272, 1993

The functional activity of compounds of the invention (i.e. whether they are antagonists, agonists or partial agonists) can readily be determined using the microphysiometer test method that follows:

Chinese Hamster Ovary (CHO) cells, expressing the human dopamine D₃ receptor, were grown on the surface of a capsule cup. Cups are assembled and placed on the microphysiometer, and buffer (Dulbecco's Modified Eagle's Medium without sodium bicarbonate and without serum) is perfused through the cup assembly until a stable baseline is achieved (4 hours). Buffer perfusion rate and solution changes were controlled by a computer. Intracellular acidification rate was measured in each of the 8 cup assemblies and recorded by the computer. Buffer containing test compound (10 nM, 100 nM, and 1 μ M) was perfused through the cup assembly for 20 min, then buffer containing quinpirole (10 nM) and test compound (same concentrations) was perfused for an additional 1 min. This was followed by a recovery period of 10-60 min where buffer alone was perfused through the cups. Quinpirole increased acidification rate. A D₃ antagonist will inhibit this increase in a concentration dependent manner.

D₃ antagonists are of potential use as antipsychotic agents for example in the treatment of schizophrenia, schizo-affective disorders, psychotic depression and mania. Conditions which may be treated by D₃ agonists include dyskinetic disorders such as

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Parkinson's disease, neuroleptic-induced parkinsonism and tardive dyskinesias, depression, anxiety, dementia, circadian rhythm disorders, and drug (e.g. cocaine) dependency

In accordance with yet another embodiment of the present invention, there is provided
5 a method of modulating the activity of dopamine D₃ receptors, said method comprising contacting cell-associated dopamine D₃ receptors with a concentration of a compound of formula I, or a physiologically acceptable salt thereof, sufficient to modulate the activity of said dopamine D₃ receptor

10 As employed herein, the phrase "modulating the activity of dopamine D₃ receptors" refers to a variety of therapeutic applications. Said therapeutic applications refer to the treatment of conditions or disorders which include dyskinetic disorders, psychoses, anxiety disorders, mood disorders, dementia, sleep disorders, substance dependence, substance abuse and nausea. The compounds are also useful for treating renal dysfunction

15 The instant invention also provides a method of treating conditions or disorders of the central nervous system comprising administering to a patient in need thereof a therapeutically effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof

20 The instant invention also provides a method of treating renal dysfunction comprising administering to a patient in need thereof a therapeutically effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof

25 The instant invention further provides a method of treating conditions or disorders of the central nervous system comprising administering to a patient in need thereof a therapeutically effective amount of a compound of formula I, or a pharmaceutically acceptable salt thereof, in conjunction with one or more D₁, D₂, D₄, D₅ or 5-HT receptor antagonists

30 In treating a patient afflicted with a condition or disorder described above, a compound of formula I can be administered in any form or mode which makes the compound bioavailable in therapeutically effective amounts, including orally, sublingually, buccally, subcutaneously, intramuscularly, intravenously, transdermally, intranasally, rectally, topically, and the like. One skilled in the art of preparing formulations can determine the proper form and mode of administration depending upon the particular characteristics of the compound
35 selected for the condition or disease to be treated, the stage of the disease, the condition of

the patient and other relevant circumstances For example, see Remington's Pharmaceutical Sciences, 18th Edition, Mack Publishing Co (1990), incorporated herein by reference

The compounds of Formula I can be administered alone or in the form of a pharmaceutical composition in combination with pharmaceutically acceptable carriers, the proportion and nature of which are determined by the solubility and chemical properties of the compound selected, the chosen route of administration, standard pharmaceutical practice and other relevant criteria

The compounds of formula I may be administered orally, for example, in the form of tablets, troches, capsules, elixirs, suspensions, solutions, syrups, wafers, chewing gums, sprinkles, and the like and may contain one or more of the following adjuvants binders such as microcrystalline cellulose, gum tragacanth or gelatin, excipients such as starch or lactose, disintegrating agents such as alginic acid, Primogel, corn starch and the like, lubricants such as magnesium stearate or Sterotex, glidants such as colloidal silicon dioxide, and sweetening agents such as sucrose or saccharin may be added or a flavoring agent such as peppermint, methyl salicylate or orange flavoring When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol or a fatty oil Other dosage unit forms may contain other various materials which modify the physical form of the dosage unit, for example, as coatings Thus, tablets or pills may be coated with sugar, shellac, or other enteric coating agents A syrup may contain, in addition to the present compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors

The compounds of Formula I may also be administered topically, and when done so the carrier may suitably comprise a solution, ointment or gel base The base, for example, may comprise one or more of petrolatum, lanolin, polyethylene glycols, bee wax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers

The solutions or suspensions may also include one or more of the following adjuvants sterile diluents such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents, antibacterial agents such as benzyl alcohol or methyl paraben, antioxidants such as ascorbic acid or sodium bisulfite, chelating agents such as ethylene diaminetetraacetic acid, buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose

vials

The highly lipophilic esters, amides and carbamates of compounds I are capable of sustained release in mammals for a period of several days or from about one to four weeks when formulated and administered as depot preparations, as for example, when injected in a properly selected pharmaceutically acceptable oil. The preferred oils are of vegetable origin such as sesame oil, cottonseed oil, corn oil, coconut oil, soybean oil, olive oil and the like, or they are synthetic esters of fatty acids and polyfunctional alcohols such as glycerol or propyleneglycol.

The depot compositions of formula I are prepared by dissolving a highly lipophilic ester, amide or carbamate of the instant invention in a pharmaceutically acceptable oil under sterile conditions. The oil is selected so as to obtain a release of the active ingredient over a desired period of time. The appropriate oil may easily be determined by consulting the prior art, or without undue experimentation by one skilled in the art.

The dosage range at which the compounds of formula I exhibit their ability to act therapeutically can vary depending upon the particular disease or condition being treated and its severity, the patient, the formulation, other underlying disease states that the patient is suffering from, and other medications that may be concurrently administered to the patient. Generally, the compounds of formula I will exhibit their therapeutic activities at dosages of between about 0.001 mg/kg of patient body weight/day to about 100 mg/kg of patient body weight/day.

In a further aspect, the present invention provides novel radiolabeled imaging agents of formula I, useful, inter alia, for imaging dopamine D₃ receptors in the CNS to diagnose CNS abnormalities.

The radiolabeled (tritiated and C-14 labeled) forms compounds of formula I are useful as radioligands to determine the binding of compounds to the dopamine D₃ receptor. They are also useful as labeled parent compounds to determine the metabolism of the compound in animals. Preferred for this purpose are compounds of formula I wherein R is group (a) with a radiolabeled ¹⁴C in the 3-position of the benzo[b]thiophene ring system, L is trifluoromethyl, p is 1, R₃ is hydrogen, n is 1, i is 0, and A is N. Particularly preferred for this purpose are compounds of formula IA. As employed herein, a "compound of formula IA" shall refer to the compound of formula I wherein R is group (a) wherein L is trifluoromethyl substituted in the 6-

position of the benzthiophene ring system and a radiolabeled ^{14}C is in the 3-position of the benzo[b]thiophene ring system, p is 1, A is N, n is 1, i is 0, and R_3 is hydrogen. Compounds of formula IA may be prepared in a manner analogous to that set forth in Example 21

5 Imbalances in dopamine production have been implicated in a variety of mental and physical disorders, such as Parkinson's disease (PD). It is thus desirable to diagnose and monitor such imbalances and to monitor the effectiveness of drugs and substances that affect brain chemistry. New and powerful imaging methods that enable one to assess the living
10 brain in vivo and thereby monitor brain chemistry and the effectiveness of drugs and substances that affect brain chemistry have been developed. Methods such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) involve administering to a patient a radioactive tracer substance comprising a ligand that binds to the presynaptic or postsynaptic neuroreceptors in the patient's brain. Emissions (primarily
15 gamma rays are emitted from the positrons or photons from the radioactive tracer) are measured. These emissions are indicative of the number and degree of occupancy of blocking of the neuroreceptors. The number of neuroreceptors and the degree of occupancy or blocking is calculated utilizing a mathematical model, and compared with an intra-person or inter-person control to determine the degree of drug response. Further treatment of the patient with drugs is based on the comparisons made. For these methods to be useful, however, a
20 ligand that has a high specificity and affinity for the desired receptor is required.

 It is believed that certain radioactive ligands may be selective for dopamine transporters and are thus potentially useful in evaluating changes in dopamine function in vivo and in vitro, especially for patients with Parkinson's disease (PD), which is characterized by a
25 selective loss of dopamine neurons in the basal ganglia and substantia nigra.

 Another aspect of this invention relates to methods for utilizing the compounds of the invention as CNS imaging agents. Imaging techniques are non-invasive diagnostic techniques that generally involve administering a compound with marker atoms that can be detected
30 externally to the mammal. Generally, these methods comprise administering to a mammal a compound of the invention, dissolved or dispersed in a suitable pharmaceutical carrier or diluent. The compound of the invention selectively binds to dopamine D_3 , thus permitting the imaging of CNS receptors and the ability to, inter alia, evaluate brain chemistry, the effectiveness of drugs, and neuronal functions. Imaging techniques suitable for practicing the
35 present invention include, but are not limited to, single photon emission computed tomography

-48-

(SPECT) and positron emission tomography (PET)

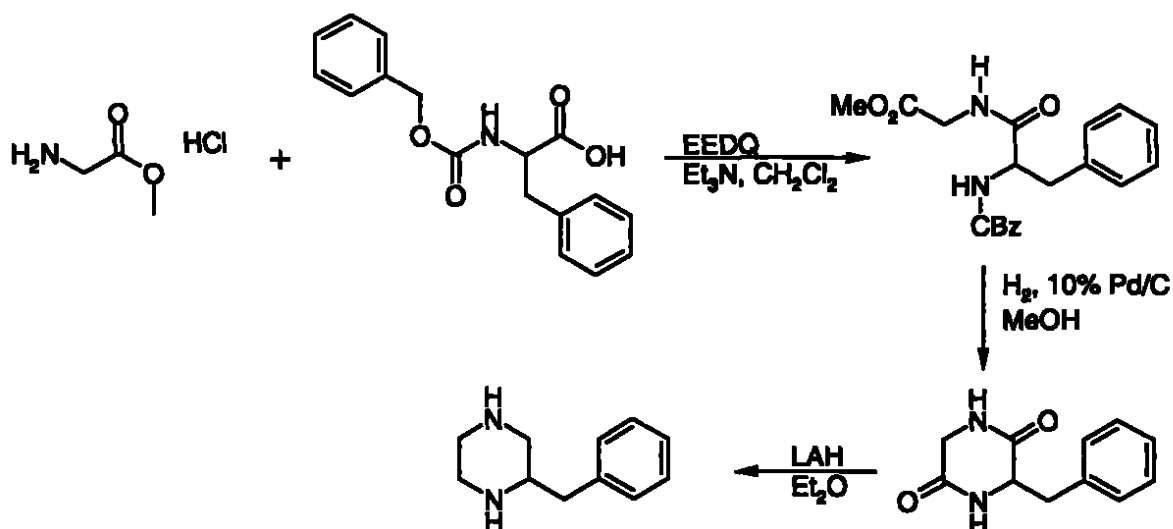
Radionuclides that are widely used in diagnostic nuclear medicine include technetium [^{99}Tc], iodine [^{123}I], carbon [^{11}C], and fluorine [^{18}F]. The radiolabeled imaging agent specifically exemplified herein contains the radionuclide ^{11}C . It should be noted that the same or similar radiochemistry reactions can be carried out using radionuclides other than ^{11}C .

The invention is further illustrated by the following non-limiting examples and tabulated information. These examples are understood to be illustrative only and are not intended to limit the scope of the present invention in any way. As used herein, the following terms have the indicated meanings: "g" refers to grams, "mmol" refers to millimoles, "ml" refers to milliliters, " $^{\circ}\text{C}$ " refers to degrees Celsius, "TLC" refers to thin layer chromatography, "LC/MS" refers to liquid chromatography mass spectrometry, "APCI" refers to atmospheric pressure chemical ionization, "mp" refers to melting point.

EXAMPLES

Example 1

5

Synthesis of Intermediate Substituted Piperazines10 **Example 1(a) Preparation of intermediate 3-benzyl-piperazine**

To a suspension of 3-benzyl-piperazine-2,5-dione (14.98 g, 73 mmol, prepared following generally the procedure of Halpern and Westley, J Org Chem 1968, 33, 864) in dry diethyl ether (500 mL) is added dropwise to a solution of lithium aluminum hydride (400 mL of a 1 M solution in diethyl ether, 400 mmol, 5.4 eq). The suspension is heated at reflux for 23 hours and then cooled to 0°C. Water (70 mL) is then cautiously added and the resulting suspension is warmed to room temperature. After 3 hours the suspension is filtered and the solid washed with diethyl ether (1 L). The filtrate is concentrated under vacuum to provide crude title compound (11.40 g, 88%) as a yellow, crystalline solid. A sample (2 g) is recrystallized from cyclohexane and then from toluene to provide the purified title compound (0.83 g) as a fine, white crystals mp 80-81°C.

20

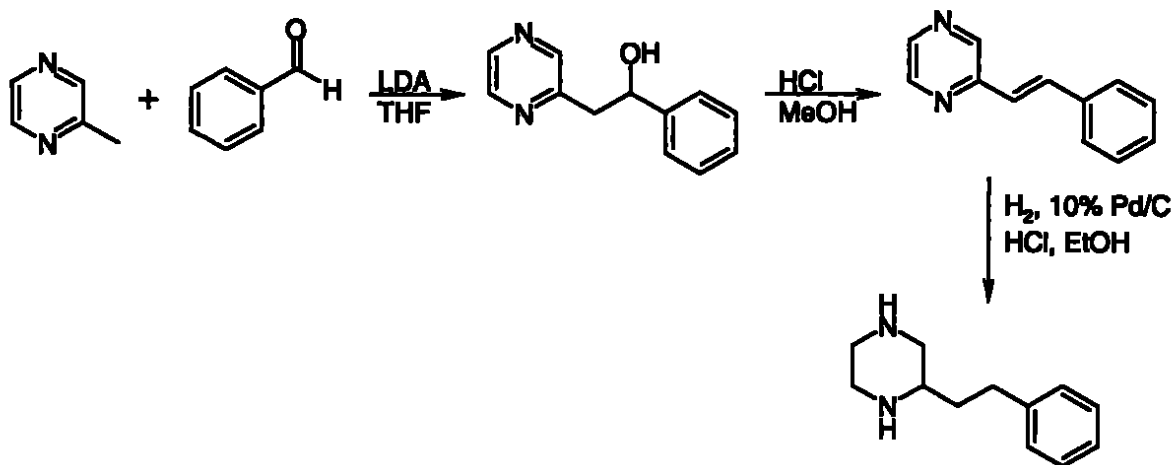
Anal Calcd For $\text{C}_{11}\text{H}_{16}\text{N}_2$

C, 74.96, H, 9.15, N, 15.89,

Found

C, 74.84, H, 9.01, N, 16.15

-50-



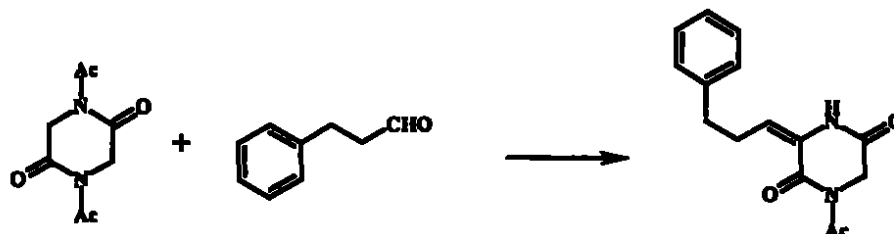
Example 1(b) To a solution of LDA (295 mL, 0.59 mol, 2 M in heptane/ THF/ ethylbenzene) in anhydrous THF (300 mL) cooled to -40°C was added 2-methylpyrazine (48.5 mL, 0.531 mol) dropwise via an addition funnel. The reaction was allowed to warm to -20°C and was stirred for 90 minutes when a solution of benzaldehyde (54 mL, 0.531 mol) in anhydrous THF (200 mL) was added dropwise via an addition funnel. After complete addition, the reaction was allowed to warm to room temperature and was stirred for 20 hours. The reaction was then cooled in an ice bath and saturated NH₄Cl (500 mL) was added. The resulting mixture was extracted with EtOAc (500 mL, 250 mL). The combined extracts were dried (Na₂SO₄), filtered and concentrated to a damp, beige solid. The product was triturated with Et₂O and collected then dried overnight to yield 56.0 g (53%) of a light brown solid, mp $81-84^{\circ}\text{C}$.

A solution of the above-obtained solid (56.0 g, 0.28 mol) in MeOH (1.1 L) and conc. HCl (290 mL) was stirred at reflux for 24 hours. The reaction was cooled to room temperature and concentrated to a dark liquid. The dark liquid was cooled in an ice bath and water (1 L) was added. The resulting solution was neutralized with a saturated solution of Na₂CO₃ and the product was extracted with EtOAc (1 L, 2 x 500 mL). The combined extracts were dried (Na₂SO₄), filtered and concentrated to yield 46 g of a dark brown solid. The solid was purified via flash column chromatography (40% EtOAc in heptane) yielding 22.7 g of the olefin as a brown foam.

A 1 L Parr shaker bottle was flushed with nitrogen and charged with 10% Pd/C (4.5 g, Degussa type) and the above-obtained olefin (20.0 g, 0.110 mol) in EtOH (450 mL). The reaction was hydrogenated at 50 psi for 3.5 hours when the reaction was filtered through a celite plug and rinsed with ethanol. The bottle was recharged with fresh 10% Pd/C (4.5 g, Degussa type), the filtrate and conc. HCl (15 mL). The reaction was hydrogenated at 50 psi for 18 hours when the reaction was diluted with warm MeOH and filtered through a plug of

celite The solid was thoroughly washed with hot MeOH and the filtrate was concentrated to yield 11.2 g (39%) of the final product as the di-HCl salt, mp 297-300

See Tetrahedron, 30, 1974 pp667-673 and Tet Lett 1979, pp4483-4486



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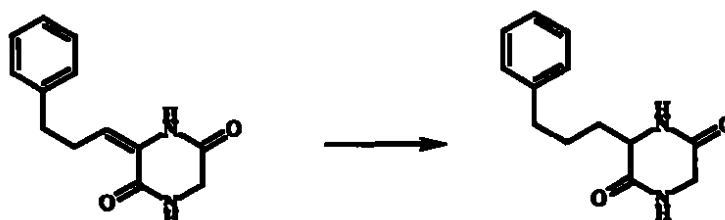
Example 1(c) DBU (14.0 g, 92 mmol) was added to a solution of the piperazine diacetate (18.2 g, 92 mmol) and aldehyde (12.3 g, 92 mmol) in 92 mL of DMF at ambient temperature. The resulting mixture was stirred at room temperature for 5h. The precipitated product was collected by filtration, providing 17.1 g of product.

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The monoacetate (17.0 g, 62.8 mmol) and hydrazine hydrate (9.4 g, 188.6 mmol) in 125 mL of DMF were stirred at room temperature for 20 h. The precipitated solid was collected by filtration, and washed with water and ethanol, leaving 13.7 g of product.

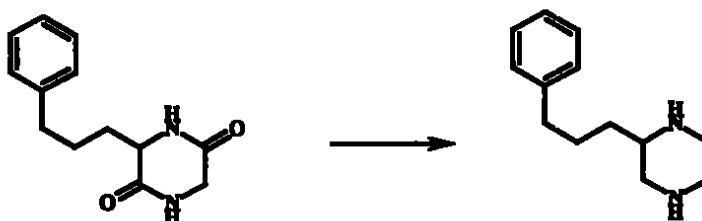
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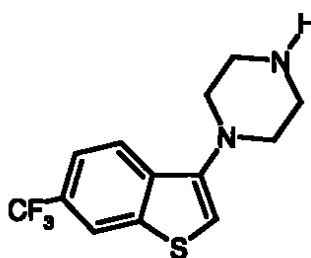
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The olefin (13.6 g, 59.1 mmol) and palladium on carbon (2.7 g, 10% Pd/C, Degussa type, 50% H₂O) in 1.2 l of methanol were shaken on a Parr hydrogenation apparatus at 40 psi of hydrogen, until hydrogen uptake ceased. The mixture was diluted with dichloromethane and filtered through celite. Concentration of the filtrate provided 12.1 g of product.

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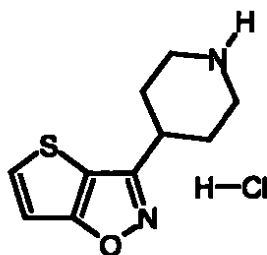
A solution of LAH (156 mL, 156 mmol, 1M in THF) was added dropwise to a 0°C solution of the piperazine dione (12.1 g, 52.1 mmol) in 100 mL of THF. The mixture was heated to reflux and stirred overnight. The mixture was cooled to 0°C and 38 mL of water in 200 mL of THF was carefully added. The resulting mixture stirred for 1 h, then it was filtered, the filter cake was washed with THF, and the filtrate was concentrated in vacuo to give 7.4 g of product.

Example 2**1-(6-(trifluoromethyl)benzo[b]thien-3-yl)piperazine hydrochloride****2a 2-Carbomethoxy-3-amino-6-trifluoromethylbenzo[b]thiophene**

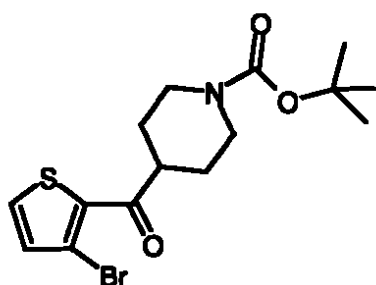
Equip a 22-L, 3-necked, round-bottom flask with a mechanical stirrer, nitrogen bubbler, and a thermocouple probe, charge with 1.20 kg (5.55 mole) of 2-nitro-4-trifluoromethylbenzonitrile, 589.3 g (496 mL, 5.55 mole) of methyl thioglycolate, and 4.3 L of NMP. Cool the resulting yellow solution to 2 °C, and add slowly, over a period of 78 min a solution prepared from 466.0 g (11.11 mole, 2.0 eq) of lithium hydroxide monohydrate in 3.36 L of water while maintaining the temperature between 2 - 20 °C. Allow the brown slurry to warm to 21 °C over a 2 h period, and then dilute with 8.0 L of water (observe exotherm -> 27 °C). Stir for 40 min and cool to 18 °C, collect the product by filtration, rinsing with 10 L of water, then air-drying at ambient temperature to give 1.295 kg (84.7% yield) of 2-carbomethoxy-3-amino-6-trifluoromethylbenzo[b]thiophene, as a light-yellow solid, 99.8% pure by HPLC assay.

2b 1-(6-(trifluoromethyl)-benzo[b]thien-3-yl)-piperazine hydrochloride

Equip a 12-L, 3-necked, round-bottom flask with a mechanical stirrer, nitrogen bubbler, and a thermocouple probe, and charge with 1.14 kg (4.14 mole) of 2-carbomethoxy-3-amino-6-trifluoromethylbenzo-[b]thiophene (Example 2a), 196.0 g (2.28 mole, 0.55 eq) of piperazine, 4.0 L of NMP, and 570 mL of xylene. Heat the solution, and hold at 170 – 180 °C for 4 h, at which time the reaction is ca. 98% complete as determined by HPLC assay. Cool the brown solution to 168 °C, and then add 1.605 kg (18.63 mole, 4.5 eq) of piperazine (temp → 109 °C) following with 1.575 kg (28.28 mole, 2.0 eq) of p-toluenesulfonic acid monohydrate (observe exotherm, 109 → 130 °C). Connect a Dean-Stark trap to the condenser, and heat the reaction to collect an azeotrope. Remove a total of 410 mL of an aqueous distillate, while allowing the pot temperature to increase from 145 to 165 °C. Monitor the progress of the reaction by GC/MS and HPLC assays. After 14 h at ca. 165 °C (>99% conversion by HPLC and GC/MS assay), cool the reaction to 30-35 °C, and then quench into an extractor that contains 5 kg of ice, 12 L of water, and 8.5 L of toluene. Separate the phases, wash the organic extract with 11 L of 0.5 N NaOH, 2 L of saturated aq. NaCl, and then extract with 8 L of 1 N HCl. Dilute the acidic aqueous extract with 1 kg of ice, and basify to pH 11.2 by adding 624 g of 50% NaOH. Extract the resulting mixture with 9.5 L of toluene. Wash the toluene extract with 2 L of saturated aqueous NaCl, dry (Na₂SO₄), and filter. Charge the filtrate into a 22 L 3-necked, round-bottomed flask (N₂, mechanical stirring, temperature control probe), and add a total of 3.7 L of 1N ethereal HCl at 20 – 27 °C so that the mixture is positive to Congo Red indicator paper. During the HCl addition, add a total of 2.5 L of toluene to improve the stirring of the thick slurry that results. Stir at ambient temperature for 40 min, filter the slurry and wash with 4.5 L of toluene. After air drying, obtain 1.165 kg (87% yield) of 3-piperazinyl-6-trifluoromethyl-benzo[b]thiophene hydrochloride as a light pink-beige solid, 99.1% pure by GC/MS assay.

Example 3**3-Piperidinyl-4-yl-thieno[2,3-d]isoxazole hydrochloride**

-54-

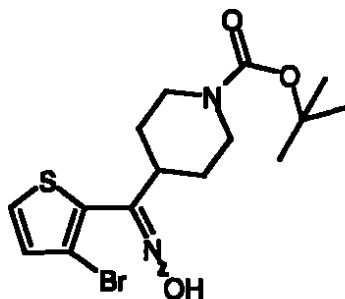
**3a 4-(3-Bromo-thiophene-2-carbonyl)-piperidine-1-carboxylic acid tert-butyl ester**

5

Stir a solution, under nitrogen, of 3-bromothiophene (21.0 mL, 0.224 mol) in tetrahydrofuran (1.0 L) at -78°C, and add a 2.0M solution of lithium diisopropylamide in heptane/tetrahydrofuran/ethylbenzene (112 mL, 0.224 mol) for 45 min. Add, dropwise, over 2 h, a solution of 4-(N-methoxy-N-methylcarboxamido)-1-piperidinecarboxylic acid 1,1-dimethylethyl ester (prepared according to US 5,134,139) (79.4 g, 0.291 mol) in tetrahydrofuran (800 mL). Stir for 2 h, add a saturated ammonium chloride solution, and stir for an additional 0.5 h. Filter the resulting solid, and pour the filtrate into water (800 mL). Extract the aqueous mixture with ether and concentrate to obtain a dark liquid. Pour the liquid into water (400 mL), add NaCl and extract the aqueous mixture with ether. Wash the extract with water, brine, and dry over Na₂SO₄. Filter and concentrate to obtain the crude product. Chromatograph the product over silica gel (pet ether/ether, 4:1) to obtain 41.5 g (50%) of white solid.

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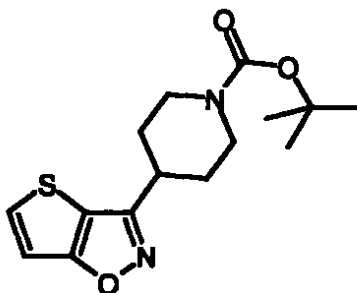
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3b 4-[(3-Bromo-thiophen-2-yl)-hydroxyimino-methyl]-piperidine-1-carboxylic acid tert-butyl ester

Stir a mixture of 4-(3-bromo-thiophene-2-carbonyl)-piperidine-1-carboxylic acid tert-butyl ester (Example 3a) (41.5 g, 0.11 mol), hydroxylamine hydrochloride (15.4 g, 0.23 mol) and pyridine (190 mL) at ambient temperature overnight. Pour the reaction into

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water (500 mL) and extract with dichloromethane (3x). Wash the combined extracts with saturated CuSO_4 solution (2x), dry (MgSO_4) and concentrate to a green solid. Dissolve the solid in toluene (175 mL) and let stand at ambient temperature for 3 h. Collect the resulting crystals that form and wash with toluene (60 mL). Concentrate the filtrate and again dissolve the residue in toluene and proceed to collect additional crystals to obtain a total yield of 25 g (58%) of the title compound as a light, green solid.



3c 4-Thieno[2,3-d]isoxazol-3-yl-piperidine-1-carboxylic acid tert-butyl ester

Add to a stirring solution of 4-[(3-bromo-thiophen-2-yl)-hydroxyimino-methyl]piperidine-1-carboxylic acid tert-butyl ester (Example 3b) (25 g, 64.2 mmol) in 2-methoxyethanol (200 mL), a solution of potassium hydroxide (7.2 g, 128.4 mmol) in water (20 mL). Heat the reaction to 60°C and then add copper powder (1.25 g). Stir at 60-70°C for 6 h and then at ambient temperature overnight. Pour the reaction mixture into water (500 mL) and extract with EtOAc (3x). Concentrate to a dark residue and purify by column chromatography over silica gel (heptane/EtOAc, 4:1) to provide 9.8 g (50%) of a white solid.

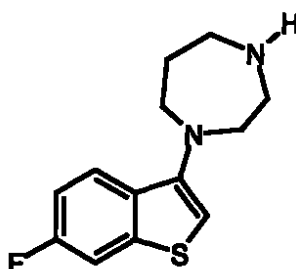
3d 3-Piperidinyl-4-yl-thieno[2,3-d]isoxazole hydrochloride

Add ethereal HCl (10 mL) to 4-thieno[2,3-d]isoxazol-3-yl-piperidine-1-carboxylic acid tert-butyl ester (Example 3c) (1.0 g, 3.2 mmol) and then methanol (1 mL) to effect solution. Permit to stand at ambient temperature for 1 h and then collect 0.34 g of white solid, mp 240-241°C. From the filtrate collect 0.25 g of additional white solid, mp 263-265°C. Both samples MS, $m/z = 209$ ($M+H$)⁺.

