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%).

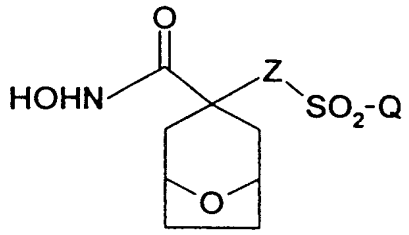
C) **3-*exo*-[4-(4-Chlorophenoxy)benzenesulfonylmethyl]-8-oxabicyclo[3.2.1] octane-3-carboxylic acid hydroxyamide**

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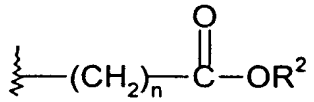
wherein Z is $>CH_2$ or $>NR^1$;

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;

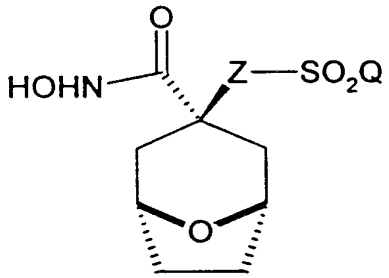
Q is (C_1-C_6)alkyl, (C_6-C_{10})aryl, (C_2-C_9)heteroaryl, (C_6-C_{10})aryloxy(C_1-C_6)alkyl, (C_6-C_{10})aryloxy(C_6-C_{10})aryl, (C_6-C_{10})aryloxy(C_2-C_9)heteroaryl, (C_6-C_{10})aryl(C_1-C_6)alkyl, (C_6-C_{10})aryl(C_6-C_{10})aryl, (C_6-C_{10})aryl(C_2-C_9)heteroaryl, (C_6-C_{10})aryl(C_6-C_{10})aryl(C_1-C_6)alkyl, (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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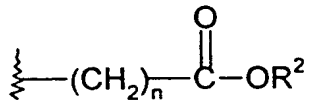
3. A compound according to claim 1, wherein Z is CH_2.

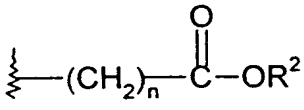
4. A compound according to claim 2, wherein Z is CH_2.

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
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Paisdor, B

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

INTERNATIONAL SEARCH REPORT

In ational 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

International 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

In ational Application No
PCT/IB 99/00503

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 December 2000 (14.12.2000)

PCT

(10) International Publication Number
WO 00/74681 A1

(51) International Patent Classification[7]: A61K 31/451, 31/4523, C07D 401/10, A61K 31/453, C07D 405/14, 401/14, 211/22, 405/12, 211/26, 417/10, A61P 9/00

(21) International Application Number: PCT/IB00/00667

(22) International Filing Date: 18 May 2000 (18.05.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9912961.1 3 June 1999 (03.06.1999) GB

(71) Applicant *(for GB only)*: **PFIZER LIMITED** [GB/GB]; Ramsgate Road, Sandwich, Kent CT13 9NJ (GB).

(71) Applicant *(for all designated States except GB, US)*: **PFIZER INC.** [US/US]; 235 East 42nd Street, New York, NY 10017 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **DACK, Kevin, Neil** [GB/GB]; Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB). **FRAY, Michael, Jonathan** [GB/GB]; Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB). **WHITLOCK, Gavin, Alistair** [GB/GB]; Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB). **LEWIS, Mark, Llewellyn** [GB/GB]; Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB). **THOMSON, Nicholas, Murray** [GB/GB]; Pfizer Central Research, Ramsgate Road, Sandwich, Kent CT13 9NJ (GB).

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

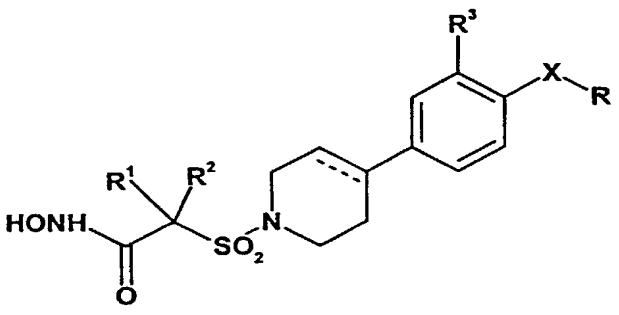
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(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).

Published:
— *With international search report.*

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).

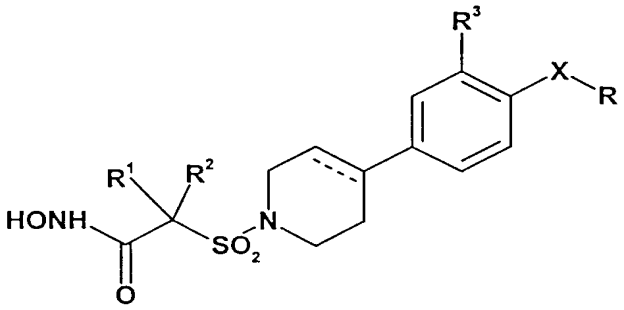
Rupture of atherosclerotic plaques by MMP-3 has also been described (see e.g. Circulation, 1997, 96, 396). Thus, MMP-3 inhibitors may find utility in the treatment of conditions caused by or complicated by embolic phenomena such as cardiac or cerebral infarctions.

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;

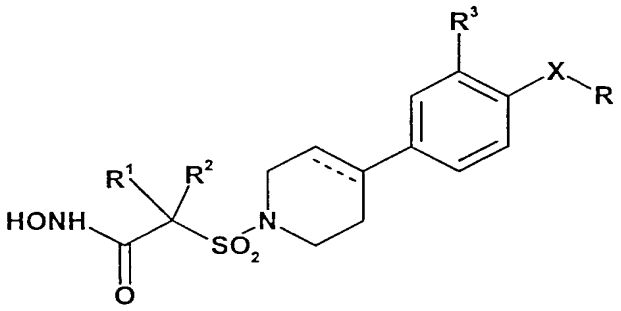
R^3 is H, halo, methyl, or methoxy;

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;

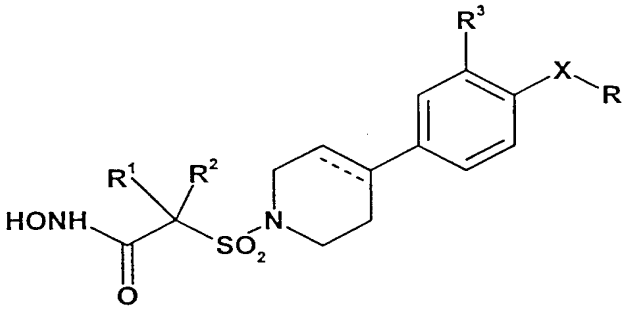
R^3 is H, halo, methyl, or methoxy;

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;

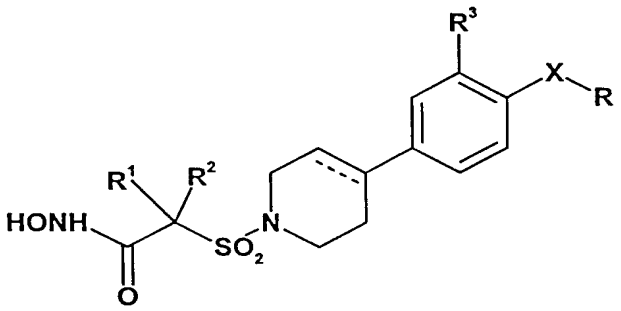
R^3 is H, halo, methyl, or methoxy;

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.

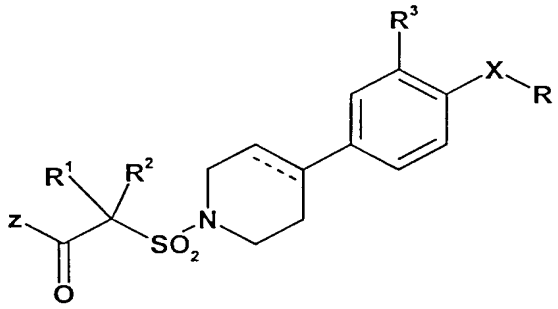
It is to be understood that the synthetic transformation methods mentioned herein may be carried out in various different sequences in order that the desired compounds can be efficiently assembled. The skilled chemist will exercise his judgement and skill as to the most efficient sequence of reactions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

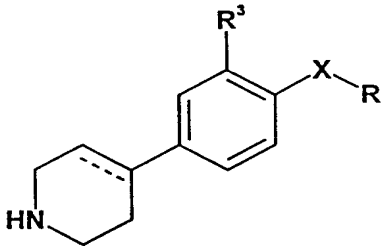
referred to as "water-soluble carbodiimide" or "WSCDI") optionally in the presence of 1-hydroxybenzotriazole or 1-hydroxy-7-aza-1H-1,2,3-benzotriazole (HOAt) and/or a catalyst such as 4-dimethylaminopyridine, or by using HOAt or a halotrisaminophosphonium salt such as bromotris(pyrrolidino)-phosphonium 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).

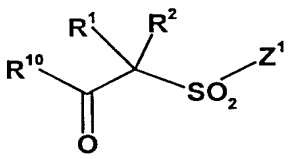
Preferably a solution of the acid (II, Z is HO) and N-ethyldiisopropylamine in a suitable solvent such as anhydrous dimethylformamide or anhydrous 1-methylpyrrolidin-2-one, under nitrogen, is treated with up to a 1.5-fold excess of HATU at about room temperature followed, after about 15 to 30 minutes,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

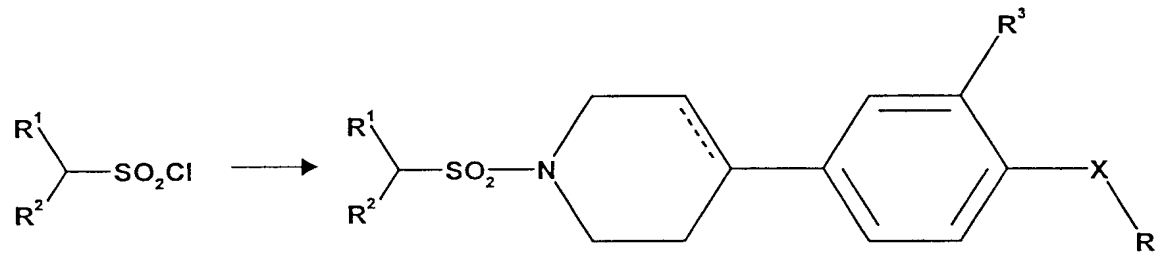
The carbanion may be generated using an appropriate base in a suitable solvent, optionally in the presence of a phase transfer catalyst (PTC). Typical base-solvent combinations may be selected from lithium, sodium or potassium hydride, lithium, sodium or potassium bis(trimethylsilyl)amide, lithium diisopropylamide 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



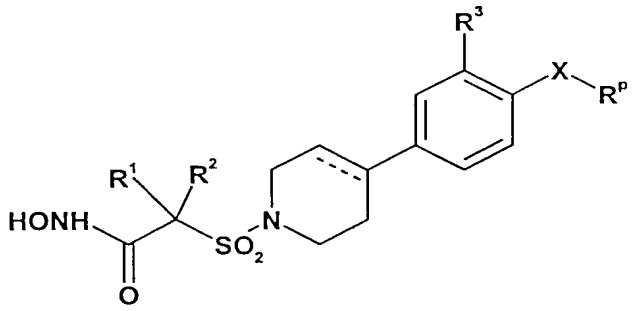
(V) (VI)

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)

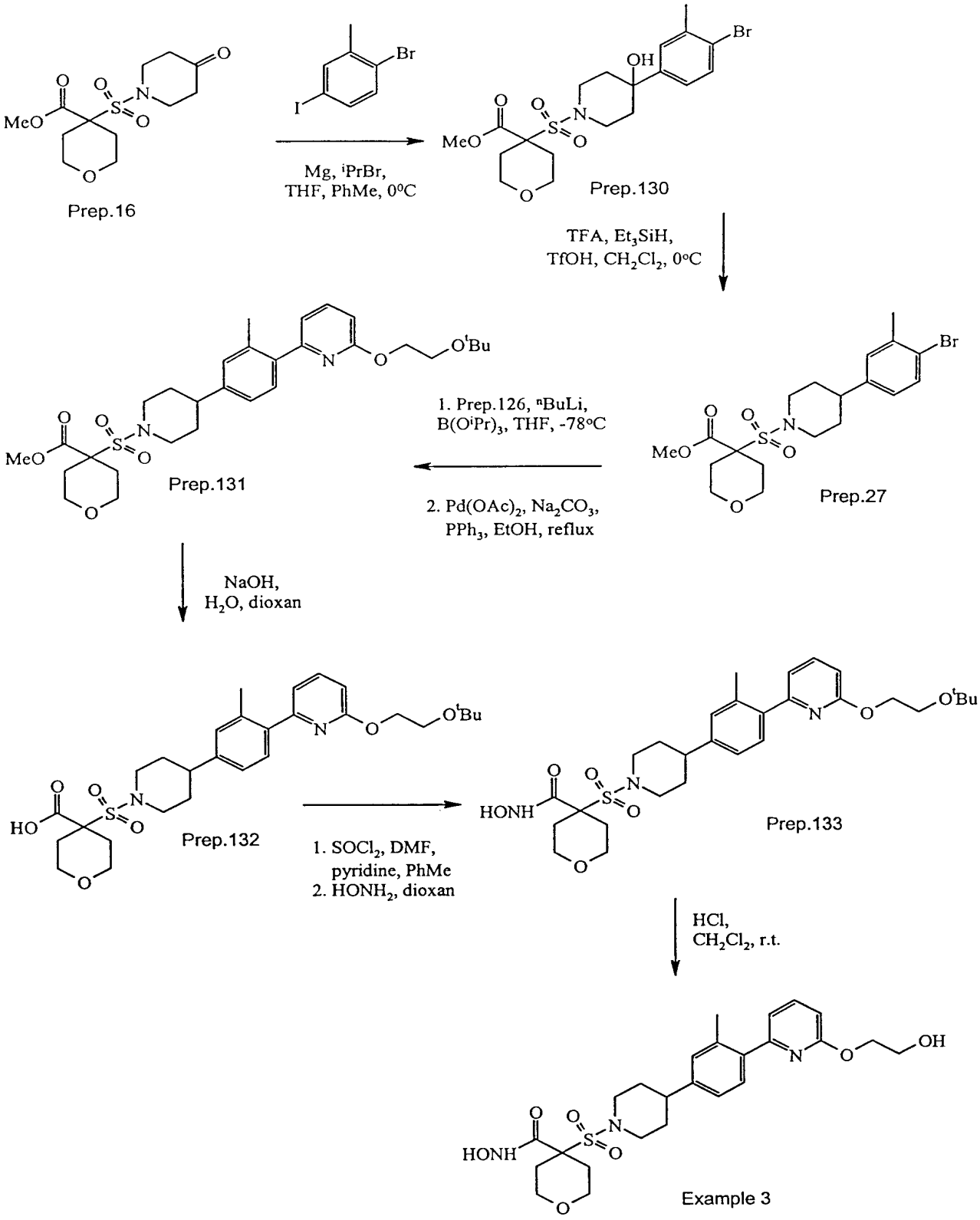
An example of a suitable OH-protecting group is the trimethylsilyl (TMS) group and the protection, reaction, deprotection sequence can be summarised by steps a) to c) below:

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).

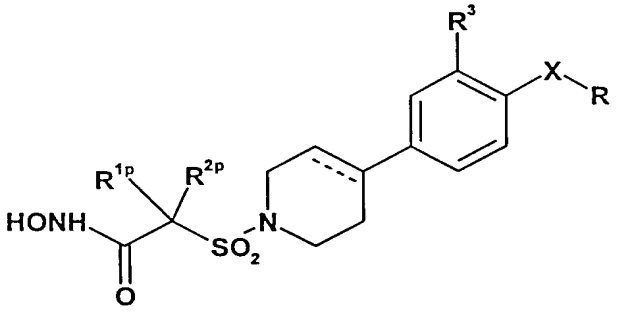

Prep.16
Mg, iPrBr,
THF, PhMe, 0°C
Prep.130
TFA, Et3SiH,
TfOH, CH2Cl2, 0°C
1. Prep.126, nBuLi,
B(OiPr)3, THF, -78°C
2. Pd(OAc)2, Na2CO3,
PPh3, EtOH, reflux
Prep.27
Prep.131
NaOH,
H2O, dioxan
Prep.132
1. SOCl2, DMF,
pyridine, PhMe
2. HONH2, dioxan
Prep.133
HCl,
CH2Cl2, r.t.
Example 3

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),

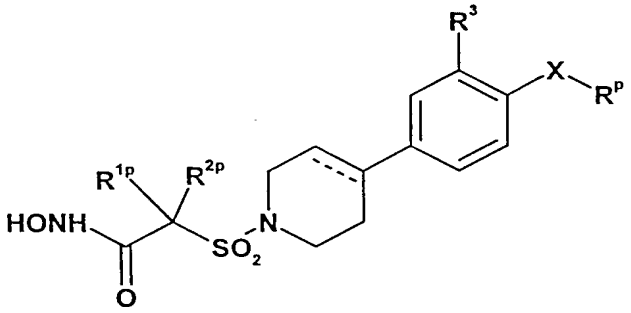
b) $NH_2OH.HCl$ (3 equiv) in DMF/pyridine or CH_2Cl_2/pyridine (3/1 to 1/1) at rt for between 4 and 20 hours.

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.

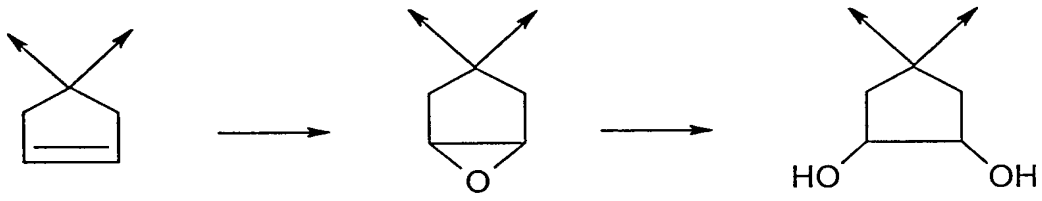
An extension of the above is where the compound of formula (I) contains a free, OH, NH and/or NH_2 group in R^1, R^2 and R (e.g. some Examples below). In thos case a suitable precursor could be the compound of formula (XIII) below:



(XIII)

where the substituents are as previously defined

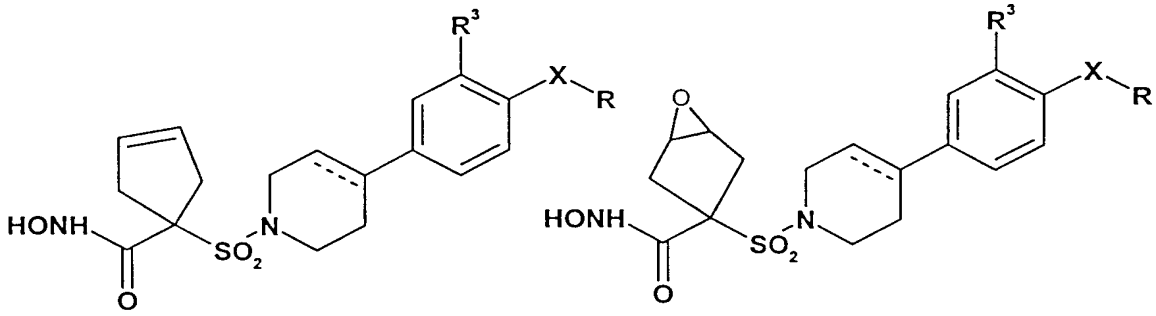
Compounds of formula (I) and appropriate intermediates thereto where R^1 and R^2 are taken together as 3,4-dihydroxycyclopentylidene can be made via the corresponding intermediacy of a corresponding cyclopent-3-enylidene moiety, viz.:



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

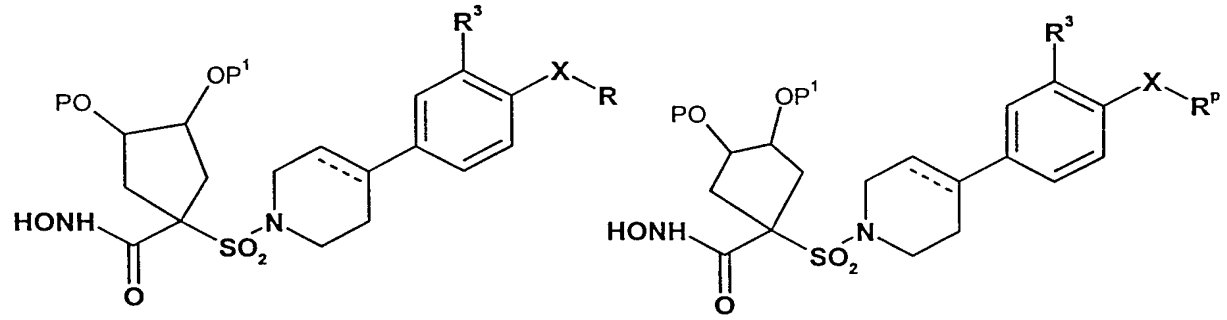
suitable choice of reagents, conditions etc., the skilled chemist can make diols with any desired stereochemistry, using well-known methods.

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.



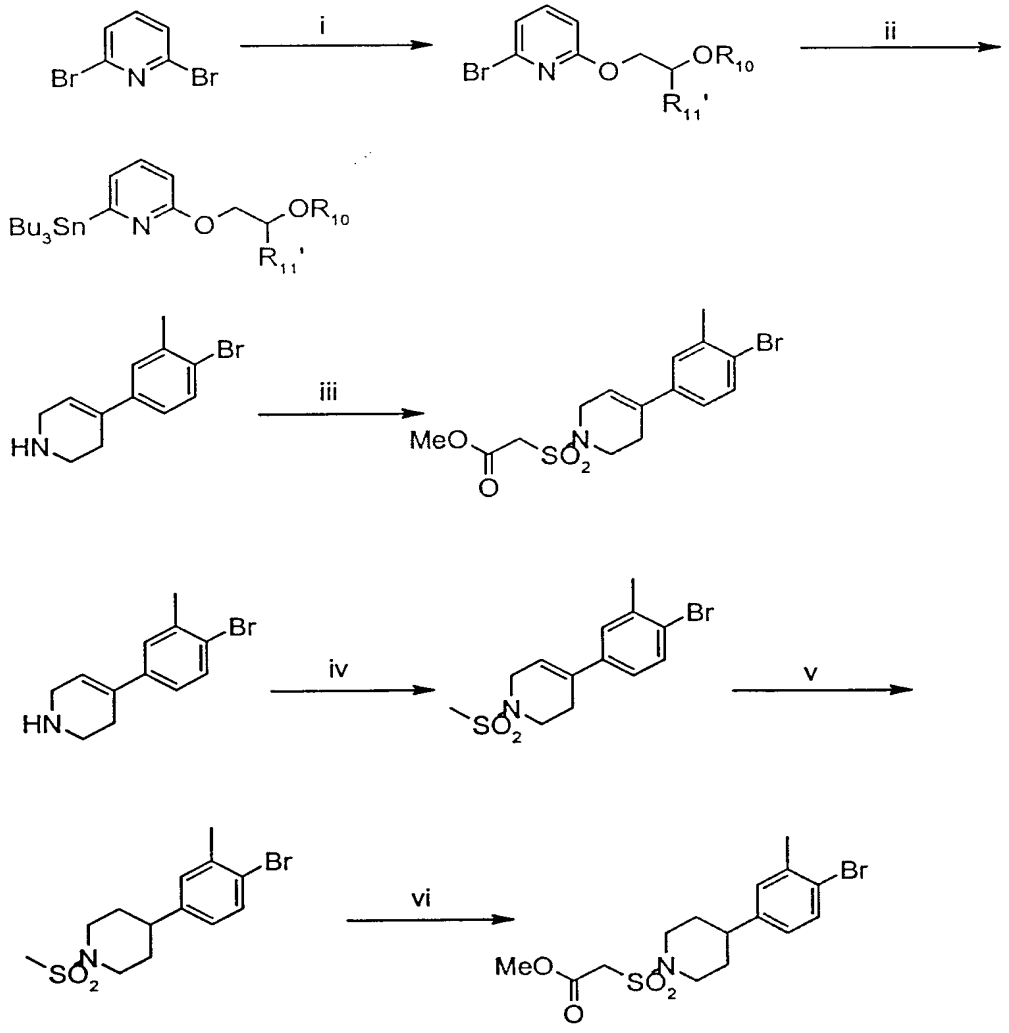
(X) (XI)

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.

