leukemias and lymphomas), tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, osteoporosis, loosening of artificial joint implants, atherosclerosis (including atherosclerotic plaque rupture), aortic aneurysm (including abdominal aortic aneurysm and brain aortic aneurysm), congestive heart failure, myocardial infarction, stroke, cerebral ischemia, head trauma, spinal cord injury, neuro-degenerative disorders (acute and chronic), autoimmune disorders, Huntington's disease, Parkinson's disease, migraine, depression, peripheral neuropathy, pain, cerebral amyloid angiopathy, nootropic or cognition enhancement, amyotrophic lateral sclerosis, multiple sclerosis, ocular angiogenesis, corneal injury, macular degeneration, abnormal wound healing, burns, diabetes, tumor invasion, tumor growth, tumor metastasis, corneal scarring, scleritis, AIDS, sepsis, septic shock and other diseases characterized by metalloproteinase activity and other diseases characterized by mammalian reprolysin activity in a mammal, including a human, comprising an amount of a compound of formula I or a pharmaceutically acceptable salt thereof effective in such treatments and a pharmaceutically acceptable carrier.

The present invention also relates to a pharmaceutical composition for the inhibition of (a) matrix metalloproteinases or other metalloproteinases involved in matrix degradation, or (b) a mammalian reprolysin (such as aggrecanase or ADAM's TS-1, 10, 12, 15 and 17, most preferably ADAM-17) in a mammal, including a human, comprising an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof.

The present invention also relates to a method for treating a condition selected from the group consisting of arthritis (including osteoarthritis and rheumatoid arthritis), inflammatory bowel disease, Crohn's disease, emphysema, chronic obstructive pulmonary disease, Alzheimer's disease, organ transplant toxicity, cachexia, allergic reactions, allergic contact hypersensitivity, cancer, tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, osteoporosis, loosening of artificial joint implants, atherosclerosis (including atherosclerotic plaque rupture), aortic aneurysm (including abdominal aortic aneurysm and brain aortic aneurysm), congestive heart failure, myocardial infarction, stroke, cerebral ischemia, head trauma, spinal cord injury, neuro-degenerative disorders (acute and chronic), autoimmune disorders, Huntington's disease, Parkinson's disease, migraine, depression, peripheral neuropathy, pain, cerebral amyloid angiopathy, nootropic or cognition enhancement, amyotrophic lateral sclerosis, multiple sclerosis, ocular angiogenesis, corneal injury, macular degeneration, abnormal wound healing, burns, diabetes, tumor invasion, tumor growth, tumor metastasis, corneal scarring, scleritis, AIDS, sepsis, septic shock and other diseases characterized by metalloproteinase activity and other diseases characterized by mammalian reprolysin activity in a mammal, including a human, comprising administering to said mammal an amount of a compound of formula I or a pharmaceutically acceptable salt thereof effective in treating such a condition.

The present invention also relates to a method for the inhibition of (a) matrix metalloproteinases or other metalloproteinases involved in matrix degradation, or (b) a mammalian reprolysin (such as aggrecanase or ADAM's TS-1, 10, 12, 15 and 17, preferably ADAM-17) in a mammal, including a human, comprising administering to said mammal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof.

This invention also encompasses pharmaceutical compositions containing prodrugs of compounds of the formula I. This invention also encompasses methods of treating or preventing disorders that can be treated or prevented by the inhibition of matrix metalloproteinases or the inhibition of mammalian reprolysin comprising administering prodrugs of compounds of the formula I. Compounds of formula I having free amino, amido, hydroxy or carboxylic groups can be converted into prodrugs. Prodrugs include compounds wherein an amino acid residue, or a polypeptide chain of two or more (e.g., two, three or four) amino acid residues which are covalently joined through peptide bonds to free amino, hydroxy or carboxylic acid groups of compounds of formula I. The amino acid residues include the 20 naturally occurring amino acids commonly designated by three letter symbols and also include, 4-hydroxyproline, hydroxylysine, demosine, isodemosine, 3-methylhistidine, norvalin, beta-alanine, gamma-aminobutyric acid, citrulline, homocysteine, homoserine, ornithine and methionine sulfone. Prodrugs also include compounds wherein carbonates, carbamates, amides and alkyl esters which are covalently bonded to the above substituents of formula I through the carbonyl carbon prodrug sidechain.

One of ordinary skill in the art will appreciate that the compounds of the invention are useful in treating a diverse array of diseases. One of ordinary skill in the art will also appreciate that when using the compounds of the invention in the treatment of a specific disease that the compounds of the invention may be combined with various existing therapeutic agents used for that disease.

For the treatment of rheumatoid arthritis, the compounds of the invention may be combined with agents such as TNF-α inhibitors such as anti-TNF monoclonal antibodies and TNF receptor immunoglobulin molecules (such as Enbrel®), low dose methotrexate, lefunimide, hydroxychloroquine, d-penicilamine, auranofin or parenteral or oral gold.

The compounds of the invention can also be used in combination with existing therapeutic agents for the treatment of osteoarthritis. Suitable agents to be used in combination include standard non-steroidal anti-inflammatory agents (hereinafter NSAID's) such as piroxicam, diclofenac, propionic acids such as naproxen, flubiprofen, fenoprofen, ketoprofen and ibuprofen, fenamates such as mefenamic acid, indomethacin, sulindac, apazone, pyrazolones such as phenylbutazone, salicylates such as aspirin, COX-2 inhibitors such as celecoxib and rofecoxib, analgesics and intraarticular therapies such as corticosteroids and hyaluronic acids such as hyalgan and synvisc.

The compounds of the present invention may also be used in combination with anticancer agents such as endostatin and angiostatin or cytotoxic drugs such as adriamycin, daunomycin, cis-platinum, etoposide, taxol, taxotere and alkaloids, such as vincristine, and antimetabolites such as methotrexate.

The compounds of the present invention may also be used in combination with cardiovascular agents such as calcium channel blockers, lipid lowering agents such as statins, fibrates, beta-blockers, Ace inhibitors, Angiotensin-2 receptor antagonists and platelet aggregation inhibitors.

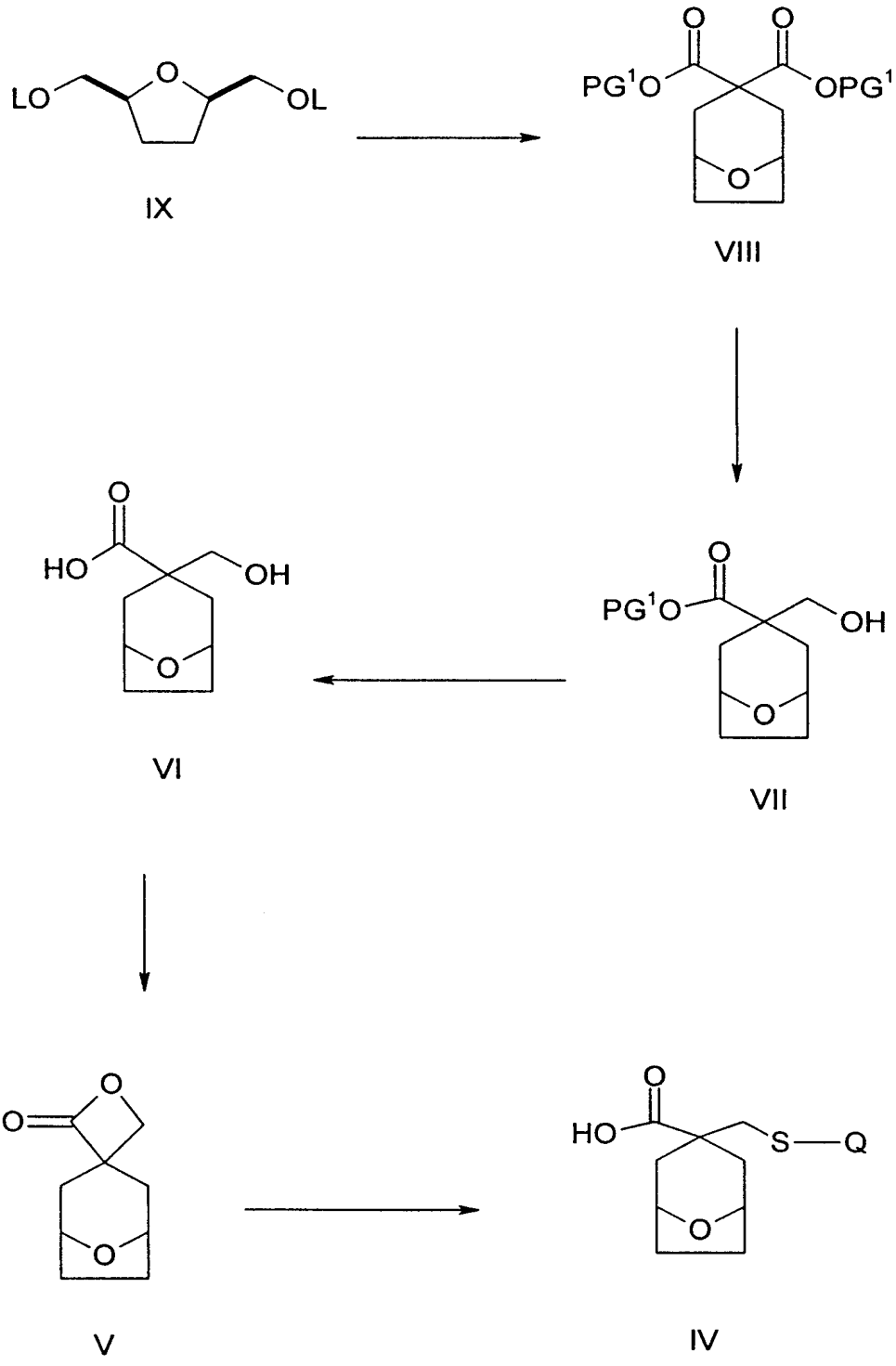
The compounds of the present invention may also be used in combination with CNS agents such as antidepressants (such as sertraline), anti-Parkinsonian drugs (such as deprenyl, L-dopa, requip, miratex, MAOB inhibitors such as selegine and rasagiline, comP inhibitors such as Tasmar, A-2 inhibitors, dopamine reuptake inhibitors, NMDA antagonists, Nicotine agonists, Dopamine agonists and inhibitors of neuronal nitric oxide synthase), and anti-Alzheimer's drugs such as Aricept, tacrine, COX-2 inhibitors, propentofylline or metryfonate.

The compounds of the present invention may also be used in combination with osteoporosis agents such as droloxifene or fosomax and immunosuppressant agents such as FK-506 and rapamycin.

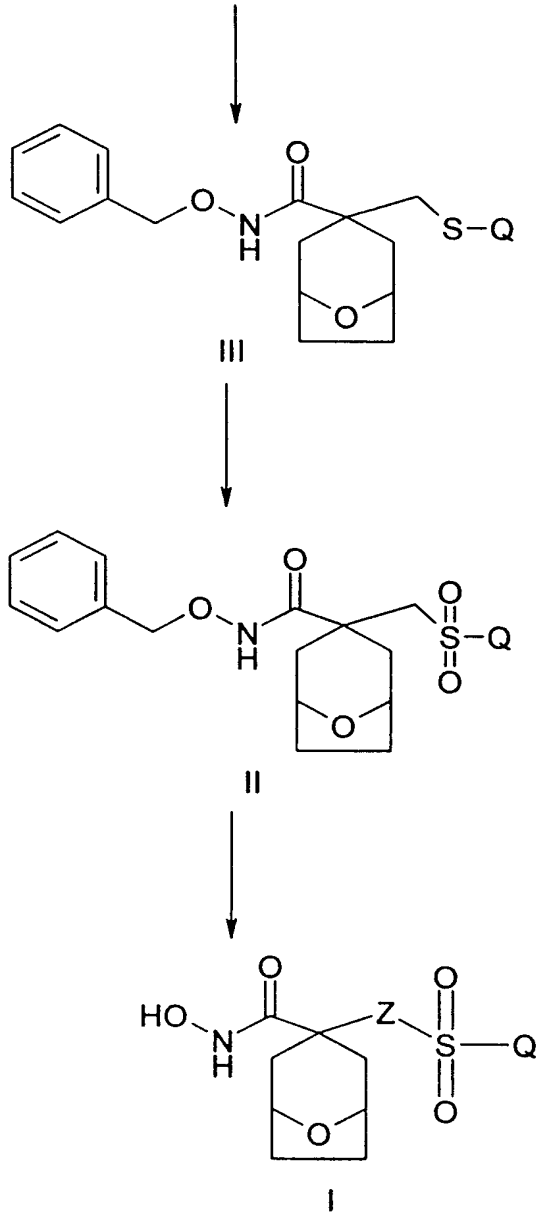
Detailed Description of the Invention

The following reaction Schemes illustrate the preparation of the compounds of the present invention. Unless otherwise indicated n, R^1, R^2, Q and Z in the reaction Schemes and the discussion that follow are defined as above.

SCHEME 1


LO
OL
O
IX
PG1O
OPG1
O
O
O
VIII
HO
OH
O
O
VI
PG1O
OH
O
VII
O
O
O
V
HO
S—Q
O
O
IV

SCHEME 1 CONTINUED



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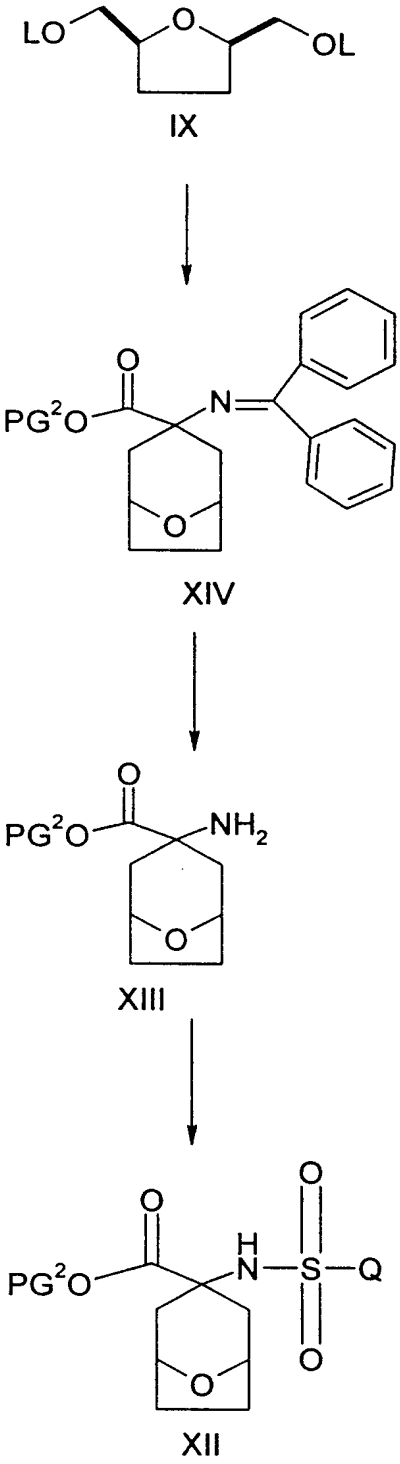
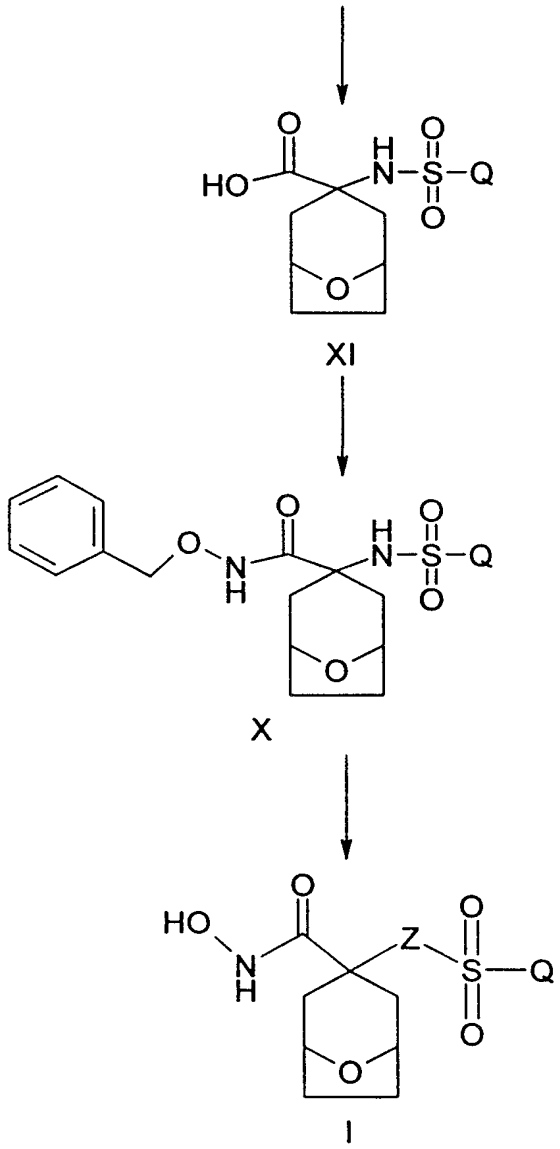


5

SCHEME 2 CONTINUED



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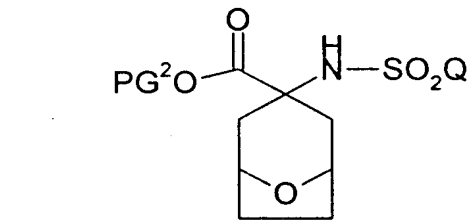
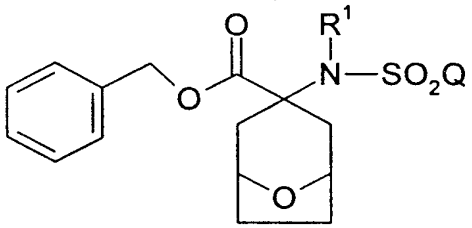
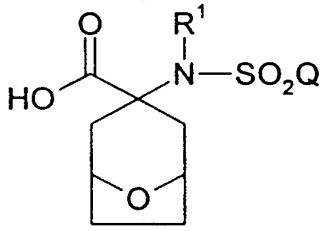


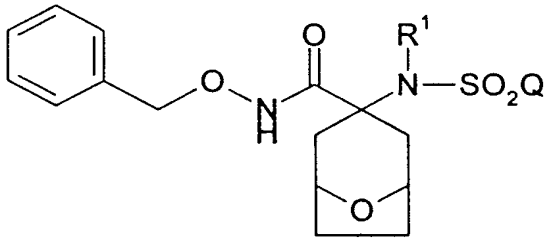
XII



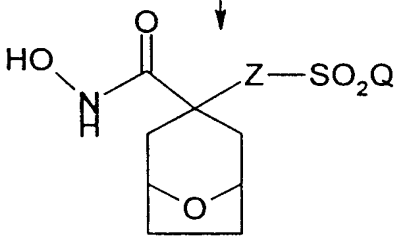
XVII



XVI

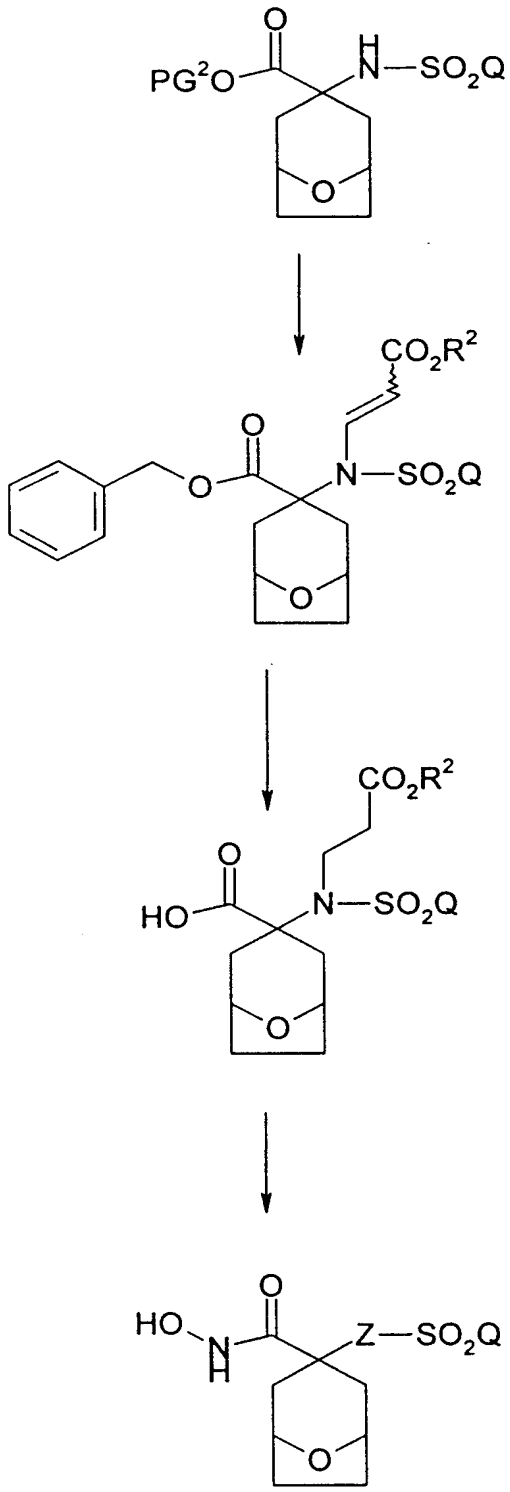


XV



I

SCHEME 4


PG2O
O
H
N—SO2Q
O
XII
CO2R2
O
O
N—SO2Q
O
XIX
CO2R2
HO
O
N—SO2Q
O
XVIII
HO
N
H
O
Z—SO2Q
O
I

Scheme 1 refers to the preparation of compounds of formula I, wherein Z is CH_2. Referring to Scheme I, a compound of the formula I is prepared from a compound of the formula II by hydrogenolysis under an atmosphere of hydrogen in the presence of a catalyst in a reaction inert solvent. Suitable catalysts include 5% palladium on barium sulfate or 5% palladium on carbon, preferably 5% palladium on barium sulfate. Suitable solvents include an alcohol such as ethanol, methanol or isopropanol, preferably methanol. The aforesaid reaction may be performed at a pressure from about 1 to about 5 atmospheres, preferably about 3 atmospheres. Suitable temperatures for the aforesaid reaction range from about 20°C (room temperature) to about 60°C, preferably the temperature may range from about 20°C to about 25°C (i.e. room temperature). The reaction is complete within about 0.5 hours to about 5 hours, preferably about 3 hours.

Compounds of the formula II can be prepared from compounds of the formula III by reaction with an oxidant in a reaction inert solvent. Suitable oxidants include meta-chloroperbenzoic acid, hydrogen peroxide or sodium perborate, preferably meta-chloroperbenzoic acid. Suitable solvents include halogenated solvents such as methylene chloride or chloroform, preferably methylene chloride. Suitable temperatures for the aforesaid reaction range from about 0°C to about 60°C, preferably the temperature may range from about 20°C to about 25°C (i.e. room temperature). The reaction is complete within about 0.5 hours to about 24 hours, preferably about 16 hours.

The compound of formula III is prepared from a compound of formula IV by reaction with O-benzylhydroxyamine hydrochloride, an activating agent, and a base in a reaction inert solvent. Suitable activating agents include (benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorophosphate or 1-(3-(dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, preferably (benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorophosphate. Suitable bases include tertiary amines such as triethylamine, diisopropylethylamine or 4-N,N-dimethylaminopyridine, preferably diisopropylethylamine. The temperature of the aforesaid reaction may range from about 0°C to about 60°C, preferably about 50°C. Suitable solvents include N,N-dimethylformamide, halogenated solvents such as methylene chloride or chloroform, or ethers such as THF or diethyl ether; preferably the solvent is N,N-dimethylformamide. The reaction is complete in about 4 hours to about 48 hours, preferably about 16 hours.

Compounds of the formula IV, can be prepared from compounds of the formula V, by reaction with a compound of the formula **QSH**, wherein Q is as defined above, in the presence of a strong base in an aprotic polar solvent. Suitable bases include sodium hydride, lithium diisopropylamide, potassium t-butoxide, sodium amide or potassium hydride, preferably sodium hydride. Suitable solvents include ethers (such as THF, diethyl ether or 1,2-

dimethoxyethane), or N, N-dimethylformamide, preferably the solvent is THF. The aforesaid reaction is conducted at about -78°C to about 0°C, preferably at about 22°C (i.e., room temperature) for a period of 30 minutes to about 24 hours, preferably about 2 hours.

Compounds of the formula V are prepared from compounds of the formula VI by dehydration in the presence of a tertiary amine base, preferably triethylamine, optionally in the presence of 4-dimethylaminopyridine, and a dehydrating agent in an inert solvent. Suitable dehydrating agents include trifluoromethanesulfonic anhydride, methanesulfonic anhydride, methanesulfonyl chloride, *p*-toluenesulfonyl chloride or benzenesulfonyl chloride, preferably benzenesulfonyl chloride. Suitable solvents include diethyl ether or dichloromethane. The reaction is performed at a temperature from about -80°C to about 0°C, preferably about 0°C. The reaction is carried out for about 10 minutes to 4 hours, preferably about 1 hour.

The compounds of the formula VI are prepared from a compound of formula VII, wherein PG^1 is methyl or ethyl, by saponification with a base, such as lithium hydroxide, in a solvent mixture. Suitable solvent mixtures include water and methanol or water, methanol and THF. The reaction is performed at a temperature from about 60°C to about 120°C, preferably at about the reflux temperature of the solvent mixture used. The reaction is carried out for about 30 minutes to 24 hours, preferably about 16 hours.

The exo-hydroxymethyl isomer of the compound of the formula VII is prepared from a compound of formula VIII. In general, a solution of a compound of formula VIII is dissolved in an inert aromatic solvent, preferably benzene or toluene, and cooled at about -40° C to -20°C, preferably about -40°C. To the cold solution is added a suitable hindered reducing agent, preferably disobutylaluminum hydride, in an inert aromatic solvent, maintaining the temperature below -25°C. After the addition is complete, the reaction is maintained below 0°C for about 3 hours. At about -15°C, a protic solvent, preferably ethanol, is added. After stirring at about -15°C for about 1 hour, sodium borohydride is added and the reaction is allowed to warm to about room temperature while stirring for a period of 2 to 24 hours, preferably about 16 hours.

The endo-hydroxymethyl isomer of the compound of the formula VII can be prepared from the exo-hydroxymethyl compound of the formula VI by a series of steps which can invert the sterochemistry about the carbon atom bearing the hydroxymethyl and carboxylic acid groups. Specifically, the exo-hydroxymethyl isomer of formula VI is first converted to the corresponding benzyl ester. Subsequent Jones oxidation of the alcohol to the carboxylic acid and alkyl ester formation (methyl or ethyl) provides an intermediate mixed benzyl alkyl ester (i.e. the exo ester is methyl or ethyl and the endo ester is benzyl). The benzyl ester is then removed by hydrogenolysis and the resulting carboxylic acid is reduced to the alcohol by diborane reduction, providing the endo-hydroxymethyl isomer of the compound of the formula VII.

The compounds formula VIII, wherein PG^1 is ethyl or methyl, are prepared from compounds of the formula IX, wherein L is methanesulfonyl, benzenesulfonyl or tosyl, by reaction with dimethyl or diethyl malonate in the presence of a strong base, such as sodium hydride, in a polar solvent, such as N,N-dimethylformamide, for a time period between about 4 hours to about 24 hours, preferably about 16 hours. The aforesaid reaction temperature is between about 70°C to about 150°C, preferably about 140° C.

Compounds of the formula IX are known or can be made by methods well known to those of ordinary skill in the art.

Compounds of the formula QSH can be prepared by reaction of an alkyl or aryl halide with sodium sulfhydride as described in Jerry March, Advanced Organic Chemistry, 360 and 589 (3rd ed., 1985). Alternatively, compounds of the formula QSH can also be prepared by reaction of an aryl diazonium salt with sodium sulfhydride as described in March id. at 601. Alternatively, compounds of the formula QSH can also be prepared by reaction of a Grignard reagent with sulfur as described in March id. at 550. Alternatively, compounds of the formula QSH can also be prepared by reduction of a sulfonyl chloride, sulfonic acid or disulfide as described in March id. at 1107 and 1110.

Scheme 2 refers to the preparation of compounds of the formula I, wherein Z is $>NR^1$, and R^1 is hydrogen. Referring to Scheme 2, compounds of formula I can be prepared from compounds of the formula X by hydrogenolysis under an atmosphere of hydrogen in the presence of a catalyst in a reaction inert solvent. Suitable catalysts include 5% palladium on barium sulfate or 5% palladium on carbon, preferably 5% palladium on barium sulfate. Suitable solvents include an alcohol such as ethanol, methanol or isopropanol, preferably methanol. The aforesaid reaction may be performed at a pressure from about 1 to about 5 atmospheres, preferably about 3 atmospheres. Suitable temperatures for the aforesaid reaction range from about 20°C (room temperature) to about 60°C, preferably the temperature may range from about 20°C to about 25°C (i.e. room temperature). The reaction is complete within about 0.5 hours to about 5 hours, preferably about 3 hours.

The compound of formula X is prepared from a compound of the formula XI by reaction with O-benzylhydroxylamine hydrochloride in the presence of a catalyst and a base in a reaction inert solvent. Suitable catalysts include (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate or 1-(3-(dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, preferably (benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorophosphate. Suitable bases include tertiary amines such as triethylamine, diisopropylethylamine or 4-N,N-dimethylaminopyridine, preferably diisopropylethylamine. The aforesaid reaction temperature is from about 0° C to about 60°C, preferably about 50° C. Suitable solvents include N,N-dimethylformamide or halogenated

solvents such as methylene chloride or chloroform; preferably, the solvent is N,N-dimethylformamide. The reaction is conducted over a period of about 4 hours to about 48 hours, preferably about 16 hours.

Compounds of the formula XI are prepared from compounds of the formula XII, wherein PG^2 is methyl or ethyl, by saponification with a base such as sodium hydroxide in a solvent mixture such as water and ethanol. The reaction is performed at a temperature from about 60°C to about 100°C, preferably at about the reflux temperature of the solvent mixture used. The reaction is carried out for about 1 day to 10 days, preferably about 6 days.

The compounds of the formula XII, wherein PG^2 is methyl or ethyl, are prepared from compounds of the formula XIII, wherein PG^2 is methyl or ethyl, by reaction with a compound of the formula QSO_2Cl in the presence of a base, such as triethylamine, and a polar solvent. Suitable solvents include N,N-dimethylformamide, tetrahydrofuran, 1,2-dimethoxyethane, dioxane, water or acetonitrile, preferably N,N-dimethylformamide. The reaction mixture is stirred at room temperature for a time period between about 1 hour to about 24 hours, preferably about 16 hours.

Compounds of the formula XIII, wherein PG^2 is methyl or ethyl, are prepared from compounds of the formula XIV, wherein PG^2 is methyl or ethyl, by hydrolysis in the presence of aqueous mineral acid and a solvent such as diethyl ether. Suitable mineral acids include hydrochloric and sulfuric acid, preferably hydrochloric acid. The reaction is carried out at a temperature ranging from about 0°C to 50°C; preferably the temperature may range from about 20°C to about 25°C (i.e. room temperature). The reaction is conducted over a period of about 2 hours to about 48 hours, preferably about 16 hours.

Compounds of the formula XIV, wherein PG^2 is methyl, ethyl or benzyl, are prepared from compounds of the formula IX, wherein L is methanesulfonyl, benzenesulfonyl or tosyl, by reaction with N-diphenylmethylene glycine, methyl, ethyl or benzyl ester, in the presence of a strong base, such as sodium hydride, in a polar solvent, such as N,N-dimethylformamide, for a time period between about 4 hours to about 24 hours, preferably about 16 hours. The aforesaid reaction temperature is between about 70°C to about 140°C, preferably about 100° C. Compounds of the formula XIV, wherein PG^2 is methyl, ethyl or benzyl, are obtained as mixtures of diastereomers which can be separated by chromatographic means.

Compounds of the formula QSO_2Cl and formula IX are known or commercially available or can be made by methods well known to those of ordinary skill in the art.

Scheme 3 refers to the preparation of compounds of the formula I, wherein Z is NR^1 and R^1 is $(C_1\text{-}C_6)$alkyl, $(C_6\text{-}C_{10})$aryl$(C_1\text{-}C_6)$alkyl, $(C_2\text{-}C_9)$heteroaryl$(C_1\text{-}C_6)$alkyl or a group of the formula $-(CH_2)_nCO_2R^2$, wherein n is 1, 3, 4, 5, or 6 and R^2 is $(C_1\text{-}C_6)$alkyl.

Referring to Scheme 3, compounds of the formula I, wherein Z is NR^1 and R^1 is (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_2-C_9)heteroaryl(C_1-C_6)alkyl or a group of the formula $-(CH_2)_nCO_2R^2$, wherein n is 1, 3, 4, 5, or 6 and R^2 is (C_1-C_6)alkyl, are prepared from compounds of the formula XV by hydrogenolysis under an atmosphere of hydrogen in the presence of a catalyst in a reaction inert solvent. Suitable catalysts include 5% palladium on barium sulfate or 5% palladium on carbon, preferably 5% palladium on barium sulfate. Suitable solvents include an alcohol such as ethanol, methanol or isopropanol, preferably methanol. The aforesaid reaction may be performed at a pressure from about 1 to about 5 atmospheres, preferably about 3 atmospheres. Suitable temperatures for the aforesaid reaction range from about 20°C (room temperature) to about 60°C, preferably the temperature may range from about 20°C to about 25°C (i.e. room temperature). The reaction is complete within about 0.5 hours to about 5 hours, preferably about 3 hours.

The compound of formula XV is prepared from a compound of the formula XVI by reaction with O-benzylhydroxylamine hydrochloride in the presence of a catalyst and a base in a reaction inert solvent. Suitable catalysts include (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate or 1-(3-(dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, preferably (benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorophosphate. Suitable bases include tertiary amines such as triethylamine, diisopropylethylamine or 4-N,N-dimethylaminopyridine, preferably diisopropylethylamine. The aforesaid reaction temperature is from about 0° C to about 60°C, preferably about 50° C. Suitable solvents include N,N-dimethylformamide or halogenated solvents such as methylene chloride or chloroform, preferably the solvent is N,N-dimethylformamide. The reaction is conducted over a period of about 4 hours to about 48 hours, preferably about 16 hours.

The compound of formula XVI is prepared from a compound of the formula XVII by removal of the benzyl protecting group. Specifically, the benzyl protecting group is removed by hydrogenolysis using palladium or palladium on carbon in a solvent such as methanol or ethanol, for a period from about 30 minutes to about 48 hours, preferably 16 hours, at a temperature of about 20°C to about 25°C (i.e., room temperature).

The compound of formula XVII is prepared from a compound of the formula XII, wherein PG^2 is benzyl, by reaction with a reactive derivative of an alcohol of the formula $\mathbf{R^1OH}$ such as the methanesulfonate, tosylate, chloro, bromo or iodo derivative, preferably the iodo derivative, in the presence of a base such as potassium carbonate or sodium hydride, preferably sodium hydride, and a polar solvent, such as N,N-dimethylformamide. The reaction mixture is stirred at room temperature for a time period between about 60 minutes to about 48 hours, preferably about 16 hours.

The compounds of formula XII, wherein PG^2 is benzyl, are prepared according to the methods of Scheme 2.

Scheme 4 refers to the preparation of compounds of formula I, wherein Z is $>NR^1$, R^1 is a group of the formula $-(CH_2)_2CO_2R^2$ (i.e. n is 2) and R^2 is $(C_1\text{-}C_6)$alkyl.

Referring to Scheme 4, compounds of said formula I are prepared from compounds of the formula XVIII, wherein R^2 is $(C_1\text{-}C_6)$alkyl, by reaction with oxalyl chloride or thionyl chloride, preferably oxalyl chloride, and a catalyst, preferably about 2% of N,N-dimethylformamide, in an inert solvent, such as methylene chloride, to form an *in situ* acid chloride that is subsequently reacted with O-trimethylsilylhydroxylamine in the presence of a base, such as pyridine, 4-N,N-dimethylaminopyridine or triethylamine, preferably pyridine. The reaction is performed at a temperature of about 22°C (i.e., room temperature) for about 1 to about 12 hours, preferably about 1 hour.

Compounds of the formula XVIII, wherein R^2 is $(C_1\text{-}C_6)$alkyl, can be prepared from compounds of the formula XIX, wherein R^2 is $(C_1\text{-}C_6)$alkyl, by reduction in a polar solvent. Suitable reducing agents include hydrogen over palladium and hydrogen over palladium on carbon, preferably hydrogen over palladium on carbon. Suitable solvents include methanol, ethanol and isopropanol, preferably ethanol. The aforesaid reaction is performed at a temperature of about 22°C (i.e., room temperature) for a period of 1 to 7 days, preferably about 2 days.

Compounds of the formula XIX, wherein R^2 is $(C_1\text{-}C_6)$alkyl, can be prepared from compounds of the formula XII, wherein PG^2 is benzyl, by Michael addition of a propiolate ester and a base in a polar solvent. Suitable propiolates are of the formula $H\text{-}C{\equiv}C\text{-}CO_2R^2$, wherein R^2 is $(C_1\text{-}C_6)$alkyl. Suitable bases include tetrabutylammonium fluoride, potassium carbonate, and cesium carbonate, preferably tetrabutylammonium fluoride. Suitable solvents include tetrahydrofuran, acetonitrile, tert-butanol and N,N-dimethylformamide, preferably tetrahydrofuran. The aforesaid reaction is performed at a temperature of about -10°C to about 60°C, preferably ranging between 0°C and about 22°C (i.e., room temperature). The compounds of formula XIX are obtained as mixtures of geometric isomers about the olefinic double bond; separation of the isomers is not necessary.

Compounds of the formula XII, wherein PG^2 is benzyl, can be prepared according to the methods of Scheme 2.

Compounds of said formula I, wherein Z is $>NR^1$, R^1 is a group of the formula $-(CH_2)_nCO_2R^2$, n is 1 to 6 and R^2 is hydrogen are prepared from compounds of formula I, wherein Z is $>NR^1$, R^1 is a group of the formula $-(CH_2)_nCO_2R^2$, n is 1 to 6 and R^2 is $(C_1\text{-}C_6)$alkyl, by saponification using a base such as sodium hydroxide in a protic solvent such as ethanol, methanol or water or a mixture such as water and ethanol, water and toluene, or water and THF.

The preferred solvent system is water and ethanol. The reaction is conducted for a period of 30 minutes to 24 hours, preferably about 2 hours.

The compounds of the formula I which are basic in nature are capable of forming a wide variety of different salts with various inorganic and organic acids. Although such salts must be pharmaceutically acceptable for administration to animals, it is often desirable in practice to initially isolate a compound of the formula I from the reaction mixture as a pharmaceutically unacceptable salt and then simply convert the latter back to the free base compound by treatment with an alkaline reagent, and subsequently convert the free base to a pharmaceutically acceptable acid addition salt. The acid addition salts of the base compounds of this invention are readily prepared by treating the base compound with a substantially equivalent amount of the chosen mineral or organic acid in an aqueous solvent medium or in a suitable organic solvent such as methanol or ethanol. Upon careful evaporation of the solvent, the desired solid salt is obtained.

The acids which are used to prepare the pharmaceutically acceptable acid addition salts of the base compounds of this invention are those which form non-toxic acid addition salts, i.e., salts containing pharmacologically acceptable anions, such as hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate or bisulfate, phosphate or acid phosphate, acetate, lactate, citrate or acid citrate, tartrate or bitartrate, succinate, maleate, fumarate, gluconate, saccharate, benzoate, methanesulfonate and pamoate [i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)] salts.

Those compounds of the formula I which are also acidic in nature, are capable of forming base salts with various pharmacologically acceptable cations. Examples of such salts include the alkali metal or alkaline-earth metal salts and particularly, the sodium and potassium salts. These salts are all prepared by conventional techniques. The chemical bases which are used as reagents to prepare the pharmaceutically acceptable base salts of this invention are those which form non-toxic base salts with the herein described acidic compounds of formula I. These non-toxic base salts include those derived from such pharmacologically acceptable cations as sodium, potassium, calcium and magnesium, etc. These salts can easily be prepared by treating the corresponding acidic compounds with an aqueous solution containing the desired pharmacologically acceptable cations, and then evaporating the resulting solution to dryness, preferably under reduced pressure. Alternatively, they may also be prepared by mixing lower alkanolic solutions of the acidic compounds and the desired alkali metal alkoxide together, and then evaporating the resulting solution to dryness in the same manner as before. In either case, stoichiometric quantities of reagents are preferably employed in order to ensure completeness of reaction and maximum product yields.

The ability of the compounds of formula I or their pharmaceutically acceptable salts (hereinafter also referred to as the compounds of the present invention) to inhibit metalloproteinases or mammalian reprolysin and, consequently, demonstrate their effectiveness for treating diseases characterized by metalloproteinase or the production of tumor necrosis factor is shown by the following in vitro assay tests.

Biological Assay

Inhibition of Human Collagenase (MMP-1)

Human recombinant collagenase is activated with trypsin. The amount of trypsin is optimized for each lot of collagenase-1 but a typical reaction uses the following ratio: 5 μg trypsin per 100 μg of collagenase. The trypsin and collagenase are incubated at room temperature for 10 minutes then a five fold excess (50 mg/10 mg trypsin) of soybean trypsin inhibitor is added.

Stock solutions (10 mM) of inhibitors are made up in dimethylsulfoxide and then diluted using the following scheme:

10 mM ------> 120 μM ------> 12 μM ------> 1.2 μM ------> 0.12 μM

Twenty-five microliters of each concentration is then added in triplicate to appropriate wells of a 96 well microfluor plate. The final concentration of inhibitor will be a 1:4 dilution after addition of enzyme and substrate. Positive controls (enzyme, no inhibitor) are set up in wells D7-D12 and negative controls (no enzyme, no inhibitors) are set in wells D1-D6.

Collagenase-1 is diluted to 240 ng/ml and 25 ml is then added to appropriate wells of the microfluor plate. Final concentration of collagenase in the assay is 60 ng/ml.

Substrate (DNP-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2) is made as a 5 mM stock in dimethylsulfoxide and then diluted to 20 μM in assay buffer. The assay is initiated by the addition of 50 ml substrate per well of the microfluor plate to give a final concentration of 10 mM.

Fluorescence readings (360 nM excitation, 460 nm emission) are taken at time 0 and then at 20 minute intervals. The assay is conducted at room temperature with a typical assay time of 3 hours

Fluorescence versus time is then plotted for both the blank and collagenase containing samples (data from triplicate determinations is averaged). A time point that provides a good signal (at least five fold over the blank) and that is on a linear part of the curve (usually around 120 minutes) is chosen to determine IC_{50} values. The zero time is used as a blank for each compound at each concentration and these values are subtracted from the 120 minute data. Data is plotted as inhibitor concentration versus % control (inhibitor fluorescence divided by fluorescence of collagenase alone x 100). IC_{50}'s are determined from the concentration of inhibitor that gives a signal that is 50% of the control.

If IC_{50}'s are reported to be less than 0.03 mM then the inhibitors are assayed at concentrations of 0.3 mM, 0.03 mM, and 0.003 mM.

Inhibition of Gelatinase (MMP-2)

Human recombinant 72 kD gelatinase (MMP-2, gelatinase A) is activated for 16-18 hours with 1mM p-aminophenyl-mercuric acetate (from a freshly prepared 100 mM stock in 0.2 N NaOH) at 4°C, rocking gently.

10 mM dimethylsulfoxide stock solutions of inhibitors are diluted serially in assay buffer (50 mM TRIS, pH 7.5, 200 mM NaCl, 5 mM $CaCl_2$, 20 μM $ZnCl_2$ and 0.02% BRIJ-35 (vol./vol.)) using the following scheme:

10 mM---→ 120 μM----→ 12 μM----→ 1.2 μM----→ 0.12 μM

Further dilutions are made as necessary following this same scheme. A minimum of four inhibitor concentrations for each compound are performed in each assay. 25 μL of each concentration is then added to triplicate wells of a black 96 well U-bottomed microfluor plate. As the final assay volume is 100 μL, final concentrations of inhibitor are the result of a further 1:4 dilution (i.e. 30 μM ----→ 3 μM ----→ 0.3 μM ----→ 0.03 μM, etc.). A blank (no enzyme, no inhibitor) and a positive enzyme control (with enzyme, no inhibitor) are also prepared in triplicate.

Activated enzyme is diluted to 100 ng/mL in assay buffer, 25 μL per well is added to appropriate wells of the microplate. Final enzyme concentration in the assay is 25 ng/mL (0.34 nM).

A five mM dimethylsulfoxide stock solution of substrate (Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2) is diluted in assay buffer to 20 μM. The assay is initiated by addition of 50 μL of diluted substrate yielding a final assay concentration of 10 μM substrate. At time zero, fluorescence reading (320 excitation; 390 emission) is immediately taken and subsequent readings are taken every fifteen minutes at room temperature with a PerSeptive Biosystems CytoFluor Multi-Well Plate Reader with the gain at 90 units.

The average value of fluorescence of the enzyme and blank are plotted versus time. An early time point on the linear part of this curve is chosen for IC_{50} determinations. The zero time point for each compound at each dilution is subtracted from the latter time point and the data then expressed as percent of enzyme control (inhibitor fluorescence divided by fluorescence of positive enzyme control x 100). Data is plotted as inhibitor concentration versus percent of enzyme control. IC_{50}'s are defined as the concentration of inhibitor that gives a signal that is 50% of the positive enzyme control.

Inhibition of Stromelysin Activity (MMP-3)

Human recombinant stromelysin (MMP-3, stromelysin-1) is activated for 20-22 hours with 2 mM p-aminophenyl-mercuric acetate (from a freshly prepared 100 mM stock in 0.2 N NaOH) at 37°C.

10 mM dimethylsulfoxide stock solutions of inhibitors are diluted serially in assay buffer (50 mM TRIS, pH 7.5, 150 mM NaCl, 10 mM $CaCl_2$ and 0.05% BRIJ-35 (vol./vol.)) using the following scheme:

10 mM---→ 120 μM----→ 12 μM----→ 1.2 μM----→ 0.12 μM

Further dilutions are made as necessary following this same scheme. A minimum of four inhibitor concentrations for each compound are performed in each assay. 25 μL of each concentration is then added to triplicate wells of a black 96 well U-bottomed microfluor plate. As the final assay volume is 100 μL, final concentrations of inhibitor are the result of a further 1:4 dilution (i.e. 30 μM ----→ 3 μM ----→ 0.3 μM ----→ 0.03 μM, etc.). A blank (no enzyme, no inhibitor) and a positive enzyme control (with enzyme, no inhibitor) are also prepared in triplicate.

Activated enzyme is diluted to 200 ng/mL in assay buffer, 25 μL per well is added to appropriate wells of the microplate. Final enzyme concentration in the assay is 50 ng/mL (0.875 nM).

A ten mM dimethylsulfoxide stock solution of substrate (Mca-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(Dnp)-NH_2) is diluted in assay buffer to 6 μM. The assay is initiated by addition of 50 μL of diluted substrate yielding a final assay concentration of 3 μM substrate. At time zero, fluorescence reading (320 excitation; 390 emission) is immediately taken and subsequent readings are taken every fifteen minutes at room temperature with a PerSeptive Biosystems CytoFluor Multi-Well Plate Reader with the gain at 90 units.

The average value of fluorescence of the enzyme and blank are plotted versus time. An early time point on the linear part of this curve is chosen for IC_{50} determinations. The zero time point for each compound at each dilution is subtracted from the latter time point and the data then expressed as percent of enzyme control (inhibitor fluorescence divided by fluorescence of positive enzyme control x 100). Data is plotted as inhibitor concentration versus percent of enzyme control. IC_{50}'s are defined as the concentration of inhibitor that gives a signal that is 50% of the positive enzyme control.

Alternatively, inhibition of stromelysin activity can be assayed using Mca-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(Dnp)-NH_2 (3 μM) under conditions similar as in inhibition of human collagenase (MMP-1).

Human stromelysin is activated for 20-24 hours at 37°C with 2 mM APMA (p-aminophenyl mercuric acetate) and is diluted to give a final concentration in the assay of 50

ng/ml. Inhibitors are diluted as for inhibition of human collagenase (MMP-1) to give final concentrations in the assay of 30 μM, 3 μM, 0.3 μM, and 0.03 μM. Each concentration is done in triplicate.

Fluorescence readings (320 nm excitation, 390 emission) are taken at time zero and then at 15 minute intervals for 3 hours.

IC_{50}'s are determined as per inhibition of human collagenase (MMP-1). If IC_{50}'s are reported to be less than 0.03 μM, then the inhibitors are assayed at final concentrations of 0.03 μM, 0.003 μM, 0.0003 μM, and 0.00003 μM.

IC_{50} values were determined in the same manner as for collagenase.

Inhibition of MMP-13

Human recombinant MMP-13 is activated with 2mM APMA (p-aminophenyl mercuric acetate) for 2.0 hours, at 37°C and is diluted to 240 ng/ml in assay buffer (50 mM Tris, pH 7.5, 200 mM sodium chloride, 5mM calcium chloride, 20mM zinc chloride, 0.02% brij 35). Twenty-five microliters of diluted enzyme is added per well of a 96 well microfluor plate. The enzyme is then diluted in a 1:4 ratio in the assay by the addition of inhibitor and substrate to give a final concentration in the assay of 60 ng/ml.

Stock solutions (10 mM) of inhibitors are made up in dimethylsulfoxide and then diluted in assay buffer as per the inhibitor dilution scheme for inhibition of human collagenase-1 (MMP-1): Twenty-five microliters of each concentration is added in triplicate to the microfluor plate. The final concentrations in the assay are 30 mM, 3mmM, 0.3m mM, and 0.03 mmM.

Substrate (Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2) is prepared as for inhibition of human collagenase (MMP-1) and 50 μl is added to each well to give a final assay concentration of 10 μM. Fluorescence readings (360 nM excitation; 450 nM emission) are taken at time 0 and every 5 minutes for 1 hour.

Positive controls and negative controls are set up in triplicate as outlined in the MMP-1 assay.

IC_{50}'s are determined as per inhibition of human collagenase (MMP-1). If IC_{50}'s are reported to be less than 0.03 mM, inhibitors are then assayed at final concentrations of 0.3 mM, 0.03 mmM, 0.003 mmM and 0.0003 mM.

Inhibition of TNF Production

The ability of the compounds or the pharmaceutically acceptable salts thereof to inhibit the production of TNF and, consequently, demonstrate their effectiveness for treating diseases involving the production of TNF is shown by the following in vitro assay:

Human mononuclear cells were isolated from anti-coagulated human blood using a one-step Ficoll-hypaque separation technique. (2) The mononuclear cells were washed three times in Hanks balanced salt solution (HBSS) with divalent cations and resuspended to a density of 2

x 10^6 /ml in HBSS containing 1% BSA. Differential counts determined using the Abbott Cell Dyn 3500 analyzer indicated that monocytes ranged from 17 to 24% of the total cells in these preparations.

180 µl of the cell suspension was aliquoted into flat bottom 96 well plates (Costar). Additions of compounds and LPS (100 ng/ml final concentration) gave a final volume of 200 µl. All conditions were performed in triplicate. After a four hour incubation at 37°C in an humidified CO_2 incubator, plates were removed and centrifuged (10 minutes at approximately 250 x g) and the supernatants removed and assayed for TNFa using the R&D ELISA Kit.

Inhibition of Soluble TNF-α Production

The ability of the compounds or the pharmaceutically acceptable salts thereof to inhibit the cellular release of TNF-α and, consequently, demonstrate their effectiveness for treating diseases involving the disregulation of soluble TNF-α is shown by the following in vitro assay:

Method for the evaluation of recombinant TNF-α Converting Enzyme Activity

Expression of recombinant TACE

A DNA fragment coding for the signal sequence, preprodomain, prodomain and catalytic domain of TACE (amino acids 1-473), can be amplified by polymerase chain reaction using a human lung cDNA library as a template. The amplified fragment is then cloned into pFastBac vector. The DNA sequence of the insert is confirmed for both the strands. A bacmid prepared using pFastBac in E. coli DH10Bac is transfected into SF9 insect cells. The virus particles is then amplified to P1, P2, P3 stages. The P3 virus is infected into both Sf9 and High Five insect cells and grown at 27°C for 48 hours. The medium is collected and used for assays and further purification.

Preparation of fluorescent quenched substrate:

A model peptidic TNF-α substrate (LY-LeucineAlanineGlutamineAlanineValine-ArginineSerine-SerineLysine(CTMR)-Arginine (LY=Lucifer Yellow; CTMR=Carboxytetramethyl-Rhodamine)) is prepared and the concentration estimated by absorbance at 560 nm (E_{560}, 60,000 M-1CM-1) according to the method of Geoghegan, KF, "Improved method for converting an unmodified peptide to an energy-transfer substrate for a proteinase." Bioconjugate Chem. **7**, 385-391 (1995). This peptide encompasses the cleavage cite on pro-TNF which is cleaved in vivo by TACE.

Expression of recombinant TACE

A DNA fragment coding for the signal sequence, preprodomain, prodomain and catalytic domain of TACE (amino acids 1-473), is amplified by polymerase chain reaction using a human lung cDNA library as a template. The amplified fragment is cloned into pFastBac vector. The DNA sequence of the insert is confirmed for both the strands. A

bacmid prepared using pFastBac in E. coli DH10Bac is transfected into SF9 insect cells. The virus particles were amplified to P1, P2, P3 stages. The P3 virus is infected into both Sf9 and High Five insect cells and grown at 27°C for 48 hours. The medium is collected and used for assays and further purification.

Enzyme reaction

The reaction, carried out in a 96 well plate (Dynatech), is comprised of 70 µl of buffer solution (25 mM Hepes-HCl, pH7.5, plus 20 uM $ZnCl_2$), 10 µl of 100 µM fluorescent quenched substrate, 10 µl of a DMSO (5%) solution of test compound, and an amount of r-TACE enzyme which will cause 50% cleavage in 60 minutes - in a total volume of 100 µl. The specificity of the enzyme cleavage at the amide bond between alanine and valine is verified by HPLC and mass spectrometry. Initial rates of cleavage are monitored by measuring the rate of increase in fluorescence at 530 nm (excitation at 409 nm) over 30 minutes. The experiment is controlled as follows: 1) for background fluorescence of substrate; 2) for fluorescence of fully cleaved substrate; 3) for fluorescence quenching or augmentation from solutions containing test compound.

Data is analyzed as follows. The rates from the non-test compound containing "control" reactions were averaged to establish the 100% value. The rate of reaction in the presence of test compound was compared to that in the absence of compound, and tabulated as "percent of non-test compound containing control. The results are plotted as "% of control" vs. the log of compound concentration and a half-maximal point or IC_{50} value determined.

All of the compounds of the invention have IC_{50} of less than 1 µM, preferably less than 50nM. Most preferred compounds of the invention are at least 100 fold less potent against r-MMP-1 than in the above TACE assay.

Human Monocyte Assay

Human mononuclear cells are isolated from anti-coagulated human blood using a one-step Ficoll-hypaque separation technique. (2) The mononuclear cells are washed three times in Hanks balanced salt solution (HBSS) with divalent cations and resuspended to a density of 2 x 10^6 /ml in HBSS containing 1% BSA. Differential counts determined using the Abbott Cell Dyn 3500 analyzer indicated that monocytes ranged from 17 to 24% of the total cells in these preparations.

180m of the cell suspension was aliquoted into flat bottom 96 well plates (Costar). Additions of compounds and LPS (100 ng/ml final concentration) gave a final volume of 200 µl. All conditions were performed in triplicate. After a four hour incubation at 37°C in an humidified CO_2 incubator, plates were removed and centrifuged (10 minutes at approximately 250 x g) and the supernatants removed and assayed for TNF-α using the R&D ELISA Kit.

Aggrecanase Assay

Primary porcine chondrocytes from articular joint cartilage are isolated by sequential trypsin and collagenase digestion followed by collagenase digestion overnight and are plated at 2 X 10^5 cells per well into 48 well plates with 5 μCi / ml ^{35}S (1000 Ci/mmol) sulphur in type I collagen coated plates. Cells are allowed to incorporate label into their proteoglycan matrix (approximately 1 week) at 37°C, under an atmosphere of 5% CO_2.

The night before initiating the assay, chondrocyte monolayers are washed two times in DMEM/ 1% PSF/G and then allowed to incubate in fresh DMEM /1% FBS overnight.

The following morning chondrocytes are washed once in DMEM/1%PSF/G. The final wash is allowed to sit on the plates in the incubator while making dilutions.

Media and dilutions can be made as described in the Table below.

Control Media	DMEM alone (control media)
IL-1 Media	DMEM + IL-1 (5 ng/ml)
Drug Dilutions	Make all compounds stocks at 10 mM in DMSO. Make a 100 uM stock of each compound in DMEM in 96 well plate. Store in freezer overnight. The next day perform serial dilutions in DMEM with IL-1 to 5 uM, 500 nM, and 50 nM. Aspirate final wash from wells and add 50 ul of compound from above dilutions to 450 ul of IL-1 media in appropriate wells of the 48 well plates. Final compound concentrations equal 500 nM, 50 nM, and 5 nM. All samples completed in triplicate with Control and IL-1 alone samples on each plate.

Plates are labeled and only the interior 24 wells of the plate are used. On one of the plates, several columns are designated as IL-1 (no drug) and Control (no IL-1, no drug). These control columns are periodically counted to monitor 35S-proteoglycan release. Control and IL-1 media are added to wells (450 ul) followed by compound (50 ul) so as to initiate the assay. Plates are incubated at 37°C, with a 5% CO_2 atmosphere.

At 40-50 % release (when CPM from IL-1 media is 4-5 times control media) as assessed by liquid scintillation counting (LSC) of media samples, the assay is terminated (9-12 hours). Media is removed from all wells and placed in scintillation tubes. Scintillate is added and radioactive counts are acquired (LSC). To solubilize cell layers, 500 ul of papain digestion buffer (0.2 M Tris, pH 7.0, 5 mM EDTA, 5 mM DTT, and 1 mg/ml papain) is added to

each well. Plates with digestion solution are incubated at 60°C overnight. The cell layer is removed from the plates the next day and placed in scintillation tubes. Scintillate is then added, and samples counted (LSC).

The percent of released counts from the total present in each well is determined. Averages of the triplicates are made with control background subtracted from each well. The percent of compound inhibition is based on IL-1 samples as 0% inhibition (100% of total counts).

For administration to mammals, including humans, for the inhibition of matrix metalloproteinases or the production of tumor necrosis factor (TNF), a variety of conventional routes may be used including oral, parenteral (e.g., intravenous, intramuscular or subcutaneous), buccal, anal and topical. In general, the active compound will be administered at dosages between about 0.1 and 25 mg/kg body weight of the subject to be treated per day, preferably from about 0.3 to 5 mg/kg. Preferably the active compound will be administered orally or parenterally. However, some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

The compounds of the present invention can be administered in a wide variety of different dosage forms, in general, the therapeutically effective compounds of this invention are present in such dosage forms at concentration levels ranging from about 5.0% to about 70% by weight.

For oral administration, tablets containing various excipients such as microcrystalline cellulose, sodium citrate, calcium carbonate, dicalcium phosphate and glycine may be employed along with various disintegrants such as starch (and preferably corn, potato or tapioca starch), alginic acid and certain complex silicates, together with granulation binders like polyvinylpyrrolidone, sucrose, gelation and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often very useful for tabletting purposes. Solid compositions of a similar type may also be employed as fillers in gelatin capsules; preferred materials in this connection also include lactose or milk sugar as well as high molecular weight polyethylene glycols. When aqueous suspensions and/or elixirs are desired for oral administration, the active ingredient may be combined with various sweetening or flavoring agents, coloring matter or dyes, and, if so desired, emulsifying and/or suspending agents as well, together with such diluents as water, ethanol, propylene glycol, glycerin and various like combinations thereof. In the case of animals, they are advantageously contained in an animal feed or drinking water in a concentration of 5-5000 ppm, preferably 25 to 500 ppm.

For parenteral administration (intramuscular, intraperitoneal, subcutaneous and intravenous use) a sterile injectable solution of the active ingredient is usually prepared.

Solutions of a therapeutic compound of the present invention in either sesame or peanut oil or in aqueous propylene glycol may be employed. The aqueous solutions should be suitably adjusted and buffered, preferably at a pH of greater than 8, if necessary and the liquid diluent first rendered isotonic. These aqueous solutions are suitable intravenous injection purposes. The oily solutions are suitable for intraarticular, intramuscular and subcutaneous injection purposes. The preparation of all these solutions under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art. In the case of animals, compounds can be administered intramuscularly or subcutaneously at dosage levels of about 0.1 to 50 mg/kg/day, advantageously 0.2 to 10 mg/kg/day given in a single dose or up to 3 divided doses.

For topical ocular administration, direct application to the affected eye may be employed in the form of a formulation as eyedrops, aerosol, gels or ointments, or can be incorporated into collagen (such as poly-2-hydroxyethylmethacrylate and co-polymers thereof), or a hydrophilic polymer shield. The materials can also be applied as a contact lens or via a local reservoir or as a subconjunctival formulation.

For intraorbital administration a sterile injectable solution of the active ingredient is usually prepared. Solutions of a therapeutic compound of the present invention in an aqueous solution or suspension (particle size less than 10 micron) may be employed. The aqueous solutions should be suitably adjusted and buffered, preferably at a pH between 5 and 8, if necessary and the liquid diluent first rendered isotonic. Small amounts of polymers can be added to increase viscosity or for sustained release (such as cellulosic polymers, Dextran, polyethylene glycol, or alginic acid). These solutions are suitable for intraorbital injection purposes. The preparation of all these solutions under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art. In the case of animals, compounds can be administered intraorbitally at dosage levels of about 0.1 to 50 mg/kg/day, advantageously 0.2 to 10 mg/kg/day given in a single dose or up to 3 divided doses.

The active compounds of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

For intranasal administration or administration by inhalation, the active compounds of the invention are conveniently delivered in the form of a solution or suspension from a pump spray container that is squeezed or pumped by the patient or as an aerosol spray presentation from a pressurized container or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container or

nebulizer may contain a solution or suspension of the active compound. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

The following Preparations and Examples illustrate the preparation of the compounds of the present invention. Melting points are uncorrected. NMR data are reported in parts per million (δ) and are referenced to the deuterium lock signal from the sample solvent (deuteriochloroform unless otherwise specified). Commercial reagents were utilized without further purification. THF refers to tetrahydrofuran. DMF refers to N,N-dimethylformamide. Chromatography refers to column chromatography performed using 32-63 mm silica gel and executed under nitrogen pressure (flash chromatography) conditions. Room or ambient temperature refers to 20-25°C. All non-aqueous reactions were run under a nitrogen atmosphere for convenience and to maximize yields. Concentration at reduced pressure means that a rotary evaporator was used.

Preparation 1:

4-(4-Fluorophenoxy)thiophenol

Lithium aluminum hydride (9.95 grams, 0.26 mole) was added in portions to a stirred solution of 4-(4-fluorophenoxy)benzenesulfonylchloride (30 grams, 0.105 mole) in tetrahydrofuran (700 mL). The resulting mixture was heated at reflux for 1.5 hours, cooled in an ice bath and quenched by addition of 10% aqueous sulfuric acid solution (100 mL). After stirring for 30 minutes, the mixture was filtered through Celite™ and the tetrahydrofuran was removed under vacuum. The residue was diluted with water and extracted with diethyl ether. The organic layer was washed with water and brine, dried over magnesium sulfate and concentrated under vacuum to provide the title compound as a white solid (23 grams, 100%).

Preparation 2

4'-Fluorobiphenyl-4-thiol

Lithium aluminum hydride (0.95 grams, 25 mmole) was added in portions to a stirred solution of 4'-fluorobiphenyl-4-sulfonylchloride (2.7 grams, 10 mmole) in tetrahydrofuran (75 mL). The resulting mixture was heated at reflux for 4 hours, cooled in an ice bath and quenched by addition of 10% aqueous sulfuric acid solution (100 mL). After stirring for 30 minutes, the mixture was filtered through Celite™ and the tetrahydrofuran was removed under vacuum. The residue was diluted with water and extracted with diethyl ether. The organic layer was washed with water and brine, dried over magnesium sulfate and concentrated under vacuum to a solid. Trituration of the solid with diethyl ether, removal of

insoluble material by filtration and concentration of the filtrate provided the title compound as a yellow solid (1.4 grams, 69%).

Preparation 3

4-(4-Chlorophenoxy)thiophenol

Lithium aluminum hydride (6.5 grams, 0.17 mole) was added in portions, maintaining gentle reflux, to a stirred solution of 4-(4-chlorophenoxy)benzenesulfonyl-chloride (20.5 grams, 68 mmole) in tetrahydrofuran (400 mL). The resulting mixture was stirred at room temperature for 2 hours, cooled in an ice bath and quenched by addition of 10% aqueous sulfuric acid solution (100 mL). After stirring for 30 minutes, the mixture was diluted with water and extracted with diethyl ether. The organic layer was washed with water and brine, dried over magnesium sulfate and concentrated under vacuum to provide the title compound as a white solid (15.9 grams, 99%).

Example 1

3-EXO-[4-(4-FLUOROPHENOXY)BENZENESULFONYLAMINO]-8-OXA-BICYCLO[3.2.1]-OCTANE-3-CARBOXYLIC ACID HYDROXYAMIDE

A) **3-(Benzhydrylideneamino)-8-oxabicyclo[3.2.1]octane-3-carboxylic acid ethyl ester**

To a suspension of sodium hydride (0.41 grams, 17.1 mmole) in N,N-dimethylformamide (50 mL) at 0°C was added dropwise a solution of N-diphenylmethylene glycine ethyl ester (2.07 grams, 7.8 mmole) in N,N-dimethylformamide (50 mL). After stirring for 30 minutes at room temperature, a solution of cis-2,5-bis(hydroxymethyl)-tetrahydrofuran ditosylate (4.1 grams, 9.3 mmole) in N,N-dimethylformamide (50 mL) was added dropwise. The reaction mixture was gradually heated to 100°C in an oil bath and stirred at this temperature overnight. The solvent was evaporated under vacuum and the residue was taken up in water and extracted twice with diethyl ether. The combined organic extracts were washed with brine, dried over magnesium sulfate and concentrated to a brown oil, from which the title compound (1.42 grams, 51%, a 3:1 mixture of exo/endo diastereomers) was isolated by chromatography on silica gel (20% ethyl acetate in hexane as eluant).

B) **3-Amino-8-oxabicyclo[3.2.1]octane-3-carboxylic acid ethyl ester hydrochloride**

A two-phase mixture of 3-(benzhydrylideneamino)-8-oxabicyclo[3.2.1]octane-3-carboxylic acid ethyl ester (1.4 grams, 3.9 mmole) in aqueous 1N hydrochloric acid solution (100 mL) and diethyl ether (100 mL) was stirred at room temperature overnight. The aqueous layer was concentrated to provide the title compound (0.70 grams, 78%, a 3:1 mixture of exo/endo diastereomers) as a pale yellow solid.

C) **3-exo-[4-(4-Fluorophenoxy)benzenesulfonylamino]-8-oxabicyclo[3.2.1]octane-3-carboxylic acid ethyl ester**

A solution of 3-amino-8-oxabicyclo[3.2.1]octane-3-carboxylic acid ethyl ester hydrochloride (690 mg, 2.9 mmole), 4-(4-fluorophenoxy)benzenesulfonylchloride (923 mg, 3.2 mmole) and triethylamine (0.9 mL, 6.5 mmole) in N,N-dimethylformamide (45 mL) was stirred at room temperature overnight. The solvent was removed under vacuum and the residue was taken up in saturated aqueous sodium bicarbonate solution. After extracting twice with methylene chloride, the combined organic layers were washed with brine, dried over magnesium sulfate and concentrated to a brown oil. The title compound (492 mg, 38%) was isolated by chromatography on silica using 1% methanol in methylene chloride as eluant.

D) **3-exo-[4-(4-Fluorophenoxy)benzenesulfonylamino]-8-oxabicyclo[3.2.1]octane-3-carboxylic acid**

Sodium hydroxide (1.5 grams, 38 mmole) was added to a solution of 3-exo-[4-(4-fluorophenoxy)benzenesulfonylamino]-8-oxabicyclo[3.2.1]octane-3-carboxylic acid ethyl ester (492 mg, 1.09 mmole) in a mixture of ethanol (10 mL) and water (10 mL). The mixture was heated at reflux for 6 days, cooled and acidified with aqueous 1N hydrochloric acid solution. The mixture was extracted with ethyl acetate and the organic layer was washed with brine, dried over magnesium sulfate and concentrated to provide the title compound (411 mg, 89%) as a tan foam.

E) **3-exo-[4-(4-Fluorophenoxy)benzenesulfonylamino]-8-oxabicyclo[3.2.1]octane-3-carboxylic acid benzyloxyamide**

To a solution of 3-exo-[4-(4-fluorophenoxy)benzenesulfonylamino]-8-oxabicyclo-[3.2.1]octane-3-carboxylic acid (411 mg, 0.98 mmole) and triethylamine (0.19 mL, 1.36 mmole) in N,N-dimethylformamide (30 mL) was added (benzotriazol-1-yloxy)tris-(dimethylamino)phoshonium hexafluoroborate (474 mg, 1.07 mmole). After stirring at room temperature for 1 hour, additional triethylamine (0.22 mL, 1.58 mmole) and O-benzylhydroxylamine hydrochloride (187 mg, 1.17 mmole) were added. The reaction mixture was stirred for 1 day at room temperature and then for 1 day at 50°C. After concentration under vacuum, the residue was dissolved in ethyl acetate and washed sequentially with aqueous 1N hydrochloric acid solution, saturated aqueous sodium bicarbonate solution, and brine. The solution was dried over magnesium sulfate and concentrated to an oil from which the title compound, a white solid (237 mg, 46%) was isolated by chromatography (50% ethyl acetate in hexane as eluant).

F) **3-exo-[4-(4-Fluorophenoxy)benzenesulfonylamino]-8-oxa-bicyclo[3.2.1]octane-3-carboxylic acid hydroxyamide**

A solution of 3-exo-[4-(4-fluorophenoxy)benzenesulfonylamino]-8-oxabicyclo-[3.2.1]octane-3-carboxylic acid benzyloxyamide (237 mg, 0.45 mmole) in methanol (25 mL) was treated with 5% palladium on barium sulfate (150 mg) and hydrogenated at 3 atmospheres pressure for 4 hours in a Parr ™ shaker. The catalyst was removed by passage through a 0.45 µm nylon filter and the filtrate was concentrated to a white foam. Crystallization from methylene chloride provided the title compound as a white solid (62 mg, 32%). A second crop (62 mg, 32%) was obtained by crystallization from ethyl acetate/hexane.

M.p. 138-141°C. ^{1}H NMR (d_6-DMSO): δ 10.50 (br s, 1 H), 8.56 (br s, 1 H), 7.67 (d, J = 8.7 Hz, 2 H), 7.66 (br s, 1 H, overlapped), 7.26-7.22 (m, 2 H), 7.16-7.12 (m, 2 H), 7.01 (d, J = 8.5 Hz, 2 H), 4.09 (br s, 2 H), 2.32 (d, J = 14.1 Hz, 2 H), 1.68-1.63 (m, 4 H), 1.51-1.48 (m, 2 H). MS: 435 m/e (M–H). Further confirmation of structure and stereochemistry was carried out by single crystal X-ray crystallography.

Example 2

3-EXO-[4-(4-FLUOROPHENOXY)BENZENESULFONYLMETHYL]-8-OXABICYCLO-[3.2.1]-OCTANE-3-CARBOXYLIC ACID HYDROXYAMIDE

A) **8-Oxabicyclo[3.2.1]octane-3,3-dicarboxylic acid diethyl ester**

Sodium hydride (2.28 grams, 95 mmole) was added in portions to a stirred solution of diethyl malonate (15 mL, 99 mmole) in N,N-dimethylformamide (400 mL). The mixture was stirred for 45 minutes at which time evolution of hydrogen was complete. A solution of cis-2,5-bis(hydroxymethyl)tetrahydrofuran ditosylate (19.0 grams, 43 mmole) in N,N-dimethylformamide (400 mL) was then added dropwise. The mixture was heated in an oil bath at 140°C overnight. After cooling to room temperature, the mixture was quenched by addition of saturated aqueous ammonium chloride solution and concentrated under vacuum. The residual oil was taken up in water and extracted with diethyl ether. The organic extract was washed with water and brine, dried over magnesium sulfate and concentrated to an oil. Distillation under vacuum afforded the title compound (7.8 grams, 71%) as a clear oil.

B) **3-exo-Hydroxymethyl-8-oxabicyclo[3.2.1]octane-3-carboxylic acid ethyl ester**

A 1.2 M solution of diisobutylaluminum hydride in toluene (62.5 mL, 75 mmole) was added dropwise to a solution of 8-oxabicyclo[3.2.1]octane-3,3-dicarboxylic acid diethyl ester (7.8 grams, 30 mmole) in toluene (80 mL) at -40°C. The mixture was allowed to warm to 0°C while stirring for a period of 3 hours. It was then cooled to -15°C and ethanol (8 mL) was

added slowly while maintaining this temperature. After stirring at -15°C for 1 hour, sodium borohydride (1.1 grams, 30 mmole) was added. The mixture was stirred at room temperature overnight and was quenched by dropwise addition of saturated aqueous sodium sulfate solution. Ethyl acetate was added and, after stirring for 20 minutes, the insoluble material was removed by filtration through Celite™. The filtrate was washed with brine, dried over magnesium sulfate and concentrated to afford the title compound (5.1 grams, 80%) as a clear oil.

C) **3-exo-Hydroxymethyl-8-oxabicyclo[3.2.1]octane-3-carboxylic acid**

Lithium hydroxide hydrate (2.5 grams, 59.5 mmole) was added to a solution of 3-exo-hydroxymethyl-8-oxabicyclo[3.2.1]octane-3-carboxylic acid ethyl ester (5.1 grams, 23.8 mmole) in a mixture of methanol (25 mL), tetrahydrofuran (25 mL) and water (2.5 mL). The mixture was heated at reflux overnight, cooled and quenched by addition of Amberlite IR-120™ ion exchange resin. After stirring for 20 minutes, the resin was removed by filtration, washing with tetrahydrofuran. Evaporation of the solvents and trituration of the residue with diethyl ether afforded the title compound (2.35 grams, 53%) as a white solid.

D) **3',8-Dioxaspiro[bicyclo[3.2.1]octane-3,1'-cyclobutane]-2'-one**

Benzenesulfonylchloride (1.7 mL, 13.5 mmole) was added dropwise to a solution of 3-exo-hydroxymethyl-8-oxabicyclo[3.2.1]octane-3-carboxylic acid (2.3 grams, 12.3 mmole), triethylamine (3.4 mL, 24.7 mmole) and 4-dimethylaminopyridine (300 mg, 2.5 mmole) in methylene chloride (50 mL) at 0°C. The mixture was stirred at 0°C for 1 hour, diluted with methylene chloride and washed with aqueous 1N hydrochloric acid solution, saturated aqueous sodium bicarbonate solution and brine. After drying over magnesium sulfate, the solvent was evaporated to provide the title compound as a white solid (1.8 grams, 90%).

E) **3-exo-[4-(4-Fluorophenoxy)phenylsulfanylmethyl]-8-oxabicyclo[3.2.1]octane-3-carboxylic acid**

A solution of 4-(4-fluorophenoxy)thiophenol (2.2 grams, 10 mmole) in tetrahydrofuran (10 mL) was added dropwise to a slurry of sodium hydride (270 mg, 11.3 mmole) in tetrahydrofuran (20 mL) at -10°C. The mixture was allowed to warm to room temperature while stirring for 30 minutes. After cooling again to -10°C, a solution of 3',8-dioxaspiro[bicyclo[3.2.1]octane-3,1'-cyclobutane]-2'-one (1.8 grams, 10 mmole) in tetrahydrofuran (20 mL) was added dropwise. The cooling bath was removed and stirring was continued at room temperature for 2 hours after which the mixture was quenched with aqueous 1N hydrochloric acid solution and extracted twice with methylene chloride. The combined organic extracts were washed with water and brine, dried over magnesium sulfate and concentrated to a solid. Recrystallization from diethyl ether/hexane afforded the title

compound (1.8 grams (47%) as a white solid. Concentration of the mother liquor followed by chromatography on silica gel (2% methanol in chloroform as eluant) gave more of the the title compound (500 mg, 13%).

F) **3-exo-[4-(4-Fluorophenoxy)phenylsulfanylmethyl]-8-oxabicyclo[3.2.1]octane-3-carboxylic acid benzyloxyamide**

To a solution of 3-exo-[4-(4-fluorophenoxy)benzenesulfanylmethyl]-8-oxabicyclo-[3.2.1]octane-3-carboxylic acid (1.0 grams, 2.6 mmole) and diisopropylethylamine (0.5 mL, 2.9 mmole) in N,N-dimethylformamide (20 mL) was added (benzotriazol-1-yloxy)tris-(dimethylamino)phoshonium hexafluoroborate (1.2 grams, 2.7 mmole). After stirring at room temperature for 2.5 hours, additional diisopropylethylamine (0.86 mL, 4.9 mmole) and O-benzylhydroxylamine hydrochloride (525 mg, 3.3 mmole) were added. The reaction mixture was stirred for 16 hours at 50°C. After concentration under vacuum, the residue was dissolved in ethyl acetate and washed sequentially with aqueous 1N hydrochloric acid solution, saturated aqueous sodium bicarbonate solution, and brine. The solution was dried over magnesium sulfate and concentrated to an oil from which the title compound, a white foam (405 mg, 32%) was isolated by chromatography (30% ethyl acetate in hexane as eluant).

G) **3-exo-[4-(4-Fluorophenoxy)phenylsulfonylmethyl]-8-oxabicyclo[3.2.1]octane-3-carboxylic acid benzyloxyamide**

Solid 57-85% meta-chloroperbenzoic acid (283 mg) was added to a solution of 3-exo-[4-(4-fluorophenoxy)phenylsulfanylmethyl]-8-oxabicyclo[3.2.1]octane-3-carboxylic acid benzyloxyamide in methylene chloride (15 mL). The resulting mixture was stirred at room temperature overnight, and was then quenched by addition of saturated aqueous sodium bisulfite solution. After dilution with methylene chloride, the organic layer was separated and washed with saturated aqueous sodium bicarbonate solution, water and brine. The organic layer was dried over magnesium sulfate and concentrated to give the title compound as a white foam (390 mg, 90%).

H) **3-exo-[4-(4-Fluorophenoxy)benzenesulfonylmethyl]-8-oxabicyclo-[3.2.1]-octane-3-carboxylic acid hydroxyamide**

A solution of 3-exo-[4-(4-fluorophenoxy)benzenesulfonylmethyl]-8-oxabicyclo-[3.2.1]octane-3-carboxylic acid benzyloxyamide (390 mg, 0.74 mmole) in methanol (20 mL) was treated with 5% palladium on barium sulfate (195 mg) and hydrogenated at 3 atmospheres pressure for 3.5 hours in a Parr ™ shaker. The catalyst was removed by passage through a 0.45 µm nylon filter and the filtrate was concentrated to a white foam. Crystallization from a mixture of ethyl acetate and hexane provided the title compound as a white solid (230 mg, 71%).

M.p. 134-139°C. ^{1}H NMR (d_6-DMSO): δ 8.55 (br s, 1 H), 7.76 (d, J = 7.5 Hz, 2 H), 7.30-7.26 (m, 2 H), 7.20-7.16 (m, 2 H), 7.09 (d, J = 7.5 Hz, 2 H), 4.13 (br s, 2 H), 3.40 (s, 2 H), 2.24 (d, J = 14.3 Hz, 2 H), 1.78-1.73 (m, 4 H), 1.57-1.55 (m, 2 H). MS m/e 434 (M–H). Further confirmation of structure and stereochemistry was carried out by single crystal X-ray crystallography.

Example 3

3-(4-PHENOXYBENZENESULFONYLMETHYL)-8-OXABICYCLO[3.2.1]OCTANE-3-CARBOXYLIC ACID HYDROXYAMIDE

Prepared according to the same procedure as Example 2, using 4-phenoxyphenylthiophenol in step E.

^{1}H NMR (d_6-DMSO): δ 8.54 (br s, 1 H), 7.75 (d, J = 8.9 Hz, 2 H), 7.44-7.40 (m, 2 H), 7.23 7.21 (m, 1 H), 7.11-7.07 (m, 4 H), 4.11 (br s, 2 H), 3.38 (s, 2 H), 2.22 (d, J = 14.3 Hz, 2 H), 1.80-1.70 (m, 4 H), 1.60-1.50 (m, 2 H). MS m/e 416 (M–H).

Example 4

3-*EXO*-(4'-FLUOROBIPHENYL-4-SULFONYLMETHYL)-8-OXABICYCLO[3.2.1]-OCTANE-3-CARBOXYLIC ACID HYDROXYAMIDE

Prepared according to the same procedure as Example 2 using 4'-fluorobiphenyl-4-thiol in step E.

^{1}H NMR (d_6-DMSO): δ 10.60 (br s, 1 H), 8.58 (br s, 1 H), 7.88-7.85 (m, 4 H), 7.81-7.78 (m, 2 H), 7.36-7.31 (m, 2 H), 4.13 (br s, 2 H), 3.47 (s, 2 H), 2.25 (d, J = 14.5 Hz, 2 H), 1.80-1.76 (m, 4 H), 1.60-1.55 (m, 2 H). MS m/e 418 (M–H).

Example 5

3-*EXO*-[4-(4-CHLOROPHENOXY)BENZENESULFONYLMETHYL]-8-OXA-BICYCLO[3.2.1] OCTANE-3-CARBOXYLIC ACID HYDROXYAMIDE

A) **3-*exo*-[4-(4-Chlorophenoxy)phenylsulfanylmethyl]-8-oxabicyclo[3.2.1]-octane-3-carboxylic acid**

4-(4-Chlorophenoxy)thiophenol (2.07 grams, 6.8 mmole) was added to a slurry of sodium hydride (180 mg, 7.5 mmole) in tetrahydrofuran (50 mL) room temperature. The mixture was allowed to stir at room temperature for 45 minutes. Solid 3',8-dioxaspiro[bicyclo[3.2.1]octane-3,1'-cyclobutane]-2'-one (1.04 grams, 6.2 mmole) was added and the reaction was stirred at room temperature overnight. The mixture was quenched with aqueous 1N hydrochloric acid solution and extracted twice with methylene chloride. The combined organic extracts were washed with brine, dried over magnesium sulfate and concentrated to a solid. Trituration with diethyl ether afforded, after filtration, the title compound as a white solid (1.47 grams, 59%).

B) **3-*exo*-[4-(4-Chlorophenoxy)phenylsulfanylmethyl]-8-oxabicyclo[3.2.1]-octane-3-carboxylic acid hydroxyamide**

To a slurry of 3-*exo*-[4-(4-chlorophenoxy)phenylsulfanylmethyl]-8-oxabicyclo-[3.2.1]octane-3-carboxylic acid (1.47 grams, 3.63 mmole) in methylene chloride (20 mL) at room temperature was added dropwise oxalyl chloride (0.8 mL, 9.2 mmole) and N,N-dimethylformamide (1 drop). The mixture was stirred at room temperature overnight. After evaporation of volatiles under vacuum, the residue was dissolved in methylene chloride (20 mL), cooled to 0°C and treated dropwise with O-trimethylsilylhydroxylamine (1.35 mL, 11.0 mmole). The resulting mixture was stirred at room temperature for 3.5 hours, cooled in an ice bath and quenched by addition of aqueous 1N hydrochloric acid solution, stirring at 0°C for an additional 30 minutes. Following dilution with ethyl acetate, the organic layer was separated, washed with water and brine, dried over magnesium sulfate and concentrated to afford the title compound as a white foam (1.52 grams, 100%).

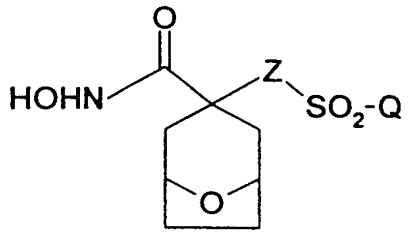
C) **3-*exo*-[4-(4-Chlorophenoxy)benzenesulfonylmethyl]-8-oxabicyclo[3.2.1] octane-3-carboxylic acid hydroxyamide**

Oxone™ (4.2 grams, 8.63 mmole) was added to a solution of 3-*exo*-[4-(4-chlorophenoxy)phenyl-sulfanylmethyl]-8-oxabicyclo[3.2.1]octane-3-carboxylic acid hydroxy-amide (1.52 grams, 3.63 mmole) in a mixture of water (30 mL), methanol (40 mL) and tetrahydrofuran (12 mL). The resulting mixture was stirred at room temperature overnight, diluted with water and extracted twice with ethyl acetate. The combined organic extracts were washed with brine, dried over magnesium sulfate and concentrated to a foam from which the title compound (846 mg, 52%) was isolated by chromatography on silica gel (4% methanol in chloroform as eluant).

^{1}H NMR (d_6-DMSO): δ 10.58 (br s, 1 H), 8.53 (br s, 1 H), 7.76 (d, J = 8.6 Hz, 2 H), 7.46 (d, J = 8.6 Hz, 2 H), 7.15-7.11 (m, 4 H), 4.11 (br s, 2 H), 3.40 (s, 2 H), 2.22 (d, J = 14.3 Hz, 2 H), 1.76-1.71 (m, 4 H), 1.57-1.55 (m, 2 H). MS m/e 450 (M–H).

CLAIMS

1. A compound of the formula



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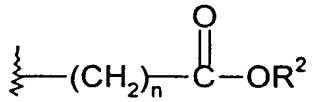
wherein Z is $>CH_2$ or $>NR^1$;

R^1 is hydrogen, (C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_2-C_9)heteroaryl(C_1-C_6)alkyl or a group of the formula



;

n is an integer from one to six;

R^2 is hydrogen or (C_1-C_6)alkyl;

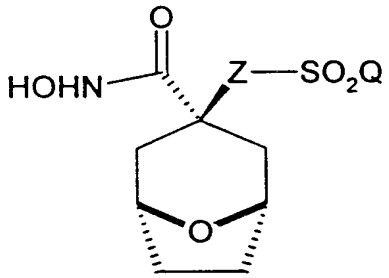
Q is (C_1-C_6)alkyl, (C_6-C_{10})aryl, (C_2-C_9)heteroaryl, (C_6-C_{10})aryloxy(C_1-C_6)alkyl, (C_6-C_{10})aryloxy(C_6-C_{10})aryl, (C_6-C_{10})aryloxy(C_2-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_6-C_{10})aryl(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_2-C_9)heteroaryl, (C_6-C_{10})aryl(C_6-C_{10})aryl(C_1-C_6)alkyl, (C_6-C_{10})aryl(C_6-C_{10})aryl(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_6-C_{10})aryl(C_2-C_9)heteroaryl, (C_2-C_9)heteroaryl(C_1-C_6)alkyl, (C_2-C_9)heteroaryl(C_6-C_{10})aryl, (C_2-C_9)heteroaryl(C_2-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkoxy(C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkoxy(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_1-C_6)alkoxy(C_2-C_9)heteroaryl, (C_2-C_9)heteroaryloxy(C_1-C_6)alkyl, (C_2-C_9)heteroaryloxy(C_6-C_{10})aryl, (C_2-C_9)heteroaryloxy(C_2-C_9)heteroaryl, (C_2-C_9)heteroaryl(C_1-C_6)alkoxy(C_1-C_6)alkyl, (C_2-C_9)heteroaryl(C_1-C_6)alkoxy(C_6-C_{10})aryl, (C_2-C_9)heteroaryl(C_1-C_6)alkoxy(C_2-C_9)heteroaryl, (C_6-C_{10})aryloxy(C_1-C_6)alkyl(C_6-C_{10})aryl, (C_6-C_{10})aryloxy(C_1-C_6)alkyl(C_2-C_9)heteroaryl, (C_2-C_9)heteroaryloxy(C_1-C_6)alkyl(C_6-C_{10})aryl or (C_2-C_9)heteroaryloxy(C_1-C_6)alkyl(C_2-C_9)heteroaryl;

wherein each (C_6-C_{10})aryl or (C_2-C_9)heteroaryl moieties of said (C_6-C_{10})aryl, (C_2-C_9)heteroaryl, (C_6-C_{10})aryloxy(C_1-C_6)alkyl, (C_6-C_{10})aryloxy(C_6-C_{10})aryl, (C_6-C_{10})aryloxy(C_2-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_6-C_{10})aryl(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_2-C_9)heteroaryl, (C_6-C_{10})aryl(C_6-C_{10})aryl(C_1-C_6)alkyl, (C_6-C_{10})aryl(C_6-C_{10})aryl(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_6-C_{10})aryl(C_2-C_9)heteroaryl, (C_2-C_9)heteroaryl(C_1-C_6)alkyl, (C_2-C_9)heteroaryl(C_6-C_{10})aryl, (C_2-C_9)heteroaryl(C_2-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkoxy(C_1-C_6)alkyl, (C_6-C_{10})aryl(C_1-C_6)alkoxy(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_1-C_6)alkoxy(C_2-C_9)heteroaryl, (C_2-C_9)heteroaryloxy(C_1-C_6)alkyl, (C_2-C_9)heteroaryloxy(C_6-C_{10})aryl, (C_2-C_9)heteroaryloxy(C_2-C_9)heteroaryl, (C_2-C_9)heteroaryl(C_1-C_6)alkoxy(C_1-C_6)alkyl, (C_2-C_9)heteroaryl(C_1-C_6)alkoxy(C_6-C_{10})aryl, $(C_2-$

C_9)heteroaryl(C_1-C_6)alkoxy(C_2-C_9)heteroaryl, (C_6-C_{10})aryloxy(C_1-C_6)alkyl(C_6-C_{10})aryl, (C_6-C_{10})aryloxy(C_1-C_6)alkyl(C_2-C_9)heteroaryl, (C_2-C_9)heteroaryloxy(C_1-C_6)alkyl(C_6-C_{10})aryl or (C_2-C_9)heteroaryloxy(C_1-C_6)alkyl(C_2-C_9)heteroaryl is optionally substituted on any of the ring carbon atoms capable of forming an additional bond by one or more substituents per ring independently selected from fluoro, chloro, bromo, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, perfluoro(C_1-C_3)alkyl, perfluoro(C_1-C_3)alkoxy and (C_6-C_{10})aryloxy;

or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1, with sterochemistry as depicted by the formula



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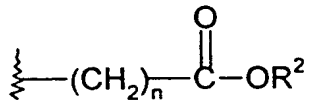
3. A compound according to claim 1, wherein Z is CH_2.

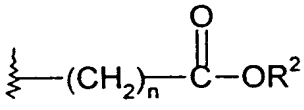
4. A compound according to claim 2, wherein Z is CH_2.

5. A compound according to claim 1, wherein Z is $>NR^1$ and R^1 is a group of the formula



and wherein n is 2.

6. A compound according to claim 2, wherein Z is $>NR^1$ and R^1 is a group of the formula



and wherein n is 2.

7. A compound according to claim 1, wherein Z is $>NR^1$ and R^1 is hydrogen.

8. A compound according to claim 2, wherein Z is $>NR^1$ and R^1 is hydrogen.

9. A compound according to claim 1, wherein Q is (C_6-C_{10})aryl, (C_2-C_9)heteroaryloxy(C_6-C_{10})aryl or (C_6-C_{10})aryloxy(C_6-C_{10})aryl, wherein each aryl or heteroaryl moiety of said (C_6-C_{10})aryl, (C_2-C_9)heteroaryloxy(C_6-C_{10})aryl or (C_6-C_{10})aryloxy(C_6-C_{10})aryl

groups may be optionally substituted with one or more substituents independently selected from fluoro, chloro, bromo, (C_1-C_6)alkyl, (C_1-C_6)alkoxy or perfluoro(C_1-C_3)alkyl.

10. A compound according to claim 2, wherein Q is (C_6-C_{10})aryl, (C_2-C_9)heteroaryloxy(C_6-C_{10})aryl or (C_6-C_{10})aryloxy(C_6-C_{10})aryl, wherein each aryl or heteroaryl moiety of said (C_6-C_{10})aryl, (C_2-C_9)heteroaryloxy(C_6-C_{10})aryl or (C_6-C_{10})aryloxy(C_6-C_{10})aryl groups may be optionally substituted with one or more substituents independently selected from fluoro, chloro, bromo, (C_1-C_6)alkyl, (C_1-C_6)alkoxy or perfluoro(C_1-C_3)alkyl.

11. A compound according to claim 3, wherein Q is (C_6-C_{10})aryl, (C_2-C_9)heteroaryloxy(C_6-C_{10})aryl or (C_6-C_{10})aryloxy(C_6-C_{10})aryl, wherein each aryl or heteroaryl moiety of said (C_6-C_{10})aryl, (C_2-C_9)heteroaryloxy(C_6-C_{10})aryl or (C_6-C_{10})aryloxy(C_6-C_{10})aryl groups may be optionally substituted with one or more substituents independently selected from fluoro, chloro, bromo, (C_1-C_6)alkyl, (C_1-C_6)alkoxy or perfluoro(C_1-C_3)alkyl.

12. A compound according to claim 5, wherein Q is (C_6-C_{10})aryl, (C_2-C_9)heteroaryloxy(C_6-C_{10})aryl or (C_6-C_{10})aryloxy(C_6-C_{10})aryl, wherein each aryl or heteroaryl moiety of said (C_6-C_{10})aryl, (C_2-C_9)heteroaryloxy(C_6-C_{10})aryl or (C_6-C_{10})aryloxy(C_6-C_{10})aryl groups may be optionally substituted with one or more substituents independently selected from fluoro, chloro, bromo, (C_1-C_6)alkyl, (C_1-C_6)alkoxy or perfluoro(C_1-C_3)alkyl.

13. A compound according to claim 7, wherein Q is (C_6-C_{10})aryl, (C_2-C_9)heteroaryloxy(C_6-C_{10})aryl or (C_6-C_{10})aryloxy(C_6-C_{10})aryl, wherein each aryl or heteroaryl moiety of said (C_6-C_{10})aryl, (C_2-C_9)heteroaryloxy(C_6-C_{10})aryl or (C_6-C_{10})aryloxy(C_6-C_{10})aryl groups may be optionally substituted with one or more substituents independently selected from fluoro, chloro, bromo, (C_1-C_6)alkyl, (C_1-C_6)alkoxy or perfluoro(C_1-C_3)alkyl.

14. A compound according to claim 8, wherein Q is (C_6-C_{10})aryl, (C_2-C_9)heteroaryloxy(C_6-C_{10})aryl or (C_6-C_{10})aryloxy(C_6-C_{10})aryl, wherein each aryl or heteroaryl moiety of said (C_6-C_{10})aryl, (C_2-C_9)heteroaryloxy(C_6-C_{10})aryl or (C_6-C_{10})aryloxy(C_6-C_{10})aryl groups may be optionally substituted with one or more substituents independently selected from fluoro, chloro, bromo, (C_1-C_6)alkyl, (C_1-C_6)alkoxy or perfluoro(C_1-C_3)alkyl.

15. A compound according to claim 1, wherein Q is phenyl, pyridyloxyphenyl or phenoxyphenyl optionally substituted with one or more substituents independently selected from fluoro, chloro, bromo, (C_1-C_6)alkyl, (C_1-C_6)alkoxy or perfluoro(C_1-C_3)alkyl.

16. A compound according to claim 2, wherein Q is phenyl, pyridyloxyphenyl or phenoxyphenyl optionally substituted with one or more substituents independently selected from fluoro, chloro, bromo, (C_1-C_6)alkyl, (C_1-C_6)alkoxy or perfluoro(C_1-C_3)alkyl.

17. A compound according to claim 3, wherein Q is phenyl, pyridyloxyphenyl or phenoxyphenyl optionally substituted with one or more substituents independently selected from fluoro, chloro, bromo, (C_1-C_6)alkyl, (C_1-C_6)alkoxy or perfluoro(C_1-C_3)alkyl.

18. A compound according to claim 5, wherein Q is phenyl, pyridyloxyphenyl or phenoxyphenyl optionally substituted with one or more substituents independently selected from fluoro, chloro, bromo, (C_1-C_6)alkyl, (C_1-C_6)alkoxy or perfluoro(C_1-C_3)alkyl.

19. A compound according to claim 7, wherein Q is phenyl, pyridyloxyphenyl or phenoxyphenyl optionally substituted with one or more substituents independently selected from fluoro, chloro, bromo, (C_1-C_6)alkyl, (C_1-C_6)alkoxy or perfluoro(C_1-C_3)alkyl.

20. A compound according to claim 8, wherein Q is phenyl, pyridyloxyphenyl or phenoxyphenyl optionally substituted with one or more substituents independently selected from fluoro, chloro, bromo, (C_1-C_6)alkyl, (C_1-C_6)alkoxy or perfluoro(C_1-C_3)alkyl.

21. A compound according to claim 1, wherein said compound is selected from the group consisting of:

3-*exo*-[4-(4-fluorophenoxy)benzenesulfonylamino]-8-oxabicyclo[3.2.1]-octane-3-carboxylic acid hydroxyamide;

3-*exo*-[4-(4-fluorophenoxy)benzenesulfonylmethyl]-8-oxabicyclo-[3.2.1]-octane-3-carboxylic acid hydroxyamide;

3-(4-phenoxybenzenesulfonylmethyl)-8-oxabicyclo[3.2.1]-octane-3-carboxylic acid hydroxyamide;

3-*exo*-(4´-fluorobiphenyl-4-sulfonylmethyl]-8-oxabicyclo-[3.2.1]-octane-3-carboxylic acid hydroxyamide; and

3-*exo*-[4-(4-chlorophenoxy)benzenesulfonylmethyl]-8-oxabicyclo[3.2.1]octane-3-carboxylic acid hydroxyamide.

22. A pharmaceutical composition for the treatment of a condition selected from the group consisting of arthritis (including osteoarthritis and rheumatoid arthritis), inflammatory bowel disease, Crohn's disease, emphysema, chronic obstructive pulmonary disease, Alzheimer's disease, organ transplant toxicity, cachexia, allergic reactions, allergic contact hypersensitivity, cancer, tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, osteoporosis, loosening of artificial joint implants, atherosclerosis (including atherosclerotic plaque rupture), aortic aneurysm (including abdominal aortic aneurysm and brain aortic aneurysm), congestive heart failure, myocardial infarction, stroke, cerebral ischemia, head trauma, spinal cord injury, neuro-degenerative disorders (acute and chronic), autoimmune disorders, Huntington's disease, Parkinson's disease, migraine, depression, peripheral neuropathy, pain, cerebral amyloid angiopathy, nootropic or cognition enhancement, amyotrophic lateral sclerosis, multiple sclerosis, ocular angiogenesis, corneal injury, macular degeneration, abnormal wound healing, burns, diabetes, tumor invasion, tumor growth, tumor metastasis, corneal scarring, scleritis, AIDS, sepsis and septic shock in a mammal, including a human, comprising an amount of a compound of claim 1 effective in such treatment and a pharmaceutically acceptable carrier.

23. A method for treating a condition selected from the group consisting of arthritis (including osteoarthritis and rheumatoid arthritis), inflammatory bowel disease, Crohn's disease, emphysema, chronic obstructive pulmonary disease, Alzheimer's disease, organ transplant toxicity, cachexia, allergic reactions, allergic contact hypersensitivity, cancer, tissue ulceration, restenosis, periodontal disease, epidermolysis bullosa, osteoporosis, loosening of artificial joint implants, atherosclerosis (including atherosclerotic plaque rupture), aortic aneurysm (including abdominal aortic aneurysm and brain aortic aneurysm), congestive heart failure, myocardial infarction, stroke, cerebral ischemia, head trauma, spinal cord injury, neuro-degenerative disorders (acute and chronic), autoimmune disorders, Huntington's disease, Parkinson's disease, migraine, depression, peripheral neuropathy, pain, cerebral amyloid angiopathy, nootropic or cognition enhancement, amyotrophic lateral sclerosis, multiple sclerosis, ocular angiogenesis, corneal injury, macular degeneration, abnormal wound healing, burns, diabetes, tumor invasion, tumor growth, tumor metastasis, corneal scarring, scleritis, AIDS, sepsis and septic shock in a mammal, including a human, comprising administering to said mammal an amount of a compound of claim 1, effective in treating such a condition.

24. A pharmaceutical composition for the treatment of a condition which can be treated by the inhibition of matrix metalloproteinases in a mammal, including a human, comprising an amount of a compound of claim 1 effective in such treatment and a pharmaceutically acceptable carrier.

25. A pharmaceutical composition for the treatment of a condition which can be treated by the inhibition of a mammalian reprolysin in a mammal, including a human, comprising an amount of a compound of claim 1 effective in such treatment and a pharmaceutically acceptable carrier.

26. A method for the inhibition of matrix metalloproteinases in a mammal, including a human, comprising administering to said mammal an effective amount of a compound of claim 1.

27. A method for the inhibition of a mammalian reprolysin in a mammal, including a human, comprising administering to said mammal an effective amount of a compound of claim 1.

INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/IB 99/00503

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D493/08 A61K31/35 //(C07D493/08,311:00,307:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 780 386 A (F. HOFFMANN-LA ROCHE AG, SWITZ.;AGOURON PHARMACEUTICALS, INC.) 1996 abstract; claims page 5, line 50 page 47; example 8 page 51 - page 52; example 11 ---	1,22-27
A	WO 96 27583 A (PFIZER ;ROBINSON RALPH P (US); RIZZI JAMES P (US)) 12 September 1996 (1996-09-12) cited in the application abstract; claims page 9, line 10 - page 11, line 6 --- -/--	1,22-27

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 August 1999	18/08/1999

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 99/00503

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,A	WO 98 30566 A (BURGESS LAURENCE EDWARD ;RIZZI JAMES PATRICK (US); PFIZER (US)) 16 July 1998 (1998-07-16) cited in the application abstract; claims page 14 - page 17; examples	1,22-27

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ternational application No.
PCT/IB 99/00503

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 23, 26, 27
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB 99/00503

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0780386 A	25-06-1997	AU 700725 B	14-01-1999
		AU 7548296 A	31-07-1997
		BR 9606134 A	03-11-1998
		CA 2193178 A	21-06-1997
		CN 1160045 A	24-09-1997
		CZ 9603740 A	14-01-1998
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		NZ 303860 A	26-08-1998
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		US 5863949 A	26-01-1999
WO 9830566 A	16-07-1998	AU 5131998 A	03-08-1998
		HR 980004 A	31-12-1998

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(25) Filing Language: English

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(74) Agents: **SPIEGEL, Allen, J.**; c/o Simpson, Alison, Urquhart-Dykes & Lord, 30 Welbeck Street, London W1M 7PG et al. (GB).

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(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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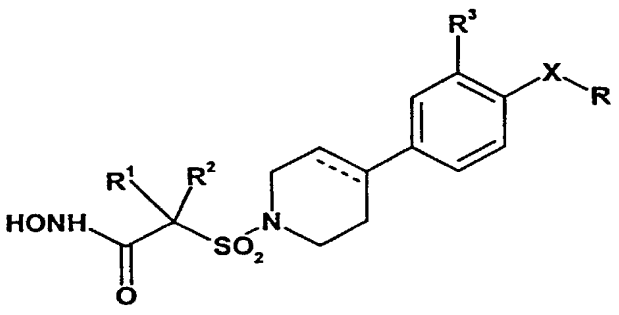
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METALLOPROTEASE INHIBITORS



(I)

(57) Abstract: Compounds of formula (I) and pharmaceutically-acceptable derivatives thereof, are matrix metalloprotease inhibitors, useful in treatment of conditions mediated by matrix metalloproteases, such as chronic dermal ulcers.