Analysis (sample mp 263-265°C)

Calc For $\text{C}_{10}\text{H}_{12}\text{N}_2\text{OS} \cdot \text{HCl}$	49.08% C	5.35% H	11.45% N
Found	49.03% C	5.29% H	11.25% N

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Example 4**1-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepane**

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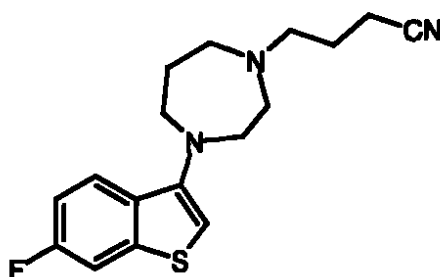
4a 3-Amino-6-fluoro-benzo[b]thiophene-2-carboxylic acid

At 50°C, add to a stirring solution of 2-carbomethoxy-3-amino-6-fluoro-benzo[b]thiophene (prepared according to US 5,143,923), (90.1 g, 0.4 mol) in H₂O (450 mL), a 50% aqueous solution of NaOH (64 g, 0.8 mol) over 2-3 min. Heat the reaction to 70-73°C and continue to stir for 3 h. Add 10% aqueous isopropanol (45 mL) and bring to reflux. Remove the isopropanol under N₂ and add H₂O (300 mL). Cool the reaction mixture to between 7-10°C and add concentrated HCl (80 mL). Add H₂O (650 mL), cool to 5-7°C, filter the resulting solid, and wash the filter cake with H₂O (2x150 mL). Dry the solid under vacuum at 35°C to obtain 80.6 g (94.7%) of solid mp 160-163°C, TLC on silica gel (dichloromethane/methanol, 3:1), R_f = 0.69.

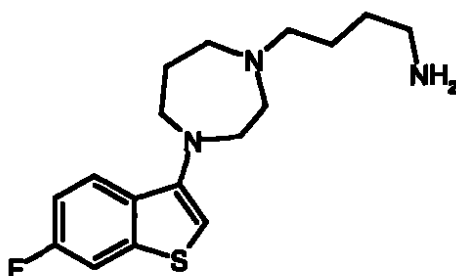
4b 1-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepane

Heat a solution of 3-amino-6-fluoro-benzo[b]thiophene-2-carboxylic acid (5.0 g, 24 mmol) in 1-methyl-2-pyrrolidinone (5 mL) to 100°C for 2 h, and then, introduce a stream of nitrogen, to cool the solution to room temperature. Add homopiperazine (9.5 g, 95 mmol) and p-toluene sulfonic acid monohydrate (9.0 g, 47 mmol) and heat the mixture to 145°C for 4 h. After that time, cool the reaction mixture to room temperature, dilute with ethyl acetate (30 mL) and wash with brine (3x15 mL). Separate the organic layer and dry over Mg SO₄. Evaporate the solvent and purify the crude product by column chromatography (SiO₂, 100 g CH₂Cl₂/MeOH 9:2, then CH₂Cl₂/MeOH/NH₄OH 9:2:0.15) to give 3.9 g (65%) of yellowish oil. LC/MS (Lichrospher 5 μ, RP-18, 250 mm) CH₃CN/ Water-gradient 20% → 100% (25 min), Flow 1.5 mL/min) t_R = 10.74 min, m/z = 250.3.

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Example 55 **4-[4-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazapan-1-yl]butyronitrile**

Add potassium carbonate (39.3 g, 284 mmol) to a solution of 1-(6-fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepane (Example 4) (23.7 g, 95 mmol) and 4-bromobutyronitrile (21.0 g, 142 mmol) in acetonitrile (400 mL) and stir the mixture under reflux for 10 h. Filter the mixture, evaporate the solvent, and dissolve the residue in ethyl acetate (EtOAc). Wash with water and saturated sodium chloride solution, and dry the organic phase over MgSO_4 . Evaporate the solvent under vacuum, and purify the crude product by column chromatography (EtOAc/MeOH 9:1) to give 12.9 g of a yellow oil. LC/MS, (LiChrospher 5 μ , RP-18, 250 mm $\text{CH}_3\text{CN}/\text{Water}$ (0.05% TFA)-gradient 2% $\rightarrow$ 98% (20 min), Flow 0.75 mL/min)
15 t_R = 9.46 min, m/z = 317.3

Example 620 **4-[4-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazapan-1-yl]butylamine**

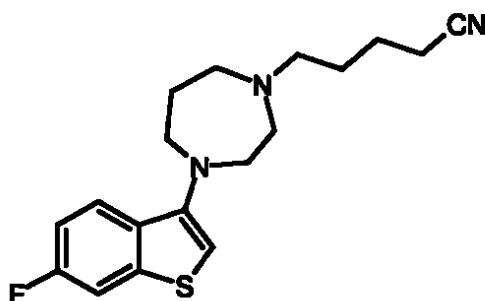
Add over 30 min, at room temperature, a solution of LiAlH_4 in diethyl ether (1M, 72.5 mL) to a solution of 4-[4-(6-fluoro-benzo[b]thiophen-3-yl)-[1,4]diazapan-1-yl]butyronitrile (Example 5) (11.5 g, 36.2 mmol) in dry diethyl ether (200 mL). Heat the solution to reflux for 5 h. After that
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time, allow the solution to cool to room temperature and carefully quench the reaction with water and aqueous sodium hydroxide solution. Separate the phases, and re-extract the aqueous phase with EtOAc. Dry the combined organic phases over MgSO_4 and remove the solvent under vacuum. Purify the crude product by column chromatography

- 5 (CH₂Cl₂/MeOH/NH₄OH 9:2:0.25) to obtain 8.9 g of a colorless oil. LC/MS, (LiChrospher 5 μ , RP-18, 250 mm CH₃CN/ Water (0.05% TFA)- gradient 2% $\rightarrow$ 98% (20 min), Flow 0.75 mL/min), t_R = 7.79 min, m/z = 321.3

Example 7

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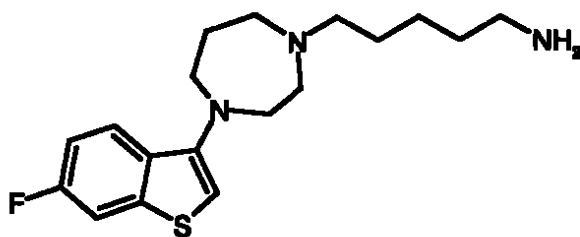


4-[4-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]pentanenitrile

- 15 Follow the procedure of Example 5, and substitute pentanenitrile for butyronitrile therein to obtain the title compound. (LiChrospher 5 μ , RP-18, 250 mm CH₃CN/ Water (0.05% TFA)- gradient 2% $\rightarrow$ 98% (20 min), Flow 0.75 mL/min) t_R = 10.4 min, m/z = 331.5

Example 8

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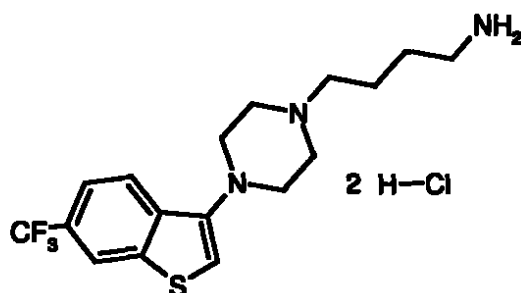
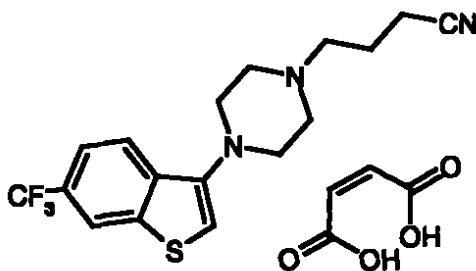


4-[4-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]pentan-1-amine

- 25 Follow the procedure of Example 6, and substitute 4-[4-(6-fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]pentanenitrile (Example 7) therein to obtain the title compound. LC/MS, (

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LiChrospher 5 μ , RP-18, 250 mm CH₃CN/ Water(0.05% TFA)- gradient 2% $\rightarrow$ 98 % (20 min),
Flow 0.75 mL/min, t_R = 8.31 min, m/z = 335.5

Example 9**4-[6-(Trifluoromethyl)-benzo[b]thien-1-yl]-piperazinebutanamine dihydrochloride****9a 4-(6-Trifluoromethyl)-benzo[b]thien-3-yl)-1-piperazinebutyl-nitrile (Z)-2-butenedioate**

Reflux a mixture of 1-(6-(trifluoromethyl)-benzo[b]thien-3-yl)-piperazine (Example 2) (10.1 g, 35.3 mmol), 4-bromobutyronitrile (6.25 g, 42.3 mmol), anhydrous potassium carbonate (8.00 g, 57.9 mmol), and anhydrous acetonitrile (80 mL) for 18 h. Filter the slurry, wash the insolubles with dichloromethane (2x150 mL), and concentrate the filtrate under vacuum. Take up the residue in dichloromethane (125 mL), wash with 5% aqueous NaOH (75 mL), water (75 mL) and dry (K₂CO₃). Concentrate under vacuum and chromatograph the crude product over silica gel (EtOAc) to obtain 10.3 g (82%) of amber oil. Add to an ethanolic solution of the oil (1.2 g, 3.40 mmol), maleic acid (400 mg, 3.45 mmol) and concentrate the solution under vacuum to receive a gum. Triturate the gum with EtOAc to afford a solid. Recrystallize the solid from methanol/EtOAc to obtain 1.01 g of white crystals, mp 158-159°C.

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Analysis

Calc for $C_{21}H_{22}F_3N_3O_4S$	53.73%C	4.72%H	8.95%N
Found	53.57%C	4.65%H	8.86%N

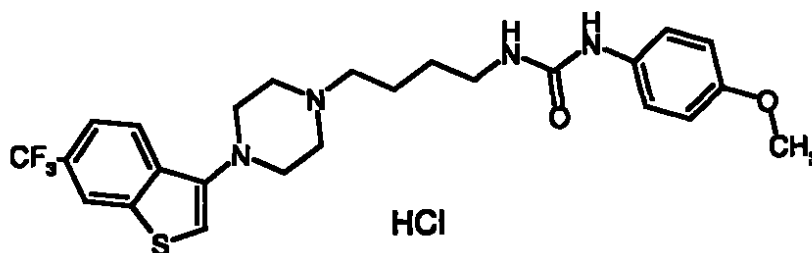
5 **9b 4-[6-(Trifluoromethyl)-benzo[b]thien-1-piperazinebutanamine dihydrochloride**

Under N_2 add, dropwise, a solution of 4-(6-trifluoromethyl)-benzo[b]thien-3-yl)-1-piperazinebutyl-nitrile (free base of Example 9a) (9.00 g, 25.5 mmol) in anhydrous tetrahydrofuran (THF, 70 mL) to a stirred, cooled ($3^\circ C$) suspension, of $LiAlH_4$ (1.06 g, 27.9 mmol) in anhydrous THF (120 mL). Maintain the temperature at $3^\circ C$ for 5 min and then stir at ambient temperature for 21 h. Cool the mixture to $0^\circ C$ and treat sequentially with H_2O (1 mL), 15% aqueous NaOH (1 mL), and H_2O (3 mL). After 20 min at room temperature, filter the mixture, wash the insolubles with dichloromethane (2x50 mL), and concentrate the filtrate under vacuum. Take the residue up in dichloromethane (150 mL), wash sequentially with 5% aqueous NaOH (75 mL), H_2O (75 mL) and then dry (K_2CO_3). Remove the solvent under vacuum and purify the residue by chromatography over silica gel (ethanol/ NH_4OH , 95/5) to obtain 5.32 g (58%) of the free base of the title compound. To a solution of the free base (689 mg) in ethanol, add ethanolic HCl until the solution is acidic (pH 2-3). Concentrate under vacuum to a gum, and triturate the gum with ethanol to obtain an off-white solid. Recrystallize the solid from MeOH/ $CHCl_3$ to obtain 485 mg of white powder, mp $256-258^\circ C$.

Analysis

Calculated for $C_{17}H_{22}F_3N_3S \cdot 2HCl$	47.45%C	5.62%H	9.76%N
Found	47.10%	5.67%H	9.62%N

Example 10

30 **1-(4-Methoxy-phenyl)-3-{4-[4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl}-urea hydrochloride**

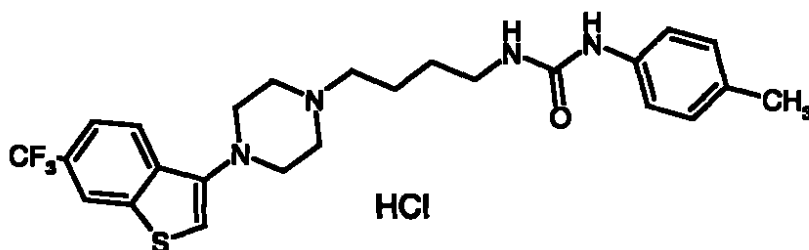
-81-

Under nitrogen, add 4-methoxyphenyl isocyanate (0.42 g, 2.8 mmol) in CHCl_3 (18 mL) to a stirring solution of 4-[6-(trifluoromethyl)-benzo[b]thien-1-piperazinebutanamine (free base of Example 9b) (1.0 g, 2.8 mmol) in CHCl_3 (10 mL). Allow the reaction to stir at ambient temperature for 18 h, filter the resulting solid, wash with CHCl_3 and obtain 635 mg of product as a white solid, mp 202-204°C. Concentrate the filtrate and obtain 1.0 g of a sticky residue. Chromatograph the residue over 40 g of silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 24/1), and obtain an additional 110 mg of product. Combine the samples, dissolve in hot EtOAc-MeOH (100 mL-2 mL), filter and after cooling add ethereal HCl to precipitate the hydrochloride salt. After 2 h collect 787 mg of the salt as a white solid, mp 228-231°C. Stir the solid in refluxing acetonitrile for 45 min, allow the mixture to cool and filter the solid to obtain 570 mg of the title compound as a white solid, mp 240-242°C.

Analysis

Calculated for $\text{C}_{25}\text{H}_{29}\text{F}_3\text{N}_4\text{O}_2\text{S}\cdot\text{HCl}$	55.29%C	5.57%H	10.32%N
Found	55.21%C	5.71%H	10.22%N

Example 11



1-(4-Methyl-phenyl)-3-{4-[4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl}-urea hydrochloride

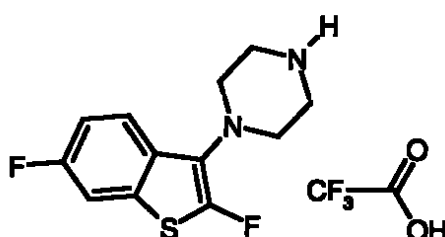
Add p-tolyl isocyanate (0.41 g, 3.1 mmol) in CHCl_3 (8 mL) to a stirring solution of 4-[6-(trifluoromethyl)-benzo[b]thien-1-piperazinebutanamine (free base of Example 9b) (1.1 g, 3.1 mmol) in CHCl_3 (8 mL). Allow the reaction to stir at ambient temperature for 63 h, filter the resulting solid, wash with CHCl_3 and obtain 677 mg of product as a beige solid. Dissolve the solid in hot EtOAc-MeOH (100 mL-2 mL), filter and after cooling add ethereal HCl to precipitate a hydrochloride salt. Collect 869 mg of the salt as a white solid, mp 231-234°C.

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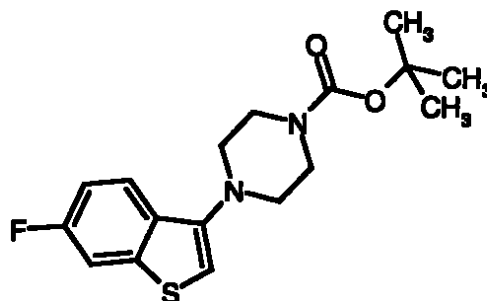
Recrystallize the solid from absolute ethanol to obtain 595 mg (37%) of the title compound as a white solid, mp 240-242°C

Analysis

5	Calculated for $C_{25}H_{29}F_3N_4OS \cdot HCl$	56.97%C	5.74%H	10.63%N
	Found	57.02%C	5.83%H	10.68%N

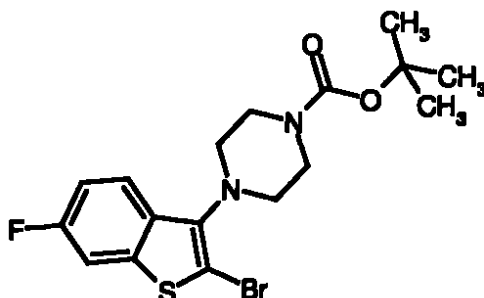
Example 12

1-(2,6-Difluoro-benzo[b]thien-3-yl)-piperazine trifluoroacetate

12a 4-(6-Fluoro-benzo[b]thiophen-3-yl)-piperazine-1-carboxylic acid tert-butyl ester

Add a solution of di-*tert*-butyl dicarbonate (5.15 g, 23.6 mmol) in $CHCl_3$ (15 mL), dropwise, over 45 min to a solution at -65°C of 1-(6-fluorobenzo[b]thiophen-3-yl)-piperazine (prepared according to US 5,143,923), (2.8 g, 11.8 mmol), 4-(dimethyl-amino)pyridine (0.16, 1.3 mmol), and diisopropylethylamine (4.3 mL, 3.2 g, 24.8 mmol) in $CHCl_3$ (50 mL). Following complete addition, stir the reaction at ambient temperature for 20 h, and then pour the reaction into a mixture of cold (5°C) 5% aqueous NaOH/EtOAc (150/150 mL). Extract the product into EtOAc, wash the extract with H_2O , brine and concentrate to a red oil. Purify the crude oil over silica gel (EtOAc), to obtain 3.6 g, of red oil, LC/MS m/z = 337 ($M+H$)⁺

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12b 4-(2-Bromo-6-fluoro-benzo[b]thiophen-3-yl)-piperazine-1-carboxylic acid tert-butyl ester

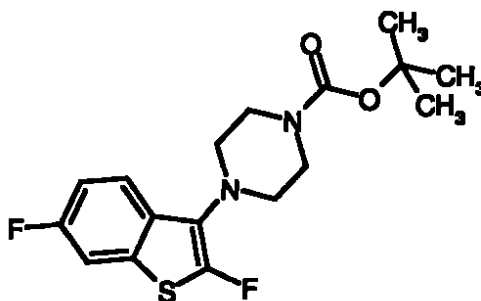
5

Add N-bromosuccinimide (0.59 g, 3.3 mmol) to a stirring solution of 4-(6-fluoro-benzo[b]thiophen-3-yl)-piperazine-1-carboxylic acid tert-butyl ester (Example 12a) (1.00 g, 2.97 mmol) in CHCl_3 (32.8 mL) and reflux for 30 min. Allow cooling to room temperature and filter. Evaporate the solvent and purify the residue by chromatography over silica gel (EtOAc/heptane, 9:1) to obtain 0.53 g (43%) of oil, MS, $m/z = 416$ ($M+H$)⁺.

10

In an alternative procedure, add N-bromosuccinimide (1.319 g, 6.62 mmol) to a stirring solution of 4-(6-fluoro-benzo[b]thiophen-3-yl)-piperazine-1-carboxylic acid tert-butyl ester (Example 12a) (2.226 g, 6.62 mmol) in CCl_4 and reflux for 2 h. Allow cooling to room temperature and filter. Evaporate the solvent and purify the residue by chromatography over silica gel (EtOAc/heptane, 9:1) to obtain 2.34 g (94%) of oil.

15



20

12c 4-(2-Fluoro-6-fluoro-benzo[b]thiophen-3-yl)-piperazine-1-carboxylic acid tert-butyl ester

At a temperature of -65°C stir, under nitrogen, a solution of the 4-(2-bromo-6-fluoro-benzo[b]thiophen-3-yl)-piperazine-1-carboxylic acid tert-butyl ester, Example 12b (15.59 g, 37.55 mmol) in anhydrous THF (247 mL) and add, dropwise, n-butyllithium in hexane (2.5M, 19.53 mL, 48.82 mmol). Stir for 30 min and then add, dropwise, N-fluorobenzenesulfonimide

25

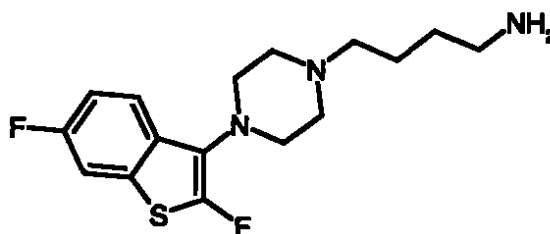
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(17.76 g, 56.33 mmol) dissolved in anhydrous THF. Stir overnight at ambient temperature, cool the reaction to 0°C, add saturated NaCl solution and then water. Extract the mixture with EtOAc (3x's), combine the extracts and wash with water and brine. Dry the extract (MgSO₄), and concentrate to obtain 11.0 g of oil. Chromatograph the oil over silica gel (ether/pet ether, 9:1) and obtain 6.28 g (52%) of red oil, MS, m/z, 354 (M+H)⁺

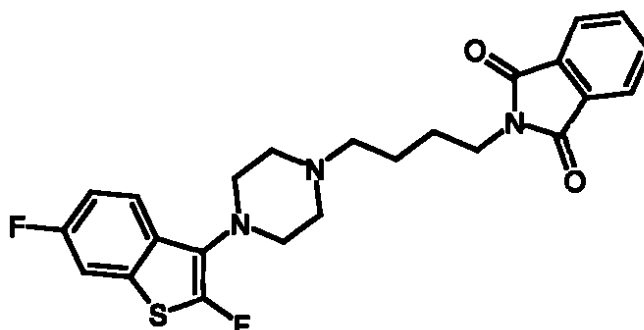
12d 1-(2,6-Difluoro-benzo[b]thien-3-yl)-piperazine trifluoroacetate

Stir a solution of 4-(2-fluoro-6-fluoro-benzo[b]thiophen-3-yl)-piperazine-1-carboxylic acid tert-butyl ester Example 12c (250 mg, 0.70 mmol) in trifluoroacetic acid (2.2 mL) at ambient temperature for 30 min. Evaporate the trifluoroacetic acid and treat the residue with ether. Stir the suspension at ambient temperature for 2 h, and filter the resulting white solid to obtain 191 mg (56%) of the trifluoroacetate salt. MS, m/z= 255 (M+H)⁺

Example 13



4-[6-(2,6-Difluoro-benzo[b]thien-1-yl)-piperazine]butanamine



13a 2-[4-[4-(6-Fluorobenzo[b]thiophen-3-yl)piperazin-1-yl]butyl]isoindole-1,3-dione

Stir and reflux under argon a mixture of 1-(2,6-difluoro-benzo[b]thien-3-yl)-piperazine (free base of Example 12d) (1.48 g, 5.8 mmol), bromobutylphthalimide (1.65 g, 5.8 mmol), triethylamine (1.2 mL) and acetonitrile (25 mL) for 4 h. Allow the reaction to cool and then

dilute with dichloromethane. Wash the organic solution with water, saturated K_2CO_3 solution and dry (K_2CO_3). Concentrate the solvent and obtain 2.55 g of solid. Chromatograph the solid over silica gel ($CH_2Cl_2/MeOH$, 49:1) to obtain 2.1 g of solid, mp 123-125°C, MS, m/z = 456 ($M+H$)⁺.

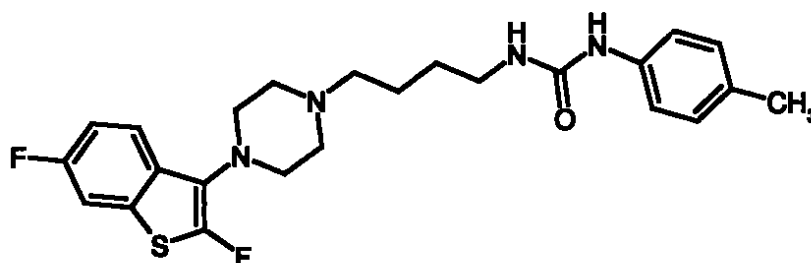
5

13b 4-[6-(2,6-Difluoro-benzo[b]thien-1-piperazinebutanamine

Stir a suspension, under argon, of 2-[4-[4-(6-fluorobenzo[b]thiophen-3-yl)piperazin-1-yl]butyl]isoindole-1,3-dione (Example 13a) (2.05 g, 4.5 mmol) in anhydrous MeOH (30 mL) and add hydrazine (0.5 mL, 15.9 mmol). Reflux for 2.5 h and allow cooling to ambient temperature. Cool the reaction in an ice bath and add 1M HCl to a pH ~1. Filter the mixture, cool the filtrate in an ice bath, and add 50% aqueous NaOH to basify. Extract the aqueous mixture with dichloromethane, wash the extract with H_2O , dry with K_2CO_3 and concentrate to obtain 1.4 g of oil, which crystallizes upon standing, LC/MS, m/z = 326 ($M+H$)⁺.

15

Example 14



20 1-(4-Methyl-phenyl)-3-{4-[4-(2,6-difluoro-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl}-urea

Add a solution of 4-methylphenyl isocyanate (43.3 mg, 0.32 mmol), in $CHCl_3$ (1-2 mL) to a solution of 4-[6-(trifluoromethyl)-benzo[b]thien-1-piperazinebutanamine (Example 13b) (3.5 mL of a 0.086M solution in $CHCl_3$, 0.31 mmol) and shake the reaction for 20 h. Filter the resulting solid or concentrate the reaction and collect the solid after trituration with EtOAc to obtain 102.7 mg of the title compound. LC/MS (Ymc005-AQ, 4x50 mm, water/ CH_3CN /acetic acid, 94:5:5 0:0.5 100% for 0.1 min then water/ CH_3CN /acetic acid, 5:0.94:5 0.5 linear gradient → 100 % (2 min, hold 4 min), Flow 1.0 mL/min) t_R = 3.22 min, m/z = 459 ($M+H$)⁺.

30

Examples 15-18

The following HPLC conditions are referred to in Examples 15-18

5 HPLC Condition I

A) 95/5/0 1% Water/Acetonitrile/Formic Acid,

B) 5/95/0 1% Water/Acetonitrile/Formic Acid

Column YMC ODS-A 4x50 mm, Flow rate 2 mL/minute

10 The initial HPLC conditions consisted of 100% (A) flowing at 2 mL/minute After the initial injection a linear gradient was performed so that at 2 minutes the HPLC conditions were 100% B These conditions were then held for 3 4 minutes at which time the system switched back to initial conditions and equilibrated for the next analysis

HPLC Condition II

15 A) 95/5/0 1% Water/Acetonitrile/Formic Acid,

B) 5/95/0 1% Water/Acetonitrile/Formic Acid

Column YMC ODS-A 2x50 mm, Flow rate=1 mL/minute

20 The initial HPLC conditions consisted of 100% (A) flowing at 0 1 mL/minute After the initial injection a linear gradient was performed so that at 2 minutes the HPLC conditions were 100% B These conditions were then held for 3 5 minutes at which time the system switched back to initial conditions and equilibrated for the next analysis

HPLC Condition III

A) 95/5/0 5% Water/Acetonitrile/Formic Acid,

25 B) 5/95/0 5% Water/Acetonitrile/Formic Acid

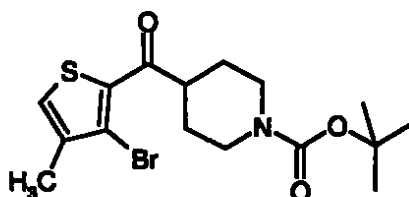
Column YMC ODS-A 4x50 mm, Flow rate=2 mL/minute

30 The initial HPLC conditions consisted of 100% (A) flowing at 2 mL/minute After the initial injection a linear gradient was performed so that at 2 minutes the HPLC conditions were 100% B These conditions were then held for 3 4 minutes at which time the system switched back to initial conditions and equilibrated for the next analysis

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Example 15**1-[4-[4-(6-Methyl-thieno[2,3-d]isoxazol-3-yl)-piperidin-1-yl]-butyl]-3-(substituted)phenyl-ureas and bis-ureas**

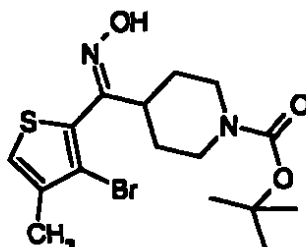
5

15a Preparation of 4-[1-(3-bromo-4-methyl-thiophen-2-yl)-methanoyl]-piperidine-1-carboxylic acid tert-butyl ester

- 10 Under inert conditions, add a 2.0 M solution (in tetrahydrofuran/n-heptane) of lithium diisopropylamide (29.65 mmol, 14.83 mL, 1.05 equivalents) to a cold (-78° C) solution of 3-bromo-4-methylthiophene (28.24 mmol, 5.00 g, 1.00 equivalents) in dry tetrahydrofuran (27.33 mL). Stir at -78° C for 1 hour and add a solution of 4-(methoxy-methyl-carbamoyl)-piperidine-1-carboxylic acid *tert*-butyl ester (28.24 mmol, 7.69 g, 1.00 equivalents), dropwise.
- 15 Continue stirring at -78° C for 3 hours. Quench the reaction mixture with saturated ammonium chloride (aqueous, 55 mL) and allow to warm to room temperature. Extract the reaction mixture with a mixture of ethyl acetate/diethyl ether (1:1, 3 x 40 mL). Combine the extracts and dry over magnesium sulfate, filter and evaporate. Purify the residue via flash column chromatography using a mixture of n-heptane/ethyl acetate (4:1) to yield a yellow, crystalline solid (9.84 g).
- 20 MS (CI, methane) *m/e* 388 (MH⁺), LC/MS (APCI), *m/e* 288 (M-100), retention time 2 min. 43 sec. Condition I.

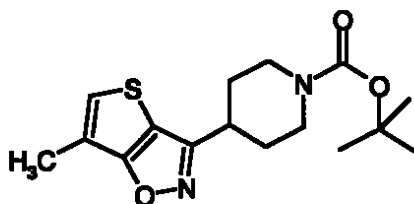
15b Preparation of 4-[1-(3-bromo-4-methyl-thiophen-2-yl)-1-hydroxyimino-methyl]-piperidine-1-carboxylic acid tert-butyl ester

25



Add ammonium hydroxide hydrochloride (50.68 mmol, 3.52 g, 2.00 equivalents) to a stirred solution of 4-[1-(3-bromo-4-methyl-thiophen-2-yl)-methanoyl]-piperidine-1-carboxylic acid tert-butyl ester (25.54 mmol, 9.84 g, 1.00 equivalents) in pyridine (47.5 mL). Stir at room temperature overnight and at 70° C for 4 hours. Cool the reaction mixture and add hydrochloric acid (3 M solution, 115 mL). Extract the reaction mixture with dichloromethane (115 mL), filter the organic layer, wash with water (100 mL), dry over magnesium sulfate, filter and evaporate. Recrystallize the resulting residue from toluene to yield a white solid (4.84 g). LC/MS (APCI), m/e 403 (MH⁺), retention time 2 min 32 sec. Condition I.