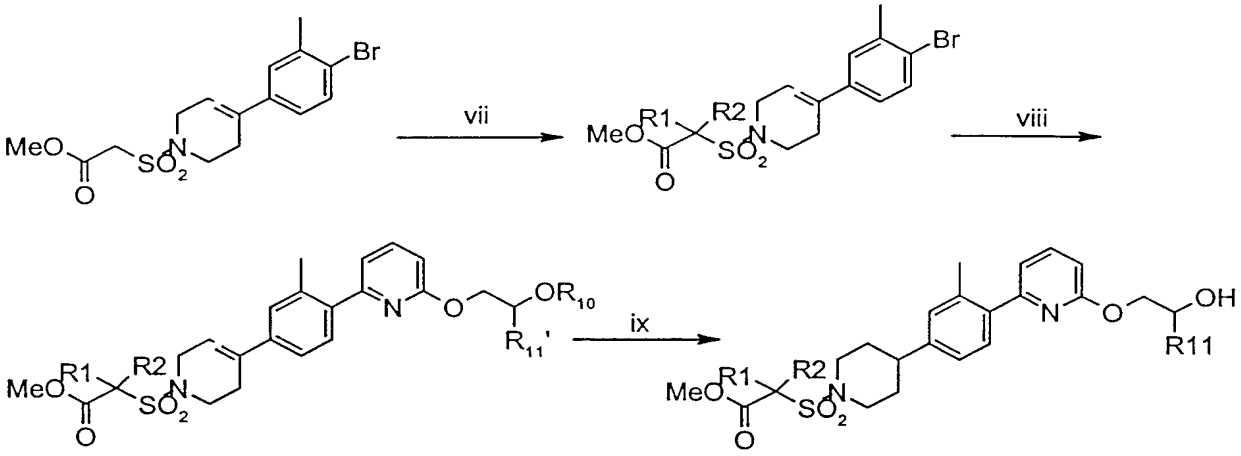
iv = $MeSO_2Cl$ (1.2 equiv), Et_3N (1.4 equiv), CH_2Cl_2, rt, for an hour.

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,

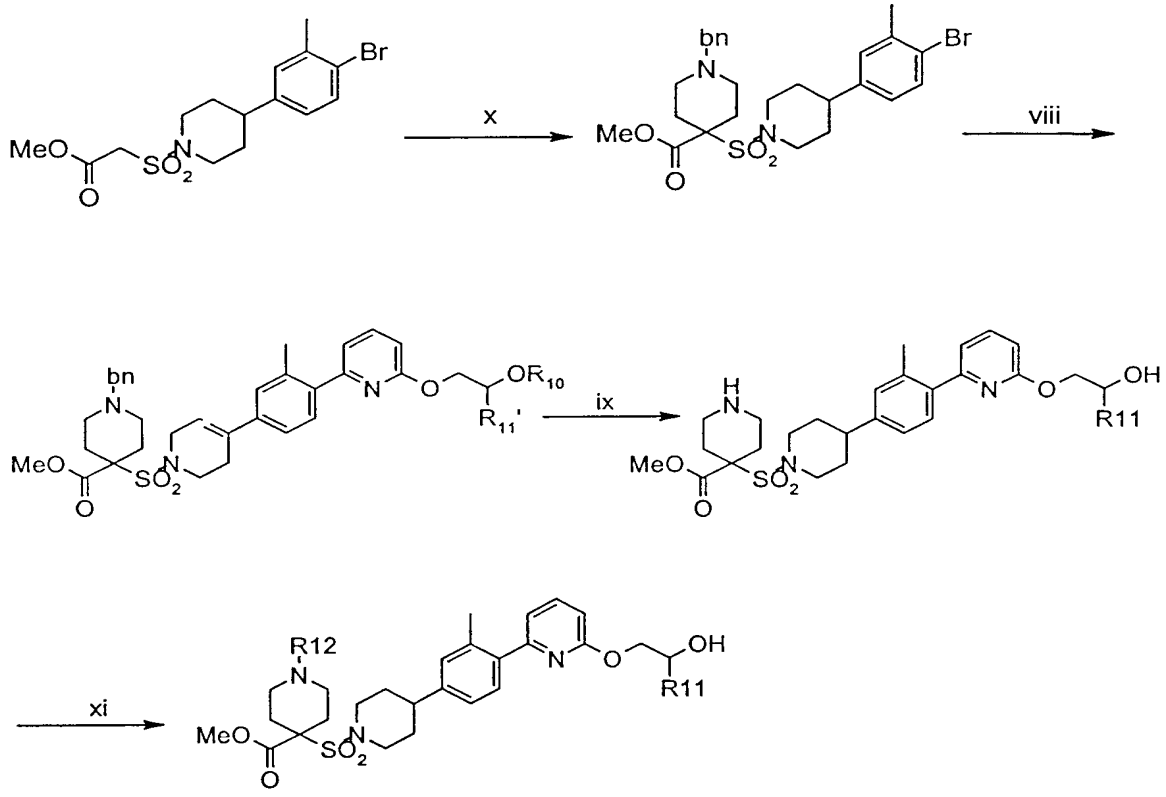
OR 10% Pd/C, in MeOH or EtOH, 3.3 atmospheres, room temperature, for 6 to 17 hours,-both methods also deprotect any benzyl group. (2N HCl, dioxan (3:1), rt, 75 mins at rt- deprotects the dioxalane)

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

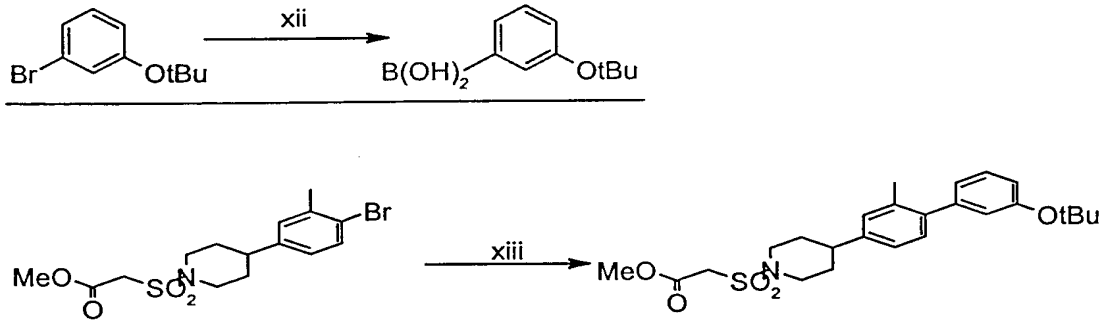
when R1R2 when taken together, are a piperidine group:

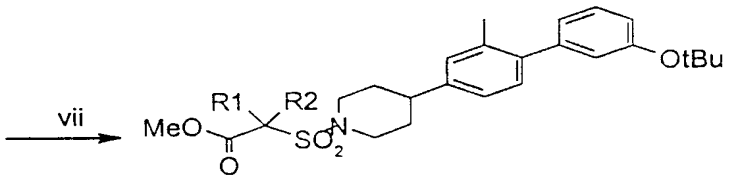


x = NaH (3 equiv), tetra-nBuNH$_4$Br (1 equiv), BnN(CH$_2$CH$_2$Cl)$_2$ (0.95 equiv), NMP, 60°C for 6 hours.

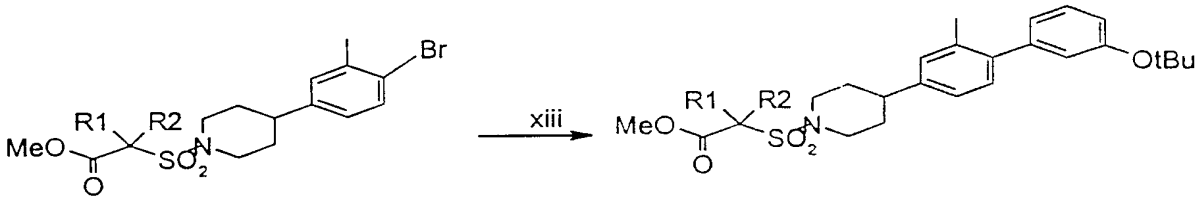
xi = When R12 is Me, formaldehyde (4 equiv), Na(OAc)$_3$BH (2 equiv), CH_2Cl_2, 20 hrs at rt.
When R12 is Boc, (Boc)$_2$O (1.05 equiv), Et_3N (1.1 equiv), CH_2Cl_2, rt for an hour.

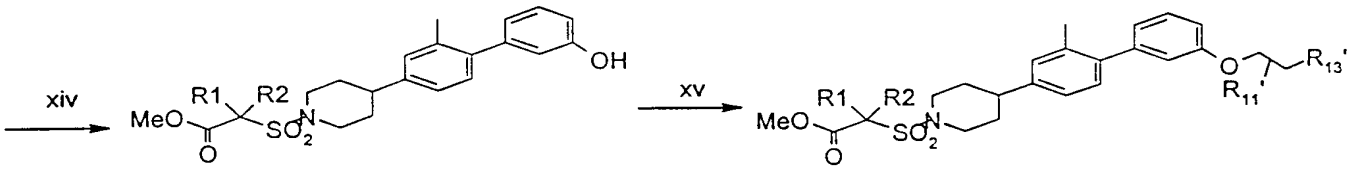
Route 2 *(Phenyl alcohols)*





Or





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

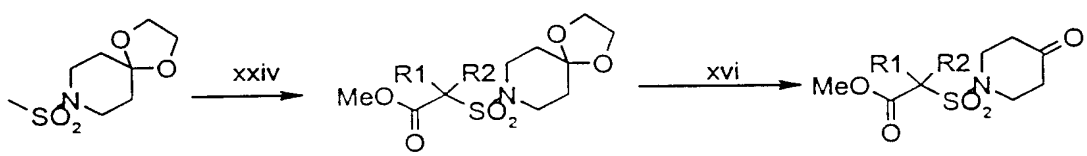
xiii = Suzuki coupling- arylboronic acid (1.2 to 1.5 equiv), CsF (2 to 2.6 equiv), $P(o\text{-}tol)_3$ (0.1 equiv), $Pd_2(dba)_2$ (0.005 equiv), DME, reflux for 6 to 50 hours.

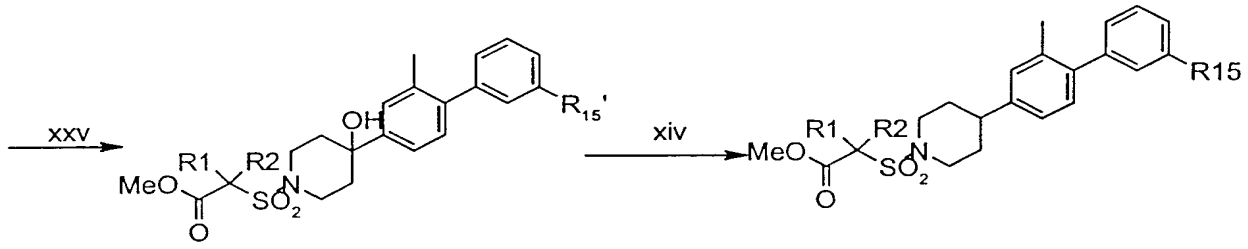
xiv = Et_3SiH (3 equiv), TFA:CH_2Cl_2 (1:1), rt for 2 to 24 hours.

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



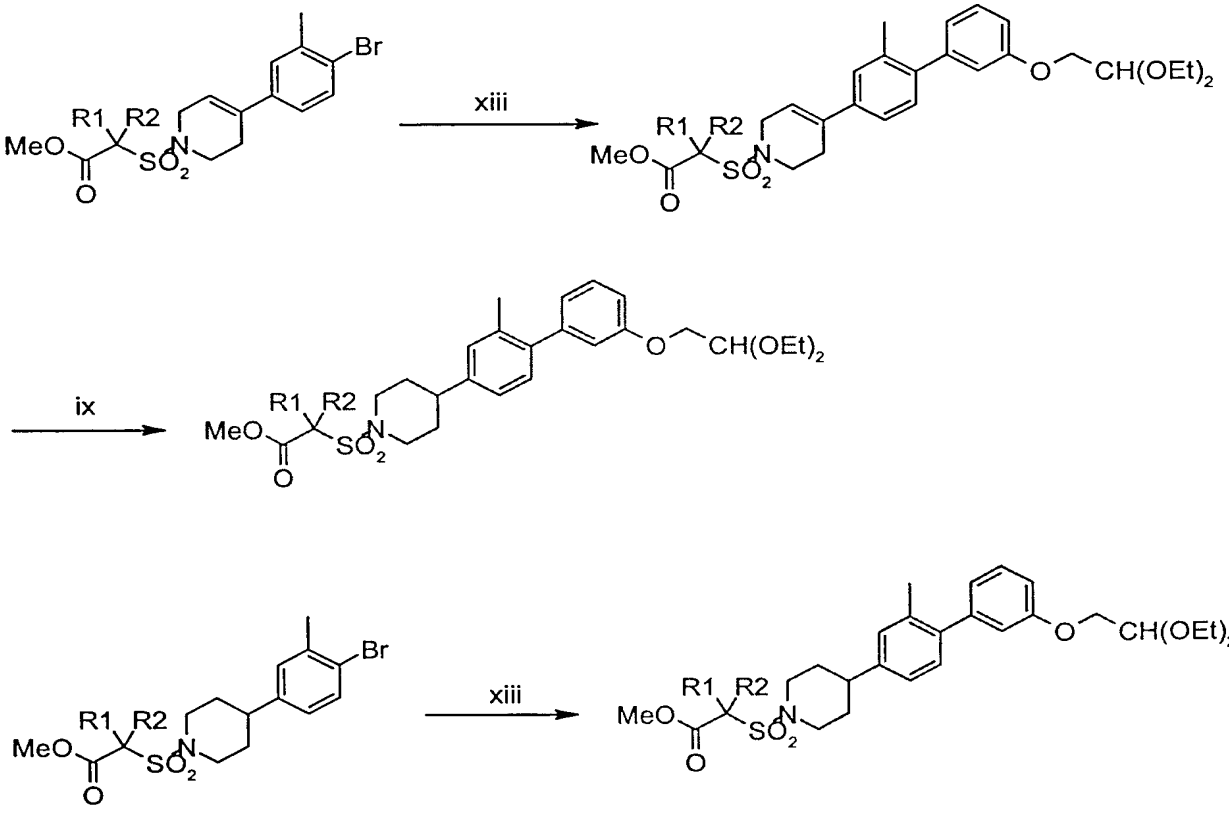


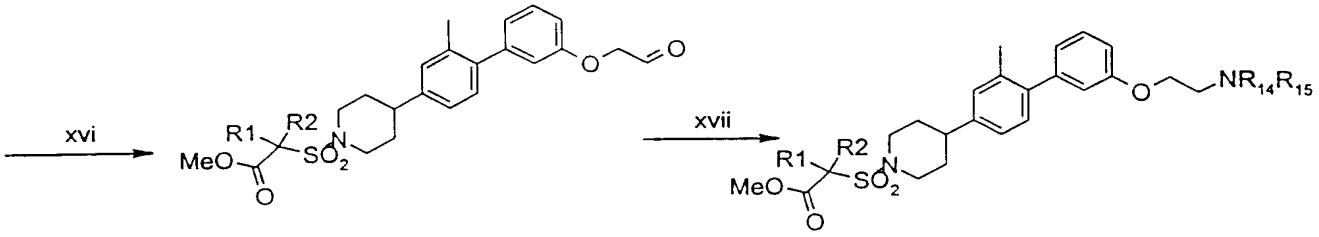
xxiv = *i*- NaH (2.2 equiv), Me_2CO_3 (5 equiv), toluene, MeOH (catalytic), 90°C, overnight.
ii- $O(CH_2CH_2Br)_2$ (1.3 equiv), NMP, 90°C, 20 hrs.

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

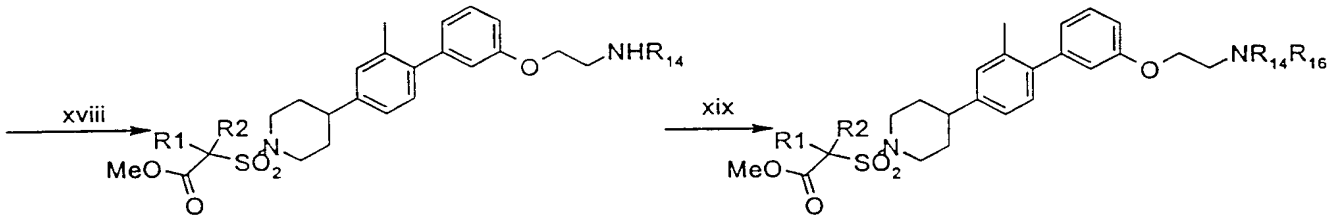
R15'-optionally protected alcohol, in prep 48 this is a t-butyl ether.
R15-OH, for prep 48.

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:



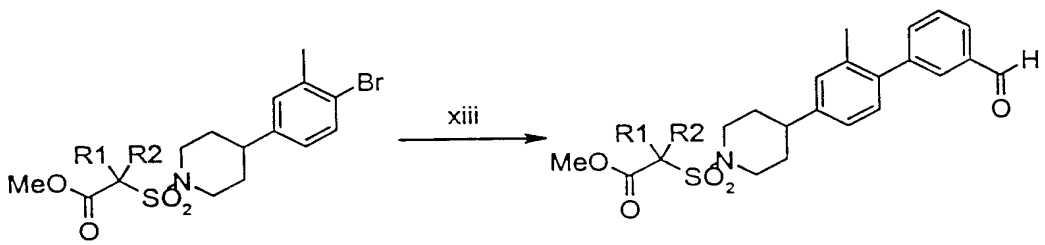
xvi = 1N HCl (1 to 2.3 equiv), acetone:dioxan (1:1), 70°C for 2 to 6 hours.

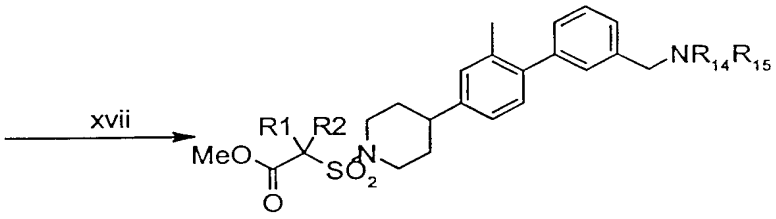
xvii = Reductive amination-amine (5.5 equiv), $Na(OAc)_3BH$ (3 to 4 equiv), CH_2Cl_2, rt, overnight.

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

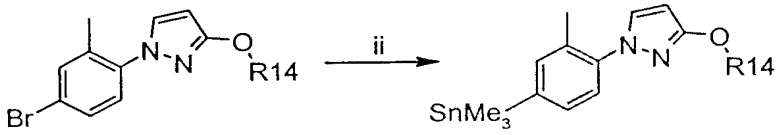
xix = When R16 is Boc,
$(Boc)_2O$ (1 to1.1 equiv), Et_3N (optional, 1 equiv), DMAP (optional, cat), CH_2Cl_2, rt, 3 hrs.

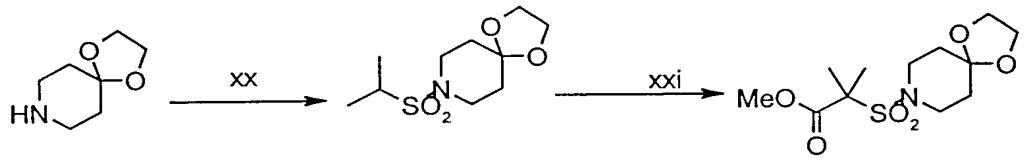
Route 4 *(aminoalkyl phenyls)*

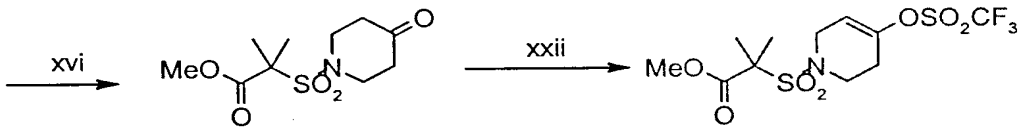


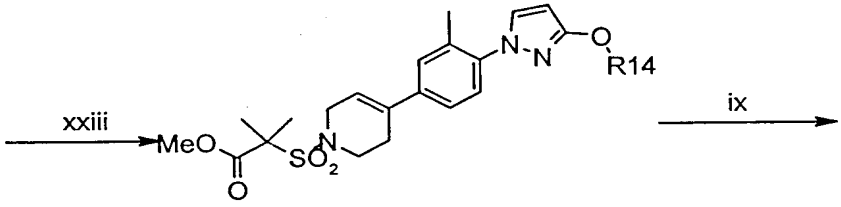


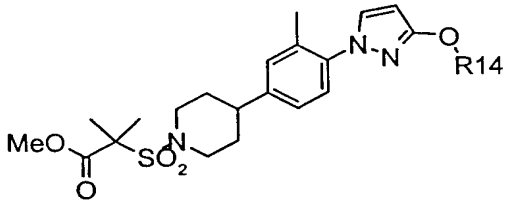
Route 5 (*Heterocycles*)











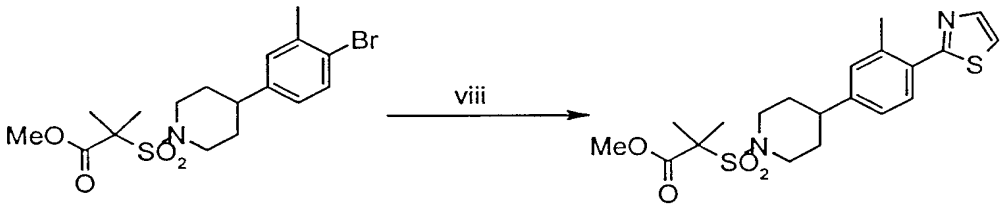
xx = iso-$PrSO_2Cl$ (1 equiv), Et_3N (1.1 equiv), CH_2Cl_2, 3hours at rt.

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

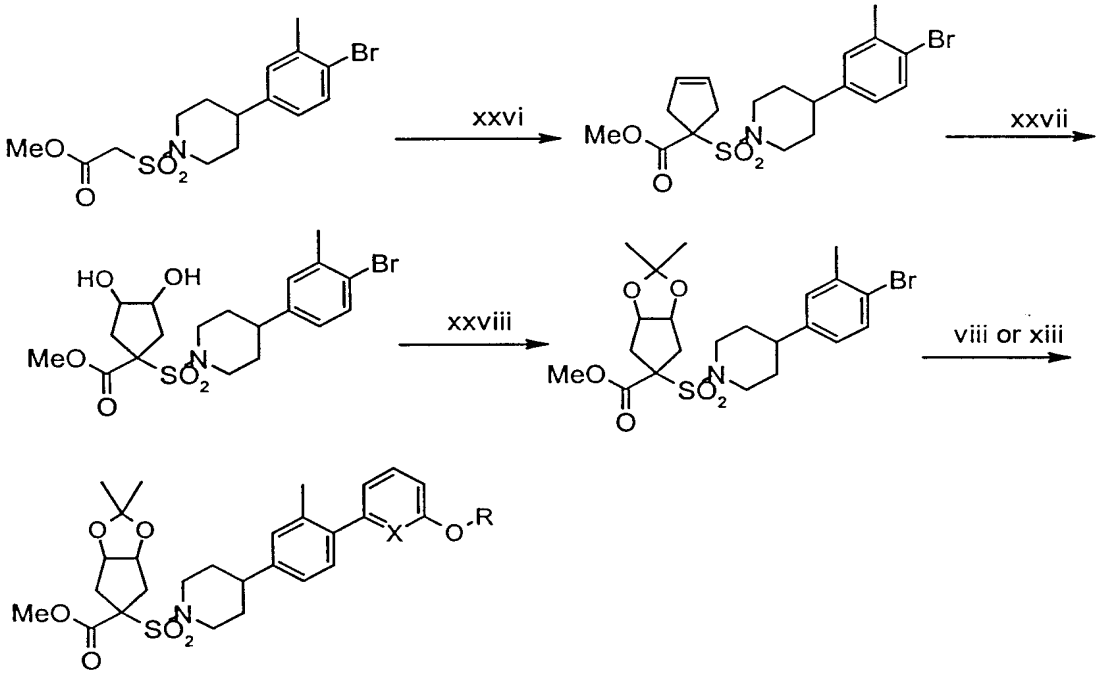
xxii = 2,6-di-t-Bu-4-Me pyridine (2.5 equiv), $(CF_3SO_2)_2O$ (2.5 equiv), CH_2Cl_2, 4°C to rt, 5 days.