WO 00/74681 A1

METALLOPROTEASE INHIBITORS

This invention relates to a series of substituted α-aminosulphonyl-acetohydroxamic acids which are inhibitors of zinc-dependent metalloprotease enzymes. In particular, the compounds are inhibitors of certain members of the matrix metalloprotease (MMP) family.

Matrix metalloproteases (MMPs) constitute a family of structurally similar zinc-containing metalloproteases, which are involved in the remodelling and degradation of extracellular matrix proteins, both as part of normal physiological processes and in pathological conditions. Since they have high destructive potential, MMPs are usually under close regulation and failure to maintain MMP regulation has been implicated as a component of a number of diseases and conditions including pathological conditions, such as atherosclerotic plaque rupture, heart failure, restenosis, periodontal disease, tissue ulceration, cancer metastasis, tumour angiogenesis, age-related macular degeneration, fibrotic disease, rheumatoid arthritis, osteoarthritis and inflammatory diseases dependent on migratory inflammatory cells.

Another important function of certain MMPs is to activate various enzymes, including other MMPs, by cleaving the pro-domains from their protease domains. Thus some MMPs act to regulate the activities of other MMPs, so that over-production of one MMP may lead to excessive proteolysis of extracellular matrix by another. Moreover, MMPs have different substrate preferences (shown in the following Table for selected family members) and different functions within normal and pathological conditions. For recent reviews of MMPs, see Current Pharmaceutical Design, 1996, 2, 624 and Exp. Opin. Ther. Patents, 1996, 6, 1305.

TABLE

Enzyme	Other Names	Preferred Substrates
MMP-1	collagenase-1; interstitial collagenase	collagens I, II, III, VII, X; gelatins
MMP-2	gelatinase A; 72kDa gelatinase	gelatins; collagens IV, V, VII, X; elastin; fibronectin; activates pro-MMP-13
MMP-3	stromelysin-1	proteoglycans; laminin; fibronectin; gelatins
MMP-8	collagenase-2; neutrophil collagenase	collagens I, II, III
MMP-9	gelatinase B; 92kDa gelatinase	gelatins; collagens IV, V; elastin
MMP-13	collagenase-3	collagens I, II, III; gelatins
MMP-14	MT-MMP-1	activates pro-MMP-2 & 13; gelatins

Excessive production of MMP-3 is thought to be responsible for pathological tissue breakdown which underlies a number of diseases and conditions. For example, MMP-3 has been found in the synovium

and cartilage of osteoarthritis and rheumatoid arthritis patients, thus implicating MMP-3 in the joint damage caused by these diseases: see Biochemistry, 1989, 28, 8691 and Biochem. J., 1989, 258, 115. MMP-13 is also thought to play an important role in the pathology of osteoarthritis and rheumatoid arthritis: see Lab. Invest., 1997, 76, 717 and Arthritis Rheum., 1997, 40, 1391.

The over-expression of MMP-3 has also been implicated in the tissue damage and chronicity of chronic wounds, such as venous ulcers, diabetic ulcers and pressure sores: see Brit. J. Dermatology, 1996, 135, 52. Collagenase-3 (MMP-13) has also recently been implicated in the pathology of chronic wounds (*J Invest Dermatol*, 1997, *109*, 96-101).

Furthermore, the production of MMP-3 may also cause tissue damage in conditions where there is ulceration of the colon (as in ulcerative colitis and Crohn's disease: see J. Immunol., 1997 158, 1582 and J. Clin. Pathol., 1994, 47, 113) or of the duodenum (see Am. J. Pathol., 1996, 148, 519).

Moreover, MMP-3 is also thought to be involved in skin diseases such as dystrophic epidermolysis bullosa (see Arch. Dermatol. Res., 1995, 287, 428) and dermatitis herpetiformis (see J. Invest. Dermatology, 1995, 105, 184).

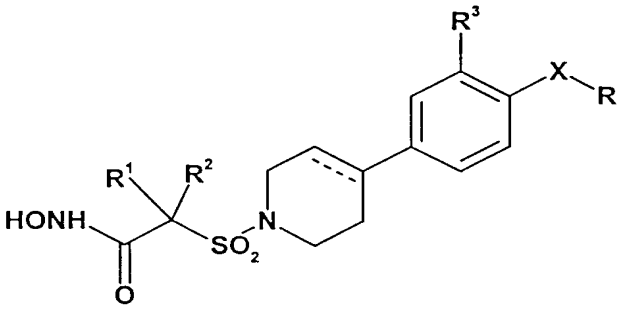
Rupture of atherosclerotic plaques by MMP-3 has also been described (see e.g. Circulation, 1997, 96, 396). Thus, MMP-3 inhibitors may find utility in the treatment of conditions caused by or complicated by embolic phenomena such as cardiac or cerebral infarctions.

Studies of human cancers have shown that MMP-2 is activated on the invasive tumour cell surface (see J. Biol.Chem., 1993, 268, 14033) and BB-94, a non-selective peptidic hydroxamate MMP inhibitor, has been reported to decrease the tumour burden and prolong the survival of mice carrying human ovarian carcinoma xenografts (see Cancer Res., 1993, 53, 2087).
Various series of MMP inhibitors have appeared in the literature which have a carbonyl moiety (CO) and a sulphone moiety (SO_2) with a two atom "spacer" interposed between them. For example, α-arylsulphonamido-substituted acetohydroxamic acids are disclosed in EP-A-0606046, WO-A-9627583 and WO-A-9719068, whilst EP-A-0780386 discloses certain related sulphone-substituted hydroxamic acids.

The compounds of the present invention represent a new class of compounds, and are inhibitors of some of the members of the MMP family. In particular, they are inhibitors of MMP-3 and/or MMP-13, with certain compounds exhibiting varying degrees of selectivity over other MMPs, such as MMP-1, MMP-2, MMP-9 and MMP-14. Thus they may be of utility in treating diseases and conditions mediated by MMPs, in particular MMP-3 and/or MMP-13.

A series of substances related to the instant invention were disclosed in International Patent Application number publication no. WO 99/29667, herein incorporated by reference in its entirety.

According to one aspect of the present invention ("A"), there is provided a compound of formula (I):



(I)

and pharmaceutically-acceptable salts thereof, and solvates thereof,

wherein

the dotted line represents an optional bond,

X is a monocyclic aromatic linker moiety selected from phenylene, pyridinylene, pyrazolylene, thiazolylene, thienylene, furylene, pyrimidinylene, pyrazinylene, pyridazinylene, pyrrolylene, oxazolylene, isoxazolylene, oxadiazolylene, thiadiazolylene, imidazolylene, triazolylene, or tetrazolylene;

R is H, C_{1-4} alkyl optionally substituted by C_{1-4} alkoxy, NR^4R^5 or OH, or R is C_{1-4} alkoxy optionally substituted by 1 or 2 substituents selected from (C_{1-4} alkyl optionally substituted by OH), C_{1-4} alkoxy, OH and NR^4R^5;

R^1 and R^2 are each independently H, C_{1-6} alkyl optionally substituted by OH or C_{1-4} alkoxy, or C_{2-6} alkenyl;

or R^1 and R^2 are taken together, with the C atom to which they are attached, to form a 3- to 7-membered ring optionally incorporating a hetero- moiety selected from O, S, SO, SO_2 and NR^6, and which 3- to 7-membered ring is optionally substituted by one or more OH;

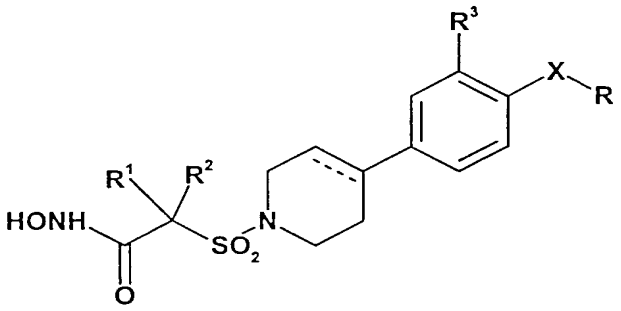
R^3 is H, halo, methyl, or methoxy;

R^4 and R^5 are each independently H or C_1 to C_6 alkyl optionally substituted by OH, C_1 to C_4 alkoxy or aryl,

or R^4 and R^5 can be taken together with the N atom to which they are attached , to form a 3- to 7-membered ring, optionally incorporating a further hetero- moiety selected from O, S, SO_2 and NR^7; and

R^6 and R^7 are each independently H or C_1 to C_4 alkyl.

According to a further aspect of the invention ("B"), there is provided a compound of formula (I):



(I)

and pharmaceutically-acceptable salts thereof, and solvates thereof,

wherein

the dotted line represents an optional bond;

X is a monocyclic aromatic linker moiety selected from pyrazolylene, thiazolylene, pyrazinylene, pyridazinylene, pyrrolylene, oxazolylene, isoxazolylene, oxadiazolylene, thiadiazolylene, imidazolylene, triazolylene, or tetrazolylene;

R is H, C_{1-4} alkyl optionally substituted by C_{1-4} alkoxy or NR^4R^5 or OH, or

C_{1-4} alkoxy optionally substituted by 1 or 2 substituents selected from (C_{1-4} alkyl optionally substituted by OH), C_{1-4} alkoxy, OH and NR^4R^5;

R^1 and R^2 are each independently H, C_{1-6} alkyl optionally substituted by OH or C_{1-4} alkoxy, or C_{2-6} alkenyl;

or R^1 and R^2 are taken, together with the C atom to which they are attached, to form a 3- to 7-membered ring optionally incorporating a hetero- moiety selected from O, S, SO, SO_2 and NR^6, and which 3- to 7-membered ring is optionally substituted by one or more OH;

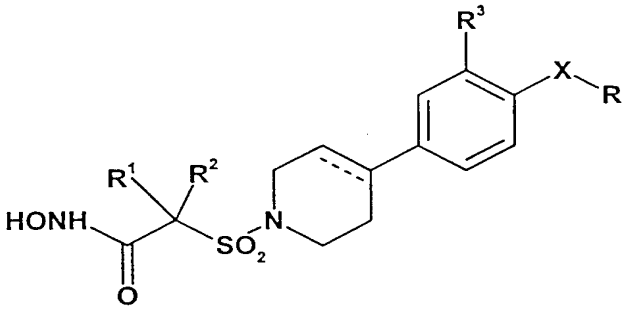
R^3 is H, halo, methyl, or methoxy;

R^4 and R^5 are each independently H or C_1 to C_6 alkyl optionally substituted by OH, C_1 to C_4 alkoxy or aryl,

or R^4 and R^5 can be taken together with the N atom to which they are attached , to form a 3- to 7-membered ring, optionally incorporating a further hetero- moiety selected from O, S, SO_2 and NR^7; and

R^6 and R^7 are each independently H or C_1 to C_4 alkyl.

According to a further aspect of the invention ("C") there is provided a compound of formula (I):



(I)

and pharmaceutically-acceptable salts thereof, and solvates thereof,

wherein

the dotted line represents an optional bond;

X is a monocyclic aromatic linker moiety selected from phenylene, pyridinylene, pyrazolylene, thiazolylene, thienylene, furylene, pyrimidinylene, pyrazinylene, pyridazinylene, pyrrolylene, oxazolylene, isoxazolylene, oxadiazolylene, thiadiazolylene, imidazolylene, triazolylene, or tetrazolylene;

R is C_{1-4} alkyl substituted by NR^4R^5, C_{1-4} alkoxy substituted by NR^4R^5, or C_{1-4} alkoxy substituted by 2 substituents selected from (C_{1-4} alkyl optionally substituted by OH), C_{1-4} alkoxy, OH and NR^4R^5;

R^1 and R^2 are each independently H, C_{1-6} alkyl optionally substituted by OH or C_{1-4} alkoxy, or C_{2-6} alkenyl;

or R^1 and R^2 are taken together, with the C atom to which they are attached, to form a 3- to 7-membered ring optionally incorporating a hetero- moiety selected from O, S, SO, SO_2 and NR^6, and which 3- to 7-membered ring is optionally substituted by one or more OH;

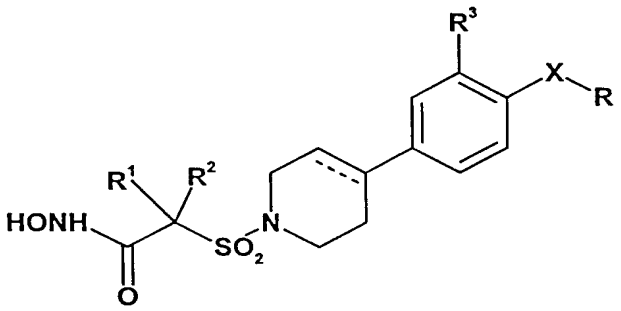
R^3 is H, halo, methyl, or methoxy;

R^4 and R^5 are each independently H or C_1 to C_6 alkyl optionally substituted by OH, C_1 to C_4 alkoxy or aryl,

or R^4 and R^5 can be taken together with the N atom to which they are attached , to form a 3- to 7-membered ring, optionally incorporating a further hetero- moiety selected from O, S, SO_2 and NR^7; and

R^6 and R^7 are each independently H or C_1 to C_4 alkyl.

According to a further aspect of the invention ("D") there is provided a compound of formula (I):



(I)

and pharmaceutically-acceptable salts thereof, and solvates thereof,

wherein

the dotted line represents an optional bond,

X is a monocyclic aromatic linker moiety selected from phenylene, pyridinylene, pyrazolylene, thiazolylene, thienylene, furylene, pyrimidinylene, pyrazinylene, pyridazinylene, pyrrolylene, oxazolylene, isoxazolylene, oxadiazolylene, thiadiazolylene, imidazolylene, triazolylene, or tetrazolylene;

R is H, C_{1-4} alkyl optionally substituted by C_{1-4} alkoxy, NR^4R^5 or OH, or

C_{1-4} alkoxy optionally substituted by 1 or 2 substituents selected from (C_{1-4} alkyl optionally substituted by OH), C_{1-4} alkoxy, OH and NR^4R^5;

R^1 and R^2 are each independently C_{1-6} alkyl substituted by OH;

or R^1 and R^2 are taken together, with the C atom to which they are attached, to form a 3- to 7-membered ring optionally incorporating a hetero- moiety selected from O, S, SO, SO_2 and NR^6, and which 3- to 7-membered ring is substituted by one or more OH;

R^3 is H, halo, methyl, or methoxy;

R^4 and R^5 are each independently H or C_1 to C_6 alkyl optionally substituted by OH, C_1 to C_4 alkoxy or aryl,

or R^4 and R^5 can be taken together with the N atom to which they are attached , to form a 3- to 7-membered ring, optionally incorporating a further hetero- moiety selected from O, S, SO_2 and NR^7; and

R^6 and R^7 are each independently H or C_1 to C_4 alkyl.

In all the above definitions A, B, C and D, unless otherwise indicated, alkyl, alkenyl, alkoxy, etc. groups having three or more carbon atoms may be straight chain or branched chain.

All the compounds of formula (I) in aspects A, B, C and D above may contain one or more chiral centres and therefore can exist as stereoisomers, i.e. as enantiomers or diastereoisomers, as well as mixtures thereof. The invention includes both the individual stereoisomers of the compounds of formula (I) and any mixture thereof. Separation of diastereoisomers may be achieved by conventional techniques, e.g. by fractional crystallisation or chromatography (including HPLC) of a diastereoisomeric mixture of a compound of formula (I) or a suitable salt or derivative thereof. An individual enantiomer of a compound of formula (I) may be prepared from a corresponding optically pure intermediate or by resolution, either by HPLC of the racemate using a suitable chiral support or, where appropriate, by fractional crystallisation of the diastereoisomeric salts formed by reaction of the racemate with a suitable optically active base or acid, as appropriate to the specific compound to be resolved. Furthermore, compound of formula (I) which contain alkenyl groups can exist as cis- or trans- geometric isomers. Again, the invention includes both the separated individual geometric isomers as well as mixtures thereof. Certain of the compounds of formula (I) may be tautomeric and all possible tautomers are included in the scope of this invention. Certain of the compounds of formula (I) may exhibit zwitterionic behaviour and all possible zwitterions are included in the scope of this invention. Also included in the invention are radiolabelled derivatives of compounds of formula (I) which are suitable for biological studies.

The pharmaceutically acceptable salts of all the compounds of the formula (I) include the acid addition and the base salts thereof. The term "pharmaceutically acceptable" means suitable for use in human or non-human animal medicine.

Suitable acid addition salts are formed from acids which form non-toxic salts and examples include the hydrochloride, hydrobromide, hydroiodide, sulphate, hydrogen sulphate, nitrate, phosphate, hydrogen phosphate, acetate, maleate, fumarate, lactate, tartrate, citrate, gluconate, succinate, benzoate, methanesulphonate, benzenesulphonate and p-toluenesulphonate salts.

Suitable base salts are formed from bases which form non-toxic salts and examples include the aluminium, calcium, lithium, magnesium, potassium, sodium, zinc, tris, meglumine, choline, olamine, diolamine, ethylenediamine, benethamine, benzathene, glucosamine, nicotinamide, ornithine, guanidine, guanine, arginine and procaine salts.

For a review on suitable salts see for example Berge et al, J. Pharm. Sci., 66, 1-19 (1977).

Solvates (e.g. hydrates) of the compounds and salts of aspects A, B, C and D of the invention are included in the invention. In some cases, the solvate may be the direct product of a reaction to make a compound or salt of the invention in a solvent, in which case no further transformation step would be necessary. In other cases, solvates may be made by methods known in the art, such as by crystallisation from a solvent.

Prodrugs of the compounds of aspects A, B, C and D of the invention, their pharmaceutically acceptable salts and solvates thereof, are also envisaged by the invention. For reference as to how to prepare prodrugs, see standard texts in this field, for example "Design of Prodrugs" ed. H.Bundgaard (1985, Elsevier, Amsterdam / New York / Oxford).

For aspects C and D of the invention, X is preferably phenylene, pyridinylene, pyrazolylene or thiazolylene.
For aspects C and D of the invention, X is more preferably 1,3-phenylene, 2,6-pyridinylene, 1,3-pyrazolylene or 2,5-thiazolylene.

For aspect B of the invention X is preferably pyrazolylene or thiazolylene.
For aspect B of the invention X is more preferably 1,3-pyrazolylene or 2,5-thiazolylene.

For aspects B and D of the invention R is preferably H, methoxy, $O(CH_2)_2OH$, $O(CH_2)_2OCH_3$, $O(CH_2)_2N(CH_3)_2$, $O(CH_2)_2NHCH_3$, $O(CH_2)_2NH_2$, CH_2NHCH_3, morpholinomethyl, 2-morpholinoethoxy, 2R-2,3-dihydroxy-1-propyloxy, 2S-2,3-dihydroxy-1-propyloxy or 1,3-dihydroxy-2-propyloxy.
For aspects B and D of the invention R is most preferably $O(CH_2)_2OH$ or $O(CH_2)_2NH_2$.

For aspect C of the invention R is preferably $O(CH_2)_2N(CH_3)_2$, $O(CH_2)_2NHCH_3$, $O(CH_2)_2NH_2$, CH_2NHCH_3, morpholinomethyl, 2-morpholinoethoxy, 2R-2,3-dihydroxy-1-propyloxy, 2S-2,3-dihydroxy-1-propyloxy or 1,3-dihydroxy-2-propyloxy.
For aspect C of the invention R is most preferably $O(CH_2)_2NH_2$.

For aspects B and C of the invention preferably R^1 and R^2 are each independently C_{1-6} alkyl optionally substituted by OH,
or R^1 and R^2 are taken together, with the C atom to which they are attached, to form a 3- to 7-membered ring optionally incorporating a hetero- moiety selected from O, S, SO, SO_2 and NR^6, and which 3- to 7-membered ring is optionally substituted by one or more OH.
For aspects B and C of the invention more preferably R^1 and R^2 are each CH_3,
or R^1 and R^2 are taken together, with the C atom to which they are attached, to form a tetrahydropyran-4-ylidene, piperidin-4-ylidene, 1-methylpiperidin-4-ylidene, or 3,4-dihydroxycyclopentylidene moiety.
For aspects B and C of the invention, yet more preferably R^1 and R^2 are taken together, with the C atom to which they are attached, to form a tetrahydropyran-4-ylidene, *cis*-3,4-dihydroxycyclopentylidene, *trans*-3,4-dihydroxycyclopentylidene or piperidin-4-ylidene moiety.
For aspects B and C of the invention, most preferably R^1 and R^2 are taken together, with the C atom to which they are attached, to form a tetrahydropyran-4-ylidene, piperidin-4-ylidene, or *cis*-3,4-dihydroxycyclopentylidene where the hydroxy substituents have a *cis*-relationship to the hydroxamate moiety.

For aspect D of the invention, R^1 and R^2 are preferably taken together, with the C atom to which they are attached, to form a 3,4-dihydroxycyclopentylidene moiety.
For aspect D of the invention, most preferably R^1 and R^2 are taken together, with the C atom to which they are attached, to form a *cis*-3,4-dihydroxycyclopentylidene group where the hydroxy substituents have a *cis*-relationship to the hydroxamate moiety.

For aspects A, B, C and D of the invention R^3 is preferably methyl.

A preferred group of substances are those selected from the compounds of the Examples and the pharmaceutically acceptable salts and solvates thereof, especially the compounds of Examples 3, 6 and 14 below, and salts and solvates thereof.

The invention further provides synthetic methods for the production of compounds, salts and solvates of the invention, which are described below and in the Examples. The skilled man will appreciate that the compounds and salts of the invention could be made by methods other than those herein described, by adaptation of the methods herein described and/or adaptation of methods known in the art, for example the art described herein. Specific art which may be mentioned includes WO 99/29667, "Comprehensive Organic Transformations" by RC Larock, VCH Publishers Inc. (1989), "Advanced Organic Chemistry" by J March, Wiley Interscience (1985), "Designing Organic Synthesis" by S Warren, Wiley Interscience (1978),

"Organic Synthesis - The Disconnection Approach" by S Warren, Wiley Interscience (1982), "Guidebook to Organic Synthesis" by RK Mackie and DM Smith, Longman (1982), "Protective Groups in Organic Synthesis" by TW Greene and PGM Wuts, John Wiley and Sons Inc. (1999), and PJ Kocienski, in "Protecting Groups", Georg Thieme Verlag (1994), references therein, and any updated versions of the aforementioned standard works.

Where desired or necessary, the compound of formula (I) can be converted into a pharmaceutically acceptable salt thereof, conveniently by mixing together solutions of a compound of formula (I) and the desired acid or base, as appropriate. The salt may be precipitated from solution and collected by filtration, or may be collected by other means such as by evaporation of the solvent. In some cases, the salt may be the direct product of a reaction to make a compound or salt of the invention in a solvent, in which case no further transformation step would be necessary.

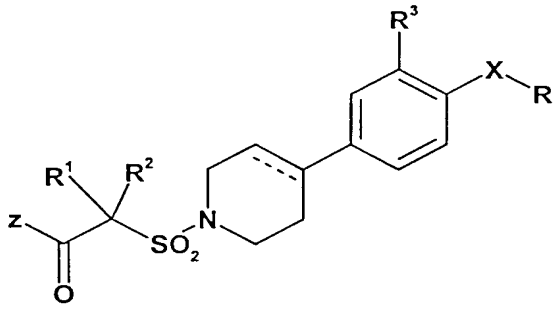
It is to be understood that the synthetic transformation methods mentioned herein may be carried out in various different sequences in order that the desired compounds can be efficiently assembled. The skilled chemist will exercise his judgement and skill as to the most efficient sequence of reactions for synthesis of a given target compound.

It will be apparent to those skilled in the art that sensitive functional groups may need to be protected and deprotected during synthesis of a compound of the invention. This may be achieved by conventional methods, for example as described in "Protective Groups in Organic Synthesis" by TW Greene and PGM Wuts, John Wiley & Sons Inc (1999).

The following methods are illustrative of the general synthetic procedures which may be adopted in order to obtain the compounds of the invention.

In the synthetic methods below, unless otherwise specified, the substituents are as defined above with reference to the compounds of formula (I) as defined above with respect to aspects A, B, C and D.

A compound of formula (I) may be prepared directly from a corresponding acid or acid derivative of formula (II):



(II)

where Z is chloro, bromo, iodo, C_{1-3} alkyloxy or HO.

When prepared directly from the ester of formula (II), where Z is C_{1-3} alkyloxy, the reaction may be carried out by treatment of the ester with hydroxylamine, preferably up to a 3-fold excess of hydroxylamine, in a suitable solvent at from about room temperature to about 85°C. The hydroxylamine is conveniently generated in situ from a suitable salt such as its hydrochloride salt by conducting the reaction in the presence of a suitable base such as an alkali metal carbonate or bicarbonate, e.g. potassium carbonate. Preferably the solvent is a mixture of methanol and tetrahydrofuran and the reaction is temperature is from about 65 to 70°C.

Alternatively, the ester (II, where Z is C_{1-3} alkyloxy) may be converted by conventional hydrolysis to the corresponding carboxylic acid (II, Z is HO) which is then transformed to the required hydroxamic acid of formula (I). [If the R, R^1 or R^2 moieties contain any free hydroxyl groups, these should be protected with groups inert to this functional group interconversion reaction sequence, and released following it, using standard methodology.]

Preferably the hydrolysis of the ester is effected under basic conditions using about 2- to 6-fold excess of an alkali metal hydroxide in aqueous solution, optionally in the presence of a co-solvent, at from about room temperature to about 85°C. Typically the co-solvent is a mixture of methanol and tetrahydrofuran or a mixture of methanol and 1,4-dioxan and the reaction temperature is from about 40 to about 70°C.

The subsequent coupling step may be achieved using conventional amide-bond forming techniques, e.g. via the acyl halide derivative (II, Z is Cl, I or Br) and hydroxylamine hydrochloride in the presence of an excess of a tertiary amine such as triethylamine or pyridine to act as acid-scavenger, optionally in the presence of a catalyst such as 4-dimethylaminopyridine, in a suitable solvent such as dichloromethane, at from about 0°C to about room temperature. For convenience, pyridine may also be used as the solvent. Such acyl halide substrates are available from the corresponding acid via conventional methods.

In particular, any one of a host of amino acid coupling variations may be used. For example, the acid of formula (II) wherein Z is HO may be activated using a carbodiimide such as 1,3-dicyclohexylcarbodiimide or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (often

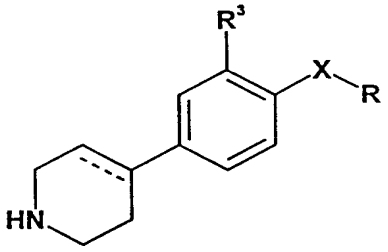
referred to as "water-soluble carbodiimide" or "WSCDI") optionally in the presence of 1-hydroxybenzotriazole or 1-hydroxy-7-aza-1H-1,2,3-benzotriazole (HOAt) and/or a catalyst such as 4-dimethylaminopyridine, or by using HOAt or a halotrisaminophosphonium salt such as bromotris(pyrrolidino)-phosphonium hexafluorophosphate. Either type of coupling is conducted in a suitable solvent such as dichloromethane, N-methylpyrrolidine (NMP)or dimethylformamide (DMF), optionally in the presence of pyridine or a tertiary amine such as N-methylmorpholine or N-ethyldiisopropylamine (for example when either the hydroxylamine or the activating reagent is presented in the form of an acid addition salt), at from about 0°C to about room temperature. Typically, from 1.1 to 2.0 molecular equivalents of the activating reagent and from 1.0 to 4.0 molecular equivalents of any tertiary amine present are employed.

Preferred reagents for mediating the coupling reaction are HOAt, WSCDI and O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU).

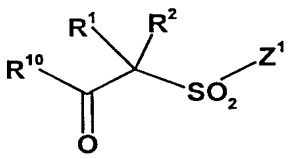
Preferably a solution of the acid (II, Z is HO) and N-ethyldiisopropylamine in a suitable solvent such as anhydrous dimethylformamide or anhydrous 1-methylpyrrolidin-2-one, under nitrogen, is treated with up to a 1.5-fold excess of HATU at about room temperature followed, after about 15 to 30 minutes, with up to about a 3-fold excess of hydroxylamine hydrochloride and up to about a 4-fold excess of N-ethyldiisopropylamine, optionally in the same solvent, at the same temperature.

More preferably the acid (II, Z is HO) is reacted with a carbodiimide, HOBt and hydroxylamine hydrochloride in pyridine in a suitable co-solvent such as dichloromethane.

An ester of formula (II, Z is C_{1-3} alkyloxy) may be prepared from an appropriate amine of formula (III) by sulphonylation with an appropriate compound of formula (IV), wherein R^{10} is C_{1-3} alkyloxy and Z^1 is a leaving group such as Br, I or Cl.



(III)



(IV)

Preferably, Z^1 is chloro.

The reaction may be effected in the presence of an appropriate base in a suitable solvent at from about 0°C to about room temperature. For example, when both R^1 and R^2 are hydrogen, an appropriate base is

1,8-diazabicyclo[5.4.0]undec-7-ene and a suitable solvent is dichloromethane. Alternatively, the base can be sodium imidazolide. An alternative method is to make a N-trialkylsilyl dervative of (III), and mix with (IV) at room temperature in tetrahydrofuran (THF) in the presence of a catalytic amount of benzenesulphonic acid (BSA).

Certain esters of formula (II, Z is C_{1-3} alkyloxy) wherein at least one of R^1 and R^2 is other than hydrogen may be conveniently obtained from the α-carbanion of an ester of formula (II) wherein at least one of R^1 and R^2 is hydrogen by conventional C-alkylation procedures using an alkylating agent of formula (VA) or (VB):

$$R^1Z^1 \text{ or } R^2Z^1 \quad (VA)$$

$$Z^2(CH_2)_qZ^3 \quad (VB),$$

where the $(CH_2)_q$ moiety of (VB) optionally incorporates a hetero- moiety selected from O, S, SO, SO_2 and NR^6, and is optionally substituted by one or more optionally protected OH, and which NR^6 group may be optionally protected, wherein R^1 and R^2 are not hydrogen, Z^2 and Z^3 may be the same or different and are suitable leaving groups such as chloro, bromo, iodo, C_1-C_4 alkanesulphonyloxy, trifluoromethanesulphonyloxy or arylsulphonyloxy (e.g. benzenesulphonyloxy or p-toluenesulphonyloxy), and q is 3, 4, 5, 6 or 7. Other conditions are outlined below - sections vii) and x).

Preferably, Z^2 and Z^3 are selected from bromo, iodo and p-toluenesulphonyloxy.

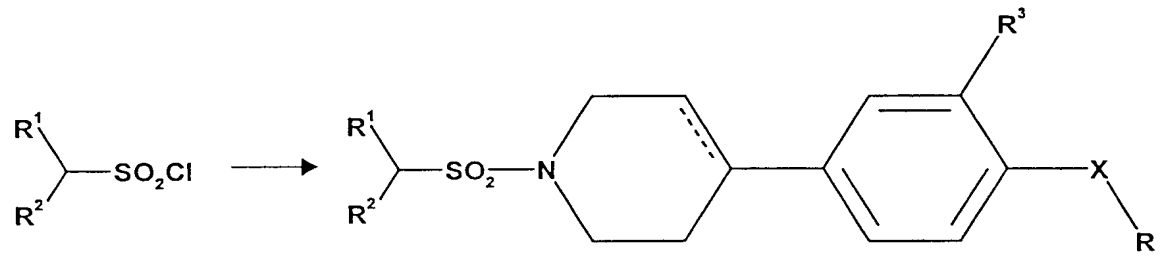
The carbanion may be generated using an appropriate base in a suitable solvent, optionally in the presence of a phase transfer catalyst (PTC). Typical base-solvent combinations may be selected from lithium, sodium or potassium hydride, lithium, sodium or potassium bis(trimethylsilyl)amide, lithium diisopropylamide and butyllithium, potassium carbonate, sodium or potassium t-butoxide, together with toluene, ether, DMSO, 1,2-dimethoxyethane, tetrahydrofuran, 1,4-dioxan, dimethylformamide, N,N-dimethylacetamide, 1-methylpyrrolidin-2-one and any mixture thereof.

Preferably the base is sodium hydride and the solvent is dimethylformamide, optionally with tetrahydrofuran as co-solvent, or 1-methylpyrrolidin-2-one. For monoalkylation up to about a 10% excess of base is employed whilst, for dialkylation, from about 2 to about 3 molar equivalents are generally appropriate.

Typically, the carbanion is generated at about room temperature, under nitrogen, and subsequently treated with the required alkylating agent at the same temperature.
Clearly, when dialkylation is required and R^1 and R^2 are different, the substituents may be introduced in tandem in a "one-pot reaction" or in separate steps.

An amine of formula (III) may be obtained by standard chemical procedures.
Other amines of formula (III), when neither commercially available nor subsequently described, can be obtained either by analogy with the processes described in the Preparations section below or by conventional synthetic procedures, in accordance with standard textbooks on organic chemistry or literature precedent, from readily accessible starting materials using appropriate reagents and reaction conditions.

Another way of making compounds of formula (II) where ZCO is an ester moiety, is via the reaction sequence



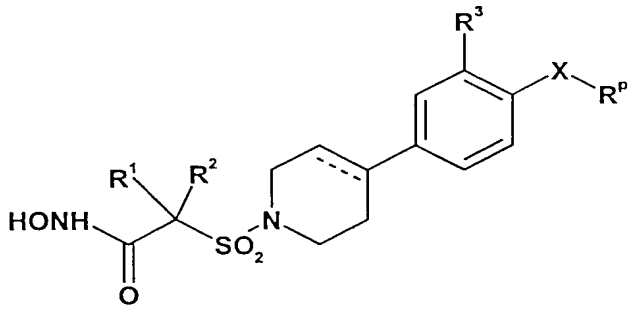
(V) (VI)

The appropriate sulphonyl chloride (V) is reacted with compound (III - see above) optionally in the presence of a base and in a suitable solvent. The resulting sulphonamide (VI) is reacted with a suitable base such as n-butyllithium, sodium hydride or potassium t-butoxide in a suitable anhydrous non-protic solvent to generate the carbanion α to the sulphonamide moiety, which is then reacted with for example dimethyl carbonate or methyl chloroformate, in suitable conditions, either of which reagent would give the compound (II) where Z is methoxy.

Compounds of formula (I) where R contains a free NH, NH_2 and/or OH group (apart from on the hydroxamic acid moiety) may conveniently be prepared from a corresponding N- or O-protected species (VII below). As such, compounds of formula (VII) where R^P is a O- and/or N-protected version of a corresponding compound of the formula (I), are included in the scope of this invention, with regard to aspects A, B, C and D of the invention and the specific compounds of formula (I) mentioned herein, such as those mentioned in the Preparations, as appropriate, below. Suitable protection / deprotection regimes are well known in the art, such as those mentioned in "Protective Groups in Organic Synthesis" by TW Greene and PGM Wuts, John Wiley & Sons Inc (1999).

Suitable OH-protecting groups and regimes include the ethers such as t-butyloxy, tri(C_{1-4})silyloxy, etc., and esters such as carbonates, sulphonates, C_{1-4} acylates, etc. mentioned by Greene and Wuts, *ibid.* chapter 2. Suitable NH-protecting groups and regimes can be found in Greene and Wuts, *ibid.* chapter 7, and include amides such as "Boc", amines such as benzyl, etc.

Compounds of formula (VII) may be made by methods described herein and /or by variation of methods described herein which the skilled man will appreciate are routine variations.



(VII)

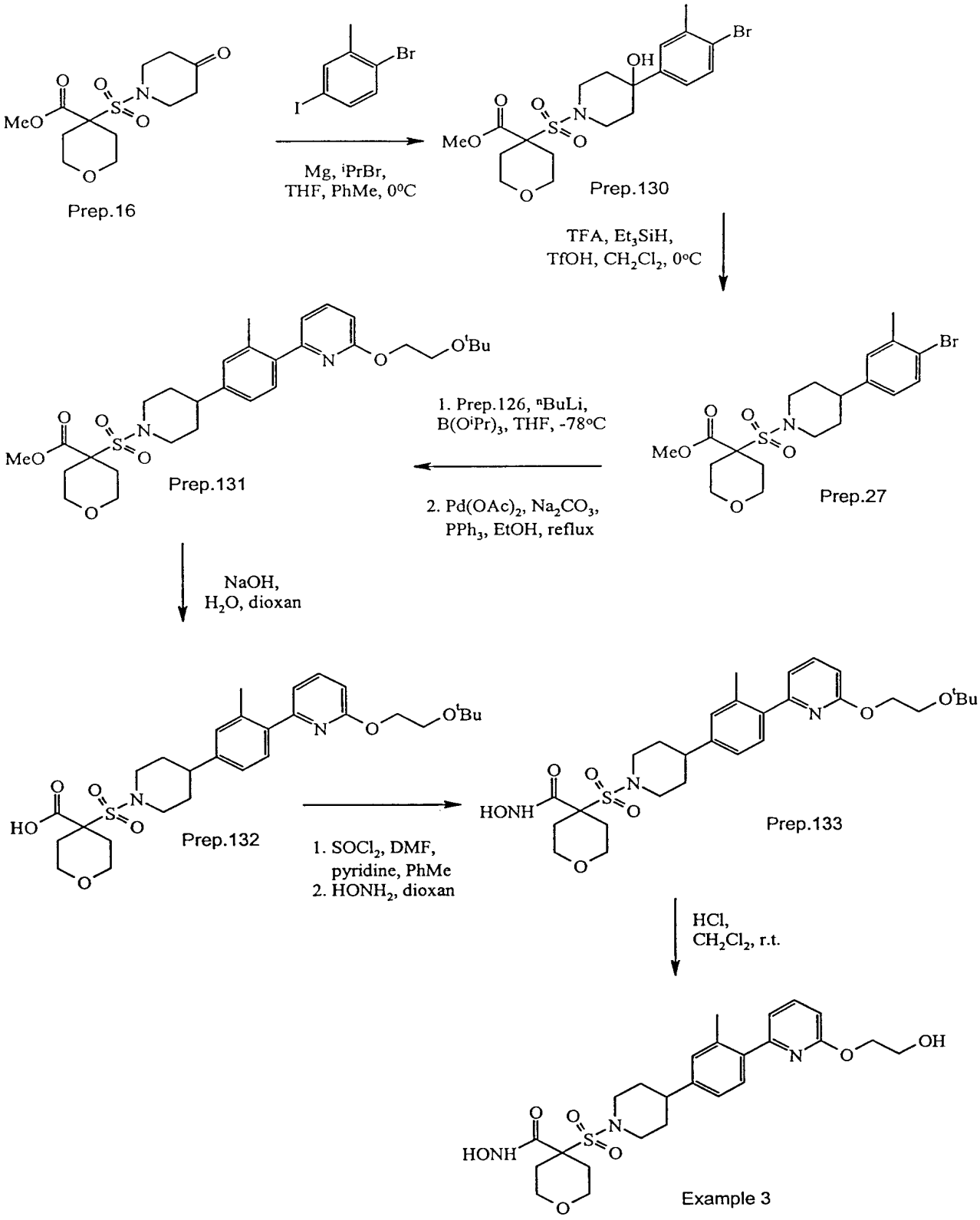
An example of a suitable OH-protecting group is the trimethylsilyl (TMS) group and the protection, reaction, deprotection sequence can be summarised by steps a) to c) below:

a) $ClSiMe_3$ (1.1 equiv per OH), WSCDI (1.1 to 1.2 equiv), HOBT or HOAT (1 to 1.1 equiv),

b) $NH_2OH.HCl$ (3 equiv) in DMF/pyridine or CH_2Cl_2/pyridine (3/1 to 1/1) at rt for between 4 and 20 hours.

c) TMS group removed by acid work-up.

Another example of a suitable OH-protecting group is the t-butyl ('Bu) group which can be carried through the synthetic process and removed in the last step of the process. An example of the route is outlined in the scheme below (in relation to the synthesis of the compound of Example 3 - via compounds of the Preparations mentioned below).

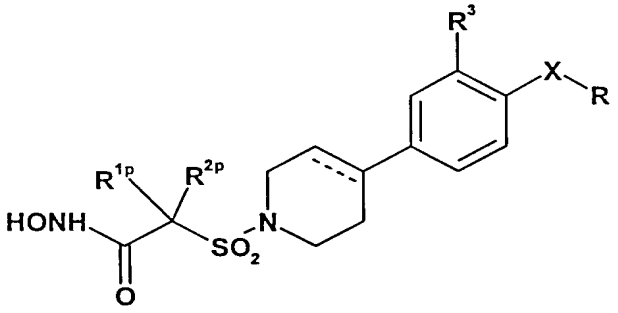

Prep.16
Mg, iPrBr,
THF, PhMe, 0°C
Prep.130
TFA, Et3SiH,
TfOH, CH2Cl2, 0°C
1. Prep.126, nBuLi,
B(OiPr)3, THF, -78°C
2. Pd(OAc)2, Na2CO3,
PPh3, EtOH, reflux
Prep.27
Prep.131
NaOH,
H2O, dioxan
Prep.132
1. SOCl2, DMF,
pyridine, PhMe
2. HONH2, dioxan
Prep.133
HCl,
CH2Cl2, r.t.
Example 3

An example of a suitable NH-protecting group is the t-butoxycarbonyl (Boc) group. This group can be introduced in standard ways, such as those delineated in the Examples and Preparations section below. After the hydroxamic acid unit has been introduced, the Boc group can be removed for example by treatment of the N-Boc compound in methanol or dichloromethane saturated with HCl gas, at room temperature for 2 to 4 hours.

Compounds of formula (I) where R^1 and/or R^2, either independently or together, contain a free NH, NH_2 and/or OH group (apart from on the hydroxamic acid moiety) may conveniently be prepared from a corresponding N- and/or O-protected species (XII below). As such, compounds of formula (XII) where R^{1p} and/or R^{2p} is a O- and/or N-protected version of a corresponding compound of the formula (I), are included in the scope of this invention, with regard to aspects A, B, C and D of the invention and the specific compounds of formula (I) mentioned herein, such as those compounds of formula (XII) mentioned in the Preparations, as appropriate, below. Suitable protection / deprotection regimes are well known in the art, such as those mentioned in "Protective Groups in Organic Synthesis" by TW Greene and PGM Wuts, John Wiley & Sons Inc (1999).

Suitable OH-protecting groups and regimes include the ethers such as t-butyloxy, tri(C_{1-4})silyloxy, etc., and esters such as carbonates, sulphonates, C_{1-4} acylates, etc. mentioned by Greene and Wuts, *ibid.* chapter 2. Suitable NH-protecting groups and regimes can be found in Greene and Wuts, *ibid.* chapter 7, and include amides such as "Boc", amines such as benzyl, etc.

Compounds of formula (XII) may be made by methods described herein and /or by variation of methods described herein which the skilled man will appreciate are routine variations.



(XII)

An example of a suitable OH-protecting group is the trimethylsilyl (TMS) group and the protection, reaction, deprotection sequence can be summarised by steps a) to c) below:

a) $ClSiMe_3$ (1.1 equiv per OH), WSCDI (1.1 to 1.2 equiv), HOBT or HOAT (1 to 1.1 equiv),

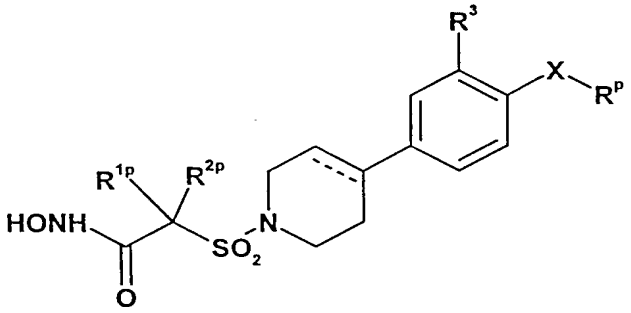
b) $NH_2OH.HCl$ (3 equiv) in DMF/pyridine or CH_2Cl_2/pyridine (3/1 to 1/1) at rt for between 4 and 20 hours.

c) TMS group removed by acid work-up.

Another example of a suitable OH-protecting group is the t-butyl ('Bu) group which can be carried through the synthetic process and removed in the last step of the process. An example of the route is outlined in the scheme below (in relation to the synthesis of the compound of Example 3 - via compounds of the Preparations mentioned below).

An example of a suitable NH-protecting group is the t-butoxycarbonyl (Boc) group. This group can be introduced in standard ways, such as those delineated in the Examples and Preparations section below. After the hydroxamic acid unit has been introduced, the Boc group can be removed for example by treatment of the N-Boc compound in methanol or dichloromethane saturated with HCl gas, at room temperature for 2 to 4 hours.

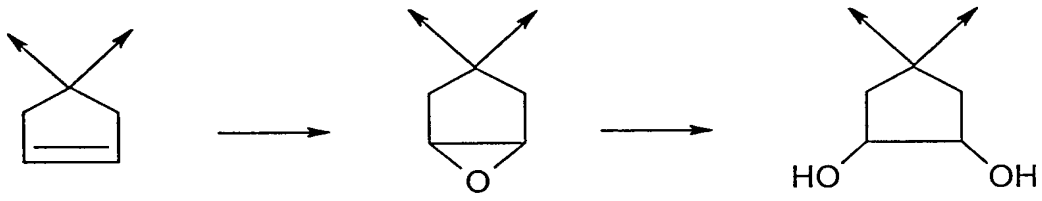
An extension of the above is where the compound of formula (I) contains a free, OH, NH and/or NH_2 group in R^1, R^2 and R (e.g. some Examples below). In thos case a suitable precursor could be the compound of formula (XIII) below:



(XIII)

where the substituents are as previously defined

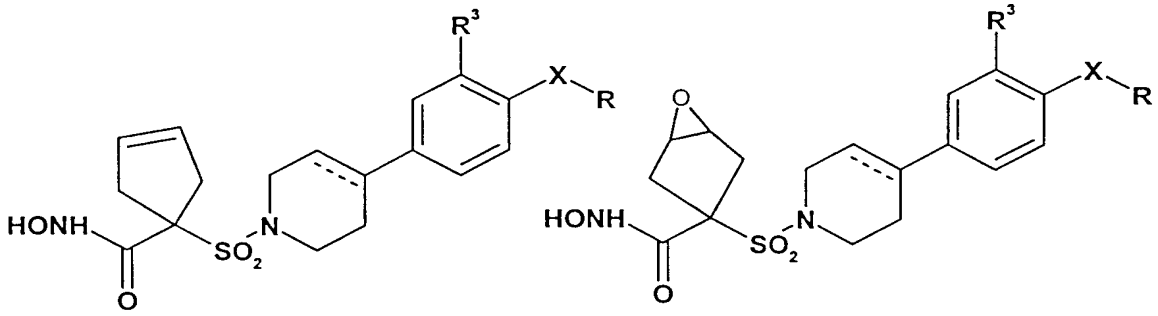
Compounds of formula (I) and appropriate intermediates thereto where R^1 and R^2 are taken together as 3,4-dihydroxycyclopentylidene can be made via the corresponding intermediacy of a corresponding cyclopent-3-enylidene moiety, viz.:



Cyclopentylidene intermediates can be epoxidised to give the corresponding epoxide using standard methods. The epoxide can be reacted in a number of different methods to give the diol product. By

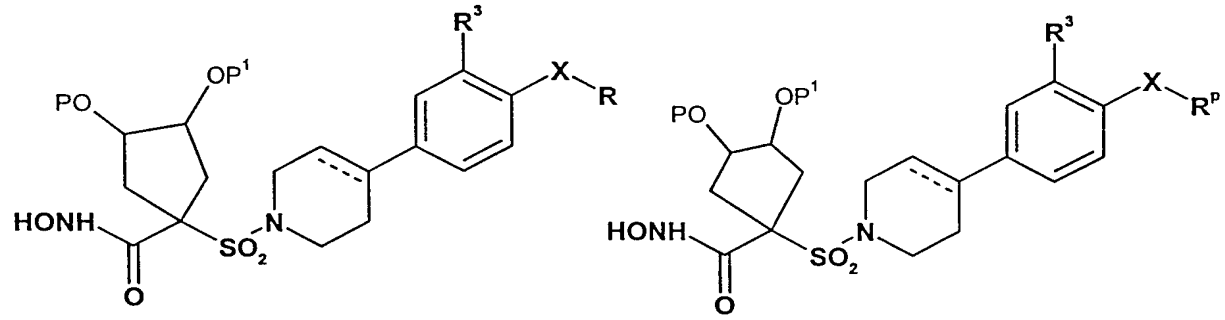
suitable choice of reagents, conditions etc., the skilled chemist can make diols with any desired stereochemistry, using well-known methods.

As such, compounds of the formula (VIII) and (IX) below are included in the scope of the invention, with regard to aspects A, B, C and D and also with respect to intermediates to appropriate individual compounds of formula (I) mentioned herein.



(VIII) (IX)

Also included in the invention are intermediates of formula (X) and (XI, where R^p is defined as above for compounds of formula (VII) wherein P and P^1 represent standard OH and 1,2-diol protecting groups mentioned in Greene and Wuts, *ibid.*, chapter 2. P and P^1 are preferably taken together and form an acetonide moiety.



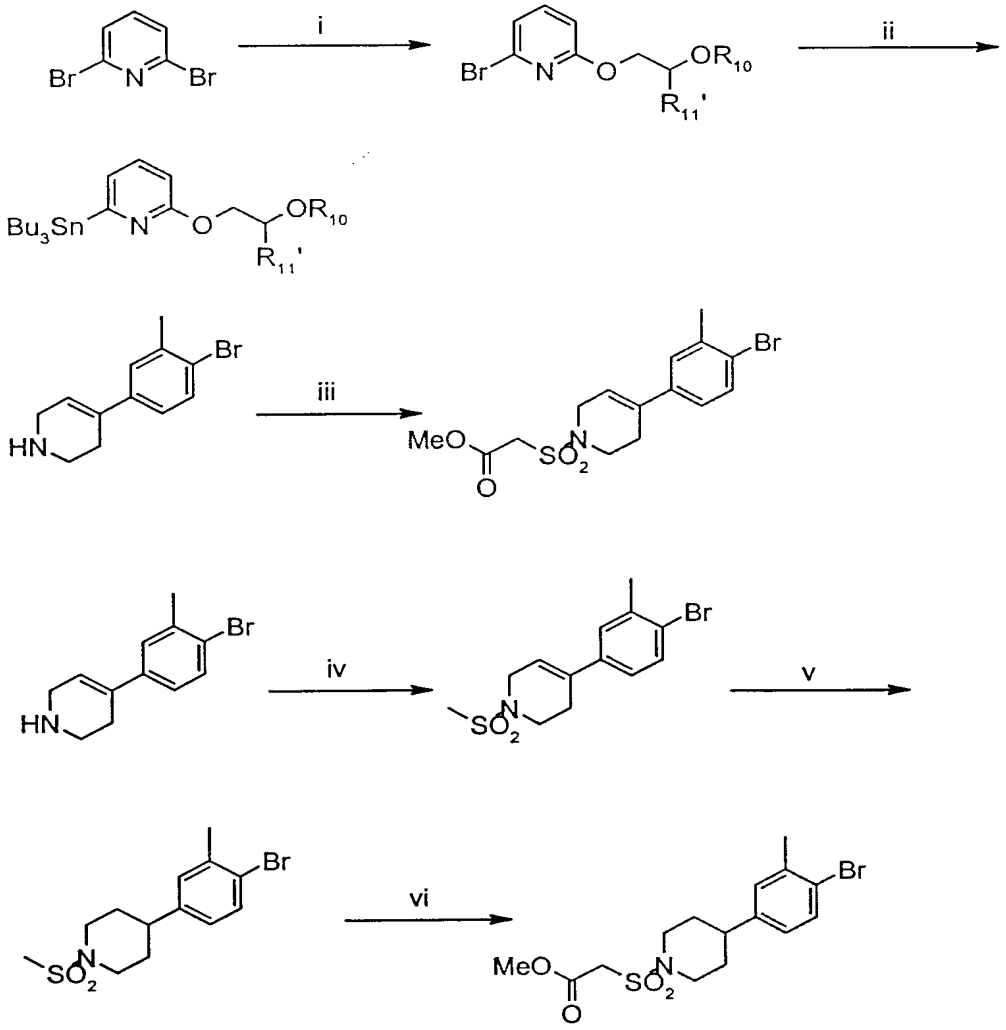
(X) (XI)

Certain specific compounds of formulae (VIII), (IX), (X) and (XI) are mentioned in the Preparations below.

Moreover, persons skilled in the art will be aware of variations of, and alternatives to, those processes described herein, including in the Examples and Preparations sections, which allow the compounds defined by formula (I) to be obtained, such as carrying out certain bond-forming or functional group interconversion reactions in different sequences.

Examples of the preparation of a number of intermediates and final compounds are outlined in the following synthetic schemes, where the abbreviations used are standard and well-known to the person skilled in the art. Routine variation of these routes can give all the required compounds of the invention.

Route 1 (*Pyridyl alcohols*)



i = NaH (1.1 equiv), $HOCH_2CHR11'OR10$ (1 equiv) in toluene, reflux for 2 to 5 hours

ii = n-BuLi (1.1 equiv), Bu_3SnCl (1.1 equiv), THF, -70°C to room temperature.
Or, $Pd(PPh_3)_4$ (0.01 to 0.05 equiv), $[SnMe_3]_2$ (1.1 equiv), dioxan, reflux for 2 to 5 hrs.

iii = BSA (0.5 equiv), $MeCO_2CH_2SO_2Cl$ (1.2 equiv), THF, rt for 18 hours.

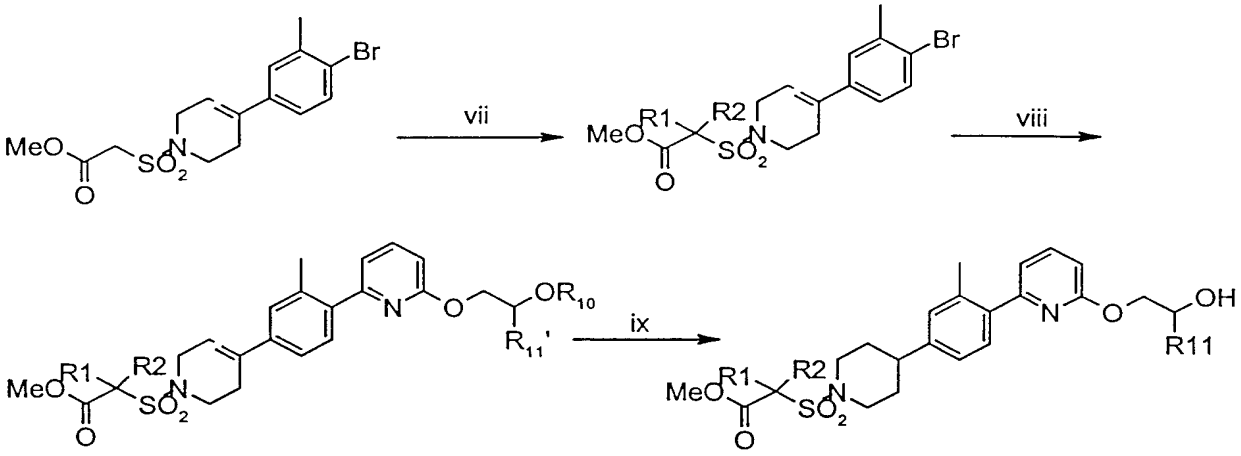
iv = $MeSO_2Cl$ (1.2 equiv), Et_3N (1.4 equiv), CH_2Cl_2, rt, for an hour.

v = Et_3SiH (3 equiv), CF_3SO_3H (0.1 equiv), TFA:CH_2Cl_2 (1:1), rt, for 1-24 hrs.

vi = NaH (2 equiv), Me_2CO_3 (4 equiv), toluene, reflux for 2 hours.

R10-alcohol protecting group- e.g. benzyl or dioxalane (for diols)

R11'-H or a protected alcohol



vii = (VB), (1.3 equiv), K_2CO_3 (3 equiv), DMSO, rt, 18-24 hours,

or KOtBu (2.5 equiv), (VA) or (VB) (excess), in THF, rt for 72 hours.

viii = Stille coupling-$Pd(PPh_3)_4$ (0.05 equiv), stannane (1.5 equiv), toluene, reflux for 4 to 20 hours.

OR $PdCl_2(PPh_3)_2$ (0.05 equiv), stannane (1.1 equiv), THF, reflux for 17 hours.

ix = NH_4^+ HCO_3^- (excess) $Pd(OH)_2/C$, AcOH, MeOH, reflux for 20 hours,

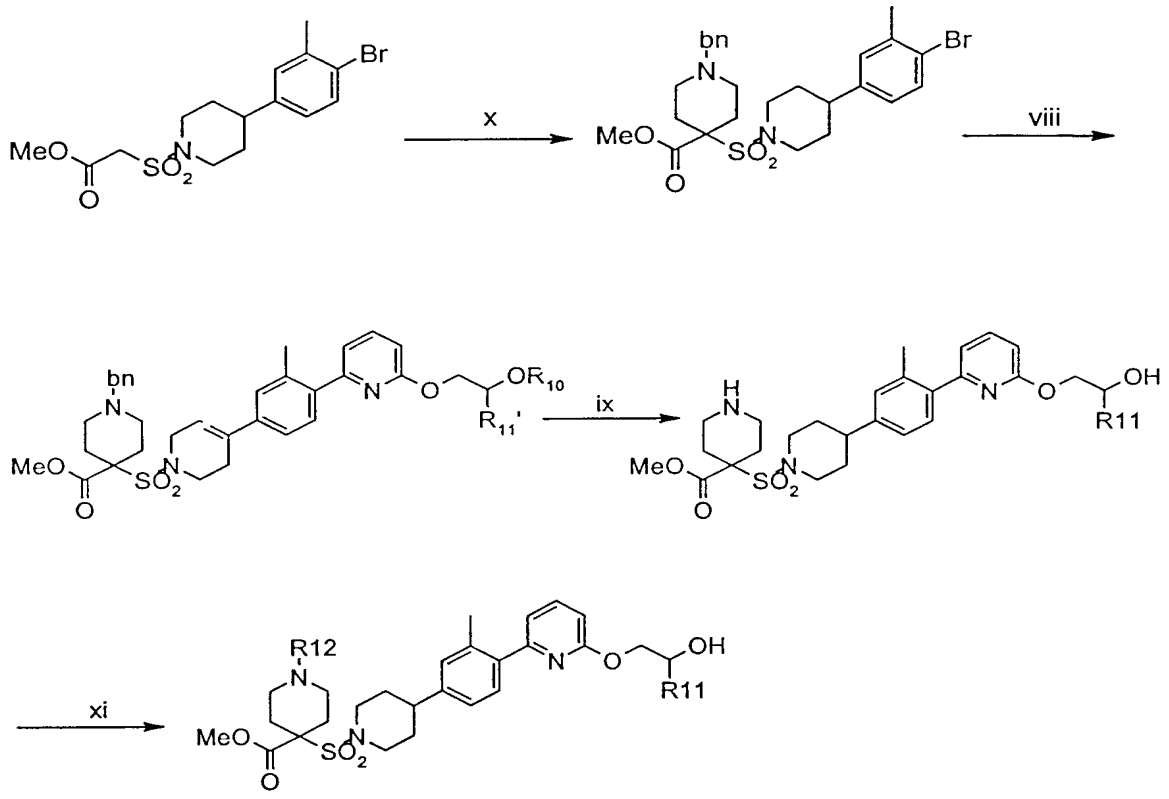
OR 10% Pd/C, in MeOH or EtOH, 3.3 atmospheres, room temperature, for 6 to 17 hours,-both methods also deprotect any benzyl group. (2N HCl, dioxan (3:1), rt, 75 mins at rt- deprotects the dioxalane)

OR $Pd(OH)_2/C$, NH_4^+ HCO_3^- (excess), in MeOH:dioxan (2.5:1), 60°C for 2 hours.

R11 = H or deprotected alcohol

Similarly

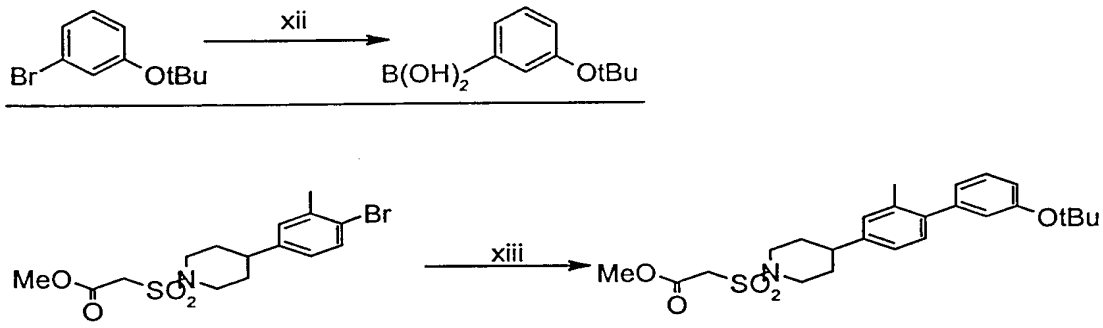
when R1R2 when taken together, are a piperidine group:

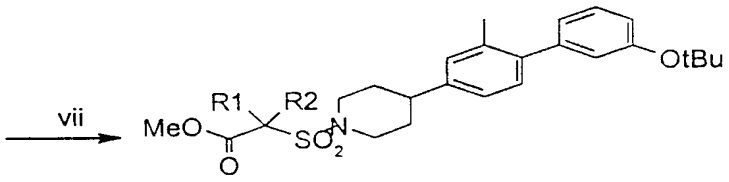


x = NaH (3 equiv), tetra-nBuNH$_4$Br (1 equiv), BnN(CH$_2$CH$_2$Cl)$_2$ (0.95 equiv), NMP, 60°C for 6 hours.

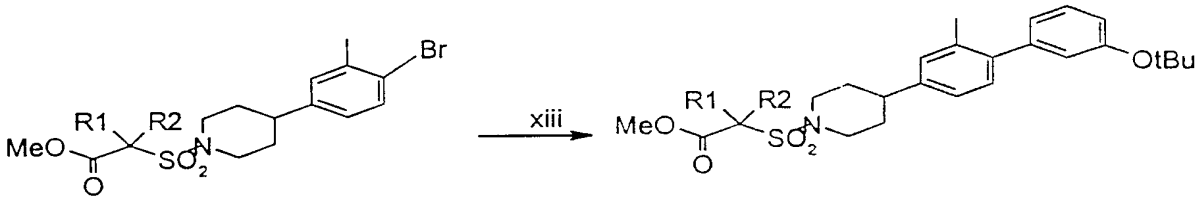
xi = When R12 is Me, formaldehyde (4 equiv), Na(OAc)$_3$BH (2 equiv), CH$_2$Cl$_2$, 20 hrs at rt.
When R12 is Boc, (Boc)$_2$O (1.05 equiv), Et$_3$N (1.1 equiv), CH$_2$Cl$_2$, rt for an hour.

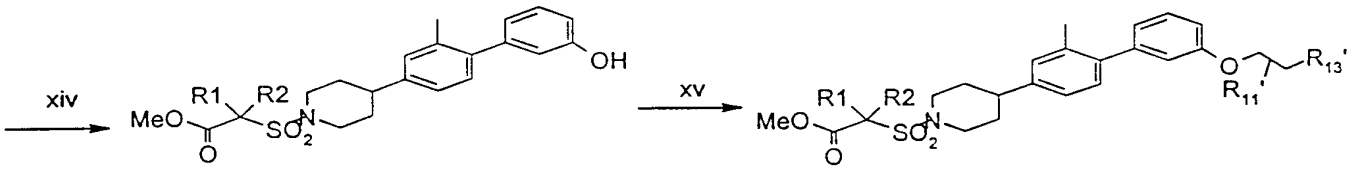
Route 2 *(Phenyl alcohols)*





Or





xii = nBuLi (1.1 equiv), $B[OCH(CH_3)_2]_3$ (1.5 equiv), THF, -70°C to rt.

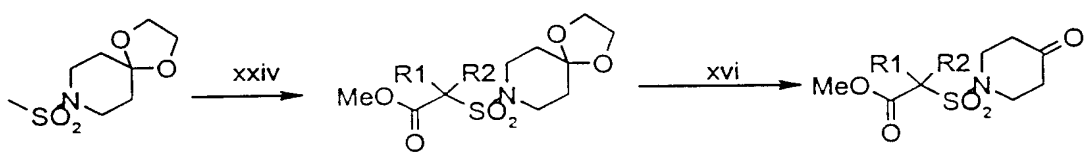
xiii = Suzuki coupling- arylboronic acid (1.2 to 1.5 equiv), CsF (2 to 2.6 equiv), $P(o\text{-}tol)_3$ (0.1 equiv), $Pd_2(dba)_2$ (0.005 equiv), DME, reflux for 6 to 50 hours.

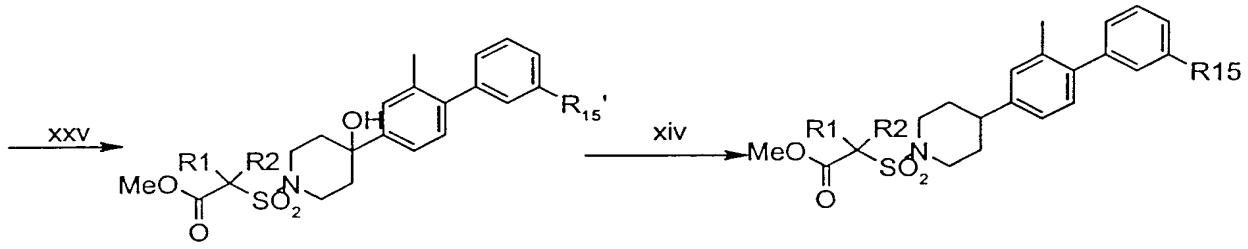
xiv = Et_3SiH (3 equiv), TFA:CH_2Cl_2 (1:1), rt for 2 to 24 hours.

xv = R/S glycidol (1 equiv), Et_3N (catalytic), MeOH, reflux for 20 hours.
OR, Mitsunobu reaction -DEAD (1.5 equiv), PPh_3 (1.5 equiv), HOCH(R11')CH_2OR13' (1.5 equiv) in THF, rt for 3 hours.
R11' is H or optionally protected alcohol
and R13' is optionally protected alcohol

For preparation 50 to 51, requires Bn deprotection using the conditions described in ix.

Alternative route



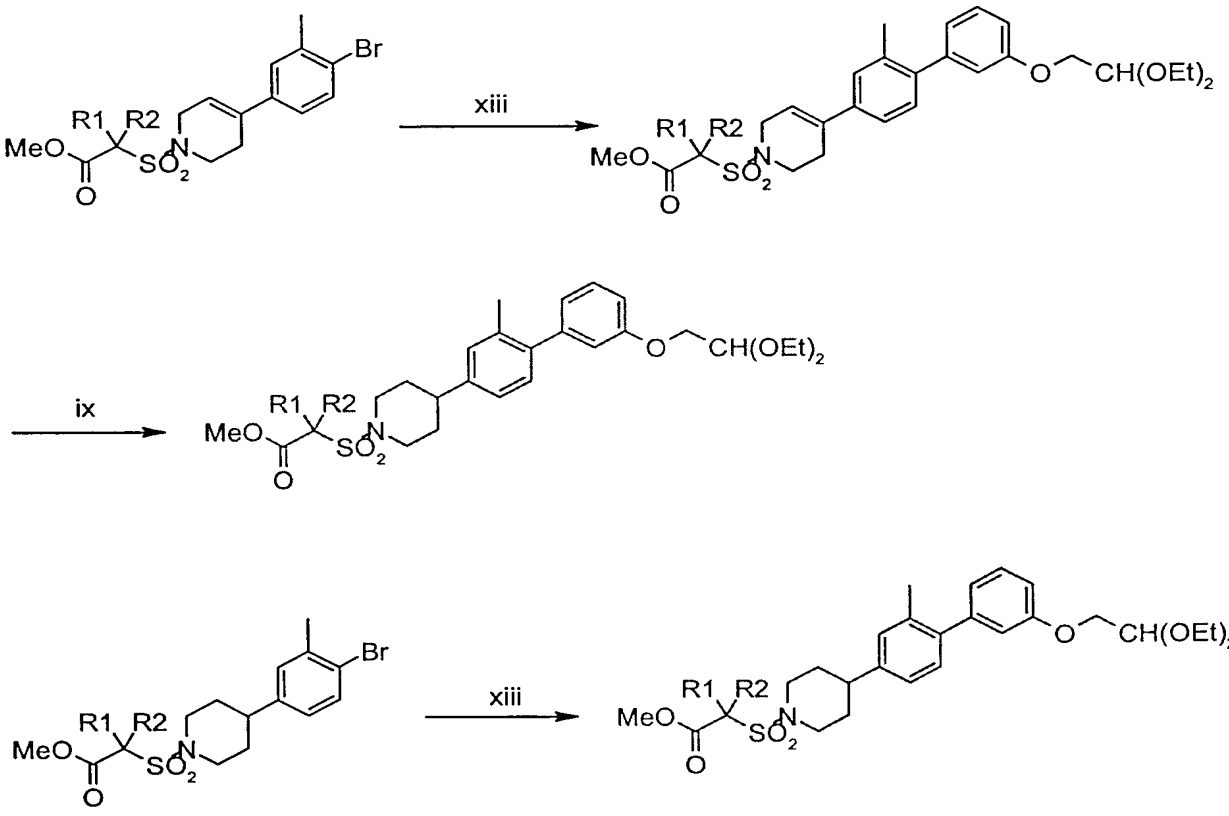


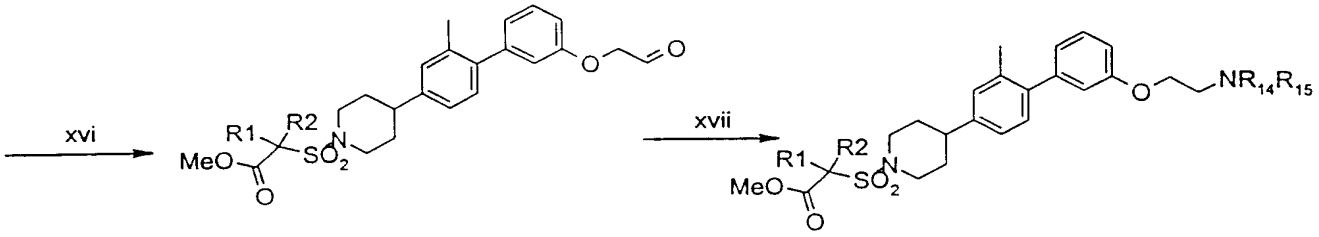
xxiv = *i*- NaH (2.2 equiv), Me_2CO_3 (5 equiv), toluene, MeOH (catalytic), 90°C, overnight.
ii- $O(CH_2CH_2Br)_2$ (1.3 equiv), NMP, 90°C, 20 hrs.

xxv = Grignard reagant (1.1equiv), THF, -78°C to rt over approx hr.

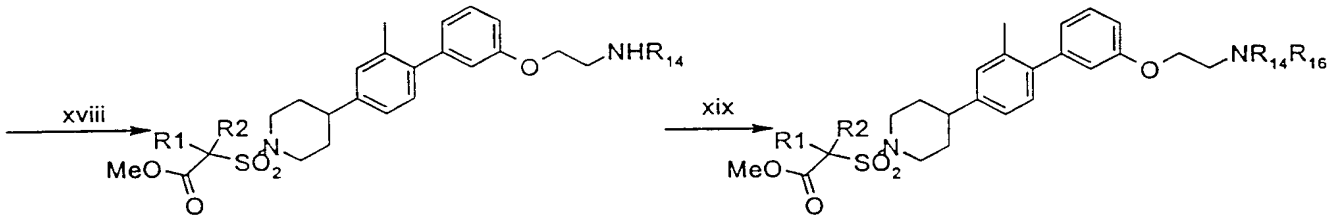
R15'-optionally protected alcohol, in prep 48 this is a t-butyl ether.
R15-OH, for prep 48.

Route 3 (*Phenyl aminoalcohols)*





When R15 is a protecting group, eg. benzyl, deprotection, followed by protection using an alternative group eg Boc, can be used as shown below:



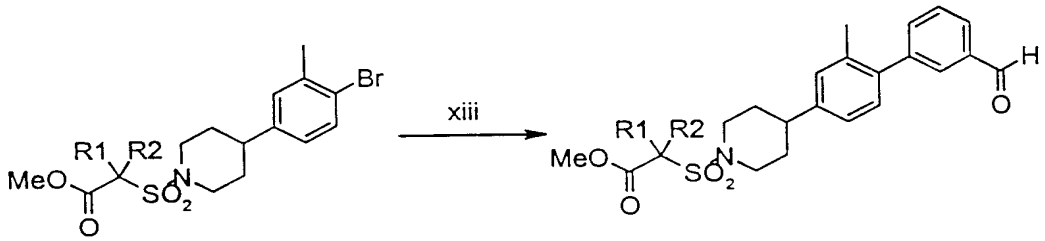
xvi = 1N HCl (1 to 2.3 equiv), acetone:dioxan (1:1), 70°C for 2 to 6 hours.

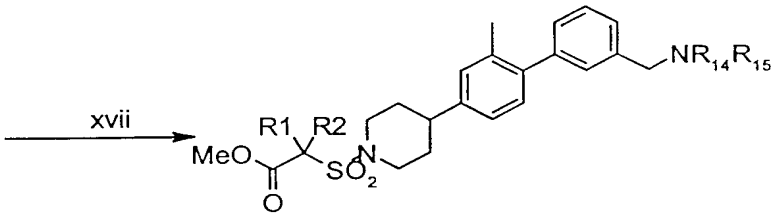
xvii = Reductive amination-amine (5.5 equiv), $Na(OAc)_3BH$ (3 to 4 equiv), CH_2Cl_2, rt, overnight.

xviii = $Pd(OH)_2/C$, MeOH, 50 psi, rt, 18 hrs.

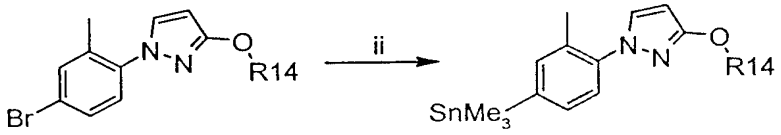
xix = When R16 is Boc,
$(Boc)_2O$ (1 to1.1 equiv), Et_3N (optional, 1 equiv), DMAP (optional, cat), CH_2Cl_2, rt, 3 hrs.

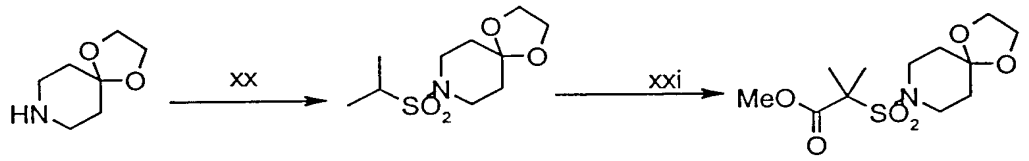
Route 4 *(aminoalkyl phenyls)*

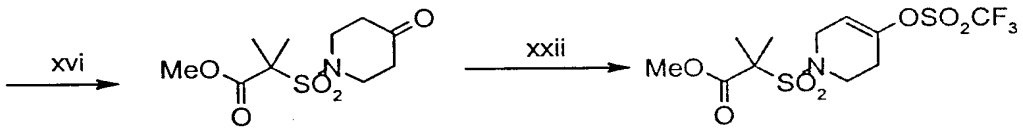


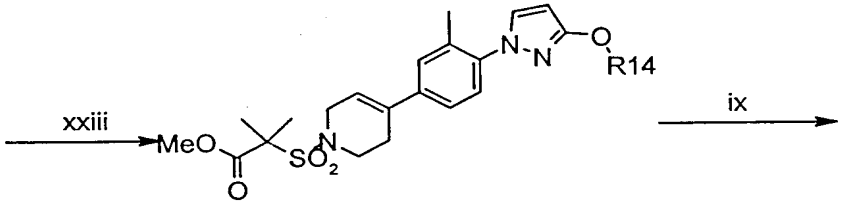


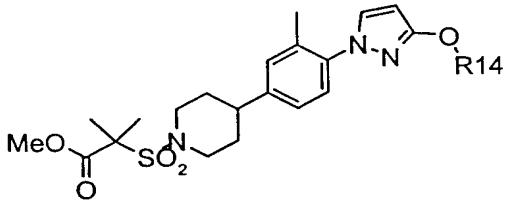
Route 5 (*Heterocycles*)











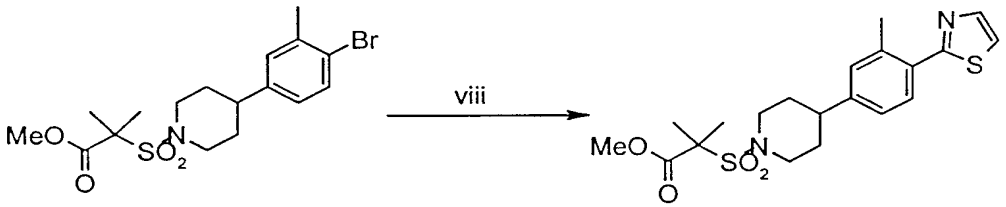
xx = iso-$PrSO_2Cl$ (1 equiv), Et_3N (1.1 equiv), CH_2Cl_2, 3hours at rt.

xxi = n-BuLi (1.1 equiv), MeOCOCl (1.2 equiv), THF -78° to rt.

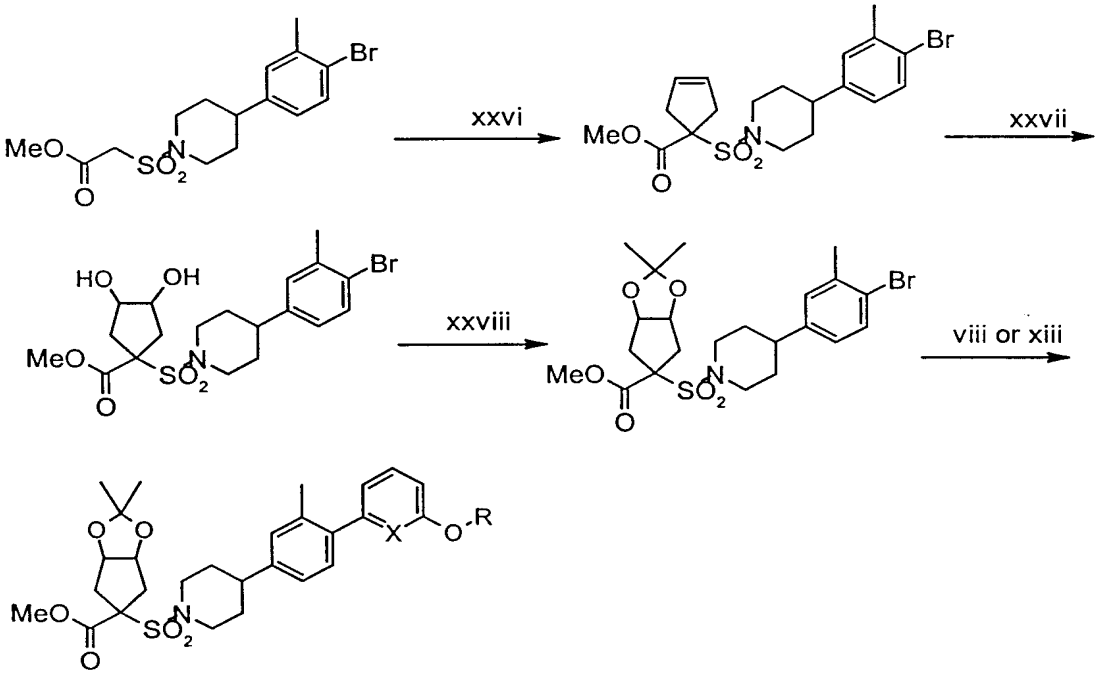
xxii = 2,6-di-t-Bu-4-Me pyridine (2.5 equiv), $(CF_3SO_2)_2O$ (2.5 equiv), CH_2Cl_2, 4°C to rt, 5 days.

xxiii = $Pd_2(dba)_3$ (0.02 equiv), vinyl triflate (1.1 equiv), Ph_3As (0.21 equiv), CuI (0.1 equiv) in NMP, 75°C for 5 hrs.

Thiazoles



Route 6 (*Cyclopentanediols*)



xxvi = NaH (1.1 equiv), tetra-nBuNH_4Br (1 equiv), $ClCH_2CHCHCH_2Cl$ (1.1 equiv), NMP, r.t for 3 hours, then NaH (1.1 equiv), 2 days.

xxvii = NMO (1.1 equiv), OsO_4 (3 mol%), dioxan/water, r.t. 18 hours
OR
(a) AgOAc (2.3 equiv), AcOH, r.t for 18 hours (b) 1N NaOH, dixoan/water

xxviii = 2,2-Dimethoxypropane (2 equiv), TsOH (0.1 equiv), DMF, 50°C for 4.5 hours.

Biological Test Methods

The biological activities of the compounds of the present invention were determined by the following test methods, which are based on the ability of the compounds to inhibit the cleavage of various fluorogenic peptides by MMPs 1, 2, 3, 9, 13 and 14.

The assays for MMPs 2, 3, 9 and 14 are based upon the original protocol described in Fed.Euro.Biochem.Soc., 1992, 296, 263, with the minor modifications described below.

Inhibition of MMP-1

Enzyme Preparation

Catalytic domain MMP-1 was prepared in Pfizer Central Research laboratories in a standard manner from sources known to the skilled person, including some of the references mentioned herein. A stock solution of MMP-1 (1μM) was activiated by the addition of aminophenylmercuric acetate (APMA), at a final concentration of 1mM, for 20 minutes at 37°C. MMP-1 was then diluted in Tris-HCl assay buffer (50mM Tris, 200mM NaCl, 5mM $CaCl_2$, 20μM $ZnSO_4$ and 0.05% Brij 35, pH 7.5) to a concentration of 10nM. The final concentration of enzyme used in the assay was 1nM.

Substrate

The fluorogenic substrate used in this assay was Dnp-Pro-β-cyclohexyl-Ala-Gly-Cys(Me)-His-Ala-Lys-(N-Me-Ala)-NH_2 as originally described in Anal. Biochem., 1993, 212, 58. The final substrate concentration used in the assay was 10μM.

Determination of Enzyme Inhibition

The test compound was dissolved in dimethyl sulphoxide and diluted with assay buffer so that no more than 1% dimethyl sulphoxide was present. Test compound and enzyme were added to each well of a 96 well plate and allowed to equilibrate for 15 minutes at 37°C in an orbital shaker prior to the addition of substrate. Plates were then incubated for 1 hour at 37°C prior to determination of fluorescence (substrate cleavage) using a fluorimeter (Fluostar; BMG LabTechnologies, Aylesbury, UK) at an excitation

wavelength of 355 nm and emission wavelength of 440 nm. The potency of inhibition was measured from the amount of substrate cleavage obtained using a range of test compound concentrations and, from the resulting dose-response curve, an IC_{50} value (the concentration of inhibitor required to inhibit 50% of the enzyme activity) was calculated.

Inhibition of MMP-2, MMP-3 and MMP-9

Enzyme Preparation

Catalytic domains MMP-2, MMP-3 and MMP-9 were prepared in Pfizer Central Research laboratories in a standard manner from sources known to the skilled person, including some of the references mentioned herein. A stock solution of MMP-2, MMP-3 or MMP-9 (1μM) was activated by the addition of APMA. For MMP-2 and MMP-9, a final concentration of 1mM APMA was added, followed by incubation for 1 hour at 37°C. MMP-3 was activated by the addition of 2mM APMA, followed by incubation for 3 hours at 37°C. The enzymes were then diluted in Tris-HCl assay buffer (100mM Tris, 100mM NaCl, 10mM $CaCl_2$ and 0.16% Brij 35, pH 7.5) to a concentration of 10nM. The final concentration of enzyme used in the assays was 1nM.

Substrate

The fluorogenic substrate used in this screen was Mca-Arg-Pro-Lys-Pro-Tyr-Ala-Nva-Trp-Met-Lys(Dnp)-NH_2 (Bachem Ltd., Essex, UK) as originally described in J.Biol.Chem., 1994, 269, 20952. This substrate was selected because it has a balanced hydrolysis rate against MMPs 2, 3 and 9 (k_{cat}/k_m of 54,000, 59,400 and 55,300 s^{-1} M^{-1} respectively). The final substrate concentration used in the assay was 5μM.

Determination of Enzyme Inhibition

The test compound was dissolved in dimethyl sulphoxide and diluted with assay buffer so that no more than 1% dimethyl sulphoxide was present. Test compound and enzyme were added to each well of a 96 well plate and allowed to equilibrate for 15 minutes at 37°C in an orbital shaker prior to the addition of substrate. Plates were then incubated for 1 hour at 37°C, prior to determination of fluorescence using a fluorimeter (Fluostar; BMG LabTechnologies, Aylesbury, UK) at an excitation wavelength of 328nm and emission wavelength of 393nm. The potency of inhibition was measured from the amount of substrate cleavage obtained using a range of test compound concentrations and, from the resulting dose-response curve, an IC_{50} value (the concentration of inhibitor required to inhibit 50% of the enzyme activity) was calculated.

Inhibition of MMP-13

Enzyme Preparation

Human recombinant MMP-13 was prepared by PanVera Corporation (Madison, Wisconsin) and characterised at Pfizer Central Research laboratories. A 1.9 mg/ml stock solution was activated with 2mM APMA for 2 hours at 37°C. MMP-13 was then diluted in assay buffer (50mM Tris, 200mM NaCl, 5mM $CaCl_2$, 20μM $ZnCl_2$ and 0.02% Brij 35, pH 7.5) to a concentration of 5.3nM. The final concentration of enzyme used in the assay was 1.3nM.

Substrate

The fluorogenic substrate used in this screen was Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-NH_2. The final substrate concentration used in the assay was 10μM.

Determination of Enzyme Inhibition

The test compound was dissolved in dimethyl sulphoxide and diluted with assay buffer so that no more than 1% dimethyl sulphoxide was present. Test compound and enzyme were added to each well of a 96 well plate. The addition of substrate to each well initiated the reaction. Fluorescence intensity was determined using a 96 well plate fluorimeter (Cytofluor II; PerSeptive Biosystems, Inc., Framingham, MA) at an excitation wavelength of 360nm and emission wavelength of 460nm. The potency of inhibition was measured from the amount of substrate cleavage obtained using a range of test compound concentrations and, from the resulting dose-response curve, an IC_{50} value (the concentration of inhibitor required to inhibit 50% of the enzyme activity) was calculated.

Inhibition of MMP-14

Enzyme Preparation

Catalytic domain MMP-14 was prepared in Pfizer Central Research laboratories in a standard manner from sources known to the skilled person, including some of the references mentioned herein. A 10μM enzyme stock solution was activated for 20 minutes at 25°C following the addition of 5μg/ml of trypsin (Sigma, Dorset, UK). The trypsin activity was then neutralised by the addition of 50μg/ml of soyabean trypsin inhibitor (Sigma, Dorset, UK), prior to dilution of this enzyme stock solution in Tris-HCl assay buffer (100mM Tris, 100nM NaCl, 10mM $CaCl_2$, 0.16% Brij 35, pH 7.5) to a concentration of 10nM. The final concentration of enzyme used in the assay was 1nM.

Substrate

The fluorogenic substrate used in this screen was Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 (Bachem Ltd., Essex, UK) as described in J.Biol.Chem., 1996, 271, 17119.

Determination of enzyme inhibition

This was performed in the same manner as described for MMPs 2, 3 and 9.

For use in mammals, including humans, the compounds of formula (I) or their salts or solvates of such compounds or salts, can be administered alone, but will generally be administered in admixture with a pharmaceutically or veterinarily acceptable diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice. For example, they can be administered orally, including sublingually, in the form of tablets containing such excipients as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavouring or colouring agents. The compound or salt could be incorporated into capsules or tablets for targetting the colon or duodenum via delayed dissolution of said capsules or tablets for a particular time following oral administration. Dissolution could be controlled by susceptibility of the formulation to bacteria found in the duodenum or colon, so that no substantial dissolution takes places before reaching the target area of the gastrointestinal tract. The compounds or salts can be injected parenterally, for example, intravenously, intramuscularly or subcutaneously. For parenteral administration, they are best used in the form of a sterile aqueous solution or suspension which may contain other substances, for example, enough salt or glucose to make the solution isotonic with blood. They can be administered topically, in the form of sterile creams, gels, suspensions, lotions, ointments, dusting powders, sprays, drug-incorporated dressings or via a skin patch. For example they can be incorporated into a cream consisting of an aqueous or oily emulsion of polyethylene glycols or liquid paraffin, or they can be incorporated into an ointment consisting of a white wax soft paraffin base, or as hydrogel with cellulose or polyacrylate derivatives or other viscosity modifiers, or as a dry powder or liquid spray or aerosol with butane/propane, HFA or CFC propellants, or as a drug-incorporated dressing either as a tulle dressing, with white soft paraffin or polyethylene glycols impregnated gauze dressings or with hydrogel, hydrocolloid, alginate or film dressings. The compound or salt could also be administered intraocularly as an eye drop with appropriate buffers, viscosity modifiers (e.g. cellulose derivatives), preservatives (e.g. benzalkonium chloride (BZK)) and agents to adjust tenicity (e.g. sodium chloride). Such formulation techniques are well-known in the art. In some instances the formulations may advantageously also contain an antibiotic. All such formulations may also contain appropriate stabilisers and preservatives.

For veterinary use, a compound of formula (I), or a veterinarily acceptable salt thereof, or a veterinarily acceptable solvate of either entity, is administered as a suitably acceptable formulation in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular animal.

Reference to treatment includes prophylaxis as well as alleviation of established conditions, or the symptoms thereof.

For oral and parenteral administration to animal (inc. human) patients, the daily dosage level of the compounds of formula (I) or their salts will be from 0.001 to 20, preferably from 0.01 to 20, more preferably from 0.1 to 10, and most preferably from 0.5 to 5 mg/kg (in single or divided doses). Thus tablets or capsules of the compounds will contain from 0.1 to 500, preferably from 50 to 200, mg of active compound for administration singly or two or more at a time as appropriate.

For topical administration to animal (inc. human) patients with chronic wounds, the daily dosage level of the compounds, in suspension or other formulation, could be from 0.001 to 30mg/ml, preferably from 0.01 to 10 mg/ml.

The physician or veterinary surgeon in any event will determine the actual dosage which will be most suitable for a an individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case; there can of course be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

Thus the invention provides a pharmaceutical composition comprising a compound of formula (I), or a pharmaceutically acceptable salt thereof, or solvate thereof, together with a pharmaceutically acceptable diluent or carrier.

The invention also provides a compound of formula (I), or a pharmaceutically acceptable salt thereof, or solvate thereof, or a pharmaceutical composition containing any of the foregoing, for use as a human medicament.

In yet another aspect, the invention provides the use of a compound of formula (I), or a pharmaceutically acceptable salt thereof, in the manufacture of a human medicament for the treatment of a condition mediated by one or more MMPs.

Moreover, the invention provides the use of a compound of formula (I), or a pharmaceutically acceptable salt thereof, for the manufacture of a human medicament for the treatment of atherosclerotic plaque rupture, myocardial infarction, heart failure, restenosis, stroke, periodontal disease, tissue ulceration, wounds, skin diseases, cancer metastasis, tumour angiogenesis, age-related macular degeneration, fibrotic disease, rheumatoid arthritis, osteoarthritis and inflammatory diseases dependent on migratory inflammatory cells.

Additionally, the invention provides a method of treating a medical condition for which a MMP inhibitor is indicated, in an animal such as a mammal (including a human being), which comprises administering to said animal a therapeutically effective amount of a compound of formula (I), or a pharmaceutically acceptable salt thereof, or a pharmaceutically acceptable solvate of either entity, or a pharmaceutical composition containing any of the foregoing.

Still further, the invention provides a method of treating atherosclerotic plaque rupture, myocardial infarction, heart failure, restenosis, stroke, periodontal disease, tissue ulceration, wounds, skin diseases, cancer metastasis, tumour angiogenesis, age-related macular degeneration, fibrotic disease, rheumatoid arthritis, osteoarthritis and inflammatory diseases dependent on migratory inflammatory cells, in a animal (including a human being), which comprises administering to said animal a therapeutically effective amount of a compound of formula (I), or a pharmaceutically or veterinarily acceptable salt thereof, or a pharmaceutically acceptable solvate of either entity, or a pharmaceutical composition containing any of the foregoing.

Biological data

The compounds of Examples 3, 4, 5, 6, 7, 10 and 14 gave the following IC_{50} values (in nM concentrations) in tests mentioned above:

MMP-3	MMP-2	MMP-1	MMP-14	MMP-9
<10	>100	>1000	>2000	>70

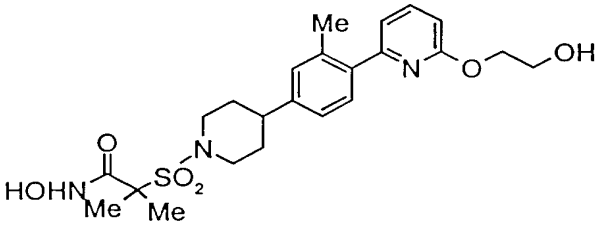
The syntheses of the compounds of the invention and of the intermediates for use therein are illustrated by the following Examples and Preparations.

EXAMPLES AND PREPARATIONS

Room temperature (rt) means 20 to 25°C. Flash chromatography refers to column chromatography on silica gel (Kieselgel 60, 230-400 mesh). Melting points are uncorrected. ^{1}H Nuclear magnetic resonance (NMR) spectra were recorded using a Bruker AC300, a Varian Unity Inova-300 or a Varian Unity Inova-400 spectrometer and were in all cases consistent with the proposed structures. Characteristic chemical shifts are given in parts-per-million downfield from tetramethylsilane using conventional abbreviations for designation of major peaks: e.g. s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Mass spectra were recorded using a Finnigan Mat. TSQ 7000 or a Fisons Intruments Trio 1000 mass spectrometer. LRMS means low resolution mass spectrum and the calculated and observed ions quoted refer to the isotopic composition of lowest mass. Hexane refers to a mixture of hexanes (hplc grade) b.p. 65-70°C. Ether refers to diethyl ether. Acetic acid refers to glacial acetic acid. 1-Hydroxy-7-aza-1H-1,2,3-benzotriazole (HOAt), N-[(dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridin-1-ylmethylene]-N-methylmethaninium hexafluorophosphate N-oxide (HATU) and 7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP) were purchased from PerSeptive Biosystems U.K. Ltd. "Me" is methyl, "Bu" is butyl, "Bn" is benzyl. Other abbreviations and terms are used in conjunction with standard chemical practice.

Example 1

N-Hydroxy 2-[(4-{4-[6-(2-hydroxyethoxy)pyridin-2-yl]-3-methylphenyl}piperidin-1-yl)sulphonyl]-2-methylpropanamide



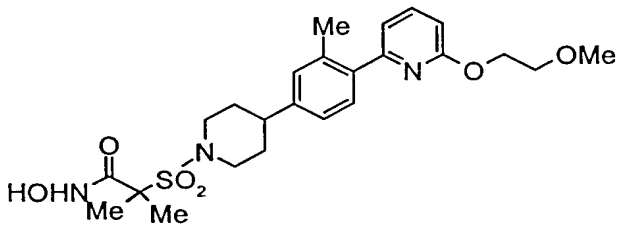
N,N-Dimethylformamide (10ml) was added to a solution of the acid from preparation 70 (430mg, 0.93mmol) in pyridine (5ml), followed by chlorotrimethylsilane (130µl, 1.03mmol) and the solution stirred for 1 ½ hours. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (215mg, 1.11mmol) and 1-hydroxybenzotriazole hydrate (130mg, 0.93mmol) were added, and the reaction stirred for a further 2 hours. Hydroxylamine hydrochloride (195mg, 2.8mmol) was then added, and the reaction stirred at room temperature overnight. The reaction mixture was acidified to pH 1 using 2N hydrochloric acid, stirred for an hour, and then the pH re-adjusted to pH 4. Water (50ml) was added, the resulting precipitate filtered, washed with water and dried under vacuum. This solid was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (90:10:1) as eluant to afford the title compound as a white solid, (220mg, 49%).

mp 137-140°C

^{1}H nmr (DMSO-d_6, 300MHz) δ: 1.50 (s, 6H), 1.61 (m, 2H), 1.80 (m, 2H), 2.36 (s, 3H), 2.68 (m, 1H), 3.05 (m, 2H), 3.72 (m, 4H), 4.25 (t, 2H), 4.79 (t, 1H), 6.76 (d, 1H), 7.05 (d, 1H), 7.17 (m, 2H), 7.35 (d, 1H), 7.76 (dd, 1H), 9.00 (s, 1H), 10.55 (s, 1H).

Example 2

N-Hydroxy 2-{[4-(4-{6-[2-(methoxy)ethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}-2-methylpropanamide



O-(7-Azabenzotriazol-1-yl)-N,N,N'N'-tetramethyluronium hexafluorophosphate (425mg, 0.95mmol) and N-ethyldiisopropylamine (150µl, 0.70mmol) were added to a solution of the acid from preparation 71

(300mg, 0.63mmol) in N,N-dimethylformamide (10ml), and the solution stirred at room temperature for 30 minutes. Hydroxylamine hydrochloride (158mg, 1.9mmol) and additional N-ethyldiisopropylamine (410µl, 1.9mmol) were added, and the reacton stirred at room temperature overnight. The reaction mixture was diluted with water (20ml), and pH 7 buffer solution (20ml), and then extracted with ethyl acetate (3x30ml). The combined organic extracts were washed with brine (3x), water (2x), then dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was triturated with di-isopropyl ether to afford the title compound as an off-white solid, (220mg, 71%).

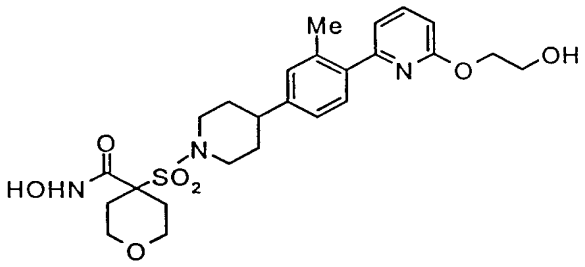
mp 134-138°C

^{1}H nmr (DMSO-d_6, 300MHz) δ: 1.48 (s, 6H), 1.61 (m 2H), 1.80 (m, 2H), 2.36 (s, 3H), 2.66 (m, 1H), 3.05 (m, 2H), 3.28 (s, 3H), 3.62 (t, 2H), 3.78 (m, 2H), 4.38 (t, 2H), 6.78 (d, 1H), 7.06 (d, 1H), 7.16 (m, 2H), 7.35 (d, 1H), 7.76 (m, 1H).

Anal. Found: C, 59.65; H, 7.12; N, 7.69. $C_{24}H_{33}N_3O_6S$;0.2i-Pr_2O requires C, 59.59; H, 7.04; N, 8.04%.

Example 3

N-Hydroxy 4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxamide



Chlorotrimethylsilane (2.1ml, 16.46mmol) was added to a solution of the acid from preparation 72 (7.55g, 14.96mmol) in N,N-dimethylformamide (150ml), and pyridine (150ml), and the solution stirred at room temperature under a nitrogen atmosphere for 1 hour. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.44g, 17.95mmol) and 1-hydroxy-7-azabenzotriazole (2.04g, 14.96mmol) were added, and stirring was continued for a further 45 minutes. Hydroxylamine hydrochloride (3.12g, 44.8mmol) was then added and the reaction stirred at room temperature for 72 hours. The reaction mixture was acidified to pH 2 using hydrochloric acid, stirred for 30 minutes, and the pH then re-adjusted to pH 4 using 1N sodium hydroxide solution. The mixture was extracted with ethyl acetate (3x), the combined organic extracts washed with brine, dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel using ethyl acetate as eluant, and recrystallised from methanol/ethyl acetate to afford the title compound as a white solid, (3.75g, 48%).

mp 193-194°C

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.61 (m, 2H), 1.79 (m, 2H), 1.92 (m, 2H), 2.36 (m 5H), 2.62 (m, 1H), 3.01 (m, 2H), 3.19 (m, 2H), 3.70 (m, 4H), 3.82 (m, 2H), 4.25 (t, 2H), 4.75 (br, t, 1H), 6.70 (d, 1H), 7.01 (d, 1H), 7.12 (m, 2H), 7.30 (d, 1H), 7.62 (dd, 1H), 9.10 (s, 1H), 10.94 (s, 1H).

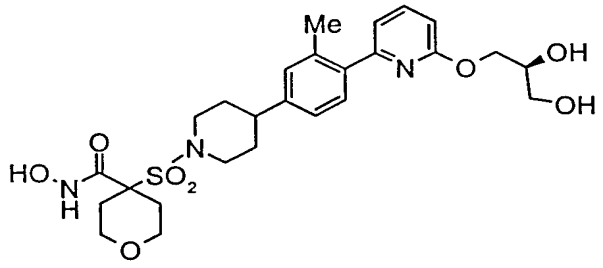
LRMS : m/z 520 $(M+1)^+$

Anal. Found: C, 57.73; H, 6.39; N, 7.99. $C_{25}H_{33}N_3O_7S$ requires C, 57.79; H, 6.40; N, 8.09%.

Alternative route: Hydrogen chloride gas was bubbled through a solution of the tert-butyl ether from preparation 133 (3.0g, 5.22mmol) in anhydrous trifluoroacetic acid (30ml) and dichloromethane (30ml) for 10 minutes, then stirred at room temperature overnight. Nitrogen gas was bubbled through the reaction mixture for 1hour and then 5N NaOH solution until the solution was pH6. The resulting precipitate was cooled to 0°C, filtered and washed with cold water. The resulting solid was dissolved in hot ethyl acetate (500ml) and the organic layer was washed with water (3x250ml) and brine (250ml) and then dried (Na_2SO_4), filtered and concentrated in vacuo. On cooling to 0°C overnight a solid formed and was filtered, washed with cold ethyl acetate and dried. The title compound was obtained as a beige solid (1.6g, 60%).

Example 4

N-Hydroxy 4-{[4-(4-{6-[(2S)-2,3-dihydroxy-1-propoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxamide



Chlorotrimethylsilane (168µl, 1.32mmol) was added to a solution of the acid from preparation 73 (318mg, 0.60mmol) in dichloromethane (6ml), and pyridine (2ml), and the solution stirred at room temperature under a nitrogen atmosphere for 1 hour. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (138mg, 0.72mmol) and 1-hydroxy-7-azabenzotriazole (90mg, 0.66mmol) were added, and stirring was continued for a further hour. Hydroxylamine hydrochloride (124mg, 1.80mmol) was added and the reaction stirred at room temperature for 2 hours. The reaction mixture was evaporated in vacuo, the residue dissolved in methanol, the solution acidified to pH 1 using hydrochloric acid (2M),

then stirred for 10 minutes. The solution was diluted with water, the pH adjusted to 6, and the resulting precipitate filtered and dried. The solid was purified by column chromatography on silica gel using dichloromethane:methanol (90:10) as eluant, and recrystallised from methanol/di-isopropyl ether to give the title compound as a white solid, (200mg, 60%).