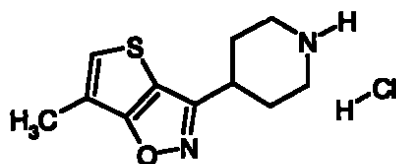
10 **15c Preparation of 4-(6-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidine-1-carboxylic acid tert-butyl ester**



Add cesium carbonate (3.72 mmol, 1.21 g, 1.50 equivalents) and copper iodide (0.25 mmol, 47 mg, 0.10 equivalents) to a stirred solution of 4-[1-(3-bromo-4-methyl-thiophen-2-yl)-1-hydroxyimino-methyl]-piperidine-1-carboxylic acid tert-butyl ester (2.48 mmol, 1.00 g, 1.00 equivalents) in 2-methoxy ethanol (25 mL). Stir the resulting mixture at room temperature overnight and filter to remove the inorganic material. Concentrate the filtrate and partition the resulting oil between ethyl acetate (75 mL) and water (25 mL). Extract the aqueous layer with ethyl acetate (2 x 75 mL) and wash the combined organic layers with saturated sodium chloride (aqueous, 25 mL), dry over magnesium sulfate, filter and evaporate. Purify the residue via flash column chromatography eluting with n-heptane:ethyl acetate (4:1) to yield a white solid (588 mg). MS (CI, methane) m/e 323 (MH⁺), LC/MS (ESI), m/e 345 (MNa⁺), retention time 2.05 minutes. Condition II.

25

15d Preparation of 6-methyl-3-piperidin-4-yl-thieno[2,3-d]isoxazole hydrochloride



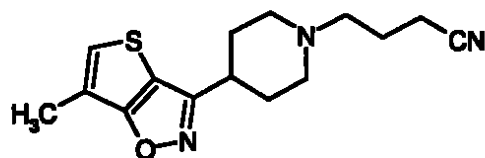
Stir a solution of 4-(6-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidine-1-carboxylic acid tert-butyl ester (8.84 mmol, 2.85 g, 1.00 equivalents) in hydrochloric acid (48.75 mL, 1 M solution in

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diethyl ether) and methanol (2 00 mL) at room temperature for 3 5 hours. Filter the suspension, collect the white solid and dry to yield the desired product (659 mg). Allow the mother liquor to age overnight, filter, collect the white solid and dry to yield additional desired product (1 252 g). LC/MS (ESI), m/e 223 (MH^+), retention time 1 14 minutes. Condition II

5

15e Preparation of 4-[4-(6-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidin-1-yl]-butyronitrile

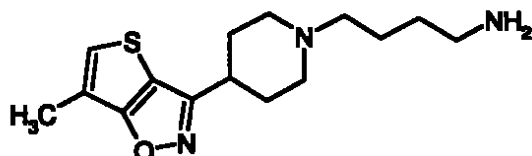


10

Add potassium carbonate (17 72 mmol, 2 45 g, 2 40 equivalents), potassium iodide (0 73 mmol, 123 mg, 0 10 equivalents), and 4-bromobutyronitrile (8 86 mmol, 0 88 mL, 1 20 equivalents) to a stirred solution of 6-methyl-3-piperidin-4-yl-thieno[2,3-d]isoxazole hydrochloride (7 38 mmol, 1 91 g, 1 00 equivalents) in acetonitrile (10 84 mL) and water (3 60 mL). Stir the resulting mixture at reflux overnight. Cool to room temperature, filter the reaction mixture and wash the solid material collected with dichloromethane and evaporate the filtrate. Take the residue up in dichloromethane (45 mL), wash with sodium hydroxide (aqueous, 18 mL, 2 M), water (18 mL), saturated sodium hydroxide (aqueous, 18 mL), dry over magnesium sulfate, filter and evaporate. Purify the residue via flash column chromatography using a gradient and eluting with a mixture of n-heptane/ethyl acetate (0 5/9 5) to ethyl acetate (100%) to yield the desired product as a brown oil (663 mg). LC/MS (ESI), m/e 290 (MH^+), retention time 1 19 minutes. Condition II

20

15f Preparation of 4-[4-(6-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidin-1-yl]-butylamine



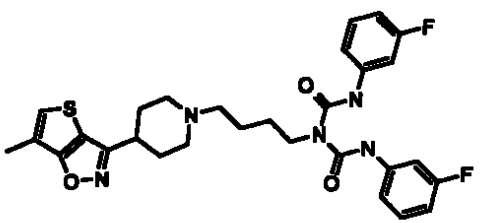
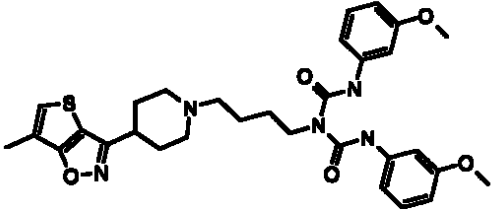
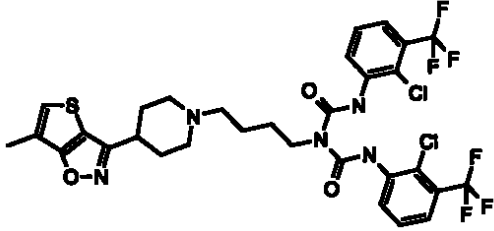
25

Under inert conditions, add lithium aluminum hydride (3 42 mmol, 3 42 mL, 1 50 equivalents, 1 0 M solution in tetrahydrofuran) to a stirred solution of 4-[4-(6-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidin-1-yl]-butyronitrile (2 28 mmol, 660 mg, 1 00 equivalents) in tetrahydrofuran (dry, 12 86 mL). Stir the resulting solution at room temperature for 2 5 hours. Quench the reaction mixture by adding water (0 16 mL), then sodium hydroxide (aqueous, 0 16 mL, 2 M solution), and then water (0 5 mL). Dilute the resulting suspension with dichloromethane (16 mL) and vigorously stir for 30 minutes. Filter the resulting mixture through a bed of celite®,

dry over magnesium sulfate, filter and evaporate to yield the desired product (457 mg) as a brown oil LC/MS (ESI), m/e 294 (MH⁺), retention time 0.56 minutes Condition II

5 The following 1-{4-[4-(6-Methyl-thieno[2,3-d]isoxazol-3-yl)-piperidin-1-yl]-butyl}-3-(substituted)phenyl-ureas and bis-ureas were prepared using 4-[4-(6-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidin-1-yl]-butylamine (15f) and carrying out the procedures found subsequent to the table

Compound Number	Product	LC/MS (ESI) Condition II
15-1		m/e 457 (MH ⁺), retention time 1.40 minutes
15-2		m/e 427 (MH ⁺), retention time 1.40 minutes
15-3		m/e 431 (MH ⁺), retention time 1.38 minutes
15-4		m/e 431 (MH ⁺), retention time 1.39 minutes
15-5		m/e 443 (MH ⁺), retention time 1.38 minutes
15-6		m/e 515 (MH ⁺), retention time 1.52 minutes
15-7		m/e 620 (MH ⁺), retention time 1.65 minutes

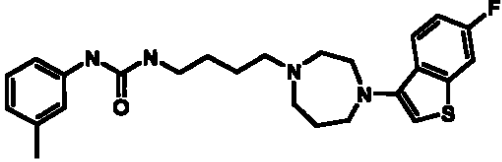
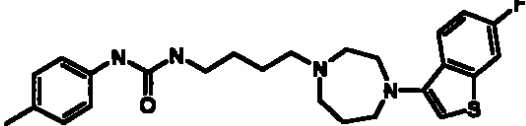
15-8		m/e 567 (MH ⁺), retention time 1.62 minutes
15-9		m/e 592 (MH ⁺), retention time 1.59 minutes
15-10		m/e 736 (MH ⁺), retention time 1.80 minutes

Add a solution of 4-[4-(6-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidin-1-yl]-butylamine (0.26 mmol, 75 mg, 1.00 equivalents) in dichloromethane (1.00 mL) to the desired isocyanate (0.50 mmol, 1.95 equivalents). Shake each reaction mixture overnight at room temperature. 5. evaporate and purify the reaction mixtures via flash column chromatograph (ISCO Combi Flash) using a gradient and eluting with ethyl acetate (100%) to a mixture of ethanol in ethyl acetate (1:5 - 8:5). Concentrate to give each desired product.

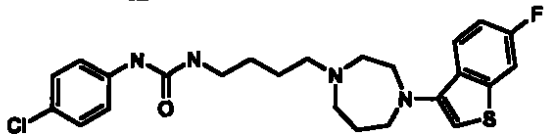
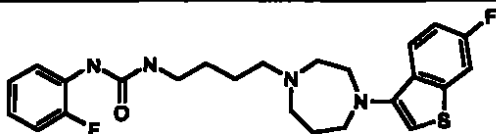
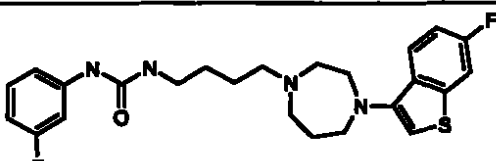
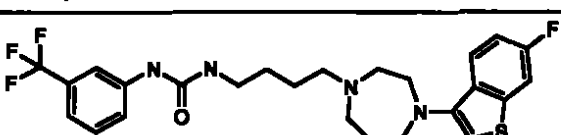
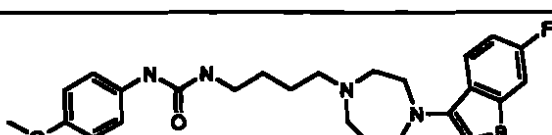
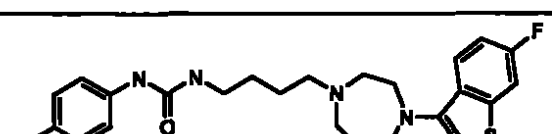
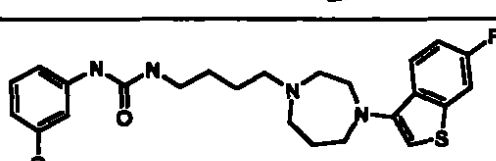
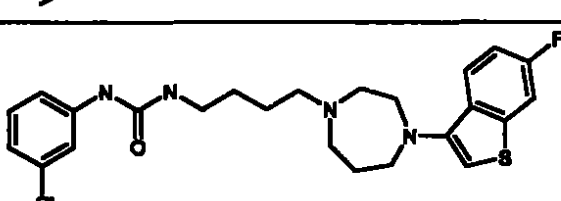
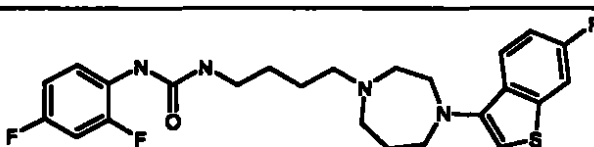
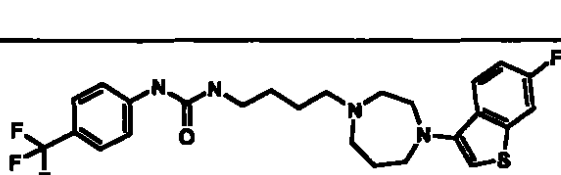
Example 16

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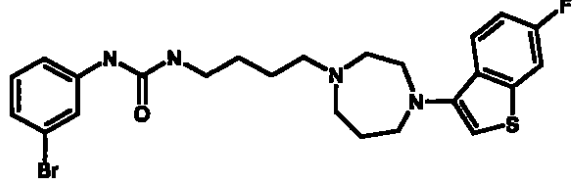
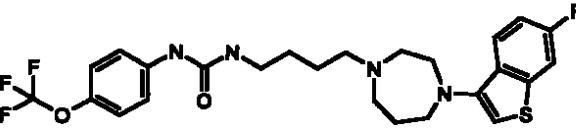
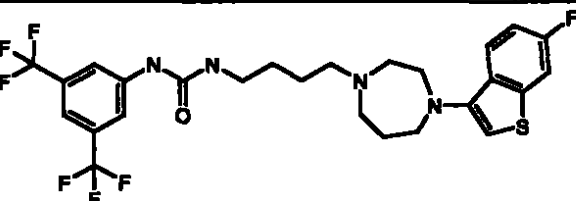
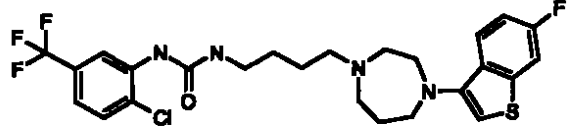
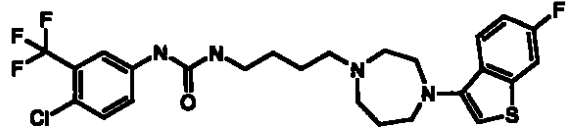
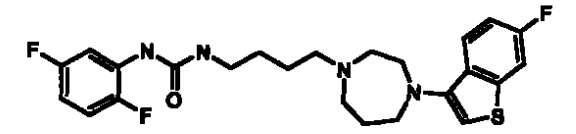
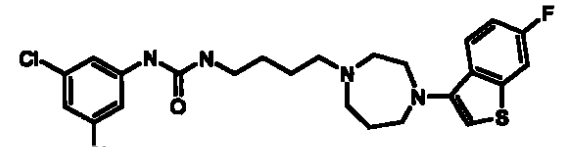
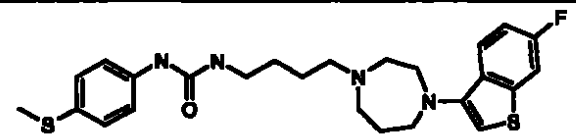
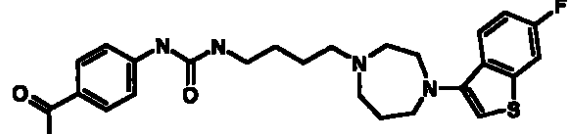
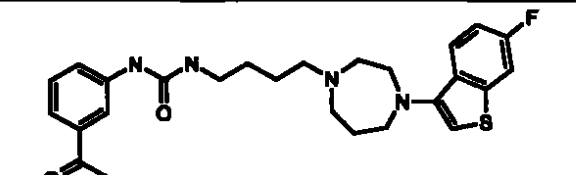
Preparation of 1-[4-[4-(6-Fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl]-3-(substituted)phenyl-ureas

Compound Number	Product	LC/MS (APCI) Condition III
16-1		m/e 455 (MH ⁺), retention time 1 min. 44 sec
16-2		m/e 455 (MH ⁺), retention time 1 min. 45 sec

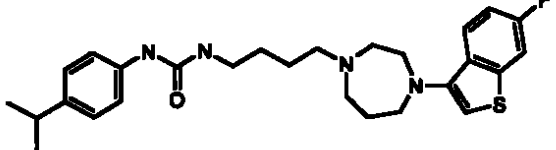
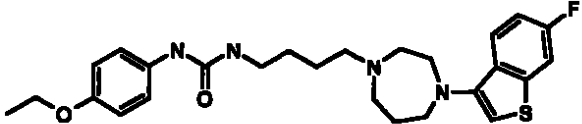
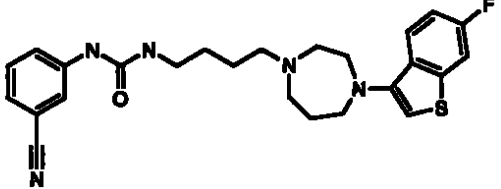
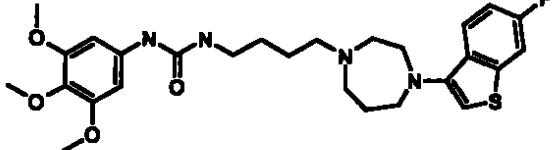
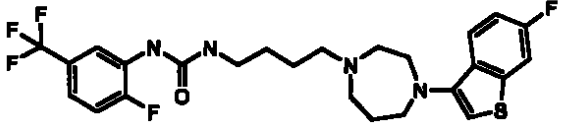
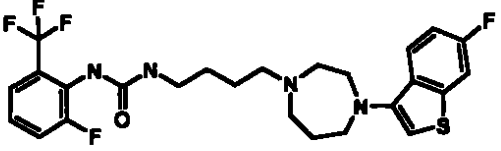
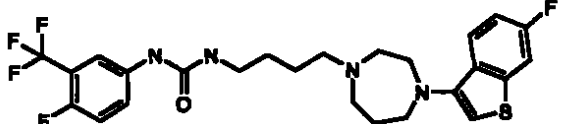
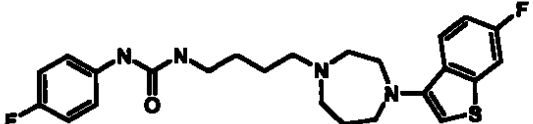
-72-

16-3		m/e 475 (MH ⁺), retention time 1 min 46 sec
16-4		m/e 459 (MH ⁺), retention time 1 min 42 sec
16-5		m/e 459 (MH ⁺), retention time 1 min 43 sec
16-6		m/e 509 (MH ⁺), retention time 1 min 47 sec
16-7		m/e 471 (MH ⁺), retention time 1 min 41 sec
16-8		m/e 519 (MH ⁺), retention time 1 min 47 sec
16-9		m/e 471 (MH ⁺), retention time 1 min 42 sec
16-10		m/e 475 (MH ⁺), retention time 1 min 46 sec
16-11		m/e 477 (MH ⁺), retention time 1 min 43 sec
16-12		m/e 509 (MH ⁺), retention time 1 min 48 sec

-73-

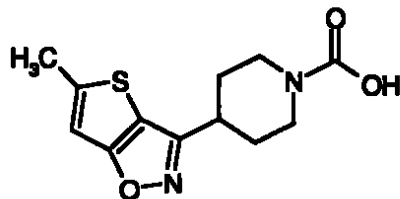
16-13		m/e 519 (MH ⁺), retention time 1 min 47 sec
16-14		m/e 525 (MH ⁺), retention time 1 min 49 sec
16-15		m/e 577 (MH ⁺), retention time 1 min 55 sec
16-16		m/e 543 (MH ⁺), retention time 1 min 51 sec
16-17		m/e 543 (MH ⁺), retention time 1 min 51 sec
16-18		m/e 477 (MH ⁺), retention time 1 min 46 sec
16-19		m/e 509 (MH ⁺), retention time 1 min 51 sec
16-20		m/e 487 (MH ⁺), retention time 1 min 46 sec
16-21		m/e 483 (MH ⁺), retention time 1 min 39 sec
16-22		m/e 483 (MH ⁺), retention time 1 min 40 sec

-74-

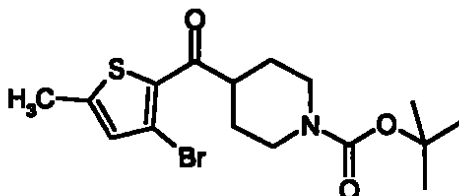
16-23		m/e 483 (MH ⁺), retention time 1 min 50 sec
16-24		m/e 485 (MH ⁺), retention time 1 min 44 sec
16-25		m/e 466 (MH ⁺), retention time 1min 43 sec
16-26		m/e 531 (MH ⁺), retention time 1 min 39 sec
16-27		m/e 527 (MH ⁺), retention time 1 min 49 sec
16-28		m/e 527 (MH ⁺), retention time 1 min 43 sec
16-29		m/e 527 (MH ⁺), retention time 1 min 50 sec
16-30		m/e 459 (MH ⁺), retention time 1 min 42 sec

The above compounds were prepared in a manner analogous to Example 15. Evaporate the pure fractions from a purification performed via flash column chromatograph (ISCO Combi Flash) to yield each compound as an oil. Dry each oil under the vacuum pump to give the final, desired compounds.

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Example 17**4-(5-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidine-1-carboxylic acid**

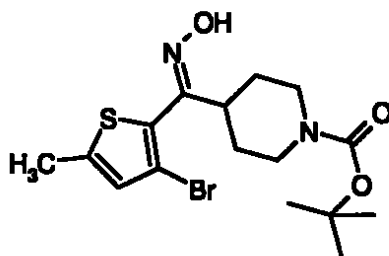
5

17a Preparation of 4-[1-(3-bromo-5-methyl-thiophen-2-yl)-methanoyl]-piperidine-1-carboxylic acid tert-butyl ester

- 10 Prepared essentially as in Example 15a except that 2-bromo-5-methyl thiophene is used as the starting material. In addition, 1.20 equivalents of lithium diisopropylamide and 1.24 equivalents of 4-(methoxy-methyl-carbamoyl)-piperidine-1-carboxylic acid *tert*-butyl ester are used for the reaction. Accordingly, stirring time of the reaction mixture may vary. Purification of the residue via flash column chromatography uses a gradient with a mixture of ethyl acetate/n-heptane (1/9) to ethyl acetate/n-heptane (2/8) to yield a yellow oil. LC/MS (ESI), *m/e* 332 (M-56) and 388 (MH⁺), retention time 2.15 minutes. Condition II.
- 15

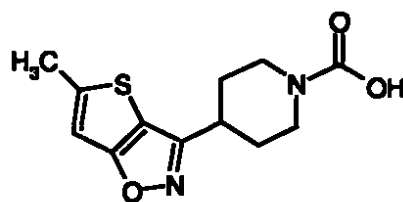
17b Preparation of 4-[1-(3-bromo-5-methyl-thiophen-2-yl)-1-hydroxyimino-methyl]-piperidine-1-carboxylic acid tert-butyl ester

20



- Prepared essentially as Example 15b except that 4-[1-(3-Bromo-5-methyl-thiophen-2-yl)-methanoyl]-piperidine-1-carboxylic acid *tert*-butyl ester is used as the starting material and the reaction mixture was stirred at 70° C for 6 hours. LC/MS (ESI), *m/e* 347 (M-56) and 403 (MH⁺), retention time 2.03 minutes. Condition II.
- 25

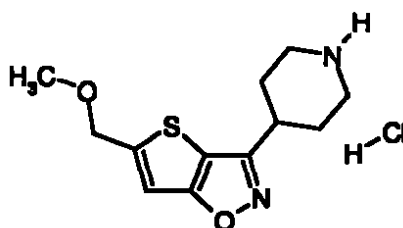
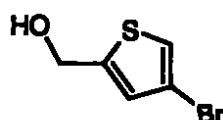
-76-

17c Preparation of 4-(5-methyl-thieno[2,3-d]isoxazol-3-yl)-piperidine-1-carboxylic acid

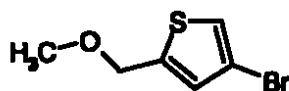
Prepared essentially as Example 15c except that 4-[1-(3-bromo-5-methyl-thiophen-2-yl)-1-hydroxyimino-methyl]-piperidine-1-carboxylic acid tert-butyl ester is used as the starting

- 5 material. Two other differences are: 1) 0.05 equivalents of copper iodide is used, and 2) no partition between ethyl acetate and water accompanied by subsequent extraction with ethyl acetate is required. Purification of the residue via flash column chromatography uses a mixture of ethyl acetate/n-heptane (1/4) to yield a white solid. LC/MS (ESI), m/e 345 (MNa⁺), retention time 2.12 minutes. Condition II

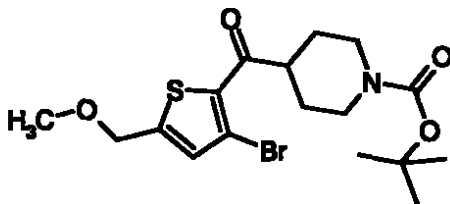
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Example 18**5-methoxymethyl-3-piperidin-4-yl-thieno[2,3-d]isoxazole hydrochloride**15 18a Preparation of (4-bromo-thiophen-2-yl)-methanol

- Under inert conditions, add sodium borohydride (13.82 mmol, 0.523 g, 2.08 equivalents) in absolute ethanol (16 mL) dropwise over a period of 15 minutes to a stirred mixture of 4-bromothiophene-2-carboxaldehyde (26.58 mmol, 5.08 g, 1.00 equivalents) in cold (0° C) absolute ethanol (32 mL). Stir the resulting mixture at room temperature for 2.5 hours and add glacial acetic acid dropwise until the effervescence ceases. Evaporate the resulting solution, take the residue up in diethyl ether (75 mL), wash with water (15 mL) and brine (15 mL) and dry over magnesium sulfate. Filter and evaporate to yield the product as a colorless
- 20 oil (5.13 g)
- 25

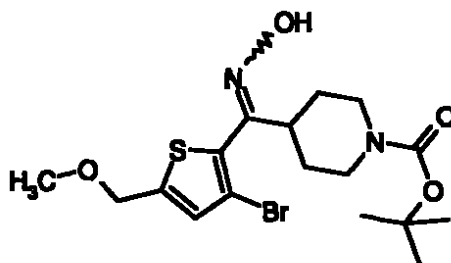
18b Preparation of 4-bromo-2-methoxymethyl-thiophene

- 5 Add sodium hydride (737 mg, 29.23 mmol, 1.10 equivalents, 95%) to a solution containing methyl iodide (1.65 mL, 26.57 mmol, 1.00 equivalents) and (4-bromo-thiophen-2-yl)-methanol (5.13 g, 26.57 mmol, 1.00 equivalents) in tetrahydrofuran (dry, 25 mL). Stir the resulting mixture at room temperature overnight and evaporate. Partition the residue between water (100 mL) and dichloromethane (100 mL). Extract the aqueous layer with dichloromethane (100 mL), combine the organic layers, dry over magnesium sulfate, filter and evaporate to yield the desired product as a yellow oil.

18c Preparation of 4-[1-(3-bromo-5-methoxymethyl-thiophen-2-yl)-methanoyl]-piperidine-1-carboxylic acid tert-butyl ester

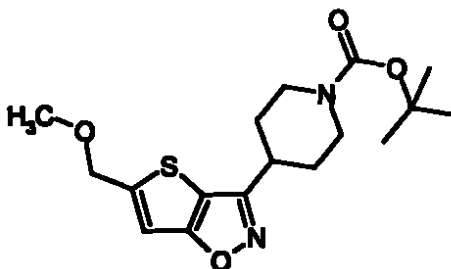
- 15 Add lithium diisopropyl amide (13.20 mL, 26.37 mmol, 1.05 equivalents) to a stirred, cold (-78° C) solution of 4-bromo-2-methoxymethyl-thiophene (5.20 g, 25.11 mmol, 1.00 equivalents) in tetrahydrofuran (dry, 24.30 mL). Stir at -78° C for 1 hour and add a solution of 4-(methoxy-methyl-carbamoyl)-piperidine-1-carboxylic acid tert-butyl ester (8.84 g, 25.11 mmol, 1.00 equivalents) in tetrahydrofuran (dry, 16.40 mL), dropwise. Stir the resulting solution at -78° C for 3 hours. Quench the reaction mixture with saturated sodium chloride (aqueous, 50 mL). Allow the resulting mixture to warm to room temperature and extract with a mixture of ethyl acetate:diethyl ether (1:1, 3 x 35 mL). Combine the extracts, dry over magnesium sulfate, filter and evaporate. Purify the residue via flash column chromatography eluting with a mixture of n-heptane:ethyl acetate (4:1) to yield the desired product as a yellow oil (9.47 g). LC/MS (ESI), m/e 362 (M-56) and 418 (MH⁺), retention time 2.08 minutes. Condition II.

18d Preparation of 4-[1-(3-bromo-5-methoxymethyl-thiophen-2-yl)-1-hydroxyimino-methyl]-piperidine-1-carboxylic acid tert-butyl ester



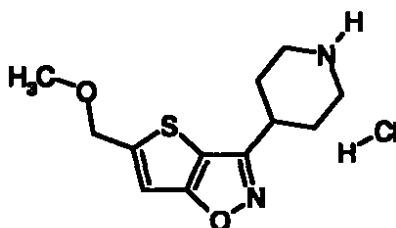
5 Add hydroxylamine hydrochloride (2.29 g, 45.27 mmol, 2.00 equivalents) to a stirred solution of 4-[1-(3-bromo-5-methoxymethyl-thiophen-2-yl)-methanoyl]-piperidine-1-carboxylic acid tert-butyl ester (9.47 g, 22.64 mmol, 1.00 equivalents) in pyridine (42.40 mL). Stir the resulting solution at room temperature overnight and then at 70° C for 4 hours. Cool the reaction mixture slightly, add hydrochloric acid (3N, 100 mL) and extract the resulting mixture with dichloromethane (100 mL). Wash the extract with water (100 mL), dry over magnesium sulfate, filter and evaporate to yield the desired product as a yellow oil (9.48 g).