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

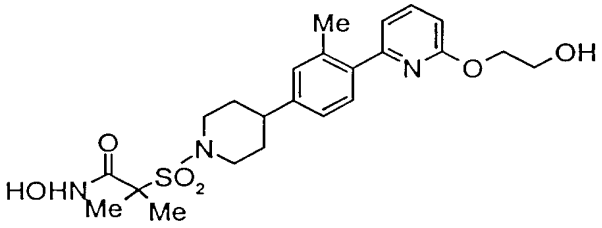
The syntheses of the compounds of the invention and of the intermediates for use therein are illustrated by the following Examples and Preparations.

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



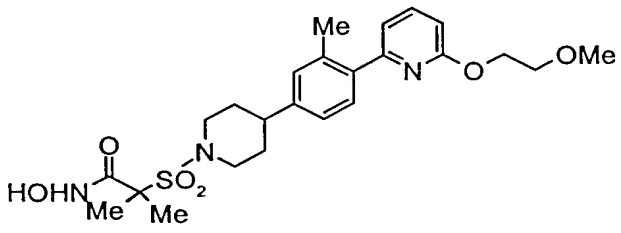
N,N-Dimethylformamide (10ml) was added to a solution of the acid from preparation 70 (430mg, 0.93mmol) in pyridine (5ml), followed by chlorotrimethylsilane (130µl, 1.03mmol) and the solution stirred for 1 ½ hours. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (215mg, 1.11mmol) and 1-hydroxybenzotriazole 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%).

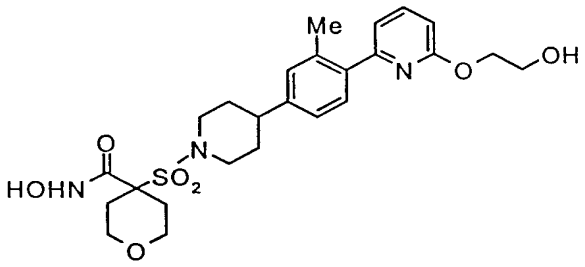
mp 134-138°C

^{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).

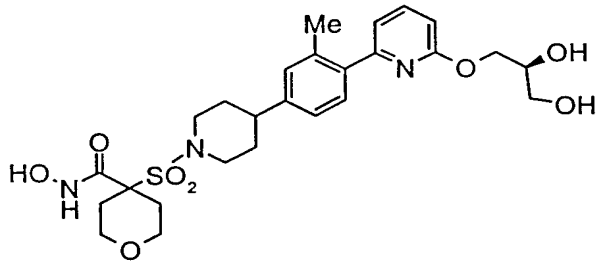
LRMS : m/z 520 $(M+1)^+$

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%).

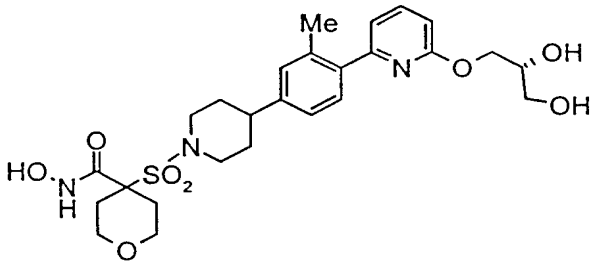
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.61 (m, 2H), 1.79 (m, 2H), 1.92 (m, 2H), 2.36 (m, 5H), 2.63 (m, 1H), 3.03 (m, 2H), 3.08-3.31 (m, 3H), 3.40 (m, 2H), 3.68-3.89 (m, 4H), 4.15 (m, 1H), 4.25 (m, 1H), 4.56 (br, s, 1H), 4.80 (br, s, 1H), 6.75 (d, 1H), 7.04 (d, 1H), 7.14 (m, 2H), 7.34 (d, 1H), 7.75 (m, 1H),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

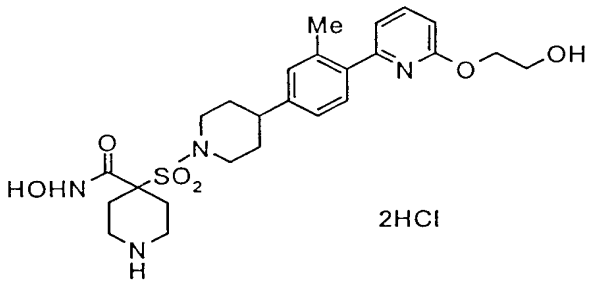
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.78 (m, 2H), 1.90 (m, 2H), 2.36 (m, 5H), 2.64 (m, 1H), 3.02 (m, 2H), 3.20 (m, 2H), 3.40 (m, 2H), 3.72 (m, 2H), 3.78 (m, 1H), 3.83 (m, 2H), 4.14 (m, 1H), 4.24 (m, 1H), 4.55 (dd, 1H), 4.80 (d, 1H), 6.75 (d, 1H), 7.03 (d, 1H), 7.15 (m, 2H), 7.32 (d 1H), 7.75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



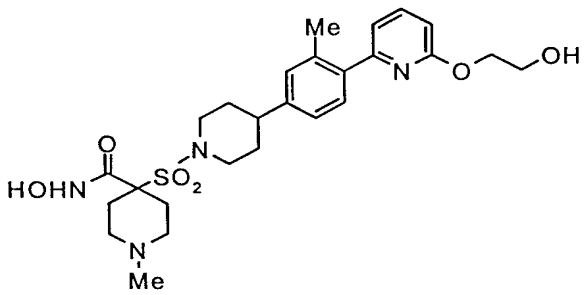
Hydrogen chloride gas was bubbled through an ice-cold solution of the hydroxamic acid from preparation 87 (135mg, 0.22mmol) in methanol (20ml), and the solution was stirred at room temperature. The reaction mixture was evaporated in vacuo, and the residue azeotroped with methanol. The solid was recrystallised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

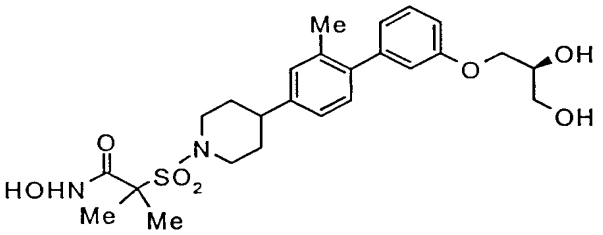
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.78 (m, 4H), 1.86 (m, 2H), 2.8 (s, 3H), 2.35 (s, 3H), 2.40 (m, 2H), 2.59-2.75 (m, 3H), 3.01 (m, 2H), 3.68 (m, 4H), 4.25 (t, 2H), 4.75 (t, 1H), 6.75 (d, 1H), 7.03 (d, 1H), 7.15 (m, 2H), 7.32 (d, 1H), 7.74 (m, 1H), 9.06 (br, s, 1H), 10.88 (br, s, 1H).

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

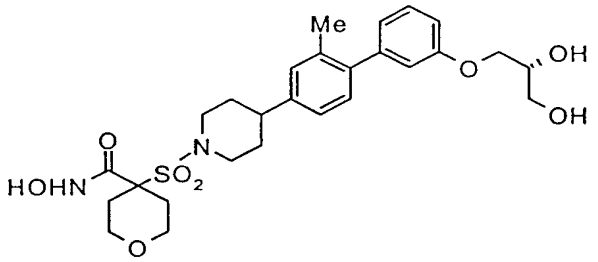
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.44 (s, 6H), 1.60 (m, 2H), 1.78 (m, 2H), 2.18 (s, 3H), 2.61 (m, 1H), 3.02 (m, 2H), 3.39 (m, 2H), 3.71 (m, 3H), 3.82 (m, 1H), 3.98 (m, 1H), 4.56 (m, 1H), 4.82 (m, 1H), 6.82 (m, 3H), 7.08 (m, 2H), 7.12 (s, 1H), 7.26 (m, 1H), 8.94 (s, 1H), 10.69 (s, 1H).

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

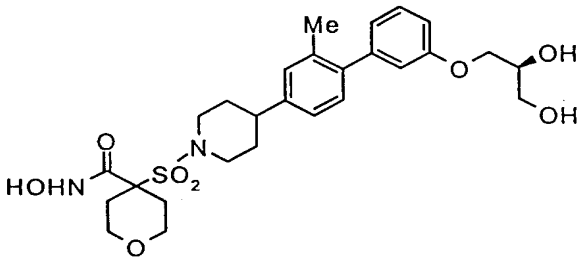
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.78 (m, 2H), 1.90 (m, 2H), 2.20 (s, 3H), 2.38 (m, 2H), 2.62 (m, 1H), 3.03 (m, 2H), 3.20 (m, 2H), 3.42 (m, 2H), 3.66-3.90 (m, 6H), 4.01 (m, 1H), 4.60 (m, 1H), 4.90 (m, 1H), 6.84 (m, 3H), 7.14 (m, 3H), 7.30 (m, 1H), 9.18 (s, 1H), 10.98 (1H, s).

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



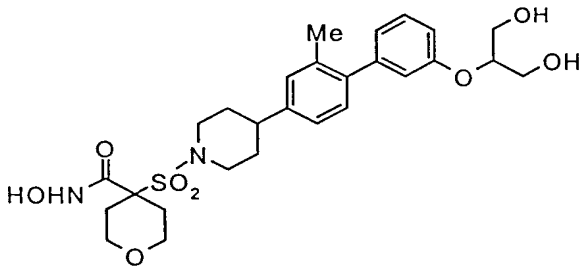
The title compound was prepared, from the acid from preparation 80, following a similar procedure to that described in example 9. The crude product was triturated with methanol/di-isopropyl ether, and the resulting precipitate filtered and dried to afford the title compound as a buff-coloured solid,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

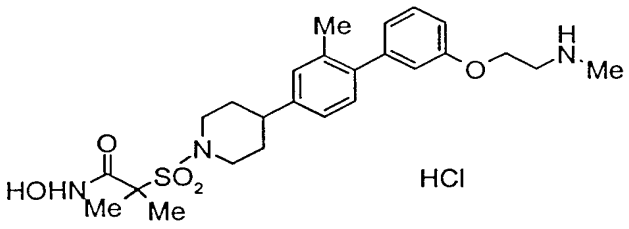
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.79 (m, 2H), 1.90 (m, 2H), 2.20 (s, 3H), 2.39 (m, 2H), 2.62 (m, 1H), 3.02 (m, 2H), 3.20 (m, 2H), 3.57 (m, 4H), 3.70 (m, 2H), 3.84 (m, 2H), 4.24 (m, 1H), 4.78 (m, 2H), 6.82 (d, 1H), 6.90 (m, 2H), 7.14 (m, 3H), 7.28 (m, 1H), 9.18 (br, s, 1H).

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

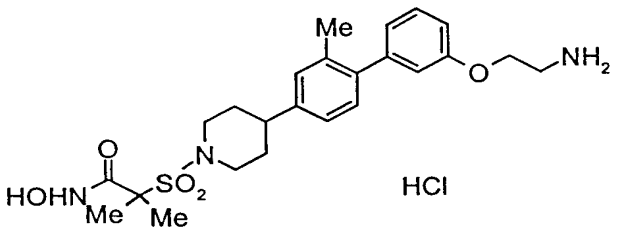
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.44 (s, 6H), 1.60 (m, 2H), 1.78 (m, 2H), 2.18 (s, 3H), 2.59 (m, 3H), 3.02 (m, 2H), 3.28 (m, 2H), 3.72 (m, 2H), 4.23 (t, 2H), 6.90 (m, 3H), 7.08 (s, 2H), 7.16 (s, 1H), 7.34 (m, 1H), 8.83 (br s, 2H), 10.80 (s, 1H).

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

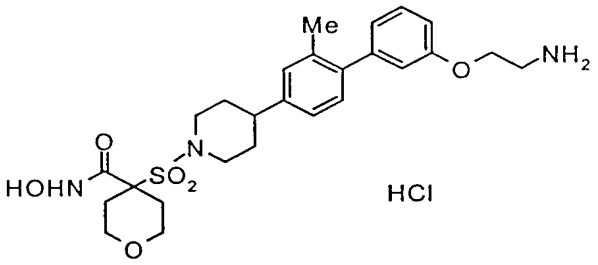
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.48 (s, 6H), 1.60 (m, 2H), 1.80 (m, 2H), 2.20 (s, 3H), 2.64 (m, 2H), 3.06 (m, 2H), 3.20 (t, 2H), 3.75 (m, 2H), 4.20 (t, 2H), 6.94 (m, 3H), 7.12 (s, 2H), 7.18 (s, 1H), 7.38 (m, 2H), 8.01 (br s, 1H), 8.99 (s, 1H).

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%).

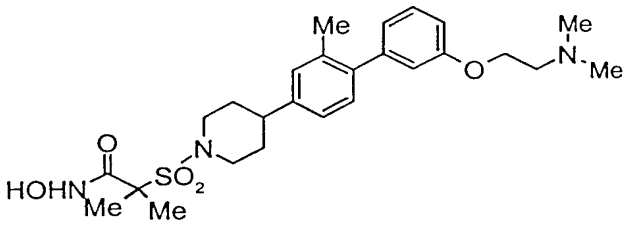
mp 208-210°C

^{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%).

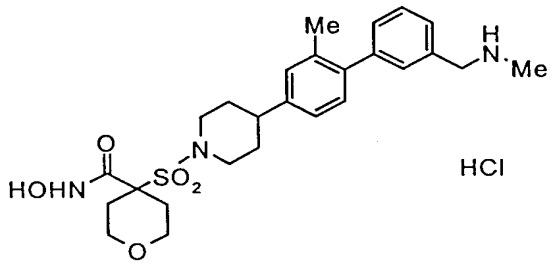
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.48 (s, 6H), 1.60 (m, 2H), 1.78 (m, 2H), 2.19 (s, 9H), 2.60 (m, 3H), 3.03 (m, 2H), 3.76 (m, 2H), 4.05 (t, 2H), 6.80 (m, 2H), 6.86 (m, 1H), 7.06 (m, 2H), 7.12 (s, 1H), 7.28 (m, 1H).

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

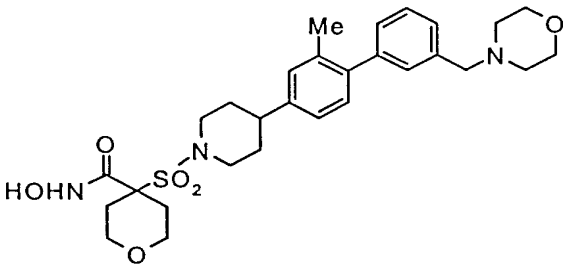
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.78 (m, 2H), 1.97 (m, 2H), 2.20 (s, 3H), 2.38 (m, 2H), 2.46 (s, 3H), 2.62 (m, 1H), 3.01 (m, 2H), 3.18 (m, 2H), 3.70 (m, 2H), 3.82 (m, 2H), 4.12 (s, 2H), 7.10 (m, 3H), 7.35 (s, 1H), 7.43 (m, 3H), 9.10 (br, s, 1H), 10.92 (s, 1H).

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

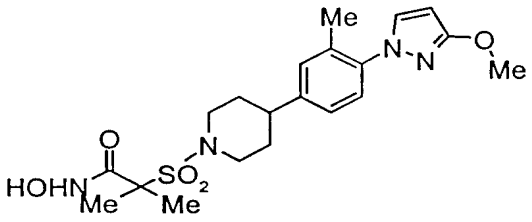
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.60 (m, 2H), 1.78 (m, 2H), 1.88 (m, 2H), 2.17 (s, 3H), 2.36 (m, 6H), 2.60 (m, 1H), 3.00 (m, 2H), 3.19 (m, 2H), 3.46 (s, 2H), 3.53 (m, 4H), 3.70 (m, 2H), 3.81 (m, 2H), 7.06 (m, 7H), 9.10 (s, 1H), 10.92 (s, 1H).

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%).

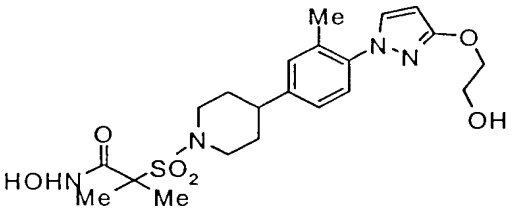
mp 172-173°C

^{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



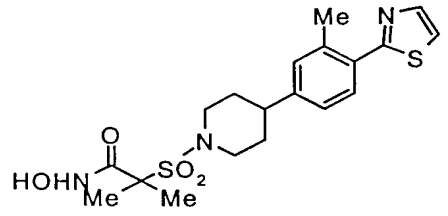
Pyridine (6ml) was added to a suspension of the acid from preparation 104 (325mg, 0.72mmol) in dichloromethane (6ml), and the solution purged with nitrogen. Chlorotrimethylsilane (858mg, 0.79mmol) was added, the solution stirred for an hour, then 1-hydroxy-7-azabenzotriazole (98mg, 0.72mmol) was added, 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%).

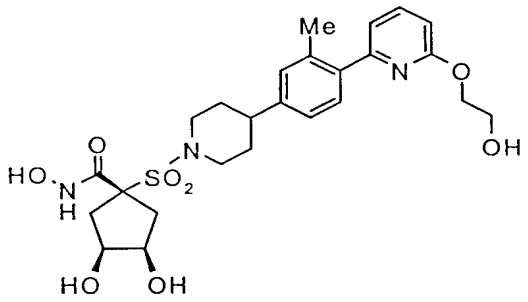
mp 199-201°C

^{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).

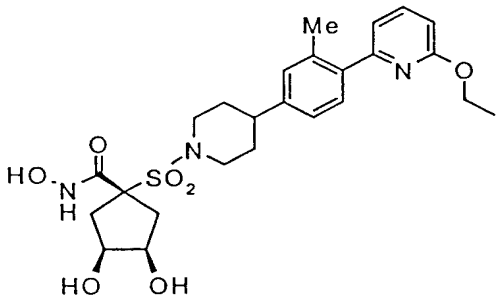
LRMS :m/z 536 $(M+1)^+$.

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).

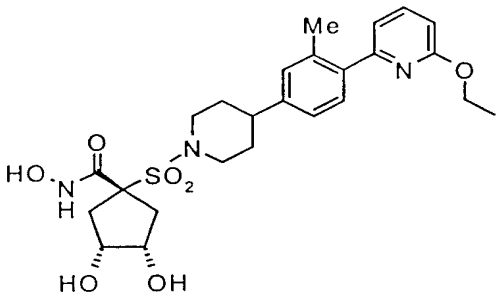
LRMS :m/z 520 $(M+1)^+$.

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).

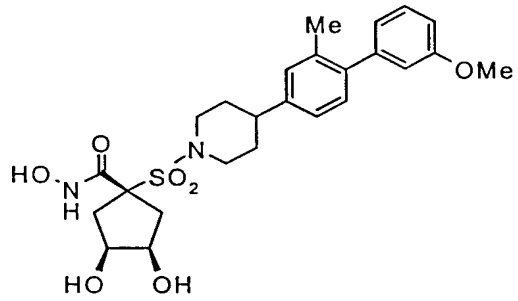
LRMS :m/z 520 $(M+1)^+$.

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).

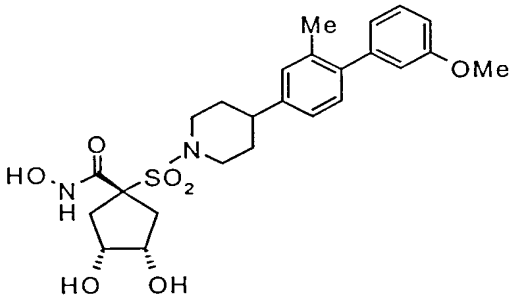
LRMS :m/z 527 $(M+23)^+$.

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).

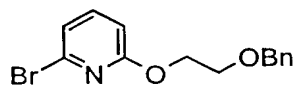
LRMS :m/z 503 $(M-1)^-$.

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

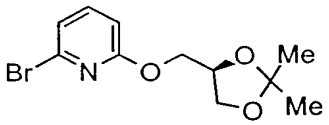


Sodium hydride (900mg, 60% dispersion in mineral oil, 22.5mmol) was added portionwise to an ice-cold solution of 2-(benzyloxy)ethanol (3.0g, 20.0mmol) in toluene (100ml), and the solution stirred for 30 minutes. 2,6-Dibromopyridine (4.75g, 20.0mmol) was added, and the reaction heated under reflux for 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

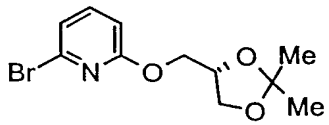


Sodium hydride (1.62g, 60% dispersion in mineral oil, 40.5mmol) was added portionwise to an ice-cooled solution of (R)-(-)-1,2-O-isopropylideneglycerol (4.86g, 36.8mmol) in toluene (100ml), and once addition was complete, the solution was allowed to warm to room temperature and stirred for 30 minutes. 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

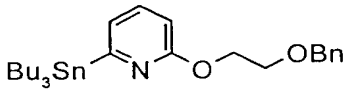


The title compound was obtained as a yellow oil (quantitative), from (S)-(-)-1,2-O-isopropylideneglycerol and 2,6-dibromopyridine, following the procedure described in preparation 2.

^{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

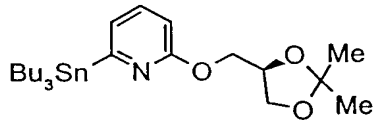


n-Butyllithium (13.8ml, 1.6M solution in hexanes, 22.0mmol) was added dropwise to a cooled (-78°C) solution of the bromide from preparation 1 (20.0mmol) in anydrous THF (100ml), so as to maintain the internal temperature <-70°C, and the solution stirred for 20 minutes. Tri-n-butyltin chloride (6.0ml, 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

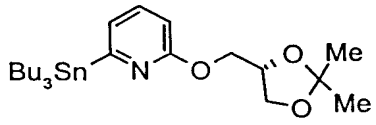


The title compound was prepared as an oil (quantitative) from the bromide of preparation 2, using a similar procedure to that described in preparation 4.

^{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

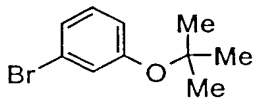


The title compound was obtained as a colourless oil (71%), from the bromide from preparation 3, following a similar procedure to that described in preparation 5.