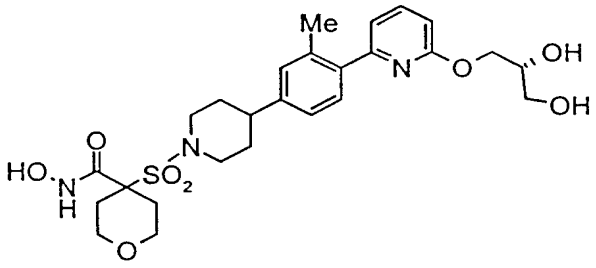
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.61 (m, 2H), 1.79 (m, 2H), 1.92 (m, 2H), 2.36 (m, 5H), 2.63 (m, 1H), 3.03 (m, 2H), 3.08-3.31 (m, 3H), 3.40 (m, 2H), 3.68-3.89 (m, 4H), 4.15 (m, 1H), 4.25 (m, 1H), 4.56 (br, s, 1H), 4.80 (br, s, 1H), 6.75 (d, 1H), 7.04 (d, 1H), 7.14 (m, 2H), 7.34 (d, 1H), 7.75 (m, 1H), 9.14 (s, 1H), 10.96 (s, 1H).

LRMS : m/z 550 $(M+1)^+$

Anal. Found: C, 50.70; H, 6.00; N, 6.93. $C_{26}H_{35}N_3O_8S;0.6H_2O$ requires C, 50.97; H, 6.21; N, 6.86%.

Example 5

N-Hydroxy 4-{[4-(4-{6-[(2R)-2,3-dihydroxy-1-propoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxamide



The title compound was prepared from the acid from preparation 74, following the procedure described in example 4. The crude product was purified by crystallisation from ethyl acetate to give an off-white solid (180mg, 58%).

mp 125-130°C

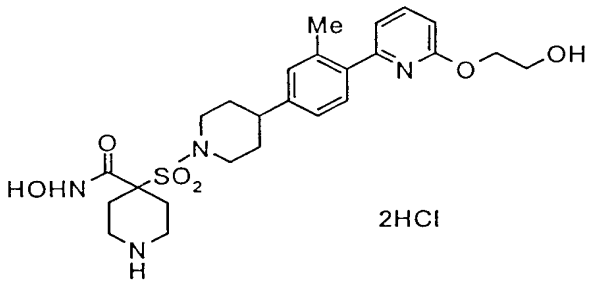
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.78 (m, 2H), 1.90 (m, 2H), 2.36 (m, 5H), 2.64 (m, 1H), 3.02 (m, 2H), 3.20 (m, 2H), 3.40 (m, 2H), 3.72 (m, 2H), 3.78 (m, 1H), 3.83 (m, 2H), 4.14 (m, 1H), 4.24 (m, 1H), 4.55 (dd, 1H), 4.80 (d, 1H), 6.75 (d, 1H), 7.03 (d, 1H), 7.15 (m, 2H), 7.32 (d 1H), 7.75 (m, 1H), 9.14 (s, 1H), 10.95 (s, 1H).

LRMS : m/z 572 $(M+23)^+$

Anal. Found: C, 55.32; H, 6.57; N, 7.28. $C_{26}H_{35}N_3O_8S;H_2O$ requires C, 55.02; H, 6.57; N, 7.40%.

Example 6

N-Hydroxy 4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}-piperidine-4-carboxamide dihydrochloride



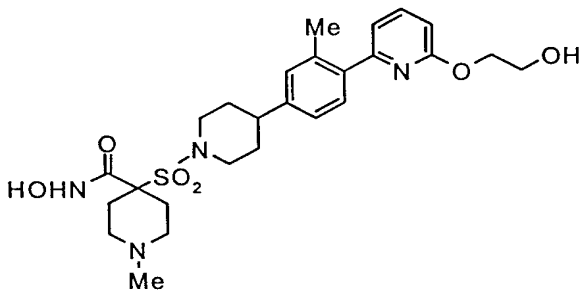
Hydrogen chloride gas was bubbled through an ice-cold solution of the hydroxamic acid from preparation 87 (135mg, 0.22mmol) in methanol (20ml), and the solution was stirred at room temperature. The reaction mixture was evaporated in vacuo, and the residue azeotroped with methanol. The solid was recrystallised from methanol/ether to afford the title compound as a white solid, (88mg, 64%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.63 (m, 2H), 1.80 (m, 2H), 2.07 (m, 2H), 2.35 (s, 3H), 2.56-2.72 (m, 5H), 2.08 (m, 2H), 2.38 (m, 2H), 3.72 (m, 4H), 4.24 (t, 2H), 4.44-4.67 (br, s, 2H), 6.76 (d, 1H), 7.04 (d, 1H), 7.17 (m, 2H), 7.34 (d, 1H), 7.75 (m, 1H), 8.97 (m, 1H), 9.18 (m, 1H).

LRMS : m/z 519 $(M+1)^+$

Example 7

N-Hydroxy 4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}-1-methyl-piperidine-4-carboxamide



The title compound was prepared from the acid from preparation 75 and hydroxylamine hydrochloride following a similar procedure to that described in example 1. The reaction mixture was acidified to pH 2 using hydrochloric acid, this mixture stirred for 45 minutes, then basified to pH 8 using sodium hydroxide solution (2N). This solution was extracted with ethyl acetate (3x), the combined organic

extracts washed with water, then brine, dried (Na_2SO_4), filtered and evaporated in vacuo. The residue was dried at 60°C, under vacuum to afford the title compound (39mg, 8%).

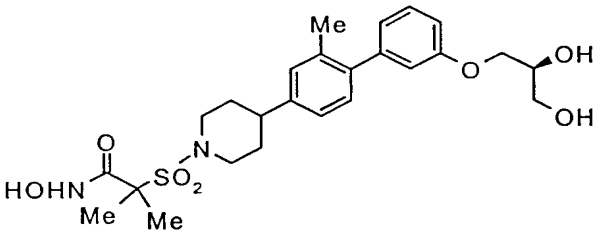
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.78 (m, 4H), 1.86 (m, 2H), 2.8 (s, 3H), 2.35 (s, 3H), 2.40 (m, 2H), 2.59-2.75 (m, 3H), 3.01 (m, 2H), 3.68 (m, 4H), 4.25 (t, 2H), 4.75 (t, 1H), 6.75 (d, 1H), 7.03 (d, 1H), 7.15 (m, 2H), 7.32 (d, 1H), 7.74 (m, 1H), 9.06 (br, s, 1H), 10.88 (br, s, 1H).

LRMS : m/z 533 $(M+1)^+$

Anal. Found: C, 57.91; H, 6.82; N, 10.24. $C_{26}H_{36}N_4O_6S;0.3H_2O$ requires C, 58.04; H, 6.86; N, 10.41%.

Example 8

N-Hydroxy 2-[4-(4-{3-[(2S)-2,3-dihydroxy-1-propoxy]phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-2-methylpropanamide



The title compound was prepared from the acid from preparation 77, following a similar procedure to that described in example 3. The crude product was recrystallised from methanol/di-isopropyl ether, to give the desired product (75mg, 24%) as a white solid. The mother liquors were evaporated in vacuo, and purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (98:2 to 95:5) to give an additional (38mg, 12%) of the desired product.
mp 152-154°C

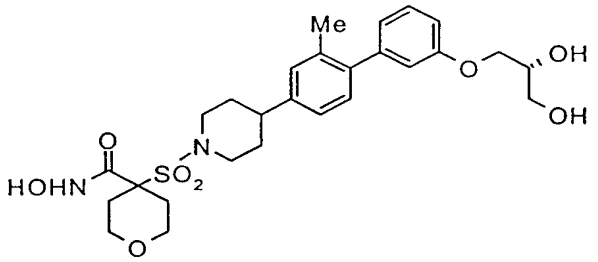
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.44 (s, 6H), 1.60 (m, 2H), 1.78 (m, 2H), 2.18 (s, 3H), 2.61 (m, 1H), 3.02 (m, 2H), 3.39 (m, 2H), 3.71 (m, 3H), 3.82 (m, 1H), 3.98 (m, 1H), 4.56 (m, 1H), 4.82 (m, 1H), 6.82 (m, 3H), 7.08 (m, 2H), 7.12 (s, 1H), 7.26 (m, 1H), 8.94 (s, 1H), 10.69 (s, 1H).

LRMS : m/z 529 $(M+23)^+$

Anal. Found: C, 58.10; H, 6.70; N, 5.09. $C_{25}H_{34}N_2O_7S;0.5MeOH$ requires C, 58.60; H, 6.94; N, 5.36%.

Example 9

N-Hydroxy 4-{4-[4-(3-[(2R)-2,3-dihydroxy-1-propoxy]phenyl)-3-methylphenyl]-piperidin-1-ylsulphonyl}-tetrahydro-(2H)-pyran-4-carboxamide



Chlorotrimethylsilane (45µl, 0.37mmol) was added to a solution of the acid from preparation 79 (90mg, 0.17mmol) in dichloromethane (2ml), and pyridine (1ml), and the solution stirred at room temperature under a nitrogen atmosphere for 1 hour. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (40mg, 0.21mmol) and 1-hydroxy-7-azabenzotriazole (26mg, 0.19mmol) were added, and stirring was continued for a further hour. Hydroxylamine hydrochloride (36mg, 0.51mmol) was then added and the reaction stirred at room temperature for a further 2 hours. The reaction mixture was diluted with methanol (5ml), acidified to pH 1 using hydrochloric acid, and the mixture stirred vigorously for an hour. The mixture was extracted with dichloromethane (3x30ml), the combined organic extracts dried (Na_2SO_4), filtered and evaporated. The residue was purified by column chromatography on silica gel using dichloromethane:methanol (90:10) as eluant to afford the title compound as an off-white solid, (40mg, 43%).

mp 141-145°C

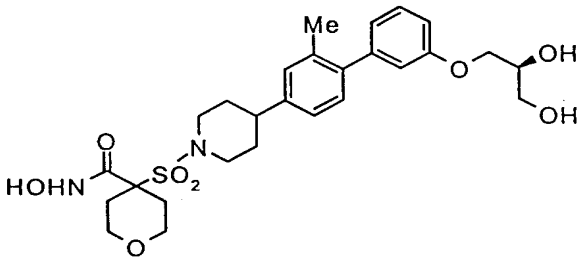
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.78 (m, 2H), 1.90 (m, 2H), 2.20 (s, 3H), 2.38 (m, 2H), 2.62 (m, 1H), 3.03 (m, 2H), 3.20 (m, 2H), 3.42 (m, 2H), 3.66-3.90 (m, 6H), 4.01 (m, 1H), 4.60 (m, 1H), 4.90 (m, 1H), 6.84 (m, 3H), 7.14 (m, 3H), 7.30 (m, 1H), 9.18 (s, 1H), 10.98 (1H, s).

LRMS : m/z 571 $(M+23)^+$

Anal. Found: C, 59.22; H, 6.80; N, 5.11. $C_{27}H_{36}N_2O_8S$ requires C, 59.11; H, 6.61; N, 5.11%.

Example 10

N-Hydroxy 4-{4-[4-(3-{(2S)-2-hydroxy-2-hydroxymethyl}ethoxyphenyl)-3-methylphenyl]-piperidin-1-ylsulphonyl}-tetrahydro-2H-pyran-4-carboxamide



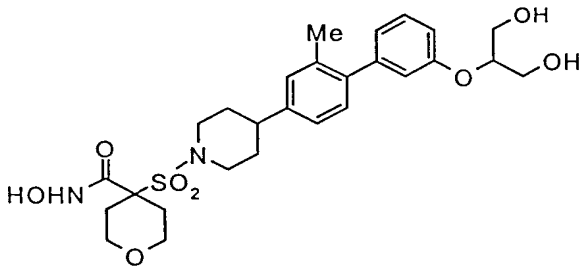
The title compound was prepared, from the acid from preparation 80, following a similar procedure to that described in example 9. The crude product was triturated with methanol/di-isopropyl ether, and the resulting precipitate filtered and dried to afford the title compound as a buff-coloured solid, (158mg, 45%).

mp 132-134°C

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.78 (m, 2H), 1.90 (m, 2H), 2.20 (s, 3H), 2.38 (m, 2H), 2.62 (m, 1H), 3.02 (m, 2H), 3.20 (m, 2H), 3.42 (dd, 2H), 3.68-3.90 (m, 6H), 4.00 (m, 1H), 4.60 (t, 1H), 4.97 (d, 1H), 6.81 (m, 2H), 6.90 (m, 1H), 7.08 (s, 2H), 7.15 (s, 1H), 7.29 (dd, 1H), 9.14 (s, 1H), 10.98 (s, 1H).

Example 11

N-Hydroxy 4-{4-[4-(3-{1,3-dihydroxy-2-propoxyphenyl)-3-methylphenyl]-piperidin-1-ylsulphonyl}-tetrahydro-2H-pyran-4-carboxamide



The title compound was obtained (25%) as a white solid, from the acid from preparation 78 and hydroxylamine hydrochloride, using a similar procedure to that described in example 9.

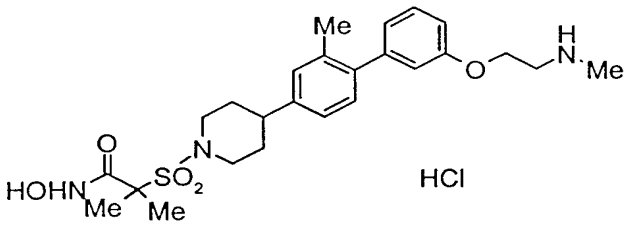
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.79 (m, 2H), 1.90 (m, 2H), 2.20 (s, 3H), 2.39 (m, 2H), 2.62 (m, 1H), 3.02 (m, 2H), 3.20 (m, 2H), 3.57 (m, 4H), 3.70 (m, 2H), 3.84 (m, 2H), 4.24 (m, 1H), 4.78 (m, 2H), 6.82 (d, 1H), 6.90 (m, 2H), 7.14 (m, 3H), 7.28 (m, 1H), 9.18 (br, s, 1H).

LRMS : m/z 570 $(M+23)^+$

Anal. Found: C, 56.98; H, 6.65; N, 5.15. $C_{27}H_{36}N_2O_8S;H_2O$ requires C, 57.22; H, 6.76; N, 4.94%.

Example 12

N-Hydroxy 2-{[4-(4-{3-[2-(methylamino)ethoxy]phenyl}-3-methylphenyl)-piperidin-1-yl]sulphonyl}-2-methylpropanamide hydrochloride



Dichloromethane saturated with hydrogen chloride (12ml) was added to a solution of the hydroxamic acid from preparation 88 (120mg, 0.2mmol) in dichloromethane (1ml), and the reaction stirred at room temperature for 4 hours. The resulting precipitate was filtered, then washed with, dichloromethane, ether, then dried under vacuum at 60°C, to afford the title compound as a solid, (90mg, 85%).

mp 180-184°C

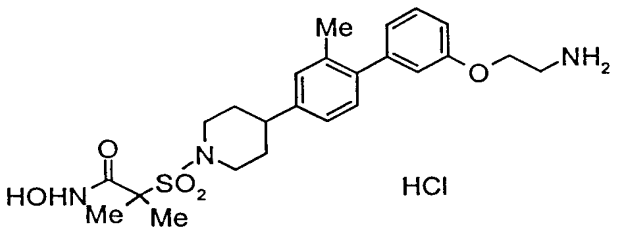
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.44 (s, 6H), 1.60 (m, 2H), 1.78 (m, 2H), 2.18 (s, 3H), 2.59 (m, 3H), 3.02 (m, 2H), 3.28 (m, 2H), 3.72 (m, 2H), 4.23 (t, 2H), 6.90 (m, 3H), 7.08 (s, 2H), 7.16 (s, 1H), 7.34 (m, 1H), 8.83 (br s, 2H), 10.80 (s, 1H).

LRMS : m/z 490 $(M+1)^+$

Anal. Found: C, 54.25; H, 6.93; N, 7.44. $C_{25}H_{35}N_3O_5S;HCl;H_2O;0.1CH_2Cl_2$ requires C, 54.56; H, 6.97; N, 7.60%.

Example 13

N-Hydroxy 2-[4-(4-{3-(2-aminoethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-2-methylpropanamide hydrochloride



The title compound was obtained as a solid (76%), from the hydroxamic acid from preparation 89, following the procedure described in example 12.

mp 204-206°C

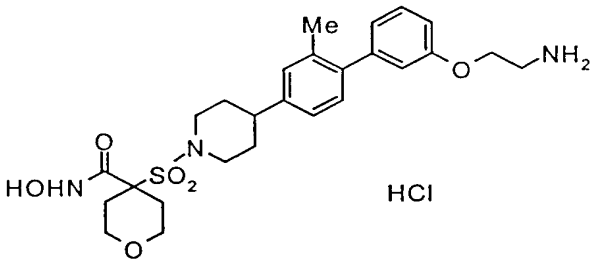
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.48 (s, 6H), 1.60 (m, 2H), 1.80 (m, 2H), 2.20 (s, 3H), 2.64 (m, 2H), 3.06 (m, 2H), 3.20 (t, 2H), 3.75 (m, 2H), 4.20 (t, 2H), 6.94 (m, 3H), 7.12 (s, 2H), 7.18 (s, 1H), 7.38 (m, 2H), 8.01 (br s, 1H), 8.99 (s, 1H).

LRMS : m/z 476 $(M+1)^+$

Anal. Found: C, 55.21; H, 6.74; N, 7.83. $C_{24}H_{33}N_3O_5S$;HCl;0.5H_2O requires C, 55.32; H, 6.77; N, 8.06%.

Example 14

N-Hydroxy 4-{[4-(-4-{6-[2-aminoethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxamide hydrochloride



A saturated solution of hydrogen chloride in dichloromethane (250ml) was added to a solution of the hydroxamic acid from preparation 90 (4.5g, 7.28mmol) in dichloromethane (30ml), and the reaction stirred at room temperature for 3 ½ hours. The mixture was cooled in an ice-bath, the resulting precipitate filtered off, and washed with dichloromethane, then ether. The solid was then dried under vacuum at 70°C to afford the title compound (3.1g, 77%).

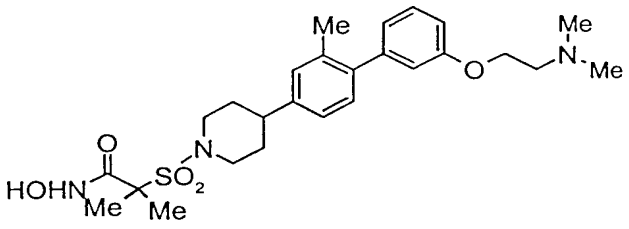
mp 208-210°C

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.78 (m, 2H), 1.90 (m, 2H), 2.19 (s, 3H), 2.38 (m, 2H), 2.62 (m, 1H), 3.02 (m, 2H), 3.19 (m, 6H), 3.70 (m, 2H), 3.83 (m, 2H), 4.18 (t, 2H), 6.92 (m, 3H), 7.06 (s, 2H), 7.17 (s, 1H), 7.35 (m, 1H), 9.12 (s, 1H).

LRMS : m/z 518 $(M+1)^+$

Example 15

N-Hydroxy 2-[4-(4-{3-(2-N,N-dimethylaminoethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-2-methylpropanamide



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (130mg, 0.68mmol) and 1-hydroxy-7-azabenzotriazole (80mg, 0.59mmol) were added to a solution of the acid from preparation 83 (270mg, 0.55mmol) in pyridine (6ml) and dichloromethane (6ml) under a nitrogen atmosphere, and the suspension stirred for 30 minutes. N,N-dimethylformamide (5ml), was added, and the reaction warmed to 50°C to obtain a solution. Hydroxylamine hydrochloride (115mg, 1.65mmol) was added and the reaction stirred at room temperature for 18 hours. The reaction mixture was partitioned between ethyl acetate (100ml) and pH 7 buffer solution (30ml), and the phases separated. The organic layer was washed with water (2x30ml), brine (30ml), dried (Na_2SO_4), filtered and evaporated in vacuo. The residue was azeotroped with toluene (3x), and ethyl acetate (2x), and dried under vacuum at 60°C, to afford the title compound as a solid, (180mg, 65%).

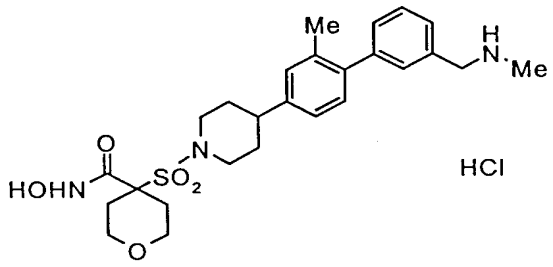
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.48 (s, 6H), 1.60 (m, 2H), 1.78 (m, 2H), 2.19 (s, 9H), 2.60 (m, 3H), 3.03 (m, 2H), 3.76 (m, 2H), 4.05 (t, 2H), 6.80 (m, 2H), 6.86 (m, 1H), 7.06 (m, 2H), 7.12 (s, 1H), 7.28 (m, 1H).

LRMS : m/z 504 $(M+1)^+$

Anal. Found: C, 60.43; H, 7.50; N, 8.08. $C_{26}H_{37}N_3O_5S;0.75H_2O$ requires C, 60.38; H, 7.50; N, 8.12%.

Example 16

N-Hydroxy 4-{[4-(4-{3-(methyl)aminomethyl}-3-methylphenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxamide hydrochloride



A solution of dichloromethane saturated with hydrogen chloride (20ml) was added to a solution of the hydroxamic acid from preparation 91 (347mg, 0.58mmol) in dichloromethane (10ml), and the solution

stirred at room temperature for 4 hours. The reaction mixture was concentrated in vacuo, and the residue triturated with hot methanol/di-isopropyl ether to give the title compound as a white solid, (202mg, 64%).

mp 213-214°C

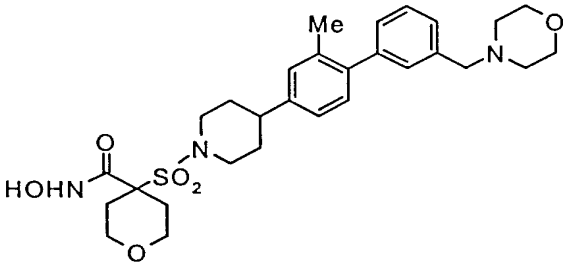
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.78 (m, 2H), 1.97 (m, 2H), 2.20 (s, 3H), 2.38 (m, 2H), 2.46 (s, 3H), 2.62 (m, 1H), 3.01 (m, 2H), 3.18 (m, 2H), 3.70 (m, 2H), 3.82 (m, 2H), 4.12 (s, 2H), 7.10 (m, 3H), 7.35 (s, 1H), 7.43 (m, 3H), 9.10 (br, s, 1H), 10.92 (s, 1H).

LRMS : m/z 502 $(M+1)^+$

Anal. Found: C, 57.16; H, 6.72; N, 7.64. $C_{26}H_{35}N_3O_5S;HCl;0.5H_2O$ reqires C, 57.08; H, 6.82; N, 7.68%.

Example 17

N-Hydroxy 4-{[4-(3-methyl-4-{3-[4-morpholinylmethyl]}phenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxamide



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (265mg, 1.38mmol) and 1-hydroxy-7-azabenzotriazole (157mg, 1.15mmol) were added to a solution of the acid from preparation 86 (625mg, 1.15mmol) in pyridine (6ml) and N,N-dimethylformamide (6ml) under a nitrogen atmosphere, and the suspension stirred for 1 hour. Hydroxylamine hydrochloride (210mg, 3.45mmol) was added and the reaction stirred at room temperature for 18 hours. The reaction mixture was partitioned between ethyl acetate and pH 7 buffer solution, the phases separated, and the aqueous layer extracted with ethyl acetate. The combined organic solutions were washed with water, brine, then dried ($MgSO_4$), filtered and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant, and recrystallised from ethyl acetate to give the desired product as a white solid, (398mg, 62%).

mp 177-179°C

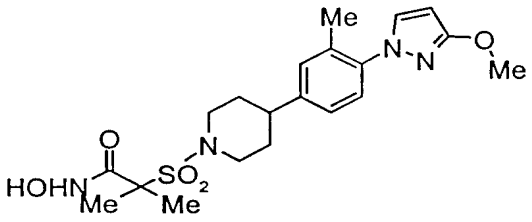
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.78 (m, 2H), 1.88 (m, 2H), 2.17 (s, 3H), 2.36 (m, 6H), 2.60 (m, 1H), 3.00 (m, 2H), 3.19 (m, 2H), 3.46 (s, 2H), 3.53 (m, 4H), 3.70 (m, 2H), 3.81 (m, 2H), 7.06 (m, 7H), 9.10 (s, 1H), 10.92 (s, 1H).

LRMS : m/z 558 $(M+1)^+$

Anal. Found: C, 62.15; H, 7.01; N, 7.40. $C_{29}H_{39}N_3O_6S$ requires C, 62.46; H, 7.05; N, 7.53%.

Example 18

N-Hydroxy 2-({4-[4-(3-methoxy-1H-pyrazol-1-yl)-3-methylphenyl]piperidin-1-yl}sulphonyl)-2-methylpropanamide



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (129mg, 0.67mmol) and 1-hydroxy-7-azabenzotriazole (76mg, 0.56mmol) were added to a solution of the acid from preparation 103 (235mg, 0.56mmol) in pyridine (1.5ml) and dichloromethane (3ml) under a nitrogen atmosphere, and the suspension stirred for 30 minutes. Hydroxylamine hydrochloride (78mg, 1.12mmol) was added and the reaction stirred at room temperature for 18 hours. The reaction mixture was poured into ethyl acetate (100ml), washed with pH 7 buffer solution (2x50ml) then dried ($MgSO_4$), filtered and evaporated in vacuo. The residual white solid was recrystallised from hot ethyl acetate, to afford the title compound as a white solid, (156mg, 64%).

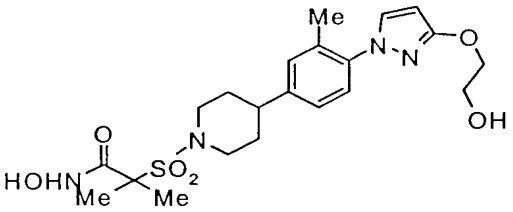
mp 172-173°C

^{1}H nmr (CD_3OD, 400MHz) δ: 1.58 (s, 6H), 1.74 (m, 2H), 1.82 (m, 2H), 2.20 (s, 3H), 2.70 (m, 1H), 3.09 (m, 2H), 3.87 (m, 5H), 5.84 (s, 1H), 7.16 (m, 1H), 7.20 (m, 2H), 7.48 (s, 1H).

Anal. Found: C, 55.04; H, 6.42; N, 12.77. $C_{20}H_{28}N_4O_5S$ requires C, 55.03; H, 6.47; N, 12.83%.

Example 19

N-Hydroxy 2-[(4-{4-[3-(2-hydroxyethoxy)-1H-pyrazol-1-yl]-3-methylphenyl}piperidin-1-yl)sulphonyl]-2-methylpropanamide



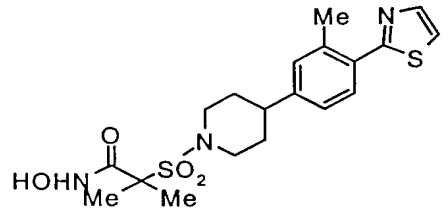
Pyridine (6ml) was added to a suspension of the acid from preparation 104 (325mg, 0.72mmol) in dichloromethane (6ml), and the solution purged with nitrogen. Chlorotrimethylsilane (858mg, 0.79mmol) was added, the solution stirred for an hour, then 1-hydroxy-7-azabenzotriazole (98mg, 0.72mmol) was added, followed by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (166.8mg, 0.87mmol), and the solution was stirred for a further hour. Hydroxylamine hydrochloride (150mg, 2.16mmol) was then added and the reaction stirred at room temperature for 17 hours. The reaction was partitioned between ethyl acetate and pH 7 buffer solution, and the pH of the mixture carefully adjusted to 3 using hydrochloric acid (2N). The layers were separated, the organic phase dried ($MgSO_4$), filtered and evaporated in vacuo, and the residue triturated with ether. The resulting white solid was filtered, then dissolved in a solution of acetic acid (10ml), water (10ml), and methanol (10ml), and this mixture stirred at room temperature for 45 minutes. The solution was poured into pH 7 buffer (300ml), extracted with ethyl acetate (3x100ml), and the combined organic extracts dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was azeotroped with toluene and ethyl acetate, and triturated several times with ether to give the title compound as a white solid, (141mg, 42%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.43 (s, 6H), 1.59 (m, 2H), 1.77 (m, 2H), 2.19 (s, 3H), 2.62 (m, 1H), 3.00 (m, 2H), 3.66 (m, 4H), 4.05 (t, 2H), 4.72 (br, t, 1H), 5.84 (s, 1H), 7.15 (m, 1H), 7.19 (m, 2H), 7.72 (s, 1H), 8.90 (s, 1H), 10.66 (s, 1H).

Anal. Fond: C, 53.85; H, 6.49; N, 11.86. $C_{21}H_{30}N_4O_6S$ requires C, 54.06; H, 6.48; N, 12.01%.

Example 20

N-Hydroxy 2-methyl-2-({4-[3-methyl-4-(1,3-thiazol-2-yl)phenyl]piperidin-1-yl}sulphonyl)propanamide



The title compound was prepared from the acid from preparation 105, following the procedure described in example 18. The crude product was crystallised from a minimum volume of methanol to give the desired product as a white solid, (58mg, 35%).

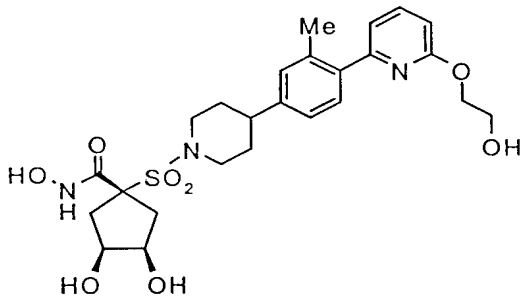
mp 199-201°C

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.45 (s, 6H), 1.60 (m, 2H), 2.44 (s, 3H), 2.65 (m, 1H), 3.01 (m, 2H), 3.14 (s, 2H), 3.72 (m, 2H), 7.18 (d, 1H), 7.20 (s, 1H), 7.61 (d, 1H), 7.75 (s, 1H), 7.90 (s, 1H), 8.82 (br, s, 1H), 10.60 (s, 1H).

Anal. Found: C, 53.51; H, 5.92; N, 9.75. $C_{19}H_{25}N_3O_4S_2$ requires C, 53.88; H, 5.95; N, 9.92%.

Example 21

(1α,3α,4α)-*N*,3,4-trihydroxy-1-[(4-{4-[6-(2-hydroxyethoxy)pyridin-2-yl]-3-methylphenyl}piperidin-1-yl)sulfonyl]cyclopentanecarboxamide



Hydrogen chloride gas was bubbled through a solution of the tert-butyl ether from preparation 121 (260mg, 0.412mmol) in trifluoroacetic acid (10ml) and dichloromethane (10ml) for 5 minutes, and the reaction was stirred for 5 $^1/_2$ hours at ambient temperature. The reaction mixture was evaporated in vacuo and the resulting oil azeotroped with toluene (x2) before partitioning between ethyl acetate (50ml) and pH7 phosphate buffer solution (40ml). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (2x50ml). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated in vacuo. The resulting solid, which contained some of the starting compound, was resubmitted to the reaction conditions. After 5 hours at ambient temperature nitrogen gas was bubbled through the reaction mixture for 15 minutes. The reaction mixture was then evaporated in vacuo and the resulting oil azeotroped with toluene (x2) before partitioning between ethyl acetate (50ml) and pH7 phosphate buffer solution (40ml). The organic layer was separated and the aqueous layer extracted with ethyl acetate (2x50ml). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated in vacuo. The resulting solid was purified by column chromatography on silica gel using dichloromethane/methanol (98:2 to 93:7) as eluant. The title compound was isolated as a white solid (30mg, 15%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.59 (m, 2H), 1.76 (m, 2H), 2.22 (m, 2H), 2.32 (s, 3H), 2.39 (m, 2H), 2.60 (m, 1H), 2.99 (t, 2H), 3.64 (m, 4H), 3.90 (s, 2H), 4.23 (m, 2H), 4.54 (s, 2H), 4.75 (t, 1H), 6.72 (d, 1H), 7.03 (d, 1H), 7.15 (m, 2H), 7.31 (d, 1H), 7.73 (t, 1H), 8.95 (s, 1H), 10.69 (s, 1H).

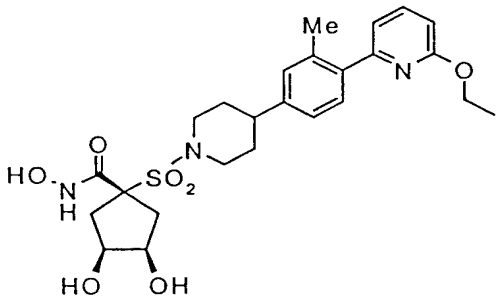
LRMS :m/z 536 $(M+1)^+$.

mp 215-218°C

Anal. Found: C, 49.73; H, 5.67; N, 6.45. $C_{25}H_{33}N_3O_8S$;TFA, 0.5MeOH requires C, 49.62; H, 5.45; N, 6.31%.

Example 22

(1α,3α,4α)-1-({4-[4-(6-ethoxypyridin-2-yl)-3-methylphenyl]piperidin-1-yl}sulfonyl)-*N*,3,4-trihydroxycyclopentanecarboxamide



2N Hydrochloric acid (2ml) was added to a solution of the dioxolane from preparation 122 in dioxan (2ml) and tetrahydrofuran (2ml) and the reaction mixture was stirred at ambient temperature for 18 hours. The reaction mixture was evaporated in vacuo and the resulting solid partitioned between pH7 phosphate buffer solution (20ml) and ethyl acetate (20ml). The aqueous layer was extracted with ethyl acetate (2x20ml) and the combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. The resulting solid was recrystalised from ethyl acetate to afford the title compound as a white solid (95mg, 70%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.25 (t, 3H), 1.58 (m, 2H), 1.76 (m, 2H), 2.22 (m, 2H), 2.35 (s, 3H), 2.38 (m, 2H), 2.60 (m, 1H), 2.99 (t, 2H), 3.66 (d, 2H), 3.85 (s, 2H), 4.25 (q, 2H), 4.61 (s, 2H), 6.71 (d, 1H), 7.03 (d, 1H), 7.12 (m, 2H), 7.31 (d, 1H), 7.72 (t, 1H), 9.00 (s, 1H), 10.78 (s, 1H).

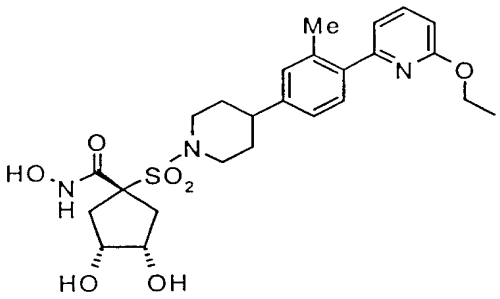
LRMS :m/z 520 $(M+1)^+$.

mp 204-205°C

Anal. Found: C, 57.42; H, 6.36; N, 7.98. $C_{25}H_{33}N_3O_7S$; 0.25 H_2O requires C, 57.29; H, 6.44; N, 8.02%.

Example 23

(1α,3β,4β)-1-({4-[4-(6-ethoxypyridin-2-yl)-3-methylphenyl]piperidin-1-yl}sulfonyl)-*N*,3,4-trihydroxycyclopentanecarboxamide



The title compound was prepared from the dioxolane from preparation 123 in a similar procedure to that described in example 22. This afforded the title compound as a white solid (50mg, 55%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.27 (t, 3H), 1.62 (m, 2H), 1.78 (m, 2H), 2.09 (m, 2H), 2.35 (s, 3H), 2.61 (m, 1H), 2.74 (m, 2H), 3.01 (t, 2H), 3.69 (m, 4H), 4.29 (q, 2H), 4.49 (s, 2H), 6.69 (d, 1H), 7.02 (d, 1H), 7.12 (m, 2H), 7.31 (d, 1H), 7.73 (t, 1H), 8.92 (s, 1H), 10.71 (s, 1H).

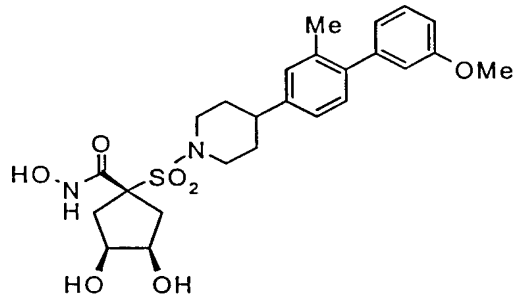
LRMS :m/z 520 $(M+1)^+$.

mp 196-197°C

Anal. Found: C, 56.83; H, 6.32; N, 7.83. $C_{25}H_{33}N_3O_7S$; 0.5 H_2O requires C, 56.80; H, 6.48; N, 7.95%.

Example 24

(1α,3α,4α)-*N*,3,4-trihydroxy-1-{4-[4-(3-methoxyphenyl)-3-methylphenyl]piperidin-1-ylsulfonyl}cyclopentanecarboxamide



2N Hydrochloric acid (2ml) was added to a solution of the dioxolane from preparation 124 in dioxan (3ml) and tetrahydrofuran (2ml) and the reaction mixture was stirred at ambient temperature for 4 hours. The reaction mixture was evaporated in vacuo and the resulting solid was partitioned between water (20ml) and ethyl acetate (20ml). The aqueous layer was extracted with ethyl acetate (2x20ml) and the combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. The resulting solid was recrystalised from ethyl acetate to afford the title compound as a white solid (60mg, 46%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.58 (m, 2H), 1.76 (m, 2H), 2.19 (s, 3H), 2.24 (m, 2H), 2.38 (m, 2H), 2.60 (m, 1H), 2.99 (t, 2H), 3.71 (m, 5H), 3.79 (s, 2H), 4.54 (s, 2H), 6.82 (m, 3H), 7.11 (m, 3H), 7.32 (t, 1H), 8.97 (s, 1H), 10.70 (s, 1H).

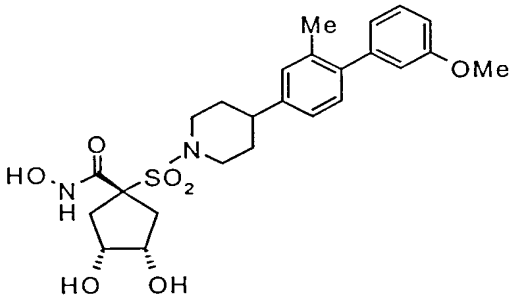
LRMS :m/z 527 $(M+23)^+$.

mp 201-202°C

Anal. Found: C, 58.85; H, 6.36; N, 5.51. $C_{25}H_{32}N_2O_7S$; 0.25 H_2O requires C, 58.98; H, 6.43; N, 5.50%.

Example 25

(1α,3β,4β)-*N*,3,4-trihydroxy-1-{4-[4-(3-methoxyphenyl)-3-methylphenyl]piperidin-1-ylsulfonyl}cyclopentanecarboxamide



The title compound was prepared from the dioxolane from preparation 125 in a similar procedure to that described in example 24. This afforded the title compound as a white solid (55mg, 50%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.59 (m, 2H), 1.76 (m, 2H), 2.17 (m, 2H), 2.19 (s, 3H), 2.60 (m, 1H), 2.71 (m, 2H), 2.99 (t, 2H), 3.70 (m, 7H), 4.61 (s, 2H), 6.82 (m, 3H), 7.12 (m, 3H), 7.32 (t, 1H), 9.00 (s, 1H), 10.82 (s, 1H).

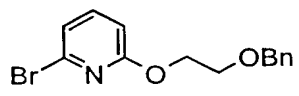
LRMS :m/z 503 $(M-1)^-$.

mp 188-189°C

Anal. Found: C, 58.97; H, 6.50; N, 5.49. $C_{25}H_{32}N_2O_7S$; 0.25 H_2O requires C, 58.98; H, 6.43; N, 5.50%.

Preparation 1

2-[2-(Benzyloxy)ethoxy]-6-bromopyridine

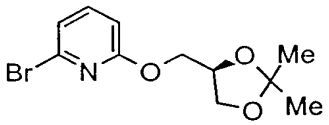


Sodium hydride (900mg, 60% dispersion in mineral oil, 22.5mmol) was added portionwise to an ice-cold solution of 2-(benzyloxy)ethanol (3.0g, 20.0mmol) in toluene (100ml), and the solution stirred for 30 minutes. 2,6-Dibromopyridine (4.75g, 20.0mmol) was added, and the reaction heated under reflux for 2 hours. The cooled mixture was diluted with water (100ml), and extracted with ethyl acetate (3x100ml). The combined organic extracts were dried ($MgSO_4$), filtered and evaporated in vacuo to give the title compound as a yellow oil, (quantitative).

^{1}H nmr ($CDCl_3$, 300MHz) δ: 3.82 (t, 2H), 4.52 (t, 2H), 4.62 (s, 2H), 6.75 (d, 1H), 7.05 (d, 1H), 7.22-7.46 (m, 6H).

Preparation 2

2-Bromo-6-{[(4R)-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}pyridine

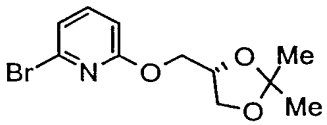


Sodium hydride (1.62g, 60% dispersion in mineral oil, 40.5mmol) was added portionwise to an ice-cooled solution of (R)-(-)-1,2-O-isopropylideneglycerol (4.86g, 36.8mmol) in toluene (100ml), and once addition was complete, the solution was allowed to warm to room temperature and stirred for 30 minutes. 2,6-Dibromopyridine (8.72g, 36.8mmol) was added, and the reaction heated under reflux for 5 hours. The cooled mixture was diluted with water, the layers separated, and the aqueous phase extracted with ether. The combined organic extracts were dried ($MgSO_4$), filtered and evaporated in vacuo to afford the title compound as a yellow oil (quantitative).

^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.39 (s, 3H), 1.45 (s, 3H), 3.83 (dd, 1H), 4.16 (dd, 1H), 4.37 (m, 2H), 4.46 (m, 1H), 6.75 (d, 1H), 7.06 (d, 1H), 7.40 (dd, 1H).

Preparation 3

2-Bromo-6-{[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}pyridine

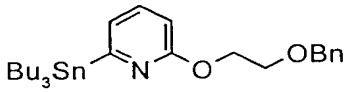


The title compound was obtained as a yellow oil (quantitative), from (S)-(-)-1,2-O-isopropylideneglycerol and 2,6-dibromopyridine, following the procedure described in preparation 2.

^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.40 (s, 3H), 1.45 (s, 3H), 3.83 (dd, 1H), 4.16 (dd, 1H), 4.37 (m, 2H), 4.48 (m, 1H), 6.76 (d, 1H), 7.06 (d, 1H), 7.41 (m/dd, 1H).

Preparation 4

2-[2-(Benzyloxy)ethoxy]-6-(tributylstannyl)pyridine

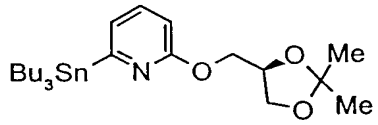


n-Butyllithium (13.8ml, 1.6M solution in hexanes, 22.0mmol) was added dropwise to a cooled (-78°C) solution of the bromide from preparation 1 (20.0mmol) in anydrous THF (100ml), so as to maintain the internal temperature <-70°C, and the solution stirred for 20 minutes. Tri-n-butyltin chloride (6.0ml, 22.0mmol) was added slowly to maintain the temperature <-70°C, and the reaction then allowed to warm to room temperature over 1 hour. The reaction was diluted with water, the mixture extracted with Et_2O (2x100ml), and the combined organic extracts dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel using pentane:Et_2O (98:2) as eluant, to afford the title compound as a colourless oil, (7.0g, 67%).

^{1}H nmr ($CDCl_3$, 300MHz) δ: 0.88 (t, 9H), 1.06 (m, 6H), 1.35 (m, 6H), 1.58 (m, 6H), 3.83 (t, 2H), 4.56 (t, 2H), 4.62 (s, 2H), 6.61 (d, 1H), 6.99 (d, 1H), 7.24-7.40 (m, 6H).

Preparation 5

2-{[(4R)-2,2-Dimethyl-1,3-dioxolan-4-yl]methoxy}-6-(tributylstannyl)pyridine

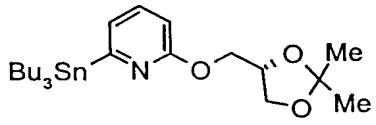


The title compound was prepared as an oil (quantitative) from the bromide of preparation 2, using a similar procedure to that described in preparation 4.

^{1}H nmr ($CDCl_3$, 300MHz) δ: 0.88 (t, 9H), 1.06 (t, 6H), 1.25-1.40 (m, 9H), 1.45 (s, 3H), 1.50-1.70 (m, 6H), 3.83 (dd, 1H), 4.15 (dd, 1H), 4.40 (m, 2H), 4.52 (m, 1H), 6.60 (d, 1H), 7.00 (d, 1H), 7.40 (dd, 1H).

Preparation 6

2-{[(4S)-2,2-Dimethyl-1,3-dioxolan-4-yl]methoxy}-6-(tributylstannyl)pyridine

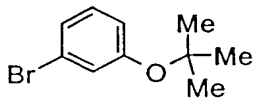


The title compound was obtained as a colourless oil (71%), from the bromide from preparation 3, following a similar procedure to that described in preparation 5.

^{1}H nmr ($CDCl_3$, 300MHz) δ: 0.89 (t, 9H), 1.07 (t, 6H), 1.35 (m, 6H), 1.40 (s, 3H), 1.48 (s, 3H), 1.58 (m, 6H), 3.83 (dd, 1H), 4.16 (dd, 1H), 4.40 (m, 2H), 4.52 (m, 1H), 6.60 (d, 1H), 7.00 (d, 1H), 7.40 (dd, 1H).

Preparation 7

3-Bromo-1-(tert-butoxy)benzene

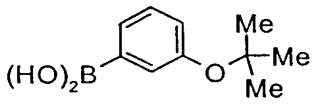


Condensed isobutylene (100ml) was added via a dry ice/acetone cold finger, to dichloromethane (70ml) at -30°C, followed by a solution of 3-bromophenol (21.5g, 125mmol) in dichloromethane (30ml). Trifluoromethanesulphonic acid (1.5g, 10.0mmol) was added dropwise, the reaction cooled to -75°C, and stirred for 2 hours. Triethylamine (1.4ml, 10.0mmol) was then added, the solution allowed to warm to room temperature and then concentrated in vacuo to remove the isobutylene. The remaining solution was washed with water, dried (Na_2SO_4), filtered and evaporated to give the desired product as a pale yellow oil, (33g, slightly impure).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.37 (s, 9H), 6.89 (d, 1H), 7.04-7.20 (m, 3H).

Preparation 8

3-(tert-Butoxy)-phenylboronic acid

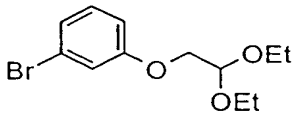


n-Butyllithium (40ml, 2.5M in hexanes, 100mmol) was added dropwise to a cooled (-78°C) solution of the bromide from preparation 7 (23.9g, 90mmol) in tetrahydrofuran (300ml), so as to maintain the temperature below -70°C. The resulting solution was stirred for 1 hour, and triisopropyl borate (30.6ml, 135mmol) was added dropwise over 10 minutes. The reaction was allowed to warm to room temperature, diluted with ether (150ml) then extracted with sodium hydroxide solution (1N). The combined aqueous layers were washed with ether and then re-acidified to pH 2 using hydrochloric acid (2N). This aqueous mixture was extracted with dichloromethane (3x200ml), the combined organic extracts dried (Na_2SO_4), filtered and concentrated in vacuo. The resulting white solid was stirred vigorously in pentane, filtered (twice) then dried under vacuum to give the title compound as a white solid, (13.1g, 75%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.39 (s, 9H), 7.19 (m, 1H), 7.37 (m, 1H), 7.79 (m, 1H), 7.88 (m, 1H).

Preparation 9

1-Bromo-3-(2,2-diethoxyethoxy)benzene

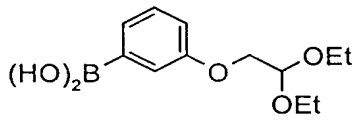


A mixture of potassium carbonate (1.5g, 10.9mmol), 3-bromophenol (1.73g, 10.0mmol) and bromoacetaldehyde diethyl acetal (1.5ml, 9.67mmol) in dimethylsulphoxide (10ml) was heated at 160°C for 1 ½ hours. The cooled reaction was partitioned between water (50ml) and ethyl acetate (100ml), and the phases separated. The aqueous layer was extracted with ethyl acetate (50ml), the combined organic solutions washed consecutively with 1N sodium hydroxide solution, water (2x), brine and then dried (Na_2SO_4), filtered and evaporated in vacuo. The residue was purified by medium pressure column chromatography on silica gel using an elution gradient of ether:pentane (0:100 to 5:95) to afford the title compound (2.01g, 72%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.22 (t, 6H), 3.60 (m, 2H), 3.75 (m, 2H), 3.97 (d, 2H), 4.80 (t, 1H), 6.82 (d, 1H), 7.07 (m, 3H).

Preparation 10

3-(2,2-Diethoxyethoxy)phenylboronic acid

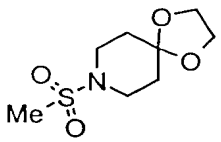


n-Butyllithium (18.5ml, 2.5M in hexanes, 46.25mmol) was added dropwise to a cooled (-78°C) solution of the bromide from preparation 9 (11.4g, 39.6mmol) in anhydrous tetrahydrofuran (100ml), so as to maintain the internal temperature <-70°C. This solution was stirred for 1 hour, then triisopropyl borate (1.13g, 6.0mmol) added slowly, and the reaction allowed to warm to room temperature over 3 hours. The mixture was cooled in an ice-bath, acidified to pH 4 using 2N hydrochloric acid, and quickly extracted with ethyl acetate (2x500ml). The combined organic extracts were washed with water and brine, dried (Na_2SO_4), filtered and evaporated in vacuo. The residual oil was purified by medium pressure column chromatography on silica gel using an elution gradient of ether:pentane (0:100 to 50:50) to afford the title compound (8.24g, 82%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.14 (t, 6H), 3.58 (m, 2H), 3.66 (m, 2H), 3.94 (d, 2H), 4.80 (t, 1H), 6.98 (m, 1H), 7.22 (m, 1H), 7.37 (m, 2H), 8.00 (s, 2H).

Preparation 11

1-Methylsulphonyl-piperidin-4-one ethylene ketal



Methanesulphonyl chloride (24.8g, 0.217mol) was added dropwise to a solution of 4-piperidone ethylene ketal (28.2g, 0.197mol) and triethylamine (30.2ml, 0.217mol) in ether (280ml), and the reaction stirred at room temperature for 3 hours. The mixture was washed consecutively with water (2x), hydrochloric acid (1N), and saturated sodium bicarbonate solution, dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was triturated with hexane, filtered and dried to give the desired product as an off-white solid (41.6g, 95%).

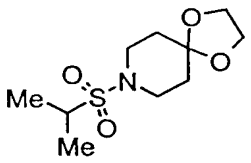
mp 107-109°C

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.78 (m, 4H), 2.75 (s, 3H), 3.32 (m, 4H), 3.92 (s, 4H).

Anal. Found: C, 43.23; H, 6.85; N, 6.23. $C_8H_{15}NO_4S$ requires C, 43.42; H, 6.83; N, 6.33%.

Preparation 12

1-Isopropylsulphonyl-piperidin-4-one ethylene ketal



Isopropylsulphonyl chloride (5.6ml, 50mmol) was added dropwise to an ice-cooled solution of 4-piperidone ethylene ketal (6.4ml, 50mmol) and triethylamine (7.7ml, 55mmol) in dichloromethane (100ml), and the reaction stirred at room temperature for 3 hours. The mixture was washed with water (2x), dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was crystallised from ether/pentane to afford the title compound as a solid, (10.55g, 85%).

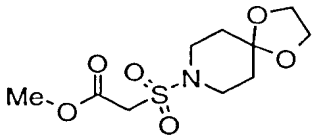
mp 66-67°C

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.34 (d, 6H), 1.77 (m, 4H), 3.18 (m, 1H), 3.43 (m, 4H), 3.98 (s, 4H).

Anal. Found: C, 48.19; H, 7.74; N, 5.50. $C_{10}H_{19}NO_4S$ requires C, 48.15; H, 7.75; N, 5.56%.

Preparation 13

Methyl 2-(1,4-dioxa-8-azaspiro[4.5]dec-8-ylsulphonyl)acetate



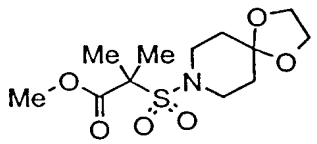
Potassium tert-butoxide (24.6g, 219mmol) was added portionwise to a solution of the ethylene ketal from preparation 11 (32.3g, 146mmol) and dimethyl carbonate (61ml, 730mmol) in tetrahydrofuan (200ml), and once addition was complete, the reaction was stirred at room temperature overnight under a nitrogen atmosphere. The reaction was poured into a mixture of hydrochloric acid (1N) and ether and the layers separated. The aqueous layer was extracted with ethyl acetate, the combined organic solutions washed with brine, dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was suspended in di-isopropyl ether, the mixture heated to reflux, cooled, and filtered, to afford the title compound as a solid, (26.7g, 65%).

^1H nmr ($CDCl_3$, 400MHz) δ: 1.77 (m, 4H), 3.42 (m, 4H), 3.78 (s, 3H), 3.92 (s, 2H), 3.95 (s, 4H).

Anal. Found: C, 42.69; H, 6.16; N, 4.93. $C_{10}H_{17}NO_6S$ requires C, 43.00; H, 6.14; N, 5.02%.

Preparation 14

Methyl 2-(1,4-dioxa-8-azaspiro[4.5]dec-8-ylsulphonyl)-2-methylpropanoate



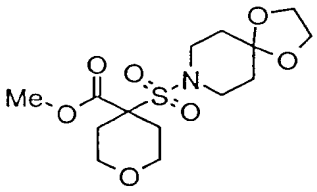
N-Butyl lithium (28ml, 1.6M in hexanes, 44.1mmol) was added dropwise to a cooled (-78°C) solution of the sulphonamide from preparation 12 (10g, 40.1mmol) in tetrahydrofuran (100ml), so as to maintain a temperature below -45°C. Once addition was complete the solution was allowed to warm to 0°C, and then recooled to -78°C. Methyl chloroformate (3.7ml, 48.1mmol) was added dropwise so as to maintain the temperature below -45°C, the reaction stirred for 30 minutes, then allowed to warm to room temperature. The reaction mixture was partitioned between ethyl acetate and water, and the layers separated. The organic phase was washed with water, dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was triturated with ether to give the title compound as a solid, (9.88g, 80%).

^1H nmr ($CDCl_3$, 400MHz) δ: 1.60 (s, 6H), 1.76 (m, 4H), 3.48 (m, 4H), 3.79 (s, 3H), 3.98 (s, 4H).

Anal. Found: C, 46.80; H, 6.87; N, 4.49. $C_{12}H_{21}NO_6S$ requires C, 46.89; H, 6.89; N, 4.56%.

Preparation 15

Methyl 4-(1,4-dioxa-8-azaspiro[4.5]dec-8-ylsulphonyl)tetrahydro-2H-pyran-4-carboxylate



Sodium hydride (880mg, 60% dispersion in mineral oil, 22mmol) was added to a solution of the sulphonamide from preparation 11 (2.21g, 10mmol) and dimethyl carbonate (4.2ml, 50mmol) in dry toluene (40ml), and the mixture heated at 90°C for 90 minutes. Tlc analysis showed starting material present, so methanol (20?l) was added, and the reaction stirred at 90°C overnight. 1-Methyl-2-pyrrolidinone (10ml) and bis(2-bromoethyl)ether (1.63ml, 13mmol) were added, and the reaction stirred for a further 20 hours at 90°C, and at room temperature for 3 days. The reaction mixture was partititoned between 1N citric acid solution and ether, and the layers separated. The organic phase was washed with water, dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was triturated with ether to give the title compound as a white solid, (1.05g, 30%).

Alternative method

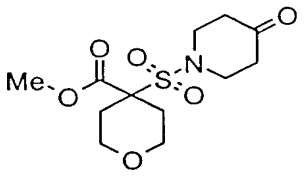
Potassium tert-butoxide (220ml, 1M in tetrahydrofuran, 220mmol) was added dropwise to a solution of the acetate from preparation 13 (27.9g, 100mmol) and bis(2-bromoethyl)ether (16.3ml, 130mmol) in tetrahydrofuran (200ml) and 1-methyl-2-pyrrolidinone (20ml), and the reaction stirred at room temperature overnight. Tlc analysis showed starting material remaining, so tetrabutylammonium iodide (3.7g, 10mmol) and sodium hydride (2.0g, 60% dispersion in mineral oil, 50mmol) were added, and the reaction stirred for a further 72 hours. Additional 1-methyl-2-pyrrolidinone (100ml), sodium hydride (4.0g, 60% dispersion in mineral oil, 100mmol) and bis(2-bromoethyl)ether (12.6ml, 100mmol) were added, and the reaction continued for a further 24 hours. The reaction was poured into a mixture of ether and 10% citric acid solution, and the layers separated. The aqueous phase was extracted with ether, the combined organic solutions washed with water, dried ($MgSO_4$), filtered and evaporated in vacuo.The residue was suspended in ether, the mixture heated to reflux, cooled and the resulting precipitate filtered, washed with ether and dried to give the title compound, (7.2g, 21%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.70 (m, 4H), 2.16 (m, 2H), 2.35 (m, 2H), 3.24 (m, 2H), 3.41 (m, 4H), 3.80 (s, 3H), 3.94 (m, 6H).

LRMS : m/z 372 $(M+23)^+$

Preparation 16

Methyl 4-(4-oxo-piperidin-1-ylsulphonyl)tetrahydro-2H-pyran-4-carboxylate



Hydrochloric acid (20ml, 1N) was added to a solution of the ethylene ketal from preparation 15 (7.1g, 20.3mmol) in acetone (20ml) and 1,4-dioxan (20ml), and the reaction stirred at 60°C for 6 hours, and then left at room temperature overnight. The reaction was neutralised by adding sodium bicarbonate portionwise, and this mixture concentrated in vacuo. The residue was diluted with water, then extracted with ethyl acetate (3x). The combined organic extracts were dried ($MgSO_4$), filtered and evaporated in vacuo.The crude product was triturated with ether/di-isopropyl ether, to give the desired product as a solid (4.1g, 66%).

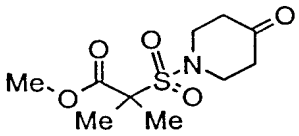
mp 158-160°C

^{1}H nmr ($CDCl_3$, 400MHz) δ: 2.18 (m, 2H), 2.38 (m, 2H), 2.48 (m, 4H), 3.26 (m, 2H), 3.60 (br, m, 4H), 3.82 (s, 3H), 3.98 (m, 2H).

Anal. Found: C, 47.14; H, 6.28; N, 4.54. $C_{12}H_{19}NO_6S$ requires C, 47.20; H, 6.27; N, 4.59%.

Preparation 17

Methyl 2-methyl-2-(4-oxo-piperidin-1-ylsulphonyl)propanoate



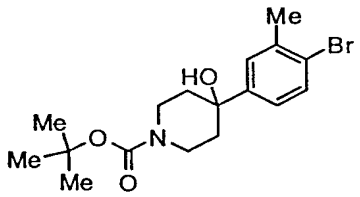
The title compound was obtained as a solid (98%) after trituration with pentane from the ethylene ketal from preparation 14, following a similar method to that described in preparation 16.

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.67 (s, 6H), 2.57 (m, 4H), 3.68 (m, 4H), 3.80 (s, 3H).

Anal. Found: C, 45.51; H, 6.52; N, 5.14. $C_{10}H_{17}NO_5S$ requires C, 45.61; H, 6.51; N, 5.32%.

Preparation 18

tert-Butyl 4-[4-(4-bromo-3-methylphenyl)-4-hydroxypiperidine-1-carboxylate



A 2.5M solution of n-butyl lithium in hexane (38ml, 94mmol) was added over about 10 minutes to a stirred mixture of 2-bromo-5-iodo-toluene (28g, 94mmol) in anhydrous ether (500ml) under nitrogen, at about -75°C. After a further 15 minutes, a solution of t-butyl 4-oxopiperidine-1-carboxylate (17 g, 85 mmol) in anhydrous tetrahydrofuran (50 ml) was added at such a rate that the reaction temperature was maintained below -60°C.

The reaction mixture was stirred at about -75°C for 1 hour, and allowed to warm to 0°C and quenched with aqueous ammonium chloride solution. The organic phase was separated, washed with water, dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was dissolved in pentane and cooled to 0°C to crystallise the title compound, which was collected by filtration as a colourless solid (20.1 g, 64%).

m.p. 102-103°C.

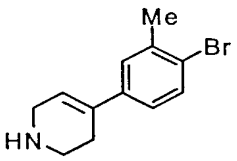
^{1}H nmr ($CDCl_3$) δ: 1.48 (s, 9H), 1.51 (s, 1H), 1.70 (d, 2H), 1.96 (m, 2H), 2.40 (s, 3H), 3.22 (t, 2H), 4.02 (m, 2H), 7.15 (dd, 1H), 7.36 (d, 1H), 7.50 (d, 1H).

LRMS :m/z 369/371 $(M+1)^+$

Anal. Found: C, 55.14; H, 6.58; N, 3.76. $C_{17}H_{24}BrNO_3$ requires C, 55.14; H, 6.53; N, 3.78%.

Preparation 19

4-(4-Bromo-3-methylphenyl)-1,2,3,6-tetrahydropyridine



Trifluoroacetic acid (100ml) was added to a stirred solution of the bromide from preparation 18 (20g, 54mmol) in dichloromethane (100 ml) at room temperature. After a further 18 hours, the reaction mixture was evaporated in vacuo and the residue basified with 2M aqueous sodium hydroxide solution to pH>12. The resulting mixture was extracted with ether, the combined extracts washed with water, dried ($MgSO_4$), filtered and evaporated under reduced pressure to yield the title compound as a low melting solid, (13.6 g, 100%).

^{1}H nmr ($CDCl_3$) δ: 1.60 (br, s, 1H), 2.40 (m, 5H), 3.10 (t, 2H), 3.52 (m, 2H), 6.10 (br, s, 1H), 7.05 (dd, 1H), 7.22 (d, 1H), 7.46 (d, 1H).

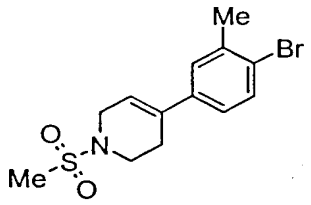
LRMS :m/z 251/253 $(M+1)^+$.

Alternative Method -

Para-toluenesulphonic acid (10.27g, 54mmol) was added to a stirred solution of the bromide from preparation 18 (10g, 27mmol) in toluene (130ml) at room temperature. The gelatinous mixture was heated to reflux in a Dean-Stark apparatus for 90 minutes, and then cooled to room temperature which resulted in a thick white precipitate. The mixture was basified with 2M sodium hydroxide solution, and extracted with ethyl acetate (3x), then the combined extracts were washed with water, dried ($MgSO_4$) and evaporated under reduced pressure to yield the title as a low melting solid, (6.8 g, 100%).

Preparation 20

4-(4-Bromo-3-methylphenyl)-1-methylsulphonyl-1,2,3,6-tetrahydropyridine



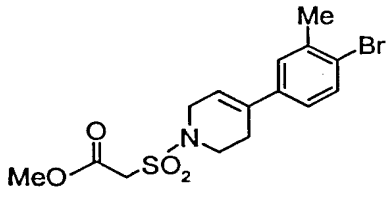
Methanesulphonyl chloride (17.5ml, 227mmol) was added dropwise to an ice-cooled solution of triethylamine (34.4ml, 247mmol) and the amine from preparation 19 (51.8g, 206mmol) in dichloromethane (400ml), and the reaction then stirred at room temperature for 1 hour. Tlc analysis showed starting material remaining, so additional methanesulphonyl chloride (1.75ml, 22.7mmol) and triethylamine (5ml, 35.9mmol) were added, and stirring continued for a further hour. The reaction was diluted with hydrochloric acid (200ml, 2N) and water (300ml), and the phases separated. The aqueous layer was extracted with dichloromethane (2x250ml) the combined organic extracts washed with brine (200ml), dried ($MgSO_4$), filtered and concentrated in vacuo. The residual solid was triturated with iso-propyl ether, filtered and dried to afford the title compound as a pale yellow solid, (65.1g, 96%).

^{1}H nmr ($CDCl_3$, 300MHz) δ: 2.40 (s, 3H), 2.62 (m, 2H), 2.85 (s, 3H), 3.54 (m, 2H), 3.95 (m, 2H), 6.04 (m, 1H), 7.04 (dd, 1H), 7.21 (m, 1H), 7.50 (d, 1H).

LRMS m/z 347, 349 $(M+18)^+$

Preparation 21

Methyl 2-[4-(4-bromo-3-methylphenyl)-1,2,3,6-tetrahydropyridin-1-ylsulphonyl]acetate



N,O-Bis(trimethylsilyl)acetamide (0.9ml, 4.0mmol) was added to a stirred solution of the amine from preparation 19 (2.0g, 7.9mmol) in anhydrous tetrahydrofuran (40ml), under nitrogen, at room temperature. A solution of methyl chlorosulphonylacetate (1.64g, 9.5mmol) in anhydrous tetrahydrofuran (15 ml) was added and the reaction mixture stirred at room temperature for 18 hours. The resulting mixture was evaporated in vacuo, and partitioned between ethyl acetate and aqueous sodium bicarbonate solution. The organic layer was separated and washed with water, dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel, using dichloromethane as eluant, followed by crystallisation from diisopropyl ether, to give the title compound as a colourless solid, (1.65 g, 55%).

m.p. 110-112°C.

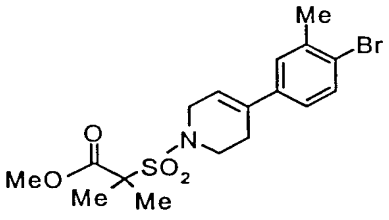
^{1}H nmr ($CDCl_3$) δ: 2.40 (s, 3H), 2.60 (m, 2H), 3.60 (t, 2H), 3.80 (s, 3H), 4.01 (s, 2H), 4.07 (m, 2H), 6.02 (br, s,1H), 7.02 (dd, 1H), 7.21 (d, 1H), 7.50 (d, 1H).

LRMS :m/z 404/406 $(M+18)^+$

Anal. Found: C, 46.32; H, 4.62; N, 3.55. $C_{15}H_{18}BrNO_4S$ requires C, 46.40; H, 4.67; N, 3.61%.

Preparation 22

Methyl 2-[4-(4-bromo-3-methylphenyl)-1,2,3,6-tetrahydropyridin-1-ylsulphonyl]-2-methyl-propanoate



Iodomethane (2ml, 32.1mmol) was added to a stirred mixture of the acetate from preparation 21 (5g, 12.9mmol) and potassium carbonate (5.4g, 39.1mmol), in anhydrous dimethylsulfoxide (50ml), under nitrogen, at room temperature. After 24 hours the reaction mixture was partitioned between ether and water, separated, and the organic layer was washed with water, dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was purified by flash chromatography, using diethyl ether:pentane (40:60 to 100:0) as eluant, followed by crystallisation from diisopropyl ether, to give the title compound as a colourless solid, (4.7 g, 87%).

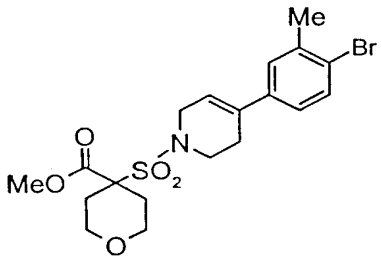
m.p. 100-101°C.

^{1}H nmr ($CDCl_3$) δ: 1.67 (s, 6H), 2.40 (s, 3H), 2.58 (m, 2H), 3.60 (t, 2H), 3.80 (s, 3H), 4.08 (m, 2H), 6.00 (br, s, 1H), 7.03 (dd, 1H), 7.21 (d, 1H), 7.49 (d, 1H).

Anal. Found: C, 49.00; H, 5.33; N, 3.28. $C_{17}H_{22}BrNO_4S$ requires C, 49.04; H, 5.33; N, 3.36%.

Preparation 23

Methyl 4-[4-(4-bromo-3-methylphenyl)-1,2,3,6-tetrahydropyridin-1-ylsulphonyl]tetrahydro-2H-pyran-4-carboxylate



Bis-2-iodoethyl ether (3.9g, 12.0mmol) was added to a stirred mixture of the acetate from preparation 21 (3.6 g, 9.3mmol) and potassium carbonate (3.8g, 27.8mmol), in anhydrous dimethylsulfoxide (50ml), under nitrogen, at room temperature. After 18 hours the reaction mixture was partitioned between diethyl ether and water, separated, and the organic layer was washed with water, dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was purified by flash chromatography, using a mixture of dichloromethane and methanol (99:1) as eluant, followed by crystallisation from diisopropyl ether, to give the title compound as a colourless solid, (3.43 g, 80%).

m.p. 128-130°C.

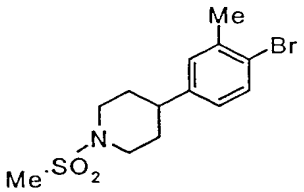
^{1}H nmr ($CDCl_3$) δ: 2.23 (m, 2H), 2.40 (s, 3H), 2.42 (m, 2H), 2.58 (m, 2H), 3.30 (m, 2H), 3.58 (m, 2H), 3.87 (s, 3H), 4.00-4.10 (m, 4H), 6.00 (br, s, 1H), 7.02 (dd, 1H), 7.21 (d, 1H), 7.49 (d, 1H).

LRMS :m/z 477 $(M+18)^+$

Anal. Found: C, 49.92; H, 5.40; N, 2.90. $C_{19}H_{24}BrNO_5S$ requires C, 49.78; H, 5.28; N, 3.06%.

Preparation 24

4-(4-Bromo-3-methylphenyl)-1-(methylsulphonyl)piperidine



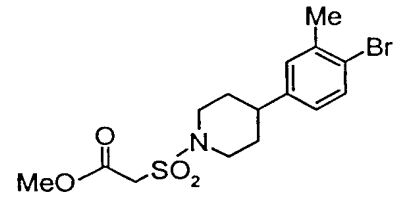
Triethylsilane (47.2ml, 296mmol), followed by trifluoromethanesulphonic acid (1.73ml, 19.7mmol) were added to a solution of the sulphonamide from preparation 20 (65.0g, 197mmol) in dichloromethane (300ml) and trifluoroacetic acid (300ml), and the reaction stirred at room temperature for an hour. Tlc analysis showed starting material remaining, so additional triethylsilane (75.2ml, 471mmol) and trifluoromethanesulphonic acid (0.86ml, 9.8mmol) were added and the reaction stirred for a further 20 hours at room temperature. The reaction was concentrated in vacuo, the residue poured into saturated aqueous potassium carbonate solution, and the mixture extracted with dichloromethane (3x650ml). The combined organic extracts were washed with brine (500ml), dried ($MgSO_4$), filtered and concentrated in vacuo. The crude product was triturated with hot methanol/hexane, filtered and dried to give the title compound (52.43g, 80%) as a buff-coloured solid.

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.78 (m, 2H), 1.90 (m, 2H), 2.37 (s, 3H), 2.52 (m, 1H), 2.77 (m, 5H), 3.94 (m, 2H), 6.83 (m, 1H), 7.02 (s, 1H), 7.42 (m, 1H).

LRMS : m/z 354, 356 $(M+23)^+$

Preparation 25

Methyl 2-[4-(4-bromo-3-methylphenyl)piperidin-1-ylsulphonyl]acetate



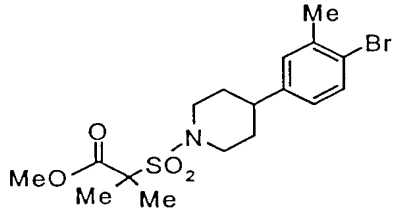
Sodium hydride (12.2g, 60% dispersion in mineral oil, 305mmol) was added to a solution of the sulphonamide from preparation 24 (50.61g, 152mmol) and dimethylcarbonate (63.8ml, 760mmol) in toluene (600ml), and the reaction heated under reflux for 1 ½ hours. The reaction was partitioned between ethyl acetate (1000ml), and cooled hydrochloric acid (600ml, 1N), and the layers separated. The aqueous layer was extracted with ethyl acetate (500ml), the combined organic extracts washed with brine (3x300ml), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was triturated with hexane, and the solid filtered. This was re-crystallised from di-isopropyl ether and dried in vacuo to give the title compound as buff-coloured crystals, (40.9g, 69%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.77 (m, 2H), 1.84 (m, 2H), 2.37 (s, 3H), 2.58 (m, 1H), 2.97 (m, 2H), 3.80 (s, 3H), 3.96 (m, 4H), 6.84 (m, 1H), 7.02 (s, 1H), 7.42 (d, 1H).

LRMS m/z 412, 414 $(M+23)^+$

Preparation 26

Methyl 2-[4-(4-bromo-3-methylphenyl)piperidin-1-ylsulphonyl]-2-methyl-propanoate



Triethylsilane (1.43ml, 9.0mmol) followed by trifluoromethanesulphonic acid (0.02ml, 0.3mmol) were added to a solution of the 1,2,3,6-tetrahydropyridine from preparation 22 (1.25g, 3.0mmol) and trifluoroacetic acid (15ml) in dichloromethane (15ml), and the reaction was stirred for an hour at room temperature. The reaction mixture was concentrated in vacuo, the residue diluted with dichloromethane (25ml), then partitioned between ethyl acetate (150ml) and saturated sodium bicarbonate solution (150ml), and the layers separated. The aqueous phase was extracted with ethyl acetate (2x35ml), the combined organic solutions dried ($MgSO_4$), filtered and evaporated in vacuo. The residual solid was triturated with di-isopropyl ether to give the title compound as a white solid, (963mg, 77%).

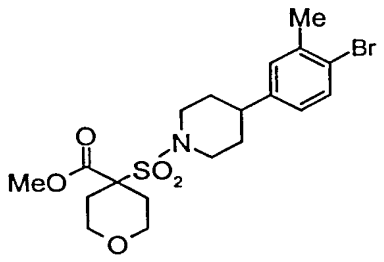
mp 103-106°C

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.52 (m, 8H), 1.77 (m, 2H), 2.28 (s, 3H), 2.63 (m, 1H), 3.00 (m, 2H), 3.70 (m, 5H), 6.98 (dd, 1H), 7.20 (s, 1H), 7.42 (dd, 1H).

Anal. Found: C, 48.42; H, 5.74; N, 3.27. $C_{17}H_{24}BrNSO_4$ requires C, 48.81; H, 5.78 N, 3.35%.

Preparation 27

Methyl 4-[4-(4-bromo-3-methylphenyl)piperidin-1-ylsulphonyl]tetrahydro-2H-pyran-4-carboxylate



Sodium hydride (60% dispersion in mineral oil, 1.16g, 29.0mmol) was added to a stirred solution of the acetate from preparation 25 (10.14 g, 26.0mmol) in N-methyl pyrrolidinone (60 ml) at ambient

temperature under nitrogen. After 45 minutes, bis-2-bromoethyl ether (4.26 ml, 33.8 mmol) was added to the stirred mixture, and after a further 150 minutes an additional portion of sodium hydride (60% dispersion in mineral oil; 1.16 g, 29 mmol) was added, and the mixture left stirring for 18 hours. The solvent was removed under reduced pressure, and the residues was partitioned between ethyl acetate and water. The organic layer was collected, washed with brine, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was crystallised from ethyl acetate and diisopropyl ether to give the title compound as a colourless solid (7.34 g, 61%). The filtrate was evaporated and purified by flash chromatography eluting with dichloromethane, and crystallisation from ethyl acetate and diisopropyl ether to give an additional batch of the title compound as a colourless solid (1.86 g, 15%). A small sample was recrystallised from ethyl acetate for further characterisation.

m.p. 162-163°C.

^{1}Hnmr ($CDCl_3$) δ: 1.65-1.83 (m, 4H), 2.20 (m, 2H), 2.38 (s, 3H), 2.40 (m, 2H), 2.57 (m, 1H), 3.00 (m, 2H), 3.29 (m, 2H), 3.85 (s, 3H), 3.87-4.00 (m, 4H), 6.83 (d, 1H), 7.02 (s, 1H), 7.41 (d, 1H).

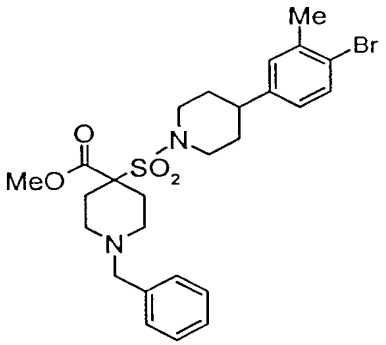
LRMS :m/z 460/462 $(M+1)^+$.

Anal. Found: C,49.49; H,5.68; N,2.93. $C_{19}H_{26}BrNO_5S$ requires C,49.57; H,5.69; N,3.04%.

Alternative Route: Triethylsilane (50ml, 0.30mol) was added dropwise over 2 min to a solution of the carbinol from preparation 130 (60g, 0.12mol) in dichloromethane (150ml) and trifluoroacetic acid (150ml), at 0°C, under nitrogen. Triflic acid (0.53ml, 6.0mmol) was added dropwise over 10 min and the resulting mixture was stirred at 0°C for 4h. Dichloromethane (300ml) and demineralised water (300ml) were added and the aqueous phase was separated. The organic phase was washed with water (200ml), saturated sodium bicarbonate solution (2x200ml) and demineralised water (200ml) and then concentrated *in vacuo* to a colourless solid. The solid was slurried in hot ethyl acetate (300ml) for 20 min and the mixture was cooled to 0°C and then filtered. The residue was dried *in vacuo* to leave the title compound as a colourless solid (53g, 92%).

Preparation 28

Methyl 1-benzyl-4-[4-(4-bromo-3-methylphenyl)piperidin-1-ylsulphonyl]-4-piperidinecarboxylate



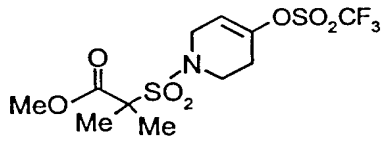
The acetate from preparation 25 (4.17g, 10.7mmol) was added portionwise to a suspension of sodium hydride (994mg, 60% dispersion in mineral oil, 33.1mmol) in 1-methyl-2-pyrrolidinone (40ml), and the resulting solution stirred for an hour. Tetra-butyl ammonium bromide (3.44g, 10.7mmol) and N-benzyl-bis-(2-chloroethyl)amine (2.73g, 10.1mmol) were added portionwise, and once addition was complete, the reaction was stirred at 60°C for 6 hours. The cooled reaction was partitioned between water and ethyl acetate, the layers separated, and the aqueous phase extracted with ethyl acetate. The combined organic extracts were washed with water, dried (Na_2SO_4), filtered and concentrated in vacuo. The crude product was purified by column chromatography on silica gel twice, using an elution gradient of dichloromethane:ether (100:0 to 90:10) to afford the title compound (3.04g, 52%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.63-1.81 (m, 4H), 1.88 (m, 2H), 2.16 (m, 2H), 2.36 (s, 3H), 2.42 (m, 2H), 2.55 (m, 1H), 2.88 (m, 2H), 2.98 (m, 2H), 3.40 (s, 2H), 3.82 (m, 5H), 6.83 (d, 1H), 7.00 (s, 1H), 7.22 (m, 5H), 7.40 (d, 1H).

LRMS m/z 549, 551 $(M+1)^+$

Preparation 29

Methyl 2-methyl-2-{4-[trifluoromethanesulphonyloxy]-1,2,3,6-tetrahydropyridin-1-ylsulphonyl}propanoate



2,6-Di-tert-butyl-4-methylpyridine (3.7g, 18mmol) was added to a solution of the ketone from preparation 17 (3.8g, 14.5mmol) in dichloromethane (50ml), and the solution then cooled to 4°C. Trifluoromethane sulphonic anhydride (2.95ml, 17.5mmol) was added dropwise, and the reaction then stirred at room temperature for 17 hours. Tlc analysis showed starting material remaining, so additional 2,6-di-tert-butyl-4-methylpyridine (3.7g, 18mmol) and trifluoromethane sulphonic anhydride (2.7ml, 16mmol) were added portionwise to the stirred reaction over the following 4 days. The mixture was then filtered, the filtrate concentrated in vacuo, and the residue triturated with ether. The resulting solid was filtered off, and the filtrate evaporated in vacuo. This crude product was purified by column

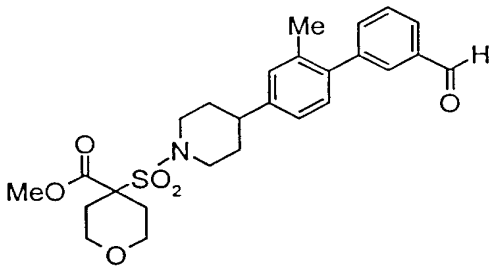
chromatography on silica gel using an elution gradient of hexane:ethyl acetate (91:9 to 50:50) to afford the title compound (4.25g, 74%) as a white solid.

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.64 (s, 6H), 2.56 (m, 2H), 3.60 (m, 2H), 3.79 (s, 3H), 4.06 (m, 2H), 5.80 (m, 1H).

Anal. Found: C, 33.62; H, 4.03; N, 3.43. $C_{11}H_{16}F_3NO_7S_2$ requires C, 33.42; H, 4.08; N, 3.54%.

Preparation 30

Methyl 2-[4-(4-{3-formylphenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]tetrahydro-2H-pyran-4-carboxylate



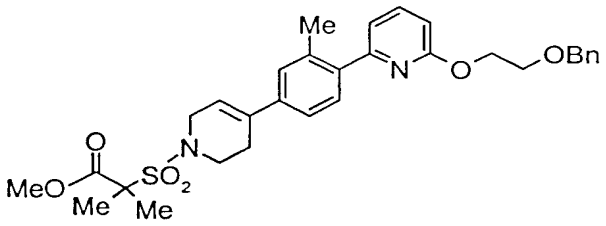
A mixture of the bromide from preparation 27 (4.02g, 8.73mmol), 3-formylphenylboronic acid (1.83g, 11.56mmol), cesium fluoride (3.46g, 22.8mmol), tris(dibenzylideneacetone)palladium (0) (430mg, 0.47mmol) and tri(o-tolyl)phosphine (284mg, 0.93mmol) in 1,2-dimethoxyethane (70ml) was heated under reflux for 6 hours. The cooled reaction was diluted with water and the mixture extracted with ethyl acetate (3x). The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:hexane (25:75 to 40:60), and triturated with di-isopropyl ether to give the title compound as a solid, (2.69g, 63%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.75-1.95 (m, 4H), 2.20 (m, 5H), 2.40 (m, 2H), 2.62 (m, 1H), 3.03 (m, 2H), 3.30 (m, 2H), 3.82-4.02 (m, 7H), 7.07 (m, 2H), 7.16 (m, 1H), 7.56 (m, 2H), 7.81 (m, 2H), 10.02 (s, 1H).

LRMS : m/z 508 $(M+23)^+$

Preparation 31

Methyl 2-[4-(4-{6-[2-benzyloxy]ethoxypyridin-2-yl}-3-methylphenyl)-1,2,3,6-tetrahydropyridin-1-ylsulphonyl]-2-methyl-propanoate



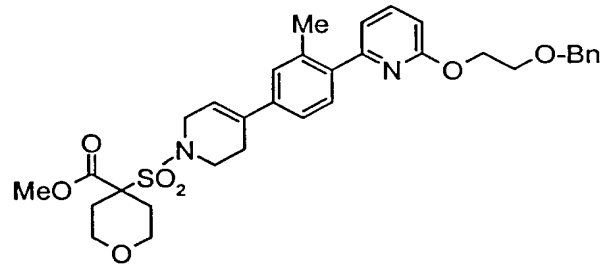
A mixture of the stannane from preparation 4 (2.8g, 5.4mmol) and the bromide from preparation 22 (1.5g, 3.62mmol), and tetrakis(triphenylphosphine)palladium (0) (205mg, 0.18mmol) in toluene (35ml) was heated under reflux overnight. The cooled mixture was evaporated in vacuo and the residue purified by column chromatography on silica gel using pentane:ethyl acetate (75:25) as eluant, to afford the title compound as a colourless oil, (1.7g, 83%).

^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.69 (s, 6H), 2.42 (s, 3H), 2.64 (m, 2H), 3.62 (t, 2H), 3.82 (m, 5H), 4.14 (m, 2H), 4.56 (t, 2H), 4.62 (s, 2H), 6.06 (s, 1H), 6.77 (d, 1H), 7.0 (d, 1H), 7.22-7.42 (m, 8H), 7.62 (m, 1H).

LRMS : m/z 565 $(M+1)^+$

Preparation 32

Methyl 4-[4-(4-{6-[2-benzyloxy]ethoxypyridin-2-yl}-3-methylphenyl)-1,2,3,6-tetrahydropyridin-1-ylsulphonyl]tetrahydro-2H-pyran-4-carboxylate



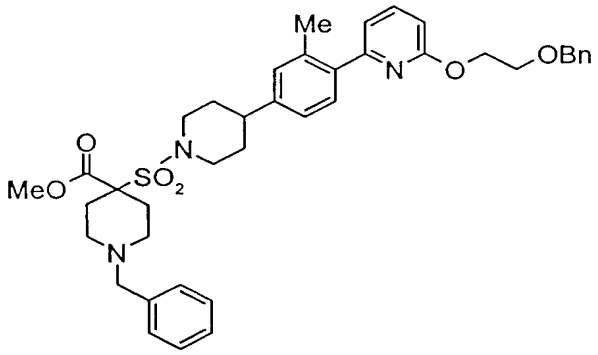
A mixture of the stannane from preparation 4 (1.74g, 3.36mmol) and the bromide from preparation 23 (1.1g, 2.4mmol) and tetrakis(triphenylphosphine)palladium (0) (138mg, 0.14mmol) in toluene (16ml) was heated under reflux for 4 hours. The cooled reaction was diluted with water, and the mixture extracted with ether (3x). The combined organic extracts were washed with brine, dried ($MgSO_4$), filtered through Arbocel® and evaporated in vacuo. The residual yellow oil was purified by column chromatography on silica gel using an elution gradient of pentane:ether (50:50 to 25:75) to afford the title compound as a pale yellow oil, (1.18g, 81%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 2.22 (m, 2H), 2.42 (m, 5H), 2.62 (m, 2H), 3.34 (m, 2H), 3.60 (m, 2H), 3.82 (t, 2H), 3.88 (s, 3H), 4.01 (m, 2H), 4.09 (m, 2H), 4.55 (t, 2H), 4.61 (s, 2H), 6.05 (m, 1H), 6.76 (d, 1H), 6.99 (d, 1H), 7.21-7.41 (m, 78H), 7.61 (m, 1H).

LRMS : m/z 607 $(M+1)^+$

Preparation 33

Methyl 1-benzyl-4-{[4-(4-{6-[2-benzyloxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}-piperidin-4-carboxylate



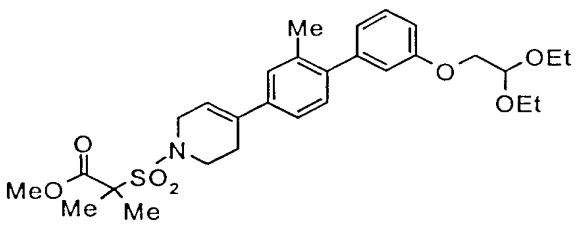
The stannane from preparation 4 (4.05g, 7.8mmol), followed by tris(triphenylphosphine) palladium (0) (410mg, 0.35mmol) were added to a solution of the bromide from preparation 28 (3.91g, 7.1mmol) in toluene (50ml), and the reaction de-gassed, then heated under a nitrogen atmosphere reflux for 7 hours. Aqueous potassium fluoride solution (20ml, 25%) was added to the cooled reaction, the mixture stirred at room temperature for 20 minutes, then filtered through Arbocel®. The filtrate was diluted with ethyl acetate, washed with brine, dried (Na_2SO_4), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel twice, using an elution gradient of ethyl acetate:hexane (40:60 to 60:40) to give the desired product as a yellow crystalline solid, (2.77g, 56%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.74-1.95 (m, 6H), 2.17 (m, 2H), 2.37 (s, 3H), 2.44 (m, 2H), 2.60 (m, 1H), 2.88 (m, 2H), 3.00 (m, 2H), 3.40 (s, 2H), 3.80 (m, 5H), 3.88 (m, 2H), 4.52 (t, 2H), 4.59 (s, 2H), 6.70 (d, 1H), 6.95 (d, 1H), 7.03 (m, 2H), 7.18-7.37 (m, 11H), 7.58 (m, 1H).

LRMS : m/z 699 $(M+1)^+$

Preparation 34

Methyl 2-[4-(4-{3-[2,2-diethoxyethoxy]phenyl}-3-methylphenyl)-1,2,3,6-tetrahydropyridin-1-ylsulphonyl]-2-methyl-propanoate



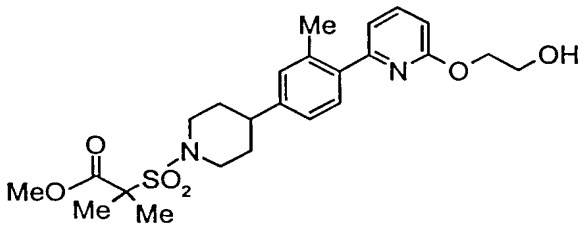
A mixture of cesium fluoride (1.81g, 11.92mmol), tri-o-tolyl phosphine (180mg, 0.59mmol), tris(dibenzylideneacetone)dipalladium (0) (280mg, 0.31mmol) and the boronic acid from preparation 10 (1.83g, 7.2mmol) and the bromide from preparation 22 (2.5g, 6.0mmol) in anhydrous 1,2-dimethoxyethane (60ml), was heated under reflux for 5 ½ h. The cooled reaction mixture was partitioned between water and ethyl acetate, and this mixture filtered through Arbocel®. The filtrate was separated, the organic phase washed with water, then brine, dried (Na_2SO_4), filtered and evaporated in vacuo. The residual green oil was purified by medium pressure column chromatography on silica gel using an elution gradient of pentane:ethyl acetate (100:0 to 85:15) to afford the title compound, (3.04g, 93%).

^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.24 (t, 6H), 1.69 (s, 6H), 2.28 (s, 3H), 2.64 (m, 2H), 3.62 (m, 4H), 3.80 (m, 5H), 4.04 (d, 2H), 4.12 (m, 2H), 4.84 (t, 1H), 6.06 (m, 1H), 6.92 (m, 3H), 7.14-7.38 (m, 4H).

LRMS : m/z 563 $(M+18)^+$

Preparation 35

Methyl 2-[(4-{4-[6-(2-hydroxyethoxy)pyridin-2-yl]-3-methylphenyl}-piperidin-1-yl)sulphonyl]-2-methyl-propanoate



A mixture of the benzyl ether from preparation 31 (1.7g, 3.0mmol), ammonium formate (3.0g, 50.0mmol), palladium hydroxide on carbon (500mg) and acetic acid (10ml) in methanol (30ml) was heated under reflux overnight. Additional ammonium formate (1.5g, 25.0mmol) and palladium hydroxide on carbon (1.5g) were added and the reaction heated under reflux for a further 72 hours. The cooled mixture was filtered through Arbocel®, and the filter pad washed well with ethyl acetate. The combined filtrates were neutralised using saturated sodium bicarbonate solution, the phases separated, and the aqueous layer extracted with ethyl acetate (2x100ml). The combined organic extracts were dried ($MgSO_4$), filtered and evaporated in vacuo to give the title compound as a colourless solid, (1.2g, 84%).

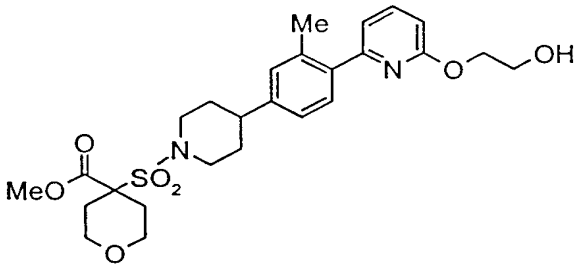
mp 108-111°C

^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.64 (s, 6H), 1.78-1.94 (m, 4H), 2.40 (s, 3H), 2.65 (m, 1H), 3.07 (m, 2H), 3.82 (s, 3H), 3.97 (m, 4H), 4.50 (t, 2H), 6.7 (d, 1H), 7.00 (d, 1H), 7.10 (m, 2H), 7.38 (d, 1H), 7.65 (m, 1H).

LRMS : m/z 477 $(M+1)^+$

Preparation 36

Methyl 4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxylate



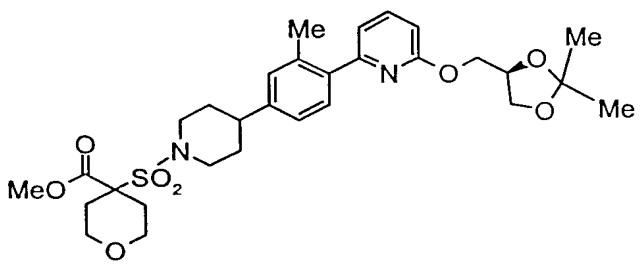
The title compound was prepared from the benzyl ether from preparation 32 in 93% yield, following a similar procedure to that described in preparation 35.

^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.70-1.95 (m, 4H), 2.22 (m, 2H), 2.40 (m, 5H), 2.64 (m, 1H), 3.06 (m, 2H), 3.34 (m, 2H), 3.92 (m, 7H), 4.00 (m, 2H), 4.50 (t, 2H), 6.78 (d, 1H), 7.00 (d, 1H), 7.10 (m, 2H), 7.38 (d, 1H), 7.65 (m, 1H).

LRMS : m/z 519 $(M+1)^+$

Preparation 37

Methyl 4-({4-[4-(6-{[(4R)-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}pyridin-2-yl)-3-methylphenyl]piperidin-1-yl}sulphonyl)tetrahydro-2H-pyran-4-carboxylate



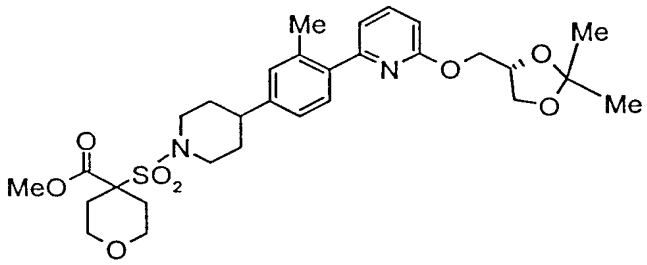
A mixture of the stannane from preparation 5 (2.0g, 4.97mmol) and the bromide from preparation 27 (1.76g, 3.82mmol) and tetrakis(triphenylphosphine)palladium (0) (242mg, 0.21mmol) in toluene (50ml) was heated under reflux for 7 hours. The cooled mixture was concentrated under reduced pressure and the residue purified by column chromatography on silica gel twice, using an elution gradient of ether:pentane (66:34 to 34:66) to give the title compound as a white solid, (1.29g, 57%).

^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.40 (s, 3H), 1.46 (s, 3H), 1.77-1.95 (m, 4H), 2.21 (m, 2H), 2.40 (m, 5H), 2.64 (m, 1H), 3.04 (m, 2H), 3.34 (m, 2H), 3.81-4.04 (m, 8H), 4.15 (dd, 1H), 4.40 (m, 2H), 4.50 (m, 1H), 6.75 (d, 1H), 7.00 (d, 1H), 7.09 (m, 2H), 7.38 (d, 1H), 7.62 (m, 1H).

LRMS : m/z 611 $(M+23)^+$

Preparation 38

Methyl 4-({4-[4-(6-{[(4S)-2,2-dimethyl-1,3-dioxolan-4-yl]methoxy}pyridin-2-yl)-3-methylphenyl]piperidin-1-yl}sulphonyl)tetrahydro-2H-pyran-4-carboxylate



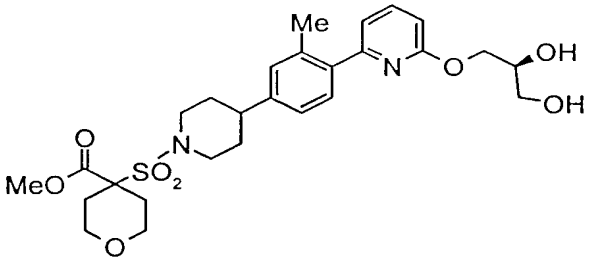
The title compound was obtained as a white solid (65%), after recrystallisation from methanol, from the stannane from preparation 6 and the bromide from preparation 27, following a similar procedure to that described in preparation 37.

^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.40 (s, 3H), 1.46 (s, 3H), 1.78-1.95 (m, 4H), 2.21 (m, 2H), 2.42 (m, 5H), 2.65 (m, 1H), 3.08 (m, 2H), 3.35 (m, 2H), 3.81-4.05 (m, 8H), 4.14 (dd, 1H), 4.40 (m, 2H), 4.50 (m, 1H), 6.76 (d, 1H), 6.99 (d, 1H), 7.08 (m, 2H), 7.38 (d, 1H), 7.62 (m, 1H).

LRMS : m/z 589 $(M+1)^+$

Preparation 39

Methyl 4-{[4-(4-{6-[(2S)-2,3-dihydroxy-1-propoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxylate

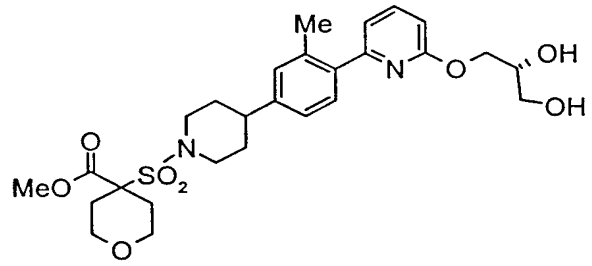


A solution of the dioxolane from preparation 37 (799mg, 1.36mmol) in 1,4-dioxan (10ml) was added to an ice-cooled solution of hydrochloric acid (30ml, 2N), and the reaction stirred for 75 minutes. The solution was poured into saturated sodium bicarbonate solution (200ml), and the resulting precipitate filtered and dried. The solid was recrystallised from ethy acetate/di-isopropyl ether, to afford the desired product as a white powder, (642mg, 86%).

^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.70-2.42 (m, 12H), 2.64 (m, 1H), 3.04 (m, 2H), 3.34 (m, 2H), 3.63 (m, 6H), 3.84-4.19 (m, 5H), 4.50 (m, 2H), 6.77 (d, 1H), 7.00 (d, 1H), 7.09 (m, 2H), 7.35 (d, 1H), 7.68 (m, 1H).

Preparation 40

Methyl 4-{[4-(-4-{6-[(2R)-2,3-dihydroxy-1-propoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxylate



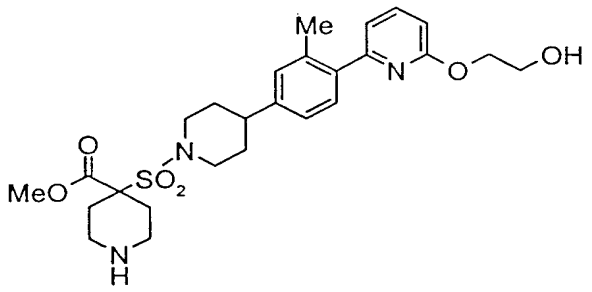
The title compound was obtained as a white crystalline solid (86%), from the dioxolane from preparation 38, following the procedure described in preparation 39.

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.76-1.92 (m, 4H), 2.21 (m, 2H), 2.40 (m, 5H), 2.50 (t, 1H), 2.64 (m, 1H), 3.06 (m, 2H), 3.34 (m, 2H), 3.64 (m, 2H), 3.72 (m, 5H), 4.00 (m, 3H), 4.12 (d, 1H), 4.50 (m, 2H), 6.78 (d, 1H), 7.01 (d, 1H), 7.10 (m, 2H), 7.36 (d, 1H), 7.68 (m, 1H).

LRMS : m/z 571 $(M+23)^+$

Preparation 41

Methyl 4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}-piperidine-4-carboxylate



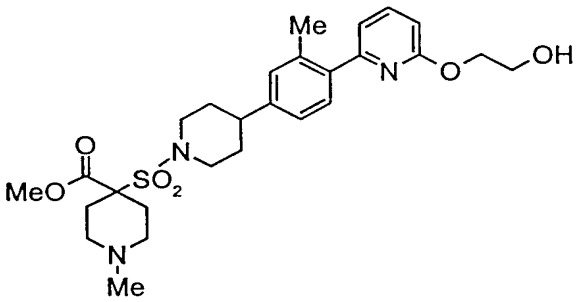
A mixture of the benzyl piperidine from preparation 33 (3.32g, 4.76mmol), ammonium formate (3.0g, 47.6mmol) and palladium hydroxide on carbon (3.32g) in a solution of acetic acid:methanol:tetrahydrofuran (2:2:1, 30ml) was heated under reflux for 2 hours. The cooled reaction was filtered through Arbocel®, washing through with tetrahydrofuran, and the filtrate concentrated in vacuo. The residue was partitoned between water and ethyl acetate, and the layers separated. The organic phase was dried (Na_2SO_4), filtered and evaporated in vacuo. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (90:10 to 85:15) to afford the title compound, (1.28g, 52%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.73-1.88 (m, 4H), 2.00 (m, 2H), 2.38 (s, 3H), 2.42-2.64 (m, 5H), 3.02 (m, 2H), 3.16 (m, 2H), 3.85 (m, 7H), 4.46 (t, 2H), 6.73 (d, 1H), 6.98 (d, 1H), 7.05 (m, 2H), 7.34 (d, 1H), 7.60 (m, 1H).

LRMS : m/z 518 $(M+1)^+$

Preparation 42

Methyl 4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}-1-methylpiperidine-4-carboxylate



Formaldehyde (0.49ml, 37 wt.% in water, 4.9mmol) was added to a solution of the piperidine from preparation 41 (634mg, 1.22mmol) in dichloromethane (30ml), and the solution was stirred vigorously at room temperature for 30 minutes. Sodium triacetoxyborohydride (519mg, 2.45mmol) was added and the reaction was stirred at room temperature for 20 hours. The reaction was washed with water, dried

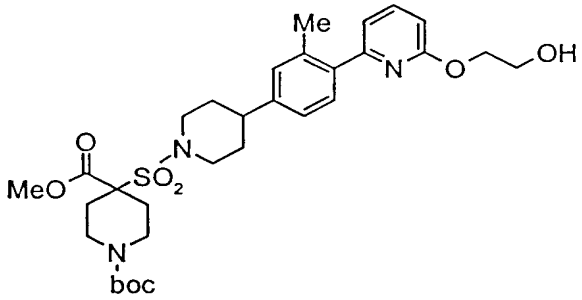
(Na_2SO_4), filtered and evaporated in vacuo. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to give the title compound (559mg, 86%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.76-1.95 (m, 6H), 2.20 (m, 5H), 2.38 (s, 3H), 2.50 (m, 2H), 2.62 (m, 1H), 2.90 (m, 2H), 3.03 (m, 2H), 3.84 (s, 3H), 3.94 (m, 4H), 4.48 (m, 2H), 6.76 (d, 1H), 6.99 (d, 1H), 7.06 (m, 2H), 7.35 (d, 1H), 7.63 (m, 1H).