18e Preparation of 4-(5-methoxymethyl-thieno[2,3-d]isoxazol-3-yl)-piperidine-1-carboxylic acid tert-butyl ester



15 Add cesium carbonate (1.13 g, 3.46 mmol, 1.50 equivalents) and copper iodide (44 mg, 0.23 mmol, 0.10 equivalents) to a stirred solution of 4-[1-(3-bromo-5-methoxymethyl-thiophen-2-yl)-1-hydroxyimino-methyl]-piperidine-1-carboxylic acid tert-butyl ester (1.00 g, 2.31 mmol, 1.00 equivalents) in 2-methoxy ethanol (23.30 mL). Stir the resulting mixture at room temperature overnight or up to 3 days and filter through celite. Evaporate the filtrate, partition the residue between ethyl acetate (70 mL) and water (23 mL) and separate. Extract the aqueous layer with ethyl acetate (3 x 70 mL), combine the organic layers, dry over magnesium sulfate, filter and evaporate. Purify the residue via flash column chromatography eluting with a mixture of hexane:ethyl acetate (4:1) to yield the desired product as a yellow oil. LC/MS (ESI), m/e 375 (MNa⁺), retention time 1.98 minutes. Condition II

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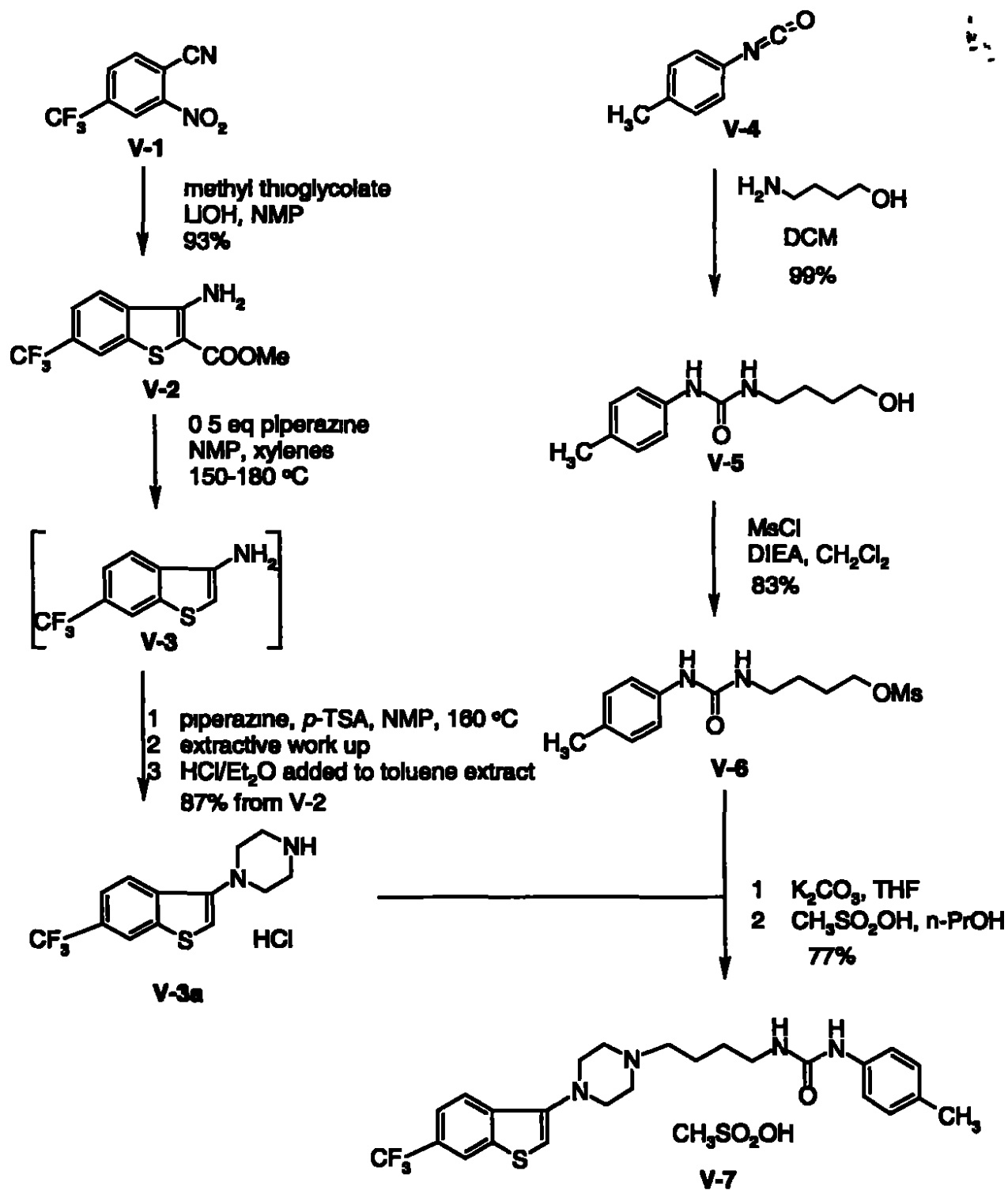
18f Preparation of 5-methoxymethyl-3-piperidin-4-yl-thieno[2,3-d]isoxazole hydrochloride

5 Stir a solution of 4-(5-methoxymethyl-thieno[2,3-d]isoxazol-3-yl)-piperidine-1-carboxylic acid tert-butyl ester (2.21 g, 6.68 mmol, 1.00 equivalents) and hydrochloric acid (1.0 M in diethyl ether, 35 mL) overnight to form a suspension. Add additional hydrochloric acid (1.0 M in diethyl ether, 10 mL). Stir the suspension overnight, filter and wash the solid with ether. Collect the solid and dry to yield the desired product as a dark blue solid. LC/MS (ESI), m/z 253 (MH^+), retention time 1.17 minutes. Condition II

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Example 19

Scheme V



General Gas chromatography/mass spectroscopy was accomplished using a HP Model 5972 system with the following conditions 0.25 mm x 30 m, HP 5MS column, cross-linked 5% Ph Me silicone, 0.25 μ film thickness, injector at 250 °C, detector at 280 °C, 50 °C for 1 min, ramp at 20 °C/min to 300 °C, 300 °C for 5 min to 10 min

5

2-Carbomethoxy-3-amino-6-trifluoromethylbenzo[b]thiophene (V-2)

A 22-L, 3-necked, round-bottom flask equipped with a mechanical stirrer, nitrogen bubbler, and a thermocouple probe, was charged with 1.20 kg (5.55 mole) of 2-nitro-4-trifluoromethylbenzonitrile, 496 mL (589.3 g, 5.55 mole) of methyl thioglycolate, and 4.3 L of NMP. The resulting yellow solution was cooled (-10 °C bath) to 1 °C, and a solution prepared from 466.0 g (11.11 mole, 2.0 eq) of lithium hydroxide monohydrate in 3.36 L of water was added over a period of 2 h while maintaining a temperature of 2 - 16 °C. The tan-orange slurry was stirred at 0 - 8 °C for 45 min, then was diluted with 8.0 L of water (T_{end} -> 21 °C). After stirring for 30 min and cooling to 14 °C, the product was collected by filtration, rinsing with 15 L of water, then air-drying at ambient temperature to give 1.43 kg (93.5% yield) of 2-carbomethoxy-3-amino-6-trifluoromethylbenzo[b]thiophene, as a light-yellow solid.

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3-Piperaziny-6-trifluoromethylbenzo[b]thiophene hydrochloride (V-3a)

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A 22-L, 3-necked, round-bottom flask equipped with a mechanical stirrer, nitrogen bubbler, and a thermocouple probe, was charged with 1.42 kg (5.17 mole) of 2-carbomethoxy-3-amino-6-trifluoromethylbenzo[b]thiophene (V-2), 245.0 g (2.84 mole, 0.55 eq) of piperazine, 5.0 L of NMP, and 720 mL of xylene. The solution was heated to and held at 165 - 176 °C for 4.5 h, at which time the reaction was ca. 97% complete as determined by hplc assay. A total of 2.00 kg (23.22 mole, 4.5 eq) of piperazine ($T > 120$ °C) and 1.97 kg (10.36 mole, 2.0 eq) of *p*-toluenesulfonic acid monohydrate (exotherm observed, 120 -> 140 °C) were added to the brown solution. A Dean-Stark trap was connected to the condenser, and the reaction was heated to collect an azeotrope. A total of 350 mL of an aqueous distillate was removed, allowing the pot temperature to increase from 140 to 158 °C. The Dean-Stark trap was disconnected. The progress of the reaction was monitored by GC/MS assays. After 12 h at ca. 158 - 162 °C (>99% conversion by GC/MS assay), the reaction was cooled to 40 °C, then quenched into an extractor that contained 6.2 kg of ice, 15 L of water, and 10.6 L of toluene. The phases were separated, and the aqueous layer was extracted with 2 L of toluene. The combined organic extract was washed with 13.7 L of 0.5 N NaOH.

followed by 2.5 L of saturated aq. NaCl, then was extracted with 10 L of 1 N HCl. The acidic aqueous extract was diluted with 1.2 kg of ice, then was basified to pH 10.7 by adding 770 g of 50% NaOH. The resulting mixture was extracted with 11 L of toluene. The toluene extract was washed with 2.5 L of saturated aq. NaCl, dried (Na₂SO₄), and filtered. The filtrate was charged into a 22 L, 3-necked, round-bottomed flask (N₂, mechanical stirring, TC probe). A total of 4.5 L of 1 N ethereal HCl was added at 20 – 27 °C until the mixture was positive to Congo Red indicator paper. After stirring at ambient temperature for 35 min, the slurry was filtered and washed with 5 L of toluene. After air drying, 1.44 kg (86.5% yield) of 3-piperaziny-6-trifluoromethyl-
benzo[b]thiophene hydrochloride (V-3a) was obtained as a light pink-beige solid.

***N*-(4-Hydroxybutyl)-*N'*-(4-tolyl) urea (V-5)**

A 12-L, 3-necked, round-bottom flask equipped with a mechanical stirrer, nitrogen bubbler, and a thermocouple probe, was charged with 8 L of methylene chloride and 367.7 g (4.13 moles, 1.10 eq.) of 4-amino-1-butanol, and the resulting solution was cooled to 0 – 5 °C. A solution of 489.3 g (3.75 moles, 1.00 eq.) of 4-tolyl isocyanate in 0.5 L of methylene chloride was added over a period of 2.5 h while maintaining a temperature of 0 – 12 °C. The white slurry was stirred for 1 h at 0 – 10 °C, then was filtered, washed with 3 L of methylene chloride, and air dried to afford 0.83 kg (99.9%) of *N*-(4-hydroxybutyl)-*N'*-(4-tolyl) urea (V-5) as a white solid.

***N*-(4-Butylmesylate)-*N'*-(4-tolyl) urea (V-6)**

A 22-L, 3-necked, round-bottom flask equipped with a mechanical stirrer, nitrogen bubbler and a thermocouple probe, was charged with 2.057 kg (9.25 moles) of *N*-(4-hydroxybutyl)-*N'*-(4-tolyl) urea (V-5), 13 L of DCM and 2.02 L (1.5 kg, 11.57 mole, 1.25 eq.) of diisopropylethylamine. The white suspension was cooled to 6 °C, and a solution of 1.22 kg (10.64 mole, 1.15 eq.) of methanesulfonyl chloride in 0.25 L of DCM was added over a period of ca. 2 h while maintaining a pot temperature of 5 – 12 °C. After stirring for 10 min at 5 – 10 °C (100% conversion by hplc assay) the light-yellow solution was quenched into an extractor that contained 9 L of 1 N HCl. The phases were separated. The organic phase was washed with 9 L of 1 N HCl, washed with 9 L of 5% aq. NaHCO₃, dried (MgSO₄), filtered and then was partially concentrated (30 °C, 150 mbar) to a mass of 6.41 kg. After stirring in an ice bath for 30 min, the resulting slurry was filtered, washed with 2 L of cold DCM, and air dried to give 2.32 kg (83.4%) of *N*-(4-butylmesylate)-*N'*-(4-tolyl) urea (V-6) as a white solid.

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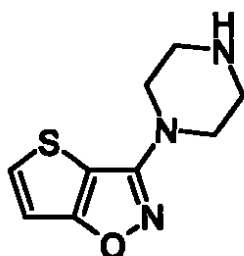
1-*p*-Tolyl-3-[4-[4-(6-trifluoromethylbenzo[*b*]thienyl-3-yl) piperazin-1-yl]butyl]-urea (V-7, free base)

A 22-L, 3-necked, round-bottom flask equipped with a mechanical stirrer, nitrogen bubbler, and a thermocouple probe was charged with 1.42 kg (4.40 mole) of V-3a, 1.757 kg (5.85 mole, 1.33 eq) of *N*-(4-butylmesylate)-*N*-(4-tolyl) urea (V-6), 4.26 L of THF, 1.23 L of water, and 1.52 kg (11.00 mole, 2.50 eq) of K₂CO₃. Using a temperature controller, the biphasic mixture was heated at 48 - 51 °C for 22 h. The resulting thick slurry was charged to a 20-gal glass-lined steel reactor along with rinses of 9.8 L of THF and 1.05 L water, and heated to reflux (65 °C). A total of 13.1 L of water was added to the biphasic mixture over a period of ca. 10 min during which the temperature dropped to ca. 60 °C. The batch was then slowly cooled (crystallization occurred at 43 °C) to 0 - 5 °C, and aged at this temperature for 30 min, then was filtered, slurry-rinsed in several portions using a cold mixture of 5 L THF/0.42 L water, and given a final rinse with 3 x 12 L of water. After air drying, 1.72 kg (79.7%) of 1-*p*-tolyl-3-[4-[4-(6-trifluoromethylbenzo[*b*]thienyl-3-yl) piperazin-1-yl]butyl]-urea (V-7, free base) as a white, fluffy solid was obtained.

1-*p*-Tolyl-3-[4-[4-(6-trifluoromethylbenzo[*b*]thienyl-3-yl) piperazin-1-yl]butyl]-urea methanesulfonate (V-7)

A 22-L, 3-necked, round-bottom flask equipped with a mechanical stirrer, nitrogen bubbler, and a thermocouple probe, was charged with 4.045 kg (8.245 mole) of V-7 (free base) and 11 L of *n*-propanol. A solution of 792.4 g (8.245, 1.00 eq) of methanesulfonic acid in 1 L of *n*-propanol was added in one portion. An exotherm was observed (T -> 39 °C), and the mixture became nearly homogeneous at the end of the addition. The mixture was heated to 92 °C, at which temperature all solids had dissolved. The solution was filtered through a coarse frit to remove a trace amount of extraneous matter. The clear, red-brown filtrate was charged into a 20-gal glass-lined steel reactor along with 3.3 L of *n*-PrOH. After slowly cooling, and seeding at 51 °C, crystallization commenced at 47 °C. Upon further crystallization and cooling to 37 °C, the slurry became very thick and was diluted with 12.1 L of *n*-PrOH. After cooling to 0 - 10 °C and stirring for 60 min, product was collected by filtering, rinsing with 5 L of cold *n*-PrOH, and drying (tray oven, 105-115 °C, 100 mm, N₂ bleed) to give 4.67 kg (96.5) of 1-*p*-tolyl-3-[4-[4-(6-trifluoromethylbenzo[*b*]thienyl-3-yl) piperazin-1-yl]butyl] urea methanesulfonate (V-7), as a white to off-white solid.

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Example 20**Synthesis of BOC protected piperazine-thienylisoxazole****5 3-Bromothiophene-2-carbaldehyde oxime**

3-Bromothiophene-2-carbaldehyde (Maybridge) (28.7 gm, 0.15 mol) in ethanol (50 ml) was added in one portion to a solution of hydroxylamine hydrochloride (13.8 gm, 0.2 mole), sodium hydroxide (8 gm, 0.2 mol) in water (30 ml) and ethanol (100 ml). The mixture was stirred at 0°C for 2 hours and was kept at 0°C overnight. The reaction mixture was diluted with cold water (600 ml), and the precipitated solids were collected by filtration to provide 20.5 gm, (67%) of product. The aqueous layer was further extracted with ethyl acetate and the combined organic layers were washed with brine, dried with magnesium sulfate, filtered and concentrated in vacuo to leave an additional 6.9 g of product.

15 3-Bromothiophene-2-hydroxymidoyl chloride

To a solution of 3-bromothiophene-2-carbaldehyde oxime (10.8 gm, 52.4 mmol), hydrogen chloride (14.5 ml, 4M in dioxane) in DMF (100 ml) was added oxone (16.9 gm, 1.05 equiv) in one portion at room temperature. The mixture was stirred at ambient temperature overnight. At the end of the reaction, DMF solution was poured into water and product was extracted into ethyl acetate. The organic solution was washed with brine, dried over magnesium sulfate, filtered and concentrated in vacuo to 12.68 gm of product which was used in the next reaction without further purification.

(4-(t-Butoxycarbonyl)piperazinyl)-3-bromo-2-thienyl methanone oxime

3-bromothiophene-2-hydroxymidoyl chloride (16.4 gm, 68 mmol) in tetrahydrofuran (THF, 70 ml) was added dropwise to a solution of N-(t-butoxycarbonyl)piperazine (14 gm, 1.1 equiv), DABCO (9.5 gm, 1.25 equiv) in DMF (100 ml) at 0°C over 25 minutes. The mixture was stirred for 3.5 hrs. At the end, the mixture was poured into water and was extracted with ethyl acetate. The organic was washed with brine and dried over magnesium sulfate. The solvent was removed on a rotary evaporator. The crude product (30.5 gm) was purified by chromatography on a Biotage cartridge (400 gm of silica gel), eluting

with methanol in dichloromethane (0-5 % of MeOH) The product thus obtained weighed 24.6 gm (85%)

(t-BOC-piperazine)-3-thienylbenzisoazole

1.

- 5 A mixture of (4-t-Butoxycarbonylpiperazinyl)-3-bromo-2-thienyl methanone oxime (10.3 gm, 26.4 mmol), cesium carbonate (10.7 gm, 32.7 mmol), and copper iodide (500 mg) in methoxyethanol (200 ml) was stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate, then washed with water. The aqueous solution was extracted three times with ethyl acetate. The organic solution (total 600 ml) was washed with brine and was
- 10 dried over magnesium sulfate then concentrated to an oil (~10 gm). This material was purified by chromatography using a Biotage cartridge (120 gm of silica gel, eluting with 0-8% Methanol in dichloromethane). The product thus obtained as light oil (5.1 gm, 62%).

Example 21

15

The following scheme exemplifies the synthesis of a radiolabeled C14 intermediate useful for the preparation of certain compounds within the scope of the present invention.

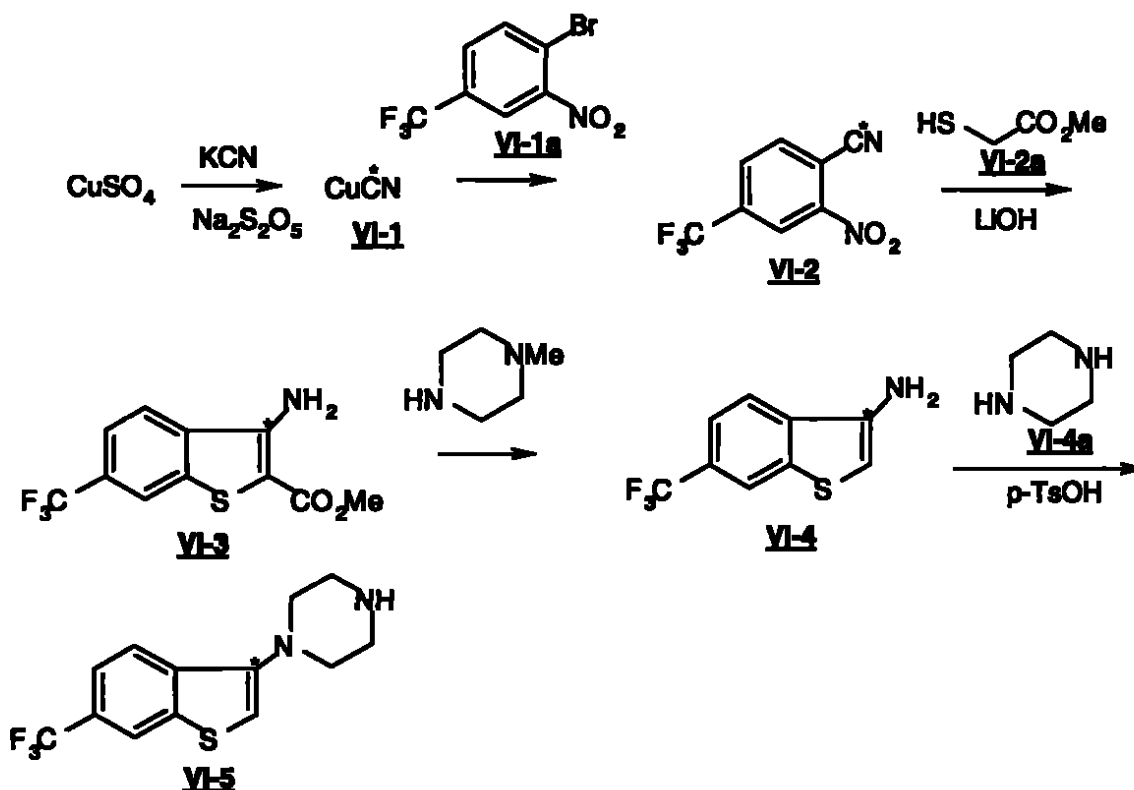
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Scheme VI

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General Analytical thin layer chromatography (TLC) was performed on E Merck TLC plates with silica gel 60 F₂₅₄ (0.25 mm). TLC plates used in the analysis of radioactive samples were scanned on a BIOSCAN system 2000 Imaging Scanner using P-10 gas (10% methane, 90% argon). Identity of the intermediates was established by co-migration in radio-TLC and/or radio-HPLC with the standard samples of unlabeled analogues. Flash chromatography was performed using silica gel with a particle size of 40–63 μm . Specific activity was determined on a Packard Minaxi Tri-Carb Liquid Scintillation Analyzer (Model 1600 TR) using Bio-Safe II as scintillation cocktail.

Purification of compounds VI-2, VI-3, VI-4, VI-5, and VI-6 was monitored by HPLC (conditions A) which was carried out on Waters 600 Controller, Waters 996 Photodiode Array Detector, Millennium Chromatography Manager and Beta-Ram Radioactive Flow Through Monitor System, Model 2 (IN/US Systems Inc.). Final purity determination of VI-7 by HPLC (conditions B) was performed on Waters Model 510 Pump, Waters 680 Gradient Controller, Waters 715 Ultra Wisp Autosampler, Waters 484 Tunable Absorbance Detector and Beta-Ram Radioactive Flow-Through Monitor System, Model 2 (IN/US Systems Inc.).

Conditions A YMC Basic 5 μ m, C18, 4 8x250 mm, mobile phase A (v/v) 50/50 acetonitrile/0.1N ammonium formate, mobile phase B (v/v) 75/25 acetonitrile/0.1N ammonium formate, flow rate 1.0 mL/min, uv detection at 254 nm

5	Gradient	Time (minutes)	%MP A	%MP B
		0	100	0
		15	100	0
		25	0	100
		30	0	100
10		35	100	0

Conditions B Ultramex 5 μ m, C8, 4.6 x 150 mm, mobile phase (v/v/v) 50/50/0.25 acetonitrile/0.05 M potassium phosphate buffer, pH 3.0/triethylamine, flow rate 1.0 mL/min, uv detection at 210 nm

15

[¹⁴C] Copper (I) Cyanide (VI-1)

A solution of copper (II) sulfate pentahydrate

(4.16 g, 16.67 mmol) in water (13.3 mL) was heated to 70 °C and a solution of sodium metabisulfite (1.94 g, 6.28 mmol) in water (3.3 mL) at 70 °C was added in one minute

20 Immediately a solution of [¹⁴C] potassium cyanide (245.5 mg, 200 mCi, 3.77 mmol, S.A. 53.0 mCi/mmol) and unlabeled potassium cyanide (0.84g, 12.9 mmol) in water (3.3 mL) at 70 °C was added in one minute. A white solid precipitated out of solution and blue color of the solution was discharged. After stirring for 10 min at 70 °C, the mixture was filtered hot and the solid was washed with hot water (15mL) and ethanol (15mL). The white solid was dried
25 under vacuum (0.1 mm Hg) for 27 h 45 min to give VI-1 (1.393 g, 186.6 mCi) in 93.3% yield

2-Nitro-4-(trifluoromethyl)-[7-¹⁴C]benzonitrile (VI-2)

To a suspension of [¹⁴C]copper (I) cyanide (VI-1) (1.393 g, 15.55 mmol, 186.6 mCi) in 1-methyl-2-pyrrolidinone (NMP, 10mL) was added 4-bromo-3-nitrobenzotrifluoride (6.33 g,

30 23.45 mmol) and the mixture was heated at 190-195 °C for 1 h. Ethyl acetate (25mL) and water (20 mL) were added at room temperature and the mixture was filtered through celite. To the filtrate more water (20mL) and ethyl acetate (25mL) were added and the aqueous layer was extracted with ethyl acetate (90mL). The organic extract was washed with Iron (III) chloride solution (50mL) prepared by dissolving iron (III) chloride (7.468 g, 46.04 mmol) in

water (50 mL) The organic extract was further washed with water (30mL), sat sodium chloride (15mL), dried (Na_2SO_4) and the solvent was removed *in vacuo*

The residue was purified by flash chromatography on silica gel (hexane/ethyl acetate, 9/1-7/3) to provide an oil which was dissolved in hexane (70 mL) The solvent was removed under reduced pressure and residue was dried under vacuum for 15 h 40 min to provide VI-2 (3.01 g, 167.13 mCi, 89.6% yield) as a yellow solid Radio-TLC (hexane/ethyl acetate, 9/1), $R_f=0.21$, HPLC (System A), RCP 99.86% (ret time, 9.2 min)

[3- ^{14}C]-3-Amino-2-carbomethoxy-6-trifluoromethylbenzo[b]thiophene (VI-3)

Nitrile (VI-2) (3.01g, 13.9 mmol, 167.13mCi) was dissolved in DMF (14mL) and methyl thioglycolate (1.78g, 15.94 mmol, 95%) was added in one minute The mixture was cooled to 0-5 °C and a solution of lithium hydroxide (0.689 g, 28.77 mmol) in water (9.2mL) was added dropwise in 12 minutes After the addition, cooling bath was removed and the mixture was stirred at room temperature for 4 hours Water (70mL) was added at 0-5 °C and the mixture was stirred for 15 min at 0-5 °C the solid was collected on a filter, washed with water (20 mL) and dried under vacuum (0.1 mm Hg) for 40 h 15 min to provide VI-3 (3.469 g, 151.24 mCi, 90.49% yield) Radio-TLC (CH_2Cl_2), $R_f=0.372$, HPLC (system A), RCP 99.92% (ret time, 16.722 min)

[3- ^{14}C]-3-Amino-6-trifluoromethylbenzo[b]thiophene (VI-4)

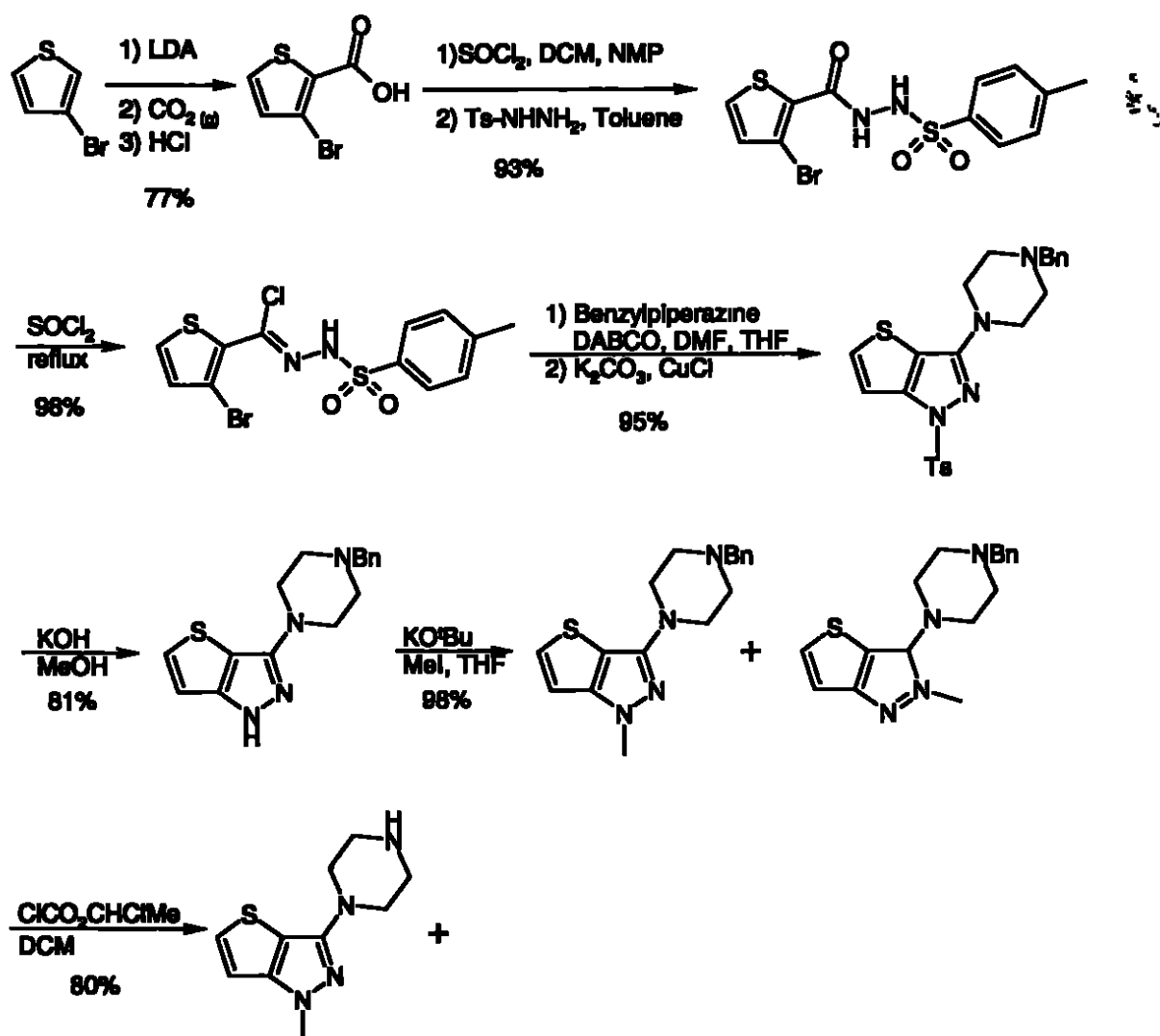
To a solution of benzo[b] thiophene (VI-3) (3.469 g, 12.6 mmol, 151.2 mCi) in NMP (14mL) was added 1-methylpiperazine (6.69g, 66.79 mmol) and the mixture was heated at 140-145 °C for 5 h The mixture was allowed to cool to room temperature, poured into water (60mL) and extracted with ethyl acetate (140 mL) The organic extract was washed with water (30 mL), sat sodium chloride (10mL), dried (Na_2SO_4) and the solvent was removed *in vacuo* The residue was purified by flash chromatography on silica gel (hexane/ethyl acetate, 1/1) to yield a greenish solid which was dried under vacuum (0.1 mm HG) for 14 h to provide VI-4 (w 66g, 146.95 mCi, 97.16% yield)) Radio-TLC (hexane/ethyl acetate, 1/5), $R_f=0.407$, HPLC (system A), RCP 99.44 % (ret time, 10.552 min)

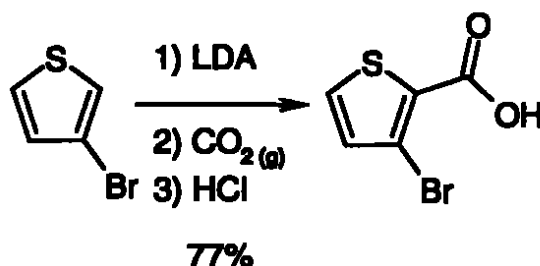
1-[6-(trifluoromethyl)benzo[b]thien-3-yl]-[3- ^{14}C]piperazine (VI-5)

To a solution of benzo[b]thiophene (VI-4) (2.66 g, 12.24 mmol, 146.95 mCi) in NMP (17mL) was added piperazine (4.309g, 50.02 mmol) and p-toluenesulfonic acid (4.76g, 25.02 mmol) at room temperature The mixture was heated at 170 °C for 20m h 24 min, allowed to cool to room temperature and poured into a solution of sodium carbonate

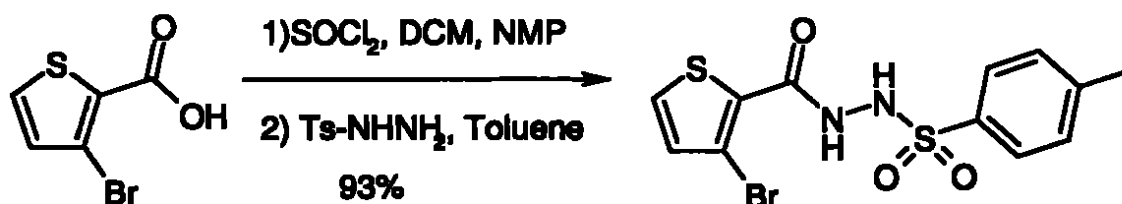
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(4.70 g, 44.3 mmol) in water (60 mL). The mixture was extracted with ethyl acetate (20 mL), dried (Na_2SO_4) and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$, 9/1/0.2) and product was dried under vacuum (0.1 mm Hg) for 11 h 50 min. Ethanol (absolute, 30 mL) was added to the product and solvent was removed under reduced pressure. The residue was dried under vacuum (0.1 mm Hg) for 24 h 55 min to provide VI-5 (3.44 g, 144.18 mCi, 98.1% yield) as an oil. Radio-TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$, 9/1/0.2), $R_f=0.46$, HPLC (system A), RCP 99.86% (ret time, 5.807 min).