^{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

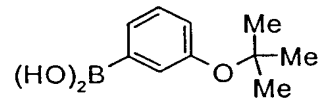


Condensed isobutylene (100ml) was added via a dry ice/acetone cold finger, to dichloromethane (70ml) at -30°C, followed by a solution of 3-bromophenol (21.5g, 125mmol) in dichloromethane (30ml). Trifluoromethanesulphonic acid (1.5g, 10.0mmol) was added dropwise, the reaction cooled to -75°C, and stirred 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

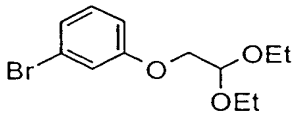


n-Butyllithium (40ml, 2.5M in hexanes, 100mmol) was added dropwise to a cooled (-78°C) solution of the bromide from preparation 7 (23.9g, 90mmol) in tetrahydrofuran (300ml), so as to maintain the temperature below -70°C. The resulting solution was stirred for 1 hour, and triisopropyl borate (30.6ml, 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

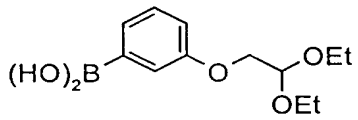


A mixture of potassium carbonate (1.5g, 10.9mmol), 3-bromophenol (1.73g, 10.0mmol) and bromoacetaldehyde diethyl acetal (1.5ml, 9.67mmol) in dimethylsulphoxide (10ml) was heated at 160°C for 1 ½ hours. The cooled reaction was partitioned between water (50ml) and ethyl acetate (100ml), and the phases 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

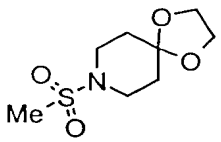


n-Butyllithium (18.5ml, 2.5M in hexanes, 46.25mmol) was added dropwise to a cooled (-78°C) solution of the bromide from preparation 9 (11.4g, 39.6mmol) in anhydrous tetrahydrofuran (100ml), so as to maintain the internal temperature <-70°C. This solution was stirred for 1 hour, then triisopropyl borate (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%).

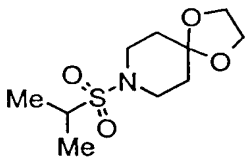
mp 107-109°C

^{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%).

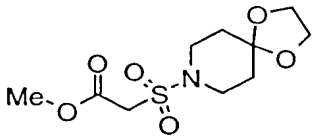
mp 66-67°C

^{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



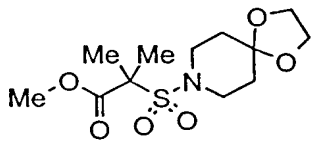
Potassium tert-butoxide (24.6g, 219mmol) was added portionwise to a solution of the ethylene ketal from preparation 11 (32.3g, 146mmol) and dimethyl carbonate (61ml, 730mmol) in tetrahydrofuan (200ml), and once addition was complete, the reaction was stirred at room temperature overnight under a nitrogen 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



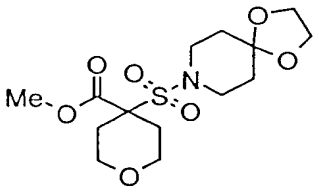
N-Butyl lithium (28ml, 1.6M in hexanes, 44.1mmol) was added dropwise to a cooled (-78°C) solution of the sulphonamide from preparation 12 (10g, 40.1mmol) in tetrahydrofuran (100ml), so as to maintain a temperature below -45°C. Once addition was complete the solution was allowed to warm to 0°C, and then 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

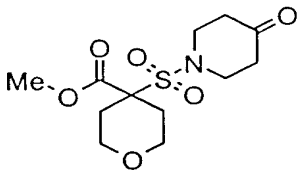
Potassium tert-butoxide (220ml, 1M in tetrahydrofuran, 220mmol) was added dropwise to a solution of the acetate from preparation 13 (27.9g, 100mmol) and bis(2-bromoethyl)ether (16.3ml, 130mmol) in tetrahydrofuran (200ml) and 1-methyl-2-pyrrolidinone (20ml), and the reaction stirred at room temperature 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%).

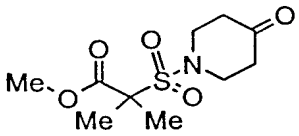
mp 158-160°C

^{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



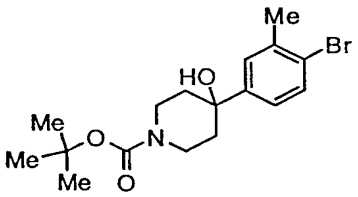
The title compound was obtained as a solid (98%) after trituration with pentane from the ethylene ketal from preparation 14, following a similar method to that described in preparation 16.

^{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.

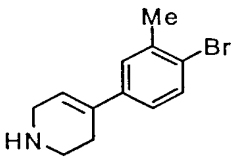
^{1}H nmr ($CDCl_3$) δ: 1.48 (s, 9H), 1.51 (s, 1H), 1.70 (d, 2H), 1.96 (m, 2H), 2.40 (s, 3H), 3.22 (t, 2H), 4.02 (m, 2H), 7.15 (dd, 1H), 7.36 (d, 1H), 7.50 (d, 1H).

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).

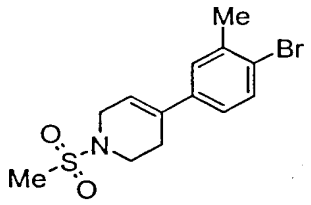
LRMS :m/z 251/253 $(M+1)^+$.

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



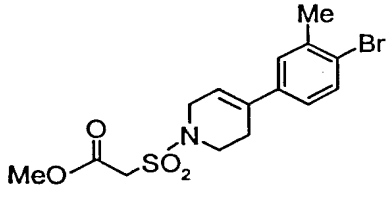
Methanesulphonyl chloride (17.5ml, 227mmol) was added dropwise to an ice-cooled solution of triethylamine (34.4ml, 247mmol) and the amine from preparation 19 (51.8g, 206mmol) in dichloromethane (400ml), and the reaction then stirred at room temperature for 1 hour. Tlc analysis showed starting material 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.

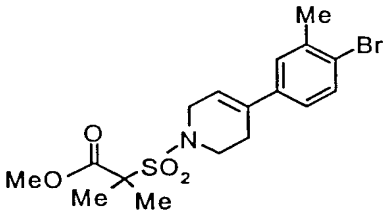
^{1}H nmr ($CDCl_3$) δ: 2.40 (s, 3H), 2.60 (m, 2H), 3.60 (t, 2H), 3.80 (s, 3H), 4.01 (s, 2H), 4.07 (m, 2H), 6.02 (br, s,1H), 7.02 (dd, 1H), 7.21 (d, 1H), 7.50 (d, 1H).

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%).

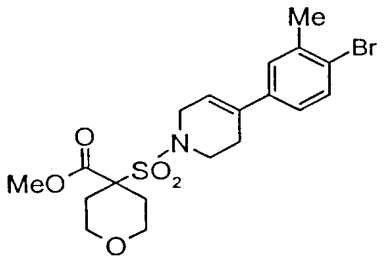
m.p. 100-101°C.

^{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.

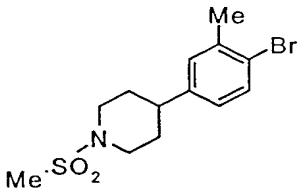
^{1}H nmr ($CDCl_3$) δ: 2.23 (m, 2H), 2.40 (s, 3H), 2.42 (m, 2H), 2.58 (m, 2H), 3.30 (m, 2H), 3.58 (m, 2H), 3.87 (s, 3H), 4.00-4.10 (m, 4H), 6.00 (br, s, 1H), 7.02 (dd, 1H), 7.21 (d, 1H), 7.49 (d, 1H).

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



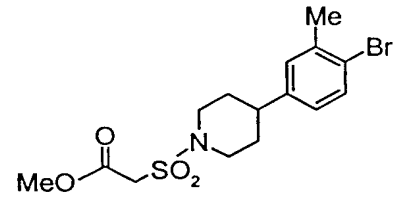
Triethylsilane (47.2ml, 296mmol), followed by trifluoromethanesulphonic acid (1.73ml, 19.7mmol) were added to a solution of the sulphonamide from preparation 20 (65.0g, 197mmol) in dichloromethane (300ml) and trifluoroacetic acid (300ml), and the reaction stirred at room temperature for an hour. Tlc 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



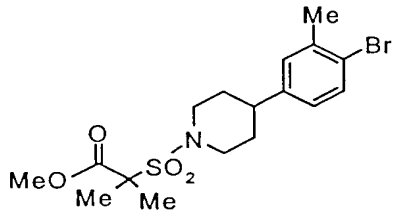
Sodium hydride (12.2g, 60% dispersion in mineral oil, 305mmol) was added to a solution of the sulphonamide from preparation 24 (50.61g, 152mmol) and dimethylcarbonate (63.8ml, 760mmol) in toluene (600ml), and the reaction heated under reflux for 1 ½ hours. The reaction was partitioned between ethyl acetate (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%).

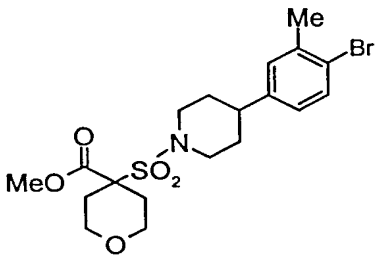
mp 103-106°C

^{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).

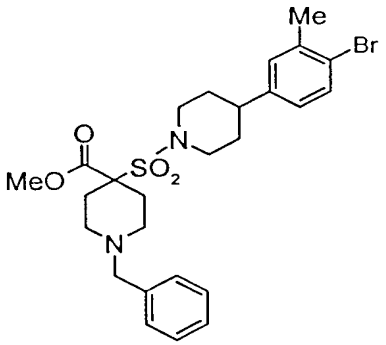
LRMS :m/z 460/462 $(M+1)^+$.

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



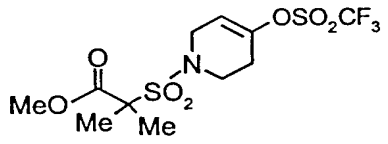
The acetate from preparation 25 (4.17g, 10.7mmol) was added portionwise to a suspension of sodium hydride (994mg, 60% dispersion in mineral oil, 33.1mmol) in 1-methyl-2-pyrrolidinone (40ml), and the resulting solution stirred for an hour. Tetra-butyl ammonium bromide (3.44g, 10.7mmol) and N-benzyl-bis-(2-chloroethyl)amine (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

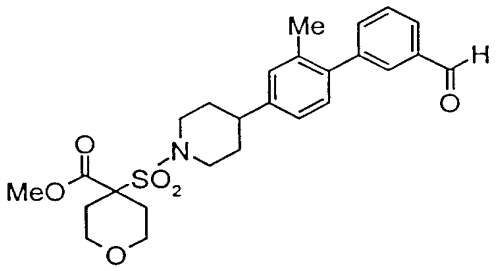
chromatography on silica gel using an elution gradient of hexane:ethyl acetate (91:9 to 50:50) to afford the title compound (4.25g, 74%) as a white solid.

^{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



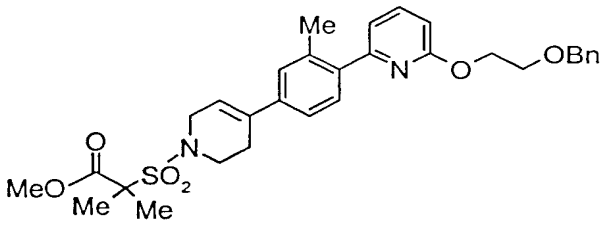
A mixture of the bromide from preparation 27 (4.02g, 8.73mmol), 3-formylphenylboronic acid (1.83g, 11.56mmol), cesium fluoride (3.46g, 22.8mmol), tris(dibenzylideneacetone)palladium (0) (430mg, 0.47mmol) and tri(o-tolyl)phosphine (284mg, 0.93mmol) in 1,2-dimethoxyethane (70ml) was heated under reflux 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



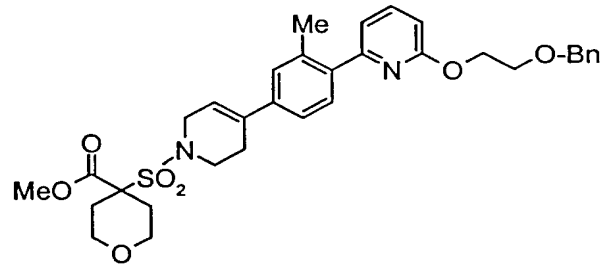
A mixture of the stannane from preparation 4 (2.8g, 5.4mmol) and the bromide from preparation 22 (1.5g, 3.62mmol), and tetrakis(triphenylphosphine)palladium (0) (205mg, 0.18mmol) in toluene (35ml) was heated under reflux overnight. The cooled mixture was evaporated in vacuo and the residue purified by 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



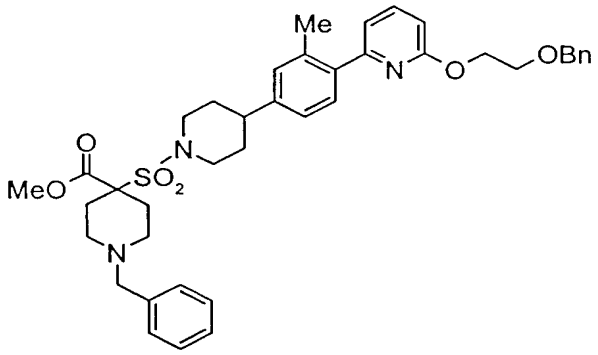
A mixture of the stannane from preparation 4 (1.74g, 3.36mmol) and the bromide from preparation 23 (1.1g, 2.4mmol) and tetrakis(triphenylphosphine)palladium (0) (138mg, 0.14mmol) in toluene (16ml) was heated under reflux for 4 hours. The cooled reaction was diluted with water, and the mixture extracted 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



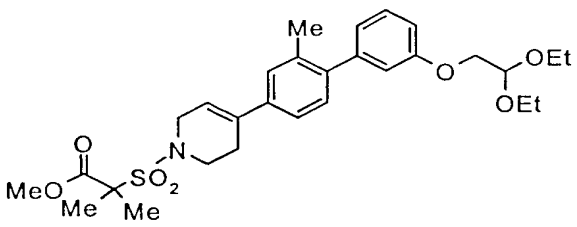
The stannane from preparation 4 (4.05g, 7.8mmol), followed by tris(triphenylphosphine) palladium (0) (410mg, 0.35mmol) were added to a solution of the bromide from preparation 28 (3.91g, 7.1mmol) in toluene (50ml), and the reaction de-gassed, then heated under a nitrogen atmosphere reflux for 7 hours. 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



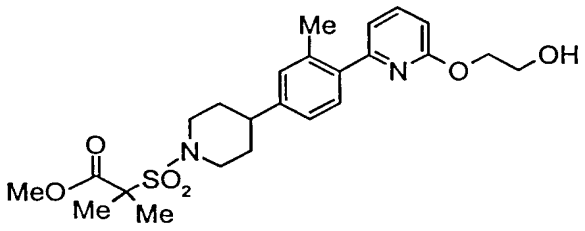
A mixture of cesium fluoride (1.81g, 11.92mmol), tri-o-tolyl phosphine (180mg, 0.59mmol), tris(dibenzylideneacetone)dipalladium (0) (280mg, 0.31mmol) and the boronic acid from preparation 10 (1.83g, 7.2mmol) and the bromide from preparation 22 (2.5g, 6.0mmol) in anhydrous 1,2-dimethoxyethane (60ml), 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%).

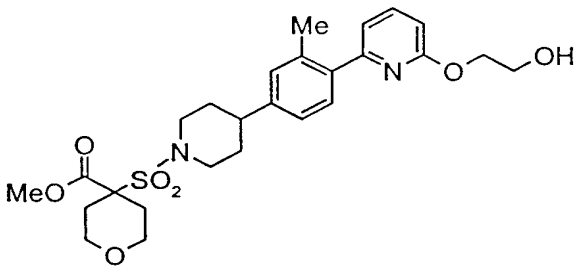
mp 108-111°C

^{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



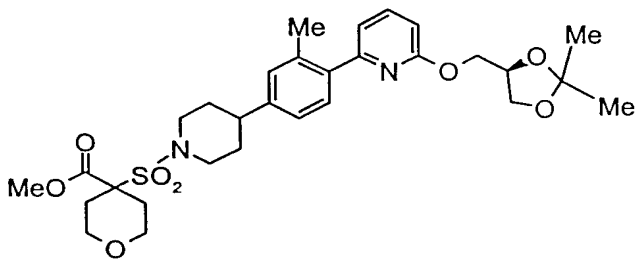
The title compound was prepared from the benzyl ether from preparation 32 in 93% yield, following a similar procedure to that described in preparation 35.

^{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



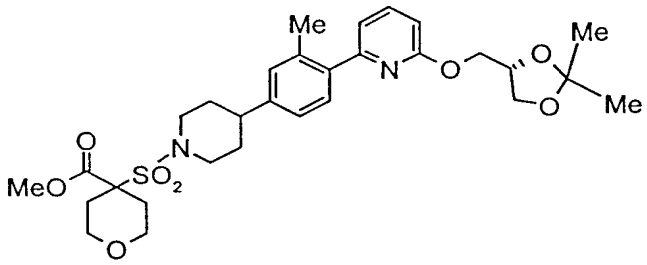
A mixture of the stannane from preparation 5 (2.0g, 4.97mmol) and the bromide from preparation 27 (1.76g, 3.82mmol) and tetrakis(triphenylphosphine)palladium (0) (242mg, 0.21mmol) in toluene (50ml) was heated under reflux for 7 hours. The cooled mixture was concentrated under reduced pressure and the 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



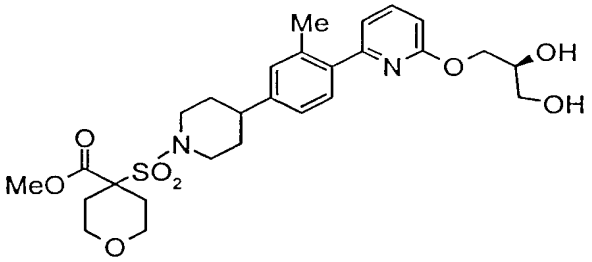
The title compound was obtained as a white solid (65%), after recrystallisation from methanol, from the stannane from preparation 6 and the bromide from preparation 27, following a similar procedure to that described in preparation 37.

^{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

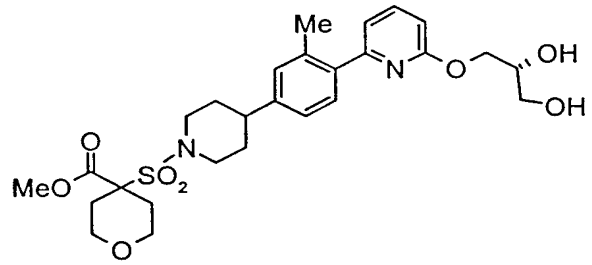


A solution of the dioxolane from preparation 37 (799mg, 1.36mmol) in 1,4-dioxan (10ml) was added to an ice-cooled solution of hydrochloric acid (30ml, 2N), and the reaction stirred for 75 minutes. The solution was poured into saturated sodium bicarbonate solution (200ml), and the resulting precipitate 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



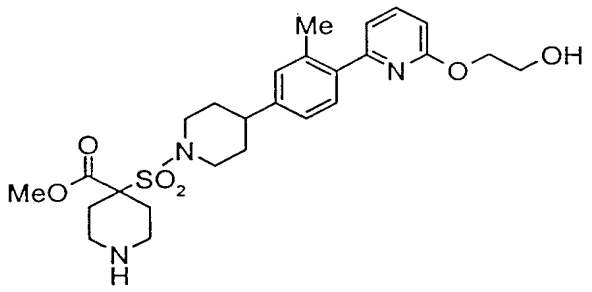
The title compound was obtained as a white crystalline solid (86%), from the dioxolane from preparation 38, following the procedure described in preparation 39.

^{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



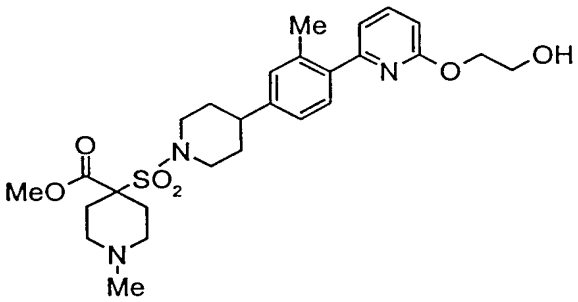
A mixture of the benzyl piperidine from preparation 33 (3.32g, 4.76mmol), ammonium formate (3.0g, 47.6mmol) and palladium hydroxide on carbon (3.32g) in a solution of acetic acid:methanol:tetrahydrofuran (2:2:1, 30ml) was heated under reflux for 2 hours. The cooled reaction was filtered through Arbocel®, 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

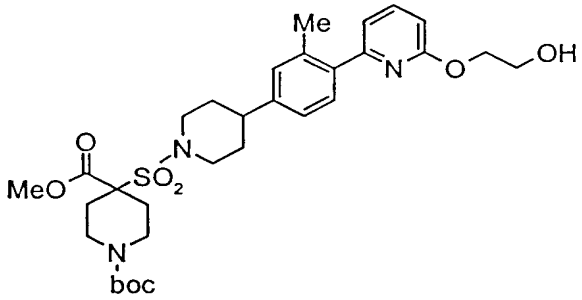
(Na_2SO_4), filtered and evaporated in vacuo. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to give the title compound (559mg, 86%).

^{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



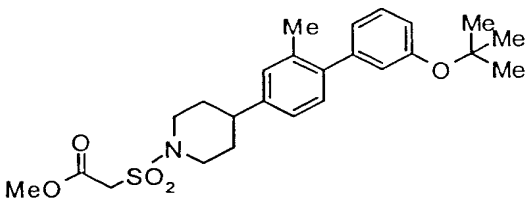
Triethylamine (175µl, 1.26mmol) was added to a solution of the amine from preparation 41 (594mg, 1.15mmol) in dichloromethane (100ml), followed by portionwise addition of di-tert-butyl dicarbonate (262mg, 1.20mmol). The reaction mixture was stirred at room temperature for an hour, then concentrated in 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



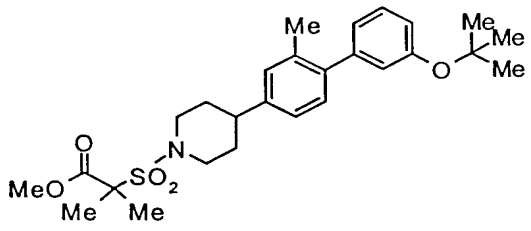
Nitrogen was bubbled through a mixture of cesium fluoride (3.71g, 24.44mmol), tri-o-tolyl phosphine (34mg, 0.11mmol), tris(dibenzylideneacetone)dipalladium (0) (50mg, 0.05mmol) the bromide from preparation 25 (4.27g, 11.0mmol) and the boronic acid from preparation 8 (3.2g, 16.5mmol) in anhydrous 1,2-dimethoxyethane (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



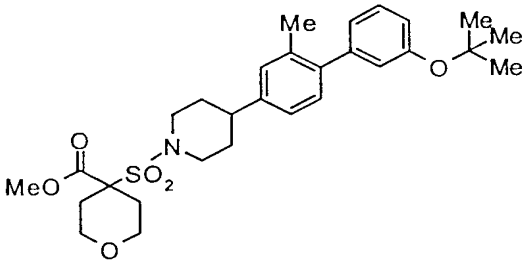
Potassium tert-butoxide (13.63ml, 1M in tetrahydrofuran, 13.63mmol) was added dropwise to a solution of the acetate from preparation 44 (2.5g, 5.45mmol) and methyl iodide (3.4ml, 54.5mmol) in tetrahydrofuran, and once addition was complete, the reaction was stirred at room temperature for 72 hours. The 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



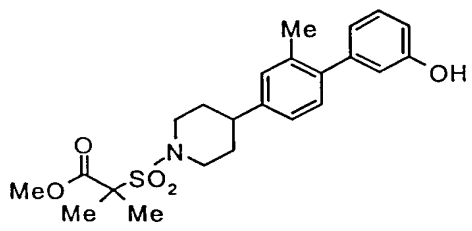
Nitrogen was bubbled through a mixture of cesium fluoride (2.19g, 14.43mmol), tri-o-tolyl phosphine (20mg, 0.065mmol), tris(dibenzylideneacetone)dipalladium (0) (30mg, 0.032mmol) and the bromide from preparation 27 (2.9g, 6.5mmol) and the boronic acid from preparation 8 (1.78g, 9.75mmol) in anhydrous 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%).