LRMS : m/z 554 $(M+23)^+$

Preparation 43

Methyl 1-(tert-butoxycarbonyl)- 4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}-4-piperidinecarboxylate



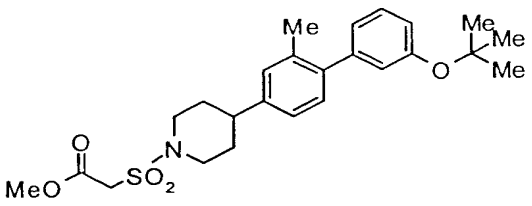
Triethylamine (175µl, 1.26mmol) was added to a solution of the amine from preparation 41 (594mg, 1.15mmol) in dichloromethane (100ml), followed by portionwise addition of di-tert-butyl dicarbonate (262mg, 1.20mmol). The reaction mixture was stirred at room temperature for an hour, then concentrated in vacuo to a volume of 20ml. The solution was diluted with ether (150ml), washed with hydrochloric acid (0.5N), brine, then dried ($MgSO_4$), filtered and evaporated in vacuo.
The residue was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to give the title compound (653mg, 92%) as a white foam.

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.42 (s, 9H), 1.75-1.90 (m, 4H), 2.01 (m, 2H), 2.38 (s, 3H), 2.45 (m, 2H), 2.63 (m, 3H), 3.02 (m, 2H), 3.50 (m, 1H), 3.87 (m, 7H), 4.17 (m, 2H), 4.46 (m, 2H), 6.75 (m, 1H), 6.98 (m, 1H), 7.05 (m, 2H), 7.35 (m, 1H), 7.62 (m, 1H).

LRMS : m/z 640 $(M+23)^+$

Preparation 44

Methyl 2-[4-(4-{3-tert-butoxyphenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]acetate



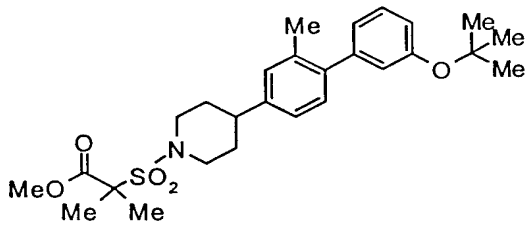
Nitrogen was bubbled through a mixture of cesium fluoride (3.71g, 24.44mmol), tri-o-tolyl phosphine (34mg, 0.11mmol), tris(dibenzylideneacetone)dipalladium (0) (50mg, 0.05mmol) the bromide from preparation 25 (4.27g, 11.0mmol) and the boronic acid from preparation 8 (3.2g, 16.5mmol) in anhydrous 1,2-dimethoxyethane (40ml). The reaction was then heated at 90°C under a nitrogen atmosphere for 50 hours. The cooled reaction mixture was diluted with ethyl acetate, the mixture washed with water (3x), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel using an elution gradient of hexane:ethyl acetate (95:5 to 50:50) to give the title compound as an oil, that crystallised on standing, (3.15g, 62%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.36 (s, 9H), 1.83 (m, 2H), 1.97 (m, 2H), 2.22 (s, 3H), 2.62 (m, 1H), 2.98 (m, 2H), 3.80 (s, 3H), 3.98 (m, 4H), 6.94 (m, 3H), 7.04 (m, 2H), 7.17 (d, 1H), 7.23 (m, 1H).

LRMS : m/z 582 $(M+23)^+$

Preparation 45

Methyl 2-[4-(4-{3-tert-butoxyphenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-2-methyl-propanoate



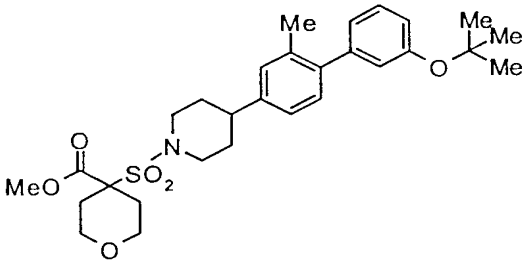
Potassium tert-butoxide (13.63ml, 1M in tetrahydrofuran, 13.63mmol) was added dropwise to a solution of the acetate from preparation 44 (2.5g, 5.45mmol) and methyl iodide (3.4ml, 54.5mmol) in tetrahydrofuran, and once addition was complete, the reaction was stirred at room temperature for 72 hours. The mixture was partitioned between ethyl acetate and water and the layers separated. The organic phase was dried ($MgSO_4$), filtered and evaporated in vacuo, to give the crude title compound, which was used without further purification (3.1g).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.36 (s, 9H), 1.63 (s, 6H), 1.77-1.94 (m, 4H), 2.22 (s, 3H), 2.63 (m, 1H), 3.05 (m, 2H), 3.80 (s, 3H), 3.95 (m, 2H), 6.90-7.10 (m, 5H), 7.18 (m, 1H), 7.24 (m, 1H).

LRMS : m/z 488 $(M+1)^+$

Preparation 46

Methyl 4-[4-(4-{3-tert-butoxyphenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylate



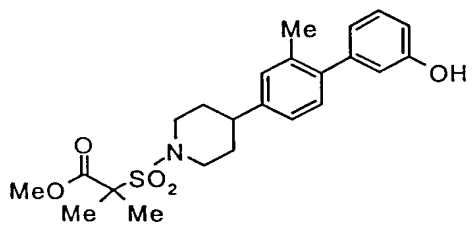
Nitrogen was bubbled through a mixture of cesium fluoride (2.19g, 14.43mmol), tri-o-tolyl phosphine (20mg, 0.065mmol), tris(dibenzylideneacetone)dipalladium (0) (30mg, 0.032mmol) and the bromide from preparation 27 (2.9g, 6.5mmol) and the boronic acid from preparation 8 (1.78g, 9.75mmol) in anhydrous 1,2-dimethoxyethane (40ml). The reaction was then heated under reflux under a nitrogen atmosphere for 24 hours. The cooled reaction was partitioned between ethyl acetate and water, the organic phase dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was triturated with di-isopropyl ether, the solid filtered and dried under vacuum, to give the desired product as a cream-coloured solid, (2.0g, 58%). The filtrate was concentrated in vacuo and the residual oil purified by column chromatography on silica gel using an elution gradient of hexane:dichloromethane:methanol (50:50:0 to 0:100:0 to 0:99:1) to provide an additional (630mg, 18%) of the title compound.

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.37 (s, 9H), 1.76-1.92 (m, 4H), 2.20 (m, 5H), 2.40 (m, 2H), 2.60 (m, 1H), 3.02 (m, 2H), 3.29 (m, 2H), 3.86 (m, 5H), 3.98 (m, 2H), 6.94 (m, 3H), 7.02 (m, 2H), 7.14 (m, 1H), 7.22 (m, 1H).

LRMS : m/z 552 $(M+23)^+$

Preparation 47

Methyl 2-[4-(4-{3-hydroxyphenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-2-methyl-propanoate



Trifluoroacetic acid (25ml) was added to a solution of the tert-butoxy ether from preparation 45 (4.8g, 9.80mmol) in dichloromethane (50ml), and the solution stirred for 4 hours. The reaction mixture was concentrated in vacuo, and the residue purified by column chromatography on silica gel, twice using an elution gradient of dichloromethane :methanol (10:0 to 95:5) to give the desired product (536mg, 13%).

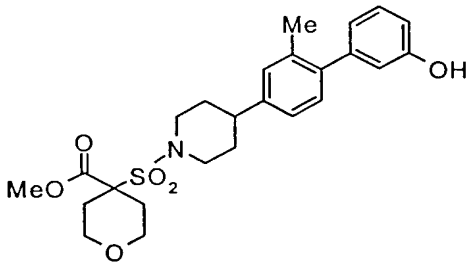
^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.62 (s, 6H), 1.76-1.92 (m, 4H), 2.22 (s, 3H), 2.62 (m, 1H), 3.04 (m, 2H), 3.78 (s, 3H), 3.95 (m, 2H), 6.78 (m, 2H), 6.83 (m, 1H), 7.03 (m, 2H), 7.15 (m, 1H), 7.21 (m, 1H).

LRMS : m/z 454 $(M+23)^+$

Anal. Found: C, 63.70; H, 6.70; N, 3.20. $C_{23}H_{29}NO_5S$ requires C, 64.01; H, 6.77; N, 3.25%.

Preparation 48

Methyl 4-[4-(4-{3-hydroxyphenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylate



Triethylsilane (2ml, 13.05mmol), followed by trifluoroacetic acid (5ml) were added to an ice-cooled solution of the tert-butyl ether from preparation 46 (2.3g, 4.35mmol) in dichloromethane (5ml) and the reaction stirred for 2 hours. The mixture was concentrated in vacuo, and the residue azeotroped with toluene. The resulting foam was triturated with di-isopropyl ether, filtered and dried to afford the title compound as a solid, (1.94g, 94%).

Alternative method

Palladium (II) acetate (300mg, 1.34mmol) and triphenylphosphine (708mg, 2.70mmol) were suspended in acetone (90ml), and sonicated for 2 minutes. The suspension was then added to a mixture of 5-bromo-2-iodotoluene (7.9g, 27mmol), and the boronic acid from preparation 8 (5.7g, 29.4mmol) in aqueous sodium carbonate (42ml, 2N). The reaction mixture was heated under reflux for 2 hours, then cooled and diluted with water (300ml). This mixture was extracted with ether (2x250ml), the combined organic extracts dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel using hexane:ether (99:1) as eluant to give 3-(4-bromo-2-methylphenyl)phenyl tert-butyl ether, 7.9g.

A solution of this intermediate ether (480mg, 1.5mmol) in tetrahydrofuran (2ml), followed by a crystal of iodine, were added to magnesium (45mg, 1.8mmol), and the mixture was heated under reflux for 2 hours. The solution was diluted with tetrahydrofuran (3ml), cooled to -78°C, and a solution of the ketone from

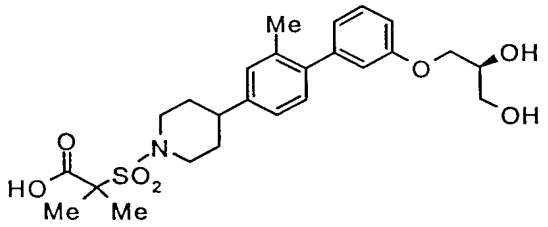
preparation 16 (425mg, 1.4mmol) in tetrahydrofuran (15ml) added dropwise.The reacton mixture was stirred at -78°C for 30 minutes, then allowed to warm to room temperature. Aqueous ammonium chloride was added, the mixture extracted with ethyl acetate (2x50ml) and the combined organic extracts were dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel using pentane:ethyl acetate (50:50) to afford methyl 4-[4-(4-{3-tert-butoxyphenyl}-3-methylphenyl)-4-hydroxypiperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylate as a clear oil, 280mg.

Triethylsilane (0.5ml, 3.14mmol), followed by trifluoroacetic acid (5ml) were added to a solution of this intermediate (350mg, 0.64mmol) in dichloromethane (5ml), and the reaction stirred at room temperature overnight. The reaction mixture was concentrated in vacuo, the residue azeotroped with toluene and the resulting solid dried under vacuum to afford the title compound, (300mg).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.74-1.90 (m, 4H), 2.20 (m, 5H), 2.40 (m, 2H), 2.62 (m, 1H), 3.02 (m, 2H), 3.29 (m, 2H), 3.87 (m, 5H), 3.98 (m, 2H), 6.77 (m, 2H), 6.83 (d, 1H), 7.02 (m, 2H), 7.15 (d, 1H), 7.21 (m, 1H).

Preparation 49

Methyl 2-[4-(4-{3-[(2S)-2,3-dihydroxypropoxy]phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-2-methyl-propanoate



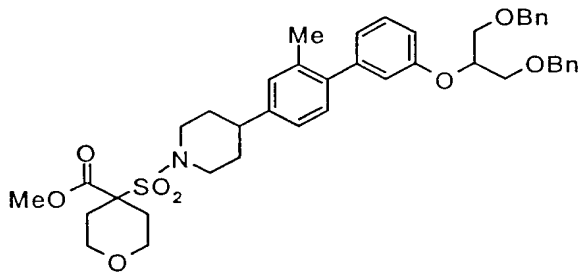
A mixture of the alcohol from preparation 47 (800mg, 1.86mmol), S-glycidol (0.12ml, 1.86mmol), and triethylamine (10μl, 0.09mmol) in methanol (10ml) was heated under reflux overnight. Tlc analysis showed starting material remaining, so the mixture was concentrated to low volume, and heated under reflux for a further 4 hours. The cooled reaction was evaporated in vacuo and the residue purified by column chromatography on silica gel using an elution gradient of hexane:ethyl acetate (91:9 to 50:50). The desired product was obtained as an oil, that gave a white foam on drying under vacuum, (391mg, 42%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.50 (s, 6H), 1.58 (m, 2H), 1.80 (m, 2H), 2.18 (s, 3H), 2.67 (m, 1H), 3.02 (m, 2H), 3.40 (m, 2H), 3.74 (m, 6H), 3.83 (m, 1H), 3.98 (m, 1H), 4.55 (m, 1H), 4.80 (m, 1H), 6.80 (m, 2H), 6.84 (m, 1H), 7.05 (m, 3H), 7.26 (m, 1H).

LRMS: m/z 528 $(M+23)^+$

Preparation 50

Methyl 4-[4-(4-{3-[1,3-dibenzyloxy-2-propoxy]phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylate

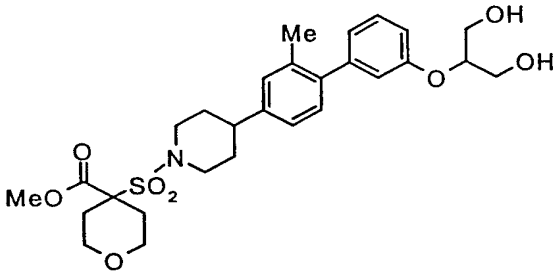


A mixture of the alcohol from preparation 48 (300mg, 0.63mmol), diethyl azodicarboxylate (150µl, 0.95mmol), triphenylphosphine (250mg, 0.95mmol), and 1,3-dibenzyloxy-2-propanol (260mg, 0.95mmol) in tetrahydrofuran (6ml), was stirred at room temperature for 3 hours. Tlc analysis showed some starting material remaining, so additional 1,3-dibenzyloxy-2-propanol (80mg, 0.3mmol), triphenyl phosphine (80mg, 0.3mmol) and diethyl azodicarboxylate (50µl, 0.32mmol) were added, and stirring was continued for an hour. The mixture was evaporated in vacuo, and the residue purified by column chromatography on silica gel using pentane:ethyl acetate (66:34) as eluant to give the title compound as a colourless oil, (400mg, 87%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.75-1.94 (m, 4H), 2.20 (m, 5H), 2.40 (m, 2H), 2.62 (m, 1H), 3.04 (m, 2H), 3.30 (m, 2H), 3.75 (m, 4H), 3.89 (m, 5H), 3.99 (m, 2H), 4.57 (m, 5H), 6.89 (m, 3H), 7.02 (m, 2H), 7.14 (d, 1H), 7.24 (m, 11H).

Preparation 51

Methyl 4-[4-(4-{3-[1,3-dihydroxy-2-propoxy]phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylate



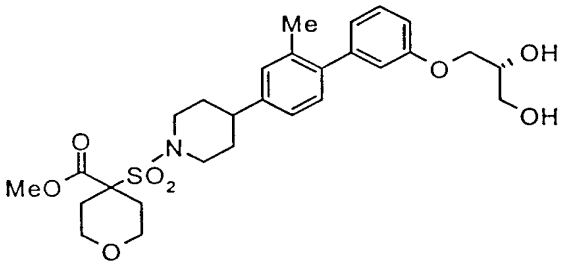
A mixture of the dibenzyl ether from preparation 50 (770mg, 1.06mmol), ammonium formate (1.4g, 11.0mmol) and palladium hydroxide on carbon (400mg) in methanol (40ml) was heated under reflux for 2 hours. Tlc analysis showed some starting material remaining, so additional palladium hydroxide (300mg) was added, and the reaction was heated under reflux overnight. The cooled mixture was filtered through Arbocel®, and the filtrate evaporated in vacuo. The crude product was purified by column

chromatography on silica gel using ethyl acetate:pentane (84:16) as eluant to afford the title compound as a white foam, (375mg, 65%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.76-1.94 (m, 6H), 2.20 (m, 5H), 2.40 (m, 2H), 2.62 (m, 1H), 3.04 (m, 2H), 3.29 (m, 2H), 3.90 (m, 10H), 3.99 (m, 2H), 6.94 (m, 3H), 7.03 (m, 2H), 7.16 (d, 1H), 7.30 (m, 1H).

Preparation 52

Methyl 4-[4-(4-{3-[(2R)-2,3-dihydroxypropoxy]phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylate



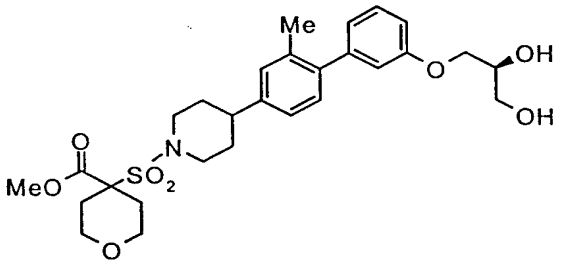
The title compound was obtained (17%) from the compound from preparation 48 and R-glycidol, following a similar procedure to that described in preparation 49.

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.75-1.97 (m, 4H), 2.20 (m, 5H), 2.40 (m, 2H), 2.61 (m, 1H), 3.02 (m, 2H), 3.28 (m, 2H), 3.58-4.14 (m, 12H), 6.84 (m, 3H), 7.02 (m, 2H), 7.15 (m, 1H), 7.26 (m, 1H).

LRMS: m/z 570 $(M+23)^+$

Preparation 53

Methyl 4-[4-(4-{3-[(2S)-2,3-dihydroxypropoxy]phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylate



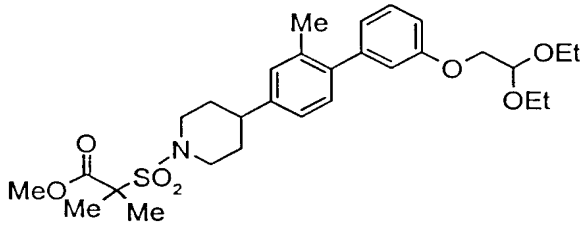
The title compound was obtained as a white solid (52%) after recrystallisation from di-isopropylether, from the alcohol of preparation 48 and S-glycidol, following a similar procedure to that described in preparation 49.

^{1}H nmr (DMSO-d_6, 300MHz) δ: 1.50-1.66 (m, 2H), 1.81 (m, 2H), 1.99 (m, 2H), 2.19-2.34 (m, 5H), 2.70 (m, 1H), 3.06 (m, 2H), 3.20 (m, 2H), 3.43 (m, 2H), 3.70-3.98 (m, 9H), 4.00 (dd, 1H), 4.60 (t, 1H), 4.90 (d, 1H), 6.80-6.95 (m, 3H), 7.15 (m, 3H), 7.31 (m, 1H).

LRMS: m/z 570 $(M+23)^+$

Preparation 54

Methyl 2-[4-(4-{3-(2,2-diethoxyethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-2-methylpropanoate



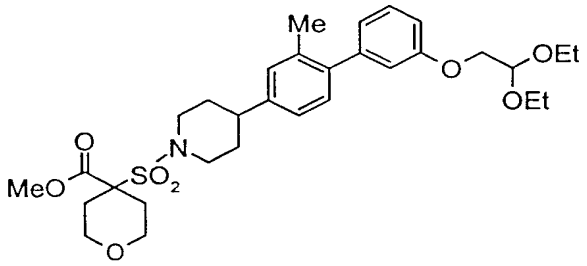
20% Palladium hydroxide on carbon (250mg) was added to a solution of the 1,2,3,6-tetrahydropyridine from preparation 34 (3.0g, 5.5mmol) and ammonium formate (1.04g, 16.5mmol) in methanol (70ml) and 1,4-dioxan (28ml), and the reaction was stirred at 60°C for 2 hours. Additional ammonium formate (1.0g, 15.8mmol) and palladium hydroxide on carbon (250mg) were added and stirring was continued for a further 2 hours. The mixture was cooled, filtered through Arbocel®, and the filter pad washed well with methanol. The combined filtrates were evaporated in vacuo and the residue partitioned between water and ether. The layers were separated, the organic phase washed with water, brine, dried ($MgSO_4$), filtered and evaporated in vacuo to give the title compound as a colourless oil, (2.8g, 93%).

^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.22 (t, 6H), 1.68 (s, 6H), 1.78-1.96 (m, 4H), 2.25 (s, 3H), 2.64 (m, 1H), 3.08 (m, 2H), 3.60-3.82 (m, 7H), 3.94-4.05 (m, 4H), 4.84 (t, 1H), 6.90 (m, 3H), 7.09 (m, 2H), 7.18 (d, 1H), 7.29 (d, 1H).

Anal. Found: C, 63.43; H, 7.75; N, 2.46. $C_{29}H_{41}NO_7S$ requires C, 63.60; H, 7.55; N, 2.56%.

Preparation 55

Methyl 4-[4-(4-{3-(2,2-diethoxyethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylate



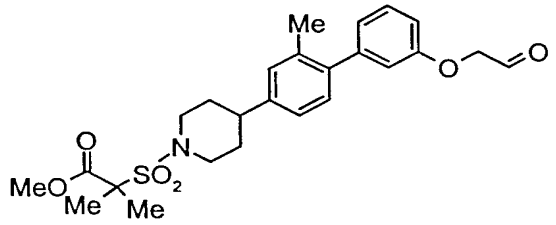
A mixture of cesium fluoride (4.3g, 28.3mmol), tri-o-tolyl phosphine (352mg, 1.15mmol), tris(dibenzylideneacetone)dipalladium (0) (535mg, 0.59mmol) and the boronic acid from preparation 10 (3.89g, 14.95mmol) and bromide from preparation 27 (5.0g, 10.86mmol) in anhydrous 1,2-dimethoxyethane (70ml), was heated under reflux for 4 ½ h. The cooled reaction mixture was concentrated in vacuo to half its volume, then partitioned between water and ethyl acetate. The layers were separated, the aqueous phase extracted with ethyl acetate (3x), and the combined organic solutions filtered through Arbocel®. The filtrate was washed with brine, dried (Na_2SO_4), filtered and evaporated in vacuo. The residual green oil was purified twice, by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (100:0 to 97:3), then triturated with di-isopropyl ether, to afford the title compound as a white solid, (2.38g, 37%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.20 (t, 6H), 1.76-1.94 (m, 4H), 2.20 (m, 5H), 2.40 (m, 2H), 2.61 (m, 1H), 3.02 (m, 2H), 3.31 (m, 2H), 3.61 (m, 2H), 3.74 (m, 2H), 3.90 (m, 5H), 4.00 (m, 3H), 4.80 (m, 1H), 6.85 (m, 3H), 7.03 (m, 2H), 7.16 (d, 1H), 7.24 (m, 2H).

LRMS: m/z 612 $(M+23)^+$

Preparation 56

Methyl 2-methyl-2-[4-(4-{3-(2-oxoethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]propanoate



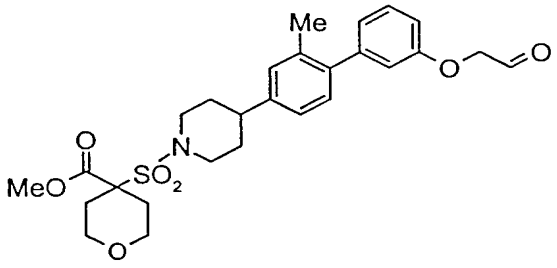
Hydrochloric acid (19ml, 1N, 19mmol) was added to a solution of the diethyl ketal from preparation 54 (4.43g, 8.1mmol) in acetone (19ml) and 1,4-dioxan (22ml), and the reaction stirred at 70°C for 2 hours. The cooled mixture was neutralised using sodium bicarbonate, concentrated in vacuo, and the residue partitioned between ether and water. The layers were separated, and the organic phase was washed with water, brine, then dried (Na_2SO_4), filtered and evaporated in vacuo. The residue was azeotroped with ethyl acetate, to afford the title compound (quantitative).

^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.67 (s, 6H), 1.78-1.96 (m, 4H), 2.26 (s, 3H), 2.66 (m, 1H), 3.09 (m, 2H), 3.82 (s, 3H), 3.98 (m, 2H), 4.60 (s, 2H), 6.86 (m, 2H), 6.98 (d, 1H), 7.09 (m, 2H), 7.17 (d, 1H), 7.35 (m, 1H), 9.90 (s, 1H).

LRMS : m/z 491 $(M+18)^+$

Preparation 57

Methyl 4-[4-(4-{3-(2-oxoethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylate



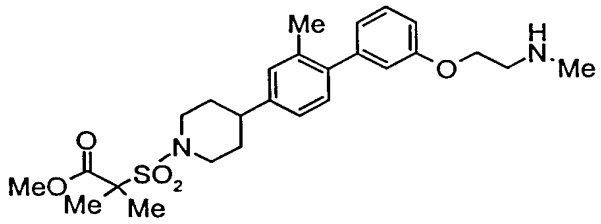
The title compound was obtained as a white foam (quantitative), from the diethyl ketal from preparation 55, following the procedure described in preparation 56.

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.77-1.93 (m, 4H), 2.21 (m, 5H), 2.40 (d, 2H), 2.62 (m, 1H), 3.02 (m, 2H), 3.30 (m, 2H), 3.88 (m, 5H), 3.99 (m, 2H), 4.57 (s, 2H), 6.83 (m, 2H), 6.94 (d, 1H), 7.03 (m, 2H), 7.15 (d, 1H), 7.30 (m, 1H), 9.83 (s, 1H).

Anal. Found: C, 61.79; H, 6.66; N, 2.46. $C_{27}H_{33}NO_7S;0.25CH_3CO_2C_2H_5;0.4H_2O$ requires C, 61.72; H, 6.62; N, 2.57%.

Preparation 58

Methyl 2-methyl-2-[4-(4-{3-(2-methylaminoethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]propanoate



Sodium triacetoxyborohydride (1.5g, 7.08mmol) was added portionwise over 1 hour to a solution of the aldehyde from preparation 56 (1.0g, 2.1mmol) and methylamine (5.8ml, 2N in tetrahydrofuran,

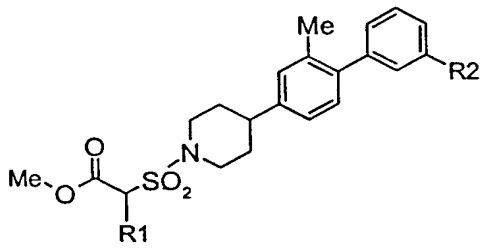
11.6mmol) in dichloromethane (50ml), and once addition was complete, the reaction was stirred at room temperature overnight. The reaction was partitioned between ethyl acetate and saturated sodium bicarbonate solution, and the layers separated, The organic phase was washed with water, brine, dried (Na_2SO_4), filtered and evaporated in vacuo to give a colourless oil. This was purified by medium pressure column chromatography on silica gel using an elution gradient of dichloromethane:methanol (100:0 to 90:10) to afford the title compound as a foam, (650mg, 63%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.62 (s, 6H), 1.76-1.90 (m, 4H), 2.22 (s, 3H), 2.56 (s, 3H), 2.61 (m, 1H), 3.04 (m, 4H), 3.78 (s, 3H), 3.95 (m, 2H), 4.12 (t, 2H), 6.83 (m, 3H), 7.03 (m, 2H), 7.14 (d, 1H), 7.24 (m, 1H).

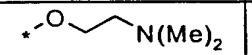
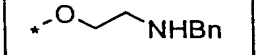
Anal. Found: C, 58.39; H, 6.90; N, 4.97. $C_{26}H_{36}N_2O_5S;0.75CH_2Cl_2$ requires C, 58.17; H, 6.84; N, 5.07%.

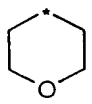
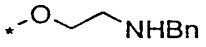
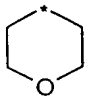
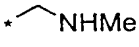
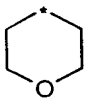
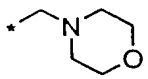
Preparations 59 to 63

The compounds of the general formula:



were prepared from the corresponding aldehydes and amines, following similar procedures to those described in preparation 58.

Prep No.	Aldehyde	R1	R2	Data
59	56	$(Me)_2$		mp 83-85°C ^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.62 (s, 6H), 1.78-1.94 (m, 4H), 2.22 (s, 3H), 2.30 (s, 6H), 2.60 (m, 1H), 2.70 t, 2H), 3.02 (m, 2H), 3.79 (s, 3H), 3.96 (m, 2H), 4.06 (t, 2H), 6.83 (m, 3H), 7.02 (m, 2H), 7.15 (d, 1H), 7.22 (m, 1H). LRMS : m/z 503 $(M+1)^+$ Anal. Found: C, 63.82; H, 7.52; N, 5.45. $C_{27}H_{38}N_2O_5S;0.1CH_2Cl_2$ requires C, 63.68; H, 7.53; N, 5.48%.
60	56	$(Me)_2$		^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.66 (s, 6H), 1.59-1.95 (m, 4H), 2.24 (s, 3H), 2.65 (m, 1H), 3.05 (m, 4H), 3.80 (s, 3H), 3.96 (m, 2H), 4.12 (t, 2H), 4.42 (d, 2H), 5.70 (br, s,

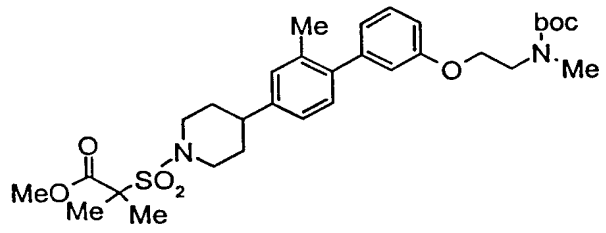
				1H), 6.85 (m, 3H), 7.07 (m, 2H), 7.17 (d, 1H), 7.24-7.38 (m, 6H). LRMS : m/z 565 $(M+1)^+$
61	57			^{1}H nmr ($CDCl_3$, 400MHz) ?: 1.75-1.92 (m, 4H), 2.20 (m, 5H), 2.40 (d, 2H), 2.62 (m, 1H), 3.00 (m, 4H), 3.28 (m, 2H), 3.88 (m, 5H), 3.99 (m, 2H), 4.09 (m, 2H), 4.40 (m, 2H), 5.60 (br s, 1H), 6.82 (m, 3H), 7.02 (m, 2H), 7.16 (d, 1H), 7.19-7.35 (m, 6H). LRMS : m/z 607 $(M+1)^+$
62[1]	30			mp 119-120°C ^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.50 (s, br, 1H), 1.75-1.92 (m, 4H), 2.20 (m, 5H), 2.40 (m, 5H), 2.61 (m, 1H), 3.02 (m, 2H), 3.30 (m, 2H), 3.75-4.01 (m, 9H), 7.01 (m, 2H), 7.16 (m, 2H), 7.24 (m, 3H). LRMS : m/z 501 $(M+1)^+$
63[2]	30			^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.75-1.94 (m, 4H), 2.20 (m, 5H), 2.40 (m, 6H), 2.61 (m, 1H), 3.02 (t, 2H), 3.30 (t, 2H), 3.50 (s, 2H), 3.66 (m, 4H), 3.87 (m, 7H), 7.02 (m, 2H), 7.16 (m, 2H), 7.26 (m, 3H). LRMS : m/z 557 $(M+1)^+$

1 = purified by crystallisation from ethyl acetate/dichloromethane/di-isopropyl ether.

2 = purified by column chromatography on silica gel using ethyl acetate:pentane (75:25) as eluant, and recrystallised from ethyl acetate.

Preparation 64

Methyl 2-[4-(4-{3-(2-[(N-tert-butoxycarbonyl)(N-methyl)amino]ethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-2-methyl-propanoate



A mixture of the compound from preparation 58 (640mg, 1.31mmol), triethylamine (180µl, 1.30mol), di-tert-butyl dicarbonate (290mg, 1.33mmol) and 4-dimethylaminopyridine (catalytic) in dichloromethane (10ml) was stirred at room temperature for 3 hours. The reaction mixture was diluted with dichloromethane (50ml), and washed with water, brine, dried (Na_2SO_4), filtered and evaporated in vacuo.

The residual oil was purified by medium pressure column chromatography on silica gel using an elution gradient of
pentane:dichloromethane:methanol (100:0:0 to 0:99.5:0.5) to afford the title compound as a gum, (590mg, 77%).

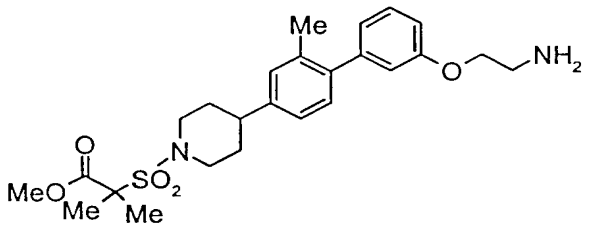
^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.42 (s, 9H), 1.62 (s, 6H), 1.77-1.90 (m, 4H), 2.22 (s, 3H), 2.63 (m, 1H), 2.97 (s, 3H), 3.03 (m, 2H), 3.58 (m, 2H), 3.78 (s, 3H), 3.95 (m, 2H), 4.08 (m, 2H), 6.82 (m, 3H), 7.04 (m, 2H), 7.16 (d, 1H), 7.25 (m, 1H).

LRMS : m/z 611 $(M+23)^+$

Anal. Found: C, 60.51; H, 7.19; N, 4.47. $C_{31}H_{44}N_2O_7S;0.4CH_2Cl_2$ requires C, 60.56; H, 7.25; N, 4.50%.

Preparation 65

Methyl 2-[4-(4-{3-(2-aminoethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-2-methyl-propanoate



A mixture of the amine from preparation 60 (1.2g, 2.12mmol) and 20% palladium hydroxide on carbon (250mg) in methanol (75ml), was hydrogenated at 50psi and room temperature for 18 hours. The reaction mixture was filtered through Arbocel®, and the filter pad washed well with methanol. The combined filtrates were evaporated in vacuo to give an oil. This was purified by medium pressure column chromatography on silica gel using an elution gradient of dichloromethane:methanol (100:0 to 90:10) to afford the title compound (610mg, 60%).

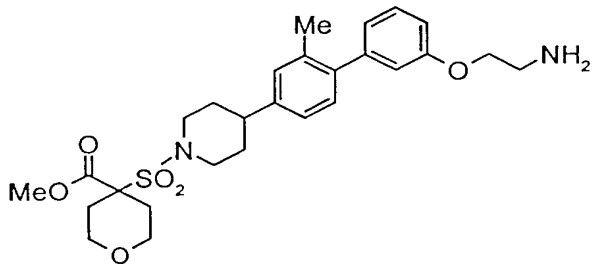
^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.66 (s, 6H), 1.78-1.97 (m, 4H), 2.28 (s, 3H), 2.66 (m, 1H), 3.10 (m, 4H), 3.82 (s, 3H), 3.99 (m, 4H), 6.88 (m, 3H), 7.10 (m, 2H), 7.19 (d, 1H), 7.30 (m, 1H).

LRMS : m/z 475 $(M+1)^+$

Anal. Found: C, 61.26; H, 7.09; N, 5.63. $C_{25}H_{34}N_2O_5S;0.25$dichloromethane requires C, 61.16; H, 7.01; N, 5.65%.

Preparation 66

Methyl 4-[4-(4-{3-(2-aminoethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylate



The title compound was obtained as a solid (65%) from the compound from preparation 61, following the procedure described in preparation 65.

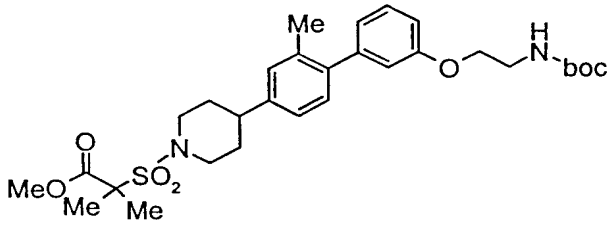
^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.76-1.92 (m, 4H), 2.20 (m, 5H), 2.40 (m, 2H), 2.62 (m, 1H), 3.04 (m, 4H), 3.30 (m, 2H), 3.88 (m, 5H), 3.98 (m, 4H), 6.82 (m, 3H), 7.03 (m, 2H), 7.16 (d, 1H), 7.22 (m, 1H).

LRMS: m/z 517 $(M+1)^+$

Anal. Found: C, 62.30; H, 6.98; N, 5.40. $C_{27}H_{36}N_2O_6S;0.05CH_2Cl_2$ requires C, 62.37; H, 6.99; N, 5.38%.

Preparation 67

Methyl 2-[4-(4-{3-(2-[(tert-butoxycarbonyl)amino]ethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-2-methyl-propanoate



The title compound was obtained as a white foam (69%) from the amine from preparation 65, following a similar procedure to that described in preparation 64.

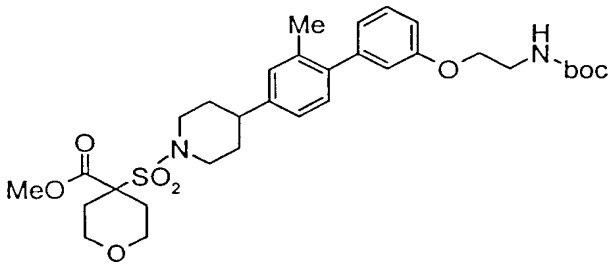
^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.44 (s, 9H), 1.65 (s, 6H), 1.78-1.95 (m, 4H), 2.25 (s, 3H), 2.64 (m, 1H), 3.08 (m, 2H), 3.55 (m, 2H), 3.81 (s, 3H), 3.97 (m, 2H), 4.04 (t, 2H), 4.99 (br, s, 1H), 6.80-6.94 (m, 3H), 7.08 (m, 2H), 7.18 (d, 1H), 7.32 (m, 1H).

LRMS : m/z 597 $(M+23)^+$

Anal. Found: C, 62.49; H, 7.46; N, 4.78. $C_{30}H_{42}N_2O_7S$ requires C, 62.69; H, 7.37; N, 4.87%.

Preparation 68

Methyl 4-[4-(4-{3-(2-[(tert-butoxycarbonyl)amino]ethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylate



Di-tert-butyl dicarbonate (300mg, 1.37mmol) was added to a solution of the amine from preparation 66 (650mg, 1.26mmol) in dichloromethane (10ml), and the reaction stirred at room temperature for 18 hours. The reaction was diluted with dichloromethane (50ml), then washed with water (2x), brine, then dried (Na_2SO_4), filtered and evaporated in vacuo. The residue was purified by medium pressure column chromatography on silica gel using an elution gradient of dichloromethane:methanol (99.5:0.5 to 99:1) to afford the title compound as a white foam, (710mg, 91%).

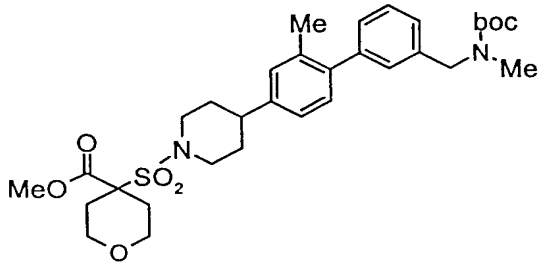
^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.40 (s, 9H), 1.78-1.92 (m, 4H), 2.20 (m, 5H), 2.40 (d, 2H), 2.61 (m, 1H), 3.02 (m, 2H), 3.30 (m, 2H), 3.50 (m, 2H), 3.88 (m, 5H), 4.00 (m, 4H), 4.86 (br s, 1H), 6.82 (m, 3H), 7.02 (m, 2H), 7.15 (d, 1H), 7.05 (m, 1H).

LRMS: m/z 639 $(M+23)^+$

Anal. Found: C, 62.15; H, 7.20; N, 4.47. $C_{32}H_{44}N_2O_8S$ requires C, 62.32; H, 7.19; N, 4.54%.

Preparation 69

Methyl 4-[4-(4-{3-([N-tert-butoxycarbonyl-N-methylamino]methyl)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylate



The title compound was prepared from the amine from preparation 62, using a similar procedure to that described in preparation 64. The crude product was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:pentane (25:75 to 50:50) and triturated with di-isopropyl ether to give the title compound as a white solid, (714mg, 65%).

mp 122-123°C.

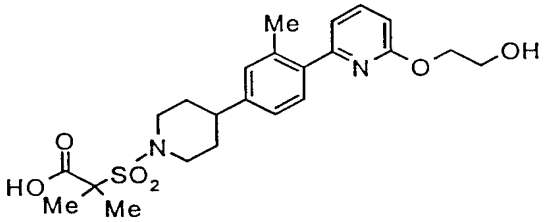
^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.42 (s, 9H), 1.75-1.92 (m, 4H), 2.20 (m, 5H), 2.40 (m, 2H), 2.61 (m, 1H), 2.82 (s, 3H), 3.03 (m, 2H), 3.30 (m, 2H), 3.85 (m, 5H), 3.99 (m, 2H), 4.42 (s, 2H), 7.03 (m, 2H), 7.17 (m, 4H), 7.35 (m, 1H).

LRMS : m/z 623 $(M+23)^+$

Anal. Found: C, 63.92; H, 7.36; N, 4.57. $C_{32}H_{44}N_2O_7S$ requires C, 63.98; H, 7.38; N, 4.66%.

Preparation 70

2-[4-{4-[6-(2-Hydroxyethoxy)pyridin-2-yl]-3-methylphenyl}-piperidin-1-ylsulphonyl]-2-methylpropanoic acid



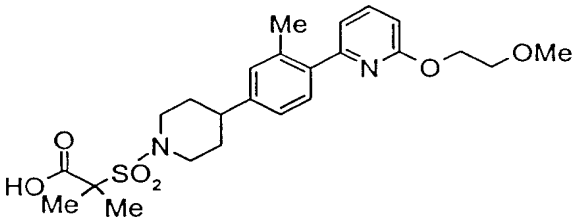
A mixture of the methyl ester from preparation 35 (4.1g, 8.6mmol) and aqueous sodium hydroxide (17ml, 1N, 17.0mmol) in methanol (50ml), was heated under reflux for 30 minutes, then cooled. The reaction was concentrated in vacuo, the residue dissolved in water (200ml), and the solution acidified to pH 4. The resulting precipitate was filtered off, washed with water, dried under vacuum, and recrystallised from ethyl acetate, to afford the title compound as a white solid, (3.15g, 79%).

^{1}H nmr (DMSO-d_6, 300MHz) δ: 1.42-1.70 (m, 8H), 1.80 (m, 2H), 2.37 (s, 3H), 2.70 (t, 1H), 3.06 (m, 2H), 3.68 (m, 2H), 3.80 (m, 2H), 4.25 (t, 2H), 4.80 (br, s, 1H), 6.77 (d, 1H), 7.06 (d, 1H), 7.17 (m, 2H), 7.35 (d, 1H), 7.77 (m, 1H), 13.38 (br, s, 1H).

Anal. Found : C, 58.35; H, 6.38; N, 5.83. $C_{23}H_{30}N_2O_6S;0.5H_2O$ requires C, 58.85; H, 6.62; N, 5.94%.

Preparation 71

2-(4-{4-[6-(2-Methoxyethoxy)pyridin-2-yl]-3-methylphenyl}-piperidin-1-ylsulphonyl)-2-methylpropanoic acid



Sodium hydride (60mg, 60% dispersion in mineral oil, 1.5mmol) was added to a solution of the methyl ester from preparation 35 (300mg, 0.63mmol) in tetrahydrofuran (10ml), and the solution stirred for 15 minutes. Methyl iodide (200µl, 3.3mmol) was added and the reaction heated under reflux for 45 minutes. Aqueous sodium hydroxide solution (2ml, 1N, 2.0mmol) and methanol (5ml) were then added, and the mixture heated under refux for a further 30 minutes. The reaction mixture was cooled to room temperature, diluted with water (20ml), and acidified to pH 4. This solution was extracted with dichloromethane (3x30ml), the combined organic extracts dried (Na_2SO_4), filtered and evaporated in vacuo to afford the title compound as a pale yellow foam, (quantitative).

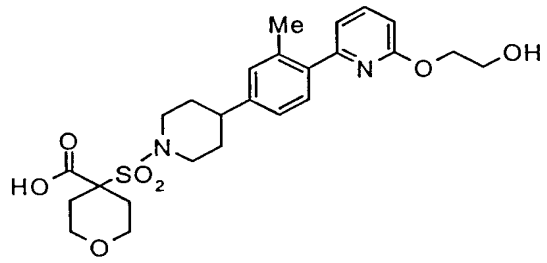
mp 142-146°C

^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.68 (s, 6H), 1.78-1.96 (m, 4H), 2.41 (s, 3H), 2.66 (m, 1H), 3.09 (m, 2H), 3.43 (s, 3H), 3.78 (t, 2H), 4.00 (m, 2H), 4.52 (t, 2H), 6.78 (d, 1H), 6.98 (d, 1H), 7.08 (m, 2H), 7.38 (d, 1H), 7.61 (d, 1H).

LRMS : m/z 433 $(M-CO_2)^+$

Preparation 72

4-[4-(4-{6-[2-Hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-ylsulphonyl]tetrahydro-2H-pyran-4-carboxylic acid



Aqueous sodium hydroxide (5.56ml, 1N, 5.56mmol) was added to a solution of the methyl ester from preparation 36 (720mg, 1.39mmol) in methanol (20ml), and the reaction heated under reflux for 3 hours, and stirred for a further 18 hours, at room temperature. The mixture was concentrated in vacuo to remove the methanol, and the solution acidified to pH 4 using acetic acid solution. This was extracted with ethyl

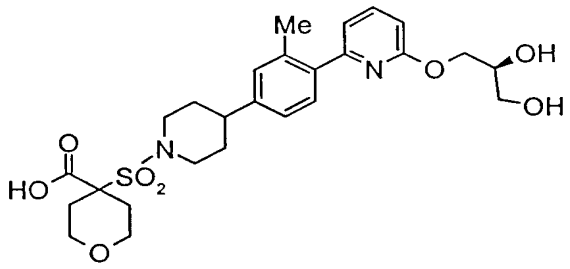
acetate (3x), the combined organic extracts washed with brine, dried ($MgSO_4$), filtered and evaporated in vacuo. The residual solid was recrystallised from ethyl acetate/di-isopropyl ether to afford the title compound as a solid, (517mg, 74%).

^{1}H nmr (DMSO-d_6, 300MHz) δ: 1.62 (m, 2H), 1.82 (m, 2H), 1.98 (m, 2H), 2.24 (m, 2H), 2.36 (s, 3H), 2.74 (m, 1H), 3.09 (t, 2H), 3.22 (m, 2H), 3.64-3.82 (m, 4H), 3.94 (dd, 2H), 4.28 (t, 2H), 4.80 (br s, 1H), 6.78 (d, 1H), 7.06 (d, 1H), 7.16 (m, 2H), 7.36 (d, 1H), 7.78 (m, 1H), 13.82 (br s, 1H).

LRMS : m/z 527 $(M+18)^+$

Preparation 73

4-[4-(4-{6-[(2S)-2,3-dihydroxy-1-propoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylic acid



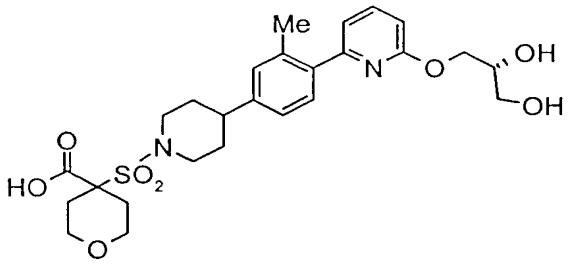
Aqueous sodium hydroxide (3.5ml, 1M, 3.5mmol) was added to a solution of the methyl ester from preparation 39 (640mg, 1.17mmol) in methanol (15ml) and 1,4-dioxan (15ml), and the reaction heated under reflux for 2 hours. Tlc analysis showed starting material remaining, so additonal sodium hydroxide (2ml, 1M, 2mmol) was added and the reaction heated under reflux for a further 3 hours. The cooled reaction mixture was concentrated under reduced pressure, the residue dissolved in water, and the pH adjusted to 4 using hydrochloric acid (2N). The resulting precipitate was filtered and dried, and the filtrate extracted with dichloromethane (2x). The combined organic extracts were dried ($MgSO_4$), filtered and evaporated in vacuo, and the product combined with the filtered solid. This was recrystallised from dichloromethane/ethyl acetate twice, to yield the title compound as a white solid, (579mg, 92%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.80 (m, 2H), 1.92 (m, 2H), 2.23 (d, 2H), 2.34 (s, 3H), 2.66 (m, 1H), 3.08 (m, 2H), 3.17-3.42 (m, 3H), 3.78 (m, 3H), 3.88 (m, 2H), 4.14 (dd, 1H), 4.26 (dd, 1H), 4.60 (br, s, 1H), 4.85 (br, s, 1H), 6.76 (d, 1H), 7.04 (d, 1H), 7.15 (m, 2H), 7.34 (m, 2H), 7.74 (dd, 1H).

LRMS : m/z 557 $(M+23)^+$

Preparation 74

4-[4-(4-{6-[(2R)-2,3-dihydroxy-1-propoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylic acid



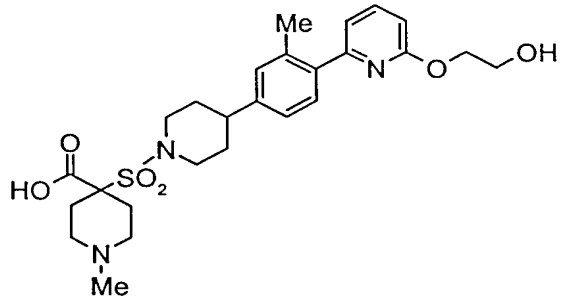
The title compound was obtained as a white solid (87%) from the methyl ester of preparation 40, following a similar procedure to that described in preparation 73.

^{1}H nmr (DMSO-d_6, 300MHz) δ: 1.61 (m, 2H), 1.80 (m, 2H), 1.96 (m, 2H), 2.24 (m, 2H), 2.36 (s, 3H), 2.70 (m, 1H), 3.06 (m, 2H), 3.14-3.44 (m, 4H), 3.78 (m, 3H), 3.93 (m, 2H), 4.14 (m, 1H), 4.26 (m, 1H), 4.59 (m, 1H), 4.84 (m, 1H), 6.76 (d, 1H), 7.06 (d, 1H), 7.15 (m, 2H), 7.35 (d, 1H), 7.76 (m, 1H), 13.80 (br, s, 1H).

LRMS : m/z 557 $(M+23)^+$

Preparation 75

4-[4-(4-{6-[2-Hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-ylsulphonyl]-1-methylpiperidine-4-carboxylic acid

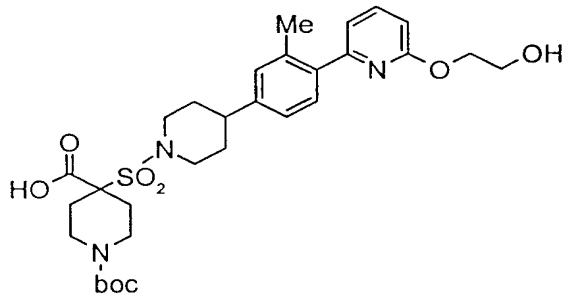


A mixture of the methyl ester from preparation 42 (200mg, 0.38mmol) and aqueous sodium hydroxide (1.5ml, 1N, 1.5mmol) in methanol (8ml) and 1,4-dioxan (8ml) was heated under reflux overnight. The cooled reaction was concentrated in vacuo, the residue acidified to pH 4 using acetic acid, and extraction with ethyl acetate attempted. A precipitate formed in the organic layer, that was filtered off, and combined with the residual solid in the separating funnel, to provide the desired compound as a white powder, (quantitative).

LRMS : m/z 518 $(M+1)^+$

Preparation 76

1-(tert-Butoxycarbonyl)- 4-[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-ylsulphonyl]-piperidine-4-carboxylic acid



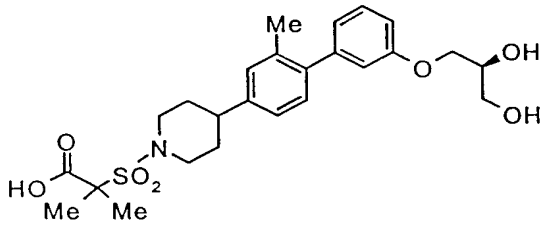
The title compound was obtained as a white solid (87%), from the methyl ester from preparation 43, following a similar procedure to that described in preparation 75.

mp 148-149°C

^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.42 (s, 9H), 1.80 (m, 4H), 2.00 (m, 2H), 2.36 (s, 3H), 2.41 (m, 2H), 2.58-2.79 (m, 4H), 3.02 (m, 4H), 3.92 (m, 5H), 4.44 (m, 2H), 6.76 (m, 1H), 6.99 (m, 1H), 7.07 (m, 2H), 7.34 (m, 1H), 7.65 (m, 1H).

Preparation 77

2-[4-(4-{3-[(2S)-2,3-Dihydroxy-1-propoxy]phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-2-methyl-propanoic acid



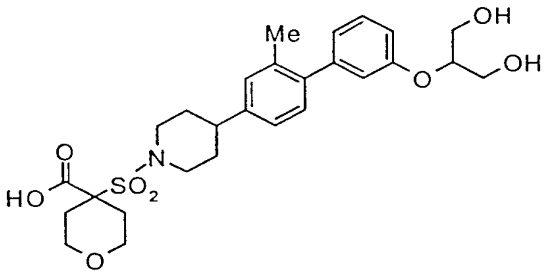
Aqueous sodium hydroxide (1.55ml, 1M, 1.55mmol) was added to a solution of the methyl ester from preparation 49 (391mg, 0.77mmol) in methanol (5ml), and the reaction stirred at room temperature overnight. The mixture was partitioned between ethyl acetate and hydrochloric acid (2N), and the phases separated. The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residual solid was triturated with di-isopropyl ether, filtered and dried under vacuum, to give the title compound as a white solid, (320mg, 85%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.48 (s, 6H), 1.59 (m, 2H), 1.79 (m, 2H), 2.18 (s, 3H), 2.64 (m, 1H), 3.04 (m, 2H), 3.40 (m, 2H), 3.78 (m, 3H), 3.82 (m, 1H), 3.98 (m, 1H), 4.57 (br, s, 1H), 4.82 (br, s, 1H), 6.80 (m, 2H), 6.85 (m, 1H), 7.05 (m, 2H), 7.12 (m, 1H), 7.27 (m, 1H), 13.25 (br, s, 1H).

Anal. Found: C, 60.77; H, 6.89; N, 2.78. $C_{25}H_{33}NO_7S$ requires C, 61.08; H, 6.77; N, 2.85%.

Preparation 78

4-[4-(4-{3-[2,3-dihydroxy-2-propoxy]phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylic acid

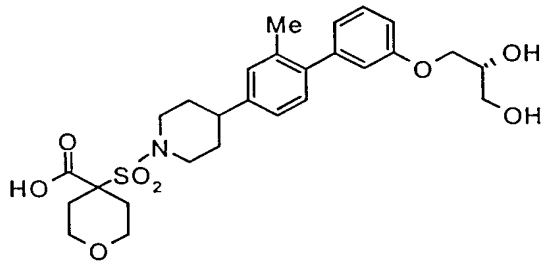


A mixture of the methyl ester from preparation 51 (370mg, 0.68mmol), aqueous sodium hydroxide (3ml, 1M, 3mmol) in methanol (5ml) and 1,4-dioxan (5ml), was heated under reflux for 6 hours. The cooled reaction was concentrated in vacuo, and then diluted with water. This aqueous solution was acidified to pH 2 using hydrochloric acid (2N), and the resulting precipitate filtered, washed with water and dried under vacuum, to give the desired product (270mg, 74%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.79 (m, 2H), 1.95 (m, 2H), 2.19 (m, 5H), 2.63 (m, 1H), 3.02 (m, 4H), 3.56 (m, 4H), 3.76 (m, 2H), 3.88 (m, 2H), 4.22 (m, 1H), 4.68 (m, 2H), 6.78-6.95 (m, 3H), 7.08 (m, 3H), 7.25 (m, 1H).

Preparation 79

4-[4-(4-{3-[(2R)-2,3-Dihydroxy-1-propoxy]phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-(2H)-pyran-4-carboxylic acid



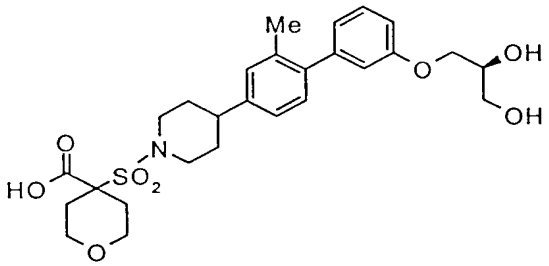
A mixture of the methyl ester from preparation 52 (110mg, 0.20mmol), aqueous sodium hydroxide (1ml, 1M, 1mmol) in methanol (5ml) and 1,4-dioxan (5ml) was heated under reflux for 2 hours. The cooled reaction was evaporated in vacuo, the residue dissolved in water and acidified to pH 1 using hydrochloric acid (1N). The resulting precipitate was filtered, the solid washed with water, and dried under vacuum to give the title compound (91mg, 85%) as a white solid.

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.80 (m, 2H), 1.94 (m, 2H), 2.20 (m, 5H), 2.65 (m, 1H), 3.05 (m, 2H), 3.18-3.48 (m, 4H), 3.77 (m, 3H), 3.88 (m, 3H), 4.00 (m, 1H), 6.81 (m, 2H), 6.89 (m, 1H), 7.10 (m, 3H), 7.30 (m, 1H).

LRMS : m/z 556 $(M+23)^+$

Preparation 80

4-[4-(4-{3-[(2S)-2,3-Dihydroxy-1-propoxy]phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-(2H)-pyran-4-carboxylic acid

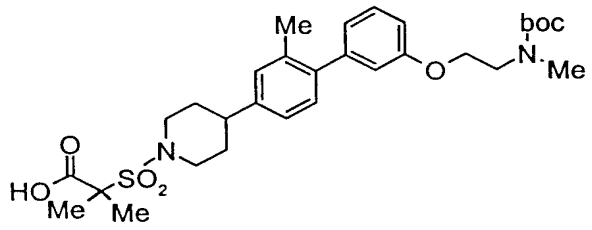


The title compound was obtained as a solid (66%) from the methyl ester from preparation 53, following the procedure described in preparation 79.

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.80 (m, 2H), 1.96 (m, 2H), 2.22 (m, 5H), 2.68 (m, 1H), 3.06 (m, 2H), 3.21 (m, 2H), 3.42 (d, 2H), 3.78 (m, 3H), 3.90 (m, 3H), 4.00 (m, 1H), 6.81 (m, 2H), 6.90 (d, 1H), 7.12 (m, 3H), 7.31 (dd, 1H).

Preparation 81

2-[4-(4-{3-(2-[N-tert-Butoxycarbonyl-N-methylamino]ethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-2-methylpropanoic acid



A mixture of the methyl ester from preparation 64 (540mg, 0.92mmol), and aqueous sodium hydroxide (6ml, 1N, 6.0mmol) in 1,4-dioxan (2.3ml) and methanol (6ml) was heated under reflux for 3 ½ h. The cooled mixture was concentrated in vacuo to remove the organic solvents, and the residual aqueous solution was acidified to pH 4 using acetic acid. This was extracted with ethyl acetate (2x), the combined organic extracts washed with water, brine, dried (Na_2SO_4), filtered and evaporated in vacuo. The residue

was azeotroped with toluene, then ethyl acetate, and finally dichloromethane to afford the title compound as a white foam, (520mg, 98%).

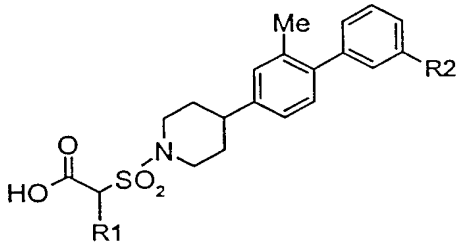
^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.41 (s, 9H), 1.64 (s, 6H), 1.78-1.94 (m, 4H), 2.22 (s, 3H), 2.63 (m, 1H), 2.97 (s, 3H), 3.06 (m, 2H), 3.59 (m, 2H), 3.98 (m, 2H), 4.08 (t, 2H), 6.83 (m, 3H), 7.04 (m, 2H), 7.16 (d, 1H), 7.26 (m, 1H).

LRMS : m/z 597 $(M+23)^+$

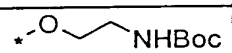
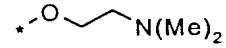
Anal. Found: C, 61.17; H, 7.27; N, 4.65. $C_{30}H_{42}N_2O_7S;0.2CH_2Cl_2$ requires C, 61.30; H, 7.22; N, 4.73%.

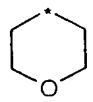
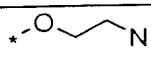
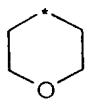
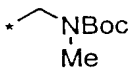
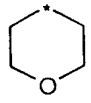
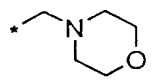
Preparations 82 to 86

The compounds of the general formula:



were prepared from the corresponding methyl esters, following similar procedures to those described in preparation 81.

Prep No.	Starting ester	R1	R2	Data
82	67	$(Me)_2$		^{1}H nmr (DMSO-d_6, 300MHz) δ: 1.36 (s, 9H), 1.50 (s, 6H), 1.62 (m, 2H), 1.81 (m, 2H), 2.20 (s, 3H), 2.68 (m, 1H), 3.06 (m, 2H), 3.28 (m, 4H), 3.80 (m, 2H), 3.98 (t, 2H), 6.80-6.99 (m, 3H), 7.14 (m, 2H), 7.30 (m, 1H). LRMS : m/z 583 $(M+23)^+$ Anal. Found: C 58.94; H, 7.02; N, 4.64. $C_{29}H_{40}N_2O_7S;0.4CH_2Cl_2$ requires C, 59.02; H, 6.94; N, 4.68%.
83[1]	59	$(Me)_2$		mp 230-232°C ^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.46 (s, 6H), 1.60 (m, 2H), 1.80 (m, 2H), 2.18 (s, 3H), 2.25 (s, 6H), 2.64 (m, 3H), 3.02 (m, 2H), 3.78 (m, 2H), 4.06 (t, 2H), 6.80 (m, 2H), 6.86 (d, 1H), 7.08 (m, 2H), 7.28 (dd, 1H). Anal. Found: C, 62.70; H, 7.37; N, 5.53. $C_{26}H_{36}N_2O_5S;0.5H_2O$ requires C, 62.75; H, 7.49; N, 5.63%.

84	68			mp 194-196°C ^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.42 (s, 9H), 1.75-1.92 (m, 4H), 2.22 (m, 5H), 2.38 (d, 2H), 2.61 (m, 1H), 3.06 (m, 2H), 3.40 (m, 2H), 3.50 (m, 2H), 3.98 (m, 6H), 6.82 (m, 3H), 7.02 (m, 2H), 7.14 (d, 1H), 7.23 (m, 1H). Anal. Found: C, 61.20; H, 7.05; N, 4.60. $C_{31}H_{42}N_2O_8S;0.25H_2O$ requires C, 61.32; H, 7.05; N, 4.61%.
85[2]	69			mp 196-197°C ^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.38 (s, 9H), 1.60 (m, 2H), 1.80 (m, 2H), 1.95 (m, 2H), 2.19 (s, 3H), 2.20 (m, 2H), 2.64 (m, 1H), 2.76 (s, 3H), 3.02 (t, 2H), 3.18 (m/t, 2H), 3.77 (m, 2H), 3.86 (m, 2H), 4.38 (s, 2H), 7.12 (m, 6H), 7.37 (m, 1H). LRMS : m/z 609 $(M+23)^+$
86[3]	63			^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.59 (m, 2H), 1.80 (m, 2H), 1.90 (m, 2H), 2.20 (m, 6H), 2.62-2.79 (m, 4H), 3.00-3.22 (m, 6H), 3.65 (m, 4H), 3.76 (m, 2H), 3.88 (m, 2H), 7.12 (m, 4H), 7.25 (m, 1H), 7.39 (m, 2H). LRMS : m/z 543 $(M+1)^+$

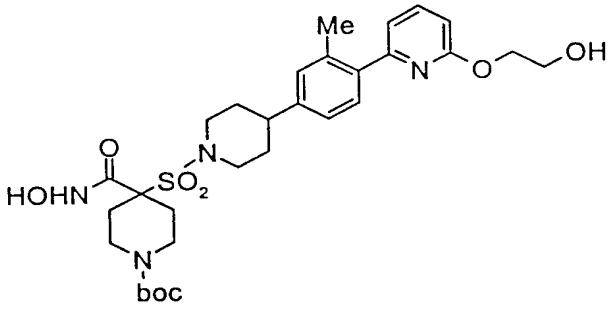
1 = isolated by filtration from aqueous acetic acid solution.

2 = recrystallised from ethyl acetate/methanol

3 = triturated with di-isopropyl ether

Preparation 87

N-Hydroxy 1-(tert-butoxycarbonyl)-4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}-piperidine-4-carboxamide



Chlorotrimethylsilane (70µl, 0.55mmol) was added to a solution of the acid from preparation 76 (300mg, 0.50mmol) in dichloromethane (4ml), and pyridine (2ml), and the solution stirred at room temperature under a nitrogen atmosphere for 1 hour. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (115mg, 0.60mmol) and 1-hydroxy-7-azabenzotriazole (75mg, 0.55mmol) were added, and stirring was continued for a further hour. Hydroxylamine hydrochloride (104mg, 1.50mmol) was added and the reaction stirred at room temperature overnight. The reaction mixture was diluted with water, the solution

acidified to pH 1 using hydrochloric acid (2M), then extracted with ethyl acetate. The combined organic solutions were washed with brine, dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was triturated with ethyl acetate, the resulting precipitate filtered and the filtrate evaporated in vacuo. The residue was recrystallised from ethyl acetate to afford the title compound (148mg, 48%) as a white solid.

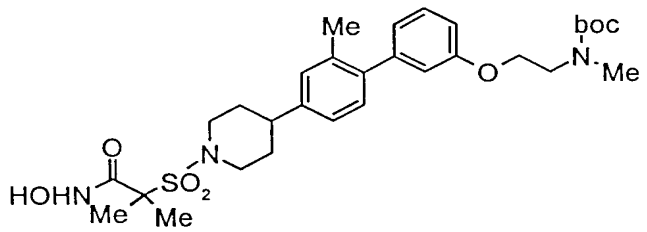
mp 180-181°C

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.39 (s, 9H), 1.55-1.81 (m, 6H), 2.36 (s, 3H), 2.42 (m, 2H), 2.62 (m, 3H), 3.03 (m, 2H), 3.70 (m, 4H), 3.95 (m, 2H), 4.24 (t, 2H), 4.78 (br, t, 1H), 6.75 (d, 1H), 7.04 (d, 1H), 7.15 (m, 2H), 7.34 (d, 1H), 7.75 (m, 1H), 9.16 (s, 1H), 11.00 (s, 1H).

LRMS : m/z 617 $(M-1)^+$

Preparation 88

N-Hydroxy 2-[4-(4-{3-(2-[(N-tert-butoxycarbony-N-methyl)amino]ethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-2-methylpropanamide



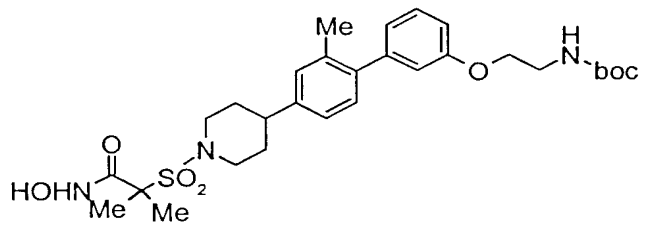
O-(7-Azabenzotriazol-1-yl)-N,N,N'N'-tetramethyluronium hexafluorophosphate (540mg, 1.42mmol) was added to a solution of the acid from preparation 81 (520mg, 0.90mmol) and N-ethyldiisopropylamine (193µl, 1.12mmol) in N-methylpyrrolidinone (10ml), and the reaction stirred at room temperature under a nitrogen atmosphere for 40 minutes. Hydroxylamine hydrochloride (210mg, 3.02mmol) and additional N-ethyldiisopropylamine (730µl, 4.23mmol) were added, and the reaction stirred at room temperature overnight. The mixture was partitioned between ethyl acetate and pH 7 buffer solution, and the layers separated. The organic phase was washed consecutively with water, brine, then dried ($NaSO_4$), filtered and evaporated in vacuo. The crude product was purified by medium pressure column chromatography on silica gel using an elution gradient of dichloromethane:methanol (99.5:0.5 to 98:2 to 80:20) to afford the title compound, (180mg, 34%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.40 (s, 9H), 1.63 (s, 6H), 1.78 (m, 2H), 1.86 (m, 2H), 2.22 (s, 3H), 2.61 (m, 1H), 2.97 (s, 3H), 3.03 (m, 2H), 3.58 (m, 2H), 3.94 (m, 2H), 4.08 (m, 2H), 6.60 (s, 1H), 6.64 (m, 2H), 7.02 (m, 2H), 7.17 (d, 1H), 7.26 (dd, 1H), 8.99 (s, 1H), 10.75 (s, 1H).

Anal. Found: C, 60.96; H, 7.33; N, 7.11. $C_{30}H_{43}N_3O_7S$ requires C, 61.10; H, 7.35; N, 7.12%.

Preparation 89

N-Hydroxy 2-[4-(4-{3-(2-[(tert-butoxycarbonyl)amino]ethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-2-methylpropionamide



The title compound was obtained (49%) from the acid from preparation 82, following a similar procedure to that described in preparation 88.

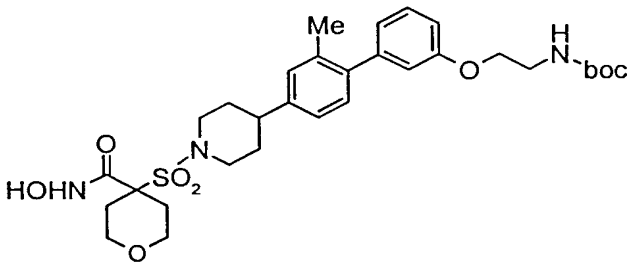
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.37 (s, 9H), 1.48 (s, 6H), 1.60 (m, 2H), 1.79 (m, 2H), 2.20 (s, 3H), 2.64 (m, 1H), 3.04 (m, 2H), 3.28 (m, 2H), 3.75 (m, 2H), 3.98 (t, 2H), 6.80-6.98 (m, 4H), 7.10 (s, 2H), 7.15 (s, 1H), 7.30 (dd, 1H), 8.99 (s, 1H), 10.55 (s, 1H).

LRMS : m/z 598 $(M+23)^+$

Anal. Found: C, 59.25; H, 7.09; N, 7.38. $C_{29}H_{41}N_3O_7S;0.1CH_2Cl_2$ requires C, 59.83; H, 7.11; N, 7.19%.

Preparation 90

N-Hydroxy 4-[4-(4-{3-(2-[(N-tert-butoxycarbonyl)amino]ethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxamide



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (260mg, 1.36mmol) and 1-hydroxy-7-azabenzotriazole (150mg, 1.1mmol) were added to a solution of the acid from preparation 84 (620mg, 1.03mmol) in pyridine (2ml) and dichloromethane (6ml), and the mixture stirred at room temperature for 30 minutes. Hydroxylamine hydrochloride (155mg, 2.25mmol) was added and the reaction stirred at room temperature for 18 h. The reaction mixture was partitioned between ethyl acetate and pH 7 buffer solution, and the layers separated. The aqueous phase was extracted with ethyl acetate, the combined

organic solutions washed again with pH 7 buffer solution, then brine, dried (Na_2SO_4), filtered and evaporated in vacuo. The residue was azeotroped with toluene, and then purified by medium pressure column chromatography on silica gel using an elution gradient of dichloromethane:methanol (100:0 to 90:10). The product was recrystallised from ethyl acetate/pentane to afford the title compound as a solid, (340mg, 53%).

mp 181-182°C

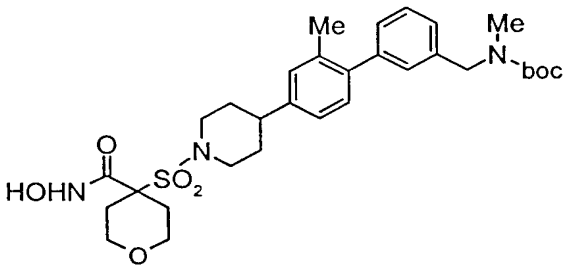
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.35 (s, 9H), 1.60 (m, 2H), 1.78 (m, 2H), 1.90 (m, 2H), 2.19 (s, 3H), 2.28 (m, 2H), 2.61 (m, 1H), 3.02 (m, 2H), 3.20 (m, 2H), 3.22 (m, 2H), 3.70 (m, 2H), 3.84 (m, 2H), 3.98 (t, 2H), 6.79-6.95 (m, 4H), 7.08 (s, 2H), 7.15 (s, 1H), 7.28 (m, 1H), 9.10 (s, 1H), 10.93 (s, 1H).

LRMS : m/z 640 $(M+23)^+$

Anal. Found: C, 60.27; H, 7.04; N, 6.63. $C_{31}H_{43}N_3O_8S$ requires C, 60.27; H, 7.02; N, 6.88%.

Preparation 91

N-Hydroxy 4-[4-(4-{3-(N-tert-butoxycarbonyl-N-methyl)aminomethyl)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxamide



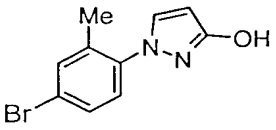
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (216mg, 1.12mmol) and 1-hydroxy-7-azabenzotriazole (128mg, 0.94mmol) were added to a solution of the acid from preparation 85 (550mg, 0.94mmol) in pyridine (2ml) and N,N dimethylformamide (6ml), and the mixture stirred at room temperature for 1 hour. Hydroxylamine hydrochloride (195mg, 2.82mmol) was added and the reaction stirred at room temperature overnight. The reaction mixture was partitioned between ethyl acetate and pH 7 buffer solution, and the layers separated. The aqueous phase was extracted with ethyl acetate (x2), the combined organic solutions washed with 2N hydrochloric acid, dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was crystallised from methanol/ethyl acetate to afford the title compound as a solid, (393mg, 70%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.36 (s, 9H), 1.59 (m, 2H), 1.78 (m, 2H), 1.88 (m, 2H), 2.18 (s, 3H), 2.27 (m, 2H), 2.61 (m, 1H), 2.76 (s, 3H), 3.00 (m, 2H), 3.18 (m, 2H), 3.68 (m, 2H), 3.82 (m, 2H), 4.38 (s, 2H), 7.09 (m, 3H), 7.18 (m, 3H), 7.38 (m, 1H), 9.10 (s, 1H), 10.92 (s, 1H).

LRMS : m/z 624 $(M+1)^+$

Preparation 92

1-(4-Bromo-2-methylphenyl)-1H-pyrazol-3-ol



Potassium tert-butoxide (20ml, 1M in tert-butanol, 20.0mmol) was added to 1-(4-bromo-2-methylphenyl)hydrazine (J.Chem.Soc. 109; 1916; 582)(2.01g, 10.0mmol) to give a dark brown suspension. Ethyl propiolate (1.02ml, 10mmol) was then added dropwise over 10 minutes, with cooling, and once addition was complete, the reaction was heated under reflux for 4 hours. The reaction was diluted with water (200ml) and this mixture washed with dichloromethane (2x50ml). The aqueous phase was acidified using hydrochloric acid (2N), extracted with dichloromethane (5x100ml), these combined organic extracts dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (98:2) as eluant, and triturated with ether/di-isopropyl ether to give the title compound (615mg, 24%) as a solid.

mp 208-210°C

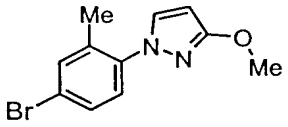
^{1}H nmr (DMSO-d_6, 400MHz) δ: 2.26 (s, 3H), 5.75 (s, 1H), 7.22 (d, 1H), 7.44 (d, 1H), 7.57 (s, 1H), 7.74 (s, 1H), 10.00 (s, 1H).

LRMS : m/z 253, 255 $(M+1)^+$

Anal.Found: C, 47.31; H, 3.52; N, 10.99. $C_{10}H_9BrN_2O$ requires C, 47.46; H, 3.58; N, 11.07%.

Preparation 93

1-(4-Bromo-2-methylphenyl)-3-methoxy-1H-pyrazole



A mixture of the pyrazole from preparation 92 (1.52g, 6.0mmol), potassium carbonate (828mg, 6.0mmol), and dimethylsulphate (624ml, 6.6mmol) in 1-methyl-2-pyrrolidinone (15ml) was heated at 90°C for 5 hours. Tlc analysis showed starting material remaining, so additional potassium carbonate (828mg, 6.0mmol) and dimethylsulphoxide (624?l, 6.6mmol) were added, and stirring continued at 90°C for a further 18 hours. The cooled reaction was poured into water (200ml), and the mixture extracted with ethyl acetate (3x100ml). The combined organic extracts were washed with brine (3x100ml), dried

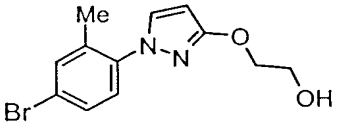
($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel using dichloromethane as the eluant, to give the desired product as a pale yellow oil, (970mg, 61%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 2.30 (s, 3H), 3.95 (s, 3H), 5.30 (s, 1H), 5.85 (s, 1H), 7.19 (d, 1H), 7.38 (m, 1H), 7.43 (s, 1H).

LRMS : m/z 267, 269 $(M+1)^+$

Preparation 94

1-(4-Bromo-2-methylphenyl)-3-(2-hydroxyethoxy)-1H-pyrazole



2-Bromoethanol (1.55ml, 21.8mmol) was added to a mixture of the alcohol from preparation 92 (2.76g, 10.9mmol) and potassium carbonate (3.01g, 21.8mmol) in N,N-dimethylformamide (50ml), and the reaction stirred at 80°C for 5 hours. The cooled mixture was concentrated in vacuo, the residue suspended in ethyl acetate (250ml), and the mixture washed with water (5x50ml). The organic phase was dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was purified by column chromatography on silica gel using dichloromethane:ether (80:20) as eluant, and crystallised from di-isopropyl ether to give the desired product as buff-coloured crystals, (1.61g, 50%).

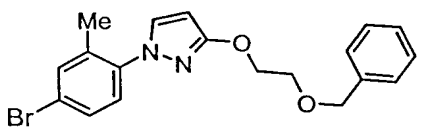
mp 104-105°C

^{1}H nmr ($CDCl_3$, 400MHz) δ: 2.24 (s, 3H), 2.58 (br, s, 1H), 3.92 (m, 2H), 4.36 (t, 2H), 5.84 (d, 1H), 7.15 (d, 1H), 7.35 (m, 2H), 7.40 (s, 1H).

Anal. Found: C, 48.38; H, 4.30; N, 9.34. $C_{12}H_{13}BrN_2O_2$ requires C, 48.50; H, 4.41; N, 9.43%.

Preparation 95

3-(2-Benzyloxyethoxy)-1-(4-bromo-2-methylphenyl)-1H-pyrazole



A solution of the alcohol from preparation 94 (1.55g, 5.2mmol) in tetrahydrofuran (12ml) was added to a suspension of sodium hydride (229mg, 60% dispersion in mineral oil, 5.73mmol) in tetrahydrofuran (10ml), and the resulting mixture stirred for 2 minutes under a nitrogen atmosphere. Benzyl bromide

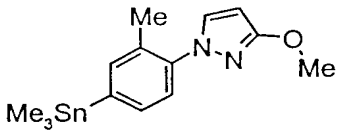
(681µl, 5.73mmol) was then added and the reaction heated under reflux for 16 hours. The cooled reaction mixture was poured into brine (70ml) and extracted with ethyl acetate (3x50ml). The combined organic solutions were dried ($MgSO_4$), filtered and concentrated in vacuo to give a yellow oil. The crude product was purified by column chromatography on silica gel using an elution gradient of hexane:ethyl acetate (90:10 to 80:20) to give the title compound as a colourless oil, (1.93g, 96%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 2.24 (s, 3H), 3.80 (t, 2H), 4.38 (t, 2H), 4.60 (s, 2H), 5.66 (s, 1H), 7.12 (d, 1H), 7.21 (m, 2H), 7.32 (m, 5H), 7.40 (s, 1H).

LRMS : m/z 409, 411 $(M+23)^+$

Preparation 96

3-Methoxy-1-[(2-methyl-4-trimethylstannyl)phenyl]-1H-pyrazole

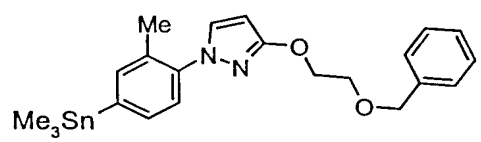


Tetrakis(triphenylphosphine)palladium (0) (30mg, 0.026mmol) was added to a solution of the bromide from preparation 93 (659mg, 2.47mmol), and hexamethylditin (889mg, 2.71mmol) in 1,4-dioxan (8ml), and nitrogen bubbled through the resulting mixture. The reaction was heated under reflux for 4 ½ hours, then tlc analysis showed starting material remaining. Additional tetrakis(triphenylphosphine)palladium (0) (48mg) was added and the reaction heated under reflux for a further 16 hours. 50% Aqueous potassium fluoride solution (5ml) was added to the cooled reaction, the mixture stirred for 15 minutes, then filtered through Arbocel®, washing through with ether. The filtrate was washed with brine (30ml), dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was purified by column chromatography on silica gel using pentane:ether (90:10) as eluant to give the title compound as a pale yellow oil, (598mg, 69%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 0.27 (s, 9H), 2.26 (s 3H), 3.92 (s, 3H), 5.80 (s, 1H), 7.21 (m, 2H), 7.35 (m, 2H).

Preparation 97

3-(2-Benzyloxyethoxy)-1-[2-methyl-4-(trimethylstannyl)phenyl]-1H-pyrazole



Tetrakis(triphenylphosphine)palladium (0) (286mg, 0.25mmol) was added to a solution of the bromide from preparation 95 (1.92g, 4.96mmol), and hexamethylditin (1.78g, 5.45mmol) in 1,4-dioxan (18ml), and nitrogen bubbled through the resulting mixture. The reaction was heated under reflux for 2 hours,

then cooled. Potassium fluoride solution (5ml, 50%) was added, the mixture stirred for 30 minutes, and filtered though Arbocel®, washing through well with ethyl acetate (150ml). The filtrate was washed with brine (2x30ml), dried ($MgSO_4$), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel using hexane:ether (84:16) to afford the desired product as a crystalline solid, (1.87g, 80%).

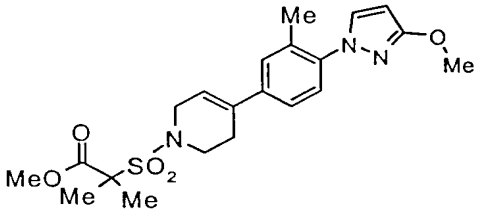
mp 50-52°C

^{1}H nmr ($CDCl_3$, 400MHz) δ: 0.28 (s, 9H), 2.24 (s, 3H), 3.80 (t, 2H), 4.40 (t, 2H), 4.60 (s, 2H), 5.82 (s, 1H), 7.22 (m, 3H), 7.33 (m, 6H).

Anal. Found: C, 56.21; H, 5.97; N, 5.95. $C_{22}H_{28}N_2O_2Sn$ requires C, 56.08; H, 5.99; N, 5.95%.

Preparation 98

Methyl 2-{4-[4-(3-methoxy-1H-pyrazol-1-yl}-3-methylphenyl]-1,2,3,6-tetrahydropyridin-1-ylsulphonyl}-2-methyl-propanoate



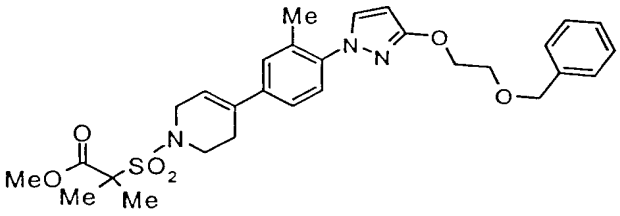
Tris(dibenzylideneacetone)dipalladium(0) (30.7mg, 0.034mmol) was added to a solution of the vinyl triflate from preparation 29 (727mg, 1.84mmol), the stannane from preparation 96 (590mg, 1.68mmol), and triphenylarsine (104mg, 0.36mmol) in 1-methyl-2-pyrrolidinone (4ml), and the solution stirred under a nitrogen atmosphere. Copper (I) iodide (16mg, 0.17mmol) was added, the solution de-gassed, and the reaction then stirred at 60°C for 30 minutes, and at 75°C for a further 4 ½ hours. Potassium fluoride solution (3ml, 50%) was added to the cooled reaction, stirring continued for 15 minutes, and the mixture filtered through Arbocel®, washing through with ethyl acetate (150ml). The filtrate was washed with water (30ml), brine (30ml), dried ($MgSO_4$), filtered and evaporated in vacuo. The residual orange foam was purified by column chromatography on silica gel using pentane:ether (50:50) to afford the title compound as a pale yellow gum, (588mg, 81%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.63 (s, 6H), 2.30 (s, 3H), 2.59 (m, 2H), 3.60 (t, 2H), 3.79 (s, 3H), 3.94 (s, 3H), 4.08 (m, 2H), 5.81 (d, 1H), 6.00 (m, 1H), 7.21 (m, 3H), 7.36 (s, 1H).

LRMS : m/z 434 $(M+1)^+$

Preparation 99

Methyl 2-{4-[4-(3-{2-benzyloxyethoxy}-1H-pyrazol-1-yl}-3-methylphenyl]-1,2,3,6-tetrahydropyridin-1-ylsulphonyl}-2-methyl-propanoate



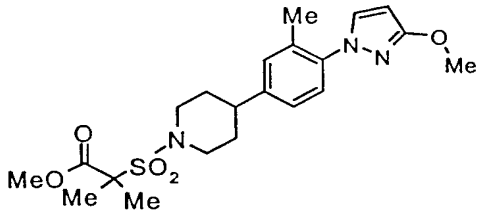
The title compound was obtained as a yellow oil (75%) from the triflate from preparation 29 and the stannane of preparation 97, using a similar method to that described in preparation 98.

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.64 (s, 6H), 2.27 (s, 3H), 2.58 (m, 2H), 3.59 (m, 2H), 3.78 (s, 3H), 3.80 (t, 2H), 4.09 (m, 2H), 4.39 (t, 2H), 4.60 (s, 2H), 5.85 (s, 1H), 6.00 (m, 1H), 7.21 (m, 4H), 7.34 (m, 5H).

LRMS : m/z 576 $(M+23)^+$

Preparation 100

Methyl 2-{4-[4-(3-methoxy-1H-pyrazol-1-yl}-3-methylphenyl]piperidin-1-ylsulphonyl}-2-methylpropanoate



10% Palladium on charcoal (60mg) was added to a solution of the 1,2,3,6-tetrahydropyridine from preparation 98 (580mg, 1.38mmol) in methanol (20ml), and the mixture hydrogenated at 50 psi and room temperature for 6 hours. Tlc analysis showed starting material remaining, so additional 10% palladium on charcoal (50mg) was added, and the mixture hydrogenated for a further 18 hours. The reaction mixture was filtered through Arbocel®, the filtrate suspended in dichloromethane (50ml), re-filtered through Arbocel®, and the filtrate evaporated in vacuo, to give the desired product as a colourless solid, (365mg, 61%).

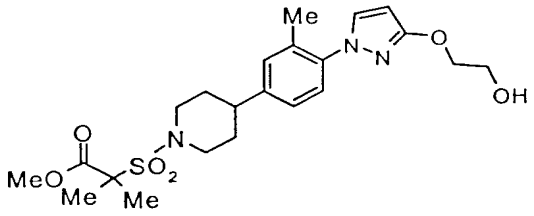
mp 109-110°C

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.61 (s, 6H), 1.75-1.86 (m, 4H), 2.25 (s, 3H), 2.62 (m, 1H), 3.02 (m, 2H), 3.79 (s, 3H), 3.94 (m, 5H), 5.80 (d, 1H), 7.06 (m, 2H), 7.21 (m, 2H).

LRMS : m/z 458 $(M+23)^+$

Preparation 101

Methyl 2-{4-[4-(3-{2-hydroxyethoxy}-1H-pyrazol-1-yl}-3-methylphenyl]piperidin-1-ylsulphonyl}-2-methylpropanoate



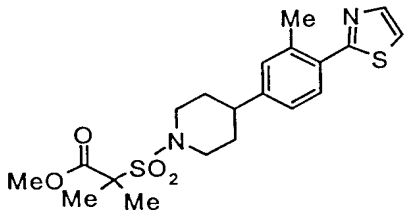
A mixture of the benzyl ether from preparation 99 (790mg, 1.42mmol) and 10% palladium on charcoal (160mg) in ethanol (35ml) was hydrogenated at 50 psi and room temperature for 17 hours. Tlc analysis showed starting material remaining, so acetic acid (2ml), and additional 10% palladium on charcoal (80mg) were added, and the reaction continued for a further 48 hours, with additional 10% palladium on charcoal (160mg) added portionwise. The reaction mixture was filtered through Arbocel®, washing through with ethanol, and the filtrate concentrated in vacuo. The residue was partitioned between ethyl acetate (100ml) and saturated sodium bicarbonate solution (100ml), the layers separated and the organic phase dried ($MgSO_4$), filtered and evaporated in vacuo to give the title compound as a colourless oil, (630mg, 95%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.46-1.62 (m, 8H), 1.80 (m, 2H), 2.19 (s, 3H), 2.71 (m, 1H), 3.02 (m, 2H), 3.10 (m, 2H), 3.62-3.79 (m, 5H), 4.10 (m, 2H), 4.60 (m, 1H), 5.84 (s, 1H), 7.12 (m, 1H), 7.19 (m, 2H), 7.69 (s, 1H).

LRMS : m/z 488 $(M+23)^+$

Preparation 102

Methyl 2-methyl-2-{4-[3-methyl-4-(1,3-thiazol-2-yl)phenyl]piperidin-1-ylsulphonyl}-propanoate



Bis(triphenylphosphine)palladium (II) chloride (49mg, 0.07mmol) was added to a solution of the bromide from preparation 26 (577mg, 1.38mmol) and 2-(trimethylstannyl)-1,3-thiazole (Synthesis, 1986, 757) (372mg, 1.5mmol) in tetrahydrofuran (3.5ml), and the resulting mixture was de-gassed, and placed

under an argon atmosphere. The reaction was heated under reflux for 17 hours. Tlc analysis showed starting material remaining, so additional 2-(trimethylstannyl)-1,3-thiazole (173mg, 0.8mmol) and bis(triphenylphosphine)palladium (II) chloride (49mg, 0.07mmol) were added, the mixture was de-gassed, and then heated under reflux for a further 17 hours. The cooled mixture was concentrated in vacuo, and the residue purified by column chromatography on silica gel using an elution gradient of hexane:ethyl acetate (91:9 to 66:34). The product was re-purified by column chromatography on silica gel using ether as eluant to give the title compound as a buff-coloured solid, (240mg, 40%).

mp 111-114°C

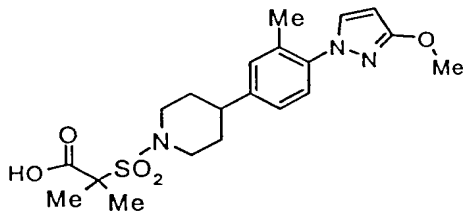
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.52 (s, 6H), 1.58 (m, 2H), 1.81 (m, 2H), 2.45 (s, 3H), 2.74 (m, 1H), 3.04 (m, 2H), 3.74 (m, 5H), 7.18 (d, 1H), 7.21 (s, 1H), 7.62 (d, 1H), 7.78 (d, 1H), 7.92 (d, 1H).

LRMS : m/z 445 $(M+23)^+$

Anal. Found: C, 56.64; H, 6.19; N, 6.55. $C_{20}H_{26}N_2S_2O_4$ requires C, 56.85; H, 6.20; N, 6.63%.

Preparation 103

2-{4-[4-(3-Methoxy-1H-pyrazol-1-yl}-3-methylphenyl]piperidin-1-ylsulphonyl}-2-methylpropanoic acid



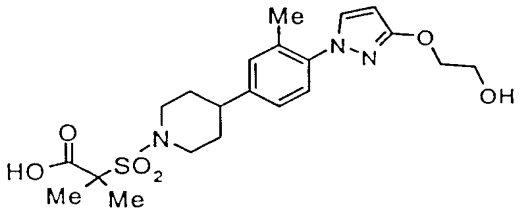
A mixture of the methyl ester from preparation 100 (355mg, 0.82mmol), and aqueous sodium hydroxide (5.9ml, 1M, 5.9mmol) in methanol (5ml) and 1,4-dioxan (5ml) was heated under reflux for 2 hours. The cooled reaction was diluted with water and acidified to pH 3 using hydrochloric acid (2N). The resulting precipitate was filtered off, washed with water, and dried under vacuum at 75°C to give the title compound as a white powder, (281mg, 82%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.63 (s, 6H), 1.70-1.90 (m, 4H), 2.24 (s, 3H), 2.62 (m, 1H), 3.04 (m, 2H), 3.90 (s, 3H), 3.98 (m, 2H), 5.80 (s, 1H), 7.04 (m, 3H), 7.32 (m, 1H).

Anal. Found: C, 56.78; H, 6.40; N, 9.71. $C_{20}H_{27}N_3O_5S$ requires C, 56.99; H, 6.46; N, 9.97%.

Preparation 104

2-{4-[4-(3-{2-Hydroxyethoxy}-1H-pyrazol-1-yl}-3-methylphenyl]piperidin-1-ylsulphonyl}-2-methylpropanoic acid



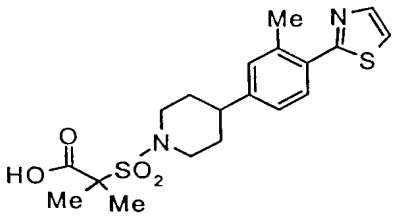
A mixture of the methyl ester from preparation 101(520mg, 1.2mmol), and aqueous sodium hydroxide (3.6ml, 1M, 3.6mmol) in 1,4-dioxan (5ml) was heated under reflux for 2 ½ hours. The cooled reaction was partitioned between water (100ml) and ethyl acetate (100ml), acidified to pH 2 using hydrochloric acid (2N), and the phases separated. The aqueous layer was extracted with ethyl acetate (2x35ml), the combined organic solutions dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was triturated with ether twice, to afford the title compound as a white solid, (338mg, 62%).

^{1}H nmr (DMSO-d_6, 300MHz) δ: 1.47 (s, 6H), 1.59 (m, 2H), 1.79 (m, 2H), 2.19 (s, 3H), 2.70 (m, 1H), 3.02 (m, 2H), 3.64 (m, 2H), 3.79 (m, 2H), 4.09 (t, 2H), 4.62 (m, 1H), 5.84 (s, 1H), 7.12 (m, 1H), 7.18 (m, 2H), 7.69 (s, 1H), 13.1 (br, s, 1H).

LRMS : m/z 474 $(M+23)^+$

Preparation 105

2-Methyl-2-{4-[3-methyl-4-(1,3-thiazol-2-yl)phenyl]piperidin-1-ylsulphonyl}-propanoic acid



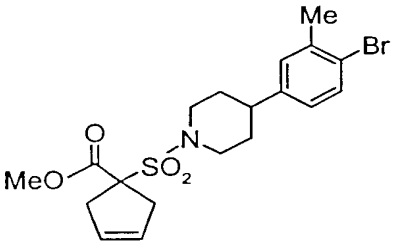
The title compound was obtained as a white solid (92%) from the methyl ester of preparation 102, following a similar procedure to that described in preparation 104.

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.47 (s, 6H), 1.60 (m, 2H), 1.80 (m, 2H), 2.45 (s, 3H), 2.70 (m, 1H), 3.03 (m, 2H), 3.78 (m, 2H), 7.18 (d, 1H), 7.21 (s, 1H), 7.63 (d, 1H), 7.78 (s, 1H), 7.92 (s, 1H), 13.37 (br, s, 1H).

Anal. Found: C, 55.28; H, 5.90; N, 6.70. $C_{19}H_{24}N_2O_4S_2$ requires C, 55.86; H, 5.92; N, 6.86%.

Preparation 106

Methyl 1-{[4-(4-bromo-3-methylphenyl)piperidin-1-yl]sulfonyl}-3-cyclopentene-1-carboxylate



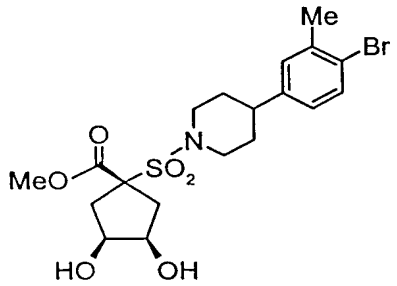
A suspension of sodium hydride (1.1g, 60% dispersion in mineral oil, 28mmol) was cooled to 0°C in anhydrous N-methyl pyrrolidinone (30ml) under nitrogen. A solution of the ester from preparation 25 (10g, 26mmol) in N-methyl pyrrolidinone (70ml) was added dropwise with stirring and the reaction mixture allowed to warm to ambient temperature over 50 minutes. 1,4-dichlorobut-2-ene (3.0ml, 28mmol) and tetrabutylammonium bromide (8.3g, 26mmol) were added to the reaction mixture and after a further 3 hours an additional portion of sodium hydride (1.1g, 60% dispersion in mineral oil, 28mmol) was added. The mixture was stirred for a further 2 days. The reaction mixture was partitioned between ethyl acetate (300ml) and water (300ml) and the layers separated. The aqueous layer was extracted with ethyl acetate (300ml) and the combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash chromatography eluting with dichloromethane to give the title compound as a white solid (7.4g, 65%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.45 (m, 2H), 1.75 (m, 2H), 2.28 (s, 3H), 2.64 (m, 1H), 2.95 (m, 4H), 3.14 (d, 2H), 3.75 (s, 3H), 3.78 (s, 2H), 5.63 (s, 2H), 6.98 (d, 1H), 7.21 (s, 1H), 7.43 (d, 1H).

LRMS :m/z 464/466 $(M+23)^+$.

Preparation 107

Methyl (1α,3α,4α)-1-{[4-(4-bromo-3-methylphenyl)piperidin-1-yl]sulfonyl}-3,4-dihydroxycyclopentanecarboxylate



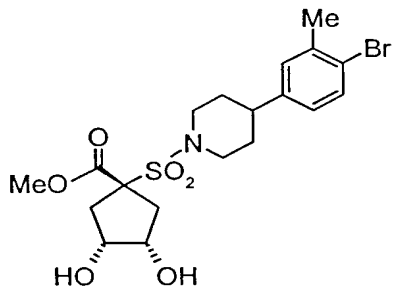
N-methylmorpholine N-oxide (580mg, 4.97mmol) and osmium tetroxide (2.5 weight % in tert-butanol, 1.1ml, 0.136mmol) were added to a solution of the cyclopentene from preparation 106 (2.0g, 4.52mmol) in dioxan (20ml), water (0.1ml), and the solution stirred at room temperature for 18 hours. The reaction mixture was partitioned between ethyl acetate (200ml) and water (300ml) and the layers separated. The aqueous layer was extracted with ethyl acetate (2x200ml), and the combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel using dichloromethane/methanol (100:0 to 97:3) as eluant to afford the title compound as a white solid (1.2g, 56%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.47 (m, 2H), 1.77 (m, 2H), 2.28 (m, 5H), 2.42 (s, 2H), 2.63 (m, 1H), 2.91 (m, 2H), 3.75 (m, 5H), 3.85 (s, 2H), 4.62 (s, 2H), 6.98 (d, 1H), 7.21 (s, 1H), 7.43 (d, 1H).

LRMS :m/z 498/500 $(M+23)^+$.

Preparation 108

Methyl (1α,3β,4β)-1-{[4-(4-bromo-3-methylphenyl)piperidin-1-yl]sulfonyl}-3,4-dihydroxycyclopentanecarboxylate



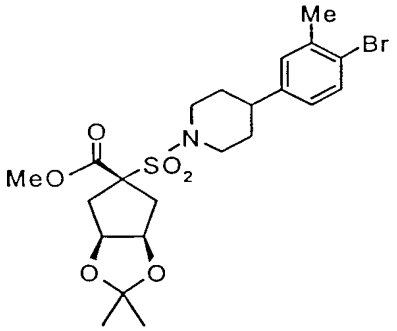
Silver acetate (2.1g, 12.46mmol) and iodine (1.5g, 5.81mmol) were added to a solution of the cyclopentene from preparation 106 (2.45g, 5.54mmol) in glacial acetic acid (125ml) and the mixture was stirred at ambient temperature for 1 hour. Wet acetic acid (2.5ml of a 1:25 water/glacial acetic acid mixture) was then added and the reaction was heated to 95°C for 3 hours and then stirred at ambient temperature for 18 hours. Sodium chloride was added to the mixture and the resulting precipitate was filtered through arbocel® and then washed with toluene. The resulting filtrate was concentrated in vacuo, azeotroped with toluene to give a solid which was triturated with diisopropyl ether. This solid was further purified by flash chromatography eluting with dichloromethane to give the intermediate monoacetate compound as a beige solid (1.35g, 50%). 1N sodium hydroxide (4ml) was added to a solution of the monoacetate intermediate in dioxan/methanol (12ml/8ml) and the reaction was stirred at ambient temperature for 1 hour. The solvent was removed under reduced pressure, and the residue was partitioned between ethyl acetate (50ml) and water (75ml), and the layers separated. The aqueous layer was extracted with ethyl acetate (2x50ml), and the combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo to give the title compound as a white solid (875mg, 70%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.55 (m, 2H), 1.87 (m, 2H), 2.18 (m, 2H), 2.30 (s, 3H), 2.63 (m, 3H), 2.98 (t, 2H), 3.72 (m, 7H), 4.68 (s, 2H), 6.98 (d, 1H), 7.22 (s, 1H), 7.43 (d, 1H).

LRMS :m/z 498/500$(M+23)^+$.

Preparation 109

Methyl (3aα,5α,6aα)-5-{[4-(4-bromo-3-methylphenyl)piperidin-1-yl]sulfonyl}-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxylate



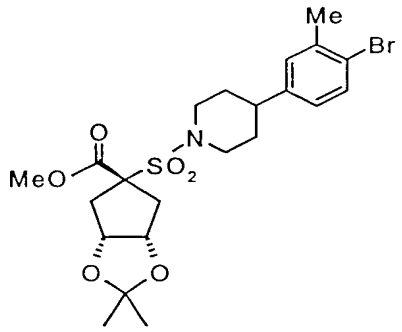
2,2-Dimethoxypropane (0.74ml, 6mmol) and p-toluenesulfonic acid (60mg, 0.3mmol) were added to a solution of the diol from preparation 107 (1.43g, 3mmol) in anhydrous dimethylformamide (10ml) under nitrogen. The reaction was warmed to 50°C for 4.5hours. The mixture was diluted with ethyl acetate (50ml) and water (40ml) and the layers separated. The aqueous layer was extracted with ethyl acetate (2x50ml), and the combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. The resulting solid was recrystalised from ethyl acetate/di-isopropyl ether to give the title compound as a white solid (1.05g, 70%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.17 (s, 3H), 1.20 (s, 3H), 1.47 (m, 2H), 1.77 (m, 2H), 2.23 (m, 2H), 2.32 (s, 3H), 2.65 (m, 3H), 2.95 (t, 2H), 3.72 (m, 5H), 4.64 (s, 2H), 6.98 (d, 1H), 7.21 (s, 1H), 7.43 (d, 1H).