Example 22



3-Bromo-thiophene-2-carboxylic acid To a solution of 3-bromothiophene (600.0 g, 3.68 mol) in THF (3 L) cooled to -72°C was added LDA (1.93 L, 3.86 mol, 2 N) slowly over 2 hours. The rate of LDA addition is such that the reaction temperature never exceeded -68°C . After complete addition, the solution is stirred for an additional 40 minutes. Diethyl ether (3 L) is then added via an addition funnel such that the temperature is maintained below -65°C . The addition funnel is then replaced with a dispersion tube and CO₂ gas is bubbled through the solution for 3 hours. Dry ice (500 g) is then added and the mixture is stirred overnight. The reaction flask is then placed in an ice bath and 6 N HCl is added slowly to prevent excessive bubbling until the pH of the solution is adjusted to 1-2. The resulting mixture is then extracted with EtOAc. The extract is washed with brine then dried over MgSO₄, filtered and evaporated. The product is dried under vacuum at room temperature yielding 585.15 g (77%) as an off-white solid.



1-(3-Bromo-thiophene-2-carboxylic acid)-2-(4-toluenesulfonyl)-hydrazine To a stirred suspension of the acid (285.53 g, 1.38 mol) in DCM (1.5 L) was added a catalytic amount of NMP (2 mL). Thionyl chloride (105.8 mL, 1.45 mol) is then added and the solution is refluxed until the solids have completely dissolved. The solution is further refluxed for 1 hour, cooled to room temperature and evaporated to afford a light, brown solid. The crude material is dried under vacuum overnight. The brown solid is taken up in toluene (3.5 L) and *p*-toluenesulfonylhydrazine (402.25 g, 2.16 mol) is added. The mixture is stirred at 100°C for 8 hours then at room temperature overnight. The resulting mixture was cooled with an ice bath and the resulting solids were collected by filtration and washed with toluene. The solids were then stirred as

a slurry in 1 N HCl for 1 hour. The solids were collected by filtration and washed with copious amounts of water. The solid was dried under vacuum at 40 °C then recrystallized from toluene / isopropyl alcohol yielding 484.28 g (93%) of the desired product.

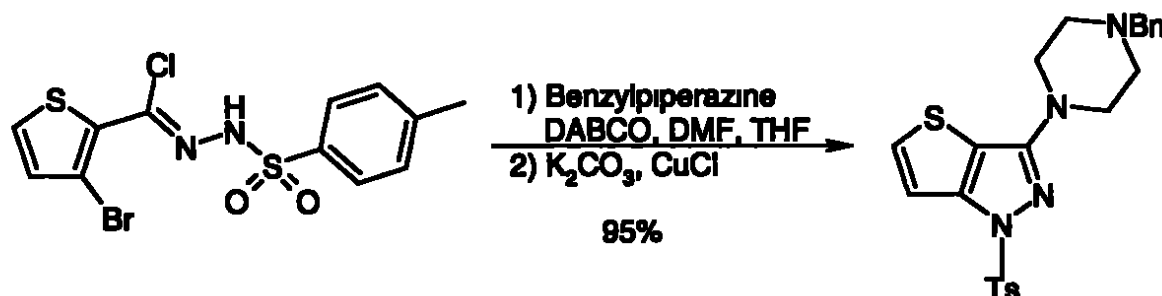
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N-((4-Methylphenyl)sulfonyl)-3-bromo-thiophene-2-carbohydrazonyl chloride

1-(3-Bromo-thiophene-2-carboxylic acid)-2-(4-toluenesulfonyl)-hydrazine (60.80 g, 0.161 mol) was added to thionyl chloride (70.5 mL, 0.966 mol). The resulting mixture was stirred at 80 °C until the mixture becomes homogenous. The solution is then stirred at 70 °C for 30 minutes and heptane (300 mL) is added over a period of 20 minutes. The solution was cooled slowly to room temperature then cooled further to 5 °C. The solids are collected by filtration, washed with heptane (3 x 100 mL) and dried under vacuum yielding 62.1 g (98%) of the desired product as an off-white solid.

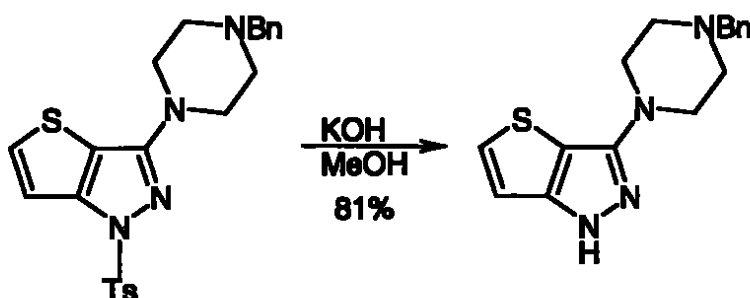
15



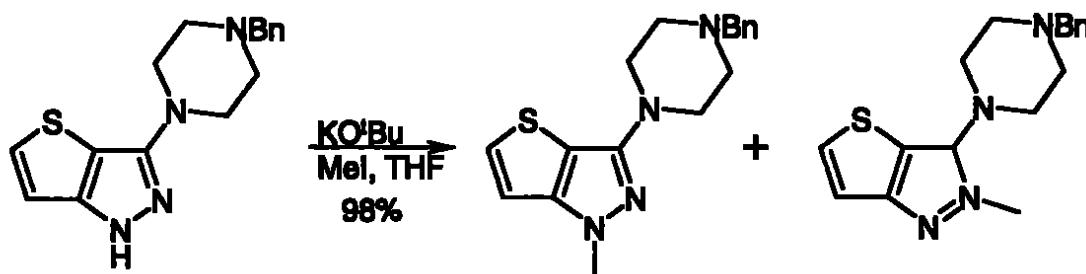
3-(4-Benzyl-piperazin-1-yl)-1-(toluene-4-sulfonyl)-1H-thieno[3,2-c]pyrazole

To a stirred solution of DABCO (14.18 g, 112.18 mol) and benzylpiperazine (35.35 g, 0.200 mol) in DMF (200 mL) cooled to -30 °C was added via cannula a solution of N-((4-Methylphenyl)sulfonyl)-3-bromo-thiophene-2-carbohydrazonyl chloride (62.1 g, 0.158 mol) in THF (100 mL). The addition is controlled to prevent the reaction temperature from exceeding -30 °C. After complete addition precipitation occurs and the mixture is then allowed to stir at room temperature overnight when K_2CO_3 (65.41 g, 0.473 mol) and CuCl (1.0 g, 0.010 mol) was added. The resulting mixture is heated to 110 °C and the THF is removed by distillation at this point. The

temperature is then increased to 140 °C and the mixture is stirred for 6 hours, cooled to room temperature and stirred overnight. The mixture was then poured over water (100 mL) and EtOAc (100 mL). The EtOAc layer is then separated and the aqueous layer is extracted with EtOAc (3 x 500 mL). The combined EtOAc layers were washed with water (500 mL) and then filtered through celite and concentrated. The solids were collected by filtration and washed with cold water then EtOAc / heptane (1 : 4) and dried under vacuum yielding 66.05 g (95%) of the desired product as an off-white solid.

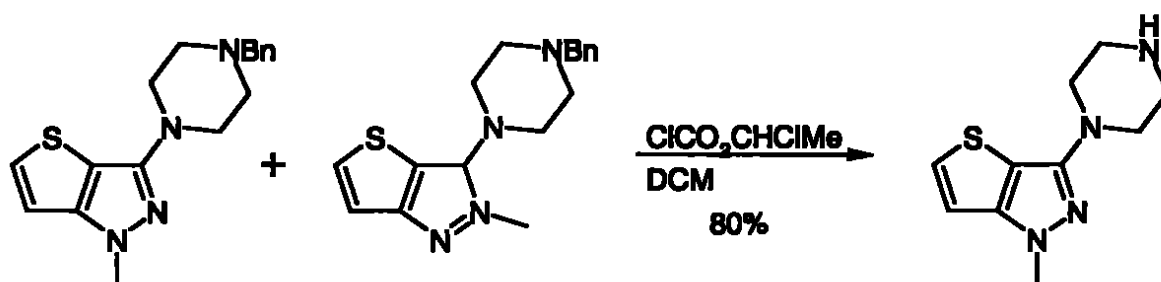


3-(4-Benzyl-piperazin-1-yl)-1H-thieno[3,2-c]pyrazole To a stirred mixture of KOH_(s) (56.09 g, 2.66 mol) in methanol (1.33 L) is added 3-(4-benzyl-piperazin-1-yl)-1-(toluene-4-sulfonyl)-1H-thieno[3,2-c]pyrazole (241 g, 0.532 mol). The mixture is heated at reflux for 1.25 hours, cooled to room temperature and evaporated. The residue is taken up in EtOAc (1 L), washed with water (2 L), dried (MgSO₄), filtered and evaporated. The residue was recrystallized from EtOAc / Heptane yielding 129 g (81%).



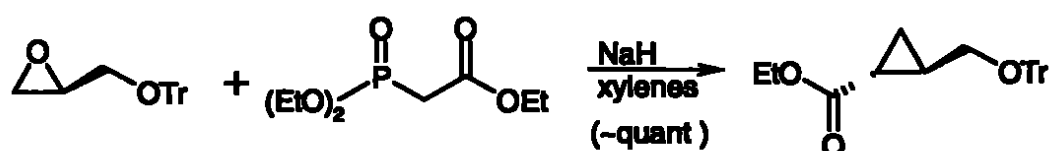
3-(4-Benzyl-piperazin-1-yl)-1-methyl-1H-thieno[3,2-c]pyrazole To a stirred solution of 3-(4-benzyl-piperazin-1-yl)-1H-thieno[3,2-c]pyrazole (318.0 g, 1.07 mol) in THF (2.5 L) was added a mixture of potassium t-butoxide (134.4 g, 1.2 mol) in THF (1.5 L) dropwise over a period of 1 hour while keeping the reaction temperature below 25 °C. After complete addition, the mixture was cooled to -30 °C and MeI (65.4 mL, 1.05 mol) was added dropwise over a period of 30 minutes. The mixture is then

slowly warmed to room temperature overnight. To the reaction mixture is slowly added saturated NaHCO_3 (1 L). The solution is then evaporated to remove the THF and the resulting aqueous mixture is taken up in EtOAc and washed with water and brine. The EtOAc extract is dried (Na_2SO_4), filtered and evaporated. The viscous concentrate is filtered through a silica gel plug with 1:1 EtOAc/heptane and evaporated yielding a viscous oil that is then dried under vacuum where it solidifies and yields 326.03 g (98%) as a 12:1 ratio of regioisomers in favor of the desired product.

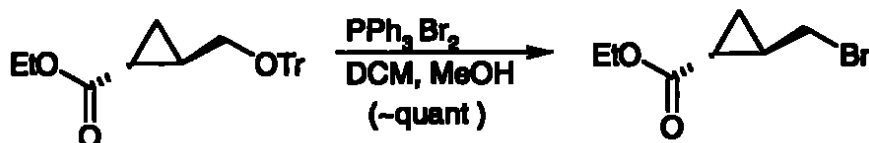


1-Methyl-3-piperazin-1-yl-1H-thieno[3,2-c]pyrazole To a solution of a mixture of 3-(4-benzylpiperazin-1-yl)-1-methyl-1H-thieno[3,2-c]pyrazole and the 2-methyl analog (189.0 g, 0.60 mol) is dissolved in DCM (1.25 L) is added 1-chloroethylchloroformate (78.6 mL, 0.72 mol). The solution is heated at reflux for 1 hour when the mixture is cooled and the solvent is removed by evaporation. The residue is taken up in methanol (1 L) and heated at reflux for 30 minutes. After cooling, the solution is treated with 1 N HCl in ether (200 mL) and an additional 1 L of ether to afford the precipitation of the product. The solid is collected via filtration and washed with cold ether. The solid is recrystallized from methanol (1 L) and the HCl salt is collected by filtration, washed with ether and dried under vacuum yielding 123.04 g (80%) of the desired product as an 80:1 mixture of regioisomers in favor of the desired regioisomer as seen by NMR.

25

Example 23

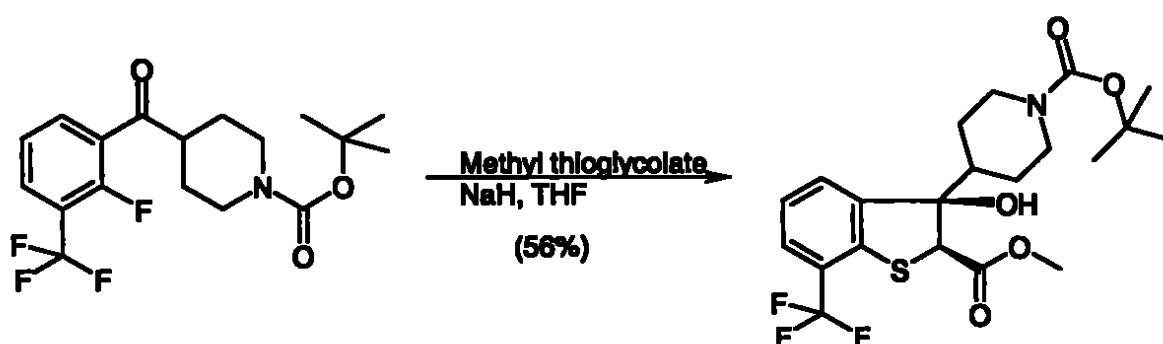
Trityloxymethyl-(1R, 2R)-cyclopropanecarboxylic acid ethyl ester To a suspension of sodium hydride (15.20 g, 380 mmol, 60% oil dispersion) in xylenes (300 mL) was added triethylphosphonoacetate (85.07 g, 379 mmol) in a controlled manner to avoid the excessive evolution of gas and to maintain the internal temperature less than 55 °C. After the complete addition, the mixture was stirred for 20 minutes when the yellow solution was added via cannula to a solution of (R)-trityl glycidyl ether (100.0 g, 316 mmol) in xylenes (300 mL). The resulting solution was heated to 125 °C for 2 hours. The resulting solution was cooled to room temperature, acidified with the addition of 10% HCl (320 mL) and extracted with EtOAc (2 x 300 mL). The combined extracts were washed with brine (100 mL), dried (MgSO₄), filtered and evaporated yielding a 175 g of a crude product as an oil. The material was carried on crude.



2R-bromomethyl-cyclopropane-1R-carboxylic acid methyl ester A solution of triphenylphosphine (124.7 g, 1.34 mol) in CH_2Cl_2 (260 mL) was cooled to 5 °C when a solution of bromine (24.4 mL, 1.34 mol) in CH_2Cl_2 (65 mL) was added over 20 minutes while the temperature was maintained below 12 °C. The mixture was stirred at 5 °C for 1 hour when 2 M $\text{HCl}/\text{Et}_2\text{O}$ (16 mL, 32 mmol) was added followed by the addition of crude trityloxymethyl-(1R, 2R)-cyclopropane carboxylic acid ethyl ester (124 g, 0.32 mol). The resulting mixture was stirred at room temperature overnight when saturated NaHCO_3 (600 mL) was added. The mixture was separated and the aqueous layer was extracted with CH_2Cl_2 (200 mL). The combined organic layers were washed with water (400 mL), dried (MgSO_4), filtered and evaporated. The residue was diluted with heptane (200 mL) and evaporated two times to remove excess CH_2Cl_2 . The residue was allowed to stand for 30 minutes when the solid impurities were removed by filtration. The filter cake was washed with heptane (2 x 400 mL). The combined organic layers were evaporated to provide 92.68 g of a crude yellow liquid. The crude liquid was distilled (BP = 80–85 °C/1.5 torr) to provide 55.19 g (84% yield for the two steps) of a colorless liquid.

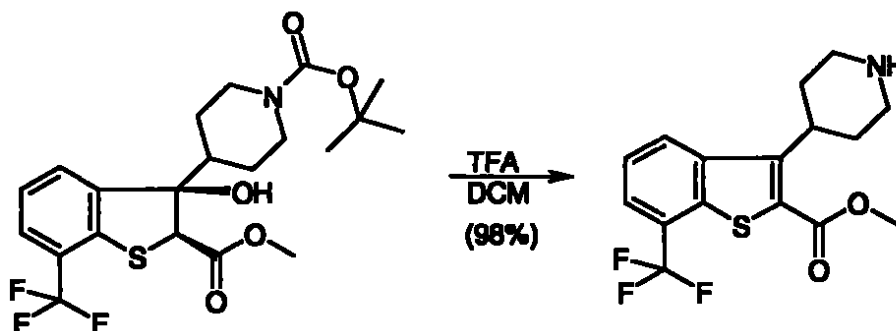
Example 24

7-Trifluoromethyl benzo[b]thienyl piperidine



4-(3-Hydroxy-2-methoxycarbonyl-7-trifluoromethyl-2,3-dihydro-benzo[b]thiophen-3-yl)-piperidine-1-carboxylic acid tert-butyl ester (MDL 832712) To a room temperature solution of 4-(2-fluoro-3-trifluoromethyl-benzoyl)-piperidine-1-carboxylic acid tert-butyl ester (9.00 g, 24.0 mmol), methyl thioglycolate (2.40 mL, 26.8 mmol), and anhydrous THF (200

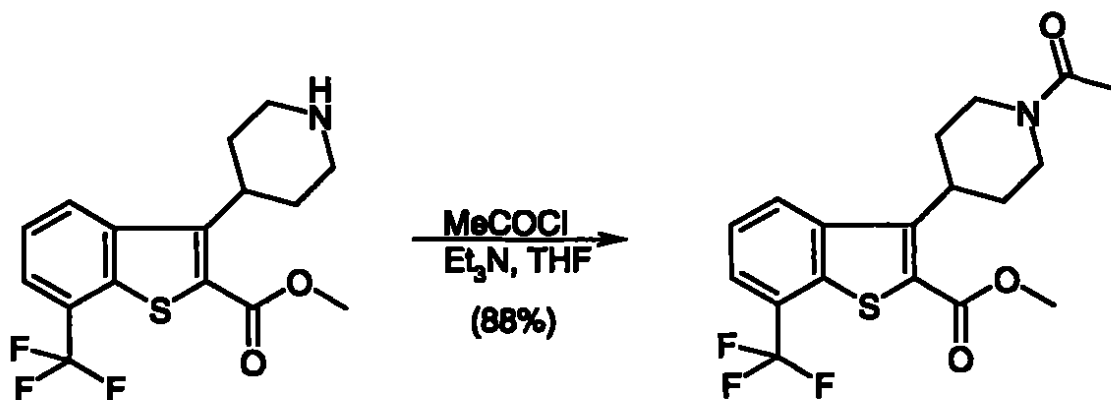
mL) under nitrogen was added NaH (1.15 g of a 80% oil dispersion, 28.7 mmol) in one portion. After the gas evolution ceased, the reaction was stirred at 55 °C. After 100 min, the reaction was cooled to room temperature and diluted with ethyl acetate (500 mL). The mixture was washed with water (300 mL) and brine (300 mL) successively, dried over magnesium sulfate, filtered, and the solvent removed to afford a sticky white solid. Trituration with 20% ethyl acetate/heptane afforded 6.20 g (56%) of the desired product as a white powder.



10 **3-(1-piperidin-4-yl)-7-trifluoromethyl-benzo[b]thiophene-2-carboxylic acid methyl ester**

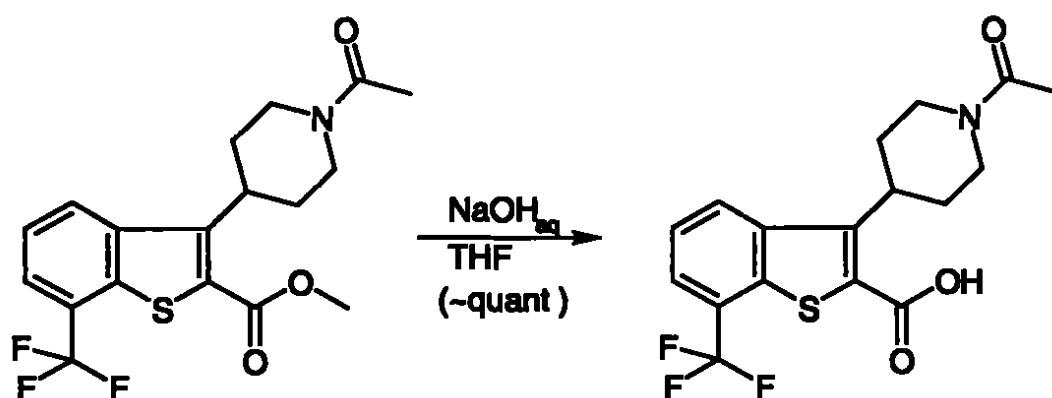
To a room temperature solution of 4-(3-hydroxy-2-methoxycarbonyl-7-trifluoromethyl-2,3-dihydro-benzo[b]thiophen-3-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (6.00 g, 13.0 mmol) in DCM (30 mL) was added TFA (30 mL) causing rapid gas evolution. After 5 min, the reaction was stirred at 40 °C for 5.5 h. After cooling to room temperature, the reaction was poured into 20% aqueous potassium carbonate (400 mL) and extracted with DCM (2 x 200 mL). The combined extracts were dried over magnesium sulfate, filtered, and the solvent removed to give a thick oil. After drying under high vacuum 4.37 g (98%) of the desired product was obtained as a white foam.

20

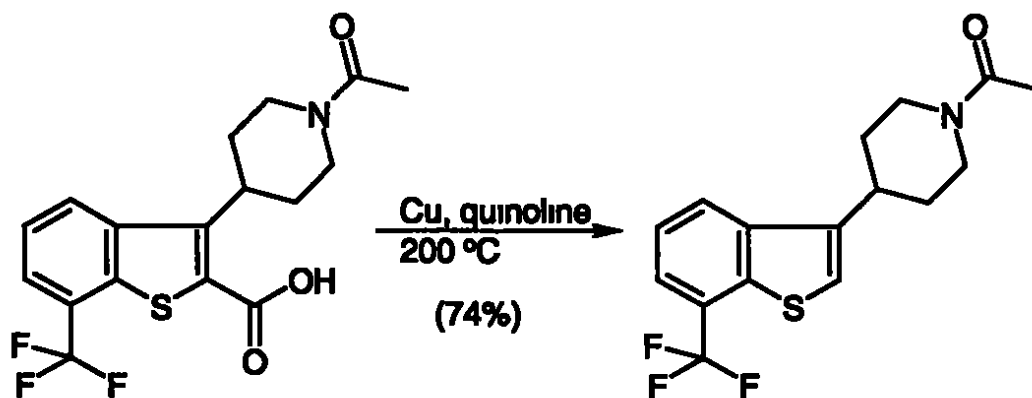


3-(1-Acetyl-piperidin-4-yl)-7-trifluoromethyl-benzo[b]thiophene-2-carboxylic acid methyl ester To a room temperature solution of 3-piperidin-4-yl-7-trifluoromethyl-

benzo[*b*]thiophene-2-carboxylic acid methyl ester (4.37 g, 12.7 mmol), triethylamine (2.70 mL, 19.4 mmol), and anhydrous THF (80 mL) under nitrogen was added acetyl chloride (1.10 mL, 15.5 mmol) in one portion and the reaction stirred at room temperature overnight. The reaction was diluted with ethyl acetate (300 mL) and washed with water (150 mL) and brine (150 mL) successively. The organic layer was dried over magnesium sulfate, filtered, and the solvent removed. The residue was chromatographed on silica, eluting with 10% methanol/ethyl acetate, to afford 4.28 g (88%) of the desired product as a white solid, mp 155.2 °C.

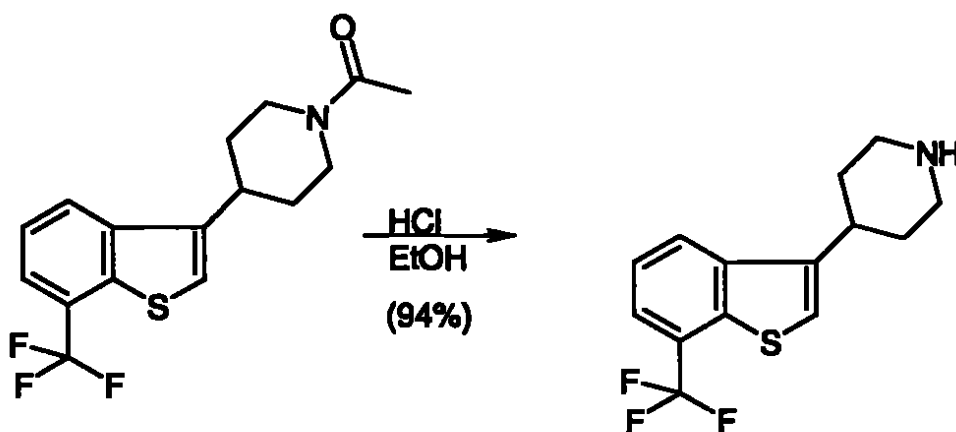


3-(1-Acetyl-piperidin-4-yl)-7-trifluoromethyl-benzo[*b*]thiophene-2-carboxylic acid To a solution of 3-(1-acetyl-piperidin-4-yl)-7-trifluoromethyl-benzo[*b*]thiophene-2-carboxylic acid methyl ester (4.10 g, 10.6 mmol) in THF (25 mL) was added 0.5 N aqueous sodium hydroxide (23.4 mL, 11.7 mmol) and the reaction stirred at room temperature. After 18 h, the reaction was acidified with 1 N HCl (200 mL) and the mixture extracted with DCM (2 x 100 mL). The organics were washed with water (100 mL), dried over magnesium sulfate, filtered, and concentrated to give 4.13 g of the desired product as a white foam.



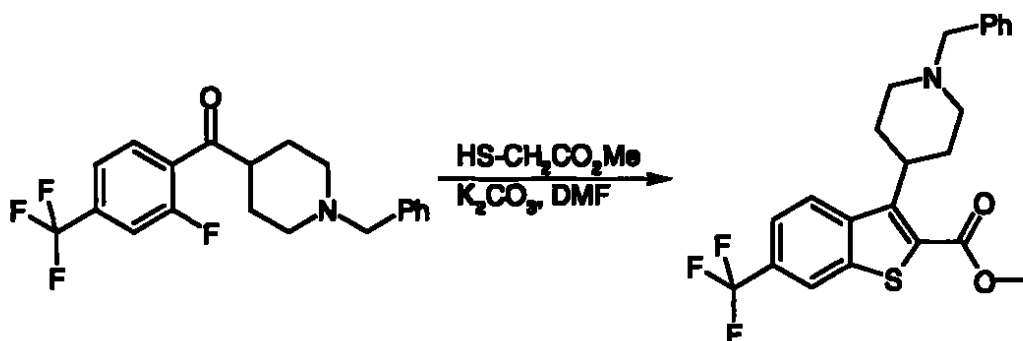
1-[4-(7-Trifluoromethyl-benzo[*b*]thiophen-3-yl)-piperidin-1-yl]-ethanone (MDL 832823)
A mixture of 3-(1-acetyl-piperidin-4-yl)-7-trifluoromethyl-benzo[*b*]thiophene-2-carboxylic acid

(4.13 g, 11.1 mmol), Cu powder (0.706 g, 11.1 mmol), and quinoline (20 mL) was heated to 200 °C under nitrogen. After 10 min, no gas evolution was observed and the reaction cooled at room temperature. The mixture was diluted with ethyl acetate (100 mL), filtered through a Celite bed and the filtrate washed with 1 N HCl (2 x 100 mL), 5% aqueous potassium carbonate (100 mL), water (100 mL), and brine (100 mL) successively. The organics were dried over magnesium sulfate, filtered, and concentrated to give an amber oil. The oil was chromatographed on silica, eluting with 10% methanol/ethyl acetate to afford 2.69 g (74%) of the desired product as a tan solid.