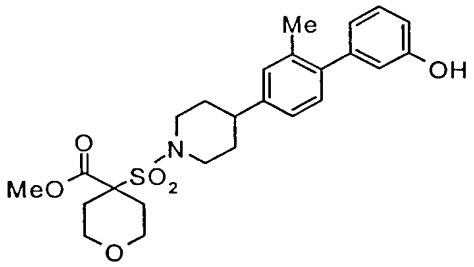
^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.62 (s, 6H), 1.76-1.92 (m, 4H), 2.22 (s, 3H), 2.62 (m, 1H), 3.04 (m, 2H), 3.78 (s, 3H), 3.95 (m, 2H), 6.78 (m, 2H), 6.83 (m, 1H), 7.03 (m, 2H), 7.15 (m, 1H), 7.21 (m, 1H).

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

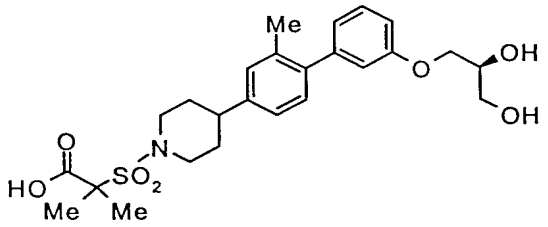
preparation 16 (425mg, 1.4mmol) in tetrahydrofuran (15ml) added dropwise.The reacton mixture was stirred at -78°C for 30 minutes, then allowed to warm to room temperature. Aqueous ammonium chloride was added, the mixture extracted with ethyl acetate (2x50ml) and the combined organic extracts were dried ($MgSO_4$), 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



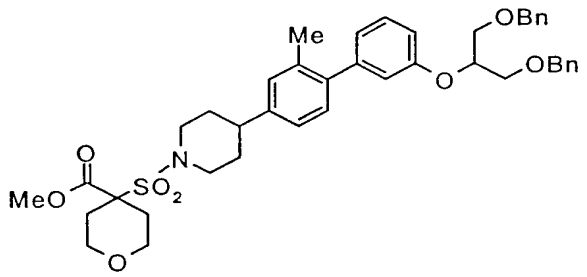
A mixture of the alcohol from preparation 47 (800mg, 1.86mmol), S-glycidol (0.12ml, 1.86mmol), and triethylamine (10μl, 0.09mmol) in methanol (10ml) was heated under reflux overnight. Tlc analysis showed starting material remaining, so the mixture was concentrated to low volume, and heated under reflux 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

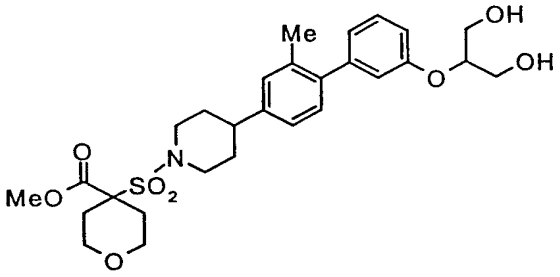


A mixture of the alcohol from preparation 48 (300mg, 0.63mmol), diethyl azodicarboxylate (150µl, 0.95mmol), triphenylphosphine (250mg, 0.95mmol), and 1,3-dibenzyloxy-2-propanol (260mg, 0.95mmol) in tetrahydrofuran (6ml), was stirred at room temperature for 3 hours. Tlc analysis showed some starting material 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



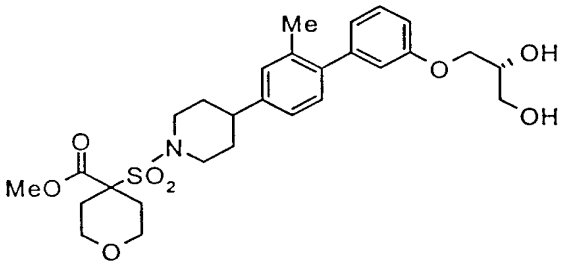
A mixture of the dibenzyl ether from preparation 50 (770mg, 1.06mmol), ammonium formate (1.4g, 11.0mmol) and palladium hydroxide on carbon (400mg) in methanol (40ml) was heated under reflux for 2 hours. Tlc analysis showed some starting material remaining, so additional palladium hydroxide (300mg) was 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



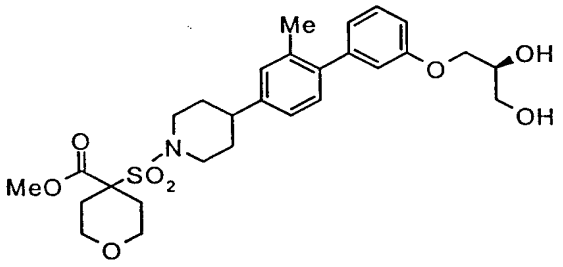
The title compound was obtained (17%) from the compound from preparation 48 and R-glycidol, following a similar procedure to that described in preparation 49.

^{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



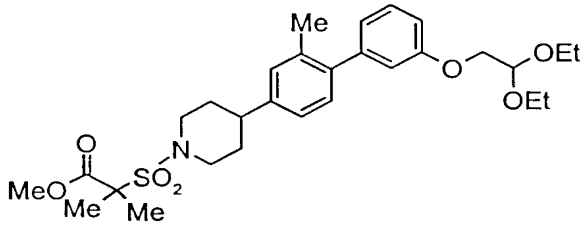
The title compound was obtained as a white solid (52%) after recrystallisation from di-isopropylether, from the alcohol of preparation 48 and S-glycidol, following a similar procedure to that described in preparation 49.

^{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



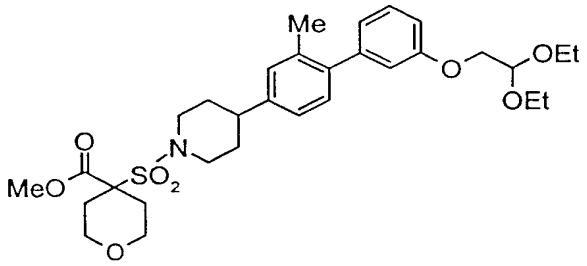
20% Palladium hydroxide on carbon (250mg) was added to a solution of the 1,2,3,6-tetrahydropyridine from preparation 34 (3.0g, 5.5mmol) and ammonium formate (1.04g, 16.5mmol) in methanol (70ml) and 1,4-dioxan (28ml), and the reaction was stirred at 60°C for 2 hours. Additional ammonium formate (1.0g, 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



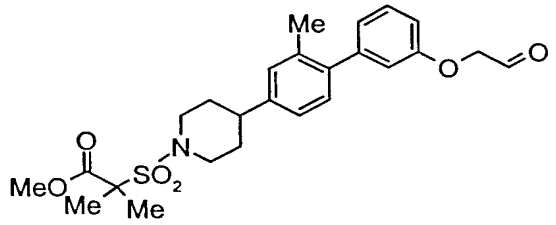
A mixture of cesium fluoride (4.3g, 28.3mmol), tri-o-tolyl phosphine (352mg, 1.15mmol), tris(dibenzylideneacetone)dipalladium (0) (535mg, 0.59mmol) and the boronic acid from preparation 10 (3.89g, 14.95mmol) and bromide from preparation 27 (5.0g, 10.86mmol) in anhydrous 1,2-dimethoxyethane (70ml), was 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



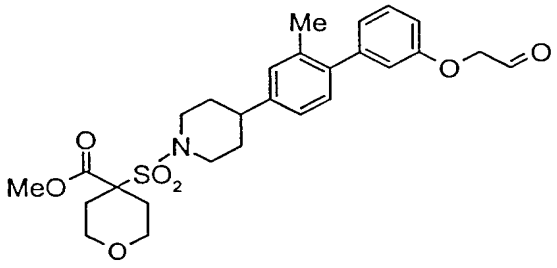
Hydrochloric acid (19ml, 1N, 19mmol) was added to a solution of the diethyl ketal from preparation 54 (4.43g, 8.1mmol) in acetone (19ml) and 1,4-dioxan (22ml), and the reaction stirred at 70°C for 2 hours. The cooled mixture was neutralised using sodium bicarbonate, concentrated in vacuo, and the residue 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



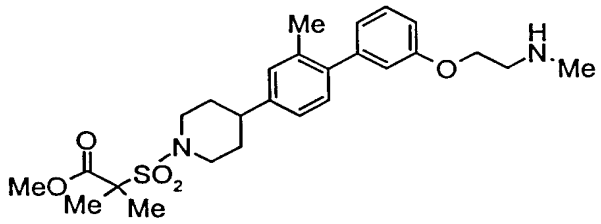
The title compound was obtained as a white foam (quantitative), from the diethyl ketal from preparation 55, following the procedure described in preparation 56.

^{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,

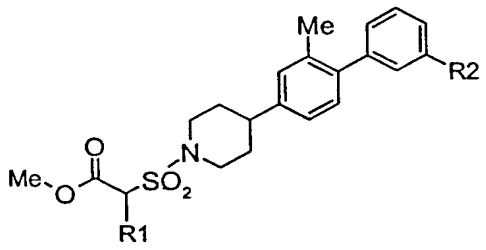
11.6mmol) in dichloromethane (50ml), and once addition was complete, the reaction was stirred at room temperature overnight. The reaction was partitioned between ethyl acetate and saturated sodium bicarbonate solution, and the layers separated, The organic phase was washed with water, brine, dried (Na_2SO_4), 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).

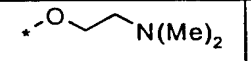
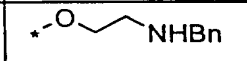
Anal. Found: C, 58.39; H, 6.90; N, 4.97. $C_{26}H_{36}N_2O_5S;0.75CH_2Cl_2$ requires C, 58.17; H, 6.84; N, 5.07%.

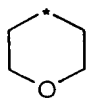
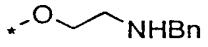
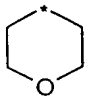
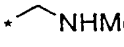
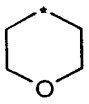
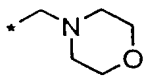
Preparations 59 to 63

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,

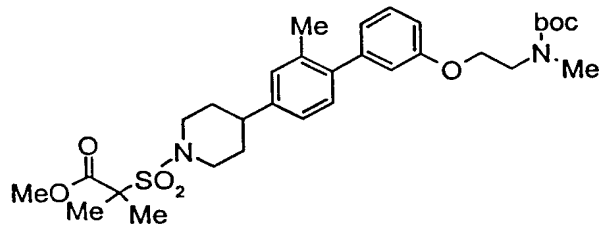
				1H), 6.85 (m, 3H), 7.07 (m, 2H), 7.17 (d, 1H), 7.24-7.38 (m, 6H). LRMS : m/z 565 $(M+1)^+$
61	57			^{1}H nmr ($CDCl_3$, 400MHz) ?: 1.75-1.92 (m, 4H), 2.20 (m, 5H), 2.40 (d, 2H), 2.62 (m, 1H), 3.00 (m, 4H), 3.28 (m, 2H), 3.88 (m, 5H), 3.99 (m, 2H), 4.09 (m, 2H), 4.40 (m, 2H), 5.60 (br s, 1H), 6.82 (m, 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%).

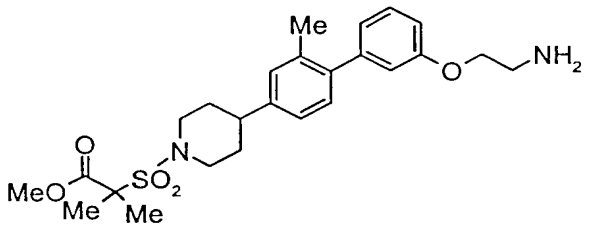
^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.42 (s, 9H), 1.62 (s, 6H), 1.77-1.90 (m, 4H), 2.22 (s, 3H), 2.63 (m, 1H), 2.97 (s, 3H), 3.03 (m, 2H), 3.58 (m, 2H), 3.78 (s, 3H), 3.95 (m, 2H), 4.08 (m, 2H), 6.82 (m, 3H), 7.04 (m, 2H), 7.16 (d, 1H), 7.25 (m, 1H).

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%).

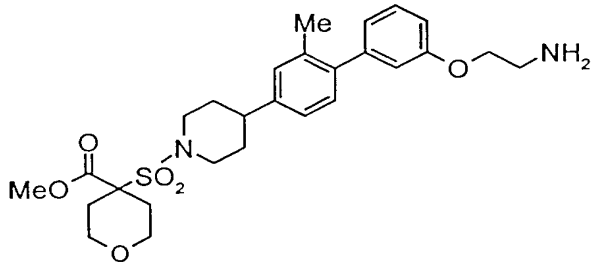
^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.66 (s, 6H), 1.78-1.97 (m, 4H), 2.28 (s, 3H), 2.66 (m, 1H), 3.10 (m, 4H), 3.82 (s, 3H), 3.99 (m, 4H), 6.88 (m, 3H), 7.10 (m, 2H), 7.19 (d, 1H), 7.30 (m, 1H).

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.

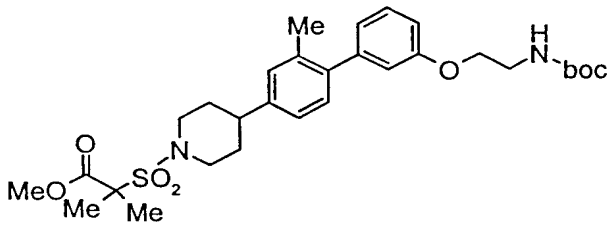
^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.76-1.92 (m, 4H), 2.20 (m, 5H), 2.40 (m, 2H), 2.62 (m, 1H), 3.04 (m, 4H), 3.30 (m, 2H), 3.88 (m, 5H), 3.98 (m, 4H), 6.82 (m, 3H), 7.03 (m, 2H), 7.16 (d, 1H), 7.22 (m, 1H).

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.

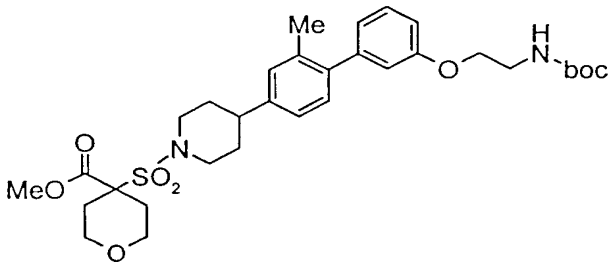
^{1}H nmr ($CDCl_3$, 300MHz) δ: 1.44 (s, 9H), 1.65 (s, 6H), 1.78-1.95 (m, 4H), 2.25 (s, 3H), 2.64 (m, 1H), 3.08 (m, 2H), 3.55 (m, 2H), 3.81 (s, 3H), 3.97 (m, 2H), 4.04 (t, 2H), 4.99 (br, s, 1H), 6.80-6.94 (m, 3H), 7.08 (m, 2H), 7.18 (d, 1H), 7.32 (m, 1H).

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%).

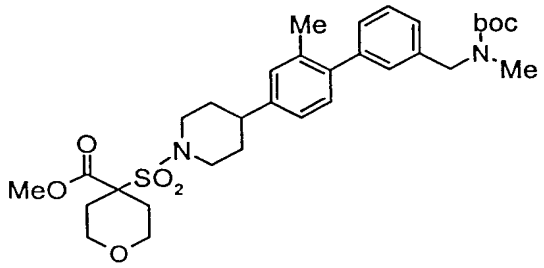
^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.40 (s, 9H), 1.78-1.92 (m, 4H), 2.20 (m, 5H), 2.40 (d, 2H), 2.61 (m, 1H), 3.02 (m, 2H), 3.30 (m, 2H), 3.50 (m, 2H), 3.88 (m, 5H), 4.00 (m, 4H), 4.86 (br s, 1H), 6.82 (m, 3H), 7.02 (m, 2H), 7.15 (d, 1H), 7.05 (m, 1H).

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.

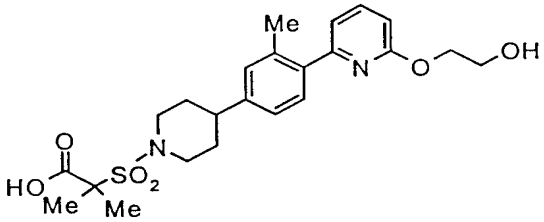
^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.42 (s, 9H), 1.75-1.92 (m, 4H), 2.20 (m, 5H), 2.40 (m, 2H), 2.61 (m, 1H), 2.82 (s, 3H), 3.03 (m, 2H), 3.30 (m, 2H), 3.85 (m, 5H), 3.99 (m, 2H), 4.42 (s, 2H), 7.03 (m, 2H), 7.17 (m, 4H), 7.35 (m, 1H).

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



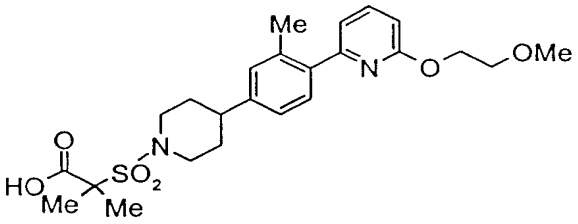
A mixture of the methyl ester from preparation 35 (4.1g, 8.6mmol) and aqueous sodium hydroxide (17ml, 1N, 17.0mmol) in methanol (50ml), was heated under reflux for 30 minutes, then cooled. The reaction was concentrated in vacuo, the residue dissolved in water (200ml), and the solution acidified to pH 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).

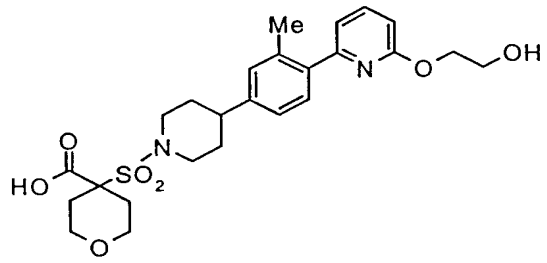
mp 142-146°C

^{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

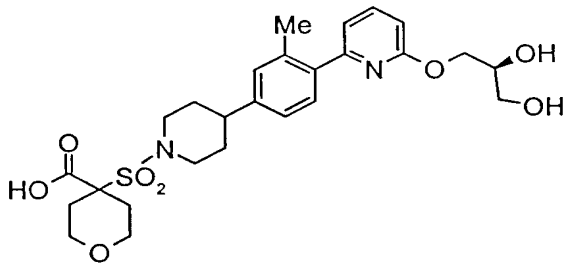
acetate (3x), the combined organic extracts washed with brine, dried ($MgSO_4$), filtered and evaporated in vacuo. The residual solid was recrystallised from ethyl acetate/di-isopropyl ether to afford the title compound as a solid, (517mg, 74%).

^{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



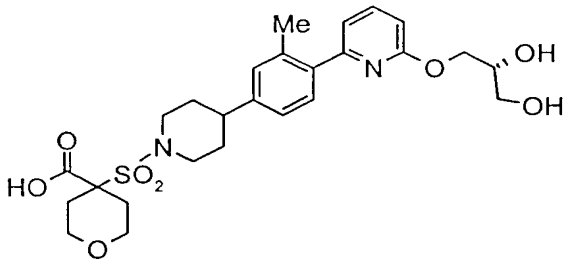
Aqueous sodium hydroxide (3.5ml, 1M, 3.5mmol) was added to a solution of the methyl ester from preparation 39 (640mg, 1.17mmol) in methanol (15ml) and 1,4-dioxan (15ml), and the reaction heated under reflux for 2 hours. Tlc analysis showed starting material remaining, so additonal sodium hydroxide (2ml, 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



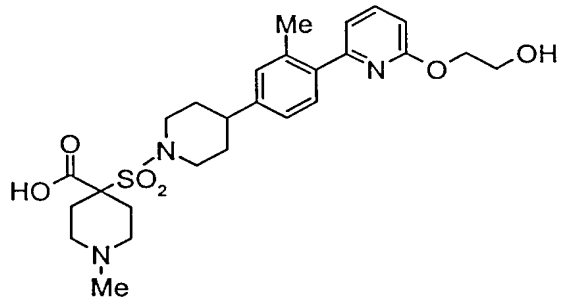
The title compound was obtained as a white solid (87%) from the methyl ester of preparation 40, following a similar procedure to that described in preparation 73.

^{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

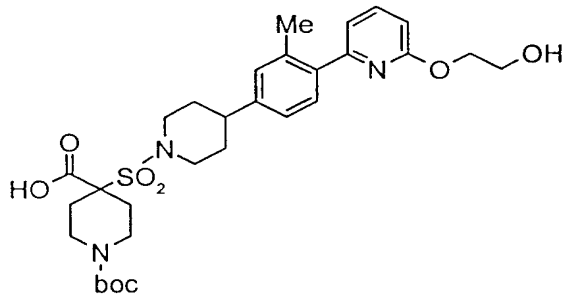


A mixture of the methyl ester from preparation 42 (200mg, 0.38mmol) and aqueous sodium hydroxide (1.5ml, 1N, 1.5mmol) in methanol (8ml) and 1,4-dioxan (8ml) was heated under reflux overnight. The cooled reaction was concentrated in vacuo, the residue acidified to pH 4 using acetic acid, and extraction 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



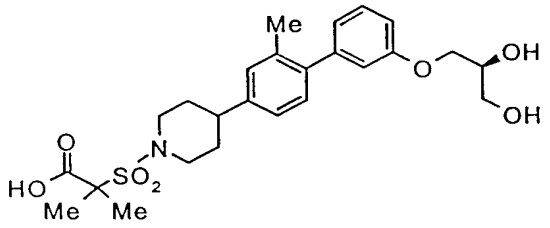
The title compound was obtained as a white solid (87%), from the methyl ester from preparation 43, following a similar procedure to that described in preparation 75.

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



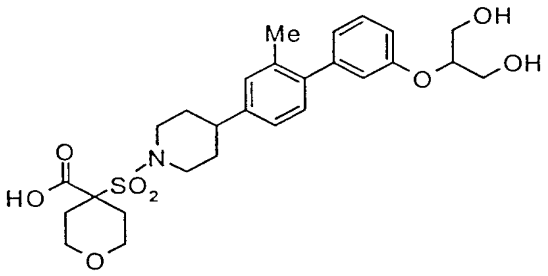
Aqueous sodium hydroxide (1.55ml, 1M, 1.55mmol) was added to a solution of the methyl ester from preparation 49 (391mg, 0.77mmol) in methanol (5ml), and the reaction stirred at room temperature overnight. The mixture was partitioned between ethyl acetate and hydrochloric acid (2N), and the phases separated. 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

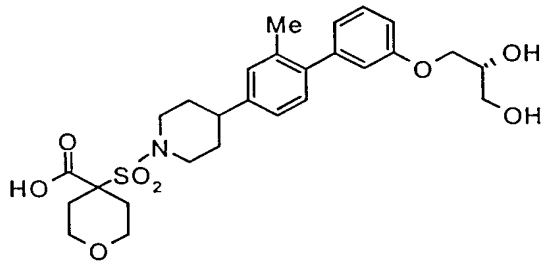


A mixture of the methyl ester from preparation 51 (370mg, 0.68mmol), aqueous sodium hydroxide (3ml, 1M, 3mmol) in methanol (5ml) and 1,4-dioxan (5ml), was heated under reflux for 6 hours. The cooled reaction was concentrated in vacuo, and then diluted with water. This aqueous solution was acidified to 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



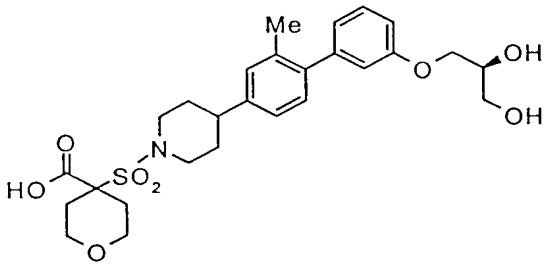
A mixture of the methyl ester from preparation 52 (110mg, 0.20mmol), aqueous sodium hydroxide (1ml, 1M, 1mmol) in methanol (5ml) and 1,4-dioxan (5ml) was heated under reflux for 2 hours. The cooled reaction was evaporated in vacuo, the residue dissolved in water and acidified to pH 1 using hydrochloric 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

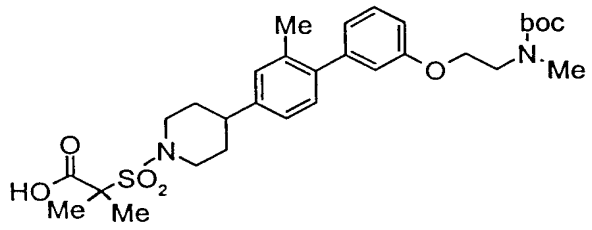


The title compound was obtained as a solid (66%) from the methyl ester from preparation 53, following the procedure described in preparation 79.