LRMS :m/z 538/540 $(M+23)^+$.

Preparation 110

Methyl (3aβ,5α,6aβ)-5-{[4-(4-bromo-3-methylphenyl)piperidin-1-yl]sulfonyl}-2,2-dimethyltetrahydro-3aH-cyclopenta[d][1,3]dioxole-5-carboxylate



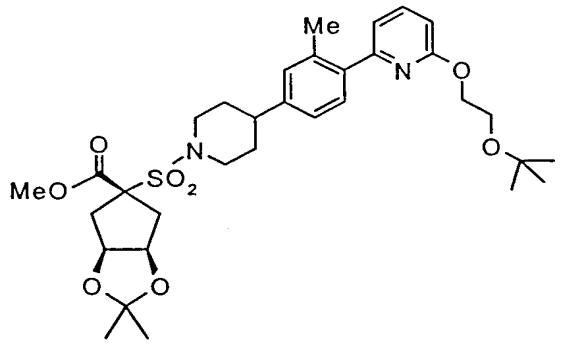
The title compound was prepared from the diol from preparation 108 in a similar procedure to that described in preparation 109. The title compound was isolated as a pale yellow solid (1.3g, 75%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.11 (s, 3H), 1.42 (s, 3H), 1.57 (m, 2H), 1.78 (m, 2H), 2.18 (m, 2H), 2.30 (s, 3H), 2.62 (m, 1H), 2.78 (m, 2H), 2.98 (t, 2H), 3.72 (m, 5H), 4.58 (m, 2H), 6.98 (d, 1H), 7.22 (s, 1H), 7.43 (d, 1H).

LRMS :m/z 538/540(M+23)$^+$.

Preparation 111

Methyl (3aα,5α,6aα)-5-{[4-(4-{6-[2-(*tert*-butoxy)ethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulfonyl}-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxylate



A mixture of the stannane from preparation 127 (2.3g, 4.78mmol) and the aryl bromide from preparation 109 (1.9g, 3.68mmol), and tetrakis(triphenylphosphine)palladium (0) (213mg, 0.18mmol) in toluene (25ml) was refluxed under nitrogen for 10 hours, then stirred at ambient temperature for 7 hours. The mixture was evaporated in vacuo and to the resulting oil was added ethyl acetate (30ml) and aqueous potassium fluoride solution (20ml) and stirred rapidly for 10 minutes. The resulting precipitate was filtered off on arbocel® washing with ethyl acetate. The filtrate was allowed to separate, and the aqueous layer extracted with ethyl acetate (30ml). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel using

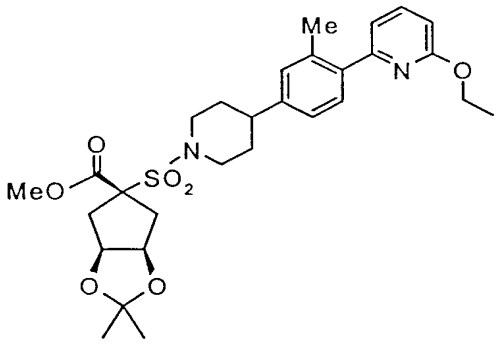
pentane:ethyl acetate (98:2 to 60:40) as eluant. The resulting solid was recrystalised from ethyl acetate to afford the title compound as a white solid, (1.4g, 60%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.13 (s, 9H), 1.17 (s, 3H), 1.20 (s, 3H), 1.57 (m, 2H), 1.80 (m, 2H), 2.23 (m, 2H), 2.32 (s, 3H), 2.69 (m, 3H), 2.95 (t, 2H), 3.60 (m, 2H), 3.72 (m, 5H), 4.29 (m, 2H), 4.68 (s, 2H), 6.73 (d, 1H), 7.03 (d, 1H) 7.15 (m, 2H), 7.31 (d, 1H), 7.75 (t, 1H).

LRMS :m/z 654 $(M+23)^+$.

Preparation 112

Methyl (3aα,5α,6aα)-5-({4-[4-(6-ethoxypyridin-2-yl)-3-methylphenyl]piperidin-1-yl}sulfonyl)-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxylate



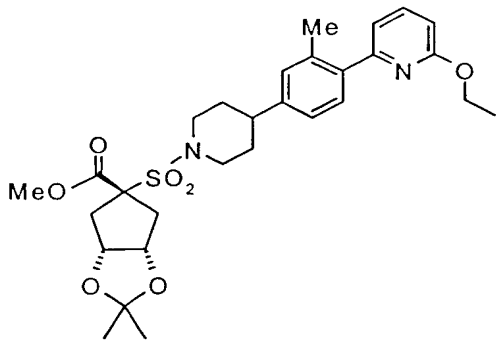
The title compound was prepared from the aryl bromide from preparation 109 and the stannane from preparation 129 in a similar procedure to that described in preparation 111. The title compound was isolated as a white solid (1.1g, 50%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.15 (s, 3H), 1.19 (s, 3H), 1.25 (t, 3H), 1.57 (m, 2H), 1.80 (m, 2H), 2.23 (m, 2H), 2.35 (s, 3H), 2.65 (m, 3H), 2.95 (t, 2H), 3.65 (m, 2H), 3.72 (m, 3H), 4.28 (q, 2H), 4.66 (d, 2H), 6.68 (d, 1H), 7.03 (d, 1H), 7.15 (m, 2H), 7.33 (d, 1H), 7.72 (t, 1H).

LRMS :m/z 581 $(M+23)^+$.

Preparation 113

Methyl (3aβ,5α,6aβ)-5-({4-[4-(6-ethoxypyridin-2-yl)-3-methylphenyl]piperidin-1-yl}sulfonyl)-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxylate



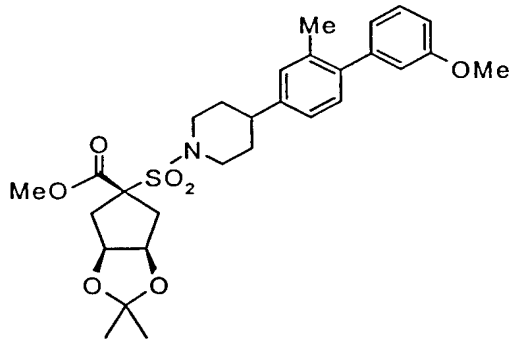
The title compound was prepared from the aryl bromide from preparation 110 and the stannane from preparation 129 in a similar procedure to that described in preparation 111. The title compound was isolated as a white foam (413mg, 60%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.21 (s, 3H), 1.28 (t, 3H), 1.42 (s, 3H), 1.57 (m, 2H), 1.80 (m, 2H), 2.18 (m, 2H), 2.35 (s, 3H), 2.65 (m, 1H), 2.80 (m, 2H), 3.00 (t, 2H), 3.75 (m, 2H), 3.77 (s, 3H), 4.28 (q, 2H), 4.56 (m, 2H), 6.68 (d, 1H), 7.03 (d, 1H), 7.15 (m, 2H), 7.35 (d, 1H), 7.72 (t, 1H).

LRMS :m/z 559 $(M+1)^+$.

Preparation 114

Methyl (3aα,5α,6aα)-5-{4-[4-(3-methoxyphenyl)-3-methylphenyl]piperidin-1-ylsulfonyl}-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxylate



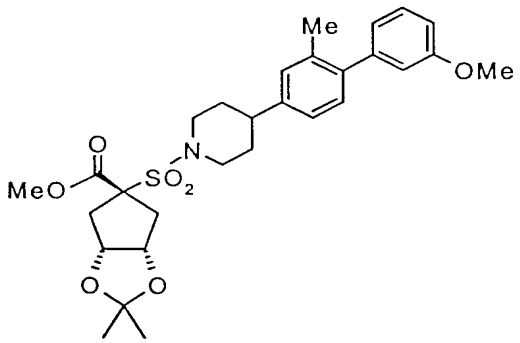
A mixture of the aryl bromide from preparation 109 (1.03, 1.99mmol), 3-methoxyphenylboronic acid (364mg, 2.40mmol), cesium fluoride (606mg, 4.00mmol), tris(dibenzylideneacetone)dipalladium(0) (91mg, 0.1mmol) and tri(o-tolyl)phosphine (61mg, 0.2mmol) in 1,2-dimethoxyethane (25ml) was heated under reflux under nitrogen for 9 hours. The cooled reaction was diluted with water and ethyl acetate, filtered through arbocel®, which was washed with water and ethyl acetate. The organic layer was separated, and washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel using pentane:ethyl acetate (95:5 to 60:40) as eluant. The title compound was obtained as a white solid (630mg, 60%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.15 (s, 3H), 1.18 (s, 3H), 1.57 (m, 2H), 1.79 (m, 2H), 2.18 (m, 5H), 2.65 (m, 3H), 2.95 (t, 2H), 3.65 (m, 8H), 4.64 (m, 2H), 6.82 (m, 3H), 7.10 (m, 3H), 7.29 (m, 1H).

LRMS :m/z 566 $(M+23)^+$.

Preparation 115

Methyl (3aβ,5α,6aβ)-5-{4-[4-(3-methoxyphenyl)-3-methylphenyl]piperidin-1-ylsulfonyl}-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxylate



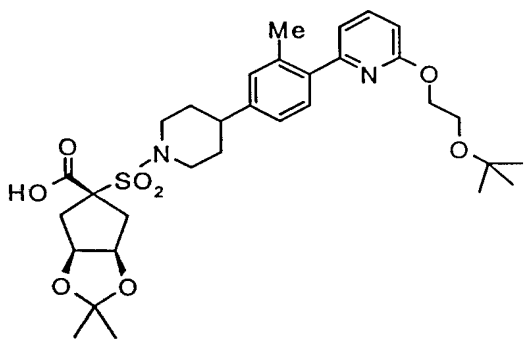
The title compound was prepared from the aryl bromide from preparation 110 in a similar procedure to that described in preparation 114 and was isolated as a white foam (310mg, 45%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.20 (s, 3H), 1.40 (s, 3H), 1.57 (m, 2H), 1.80 (m, 2H), 2.18 (m, 5H), 2.67 (m, 1H), 2.81 (m, 2H), 2.95 (t, 2H), 3.75 (m, 8H), 4.57 (m, 2H), 6.82 (m, 3H), 7.10 (m, 3H), 7.29 (m, 1H).

LRMS :m/z 566 $(M+23)^+$.

Preparation 116

(3aα,5α,6aα)-5-{[4-(4-{6-[2-(*tert*-butoxy)ethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulfonyl}-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxylic acid



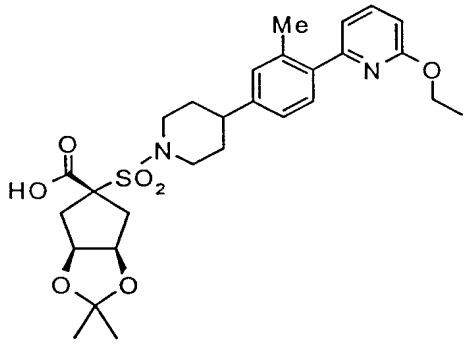
A mixture of the methyl ester from preparation 111 (1.4g, 2.22mmol) and aqueous sodium hydroxide (5.5ml, 2N, 11.1mmol) in methanol (7ml) and dioxan (7ml) was heated under reflux for 1hour, then allowed to cool. The reaction was concentrated in vacuo, the residue dissolved in water (20ml), and the solution acidified to pH 4 with glacial acetic acid. The aqueous was extracted with ethyl acetate (2x 50ml) and the collected organic layers dried (Na_2SO_4), filtered and concentrated in vacuo. The resulting oily solid was azeotroped with toluene then triturated with cold ethyl acetate to afford the title compound as a white solid (1.0g, 75%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.13 (s, 9H), 1.16 (s, 3H), 1.28 (s, 3H), 1.57 (m, 2H), 1.75 (m, 2H), 2.26 (m, 5H), 2.59 (m, 3H), 3.05 (t, 2H), 3.60 (m, 2H), 3.72 (d, 2H), 4.28 (m, 2H), 4.58 (m, 2H), 6.73 (d, 1H), 7.03 (d, 1H), 7.15 (m, 2H), 7.31 (d, 1H), 7.75 (t, 1H) 12.9 (s, 1H).

LRMS :m/z 617 $(M+1)^+$.

Preparation 117

(3aα,5α,6aα)-5-({4-[4-(6-ethoxypyridin-2-yl)-3-methylphenyl]piperidin-1-yl}sulfonyl)-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxylic acid



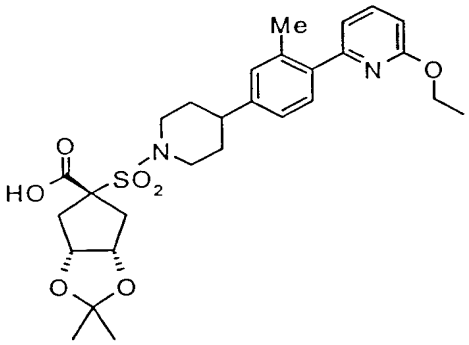
A mixture of the methyl ester from preparation 112 (780mg, 1.40mmol) and aqueous sodium hydroxide (3.5ml, 2N, 6.98mmol) were dissolved in methanol (5ml) and dioxan (5ml) and were heated under reflux for 1.5 hour, then allowed to cool. The reaction was concentrated in vacuo, the residue dissolved in water (20ml), and the solution acidified to pH 4 with glacial acetic acid. The resulting mixture was extracted with ethyl acetate (2x 50ml) and the collected organic layers dried (Na_2SO_4), filtered and concentrated in vacuo. This afforded the title compound as a white solid (240mg, 85%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 0.93 (s, 3H), 1.14 (m, 6H), 1.41 (m, 2H), 1.58 (m, 2H), 2.01 (m, 2H), 2.13 (s, 3H), 2.43 (m, 3H), 2.78 (m, 2H), 3.50 (m, 2H), 4.08 (m, 2H), 4.43 (m, 2H), 6.48 (m, 1H), 6.80 (d, 1H), 6.91 (m, 2H), 7.10 (m, 1H), 7.51 (m, 1H) 13.10 (s, 1H).

LRMS :m/z 545 $(M+1)^+$.

Preparation 118

(3aβ,5α,6aβ)-5-({4-[4-(6-ethoxypyridin-2-yl)-3-methylphenyl]piperidin-1-yl}sulfonyl)-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxylic acid



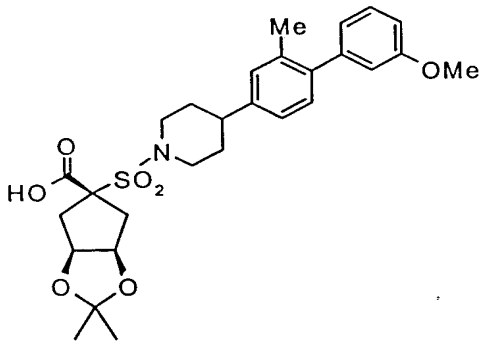
The title compound was prepared from the methyl ester from preparation 113 in a similar procedure to that described in preparation 117 and was isolated as a white foam (250mg, 65%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.21 (s, 3H), 1.28 (t, 3H), 1.42 (s, 3H), 1.61 (m, 2H), 1.80 (d, 2H), 2.18 (m, 2H), 2.35 (s, 3H), 2.65 (m, 1H), 2.80 (m, 2H), 3.00 (t, 2H), 3.78 (d, 2H), 4.28 (q, 2H), 4.56 (m, 2H), 6.68 (d, 1H), 7.01 (d, 1H), 7.15 (m, 2H), 7.35 (d, 1H), 7.72 (t, 1H), 13.65 (s, 1H).

LRMS :m/z 545 $(M+1)^+$.

Preparation 119

(3aα,5α,6aα)-5-{4-[4-(3-methoxyphenyl)-3-methylphenyl]piperidin-1-ylsulfonyl}-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxylic acid



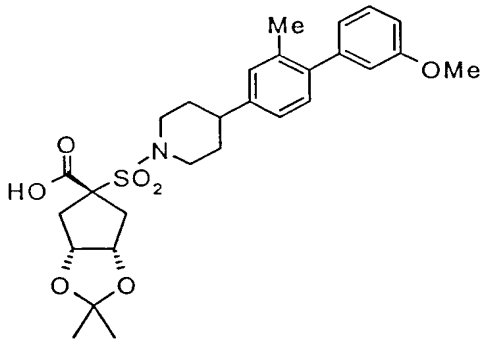
A mixture of the methyl ester from preparation 114 (630mg, 1.16mmol) and aqueous sodium hydroxide (3.0ml, 2N, 5.80mmol) were dissolved in methanol (5ml) and dioxan (5ml) and heated under reflux for 1hour, then allowed to cool. The reaction was concentrated in vacuo, the residue dissolved in water (20ml), and the solution acidified to pH 1 with 2N hydrochloric acid. The resulting mixture was extracted with ethyl acetate (2x 50ml) and the collected organic layers dried (Na_2SO_4), filtered and concentrated in vacuo. This afforded the title compound as a white solid (500mg, 83%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.13 (s, 3H), 1.22 (s, 3H), 1.58 (m, 2H), 1.79 (m, 2H), 2.18 (m, 5H), 2.62 (m, 3H), 2.97 (t, 2H), 3.71 (m, 5H), 4.64 (m, 2H), 6.82 (m, 3H), 7.06 (m, 2H), 7.14 (s, 1H), 7.29 (t, 1H).

LRMS :m/z 528 $(M-1)^-$.

Preparation 120

(3aβ,5α,6aβ)-5-{4-[4-(3-methoxyphenyl)-3-methylphenyl]piperidin-1-ylsulfonyl}-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxylic acid



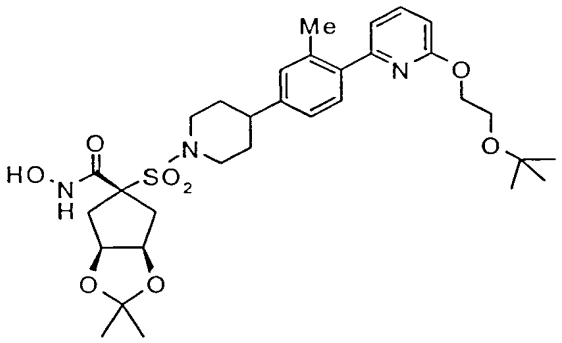
The title compound was prepared from the methyl ester from preparation 115 in a similar procedure to that described in preparation 119 and was isolated as a white foam (250mg, 85%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.20 (s, 3H), 1.40 (s, 3H), 1.58 (m, 2H), 1.80 (m, 2H), 2.15 (m, 2H), 2.18 (s, 3H), 2.65 (m, 1H), 2.78 (m, 2H), 2.99 (t, 2H), 3.77 (m, 5H), 4.56 (m, 2H), 6.82 (m, 3H), 7.10 (m, 3H), 7.29 (t, 1H), 13.78 (s, 1H).

LRMS :m/z 528 $(M-1)^-$.

Preparation 121

(3aα,5α,6aα)-*N*-hydroxy-5-{[4-(4-{6-[2-(*tert*-butoxy)ethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulfonyl}-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxamide



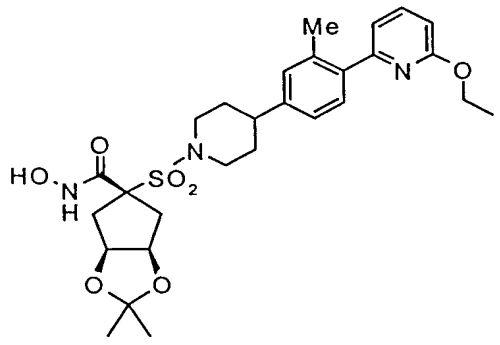
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (190mg, 0.973mmol) and 1-hydroxy-7-azabenzotriazole (121mg, 0.892mmol) were added to a solution of the acid from preparation 116 (500mg, 0.811mmol) in N,N-dimethylformamide (6ml) and pyridine (3ml) and the reaction was stirred under nitrogen for 50 minutes. Hydroxylamine hydrochloride (170mg, 2.43mmol) was then added, and the reaction stirred at room temperature overnight. The reaction was diluted with ethyl acetate (50ml) and washed with pH 7 phosphate buffer solution (30ml). The aqueous layer was extracted with ethyl acetate (2x 50ml) and the combined organic extracts were washed with brine, then water, dried (Na_2SO_4), filtered and concentrated in vacuo. The resulting solid was recrystallised from ethyl acetate to afford the title compound as a white solid (260mg, 50%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.15 (s, 9H), 1.16 (s, 3H), 1.20 (s, 3H), 1.59 (m, 2H), 1.75 (m, 2H), 2.17 (m, 2H), 2.31 (s, 3H), 2.59 (m, 1H), 2.66 (d, 2H), 2.99 (t, 2H), 3.59 (m, 2H), 3.64 (d, 2H), 4.28 (m, 2H), 4.62 (m, 2H), 6.72 (d, 1H), 7.03 (d, 1H), 7.15 (m, 2H), 7.29 (d, 1H), 7.70 (t, 1H), 8.85 (s, 1H), 10.82 (s, 1H).

LRMS :m/z 632 $(M+1)^+$.

Preparation 122

(3aα,5α,6aα)-*N*-hydroxy-5-({4-[4-(6-ethoxypyridin-2-yl)-3-methylphenyl]piperidin-1-yl}sulfonyl)-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxamide



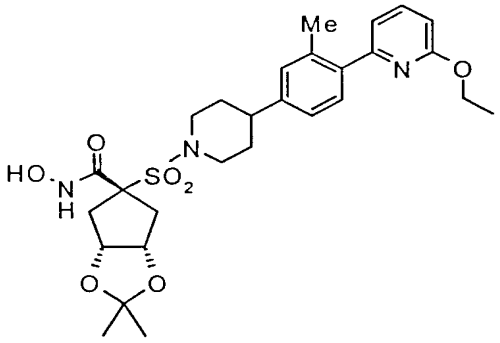
The title compound was prepared from the acid from preparation 117 in a similar procedure to that described in preparation 121, and was isolated as a white solid (150mg, 60%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.13 (s, 3H), 1.21 (s, 3H), 1.25 (t, 3H), 1.61 (m, 2H), 1.76 (m, 2H), 2.18 (m, 2H), 2.32 (s, 3H), 2.60 (m, 1H), 2.77 (d, 2H), 2.99 (t, 2H), 3.63 (d, 2H), 4.25 (q, 2H), 4.63 (m, 2H), 6.68 (d, 1H), 7.02 (d, 1H), 7.14 (m, 2H), 7.30 (d, 1H), 7.71 (t, 1H), 8.86 (s, 1H), 10.82 (s, 1H).

LRMS :m/z 560 $(M+1)^+$.

Preparation 123

(3aβ,5α,6aβ)-*N*-hydroxy-5-({4-[4-(6-ethoxy-pyridin-2-yl)-3-methylphenyl]piperidin-1-yl}sulfonyl)-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxamide



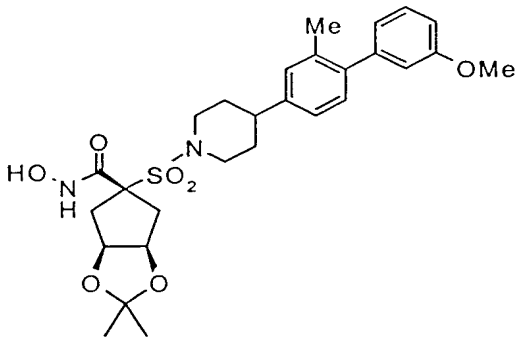
The title compound was prepared from the acid from preparation 118 in a similar procedure to that described in preparation 121. The title compound was isolated after column chromatography (using dichloromethane/methanol 99:1 as eluant) as a white solid (107mg, 45%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.20 (s, 3H), 1.28 (t, 3H), 1.40 (s, 3H), 1.61 (m, 2H), 1.80 (d, 2H), 2.05 (m, 2H), 2.30 (s, 3H), 2.62 (m, 1H), 2.97 (m, 4H), 3.70 (d, 2H), 4.28 (q, 2H), 4.45 (m, 2H), 6.68 (d, 1H), 7.01 (d, 1H), 7.15 (m, 2H), 7.32 (d, 1H), 7.72 (t, 1H), 9.00 (s, 1H), 10.39 (s, 1H).

LRMS :m/z 560 $(M+1)^+$.

Preparation 124

(3aα,5α,6aα)-*N*-hydroxy-5-{4-[4-(3-methoxyphenyl)-3-methylphenyl]piperidin-1-ylsulfonyl}-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxamide

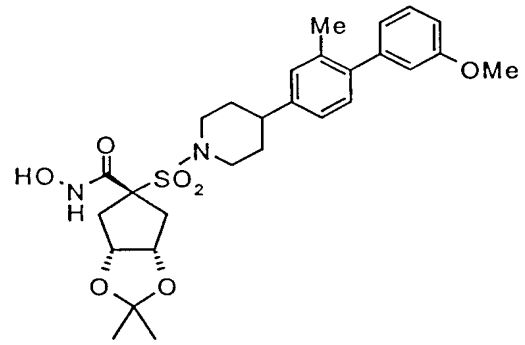


The title compound was prepared from the acid from preparation 119 in a similar procedure to that described in preparation 121, and was isolated as a white solid (110mg, 43%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.13 (s, 3H), 1.22 (s, 3H), 1.58 (m, 2H), 1.77 (m, 2H), 2.18 (m, 5H), 2.58 (m, 1H), 2.75 (d, 2H), 2.98 (t, 2H), 3.65 (d, 2H), 3.75 (s, 3H), 4.63 (m, 2H), 6.82 (m, 3H), 7.08 (s, 2H), 7.15 (s, 1H), 7.28 (t, 1H), 8.85 (s, 1H), 10.82 (s, 1H).

Preparation 125

(3aβ,5α,6aβ)-*N*-hydroxy-5-{4-[4-(3-methoxyphenyl)-3-methylphenyl]piperidin-1-ylsulfonyl}-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxamide



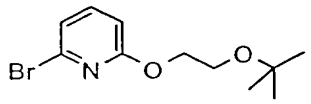
The title compound was prepared from the acid from preparation 120 in a similar procedure to that described in preparation 121. The title compound was isolated after column chromatography (using dichloromethane/methanol 98:2 as eluant) as a white solid (130mg, 50%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.20 (s, 3H), 1.40 (s, 3H), 1.58 (m, 2H), 1.78 (m, 2H), 2.05 (m, 2H), 2.18 (s, 3H), 2.60 (m, 1H), 2.95 (m, 4H), 3.67 (m, 2H), 3.74 (s, 3H), 4.42 (m, 2H), 6.82 (m, 3H), 7.08 (s, 2H), 7.13 (s, 1H), 7.29 (t, 1H), 9.09 (s, 1H), 10.49 (s, 1H).

LRMS :m/z 543 $(M-1)^-$.

Preparation 126

2-[2-(*tert*-butoxy)ethoxy]-6-bromopyridine



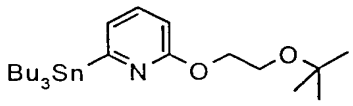
Sodium hydride (6.8g, 60% dispersion in mineral oil, 0.169mol) was added portionwise to an ice-cold solution of 2-(tert-butoxy)ethanol (20.0g, 0.169mol) in toluene (500ml) under nitrogen, and the solution stirred for 30 minutes whilst warming to ambient temperature. 2,6-Dibromopyridine (40.0, 0.169mol) was added, and the reaction heated under reflux for 3 hours. The mixture was allowed to cool to ambient temperature and was diluted with water (1000ml), and extracted with ethyl acetate (2x400ml). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated in vacuo to give the title compound as a yellow oil (quantitative).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.21 (s, 9H), 3.67 (t, 2H), 4.40 (t, 2H), 6.68 (d, 1H), 7.05 (d, 1H), 7.38 (t, 1H).

LRMS :m/z 296/298 $(M+23)^+$.

Preparation 127

2-[2-(*tert*-butoxy)ethoxy]-6-(tributylstannyl)pyridine



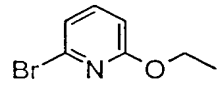
n-Butyllithium (71ml, 2.5M solution in hexanes, 0.177mol) was added dropwise to a cooled (-78°C) solution of the bromide from preparation 126 (46.3g, 0.169mol) in anhydrous THF (1000ml) under nitrogen, so as to maintain the internal temperature <-70°C, and the solution stirred for 10 minutes. Tri-n-butyltin chloride (48ml, 0.177mol) was added slowly to maintain the internal temperature <-70°C, and the reaction was then allowed to warm to room temperature over 1 hour. The reaction was diluted with water (1000ml), the mixture extracted with Et_2O (2x1000ml), and the combined organic extracts dried (Na_2SO_4), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel using pentane:Et_2O (100:1 to 98:2) as eluant, to afford the title compound as a colourless oil, (45.5g, 55%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 0.86 (t, 9H), 1.04 (m, 6H), 1.21 (s, 9H), 1.35 (m, 6H), 1.58 (m, 6H), 3.69 (t, 2H), 4.43 (t, 2H), 6.58 (d, 1H), 6.97 (m, 1H), 7.37 (m, 1H).

LRMS :m/z 506/508 $(M+23)^+$.

Preparation 128

2-bromo-6-ethoxypyridine



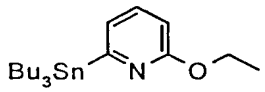
Sodium ethoxide (1.5g, 63mmol sodium, in ethanol (30ml)) was added to 2,6-dibromopyridine (15g, 63mmol) in toluene (150ml) at ambient temperature under nitrogen, and the reaction heated under reflux for 5 hours. The cooled mixture was diluted with water (100ml), and extracted with ethyl acetate (2x100ml). The combined organic extracts were dried (Na_2SO_4), filtered and evaporated in vacuo. The residue was purified by column chromatography on silica gel using pentane/ethyl acetate (100:0 to 95:5) as eluant to give the title compound as a yellow oil, (quantitative).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.37 (t, 3H), 4.35 (q, 2H), 6.62 (d, 1H), 7.01 (d, 1H), 7.38 (t, 1H).

LRMS :m/z 202/204 $(M+1)^+$.

Preparation 129

2-ethoxy-6-(tributylstannyl)pyridine



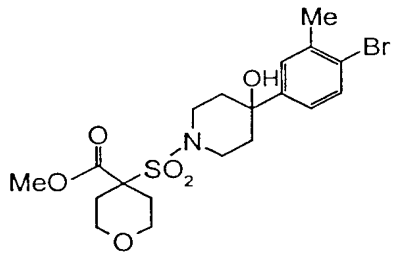
The title compound was prepared from the bromide from preparation 128 in a similar procedure to that described in preparation 127, and was isolated as a colourless oil (1.3g, 6%).

^{1}H nmr ($CDCl_3$, 400MHz) δ: 0.86 (t, 9H), 1.04 (m, 6H), 1.36 (m, 9H), 1.57 (m, 6H), 4.38 (q, 2H), 6.52 (d, 1H), 6.95 (m, 1H), 7.37 (m, 1H).

LRMS :m/z 434/436 $(M+23)^+$.

Preparation 130

Methyl 4-{[4-(4-bromo-3-methylphenyl)-4-hydroxy-1-piperidin-1-yl]sulfonyl}tetrahydro-2*H*-pyran-4-carboxylate

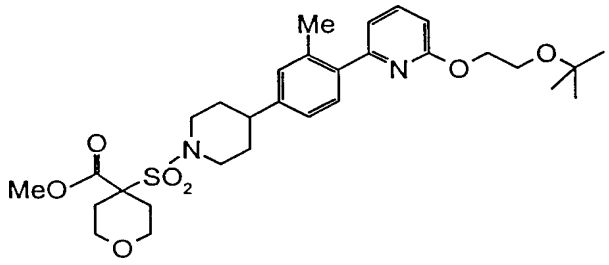


Iso-propylbromide (20ml, 0.21mol) was added dropwise over 1h to a stirred mixture of magnesium (4.7g, 0.19mol) in THF (50ml) and toluene (50ml), under nitrogen. The mixture was stirred at room temperature for 1 hour and then cooled to 0°C. A solution of 2-bromo-5-iodotoluene (57g, 0.19mol) in toluene (50ml) was added dropwise over 30 min, between 0 and 5°C, and the mixture was stirred at 0°C for 30min. The mixture was then added dropwise over 45 min to a stirred suspension the ketone from preparation 16 (50g, 0.16mol) in toluene (250ml), between 0 and 5°C, under nitrogen. The resulting mixture was stirred at 0°C for 1 hour and then citric acid solution (10%, 400ml) and ethyl acetate (200ml) were added. The organic phase was separated and the aqueous phase was re-extracted with ethyl acetate (2x200ml). The combined organic phases were washed with water (200ml) and concentrated *in vacuo* to a solid which was purified by re-crystallisation from toluene (500ml) to give the title compound as a colourless solid (66g, 84%).

^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.70-1.77 (m, 2H), 2.02-2.26 (m, 4H), 2.38-2.42 (m, 5H), 3.30 (t, 2H), 3.45 (t, 2H), 3.67-3.75 (m, 2H), 3.88 (s, 3H), 3.99 (dd, 2H), 7.14 (dd, 1H), 7.31 (d, 1H), 7.50 (d, 1H).

Preparation 131

Methyl 4-{[4-(4-{6-[2-(*tert*-butoxy)ethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulfonyl}tetrahydro-2*H*-pyran-4-carboxylate



A solution of n-butyllithium in hexanes (2.5M, 3.1ml, 7.7mmol) was added dropwise over 5 min to a solution of the bromopyridine from preparation 126 (2.0g, 7.3mmol) in THF (20ml) at –78°C, under

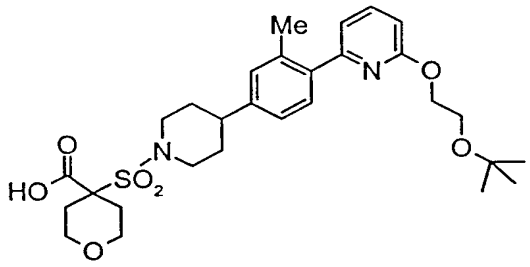
nitrogen. The mixture was stirred at –78°C for 10 min and then tri-*iso*-propylborate (1.9ml, 8.0mmol) was added dropwise over 10 min. The mixture was stirred at –78°C for 10 min and then allowed to warm to room temperature over 1 hour. The aryl bromide from preparation 27 (2.7g, 5.8mmol), palladium acetate (82mg, 0.36mmol), triphenylphosphine (191mg, 0.73mmol), ethanol (20ml) and aqueous sodium carbonate (2M, 20ml) were added and the mixture was heated to reflux for 4 hours, under nitrogen, and then cooled. Ethyl acetate (50ml) and demineralised water (50ml) were added and the organic phase was separated. The aqueous phase was re-extracted with ethyl acetate (2x30ml) and the combined organic phases were washed with demineralised water (50ml) and then concentrated *in vacuo* to a solid. Purification by re-crystallisation from methanol (30ml) gave the title compound as a colourless solid (2.0g, 60%).

^{1}H nmr (CD_3OD, 300MHz) δ: 1.12 (s, 9H), 1.50-1.69 (m, 2H), 1.72-1.88 (m, 2H), 1.91-2.05, (m, 2H), 2.24-2.30 (m, 2H), 2.34 (m, 3H), 2.65-2.78 (m, 1H), 3.00-3.23 (m, 4H), 3.61 (t, 2H), 3.70-3.78 (m, 2H), 3.80 (s, 3H), 3.87-3.95 (m, 2H), 4.30 (t, 2H), 6.74 (d, 1H), 7.05 (d, 1H), 7.10-7.17 (m, 2H), 7.33 (d, 1H), 7.73 (t, 1H).

LCMS :m/z 575 $(M+H)^+$

Preparation 132

4-{[4-(4-{6-[2-tert-butoxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulfonyl}-tetrahydro-2H-pyran-4-carboxylic acid



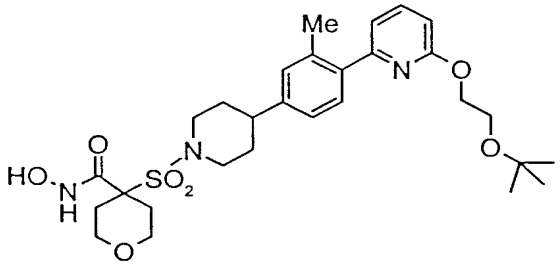
A mixture of the methyl ester from preparation 131 (9.1g, 16.0mmol) and aqueous sodium hydroxide (80ml, 1N, 80.0mmol) in dioxan (250ml) were heated under reflux for 2 hours. Methanol (100ml) and aqueous sodium hydroxide (40ml, 1N, 40.0mmol) were added and the mixture refluxed for a further 2 hours, then allowed to cool to ambient temperature. The reaction was concentrated in vacuo, the residue dissolved in water (200ml), and the solution acidified to pH 4 with glacial acetic acid. The aqueous layer was extracted with ethyl acetate (2x 200ml) and the combined organic extracts were washed with brine (200ml), then water (2x200ml), dried (Na_2SO_4), filtered and concentrated in vacuo. The resulting oily solid was azeotroped with toluene then triturated with cold di-isopropyl ether to afford the title compound as a pale yellow solid (7.66g, 85%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.13 (s, 9H), 1.61 (m, 2H), 1.79 (m, 2H), 1.95 (m, 2H), 2.22 (d, 2H), 2.32 (s, 3H), 2.66 (m, 1H), 3.05 (t, 2H), 3.20 (t, 2H), 3.60 (t, 2H), 3.76 (d, 2H), 3.88 (m, 2H), 4.28 (t, 2H), 6.73 (d, 1H), 7.03 (d, 1H), 7.12 (m, 2H), 7.31 (d, 1H), 7.75 (t, 1H), 13.77 (s, 1H).

LRMS :m/z 583 $(M+23)^+$.

Preparation 133

N-Hydroxy-4-[(4-{4-[6-(2-*tert*-butoxyethoxy)pyridin-2-yl]-3-methylphenyl}piperidin-1-yl)sulfonyl]tetrahydro-2*H*-pyran-4-carboxamide



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.15g, 16.0mmol) and 1-hydroxy-7-azabenzotriazole (2.05g, 15.0mmol) were added to a solution of the acid from preparation 132 (7.66g, 14mmol) in anhydrous dichloromethane (80ml) and pyridine (80ml) and the reaction was stirred under nitrogen for 1hour. Hydroxylamine hydrochloride (2.85g, 41.0mmol) was then added, and the reaction stirred at room temperature overnight. The reaction was diluted with dichloromethane (200ml) and washed with pH 7 phosphate buffer solution (200ml). The aqueous layer was extracted with dichloromethane (2x 200ml) and the combined organic extracts were washed with dilute aqueous acetic acid (150ml), brine (150ml), then water (150ml), dried (Na_2SO_4), filtered and concentrated in vacuo. The resulting solid was azeotroped with toluene and then recrystallised from ethyl acetate and di-isopropyl ether to afford the title compound as a white solid (6.3g, 75%).

^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.13 (s, 9H), 1.61 (m, 2H), 1.78 (m, 2H), 1.91 (m, 2H), 2.37 (m, 5H), 2.62 (m, 1H), 3.05 (t, 2H), 3.20 (t, 2H), 3.60 (t, 2H), 3.73 (d, 2H), 3.83 (m, 2H), 4.28 (t, 2H), 6.73 (d, 1H), 7.03 (d, 1H), 7.12 (m, 2H), 7.31 (d, 1H), 7.72 (t, 1H), 9.05 (s, 1H), 10.90 (s, 1H).

LRMS :m/z 598 $(M+23)^+$.

CLAIMS

1. N-Hydroxy 4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxamide and the pharmaceutically acceptable salts thereof, and solvates thereof.

2. A compound selected from :
N-hydroxy 2-[(4-{4-[6-(2-hydroxyethoxy)pyridin-2-yl]-3-methylphenyl}piperidin-1-yl)sulphonyl]-2-methylpropanamide;
N-hydroxy 2-{[4-(4-{6-[2-(methoxy)ethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}-2-methylpropanamide;
N-hydroxy 4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxamide;
N-hydroxy 4-{[4-(4-{6-[(2S)-2,3-dihydroxy-1-propoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxamide;
N-hydroxy 4-{[4-(4-{6-[(2R)-2,3-dihydroxy-1-propoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxamide;
N-hydroxy 4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}-piperidine-4-carboxamide dihydrochloride;
N-hydroxy 4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}-1-methyl-piperidine-4-carboxamide;
N-hydroxy 2-[4-(4-{3-[(2S)-2,3-dihydroxy-1-propoxy]phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-2-methylpropanamide;
N-hydroxy 4-{4-[4-(3-[(2R)-2,3-dihydroxy-1-propoxy]phenyl)-3-methylphenyl]-piperidin-1-ylsulphonyl}-tetrahydro-(2H)-pyran-4-carboxamide;
N-hydroxy 4-{4-[4-(3-{(2S)-2-hydroxy-2-hydroxymethyl}ethoxyphenyl)-3-methylphenyl]-piperidin-1-ylsulphonyl}-tetrahydro-2H-pyran-4-carboxamide;
N-hydroxy 4-{4-[4-(3-{1,3-dihydroxy-2-propoxyphenyl)-3-methylphenyl]-piperidin-1-ylsulphonyl}-tetrahydro-2H-pyran-4-carboxamide;
N-hydroxy 2-{[4-(4-{3-[2-(methylamino)ethoxy]phenyl}-3-methylphenyl)-piperidin-1-yl]sulphonyl}-2-methylpropanamide hydrochloride;
N-hydroxy 2-[4-(4-{3-(2-aminoethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-2-methylpropanamide hydrochloride;
N-hydroxy 4-{[4-(-4-{6-[2-aminoethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxamide hydrochloride;
N-hydroxy 2-[4-(4-{3-(2-N,N-dimethylaminoethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-2-methylpropanamide;
N-hydroxy 4-{[4-(4-{3-(methyl)aminomethyl}-3-methylphenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxamide hydrochloride;

N-hydroxy 4-{[4-(3-methyl-4-{3-[4-morpholinylmethyl]}phenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxamide;

N-hydroxy 2-({4-[4-(3-methoxy-1H-pyrazol-1-yl)-3-methylphenyl]piperidin-1-yl}sulphonyl)-2-methylpropanamide;

N-hydroxy 2-[(4-{4-[3-(2-hydroxyethoxy)-1H-pyrazol-1-yl]-3-methylphenyl}piperidin-1-yl)sulphonyl]-2-methylpropanamide;

N-hydroxy 2-methyl-2-({4-[3-methyl-4-(1,3-thiazol-2-yl)phenyl]piperidin-1-yl}sulphonyl)propanamide;

(1α,3α,4α)-*N*,3,4-trihydroxy-1-[(4-{4-[6-(2-hydroxyethoxy)pyridin-2-yl]-3-methylphenyl}piperidin-1-yl)sulfonyl]cyclopentanecarboxamide;

(1α,3α,4α)-1-({4-[4-(6-ethoxypyridin-2-yl)-3-methylphenyl]piperidin-1-yl}sulfonyl)-*N*,3,4-trihydroxycyclopentanecarboxamide;

(1α,3β,4β)-1-({4-[4-(6-ethoxypyridin-2-yl)-3-methylphenyl]piperidin-1-yl}sulfonyl)-*N*,3,4-trihydroxycyclopentanecarboxamide;

(1α,3α,4α)-*N*,3,4-trihydroxy-1-{4-[4-(3-methoxyphenyl)-3-methylphenyl]piperidin-1-ylsulfonyl}cyclopentanecarboxamide; and

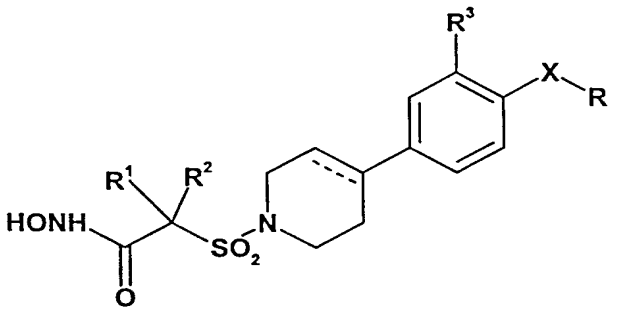
(1α,3β,4β)-*N*,3,4-trihydroxy-1-{4-[4-(3-methoxyphenyl)-3-methylphenyl]piperidin-1-ylsulfonyl}cyclopentanecarboxamide,

and the pharmaceutically acceptable salts thereof, and solvates thereof.

3. N-Hydroxy 4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}-piperidine-4-carboxamide and the pharmaceutically acceptable salts thereof, and solvates thereof.

4. N-Hydroxy 4-{[4-(-4-{6-[2-aminoethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxamide and the pharmaceutically acceptable salts thereof, and solvates thereof.

5. A compound of formula (I):



(I)

and pharmaceutically-acceptable salts thereof, and solvates thereof,

wherein

the dotted line represents an optional bond;

X is a monocyclic aromatic linker moiety selected from pyrazolylene, thiazolylene, pyrazinylene, pyridazinylene, pyrrolylene, oxazolylene, isoxazolylene, oxadiazolylene, thiadiazolylene, imidazolylene, triazolylene, or tetrazolylene;

R is H, C_{1-4} alkyl optionally substituted by C_{1-4} alkoxy or NR^4R^5 or OH, or

C_{1-4} alkoxy optionally substituted by 1 or 2 substituents selected from (C_{1-4} alkyl optionally substituted by OH), C_{1-4} alkoxy, OH and NR^4R^5;

R^1 and R^2 are each independently H, C_{1-6} alkyl optionally substituted by OH or C_{1-4} alkoxy, or C_{2-6} alkenyl;

or R^1 and R^2 are taken, together with the C atom to which they are attached, to form a 3- to 7-membered ring optionally incorporating a hetero- moiety selected from O, S, SO, SO_2 and NR^6, and which 3- to 7-membered ring is optionally substituted by one or more OH;

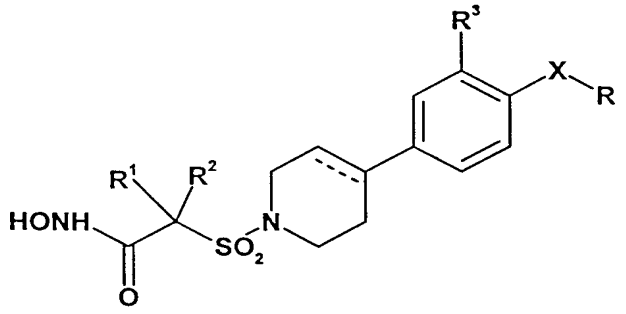
R^3 is H, halo, methyl, or methoxy;

R^4 and R^5 are each independently H or C_1 to C_6 alkyl optionally substituted by OH, C_1 to C_4 alkoxy or aryl,

or R^4 and R^5 can be taken together with the N atom to which they are attached , to form a 3- to 7-membered ring, optionally incorporating a further hetero- moiety selected from O, S, SO_2 and NR^7; and

R^6 and R^7 are each independently H or C_1 to C_4 alkyl.

6. A compound of formula (I):



(I)

and pharmaceutically-acceptable salts thereof, and solvates thereof,

wherein

the dotted line represents an optional bond;

X is a monocyclic aromatic linker moiety selected from phenylene, pyridinylene, pyrazolylene, thiazolylene, thienylene, furylene, pyrimidinylene, pyrazinylene, pyridazinylene, pyrrolylene, oxazolylene, isoxazolylene, oxadiazolylene, thiadiazolylene, imidazolylene, triazolylene, or tetrazolylene;

R is C_{1-4} alkyl substituted by NR^4R^5, C_{1-4} alkoxy substituted by NR^4R^5, or C_{1-4} alkoxy substituted by 2 substituents selected from (C_{1-4} alkyl optionally substituted by OH), C_{1-4} alkoxy, OH and NR^4R^5;

R^1 and R^2 are each independently H, C_{1-6} alkyl optionally substituted by OH or C_{1-4} alkoxy, or C_{2-6} alkenyl;

or R^1 and R^2 are taken together, with the C atom to which they are attached, to form a 3- to 7-membered ring optionally incorporating a hetero- moiety selected from O, S, SO, SO_2 and NR^6, and which 3- to 7-membered ring is optionally substituted by one or more OH;

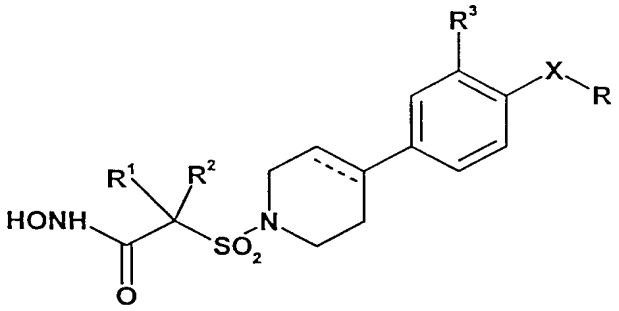
R^3 is H, halo, methyl, or methoxy;

R^4 and R^5 are each independently H or C_1 to C_6 alkyl optionally substituted by OH, C_1 to C_4 alkoxy or aryl,

or R^4 and R^5 can be taken together with the N atom to which they are attached , to form a 3- to 7-membered ring, optionally incorporating a further hetero- moiety selected from O, S, SO_2 and NR^7,

and R^6 and R^7 are each independently H or C_1 to C_4 alkyl.

7. A compound of formula (I):



(I)

and pharmaceutically-acceptable salts thereof, and solvates thereof,

wherein

the dotted line represents an optional bond,

X is a monocyclic aromatic linker moiety selected from phenylene, pyridinylene, pyrazolylene, thiazolylene, thienylene, furylene, pyrimidinylene, pyrazinylene, pyridazinylene, pyrrolylene, oxazolylene, isoxazolylene, oxadiazolylene, thiadiazolylene, imidazolylene, triazolylene, or tetrazolylene;

R is H, C_{1-4} alkyl optionally substituted by C_{1-4} alkoxy, NR^4R^5 or OH, or

C_{1-4} alkoxy optionally substituted by 1 or 2 substituents selected from (C_{1-4} alkyl optionally substituted by OH), C_{1-4} alkoxy, OH and NR^4R^5;

R^1 and R^2 are each independently C_{1-6} alkyl substituted by OH;

or R^1 and R^2 are taken together, with the C atom to which they are attached, to form a 3- to 7-membered ring optionally incorporating a hetero- moiety selected from O, S, SO, SO_2 and NR^6, and which 3- to 7-membered ring is substituted by one or more OH;

R^3 is H, halo, methyl, or methoxy;

R^4 and R^5 are each independently H or C_1 to C_6 alkyl optionally substituted by OH, C_1 to C_4 alkoxy or aryl,

or R^4 and R^5 can be taken together with the N atom to which they are attached , to form a 3- to 7-membered ring, optionally incorporating a further hetero- moiety selected from O, S, SO_2 and NR^7, and

R^6 and R^7 are each independently H or C_1 to C_4 alkyl.

8. A compound, salt or solvate according to claim 6 or claim 7 where X is phenylene, pyridinylene, pyrazolylene or thiazolylene.

9. A compound, salt or solvate according to claim 8 wherein X is 1,3-phenylene, 2,6-pyridinylene, 1,3-pyrazolylene or 2,5-thiazolylene.

10. A compound, salt or solvate according to claim 5 wherein X is pyrazolylene or thiazolylene.

11. A compound, salt or solvate according to claim 10 wherein X is 1,3-pyrazolylene or 2,5-thiazolylene.

12. A compound, salt or solvate according to claim 5 or claim 7 wherein R is H, methoxy, $O(CH_2)_2OH$, $O(CH_2)_2OCH_3$, $O(CH_2)_2N(CH_3)_2$, $O(CH_2)_2NHCH_3$, $O(CH_2)_2NH_2$, CH_2NHCH_3, morpholinomethyl, 2-morpholinoethoxy, 2R-2,3-dihydroxy-1-propyloxy, 2S-2,3-dihydroxy-1-propyloxy or 1,3-dihydroxy-2-propyloxy.

13. A compound, salt or solvate according to claim 12 wherein R is $O(CH_2)_2OH$ or $O(CH_2)_2NH_2$.

14. A compound, salt or solvate according to claim 6 wherein R is $O(CH_2)_2N(CH_3)_2$, $O(CH_2)_2NHCH_3$, $O(CH_2)_2NH_2$, CH_2NHCH_3, morpholinomethyl, 2-morpholinoethoxy, 2R-2,3-dihydroxy-1-propyloxy, 2S-2,3-dihydroxy-1-propyloxy or 1,3-dihydroxy-2-propyloxy.

15. A compound, salt or solvate according to claim 14 wherein R is $O(CH_2)_2NH_2$.

16. . A compound, salt or solvate according to claim 5 or claim 6 wherein R^1 and R^2 are each independently C_{1-6} alkyl optionally substituted by OH,
or R^1 and R^2 are taken together, with the C atom to which they are attached, to form a 3- to 7-membered ring optionally incorporating a hetero- moiety selected from O, S, SO, SO_2 and NR^6, and which 3- to 7-membered ring is optionally substituted by one or more OH.

17. A compound, salt or solvate according to claim 16 wherein R^1 and R^2 are each CH_3,
or R^1 and R^2 are taken together, with the C atom to which they are attached, to form a tetrahydropyran-4-ylidene, piperidin-4-ylidene, 1-methylpiperidin-4-ylidene, or 3,4-dihydroxycyclopentylidene moiety.

18. A compound, salt or solvate according to claim 17 wherein R^1 and R^2 are taken together, with the C atom to which they are attached, to form a tetrahydropyran-4-ylidene, *cis*-3,4-dihydroxycyclopentylidene, *trans*-3,4-dihydroxycyclopentylidene or piperidin-4-ylidene moiety.

19. A compound, salt or solvate according to claim 18 wherein R^1 and R^2 are taken together, with the C atom to which they are attached, to form a tetrahydropyran-4-ylidene, piperidin-4-ylidene, or *cis*-3,4-

dihydroxycyclopentylidene where the hydroxy substituents have a *cis*-relationship to the hydroxamate moiety.

20. A compound, salt or solvate according to claim 7 wherein R^1 and R^2 are taken together, with the C atom to which they are attached, to form a 3,4-dihydroxycyclopentylidene moiety.

21. A compound, salt or solvate according to claim 20 wherein R^1 and R^2 are taken together, with the C atom to which they are attached, to form a *cis*-3,4-dihydroxycyclopentylidene group where the hydroxy substituents have a *cis*-relationship to the hydroxamate moiety.

22. A compound, salt or solvate according to any one of claims 5 to 21 wherein R^3 is methyl and the optional double bond depicted as a dotted line in formula (I) is absent.

23. A pharmaceutical composition comprising a substance according to any one of claims 1 to 22 and a pharmaceutically acceptable diluent, adjuvant or carrier.

24. A substance according to any one of claims 1 to 22 for use as a medicament.

25. The use of a substance according to any one of claims 1 to 22 in the manufacture of a medicament for the treatment of a MMP-mediated disease, condition or process.

26. A method of treatment of a MMP-mediated disease, condition or process comprising administration of an effective amount of a substance according to any one of claim 1 to 22.

27. A compound selected from:
methyl 4-(4-oxo-piperidin-1-ylsulphonyl)tetrahydro-2H-pyran-4-carboxylate;
methyl 4-{[4-(4-bromo-3-methylphenyl)-4-hydroxy-1-piperidin-1-yl]sulfonyl}tetrahydro-2*H*-pyran-4-carboxylate;
methyl 4-{[4-(4-{6-[2-(*tert*-butoxy)ethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulfonyl}tetrahydro-2*H*-pyran-4-carboxylate;
4-{[4-(4-{6-[2-tert-butoxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulfonyl}-tetrahydro-2H-pyran-4-carboxylic acid; and
N-hydroxy-4-[(4-{4-[6-(2-*tert*-butoxyethoxy)pyridin-2-yl]-3-methylphenyl}piperidin-1-yl)sulfonyl]tetrahydro-2*H*-pyran-4-carboxamide.

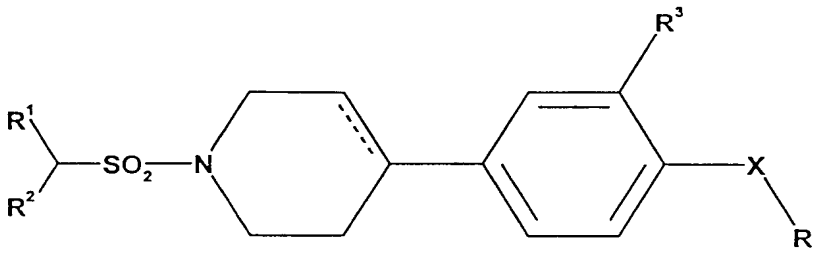
28. A compound selected from:
N-hydroxy 1-(tert-butoxycarbonyl)-4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}-piperidine-4-carboxamide;
1-(tert-butoxycarbonyl)- 4-[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-ylsulphonyl]-piperidine-4-carboxylic acid;

methyl 1-(tert-butoxycarbonyl)- 4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}-4-piperidinecarboxylate;
methyl 4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}-piperidine-4-carboxylate;
methyl 1-benzyl-4-{[4-(4-{6-[2-benzyloxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}-piperidin-4-carboxylate;
methyl 1-benzyl-4-[4-(4-bromo-3-methylphenyl)piperidin-1-ylsulphonyl]-4-piperidinecarboxylate; and
methyl 2-[4-(4-bromo-3-methylphenyl)piperidin-1-ylsulphonyl]acetate

29. A compound selected from:
N-hydroxy 4-[4-(4-{3-(2-[(N-tert-butoxycarbonyl)amino]ethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxamide;
Preparation 84;
methyl 4-[4-(4-{3-(2-[(tert-butoxycarbonyl)amino]ethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylate;
methyl 4-[4-(4-{3-(2-aminoethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylate;
Preparation 61;
methyl 4-[4-(4-{3-(2-oxoethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylate; and
methyl 4-[4-(4-{3-(2,2-diethoxyethoxy)phenyl}-3-methylphenyl)-piperidin-1-ylsulphonyl]-tetrahydro-2H-pyran-4-carboxylate.

30. A compound selected from:
4-[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-ylsulphonyl]tetrahydro-2H-pyran-4-carboxylic acid;
methyl 4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}tetrahydro-2H-pyran-4-carboxylate;
methyl 4-[4-(4-{6-[2-benzyloxy]ethoxypyridin-2-yl}-3-methylphenyl)-1,2,3,6-tetrahydropyridin-1-ylsulphonyl]tetrahydro-2H-pyran-4-carboxylate; and
methyl 4-[4-(4-bromo-3-methylphenyl)-1,2,3,6-tetrahydropyridin-1-ylsulphonyl]tetrahydro-2H-pyran-4-carboxylate.

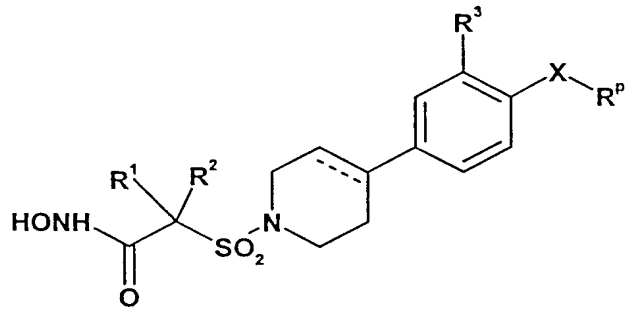
31. A compound of formula (VI):



(VI)

wherein the substituents R^1, R^2, R^3, X and R are as defined above in relation to any one of claims 5, 6 or 7.

32. A compound of formula (VII):

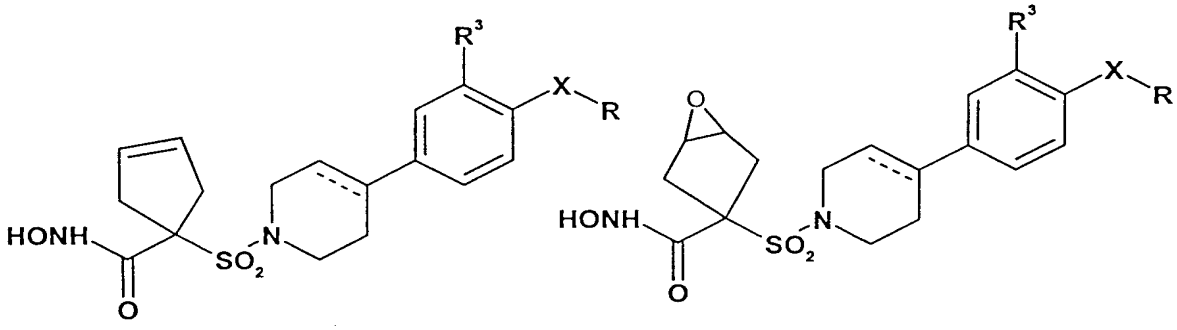


(VII)

wherein R^1, R^2, R^3 and X are as defined in any one of claims 5, 6 or 7, and where R^p is a NH- and/or OH-protected version of the corresponding compound of formula (I) as defined in any one of claims 5, 6 or 7, and where the corresponding compound of formula (I) as defined in any one of claims 5, 6 or 7 contains a free NH, NH_2 or OH group.

33. A process for making a compound of formula (I) as defined in any one of claims 5, 6 or 7 where R contains a free NH, NH_2 or OH group, which comprises deprotecting a corresponding compound of formula (VII) as defined in claim 32.

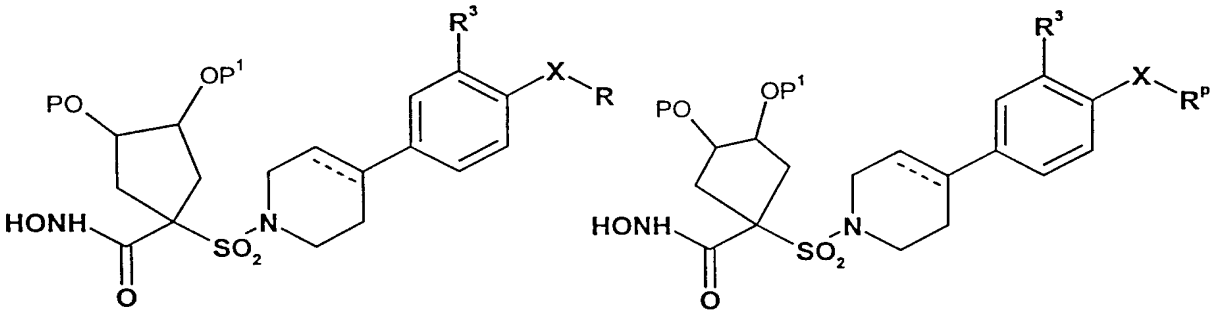
34. A compound of formula (VIII) or (IX):



(VIII) (IX)

where R^3, X and R are as defined in any one of claims 5, 6 or 7.

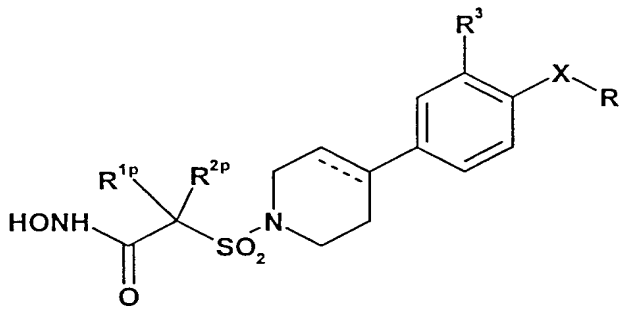
35. A compound of formula (X) or (XI) :



(X) (XI)

wherein R^3, X and R are as defined in any one of claims 5, 6 or 7, R^p is as defined in claim 32, and P and P^1 are OH-protecting groups which may be taken independently or together.

36. A compound of formula (XII):

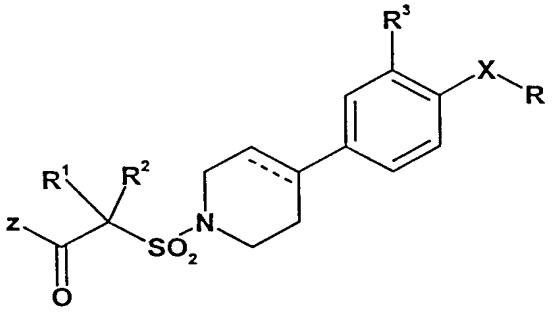


(XII)

wherein R^3, X and R are as defined in any one of claims 5, 6 or 7 and R^{1p} and R^{2p} is a N- and/or O-protected precursor which, on deprotection would give a corresponding compound of formula (I) as defined in the corresponding claim 5, 6 or 7.

37. A process for making a compound of formula (I) as defined in any one of claims 5, 6 or 7 where R^1 and/or R^2 contains a free NH, NH_2 or OH group, which comprises deprotecting a corresponding compound of formula (XII) as defined in claim 36.

38. A compound of formula (II) :

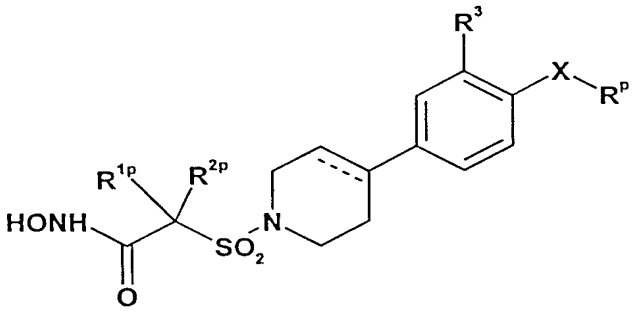


(II)

where R^1, R^2, R^3, X and R are as defined in any one of claims 5, 6 or 7, and where Z is a leaving group such as chloro, bromo, iodo, C_{1-3} alkyloxy or HO.

39. A process for making a compound of formula (I) as defined in any one of claims 5, 6 or 7, which comprises reaction of a compound of formula (II) as defined in claim 38 with hydroxylamine.

40. A compound of formula (XIII):



(XIII)

wherein R^3, X and R are as defined in any one of claims 5, 6 or 7 and R^{1p} , R^{2p} and R are independently a N- and/or O-protected precursor which, on deprotection would give a corresponding compound of formula (I) as defined in the corresponding claims 5, 6 or 7 where R^1, R^2 and R contain a free NH, NH_2 and/or OH group.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/IB 00/00667

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/451 A61K31/4523 C07D401/10 A61K31/453 C07D405/14
C07D401/14 C07D211/22 C07D405/12 C07D211/26 C07D417/10
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	EP 0 780 386 A (HOFFMANN LA ROCHE ;AGOURON PHARMA (US)) 25 June 1997 (1997-06-25) cited in the application abstract	
A	EP 0 606 046 A (CIBA GEIGY AG) 13 July 1994 (1994-07-13) cited in the application abstract	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 August 2000	16/08/2000

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

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(74) Agent: MAYER BROWN ROWE & MAW LLP; P.O.Box 2828, Chicago, Illinois 60609-2828 (GB).

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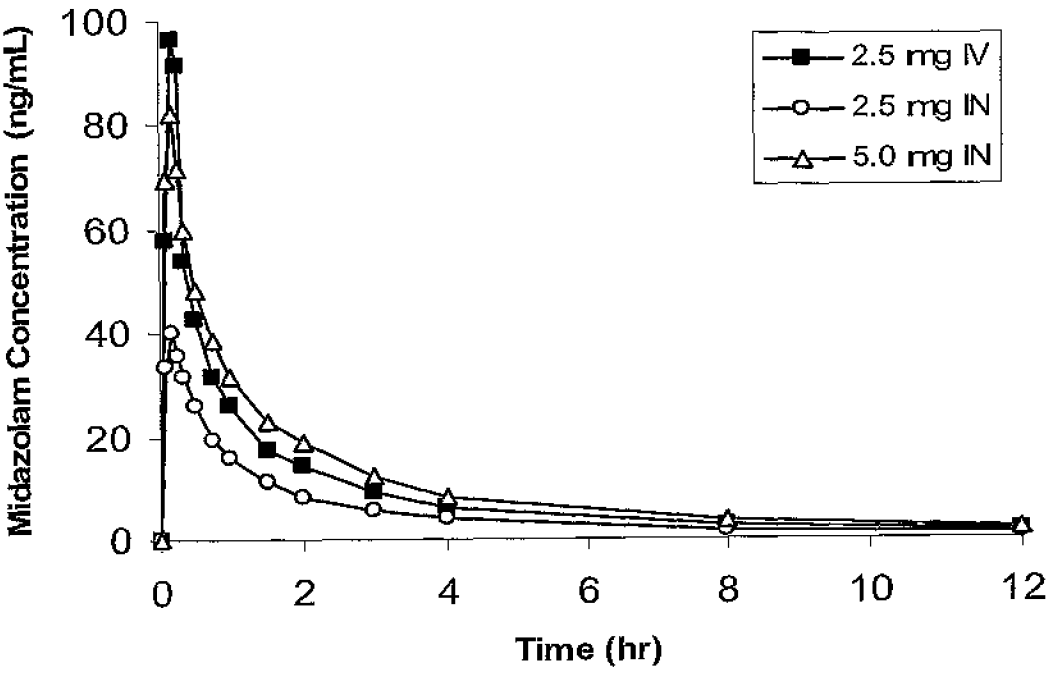
[Continued on next page]

(54) Title: INTRANASAL BENZODIAZEPINE COMPOSITIONS



(57) Abstract: A pharmaceutical composition for intranasal administration to a mammal. The pharmaceutical composition comprises an effective amount of a benzodiazepine or pharmaceutically acceptable salt thereof; and a nasal carrier. In some embodiments, the pharmaceutical composition when administered intranasally produces a rapid physiological response. Pharmaceutical compositions may also include at least one or more sweeteners, flavoring agents, masking agents or combinations thereof.

WO 2005/089768 A1

Published:
— *with international search report*
— *with amended claims*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTRANASAL BENZODIAZEPINE COMPOSITIONS

This application is a continuation-in-part of U.S. application Serial No. 10/418,260 filed April 15, 2003, which is a continuation application of U.S. application Serial No. 09/790,199 filed February 20, 2001, now U.S. Patent No. 6,610,271. The entire disclosure of these applications is herein incorporated by reference.

BACKGROUND

Benzodiazepines have been used to prevent or treat a wide variety of clinical conditions based on their anxiolytic, hypnotic, anticonvulsant, and antispastic properties. Some benzodiazepines have also demonstrated efficacy for their antipanic, antidepressant, amnestic, and anesthetic effects.

Chlordiazepoxide and diazepam, the earliest benzodiazepines, have the classic 1,4-diazepine ring structure and also a 5-aryl substituent ring fused to a benzene ring. A number of modifications to the 1,4-diazepine structure led to compounds such as midazolam, which is a short-acting benzodiazepine that has an imidazo ring fused to the diazepine ring, and alprazolam and triazolam, which have a triazolo ring fused to the diazepine ring. There are other compounds that do not have the classic benzodiazepine structure, yet still have the anxiolytic or sedative effects associated with some of the benzodiazepines. These other compounds include for example, zopiclone, zolpidem, abecarnil, and bretazenil.

The therapeutic effects of benzodiazepines and other compounds, in part, result from enhancing the actions of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) at its receptor. Benzodiazepines work at the GABA receptor and cause GABA to produce a more rapid pulsatile opening of the chloride channel causing an influx of chloride into the cell.

Benzodiazepines have different onset and duration of action, making them useful in treating a variety of different clinical conditions. Benzodiazepines with short onset and duration of action may be useful when an immediate effect is needed (e.g., for outpatient surgical and diagnostic procedures), although longer duration of action may be

desired (e.g., in treatment of sleep-maintenance disturbances or for seizure control). Some benzodiazepines have been used to treat anxiety, schizophrenia, phobias, sleep and depressive disorders. Used alone or in combination with neuroleptics, benzodiazepines have proved valuable for management of various psychiatric emergencies involving agitation or hostility. Intravenous diazepam is frequently a life saving drug in various convulsive emergencies, such as status epilepticus or tetanus spasms. Benzodiazepines frequently bring substantial relief of spasticity and involuntary movement disorders, such as, choreas, myoclonus, and some dyskinesias and dystonias associated with use of neuroleptic medications. Benzodiazepines are also effective in managing acute withdrawal from alcohol. When administered prior to surgical procedures, benzodiazepines reduce anxiety, provide sedation, facilitate anesthetic induction, and produce amnesia for the events surrounding induction. In the treatment of cancer, lorazepam and other benzodiazepines can help to control nausea and vomiting associated with chemotherapy.

Although benzodiazepines can be used to treat a wide variety of conditions, a patient's non-compliance or failure to take medication as prescribed, has been linked to inadequate treatment of many conditions. Some benzodiazepines are available by injections (e.g., intravenous (IV), intramuscular (IM) or subcutaneous injection). The intravenous route is normally regarded as one of the most in-convenient routes to administer medication. Intravenous administration may cause non-compliance, because not only do patients fear getting the injection, but unpleasant experiences such as pain, irritation and infection resulting at the injection site may also lead to non-compliance.

The intranasal route is currently receiving special interest for administering benzodiazepines. When medication is administered via the intranasal route, the medication is applied to the nasal mucosa where it is absorbed. The extensive network of blood capillaries under the nasal mucosa is particularly suited to provide rapid and effective systemic absorption of drugs. The intranasal route of administration should achieve similar dose to plasma concentration (bioavailability) and efficacy to that of the intravenous route.

Intranasal administration of medication provides numerous advantages over the intravenous route. The principal advantages of intranasal route are non-invasive delivery,

rapid drug absorption, and convenience. The intravenous route, unlike the intranasal route, requires sterilization of hypodermic syringes and, in the institutional setting, leads to concerns among medical personnel about the risk of contracting disease if they are accidentally stuck by a contaminated needle. Strict requirements for the safe disposal of needles and syringes have also been imposed.

In contrast, intranasal administration requires little time on the part of the patient and attending medical personnel, and is far less burdensome on the institution than injectable routes. There is no significant risk of infection of the patient or medical personnel in the institutional setting when dealing with the intranasal delivery of medication.

A second important advantage of intranasal administration over intravenous is patient acceptance of the intranasal delivery route. In some cases, the injections cause burning edema, swelling, turgidity, hardness and soreness. In contrast, intranasal administration is perceived as non-invasive, is not accompanied by pain, has no after-effects and produces a prompt means of treating a wide variety of medical conditions. This is of particular advantage when the patient is a child. Many, if not most, patients experience anxiety and exhibit symptoms of stress when faced with hypodermic injections via the IM or IV routes. Further, most people have some familiarity with nasal sprays in the form of over-the-counter decongestants for alleviating the symptoms of colds and allergies that they or a family member have used routinely. Another important consideration is that the patient can self-administer the prescribed dosage(s) of nasal spray without the need for trained medical personnel.

There are different intranasal benzodiazepine compositions known in the pharmaceutical arts. However, some intranasal benzodiazepine compositions have poor absorption or delayed time to peak plasma concentration, which is not appropriate, for prevention or treatment of some clinical conditions. Other prior art benzodiazepine formulations do not enhance patient compliance. For example, some intranasal midazolam formulations are produced at a pH that often causes nasal irritation and burning.

Based on the above, there is a need for intranasal benzodiazepine compositions with improved properties, such as for example, rapid absorption and time to peak

concentration. There is also a need for intranasal compositions that improve patient compliance.

SUMMARY

In various embodiments, pharmaceutical compositions for intranasal administration to a mammal are provided. The pharmaceutical composition comprises an effective amount of a benzodiazepine or pharmaceutically acceptable salt thereof and a nasal carrier. In various embodiments, the pharmaceutical composition, when administered intranasally, produce a rapid physiological response.

In various embodiments, a pharmaceutical composition is provided for intranasal administration comprising: an effective amount of a benzodiazepine or pharmaceutically acceptable salt thereof; a nasal carrier; and at least one or more sweeteners, flavoring agents, or masking agents or combinations thereof.

In various embodiments, a pharmaceutical composition is provided for intranasal administration to a mammal comprising: an effective amount of midazolam or pharmaceutically acceptable salt thereof, polyethylene glycol, and propylene glycol.

In various embodiments, a method of treating a mammal in need of rapid sedation, anxiolysis, amnesia, or induction of anesthesia is provided comprising intranasally administering to the mammal an effective amount of a pharmaceutical composition comprising midazolam or pharmaceutically acceptable salt thereof; and a nasal carrier; wherein the rapid sedation, anxiolysis, amnesia, or induction of anesthesia occurs within 5 minutes after intranasal administration.

In various embodiments, a method of treating a mammal in need of rapid sedation, anxiolysis, amnesia, or induction of anesthesia is provided comprising intranasally administering to the mammal an effective amount of a pharmaceutical composition comprising midazolam or pharmaceutically acceptable salt thereof; a nasal carrier; and at least one or more sweeteners, flavoring agents, or masking agents or combinations thereof.

In various embodiments, a method of making a pharmaceutical composition for intranasal administration is provided comprising adding at least one or more sweeteners, flavoring agents, or masking agents or combinations thereof to a pharmaceutical

composition comprising midazolam or pharmaceutically acceptable salt thereof, and a nasal carrier so as to make the pharmaceutical composition.

For a better understanding of various embodiments, reference is made to the following description taken in conjunction with the examples, the scope of which is set forth in the appended claims.

BRIEF DESCRIPTION OF THE FIGURES

Preferred embodiments have been chosen for purposes of illustration and description, but are not intended in any way to restrict the scope of the claims. The preferred embodiments are shown in the accompanying figures, wherein:

Figure 1 is a graphic representation of mean blood plasma concentration (n=12) of midazolam in plasma versus time for three different midazolam compositions over a four-hour period.

Figure 2 is a graphic representation of mean blood plasma concentration (n=12) of midazolam in plasma versus time for three different midazolam compositions over a twelve-hour period.

Figure 3 is a graphic representation of mean blood plasma concentration (n=17) of midazolam in plasma versus time for three different midazolam compositions over a four-hour period.

Figure 4 is a graphic representation of mean blood plasma concentration (n=17) of midazolam in plasma versus time for three different midazolam compositions over a twelve-hour period.

DETAILED DESCRIPTION

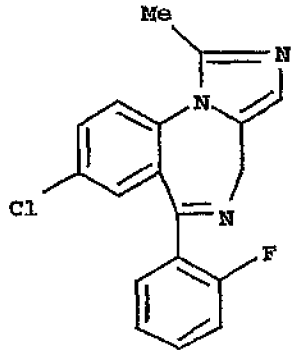
Various embodiments will now be described. These embodiments are presented to aid in an understanding of the claims and are not intended to, and should not be construed to, limit the claims in any way. All alternatives, modifications and equivalents that may become obvious to those of ordinary skill on reading the disclosure are included within the spirit and scope of the claims.

The pharmaceutical composition comprise benzodiazepine or other compounds. Benzodiazepines, as used herein, include but are not limited to alprazolam, brotizolam, chlordiazepoxide, clobazepam, clonazepam, clorazepate, demoxepam, diazepam, estazolam, flurazepam, quazepam, halazepam, lorazepam, midazolam, nitrazepam, nordazapam, oxazepam, prazepam, quazepam, temazepam, triazolam, zolpidem, zaleplon or combinations thereof. Other compounds that have anxiolytic or sedative effects of some benzodiazepines include, for example, zopiclone, zolpidem, abecarnil, and bretazenil.

In various embodiments, the benzodiazepine may be in free form or in pharmaceutically acceptable salt or complex form. Some examples of pharmaceutically acceptable salts of benzodiazepines include those salt-forming acids and bases that do not substantially increase the toxicity of the compound. Some examples of suitable salts include salts of alkali metals such as magnesium, potassium and ammonium. Salts of mineral acids such as hydrochloric, hydriodic, hydrobromic, phosphoric, metaphosphoric, nitric and sulfuric acids, as well as salts of organic acids such as tartaric, acetic, citric, malic, benzoic, glycollic, gluconic, gulonic, succinic, arylsulfonic, e.g. p-toluenesulfonic acids, and the like.

In various embodiments, pharmaceutical compositions are provided for intranasal administration comprising midazolam or pharmaceutically acceptable salts thereof. In various embodiments, the pharmaceutical composition comprises midazolam hydrochloride. Midazolam includes 8-chloro-6-(2-fluorophenyl)-1-methyl- 4H-Imidazo-[1,5-a][1,4]benzodiazepine, [CAS 59467-70-8]. The molecular weight of midazolam is 325.8.

Midazolam has the molecular formula: $C_{18}H_{13}ClFN_3$ and exhibits the following general structure:



In various embodiments, the pharmaceutical compositions comprise a benzodiazepine or pharmaceutically acceptable salt thereof and a nasal carrier. As used herein, "nasal carrier" includes a solution, emulsion, suspension, or powder designed for delivery of the benzodiazepine or other compound to the nasal mucosa. The nasal carrier may include a diluent suitable for application to the nasal mucosa. Suitable diluents include aqueous or non-aqueous diluents or combinations thereof. Examples of aqueous diluents include, but are not limited to, saline, water, dextrose or combinations thereof. Non-aqueous diluents include, but are not limited to, alcohols, particularly polyhydroxy alcohols such as propylene glycol, polyethylene glycol, glycerol, and vegetable or mineral oils or combinations thereof. These aqueous and/or non-aqueous diluents can be added in various concentrations and combinations to form solutions, suspensions, oil-in-water emulsions or water-in-oil emulsions.

In various embodiments, the nasal carrier comprises polyethylene glycol and propylene glycol. In various embodiments; the polyethylene glycol constitutes from about 15% to about 25% by volume and the propylene glycol constitutes from about 75% to about 85% by volume of the composition. In various embodiments, the polyethylene glycol has an average molecular weight of about 400. In various embodiments, the ratio of polyethylene glycol to propylene glycol is about one to about four.

The nasal carrier, in some embodiments, may also contain excipients such as antioxidants, chemical preservatives, buffering agents, surfactants and/or agents that

increase viscosity. Antioxidants are substances that prevent oxidation of the formulations. Suitable antioxidants for use in the pharmaceutical composition, if one is employed, includes but is not limited to, butylated hydroxytoluene, butylated hydroxyanisole, potassium metabisulfite, and the like.

In various embodiments, the composition contains a preservative that is chosen in quantities that preserve the composition, but preferably does not cause irritation to the nasal mucosa. Suitable preservatives for use in some embodiments include, but is not limited to, benzalkonium chloride, methyl, ethyl, propyl or butylparaben, benzyl alcohol, phenylethyl alcohol, benzethonium, or combination thereof. Typically, the preservative is added to the compositions in quantities of from about 0.01% to about 0.5% by weight.

In some embodiments, the formulation is preservative-free. As used herein, preservative-free includes compositions that do not contain any preservative. Thus, the composition does not contain, for example, benzalkonium chloride, methyl, ethyl, propyl or butylparaben, benzyl alcohol, phenylethyl alcohol, or benzethonium.

If a buffering agent is employed in the composition, it is chosen in quantities that preferably do not irritate the nasal mucosa. Buffering agents include agents that reduce pH changes. Some buffering agents that may be used in the pharmaceutical composition include, but are not limited to, salts of citrate, acetate, or phosphate, for example, sodium citrate, sodium acetate, sodium phosphate, and/or combinations thereof. Typically, the buffer is added to the compositions in quantities of from about 0.01% to about 3% by weight.

When one or more surfactants are employed, the amount present in the compositions will vary depending on the particular surfactant chosen, the particular mode of administration (e.g. drop or spray) and the effect desired. In general, however, the amount present will be in the order of from about 0.1 mg/ml to about 10 mg/ml, in various embodiments, about 0.5 mg/ml to 5 mg/ml and, in various embodiments, about 1 mg/ml is used.

In various embodiments, the pharmaceutical composition may include one or more agents that increase viscosity, which are chosen in quantities that preferably do not irritate the nasal mucosa and increase nasal retention time. Some agents that increase viscosity include, but are not limited to, methylcellulose, carboxymethylcellulose sodium,

ethylcellulose, carrageenan, carbopol, and/or combinations thereof. In various embodiments, an agent used to increase viscosity and increase nasal retention time is methylcellulose or carbopol. Typically, the agent that increases viscosity may be added to the compositions in quantities of from about 0.1% to about 10% by weight.

To reduce the bitter taste of the intranasal composition and/or enhance patient compliance, in various embodiments, one or more sweeteners or flavoring agents or masking agents are employed. The sweetener or flavoring agent or masking agent includes any agent that sweetens or provides flavor to the pharmaceutical composition: The sweetener or flavoring agent or masking agent will mask the bitter or bad taste that may occur if the pharmaceutical composition drips back into the mouth after intranasal administration. By addition of a sweetener or flavoring agent or masking agent to the intranasal composition, any barrier that a patient may have to taking the intranasal composition because of unpleasant taste is reduced. By adding a sweetener, flavoring agent or masking agent to the intranasal pharmaceutical composition, patient compliance is enhanced or improved.

As used herein, one or more sweeteners or flavoring agents or masking agents include, but are not limited to, acacia syrup, anethole, anise oil, aromatic elixir, benzaldehyde, benzaldehyde elixir, cyclodextrins, compound, caraway, caraway oil, cardamom oil, cardamom seed, cardamom spirit, compound, cardamom tincture, compound, cherry juice, cherry syrup, cinnamon, cinnamon oil, cinnamon water, citric acid, citric acid syrup, clove oil, cocoa, cocoa syrup, coriander oil, dextrose, eriodictyon, eriodictyon fluidextract, eriodictyon syrup, aromatic, ethylacetate, ethyl vanillin, fennel oil, ginger, ginger fluidextract, ginger oleoresin, dextrose, glucose, sugar, maltodextrin, glycerin, glycyrrhiza, glycyrrhiza elixir, glycyrrhiza extract, glycyrrhiza extract pure, glycyrrhiza fluidextract, glycyrrhiza syrup, honey, iso-alcoholic elixir, lavender oil, lemon oil, lemon tincture, mannitol, methyl salicylate, nutmeg oil, orange bitter, elixir, orange bitter, oil, orange flower oil, orange flower water, orange oil, orange peel, bitter, orange peel sweet, tincture, orange spirit, compound, orange syrup, peppermint, peppermint oil, peppermint spirit, peppermint water, phenylethyl alcohol, raspberry juice, raspberry syrup, rosemary oil, rose oil, rose water, rose water, stronger, saccharin, saccharin calcium, saccharin sodium, sarsaparilla syrup, sarsaparilla compound, sorbitol

solution, spearmint, spearmint oil, sucrose, sucralose, syrup, thyme oil, tolu balsam, tolu balsam syrup, vanilla, vanilla tincture, vanillin, or wild cherry syrup, or combinations thereof.

In various embodiments, the sweetener is saccharin, sodium saccharin, xylitol, mannitol, glycerin, sorbitol, sucralose, maltodextrin, sucrose, aspartame, acesulfame potassium, dextrose, glycosides, maltose, sweet orange oil, dextrose, glucose, or honey or combinations thereof. Some flavoring agents to use in various embodiments include, but are not limited to, glycerin, wintergreen oil, peppermint oil, peppermint water, peppermint spirit, menthol, or syrup, or combinations thereof. In various embodiments, the masking agents do not make contact with the taste buds. In various embodiments, the masking agent includes, but is not limited to, cyclodextrins, cyclodextrins emulsions, cyclodextrins particles, or cyclodextrin complexes, or combinations thereof.

To reduce burning, if it occurs, the composition may contain an anesthetic agent. Some anesthetic agents include, but are not limited to, lidocaine, prilocaine, procaine, benzocaine tetracaine, chloroprocaine, or pharmaceutically acceptable salts thereof or combinations thereof.

The pharmaceutical compositions, in different embodiments, may also include additional ingredients, such as pharmaceutically acceptable surfactants, co-solvents, adhesives, agents to adjust the pH and osmolarity. The pharmaceutical compositions are not limited to any particular pH. However, generally for nasal administration a mildly acid pH will be preferred. The pH ranges from about 3 to 6 in some embodiments, in other embodiments, pH ranges are from about 3 to about 5, and in other embodiments pH ranges are from about 4 to about 5. If the adjustment of the pH is needed, it can be achieved by the addition of an appropriate acid, such as hydrochloric acid, or base, such as for example, sodium hydroxide.

The pharmaceutical composition in some embodiments can be made, for example, by mixing the benzodiazepine with the nasal carrier and/or a sweetener, flavoring agent, or masking agent or combinations thereof at, for example, room temperature under aseptic conditions to form a mixture. In other embodiments, the mixture is filtered, for example, by a 0.22 micron filter. It will be understood by those of ordinary skill in the art that the order of mixing is not critical, and various embodiments include without

limitation mixing of the composition in any order. In various embodiments, the pharmaceutical composition is a sterile solution or suspension.

Pharmaceutical compositions can be administered intranasally by nasal spray, drop, solution, suspension, gel, and the like. Intranasal administration is an art-recognized term and includes, but is not limited to, administration of the composition into the nasal cavity.

When the pharmaceutical composition is a liquid, volumes of the liquid that may be absorbed through the nasal mucosa include, for example, from about 0.025ml to about 2ml or from about 0.25ml to 1ml, or from about 0.05ml to about 15ml in an adult and smaller volumes for children. However, the pharmaceutical compositions are not limited to any one particular volume.

Devices for intranasal delivery are known in the art. Some devices suitable for use with the pharmaceutical compositions are available from, for example, Pfeiffer of America of Princeton, New Jersey and Valois of America, Inc. of Greenwich, Connecticut. These devices are preferred because they have the capability of consistently delivering the pharmaceutical composition. These devices are easily operable by the patient, leave virtually no benzodiazepine remaining in the device after use and can thereafter be discarded without concern that others may abuse the benzodiazepine or other controlled substance.

In various embodiments, the intranasal delivery device may be modified, for example, by increasing the size of the discharge orifice in the nose piece of the applicator to about 0.07 mm for non-aqueous compositions that comprise, for example, polyethylene glycol and/or propylene glycol, in order to accommodate higher viscosity compositions. For aqueous compositions, the diameter can be, for example, from about 0.05 mm in diameter. The intranasal delivery device may also contain a swirl chamber. The applicator components may also be sterilized by methods well known in the art.

The intranasal delivery device may be filled with single or multidose amounts of benzodiazepines. In various embodiments, the device is filled with one single dose of benzodiazepine. In some embodiments, the container holding the pharmaceutical composition and its sealing means are sterilizable, in some embodiments, at least parts of the device that are in contact with the pharmaceutical composition is constructed and

assembled in a configuration that can be sterilized. Devices with one or more unit-dose(s) can be sterilized either before or after packaging, employing methods and technology that are well known in the art. Individual devices can be packaged, sterilized and shipped; alternatively, entire shipping and storage packages can be sterilized at once, and the devices removed individually for dispensing, without affecting the sterility of the remaining units.

The amount of benzodiazepine or other compound that can be intranasally administered in accordance with the composition and methods will depend on the particular benzodiazepine chosen, the condition to be treated, the desired frequency of administration and the effect desired. Some medical or veterinary symptoms, syndromes, conditions or diseases that benzodiazepines or other compounds are useful in preventing or treating include, but are not limited to, anxiety, panic attacks, schizophrenia, phobias, sleep disorders (e.g. insomnia) and depressive disorders, agitation, hostility, epilepsy, convulsion, spasticity, involuntary movements, or alcohol withdrawal or combinations thereof. Benzodiazepines or other compounds may be used as adjuncts in medical and dental procedures, such as for example, reducing anxiety before surgical anesthesia, providing sedation, facilitating anesthesia induction, producing amnesia, or to control nausea and vomiting.

In various embodiments, the pharmaceutical composition comprises midazolam and is administered to a mammal in need of rapid sedation, anxiolysis, amnesia, or anesthesia induction. As used herein, an effective amount of benzodiazepine or other compound includes that amount effective to achieve the relief or palliation of symptoms, condition and/or diseases that need benzodiazepine therapy. Maximal dosage of the pharmaceutical composition for a mammal is the highest dosage that elicits the desirable response, which does not cause undesirable or intolerable side effects. The minimal dose of the benzodiazepine is the lowest dose that achieves the desired result. In any event, the practitioner is guided by skill and knowledge in the field, and the present invention includes without limitation dosages that are effective to achieve the desired effect in the mammal. Doses of benzodiazepines suitable for intranasal administration, include but are not limited to, from about 0.1mg to about 30mg. For example, doses of midazolam

HCL for intranasal administration include, but are not limited to, from about 0.1mg to about 20 mg.

In various embodiments, it has been surprisingly discovered that pharmaceutical compositions comprising midazolam, when intranasally administered, have rapid absorption and time to peak (T_{max}) leading to rapid onset than midazolam administered by the IV route. For example, the T_{max} for intranasally administered midazolam was in some cases about 5 minutes, while the T_{max} for midazolam administered IV was about 15 minutes. In various embodiments, the pharmaceutical composition comprising midazolam achieves a maximum plasma concentration (C_{max}) of about 40ng/mL from a 2.5mg dose or about 80ng/mL from a 5mg dose after intranasal administration. In various embodiments, the ratio of the AUC for intranasal midazolam to AUC of for midazolam after an equivalent dose of intravenous midazolam is at least about 1:1.7.

In various embodiments, the benzodiazepine is administered to a mammal suffering from a condition and/or disease that requires benzodiazepine treatment. Mammals include, for example, humans, as well as pet animals such as dogs and cats, laboratory animals, such as rats and mice, and farm animals, such as horses and cows.

In various embodiments, a method of treating a mammal in need of rapid sedation, anxiolysis, amnesia, or induction of anesthesia is provided. The method comprises intranasally administering to the mammal an effective amount of a pharmaceutical composition comprising midazolam or pharmaceutically acceptable salt thereof in a nasal carrier. The pharmaceutical composition may also contain a sweetener, masking agent or flavoring agent. In various embodiments, the pharmaceutical composition comprising midazolam is intranasally administered to the mammal and the composition is metabolized by the mammal and achieves a 1-hydroxymidazolam plasma level of about 1 to about 8 nanograms/ml.

EXAMPLES

The examples below demonstrate improved absorption, rapid time to reached peak concentrations, and good bioavailability of the various compositions. The examples also show midazolam compositions that include, for example, sweeteners, which improve patient compliance by reducing the unpleasant taste after intranasal administration.

Example 1

This example compares 5.0 mg midazolam (MZ) after intranasal (IN), intramuscular (IM) and intravenous (IV) administration in 12 healthy male and female subjects.

Subjects

Twelve, nonsmoking, healthy subjects (6 male, 6 female) between the ages of 20 and 29 years (mean 22.3 years) and weighing 132 to 202 lbs. (mean 157 lbs.) participated in this inpatient study after giving informed consent. Eleven of the volunteers who enrolled in the study were Caucasian and one was Asian. Study participants were selected based on inclusion/exclusion criteria, medical history, physical and nasal exams, vital signs, laboratory tests, and other procedures as outlined in the protocol. Subjects were within ± 20% of ideal body weight in relation to height and elbow breadth and weighed at least 60 kg (132 lbs). The subjects were in good health and had no clinically significant previous nasal surgery or polyps or other physical abnormalities of the nose, cardiovascular, gastrointestinal, renal, hepatic, pulmonary or hematological disease. Subjects who had a history of cerebral trauma with sequelae, hypotension, heart failure, cardiac conduction defect, chronic respiratory disease, bleeding tendency, glaucoma, and a formal diagnosis of sleep apnea or a history of alcohol or substance abuse were excluded. Subjects abstained from alcohol and caffeine containing beverages 48 hours before the dosing period and during the study. Subjects were asked to abstain from prescription and non-prescription drugs that might interact with MZ metabolism or nasal physiology from the date of screening until the end of the study. Subjects had to demonstrate their ability to perform the pharmacodynamic (PD) assessments during the screening evaluation. Informed consent was obtained and this study was conducted according to the applicable guidelines for Good Clinical Practice.

IV and IM Formulations

The intravenous (IV) and intramuscular (IM) solutions were prepared for administration in the University of Kentucky Hospital Investigational Drug Service Pharmacy using commercially available MZ (Versed® Injection by Hoffman-LaRoche). MZ (5 mL of 1.0 mg/mL) sterile solution was diluted to 10 mL with normal saline for a total volume of

10 mL to be infused over 15 minutes. The 5.0 mg IM MZ (1 mL of 5.0 mg/1.0 mL) was administered without dilution.

IN Formulation of MZ

The 25 mg/mL IN MZ formulation was prepared under GMP conditions in the University of Kentucky College of Pharmacy Center for Pharmaceutical Science and Technology (CPST). The IN formulation comprised midazolam 25 mg; polyethylene glycol 400, USP 0.18 mL; butylated hydroxytoluene, NF 0.10 mg; saccharin powder, NF 1.00 mg; propylene glycol, USP Q.S. to 1.00 mL. The formulation provided 2.5 mg of MZ in 0.1 mL spray from a modified version of the commercially available, single-dose, metered sprayer (unit dose spray pumps, Pfeiffer of America, Princeton, NJ). Each subject received a single spray in each nostril for a total of 5.0 mg.

Protocol

An open-label, randomized, three-way crossover study design was used. Treatment assignments were in the random order generated by a statistician. The three treatments were: Treatment A: 5.0 mg (5 mL of 1.0 mg/mL) IV MZ infused over 15 minutes, Treatment B: 5.0 mg intramuscular MZ (5.0 mg/1.0 mL), and Treatment C: 5.0 mg intranasal MZ solution (2.5 mg/100 μL per sprayer). The three treatments were separated by six-day washout periods. PK blood samples were drawn following each dose. MZ (5 mL of 1.0 mg/mL) sterile solution was diluted to 10 mL with normal saline for a total volume of 10 mL and infused over 15 minutes by a nurse using a stopwatch. IN MZ doses were administered by a physician using Pfeiffer modified unit dose sprayers (Pfeiffer of America, Princeton NJ). The 5.0 mg IM MZ (5.0 mg/1.0 mL) was administered without dilution. Drug administration occurred in the morning following an overnight fast of at least 8 hours. The subjects continued to fast for 2 hours after dosing. Water was allowed except within two hours before or after drug administration. Subjects were allowed juice, 360 mL, at least 2 hours prior to dosing for each dose. Subjects were awakened 1 hour prior to dosing for performance of PD testing. Blood samples were collected in 10 mL Vacutainer® tubes containing the anticoagulant sodium heparin. Serial blood samples were obtained by venipuncture according to the following schedule: 0 (pre-dose), 5, 10, 20, 30, and 45 minutes, and 1, 1.5, 2, 3, 4, 8, and 12 hours following

MZ administration. Actual sampling times were used in PK analysis. After collection, the blood was centrifuged in a refrigerated centrifuge at 4°C to separate the plasma and the cells, and the plasma was transferred to polypropylene tubes. The plasma was stored at or below –20°C at the study site until shipped to Kansas City Analytical Services, Inc. (KCAS) in Shawnee, Kansas.

LC/MS/MS Assay for MZ and α-hydroxymidazolam

The sample analysis was conducted for MZ and α-hydroxymidazolam using a PE/Sciex API III + LC/MS/MS system in MRM mode by KCAS in Shawnee, KS. Concentrations less than 0.50 ng/mL were reported as below quantitation limit (BQL). Samples with concentrations greater than 500 ng/mL were reanalyzed using a dilution so that the assayed concentration was within the range of 0.50 to 500.0 ng/mL.

Pharmacokinetic (PK) Data Analysis

PK parameters were determined using standard noncompartmental methods with log-linear least square regression analysis to determine the elimination rate constants (WinNonlin, Pharsight Corp., Palo Alto, CA). The areas under the concentration versus time curves from time zero to infinity ($AUC_{0-\infty}$) were calculated using a combination of the linear and logarithmic trapezoidal rules, with extrapolation to infinity by dividing the last measurable serum concentration by the elimination rate constant (λ_z) (Proost, 1985). Values for the maximum concentration (C_{max}) and time to C_{max} (T_{max}) were determined by WinNonlin. The elimination half-life was determined from $0.693/\lambda_z$. Clearance (CL/F) was determined by dividing the dose by $AUC_{0-\infty}$. Volumes of distribution for elimination (V_z/F) and at steady state (V_{ss}) were determined by moment curves (Gibaldi and Perrier, 1982). V_z/F was calculated as Dose/(λ_z* $AUC_{0-\infty}$). V_{ss} was calculated as CL * MRT for IV data. The absolute bioavailability (F) for the IN and IM dosage forms was determined by $F = AUC_{IN,0-\infty}/ AUC_{IV,0-\infty}$. and $F = AUC_{IM,0-\infty}/ AUC_{IV,0-\infty}$, respectively. Relative bioavailability of the IN compared to the IM dose was calculated by $AUC_{IN,0-\infty}/ AUC_{IM,0-\infty}$.. Mean plasma concentrations were calculated for graphical evaluation only. The calculations included data from samples with measurable concentrations drawn within 5% of the expected sampling time.

Statistical Data Analysis

Statistical analyses were performed with Statistical Analysis System PC-SAS version 6.12. The statistical tests were 2-sided with a critical level of 0.05. An analysis of variance (ANOVA) with factors sequence, subject(sequence), treatment and period was performed for log-transformed AUC and C_{max}. The least square geometric means from the ANOVA were used to calculate the ratios and their 90% confidence intervals between treatment groups for AUC and Cmax. The carryover effect for the three treatments was analyzed using an ANOVA of log-transformed AUC and C_{max}. The difference in T_{max} values between the IN and IM treatments was compared using an ANOVA of rank-transformed T_{max}. The ANOVA model included factors sequence, subject(sequence), treatment and period. The gender effect for all three treatments was analyzed using an ANOVA of log-transformed AUC and C_{max} with factors gender, treatment and period.

Results of Example 1

Twelve subjects completed the study without clinically significant or serious adverse events. There were no clinically relevant changes in physical examination, nasal evaluations, or laboratory tests. The principal investigator's review of the data indicated that, in general, doses of the study drug were well tolerated and events were mild to moderate and temporary (2-90 minutes). Two of twelve subjects noted mild dizziness that lasted 35 and 50 minutes. Three of twelve subjects noted blurred vision that lasted 5-90 minutes. No subjects experienced respiratory depression, apnea, laryngospasm, bronchospasm or wheezing. The mean plasma concentration versus time curve profiles over the first 4 hours and the entire 12 hours for the three doses are shown in Figures 1 and 2. Figure 1 shows that absorption of MZ following IN administration was very rapid. MZ concentrations reached a peak in 2 individuals at 5 min and in 8 of 12 individuals in 10 min or less. No secondary or late bumps indicating absorption from swallowing the IN dose were observed in the plasma concentration time curves. Table 1 summarizes PK data for the three treatments. Median T_{max} values were 10 and 30 min for the IN and IM doses, respectively. C_{max} values after the IN dose were higher than those after the IM dose and occurred consistently earlier. Relative bioavailability of the IM to IN dose was on average 79%. Unfortunately, the absolute bioavailability of MZ by the IN and IM

routes in Table 1 is overestimated due to the underestimation of the $AUC_{0-\infty}$ for the IV dose. The $AUC_{0-\infty}$ given for the IV dose underestimates the true $AUC_{0-\infty}$ because the area around the C_{max} (which would have been at the end of the 15 minute infusion) was not captured in this study. However, the data for the IM dose are accurate and acceptable for making conclusions regarding the relative bioavailability of the IN dose compared to the IM dose. The high relative bioavailability of the IN to IM dose confirms that bioavailability was good for MZ administered by the IN route.

Table 1.