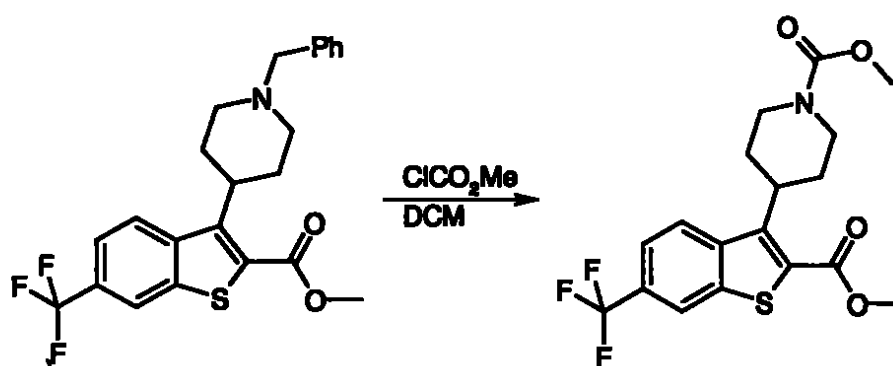
4-(7-(Trifluoromethyl)-benzo[b]thiophen-3-yl)-piperidine A mixture of 1-[4-(7-(trifluoromethyl)-benzo[b]thiophen-3-yl)-piperidin-1-yl]-ethanone (2.95 g, 9.01 mmol), concentrated HCl (30 mL), and ethanol was heated at 80 °C for 18 h. After cooling to room temperature, the reaction was basified with 20% aqueous potassium carbonate (150 mL) and the mixture extracted with DCM (2 x 100 mL). The organics were washed with water (100 mL), dried over potassium carbonate, filtered, and concentrated to give 2.42 g (94%) of the desired product as an amber waxy solid.

Example 25

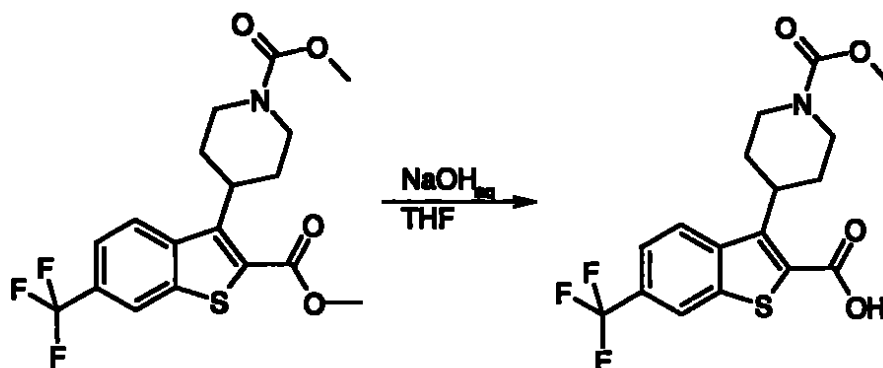


3-(1-Benzyl-piperidin-4-yl)-6-trifluoromethyl-benzo[b]thiophene-2-carboxylic acid

methyl ester (MDL 833803) To a room temperature solution of (1-benzyl-piperidin-4-yl)-(2-fluoro-4-trifluoromethyl-phenyl)-methanone (7.5 g, 20.5 mmol), methyl thioglycolate (2.0 mL, 22.5 mmol), and DMF (100 mL) was added K_2CO_3 (5.65 g, 41.0 mmol). The reaction was stirred at 60 °C for 24 hours, cooled to room temperature and diluted with ethyl acetate (500 mL). The mixture was washed with water (2 x 300 mL) and brine (300 mL) successively, dried over magnesium sulfate, filtered, and the solvent removed to afford an oil. The oil was purified via chromatography (30% EtOAc in heptane) yielding 5.91 g (67%) as a solid.

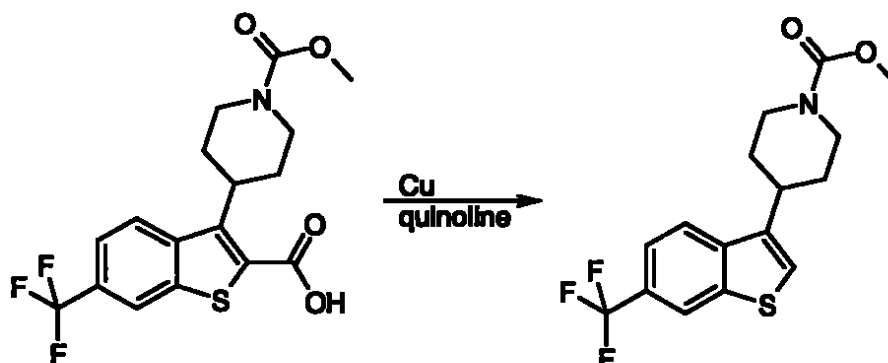
**4-(2-Methoxycarbonyl-6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperidine-1-**

carboxylic acid methyl ester To a solution of 3-(1-benzyl-piperidin-4-yl)-6-trifluoromethyl-benzo[b]thiophene-2-carboxylic acid methyl ester (5.9 g, 13.6 mmol) in DCM (50 mL) was added methyl chloroformate (1.26 mL, 16.3 mmol) drop-wise. The resulting solution was stirred overnight when the volatiles were removed *in vacuo*. The residue was washed with heptane to yield 4.2 g (77%) of the desired product as a white solid.

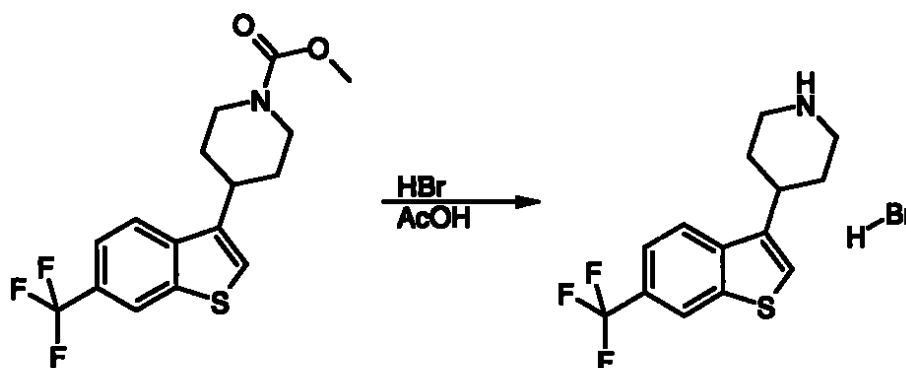
**4-(2-Carboxy-6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperidine-1-carboxylic**

acid methyl ester To a stirred solution of 4-(2-methoxycarbonyl-6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperidine-1-carboxylic acid methyl ester (1.1 g, 2.7 mmol) in THF (7.0 mL) was added 1 N NaOH (2.97 mL). The resulting mixture was stirred at

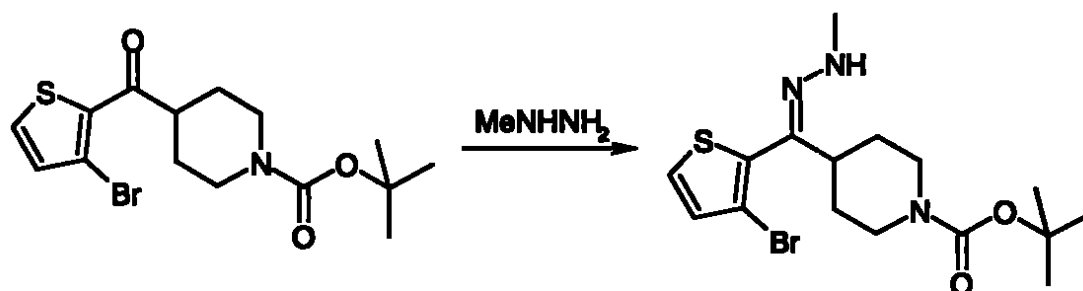
room temperature overnight when the mixture was diluted with water (50 mL) and washed with ether (100 mL). The aqueous layer was acidified with the addition of 3 N HCl and the product was extracted with EtOAc (2 x 150 mL). The combined organic layers were washed with brine (50 mL), dried (MgSO₄), filtered and evaporated yielding 960 mg (92%) of the desired product as a white solid.



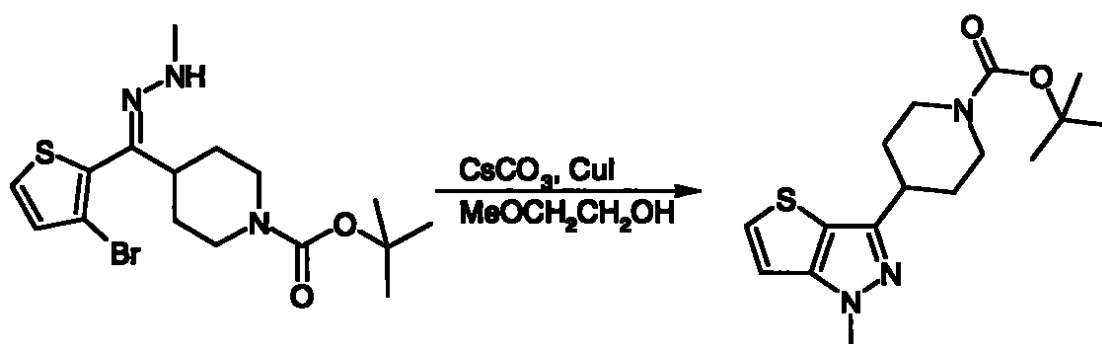
4-(6-Trifluoromethyl-benzo[b]thiophen-3-yl)-piperidine-1-carboxylic acid methyl ester A mixture of 4-(2-carboxy-6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperidine-1-carboxylic acid methyl ester (4.3 g, 11.1 mmol) and copper (705 mg, 11.1 mmol) in quinoline (28 mL) was heated at 200 °C for 45 minutes. Upon cooling to room temperature the mixture was diluted with EtOAc (50 mL) and filtered. The filtrate was washed with 5% HCl (2 x 20 mL), water (20 mL) and brine (20 mL), dried (MgSO₄), filtered and evaporated. The residue was separated via chromatography (30% EtOAc in heptane) yielding 3.14 g (82%) of the desired product as a white solid.



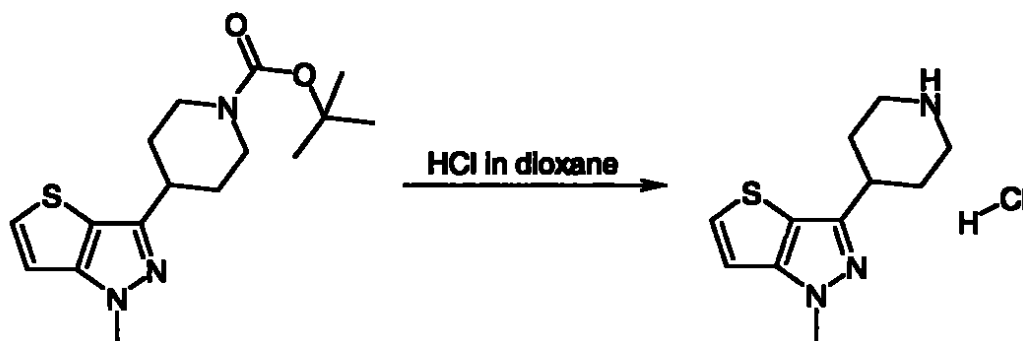
4-(6-Trifluoromethyl-benzo[b]thiophen-3-yl)-piperidine hydrobromide A mixture of 4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperidine-1-carboxylic acid methyl ester (3.1 g, 9.0 mmol) in HBr (45 mL, 30% in acetic acid) was stirred at room temperature for 20 hours when the volatiles were removed *in vacuo*. The residue was washed with EtOAc and the product was collected by filtration yielding 3.09 g (94%) of the desired product as a white solid.

Example 26

- 5 **4-[(3-Bromo-thiophen-2-yl)-(methyl-hydrazono)- methyl]-piperidine-1-carboxylic acid *tert*-butyl ester** A mixture of 4-(thiophene-2-carbonyl)-piperidine-1-carboxylic acid *tert*-butyl ester (1.96 g, 5.2 mmol) in methylhydrazine (2 mL) was heated at 75 °C overnight. The excess methyl hydrazine was then removed with a vacuum pump. The residue was purified by chromatography (eluted with 0-8% of MeOH in DCM) yielding
- 10 0.95 g (45%) of the desired product



- 4-(1-Methyl-1H-thieno[3,2-c]pyrazol-3-yl)-piperidine-1-carboxylic acid *tert*-butyl ester** 4-[(3-Bromo-thiophen-2-yl)-(methyl-hydrazono)- methyl]-piperidine-1-
- 15 carboxylic acid *tert*-butyl ester (700 mg, 1.74 mmol) was mixed with CuI (20 mg), CsCO₃ (650 mg, 1.15 eq) in methoxyethanol (10 mL). The mixture was heated to 70 °C for 2 hr, then stirred overnight at room temperature. The solvent was stripped on rotary evaporator. The residue was extracted into EtOAc, then washed with brine and concentrated down to an oil. This oil was purified via chromatography (eluted with 0-
- 20 10% MeOH in DCM) yielding 520 mg (68%) of the desired product.



1-Methyl-3-(piperidin-4-yl)-1H-thieno[3,2-c]pyrazole hydrochloride

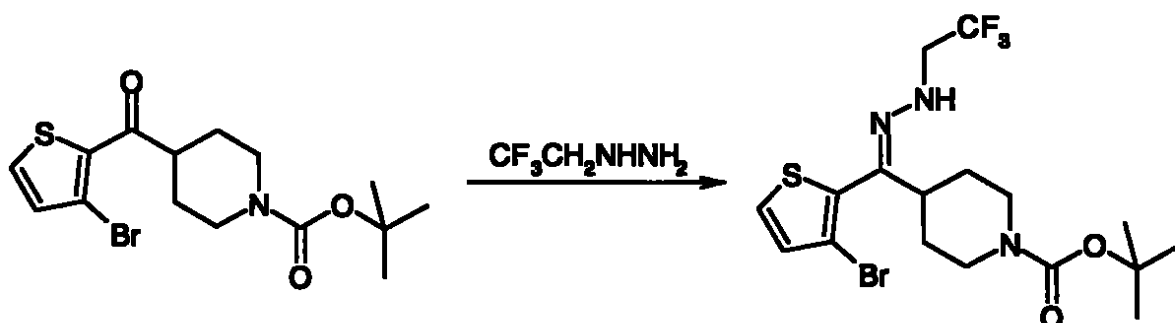
(A002436287A) 4-(1-Methyl-1H-thieno[3,2-c]pyrazol-3-yl)-piperidine-1-carboxylic

acid *tert*-butyl ester (520 mg, 1.6 mmol) was stirred at room temperature in a solution

5 of HCl (5 mL, 4N HCl in dioxane) for 4 hours. The volatiles were removed *in vacuo* and the residue was triturated with ether (twice) to yield off white solids 304 mg (74%) as the desired hydrochloride salt.

10

Example 27



15

4-((3-Bromo-thiophen-2-yl)-[(2,2,2-trifluoro-ethyl)-hydrazone]-methyl)-

piperidine-1-carboxylic acid *tert*-butyl ester To a mixture of 4-(thiophene-2-

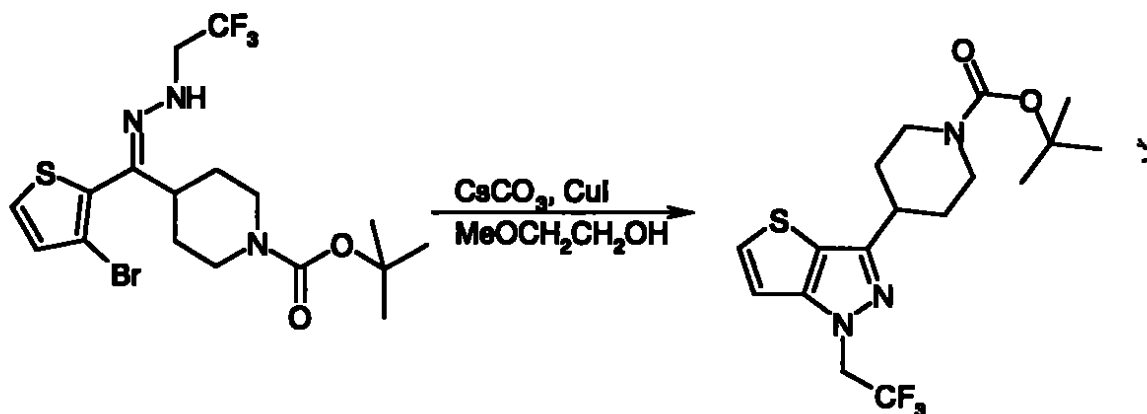
carbonyl)-piperidine-1-carboxylic acid *tert*-butyl ester (2.34 g, 6.24 mmol) in n-butanol

(20 mL) was added trifluoroethylhydrazine (2.43 g, 12.4 mmol). The resulting mixture

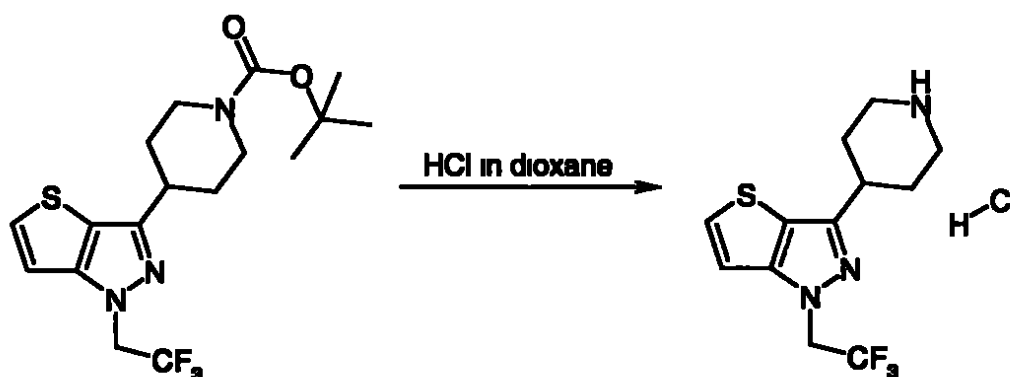
20 was heated at 110 °C overnight. The volatiles were then removed *in vacuo*. The

residue was purified by chromatography (eluted with 0-10% MeOH in DCM) yielding

2.41 g (92%) of the desired product.

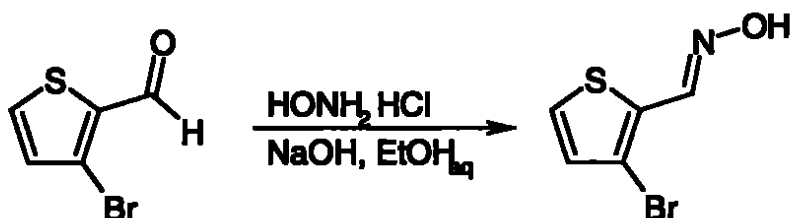


4-[1-(2,2,2-Trifluoro-ethyl)-1H-thieno[3,2-c]pyrazol-3-yl]-piperidine-1-carboxylic acid tert-butyl ester 4-((3-Bromo-thiophen-2-yl)-[(2,2,2-trifluoro-ethyl)-hydrazono]-methyl)-piperidine-1-carboxylic acid tert-butyl ester (2.34 g, 4.98 mmol) was mixed with CuI (50 mg), CsCO_3 (1.9 g, 1.2 eq) in methoxyethanol (25 mL). The mixture was heated to 75 °C for 1 hour. The mixture was then diluted with EtOAc and filtered. The filtrate was evaporated and the residue was purified via chromatography (eluted with 0-10% MeOH in DCM) yielding 2.03 g (>95%) of the desired product.



3-(4-(1-(2,2,2-trifluoro-ethyl)-1H-thieno[3,2-c]pyrazol-3-yl)-piperidine-1-carboxylic acid tert-butyl ester (1.9 g, 4.87 mmol) was stirred at room temperature in a solution of HCl (6 mL, 4N HCl in dioxane) for 4 hours. The volatiles were removed *in vacuo* and the residue was triturated with ether (twice) to yield off white solids 2.1 g (74%) as the desired hydrochloride salt.

Example 28

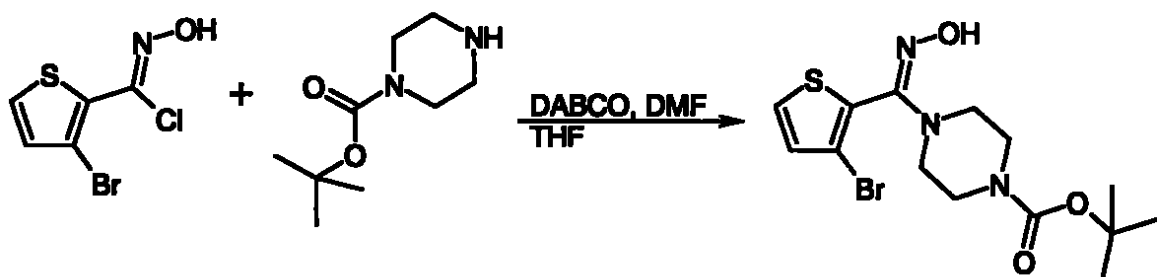


3-Bromo-thiophene-2-carbaldehyde oxime 3-Bromothiophene-2-carbaldehyde

(28.7 g, 0.15 mol) in ethanol (50 mL) was added in one portion to a solution of hydroxylamine hydrochloride (13.8 g, 0.2 mol), sodium hydroxide (8 g, 0.2 mol) in water (30 mL) and ethanol (100 mL). The mixture was stirred at 0 °C for 2 hours and was kept at 0 °C overnight when a precipitate formed. The mixture was diluted with cold water (600 mL) and the solid was collected by filtration yielding 20.5 g, (67%). The aqueous solution was further extracted with ethyl acetate. The organic solution was washed with brine, dried with magnesium sulfate, filtered and evaporated yielding 6.9 g of additional product as a light yellow solid. The total yield was 27.4 g (89%).

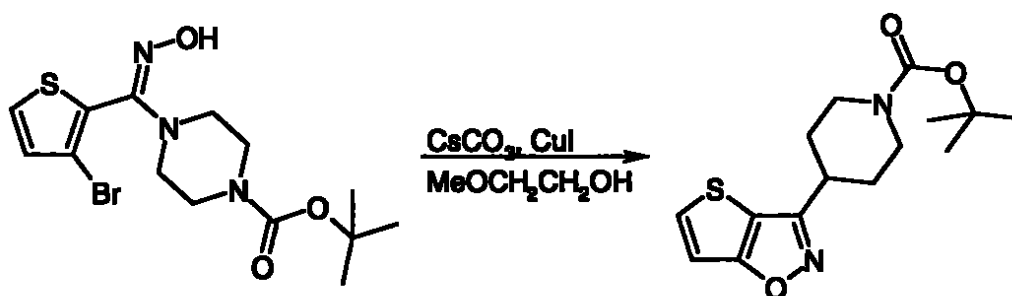


3-Bromo-thiophene-2-(chloro-carbaldehyde) oxime To the solution of 3-bromothiophene-2-carbaldehyde oxime (10.8 g, 52.4 mmol), hydrogen chloride (14.5 mL, 4M in dioxane) in DMF (100 mL) was charged with *oxone* (16.9 g, 1.05 equiv) in one portion at room temperature. The mixture was stirred at room temp overnight when the solution was poured in to water and extracted with ethyl acetate. The organic solution was washed with brine and dried over magnesium sulfate, filtered and evaporated to dryness to give a yellow solid (12.68 g, quantitative by weight) which was used in the next reaction without further purification.

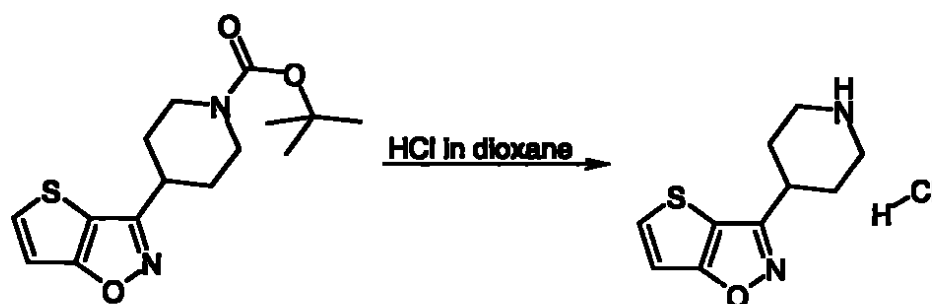


4-[(3-Bromo-thi phen-2-yl)-hydroxylmino-methyl]-piperazine)-1-carboxylic acid

***tert*-butyl ester** A solution of 3-bromo-thiophene-2-(chloro-carbaldehyde) oxime (16.4 g, 68 mmol) in THF (70 mL) was added drop-wise to a solution of N-(*t*-butoxycarbonyl)piperazine (14 g, 1.1 equiv), DABCO (9.5 g, 1.25 equiv) in DMF (100 mL) at 0 °C over 25 minutes. The mixture was stirred at 0 °C for 3.5 hours when the mixture was poured into water and was extracted with ethyl acetate. The organic layer was washed with brine and dried over magnesium sulfate, filtered and evaporated. The crude product (30.5 g) was purified via chromatography (eluted with 0-5 % of MeOH in DCM) yielding 24.6 g (85%) of the desired product.

**4-Thieno[2,3-*d*]isoxazol-3-yl-piperazine-1-carboxylic acid *tert*-butyl ester**

A mixture of 4-[(3-bromo-thiophen-2-yl)-hydroxylmino-methyl]-piperazine)-1-carboxylic acid *tert*-butyl ester (10.3 g, 26.4 mmol), cesium carbonate (10.7 g, 32.7 mmol), copper iodide (500 mg) in methoxyethanol (200 mL) was stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate and washed with water. The aqueous solution was extracted three times with ethyl acetate. The combined organic layers (total 600 mL) were washed with brine, dried over magnesium sulfate, filtered and evaporated. The residue was purified via chromatography (120 g of silica gel, eluted with 0-8% Methanol in dichloromethane) yielding 5.1 g (62%) of the desired product as light oil.

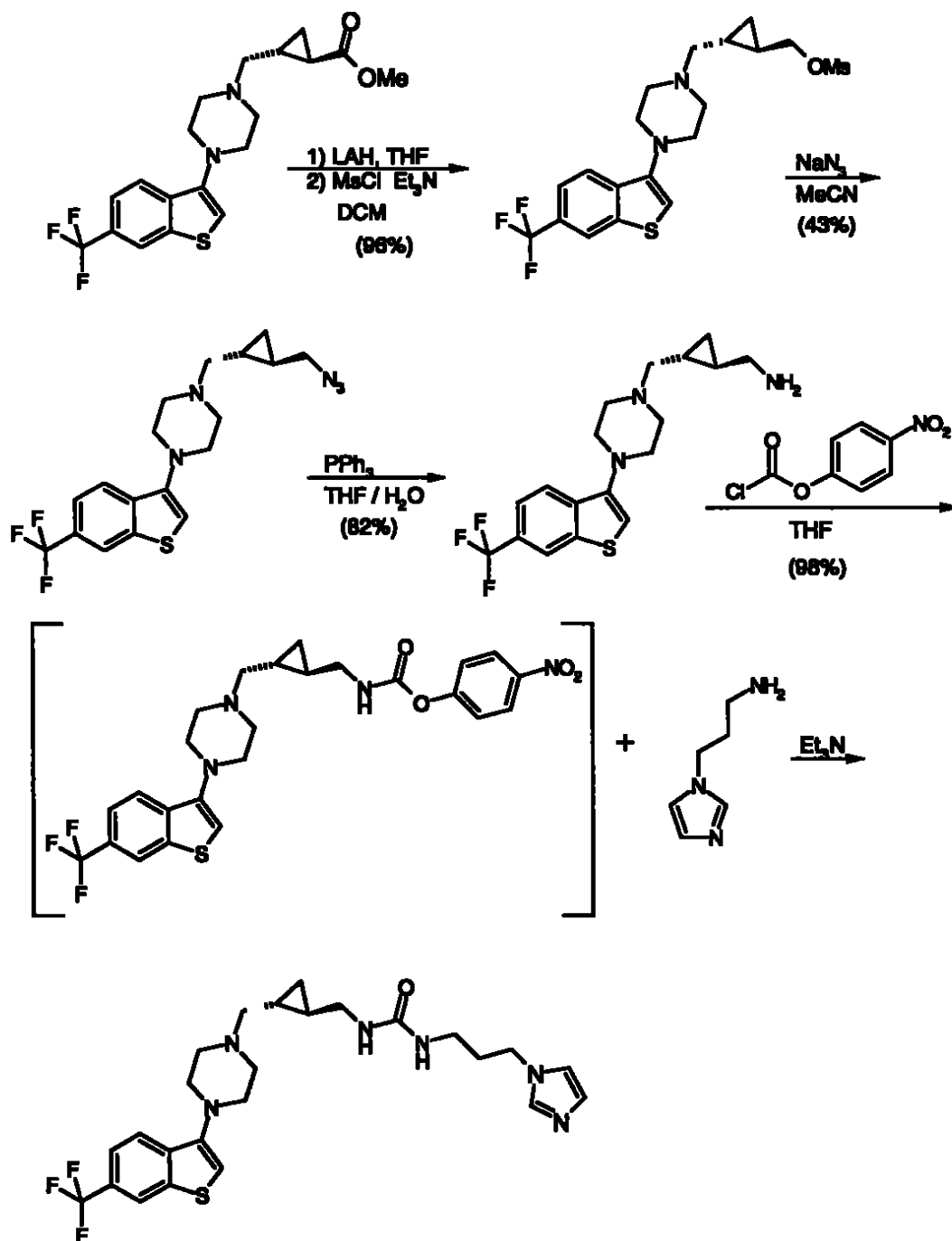
**3-Piperazin-1-yl-thieno[2,3-*d*]isoxazole. 4-Thieno[2,3-*d*]isoxazol-3-yl-piperazine-1-**

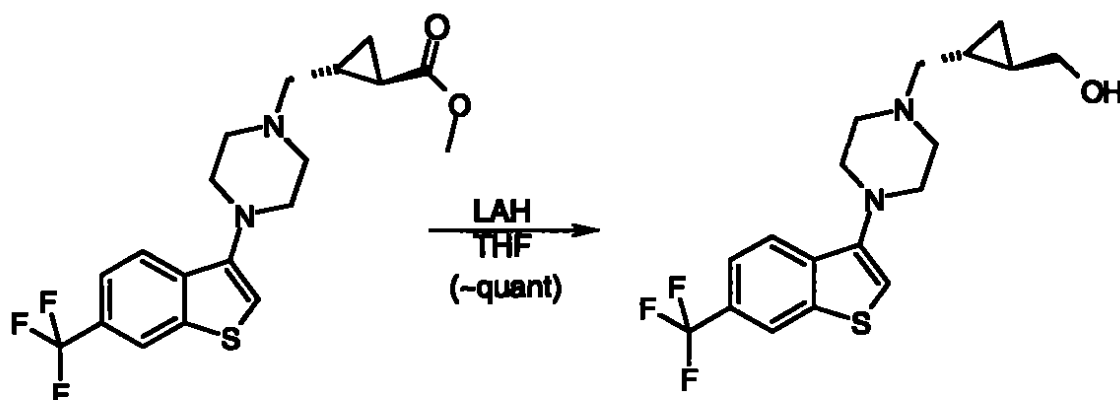
carboxylic acid *tert*-butyl ester (5.0 g, 16.2 mmol) was stirred at room temperature in

a solution of HCl (25 mL, 4N HCl in dioxane) for 4 hours. The volatiles were removed *in vacuo* and the residue was triturated with ether (twice) to yield off white solids 3.3 g (84%) as the desired hydrochloride salt.