^{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%).

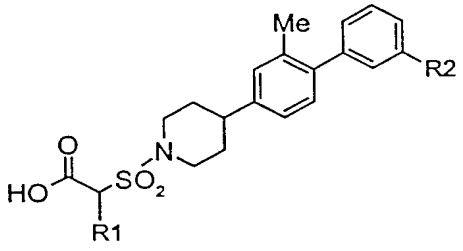
^{1}H nmr ($CDCl_3$, 400MHz) δ: 1.41 (s, 9H), 1.64 (s, 6H), 1.78-1.94 (m, 4H), 2.22 (s, 3H), 2.63 (m, 1H), 2.97 (s, 3H), 3.06 (m, 2H), 3.59 (m, 2H), 3.98 (m, 2H), 4.08 (t, 2H), 6.83 (m, 3H), 7.04 (m, 2H), 7.16 (d, 1H), 7.26 (m, 1H).

LRMS : m/z 597 $(M+23)^+$

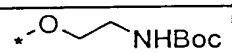
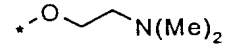
Anal. Found: C, 61.17; H, 7.27; N, 4.65. $C_{30}H_{42}N_2O_7S;0.2CH_2Cl_2$ requires C, 61.30; H, 7.22; N, 4.73%.

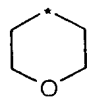
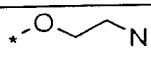
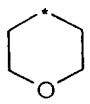
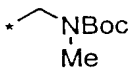
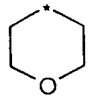
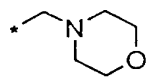
Preparations 82 to 86

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 $(M+1)^+$

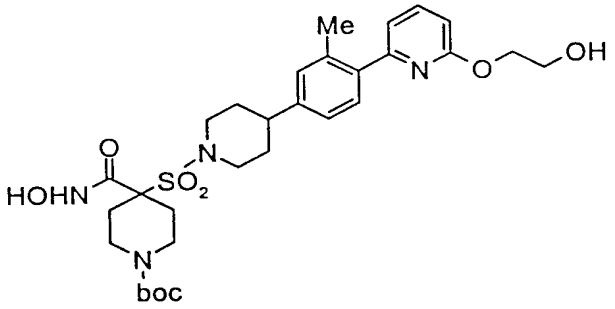
1 = isolated by filtration from aqueous acetic acid solution.

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.

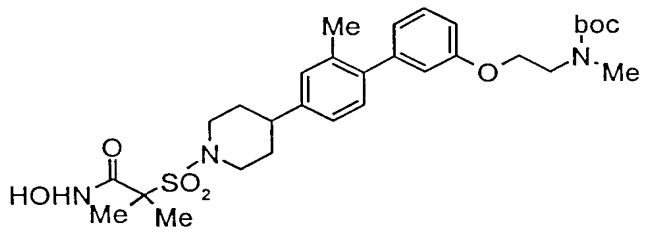
mp 180-181°C

^{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



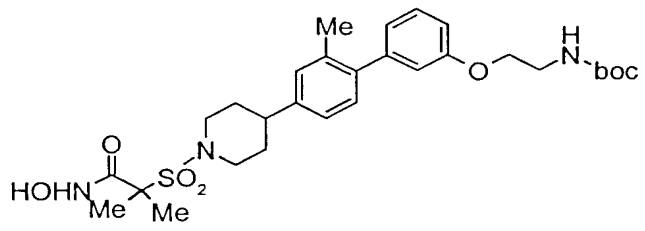
O-(7-Azabenzotriazol-1-yl)-N,N,N'N'-tetramethyluronium hexafluorophosphate (540mg, 1.42mmol) was added to a solution of the acid from preparation 81 (520mg, 0.90mmol) and N-ethyldiisopropylamine (193µl, 1.12mmol) in N-methylpyrrolidinone (10ml), and the reaction stirred at room temperature under a nitrogen 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.

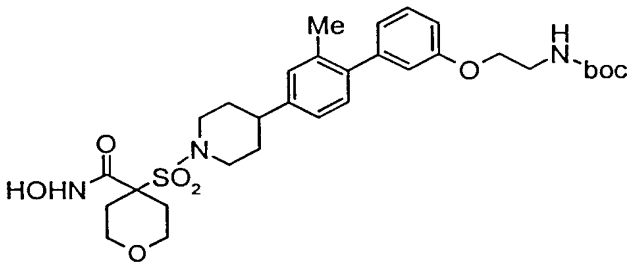
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.37 (s, 9H), 1.48 (s, 6H), 1.60 (m, 2H), 1.79 (m, 2H), 2.20 (s, 3H), 2.64 (m, 1H), 3.04 (m, 2H), 3.28 (m, 2H), 3.75 (m, 2H), 3.98 (t, 2H), 6.80-6.98 (m, 4H), 7.10 (s, 2H), 7.15 (s, 1H), 7.30 (dd, 1H), 8.99 (s, 1H), 10.55 (s, 1H).

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

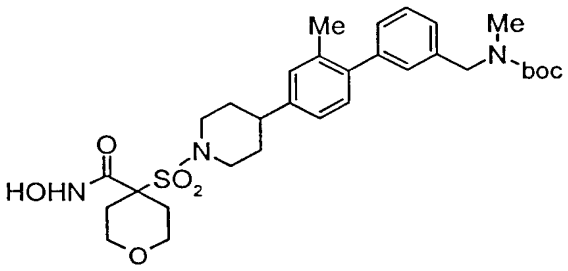
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.35 (s, 9H), 1.60 (m, 2H), 1.78 (m, 2H), 1.90 (m, 2H), 2.19 (s, 3H), 2.28 (m, 2H), 2.61 (m, 1H), 3.02 (m, 2H), 3.20 (m, 2H), 3.22 (m, 2H), 3.70 (m, 2H), 3.84 (m, 2H), 3.98 (t, 2H), 6.79-6.95 (m, 4H), 7.08 (s, 2H), 7.15 (s, 1H), 7.28 (m, 1H), 9.10 (s, 1H), 10.93 (s, 1H).

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



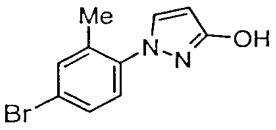
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (216mg, 1.12mmol) and 1-hydroxy-7-azabenzotriazole (128mg, 0.94mmol) were added to a solution of the acid from preparation 85 (550mg, 0.94mmol) in pyridine (2ml) and N,N dimethylformamide (6ml), and the mixture stirred at room temperature for 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

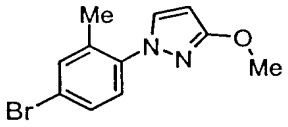
^{1}H nmr (DMSO-d_6, 400MHz) δ: 2.26 (s, 3H), 5.75 (s, 1H), 7.22 (d, 1H), 7.44 (d, 1H), 7.57 (s, 1H), 7.74 (s, 1H), 10.00 (s, 1H).

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

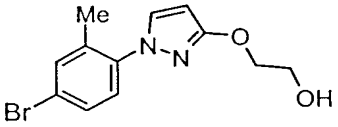
($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel using dichloromethane as the eluant, to give the desired product as a pale yellow oil, (970mg, 61%).

^{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%).

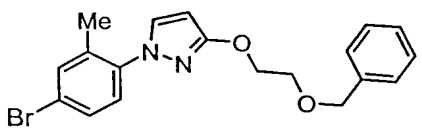
mp 104-105°C

^{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

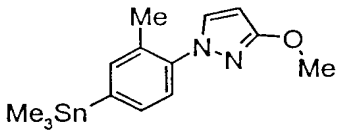
(681µl, 5.73mmol) was then added and the reaction heated under reflux for 16 hours. The cooled reaction mixture was poured into brine (70ml) and extracted with ethyl acetate (3x50ml). The combined organic solutions were dried ($MgSO_4$), filtered and concentrated in vacuo to give a yellow oil. The crude 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

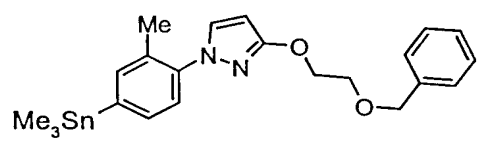


Tetrakis(triphenylphosphine)palladium (0) (30mg, 0.026mmol) was added to a solution of the bromide from preparation 93 (659mg, 2.47mmol), and hexamethylditin (889mg, 2.71mmol) in 1,4-dioxan (8ml), and nitrogen bubbled through the resulting mixture. The reaction was heated under reflux for 4 ½ hours, 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%).

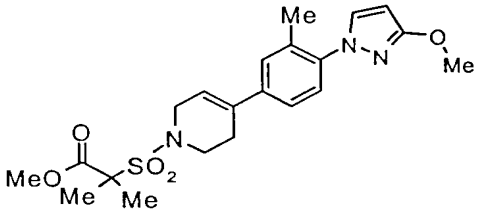
mp 50-52°C

^{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



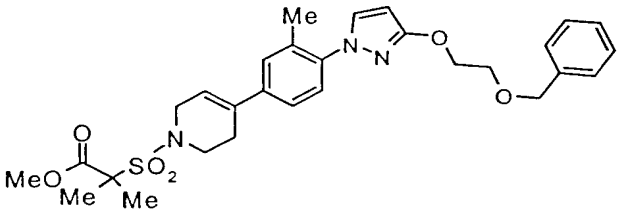
Tris(dibenzylideneacetone)dipalladium(0) (30.7mg, 0.034mmol) was added to a solution of the vinyl triflate from preparation 29 (727mg, 1.84mmol), the stannane from preparation 96 (590mg, 1.68mmol), and triphenylarsine (104mg, 0.36mmol) in 1-methyl-2-pyrrolidinone (4ml), and the solution stirred under 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



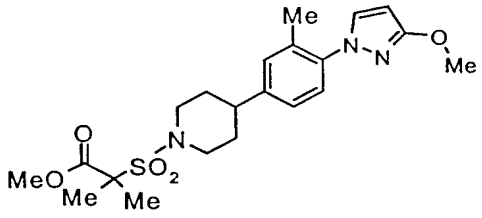
The title compound was obtained as a yellow oil (75%) from the triflate from preparation 29 and the stannane of preparation 97, using a similar method to that described in preparation 98.

^{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%).

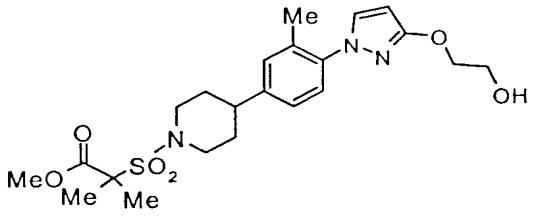
mp 109-110°C

^{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



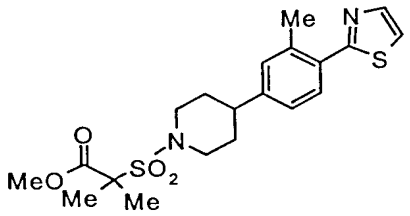
A mixture of the benzyl ether from preparation 99 (790mg, 1.42mmol) and 10% palladium on charcoal (160mg) in ethanol (35ml) was hydrogenated at 50 psi and room temperature for 17 hours. Tlc analysis showed starting material remaining, so acetic acid (2ml), and additional 10% palladium on charcoal (80mg) 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

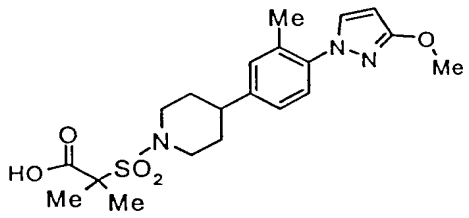
^{1}H nmr (DMSO-d_6, 400MHz) δ: 1.52 (s, 6H), 1.58 (m, 2H), 1.81 (m, 2H), 2.45 (s, 3H), 2.74 (m, 1H), 3.04 (m, 2H), 3.74 (m, 5H), 7.18 (d, 1H), 7.21 (s, 1H), 7.62 (d, 1H), 7.78 (d, 1H), 7.92 (d, 1H).

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



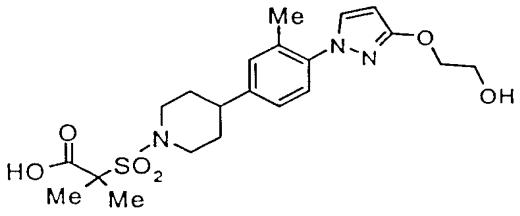
A mixture of the methyl ester from preparation 100 (355mg, 0.82mmol), and aqueous sodium hydroxide (5.9ml, 1M, 5.9mmol) in methanol (5ml) and 1,4-dioxan (5ml) was heated under reflux for 2 hours. The cooled reaction was diluted with water and acidified to pH 3 using hydrochloric acid (2N). The resulting 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



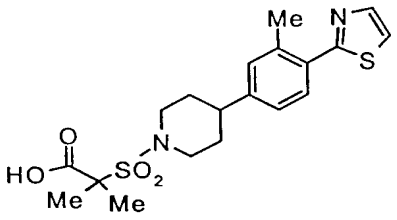
A mixture of the methyl ester from preparation 101(520mg, 1.2mmol), and aqueous sodium hydroxide (3.6ml, 1M, 3.6mmol) in 1,4-dioxan (5ml) was heated under reflux for 2 ½ hours. The cooled reaction was partitioned between water (100ml) and ethyl acetate (100ml), acidified to pH 2 using hydrochloric acid (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



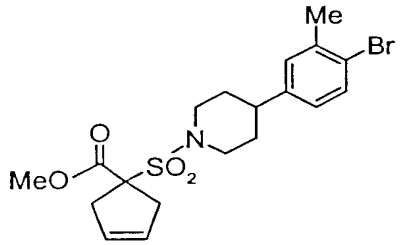
The title compound was obtained as a white solid (92%) from the methyl ester of preparation 102, following a similar procedure to that described in preparation 104.

^{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



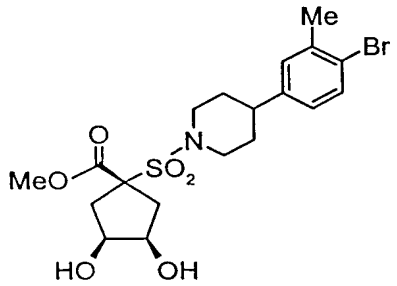
A suspension of sodium hydride (1.1g, 60% dispersion in mineral oil, 28mmol) was cooled to 0°C in anhydrous N-methyl pyrrolidinone (30ml) under nitrogen. A solution of the ester from preparation 25 (10g, 26mmol) in N-methyl pyrrolidinone (70ml) was added dropwise with stirring and the reaction mixture 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



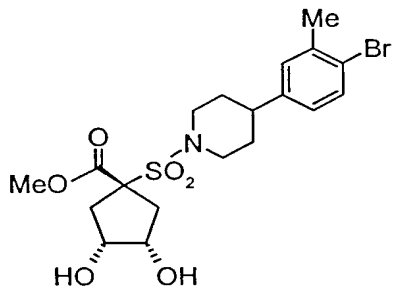
N-methylmorpholine N-oxide (580mg, 4.97mmol) and osmium tetroxide (2.5 weight % in tert-butanol, 1.1ml, 0.136mmol) were added to a solution of the cyclopentene from preparation 106 (2.0g, 4.52mmol) in dioxan (20ml), water (0.1ml), and the solution stirred at room temperature for 18 hours. The reaction 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



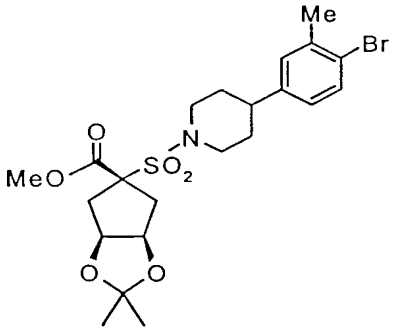
Silver acetate (2.1g, 12.46mmol) and iodine (1.5g, 5.81mmol) were added to a solution of the cyclopentene from preparation 106 (2.45g, 5.54mmol) in glacial acetic acid (125ml) and the mixture was stirred at ambient temperature for 1 hour. Wet acetic acid (2.5ml of a 1:25 water/glacial acetic acid mixture) 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



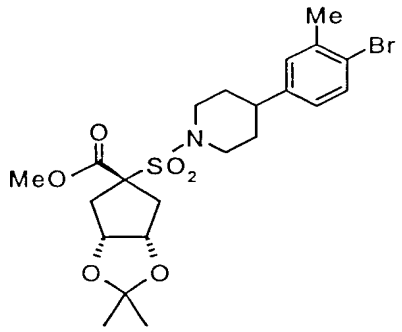
2,2-Dimethoxypropane (0.74ml, 6mmol) and p-toluenesulfonic acid (60mg, 0.3mmol) were added to a solution of the diol from preparation 107 (1.43g, 3mmol) in anhydrous dimethylformamide (10ml) under nitrogen. The reaction was warmed to 50°C for 4.5hours. The mixture was diluted with ethyl acetate (50ml) 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



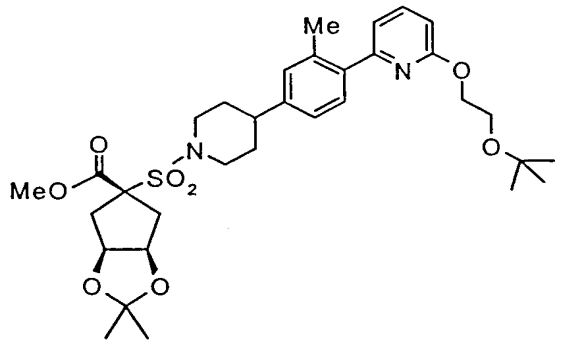
The title compound was prepared from the diol from preparation 108 in a similar procedure to that described in preparation 109. The title compound was isolated as a pale yellow solid (1.3g, 75%).

^{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

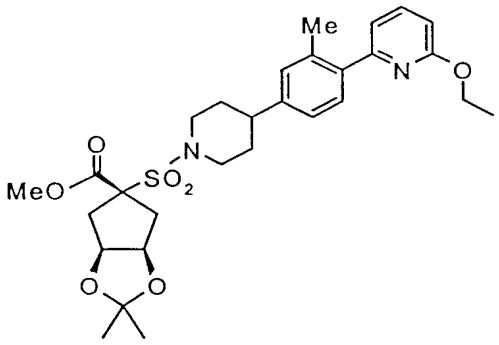
pentane:ethyl acetate (98:2 to 60:40) as eluant. The resulting solid was recrystalised from ethyl acetate to afford the title compound as a white solid, (1.4g, 60%).

^{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



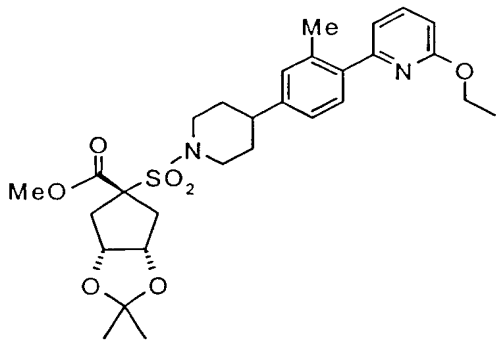
The title compound was prepared from the aryl bromide from preparation 109 and the stannane from preparation 129 in a similar procedure to that described in preparation 111. The title compound was isolated as a white solid (1.1g, 50%).

^{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



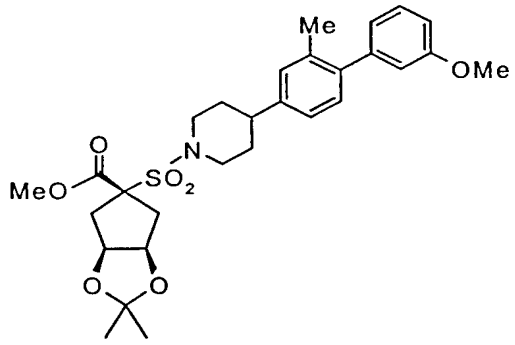
The title compound was prepared from the aryl bromide from preparation 110 and the stannane from preparation 129 in a similar procedure to that described in preparation 111. The title compound was isolated as a white foam (413mg, 60%).

^{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



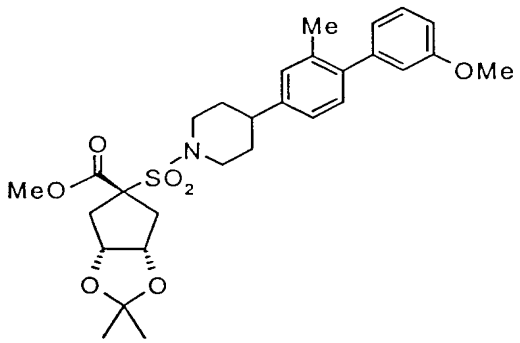
A mixture of the aryl bromide from preparation 109 (1.03, 1.99mmol), 3-methoxyphenylboronic acid (364mg, 2.40mmol), cesium fluoride (606mg, 4.00mmol), tris(dibenzylideneacetone)dipalladium(0) (91mg, 0.1mmol) and tri(o-tolyl)phosphine (61mg, 0.2mmol) in 1,2-dimethoxyethane (25ml) was heated under reflux 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



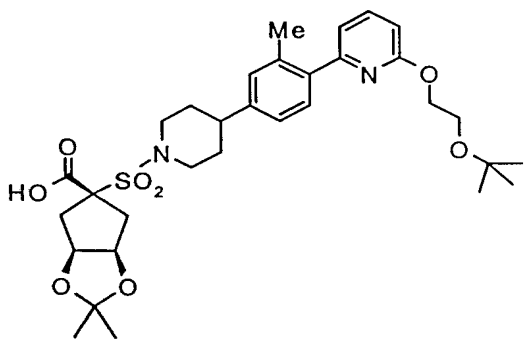
The title compound was prepared from the aryl bromide from preparation 110 in a similar procedure to that described in preparation 114 and was isolated as a white foam (310mg, 45%).

^{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



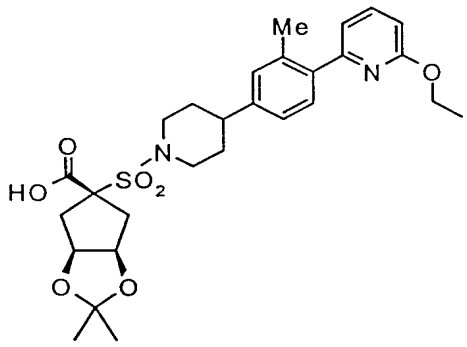
A mixture of the methyl ester from preparation 111 (1.4g, 2.22mmol) and aqueous sodium hydroxide (5.5ml, 2N, 11.1mmol) in methanol (7ml) and dioxan (7ml) was heated under reflux for 1hour, then allowed to cool. The reaction was concentrated in vacuo, the residue dissolved in water (20ml), and the solution 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



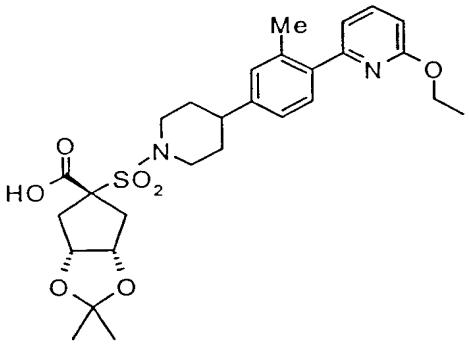
A mixture of the methyl ester from preparation 112 (780mg, 1.40mmol) and aqueous sodium hydroxide (3.5ml, 2N, 6.98mmol) were dissolved in methanol (5ml) and dioxan (5ml) and were heated under reflux for 1.5 hour, then allowed to cool. The reaction was concentrated in vacuo, the residue dissolved in water (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



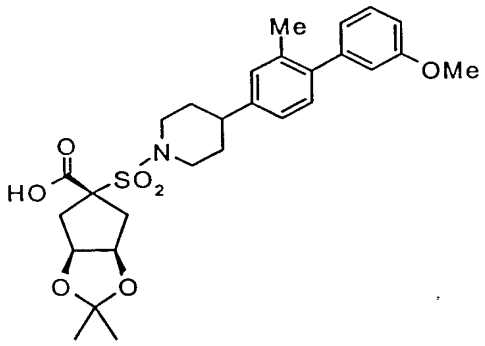
The title compound was prepared from the methyl ester from preparation 113 in a similar procedure to that described in preparation 117 and was isolated as a white foam (250mg, 65%).