Mean (CV as a %) Single Dose MZ Pharmacokinetic (PK) Parameters Following Administration of 5.0 mg Intravenous (IV), Intramuscular (IM) and Intranasal (IN) MZ in Healthy Subjects (n=12)

1.1. PK Parameter	IV (5.0 mg)	IM (5.0 mg)	IN (5.0 mg)
T_{max} (min)*	10 (5-31)	30 (20-60)	10 (5-20)
C_{max} (ng/mL)	167.3 (28.9)	58.7 (49.7)	80.0 (20.8)
$t_{1/2}$ (hr)	3.14 (23.0)	4.17 (50.2)	3.25 (29.8)
AUC_{0-t} (ng•hr/mL)	178.1 (17.1)	152.3 (25.8)	126.7 (20.6)
$AUC_{0-\infty}$ (ng•hr/mL)	186.4 (16.5)	174.6 (22.1)	133.8 (19.4)
MRT (hr)	2.88 (20.2)	5.48 (48.9)	3.33 (27.4)
CL/F or CL_{ss}/F(L/hr)	27.5 (17.8)	30.1 (24.6)	38.6 (19.2)
V_{ss} (L)	78.8 (23.3)	-	-
V_z /F (L)	123.4 (26.1)	177.9 (51.7)	182.3 (39.0)
F (%)**	*assume 100%*	*93.4 (12.4)*	*72.5 (16.8)*
Relative F (IM/IN) (%)	-	-	79.2 (23.7)

*median and range given for T_{max};
**see above for discussion of F.-

No significant gender differences were found for $AUC_{0-\infty}$ and C_{max} values (P >.1). The gender effect was significant for AUC_{0-t} values (P= 0.0452, M > F). Larger differences in AUC_{0-t} between males and females were observed for the IM formulation. The differences were smaller for the IN formulation (12%). Data were combined for analysis of treatment effects. A significantly shorter T_{max} was observed for the IN formulation compared to the IM formulation (p=0.0001). T_{max} and C_{max} were not captured at the end of the infusion for the IV dose. Statistical analysis of carryover effect

on log transformed $AUC_{0-\infty}$, AUC_{0-t} and C_{max} for the two IN treatments was performed. P-values from an ANOVA with factors sequence, subject (sequence), treatment and period for sequence BC and CB were >0.1, so the carryover effects were not significant and this implies the validity of the analyses in Table 2.

Table 2 summarizes the ratios and 90% confidence intervals (CI) of C_{max} and AUCs after Treatments A, B and C. AUC_{0-t} and $AUC_{0-\infty}$ were more comparable between the IM and IV treatments (B/A) than between the IV and IN (C/A) treatments. However, C_{max} values were almost 50% higher after Treatment C (IN) compared to Treatment B (IM).

Table 2.
Summary of Ratios of Least Squares Geometric Means and 90% Confidence Intervals

Parameter	1.1.2. Treatment Group Geometric Means			B/A (IM/IV)	C/A (IN/IV)	C/B (IN/IM)
	5 mg MZ IV (A)	5 mg MZ IM (B)	5 mg MZ IN (C)	Ratio (90%CI)	Ratio (90%CI)	Ratio (90%CI)
$AUC_{0-\infty}$ (ng•hr/mL)	184.01	170.51	131.58	0.93 (0.85-1.01)	0.72 (0.65-0.78)	0.77 (0.71-0.84)
AUC_{0-t} (ng•hr/mL)	175.72	147.81	124.29	0.84 (0.77-0.92)	0.71 (0.65-0.77)	0.84 (0.77-0.92)
C_{max} (ng/mL)	159.02	53.28	78.35	0.34 (0.26-0.43)	0.49 (0.38-0.63)	1.47 (1.15-1.88)

CI = Confidence Intervals
Least squares geometric means are from an ANOVA with with factors sequence, subject(sequence), treatment and period for log-transformed AUCs and C_{max}.

The 1-hydroxymidazolam metabolite concentrations were consistently lower than those of the parent drug.

Discussion

The pharmacokinetics of MZ were evaluated in 12 healthy male and female volunteers after single 5.0 mg doses of IV, IM and IN MZ. All subjects completed the study without clinically significant or serious adverse events. The pharmacokinetics of MZ were consistent with rapid but relatively short duration of action. The mean absolute bioavailability of IN MZ would be predicted to be around 65% assuming that about 7% of the IV AUC was missed. The mean relative bioavailability compared to the IM dose was 79%. Less than complete bioavailability after the IN administration may be explained by metabolism during absorption across the nasal mucosa or simply incomplete absorption and swallowing. There was no evidence of swallowing. Plasma clearance and volumes of distribution were high. The IN formulation of MZ had rapid absorption (median peak times of 10 min). In comparison with IM administration, the IN formulation had earlier and higher peak plasma concentrations.

Conclusion

Intravenously administered MZ distributes extensively and rapidly in the body. A total systemic clearance of 28 L/hr indicates that MZ is a highly cleared drug. The IN formulation of MZ had rapid absorption and reached peak concentrations significantly more rapidly than the IM dose. Absolute bioavailability of MZ from the IN dosage form was good and supports further investigation of this dosage form for clinical use. Relative bioavailability compared to the IM dose was 79.2% (23.7 %CV). No treatment emergent adverse events were observed during the conduct of this protocol that would preclude further study of MZ in healthy subjects. Adverse events were mild and expected for this drug. As evidenced by the lack of cardiovascular and respiratory adverse events, all the subjects tolerated the drug well.

Example 2

This study compares the pharmacokinetics of midazolam (MZ) after administration of 2.5 and 5.0 mg intranasal (IN) MZ and 2.5 mg intravenous (IV) MZ in 18 healthy male and female subjects.

Subjects

Eighteen, nonsmoking, healthy subjects (9 male, 9 female) between the ages of 20 and 29 years (mean 22.3 years) and weighing 60 to 92 kg (mean 71 kg) participated in this inpatient study after giving informed consent. Seventeen of the volunteers who enrolled in the study were Caucasian and one was African-American. Seventeen subjects completed the study. Study participants were selected based on inclusion/exclusion criteria, medical history, physical and nasal exams, vital signs, laboratory tests, and other procedures as outlined in the protocol. Subjects were within ± 25% of ideal body weight in relation to height and elbow breadth and weighed at least 60 kg (132 lbs). The subjects were in good health, between 18 and 45 years of age and had no clinically significant previous nasal surgery or polyps or other physical abnormalities of the nose, vital signs, cardiovascular, gastrointestinal, renal, hepatic, pulmonary, hematological or neurological disease. Subjects who had a history of a seizure disorder, cerebral trauma with sequelae, hypotension, heart failure, cardiac conduction defect, chronic respiratory disease, bleeding tendency, narrow-angle glaucoma, a formal diagnosis of sleep apnea, a current formal diagnosis of depressive disorder or psychosis or a medical diagnosis of alcohol or substance abuse were excluded. Subjects with a known history of Gilbert's Syndrome or with any other etiology for an increased serum total bilirubin level and subjects with any other clinical condition that might affect the absorption, distribution, biotransformation, or excretion of the drug (e.g., acute respiratory illness, allergic rhinitis, etc.) or were allergic to MZ or formulation components were excluded. Subjects who had a history of regular sedative/hypnotic medication use (i.e., at least once per week) or who had taken any sedative/hypnotic medications within the 2 weeks prior to study drug administration were excluded. Subjects abstained from alcohol and caffeine containing beverages 48 hours before the dosing period and during the study. Subjects were asked to abstain from prescription and non-prescription medication, vaccines, herbal and nutritional supplements that might interact with MZ metabolism or nasal physiology within 7 days of dosing and during the study.

IV Formulation

The intravenous (IV) solutions were prepared for administration in the University of Kentucky Hospital Investigational Drug Service Pharmacy using commercially available MZ (Versed® Injection by Hoffman-LaRoche). MZ (0.5 mL of 5.0 mg/mL) sterile solution was diluted to 10 mL with normal saline for a total volume of 10 mL to be infused over 15 minutes.

IN Formulation of MZ

The 25 mg/mL IN MZ formulation was prepared under GMP conditions in the University of Kentucky College of Pharmacy Center for Pharmaceutical Science and Technology (CPST). The IN formulation contained midazolam 25 mg; polyethylene glycol 400, USP 0.18 mL; butylated hydroxytoluene, NF 0.10 mg; saccharin powder, NF 1.00 mg; propylene glycol, USP Q.S. to 1.00 mL. The formulation provided 2.5 mg of MZ in 0.1 mL spray from a modified version of the commercially available, single-dose, metered sprayer (unit dose spray pumps, Pfeiffer of America, Princeton, NJ). Each subject received a single spray in one nostril for a 2.5 mg dose or a single spray in each nostril for a total of 5.0 mg.

Protocol

An open-label, randomized, three-way crossover study design was used. Treatment assignments were in the random order generated by a statistician. The three treatments were: Treatment A: 2.5 mg (5 mL of 1.0 mg/mL) IV MZ infused over 15 minutes, Treatment B: 2.5 mg intranasal MZ solution, one 2.5 mg/100 µL sprayer, and Treatment C: 5.0 mg intranasal MZ solution, two 2.5 mg/100 µL sprayers, one sprayer per naris. The three treatments were separated by six-day washout periods. PK blood samples were drawn following each dose. MZ (5 mL of 1.0 mg/mL) sterile solution was diluted to 10 mL with normal saline for a total volume of 10 mL and infused over 15 minutes by a nurse using a stopwatch. IN MZ doses were administered by a physician using Pfeiffer modified unit dose sprayers (Pfeiffer of America, Princeton NJ). Drug administration occurred in the morning following an overnight fast of at least 8 hours. The subjects continued to fast for 2 hours after dosing. Water was allowed except within two hours

before or after drug administration. Subjects were allowed juice, 240 mL, at least 2 hours prior to dosing for each dose. Grapefruit juice was not allowed during the study. Blood samples were collected in 10 mL Vacutainer® tubes containing the anticoagulant sodium heparin. Serial blood samples were obtained by venipuncture according to the following schedule: 0 (pre-dose), 5, 10, 15, 20, 30, and 45 minutes, and 1, 1.5, 2, 3, 4, 8, and 12 hours following MZ administration. Actual sampling times were used in PK analysis. After collection, the blood was centrifuged in a refrigerated centrifuge at 4°C to separate the plasma and the cells, and the plasma was transferred to polypropylene tubes. The plasma was stored at or below –20°C at the study site until shipped to Kansas City Analytical Services, Inc. (KCAS) in Shawnee, Kansas.

LC/MS/MS Assay for MZ and α-hydroxymidazolam

The sample analysis was conducted for MZ and α-hydroxymidazolam using a PE/Sciex API III + LC/MS/MS system in MRM mode by KCAS in Shawnee, KS. Concentrations less than 0.50 ng/mL were reported as below quantitation limit (BQL). Samples with concentrations greater than 500 ng/mL were reanalyzed using a dilution so that the assayed concentration was within the range of 0.50 to 500.0 ng/mL.

Pharmacokinetic (PK) Data Analysis

IN doses were determined by weighing the nasal spray pumps before and after dosing. These weights and the concentrations of the IN solutions (2.5 mg/mL, density 1.056) were used to confirm each subject's dose and to evaluate delivery. The dose weights were not used for PK analysis. PK parameters were determined using standard noncompartmental methods with log-linear least square regression analysis to determine the elimination rate constants (WinNonlin, Pharsight Corp., Palo Alto, CA). The areas under the concentration versus time curves from time zero to infinity ($AUC_{0-\infty}$) were calculated using a combination of the linear and logarithmic trapezoidal rules, with extrapolation to infinity by dividing the last measurable serum concentration by the elimination rate constant (λ_z) (Proost, 1985). Values for the maximum concentration (C_{max}) and time to C_{max} (T_{max}) were determined by WinNonlin. The elimination half-life was determined from $0.693/\lambda_z$. Clearance (CL/F) was determined by dividing the dose

by $AUC_{0-\infty}$. Volumes of distribution for elimination (V_z/F) and at steady state (V_{ss}) were determined by moment curves (Gibaldi and Perrier, 1982). V_z/F was calculated as Dose/(λ_z* $AUC_{0-\infty}$). V_{ss} was calculated as CL * MRT for IV data. The absolute bioavailability (F) for the IN dosage form was determined by $F = AUC_{IN,0-\infty}/ AUC_{IV,0-\infty}$. Mean plasma concentrations were calculated for graphical evaluation only. The calculations included data from samples with measurable concentrations drawn within 5% of the expected sampling time.

Pharmacodynamic (PD) Data Analysis

Self-report measures were collected using Visual Analog Scales (VAS) and the Stanford Sleepiness Scale (SSS). The VAS and SSS were administered at 0 (pre-dose), 10, 20, 30, and 45 minutes, and 1, 1.5, 2, 3, 4, 6, 8, and 12 hours after initiation of the IV dose and administration of the IN doses. Observer Sedation Rating was also performed. The observer for each subject rated the degree of sedation using a qualitative categorical measure of sedation at 0 (pre-dose), 5, 10, 20, 30, and 45 minutes, and 1, 1.5, 2, 3, 4, 6, 8, and 12 hours after initiation of the IV dose and administration of the IN doses. The Observer's Assessment of Alertness/Sedation Scale was used to rate sedation at the above time points. The OAA/S Scale is composed of the following categories: responsiveness, speech, facial expression, and eyes. Subjects were evaluated in each category. The OAA/S was scored in two ways. A composite score was documented as the lowest score in any one of the four assessment categories. A sum score was calculated as the sum of the four category scores. Dependent variables were analyzed as a function of treatment. Analyses of peak effects, time to peak effects, and AUCs, using linear trapezoidal rules, were also evaluated. Separate AUC analyses were completed for AUC between baseline and 4 hours after dose (AUC4, over the duration of peak effects) as well as between baseline and last measurable point and 12 hours after dose (AUCall and AUC12, respectively).

Statistical Data Analysis

Statistical analyses were performed with PC-SAS (version 6.12, SAS Institute, Cary, North Carolina). The statistical tests for PK parameters were 2-sided with a critical level of 0.05 unless specified otherwise. An analysis of variance (ANOVA) with factors

sequence, subject(sequence), treatment and period was performed for log-transformed AUC and C_{max}. The least square geometric means from the ANOVA were used to calculate the ratios and their 90% confidence intervals between treatment groups for AUC and C_{max}. The carryover effect for the three treatments was also assessed using the ANOVA. The gender effect for all three treatments was analyzed using an ANOVA of log-transformed AUC and C_{max} with factors gender, treatment and period.
One subject 216's data for Treatment B was included in the summary statistics of PK parameters. However, Subjects 216 (with outlier for Treatment B) and 218 (early withdrawal) were excluded from the PK analyses for evaluable subjects.

Effects of treatment on each PD parameter were tested using ANOVA with factors sequence, subject(sequence), treatment and period. The carryover effects for the treatment PD effects were also assessed using ANOVA. In some cases, significant carryover was found but this was expected because repetition of tests has been shown to produce performance changes.

PK Results of Example 2

Seventeen subjects completed the study without clinically significant or serious adverse events. One subject received a single 2.5 mg IN dose and then did not return for subsequent treatments. There were no clinically relevant changes in physical examination, nasal evaluations, or laboratory tests. The principal investigator's review of the data indicated that, in general, doses of the study drug were well tolerated and events were mild to moderate and temporary. There were 1, 2 and no reports of dizziness after the 2.5 mg IV, 2.5 mg IN and 5.0 mg IN doses, respectively. Dizziness lasted up to 86 minutes. Three out of eighteen subjects noted blurred or double vision that lasted 5-40 minutes. No subjects experienced respiratory depression, apnea, laryngospasm, bronchospasm or wheezing.

The mean plasma concentration versus time curve profiles over the first 4 hours and the entire 12 hours for the three treatments are shown in Figures 3 and 4. Figure 3 shows that the absorption of MZ following IN administration was very rapid.

MZ concentrations reached a peak at 5 min in one-quarter to one-third of the individuals for the two IN treatments. Median T_{max} values were 10 min (range 5 to 20 min) for the 2.5 mg and 5.0 mg IN doses. Three individuals had C_{max} values after the 5.0 mg IN dose that were higher than the C_{max} after the 15 minute, 2.5 mg IV infusion. One subject had plasma concentrations that were low and they increased and decreased with no pattern. His elimination rate constant was indeterminant as a result. The concentrations ranged from 1.15 to 3.16 ng/mL over the 4 hour period and then dropped to below quantifiable limits.

Table 3 summarizes PK data for the three treatments. T_{max} values were not significantly different for the two IN treatments (P>0.2).

Table 3.
Mean (CV as a %) Single Dose MZ Pharmacokinetic (PK) Parameters Following Administration of Intravenous (IV) and Intranasal (IN) MZ in Healthy Subjects

1.2. PK Parameter	Treatment A 2.5 mg IV	Treatment B 2.5 mg IN	Treatment C 5.0 mg IN
T_{max} (min)*	15 (10-15)	10 (5-20)	10 (5-20)
C_{max} (ng/mL)	108.5 (13.5)	44.5 (38.4)	83.9 (28.9)
$t_{1/2}$ (hr)	4.03 (33.8)	4.00 (33.4)	4.07 (34.2)
AUC_{0-t} (ng•hr/mL)	109.2 (12.1)	65.8 (31.9)	130.9 (24.7)
$AUC_{0-\infty}$ (ng•hr/mL)	119.3 (14.1)	72.6 (30.6))	143.6 (24.5)
MRT (hr)	3.70 (31.7)	4.18 (33.8)	4.18 (28.3)
CL or CL/F(L/hr)	21.4 (14.3)	43.9 (93.9)	37.0 (26.6)
V_{ss} (L)	77.3 (25.2)	-	-

The actual doses administered presented were determined by weighing the pumps before and after dosing. They were lower that the intended doses, on average, by about 16% (Table 4). The range was from 38% below to 20% above the intended dose.

Table 4.
Mean (CV as a %) Dose Weights Following Administration of Intranasal (IN) MZ in Healthy Subjects

1.3.1 N Dose	N	Mean	%CV	Min	Max	% of Dose
2.5 mg	16	2.09	12.9	1.60	2.50	83.7
5.0 mg	17	4.22	7.98	3.77	5.21	84.4

Absolute bioavailability of the MZ was, on average, 60-61% for the IN doses. However, the absolute bioavailability of MZ by the IN routes in Table 3 is underestimated due to the less than expected dose delivery of the nasal sprayers. The dose weight data that are given in Table 4 show that on average, the delivered dose in this study was about 84% of the planned dose. Recalculating the bioavailability based on the actual doses administered (by weight) would make the bioavailability about 72% for the IN doses. No significant gender differences were found for $AUC_{0-\infty}$ and C_{max} values ($P > 0.1$). The gender effect was significant for dose-normalized AUC_{0-t} values ($P = 0.0371$, M > F). Data were combined for analysis of treatment effects. Statistical analysis of carryover effect on log transformed $AUC_{0-\infty}$, AUC_{0-t} and C_{max} for the two IN treatments was performed. P-values from an ANOVA with factors sequence, subject(sequence), treatment and period for sequence were >0.3, so the carryover effects were not significant and this implies the validity of the analyses in Table 5.

Table 5 summarizes the ratios and 90% confidence intervals (CI) of C_{max} and AUCs after Treatments A, B and C. The ratio of dose normalized C_{max} and AUC values were near unity after Treatment C (IN) compared to Treatment B (IN), as expected.

Table 5.
Summary of Ratios of Least Squares Geometric Means and 90% Confidence Intervals (Dose Normalized Parameters)

Parameter	Treatment Group Geometric Means			B/A (IN/IV)	C/A (IN/IV)	C/B (IN/IN)
	2.5 mg MZ IV (A)	2.5 mg MZ IN (B)	5.0 mg MZ IN (C)	Ratio (90%CI)	Ratio (90%CI)	Ratio (90%CI)
$AUC_{0-\infty}$ (ng•hr/mL)	47.80	29.13	28.42	0.61 (0.54-0.69)	0.59 (0.52-0.67)	0.98 (0.86-1.11)
AUC_{0-t} (ng•hr/mL)	43.67	26.31	25.75	0.60 (0.53-0.68)	0.59 (0.52-0.67)	0.98 (0.86-1.11)
C_{max} (ng/mL)	42.15	17.12	15.93	0.41 (0.34-0.48)	0.38 (0.32-0.45)	0.93 (0.78-1.11)

CI = Confidence Intervals
Log-transformed data are analyzed using an ANOVA with factors sequence, subject(sequence), treatment and period.
Dose normalized data are used (2.5 or 5.0 mg).

The α-hydroxymidazolam metabolite concentrations were consistently lower than those of the parent drug.

PD Results of Example 2

Table 6 summarizes analyses of PD VAS ratings. Cmax (peak effects), time to peak effects (Tmax), and areas under the ratings curves are given (AUC4, AUC12 and AUCall) for the VAS ratings. VAS parameters that showed statistical significance and their *P* values are listed in alphabetical order above the break in Table 6. These ratings illustrate the typical effects of dose and route on MZ PD. On 30 out of 40 measures, the order of magnitude of effects were identical with IV producing the greatest effects followed by the higher IN dose and then the lower IN dose. There were many trends in these data, however, only ratings of 10 parameters out of 40 reached significance. No differences were obtained on Tmax. No parameters for "willing to take drug again," "anxious" or "stimulated" reached significance. Due to the large number of missing values, the results from VAS ratings should be interpreted with caution. These statistical comparisons are presented for their usefulness in future study design.

Table 6.
Mean (SD) midazolam PD parameters following Treatments A, B and C

Parameter	Variable Name	2.5 mg MZ IV Treatment A	2.5 mg MZ IN Treatment B	5.0 mg MZ IN Treatment C	*P* Value
			P < 0.05		
fatigue	AUC12	158.88 (149.98)	75.24 (58.97)	108.71 (76.91)	0.0213
fatigue	AUC4	86.24 (53.67)	48.47 (38.88)	72.46 (46.95)	0.0054
fatigue	AUCall	140.83 (137.18)	78.79 (57.56)	99.45 (71.32)	0.0200
fatigue	Cmax	53.59 (22.17)	36.72 (21.14)	48.29 (20.68)	0.0080
Feel	AUC12	87.58 (53.23)	64.85 (44.60)	95.54 (54.92)	0.0430
Feel	AUC4	64.88 (33.38)	48.05 (34.88)	75.37 (47.54)	0.0211
Feel	Cmax	56.06 (17.52)	40.22 (26.43)	59.41 (21.11)	0.0085
High	Cmax	46.35 (26.07)	27.39 (18.08)	38.53 (22.61)	0.0053
Like	Cmax	61.31 (22.98)	47.38 (22.75)	70.00 (19.47)	0.0264
Sedate	Cmax	55.85 (19.27)	40.22 (22.70)	52.35 (13.60)	0.0157
			P > 0.05		
anxious	AUC12	54.79 (66.56)	46.18 (72.33)	54.76 (84.30)	0.4220
anxious	AUC4	29.78 (28.52)	18.64 (17.40)	25.95 (32.12)	0.0849
anxious	AUCall	53.76 (61.44)	49.71 (72.20)	52.94 (77.28)	0.2931
anxious	Cmax	26.79 (25.29)	15.36 (15.71)	19.26 (20.76)	0.1023
anxious	Tmax	0.51 (0.56)	1.52 (2.89)	2.41 (3.55)	0.0978
fatigue	Tmax	0.78 (0.50)	1.10 (1.58)	1.11 (1.44)	0.6626
Feel	AUCall	86.43 (53.96)	68.76 (46.02)	86.98 (54.25)	0.0646
Feel	Tmax	0.72 (0.52)	0.74 (0.92)	0.69 (0.50)	0.9469
High	AUC12	66.81 (38.03)	54.74 (42.79)	62.24 (51.11)	0.2549
High	AUC4	48.28 (30.67)	32.82 (25.47)	45.52 (38.64)	0.1299
High	AUCall	66.33 (41.50)	58.14 (45.62)	61.94 (49.98)	0.3256
High	Tmax	1.06 (2.85)	0.54 (0.69)	1.40 (2.83)	0.5662
Like	AUC12	339.54 (321.48)	270.93 (290.84)	309.07 (234.08)	0.6696
Like	AUC4	126.94 (78.08)	106.78 (86.06)	119.62 (64.44)	0.6350
Like	AUCall	288.98 (293.37)	246.16 (268.39)	253.80 (224.50)	0.8362
Like	Tmax	2.52 (3.01)	1.08 (1.83)	2.07 (2.81)	0.2344
Sedate	AUC12	95.76 (79.62)	68.43 (56.69)	99.77 (68.40)	0.0702
Sedate	AUC4	71.42 (46.70)	52.29 (45.44)	70.24 (40.68)	0.1273
Sedate	AUCall	93.47 (73.41)	73.15 (55.43)	92.02 (64.32)	0.0931
Sedate	Tmax	0.75 (0.50)	0.53 (0.47)	0.62 (0.37)	0.2946
Stim	AUC12	172.23 (195.40)	148.22 (182.23)	187.20 (201.87)	0.3108
Stim	AUC4	67.07 (52.47)	56.53 (53.38)	67.09 (50.51)	0.5830
Stim	AUCall	187.40 (196.21)	157.54 (177.94)	184.74 (192.46)	0.5364
Stim	Cmax	40.00 (21.78)	33.36 (20.50)	41.41 (22.03)	0.3008
Stim	Tmax	1.62 (2.90)	1.74 (2.86)	2.69 (3.93)	0.3200
Will	AUC12	739.92 (396.94)	690.63 (392.61)	714.86 (368.68)	0.5568
Will	AUC4	241.10 (102.12)	215.94 (122.09)	233.52 (108.09)	0.6826
Will	AUCall	699.79 (385.66)	638.61 (400.75)	704.00 (379.58)	0.5389
Will	Cmax	79.44 (16.62)	76.25 (25.26)	80.26 (20.05)	0.9669
Will	Tmax	3.09 (4.06)	2.73 (3.37)	2.76 (3.90)	0.9608

P values from ANOVA. Note: These ratings are not the same as the similarly named PK parameters. Units for parameters: Tmax (hr), Cmax (rating score), AUC4, AUC12 and AUCall (rating*hour).

Discussion

The pharmacokinetics of MZ were evaluated in healthy male and female volunteers after single 2.5 mg and 5.0 mg doses of IV and IN MZ. Seventeen out of eighteen subjects completed the study without clinically significant or serious adverse events. One male subject dropped out for scheduling reasons after receiving one treatment. The pharmacokinetics of MZ were consistent with rapid absorption (median peak times of 10 minutes after IN administration). but relatively short duration of action. The mean absolute bioavailability of IN MZ was approximately 60-61%. However, based on actual dose delivery weights, bioavailability was about 72% for the IN doses. The 84% delivery of doses was most likely because of under filling of sprayers during manufacturing. The remainder of the incomplete bioavailability after the IN administration may be explained by metabolism during absorption across the nasal mucosa or simply, incomplete absorption and swallowing. There was no evidence of swallowing but that would be expected due to the low oral bioavailability of MZ. Plasma clearance and volumes of distribution were high, as expected for MZ.

PD analyses indicated clearly that all three treatments produced changes in subjective ratings of sleep scores, VAS ratings and observer ratings. The intensity of the PD effects was greatest over the first 2 hours following dose administration. The order of magnitude of effects on all PD outcome measures were not always identical but in most cases, IV produced the largest or a similar duration/magnitude of effects compared to the high dose of IN MZ which was followed by the low IN MZ dose. The peak time of effects did not differ statistically between IV and IN doses. The onset did not vary with dose as much as the duration of effect did, as determined through the AUC analyses.

Conclusion

Intravenously administered MZ distributes extensively and rapidly in the body. A total systemic clearance of 21 L/hr indicates that MZ is a highly cleared drug. The IN formulation of MZ had rapid absorption with median times of 10 minutes to achieve peak concentrations . The rise in plasma concentrations matched the IV infusion in some cases. The α-hydroxymidazolam metabolite concentrations were consistently lower than those of the parent drug. The absolute bioavailability of MZ from the IN dosage form

was approximately 60% and supports further investigation of this dosage form for clinical use. PD analyses indicated clearly that all three treatments produced changes in subjective ratings of sleep scores, VAS ratings and observer ratings. The intensity of the PD effects was greatest over the first 2 hours following dose administration.

No treatment emergent adverse events were observed during the conduct of this protocol that would preclude further study of MZ in healthy subjects. Adverse events were unremarkable and expected for this drug. As evidenced by the lack of cardiovascular and respiratory adverse events, all the subjects tolerated the drug well.

Having now generally described the embodiments, the same may be more readily understood through the following reference to the following example, which is provided by way of illustration and is not intended to limit the present invention unless specified.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition for intranasal administration comprising: an effective amount of a benzodiazepine or pharmaceutically acceptable salt thereof; a nasal carrier; and at least one or more sweeteners, flavoring agents, or masking agents or combinations thereof.

2. A pharmaceutical composition according to claim 1, wherein the benzodiazepine is alprazolam, brotizolam, chlordiazepoxide, clobazepam, clonazepam, clorazepate, demoxepam, diazepam, estazolam, flurazepam, quazepam, halazepam, lorazepam, midazolam, nitrazepam, nordazapam, oxazepam, prazepam, quazepam, temazepam, triazolam, zolpidem, zaleplon or combinations thereof.

3. A pharmaceutical composition according to claim 2, wherein the benzodiazepine is midazolam.

4. A pharmaceutical composition according to claim 3, wherein the volume of the composition is about 0.1 ml.

5. A pharmaceutical composition according to claim 3, wherein the composition is preservative free.

6. A pharmaceutical composition according to claim 3, wherein the composition contains a buffer.

7. A pharmaceutical composition according to claim 3, wherein the composition is a sterile solution or suspension.

8. A pharmaceutical composition according to claim 3, wherein the composition contains an anesthetic agent.

9. A pharmaceutical composition according to claim 1, wherein the one or more sweeteners, flavoring agents or masking agents is saccharin, sodium saccharin, xylitol, mannitol, sorbitol, sucrose, sucralose, maltodextrin, aspartame, acesulfame potassium, dextrose, glycosides, maltose, sweet orange oil, glycerin, wintergreen oil, peppermint oil, peppermint water, peppermint spirit, menthol, or combinations thereof.

10. A pharmaceutical composition according to claim 1, wherein the composition has a pH of about 5.0.

11. A pharmaceutical composition for intranasal administration to a mammal: comprising: an effective amount of midazolam or pharmaceutically acceptable salt thereof, polyethylene glycol, saccharin powder, and propylene glycol.

12. A pharmaceutical composition according to claim 11, wherein the polyethylene glycol comprises from about 15% to about 25% by volume and the propylene glycol constitutes from about 75% to about 85% by volume of the composition.

13. A pharmaceutical composition according to claim 11, wherein the composition contains a preservative.

14. A pharmaceutical composition according to claim 11, wherein the composition is preservative-free.

15. A pharmaceutical composition according to claim 11, wherein the composition contains an anesthetic agent.

16. A pharmaceutical composition according to claim 11, wherein the composition achieves a time to maximum plasma concentration (T_{max}) within about 5 minutes to about 20 minutes after intranasal administration.

17. A pharmaceutical composition according to claim 11, wherein the composition achieves a time to maximum plasma concentration (T_{max}) within about 5 minutes after intranasal administration.

18. A pharmaceutical composition according to claim 11, wherein the composition achieves a maximum plasma concentration (C_{max}) of about 40ng/mL from a 2.5mg dose or about 80ng/mL from a 5mg dose after intranasal administration.

19. A pharmaceutical composition according to claim 18, wherein the ratio of the AUC for intranasal midazolam to AUC of for midazolam after an equivalent dose of intravenous midazolam is at least about 1:1.7.

20. A method of treating a mammal in need of rapid sedation, anxiolysis, amnesia, or induction of anesthesia comprising intranasally administering to the mammal an effective amount of a pharmaceutical composition comprising midazolam or pharmaceutically acceptable salt thereof; and a nasal carrier; wherein the rapid sedation, anxiolysis, amnesia, or induction of anesthesia occurs within 5 minutes after intranasal administration.

21. A method of treating a mammal in need of rapid sedation, anxiolysis, amnesia, or induction of anesthesia comprising intranasally administering to the mammal an effective amount of a pharmaceutical composition comprising midazolam or pharmaceutically acceptable salt thereof; a nasal carrier; and at

least one or more sweeteners, flavoring agents, or masking agents or combinations thereof.

22. A method according to claim 21, wherein the at least one sweetener, flavoring agent or masking agent is saccharin, sodium saccharin, xylitol, mannitol, sorbitol, sucrose, aspartame, acesulfame potassium, dextrose, glycosides, maltose, sweet orange oil, glycerin, wintergreen oil, peppermint oil, peppermint water, peppermint spirit, menthol, or combinations thereof.

23. A method according to claim 21, wherein the rapid sedation, anxiolysis, amnesia, or induction of anesthesia occurs within 5 minutes after intranasal administration.

24. A method according to claim 21, wherein the rapid sedation, anxiolysis, amnesia, or induction of anesthesia occurs at a time to maximum plasma concentration (T_{max}) of within 5 minutes after intranasal administration.

25. A method according to claim 21, wherein the pharmaceutical composition achieves a 1-hydroxymidazolam plasma level of about 1 to about 8 nanograms/ml after intranasal administration.

26. A method of making a pharmaceutical composition for intranasal administration comprising adding at least one or more sweeteners, flavoring agents, or masking agents or combinations thereof to a pharmaceutical composition comprising midazolam or pharmaceutically acceptable salt thereof, and a nasal carrier so as to make the pharmaceutical composition.

AMENDED CLAIMS
received by the International Bureau on 30 August 2005 (30.08.05): original claims 1-26 have been replaced by amended claims 1-20.

1. A pharmaceutical composition for intranasal administration comprising midazolam or a pharmaceutically acceptable salt thereof and a non-aqueous liquid nasal carrier, wherein upon intranasal administration of the composition to a group of human subjects in an amount of the composition sufficient to provide about 2.5 mg of midazolam per subject, the subjects exhibit a mean maximum plasma midazolam concentration (T_{max}) of at least about 40 ng/ml.
2. The pharmaceutical composition of claim 1, wherein the non-aqueous liquid nasal carrier comprises polyethylene glycol.
3. The pharmaceutical composition of claim 2, wherein the polyethylene glycol has an average molecular weight of about 400.
4. The pharmaceutical composition of claim 2, wherein the non-aqueous liquid nasal carrier further comprises propylene glycol.
5. The pharmaceutical composition of claim 4, wherein the polyethylene glycol constitutes about 20% of the composition by volume and the propylene glycol about 80% of the composition by volume.
6. The pharmaceutical composition of claim1 further comprising a sweetener.
7. The pharmaceutical composition of claim 6 wherein the sweetener is selected from saccharin, aspartame or mixtures thereof.
8. The pharmaceutical composition of claim 1 further comprising a preservative.
9. The pharmaceutical composition of claim 1 wherein upon intranasal administration of the composition to a group of human subjects, the subjects exhibit an average maximum plasma concentration (C_{max}) of midazolam at any time within about 5 minutes after administration.
10. The pharmaceutical composition of claim 9 wherein upon intranasal administration of the composition to a group of human subjects, the subjects exhibit an average AUC plasma concentration of midazolam of about 12 ng*hr/ml to about 100 ng*hr/ml.
11. The pharmaceutical composition of claim 1 wherein upon intranasal administration of the composition to a group of human subjects, the subjects

exhibit an average absolute bioavailability of midazolam, as a percentage of the total weight of midazolam delivered, of at least about 60%.

12. The pharmaceutical composition of claim 1 wherein upon intranasal administration of the composition to a group of human subjects, the subjects exhibit an average absolute bioavailability of midazolam, as a percentage of the total weight of midazolam delivered, of at least about 70%.

13. The pharmaceutical composition of claim 9 wherein upon intranasal administration of the composition to a group of human subjects, the subjects exhibit an α-hydroxymidazolam plasma concentrations of about 1 to about 8 ng/ml.

14. A method of treating a mammal in need of rapid sedation, anxiolysis, amnesia, or induction of anesthesia comprising intranasally administering to the mammal an effective amount of a pharmaceutical composition comprising midazolam or pharmaceutically acceptable salt thereof and a non-aqueous liquid nasal carrier, wherein the rapid sedation, anxiolysis, amnesia, or induction of anesthesia occurs within about 5 minutes after intranasal administration.

15. A method of treating a mammal in need of rapid sedation, anxiolysis, amnesia, or induction of anesthesia comprising intranasally administering to the mammal an effective amount of a pharmaceutical composition comprising midazolam or pharmaceutically acceptable salt thereof, a non-aqueous liquid nasal carrier, and at least one sweetener, flavoring agent, masking agent or combination thereof.

16. The method of claim 15, wherein the at least one sweetener, flavoring agent or masking agent is selected from saccharin, sodium saccharin, xylitol, mannitol, sorbitol, sucrose, aspartame, acesulfame potassium, dextrose, glycosides, maltose, sweet orange oil, glycerin, wintergreen oil, peppermint oil, peppermint water, peppermint spirit, menthol, or combinations thereof.

17. The method of claim 15, wherein the rapid sedation, anxiolysis, amnesia, or induction of anesthesia occurs within about 5 minutes after intranasal administration.

18. The method of claim 15, wherein the rapid sedation, anxiolysis, amnesia, or induction of anesthesia occurs within about 5 minutes after intranasal administration.

19. The method of claim 15, wherein upon intranasal administration of the pharmaceutical composition to a subject, the subject exhibits a mean α-hydroxymidazolam plasma concentration of about 1 to about 8 ng/ml.

20. A method of making a pharmaceutical composition for intranasal administration comprising the steps combining midazolam or pharmaceutically acceptable salt thereof, a non-aqueous liquid carrier, and one or more sweeteners, flavoring agents, or masking agents or combinations thereof to make the pharmaceutical composition.

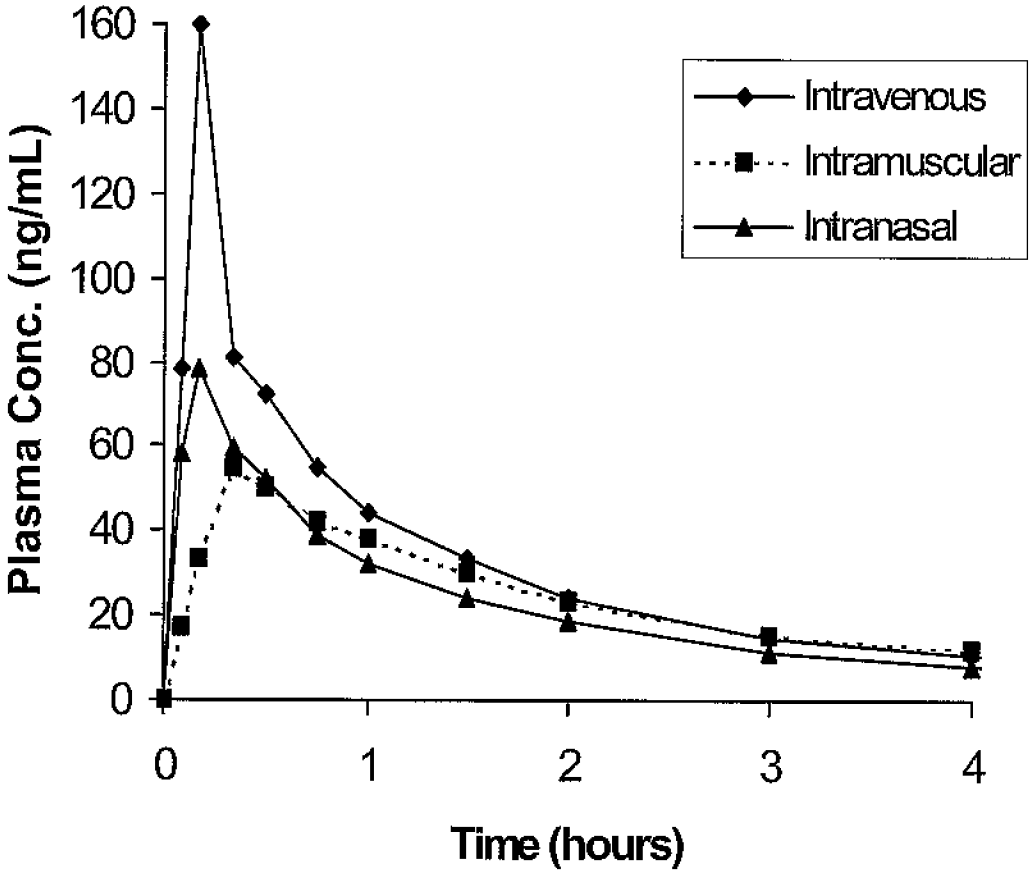


Figure 1. Mean (n=12) plasma midazolam concentration versus time profiles following 5.0 mg midazolam treatments A, B and C (IV, IM and IN, respectively).

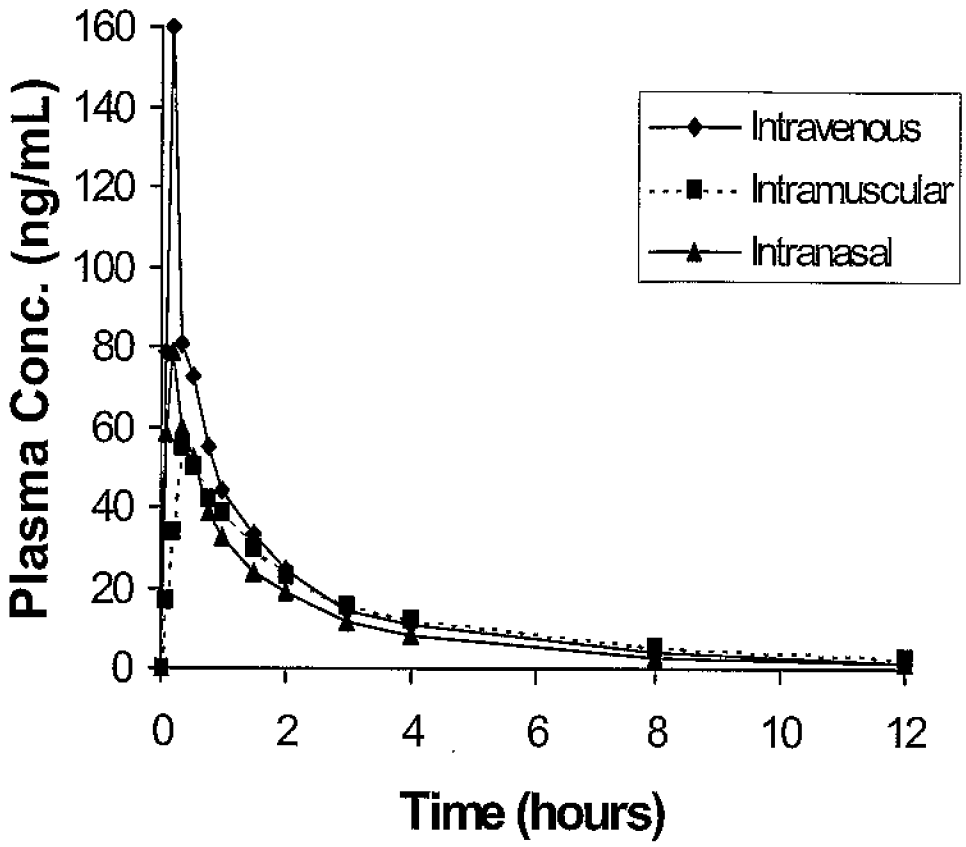


Figure 2. Mean (n=12) plasma midazolam concentration versus time profiles following 5.0 mg midazolam treatments A, B and C (IV, IM and IN, respectively).

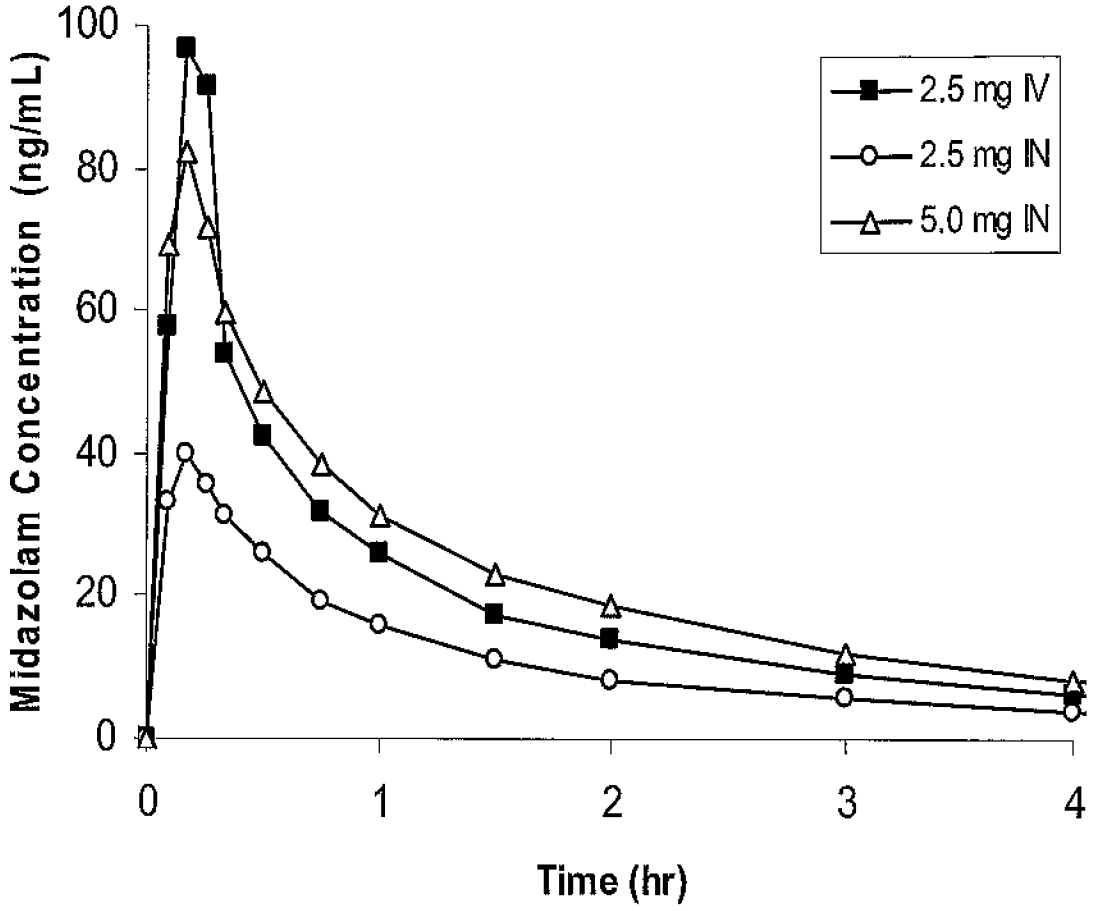


Figure 3. Mean (n=17) plasma midazolam concentration versus time profiles following treatments A, B and C (IV, IM and IN, respectively).

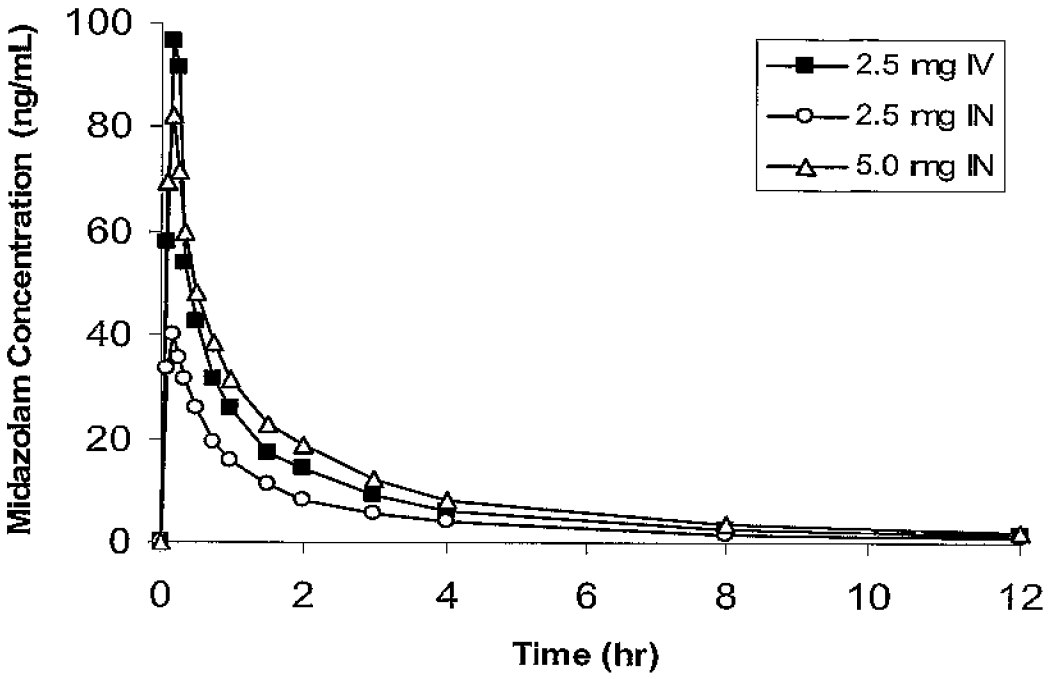


Figure 4. Mean (n=17) plasma midazolam concentration versus time profiles following treatments A, B and C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/08090

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 31/55; A61L 9/14
US CL : 514/219, 220; 424/45, 434
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/219, 220; 424/45, 434

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	RE 36,744 (GOLDBERG) 20 June 2000 (20.06.2000), entire document.	1-8, 10, 11, 13, 14, 16-21, 23, 24 --- 9, 11, 12, 15, 22, 25, 1-26
X	US 5,693,608 (BECHGAARD et al) 02 December 1997 (02.12.1997), column 7, lines 35 - 52.	
Y	US 5,474,759 (FASSBERG et al.), 12 December 1995 (12.12.1995), column 3, line 1 - column 4, line 50.	9, 22, 21, 26
X	WEBER, F. et al., Premedication with nasal s-ketamine and midazolam provides good conditions for induction of anesthesia in preschool children, Can. J. Anesth., 2003 50:5, pages 470-475.	1-4, 8, 20-, 21, 23-26

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 June 2005 (12.06.2005)	30 JUN 2005
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Sreeni Padmanabhan Telephone No. 703-308-1234

Form PCT/ISA/210 (second sheet) (January 2004)

Continuation of B. FIELDS SEARCHED Item 3:
WEST, GOOGLE, MEDLINE/PUBMED
benzodiazepine, nasal, midazolam, polyethylene glycol, propylene glycol, sedation anxiolysis amnesia anesthesia, AUC

AMENDED CLAIMS

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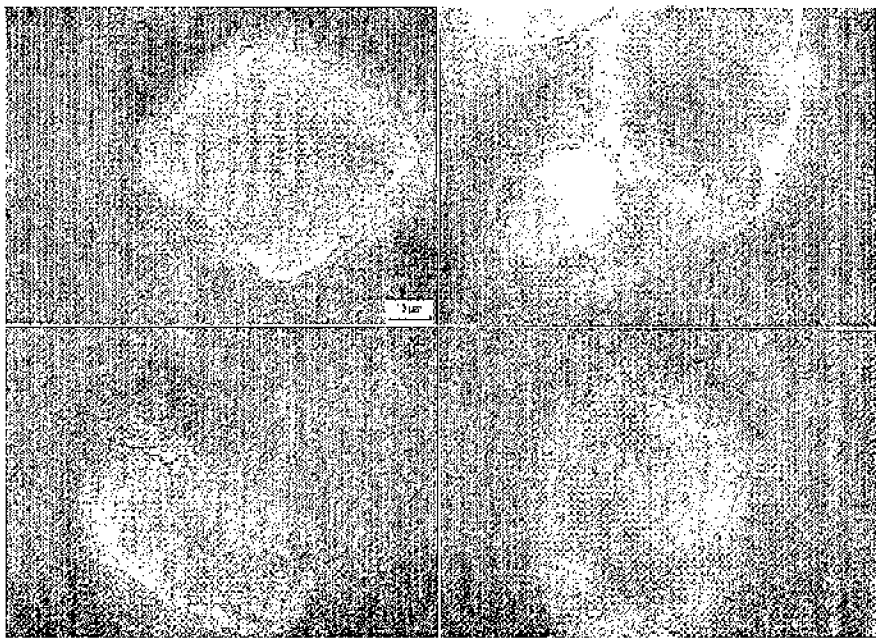
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: INJECTABLE NANOPARTICULATE OLANZAPINE FORMULATIONS

Olanzapine crystals prior to particle size reduction



(57) Abstract: Described are injectable formulations of nanoparticulate olanzapine that produce a prolonged duration of action upon administration, and methods of making and using such formulations. The injectable formulations comprise nanoparticulate olanzapine.

WO 2006/055603 A2

INJECTABLE NANOPARTICULATE OLANZAPINE FORMULATIONS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention is directed to novel delivery systems for psychotropic agents that ensure better patient compliance and therefore improved therapeutic efficacy and better overall mental health for the patient. More specifically, the present invention comprises injectable nanoparticulate olanzapine formulations having a prolonged duration of action.

Background of Invention

A. Background Regarding Olanzapine

Currently there are many drugs available for the treatment of disorders of the central nervous system. Among these drugs is a category known as antipsychotics for treating serious mental conditions such as schizophrenia and schizophreniform illness. The drugs available for such conditions are often associated with undesirable side effects, and there is a need for better products that control or eliminate the symptoms in a safer and more effective way. Furthermore, many patients do not respond or only partially respond to present drug treatment, and estimates of such partial-or non-responders vary between 40% and 80% of those treated.

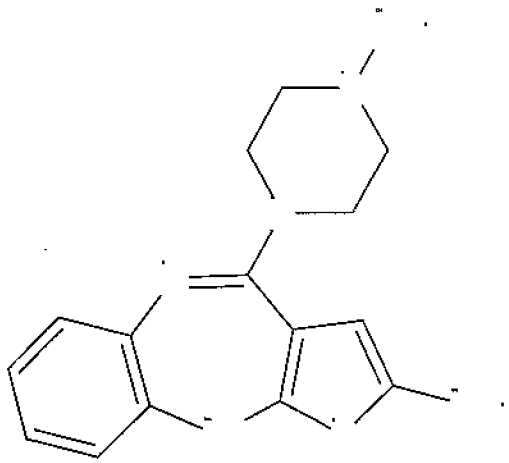
Since antipsychotics were introduced it has been observed that patients are liable to suffer from drug-induced extra pyramidal symptoms, which include drug-induced Parkinsonism, acute dystonic reactions, akathisia, tardive dyskinesia, and tardive dystonia. The Simpson Angus Scale, Barnes Akathisia Rating Scale, and Abnormal Involuntary Movement Scale (AIMS) are well known scales for assessing extra pyramidal symptoms. The great majority of drugs available for treatment of schizophrenia are prone to produce these extra pyramidal side effects when used at dosages that yield a beneficial effect on the

symptoms of the disease. The severity of adverse events and/or lack of efficacy in a considerable number of patients frequently result in poor compliance or termination of treatment.

Many of the drugs are associated with a sedative effect and may also have an undesirable influence on the affective symptoms of the disease, causing depression. In some instances long term use of the drug leads to irreversible conditions, such as the tardive dyskinesia and tardive dystonia referred to above. This, coupled with the fact that many of the patients in need of such drugs are not in full control of their mental faculties, often results in poor patient compliance and diminished therapeutic effect. A dosage form of such a drug having prolonged activity, and therefore requiring less frequent administrations, is highly desirable. This is because such a dosage form would minimize complications caused by patients missing or failing to take a dose.

A widely used and popular anti-psychotic drug useful in the treatment of disorders of the central nervous system is olanzapine, which is commercially available as Zyprexa® (Eli Lilly, Indianapolis, Ind.). Zyprexa® is available in both orally administered tablets and intramuscular injection formulations.

Olanzapine has the chemical name 2-methyl-4-(4-methyl-1-piperazinyl)-*10*H-thieno[2,3-*b*][1,5]benzodiazepine ($C_{17}H_{20}N_4S$), a molecular weight of 312.439, and the following chemical structure:



Olanzapine is a yellow crystalline solid which is practically insoluble in water. The compound is disclosed and claimed in U.S. Patent No. 5,229,382 to Chakrabarti et al., which is incorporated herein by reference.

Olanzapine is an antagonist of dopamine at D-1 and D-2 receptors, and in addition has antimuscarinic, anti-cholinergic properties, and is an antagonist for 5HT-2 receptor sites. The compound also has antagonist activity at noradrenergic alpha-receptors. These properties indicate that the compound is a potential neuroleptic with relaxant, anxiolytic, or anti-emetic properties, and is useful in treating psychotic conditions such as schizophrenia, schizophreniform diseases, and acute mania. At lower doses the compound is indicated for use in the treatment of mild anxiety states.

Olanzapine is a selective monoaminergic antagonist with high affinity binding to the following receptors serotonin 5HT $_{2A/2C}$ (K_I = 4 and 11nM, respectively), dopamine D $_{1-4}$ (K_I=11-31 $_I$ 25 nM), histamine H $_I$ (K_I =7nM), and adrenergic (alpha) $_1$ receptors (K_I = nM) GABA $_A$, BZD, and (beta) adrenergic receptors (K_I > 10 μM).

The mechanism of action of olanzapine, as with other drugs having efficacy in schizophrenia is unknown. However, it has been proposed that this drug's efficacy in schrizophrenia is mediated through a combination of dopamine and serotonin type 2 (5HT $_2$) antagonism. The mechanism of action of olanzapine in the treatment of acute manic episodes associated with Bipolar 1 Disorder is unknown.

Antagonism at receptor other than dopamine and 5HT $_2$ with similar receptor affinities may explain some of the other therapeutic and side effect of olanzapine. Olanzapine's antagonism of muscorinic M $_{1-5}$ receptors explains its anticholinergic effects. Olanzapine's antagonism of histamine H $_1$ receptors may explain somnolence observed with this drug. Olanzapine's antagonism of adrenergic (alpha) receptors may explain orthostatic hypotension observed with this drug.

B. Background Regarding Nanoparticulate Drugs

Bioavailability is the degree to which a drug becomes available to the target tissue after administration. Many factors can affect bioavailability including the dosage form and various properties, e.g., dissolution rate of the drug. Poor bioavailability is a significant problem encountered in the development of pharmaceutical compositions, particularly those containing an active ingredient that is poorly soluble in water. Poorly water soluble drugs tend to be unsafe for intravenous administration techniques, which are used primarily in conjunction with fully soluble drug substances.

It is known that the rate of dissolution of a particulate drug can increase with increasing surface area, i.e., decreasing particle size. Consequently, methods of making finely divided drugs have been studied and efforts have been made to control the size and size range of drug particles in pharmaceutical compositions. U.S. Patent No. 5,145,684 to Liversidge et. al., which is herein incorporated by reference, discloses particles of a drug substance having a non-crosslinked surface stabilizer absorbed on the surface thereof and methods for the preparation thereof. This patent does not teach or suggest nanoparticulate compositions of olanzapine.

Methods of making nanoparticulate compositions are described, for example, in U.S. Patent Nos. 5,518,187 and 5,862,999, both for "Method of Grinding Pharmaceutical Substances;" U.S. Patent No. 5,718,388, for "Continuous Method of Grinding Pharmaceutical Substances;" and U.S. Patent No. 5,510,118 for "Process of Preparing Therapeutic Compositions Containing Nanoparticles." These patents do not describe methods of making nanoparticulate olanzapine.

Nanoparticulate compositions are also described, for example, in U.S. Patent Nos. 5,298,262 for "Use of Ionic Cloud Point Modifiers to Prevent Particle Aggregation During Sterilization;" 5,302,401 for "Method to Reduce Particle Size Growth During Lyophilization;" 5,336,507 for "Use of Charged

Phospholipids to Reduce Nanoparticle Aggregation;" 5,340,564 for "Formulations Comprising Olin 10-G to Prevent Particle Aggregation and Increase Stability;" 5,346,702 for "Use of Non-Ionic Cloud Point Modifiers to Minimize Nanoparticulate Aggregation During Sterilization;" 5,352,459 for "Use of Purified Surface Modifiers to Prevent Particle Aggregation During Sterilization;" 5,399,363 and 5,494,683, both for "Surface Modified Anticancer Nanoparticles;" 5,429,824 for "Use of Tyloxapol as a Nanoparticulate Stabilizer;" 5,470,583 for "Method of Preparing Nanoparticle Compositions Containing Charged Phospholipids to Reduce Aggregation;" 5,518,738 for "Nanoparticulate NSAID Formulations;" 5,552,160 for "Surface Modified NSAID Nanoparticles;" 5,560,931 for "Formulations of Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" 5,565,188 for "Polyalkylene Block Copolymers as Surface Modifiers for Nanoparticles;" 5,569,448 for "Sulfated Non-ionic Block Copolymer Surfactant as Stabilizer Coatings for Nanoparticle Compositions;" 5,571,536 for "Formulations of Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" 5,573,783 for "Redispersible Nanoparticulate Film Matrices With Protective Overcoats;" 5,580,579 for "Site-specific Adhesion Within the GI Tract Using Nanoparticles Stabilized by High Molecular Weight, Linear Poly(ethylene Oxide) Polymers;" 5,585,108 for "Formulations of Oral Gastrointestinal Therapeutic Agents in Combination with Pharmaceutically Acceptable Clays;" 5,587,143 for "Butylene Oxide-Ethylene Oxide Block Copolymers Surfactants as Stabilizer Coatings for Nanoparticulate Compositions;" 5,591,456 for "Milled Naproxen with Hydroxypropyl Cellulose as Dispersion Stabilizer;" 5,622,938 for "Sugar Based Surfactant for Nanocrystals;" 5,718,919 for "Nanoparticles Containing the R(-)Enantiomer of Ibuprofen;" 5,747,001 for "Aerosols Containing Beclomethasone Nanoparticle Dispersions;" 5,834,025 for "Reduction of Intravenously Administered Nanoparticulate Formulation Induced Adverse

Physiological Reactions;" 6,045,829 "Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors Using Cellulosic Surface Stabilizers;" 6,068,858 for "Methods of Making Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors Using Cellulosic Surface Stabilizers;" 6,153,225 for "Injectable Formulations of Nanoparticulate Naproxen;" 6,165,506 for "New Solid Dose Form of Nanoparticulate Naproxen;" 6,221,400 for "Methods of Treating Mammals Using Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors;" 6,264,922 for "Nebulized Aerosols Containing Nanoparticle Dispersions;" 6,267,989 for "Methods for Preventing Crystal Growth and Particle Aggregation in Nanoparticle Compositions;" 6,270,806 for "Use of PEG-Derivatized Lipids as Surface Stabilizers for Nanoparticulate Compositions;" 6,316,029 for "Rapidly Disintegrating Solid Oral Dosage Form," 6,375,986 for "Solid Dose Nanoparticulate Compositions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate," 6,428,814 for "Bioadhesive nanoparticulate compositions having cationic surface stabilizers;" 6,431,478 for "Small Scale Mill;" 6,432,381 for "Methods for Targeting Drug Delivery to the Upper and/or Lower Gastrointestinal Tract," 6,592,903 for "Nanoparticulate Dispersions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate," 6,582,285 for "Apparatus for sanitary wet milling;" 6,656,504 for "Nanoparticulate Compositions Comprising Amorphous Cyclosporine;" 6,742,734 for "System and Method for Milling Materials;" 6,745,962 for "Small Scale Mill and Method Thereof;" 6,811,767 for "Liquid droplet aerosols of nanoparticulate drugs;" and 6,908,626 for "Compositions having a combination of immediate release and controlled release characteristics;" all of which are specifically incorporated by reference. In addition, U.S. Patent Application No. 20020012675 A1, published on January 31, 2002, for "Controlled Release Nanoparticulate Compositions,"

and WO 02/098565 for "System and Method for Milling Materials," describe nanoparticulate active agent compositions, and are specifically incorporated by reference. None of these references describe nanoparticulate compositions of olanzapine.

Amorphous small particle compositions are described, for example, in U.S. Patent Nos. 4,783,484 for "Particulate Composition and Use Thereof as Antimicrobial Agent;" 4,826,689 for "Method for Making Uniformly Sized Particles from Water-Insoluble Organic Compounds;" 4,997,454 for "Method for Making Uniformly-Sized Particles From Insoluble Compounds;" 5,741,522 for "Ultrasmall, Non-aggregated Porous Particles of Uniform Size for Entrapping Gas Bubbles Within and Methods;" and 5,776,496, for "Ultrasmall Porous Particles for Enhancing Ultrasound Back Scatter." These references do not describe nanoparticulate olanzapine.

There is a need in the art for nanoparticulate olanzapine formulations which overcome these and other problems associated with prior conventional olanzapine formulations. The present invention satisfies these needs.

SUMMARY OF THE INVENTION

The present invention relates to injectable nanoparticulate olanzapine compositions. The compositions comprise olanzapine and at least one surface stabilizer, which is preferably adsorbed on or associated with the surface of the olanzapine particles. The nanoparticulate olanzapine particles have an effective average particle size of less than about 5 microns. The surface stabilizer is present in an amount sufficient to maintain the olazapine at an effective average particle size that maintains the efficacy of the drug over a period of time, such as about one week or greater than about one week. The nanoparticle size of the olanzapine particles can be manipulated to give the desirable blood profile and

duration of action when administered by either intramuscular (IM) or subcutaneous (SC) routes.

Long acting anti-psychotics are preferred, as the patient population treated with such drugs can suffer from poor patient compliance, resulting in diminished therapeutic effect for the administered drug. Drugs requiring multiple daily administration, or even daily administration, are not preferred for this patient population. A simpler dosage form, such as a once-weekly dosage form, can result in dramatically improved patient compliance, and consequently improved quality of life. Advantages and properties of the compositions of the invention are described herein.

Another aspect of the invention is directed to pharmaceutical compositions comprising a nanoparticulate olanzapine composition of the invention. The pharmaceutical compositions preferably comprise olanzapine, at least one surface stabilizer, and at least one pharmaceutically acceptable carrier, as well as any desired excipients.

The invention further discloses a method of making a nanoparticulate olanzapine composition. Such a method comprises contacting olanzapine and at least one surface stabilizer for a time and under conditions sufficient to provide a nanoparticulate olanzapine composition. The one or more surface stabilizers can be contacted with olanzapine either before, preferably during, or after size reduction of the olanzapine.

The present invention is also directed to methods of treatment using the injectable nanoparticulate olanzapine compositions of the invention for, for example, psychotropic therapy and the treatment of central nervous system disorders. In one embodiment of the invention, intramuscular or subcutaneous injection of olanzapine is utilized. The administration of the drug in this manner allows for the formation of an intramuscular or subcutaneous depot of olanzapine which slowly releases the drug into the patient's system over a longer period of

time than if administered orally. The period of time over which the drug is released is preferably up to about one week, from about two weeks to about six weeks, and from about two weeks to about twelve weeks. Additional time periods of efficacy are described herein. This allows for improved patient compliance with enhanced therapeutic outcomes. Moreover, injectable formulations of olanzapine result in a significantly shorter response time as compared to oral administration. While current conventional formulations of olanzapine can be formulated for injection (i.e., Zyprexa®), such conventional injectable olanzapine formulations are difficult to prepare due to the low water solubility of the drug.

In psychotropic therapy and the treatment of central nervous system disorders, it is important to provide an olanzapine dosage form that delivers the required therapeutic amount of the drug *in vivo* and renders the drug bioavailable in a rapid and consistent manner. The nanoparticulate olanzapine formulations of the present invention achieve those goals through the formation of a drug depot, preferably following intramuscular injection. The depot slowly releases the drug into the bloodstream at almost zero order kinetics for about one (1) to about twelve (12) weeks through control of the nanoparticle size of the drug. Different nanoparticle sizes will dissolve at different rates, and will therefore release the drug to the bloodstream from the depot at different release rates.

Both the foregoing general description and the following brief description of the drawings and detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed. Other objects, advantages, and novel features will be readily apparent to those skilled in the art from the following detailed description of the invention.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1: Shows an electron micrograph of unmilled olanzapine.

Figure 2: Shows an electron micrograph of a milled nanoparticulate olanzapine formulation.

Figure 3: Shows an electron micrograph of a milled nanoparticulate olanzapine formulation.

Figure 4: Graphically shows the plasma concentration (ng/mL) of olanazpine over a six hour time period following intramuscular administration to six male dogs of a nanoparticulate olanzapine formulation.

Figure 5: Graphically shows the plasma concentration (ng/mL) of olanazpine over a six hour time period following intramuscular administration to six male dogs of a nanoparticulate olanzapine formulation.

Detailed Description of Invention

The invention provides injectable nanoparticulate olanzapine formulations that can comprise high drug concentrations in low injection volumes, with durations of action that can be controlled to give efficacious blood levels through manipulation of particle size and hence dissolution for periods of about one week or greater.

In other embodiments of the invention, compositions of the invention provide efficacious levels of drug from about one week to about two weeks, from about one week to about three weeks, from about one week to about four weeks, from about one week to about five weeks, from about one week to about six weeks, from about one week to about seven weeks, from about one week to about eight weeks, from about one week to about nine weeks, from about one week to about ten weeks, from about one week to about eleven weeks, from about one week to about twelve weeks, and any combination thereof, such as from about two weeks to about six weeks, from about three weeks to about four weeks, from about three weeks to about seven weeks, etc.

The composition of the invention is administered via injection, such as by intramuscular or subcutaneously, to form a drug depot. The drug depot results in efficacious levels of drug up to about one week or greater.

As taught in U.S. Patent No. 5,145,684, not every combination of surface stabilizer and active agent will result in a stable nanoparticulate composition. It was surprisingly discovered that stable, injectable, nanoparticulate olanzapine formulations can be made.

The current formulations of olanzapine suffer from the following problems: (1) the poor solubility of the drug results in a relatively low bioavailability; (2) dosing must be repeated several times each day; and (3) a wide variety of side effects are associated with the current dosage forms of the drug.

The present invention overcomes problems encountered with the prior art olanzapine formulations. Specifically, the nanoparticulate olanzapine formulations of the invention may offer the following advantages: (1) a decrease in the frequency of dosing and/or prolonged therapeutic levels of drug following dosing; (2) faster onset of action; (3) smaller doses of olanzapine required to obtain the same pharmacological effect; (4) increased bioavailability; (5) improved performance characteristics for intravenous, subcutaneous, or intramuscular injection, such as higher dose loading and smaller liquid dose volumes; (6) improved pharmacokinetic profiles, such as improved C_{max} and AUC profiles; (7) substantially similar or bioequivalent pharmacokinetic profiles of the nanoparticulate olanzapine compositions when administered in the fed versus the fasted state; (8) bioadhesive olanzapine formulations, which can coat the desired site of application and be retained for a period of time, thereby increasing the efficacy of the drug as well as eliminating or decreasing the frequency of dosing; (9) high redispersibility of the nanoparticulate olanzapine particles present in the compositions of the invention following administration;

(10) low viscosity liquid nanoparticulate olanzapine dosage forms can be made; (11) the nanoparticulate olanzapine compositions can be used in conjunction with other active agents; (12) the nanoparticulate olanzapine compositions can be sterile filtered; (13) the nanoparticulate olanzapine compositions are suitable for parenteral administration; and (14) the nanoparticulate olanzapine compositions do not require organic solvents or pH extremes.

A preferred dosage form of the invention is a liquid injectable formulation. However, the composition may also be formulated in a powder or solid for reconstitution prior to injectable administration, such as by lyophilization. The dosage form can be, for example, controlled release dosage form, delayed release dosage form, extended release dosage form, pulsatile release dosage form, mixed immediate release and controlled release dosage form, or a combination thereof.

The present invention is described herein using several definitions, as set forth below and throughout the application.

As used herein, "about" will be understood by persons of ordinary skill in the art and will vary to some extent on the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art given the context in which it is used, "about" will mean up to plus or minus 10% of the particular term.

"Conventional" or "non-nanoparticulate active agent" shall mean an active agent which is solubilized or which has an effective average particle size of greater than about 5 microns. Nanoparticulate active agents as defined herein have an effective average particle size of less than about 5 microns.

"Poorly water soluble drugs" as used herein means those having a solubility of less than about 30 mg/ml, preferably less than about 20 mg/ml, preferably less than about 10 mg/ml, or preferably less than about 1 mg/ml.

As used herein with reference to stable drug particles, 'stable' includes, but is not limited to, one or more of the following parameters: (1) that the olanzapine particles do not appreciably flocculate or agglomerate due to interparticle attractive forces, or otherwise significantly increase in particle size over time; (2) that the physical structure of the olanzapine particles is not altered over time, such as by conversion from an amorphous phase to crystalline phase; (3) that the olanzapine particles are chemically stable; and/or (4) where the olanzapine has not been subject to a heating step at or above the melting point of the olanzapine in the preparation of the nanoparticles of the invention.

'Therapeutically effective amount' as used herein with respect to a drug dosage, shall mean that dosage that provides the specific pharmacological response for which the drug is administered in a significant number of subjects in need of such treatment. It is emphasized that 'therapeutically effective amount,' administered to a particular subject in a particular instance will not always be effective in treating the diseases described herein, even though such dosage is deemed a 'therapeutically effective amount' by those skilled in the art. It is to be further understood that drug dosages are, in particular instances, measured as injectable dosages.

Enhanced pK Profiles

The invention also preferably provides olanzapine compositions having a desirable pharmacokinetic profile when administered to mammalian subjects. The desirable pharmacokinetic profile of the olanzapine compositions preferably includes, but is not limited to: (1) a C_{max} for olanzapine, when assayed in the plasma of a mammalian subject following administration, that is preferably greater than the C_{max} for a non-nanoparticulate olanzapine formulation (*e.g.*, Zyprexa®), administered at the same dosage; and/or (2) an AUC for olanzapine, when assayed in the plasma of a mammalian subject following administration,

that is preferably greater than the AUC for a non-nanoparticulate olanzapine formulation (*e.g.*, Zyprexa®), administered at the same dosage. The desirable pharmacokinetic profile, as used herein, is the pharmacokinetic profile measured after the initial injectable dose of olanzapine.

Conventional olanzapine (*e.g.*, Zyprexa®), reaches peak plasma levels in 5-8 hours, and has a half-life of about 35 hours, depending on metabolism.

A preferred injectable olanzapine composition of the invention exhibits in comparative pharmacokinetic testing with a non-nanoparticulate olanzapine formulation of (*e.g.*, Zyprexa®), administered at the same dosage, a C_{max} which is at least about 50%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500%, at least about 600%, at least about 700%, at least about 800%, at least about 900%, at least about 1000%, at least about 1100%, at least about 1200%, at least about 1300%, at least about 1400%, at least about 1500%, at least about 1600%, at least about 1700%, at least about 1800%, or at least about 1900% greater than the C_{max} exhibited by the non-nanoparticulate olanzapine formulation.

A preferred injectable olanzapine composition of the invention exhibits in comparative pharmacokinetic testing with a non-nanoparticulate olanzapine formulation (*e.g.*, Zyprexa®), administered at the same dosage, an AUC which is at least about 25%, at least about 50%, at least about 75%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 225%, at least about 250%, at least about 275%, at least about 300%, at least about 350%, at least about 400%, at least about 450%, at least about 500%, at least about 550%, at least about 600%, at least about 750%, at least about 700%, at least about 750%, at least about 800%, at least about 850%, at least about 900%, at least about 950%, at least about 1000%, at least about 1050%, at least about 1100%, at least about 1150%, or at least about 1200%

greater than the AUC exhibited by the non-nanoparticulate olanzapine formulation.

Combination Pharmacokinetic Profile Compositions

In yet another embodiment of the invention, a first nanoparticulate olanzapine composition providing a desired pharmacokinetic profile is co-administered, sequentially administered, or combined with at least one other olanzapine composition that generates a desired different pharmacokinetic profile. More than two olanzapine compositions can be co-administered, sequentially administered, or combined. While the first olanzapine composition has a nanoparticulate particle size, the additional one or more olanzapine compositions can be nanoparticulate, solubilized, or have a microparticulate particle size.

The second, third, fourth, *etc.*, olanzapine compositions can differ from the first, and from each other, for example: (1) in the effective average particle sizes of olanzapine; or (2) in the dosage of olanzapine. Such a combination composition can reduce the dose frequency required.

If the second olanzapine composition has a nanoparticulate particle size, then preferably the olanzapine particles of the second composition have at least one surface stabilizer associated with the surface of the drug particles. The one or more surface stabilizers can be the same as or different from the surface stabilizer(s) present in the first olanzapine composition.

Preferably where co-administration of a "fast-acting" formulation and a "longer-lasting" formulation is desired, the two formulations are combined within a single composition, for example a dual-release composition.

A. Olanazpine Compositions

The invention provides compositions comprising nanoparticulate olanzapine particles and at least one surface stabilizer. The surface stabilizers are preferably adsorbed to or associated with the surface of the olanzapine particles. Surface stabilizers useful herein do not chemically react with the olanzapine particles or itself. Preferably, individual molecules of the surface stabilizer are essentially free of intermolecular cross-linkages. The compositions can comprise two or more surface stabilizers.

The present invention also includes nanoparticulate olanzapine compositions together with one or more non-toxic physiologically acceptable carriers, adjuvants, or vehicles, collectively referred to as carriers. The compositions can be formulated for parenteral injection (*e.g.*, intravenous, intramuscular, or subcutaneous).

Olanzapine can be in a crystalline phase, an amorphous phase, a semi-crystalline phase, a semi-amorphous phase, or a mixtures thereof.

Illustrative but not limiting compositions comprise, based on % w/w:

Olanzapine	5 – 50%
Surface stabilizer	0.1 – 50%
preservatives (Optional)	0.05 - 0.25%
pH adjusting agent	pH about 6 to about 7
water for injection	q.s.

1. Surface Stabilizers

The choice of a surface stabilizer for olanzapine is non-trivial and required experimentation to realize a desirable formulation. Combinations of more than one surface stabilizer can be used in the invention. Useful surface stabilizers which can be employed in the invention include, but are not limited to, known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products, and

surfactants. Surface stabilizers include nonionic, ionic, anionic, cationic, and zwitterionic surfactants.

Preferred surface stabilizers include, but are not limited to, a polysorbate, such as Tween 80, benzalkonium chloride, and combinations thereof.

Representative examples of useful surface stabilizers include but are not limited to Low viscosity hydroxypropyl cellulose (HPC or HPC-SL); hydroxypropyl methyl cellulose (HPMC); hydroxymethyl cellulose (HMC); ethycellulose; povidone; Pluronics; sodium deoxycholate; PEG-Phospholipids; Tyloxapol and other approved tritons, polyvinylpyrrolidone, sodium lauryl sulfate, dioctylsulfosuccinate, gelatin, casein, lecithin (phosphatides), dextran, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers (*e.g.*, macrogol ethers such as cetomacrogol 1000), polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters (*e.g.*, the commercially available Tweens® such as *e.g.*, Tween 20® and Tween 80® (ICI Speciality Chemicals)); polyethylene glycols (*e.g.*, Carbowaxs 3550® and 934® (Union Carbide)), polyoxyethylene stearates, colloidal silicon dioxide, phosphates, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminium silicate, triethanolamine, polyvinyl alcohol (PVA), 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde (also known as tyloxapol, superione, and triton), poloxamers (*e.g.*, Pluronics F68® and F108®, which are block copolymers of ethylene oxide and propylene oxide); poloxamines (*e.g.*, Tetronic 908®, also known as Poloxamine 908®, which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine (BASF Wyandotte Corporation, Parsippany, N.J.)); Tetronic 1508® (T-1508) (BASF Wyandotte Corporation),

Tritons X-200®, which is an alkyl aryl polyether sulfonate (Rohm and Haas); Crodestas F-110®, which is a mixture of sucrose stearate and sucrose distearate (Croda Inc.); p-isononylphenoxypoly-(glycidol), also known as Olin-lOG® or Surfactant 10-G® (Olin Chemicals, Stamford, CT); Crodestas SL-40® (Croda, Inc.); and SA9OHCO, which is $C_{18}H_{37}CH_2(CON(CH_3)$-$CH_2(CHOH)_4(CH_2OH)_2$ (Eastman Kodak Co.); decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; PEG-derivatized phospholipid, PEG- derivatized cholesterol, PEG- derivatized cholesterol derivative, PEG- derivatized vitamin A, PEG- derivatized vitamin E, lysozyme, random copolymers of vinyl pyrrolidone and vinyl acetate, and the like.

Povidone Polymers

In one embodiment of the invention, a povidone polymer is utilized as a surface stabilizer. Povidone polymers for injectable compositions preferably have a molecular weight of less than about 40,000 daltons. Povidone polymers, also known as polyvidon(e), povidonum, PVP, and polyvinylpyrrolidone, are sold under the trade names Kollidon® (BASF Corp.) and Plasdone® (ISP Technologies, Inc.). They are polydisperse macromolecular molecules, with a chemical name of 1-ethenyl-2-pyrrolidinone polymers and 1-vinyl-2-pyrrolidinone polymers. Povidone polymers are produced commercially as a series of products having mean molecular weights ranging from about 10,000 to about 700,000 daltons. To be useful as a surface modifier for a drug compound to be administered to a mammal, the povidone polymer must have a molecular

weight of less than about 40,000 daltons, as a molecular weight of greater than 40,000 daltons would have difficulty clearing the body.

Povidone polymers are prepared by, for example, Reppe's process, comprising: (1) obtaining 1,4-butanediol from acetylene and formaldehyde by the Reppe butadiene synthesis; (2) dehydrogenating the 1,4-butanediol over copper at 200° to form γ-butyrolactone; and (3) reacting γ-butyrolactone with ammonia to yield pyrrolidone. Subsequent treatment with acetylene gives the vinyl pyrrolidone monomer. Polymerization is carried out by heating in the presence of H_2O and NH_3. *See The Merck Index*, 10th Edition, pp. 7581 (Merck & Co., Rahway, NJ, 1983).

The manufacturing process for povidone polymers produces polymers containing molecules of unequal chain length, and thus different molecular weights. The molecular weights of the molecules vary about a mean or average for each particular commercially available grade. Because it is difficult to determine the polymer's molecular weight directly, the most widely used method of classifying various molecular weight grades is by K-values, based on viscosity measurements. The K-values of various grades of povidone polymers represent a function of the average molecular weight, and are derived from viscosity measurements and calculated according o Fikentscher's formula.

The weight-average of the molecular weight, Mw, is determined by methods that measure the weights of the individual molecules, such as by light scattering. Table 1 provides molecular weight data for several commercially available povidone polymers, all of which are soluble.

TABLE 1

Povidone	K-Value	Mv (Daltons)**	Mw (Daltons)**	Mn (Daltons)**
Plasdone C-15®	17 ± 1	7,000	10,500	3,000
Plasdone C-30®	30.5 ± 1.5	38,000	62,500*	16,500
Kollidon 12 PF®	11-14	3,900	2,000-3,000	1,300
Kollidon 17 PF®	16-18	9,300	7,000-11,000	2,500
Kollidon 25®	24-32	25,700	28,000-34,000	6,000

*Because the molecular weight is greater than 40,000 daltons, this povidone polymer is not useful as a surface stabilizer for a drug compound to be administered parenterally (*i.e.*, injected).
**Mv is the viscosity-average molecular weight, Mn is the number-average molecular weight, and Mw is the weight average molecular weight. Mw and Mn were determined by light scattering and ultra-centrifugation, and Mv was determined by viscosity measurements.

Based on the data provided in Table 1, exemplary preferred commercially available povidone polymers for injectable compositions include, but are not limited to, Plasdone C-15®, Kollidon 12 PF®, Kollidon 17 PF®, and Kollidon 25®.

Cationic Surface Stabilizers

Depending upon the desired method of administration, bioadhesive formulations of nanoparticulate olanzapine can be prepared by selecting one or more cationic surface stabilizers that impart bioadhesive properties to the resultant composition. Useful cationic surface stabilizers are described below.

Examples of useful cationic surface stabilizers include, but are not limited to, polymers, biopolymers, polysaccharides, cellulosics, alginates, phospholipids, and nonpolymeric compounds, such as zwitterionic stabilizers, poly-n-methylpyridinium, anthryul pyridinium chloride, cationic phospholipids, chitosan, polylysine, polyvinylimidazole, polybrene, polymethylmethacrylate trimethylammoniumbromide bromide (PMMTMABr), hexyldesyltrimethylammonium bromide (HDMAB), polyvinylpyrrolidone-2-

dimethylaminoethyl methacrylate dimethyl sulfate, 1,2 Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-[Amino(Polyethylene Glycol)2000] (sodium salt) (also known as DPPE-PEG(2000)-Amine Na) (Avanti Polar Lipids, Alabaster, Al), Poly(2-methacryloxyethyl trimethylammonium bromide) (Polysciences, Inc., Warrington, PA) (also known as S1001), poloxamines such as Tetronic 908®, also known as Poloxamine 908®, which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine (BASF Wyandotte Corporation, Parsippany, N.J.), lysozyme, long-chain polymers such as alginic acid, carrageenan (FMC Corp.), and POLYOX (Dow, Midland, MI).

Other useful cationic stabilizers include, but are not limited to, cationic lipids, sulfonium, phosphonium, and quarternary ammonium compounds, such as stearyltrimethylammonium chloride, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride or bromide, coconut methyl dihydroxyethyl ammonium chloride or bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride or bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride or bromide, coconut dimethyl hydroxyethyl ammonium chloride or bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride or bromide, lauryl dimethyl (ethenoxy)$_4$ ammonium chloride or bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts and dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt and/or an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl

ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride and dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12}, C_{15}, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride (ALIQUAT 336™), POLYQUAT 10™, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters (such as choline esters of fatty acids), benzalkonium chloride, stearalkonium chloride compounds (such as stearyltrimonium chloride and Di-stearyldimonium chloride), cetyl pyridinium bromide or chloride, halide salts of quaternized polyoxyethylalkylamines, MIRAPOL™ and ALKAQUAT™ (Alkaril Chemical Company), alkyl pyridinium salts; amines, such as alkylamines, dialkylamines, alkanolamines, polyethylenepolyamines, N,N-dialkylaminoalkyl acrylates, and vinyl pyridine, amine salts, such as lauryl amine acetate, stearyl amine acetate, alkylpyridinium salt, and alkylimidazolium salt, and amine oxides; imide azolinium salts; protonated quaternary acrylamides; methylated quaternary polymers, such as poly[diallyl dimethylammonium chloride] and poly-[N-methyl vinyl pyridinium chloride]; and cationic guar.

Such exemplary cationic surface stabilizers and other useful cationic surface stabilizers are described in J. Cross and E. Singer, *Cationic Surfactants: Analytical and Biological Evaluation* (Marcel Dekker, 1994); P. and D. Rubingh (Editor), *Cationic Surfactants: Physical Chemistry* (Marcel Dekker, 1991); and J. Richmond, *Cationic Surfactants: Organic Chemistry,* (Marcel Dekker, 1990).

Nonpolymeric cationic surface stabilizers are any nonpolymeric compound, such as benzalkonium chloride, a carbonium compound, a phosphonium compound, an oxonium compound, a halonium compound, a cationic organometallic compound, a quarternary phosphorous compound, a pyridinium compound, an anilinium compound, an ammonium compound, a hydroxylammonium compound, a primary ammonium compound, a secondary ammonium compound, a tertiary ammonium compound, and quarternary ammonium compounds of the formula $NR_1R_2R_3R_4^{(+)}$. For compounds of the formula $NR_1R_2R_3R_4^{(+)}$:

(i) none of R_1-R_4 are CH_3;

(ii) one of R_1-R_4 is CH_3;

(iii) three of R_1-R_4 are CH_3;

(iv) all of R_1-R_4 are CH_3;

(v) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 is an alkyl chain of seven carbon atoms or less;

(vi) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 is an alkyl chain of nineteen carbon atoms or more;

(vii) two of R_1-R_4 are CH_3 and one of R_1-R_4 is the group $C_6H_5(CH_2)_n$, where n>1;

(viii) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 comprises at least one heteroatom;

(ix) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 comprises at least one halogen;

(x) two of R_1-R_4 are CH_3, one of R_1-R_4 is $C_6H_5CH_2$, and one of R_1-R_4 comprises at least one cyclic fragment;

(xi) two of R_1-R_4 are CH_3 and one of R_1-R_4 is a phenyl ring; or

(xii) two of R_1-R_4 are CH_3 and two of R_1-R_4 are purely aliphatic fragments.

Such compounds include, but are not limited to, behenalkonium chloride, benzethonium chloride, cetylpyridinium chloride, behentrimonium chloride, lauralkonium chloride, cetalkonium chloride, cetrimonium bromide, cetrimonium chloride, cethylamine hydrofluoride, chlorallylmethenamine chloride (Quaternium-15), distearyldimonium chloride (Quaternium-5), dodecyl dimethyl ethylbenzyl ammonium chloride(Quaternium-14), Quaternium-22, Quaternium-26, Quaternium-18 hectorite, dimethylaminoethylchloride hydrochloride, cysteine hydrochloride, diethanolammonium POE (10) oletyl ether phosphate, diethanolammonium POE (3)oleyl ether phosphate, tallow alkonium chloride, dimethyl dioctadecylammoniumbentonite, stearalkonium chloride, domiphen bromide, denatonium benzoate, myristalkonium chloride, laurtrimonium chloride, ethylenediamine dihydrochloride, guanidine hydrochloride, pyridoxine HCl, iofetamine hydrochloride, meglumine hydrochloride, methylbenzethonium chloride, myrtrimonium bromide, oleyltrimonium chloride, polyquaternium-1, procainehydrochloride, cocobetaine, stearalkonium bentonite, stearalkoniumhectonite, stearyl trihydroxyethyl propylenediamine dihydrofluoride, tallowtrimonium chloride, and hexadecyltrimethyl ammonium bromide.

Most of these surface stabilizers are known pharmaceutical excipients and are described in detail in the *Handbook of Pharmaceutical Excipients*, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain (The Pharmaceutical Press, 2000), specifically incorporated by reference.

The surface stabilizers are commercially available and/or can be prepared by techniques known in the art.

While applicants do not wish to be bound by theoretical mechanisms, it is believed that the stabilizer hinders the flocculation and/or agglomeration of the olanzapine particles by functioning as a mechanical or steric barrier between the

particles, minimizing the close, interparticle approach necessary for agglomeration and flocculation.

2. Excipients

Exemplary preservatives include methylparaben (about 0.18% based on % w/w), propylparaben (about 0.02% based on % w/w), phenol (about 0.5% based on % w/w), and benzyl alcohol (up to 2% v/v). An exemplary pH adjusting agent is sodium hydroxide, and an exemplary liquid carrier is sterile water for injection. Other useful preservatives, pH adjusting agents, and liquid carriers are well-known in the art.

3. Nanoparticulate Olanzapine Particle Size

As used herein, particle size is determined on the basis of the weight average particle size as measured by conventional particle size measuring techniques well known to those skilled in the art. Such techniques include, for example, sedimentation field flow fractionation, photon correlation spectroscopy, light scattering, and disk centrifugation.

The compositions of the invention comprise olanzapine nanoparticles which have an effective average particle size of less than about 5 microns. In other embodiments of the invention, the olanzapine particles have a size of less than about 4900 nm, less than about 4800 nm, less than about 4700 nm, less than about 4600 nm, less than about 4500 nm, less than about 4400 nm, less than about 4300 nm, less than about 4200 nm, less than about 4100 nm, less than about 4 microns, less than about 3900 nm, less than about 3800 nm, less than about 3700 nm, less than about 3600 nm, less than about 3500 nm, less than about 3400 nm, less than about 3300 nm, less than about 3200 nm, less than about 3100 nm, less than about 3 microns, less than about 2900 nm, less than about 2800 nm, less than about 2700 nm, less than about 2600 nm, less than

about 2500 nm, less than about 2400 nm, less than about 2300 nm, less than about 2200 nm, less than about 2100 nm, less than about 2000 nm, less than about 1900 nm, less than less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 140 nm, less than about 130 nm, less than about 120 nm, less than about 110 nm, less than about 100 nm, less than about 90 nm, less than about 80 nm, less than about 70 nm, less than about 60 nm, or less than about 50 nm, when measured by the above-noted techniques.

By "an effective average particle size of less than about 5 microns" it is meant that at least 50% of the nanoparticulate olanzapine particles have a weight average particle size of less than about 5 microns, when measured by the above-noted techniques. In other embodiments of the invention, at least about 70%, at least about 90%, at least about 95%, or at least about 99% of the nanoparticulate olanzapine particles have a particle size of less than the effective average, by weight, *i.e.,* less than about 5 microns, less than about 4900 nm, less than less than about 4800 nm, less than about 4700 nm, *etc.* (as listed in the paragraph above).

If the nanoparticulate olanzapine composition is combined with a microparticulate olanzapine or non-olanzapine active agent composition, then such a composition is either solubilized or has an effective average particle size of greater than about 5 microns. By "an effective average particle size of greater than about 5 microns" it is meant that at least 50% of the microparticulate olanzapine or non-olanzapine active agent particles have a particle size of greater than about 5 microns, by weight, when measured by the above-noted techniques.

In other embodiments of the invention, at least about 70%, at least about 90%, at least about 95%, or at least about 99%, by weight, of the microparticulate olanzapine or non-olanzapine active agent particles have a particle size greater than about 5 microns.

In the present invention, the value for D50 of a nanoparticulate olanzapine composition is the particle size below which 50% of the olanzapine particles fall, by weight. Similarly, D90 and D99 are the particle sizes below which 90% and 99%, respectively, of the olanzapine particles fall, by weight.

4. Concentration of Nanoparticulate Olanzapine and Surface Stabilizers

The relative amounts of olanzapine and one or more surface stabilizers can vary widely. The optimal amount of the individual components can depend, for example, upon the hydrophilic lipophilic balance (HLB), melting point, and the surface tension of water solutions of the stabilizer, *etc.*

The concentration of olanzapine can vary from about 99.5% to about 0.001%, from about 95% to about 0.1%, from about 90% to about 0.5%, or from about 5.0% to about 50%, by weight, based on the total combined dry weight of the olanzapine and at least one surface stabilizer, not including other excipients.

The concentration of the at least one surface stabilizer can vary from about 0.5% to about 99.999%, from about 5.0% to about 99.9%, from about 10% to about 99.5%, or from about 0.1 to about 50%, by weight, based on the total combined dry weight of the olanzapine and at least one surface stabilizer, not including other excipients.

5. Additional Active Agents

The invention encompasses the nanoparticulate olanzapine compositions of the invention formulated or co-administered with one or more non-olanzapine active agents. Methods of using such combination compositions are also

encompassed by the invention. The non- olanzapine active agents can be present in a crystalline phase, an amorphous phase, a semi-crystalline phase, a semi-amorphous phase, or a mixture thereof.

The compound to be administered in combination with a nanoparticulate olanzapine composition of the invention can be formulated separately from the nanoparticulate olanzapine composition or co-formulated with the nanoparticulate olanzapine composition. Where a nanoparticulate olanzapine composition is co-formulated with a second active agent, the second active agent can be formulated in any suitable manner, such as immediate-release, rapid-onset, sustained-release, or dual-release form.

Such non-olanzapine active agents can be, for example, a therapeutic agent. A therapeutic agent can be a pharmaceutical agent, including a biologic. The active agent can be selected from a variety of known classes of drugs, including, for example, amino acids, proteins, peptides, nucleotides, anti-obesity drugs, central nervous system stimulants, carotenoids, corticosteroids, elastase inhibitors, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, such as NSAIDs and COX-2 inhibitors, anthelmintics, anti-arrhythmic agents, antibiotics (including penicillins), anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytics, sedatives (hypnotics and neuroleptics), astringents, alpha-adrenergic receptor blocking agents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants (expectorants and mucolytics), diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics (antiparkinsonian agents), haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates,

prostaglandins, radio-pharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, and xanthines.

Examples of secondary active agents particularly useful in the compositions of the invention include, but are not limited to, antidepressants. Examples of classes of useful antidepressants include, but are not limited to, selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants, and monoamine oxidase Inhibitors (MAOI's). Examples of antidepressants include, but are not limited to, citalopram (Celexa®), escitalopram HB (Lexapro®), fluoxetine hydrochloride (Prozac®), paroxetine (Paxil®), fluvoxamine (Luvox®), sertraline (Zoloft®), venlafaxine (Effexor®), amitriptyline (Elavil®), desipramine, nortriptyline, duloxetine (Cymbalta®), mirtazepine (Remeron®), phenelzine (Nardil®), tranylcypromine (Parnate®), nefazodone (Serzone®), trazodone, and bupropion (Wellbutrin®). A particularly useful antidepressant is fluoxetine (Prozac®).

B. Methods of Making Injectable Olanzapine Formulations

In another aspect of the invention there is provided a method of preparing the injectable nanoparticulate olanzapine formulations of the invention. The method comprises of one of the following methods: attrition, precipitation, evaporation, or combinations of these. Exemplary methods of making nanoparticulate compositions are described in U.S. Patent No. 5,145,684. Methods of making nanoparticulate compositions are also described in U.S. Patent No. 5,518,187 for "Method of Grinding Pharmaceutical Substances;" U.S. Patent No. 5,718,388 for "Continuous Method of Grinding Pharmaceutical Substances;" U.S. Patent No. 5,862,999 for "Method of Grinding Pharmaceutical Substances;" U.S. Patent No. 5,665,331 for "Co-Microprecipitation of Nanoparticulate Pharmaceutical Agents with Crystal Growth Modifiers;" U.S.

Patent No. 5,662,883 for "Co-Microprecipitation of Nanoparticulate Pharmaceutical Agents with Crystal Growth Modifiers;" U.S. Patent No. 5,560,932 for "Microprecipitation of Nanoparticulate Pharmaceutical Agents;" U.S. Patent No. 5,543,133 for "Process of Preparing X-Ray Contrast Compositions Containing Nanoparticles;" U.S. Patent No. 5,534,270 for "Method of Preparing Stable Drug Nanoparticles;" U.S. Patent No. 5,510,118 for "Process of Preparing Therapeutic Compositions Containing Nanoparticles;" and U.S. Patent No. 5,470,583 for "Method of Preparing Nanoparticle Compositions Containing Charged Phospholipids to Reduce Aggregation," all of which are specifically incorporated by reference.

Following milling, homogenization, precipitation, *etc.*, the resultant nanoparticulate olanzapine composition can be utilized a liquid dosage formulation for injectable administration.

In one embodiment of the invention, the olanzapine particles are reduced to an effective average particle size of less than about 600 nm. Preferably, the effective average particle size of the nanoparticulate olanzapine is less than about 450 nm, more preferably less than about 300 nm, even more preferably less than about 250 nm, and most preferably less than about 100 nm. The pH of the liquid dispersion media is preferably maintained within the range of from about 3.0 to about 8.0, or about 5.0 to about 7.5, more preferably, at a pH of about 7.4, during the size reduction process. Preferably, the dispersion media used for the size reduction process is aqueous. However, any media in which olanzapine is poorly soluble and dispersible can be used as a dispersion media. Non-aqueous examples of dispersion media include, but are not limited to, aqueous salt solutions, safflower oil and solvents such as ethanol, t-butanol, hexane, and glycol.

Effective methods of providing mechanical force for particle size reduction of olanzapine include ball milling, media milling, and homogenization,

for example, with a Microfluidizer® (Microfluidics Corp.). Ball milling is a low energy milling process that uses milling media, drug, stabilizer, and liquid. The materials are placed in a milling vessel that is rotated at optimal speed such that the media cascades and reduces the drug particle size by impaction. The media used must have a high density as the energy for the particle reduction is provided by gravity and the mass of the attrition media.

Media milling is a high energy milling process. Drug, stabilizer, and liquid are placed in a reservoir and recirculated in a chamber containing media and a rotating shaft/impeller. The rotating shaft agitates the media which subjects the drug to impaction and sheer forces, thereby reducing the drug particle size.

Homogenization is a technique that does not use milling media. Drug, stabilizer, and liquid (or drug and liquid with the stabilizer added after particle size reduction) constitute a process stream propelled into a process zone, which in the Microfluidizer® is called the Interaction Chamber. The product to be treated is inducted into the pump, and then forced out. The priming valve of the Microfluidizer® purges air out of the pump. Once the pump is filled with product, the priming valve is closed and the product is forced through the interaction chamber. The geometry of the interaction chamber produces powerful forces of sheer, impact, and cavitation which are responsible for particle size reduction. Specifically, inside the interaction chamber, the pressurized product is split into two streams and accelerated to extremely high velocities. The formed jets are then directed toward each other and collide in the interaction zone. The resulting product has very fine and uniform particle or droplet size. The Microfluidizer® also provides a heat exchanger to allow cooling of the product. U.S. Patent No. 5,510,118, which is specifically incorporated by reference, refers to a process using a Microfluidizer® resulting in nanoparticulate particles.

Olanzapine can be added to a liquid medium in which it is essentially insoluble to form a premix. The concentration of the olanzapine in the liquid

medium can vary from about 5 to about 60%, and preferably is from about 15 to about 50% (w/v), and more preferably about 20 to about 40%. The surface stabilizer can be present in the premix, it can be during particle size reduction, or it can be added to the drug dispersion following particle size reduction. The concentration of the surface stabilizer can vary from about 0.1 to about 50%, and preferably is from about 0.5 to about 20%, and more preferably from about 1 to about 10%, by weight.

The premix can be used directly by subjecting it to mechanical means to reduce the average olanzapine particle size in the dispersion to the desired size, preferably less than about 5 microns. It is preferred that the premix be used directly when a ball mill is used for attrition. Alternatively, olanzapine and the surface stabilizer can be dispersed in the liquid media using suitable agitation, *e.g.*, a Cowles type mixer, until a homogeneous dispersion is observed in which there are no large agglomerates visible to the naked eye. It is preferred that the premix be subjected to such a premilling dispersion step when a recirculating media mill is used for attrition.

The mechanical means applied to reduce the olanzapine particle size conveniently can take the form of a dispersion mill. Suitable dispersion mills include a ball mill, an attritor mill, a vibratory mill, and media mills such as a sand mill and a bead mill. A media mill is preferred due to the relatively shorter milling time required to provide the desired reduction in particle size. For media milling, the apparent viscosity of the premix is preferably from about 100 to about 1000 centipoise, and for ball milling the apparent viscosity of the premix is preferably from about 1 up to about 100 centipoise. Such ranges tend to afford an optimal balance between efficient particle size reduction and media erosion but are in no way limiting

The attrition time can vary widely and depends primarily upon the particular mechanical means and processing conditions selected. For ball mills,

processing times of up to five days or longer may be required. Alternatively, processing times of less than 1 day (residence times of one minute up to several hours) are possible with the use of a high shear media mill.

The olanzapine particles must be reduced in size at a temperature which does not significantly degrade olanzapine. Processing temperatures of less than about 30° to less than about 40°C are ordinarily preferred. If desired, the processing equipment can be cooled with conventional cooling equipment. Control of the temperature, *e.g.*, by jacketing or immersion of the milling chamber with a cooling liquid, is contemplated. Generally, the method of the invention is conveniently carried out under conditions of ambient temperature and at processing pressures which are safe and effective for the milling process. Ambient processing pressures are typical of ball mills, attritor mills, and vibratory mills.

Grinding Media

The grinding media can comprise particles that are preferably substantially spherical in shape, *e.g.*, beads, consisting essentially of polymeric resin or glass or Zirconium Silicate or other suitable compositions. Alternatively, the grinding media can comprise a core having a coating of a polymeric resin adhered thereon.