Example 29

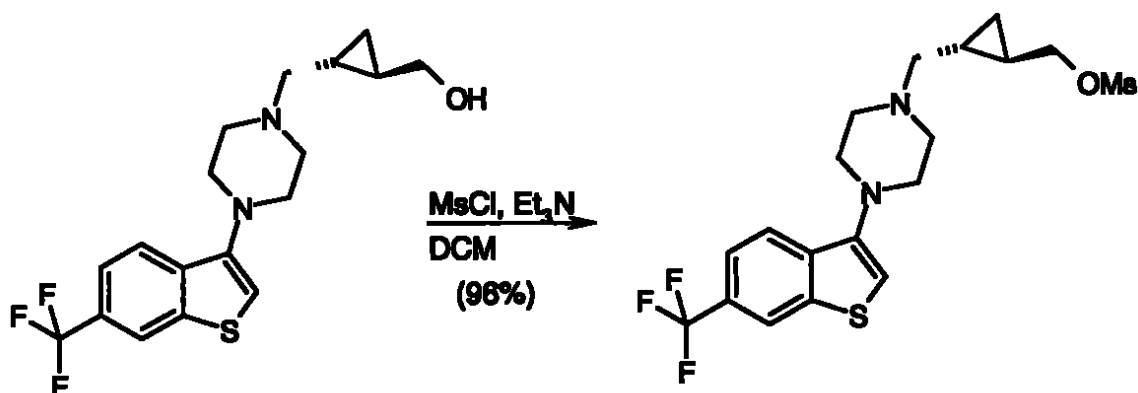
- 5 **Synthesis of 1-(3-imidazol-1-yl-propyl)-3-((1R, 2R)-2-[4-(6-trifluoromethyl-benzo[*b*]thiophen-3-yl)-piperazin-1-ylmethyl]-cyclopropylmethyl)-urea (MDL 833306)**





{{(1R, 2R)-2-[4-(6-Trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-ylmethyl]-cyclopropyl)-methanol

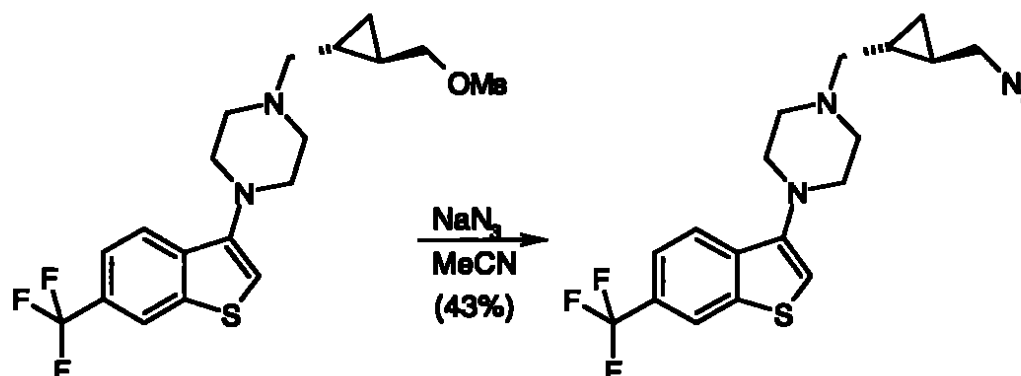
To a stirred solution of 2R-[4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-ylmethyl]-1R-cyclopropanecarboxylic acid methyl ester (5.0 g, 12.5 mmol) in THF (75 mL) cooled to 0 °C was added lithium aluminum hydride (18.75 mL, 18.75 mmol, 1.0 M in THF) drop-wise. The resulting mixture was stirred at 0 °C for 2 hours when water (1 mL), 2 N NaOH (1 mL) and water (3 mL) was added sequentially. The resulting mixture was diluted with DCM (90 mL) and filtered through a celite plug. The aluminum salts were thoroughly washed with DCM and the filtrate was dried (MgSO₄), filtered and evaporated yielding 4.6 g of the desired product.



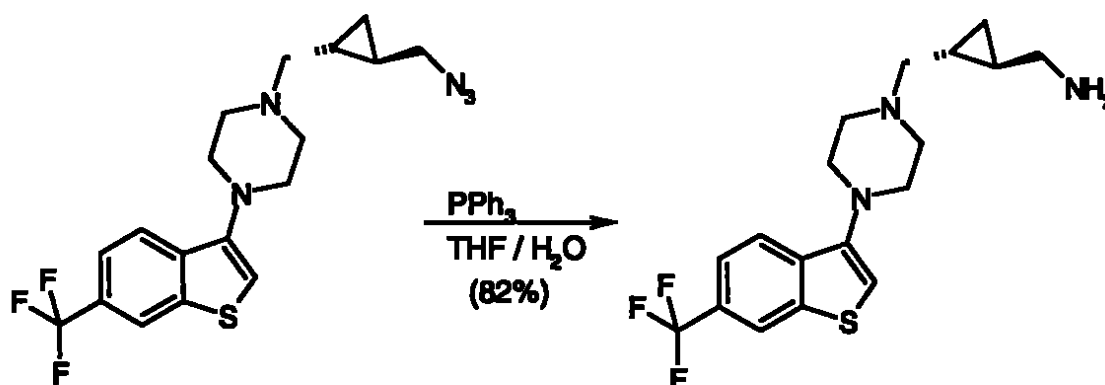
Methanesulfonic acid ((1R, 2R)-2-[4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-ylmethyl]-cyclopropyl)methyl ester

To a stirred solution of ((1R, 2R)-2-[4-(6-Trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-ylmethyl]-cyclopropyl)-methanol (3.712 g, 10.03 mmol) in Et₃N (8.5 mL, 61.1 mmol) and anhydrous CH₂Cl₂ (100 mL) at 0 °C under N₂ was added dropwise CH₃SO₂Cl (930 µL, 12.02 mmol). Stirring was continued at 0 °C for 2.5 h. The reaction was quenched with H₂O (20 mL). A solution of K₂CO₃ (4.28 g, 31.01 mmol) in H₂O (60 mL) was then added. The resulting mixture was stirred at rt for 15 min, then extracted with CH₂Cl₂,

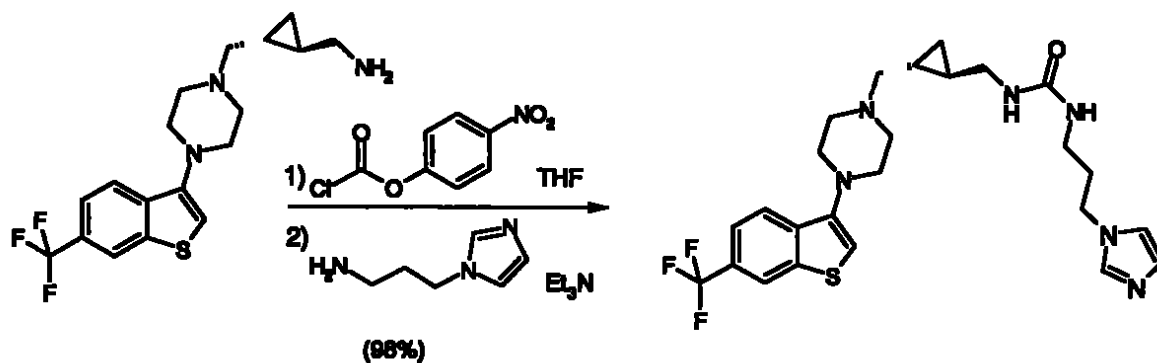
washed with brine, dried over Na_2SO_4 . Filtration, concentration and drying afforded the desired product (4.301 g, 96%)



- 5 **1-((1R,2R)-2-Azidomethyl-cyclopropylmethyl)-4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazine** A mixture of Methanesulfonic acid (1R, 2R)-2-[4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-ylmethyl]-cyclopropylmethyl ester (3.995 g, 8.92 mmol), NaN_3 (1.16 g, 17.85 mmol) and anhydrous CH_3CN (60 mL) was stirred at 47 °C under N_2 for 4 h, then an additional quantity of NaN_3 (580 mg, 8.92 mmol) was added. Stirring was continued at 47 °C for a further 4 h. After cooling to rt, the mixture was filtered through Celite 545, washed with CH_3CN . The combined filtrate and washings were concentrated and then separated by Prep LC (heptane/EtOAc—70/30, 60/40, 50/50, 40/60, 30/70, 20/80, 100% EtOAc) to give the desired product (1.5 g, 43%).



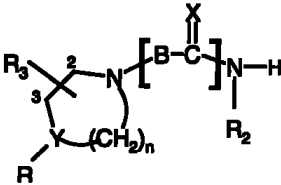
- 15 **C-((1R,2R)-2-[4-(6-Trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-ylmethyl]-cyclopropyl)methylamine** A solution of 1-((1R,2R)-2-Azidomethyl-cyclopropylmethyl)-4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazine (1.495 g, 3.78 mmol), PPh_3 (3.97 g, 15.15 mmol) and H_2O (273 μL , 15.17 mmol) in THF (30 mL) was stirred at 40 °C under N_2 for 18 h, then at 55 °C for 23 h. After cooling to rt, the mixture was concentrated, and then flash chromatographed (100% EtOAc, then MeOH / CH_2Cl_2 / Et_3N —60/40/10) to provide the desired product (1.14 g, 82%).



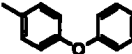
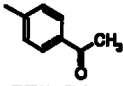
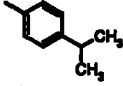
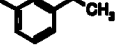
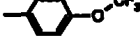
1-(3-imidazol-1-yl-propyl)-3-((1R, 2R)-2-[4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-ylmethyl]-cyclopropyl)methyl-urea (MDL 833306) A solution of C-

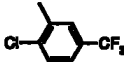
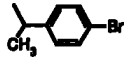
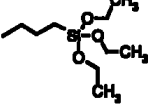
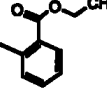
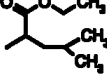
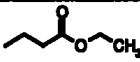
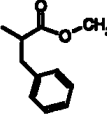
- 5 {(1R,2R)-2-[4-(6-Trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-ylmethyl]-cyclopropyl)methylamine (30 mg , 0.081 mmol) and 4-nitrophenyl chloroformate (18 mg , 0.089 mmol) in anhydrous THF (3 mL) was stirred at rt under N₂ for 1 h. Then Et₃N (113 μ L, 0.81 mmol) was added, followed by 3-imidazol-1-yl-propylamine (39 μ L , 0.33 mmol). Stirring was continued at 35 $^{\circ}$ C for 2 h. The reaction mixture was
- 10 filtered. The filtrate was concentrated, then separated by Prep LC (MeOH / CH₂Cl₂ - 5:95, 10:90, 15:85, 20:80, 25:75, 30:70) to give the target compound (41 mg , 98 %)

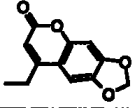
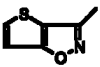
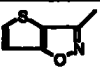
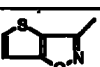
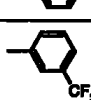
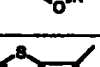
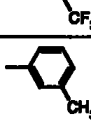
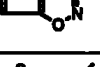
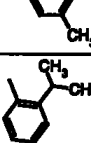
TABLE III

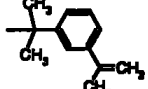
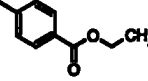
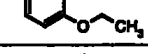
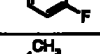
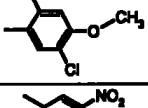
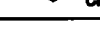


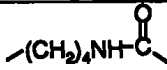
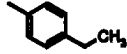
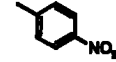
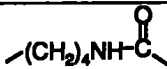
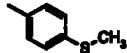
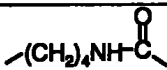
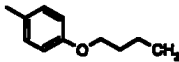
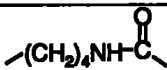
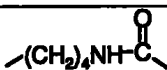
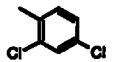
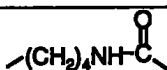
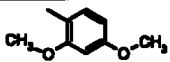
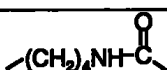
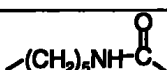
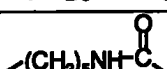
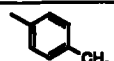
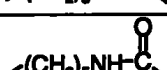
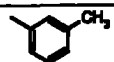
COMPOUND NUMBER	R	n		R ₂	R ₃	Y	d3K ₁
815383		2			H	CH	190
815384		2			H	CH	9
815385		2			H	CH	4
815386		2			H	CH	18
815387		2			H	CH	28
815388		2			H	CH	12

815389		2	$-(CH_2)_4NH-C(=O)-$		H	CH	30
815390		2	$-(CH_2)_4NH-C(=O)-$		H	CH	52
815391		2	$-(CH_2)_4NH-C(=O)-$		H	CH	16
815392		2	$-(CH_2)_4NH-C(=O)-$		H	CH	24
815393		2	$-(CH_2)_4NH-C(=O)-$		H	CH	37
815394		2	$-(CH_2)_4NH-C(=O)-$		H	CH	13
815395		2	$-(CH_2)_4NH-C(=O)-$		H	CH	110
815934		2	$-(CH_2)_4NH-C(=O)-$		H	CH	419
815935		2	$-(CH_2)_4NH-C(=O)-$		H	CH	538

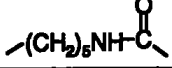
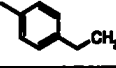
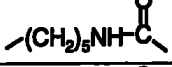
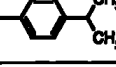
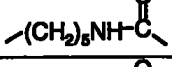
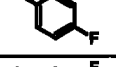
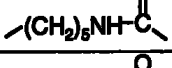
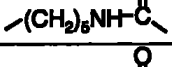
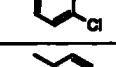
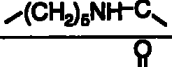
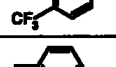
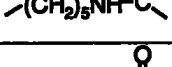
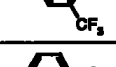
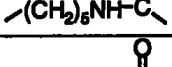
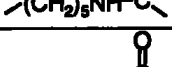
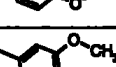
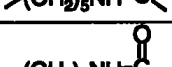
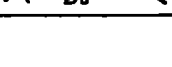
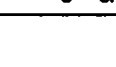
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815938		2	$-(CH_2)_4NH-C(=O)-$		H	CH	153
815939		2	$-(CH_2)_4NH-C(=O)-$		H	CH	19 1
815940		2	$-(CH_2)_4NH-C(=O)-$		H	CH	140
815941		2	$-(CH_2)_4NH-C(=O)-$		H	CH	128
815942		2	$-(CH_2)_4NH-C(=O)-$		H	CH	41 7
815943		2	$-(CH_2)_4NH-C(=O)-$		H	CH	198
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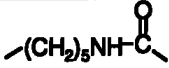
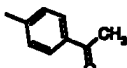
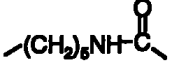
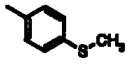
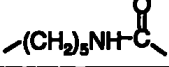
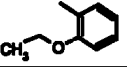
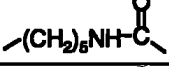
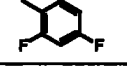
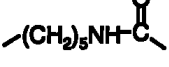
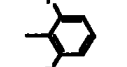
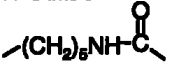
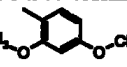
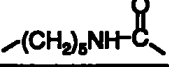
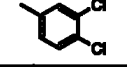
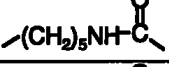
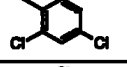
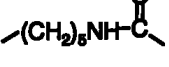
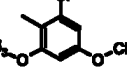
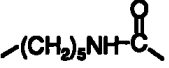
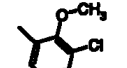
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815946		2	$-(CH_2)_4NH-C(=O)-$	$-(CH_2)_7CH_3$	H	CH	28 3
815947		2	$-(CH_2)_4NH-C(=O)-$		H	CH	26
815948		2	$-(CH_2)_4NH-C(=O)-$		H	CH	6 04
815949		2	$-(CH_2)_4NH-C(=O)-$		H	CH	13 6
815950		2	$-(CH_2)_4NH-C(=O)-$		H	CH	4 59
815951		2	$-(CH_2)_4NH-C(=O)-$		H	CH	2 23
815952		2	$-(CH_2)_4NH-C(=O)-$		H	CH	27

815953		2	$-(CH_2)_4NH-C(=O)-$		H	CH	67 9
815954		2	$-(CH_2)_4NH-C(=O)-$		H	CH	14 9
81595		2	$-(CH_2)_4NH-C(=O)-$		H	CH	23 5
81595		2	$-(CH_2)_4NH-C(=O)-$		H	CH	5 24
816210		2	$-(CH_2)_5NH-C(=O)-$		H	CH	187
816557		2	$-(CH_2)_4NH-C(=O)-$		H	CH	20 7
816558		2	$-(CH_2)_4NH-C(=O)-$		H	CH	33 3
816559		2	$-(CH_2)_4NH-C(=O)-$		H	CH	13 1
816560		2	$-(CH_2)_4NH-C(=O)-$		H	CH	10 9

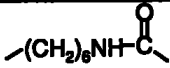
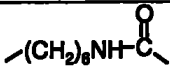
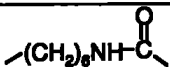
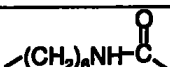
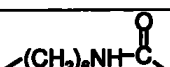
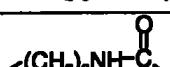
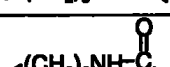
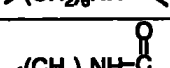
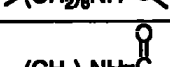
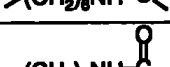
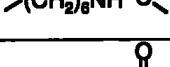
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816566		2			H	CH	95 1
816567		2			H	CH	73 8
816568		2			H	CH	11 2
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241

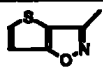
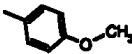
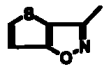
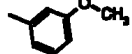
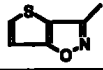
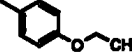
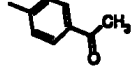
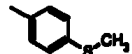
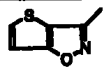
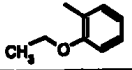
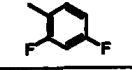
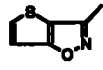
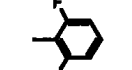
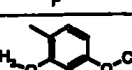
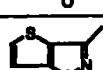
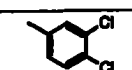
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816756		2			H	CH	96 1
816757		2			H	CH	92 6
816758		2			H	CH	116
816759		2			H	CH	107
816760		2			H	CH	121
816761		2			H	CH	82 9

816762		2			H	CH	57 1
816763		2			H	CH	78 9
816764		2			H	CH	85 4
816765		2			H	CH	104
816766		2			H	CH	42 9
816767		2			H	CH	60 1
816768		2			H	CH	23 4
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816770		2			H	CH	28 2
816771		2			H	CH	74 1

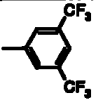
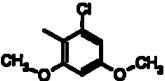
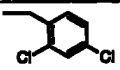
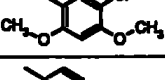
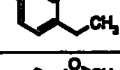
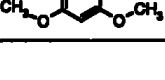
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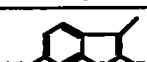
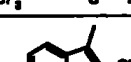
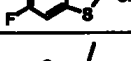
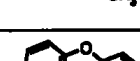
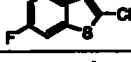
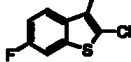
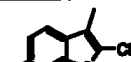
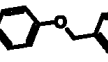
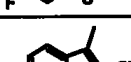
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816862		2			H	CH	19
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816864		2			H	CH	21 5
816865		2			H	CH	11 9
816866		2			H	CH	8 25
816867		2			H	CH	7 38
816868		2			H	CH	58 6
816869		2			H	CH	23 5
816870		2			H	CH	17 6

245

816871		2	$-(CH_2)_6NH-C(=O)-$		H	CH	17.3
816872		2	$-(CH_2)_6NH-C(=O)-$		H	CH	19
816873		2	$-(CH_2)_6NH-C(=O)-$		H	CH	22
816874		2	$-(CH_2)_6NH-C(=O)-$		H	CH	6.84
816875		2	$-(CH_2)_6NH-C(=O)-$		H	CH	35.4
816876		2	$-(CH_2)_6NH-C(=O)-$		H	CH	46.9
816877		2	$-(CH_2)_6NH-C(=O)-$		H	CH	19.1
816878		2	$-(CH_2)_6NH-C(=O)-$		H	CH	33.8
816879		2	$-(CH_2)_6NH-C(=O)-$		H	CH	47
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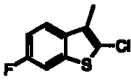
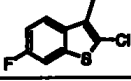
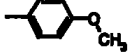
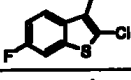
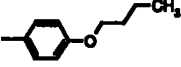
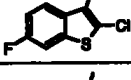
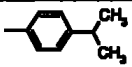
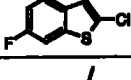
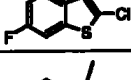
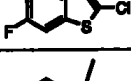
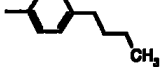
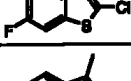
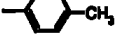
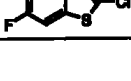
246

816881		2	$-(CH_2)_6NH-C(=O)-$		H	CH	23 8
816882		2	$-(CH_2)_6NH-C(=O)-$		H	CH	58 6
816883		2	$-(CH_2)_6NH-C(=O)-$		H	CH	35 5
816968		2	$-(CH_2)_6NH-C(=O)-$		H	CH	24 2
817075		2	$-(CH_2)_4NH-C(=O)-$		H	CH	12 7
818280		2	$-(CH_2)_3NH-C(=O)-$		H	CH	217
818283		2	$-(CH_2)_3NH-C(=O)-$		H	CH	131
818308		2	$-(CH_2)_3NH-C(=O)-$		H	CH	351
818309		2	$-(CH_2)_3NH-C(=O)-$		H	CH	390
818310		2	$-(CH_2)_3NH-C(=O)-$		H	CH	133

818316		2	$-(CH_2)_3NH-C(=O)-$		H	CH	426
818318		2	$-(CH_2)_3NH-C(=O)-$		H	CH	384
818426		2	$-(CH_2)_3NH-C(=O)-$		H	CH	158
817510		2	$-(CH_2)_4NH-C(=O)-$		H	N	0 665
815158		2	$-(CH_2)_4NH-C(=O)-$		H	N	7 4
815159		2	$-(CH_2)_4NH-C(=O)-$		H	N	85 4
815160		2	$-(CH_2)_4NH-C(=O)-$		H	N	192
815161		2	$-(CH_2)_4NH-C(=O)-$		H	N	75 9
815162		2	$-(CH_2)_4NH-C(=O)-$		H	N	38
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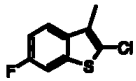
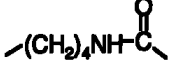
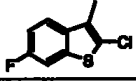
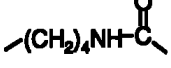
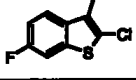
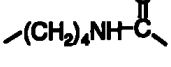
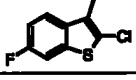
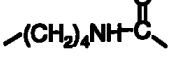
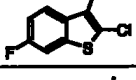
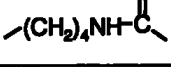
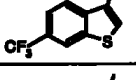
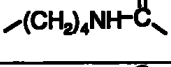
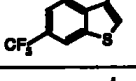
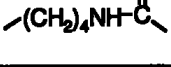
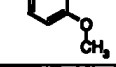
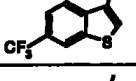
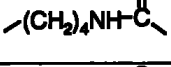
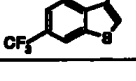
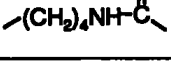
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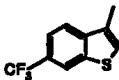
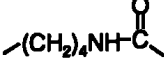
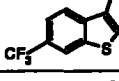
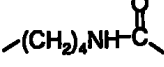
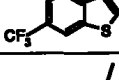
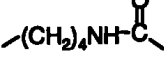
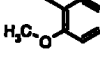
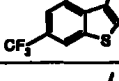
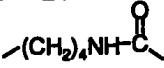
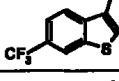
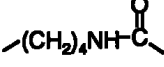
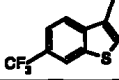
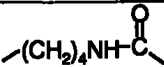
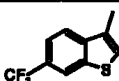
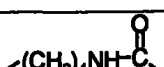
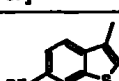
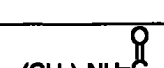
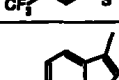
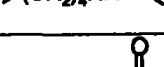
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815166		2	$(CH_2)_4NH-C(=O)-$		H	N	37
815167		2	$(CH_2)_4NH-C(=O)-$		H	N	48
815168		2	$(CH_2)_4NH-C(=O)-$		H	N	33 4
815169		2	$(CH_2)_4NH-C(=O)-$		H	N	11 2
815170		2	$(CH_2)_4NH-C(=O)-$		H	N	17 7
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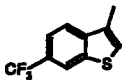
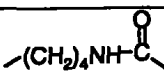
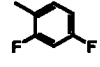
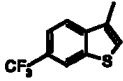
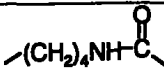
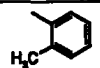
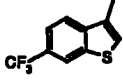
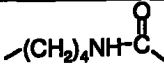
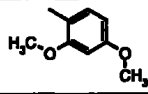
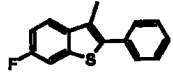
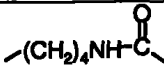
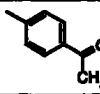
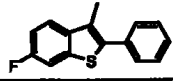
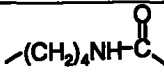
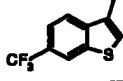
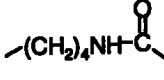
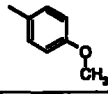
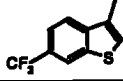
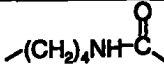
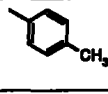
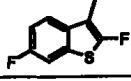
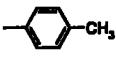
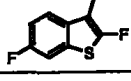
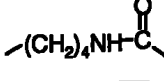
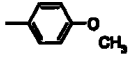
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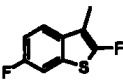
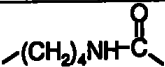
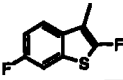
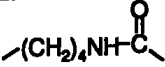
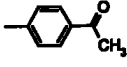
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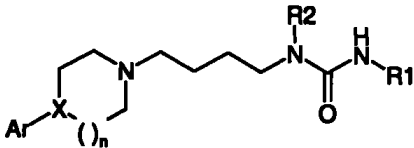
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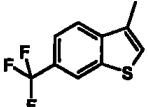
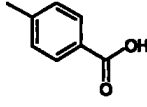
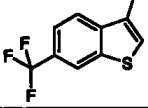
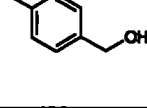
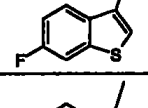
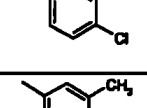
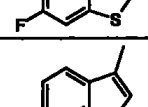
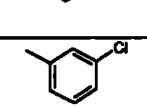
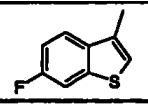
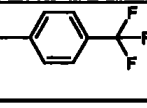
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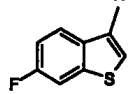
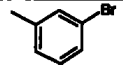
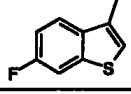
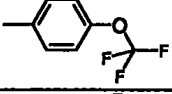
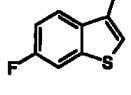
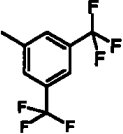
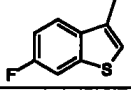
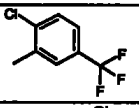
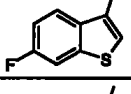
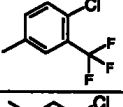
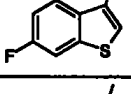
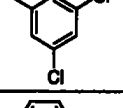
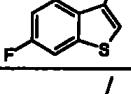
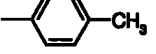
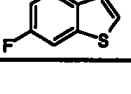
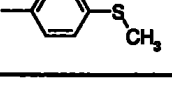
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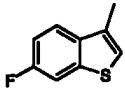
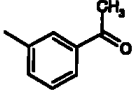
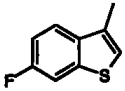
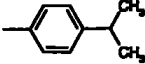
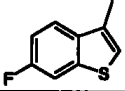
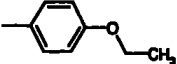
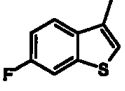
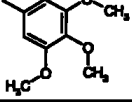
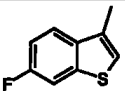
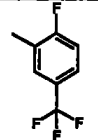
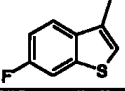
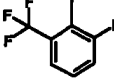
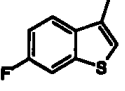
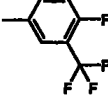


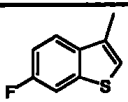
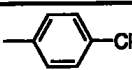
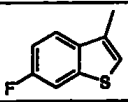
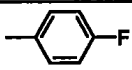
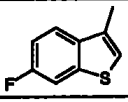
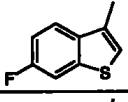
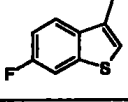
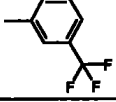
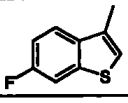
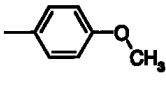
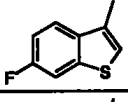
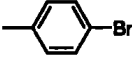
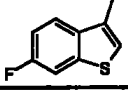
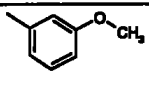
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829192	4 03	2	N			H
829193	7 86	2	N			H
829195	21 1	2	N			H