^{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



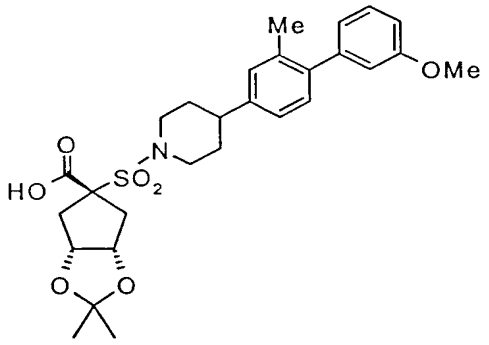
A mixture of the methyl ester from preparation 114 (630mg, 1.16mmol) and aqueous sodium hydroxide (3.0ml, 2N, 5.80mmol) were dissolved in methanol (5ml) and dioxan (5ml) and heated under reflux for 1hour, then allowed to cool. The reaction was concentrated in vacuo, the residue dissolved in water (20ml), 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



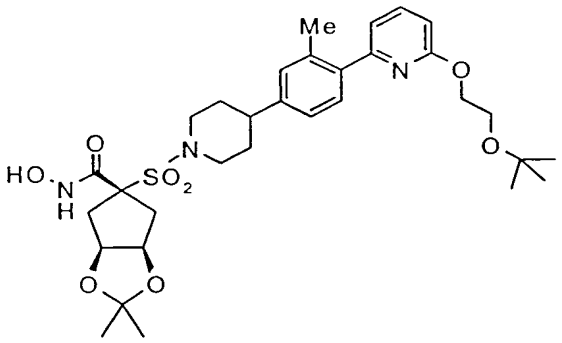
The title compound was prepared from the methyl ester from preparation 115 in a similar procedure to that described in preparation 119 and was isolated as a white foam (250mg, 85%).

^{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



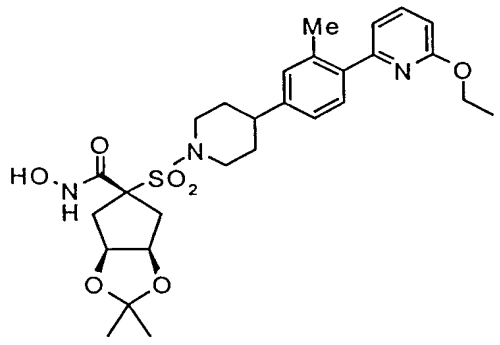
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (190mg, 0.973mmol) and 1-hydroxy-7-azabenzotriazole (121mg, 0.892mmol) were added to a solution of the acid from preparation 116 (500mg, 0.811mmol) in N,N-dimethylformamide (6ml) and pyridine (3ml) and the reaction was stirred under nitrogen 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



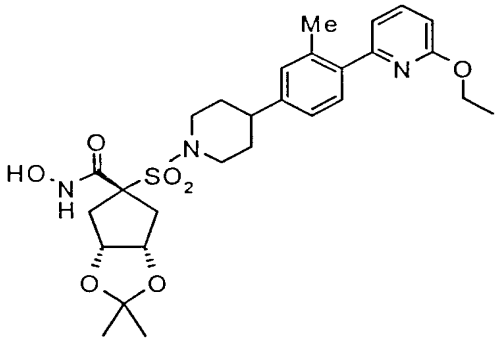
The title compound was prepared from the acid from preparation 117 in a similar procedure to that described in preparation 121, and was isolated as a white solid (150mg, 60%).

^{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



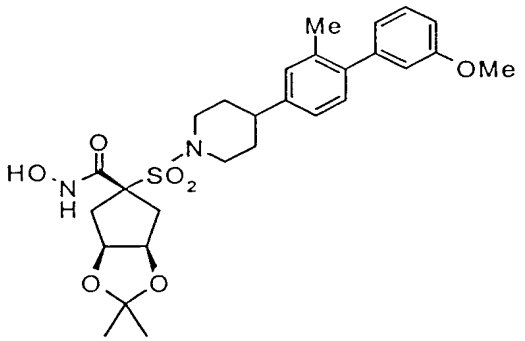
The title compound was prepared from the acid from preparation 118 in a similar procedure to that described in preparation 121. The title compound was isolated after column chromatography (using dichloromethane/methanol 99:1 as eluant) as a white solid (107mg, 45%).

^{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

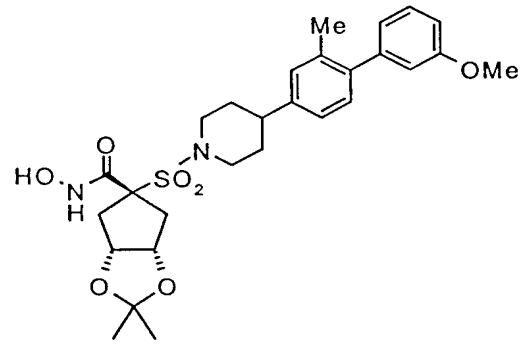


The title compound was prepared from the acid from preparation 119 in a similar procedure to that described in preparation 121, and was isolated as a white solid (110mg, 43%).

^{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



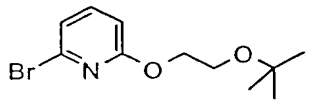
The title compound was prepared from the acid from preparation 120 in a similar procedure to that described in preparation 121. The title compound was isolated after column chromatography (using dichloromethane/methanol 98:2 as eluant) as a white solid (130mg, 50%).

^{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



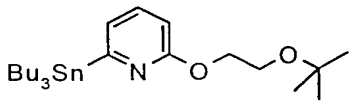
Sodium hydride (6.8g, 60% dispersion in mineral oil, 0.169mol) was added portionwise to an ice-cold solution of 2-(tert-butoxy)ethanol (20.0g, 0.169mol) in toluene (500ml) under nitrogen, and the solution stirred for 30 minutes whilst warming to ambient temperature. 2,6-Dibromopyridine (40.0, 0.169mol) 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



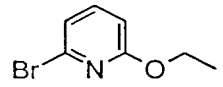
n-Butyllithium (71ml, 2.5M solution in hexanes, 0.177mol) was added dropwise to a cooled (-78°C) solution of the bromide from preparation 126 (46.3g, 0.169mol) in anhydrous THF (1000ml) under nitrogen, so as to maintain the internal temperature <-70°C, and the solution stirred for 10 minutes. Tri-n-butyltin 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



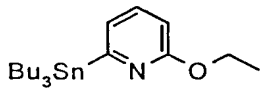
Sodium ethoxide (1.5g, 63mmol sodium, in ethanol (30ml)) was added to 2,6-dibromopyridine (15g, 63mmol) in toluene (150ml) at ambient temperature under nitrogen, and the reaction heated under reflux for 5 hours. The cooled mixture was diluted with water (100ml), and extracted with ethyl acetate (2x100ml). 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



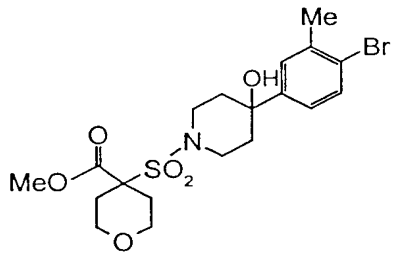
The title compound was prepared from the bromide from preparation 128 in a similar procedure to that described in preparation 127, and was isolated as a colourless oil (1.3g, 6%).

^{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

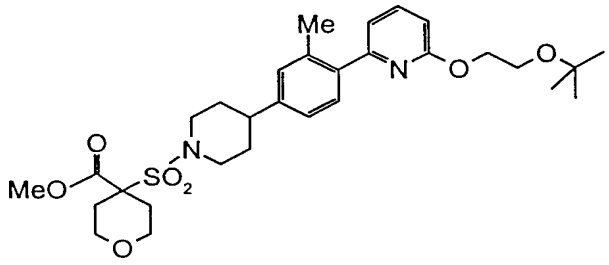


Iso-propylbromide (20ml, 0.21mol) was added dropwise over 1h to a stirred mixture of magnesium (4.7g, 0.19mol) in THF (50ml) and toluene (50ml), under nitrogen. The mixture was stirred at room temperature for 1 hour and then cooled to 0°C. A solution of 2-bromo-5-iodotoluene (57g, 0.19mol) in toluene (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

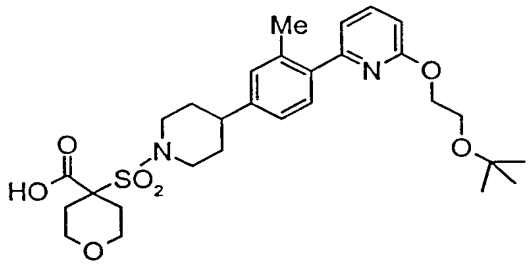
nitrogen. The mixture was stirred at –78°C for 10 min and then tri-*iso*-propylborate (1.9ml, 8.0mmol) was added dropwise over 10 min. The mixture was stirred at –78°C for 10 min and then allowed to warm to room temperature over 1 hour. The aryl bromide from preparation 27 (2.7g, 5.8mmol), palladium 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



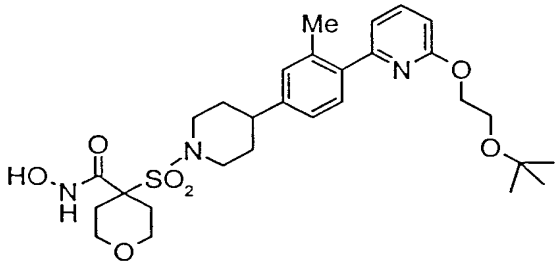
A mixture of the methyl ester from preparation 131 (9.1g, 16.0mmol) and aqueous sodium hydroxide (80ml, 1N, 80.0mmol) in dioxan (250ml) were heated under reflux for 2 hours. Methanol (100ml) and aqueous sodium hydroxide (40ml, 1N, 40.0mmol) were added and the mixture refluxed for a further 2 hours, then 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

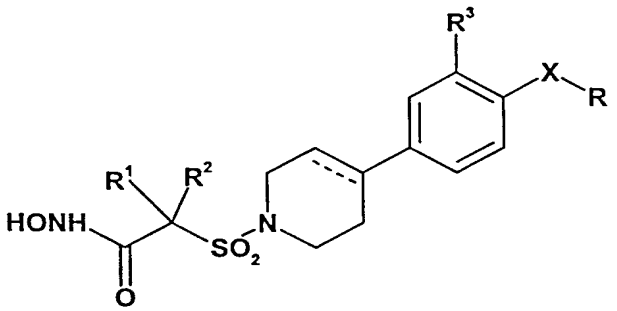
(1α,3β,4β)-*N*,3,4-trihydroxy-1-{4-[4-(3-methoxyphenyl)-3-methylphenyl]piperidin-1-ylsulfonyl}cyclopentanecarboxamide,

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;

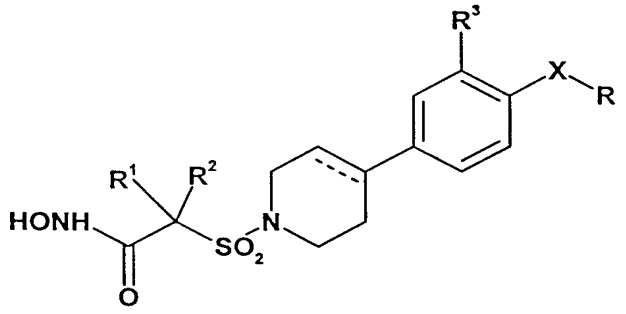
R^3 is H, halo, methyl, or methoxy;

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;

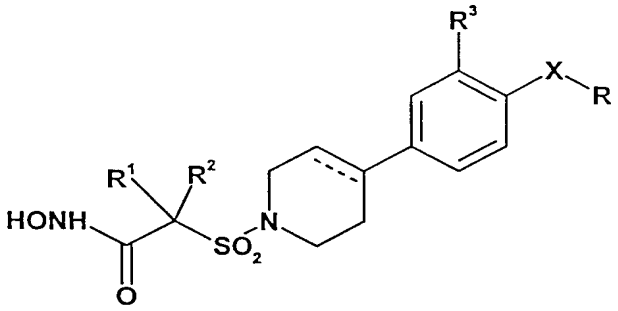
R^3 is H, halo, methyl, or methoxy;

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.

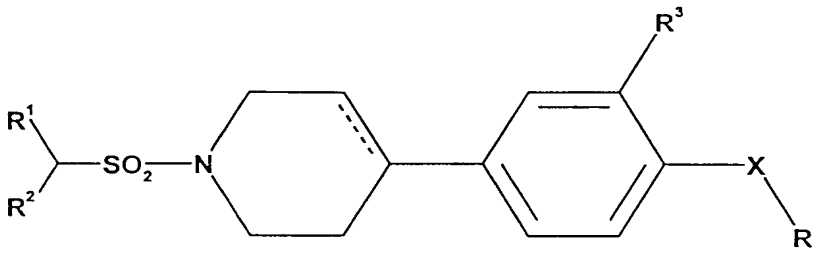
28. A compound selected from:
N-hydroxy 1-(tert-butoxycarbonyl)-4-{[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-yl]sulphonyl}-piperidine-4-carboxamide;
1-(tert-butoxycarbonyl)- 4-[4-(4-{6-[2-hydroxyethoxy]pyridin-2-yl}-3-methylphenyl)piperidin-1-ylsulphonyl]-piperidine-4-carboxylic 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.

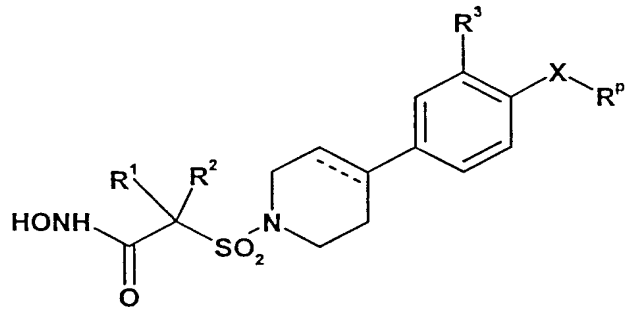
31. A compound of formula (VI):



(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):

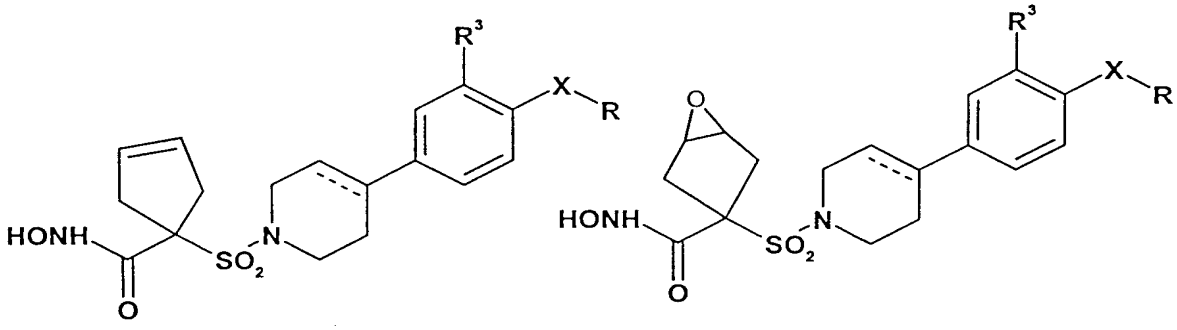


(VII)

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.

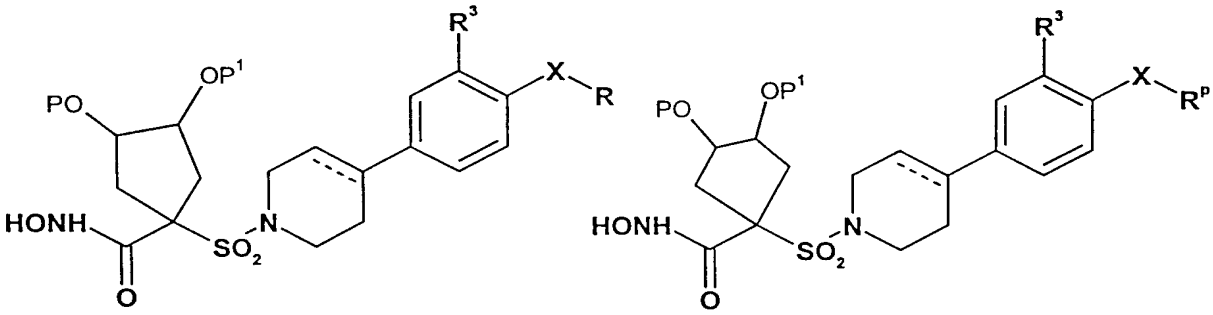
34. A compound of formula (VIII) or (IX):



(VIII) (IX)

where R^3, X and R are as defined in any one of claims 5, 6 or 7.

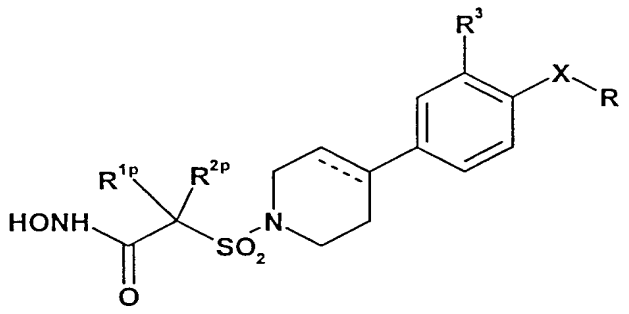
35. A compound of formula (X) or (XI) :



(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):

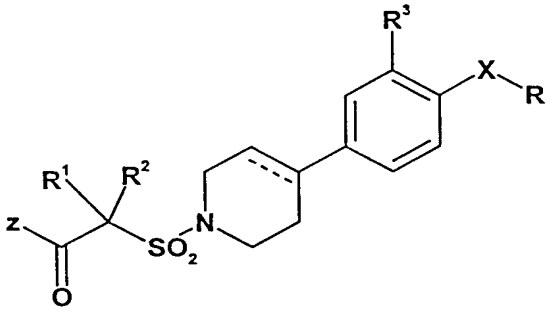


(XII)

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) :

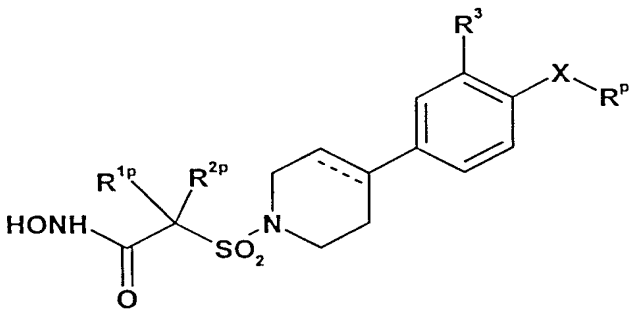


(II)

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
A61P9/00

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 7 C07D A61K A61P

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

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 29667 A (WHITLOCK GAVIN ALISTAIR ;DACK KEVIN NEIL (GB); PFIZER LTD (GB); PF) 17 June 1999 (1999-06-17) cited in the application examples	1-7
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.

° 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
4 August 2000	16/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	De Jong, B

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INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/IB 00/00667

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9929667 A	17-06-1999	AU 1230199 A	28-06-1999
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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 May 2005 (19.05.2005)

PCT

(10) International Publication Number
WO 2005/044234 A2

(51) International Patent Classification[7]: A61K 9/14

(21) International Application Number:
PCT/US2004/036337

(22) International Filing Date:
2 November 2004 (02.11.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/517,106 5 November 2003 (05.11.2003) US

(71) Applicant *(for all designated States except US)*: **ELAN PHARMA INTERNATIONAL, LTD.** [IE/IE]; WIL House, Shannon Business Park, Shannon, County Clare (IE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CUNNINGHAM, James** [US/US]; 903 Charleston Greene, Malvern, PA 19355 (US). **LIVERSIDGE, Elaine, Merisko** [US/US]; 258 Colwyn Terrace, West Chester, PA 19380 (US).

(74) Agents: **SIMKIN, Michele, M.** et al.; Foley & Lardner LLP, Washington Harbour, 3000 K Street, N.W. Suite 500, Washington, DC 20007-5101 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ,*

[Continued on next page]

(54) Title: NANOPARTICULATE COMPOSITIONS HAVING A PEPTIDE AS A SURFACE STABILIZER

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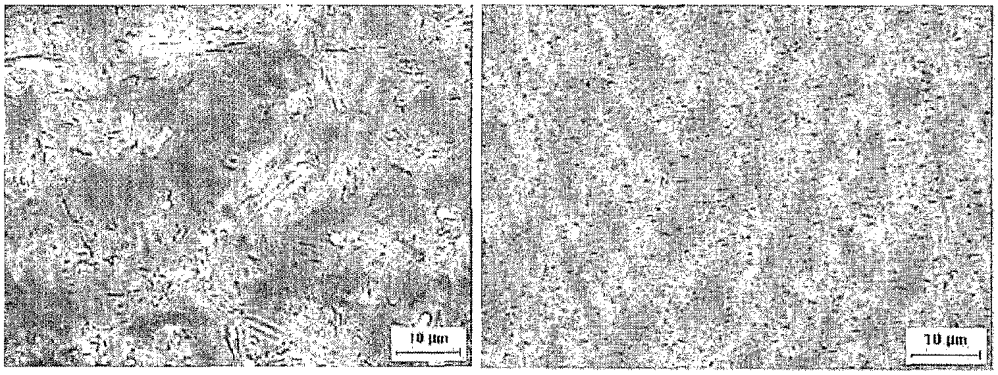


(57) Abstract: The present invention is directed to nanoparticulate active agent compositions comprising at least one peptide as a surface stabilizer. Also encompassed by the invention are pharmaceutical compositions comprising a nanoparticulate active agent composition of the invention and methods of making and using such nanoparticulate and pharmaceutical compositions.

WO 2005/044234 A2

CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

Published:

— *without international search report and to be republished upon receipt of that report*

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.

NANOPARTICULATE COMPOSITIONS HAVING A PEPTIDE AS A SURFACE STABILIZER

FIELD OF THE INVENTION

The present invention is directed to nanoparticulate active agent compositions having a peptide adsorbed onto or associated with the surface of the active agent as a surface stabilizer, and methods of making and using such compositions.

BACKGROUND OF THE INVENTION

Nanoparticulate active agent compositions, first described in U.S. Patent 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 describes the use of a variety of surface stabilizers for nanoparticulate compositions. The use of a peptide as a surface stabilizer for nanoparticulate active agent compositions is not described by the '684 patent.

The '684 patent describes a method of screening active agents to identify useful surface stabilizers that enable the production of a nanoparticulate composition. Not all surface stabilizers will function to produce a stable, non-agglomerated nanoparticulate composition for all active agents. Moreover, known surface stabilizers may be unable to produce a stable, non-agglomerated nanoparticulate composition for certain active agents. Thus, there is a need in the art to identify new surface stabilizers useful in making nanoparticulate active agent compositions. Additionally, such new surface stabilizers may have superior properties over prior known surface stabilizers.

Methods of making nanoparticulate active agent 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."