In general, suitable polymeric resins are chemically and physically inert, substantially free of metals, solvent, and monomers, and of sufficient hardness and friability to enable them to avoid being chipped or crushed during grinding. Suitable polymeric resins include crosslinked polystyrenes, such as polystyrene crosslinked with divinylbenzene; styrene copolymers; polycarbonates; polyacetals, such as Delrin® (E.I. du Pont de Nemours and Co.); vinyl chloride polymers and copolymers; polyurethanes; polyamides; poly(tetrafluoroethylenes), *e.g.*, Teflon®(E.I. du Pont de Nemours and Co.), and

other fluoropolymers; high density polyethylenes; polypropylenes; cellulose ethers and esters such as cellulose acetate; polyhydroxymethacrylate; polyhydroxyethyl acrylate; and silicone-containing polymers such as polysiloxanes and the like. The polymer can be biodegradable. Exemplary biodegradable polymers include poly(lactides), poly(glycolide) copolymers of lactides and glycolide, polyanhydrides, poly(hydroxyethyl methacylate), poly(imino carbonates), poly(N-acylhydroxyproline)esters, poly(N-palmitoyl hydroxyproline) esters, ethylene-vinyl acetate copolymers, poly(orthoesters), poly(caprolactones), and poly(phosphazenes). For biodegradable polymers, contamination from the media itself advantageously can metabolize *in vivo* into biologically acceptable products that can be eliminated from the body.

The grinding media preferably ranges in size from about 0.01 to about 3 mm. For fine grinding, the grinding media is preferably from about 0.02 to about 2 mm, and more preferably from about 0.03 to about 1 mm in size.

The polymeric resin can have a density from about 0.8 to about 3.0 g/cm^3.

In one embodiment of the invention, the olanzapine particles are made continuously. Such a method comprises continuously introducing olanzapine into a milling chamber, contacting the olanzapine with grinding media while in the chamber to reduce the olanzapine particle size, and continuously removing the nanoparticulate olanzapine from the milling chamber.

The grinding media can be separated from the milled nanoparticulate olanzapine using conventional separation techniques, in a secondary process such as by simple filtration, sieving through a mesh filter or screen, and the like. Other separation techniques such as centrifugation may also be employed. Alternatively, a screen can be utilized during the milling process to remove the grinding media following completion of particle size reduction.

Sterile Product Manufacturing

Development of injectable compositions requires the production of a sterile product. The manufacturing process of the present invention is similar to typical known manufacturing processes for sterile suspensions. A typical sterile suspension manufacturing process flowchart is as follows:

Media Conditioning
↓
Compounding
↓
Particle Size Reduction
↓
Vial Filling
↓
Lyophilization and/or Terminal Sterilization

As indicated by the optional steps in parentheses, some of the processing is dependent upon the method of particle size reduction and/or method of sterilization. For example, media conditioning is not required for a milling method that does not use media. If terminal sterilization is not feasible due to chemical and/or physical instability, aseptic processing can be used.

C. Method of Treatment

Yet another aspect of the present invention provides a method of treating a mammal, including a human, of disorders of the central nervous system including, but not limited to psychiatric treatment. Such treatment comprises administering to the subject the injectable nanoparticulate olanzapine formulation of the invention. As used herein, the term "subject" is used to mean an animal,

preferably a mammal, including a human or non-human. The terms patient and subject may be used interchangeably.

Examples of disorders that can be treated with olanzapine include, but are not limited to, schizophrenia and related psychoses, bipolar mania and/or bipolar disorder, seizures, obsessive/compulsive disorders, generalized anxiety disorder, post traumatic distress syndrome, extreme shyness, diabetic nerve pain, smoking cessation, and depression.

Particularly advantageous features of the present invention include that the pharmaceutical formulation of the invention exhibits a prolonged duration of action that can be controlled upon administration, and produces minimal or no pain or irritation upon administration. For example, compositions of the invention can provide efficacious levels of drug for up to about one week, from about two to about six weeks, or from about two to about twelve weeks. In addition, the injectable formulation of the invention can provide a high olanzapine concentration in a small volume to be injected. A general protocol for administration thereof comprises an intramuscular or subcutaneous bolus injection of olanzapine.

Conventional olanzapine (Zyprexa®) has a starting single evening dose of 10 mg. The usual maximum dose should be 20 mg. For treatment of psychoses, such as schizophrenia, the adult dosage is 5-10 mg/day initially, with a target dose of 10 mg/day within several days.

Olanzapine shows mesolimbic sensitivity, blocks conditioned avoidance at lower doses than those inducing catalepsy, substitutes for clozapine in a drug discrimination assay, produces a modest rise in prolactin, produces few extrapyramidal side effects, and reduces positive and negative symptoms of schizophrenia as efficaciously as clozapine. However, despite this 'atypical' profile, olanzapine has a weaker alpha-2 blockade than clozapine or risperidone. It has relatively high affinity for muscarinic, 5HT - 2, and D1, D2 and D4

receptors. Trials suggest a good response in schizophrenia with few extrapyramidal side effects (EPSEs).

Compositions suitable for parenteral injection may comprise physiologically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents, or vehicles including water, ethanol, polyols (propyleneglycol, polyethylene-glycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

The nanoparticulate compositions may also contain adjuvants such as preserving, wetting, emulsifying, and dispensing agents. Prevention of the growth of microorganisms can be ensured by various antibacterial and antifungal agents, such as parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, such as aluminum monostearate and gelatin.

One of ordinary skill will appreciate that effective amounts of olanzapine can be determined empirically and can be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt, ester, or prodrug form. Actual dosage levels of olanzapine in the nanoparticulate compositions of the invention may be varied to obtain an amount of olanzapine that is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage level therefore depends upon the desired

therapeutic effect, the route of administration, the potency of the administered olanzapine, the desired duration of treatment, and other factors.

Dosage unit compositions may contain such amounts of such submultiples thereof as may be used to make up the daily dose. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors: the type and degree of the cellular or physiological response to be achieved; activity of the specific agent or composition employed; the specific agents or composition employed; the age, body weight, general health, sex, and diet of the patient; the time of administration, route of administration, and rate of excretion of the agent; the duration of the treatment; drugs used in combination or coincidental with the specific agent; and like factors well known in the medical arts.

The following examples are given to illustrate the present invention. It should be understood, however, that the spirit and scope of the invention is not to be limited to the specific conditions or details described in these examples but should only be limited by the scope of the claims that follow. All references identified herein, including U.S. patents, are hereby expressly incorporated by reference.

Example 1

The purpose of this example is to illustrate the procedure for identifying a suitable nanoparticulate formulation of olanzapine.

The study can be conducted by screening eleven surface stabilizers to identify the most suitable stabilizer for parenteral administration of olanzapine. The dispersions can be formulated at 40% solids to 2.4% surface stabilizer.

TABLE 2

Surface Stabilizer
Plasdone C15® (polyvinylpyrrolidone)
Kollidon 17PF® (a polyvinylpyrrolidone polymer)
Povidone K30® (a polyvinylpyrrolidone polymer)
Tyloxapol
Pluronic F68® (a high molecular weight polyoxyalkylene ether)
Pluronic F108® (a high molecular weight polyoxyalkylene ether)
Tween 80® (a polyoxyethylene sorbitan fatty acid ester)
dioctylsulfosuccinate (CAS No. 577-11-7; aka Docusate Sodium)
B20-5000® (a triblock copolymer surface modifier)
B20-5000-sulfonated (a triblock copolymer surface modifier)
lecithin (CAS No. 8002-43-5)
Povidone K30® and Pluronic F108®

Such combinations may produce stable dispersions of differing nanoparticulate size that will have differing durations of action when administered. Preclinical and clinical studies will identify the optimum formulation and size associated with the desired prolonged duration of action.

Example 2

The purpose of this example was to prepare a nanoparticulate formulation of olanzapine.

The particle size of olanzapine drug crystals was first measured prior to incorporation into a nanoparticulate formulation. The particle size, as measured using a Horiba LA 910 particle size analyzer (Horiba Instruments, Irvine, CA),

was a mean of 137.08 microns, and a D90 of less than 335.59 microns. See Fig. 1.

An aqueous dispersion of 10% olanzapine (Camida LLC, Newark, NJ), combined with 1% Tween 80, 0.1% benzalkonium chloride, and 20% dextrose, was milled in a NanoMill® 0.01 (Elan Drug Delivery), along with 500 micron PolyMill® grinding media (Dow Chemical) (50-89% media load). The mixture was milled at a speed of 1009 – 5500 rpms, at a temperature of 5-10°C, for about 30 min.

Following milling, the particle size of the milled olanzapine particles was measured, in deionized distilled water, using a Horiba LA 910 particle size analyzer. The median milled olanzapine particle size was 347 nm, with a mean size of 606 nm, a D90 of 1.28 microns, and a D83 of less than 1 micron. See Fig. 2.

Example 3

The purpose of this example was to prepare a nanoparticulate formulation of olanzapine.

An aqueous dispersion of 30% olanzapine (Camida LLC, Newark, NJ), combined with 2.5% Tween 80, was milled in a NanoMill® 0.01 (Elan Drug Delivery), along with 500 micron PolyMill® grinding media (Dow Chemical) (50-89% media load). The mixture was milled at a speed of 1009 – 5500 rpms, at a temperature of 5-10°C, for about 30 min.

Following milling, the particle size of the milled olanzapine particles was measured, in deionized distilled water, using a Horiba LA 910 particle size analyzer. The median milled olanzapine particle size was 990 nm, with a mean size of 1.136 nm, a D90 of 2.07 microns, and a D50 of less than 1 micron. See Fig. 3.

Example 4

The purpose of this example was to determine the *in vivo* characteristics of the nanoparticulate olanzapine formulation prepared in Example 2.

An *in vivo* study, utilizing male beagle dogs, was conducted to determine the therapeutic levels of olanazapine present *in vivo* over a period of time following intramuscular (IM) administration of the nanoparticulate olanazapine formulation prepared in Example 2. Six dogs were given a single intramuscular dose of 10 mg/kg (about 100 mg/animal), which is about 10x the daily dose in humans. Blood samples were taken at t = 0, 0.5, 1, 2, 4, 8, 24, and 49 hours post administration, and 4, 7, 14, and 28 days post administration. The plasma concentration (ng/ml) over a 168 hr period is shown in Fig. 4. As shown in Fig. 4, therapeutic levels of olanzapine, of 5 to 22 ng/ml, were present *in vivo* for over a 168 hr period. Fig. 5 further demonstrates that for all animals dosed, therapeutic levels of olanzapine, of 5to 22 ng/ml, were present *in vivo* for over a 168 hr period.

In addition to demonstrating that the injectable olazapine formulations of the invention produce measurable and detectable levels of drug in the plasma for more than seven days following administration, this example further demonstrates: (1) that the olanzapine formulation prepared as in Example 2 is syringeable with a 23 gauge needle; and (2) that the olanzapine formulation prepared as in Example 2 is well tolerated by mammals.

* * * *

It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention

provided they come within the scope of the appended claims and their equivalents.

What We Claim Is:

1. An injectable nanoparticulate olanzapine composition comprising:

(a) olanzapine nanoparticles having an effective average particle size that results in a therapeutic efficacy of about one week or greater;

(b) at least one surface stabilizer; and

(c) a pharmaceutically acceptable carrier.

2. The composition of claim 1, wherein the composition is administered via intramuscular or subcutaneous injection so as to form a depot.

3. The composition of claim 2, wherein the depot releases the olanzapine at therapeutic levels for a period of time from about two to about six weeks.

4. The composition of claim 1, wherein the depot releases the olanzapine at therapeutic levels for a period of time from about two to about twelve weeks.

5. The composition of claim 1, wherein the depot releases the olanzapine at therapeutic levels for a period of time selected from the group consisting of one week to about two weeks, from about one week to about three weeks, from about one week to about four weeks, from about one week to about five weeks, from about one week to about six weeks, from about one week to about seven weeks, from about one week to about eight weeks, from about one week to about nine weeks, from about one week to about ten weeks, from about one week to about eleven weeks, from about one week to about twelve weeks, and combinations thereof.

6. The composition of claim 1, wherein the olanzapine is selected from the group consisting of a crystalline phase, an amorphous phase, a semi-crystalline phase, a semi-amorphous phase, and mixtures thereof.

7. The composition of claim 1, wherein the effective average particle size of the olanzapine particles is less than about 5 microns.

8. The composition of claim 7, wherein the effective average particle size of the olanzapine particles is selected from the group consisting of less than about 4900 nm, less than about 4800 nm, less than about 4700 nm, less than about 4600 nm, less than about 4500 nm, less than about 4400 nm, less than about 4300 nm, less than about 4200 nm, less than about 4100 nm, less than about 4 microns, less than about 3900 nm, less than about 3800 nm, less than about 3700 nm, less than about 3600 nm, less than about 3500 nm, less than about 3400 nm, less than about 3300 nm, less than about 3200 nm, less than about 3100 nm, less than about 3 microns, less than about 2900 nm, less than about 2800 nm, less than about 2700 nm, less than about 2600 nm, less than about 2500 nm, less than about 2400 nm, less than about 2300 nm, less than about 2200 nm, less than about 2100 nm, less than about 2000 nm, less than about 1900 nm, less than less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 140 nm, less than about 130 nm, less than about 120 nm, less than about 110 nm, less than about 100 nm, less than about 90 nm, less than about 80 nm, less than about 70 nm, less than about 60 nm, and less than about 50 nm.

9. The composition of claim 1, wherein:

(a) the olanzapine is present in an amount selected from the group consisting of from about 99.5% to about 0.001%, from about 95% to about 0.1%, from about 90% to about 0.5%, and from about 5.0% to about 50%, by weight, based on the total combined weight of the olanzapine and at least one surface stabilizer, not including other excipients; and

(b) the at least one surface stabilizer is present in an amount selected from the group consisting of from about 0.5% to about 99.999% by weight, from about 5.0% to about 99.9% by weight, from about 10% to about 99.5%, and from about 0.1 to about 50%, by weight, based on the total combined dry weight of the olanzapine and at least one surface stabilizer, not including other excipients.

10. The composition of claim 1, wherein the surface stabilizer is selected from the group consisting of a non-ionic surface stabilizer, an ionic surface stabilizer, a anionic surface stabilizer, a cationic surface stabilizer, and a zwitterionic surface stabilizer.

11. The composition of claim 1, wherein at least one surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose,

hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; lysozyme, PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, random copolymers of vinyl acetate and vinyl pyrrolidone, cationic polymers, cationic biopolymers, cationic polysaccharides, cationic cellulosics, cationic alginates, cationic nonpolymeric compounds, cationic phospholipids, cationic lipids, polymethylmethacrylate trimethylammonium bromide, sulfonium compounds, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, hexadecyltrimethyl ammonium bromide, phosphonium compounds, quarternary ammonium compounds, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium chloride bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl

ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl (ethenoxy)$_4$ ammonium chloride, lauryl dimethyl (ethenoxy)$_4$ ammonium bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12} trimethyl ammonium bromides, C_{15} trimethyl ammonium bromides, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, POLYQUAT 10™, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, MIRAPOL™, ALKAQUAT™, alkyl pyridinium salts; amines, amine salts, amine oxides, imide azolinium salts, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

12. The composition of claim 1, comprising a surface stabilizer selected from the group consisting of a polysorbate, benzalkonium chloride, dextrose, and a combination thereof.

13. The composition of claim 1, further comprising at least one additional olanzapine composition having an effective average particle size which is different that the effective average particle size of the olanzapine composition of claim 1.

14. The composition of claim 1, additionally comprising one or more non-olanzapine active agents.

15. The composition of claim 14, wherein at least one non-olanzapine agent is an antidepressant.

16. The composition of claim 15, wherein the antidepressant is fluoxetine.

17. The composition of claim 1, wherein the composition is syringeable with a 23 gauge needle.

18. The composition of claim 1, which is well tolerated by a mammal.

19. A method of making an injectable nanoparticulate olanzapine composition that produces an intramuscular depot upon administration comprising:

contacting particles of olanzapine or a salt thereof with at least one surface stabilizer for a time and under conditions sufficient to provide a

olanzapine composition having an effective average particle size that results in a therapeutic efficacy of about one week or greater.

20. The method of claim 19, wherein the contacting comprises grinding, wet grinding, homogenizing, or a combination thereof.

21. The method of claim 19, wherein the effective average particle size of the olanzapine particles is less than about 5 microns.

22. The composition of claim 21, wherein the effective average particle size of the olanzapine particles is selected from the group consisting of less than about 4900 nm, less than about 4800 nm, less than about 4700 nm, less than about 4600 nm, less than about 4500 nm, less than about 4400 nm, less than about 4300 nm, less than about 4200 nm, less than about 4100 nm, less than about 4 microns, less than about 3900 nm, less than about 3800 nm, less than about 3700 nm, less than about 3600 nm, less than about 3500 nm, less than about 3400 nm, less than about 3300 nm, less than about 3200 nm, less than about 3100 nm, less than about 3 microns, less than about 2900 nm, less than about 2800 nm, less than about 2700 nm, less than about 2600 nm, less than about 2500 nm, less than about 2400 nm, less than about 2300 nm, less than about 2200 nm, less than about 2100 nm, less than about 2000 nm, less than about 1900 nm, less than less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 140 nm, less than about 130 nm, less than about 120 nm, less than about

110 nm, less than about 100 nm, less than about 90 nm, less than about 80 nm, less than about 70 nm, less than about 60 nm, and less than about 50 nm.

23. A method for the treatment of a subject for disorders of the central nervous system comprising administering to the subject an effective amount of an injectable composition comprising:

(a) olanzapine nanoparticles having an effective average particle size of that results in a therapeutic efficacy of about one week or greater;

(b) at least one surface stabilizer;

(c) at least one pharmaceutically acceptable carrier.

24. The method of claim 23, wherein the effective average particle size of the olanzapine particles is less than about 5 microns.

25. The method of claim 24, wherein the effective average particle size of the olanzapine particles is selected from the group consisting of less than about 4900 nm, less than about 4800 nm, less than about 4700 nm, less than about 4600 nm, less than about 4500 nm, less than about 4400 nm, less than about 4300 nm, less than about 4200 nm, less than about 4100 nm, less than about 4 microns, less than about 3900 nm, less than about 3800 nm, less than about 3700 nm, less than about 3600 nm, less than about 3500 nm, less than about 3400 nm, less than about 3300 nm, less than about 3200 nm, less than about 3100 nm, less than about 3 microns, less than about 2900 nm, less than about 2800 nm, less than about 2700 nm, less than about 2600 nm, less than about 2500 nm, less than about 2400 nm, less than about 2300 nm, less than about 2200 nm, less than about 2100 nm, less than about 2000 nm, less than about 1900 nm, less than less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than

about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 140 nm, less than about 130 nm, less than about 120 nm, less than about 110 nm, less than about 100 nm, less than about 90 nm, less than about 80 nm, less than about 70 nm, less than about 60 nm, and less than about 50 nm.

26. The method of claim 23, wherein the depot releases the olanzapine at therapeutic levels for a period of time from about two to about six weeks.

27. The method of claim 23, wherein the depot releases the olanzapine at therapeutic levels for a period of time from about two to about twelve weeks.

28. The method of claim 23, wherein the depot releases the olanzapine at therapeutic levels for a period of time selected from the group consisting of one week to about two weeks, from about one week to about three weeks, from about one week to about four weeks, from about one week to about five weeks, from about one week to about six weeks, from about one week to about seven weeks, from about one week to about eight weeks, from about one week to about nine weeks, from about one week to about ten weeks, from about one week to about eleven weeks, from about one week to about twelve weeks, and combinations thereof.

29. The method of claim 23, wherein the AUC of the olanzapine, when assayed in the plasma of a mammalian subject following injectable administration, is greater than the AUC for a non-nanoparticulate olanzapine formulation, administered at the same dosage.

30. The method of claim 29, wherein the AUC is selected from the group consisting of at least about 25%, at least about 50%, at least about 75%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 225%, at least about 250%, at least about 275%, at least about 300%, at least about 350%, at least about 400%, at least about 450%, at least about 500%, at least about 550%, at least about 600%, at least about 750%, at least about 700%, at least about 750%, at least about 800%, at least about 850%, at least about 900%, at least about 950%, at least about 1000%, at least about 1050%, at least about 1100%, at least about 1150%, or at least about 1200% greater than the AUC exhibited by the non-nanoparticulate formulation of olanzapine, administered at the same dosage.

31. The method of claim 23, wherein the method is used to treat an indication selected from the group consisting of schizophrenia and related psychoses, bipolar mania, bipolar disorder, seizures, obsessive/compulsive disorders, generalized anxiety disorder, post traumatic distress syndrome, extreme shyness, diabetic nerve pain, smoking cessation, and depression.

FIGURE 1: Olanzapine crystals prior to particle size reduction

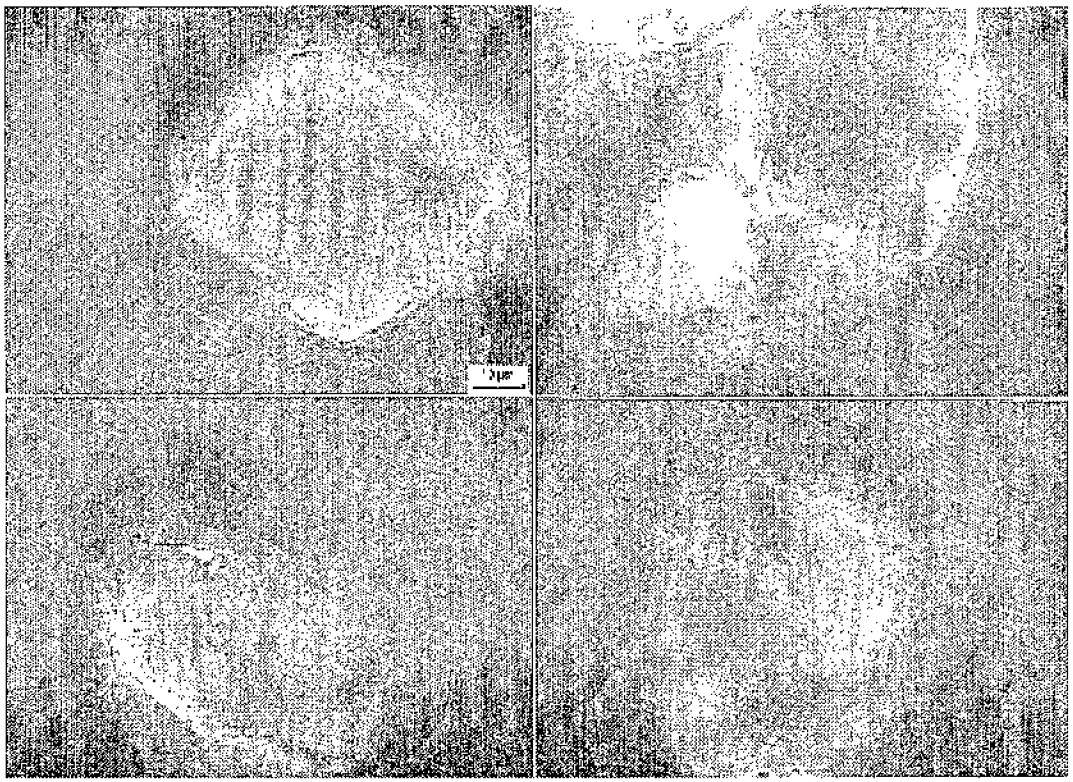


FIGURE 2: Olanzapine crystals following particle size reduction

FIGURE 3: Olanzapine crystals following particle size reduction

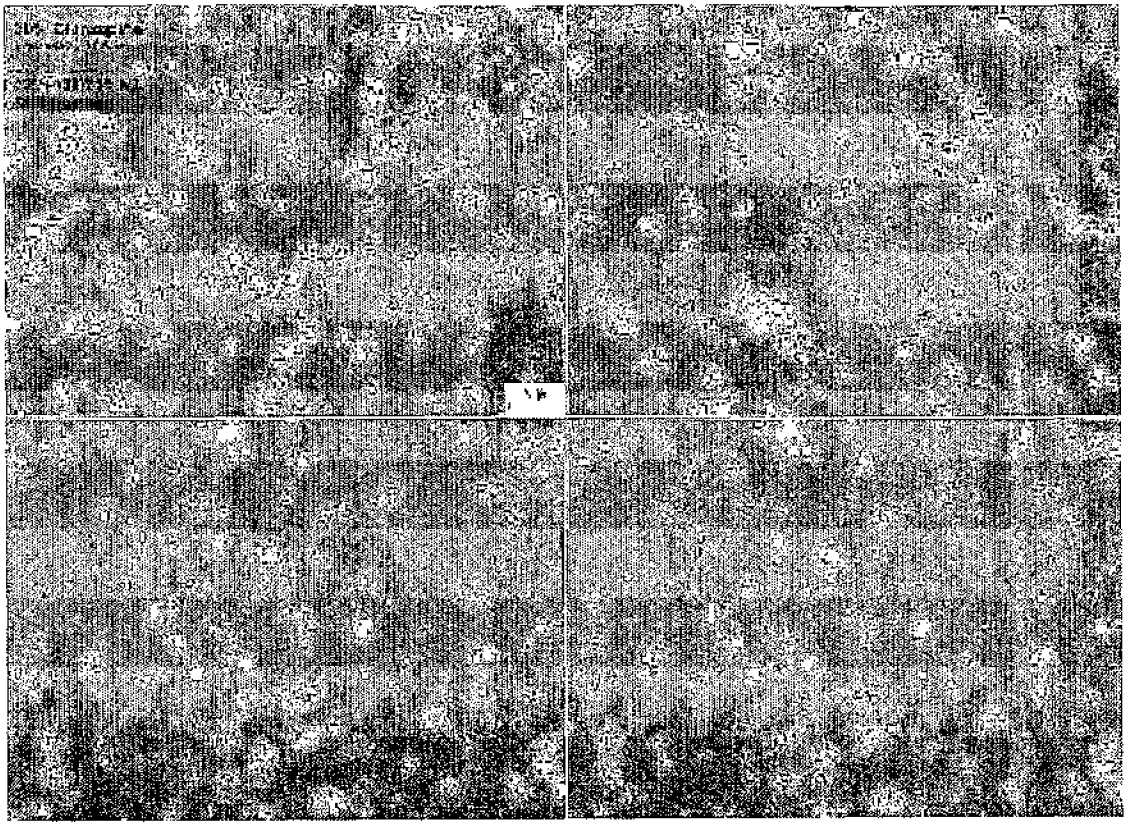


FIGURE 4

NanoOlanzapine Dog Study
Dosing (IM @ 10mg/kg ~100mg per animal)

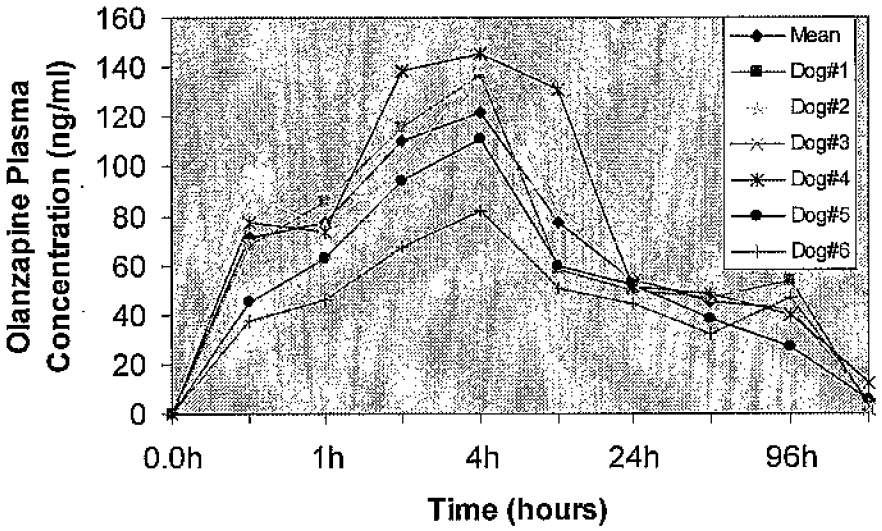
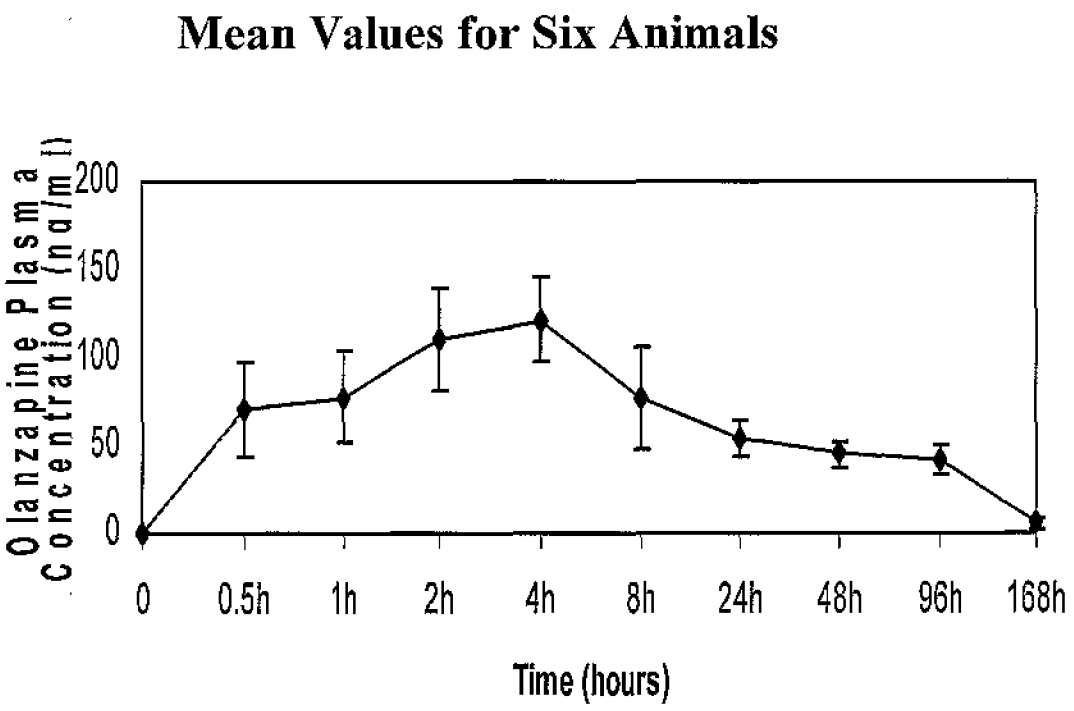


FIGURE 5

NanoOlanzapine Dog Study
Dosing (IM @ 10mg/kg ~100mg per animal)
Dose 10X the daily dose in man & well tolerated



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(54) Title: AEROSOL AND INJECTABLE FORMULATIONS OF NANOPARTICULATE BENZODIAZEPINE

(57) Abstract: Described are nanoparticulate formulations of a benzodiazepine, such as lorazepam, that does not require the presence of polyethylene glycol and propylene glycol as stabilizers, and methods of making and using such formulations. The formulations are particularly useful in aerosol and injectable dosage forms, and comprise nanoparticulate benzodiazepine, such as lorazepam, and at least one surface stabilizer. The formulations are useful in the treatment of status epilepticus, treatment of irritable bowel syndrome, sleep induction, acute psychosis, and as a pre-anesthesia medication.

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AEROSOL AND INJECTABLE FORMULATIONS OF NANOPARTICULATE BENZODIAZEPINE

FIELD OF THE INVENTION

The present invention is directed to aerosol and injectable formulations of nanoparticulate benzodiazepine, and preferably, nanoparticulate lorazepam. The compositions of the invention are useful in treating status epilepticus, sleep induction, acute psychosis, irritable bowel syndrome, and for pre-anesthesia medication. Also encompassed by the invention are methods of making and using such compositions.

BACKGROUND OF THE INVENTION

I. Administration Routes for Drugs

The route of administration of a drug substance can be critical to its pharmacological effectiveness. Various routes of administration exist, and all have their own advantages and disadvantages. Oral drug delivery of tablets, capsules, liquids, and the like is the most convenient approach to drug delivery, but many drug compounds are not amenable to oral administration. For example, modern protein drugs which are unstable in the acidic gastric environment or which are rapidly degraded by proteolytic enzymes in the digestive tract are poor candidates for oral administration. Similarly, poorly water soluble compounds which do not dissolve rapidly enough to be orally absorbed are likely to be ineffective when given as oral dosage forms. Oral administration can also be undesirable because drugs which are administered orally are generally distributed to all tissues in the body, and not just to the intended site of pharmacological activity. Alternative types of systemic administration are subcutaneous or intravenous injection. This approach avoids the gastrointestinal tract and therefore can be an effective route for delivery of proteins and peptides. However, these routes of administration have a low rate of patient compliance, especially for drugs such as insulin which must be administered one or more times daily. Additional alternative methods of drug delivery have been developed including transdermal, rectal, vaginal, intranasal, and pulmonary delivery.

Nasal drug delivery relies on inhalation of an aerosol through the nose so that active drug substance can reach the nasal mucosa. Drugs intended for systemic activity can be absorbed into the bloodstream because the nasal mucosa is highly vascularized. Alternatively, if the drug is intended to act topically, it is delivered directly to the site of activity and does not have to distribute throughout the body; hence, relatively low doses may be used. Examples of such drugs are decongestants, antihistamines, and anti-inflammatory steroids for seasonal allergic rhinitis.

Pulmonary drug delivery relies on inhalation of an aerosol through the mouth and throat so that the drug substance can reach the lung. For systemically active drugs, it is desirable for the drug particles to reach the alveolar region of the lung, whereas drugs which act on the smooth muscle of the conducting airways should preferentially deposit in the bronchiole region. Such drugs can include beta-agonists, anti cholinergics, and corticosteroids.

A. Droplet/Particle Size Determines Deposition Site

In developing a therapeutic aerosol, the aerodynamic size distribution of the inhaled particles is the single most important variable in defining the site of droplet or particle deposition in the patient; in short, it will determine whether drug targeting succeeds or fails. *See* P. Byron, "Aerosol Formulation, Generation, and Delivery Using Nonmetered Systems," *Respiratory Drug Delivery*, 144-151, 144 (CRC Press, 1989). Thus, a prerequisite in developing a therapeutic aerosol is a preferential particle size. The deposition of inhaled aerosols involves different mechanisms for different size particles. D. Swift (1980); Parodi et al., "Airborne Particles and Their Pulmonary Deposition," in *Scientific Foundations of Respiratory Medicine*, Scaddings et al. (eds.), pp. 545-557 (W. B. Saunders, Philadelphia, 1981); J. Heyder, "Mechanism of Aerosol Particle Deposition," *Chest*, 80:820-823 (1981).

Generally, inhaled particles are subject to deposition by one of two mechanisms: impaction, which usually predominates for larger particles, and sedimentation, which is prevalent for smaller particles. Impaction occurs when the momentum of an inhaled particle is large enough that the particle does not follow the air stream and encounters a physiological surface. In contrast, sedimentation occurs primarily in the deep lung when very small particles which have traveled with the inhaled air stream encounter physiological surfaces as

a result of random diffusion within the air stream. For intranasally administered drug compounds which are inhaled through the nose, it is desirable for the drug to impact directly on the nasal mucosa; thus, large (ca. 5 to 100 μm) particles or droplets are generally preferred for targeting of nasal delivery.

Pulmonary drug delivery is accomplished by inhalation of an aerosol through the mouth and throat. Particles having aerodynamic diameters of greater than about 5 microns generally do not reach the lung; instead, they tend to impact the back of the throat and are swallowed and possibly orally absorbed. Particles having diameters of about 2 to about 5 microns are small enough to reach the upper- to mid-pulmonary region (conducting airways), but are too large to reach the alveoli. Even smaller particles, *i.e.*, about 0.5 to about 2 microns, are capable of reaching the alveolar region. Particles having diameters smaller than about 0.5 microns can also be deposited in the alveolar region by sedimentation, although very small particles may be exhaled.

B. Devices Used For Nasal And Pulmonary Drug Delivery

Drugs intended for intranasal delivery (systemic and local) can be administered as aqueous solutions or suspensions, as solutions or suspensions in halogenated hydrocarbon propellants (pressurized metered-dose inhalers), or as dry powders. Metered-dose spray pumps for aqueous formulations, pMDIs, and DPIs for nasal delivery are available from, for example, Valois of America or Pfeiffer of America.

Drugs intended for pulmonary delivery can also be administered as aqueous formulations, as suspensions or solutions in halogenated hydrocarbon propellants, or as dry powders. Aqueous formulations must be aerosolized by liquid nebulizers employing either hydraulic or ultrasonic atomization, propellant-based systems require suitable pressurized metered-dose inhalers (pMDIs), and dry powders require dry powder inhaler devices (DPIs) which are capable of dispersing the drug substance effectively. For aqueous and other non-pressurized liquid systems, a variety of nebulizers (including small volume nebulizers) are available to aerosolize the formulations. Compressor-driven nebulizers incorporate jet technology and use compressed air to generate the liquid aerosol. Such devices are commercially available from, for example, Healthdyne Technologies, Inc.; Invacare, Inc.; Mountain Medical Equipment, Inc.; Pari Respiratory, Inc.; Mada Medical, Inc.; Puritan-

Bennet; Schuco, Inc., DeVilbiss Health Care, Inc.; and Hospitak, Inc. Ultrasonic nebulizers rely on mechanical energy in the form of vibration of a piezoelectric crystal to generate inhalable liquid droplets and are commercially available from, for example, Omron Heathcare, Inc. and DeVilbiss Health Care, Inc.

A propellant driven inhaler (pMDI) releases a metered dose of medicine upon each actuation. The medicine is formulated as a suspension or solution of a drug substance in a suitable propellant such as a halogenated hydrocarbon. pMDIs are described in, for example, Newman, S. P., Aerosols and the Lung, Clarke et al., eds., pp. 197-224 (Butterworths, London, England, 1984).

Dry powder inhalers (DPIs), which involve deaggregation and aerosolization of dry powders, normally rely upon a burst of inspired air that is drawn through the unit to deliver a drug dosage. Such devices are described in, for example, U.S. Pat. No. 4,807,814 to Douche et al., which is directed to a pneumatic powder ejector having a suction stage and an injection stage; SU 628930 (Abstract), describing a hand-held powder disperser having an axial air flow tube; Fox et al., *Powder and Bulk Engineering*, pages 33-36 (March 1988), describing a venturi eductor having an axial air inlet tube upstream of a venturi restriction; EP 347 779, describing a hand-held powder disperser having a collapsible expansion chamber, and U.S. Pat. No. 5,785,049 to Smith et al., directed to dry powder delivery devices for drugs.

C. Problems With Conventional Aerosol And Injectable Compositions And Methods

Conventional techniques are extremely inefficient in delivering agents to the lung for a variety of reasons. Prior to the present invention, attempts to develop inhalable aqueous suspensions of poorly water soluble drugs have been largely unsuccessful. For example, it has been reported that ultrasonic nebulization of a suspension containing fluorescein and latex drug spheres, representing insoluble drug particles, resulted in only 1% aerosolization of the particles, while air-jet nebulization resulted in only a fraction of particles being aerosolized (Susan L. Tiano, "Functionality Testing Used to Rationally Assess Performance of a Model Respiratory Solution or Suspension in a Nebulizer," Dissertation Abstracts International, 56/12-B, pp. 6578 (1995)). Another problem encountered with nebulization of liquid formulations prior to the present invention was the long (4–20 min) period of time

required for administration of a therapeutic dose. Long administration times are required because conventional liquid formulations for nebulization are very dilute solutions or suspensions of micronized drug substance. Prolonged administration times are undesirable because they lessen patient compliance and make it difficult to control the dose administered. Lastly, aerosol formulations of micronized drug are not feasible for deep lung delivery of insoluble compounds because the droplets needed to reach the alveolar region (0.5 to 2 microns) are too small to accommodate micronized drug crystals, which are typically 2–3 microns or more in diameter.

Conventional pMDIs are also inefficient in delivering drug substance to the lung. In most cases, pMDIs consist of suspensions of micronized drug substance in halogenated hydrocarbons such as chlorofluorocarbons (CFCs) or hydrofluoroalkanes (HFAs). Actuation of the pMDI results in delivery of a metered dose of drug and propellant, both of which exit the device at high velocities because of the propellant pressures. The high velocity and momentum of the drug particles results in a high degree of oropharyngeal impaction as well as loss to the device used to deliver the agent. These losses lead to variability in therapeutic agent levels and poor therapeutic control. In addition, oropharyngeal deposition of drugs intended for topical administration to the conducting airways (such as corticosteroids) can lead to systemic absorption with resultant undesirable side effects. Additionally, conventional micronization (air-jet milling) of pure drug substance can reduce the drug particle size to no less than about 2-3 microns. Thus, the micronized material typically used in pMDIs is inherently unsuitable for delivery to the alveolar region and is not expected to deposit below the central bronchiole region of the lung.

Prior to the present invention, delivery of dry powders to the lung typically used micronized drug substance. In the dry powder form, micronized substances tend to have substantial interparticle electrostatic attractive forces which prevent the powders from flowing smoothly and generally make them difficult to disperse. Thus, two key challenges to pulmonary delivery of dry powders are the ability of the device to accurately meter the intended dose and the ability of the device to fully disperse the micronized particles. For many devices and formulations, the extent of dispersion is dependent upon the patient's inspiration rate, which itself may be variable and can lead to a variability in the delivered dose.

Delivery of drugs to the nasal mucosa can also be accomplished with aqueous, propellant-based, or dry powder formulations. However, absorption of poorly soluble drugs can be problematic because of mucociliary clearance which transports deposited particles from the nasal mucosa to the throat where they are swallowed. Complete clearance generally occurs within about 15–20 minutes. Thus, poorly soluble drugs which do not dissolve within this time frame are unavailable for either local or systemic activity.

As described below in the Background of Nanoparticulate Active Agent Compositions, several published U.S. patents and patent applications describe aerosols of nanoparticulate drugs. However, none of these documents describe aerosols of a nanoparticulate benzodiazepine, such as lorazepam.

II. Background Regarding Lorazepam

Lorazepam is a benzodiazepine. It is also known as 7-Chloro-5-(2-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one. Its molecular formula is $C_{15}H_{10}Cl_2N_2O_2$, and it has a molecular weight of 321.16. Lorazepam has only slight solubility in water, *i.e.*, 0.08 mg/mL. United States Patent No. 6,699,849 to Loftsson et al., which is specifically incorporated by reference, refers to lorazepam and benzodiazepine. Lorazepam is a controlled substance. *Merck Index*, Thirteenth Ed., p. 999 (Merck & Co., Whitehouse Station, N.J. 2001). As pharmaceutically acceptable salts including organic salts or esters of lorazepam can be employed as a substitute for lorazepam, the references below to lorazepam are also intended to include lorazepam salts and esters and mixtures thereof.

Because of lorazepam's low water solubility, it is generally formulated for oral administration. However, oral administration of lorazepam has disadvantages. For example, lorazepam is susceptible to enzymatic degradation by glucuronyl transferase enzyme in the intestine or in the intestinal mucosa, as disclosed in United States Patent No. 6,692,766 to Rubinstein et al., which is incorporated by reference. Sterile lorazepam typically includes a preservative such as benzyl alcohol and requires refrigeration. Lorazepam delivered orally may have a slow absorption and onset of action.

Injectable formulations of lorazepam are preferable over oral administration doses because intravenous (IV) or intramuscular (IM) administration of a drug results in a significantly shorter response time as compared to oral administration. Moreover, injectable

formulations of pain medication are also preferable for post-operative health care, where oral administration may not be feasible. Injectable formulations of lorazepam are particularly preferred, as lorazepam is not addictive, in contrast to other injectable formulations of drugs, such as morphine and ketorolac (Toradol®).

However, injectable lorazepam formulations are difficult to formulate due to the low water-solubility of lorazepam. Moreover, current injectable formulations of lorazepam are undesirable because the formulations must include polyethylene glycol and propylene glycol as solubilizers, which can result in pain at the injection site.

III. Background Regarding Nanoparticulate Active Agent Compositions

Nanoparticulate compositions, first described in U.S. Pat. No. 5,145,684 ("the '684 patent"), are particles consisting of a poorly soluble therapeutic or diagnostic agent having adsorbed onto or associated with the surface thereof a non-crosslinked surface stabilizer. The '684 patent also describes methods of making such nanoparticulate compositions but does not describe compositions comprising a benzodiazepine, such as lorazepam, in nanoparticulate form. Methods of making nanoparticulate compositions are described, for example, in U.S. Pat. Nos. 5,518,187 and 5,862,999, both for "Method of Grinding Pharmaceutical Substances;" U.S. Pat. No. 5,718,388, for "Continuous Method of Grinding Pharmaceutical Substances;" and U.S. Pat. No. 5,510,118 for "Process of Preparing Therapeutic Compositions Containing Nanoparticles."

Nanoparticulate compositions are also described, for example, in U.S. Pat. No. 5,298,262 for "Use of Ionic Cloud Point Modifiers to Prevent Particle Aggregation During Sterilization;" U.S. Pat. No. 5,302,401 for "Method to Reduce Particle Size Growth During Lyophilization;" U.S. Pat. No. 5,318,767 for "X-Ray Contrast Compositions Useful in Medical Imaging;" U.S. Pat. No. 5,326,552 for "Novel Formulation For Nanoparticulate X-Ray Blood Pool Contrast Agents Using High Molecular Weight Non-ionic Surfactants;" U.S. Pat. No. 5,328,404 for "Method of X-Ray Imaging Using Iodinated Aromatic Propanedioates;" U.S. Pat. No. 5,336,507 for "Use of Charged Phospholipids to Reduce Nanoparticle Aggregation;" U.S. Pat. No. 5,340,564 for "Formulations Comprising Olin 10-G to Prevent Particle Aggregation and Increase Stability;" U.S. Pat. No. 5,346,702 for "Use of Non-Ionic Cloud Point Modifiers to Minimize Nanoparticulate Aggregation During

Sterilization;" U.S. Pat. No. 5,349,957 for "Preparation and Magnetic Properties of Very Small Magnetic-Dextran Particles;" U.S. Pat. No. 5,352,459 for "Use of Purified Surface Modifiers to Prevent Particle Aggregation During Sterilization;" U.S. Pat. Nos. 5,399,363 and 5,494,683, both for "Surface Modified Anticancer Nanoparticles;" U.S. Pat. No. 5,401,492 for "Water Insoluble Non-Magnetic Manganese Particles as Magnetic Resonance Enhancement Agents;" U.S. Pat. No. 5,429,824 for "Use of Tyloxapol as a Nanoparticulate Stabilizer;" U.S. Pat. No. 5,447,710 for "Method for Making Nanoparticulate X-Ray Blood Pool Contrast Agents Using High Molecular Weight Non-ionic Surfactants;" U.S. Pat. No. 5,451,393 for "X-Ray Contrast Compositions Useful in Medical Imaging;" U.S. Pat. No. 5,466,440 for "Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents in Combination with Pharmaceutically Acceptable Clays;" U.S. Pat. No. 5,470,583 for "Method of Preparing Nanoparticle Compositions Containing Charged Phospholipids to Reduce Aggregation;" U.S. Pat. No. 5,472,683 for "Nanoparticulate Diagnostic Mixed Carbamic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" U.S. Pat. No. 5,500,204 for "Nanoparticulate Diagnostic Dimers as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" U.S. Pat. No. 5,518,738 for "Nanoparticulate NSAID Formulations;" U.S. Pat. No. 5,521,218 for "Nanoparticulate Iododipamide Derivatives for Use as X-Ray Contrast Agents;" U.S. Pat. No. 5,525,328 for "Nanoparticulate Diagnostic Diatrizoxy Ester X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" U.S. Pat. No. 5,543,133 for "Process of Preparing X-Ray Contrast Compositions Containing Nanoparticles;" U.S. Pat. No. 5,552,160 for "Surface Modified NSAID Nanoparticles;" U.S. Pat. No. 5,560,931 for "Formulations of Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" U.S. Pat. No. 5,565,188 for "Polyalkylene Block Copolymers as Surface Modifiers for Nanoparticles;" U.S. Pat. No. 5,569,448 for "Sulfated Non-ionic Block Copolymer Surfactant as Stabilizer Coatings for Nanoparticle Compositions;" U.S. Pat. No. 5,571,536 for "Formulations of Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" U.S. Pat. No. 5,573,749 for "Nanoparticulate Diagnostic Mixed Carboxylic Anydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" U.S. Pat. No. 5,573,750 for "Diagnostic Imaging X-Ray Contrast Agents;" U.S. Pat. No. 5,573,783 for "Redispersible Nanoparticulate Film Matrices With Protective Overcoats;" U.S. Pat. No. 5,580,579 for "Site-specific Adhesion Within the

GI Tract Using Nanoparticles Stabilized by High Molecular Weight, Linear Poly(ethylene Oxide) Polymers;" U.S. Pat. No. 5,585,108 for "Formulations of Oral Gastrointestinal Therapeutic Agents in Combination with Pharmaceutically Acceptable Clays;" U.S. Pat. No. 5,587,143 for "Butylene Oxide-Ethylene Oxide Block Copolymers Surfactants as Stabilizer Coatings for Nanoparticulate Compositions;" U.S. Pat. No. 5,591,456 for "Milled Naproxen with Hydroxypropyl Cellulose as Dispersion Stabilizer;" U.S. Pat. No. 5,593,657 for "Novel Barium Salt Formulations Stabilized by Non-ionic and Anionic Stabilizers;" U.S. Pat. No. 5,622,938 for "Sugar Based Surfactant for Nanocrystals;" U.S. Pat. No. 5,628,981 for "Improved Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents and Oral Gastrointestinal Therapeutic Agents;" U.S. Pat. No. 5,643,552 for "Nanoparticulate Diagnostic Mixed Carbonic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" U.S. Pat. No. 5,718,388 for "Continuous Method of Grinding Pharmaceutical Substances;" U.S. Pat. No. 5,718,919 for "Nanoparticles Containing the R(-)Enantiomer of Ibuprofen;" U.S. Pat. No. 5,747,001 for "Aerosols Containing Beclomethasone Nanoparticle Dispersions;" U.S. Pat. No. 5,834,025 for "Reduction of Intravenously Administered Nanoparticulate Formulation Induced Adverse Physiological Reactions;" U.S. Pat. No. 6,045,829 "Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors Using Cellulosic Surface Stabilizers;" U.S. Pat. No. 6,068,858 for "Methods of Making Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors Using Cellulosic Surface Stabilizers;" U.S. Pat. No. 6,153,225 for "Injectable Formulations of Nanoparticulate Naproxen;" U.S. Pat. No. 6,165,506 for "New Solid Dose Form of Nanoparticulate Naproxen;" U.S. Pat. No. 6,221,400 for "Methods of Treating Mammals Using Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors;" U.S. Pat. No. 6,264,922 for "Nebulized Aerosols Containing Nanoparticle Dispersions;" U.S. Pat. No. 6,267,989 for "Methods for Preventing Crystal Growth and Particle Aggregation in Nanoparticle Compositions;" U.S. Pat. No. 6,270,806 for "Use of PEG-Derivatized Lipids as Surface Stabilizers for Nanoparticulate Compositions;" U.S. Pat. No. 6,316,029 for "Rapidly Disintegrating Solid Oral Dosage Form," U.S. Pat. No. 6,375,986 for "Solid Dose Nanoparticulate Compositions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate;" U.S. Pat. No. 6,428,814 for "Bioadhesive

Nanoparticulate Compositions Having Cationic Surface Stabilizers;" U.S. Pat. No. 6,431,478 for "Small Scale Mill;" U.S. Pat. No. 6,432,381 for "Methods for Targeting Drug Delivery to the Upper and/or Lower Gastrointestinal Tract;" U.S. Pat. No. 6,582,285 for "Apparatus for Sanitary Wet Milling;" and U.S. Pat. No. 6,592,903 for "Nanoparticulate Dispersions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate;" 6,656,504 for "Nanoparticulate Compositions Comprising Amorphous Cyclosporine;" 6,742,734 for "System and Method for Milling Materials;" 6,745,962 for "Small Scale Mill and Method Thereof;" 6,811,767 for "Liquid droplet aerosols of nanoparticulate drugs;" and 6,908,626 for "Compositions having a combination of immediate release and controlled release characteristics;" all of which are specifically incorporated by reference. In addition, U.S. patent application Ser. No. 20020012675 A1, published on Jan. 31, 2002, for "Controlled Release Nanoparticulate Compositions" and WO 02/098565 for "System and Method for Milling Materials," describe nanoparticulate compositions, and are specifically incorporated by reference.

In particular, documents referring to aerosols of nanoparticulate drugs include U.S. Pat. No. 5,747,001 for "Aerosols Containing Beclomethasone Nanoparticle Dispersions" and U.S. Pat. No. 6,264,922 for "Nebulized Aerosols Containing Nanoparticle Dispersions," and documents referring to injectable compositions of nanoparticulate drugs include U.S. Pat. No. 6,153,225 for "Injectable Formulations of Nanoparticulate Naproxen," and U.S. Pat. Nos. 5,399,363 and 5,494,683, both for "Surface Modified Anticancer Nanoparticles." None of these documents describe injectable or aerosol compositions of a nanoparticulate benzodiazepine, such as lorazepam.

Amorphous small particle compositions are described, for example, in U.S. Pat. No. 4,783,484 for "Particulate Composition and Use Thereof as Antimicrobial Agent;" U.S. Pat. No. 4,826,689 for "Method for Making Uniformly Sized Particles from Water-Insoluble Organic Compounds;" U.S. Pat. No. 4,997,454 for "Method for Making Uniformly-Sized Particles From Insoluble Compounds;" U.S. Pat. No. 5,741,522 for "Ultrasmall, Non-aggregated Porous Particles of Uniform Size for Entrapping Gas Bubbles Within and Methods;" and U.S. Pat. No. 5,776,496, for "Ultrasmall Porous Particles for Enhancing Ultrasound Back Scatter" all of which are specifically incorporated herein by reference.

There remains a need in the art for improved dosage forms of benzodiazepines, such

as lorazepam. The present invention satisfies this need.

SUMMARY OF THE INVENTION

The present invention is directed to the surprising and unexpected discovery of new aerosol and injectable dosage forms of a nanoparticulate benzodiazepine, such as lorazepam. The formulations comprises a nanoparticulate benzodiazepine, such as nanoparticulate lorazepam, having an effective average particle size of less than about 2000 nm. The nanoparticulate benzodiazepine, such as lorazepam, preferably has at least one surface stabilizer either adsorbed onto or associated with the surface of the benzodizepine. In one embodiment of the invention, the surface stabilizer is a povidone polymer. Because lorazepam is practically insoluble in water, significant bioavailability can be problematic.

In one embodiment there is provided an aerosol that delivers an optimal dosage of a benzodiazepine, such as lorazepam. The aerosols of the invention do not require a preservative such as benzyl alcohol, which affects lorazepam stability.

In another embodiment, a safe and effective injectable formulation of a benzodiazepine, such as lorazepam, is provided. The injectable formulation eliminates the need for propylene glycol and polyethylene glycol, such as polyoxyl 60 hydrogenated castor oil (HCO-60), as solubilizers for injectable lorazepam compositions, and solves the problem of the insolubility of lorazepam in water. This is beneficial, as in convention non-nanoparticulate injectable benzodiazepine formulations comprising polyoxyl 60 hydrogenated castor oil as a solubilizer, the presence of this solubilizer can lead to anaphylactic shock (i.e., severe allergic reaction) and death. The injectable dosage forms of the invention surprisingly deliver the required therapeutic amount of the drug *in vivo*, and render the drug bioavailable in a rapid and constant manner, which is required for effective human therapy. Moreover, the invention provides for compositions comprising high concentrations of a benzodiazepine, such as lorazepam, in low injection volumes, with rapid drug dissolution upon administration.

The present invention is also directed to aqueous, propellant-based, and dry powder aerosols of a nanoparticulate benzodiazepine, such as lorazepam, for pulmonary and nasal delivery, in which essentially every inhaled particle contains at least one nanoparticulate benzodiazepine, such as lorazepam, nanoparticle. The nanoparticulate benzodiazepine, such

as lorazepam, is highly water-insoluble. Preferably, the nanoparticulate benzodiazepine, such as lorazepam, has an effective average particle size of less than about 2 microns. Nanoparticulate aerosol formulations are described in U.S. Patent No. 6,811,767 to Bosch et al., specifically incorporated by reference. Non-aerosol preparations of submicron sized water-insoluble drugs are described in U.S. Pat. No. 5,145,684 to Liversidge et al., specifically incorporated herein by reference.

The invention also includes the following embodiments directed to aerosol formulations of a benzodiazepine, such as lorazepam. One embodiment of the invention is directed to aqueous aerosols of nanoparticulate dispersion of a benzodiazepine, such as lorazepam. Another embodiment of the invention is directed to dry powder aerosol formulations comprising a benzodiazepine, such as lorazepam, for pulmonary and/or nasal administration. Yet another embodiment of the invention is directed to a process and composition for propellant-based systems comprising a nanoparticulate benzodiazepine, such as lorazepam.

The nanoparticulate benzodiazepine, such as lorazepam, formulations of the invention may optionally include one or more pharmaceutically acceptable excipients, such as non-toxic physiologically acceptable liquid carriers, pH adjusting agents, or preservatives.

In another aspect of the invention there is provided a method of preparing the nanoparticulate benzodiazepine, such as lorazepam, injectable and aerosol formulations of the invention. The nanoparticulate dispersions used in making aerosol and injectable nanoparticulate benzodiazepine compositions can be made by wet milling, homogenization, precipitation, or supercritical fluid methods known in the art. An exemplary method comprises: (1) dispersing a benzodiazepine, such as lorazepam, in a liquid dispersion media; and (2) mechanically reducing the particle size of the benzodiazepine to the desired effective average particle size, e.g., less than about 2000 nm. At least one surface stabilizer can be added to the dispersion media either before, during, or after particle size reduction of the benzodiazepine. In one embodiment for the injectable composition, the surface stabilizer is a povidone polymer with a molecular weight of less than about 40,000 daltons. Preferably, the liquid dispersion media is maintained at a physiologic pH, for example, within the range of from about 3 to about 8, during the size reduction process. The nanoparticulate benzodiazepine dispersion can be used as an injectable formulation.

Dry powders comprising a nanoparticulate benzodiazepine, such as lorazepam, can be made by spray drying or freeze-drying aqueous dispersions of the nanoparticles. The dispersions used in these systems may or may not comprise dissolved diluent material prior to drying. Additionally, both pressurized and non-pressurized milling operations can be employed to make nanoparticulate benzodiazepine, such as lorazepam, compositions in non-aqueous systems.

In yet another aspect of the invention, there is provided a method of treating a subject in need with the injectable and/or aerosol nanoparticulate benzodiazepine, such as lorazepam, compositions of the invention. In an exemplary method, therapeutically effective amount of an injectable or aerosol nanoparticulate benzodiazepine composition of the invention is administered to a subject in need. The methods of the invention encompass treating a subject for status epilepticus, treatment of irritable bowel syndrome, sleep induction, acute psychosis, and pre-anesthesia medication. Diagnostic methods, comprising imaging of the administered dosage form, are also encompassed by the invention.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed. Other objects, advantages, and novel features will be readily apparent to those skilled in the art from the following detailed description of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The compositions of the invention encompass a nanoparticulate benzodiazepine, such as lorazepam, having an effective average particle size of less than about 2000 nm. For the injectable compositions, the nanoparticulate benzodiazepine, such as lorazepam, preferably has an effective average particle size of less than about 600 nm. For the aerosol compositions, the nanoparticulate benzodiazepine, such as lorazepam, has an effective average particle size of less than about 2000 nm. In one embodiment of the invention, the nanoparticulate benzodiazepine particles have at least one surface stabilizer either adsorbed onto or associated with the surface of the drug particles. The compositions are formulated into either an aerosol dosage form or an injectable dosage form. The aerosol dosage form can be either an aqueous aerosol or a dry powder aerosol.

Using the nanoparticulate benzodiazepine aerosol compositions of the invention, an essentially water-insoluble benzodiazepine, such as lorazepam, can be delivered to the deep lung. This is either not possible or extremely difficult using aerosol formulations of a micronized water-insoluble benzodiazepine. Deep lung delivery is necessary for benzodiazepine, such as lorazepam, compositions that are intended for systemic administration because deep lung delivery allows rapid absorption of the drug into the bloodstream by the alveoli, thus enabling rapid onset of action.

The present invention increases the number of benzodiazepine, such as lorazepam, particles per unit dose and results in distribution of a nanoparticulate benzodiazepine, such as lorazepam, over a larger physiological surface area as compared to the same quantity of a delivered micronized benzodiazepine, such as lorazepam. For systemic delivery by the pulmonary route, this approach takes maximum advantage of the extensive surface area presented in the alveolar region — thus producing more favorable benzodiazepine, such as lorazepam, delivery profiles, such as a more complete absorption and rapid onset of action.

Moreover, in contrast to micronized aqueous aerosol dispersions, aqueous dispersions of a water-insoluble nanoparticulate benzodiazepine, such as lorazepam, can be nebulized ultrasonically. Micronized drug is too large to be delivered efficiently by an ultrasonic nebulizer.

Droplet size determines *in vivo* deposition of a benzodiazepine, *i.e.*, very small particles, about <2 microns, are delivered to the alveoli; larger particles, about 2 to about 10 microns, are delivered to the bronchiole region; and for nasal delivery, particles of about 5 to about 100 microns are preferred. Thus, the ability to obtain very small benzodiazepine, such as lorazepam, particle sizes which can "fit" in a range of droplet sizes allows more effective and more efficient (*i.e.*, benzodiazepine uniformity) targeting to the desired delivery region. This is not possible using micronized benzodiazepine, as the particle size of benzodiazepine is too large to target areas such as the alveolar region of the lung. Moreover, even when micronized benzodiazepine is incorporated into larger droplet sizes, the resultant aerosol formulation is heterogeneous (*i.e.*, not all droplets contain benzodiazepine), and does not result in the rapid and efficient benzodiazepine delivery enabled by the nanoparticulate aerosol benzodiazepine, such as lorazepam, formulations of the invention.

The present invention also enables the aqueous aerosol delivery of high doses of benzodiazepine, such as lorazepam, in an extremely short time period, *i.e.*, 1–2 seconds (1 puff). This is in contrast to the conventional 4–20 min. administration period observed with pulmonary aerosol formulations of micronized drug. Furthermore, the dry aerosol nanoparticulate benzodiazepine, such as lorazepam, powders of the present invention are spherical and can be made smaller than micronized material, thereby producing aerosol compositions having better flow and dispersion properties, and capable of being delivered to the deep lung.

Finally, the aerosol benzodiazepine, such as lorazepam, compositions of the present invention enable rapid nasal delivery. Nasal delivery of such aerosol compositions will be absorbed more rapidly and completely than micronized aerosol compositions before being cleared by the mucociliary mechanism.

The dosage forms of the present invention may be provided in formulations which exhibit a variety of release profiles upon administration to a patient including, for example, an IR formulation, a CR formulation that allows once per day administration, and a combination of both IR and CR formulations. Because CR forms of the present invention can require only one dose per day (or one dose per suitable time period, such as weekly or monthly), such dosage forms provide the benefits of enhanced patient convenience and compliance. The mechanism of controlled-release employed in the CR form may be accomplished in a variety of ways including, but not limited to, the use of erodable formulations, diffusion-controlled formulations, and osmotically-controlled formulations.

Advantages of the nanoparticulate benzodiazepine formulations of the invention over conventional forms of a benzodiazepine, such as lorazepam (e.g., non-nanoparticulate or solubilized dosage forms) include, but are not limited to: (1) increased water solubility; (2) increased bioavailability; (3) smaller dosage form size due to enhanced bioavailability; (4) lower therapeutic dosages due to enhanced bioavailability; (5) reduced risk of unwanted side effects due to lower dosing; and (6) enhanced patient convenience and compliance. A further advantage of the injectable nanoparticulate benzodiazepine formulation of the present invention over conventional forms of injectable benzodiazepines, such as lorazepam, is the elimination of the need to use polyoxyl 60 hydrogenated castor oil (HCO-60) as a solubilizer. A further advantage of the aerosol nanoparticulate benzodiazepines, such as lorazepam, is a

reduced risk of unwanted side effects.

The present invention also includes nanoparticulate benzodiazepine, such as lorazepam, compositions, together with one or more non-toxic physiologically acceptable carriers, adjuvants, or vehicles, collectively referred to as carriers. The compositions can be formulated for parenteral injection (*e.g.*, intravenous, intramuscular, or subcutaneous) or aerosol delivery. The aerosols can be used for any suitable delivery, such as pulmonary or nasal delivery.

The present invention is described herein using several definitions, as set forth below and throughout the application.

The term "effective average particle size of less than about 2000 nm", as used herein means that at least 50% of the benzodiazepine, such as lorazepam, particles have a size, by weight, of less than about 2000 nm, when measured by, for example, sedimentation field flow fractionation, photon correlation spectroscopy, light scattering, disk centrifugation, and other techniques known to those of skill in the art.

As used herein, "about" will be understood by persons of ordinary skill in the art and will vary to some extent on the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art given the context in which it is used, "about" will mean up to plus or minus 10% of the particular term.

As used herein with reference to a stable benzodiazepine, such as lorazepam, particle connotes, but is not limited to one or more of the following parameters: (1) benzodiazepine particles do not appreciably flocculate or agglomerate due to interparticle attractive forces or otherwise significantly increase in particle size over time; (2) that the physical structure of the benzodiazepine particles is not altered over time, such as by conversion from an amorphous phase to a crystalline phase; (3) that the benzodiazepine particles are chemically stable; and/or (4) where the benzodiazepine has not been subject to a heating step at or above the melting point of the benzodiazepine in the preparation of the nanoparticles of the present invention.

The term "conventional" or "non-nanoparticulate" active agent or benzodiazepine, such as lorazepam, shall mean an active agent, such as lorazepam, which is solubilized or which has an effective average particle size of greater than about 2000 nm. Nanoparticulate active agents as defined herein have an effective average particle size of less than about 2000

nm.

The phrase "poorly water soluble drugs" as used herein refers to those drugs that have a solubility in water of less than about 30 mg/ml, preferably less than about 20 mg/ml, preferably less than about 10 mg/ml, or preferably less than about 1 mg/ml.

As used herein, the phrase "therapeutically effective amount" shall mean that drug dosage that provides the specific pharmacological response for which the drug is administered in a significant number of subjects in need of such treatment. It is emphasized that a therapeutically effective amount of a drug that is administered to a particular subject in a particular instance will not always be effective in treating the conditions/diseases described herein, even though such dosage is deemed to be a therapeutically effective amount by those of skill in the art.

The term "particulate" as used herein refers to a state of matter which is characterized by the presence of discrete particles, pellets, beads or granules irrespective of their size, shape or morphology. The term "multiparticulate" as used herein means a plurality of discrete, or aggregated, particles, pellets, beads, granules or mixture thereof irrespective of their size, shape or morphology.

The term "modified release" as used herein in relation to the composition according to the invention means release which is not immediate release and is taken to encompass controlled release, sustained release, and delayed release.

The term "time delay" as used herein refers to the duration of time between administration of the composition and the release of benzodiazepine, such as lorazepam, from a particular component.

The term "lag time" as used herein refers to the time between delivery of active ingredient from one component and the subsequent delivery of benzodiazepine, such as lorazepam, from another component.

I. Preferred Characteristics of the Nanoparticulate Benzodiazepine Compositions

There are a number of enhanced pharmacological characteristics of the nanoparticulate benzodiazepine, such as lorazepam, compositions of the present invention.

A. Increased Bioavailability

The benzodiazepine, such as lorazepam, formulations of the present invention exhibit increased bioavailability at the same dose of the same benzodiazepine, such as lorazepam, and require smaller doses as compared to prior conventional benzodiazepine, such as lorazepam, formulations.

Moreover, a nanoparticulate benzodiazepine, such as lorazepam, dosage form requires less drug to obtain the same pharmacological effect observed with a conventional microcrystalline benzodiazepine, such as lorazepam, dosage form. Therefore, the nanoparticulate benzodiazepine, such as lorazepam, dosage form has an increased bioavailability as compared to the conventional microcrystalline benzodiazepine, such as lorazepam, dosage form.

B. The Pharmacokinetic Profiles of the Benzodiazepine Compositions of the Invention are not Affected by the Fed or Fasted State of the Subject Ingesting the Compositions

The compositions of the present invention encompass a benzodiazepine, such as lorazepam, wherein the pharmacokinetic profile of the benzodiazepine is not substantially affected by the fed or fasted state of a subject ingesting the composition. This means that there is little or no appreciable difference in the quantity of drug absorbed or the rate of drug absorption when the nanoparticulate benzodiazepine, such as lorazepam, compositions are administered in the fed versus the fasted state.

Benefits of a dosage form which substantially eliminates the effect of food include an increase in subject convenience, thereby increasing subject compliance, as the subject does not need to ensure that they are taking a dose either with or without food. This is significant, as with poor subject compliance with a benzodiazepine, such as lorazepam,, an increase in the medical condition for which the drug is being prescribed may be observed.

The invention also preferably provides a benzodiazepine, such as lorazepam, compositions having a desirable pharmacokinetic profile when administered to mammalian subjects. The desirable pharmacokinetic profile of the benzodiazepine, such as lorazepam, compositions preferably includes, but is not limited to: (1) a C_{max} for benzodiazepine, when assayed in the plasma of a mammalian subject following administration, that is preferably greater than the C_{max} for a non-nanoparticulate benzodiazepine formulation administered at

the same dosage; and/or (2) an AUC for benzodiazepine, when assayed in the plasma of a mammalian subject following administration, that is preferably greater than the AUC for a non-nanoparticulate benzodiazepine formulation, administered at the same dosage; and/or (3) a Tmax for benzodiazepine, when assayed in the plasma of a mammalian subject following administration, that is preferably less than the Tmax for a non-nanoparticulate benzodiazepine formulation, administered at the same dosage. The desirable pharmacokinetic profile, as used herein, is the pharmacokinetic profile measured after the initial dose of the benzodiazepine.

In one embodiment, a preferred benzodiazepine, such as lorazepam, composition exhibits in comparative pharmacokinetic testing with a non-nanoparticulate benzodiazepine, such as lorazepam, formulation, administered at the same dosage, a T_{max} not greater than about 90%, not greater than about 80%, not greater than about 70%, not greater than about 60%, not greater than about 50%, not greater than about 30%, not greater than about 25%, not greater than about 20%, not greater than about 15%, not greater than about 10%, or not greater than about 5% of the T_{max} exhibited by the non-nanoparticulate benzodiazepine, such as lorazepam, formulation.

In another embodiment, the benzodiazepine, such as lorazepam, composition of the invention exhibits in comparative pharmacokinetic testing with a non-nanoparticulate benzodiazepine, such as lorazepam, formulation, administered at the same dosage, a C_{max} which is at least about 50%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500%, at least about 600%, at least about 700%, at least about 800%, at least about 900%, at least about 1000%, at least about 1100%, at least about 1200%, at least about 1300%, at least about 1400%, at least about 1500%, at least about 1600%, at least about 1700%, at least about 1800%, or at least about 1900% greater than the C_{max} exhibited by the non-nanoparticulate benzodiazepine, such as lorazepam, formulation.

In yet another embodiment, the benzodiazepine, such as lorazepam, composition of the invention exhibits in comparative pharmacokinetic testing with a non-nanoparticulate benzodiazepine, such as lorazepam, formulation, administered at the same dosage, an AUC which is at least about 25%, at least about 50%, at least about 75%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 225%, at least about 250%, at least about 275%, at least about 300%, at least about

350%, at least about 400%, at least about 450%, at least about 500%, at least about 550%, at least about 600%, at least about 750%, at least about 700%, at least about 750%, at least about 800%, at least about 850%, at least about 900%, at least about 950%, at least about 1000%, at least about 1050%, at least about 1100%, at least about 1150%, or at least about 1200% greater than the AUC exhibited by the non-nanoparticulate benzodiazepine, such as lorazepam, formulation.

C. Bioequivalency of the Benzodiazepine Compositions of the Invention When Administered in the Fed Versus the Fasted State

The invention also encompasses a composition comprising a nanoparticulate benzodiazepine, such as lorazepam, in which administration of the composition to a subject in a fasted state is bioequivalent to administration of the composition to a subject in a fed state.

The difference in absorption of the compositions comprising the nanoparticulate benzodiazepine, such as lorazepam, when administered in the fed versus the fasted state, is preferably less than about 100%, less than about 90%, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 35%, less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, or less than about 3%.

In one embodiment of the invention, the invention encompasses nanoparticulate benzodiazepine, such as lorazepam, wherein administration of the composition to a subject in a fasted state is bioequivalent to administration of the composition to a subject in a fed state, in particular as defined by C_{max} and AUC guidelines given by the U.S. Food and Drug Administration and the corresponding European regulatory agency (EMEA). Under U.S. FDA guidelines, two products or methods are bioequivalent if the 90% Confidence Intervals (CI) for AUC and C_{max} are between 0.80 to 1.25 (T_{max} measurements are not relevant to bioequivalence for regulatory purposes). To show bioequivalency between two compounds or administration conditions pursuant to Europe's EMEA guidelines, the 90% CI for AUC must be between 0.80 to 1.25 and the 90% CI for C_{max} must between 0.70 to 1.43.

D. Dissolution Profiles of the Benzodiazepine Compositions of the Invention

The benzodiazepine, such as lorazepam, compositions of the present invention have unexpectedly dramatic dissolution profiles. Rapid dissolution of an administered active agent is preferable, as faster dissolution generally leads to faster onset of action and greater bioavailability. To improve the dissolution profile and bioavailability of benzodiazepine, such as lorazepam,, it is useful to increase the drug's dissolution so that it could attain a level close to 100%.

The benzodiazepine, such as lorazepam, compositions of the present invention preferably have a dissolution profile in which within about 5 minutes at least about 20% of the composition is dissolved. In other embodiments of the invention, at least about 30% or about 40% of the benzodiazepine, such as lorazepam, composition is dissolved within about 5 minutes. In yet other embodiments of the invention, preferably at least about 40%, about 50%, about 60%, about 70%, or about 80% of the benzodiazepine, such as lorazepam, composition is dissolved within about 10 minutes. Finally, in another embodiment of the invention, preferably at least about 70%, about 80%, about 90%, or about 100% of the benzodiazepine, such as lorazepam, composition is dissolved within about 20 minutes.

Dissolution is preferably measured in a medium which is discriminating. Such a dissolution media will produce two very different dissolution curves for two products having very different dissolution profiles in gastric juices, *i.e.*, the dissolution medium is predictive of *in vivo* dissolution of a composition. An exemplary dissolution medium is an aqueous medium containing the surfactant sodium lauryl sulfate at 0.025 M. Determination of the amount dissolved can be carried out by spectrophotometry. The rotating blade method (European Pharmacopoeia) can be used to measure dissolution.

E. Redispersibility Profiles of the Benzodiazepine Compositions of the Invention

An additional feature of the benzodiazepine, such as lorazepam, compositions of the present invention is that the compositions redisperse such that the effective average particle size of the redispersed benzodiazepine, such as lorazepam, particles is less than about 2 microns. This is significant, as if upon administration the nanoparticulate benzodiazepine, such as lorazepam, compositions of the invention did not redisperse to a nanoparticulate

particle size, then the dosage form may lose the benefits afforded by formulating the benzodiazepine, such as lorazepam, into a nanoparticulate particle size. A nanoparticulate size suitable for the present invention is an effective average particle size of less than about 2000 nm. In another embodiment, a nanoparticulate size suitable for the present invention is an effective average particle size of less than about 600 nm

Indeed, the nanoparticulate active agent compositions of the present invention benefit from the small particle size of the active agent; if the active agent does not redisperse into a small particle size upon administration, then "clumps" or agglomerated active agent particles are formed, owing to the extremely high surface free energy of the nanoparticulate system and the thermodynamic driving force to achieve an overall reduction in free energy. With the formation of such agglomerated particles, the bioavailability of the dosage form may fall well below that observed with the liquid dispersion form of the nanoparticulate active agent.

Moreover, the nanoparticulate benzodiazepine, such as lorazepam, compositions of the invention exhibit dramatic redispersion of the nanoparticulate benzodiazepine, such as lorazepam, particles upon administration to a mammal, such as a human or animal, as demonstrated by reconstitution/redispersion in a biorelevant aqueous media such that the effective average particle size of the redispersed benzodiazepine, such as lorazepam, particles is less than about 2 microns. Such biorelevant aqueous media can be any aqueous media that exhibit the desired ionic strength and pH, which form the basis for the biorelevance of the media. The desired pH and ionic strength are those that are representative of physiological conditions found in the human body. Such biorelevant aqueous media can be, for example, aqueous electrolyte solutions or aqueous solutions of any salt, acid, or base, or a combination thereof, which exhibit the desired pH and ionic strength.

Biorelevant pH is well known in the art. For example, in the stomach, the pH ranges from slightly less than 2 (but typically greater than 1) up to 4 or 5. In the small intestine the pH can range from 4 to 6, and in the colon it can range from 6 to 8. Biorelevant ionic strength is also well known in the art. Fasted state gastric fluid has an ionic strength of about 0.1M while fasted state intestinal fluid has an ionic strength of about 0.14. *See e.g.*, Lindahl et al., "Characterization of Fluids from the Stomach and Proximal Jejunum in Men and Women," *Pharm. Res., 14* (4): 497-502 (1997).

It is believed that the pH and ionic strength of the test solution is more critical than

the specific chemical content. Accordingly, appropriate pH and ionic strength values can be obtained through numerous combinations of strong acids, strong bases, salts, single or multiple conjugate acid-base pairs (*i.e.*, weak acids and corresponding salts of that acid), monoprotic and polyprotic electrolytes, *etc.*

Representative electrolyte solutions can be, but are not limited to, HCl solutions, ranging in concentration from about 0.001 to about 0.1 M, and NaCl solutions, ranging in concentration from about 0.001 to about 0.1 M, and mixtures thereof. For example, electrolyte solutions can be, but are not limited to, about 0.1 M HCl or less, about 0.01 M HCl or less, about 0.001 M HCl or less, about 0.1 M NaCl or less, about 0.01 M NaCl or less, about 0.001 M NaCl or less, and mixtures thereof. Of these electrolyte solutions, 0.01 M HCl and/or 0.1 M NaCl, are most representative of fasted human physiological conditions, owing to the pH and ionic strength conditions of the proximal gastrointestinal tract.

Electrolyte concentrations of 0.001 M HCl, 0.01 M HCl, and 0.1 M HCl correspond to pH 3, pH 2, and pH 1, respectively. Thus, a 0.01 M HCl solution simulates typical acidic conditions found in the stomach. A solution of 0.1 M NaCl provides a reasonable approximation of the ionic strength conditions found throughout the body, including the gastrointestinal fluids, although concentrations higher than 0.1 M may be employed to simulate fed conditions within the human GI tract.

Exemplary solutions of salts, acids, bases or combinations thereof, which exhibit the desired pH and ionic strength, include but are not limited to phosphoric acid/phosphate salts + sodium, potassium and calcium salts of chloride, acetic acid/acetate salts + sodium, potassium and calcium salts of chloride, carbonic acid/bicarbonate salts + sodium, potassium and calcium salts of chloride, and citric acid/citrate salts + sodium, potassium and calcium salts of chloride.

In other embodiments of the invention, the redispersed benzodiazepine, such as lorazepam, particles of the invention (redispersed in an aqueous, biorelevant, or any other suitable media) have an effective average particle size of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 650 nm, less than about 600 nm, less than about 550 nm, less

than about 500 nm, less than about 450 nm, less than about 400 nm, less than about 350 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, or less than about 50 nm, as measured by light-scattering methods, microscopy, or other appropriate methods. Such methods suitable for measuring effective average particle size are known to a person of ordinary skill in the art.

Redispersibility can be tested using any suitable means known in the art. *See e.g.,* the example sections of U.S. Patent No. 6,375,986 for "Solid Dose Nanoparticulate Compositions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate."

F. Benzodiazepine Compositions Used in Conjunction with Other Active Agents

The benzodiazepine, such as lorazepam, compositions of the invention can additionally comprise one or more compounds useful in the condition to be treated. Examples of such other active agents include, but are not limited to, antidepressants, steroids, antiemetics, antinauseants, spasmolytics, antipsychotics, opioids, carbidopa/levodopa or dopamine agonists, anesthetics, and narcotics.

Examples of antidepressants include, but are not limited to, selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (tricyclics). SSRIs include drugs such as escitalopram (brand name: Lexapro) citalopram (brand name: Celexa), fluoxetine (brand name: Prozac), paroxetine (brand name: Paxil) and sertraline (brand name: Zoloft). Tricyclics include amitriptyline (brand name: Elavil), desipramine (brand name: Norpramin), imipramine (brand name: Tofranil) and nortriptyline (brand names: Aventyl, Pamelor). Other antidepressants exist that have different ways of working than the SSRIs and tricylics. Commonly used ones are venlafaxine (brand name: Effexor), nefazadone (brand name: Serzone), bupropion (brand name: Wellbutrin), mirtazapine (brand name: Remeron) and trazodone (brand name: Desyrel). Less commonly used are the monomine oxidase inhibitors (MAOIs), such as phenelzine (brand name: Nardil) and tranylcypromine (brand name: Parnate).

Examples of steroids include, but are not limited to, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone,

and triamcinolone.

Examples of antiemetics or antinauseants include, but are not limited to, promethazine (Phenergan®), metoclopramide (Reglan®), cyclizine (Merezine®), diphenhydramine (Benadryl®), meclizine (Antivert®, Bonine®), chlorpromazine (Thorazine®), droperidol (Inapsine®), hydroxyzine (Atarax®, Vistaril®), prochlorperazine (Compazine®), trimethobenzamide (Tigan®), cisapride; h2-receptor antagonists, such as nizatidine, ondansetron (Zofran®), corticosteriods, 5-Hydroxytryptamine antagonists, such as dolasetron (Anzemet®), granisetron (Kytril®), ondansetron (Zofran®), tropisetron; dopamine antagonists, such as domperidone (Motilium®), droperidol (Inapsine®), haloperidol (Haldol®), chlorpromazine (Thorazine®); Antihistamines (5HT2 receptor antagonists), such as cyclizine (Antivert®, Bonine®, Dramamine®, Marezine®, Meclicot®, Medivert®), diphenhydramine, dimenhydrinate (Alavert®, Allegra®, Dramanate®) dimenhydrinate (Driminate®); and cannabinoids, such as marijuana and marinol.

Examples of spasmolytics or antispasmodics include, but are not limited to, methocarbamol, guaifenesin, diazepam, dantrolene, phenytoin, tolterodine, oxybutynin, flavoxate, and emepronium.

Examples of antipsychotics include, but are not limited to, clozapine (Clozaril®), risperidone (Risperdal®), olanzapine (Zyprexa®), quetiapine (Seroquel®), ziprasidone (Geodon®), and aripiprazole (Abilify®).

Examples of opioids include, but are not limited to, (1) opium alkaloids, such as morphine (Kadian®, Avinza®), codeine, and thebaine; (2) semisynthetic opioid derivatives, such as diamorphine (heroin), oxycodone (OxyContin®, Percodan®, Percocet®), hydrocodone, dihydrocodeine, hydromorphine, oxymorphone, and nicomorphine; (3) synthetic opioids, such as (a) pheylheptylamines, including methadone and levo-alphacetylmethadol (LAAM), (b) phenylpiperidines, including pethidine (meperidine), fentanyl, alfentanil, sufentanil, remifentanil, ketobemidone, and carfentanyl, (c) diphenylpropylamine derivatives, such as propoxyphene, dextropropoxyphene, dextromoramide, bezitramide, and piritramide, (d) benzomorphan derivatives, such as pentazocine and phenzocine, (e) oripavine derivatives, such as buprenorphine, (f) morphinan derivatives, such as butorphanol and nalbufine, and miscellaneous other synthetic opioids, such as dezocine, etorphine, tilidine, tramadol, loperamide, and diphenoxylate (Lomotil®).

Examples of carbidopa/levodopa or dopamine agonists include, but are not limited to, ropinirole, pramipexole and cabergoline, bromocriptine mesylate (Parlodel®), pergolide mesylate (Permax®), pramipexole dihydrochloride (Mirapex®), and ropinirole hydrochloride (Requip™).

Examples of anesthetics include, but are not limited to, enflurane, halothane, isoflurane, methoxyflurane, nitrous oxide, etomidate, ketamine, methohexital, propofol, and thiopental.

II. Compositions

The invention provides compositions comprising nanoparticulate benzodiazepine, such as lorazepam, particles and at least one surface stabilizer. The surface stabilizers are preferably adsorbed to or associated with the surface of the benzodiazepine, such as lorazepam, particles. Surface stabilizers useful herein do not chemically react with the benzodiazepine, such as lorazepam, particles or itself. Preferably, individual molecules of the surface stabilizer are essentially free of intermolecular cross-linkages. In another embodiment, the compositions of the present invention can comprise two or more surface stabilizers.

The present invention also includes nanoparticulate benzodiazepine, such as lorazepam, compositions together with one or more non-toxic physiologically acceptable carriers, adjuvants, or vehicles, collectively referred to as carriers. The compositions can be formulated for parenteral injection (*e.g.*, intravenous, intramuscular, or subcutaneous) or aerosol delivery. In certain embodiments of the invention, the nanoparticulate benzodiazepine, such as lorazepam, formulations are in an injectable form or an aerosol dosage form.

A. Benzodiazepine Particles

The invention is practiced with a benzodiazepine, such as lorazepam. The benzodiazepine, such as lorazepam, is preferably present in an essentially pure form, is poorly soluble, and is dispersible in at least one liquid media. By "poorly soluble," it is meant that the benzodiazepine, such as lorazepam, has a solubility in the liquid dispersion

media of less than about 10 mg/mL, and preferably of less than about 1 mg/mL. As noted above, the solubility of lorazepam in water is 0.08 mg/mL.

The drug can be selected from a variety of benzodiazepines for treatment of status epilepticus, treatment of irritable bowel syndrome, sleep induction, acute psychosis, and pre-anesthesia medications. Preferable drug classes are benzodiazepine, such as lorazepam, and pharmaceutically acceptable salts and esters of lorazepam. Benzodiazepines of particular interest are alprazolam, brotizolam, chlordiazepoxide, clobazam, clonazepam, clorazepam, demoxazepam, flumazenil, flurazepam halazepam, midazolam, nordazepam, medazepam, diazepam, nitrazepam oxazepam, midazepam, lorazepam, prazepam, quazepam, triazolam, temazepam, and loprazolam. Particularly preferred benzodiazepines are alprazolam, midazolam, clonazepam, lorazepam, and triazolam. The preferred benzodiazepine is lorazepam. A description of these classes of benzodiazepines and a listing of species within each class can be found in Martindale, The Extra Pharmacopoeia, Twenty-ninth Edition (The Pharmaceutical Press, London, 1989), specifically incorporated by reference. The drugs are commercially available and/or can be prepared by techniques known in the art.

"Pharmaceutically acceptable" as used herein refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

"Pharmaceutically acceptable salts and esters" as used herein refers to derivatives wherein the benzediazepine, such as lorazepam, is modified by making acid or base salts thereof. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. The pharmaceutically acceptable salts include the conventional non-toxic salts or the quarternary ammonium salts of the benzodiazepine and preferably, lorazepam formed, for example, from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic,

benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like.

B. Surface Stabilizers

Suitable surface stabilizers can be selected from known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products, and surfactants. Preferred surface stabilizers include nonionic, ionic, cationic, anionic, and zwitterionic surfactants. A preferred surface stabilizer for an injectable nanoparticulate benzodiazepine formulation is a povidone polymer. Two or more surface stabilizers can be used in combination.

Representative examples of surface stabilizers include hydroxypropyl methylcellulose (now known as hypromellose), hydroxypropylcellulose, polyvinylpyrrolidone, sodium lauryl sulfate, dioctylsulfosuccinate, gelatin, casein, lecithin (phosphatides), dextran, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers (e.g., macrogol ethers such as cetomacrogol 1000), polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters (e.g., the commercially available Tweens® such as e.g., Tween 20® and Tween 80® (ICI Speciality Chemicals)); polyethylene glycols (e.g., Carbowaxes 3550® and 934® (Union Carbide)), polyoxyethylene stearates, colloidal silicon dioxide, phosphates, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol (PVA), 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde (also known as tyloxapol, superione, and triton), poloxamers (e.g., Pluronics F68® and F108®, which are block copolymers of ethylene oxide and propylene oxide); poloxamines (e.g., Tetronic 908®, also known as Poloxamine 908®, which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine (BASF Wyandotte Corporation, Parsippany, N.J.)); Tetronic 1508® (T-1508) (BASF Wyandotte Corporation), Tritons X-200®, which is an alkyl aryl polyether sulfonate (Rohm and Haas); Crodestas F-110®, which is a mixture of sucrose stearate and sucrose distearate (Croda Inc.); p-isononylphenoxypoly-(glycidol), also

known as Olin-lOG® or Surfactant 10-G® (Olin Chemicals, Stamford, CT); Crodestas SL-40® (Croda, Inc.); and SA9OHCO, which is C18H37CH2(CON(CH3)-CH2(CHOH)4(CH20H)2 (Eastman Kodak Co.); decanoyl-N-methylglucamide; n-decyl (-D-glucopyranoside; n-decyl (-D-maltopyranoside; n-dodecyl (-D-glucopyranoside; n-dodecyl (-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-(-D-glucopyranoside; n-heptyl (-D-thioglucoside; n-hexyl (-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl (-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-(-D-glucopyranoside; octyl (-D-thioglucopyranoside; PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, PEG-vitamin E, lysozyme, random copolymers of vinyl pyrrolidone and vinyl acetate, and the like.

Examples of useful cationic surface stabilizers include, but are not limited to, polymers, biopolymers, polysaccharides, cellulosics, alginates, phospholipids, and nonpolymeric compounds, such as zwitterionic stabilizers, poly-n-methylpyridinium, anthryul pyridinium chloride, cationic phospholipids, chitosan, polylysine, polyvinylimidazole, polybrene, polymethylmethacrylate trimethylammoniumbromide bromide (PMMTMABr), hexyldesyltrimethylammonium bromide (HDMAB), and polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate. Other useful cationic stabilizers include, but are not limited to, cationic lipids, sulfonium, phosphonium, and quarternary ammonium compounds, such as stearyltrimethylammonium chloride, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride or bromide, coconut methyl dihydroxyethyl ammonium chloride or bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride or bromide, C12-15dimethyl hydroxyethyl ammonium chloride or bromide, coconut dimethyl hydroxyethyl ammonium chloride or bromide, myristyl trimethyl ammonium methyl sulfate, lauryl dimethyl benzyl ammonium chloride or bromide, lauryl dimethyl (ethenoxy)4 ammonium chloride or bromide, N-alkyl (C12-18)dimethylbenzyl ammonium chloride, N-alkyl (C14-18)dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C12-14) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts and dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt and/or an ethoxylated trialkyl ammonium salt,

dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C12-14) dimethyl 1-naphthylmethyl ammonium chloride and dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C12, C15, C17 trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride (ALIQUAT 336), POLYQUAT, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters (such as choline esters of fatty acids), benzalkonium chloride, stearalkonium chloride compounds (such as stearyltrimonium chloride and distearyldimonium chloride), cetyl pyridinium bromide or chloride, halide salts of quaternized polyoxyethylalkylamines, MIRAPOL and ALKAQUAT (Alkaril Chemical Company), alkyl pyridinium salts; amines, such as alkylamines, dialkylamines, alkanolamines, polyethylenepolyamines, N,N-dialkylaminoalkyl acrylates, and vinyl pyridine, amine salts, such as lauryl amine acetate, stearyl amine acetate, alkylpyridinium salt, and alkylimidazolium salt, and amine oxides; imide azolinium salts; protonated quaternary acrylamides; methylated quaternary polymers, such as poly[diallyl dimethylammonium chloride] and poly-[N-methyl vinyl pyridinium chloride]; and cationic guar.

Such exemplary cationic surface stabilizers and other useful cationic surface stabilizers are described in J. Cross and E. Singer, Cationic Surfactants: Analytical and Biological Evaluation (Marcel Dekker, 1994); P. and D. Rubingh (Editor), Cationic Surfactants: Physical Chemistry (Marcel Dekker, 1991); and J. Richmond, Cationic Surfactants: Organic Chemistry, (Marcel Dekker, 1990).

Nonpolymeric surface stabilizers are any nonpolymeric compound, such benzalkonium chloride, a carbonium compound, a phosphonium compound, an oxonium compound, a halonium compound, a cationic organometallic compound, a quarternary phosphorous compound, a pyridinium compound, an anilinium compound, an ammonium compound, a hydroxylammonium compound, a primary ammonium compound, a secondary

ammonium compound, a tertiary ammonium compound, and quarternary ammonium compounds of the formula NR1R2R3R4(+). For compounds of the formula NR1R2R3R4(+):

(i) none of R1-R4 are CH3;

(ii) one of R1-R4 is CH3;

(iii) three of R1-R4 are CH3;

(iv) all of R1-R4 are CH3;

(v) two of R1-R4 are CH3, one of R1-R4 is C6H5CH2, and one of R1-R4 is an alkyl chain of seven carbon atoms or less;

(vi) two of R1-R4 are CH3, one of R1-R4 is C6H5CH2, and one of R1-R4 is an alkyl chain of nineteen carbon atoms or more;

(vii) two of R1-R4 are CH3 and one of R1-R4 is the group C6H5(CH2)n, where n>1;

(viii) two of R1-R4 are CH3, one of R1-R4 is C6H5CH2, and one of R1-R4 comprises at least one heteroatom;

(ix) two of R1-R4 are CH3, one of R1-R4 is C6H5CH2, and one of R1-R4 comprises at least one halogen;

(x) two of R1-R4 are CH3, one of R1-R4 is C6H5CH2, and one of R1-R4 comprises at least one cyclic fragment;

(xi) two of R1-R4 are CH3 and one of R1-R4 is a phenyl ring; or

(xii) two of R1-R4 are CH3 and two of R1-R4 are purely aliphatic fragments.

Such compounds include, but are not limited to, behenalkonium chloride, benzethonium chloride, cetylpyridinium chloride, behentrimonium chloride, lauralkonium chloride, cetalkonium chloride, cetrimonium bromide, cetrimonium chloride, cethylamine hydrofluoride, chlorallylmethenamine chloride (Quaternium-15), distearyldimonium chloride (Quaternium-5), dodecyl dimethyl ethylbenzyl ammonium chloride (Quaternium-14), Quaternium-22, Quaternium-26, Quaternium-18 hectorite, dimethylaminoethylchloride hydrochloride, cysteine hydrochloride, diethanolammonium POE (10) oletyl ether phosphate, diethanolammonium POE (3)oleyl ether phosphate, tallow alkonium chloride, dimethyl dioctadecylammoniumbentonite, stearalkonium chloride, domiphen bromide, denatonium benzoate, myristalkonium chloride, laurtrimonium chloride, ethylenediamine dihydrochloride, guanidine hydrochloride, pyridoxine HCl, iofetamine hydrochloride,

meglumine hydrochloride, methylbenzethonium chloride, myrtrimonium bromide, oleyltrimonium chloride, polyquaternium-1, procainehydrochloride, cocobetaine, stearalkonium bentonite, stearalkoniumhectonite, stearyl trihydroxyethyl propylenediamine dihydrofluoride, tallowtrimonium chloride, and hexadecyltrimethyl ammonium bromide.

Most of these surface stabilizers are known pharmaceutical excipients and are described in detail in the *Handbook of Pharmaceutical Excipients*, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain (The Pharmaceutical Press, 2000), specifically incorporated herein by reference.

Povidone Polymers

Povidone polymers are preferred surface stabilizers for use in formulating an injectable nanoparticulate benzodiazepine, such as lorazepam, formulations. Povidone polymers, also known as polyvidon(e), povidonum, PVP, and polyvinylpyrrolidone, are sold under the trade names Kollidon® (BASF Corp.) and Plasdone® (ISP Technologies, Inc.). They are polydisperse macromolecular molecules, with a chemical name of 1-ethenyl-2-pyrrolidinone polymers and 1-vinyl-2-pyrrolidinone polymers. Povidone polymers are produced commercially as a series of products having mean molecular weights ranging from about 10,000 to about 700,000 daltons. To be useful as a surface modifier for a drug compound to be administered to a mammal, the povidone polymer must have a molecular weight of less than about 40,000 daltons, as a molecular weight of greater than 40,000 daltons would have difficulty clearing the body.

Povidone polymers are prepared by, for example, Reppe's process, comprising: (1) obtaining 1,4-butanediol from acetylene and formaldehyde by the Reppe butadiene synthesis; (2) dehydrogenating the 1,4-butanediol over copper at 200° to form γ-butyrolactone; and (3) reacting γ-butyrolactone with ammonia to yield pyrrolidone. Subsequent treatment with acetylene gives the vinyl pyrrolidone monomer. Polymerization is carried out by heating in the presence of H_2O and NH_3. *See The Merck Index*, 10th Edition, pp. 7581 (Merck & Co., Rahway, NJ, 1983).

The manufacturing process for povidone polymers produces polymers containing molecules of unequal chain length, and thus different molecular weights. The molecular weights of the molecules vary about a mean or average for each particular commercially

available grade. Because it is difficult to determine the polymer's molecular weight directly, the most widely used method of classifying various molecular weight grades is by K-values, based on viscosity measurements. The K-values of various grades of povidone polymers represent a function of the average molecular weight, and are derived from viscosity measurements and calculated according to Fikentscher's formula.

The weight-average of the molecular weight, Mw, is determined by methods that measure the weights of the individual molecules, such as by light scattering. Table 1 provides molecular weight data for several commercially available povidone polymers, all of which are soluble.

TABLE 1

Povidone	K-Value	Mv (Daltons)**	Mw (Daltons)**	Mn (Daltons)**
Plasdone C-15®	17 ± 1	7,000	10,500	3,000
Plasdone C-30®	30.5 ± 1.5	38,000	62,500*	16,500
Kollidon 12 PF®	11-14	3,900	2,000-3,000	1,300
Kollidon 17 PF®	16-18	9,300	7,000-11,000	2,500
Kollidon 25®	24-32	25,700	28,000-34,000	6,000

*Because the molecular weight is greater than 40,000 daltons, this povidone polymer is not useful as a surface stabilizer for a drug compound to be administered parenterally (*i.e.*, injected).

**Mv is the viscosity-average molecular weight, Mn is the number-average molecular weight, and Mw is the weight average molecular weight. Mw and Mn were determined by light scattering and ultra-centrifugation, and Mv was determined by viscosity measurements.

Based on the data provided in Table 1, exemplary preferred commercially available povidone polymers include, but are not limited to, Plasdone C-15®, Kollidon 12 PF®, Kollidon 17 PF®, and Kollidon 25®.

C. Nanoparticulate Benzodiazepine Particle Size

As used herein, particle size is determined on the basis of the weight average particle size as measured by conventional particle size measuring techniques well known to those skilled in the art. Such techniques include, for example, sedimentation field flow fractionation, photon correlation spectroscopy, light scattering, and disk centrifugation.

Compositions of the invention comprise benzodiazepine, such as lorazepam, nanoparticles having an effective average particle size of less than about 2000 nm (*i.e.*, 2

microns). In other embodiments of the invention, the benzodiazepine, such as lorazepam, nanoparticles have an effective average particle size of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 650 nm, less than about 600 nm, less than about 550 nm, less than about 500 nm, less than about 450 nm, less than about 400 nm, less than about 350 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, or less than about 50 nm, as measured by light-scattering methods, microscopy, or other appropriate methods.

In another embodiment, the nanoparticulate compositions of the present invention, and the injectable nanoparticulate compositions in particular, comprise benzodiazepine, such as lorazepam, nanoparticles that have an effective average particles size of less than about 600 nm. In other embodiments, the effective average particle size is less than about 550 nm, less than about 500 nm, less than about 450 nm, less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, or less than about 50 nm.

An "effective average particle size of less than about 2000 nm" means that at least 50% of the benzodiazepine, such as lorazepam, particles have a particle size less than the effective average, by weight, *i.e.*, less than about 2000 nm. If the "effective average particle size" is less than about 1900 nm, then at least about 50% of the benzodiazepine, such as lorazepam, particles have a size of less than about 1900 nm, when measured by the above-noted techniques. The same is true for the other particle sizes referenced above. In other embodiments, at least about 70%, at least about 90%, at least about 95%, or at least about 99% of the benzodiazepine, such as lorazepam, particles have a particle size less than the effective average, *i.e.*, less than about 2000 nm, about 1900 nm, about 1800 nm, *etc.*.

In the present invention, the value for D50 of a nanoparticulate benzodiazepine, such as lorazepam, composition is the particle size below which 50% of the benzodiazepine, such as lorazepam, particles fall, by weight. Similarly, D90 is the particle size below which 90% of the benzodiazepine, such as lorazepam, particles fall, by weight.

D. Concentration of Nanoparticulate Benzodiazepine and Surface Stabilizers

The relative amounts of benzodiazepine, such as lorazepam, and one or more surface stabilizers can vary widely. The optimal amount of the individual components depends, for example, upon physical and chemical attributes of the surface stabilizer(s) and benzodiazepine selected, such as the hydrophilic lipophilic balance (HLB), melting point, and the surface tension of water solutions of the stabilizer and benzodiazepine, *etc.*

Preferably, the concentration of benzodiazepine, such as lorazepam, can vary from about 99.5% to about 0.001%, from about 95% to about 0.1%, or from about 90% to about 0.5%, by weight, based on the total combined weight of the benzodiazepine and at least one surface stabilizer, not including other excipients. Higher concentrations of the active ingredient are generally preferred from a dose and cost efficiency standpoint.

Preferably, the concentration of surface stabilizer can vary from about 0.5% to about 99.999%, from about 5.0% to about 99.9%, or from about 10% to about 99.5%, by weight, based on the total combined dry weight of benzodiazepine, such as lorazepam, and at least one surface stabilizer, not including other excipients.

E. Other Pharmaceutical Excipients

Pharmaceutical compositions of the invention may also comprise one or more binding agents, filling agents, lubricating agents, suspending agents, sweeteners, flavoring agents, preservatives, buffers, wetting agents, disintegrants, effervescent agents, and other excipients depending upon the route of administration and the dosage form desired. Such excipients are well known in the art.

Examples of filling agents are lactose monohydrate, lactose anhydrous, and various starches; examples of binding agents are various celluloses and cross-linked polyvinylpyrrolidone, microcrystalline cellulose, such as Avicel® PH101 and Avicel® PH102, microcrystalline cellulose, and silicified microcrystalline cellulose (ProSolv SMCC™).

Suitable lubricants, including agents that act on the flowability of the powder to be compressed, are colloidal silicon dioxide, such as Aerosil® 200, talc, stearic acid, magnesium stearate, calcium stearate, and silica gel.

Examples of sweeteners are any natural or artificial sweetener, such as sucrose, xylitol, sodium saccharin, cyclamate, aspartame, and acsulfame. Examples of flavoring

agents are Magnasweet® (trademark of MAFCO), bubble gum flavor, and fruit flavors, and the like.

Examples of preservatives are potassium sorbate, methylparaben, propylparaben, benzoic acid and its salts, other esters of parahydroxybenzoic acid such as butylparaben, alcohols such as ethyl or benzyl alcohol, phenolic compounds such as phenol, and quarternary compounds such as benzalkonium chloride.

Suitable diluents include pharmaceutically acceptable inert fillers, such as microcrystalline cellulose, lactose, dibasic calcium phosphate, saccharides, and/or mixtures of any of the foregoing. Examples of diluents include microcrystalline cellulose, such as Avicel® PH101 and Avicel® PH102; lactose such as lactose monohydrate, lactose anhydrous, and Pharmatose® DCL21; dibasic calcium phosphate such as Emcompress®; mannitol; starch; sorbitol; sucrose; and glucose.

Suitable disintegrants include lightly crosslinked polyvinyl pyrrolidone, corn starch, potato starch, maize starch, and modified starches, croscarmellose sodium, cross-povidone, sodium starch glycolate, and mixtures thereof.