829196	10 9	2	N			H
829197	34 8	2	N			H
829198	65 6	2	N			H
829199	6 51	2	N			H
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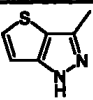
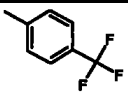
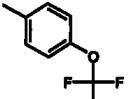
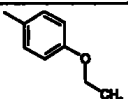
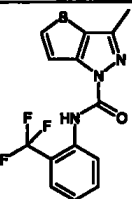
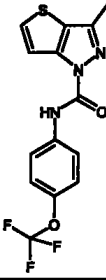
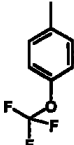
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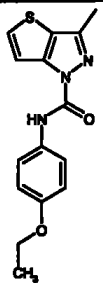
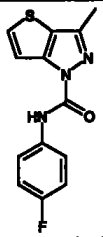
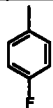
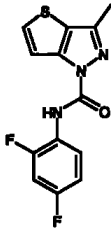
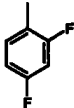
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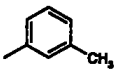
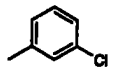
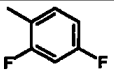
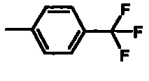
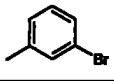
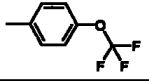
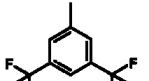
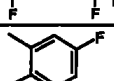
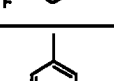
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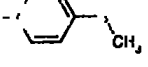
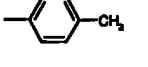
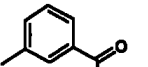
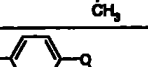
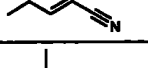
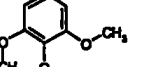
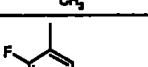
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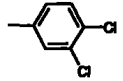
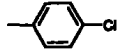
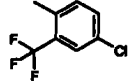
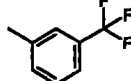
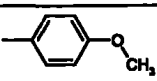
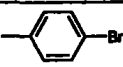
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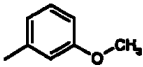
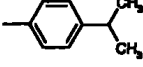
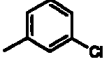
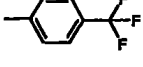
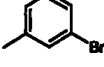
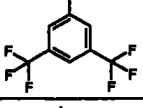
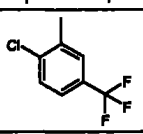
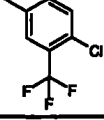
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825171	496	1	N			H

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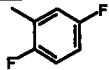
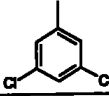
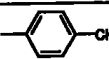
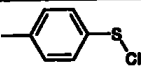
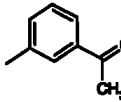
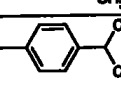
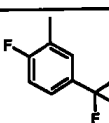
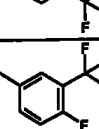
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829370	4 91	1	N			H
829371	55 2	1	N			H
829372	31 9	1	N			H
829373	14 9	1	N			H
829374	103	1	N			H
829375	176	1	N			H
829377	87 9	1	N			H
829378	184	1	N			H

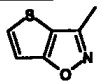
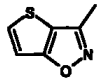
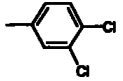
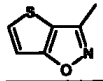
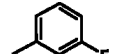
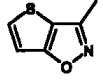
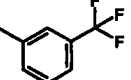
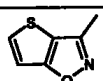
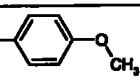
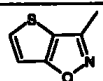
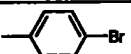
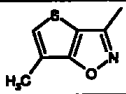
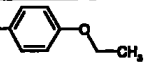
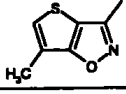
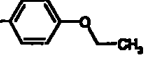
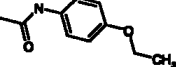
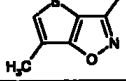
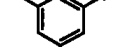
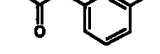
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829380	26 9	1	N			H
829381	46 5	1	N			H
829382	42 7	1	N			H
829383	102	1	N			H
829384	190	1	N			H
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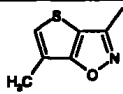
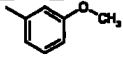
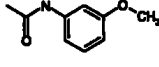
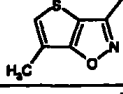
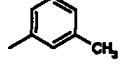
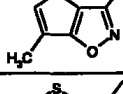
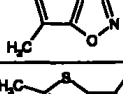
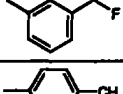
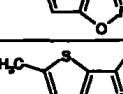
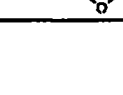
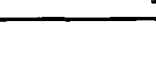
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829389	50 5	1	N			H
829390	37 5	1	N			H
829392	487	1	N			H
829393	190	1	N			H
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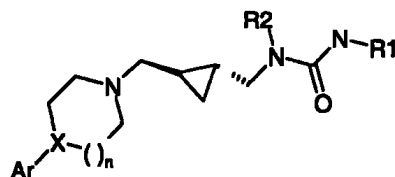
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829409	379	2	N			H
829411	670	2	N			H
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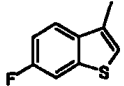
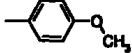
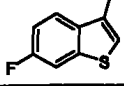
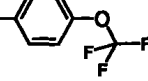
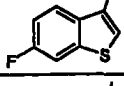
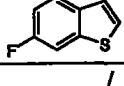
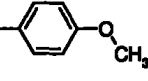
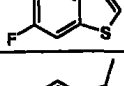
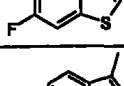
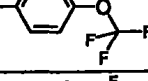
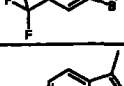
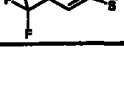
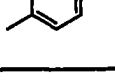
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829417	338	2	N			H
829418	529	2	N			H
829419	138	2	N			H
829420	233	2	N			H
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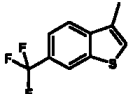
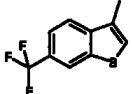
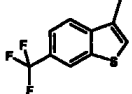
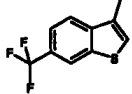
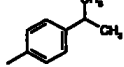
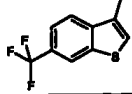
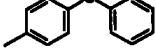
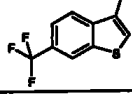
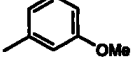
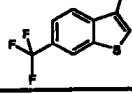
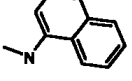
829428	747	2	N			H
829429	111	2	N			H
829431	153	2	N			H
829432	226	2	N			H
829433	216	2	N			H
829434	197	2	N			H
829873	27 7	1	CH			H
829874	360	1	CH			
829875	94 7	1	CH			

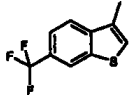
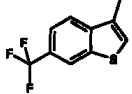
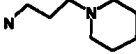
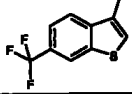
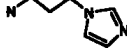
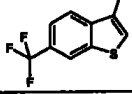
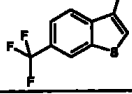
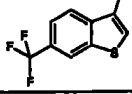
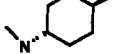
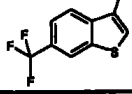
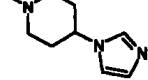
829876	77 1	1	CH			
829878	8 78	1	CH			H
829879	354	1	CH			H
829882	143	1	CH			H
830517	9 04	1	CH			H
830518	9 16	1	CH			H

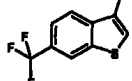
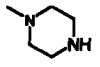
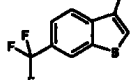
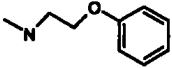
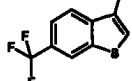
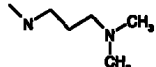
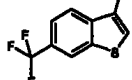
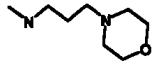
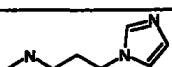
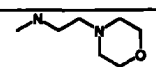
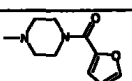


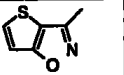
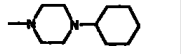
MDL#	D3 KI (nM)	Chirality	n	X	Ar	R1	R2
830389	2 97	Racemic	2	N			CH ₂ CH=CH ₂
830400	41 3	Racemic	2	N			Bn
830401	29 7	Racemic	2	N			Bn
830408	3 43	Racemic	2	N			H
830409	47 4	Racemic	2	N			H
830410	137	Racemic	2	N			H
832786	61 56	R,R	1	N			H
832810	33 5	R,R	1	N			H

2/6

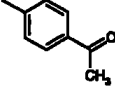
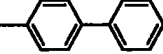
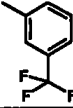
832811	18 2	R,R	1	N			H
832812	39 5	R,R	1	N			H
832813	50 84	R,R	1	N			H
832819	45 24	R,R	1	N			H
832820	3 9	R,R	1	N			H
833203	18 19	R,R	1	N			H
833209	21 95	R,R	1	N			H

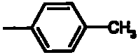
833221	25.2	R,R	1	N			H
833258	0 39	R,R	1	N			H
833306	0 238	R,R	1	N			H
833331	18 1	R,R	1	N			H
833332	17 5	R,R	1	N			H
833356	29 1	R,R	1	N			H
833357	2 95	R,R	1	N			H

833498	3 32	R,R	1	N			H
833499	34 43	R,R	1	N			H
833618	0 88	R,R	1	N			H
833713	8 12	R,R	1	N			H
831498	43 6	Racemic	1	CH		t-Bu	H
831499	66 5	Racemic	1	CH			H
831669	172	Racemic	1	CH			H
831670	159	Racemic	1	CH		NH-(CH ₂) ₂ Ph	H
831672	153	Racemic	1	CH			H

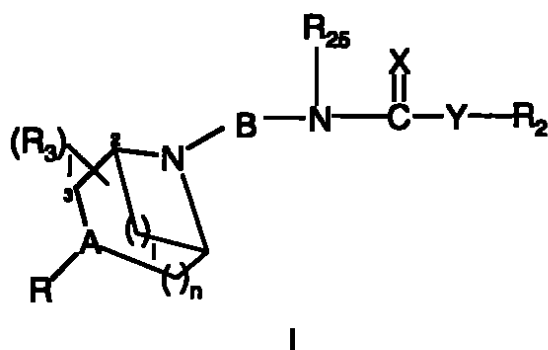
831700	41 5	Racemic	1	CH			H
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MDL #	D3 KI (nM)	R
817228	179	
817229	365	
817230	182	
817231	433	
817233	146	

817234	256	
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1 A compound of the formula (I)



wherein

A is CH or N,

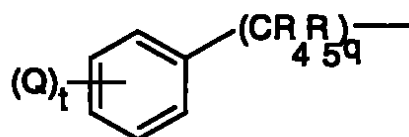
Y is O or NR₁

wherein R₁ is

a) hydrogen,

b) C₁-C₈alkyl, or

c)



wherein

each Q is independently hydrogen, C₁-C₈alkyl, halogen or trifluoromethyl,

each R₄ and R₅ is independently hydrogen or C₁-C₈alkyl,

t is 0 or 1, and

q is 0 or 1,

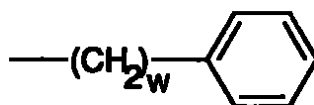
j is 0, 1 or 2,

n is 1 or 2,

when n is 1, i is 0 or 2,

when n is 2, i is 0,

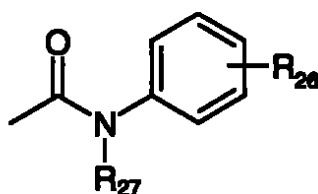
R₃ is hydrogen, C₁-C₈alkyl or



wherein w is 1, 2 or 3,

X is O or S,

R₂₅ is hydrogen or



5

wherein R₂₇ is hydrogen or C₁-C₆alkyl and R₂₆ is hydrogen,

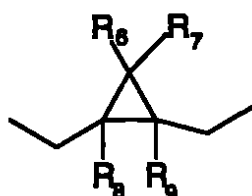
C₁-C₆alkoxy, halogen or C₁-C₆alkyl that is unsaturated or saturated,

-B- is a group selected from (a) - (e)

(a) $-(CR_{21}R_{22})_m-$

10

(b)

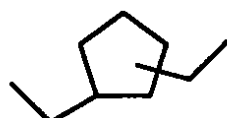


(c)

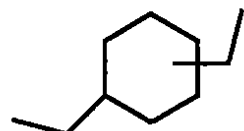


15

(d)



(e)



20

wherein

m is 3, 4, 5, or 6,

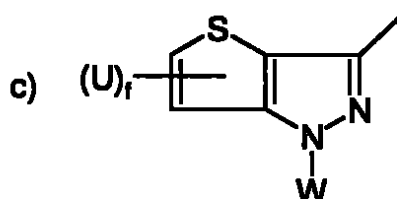
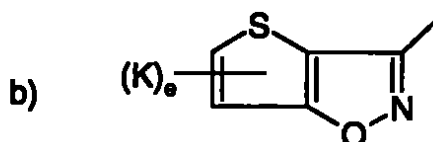
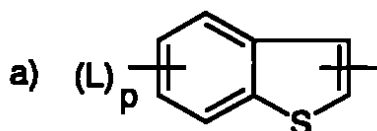
each R₂₁ and R₂₂ is independently hydrogen, C₁-C₆alkyl or

$-(CH_2)_aOH$ wherein a is 0, 1 or 2,

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R_6 , R_7 , R_8 and R_9 are each independently hydrogen, halogen or C_1 - C_3 alkyl with the proviso that when R_8 is C_1 - C_3 alkyl, R_9 is not C_1 - C_3 alkyl,

5 R is a group selected from (a) - (c)

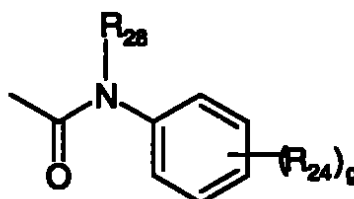


wherein

each L is independently hydrogen, C_1 - C_8 alkyl, halogen or trifluoromethyl,

10 each U is independently hydrogen, C_1 - C_8 alkyl, CHO, halogen, $-(CH_2)_bOH$, or $-(CH_2)_bOC_1$ - C_8 alkyl wherein b is 1 or 2,

each K is independently hydrogen, C_1 - C_8 alkyl, CHO, halogen, $-(CH_2)_bOH$, or $-(CH_2)_bOC_1$ - C_8 alkyl wherein b is as hereinbefore defined, W is hydrogen or



15

wherein R_{28} is hydrogen or C_1 - C_8 alkyl,

each R_{24} is independently trifluoromethyl, trifluoromethoxy, C_1 - C_8 alkoxy or halogen, and g is 0, 1 or 2,

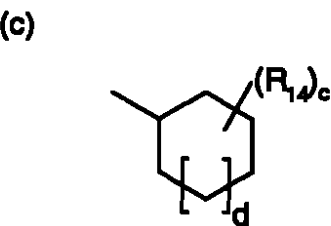
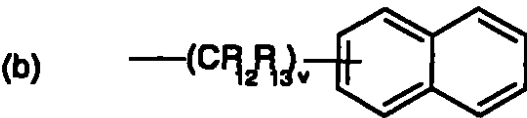
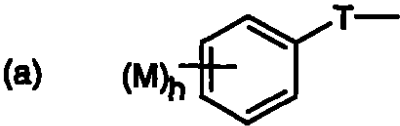
20

p is 0, 1 or 2,

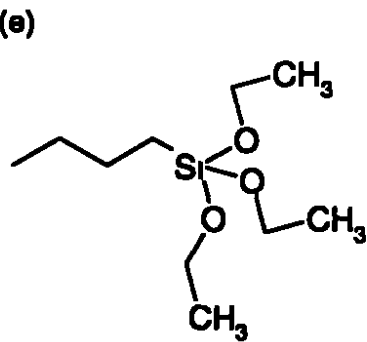
e is 0, 1 or 2,

f is 0, 1, or 2,

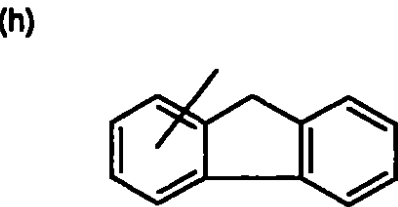
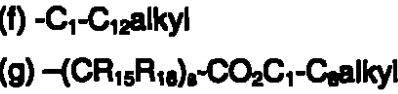
R_2 is a group selected from (a) - (t)



5



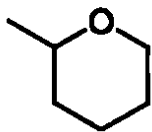
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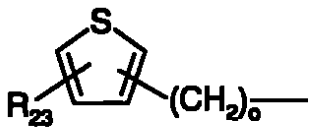
15



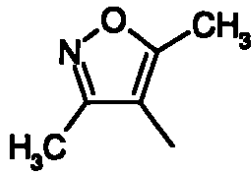
-152-



(j)



(k)

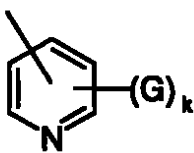


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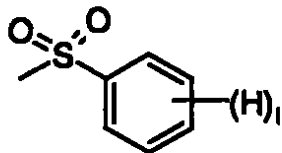
- (l) $-\text{CH}_2\text{CH}_2\text{SO}_2\text{CH}_3$
- (m) $-(\text{CR}_{17}\text{R}_{18})_r\text{OC}_1\text{-C}_2\text{alkyl}$
- (n) $-\text{CH}_2\text{CH}_2\text{OPh}$
- (o) $-(\text{CR}_{19}\text{R}_{20})_q\text{CZ}_3$
- (p) $-\text{CO}_2\text{Ph}$
- (q) $-\text{COCV}_3$

10

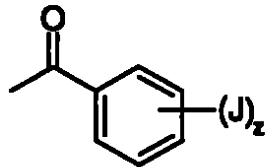
(r)



(s)



(t)



wherein

15

- T- is selected from (i) or (ii)
- (i) $-(\text{CR}_{10}\text{R}_{11})_u-$
wherein
u is 0, 1 or 2, and

-153-

each R_{10} and R_{11} is independently hydrogen or C_1 - C_8 alkyl,

(ii)



5 each M, G, H and J is independently selected from

- (1) hydrogen,
- (2) halogen,
- (3) C_1 - C_8 alkyl,
- (4) C_1 - C_8 alkoxy,
- 10 (5) phenoxy,
- (6) phenyl,
- (7) trifluoromethyl,
- (8) trifluoromethoxy,
- (9) $-CO_2-R_{19}$ wherein R_{19} is hydrogen or
- 15 C_1 - C_8 alkyl,
- (10) $-COC_1$ - C_8 alkyl,
- (11) hydroxy,
- (12) $-(CH_2)-OR_{20}$ wherein R_{20} is hydrogen or
- C_1 - C_8 alkyl,
- 20 (13) $-C(CH_2)CH_3$,
- (14) $-NO_2$,
- (15) $-SCH_3$,
- (16) benzyloxy, and
- (17) $-CN$,

25

each R_{12} and R_{13} is independently hydrogen or C_1 - C_8 alkyl,
v is 0 or 1,

R_{14} is hydrogen or C_1 - C_4 alkyl,

c is 0, 1 or 2,

30

d is 0 or 1,

each R_{15} and R_{16} is independently hydrogen or C_1 - C_8 alkyl,

s is 0, 1, 2, 3, 4 or 5,

o is 1 or 2,

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each R_{17} and R_{18} is independently hydrogen, C_1 - C_8 alkyl or CO_2C_1 - C_2 alkyl,

r is 2 or 3,

each R_{19} and R_{20} is independently hydrogen or

C_1 - C_2 alkyl,

R_{23} is hydrogen or C_1 - C_4 alkyl,

Z is chlorine or fluorine,

q is 0 or 1,

V is chlorine or fluorine, and

h , k , l , and z are 0, 1, 2, or 3

2 A compound according to claim 1 wherein X is O, Y is NH and $-B-$ is group (a)

3 A compound according to claim 2 wherein R_2 is group (a)

4 A compound according to claim 3 wherein m is 4, M is hydrogen, C_1 - C_8 alkyl, C_1 - C_8 alkoxy, or phenyl and T is (i) wherein u is 0 or 1

5 A compound according to claim 2 wherein R_2 is group (c)

6 A compound according to claim 5 wherein wherein m is 4, d is 1, and R_{14} is C_1 - C_8 alkyl

7 A compound according to claim 1 wherein X is O, Y is NH and $-B-$ is group (b)

8 A compound according to claim 6 wherein R_2 is group (a)

9 A compound according to claim 7 wherein R_6 , R_7 , R_8 or R_9 are hydrogen, M is hydrogen, C_1 - C_8 alkyl, C_1 - C_8 alkoxy or phenyl, and T is 0 or 1

10 A compound according to claim 6 wherein R_2 is group (c)

11 A compound according to claim 9 wherein R_6 , R_7 , R_8 or R_9 are hydrogen, d is 1, and R_{14} is C_1 - C_8 alkyl

12 The compound of claim 1 which is 1-(4-methoxy-phenyl)-3-{4-[4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl}-urea

13 The compound of claim 1 which is 1-p-tolyl-3-{4-[4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl}-urea

14 The compound of claim 1 which is 1-{4-[4-(2,6-difluoro-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl}-3-p-tolyl-urea

10 15 The compound of claim 1 which is 1-{4-[4-(2-chloro-6-fluoro-benzo[b]thiophen-3-yl)-piperazin-1-yl]-butyl}-3-(4-chloro-phenyl)-urea

16 The compound of claim 1 which is 1-(4-acetyl-phenyl)-3-[4-(4-thieno[2,3-d]isoxazol-3-yl-piperidin-1-yl)-butyl]-urea

15

17 The compound of claim 1 which is 1-(4-ethoxy-phenyl)-3-[4-(4-thieno[2,3-d]isoxazol-3-yl-piperidin-1-yl)-butyl]-urea

18 The compound of claim 1 which is 1-(4-ethoxy-phenyl)-3-{4-[4-(6-fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl}-urea

20

19 The compound of claim 1 which is 1-{4-[4-(6-fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl}-3-(2-fluoro-phenyl)-urea

25 20 The compound of claim 1 which is 1-{4-[4-(6-fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl}-3-(3-trifluoromethyl-phenyl)-urea

21 The compound of claim 1 which is 1-{4-[4-(6-fluoro-benzo[b]thiophen-3-yl)-[1,4]diazepan-1-yl]-butyl}-3-(4-methoxy-phenyl)-urea

30

22 The compound of claim 1 which is 1-(3-imidazol-1-yl-propyl)-3-((1R, 2R)-2-[4-(6-trifluoromethyl-benzo[b]thiophen-3-yl)-piperazin-1-ylmethyl]-cyclopropylmethyl)-urea

23 A method of modulating the activity of dopamine D₃ receptors, said method comprising contacting cell-associated dopamine D₃ receptors with a concentration of a compound of claim 1

5 24 A method of treating conditions or disorders of the central nervous system comprising administering to a patient in need thereof a therapeutically effective amount of a compound of claim 1

10 25 The method of claim 23, wherein the central nervous system disorder is selected from Psychotic Disorders, Substance Dependence, Substance Abuse, Dyskinetic Disorders, Dementia, Anxiety Disorders, Sleep Disorders, Circadian Rhythm Disorders, Mood Disorders and Nausea

15 26 The method of claim 24 wherein the Psychotic Disorder is Schizophrenia

27 The method of claim 24 wherein the compound of claim 1 is administered in conjunction with one or more dopamine D₁, D₂, D₄, D₅, or 5HT receptor antagonists

20 28 A pharmaceutical composition comprising an effective amount of a compound of claim 1 with a pharmaceutically-acceptable carrier or diluent

25 29 A pharmaceutical composition comprising an effective amount of a compound of claim 1 with a pharmaceutically-acceptable carrier or diluent in conjunction with one or more dopamine D₁, D₂, D₄, D₅ or 5HT receptor antagonists

30 30 A depot pharmaceutical composition, which comprises a pharmaceutically acceptable carrier and a therapeutically effective amount of the compound of claim 1, wherein the compound contains an acylated hydroxy group, or an acylated amino group

31 The depot pharmaceutical composition of claim 29, wherein the hydroxy group is acylated, or the amino group is acylated with (C₄-C₁₈)alkanoyl group or a (C₄-C₁₈)alkoxycarbonyl group

35 32 The composition of claim 29 which contains a pharmaceutically acceptable oil

33 The composition of claim 31 wherein the oil is selected from the group consisting of coconut oil, peanut oil, sesame oil, cotton seed oil, corn oil, soybean oil, olive oil, and synthetic esters of fatty acids and polyfunctional alcohols

5 34 A method for providing a long acting antipsychotic effect, which comprises injecting into a mammal an amount of the composition of claim 29 sufficient to produce a long acting antipsychotic effect

10 35 A method for providing a long acting antipsychotic effect, which comprises injecting into a mammal an amount of the composition of claim 30 sufficient to produce a long acting antipsychotic effect

15 36 A method for providing a long acting antipsychotic effect, which comprises injecting into a mammal an amount of the composition of claim 31 sufficient to produce a long acting antipsychotic effect

37 A compound of claim 1 wherein one or more of the atoms contained therein is a radionuclide

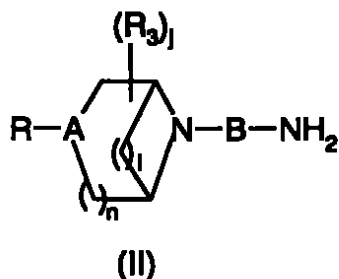
20 38 A compound of claim 1 wherein R is group (a) with a radiolabeled ^{14}C in the 3-position of the benzo[b]thiophene ring system, L is trifluoromethyl, p is 1, R_3 is hydrogen, n is 1, r is 0, and A is N

25 39 A diagnostic method for monitoring neuronal functions in a mammal comprising introducing into a mammal a radiolabeled compound according to claim 36

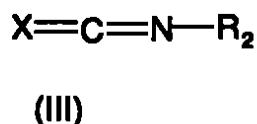
40 The method of claim 38 wherein said diagnostic method is performed using single photon emission computed tomography (SPECT) or positron emission tomography (PET)

30 41 A process for preparing a compound of claim 1 wherein Y is N which comprises reacting a compound of formula (II)

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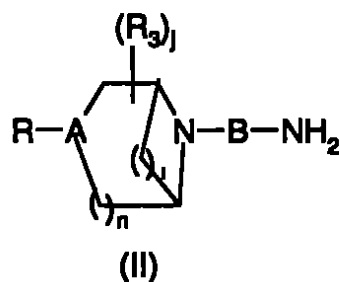


wherein R, R₃, j, n, i, B and A are as defined in formula I of claim 1
with a compound of formula (III)

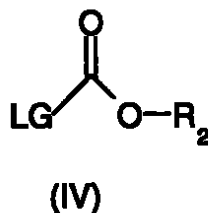


5 wherein X and R₂ are as defined in formula I of claim 1

42 A process for preparing a compound of formula I wherein Y is O which comprises reacting a compound of formula (II)



10 wherein R, R₃, j, n, i, B and A are as defined in formula I of claim 1
with a compound of formula IV



wherein "LG" is a suitable leaving group selected from bromine, chlorine or iodine and R₂ is
as defined in formula I of claim 1

15

43 A process for preparing compounds of formula I which comprises reacting a
compound of formula (V)

REQUISITOS NECESARIOS PARA PRESENTACIÓN Y AGILIZACIÓN DEL TRAMITE

USUARIO RADICADOR

PATENTE DE INVENCION

MODELO DE UTILIDAD

- | | | |
|---|-----|-----|
| • Indicación de que se solicita la concesión de una patente | [] | [] |
| • Datos de identificación del solicitante o de la persona que presenta la solicitud | [] | [] |
| • Descripción de la invención | [] | [] |
| • Dibujos de ser estos pertinentes | [] | [] |
| • Comprobante de Pago de las tasas establecidas | [] | [] |

COMPLETA []

INCOMPLETA

[] []

DISEÑO INDUSTRIAL

- | | | |
|---|-----|-----|
| • Indicación de que se solicita el registro de Diseño Industrial | [] | [] |
| • Datos de identificación del solicitante o de la persona que presenta la solicitud | [] | [] |
| • Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño | [] | [] |
| • Comprobante de pago de las tasas establecidas | [] | [] |

COMPLETA []

INCOMPLETA

[] []

ESQUEMA DE TRAZADO

- | | | |
|---|-----|-----|
| • Indicación de que se solicita el registro de un Esquema de trazado | [] | [] |
| • Datos de identificación del solicitante o de la persona que presenta la solicitud | [] | [] |
| • Representación gráfica del esquema de trazado | [] | [] |
| • Comprobante de pago de las tasas establecidas | [] | [] |

COMPLETA

INCOMPLETA

[] []

Firma Responsable

Radicación 82013030 00000000 Folios 20
Fecha (MM/DD) 2002-02-15 16:25:49
Trámite 002 PATENTES D 1 REGISTRO/ 411 PRESENTA
Dependencia 2020 DIVISION DE MARCAS Y DISEÑOS

Controlado por el
Departamento de
Industria y Comercio

2020
Bogotá, D C

Doctor
GUSTAVO HOLLMANN RESTREPO

Oficio No 09640

REFERENCIA	Radicación	02-13030
	Trámite	002
	Evento	001
	Actuación	430
	Folios	001

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica que

- Debe allegar copia del documento en que consta la cesión del derecho a la patente otorgado por los inventores al solicitante o a su causante (art 26-k, Dec 486/2000)
- Debe indicarse el lugar de constitución de la sociedad solicitante (Art. 27-c, D 486/00)
- Debe allegar el poder otorgado al abogado que aparece en la solicitud como apoderado, con el lleno de los requisitos exigidos en el Código de Procedimiento Civil. Cuando se requiera debe allegarse la sustitución del poder, otorgada por quien aparece como apoderado principal. Adicionalmente, deberá presentar el documento que acredita la existencia y representación legal de la sociedad solicitante.
- Debe aportar la traducción de la solicitud presentada como solicitud colombiana.

En cumplimiento del artículo 39 de la Decisión 486, en concordancia con el artículo 85 de la misma decisión, se le requiere para que dentro del término de dos (2) meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el artículo 8 de la Resolución 210 del 15 de enero del 2001.

ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de fecha

21 MAR. 2002

Se recuerda al interesado que debe presentar dentro de los dieciséis meses siguientes contados a partir de la fecha de presentación de la solicitud cuya prioridad se invoca en los casos en que haya lugar la traducción simple del capítulo reivindicatorio. El incumplimiento de este plazo acarreará la pérdida de la prioridad invocada.

202027

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