Examples of effervescent agents are effervescent couples, such as an organic acid and a carbonate or bicarbonate. Suitable organic acids include, for example, citric, tartaric, malic, fumaric, adipic, succinic, and alginic acids and anhydrides and acid salts. Suitable carbonates and bicarbonates include, for example, sodium carbonate, sodium bicarbonate, potassium carbonate, potassium bicarbonate, magnesium carbonate, sodium glycine carbonate, L-lysine carbonate, and arginine carbonate. Alternatively, only the sodium bicarbonate component of the effervescent couple may be present.

F. Aerosol Formulations of Nanoparticulate Benzodiazepines

The compositions of the invention encompass aerosols comprising a nanoparticulate benzodiazepine, such as lorazepam. Aerosols can be defined as colloidal systems comprising very finely divided liquid droplets or dry particles dispersed in and surrounded by a gas. Both liquid and dry powder aerosol compositions are encompassed by the invention.

Aerosols intended for delivery to the nasal mucosa are inhaled through the nose. For optimal delivery to the nasal cavities, droplet or aggregate dry powder particle sizes of about 5 to about 100 microns are useful, with droplet or aggregate dry powder particle sizes of

about 30 to about 60 microns being preferred. The nanoparticulate benzodiazepine particles are either suspended in the liquid droplet for an aqueous dispersion aerosol, or comprised in the aggregate dry powder particles for a dry powder aerosol. For nasal delivery, a larger inhaled particle size is desired to maximize impaction on the nasal mucosa and to minimize or prevent pulmonary deposition of the administered formulation. Inhaled particles may be defined as (1) liquid droplets comprising a suspended benzodiazepine particle, such as lorazepam, (2) dry particles of a benzodiazepine, such as lorazepam, (3) dry powder aggregates of a nanoparticulate benzodiazepine, such as lorazepam, or (4) dry particles of a diluent which comprise an embedded benzodiazepine, such as lorazepam, nanoparticles.

For delivery to the upper respiratory region, inhaled particle sizes of about 2 to about 10 microns are preferred. More preferred is about 2 to about 6 microns. Delivery to the upper respiratory region may be desirable for a nanoparticulate benzodiazepine, such as lorazepam nanoparticles, that are to act locally. This is because a nanoparticulate benzodiazepine, such as lorazepam, deposited in the upper respiratory tract can dissolve and act on the smooth muscle of the airway, rather than being absorbed into the bloodstream of the patient. However, the goal for an inhaled benzodiazepine, such as lorazepam, is systemic delivery, such as in cases of a benzodiazepine, such as lorazepam, which are not amenable to oral administration. It is preferred that a benzodiazepine, such as lorazepam, which is intended for systemic administration, be delivered to the alveolar region of the lung because 99.99% of the available surface area for a benzodiazepine, such as lorazepam, absorption is located in the peripheral alveoli. Thus, with administration to the alveolar region, rapid absorption can be realized. For delivery to the deep lung (alveolar) region, inhaled particle sizes of less than about 2 microns are preferred.

1. Concentration of Nanoparticulate Benzodiazepine

For aqueous aerosol formulations, nanoparticulate benzodiazepine, such as lorazepam, nanoparticles are present at a concentration of about 0.05 mg/mL up to about 600 mg/mL. For dry powder aerosol formulations, nanoparticulate benzodiazepine, such as lorazepam, nanoparticles are present at a concentration of about 0.05 mg/g up to about 990 mg/g, depending on the desired dosage. Concentrated nanoparticulate aerosols, defined as comprising a nanoparticulate benzodiazepine, such as lorazepam, at a concentration of about

10 mg/mL up to about 600 mg/mL for aqueous aerosol formulations, and about 10 mg/g up to about 990 mg/g for dry powder aerosol formulations, are specifically encompassed by the present invention. More concentrated aerosol formulations enable the delivery of large quantities of a nanoparticulate benzodiazepine, such as nanoparticulate lorazepam, to the lung in a very short period of time, thereby providing effective delivery to appropriate areas of the lung or nasal cavities in short administration times, *i.e.*, less than about 15 seconds as compared to administration times of up to 4 to 20 minutes as found in conventional pulmonary nebulizer therapies.

2. Aqueous Aerosols

The present invention encompasses aqueous formulations comprising nanoparticulate benzodiazepine, such as lorazepam, nanoparticles. Aqueous formulations of the invention comprise colloidal dispersions of a poorly water-soluble nanoparticulate benzodiazepine, such as lorazepam, in an aqueous vehicle which are aerosolized using air-jet or ultrasonic nebulizers. The advantages of the invention can best be understood by comparing the sizes of nanoparticulate and conventional micronized benzodiazepine, such as lorazepam, particles with the sizes of liquid droplets produced by conventional nebulizers. Conventional micronized material is generally about 2 to about 5 microns or more in diameter and is approximately the same size as the liquid droplet size produced by medical nebulizers. In contrast, nanoparticulate benzodiazepine, such as lorazepam, are substantially smaller than the droplets in such an aerosol. Thus, aerosols comprising nanoparticulate benzodiazepine, such as lorazepam, improve drug delivery efficiency. Such aerosols comprise a higher number of nanoparticles per unit dose, resulting in each aerosolized droplet containing active benzodiazepine, such as lorazepam.

Thus, with administration of the same dosages of nanoparticulate and micronized benzodiazepine, such as lorazepam, more lung or nasal cavity surface area is covered by the aerosol formulation comprising a nanoparticulate benzodiazepine, such as lorazepam.

Another advantage of the invention is that the compositions of the invention permit a poorly water-soluble benzodiazepine, such as lorazepam, to be delivered to the deep lung. Conventional micronized drug substance is too large to reach the peripheral lung regardless of the size of the droplet produced by the nebulizer, but the present invention permits

nebulizers which generate very small (about 0.5 to about 2 microns) aqueous droplets to deliver a poorly water-soluble benzodiazepine, such as lorazepam, in the form of nanoparticles to the alveoli. One example of such devices is the Circular™ aerosol (Westmed Corp., Tucson, Ariz.).

Yet another advantage of the invention is that ultrasonic nebulizers can be used to deliver a poorly water-soluble benzodiazepine, such as lorazepam, to the lung. Unlike conventional micronized material, nanoparticulate benzodiazepine, such as lorazepam, are readily aerosolized and show good *in vitro* deposition characteristics. A specific advantage of the invention is that it permits poorly water-soluble benzodiazepine, such as lorazepam, to be aerosolized by ultrasonic nebulizers which require a nanoparticulate benzodiazepine, such as lorazepam, to pass through very fine orifices to control the size of the aerosolized droplets. While conventional drug material would be expected to occlude the pores, such nanoparticulates are much smaller and can pass through the pores without difficulty.

Another advantage of the invention is the enhanced rate of dissolution of a poorly water-soluble benzodiazepine, such as lorazepam, which is practically insoluble in water. Since dissolution rate is a function of the total surface area of a benzodiazepine, such as lorazepam, to be dissolved, a more finely divided benzodiazepine (*e.g.*, nanoparticles) have much faster dissolution rates than conventional micronized drug particles. This can result in more rapid absorption of an inhaled benzodiazepine, such as lorazepam. For a nasally administered benzodiazepine, such as lorazepam, it can result in more complete absorption of the dose, since with a nanoparticulate dose of the benzodiazepine, such as lorazepam, the nanoparticles can dissolve rapidly and completely before being cleared by the mucociliary mechanism.

3. Dry Powder Aerosol Formulations

Another embodiment of the invention is directed to dry powder aerosol formulations comprising a benzodiazepine, such as lorazepam, for pulmonary and/or nasal administration. Dry powders, which can be used in both DPIs and pMDIs, can be made by spray-drying an aqueous nanoparticulate dispersion of a benzodiazepine, such as lorazepam. Alternatively, dry powders comprising a nanoparticulate benzodiazepine, such as lorazepam, can be made by freeze-drying dispersions of the nanoparticles. Combinations of the spray-dried and

freeze-dried nanoparticulate powders can be used in DPIs and pMDIs. For dry powder aerosol formulations, a nanoparticulate benzodiazepine, such as lorazepam, may be present at a concentration of about 0.05 mg/g up to about 990 mg/g. In addition, the more concentrated aerosol formulations (*i.e.*, for dry powder aerosol formulations about 10 mg/g up to about 990 mg/g) have the additional advantage of enabling large quantities of a benzodiazepine, such as lorazepam, to be delivered to the lung in a very short period of time, *e.g.*, about 1 to about 2 seconds (1 puff).

The invention is also directed to dry powders which comprise nanoparticulate compositions for pulmonary or nasal delivery. The powders may comprise inhalable aggregates of a nanoparticulate benzodiazepine, such as lorazepam, or inhalable particles of a diluent which comprises at least one embedded benzodiazepine, such as lorazepam. Powders comprising a nanoparticulate benzodiazepine, such as lorazepam, can be prepared from aqueous dispersions of nanoparticles by removing the water by spray-drying or lyophilization (freeze drying). Spray-drying is less time consuming and less expensive than freeze-drying, and therefore more cost-effective. However, certain benzodiazepines, such as lorazepam, benefit from lyophilization rather than spray-drying in making dry powder formulations.

Dry powder aerosol delivery devices must be able to accurately, precisely, and repeatably deliver the intended amount of benzodiazepine, such as lorazepam. Moreover, such devices must be able to fully disperse the dry powder into individual particles of a respirable size. Conventional micronized drug particles of 2–3 microns in diameter are often difficult to meter and disperse in small quantities because of the electrostatic cohesive forces inherent in such powders. These difficulties can lead to loss of drug substance to the delivery device as well as incomplete powder dispersion and sub-optimal delivery to the lung. Many drug compounds, particularly a benzodiazepine, such as lorazepam, are intended for deep lung delivery and systemic absorption. Since the average particle sizes of conventionally prepared dry powders are usually in the range of 2–3 microns, the fraction of material which actually reaches the alveolar region may be quite small. Thus, delivery of micronized dry powders to the lung, especially the alveolar region, is generally very inefficient because of the properties of the powders themselves.

The dry powder aerosols which comprise nanoparticulate benzodiazepine, such as lorazepam, can be made smaller than comparable micronized drug substance and, therefore,

are appropriate for efficient delivery to the deep lung. Moreover, aggregates of nanoparticulate benzodiazepine, such as lorazepam, are spherical in geometry and have good flow properties, thereby aiding in dose metering and deposition of the administered composition in the lung or nasal cavities.

Dry nanoparticulate compositions can be used in both DPIs and pMDIs. (In this invention, "dry" refers to a composition having less than about 5% water.)

a. Spray-dried powders comprising a nanoparticulate benzodiazepine

Powders comprising a nanoparticulate benzodiazepine, such as lorazepam, can be made by spray-drying aqueous dispersions of a nanoparticulate benzodiazepine, such as lorazepam, and a surface stabilizer to form a dry powder which comprises aggregated nanoparticulate benzodiazpine, such as lorazepam. The aggregates can have a size of about 1 to about 2 microns which is suitable for deep lung delivery. The aggregate particle size can be increased to target alternative delivery sites, such as the upper bronchial region or nasal mucosa by increasing the concentration of a benzodiazepine, such as lorazepam, in the spray-dried dispersion or by increasing the droplet size generated by the spray dryer.

Alternatively, the aqueous dispersion of a nanoparticulate benzodiazepine, such as lorazepam, and surface stabilizer can comprise a dissolved diluent such as lactose or mannitol which, when spray dried, forms inhalable diluent particles, each of which comprises at least one embedded benzodiazepine, such as lorazepam, nanoparticle and surface stabilizer. The diluent particles with an embedded benzodiazepine, such as lorazepam, nanoparticles can have a particle size of about 1 to about 2 microns, suitable for deep lung delivery. In addition, the diluent particle size can be increased to target alternate delivery sites, such as the upper bronchial region or nasal mucosa by increasing the concentration of dissolved diluent in the aqueous dispersion prior to spray drying, or by increasing the droplet size generated by the spray dryer.

Spray-dried powders can be used in DPIs or pMDIs, either alone or combined with freeze-dried nanoparticulate active agent powder. In addition, spray-dried powders comprising a nanoparticulate benzodiazepine, such as lorazepam, can be reconstituted and used in either jet or ultrasonic nebulizers to generate aqueous dispersions having respirable

droplet sizes, where each droplet comprises at least one nanoparticulate benzodiazepine, such as lorazepam. Concentrated nanoparticulate dispersions may also be used in these aspects of the invention.

b. Freeze-Dried Powders Comprising a Nanoparticulate Benzodiazepine

Nanoparticulate benzodiazepine, such as lorazepam, dispersions can also be freeze-dried to obtain powders suitable for nasal or pulmonary delivery. Such powders may comprise aggregated nanoparticulate benzodiazepine, such as lorazepam, having a surface stabilizer. Such aggregates may have sizes within a respirable range, *i.e.*, about 2 to about 5 microns. Larger aggregate particle sizes can be obtained for targeting alternate delivery sites, such as the nasal mucosa.

Freeze dried powders of the appropriate particle size can also be obtained by freeze drying aqueous dispersions of benzodiazepine, such as lorazepam, and surface stabilizer, which additionally may comprise a dissolved diluent such as lactose or mannitol. In these instances the freeze dried powders comprise respirable particles of diluent, each of which comprises at least one embedded nanoparticulate benzodiazepine, such as lorazepam.

Freeze-dried powders can be used in DPIs or pMIs, either alone or combined with spray-dried nanoparticulate powder. In addition, freeze-dried powders containing a nanoparticulate benzodiazepine, such as lorazepam, can be reconstituted and used in either jet or ultrasonic nebulizers to generate aqueous dispersions having respirable droplet sizes, where each droplet comprises at least one nanoparticulate benzodiazepine, such as lorazepam. Concentrated nanoparticulate dispersions may also be used in these aspects of the invention.

c. Propellant-Based Aerosols

Yet another embodiment of the invention is directed to a process and composition for propellant-based systems comprising a nanoparticulate benzodiazepine, such as lorazepam. Such formulations may be prepared by wet milling the coarse benzodiazepine, and preferably, lorazepam particles and surface stabilizer in liquid propellant, either at ambient pressure or under high pressure conditions. Alternatively, dry powders comprising a

nanoparticulate benzodiazepine, such as lorazepam, may be prepared by spray-drying or freeze-drying aqueous dispersions of a nanoparticulate benzodiazepine, such as lorazepam, with the resultant powders dispersed into suitable propellants for use in conventional pMDIs. Such nanoparticulate pMDI formulations can be used for either nasal or pulmonary delivery. For pulmonary administration, such formulations afford increased delivery to the deep lung regions because of the small (*i.e.*, about 1 to about 2 microns) particle sizes available from these methods. Concentrated aerosol formulations can also be employed in pMDIs.

Another embodiment of the invention is directed to a process and composition for propellant-based MDIs containing nanoparticulate benzodiazepine, such as lorazepam. pMDIs can comprise either the discrete nanoparticles and surface stabilizer, aggregates of the nanoparticles and surface stabilizer, or diluent particles comprising the embedded nanoparticles. pMDIs can be used for targeting the nasal cavity, the conducting airways of the lung, or the alveoli. Compared to conventional formulations, the present invention affords increased delivery to the deep lung regions because the inhaled nanoparticles are smaller than conventional micronized material (<2 microns) and are distributed over a larger mucosal or alveolar surface area as compared to miconized drugs.

The nanoparticulate drug pMDIs of the invention can utilize either chlorinated or non-chlorinated propellants. Concentrated nanoparticulate aerosol formulations can also be employed in pMDIs.

In a non-aqueous, non-pressurized milling system, a non-aqueous liquid which has a vapor pressure of 1 atm or less at room temperature is used as a milling medium and may be evaporated to yield a dry nanoparticulate benzodiazepine, and preferably, lorazepam nanoparticles and surface modifier. The non-aqueous liquid may be, for example, a high-boiling halogenated hydrocarbon. The dry nanoparticulate benzodiazepine, and preferably, lorazepam nanoparticle composition thus produced may then be mixed with a suitable propellant or propellants and used in a conventional pMDI.

Alternatively, in a pressurized milling operation, a non-aqueous liquid which has a vapor pressure >1 atm at room temperature is used as a milling medium for making a nanoparticulate benzodiazepine, such as lorazepam, and surface stabilizer composition. Such a liquid may be, for example, a halogenated hydrocarbon propellant which has a low boiling point. The resultant nanoparticulate composition can then be used in a conventional pMDI

without further modification, or can be blended with other suitable propellants. Concentrated aerosols may also be made by such methods.

G. Injectable Nanoparticulate Benzodiazepine Formulations

The invention provides injectable nanoparticulate benzodiazepine, such as lorazepam, formulations that can comprise high drug concentrations in low injection volumes, with rapid drug dissolution upon administration. In addition, the injectable nanoparticulate benzodiazepine, such as lorazepam, formulations of the invention eliminate the need to use polyoxyl 60 hydrogenated castor oil (HCO-60) as a solubilizer. An exemplary injectable composition comprises, based on % w/w:

benzodiazepine (such as lorazepam)	5 – 50%
povidone polymer	0.1 – 50%
preservatives	0.05 - 0.25%
pH adjusting agent	pH about 6 to about 7
water for injection	q.s.

Exemplary preservatives include methylparaben (about 0.18% based on % w/w), propylparaben (about 0.02% based on % w/w), phenol (about 0.5% based on % w/w), and benzyl alcohol (up to 2% v/v). An exemplary pH adjusting agent is sodium hydroxide, and an exemplary liquid carrier is sterile water for injection. Other useful preservatives, pH adjusting agents, and liquid carriers are well-known in the art.

III. Methods Of Making the Benzodiazepine Formulations

Nanoparticulate benzodiazepine, such as lorazepam, compositions can be made using any suitable method known in the art such as, for example, milling, homogenization, precipitation, or supercritical fluid techniques. Exemplary methods of making nanoparticulate compositions are described in U.S. Patent No. 5,145,684. Methods of making nanoparticulate compositions are also described in U.S. Patent No. 5,518,187 for "Method of Grinding Pharmaceutical Substances;" U.S. Patent No. 5,718,388 for "Continuous Method of Grinding Pharmaceutical Substances;" U.S. Patent No. 5,862,999 for "Method of Grinding Pharmaceutical Substances;" U.S. Patent No. 5,665,331 for "Co-

Microprecipitation of Nanoparticulate Pharmaceutical Agents with Crystal Growth Modifiers;" U.S. Patent No. 5,662,883 for "Co-Microprecipitation of Nanoparticulate Pharmaceutical Agents with Crystal Growth Modifiers;" U.S. Patent No. 5,560,932 for "Microprecipitation of Nanoparticulate Pharmaceutical Agents;" U.S. Patent No. 5,543,133 for "Process of Preparing X-Ray Contrast Compositions Containing Nanoparticles;" U.S. Patent No. 5,534,270 for "Method of Preparing Stable Drug Nanoparticles;" U.S. Patent No. 5,510,118 for "Process of Preparing Therapeutic Compositions Containing Nanoparticles;" and U.S. Patent No. 5,470,583 for "Method of Preparing Nanoparticle Compositions Containing Charged Phospholipids to Reduce Aggregation," all of which are specifically incorporated herein by reference.

The resultant nanoparticulate benzodiazepine, such as lorazepam, compositions or dispersions can be utilized in injectable, aerosol dosage formulations, controlled release formulations, lyophilized formulations, delayed release formulations, extended release formulations, pulsatile release formulations, mixed immediate release and controlled release formulations, *etc.*

Consistent with the above disclosure, provided herein is a method of preparing the nanoparticulate benzodiazepine, such as lorazepam, formulations of the invention. The method comprises the steps of: (1) dispersing a benzodiazepine, such as lorazepam, in a liquid dispersion media; and (2) mechanically reducing the particle size of the benzodiazepine, such as lorazepam, to the desired effective average particle size, such as less than about 2000 nm or less than about 600 nm. A surface stabilizer can be added before, during, or after particle size reduction of the benzodiazepine, such as lorazepam. The liquid dispersion media can be maintained at a physiologic pH, for example, within the range of from about 3.0 to about 8.0 during the size reduction process; more preferably within the range of from about 5.0 to about 7.5 during the size reduction process. The dispersion media used for the size reduction process is preferably aqueous, although any media in which the benzodiazepine, such as lorazepam, is poorly soluble and dispersible can be used, such as safflower oil, ethanol, t-butanol, glycerin, polyethylene glycol (PEG), hexane, or glycol.

Effective methods of providing mechanical force for particle size reduction of a benzodiazepine, such as lorazepam, include ball milling, media milling, and homogenization, for example, with a Microfluidizer® (Microfluidics Corp.). Ball milling is a low energy

milling process that uses milling media, drug, stabilizer, and liquid. The materials are placed in a milling vessel that is rotated at optimal speed such that the media cascades and reduces the drug particle size by impaction. The media used must have a high density as the energy for the particle reduction is provided by gravity and the mass of the attrition media.

Media milling is a high energy milling process. Drug, stabilizer, and liquid are placed in a reservoir and recirculated in a chamber containing media and a rotating shaft/impeller. The rotating shaft agitates the media which subjects the drug to impaction and sheer forces, thereby reducing the drug particle size.

Homogenization is a technique that does not use milling media. Drug, stabilizer, and liquid (or drug and liquid with the stabilizer added after particle size reduction) constitute a process stream propelled into a process zone, which in the Microfluidizer® is called the Interaction Chamber. The product to be treated is inducted into the pump, and then forced out. The priming valve of the Microfluidizer® purges air out of the pump. Once the pump is filled with product, the priming valve is closed and the product is forced through the interaction chamber. The geometry of the interaction chamber produces powerful forces of sheer, impact, and cavitation which are responsible for particle size reduction. Specifically, inside the interaction chamber, the pressurized product is split into two streams and accelerated to extremely high velocities. The formed jets are then directed toward each other and collide in the interaction zone. The resulting product has very fine and uniform particle or droplet size. The Microfluidizer® also provides a heat exchanger to allow cooling of the product. U.S. Patent No. 5,510,118, which is specifically incorporated by reference, refers to a process using a Microfluidizer®.

Using a particle size reduction method, the particle size of benzodiazepine, such as lorazepam, is reduced to the desired effective average particle size, such as less than about 2000 nm for the aerosol formulation, and less than about 600 nm for the injectable formulation.

The benzodiazepine, such as lorazepam, can be added to a liquid media in which it is essentially insoluble to form a premix. The concentration of the benzodiazepine, such as lorazepam, in the liquid media can vary from about 5 to about 60%, and preferably is from about 15 to about 50% (w/v), and more preferably about 20 to about 40%. The surface stabilizer can be present in the premix or it can be added to the drug dispersion following

particle size reduction. The concentration of the surface stabilizer can vary from about 0.1 to about 50%, and preferably is from about 0.5 to about 20%, and more preferably from about 1 to about 10%, by weight.

The premix can be used directly by subjecting it to mechanical means to reduce the average benzodiazepine, such as lorazepam, particle size in the dispersion to less than about 2000 nm. It is preferred that the premix be used directly when a ball mill is used for attrition. Alternatively, the benzodiazepine, such as lorazepam, and at least one surface stabilizer can be dispersed in the liquid media using suitable agitation, *e.g.*, a Cowles type mixer, until a homogeneous dispersion is observed in which there are no large agglomerates visible to the naked eye. It is preferred that the premix be subjected to such a premilling dispersion step when a recirculating media mill is used for attrition.

The mechanical means applied to reduce the benzodiazepine, such as lorazepam, particle size conveniently can take the form of a dispersion mill. Suitable dispersion mills include a ball mill, an attritor mill, a vibratory mill, and media mills such as a sand mill and a bead mill. A media mill is preferred due to the relatively shorter milling time required to provide the desired reduction in particle size. For media milling, the apparent viscosity of the premix is preferably from about 100 to about 1000 centipoise, and for ball milling the apparent viscosity of the premix is preferably from about 1 up to about 100 centipoise. Such ranges tend to afford an optimal balance between efficient particle size reduction and media erosion.

The attrition time can vary widely and depends primarily upon the particular mechanical means and processing conditions selected. For ball mills, processing times of up to five days or longer may be required. Alternatively, processing times of less than 1 day (residence times of one minute up to several hours) are possible with the use of a high shear media mill.

The benzodiazepine, such as lorazepam, particles can be reduced in size at a temperature which does not significantly degrade the benzodiazepine, such as lorazepam. Processing temperatures of less than about 30 to less than about 40°C are ordinarily preferred. If desired, the processing equipment can be cooled with conventional cooling equipment. Control of the temperature, *e.g.*, by jacketing or immersion of the milling chamber in ice water, is contemplated. Generally, the method of the invention is

conveniently carried out under conditions of ambient temperature and at processing pressures which are safe and effective for the milling process. Ambient processing pressures are typical of ball mills, attritor mills, and vibratory mills.

Grinding Media

The grinding media can comprise particles that are preferably substantially spherical in shape, *e.g.*, beads, consisting essentially of polymeric resin. Alternatively, the grinding media can comprise a core having a coating of a polymeric resin adhered thereon. The polymeric resin can have a density from about 0.8 to about 3.0 g/cm^3.

In general, suitable polymeric resins are chemically and physically inert, substantially free of metals, solvent, and monomers, and of sufficient hardness and friability to enable them to avoid being chipped or crushed during grinding. Suitable polymeric resins include crosslinked polystyrenes, such as polystyrene crosslinked with divinylbenzene; styrene copolymers; polycarbonates; polyacetals, such as Delrin® (E.I. du Pont de Nemours and Co.); vinyl chloride polymers and copolymers; polyurethanes; polyamides; poly(tetrafluoroethylenes), *e.g.*, Teflon®(E.I. du Pont de Nemours and Co.), and other fluoropolymers; high density polyethylenes; polypropylenes; cellulose ethers and esters such as cellulose acetate; polyhydroxymethacrylate; polyhydroxyethyl acrylate; and silicone-containing polymers such as polysiloxanes and the like. The polymer can be biodegradable. Exemplary biodegradable polymers include poly(lactides), poly(glycolide) copolymers of lactides and glycolide, polyanhydrides, poly(hydroxyethyl methacylate), poly(imino carbonates), poly(N-acylhydroxyproline)esters, poly(N-palmitoyl hydroxyproline) esters, ethylene-vinyl acetate copolymers, poly(orthoesters), poly(caprolactones), and poly(phosphazenes). For biodegradable polymers, contamination from the media itself advantageously can metabolize *in vivo* into biologically acceptable products that can be eliminated from the body.

The grinding media preferably ranges in size from about 0.01 to about 3 mm. For fine grinding, the grinding media is preferably from about 0.02 to about 2 mm, and more preferably from about 0.03 to about 1 mm in size.

In a preferred grinding process the particles are made continuously. Such a method comprises continuously introducing a benzodiazepine, such as lorazepam, into a milling

chamber, contacting the benzodiazepine, such as lorazepam, with grinding media while in the chamber to reduce the benzodiazepine particle size, and continuously removing the nanoparticulate benzodiazepine from the milling chamber.

The grinding media is separated from the milled nanoparticulate benzodiazepine, such as lorazepam, using conventional separation techniques, in a secondary process such as by simple filtration, sieving through a mesh filter or screen, and the like. Other separation techniques such as centrifugation may also be employed.

Sterile Product Manufacturing

Development of injectable compositions requires the production of a sterile product. The manufacturing process of the present invention is similar to typical known manufacturing processes for sterile suspensions. A typical sterile suspension manufacturing process flowchart is as follows:

(Media Conditioning)
↓
Compounding
↓
Particle Size Reduction
↓
Vial Filling
↓
(Lyophilization) and/or (Terminal Sterilization)

As indicated by the optional steps in parentheses, some of the processing is dependent upon the method of particle size reduction and/or method of sterilization. For example, media conditioning is not required for a milling method that does not use media. If terminal sterilization is not feasible due to chemical and/or physical instability, aseptic processing can be used.

Aerosol Formulations

A nanoparticulate benzodiazepine, such as lorazepam, composition for aerosol administration can be made by, for example, by (1) nebulizing an aqueous dispersion of nanoparticulate benzodiazepine, such as lorazepam, obtained by milling, homogenization, precipitation, or supercritical fluid processes; (2) aerosolizing a dry powder of aggregates of nanoparticulate benzodiazepine, such as lorazepam, and surface modifier (the aerosolized composition may additionally contain a diluent); or (3) aerosolizing a suspension of a nanoparticulate benzodiazepine, such as lorazepam, aggregates in a non-aqueous propellant. The aggregates of nanoparticulate benzodiazepine, such as lorazepam, and surface stabilizer, which may additionally contain a diluent, can be made in a non-pressurized or a pressurized non-aqueous system. Concentrated aerosol formulations may also be made by such methods.

A. Aqueous Milling to Obtain Nanoparticulate Benzodiazepine Dispersions

In an exemplary aqueous milling process, benzodiazepine, such as lorazepam, particles are dispersed in a liquid dispersion media and mechanical means is applied in the presence of grinding media to reduce the particle size of the benzodiazepine, such as lorazepam, to the desired effective average particle size. The particles can be reduced in size in the presence of one or more surface stabilizers. Alternatively, the particles can be contacted with one or more surface stabilizer either before or after attrition. Other compounds, such as a diluent, can be added to the benzodiazepine, such as lorazepam, and surface stabilizer composition during the size reduction process. Dispersions can be manufactured continuously or in a batch mode.

B. Precipitation to Obtain Nanoparticulate Benzodiazepine Compositions

Another method of forming the desired nanoparticle dispersion is by microprecipitation. This is a method of preparing stable dispersions of nanoparticulate benzodiazepine, such as lorazepam, in the presence of one or more surface stabilizers and one or more colloid stability enhancing surface active agents free of any trace toxic solvents or solubilized heavy metal impurities. Such a method comprises, for example, (1) dissolving the benzodiazepine, such as lorazepam, in a suitable solvent with mixing; (2) adding the formulation from step (1) with mixing to a solution comprising at least one surface stabilizer

to form a clear solution; and (3) precipitating the formulation from step (2) with mixing using an appropriate nonsolvent. The method can be followed by removal of any formed salt, if present, by dialysis or diafiltration and concentration of the dispersion by conventional means. The resultant nanoparticulate benzodiazepine, such as lorazepam, dispersion can be utilized in liquid nebulizers or processed to form a dry powder for use in a DPI or pMDI.

C. Non-Aqueous Non-Pressurized Milling System

In a non-aqueous, non-pressurized milling system, a non-aqueous liquid having a vapor pressure of about 1 atm or less at room temperature and in which the benzodiazepine, such as lorazepam, is essentially insoluble is used as a wet milling media to make a nanoparticulate benzodiazepine, such as lorazepam, composition. In such a process, a slurry of benzodiazepine, such as lorazepam, and surface stabilizer is milled in the non-aqueous media to generate nanoparticulate benzodiazepine, such as lorazepam. Examples of suitable non-aqueous media include ethanol, trichloromonofluoromethane, (CFC-11), and dichlorotetrafluoroethane (CFC-114). An advantage of using CFC-11 is that it can be handled at only marginally cool room temperatures, whereas CFC-114 requires more controlled conditions to avoid evaporation. Upon completion of milling the liquid medium may be removed and recovered under vacuum or heating, resulting in a dry nanoparticulate benzodiazepine, and preferably, lorazepam nanoparticle composition. The dry composition may then be filled into a suitable container and charged with a final propellant. Exemplary final product propellants, which ideally do not contain chlorinated hydrocarbons, include HFA-134a (tetrafluoroethane) and HFA-227 (heptafluoropropane). While non-chlorinated propellants may be preferred for environmental reasons, chlorinated propellants may also be used in this aspect of the invention.

D. Non-Aqueous Pressurized Milling System

In a non-aqueous, pressurized milling system, a non-aqueous liquid media having a vapor pressure significantly greater than 1 atm at room temperature is used in the milling process to make nanoparticulate benzodiazepine, such as lorazepam, compositions. If the milling media is a suitable halogenated hydrocarbon propellant, the resultant dispersion may be filled directly into a suitable pMDI container. Alternately, the milling media can be

removed and recovered under vacuum or heating to yield a dry benzodiazepine, such as lorazepam, nanoparticulate composition. This composition can then be filled into an appropriate container and charged with a suitable propellant for use in a pMDI.

E. Spray-Dried Powder Aerosol Formulations

Spray drying is a process used to obtain a powder comprising nanoparticulate drug particles following particle size reduction of the benzodiazepine, such as lorazepam, in a liquid media. In general, spray-drying is used when the liquid media has a vapor pressure of less than about 1 atm at room temperature. A spray-dryer is a device which allows for liquid evaporation and powder collection. A liquid sample, either a solution or suspension, is fed into a spray nozzle. The nozzle generates droplets of the sample within a range of about 20 to about 100 μm in diameter which are then transported by a carrier gas into a drying chamber. The carrier gas temperature is typically between about 80 and about 200 degrees C. The droplets are subjected to rapid liquid evaporation, leaving behind dry particles which are collected in a special reservoir beneath a cyclone apparatus.

If the liquid sample comprises an aqueous dispersion of a nanoparticulate benzodiazepine, such as lorazepam, and surface stabilizer, the collected product will comprise spherical aggregates of the nanoparticulate benzodiazepine, such as lorazepam. If the liquid sample comprises an aqueous dispersion of nanoparticles in which an inert diluent material was dissolved (such as lactose or mannitol), the collected product will comprise diluent (*e.g.*, lactose or mannitol) particles which comprise embedded nanoparticulate benzodiazepine, such as lorazepam. The final size of the collected product can be controlled and depends on the concentration of nanoparticulate benzodiazepine, such as lorazepam, and/or diluent in the liquid sample, as well as the droplet size produced by the spray-dryer nozzle. For deep lung delivery it is desirable for the collected product size to be less than about 2 microns in diameter, for delivery to the conducting airways it is desirable for the collected product size to be about 2 to about 6 microns in diameter, and for nasal delivery a collected product size of about 5 to about 100 microns is preferred. Collected products may then be used in conventional DPIs for pulmonary or nasal delivery, dispersed in propellants for use in pMDIs, or the particles may be reconstituted in water for use in nebulizers.

In some instances, it may be desirable to add an inert carrier to the spray-dried material to improve the metering properties of the final product. This may especially be the case when the spray dried powder is very small (less than about 5 microns) or when the intended dose is extremely small, whereby dose metering becomes difficult. In general, such carrier particles (also known as bulking agents) are too large to be delivered to the lung and simply impact the mouth and throat and are swallowed. Such carriers typically consist of sugars such as lactose, mannitol, or trehalose. Other inert materials, including polysaccharides and cellulosics, may also be useful as carriers.

Spray-dried powders comprising nanoparticulate benzodiazepine, such as lorazepam, may used in conventional DPIs, dispersed in propellants for use in pMDIs, or reconstituted in a liquid media for use with nebulizers.

F. Freeze-Dried Nanoparticulate Compositions

For a benzodiazepine that is denatured or destabilized by heat, such as having a low melting point (*i.e.*, about 70 to about 150 degrees C.), or, for example, biologics, sublimation is preferred over evaporation to obtain a dry powder nanoparticulate composition. This is because sublimation avoids the high process temperatures associated with spray-drying. In addition, sublimation, also known as freeze-drying or lyophilization, can increase the shelf stability of a benzodiazepine, particularly for biological products. Freeze-dried particles can also be reconstituted and used in nebulizers. Aggregates of freeze-dried nanoparticulate benzodiazepine, such as lorazepam, can be blended with either dry powder intermediates or used alone in DPIs and pMDIs for either nasal or pulmonary delivery.

Sublimation involves freezing the product and subjecting the sample to strong vacuum conditions. This allows for the formed ice to be transformed directly from a solid state to a vapor state. Such a process is highly efficient and, therefore, provides greater yields than spray-drying. The resultant freeze-dried product contains benzodiazepine, such as lorazepam, and at least one surface stabilizer. The benzodiazepine, such as lorazepam, is typically present in an aggregated state and can be used for inhalation alone (either pulmonary or nasal), in conjunction with diluent materials (lactose, mannitol, *etc.*), in DPIs or pMDIs, or reconstituted for use in a nebulizer.

IV

IV. Method of Treatment

In human therapy, it is important to provide a benzodiazepine, such as lorazepam, dosage form that delivers the required therapeutic amount of the drug *in vivo*, and that renders the drug bioavailable in a constant manner. Thus, another aspect of the present invention provides a method of treating a mammal, including a human, requiring status epilepticus treatment, irritable bowel syndrome treatment, sleep induction, acute psychosis, or pre-anesthesia medication using a nanoparticulate benzodiazepine, such as lorazepam, formulation of the invention. Such methods comprise the step of administering to a subject a therapeutically effective amount of a nanoparticulate benzodiazepine, such as lorazepam, formulation of the present invention. In one embodiment, the nanoparticulate benzodiazepine, such as lorazepam, formulation is an injectable formulation. In another embodiment, the nanoparticulate benzodiazepine, such as lorazepam, formulation is an aerosol formulation. Particularly advantageous features of the present invention include that the pharmaceutical formulation of the invention does not require the presence of polyethylene glycol and propylene glycol as stabilizers. In addition, the injectable formulation of the invention can provide a high lorazepam concentration in a small volume to be injected. A general protocol for injectable administration comprises a bolus injection of a benzodiazepine, such as lorazepam, with one continuous fast injection, rather than a slow infusion of the drug.

The benzodiazepine, such as lorazepam, compositions of the invention can be used for pulmonary or intranasal delivery. Pulmonary and intranasal delivery are particularly useful for the delivery of benzodiazepine, and preferably, lorazepam which is difficult to deliver by other routes of administration. Pulmonary or intranasal delivery is effective both for systemic delivery and for localized delivery to treat diseases of the air cavities.

The aerosols of the present invention, both aqueous and dry powder, are particularly useful in the treatment of respiratory-related illnesses such as asthma, emphysema, respiratory distress syndrome, chronic bronchitis, cystic fibrosis, chronic obstructive pulmonary disease, organ-transplant rejection, tuberculosis and other infections of the lung, fugal infections, respiratory illness associated with acquired immune deficiency syndrome, oncology, and systemic administration of an anti-emetic, analgesic, cardiovascular agent, *etc.*

The formulations and method result in improved lung and nasal surface area coverage by the administered benzodiazepine, such as lorazepam.

In addition, the aerosols of the invention, both aqueous and dry powder, can be used in a method for diagnostic imaging. Such a method comprises administering to the body of a test subject in need of a diagnostic image an effective contrast-producing amount of the nanoparticulate aerosol diagnostic image contrast composition. Thereafter, at least a portion of the body containing the administered contrast agent is exposed to x-rays or a magnetic field to produce an x-ray or magnetic resonance image pattern corresponding to the presence of the contrast agent. The image pattern can then be visualized.

"Therapeutically effective amount" is used herein with respect to a drug dosage, shall mean that dosage that provides the specific pharmacological response for which the drug is administered in a significant number of subjects in need of such treatment. It is emphasized that 'therapeutically effective amount,' administered to a particular subject in a particular instance will not always be effective in treating the diseases described herein, even though such dosage is deemed a "therapeutically effective amount" by those skilled in the art. "Therapeutically effective amount" also includes an amount that is effective for prophylaxis. It is to be further understood that drug dosages are, in particular instances, measured as oral dosages, or with reference to drug levels as measured in blood.

One of ordinary skill will appreciate that effective amounts of a benzodiazepine, such as lorazepam, can be determined empirically and can be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt, ester, or prodrug form. Actual dosage levels of benzodiazepine, such as lorazepam, in the aerosol and injectable compositions of the invention may be varied to obtain an amount of benzodiazepine, such as lorazepam, that is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage level therefore depends upon the desired therapeutic effect, the route of administration, the potency of the administered benzodiazepine, such as lorazepam,, the desired duration of treatment, and other factors.

Dosage unit compositions may contain such amounts of such submultiples thereof as may be used to make up the daily dose. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors: the type and degree of the cellular or physiological response to be achieved; activity of the specific agent or

composition employed; the specific agents or composition employed; the age, body weight, general health, sex, and diet of the patient; the time of administration, route of administration, and rate of excretion of the agent; the duration of the treatment; drugs used in combination or coincidental with the specific agent; and like factors well known in the medical arts.

It will be apparent to those skilled in the art that various modifications and variations can be made in the compositions, methods, and uses of the present invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

The following prophetic example is given to illustrate the present invention. It should be understood, however, that the spirit and scope of the invention is not to be limited to the specific conditions or details described in this example but should only be limited by the scope of the claims that follow. All references identified herein, including U.S. patents, are hereby expressly incorporated by reference.

Example 1

The purpose of this example was to prepare a nanoparticulate benzodiazepine, such as lorazepam, formulation.

An aqueous dispersion of 10% (w/w) lorazepam, combined with 2% (w/w) polyvinylpyrrolidone (PVP) K29/32 and 0.05% (w/w) dioctylsulfosuccinate (DOSS), could be milled in a 10 ml chamber of a NanoMill® 0.01 (NanoMill Systems, King of Prussia, PA; see e.g., U.S. Patent No. 6,431,478), along with 500 micron PolyMill® attrition media (Dow Chemical Co.) (89% media load). In an exemplary process, the mixture could be milled at a speed of 2500 rpms for 60 minutes.

Following milling, the particle size of the milled lorazepam particles can be measured, in deionized distilled water, using a Horiba LA 910 particle size analyzer. The initial mean milled lorazepam particle size is expected to be less than 2000 nm.

We Claim:

1. A nanoparticulate composition comprising:

(a) a benzodiazepine having an effective average particle size of less than about 2000 nm, wherein the benzodiazepine is selected from the group consisting of alprazolam, brotizolam, chlordiazepoxide, clobazam, clonazepam, clorazepam, demoxazepam, flumazenil, flurazepam, halazepam, midazolam, nordazepam, medazepam, diazepam, nitrazepam, oxazepam, midazepam, lorazepam, prazepam, quazepam, triazolam, temazepam, loprazolam, pharmaceutically acceptable salts and esters thereof, and mixtures thereof; and

(b) at least one surface stabilizer.

2. The composition of claim 1, wherein the surface stabilizer is selected from the group consisting of a nonionic surfactant, an ionic surfactant, a cationic surfactant, an anionic surfactant, and a zwitterionic surfactant.

3. The composition of claim 1 or claim 2, wherein the surface stabilizer is selected from the group consisting of hypromellose, hydroxypropylcellulose, polyvinylpyrrolidone, sodium lauryl sulfate, dioctylsulfosuccinate, gelatin, casein, lecithin, dextran, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, tyloxapol, poloxamers, poloxamines, Tetronic 1508®, an alkyl aryl polyether sulfonate, a mixture of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), Crodestas SL-40® (Croda, Inc.); and SA9OHCO, decanoyl-N-methylglucamide; n-decyl (-D-glucopyranoside; n-decyl (-D-maltopyranoside; n-dodecyl (-D-glucopyranoside; n-dodecyl (-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-(-D-glucopyranoside; n-heptyl (-D-thioglucoside; n-hexyl (-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl (-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-(-D-glucopyranoside; octyl (-D-thioglucopyranoside; PEG-

phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, PEG-vitamin E, lysozyme, random copolymers of vinyl pyrrolidone and vinyl acetate, cationic polymers, cationic biopolymers, cationic polysaccharides, cationic cellulosics, cationic alginates, cationic phospholipids, cationic nonpolymeric compounds, poly-n-methylpyridinium, anthryul pyridinium chloride, cationic phospholipids, chitosan, polylysine, polyvinylimidazole, polybrene, polymethylmethacrylate trimethylammoniumbromide bromide, hexyldesyltrimethylammonium bromide, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, cationic lipids, sulfonium, phosphonium, quarternary ammonium compounds, stearyltrimethylammonium chloride, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium bromide, C12-15dimethyl hydroxyethyl ammonium chloride, C12-15dimethyl hydroxyethyl ammonium bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulfate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl (ethenoxy)4 ammonium chloride, lauryl dimethyl (ethenoxy)4 ammonium bromide, N-alkyl (C12-18)dimethylbenzyl ammonium chloride, N-alkyl (C14-18)dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C12-14) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C12-14) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C12, C15, C17 trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride, dimethyl ammonium chlorides, alkyldimethylammonium

halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, POLYQUAT, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, MIRAPOL, ALKAQUAT, alkyl pyridinium salts, amines, alkylamines, dialkylamines, alkanolamines, polyethylenepolyamines, N,N-dialkylaminoalkyl acrylates, vinyl pyridine, amine salts, lauryl amine acetate, stearyl amine acetate, alkylpyridinium salt, alkylimidazolium salt, amine oxides, imide azolinium salts, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

4. The composition of any one of claims 1 to 3, wherein the nanoparticulate benzodiazepine particles have an effective average particle size selected from the group consisting of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 650 nm, less than about 600 nm, less than about 550 nm, less than about 500 nm, less than about 450 nm, less than about 400 nm, less than about 350 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

5. The composition of any one of claims 1 to 4, wherein the composition is formulated into an aerosol of an aqueous dispersion of the composition of claim 1, wherein essentially each droplet of the aerosol comprises at least one nanoparticulate benzodiazepine particle, wherein:

(a) the benzodiazepine has a solubility in the aqueous dispersion of less than about 10 mg/mL; and

(b) the droplets of the aerosol have a mass median aerodynamic diameter (MMAD) less than or equal to about 100 microns.

6. The aerosol composition of claim 5, wherein the benzodiazepine is present in a concentration selected from the group consisting of from about 0.05 mg/mL up to about 600 mg/mL, about 10 mg/mL or more, about 100 mg/mL or more, about 200 mg/mL or more, about 400 mg/mL or more, and about 600 mg/mL.

7. The aerosol composition of claim 5 or claim 6, wherein the composition is suitable for administration of the benzodiazepine dosage in about 15 seconds or less.

8. The aerosol composition of any one of claims 5 to 7, wherein the droplets of the aerosol have a mass median aerodynamic diameter (MMAD) selected from the group consisting of about 2 to about 10 microns, about 2 to about 6 microns, less than about 2 microns, about 5 to about 100 microns, and about 30 to about 60 microns.

9. The composition of any one of claims 1 to 4, formulated into an injectable composition.

10. The injectable composition of claim 9, comprising as a surface stabilizer a povidone polymer.

11. The injectable composition of claim 10, wherein the povidone polymer has a molecular weight of about 40,000 daltons or less.

12. The injectable composition of any one of claims 9 to 11, wherein the effective average particle size of the benzodiazepine particles is less than about 600 nm.

13. A method of treating a subject in need comprising administering to the subject a nanoparticulate benzodiazepine composition comprising:

(a) a benzodiazepine having an effective average particle size of less than about 2000 nm, wherein the benzodiazepine is selected from the group consisting of alprazolam, brotizolam, chlordiazepoxide, clobazam, clonazepam, clorazepam, demoxazepam, flumazenil, flurazepam, halazepam, midazolam, nordazepam, medazepam, diazepam,

nitrazepam, oxazepam, midazepam, lorazepam, prazepam, quazepam, triazolam, temazepam, loprazolam, pharmaceutically acceptable salts and esters thereof, and mixtures thereof; and

(b) at least one surface stabilizer.

14. The method of claim 13, wherein the surface stabilizer is selected from the group consisting of a nonionic surfactant, an ionic surfactant, a cationic surfactant, an anionic surfactant, and a zwitterionic surfactant.

15. The method of claim 13 or claim 14, wherein the surface stabilizer is selected from the group consisting of hypromellose, hydroxypropylcellulose, polyvinylpyrrolidone, sodium lauryl sulfate, dioctylsulfosuccinate, gelatin, casein, lecithin, dextran, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, tyloxapol, poloxamers, poloxamines, Tetronic 1508®, an alkyl aryl polyether sulfonate, a mixture of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), Crodestas SL-40® (Croda, Inc.); and SA9OHCO, decanoyl-N-methylglucamide; n-decyl (-D-glucopyranoside; n-decyl (-D-maltopyranoside; n-dodecyl (-D-glucopyranoside; n-dodecyl (-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-(-D-glucopyranoside; n-heptyl (-D-thioglucoside; n-hexyl (-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl (-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-(-D-glucopyranoside; octyl (-D-thioglucopyranoside; PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, PEG-vitamin E, lysozyme, random copolymers of vinyl pyrrolidone and vinyl acetate, cationic polymers, cationic biopolymers, cationic polysaccharides, cationic cellulosics, cationic alginates, cationic phospholipids, cationic nonpolymeric compounds, poly-n-methylpyridinium, anthryul pyridinium chloride, cationic phospholipids, chitosan, polylysine, polyvinylimidazole, polybrene, polymethylmethacrylate trimethylammoniumbromide

bromide, hexyldesyltrimethylammonium bromide, polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, cationic lipids, sulfonium, phosphonium, quarternary ammonium compounds, stearyltrimethylammonium chloride, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium bromide, C12-15dimethyl hydroxyethyl ammonium chloride, C12-15dimethyl hydroxyethyl ammonium bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulfate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl (ethenoxy)4 ammonium chloride, lauryl dimethyl (ethenoxy)4 ammonium bromide, N-alkyl (C12-18)dimethylbenzyl ammonium chloride, N-alkyl (C14-18)dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C12-14) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C12-14) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C12, C15, C17 trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride, dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, POLYQUAT, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, MIRAPOL, ALKAQUAT, alkyl pyridinium salts, amines,

alkylamines, dialkylamines, alkanolamines, polyethylenepolyamines, N,N-dialkylaminoalkyl acrylates, vinyl pyridine, amine salts, lauryl amine acetate, stearyl amine acetate, alkylpyridinium salt, alkylimidazolium salt, amine oxides, imide azolinium salts, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

16. The method of any one of claims 13 to 15, wherein the nanoparticulate benzodiazepine particles have an effective average particle size selected from the group consisting of less than about 1900 nm, less than about 1800 nm, less than about 1700 nm, less than about 1600 nm, less than about 1500 nm, less than about 1400 nm, less than about 1300 nm, less than about 1200 nm, less than about 1100 nm, less than about 1000 nm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 650 nm, less than about 600 nm, less than about 550 nm, less than about 500 nm, less than about 450 nm, less than about 400 nm, less than about 350 nm, less than about 300 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 100 nm, less than about 75 nm, and less than about 50 nm.

17. The method of any one of claims 13 to 16, wherein the composition is formulated into an aerosol of an aqueous dispersion of the composition of claim 1, wherein essentially each droplet of the aerosol comprises at least one nanoparticulate benzodiazepine particle, wherein:

(a) the benzodiazepine has a solubility in the aqueous dispersion of less than about 10 mg/mL; and

(b) the droplets of the aerosol have a mass median aerodynamic diameter (MMAD) less than or equal to about 100 microns.

18. The method of claim 17, wherein the benzodiazepine is present in a concentration selected from the group consisting of from about 0.05 mg/mL up to about 600 mg/mL, about 10 mg/mL or more, about 100 mg/mL or more, about 200 mg/mL or more, about 400 mg/mL or more, and about 600 mg/mL.

19. The method of claim 17 or claim 18, wherein the composition is suitable for administration of the benzodiazepine dosage in about 15 seconds or less.

20. The method of any one of claims 17 to 19, wherein the droplets of the aerosol have a mass median aerodynamic diameter (MMAD) selected from the group consisting of about 2 to about 10 microns, about 2 to about 6 microns, less than about 2 microns, about 5 to about 100 microns, and about 30 to about 60 microns.

21. The method of any one of claims 13 to 16, wherein the composition is formulated into an injectable dosage form.

22. The method of claim 21, comprising as a surface stabilizer a povidone polymer.

23. The method of claim 22, wherein the povidone polymer has a molecular weight of about 40,000 daltons or less.

24. The method of any one of claims 21 to 23, wherein the effective average particle size of the benzodiazepine particles is less than about 600 nm.

Electronic Acknowledgement Receipt

EFS ID:	15523019
Application Number:	12413439
International Application Number:	
Confirmation Number:	9049
Title of Invention:	ADMINISTRATION OF BENZODIAZEPINE COMPOSITIONS
First Named Inventor/Applicant Name:	Steve Cartt
Customer Number:	21971
Filer:	Matthew Virgil Grumbling/Heather Glasson
Filer Authorized By:	Matthew Virgil Grumbling
Attorney Docket Number:	35401-716.201
Receipt Date:	15-APR-2013
Filing Date:	27-MAR-2009
Time Stamp:	18:28:37
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	35401_716_201_Transmtl_041513.pdf	326248 603dac15e3988d3ff73980d36aa8a6130b6e576a	no	4

Warnings:	
Information:	

2	Information Disclosure Statement (IDS) Form (SB08)	35401_716_201_IDS_041513.pdf	396027 7b0f0d11aaeb4c6c9f528c75d8345c39baff4e6d	no	3
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
3	Other Reference-Patent/App/Search documents	USProvAppl60148464.pdf	4216678 e3e77c38c70677ba3787ffeac27e3fe3d1b32ba8	no	93
Warnings:					
Information:					
4	Foreign Reference	EP780386.pdf	7346493 64aa58aa2f1137f726ff8788544b92fd3caa70d4	no	86
Warnings:					
Information:					
5	Foreign Reference	EP818442.pdf	1148513 08774915e8a186879aab67908f8069af53bb079f	no	22
Warnings:					
Information:					
6	Foreign Reference	EP945485.pdf	1225922 e3ae956dd39f84eb0216ef8d024db50f251edf4f	no	26
Warnings:					
Information:					
7	Foreign Reference	EP1004578.pdf	2457315 677cae9be5f6bfdaf20556b94b45bf6a88d503c0	no	26
Warnings:					
Information:					
8	Foreign Reference	EP606046.pdf	5415758 a23f0e58e0785d0b17089d7eec2f0242c968b0cd	no	43
Warnings:					
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9	Foreign Reference	EP931788.pdf	1166301 7fc9455ce921384b1c7b0edcb07c2b31d52b9f4e	no	25
Warnings:					
Information:					
10	Foreign Reference	JP2003_505403.pdf	1045392 f14baf9d05bdc34cdf2a5bcb53a5df29767db222	no	73

Warnings:					
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11	Foreign Reference	JP2005_508939.pdf	1567369 4c0e038db5fb6034d4086f83b7cbbdca50350a18	no	76
Warnings:					
Information:					
12	Foreign Reference	JP2007_510722.pdf	2206873 52352de018ecb0950de7da4bca65b310ccd7651c	no	110
Warnings:					
Information:					
13	Foreign Reference	WO90_05719.pdf	2689362 17218eca0467a2b4ebb50bd7122c895d47c758cd	no	84
Warnings:					
Information:					
14	Foreign Reference	WO96_27583.pdf	2520695 fbc0453fded7e011fcf884125b197d0a5f35d1a6	no	71
Warnings:					
Information:					
15	Foreign Reference	WO96_33172.pdf	1740185 89915dc892ae1a11c67e0268ec6baa28d2bf81c2	no	50
Warnings:					
Information:					
16	Foreign Reference	WO98_03516.pdf	1615821 5f42696aade44e3922a3e35a94787bfaa64996b4	no	48
Warnings:					
Information:					
17	Foreign Reference	WO98_07697.pdf	2089211 5e165ce9d3900921df70ad9d47f87296e8db60d0	no	51
Warnings:					
Information:					
18	Foreign Reference	WO98_30566.pdf	1155452 4ab27f64d4470f2d252a92db3798a0c4cf81ef04	no	27
Warnings:					
Information:					
19	Foreign Reference	WO98_33768.pdf	1459018 [illegible]fb68e	no	34

Warnings:					
Information:					
20	Foreign Reference	WO98_34915.pdf	2284898 910c823a80be23803bb2f04438fb5a23c7370c1b	no	50
Warnings:					
Information:					
21	Foreign Reference	WO98_34918.pdf	2393044 c692249a3c2265012f51699bc29461db8e30bcf0	no	63
Warnings:					
Information:					
22	Foreign Reference	WO1999_007675.pdf	1035577 84d15cf9ad60d750f2df9245d14234aa5e57d114	no	26
Warnings:					
Information:					
23	Foreign Reference	WO99_29667.pdf	5674289 d0b4257062859b327e9f083c0cd6afd5aa15a33f	no	149
Warnings:					
Information:					
24	Foreign Reference	WO99_52889.pdf	1755491 751d474d43e0c65013ba7f6c5e1f76b2239e17cd	no	36
Warnings:					
Information:					
25	Foreign Reference	WO99_52910.pdf	2399490 77a59da608ac54f2645a0d58f4a1f517f72b02b	no	52
Warnings:					
Information:					
26	Foreign Reference	WO2000_74681.pdf	4935004 ffbe67d8b3aafa4aaedb49fa795e38ec9db18fd7	no	141
Warnings:					
Information:					
27	Foreign Reference	WO2005_089768.pdf	1430921 02db9db78198e981667f7a669801fa552a30221d	no	47
Warnings:					
Information:					
28	Foreign Reference	WO2006_055603.pdf	1748085 [illegible]4dfc98	no	58

Warnings:					
Information:					
29	Foreign Reference	WO2006_088894.pdf	2498937 a965c46f0e9b53d3c06a380b36d2eb8982fa31fd	no	65
Warnings:					
Information:					
			Total Files Size (in bytes):	67944369	

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New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor: Steve Cartt	Group Art Unit: 1612
Serial Number: 12/413,439	Examiner: Adam Milligan
Filing Date: 03/27/2009	**CONFIRMATION NO: 9049**
Title: ADMINISTRATION OF BENZODIAZEPINE COMPOSITIONS	

FILED ELECTRONICALLY ON: April 15, 2013

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

INFORMATION DISCLOSURE STATEMENT UNDER 37 CFR §1.97

Madam:

An Information Disclosure Statement along with attached PTO/SB/08 is hereby submitted. A copy of each listed publication is submitted, if required, pursuant to 37 CFR §§1.97-1.98, as indicated below.

The Examiner is requested to review the information provided and to make the information of record in the above-identified application. The Examiner is further requested to initial and return the attached PTO/SB/08 in accordance with MPEP §609.

The right to establish the patentability of the claimed invention over any of the information provided herewith, and/or to prove that this information may not be prior art, and/or to prove that this information may not be enabling for the teachings purportedly offered, is hereby reserved.

This statement is not intended to represent that a search has been made or that the information cited in the statement is, or is considered to be, prior art or material to patentability as defined in §1.56.

A. ☒ *37 CFR §1.97(b).* This Information Disclosure Statement should be considered by the Office because:

☐ (1) It is being filed within 3 months of the filing date of a national application and is other than a continued prosecution application under §1.53(d);

-- OR --

☐ (2) It is being filed within 3 months of entry of the national stage as set forth in §1.491 in an international application;

-- OR --

☐ (3) It is being filed before the mailing of a first Office action on the merits;

-- OR --

☒ (4) It is being filed before the mailing of a first Office action after the filing of a request for continued examination under §1.114.

B. ☐ *37 CFR §1.97(c).* Although this Information Disclosure Statement is being filed after the period specified in *37 CFR §1.97(b),* above, it is filed before the mailing date of the earlier of (1) a final office action under §1.113, (2) a notice of allowance under §1.311, or (3) an action that otherwise closes prosecution on the merits, this Information Disclosure Statement should be considered because it is accompanied by one of:

☐ a statement as specified in §1.97(e) provided concurrently herewith;

-- OR --

☐ a fee of $180.00 as set forth in §1.17(p) authorized below, enclosed, or included with the payment of other papers filed together with this statement.

C. ☐ *37 CFR §1.97(d).* Although this Information Disclosure Statement is being filed after the mailing date of the earlier of (1) a final office action under §1.113 or (2) a notice of allowance under §1.311, it is being filed before payment of the issue fee and should be considered because it is accompanied by:

i. a statement as specified in §1.97(e);

-- AND --

ii. a fee of $180.00 as set forth in §1.17(p) is authorized below, enclosed, or included with the payment of other papers filed together with this Statement.

D. ☐ *37 CFR §1.97(e).* Statement.

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§1.97(c);

-- AND/OR --

☐ A statement is provided herewith to satisfy the requirement under 37 CFR §§1.97(d);

-- AND/OR --

☐ A copy of a dated communication from a foreign patent office clearly showing that the information disclosure statement is being submitted within 3 months of the filing date on the communication is provided in lieu of a statement under 37 C.F.R. § 1.97(e)(1) as provided for under MPEP 609.04(b) V.

E. ☐ *Statement Under 37 C.F.R. §1.704(d).* Each item of information contained in the information disclosure statement was first cited in a communication from a foreign patent office in a counterpart application that was received by an individual designated in § 1.56(c) not more than thirty (30) days prior to the filing of this information disclosure statement. This statement is made pursuant to the requirements of 37 C.F.R. §1.704(d) to avoid reduction of the period of adjustment of the patent term for Applicant(s) delay.

F. ☒ *37 CFR §1.98(a)(2).* The content of the Information Disclosure Statement is as follows:

☐ Copies of each of the references listed on the attached Form PTO/SB/08 are enclosed herewith.

-- OR --

☒ Copies of U.S. Patent Documents (issued patents and patent publications) listed on the attached Form PTO/SB/08 are NOT enclosed.

-- AND/OR --

☒ Copies of Foreign Patent Documents and/or Non Patent Literature Documents listed on the attached Form PTO/SB/08 are enclosed in accordance with 37 CFR §1.98 (a)(2).

-- AND/OR --

☐ Copies of pending unpublished U.S. patent applications are enclosed in accordance with 37 CFR §1.98(a)(2)(iii).

G. ☒ *37 CFR §1.98(a)(3).* The Information Disclosure Statement includes non-English patents and/or references.

☐ Pursuant to 37 CFR §1.98(a)(3)(i), a concise explanation of the relevance of each patent, publication or other information provided that is not in English is provided herewith.

☐ Pursuant to MPEP 609(B), an English language copy of a foreign search report is submitted herewith to satisfy the requirement for a concise explanation where non-English language information is cited in the search report.

-- OR --

☐ A concise explanation of the relevance of each patent, publication or other information provided that is not in English is as follows: _____

☒ Pursuant to 37 CFR §1.98(a)(3)(ii), a copy of a translation, or a portion thereof, of the non-English language reference(s) is provided herewith.

H. ☐ *37 CFR §1.98(d).* Copies of patents, publications and pending U.S. patent applications, or other information specified in 37 C.F.R. § 1.98(a) are not provided herewith because:

☐ Pursuant to 37 CFR §1.98(d)(1) the information was previously submitted in an Information Disclosure Statement, or cited by examiner, for another application under which this application claims priority for an earlier effective filing date under 35 U.S.C. 120.

Application in which the information was submitted: _____

Information Disclosure Statement(s) filed on: _____

AND

☐ The information disclosure statement submitted in the earlier application complied with paragraphs (a) through (c) of 37 CFR §1.98.

I. ☒ *Fee Authorization.* The Commissioner is hereby authorized to charge the above-referenced fees of $0.00 and charge any additional fees or credit any overpayment associated with this communication to Deposit Account No. 23-2415 (Docket No. 35401-716.201).

Respectfully submitted,

WILSON SONSINI GOODRICH & ROSATI

Dated: April 15, 2013

By: /Matthew V. Grumbling/
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Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	12/413,439
				Filing Date	03/27/2009
				First Named Inventor	Steve Cartt
				Art Unit	1612
				Examiner Name	Milligan, Adam C.
Sheet	1	of	9	Attorney Docket Number	35401-716.201

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	1.	US- 2002-0110524 Al	8/15/2002	Cowan, S. M. L. et al.	
	2.	US- 2002-0141971 Al	10/3/2002	William H. Frey, II	
	3.	US- 2003-0017203 Al	6/23/2003	Crotts et al.	
	4.	US- 2003-0040497 Al	2/27/2003	Teng et al.	
	5.	US- 2003-0087820 Al	5/1/2003	Young et al.	
	6.	US- 2003-0100755 Al	5/29/2003	Sham et al.	
	7.	US- 2003-0118547 Al	6/1/2303	Vandenberg, G. W.	
	8.	US- 2003-0118594 Al	6/26/2003	Nag et al.	
	9.	US- 2003-0158206 Al	8/21/2003	Billotte et al.	
	10.	US- 2003-0170206 Al	9/11/2003	Rasmussen et al.	
	11.	US- 2004-0115135 Al	6/17/2004	Quay	
	12.	US- 2004-0126358 Al	7/1/2004	Warne et al.	
	13.	US- 2004-0147473 Al	7/29/2004	Warriell, Jr.	
	14.	US- 2004-0258663 Al	12/23/2004	Quay & El-Shafy	
	15.	US-2004-141923 A1	07/22/2004	Dugger et al.	
	16.	US- 2005-0130260 Al	6/16/2005	Linden et. al.	
	17.	US- 2005-0234101 Al	10/20/2005	Stenkamp et al.	
	18.	US- 2006-0045869 A1	03/02/2006	Meezan et al.	
	19.	US- 2006-0046969 Al	3/2/2006	Maggio, E. T.	
	20.	US- 2006-0106227 Al	5/18/2006	Reddy et al.	
	21.	US- 2006-0147386 Al	07-06-2006	Wermling, D. P.	
	22.	US- 2007-0059254 Al	3/15/2007	Nikhilesh N. Singh	
	23.	US- 2007-0098805 Al	5/3/2007	Gary G. Liversidge	
	24.	US- 2007-0298010 Al	12/27/2007	Maggio, E. T.	
	25.	US- 2008-0268032 Al	10/30/2008	Edward T. Maggio	
	26.	US- 2009-0258865 Al	10/15/2009	Cartt et al.	
	27.	US- 2010-0203119 Al	8/12/2010	Leane et al.	

Examiner Signature		Date Considered	

**EXAMINER:* *Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy* of this form with next communication to applicant. [1]Applicant's unique citation designation number (optional). [2]See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3]Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4]For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5]Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6]Applicant is to place a check mark here if English language Translation is attached.

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

Substitute for form 1449/PTO **INFORMATION DISCLOSURE STATEMENT BY APPLICANT** *(Use as many sheets as necessary)*				***Complete if Known***	
				Application Number	12/413,439
				Filing Date	03/27/2009
				First Named Inventor	Steve Cartt
				Art Unit	1612
				Examiner Name	Milligan, Adam C.
Sheet	2	of	9	Attorney Docket Number	35401-716.201

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	28.	US- 2010-0209485 Al	8/19/2010	Edward T. Maggio	
	29.	US- 3,547,828	12/15/1970	Mansfield et al.	
	30.	US- 3,849,341	11/19/1974	Lambeiti, V.	
	31.	US- 4,397,951	8/9/1983	Taki et al.	
	32.	US- 4,748,158	5/31/1988	Biermann ***et*** al.	
	33.	US- 4,868,289	9/1/1989	Magnusson et al.	
	34.	US- 4,921,838	5/1/1990	Catsimpoolas et al.	
	35.	US- 5,182,258	1/1/1993	Chiou	
	36.	US- 5,192,528	3/3/1993	Radhakrishnan et al.	
	37.	US- 5,236,707	8/17/1993	William E. Stewart, II	
	38.	US- 5,268,461	12/7/1993	Shoji et al.	
	39.	US- 5,308,531	5/3/1994	Urfer et al.	
	40.	US- 5,317,010	5/31/1994	Pang et al.	
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				First Named Inventor	Steve Cartt
				Art Unit	1612
				Examiner Name	Milligan, Adam C.
Sheet	3	of	9	Attorney Docket Number	35401-716.201

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Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	56.	US- 6,495,498	12/17/2002	Niemiec et al.	
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	62.	EP-0396777 A1	11/14/1990	OTSUKA PHARMA CO LTD		
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Examiner Signature		Date Considered	

**EXAMINER:* *Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy* of this form with next communication to applicant. [1]Applicant's unique citation designation number (optional). [2]See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3]Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4]For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5]Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. [6]Applicant is to place a check mark here if English language Translation is attached.

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(54) **INTERFERON PREPARATION FOR NASAL ADMINISTRATION.**

(57) The invention provides preparation for nasal administration which contains a pharmacologically effective amount of interferon and a fatty acid ester of sugar.

EP 0 396 777 A1

DESCRIPTION

Interferon Preparation for Nasal Administration

Technical Field

The present invention relates to interferon preparations for nasal administration, and more particularly to an interferon preparation for nasal administration which is applied by dropping or spraying into the nasal cavity in the form of a powder or liquid.

Background Art

Interferons (IFNs) are bioactive substances which display various clinical effects such as antiviral effect, antitumor effect and immunological enhancement effect and so have been developed as antiviral agents, anticancer agents, etc. However, such pharmaceutical preparations which contain IFNs as active components are limited to injectable forms such as intravenous injection, intramuscular injection and subcutaneous injection, since IFNs are polypeptides to be immediately inactivatd by protease, etc. and are difficult to absorb through the gastrointestinal tract, skin and mucosa due to their large molecular weight.

Administration of such injections not only gives severe pain to patients but also makes home care difficult, hence very inconvenient. Therefore, it is desired in this field to research and develop more

convenient dosage forms other than injections. Preparations for nasal administration are exemplified as such dosage forms and disclosed in Unexamined Japanese Patent Publication No. 207226/87, etc. However, the absorbefacients themselves used in such preparations largely stimulate nasal mucosa and thus have not been yet usable for nasal preparation in spite of their relatively large IFN-absorption promoting effect.

Disclosure of Invention

The present invention provides IFN preparations for nasal administration which can be effectively absorbed through the nasal mucosa and have little or no likelihood of stimulating the nasal mucosa for use with high safety.

The present invention relates to preparations for nasal administration comprising an IFN in a pharmacologically effective amount and a sucrose-fatty acid ester.

The inventors have found that, if sucrose fatty acid esters are used as absorbefacients together with IFN, IFN can be very efficiently absorbed through the nasal mucosa, and that the IFN preparation thus obtained for nasal administration does not substantially impair the nasal mucosa but is usable with extremely high safety to the living body. The present invention has been accomplished based on the above novel findings.

In the nasal preparation of the present invention, a solution or powder containing an active substance is inhaled into the nasal cavity using an appropriate instrument such as a spray bomb, aerosol bomb or nebulizer aspirator, whereby the active substance is adhered to and absorbed through the nasal mucosa. The present invention provides a nasal preparation comprising an IFN as the above-mentioned active substance, and more particularly a nasal preparation which contains a specific absorbefacient to efficiently promote the absorption of the IFN and which hardly stimulates the nasal mucosa substantially.

The IFN to be used in the present invention is at least one compound selected from the group comprising interferon-α (IFN-α), interferon-β (IFN-β) and interferon-γ (IFN-γ), which are already known. The method for preparing the present preparation for nasal administration is applicable to various bioactive substances as well as IFNs. Such bioactive substances include hormones such as insulins, calcitonins, luteohormone-releasing hormones, growth hormones, oxytocins, vasopressins, desmopressins; vaccines such as influenzae vaccines, hepatitis B vaccines, whooping cough vaccines; enzyme proteins such as urokinases, tissue plasminogen activators; bioactive proteins such as interleukins, tumor necrosis factors

(TNF), immunoglobulins, blood coagulation factors VIII; etc.

The absorbefacient to be used in the present invention is at least one sucrose fatty acid ester. Such sucrose fatty acid esters are preferably selected from those having 8 to 22 carbon atoms in their fatty acid moiety and more preferably those having 12 to 18 carbon atoms in their fatty acid moiety. These sucrose fatty acid esters are known and any of them can be used. Fatty acids constituting these esters may be saturated or unsaturated and straight- or branched-chain ones, and are particularly preferably straight-chain saturated fatty acids.

The amounts of IFN and sucrose fatty acid ester (absorbefacient) to be contained in the present nasal preparation are somewhat variable and can be appropriately decided depending on the kind of these components. IFN is generally used in a concentration of about 10^3 to about 10^9 IU per preparation. Sucrose fatty acid esters are used generally in a concentration of about 0.05 to about 10 w/v%, preferably about 0.1 to about 5.0 w/v%.

The nasal preparation of the present invention can be prepared by conventional methods suitable for the respective dosage forms. For example, preparations of the liquid type can be formulated by properly mixing IFN and

sucrose fatty acid ester (absorbefacient) and dissolving, emulsifying or dispersing the mixture in water or in an aqueous solution containing an organic solvent which is generally acceptable for use in nasal preparations. If desired, various pharmaceutically acceptable additives can be added which include, for example, buffers, stabilizing agents, preservatives, isotonic agents and viscosity increasing agents. Preparations of the solid type such as powder can also be prepared by a conventional method of pulverization.

The nasal preparation of the present invention thus obtained, like conventional nasal preparations, is intranasally given to the patient in an effective dose to exhibit the desired pharmacological effect. Particularly the present preparation can be administered in a relatively large amount because it is markedly less stimulant to the nasal mucosa, whereas the preparation displays a sufficient pharmacological effect even in a relatively small amount, since it is rendered highly absorbable by the addition of the specific absorbefacient.

Examples

The present invention will be described in more detail with reference to examples, comparative examples and test examples, but is not limited by these examples.

1) IFN-α standard solution (manufactured by

Hayashibara Biochemical Laboratories):

A standard solution wherein IFN derived from human BALL-1 cells (specific activity of 50000000 IU/mg) is dissolved in an amount of 30000000 IU/ml in a phosphate buffer (pH 6) containing human serum albumin (0.1 w/v%) as a stabilizer.

2) IFN-β standard preparation (manufactured by Toray Industries, Inc.):

A lyophilized preparation containing 3000000 IU per vial of IFN derived from human fibloblasts (specific activity of 10000000 IU/mg) and 9 mg per vial of human serum albumin as a stabilizer.

3) IFN-γ standard solution (manufactured by Hayashibara Biochemical Laboratories):

A standard solution wherein IFN derived from human HBL-38 cells (specific activity of 10000000 IU/mg) is dissolved in an amount of 30000000 IU/ml in a phosphate buffer (pH 7.2) containing human serum albumin (0.1 w/v%) as a stabilizer.

4) Sucrose fatty acid esters:

Sucorose lauric acid ester, sucrose palmitic acid ester, sucrose stearic acid ester and sucrose oleic acid ester (all are manufactured by Mitsubishi Chemical Food Co.,).

Example 1

A 50 mg quantity of sucrose lauric acid ester was dissolved in 5 ml of IFN-α standard solution, and D-mannitol was added thereto. The mixture was adjusted to osmotic ratio of 2 and lyophilized to afford a nasal preparation according to the present invention.

This preparation is given as dissolved in 10 ml of water at use.

Example 2

A 5 ml portion of 1 w/v% sucrose palmitic acid ester solution was added to 5 ml of IFN-α solution, followed by isotonization with D-mannitol. A nasal preparation according to the present invention was thus obtained.

Example 3

The lyophilized powder of IFN-α standard solution was added to 0.5 w/v% sucrose stearic acid ester solution to prepare a solution of 15000000 IU/ml. This solution was isotonized with D-mannitol, giving a nasal preparation of the invention.

Example 4

A 5 ml portion of 1 w/v% sucrose stearic acid ester solution was added to 5 ml of IFN-γ standard solution, followed by isotonization with D-mannitol. A nasal preparation of the present invention was thus obtained.

Example 5

A 0.2 ml portion of 0.5 w/v% sucrose stearic acid ester was added to IFN-β standard solution, giving a nasal preparation of the present invention.

Example 6

A 5 ml portion of 1 w/v% sucrose oleic acid ester solution was added to 5 ml of IFN-α solution, and the mixture was isotonized with D-mannitol, giving a nasal preparation of the invention.

Example 7

A 50 mg quantity of sucrose stearic acid ester was dissolved in 5 ml of IFN-α standard solution, and the solution was isotonized with D-mannitol, followed by lyophilization. A nasal preparation was thus obtained according to the invention.

This preparation is to be given as dissolved in 5 ml of water.

Comparative Example 1

A 5ml portion of water was added to 5 ml of IFN-α standard solution, and the mixture was isotonized with D-mannitol to give a nasal preparation for comparison.

Comparative Example 2

A 50 mg quantity of polyoxyethylene (9) lauryl ether (BL-9EX) was dissolved in 5 ml of IFN-α standard solution. The solution was isotonized with sodium

chloride, giving a nasal preparation for comparison.

Comparative Example 3

A 50 mg quantity of sodium glycocholate was dissolved in 5 ml of IFN-α standard solution. The solution was isotonized with sodium chloride, giving a nasal preparation for comparison.

Comparative Example 4

A 50 mg quantity of saponin was dissolved in 5 ml of IFN-α standard solution. The solution was isotonized with sodium chloride, giving a nasal preparation for comparison.

Test Example 1

IFN-absorption promoting effect

Male Sprague-Dawley rats (weighing 200-300 g) were anesthetized intraperitoneally with pentobarbital (50 mg/kg). Subsequently, the cervix was opened and a polyethylene tube was inserted into the trachea to rescue the airway. A part of the esophagus was opened, and a polyethylene tube whose end was stoppered was inserted thereinto in the direction of the postnaris to stop the postnaris.

Each of the preparations of the above examples and reference examples was intranasally administered in a dose of 0.1 ml/kg. Blood was then drawn periodically from jugular to determine antiviral activity of IFN in serum.

The result is shown in Table 1.

Table 1

	Change in serum IFN levels (IU/ml)					
	0 min	15 min	30 min	60 min	90 min	120 min
Example 1	N.D.	548	622	252	156	127
Example 2	N.D.	180	375	316	228	232
Example 3	N.D.	349	376	400	369	138
Example 4	N.D.	43	114	138	108	71
Example 5	N.D.	29	59	113	83	75
Example 7	N.D.	880	1102	783	436	271
Comparative Example 1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Comparative Example 2	N.D.	402	548	488	388	372
Comparative Example 3	N.D.	235	271	199	86	43
Comparative Example 4	N.D.	374	300	226	213	210

In the above table, N.D. represents "not detectable". Each value given is the mean of three test values.

The result shows that with all the nasal preparations of the present invention comprising a sucrose C_{12-18}-fatty acid ester, IFN is absorbed in a larger amount than in the case with the comparative preparation containing only IFN (Comparative example 1) and is absorbed in as great an amount as or in a larger amount

than in the case of the comparative preparations comprising other conventional absorbefacient (Comparative Examples 2 to 4).

Test Example 2

Protein Dissolution by Absorbefacient

The absorbefacients used were tested for the stimulant effect on the nasal mucosa by determining protein dissolution according to the following in situ recirculation technique.

Male Sprague-Dawley rats (weighing about 200-300 g) were anesthetized intraperitoneally with pentobarbital (50 mg/kg). Subsequently, the cervix was opened and a polyethylene tube was inserted into the trachea to rescue the airway. A part of the esophagus was opened, and a polyethylene tube whose end was stoppered was inserted thereinto in the direction of the postnaris to connect the tube to the postnasal. The preparations of the examples and comparative examples were diluted 2-fold with physiological saline. (In these dilutions, the absorbefacient was contained in a constant concentration of 0.5 w/v% (minimum concentration for efficient absorption of IFN.) A 20 ml portion of each dilution was transfused at a flow rate of 1 ml/min using a transfusion pump and perfused through the nasal cavity for 30 minutes. A part of the perfusate was sampled 15 minutes

and 30 minutes after starting the perfusion to determine protein dissolution in the perfusion liquid. The degree of stimulating effect on the nasal mucosa was thus evaluated.

The result is shown in Table 2.

Table 2

	Protein Dissolution (μg/0.1 ml)	
	15 minutes	30 minutes
Example 1	50.3	88.2
Example 2	23.5	32.4
Example 3	17.4	30.8
Example 6	15.5	28.2
Comparative Example 1	13.0	19.8
Comparative Example 2	51.9	91.8
Comparative Example 3	54.2	119.4
Comparative Example 4	114.7	192.7

The result reveals that all the nasal preparations of the present invention comprising sucrose C_{12-18} fatty acid ester causes slightly greater stimulation than the comparative preparation containing only IFN (Comparative example 1) but are much less stimulant than the comparative preparations comprising other conventional absorbefacient (Comparative examples 2 to 4).

CLAIMS:

1. A preparation for nasal administration comprising an IFN in a pharmacologically effective amount and a sucrose fatty acid ester.

2. A preparation according to claim 1 wherein the sucrose fatty acid ester is an ester of C_{8-22} fatty acids.

3. A preparation according to claim 1 wherein the sucrose fatty acid ester is an ester of C_{12-18} fatty acids.

4. A preparation according to claim 1 wherein the sucrose fatty acid ester is contained in a concentration of 0.05-10 w/v%.

5. A preparation according to claim 1 wherein the sucrose fatty acid ester is contained in a concentration of 0.01-5.0 w/v%.

INTERNATIONAL SEARCH REPORT

International Application No PCT/JP89/01156

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

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II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	A61K37/66, 9/12, 9/14, A61K47/26

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	JP, A, 59-163313 (Teijin Ltd.), 14 September 1984 (14. 09. 84), Claim & EP, A, 122036	1 - 5
A	JP, A, 62-281813 (Delaware Chemicals Corp.), 7 December 1987 (07. 12. 87), Claim & EP, A, 242643	1 - 5
A	JP, A, 63-115822 (Teijin Ltd.), 20 May 1988 (20. 05. 88), Claim (Family: none)	1 - 5

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

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(54) Stabilized teriparatide solutions

(57) A stabilized pharmaceutical composition in the form of a solution for parenteral administration of a parathyroid hormone is described wherein the therapeutically active ingredient is stabilized with a buffer and a polyol. Preferred preparations contain in an aqueous solution human PTH(1-34), mannitol, an acetate or tartrate buffering agent and m-cresol or benzyl alcohol as a preservative.

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Description

TECHNICAL FIELD

[0001] This invention relates to pharmaceutical compositions containing a parathyroid hormone. More particularly, the invention relates to teriparatide, PTH(1-34), stabilized solution formulations

BACKGROUND OF THE INVENTION

[0002] Parathyroid hormone (PTH) is a secreted, 84 amino acid product of the mammalian parathyroid gland that controls serum calcium levels through its action on various tissues, including bone. Studies in humans with certain forms of PTH have demonstrated an anabolic effect on bone, and have prompted significant interest in its use for the treatment of osteoporosis and related bone disorders.

[0003] Using the N-terminal 34 amino acids of the bovine and human hormone for example, which by all published accounts are deemed biologically equivalent to the full length hormone, it has been demonstrated in humans that parathyroid hormone enhances bone growth particularly when administered in pulsatile fashion by the subcutaneous route. A slightly different form of PTH, human PTH(1-38) has shown similar results.

[0004] PTH preparations have been reconstituted from fresh or lyophilized hormone, and incorporate various forms of carrier, excipient and vehicle. Most are prepared in water-based vehicles such as saline, or water acidified typically with acetic acid to solubilize the hormone. The majority of reported formulations also incorporate albumin as a stabilizer (see for example Reeve at al., Br. Med. J., 1980, 280:6228; Reeve at al., Lancet, 1976, 1:1035; Reeve at al., Calcif. Tissue Res., 1976, 21:469; Hodsman et al., Bone Miner; 1990, 9(2):137; Tsai et al., J. Clin. Endocrinol Metab., 1989,69 (5): 1024; Isaac et al., Horm. Metab. Res., 1980, 12(9):487; Law et al., J. Clin Invest. 1983, 72(3):1106; and Hulter, J. Clin Hypertens, 1986, 2(4):360). Other reported formulations have incorporated an excipient such as mannitol, which is present either with the lyophilized hormone or in the reconstitution vehicle. Formulations representative of those employed for human studies include a human PTH(1-34) (SEQ ID NO: 2) preparation consisting, upon reconstitution, of mannitol, heat inactivated human serum albumin, and caproic acid (a protease inhibitor) as absorption enhancer (see Reeve at al., 1976, Calcif. Tissue Res., 21, Suppl., 469-477); a human PTH(1-38) preparation reconstituted into a saline vehicle (see Hodsman et al., 1991, 14(1), 67-83); and a bovine PTH(1-34) preparation in aqueous vehicle pH adjusted with acetic acid and containing albumin. There is also an International Reference preparation which for human PTH (1-84) (SEQ ID NO: 1) consists of 100 ng of hormone ampouled with 250 μg human serum albumin and 1.25 mg lactose (1981), and for bovine PTH (1-84) consists of 10 μg lyophilized hormone in 0.01M acetic acid and 0.1% w/v mannitol (see Martindale, The Extra Pharmacoepia, The Pharmaceutical Press, London, 29th Edition, 1989 at p. 1338).

[0005] A recent attempt at improving the stability for the lyophilized preparation of h-PTH(1-34) (SEQ ID NO: 2) is reported in EP 619 119 with a combination of sugar and sodium chloride. Also U.S. Pat. No. 5,496,801 describes a freeze-dried composition for the natural hormone, PTH(1-84), containing mannitol as an excipient and a citrate source as a non-volatile buffering agent.

[0006] Commercial exploitation of parathyroid hormone requires the development of a formulation that is acceptable in terms of storage stability and ease of preparation. Because it is a protein and thus far more labile than the traditionally small molecular weight drugs, however, the formulation of parathyroid hormone presents challenges not commonly encountered by the pharmaceutical industry. Furthermore, like other proteins that have been formulated successfully, PTH is particularly sensitive to oxidation, deamidation and hydrolysis, and requires that its N-terminal and C-terminal sequences remain intact in order to preserve bioactivity.

[0007] It is an object of the present invention to provide a pharmaceutically useful PTH preparation, particularly one comprising, as active ingredient, teriparatide, PTH(1-34) (SEQ ID NO: 2).

SUMMARY OF THE INVENTION

[0008] The present invention provides a pharmaceutical composition in the form of a stabilized solution containing a parathyroid hormone (PTH) in a therapeutically effective amount. The solution is storage stable and, in sterile form, may be stored in vials or cartridges ready for parenteral administration in human patients. The advantages of the present solution is the elimination of the need for lyophilization.

[0009] Accordingly, the present invention is a parathyroid hormone solution including:

(a) a therapeutically effective amount of a parathyroid hormone;
(b) an effective amount of a stabilizing agent;
(c) a buffering agent in an amount sufficient to maintain the pH of the composition within a range of about 3-7; and
(d) the balance being water.

[0010] This solution may, if desired, undergo lyophilization to form a freeze-dried powder containing not more than 2% water by weight.

[0011] Another aspect of the present invention is a parathyroid hormone solution including:

(a) a therapeutically effective amount of a parathyroid hormone;
(b) from about 1 to 20 wt-% of a stabilizing agent;
(c) a buffering agent in an amount sufficient to maintain the pH of the composition within a range of about 3-7 and selected from an acetate or tartrate source;
(d) from about 0.1 to 2 wt-% of a parenterally acceptable preservative; and
(e) the balance being water.

[0012] Still another aspect of the present invention is a pharmaceutical composition in the form of a freeze-dried powder prior to reconstitution including:

(a) a therapeutically effective amount of a fragmented parathyroid hormone selected from the group consisting of PTH (1-34), PTH (1-37), PTH (1-38), and PTH (1-41);
(b) an effective amount of a stabilizing agent;
(c) a buffering agent in an amount sufficient to maintain the pH of the composition within a range of about 3-7; and
(d) less than 2% water by weight.

DETAILED DESCRIPTION

[0013] The invention relates to parathyroid hormone solutions that exhibit storage stability in terms of hormone composition and activity.

[0014] As active ingredient, the composition or solution may incorporate the full length, 84 amino acid form of parathyroid hormone, particularly the human form, hPTH (1-84) (SEQ ID NO: 1), obtained either recombinantly, by peptide synthesis or by extraction from human fluid. See, for example, U.S. Pat. No. 5,208,041, incorporated herein by reference. The amino acid sequence for hPTH (1-84) is reported by Kimura et al. in Biochem. Biophys. Res. Comm., 114 (2):493 (SEQ ID NO: 1).

[0015] The composition or solution may also incorporate as active ingredient fragments or variants of fragments of human PTH or of rat, porcine or bovine PTH that have human PTH activity as determined in the ovarectomized rat model of osteoporosis reported by Kimmel et al., Endocrinology, 1993, 32(4):1577.

[0016] The parathyroid hormone fragments desirably incorporate at least the first 34 N-terminal residues, such as PTH(1-34) (SEQ ID NO: 2), PTH(1-37), PTH(1-38) and PTH(1-41). Alternatives in the form of PTH variants incorporate from 1 to 5 amino acid substitutions that improve PTH stability and half-life, such as the replacement of methionine residues at positions 8 and/or 18 with leucine or other hydrophobic amino acid that improves PTH stability against oxidation and the replacement of amino acids in the 25-27 region with trypsin-insensitive amino acids such as histidine or other amino acid that improves PTH stability against protease. These forms of PTH are embraced by the term "parathyroid hormone" as used generically herein. The preferred hormone is human PTH(1-34) (SEQ ID NO: 2) also known as teriparatide. The hormones may be obtained by known recombinant or synthetic methods, such as described in U.S. Pat. No. 4,086,196, incorporated herein by reference.

[0017] The stabilizing agent incorporated into the solution or composition includes a polyol which includes a saccharide, preferably a monosaccharide or disaccharide, e.g., glucose, trehalose, raffinose, or sucrose; a sugar alcohol such as, for example, mannitol, sorbitol or inositol, and a polyhydric alcohol such as glycerine or propylene glycol or mixtures thereof. A preferred polyol is mannitol or propylene glycol. The concentration of polyol may range from about 1 to about 20 wt-%, preferably about 3 to 10 wt-% of the total solution.

[0018] The buffering agent employed in the solution or composition of the present invention may be any acid or salt combination which is pharmaceutically acceptable and capable of maintaining the aqueous solution at a pH range of 3 to 7, preferably 3-6. Useful buffering systems are, for example, acetate, tartrate or citrate sources. Preferred buffer systems are acetate or tartrate sources, most preferred is an acetate source. The concentration of buffer may be in the range of about 2 mM to about 500 mM, preferably about 2 mM to 100 mM.

[0019] The stabilized solution or composition of the present invention may also include a parenterally acceptable preservative. Such preservatives include, for example, cresols, benzyl alcohol, phenol, benzalkonium chloride, benzethonium chloride, chlorobutanol, phenylethyl alcohol, methyl paraben, propyl paraben, thimerosal and phenylmercuric nitrate and acetate. A preferred preservative is m-cresol or benzyl alcohol; most preferred is m-cresol. The amount of preservative employed may range from about 0.1 to about 2 wt-%, preferably about 0.3 to about 1.0 wt-% of the total solution.

[0020] Thus, the present invention has provided, for example, a stabilized teriparatide solution containing mannitol,

acetate and m-cresol with a predicted shelf-life of over 15 months at 5°C.

[0021] The parathyroid hormone compositions of the present invention may, if desired, be provided in a powder form containing not more than 2% water by weight, that results from the freeze-drying of a sterile, aqueous hormone solution prepared by mixing the selected parathyroid hormone, a buffering agent and a stabilizing agent as above described. Especially useful as a buffering agent when preparing lyophilized powders is a tartrate source. Particularly useful stabilizing agents include glycine, mannitol, sucrose, trehalose, raffinose or a mixture thereof.

[0022] The PTH solution and composition of the present invention incorporate PTH in a medically effective amount, a term used with reference to amounts useful either therapeutically or in medical diagnosis. The particular amount of parathyroid hormone incorporated in the preparation can be pre-determined based on the type of PTH selected and on the intended end-use of the preparation. In one application, the preparations are exploited for therapeutic purposes, and particularly for the treatment of osteoporosis. Osteoporosis therapy entails administration of the reconstituted preparation by injection, desirably subcutaneous injection, in unit doses that reflect the prescribed treatment regimen but are, by way of example, for human PTH(1-34) (SEQ ID NO: 2), within the range from 25 μg PTH/mL of injected solution to 1000 μg/mL of injected solution per patient, with injection volumes being desirably from 0.02 to 1.3 mL. Accordingly, the purified PTH is desirably incorporated with the buffering agent and excipient to form an aqueous solution containing PTH in a concentration range from 25 μg/mL to 1000 μg/mL, preferably 100 μg/mL to 500 μg/mL, which is then sterile-filtered and filled into a vial or cartridge for use.

[0023] Once the preparation is obtained as an aqueous solution containing desired amounts and concentrations of the buffering agent, excipient and PTH, individual vials are filled with the solution to the desired volume. The advantage of the present invention is that the above solution may be prepared with sterile water without the need to undergo a freeze-drying process.

[0024] In an alternative embodiment of the invention, the preparations are provided in a form that yields a unit container of 100-500 μg human PTH(1-34) (SEQ ID NO: 2) upon reconstitution into about 1 mL (0.8-1.2 mL) of the reconstitution vehicle, and the vials are accordingly loaded with about 1 mL of the aqueous PTH preparation, for subsequent freeze-drying.

[0025] In a preferred alternative embodiment of the invention, the PTH preparation subjected to freeze-drying comprises from 25 to 1000 μg/mL of human PTH(1-34) (SEQ ID NO: 2), from 2 to 8% by weight of mannitol, and a tartrate source in an amount capable of buffering the preparation to within the range from 3.0 to 6.5 upon reconstitution in sterile water. In specific embodiments of the invention, the tartrate buffering agent is incorporated in an amount sufficient to buffer the pH to 3.5 to 5.5.

[0026] In addition to their therapeutic use, the present PTH composition can be formulated and administered to aid in medical diagnosis, and particularly to assist in establishing the diagnosis of hypoparathyroidism and pseudohypoparathyroidism in hypocalcemic patients. Except for the dose of PTH, the composition of the PTH preparation will remain as described herein for therapeutic use. An intravenously infused, single dose of human PTH(1-34) (SEQ ID NO: 2) that is equal to 200 International Units of PTH activity is appropriate for this diagnostic purpose. Diagnosis is then made by determining the effect of administered PTH or urinary cAMP levels, with cAMP elevation being indicative of the hypoparathyroidism condition, rather than its pseudoform.

[0027] The examples which follow are illustrative of the invention and are not intended to be limiting.

EXAMPLES

Example 1

[0028] 0.1 mg rhPTH (1-34) (SEQ ID NO: 2), 50 mg mannitol, 2.5 mg m-cresol, 0.52 mg acetic acid and 0.12 mg sodium acetate were mixed into a solution with 1 ml of distilled water.

Example 2

[0029] 0.25 mg rhPTH (1-34) (SEQ ID NO: 2), 45.4 mg mannitol, 3 mg m-cresol, 0.41 mg acetic acid and 0.1 mg sodium acetate were mixed into a solution with 1 ml of distilled water.

[0030] The formulations of the present invention, Examples 1 and 2 were compared to solutions containing no stabilizer, 0.9% NaCl, 20 mM acetate and 10 mM acetate as primary stabilizer. The stability was measured by determining the amount in % of rhPTH (1-34) (SEQ ID NO: 2) remaining after a certain time. The measurement was made by HPLC. The results are shown in Tables 1 and 2.

Table 1

Effect of Primary Stabilizer on Chemical Stability of rhPTH (1-34) at 50°C				
	Water	0.9% NaCl	20 mM acetate	10 mM acetate
Time, days	% Remaining			
Initial	100	100	100	100
7	74	81	84	80
14	55	58	67	71

Table 2

Comparison of Stability of rhPTH (1-34) at 30°C				
	20 mM acetate	10 mM acetate	Example 1	Example 2
Time, days	% Remaining			
Initial	100	100	100	100
7	96	94	100	--
14	94	92	96	100
21	90	93	97	--
30	--	81	96	96

Example 3

[0031] The following experiment was carried out to show that lyophilized powder formulations prepared from stabilized solutions of the present invention are more stable than a control which was prepared from PTH(1-34) and mannitol alone.

[0032] A control solution and solutions for samples A through O were prepared as previously described with the ingredients and concentrations shown in Table 3. The solutions were then freeze-dried and the resulting lyophilized powder formulations were stored at 40°C for a one month period. The amount of PTH(1-34) remaining in each sample was then measured by HPLC. The results are shown in Table 3.

Table 3

Stability of PTH(1-34) Lyophilized Formulations at 40°C for One Month						
Sample	PTH(1-34) mg/mL	Bulking Agent	Bulking Agent Conc. mg/mL	Buffer	Buffer. Conc. mM	%PTH Remaining
Control	0.2	mannitol	40	--	--	78
A	0.5	mannitol	30	acetate	5	90
B	0.5	glycine	30	acetate	5	98
C	0.5	sucrose	30	acetate	5	98
D	0.5	trehalose	30	acetate	5	97
E	0.5	raffinose	30	acetate	5	99
F	0.75	mannitol	30	tartrate	15	95
G	1.5	sucrose & mannitol	5/25	tartrate	5	99
H	0.75	sucrose & mannitol	5/25	tartrate	15	99
I	1.5	mannitol	30	tartrate	5	96

Table 3 (continued)

Stability of PTH(1-34) Lyophilized Formulations at 40°C for One Month						
Sample	PTH(1-34) mg/mL	Bulking Agent	Bulking Agent Conc. mg/mL	Buffer	Buffer. Conc. mM	%PTH Remaining
J	1.5	sucrose	30	tartrate	15	100
K	1.5	mannitol	30	tartrate	15	99
L	0.75	sucrose	30	tartrate	15	100
M	0.75	sucrose	30	tartrate	5	100
N	1.5	sucrose & mannitol	5/25	tartrate	15	99
O	1.5	sucrose & mannitol	5/25	acetate	5	91*

*the stability at 2 months was 96%

SEQUENCE LISTING

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<120> STABILIZED TERIPARATIDE SOLUTIONS

<130> 3797.16WO01

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<150> 60/069,075
<151> 1997-12-09

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Ser	Met	Glu	Arg	Val	Glu	Trp	Leu	Arg	Lys	Lys	Leu	Gln	Asp	Val	His
		20					25					30			

Asn	Phe	Val	Ala	Leu	Gly	Ala	Pro	Leu	Ala	Pro	Arg	Asp	Ala	Gly	Ser
		35					40					45			

Gln	Arg	Pro	Arg	Lys	Lys	Glu	Asp	Asn	Val	Leu	Val	Glu	Ser	His	Glu
	50					55					60				

Lys	Ser	Leu	Gly	Glu	Ala	Asp	Lys	Ala	Asn	Val	Asp	Val	Leu	Thr	Lys
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Ala	Lys	Ser	Gln

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Asn Phe

Claims

1. A parathyroid hormone solution comprising:

   (a) a therapeutically effective amount of a parathyroid hormone;
   (b) an effective amount of a stabilizing agent;
   (c) a buffering agent in an amount sufficient to maintain the pH of the composition within a range of about 3-7; and
   (d) the balance being water.

2. The solution of claim 1, wherein the hormone is a fragmented hormone selected from the group consisting of PTH (1-34), PTH(1-37), PTH(1-38), and PTH(1-41).

3. The solution of claim 1, wherein the hormone is human PTH(1-34) (SEQ ID NO: 2).

4. The solution of claim 1, wherein the hormone is human PTH(1-84) (SEQ ID NO: 1).

5. The solution of claim 1, wherein the stabilizing agent is a polyol.

6. The solution of claim 5, wherein the polyol is mannitol.

7. The solution of claim 5, wherein the polyol is propylene glycol.

8. The solution of claim 1, wherein the buffering agent is an acetate or tartrate source.

9. The solution of claim 8, wherein the buffering agent is acetate.

10. The solution of claim 1, which further comprises a parenterally acceptable preservative.

11. The solution of claim 10, wherein the preservative is m-cresol or benzyl alcohol.

12. The solution of claim 11, wherein the preservative is m-cresol.

13. A composition according to claim 1, in the form of a freeze-dried powder containing less than 2% water by weight.

14. A parathyroid hormone solution comprising:

   (a) a therapeutically effective amount of a parathyroid hormone;
   (b) from about 1 to 20 wt-% of a stabilizing agent;
   (c) a buffering agent in an amount sufficient to maintain the pH of the composition within a range of about 3-7 and selected from an acetate or tartrate source;
   (d) from about 0.1 to 2 wt-% of a parenterally acceptable preservative; and
   (e) the balance being water.

15. The solution of claim 14, wherein the hormone is PTH(1-84).

16. The solution of claim 14, wherein the hormone is selected from the group consisting of PTH(1-34), PTH(1-37), PTH(1-38), and PTH(1-41).

17. The solution of claim 16, wherein the hormone is human PTH(1-34) (SEQ ID NO: 2).

18. The solution of claim 14, wherein the stabilizing agent is a polyol in an amount of about 3 to about 10 wt-%.

19. The solution of claim 14, wherein the preservative is m-cresol or benzylalcohol in an amount of about 0.3 to about 1.0 wt-%.

20. A pharmaceutical composition in the form of a freeze-dried powder comprising:

(a) a therapeutically effective amount of a fragmented parathyroid hormone selected from the group consisting of PTH(1-34), PTH(1-37), PTH(1-38), and PTH(1-41);
(b) an effective amount of a stabilizing agent;
(c) a buffering agent in an amount sufficient to maintain the pH of the composition within a range of about 3-7; and
(d) less than 2 wt-% water by weight.

21. The composition of claim 20, wherein the hormone is human PTH(1-34) (SEQ ID NO: 2).

22. The composition of claim 20, wherein the stabilizing agent is selected from the group consisting of glycine, mannitol, sucrose, trehalose, raffinose and a mixture thereof.

23. The composition of claim 20, wherein the buffering agent is an acetate or tartrate source.

24. The composition of claim 23, wherein the buffering agent is a tartrate source.

25. The composition of claim 20, wherein the stabilizing agent is in an amount of about 1 to about 20 wt-%.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 03 10 4219

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Y,D	WO 95 17207 A (ALLELIX) 29 June 1995 (1995-06-29) * claims * * page 5, line 3 - line 22 * * page 6, line 4 - line 7 * * page 6, line 11 - page 7, line 2 * ---	1,5-8, 13,14, 20-24	A61K38/29 A61K47/10 A61K9/08 A61K47/26
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Y	EP 0 302 772 A (TOYO JOZO) 8 February 1989 (1989-02-08) * claims 1,2,4,5,7,8 * * page 3, line 21 - line 30 * * page 3, line 32 - line 50 * * example 12 * ---	1,5-8, 13,14, 20-24	TECHNICAL FIELDS SEARCHED (Int.Cl.7) A61K
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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	17 March 2004	Allnutt, S

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EPO FORM 1503 03.82 (P04C01)

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EUROPEAN SEARCH REPORT

Application Number
EP 03 10 4219

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Y	CHEMICAL ABSTRACTS, vol. 111, no. 26, 25 December 1989 (1989-12-25) Columbus, Ohio, US; abstract no. 239514, XP002105393 * abstract * & JP 01 016799 A (TOYO JOZO) 20 January 1989 (1989-01-20) -----	1,5-8, 13,14, 20-24	

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	17 March 2004	Allnutt, S

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& : member of the same patent family, corresponding document

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A61K 9/02

(21)Application number : 62-311680

(22)Date of filing : 08.12.1987

(71)Applicant : TAIHO YAKUHIN KOGYO KK

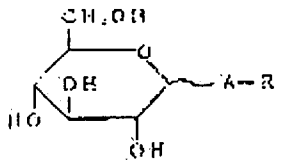
(72)Inventor : MURANISHI SHOZO

(54) TRANSMUCOUS ABSORBEFACIENT

(57)Abstract:

PURPOSE: To obtain a transmucous absorbefacient for a hardly absorbable drug, by using a (thio)ether compound of monosaccharides or disaccharides as an active component.

CONSTITUTION: The objective transmucous absorbefacient contains an ether compound or thioether compound of monosaccharides (e.g. L-arabinose or D-mannose) or disaccharides (e.g. maltose) and a 6W18C aliphatic hydrocarbon, preferably a compound of formula I or formula II (A is O or S; R is 6W18C aliphatic hydrocarbon group). The amount of drug component in a mucous administration drug containing said absorbefacient is preferably 0.1W20wt.%, especially 0.5W5wt.%. The (thio)ether compound is e.g. n-octyl-β-D-(thio) glucopyranoside or n-lauryl-β-D-maltopyranoside.



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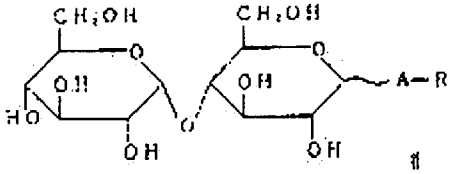

II

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