Nanoparticulate active agent 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;" 5,318,767 for "X-Ray Contrast Compositions Useful in Medical Imaging;" 5,326,552 for "Novel Formulation For Nanoparticulate X-Ray Blood Pool Contrast Agents Using High Molecular Weight Non-ionic Surfactants;" 5,328,404 for "Method of X-Ray Imaging Using Iodinated Aromatic Propanedioates;" 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;" 5,349,957 for "Preparation and Magnetic Properties of Very Small Magnetic-Dextran Particles;" 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;" 5,401,492 for "Water Insoluble Non-Magnetic Manganese Particles as Magnetic Resonance Enhancement Agents;" 5,429,824 for "Use of Tyloxapol as a Nanoparticulate Stabilizer;" 5,447,710 for "Method for Making Nanoparticulate X-Ray Blood Pool Contrast Agents Using High Molecular Weight Non-ionic Surfactants;" 5,451,393 for "X-Ray Contrast Compositions Useful in Medical Imaging;" 5,466,440 for "Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents in Combination with Pharmaceutically Acceptable Clays;" 5,470,583 for "Method of Preparing Nanoparticle Compositions Containing Charged Phospholipids to Reduce Aggregation;" 5,472,683 for "Nanoparticulate Diagnostic Mixed Carbamic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,500,204 for "Nanoparticulate Diagnostic Dimers as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,518,738 for "Nanoparticulate NSAID Formulations;" 5,521,218 for "Nanoparticulate Iododipamide Derivatives for Use as X-Ray Contrast Agents;" 5,525,328 for "Nanoparticulate Diagnostic Diatrizoxy Ester X-Ray Contrast Agents for Blood Pool

and Lymphatic System Imaging;" 5,543,133 for "Process of Preparing X-Ray Contrast Compositions Containing Nanoparticles;" 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;" 5,573,749 for "Nanoparticulate Diagnostic Mixed Carboxylic Anydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,573,750 for "Diagnostic Imaging X-Ray Contrast Agents;" 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;" 5,593,657 for "Novel Barium Salt Formulations Stabilized by Non-ionic and Anionic Stabilizers;" 5,622,938 for "Sugar Based Surfactant for Nanocrystals;" 5,628,981 for "Improved Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents and Oral Gastrointestinal Therapeutic Agents;" 5,643,552 for "Nanoparticulate Diagnostic Mixed Carbonic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,718,388 for "Continuous Method of Grinding Pharmaceutical Substances;" 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," Patent No. 6,582,285 for "Apparatus for Sanitary Wet Milling;" 6,592,903 for "Nanoparticulate Dispersions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate," 6,742,734 for "System and Method for Milling Materials," and 6,745,962 for "Small Scale Mill and Method Thereof," 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 active agent compositions comprising a peptide surface stabilizer.

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."

There is a need in the art for new surface stabilizers useful in preparing nanoparticulate active agent compositions. The present invention satisfies this need.

SUMMARY OF THE INVENTION

The present invention is directed to nanoparticulate compositions comprising at least one active agent and at least one peptide as a surface stabilizer adsorbed on to, or associated with, the surface of the active agent.

Another aspect of the invention is directed to pharmaceutical compositions comprising a nanoparticulate active agent composition of the invention. The pharmaceutical compositions preferably comprise at least one active agent, at least one peptide, and a pharmaceutically acceptable carrier, as well as any desired excipients.

In yet another embodiment, the invention is directed to bioadhesive nanoparticulate active agent compositions comprising at least one cationic peptide as a surface stabilizer, or at least one non-cationic peptide surface stabilizer in combination with at least one secondary cationic surface stabilizer. Such compositions can coat the gut, or the desired site of application, and be retained for a period of time, thereby increasing the efficacy of the active agent as well as eliminating or decreasing the frequency of dosing.

This invention further discloses a method of making a nanoparticulate active agent composition having a peptide surface stabilizer adsorbed on or associated with the surface of the active agent. Such a method comprises contacting an active agent with at least one peptide for a time and under conditions sufficient to provide a Nanoparticle active agent/peptide composition. The peptide surface stabilizer can be contacted with the active agent either before, preferably during, or after size reduction of the active agent.

The present invention is further directed to a method of treatment comprising administering to a mammal a therapeutically effective amount of a nanoparticulate active agent/peptide composition according to the invention.

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.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1: Shows representative photomicrographs of nystatin crystals before (Fig. 1A) and after (Fig. 1B) milling;

FIGURE 2: Shows the results of monitoring the particle size stability over time at 5°C (solid line), 25°C (dashed line), and 40°C (dotted line) for a nanoparticulate nystatin composition comprising the peptide poly(Lysine, Tryptophan) 4:1 hydrobromide as a surface stabilizer; and

FIGURE 3: Shows representative micrographs of cells with anionic particles (Fig. 3A) and cationic particles (Fig. 3B).

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to compositions comprising nanoparticulate active agents having at least one peptide as a surface stabilizer adsorbed on or associated with the surface thereof, and methods of making and using such nanoparticulate compositions.

As taught in the '684 patent, not every combination of surface stabilizer and active agent will result in a stable nanoparticulate composition. The discovery of the present invention is surprising in that peptides are biological compounds having secondary and tertiary structures which are critical to the activity of the peptide. It was surprising that such a compound could be successfully used to stabilize a

nanoparticulate active agent. Moreover, it was even more surprising that milling of a peptide surface stabilizer did not change the activity or function of the peptide.

A "peptide" is defined as any compound consisting of two or more amino acids where the alpha carboxyl group of one is bound to the alpha amino group of another. A polypeptide is a long peptide chain. A protein is a large macromolecule composed of one or more polypeptide chains. In the context of the present invention, "peptide" refers to a peptide or a polypeptide, but not a protein.

A striking characteristic of peptides is that they have well-defined three dimensional structures. Peptides fold into compact structures with nominal bond lengths. The strong tendency of hydrophobic amino acid residues to flee from water drives the folding of soluble peptides.

A stretched-out or randomly arranged polypeptide chain is devoid of biological activity. This is because the function of a peptide arises from conformation, which is the three dimensional arrangement of atoms in a structure. *See e.g.*, L. Stryer, *Biochemistry,* 3rd Edition, p. 1-41 (W.H. Freeman & Co., NY, 1988). Amino acid sequences are important because they specify the conformation of peptides. *Id.*

Peptides have several different defined structures, including a primary, secondary, and tertiary structure. The primary structure of a peptide is generally the amino acid sequence of the peptide and the location of disulfides. *See e.g.*, L. Stryer, *Biochemistry,* 3rd Edition, p. 31 (W.H. Freeman & Co., NY, 1988). Secondary structure refers to the spatial arrangement of amino acid residues that are near one another in the linear sequence. Examples of these steric relationships are structures known as an alpha helix, a beta pleated sheet, and a collagen helix. *Id.* Tertiary structure refers to the spatial arrangement of amino acid residues in a peptide or polypeptide that are far apart in the linear sequence.

Proteins, comprising multiple polypeptide chains, also have a quaternary structure, which refers to the spatial arrangement of the polypeptide subunits and the nature of their contacts. *Id.*

It was very surprising that such complex compounds as peptides and polypeptides could be successfully utilized as a surface stabilizer for a nanoparticulate active agent. In addition to enabling the use of a new class of surface stabilizers for nanoparticulate active agents, this discovery is significant as the peptide surface stabilizer in the compositions of the invention may also have therapeutic or diagnostic properties. This is in contrast to prior art nanoparticulate active agent compositions, in which the surface stabilizer is generally a surfactant, which lacks such therapeutic or diagnostic properties.

The nanoparticulate active agent compositions of the invention may also offer the following advantages as compared to prior conventional or non-nanoparticulate active agent compositions: (1) faster onset of action; (2) a potential decrease in the frequency of dosing; (3) smaller doses of active agent required to obtain the same pharmacological effect; (4) increased bioavailability; (5) an increased rate of dissolution; (6) improved performance characteristics for oral, intravenous, subcutaneous, or intramuscular injection, such as higher active agent dose loading and smaller tablet or liquid dose volumes; (7) improved pharmacokinetic profiles, such as improved T_{max}, C_{max}, and AUC profiles; (8) substantially similar or bioequivalent pharmacokinetic profiles of the nanoparticulate active agent compositions when administered in the fed versus the fasted state; (9) bioadhesive active agent compositions, which can coat the gut or the desired site of application and be retained for a period of time, thereby increasing the efficacy of the active agent as well as eliminating or decreasing the frequency of dosing; (10) high redispersibility of the nanoparticulate active agent particles present in the compositions of the invention following administration; (11) the nanoparticulate active agent compositions can be formulated in a dried form which readily redisperses; (12) low viscosity liquid nanoparticulate active agent dosage forms can be made; (13) for liquid nanoparticulate active agent compositions having a low viscosity - better subject compliance due to the perception of a lighter formulation which is easier to consume and digest; (14) for liquid nanoparticulate active agent compositions having a low viscosity - ease of dispensing because one can use a cup or a syringe; (15) the nanoparticulate active

agent compositions can be used in conjunction with other active agents; (16) the nanoparticulate active agent compositions can be sterile filtered; (17) the nanoparticulate active agent compositions are suitable for parenteral administration; and (18) the nanoparticulate active agent compositions do not require organic solvents or pH extremes.

A preferred dosage form of the invention is a solid dosage form, although any pharmaceutically acceptable dosage form can be utilized. Exemplary dosage forms include, but are not limited to, tablets, capsules, sachets, lozenges, powders, pills, granules, liquid dispersions, oral suspensions, gels, aerosols (including nasal and pulmonary), ointments, and creams.

The dosage form of the invention can be, for example, a fast melt dosage form, controlled release dosage form, lyophilized 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.

In addition, the compositions of the invention can be formulated for any suitable administration route, such as oral, pulmonary, rectal, opthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, or topical administration.

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 2 microns. Nanoparticulate active agents as defined herein have an effective average particle size of less than about 2 microns.

"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" as used herein refers to derivatives wherein the parent compound 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 quaternary ammonium salts of the parent compound 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.

"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. Such drugs tend to be eliminated from the gastrointestinal tract before being absorbed into the circulation.

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 active agent 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 active agent particles is not altered over time, such as by conversion from an amorphous phase to crystalline phase; (3) that the active agent

particles are chemically stable; and/or (4) where the active agent has not been subject to a heating step at or above the melting point of the active agent in the preparation of the nanoparticles of the invention.

"Therapeutically effective amount" as used herein with respect to an active agent dosage, shall mean that dosage that provides the specific pharmacological response for which the active agent 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 active agent dosages are, in particular instances, measured as oral dosages, or with reference to active agent levels as measured in blood.

I. Preferred Characteristics of the Nanoparticulate Active Agent Compositions of the Invention

A. Increased Bioavailability, Frequency of Dosing, and Dosage Quantity

The nanoparticulate active agent compositions of the invention, having at least one peptide as a surface stabilizer, may preferably exhibit increased bioavailability and require smaller doses as compared to prior non-nanoparticulate compositions of the same active agent administered at the same dose.

Any active agent can have adverse side effects. Thus, lower doses of an active agent that can achieve the same or better therapeutic effects as those observed with larger doses of a non-nanoparticulate composition of the same active agent are desired. Such lower doses may be realized with the nanoparticulate active agent compositions of the invention because the nanoparticulate active agent compositions may exhibit greater bioavailability as compared to non-nanoparticulate compositions of the same active agent, which means that smaller doses of the active agent are likely required to obtain the desired therapeutic effect.

The nanoparticulate active agent compositions of the invention may be administered less frequently and at lower doses, as compared to conventional non-nanoparticulate compositions of the same active agent, in dosage forms such as liquid dispersions, powders, sprays, aerosols (pulmonary and nasal), solid re-dispersable dosage forms, gels, ointments, creams, *etc.* of the nanoparticulate active agent. Lower dosages can be used because the small particle size of the active agent particles ensure greater absorption, and in the case of bioadhesive nanoparticulate active agent compositions, the active agent is retained at the desired site of application for a longer period of time as compared to conventional, non-nanoparticulate active agent dosage forms.

In one embodiment of the invention, the therapeutically effective amount of the nanoparticulate active agent compositions is 1/6, 1/5, ¼, 1/3rd, or ½ of the therapeutically effective amount of a non-nanoparticulate composition of the same active agent.

Such lower doses are preferred as they may decrease or eliminate adverse effects of the active agent. In addition, such lower doses decrease the cost of the dosage form and may increase patient compliance.

B. Pharmacokinetic Profiles of the Nanoparticulate Active Agent Compositions of the Invention

The invention also preferably provides nanoparticulate active agent compositions, having at least one peptide as a surface stabilizer, and having a desirable pharmacokinetic profile when administered to mammalian subjects. The desirable pharmacokinetic profile of the active agent compositions preferably includes, but is not limited to: (1) a T_{max} for an active agent, when assayed in the plasma of a mammalian subject following administration, that is preferably less than the T_{max} for a non-nanoparticulate composition of the same active agent, administered at the same dosage; (2) a C_{max} for an active agent, when assayed in the plasma of a mammalian subject following administration, that is preferably greater than the C_{max} for a non-nanoparticulate composition of the same active agent, administered at the same dosage; and/or (3) an AUC for an active agent, when assayed in the plasma of a

mammalian subject following administration, that is preferably greater than the AUC for a non-nanoparticulate composition of the same active agent, administered at the same dosage.

The desirable pharmacokinetic profile, as used herein, is the pharmacokinetic profile measured after the initial dose of the active agent. The compositions can be formulated in any way as described herein and as known to those of skill in the art.

A preferred active agent composition of the invention, comprising at least one peptide as a surface stabilizer, exhibits in comparative pharmacokinetic testing with a non-nanoparticulate composition of the same active agent, administered at the same dosage, a T_{max} not greater than about 100%, 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 40%, 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 active agent composition. This shorter T_{max} translates into a faster onset of therapeutic activity.

A preferred active agent composition of the invention, comprising at least one peptide as a surface stabilizer, exhibits in comparative pharmacokinetic testing with a non-nanoparticulate composition of the same active agent, administered at the same dosage, a C_{max} which is at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 110%, at least about 120%, at least about 130%, at least about 140%, at least about 150%, at least about 160%, at least about 170%, at least about 180%, at least about 190%, or at least about 200% greater than the C_{max} exhibited by the non-nanoparticulate active agent composition.

A preferred active agent composition of the invention, comprising at least one peptide as a surface stabilizer, exhibits in comparative pharmacokinetic testing with a non-nanoparticulate composition of the same active agent, administered at the same

dosage, an AUC which is at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 110%, at least about 120%, at least about 130%, at least about 140%, at least about 150%, at least about 160%, at least about 170%, at least about 180%, at least about 190%, or at least about 200% greater than the AUC exhibited by the non-nanoparticulate active agent formulation.

Any formulation giving the desired pharmacokinetic profile is suitable for administration according to the present methods.

C. The Pharmacokinetic Profiles of the Nanoparticulate Active Agent Compositions of the Invention are Preferably not Substantially Affected by the Fed or Fasted State of the Subject Ingesting the Compositions

The invention encompasses nanoparticulate active agent compositions, comprising at least one peptide as a surface stabilizer, wherein preferably the pharmacokinetic profile of the active agent is not substantially affected by the fed or fasted state of a subject ingesting the composition. This means that there is no substantial difference in the quantity of active agent absorbed or the rate of active agent absorption when the nanoparticulate active agent compositions are administered in the fed versus the fasted state. Thus, the nanoparticulate active agent compositions of the invention can preferably substantially eliminate the effect of food on the pharmacokinetics of the active agent.

In another embodiment of the invention, the pharmacokinetic profile of the active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, when administered to a mammal in a fasted state, is bioequivalent to the pharmacokinetic profile of the same nanoparticulate active agent composition administered at the same dosage, when administered to a mammal in a fed state.

"Bioequivalency" is preferably established by a 90% Confidence Interval (CI) of between 0.80 and 1.25 for both C_{max} and AUC under U.S. Food and Drug Administration (USFDA) regulatory guidelines, or a 90% CI for AUC of between

0.80 to 1.25 and a 90% CI for C_{max} of between 0.70 to 1.43 under the European Medicines Evaluation Agency (EMEA) regulatory guidelines (T_{max} is not relevant for bioequivalency determinations under USFDA and EMEA regulatory guidelines).

Preferably the difference in AUC (*e.g.*, absorption) of the nanoparticulate active agent composition of the invention, comprising at least one peptide as a surface stabilizer, when administered in the fed versus the fasted state, is 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 addition, preferably the difference in C_{max} of the nanoparticulate active agent composition of the invention, comprising at least one peptide as a surface stabilizer, when administered in the fed versus the fasted state, is 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%.

Finally, preferably the difference in the T_{max} of the nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, when administered in the fed versus the fasted state, is 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 30%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 3%, or essentially no difference.

Benefits of a dosage form that 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.

D. Redispersibility Profiles of the Nanoparticulate Active Agent Compositions of the Invention

An additional feature of the nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, comprising at least one peptide as a surface stabilizer, is that the compositions redisperse such that the effective average particle size of the redispersed active agent particles is less than about 2 microns. This is significant, as if upon administration the nanoparticulate active agent particles present in the compositions of the invention did not redisperse to a substantially nanoparticulate particle size, then the dosage form may lose the benefits afforded by formulating the active agent into a nanoparticulate particle size.

This is because the nanoparticulate active agent compositions of the invention benefit from the small particle size of the active agent; if the nanoparticulate active agent particles do not redisperse into the small particle sizes upon administration, then "clumps" or agglomerated active agent particles are formed. With the formation of such agglomerated particles, the bioavailability of the dosage form may fall.

Moreover, the nanoparticulate active agent compositions of the invention exhibit dramatic redispersion of the active agent particles upon administration to a mammal, such as a human or animal, as demonstrated by reconstitution in a biorelevant aqueous media. 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 active agent 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 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 100 nm, less than about 75 nm, or less than about 50 nm, as measured by light-scattering methods, microscopy, or other appropriate methods.

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."

E. Bioadhesive Nanoparticulate Active Agent Compositions

Bioadhesive nanoparticulate active agent compositions of the invention comprise at least one cationic peptide surface stabilizer, or in addition to at least one non-cationic peptide as a surface stabilizer, at least one secondary non-peptide cationic surface stabilizer. Exemplary non-peptide cationic surface stabilizers are described in more detail below. Bioadhesive formulations of active agents exhibit exceptional bioadhesion to biological surfaces, such as mucous and skin.

Cationic surface stabilizers generally confer relatively large, positive zeta potentials to particles on which they adsorb or associate. To increase the bioadhesive properties of a nanoparticulate composition, two or more cationic surface stabilizers can be utilized.

In the case of bioadhesive nanoparticulate active agent compositions, the term "bioadhesion" is used to describe the adhesion between the nanoparticulate active agent compositions and a biological substrate (*i.e.*, gastrointestinal mucin, lung tissue,

nasal mucosa, *etc.*). *See e.g.,* U.S. Patent No. 6,428,814 for "Bioadhesive Nanoparticulate Compositions Having Cationic Surface Stabilizers," which is specifically incorporated by reference.

There are basically two mechanisms which may be responsible for this bioadhesion phenomena: mechanical or physical interactions and chemical interactions. The first of these, mechanical or physical mechanisms, involves the physical interlocking or interpenetration between a bioadhesive entity and the receptor tissue, resulting from a good wetting of the bioadhesive surface, swelling of the bioadhesive polymer, penetration of the bioadhesive entity into a crevice of the tissue surface, or interpenetration of bioadhesive composition chains with those of the mucous or other such related tissues. The second possible mechanism of bioadhesion incorporates forces such as ionic attraction, dipolar forces, van der Waals interactions, and hydrogen bonds. It is this form of bioadhesion which is primarily responsible for the bioadhesive properties of the nanoparticulate active agent compositions of the invention. However, physical and mechanical interactions may also play a secondary role in the bioadhesion of such nanoparticulate active agent compositions.

The bioadhesive active agent compositions of the invention are useful in any situation in which it is desirable to apply the compositions to a biological surface. The bioadhesive active agent compositions preferably coat the targeted surface in a continuous and uniform film that is invisible to the naked human eye.

A bioadhesive nanoparticulate active agent composition slows the transit of the composition, and some active agent particles would also most likely adhere to tissue other than the mucous cells and therefore give a prolonged exposure to the active agent, thereby increasing absorption and the bioavailability of the administered dosage.

The adhesion exhibited by the inventive compositions means that nanoparticulate active agent particles are not easily washed off, rubbed off, or otherwise removed from the biological surface for an extended period of time. The period of time in which a biological cell surface is replaced is the factor that limits

retention of the bioadhesive nanoparticulate active agent particles to that biological surface.

F. Low Viscosity Active Agent Dosage Forms

A liquid dosage form of a conventional microcrystalline or non-nanoparticulate active agent composition would be expected to be a relatively large volume, highly viscous substance which would not be well accepted by patient populations. Moreover, viscous solutions can be problematic in parenteral administration because these solutions require a slow syringe push and can stick to tubing. In addition, conventional formulations of poorly water-soluble active agents tend to be unsafe for intravenous administration techniques, which are used primarily in conjunction with highly water-soluble substances.

Liquid dosage forms of the nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, provide significant advantages over a liquid dosage form of a conventional microcrystalline or solubilized active agent composition. The low viscosity and silky texture of liquid dosage forms of the nanoparticulate active agent compositions of the invention result in advantages in both preparation and use. These advantages include, for example: (1) better subject compliance due to the perception of a lighter formulation which is easier to consume and digest; (2) ease of dispensing because one can use a cup or a syringe; (3) potential for formulating a higher concentration of active agent resulting in a smaller dosage volume and thus less volume for the subject to consume; and (4) easier overall formulation concerns.

Liquid active agent dosage forms that are easier to consume are especially important when considering juvenile patients, terminally ill patients, and elderly patients. Viscous or gritty formulations, and those that require a relatively large dosage volume, are not well tolerated by these patient populations. Liquid oral dosage forms can be particularly preferably for patient populations who have difficulty consuming tablets, such as infants and the elderly.

The viscosities of liquid dosage forms of a nanoparticulate active agent according to the invention are preferably less than about 1/200, less than about 1/175, less than about 1/150, less than about 1/125, less than about 1/100, less than about 1/75, less than about 1/50, or less than about 1/25 of a liquid oral dosage form of a non-nanoparticulate composition of the same active agent, at about the same concentration per ml of active agent.

Typically liquid nanoparticulate active agent dosage forms of the invention, comprising at least one peptide as a surface stabilizer, have a viscosity at a shear rate of 0.1 (1/s) measured at 20°C, is from about 2000 mPa s to about 1 mPa s, from about 1900 mPa·s to about 1 mPa·s, from about 1800 mPa·s to about 1 mPa·s, from about 1700 mPa·s to about 1 mPa·s, from about 1600 mPa·s to about 1 mPa·s, from about 1500 mPa·s to about 1 mPa·s, from about 1400 mPa·s to about 1 mPa·s, from about 1300 mPa·s to about 1 mPa·s, from about 1200 mPa·s to about 1 mPa·s, from about 1100 mPa·s to about 1 mPa·s, from about 1000 mPa·s to about 1 mPa·s, from about 900 mPa·s to about 1 mPa·s, from about 800 mPa·s to about 1 mPa·s, from about 700 mPa·s to about 1 mPa·s, from about 600 mPa·s to about 1 mPa·s, from about 500 mPa·s to about 1 mPa·s, from about 400 mPa·s to about 1 mPa·s, from about 300 mPa·s to about 1 mPa·s, from about 200 mPa·s to about 1 mPa·s, from about 175 mPa·s to about 1 mPa·s, from about 150 mPa·s to about 1 mPa·s, from about 125 mPa·s to about 1 mPa·s, from about 100 mPa·s to about 1 mPa·s, from about 75 mPa·s to about 1 mPa·s, from about 50 mPa·s to about 1 mPa·s, from about 25 mPa·s to about 1 mPa·s, from about 15 mPa·s to about 1 mPa·s, from about 10 mPa·s to about 1 mPa·s, or from about 5 mPa·s to about 1 mPa·s. Such a viscosity is much more attractive for subject consumption and may lead to better overall subject compliance.

Viscosity is concentration and temperature dependent. Typically, a higher concentration results in a higher viscosity, while a higher temperature results in a lower viscosity. Viscosity as defined above refers to measurements taken at about 20°C. (The viscosity of water at 20°C is 1 mPa s.) The invention encompasses equivalent viscosities measured at different temperatures.

Another important aspect of the invention is that the nanoparticulate active agent compositions of the invention, formulated into a liquid dosage form, are not turbid. "Turbid," as used herein refers to the property of particulate matter that can be seen with the naked eye or that which can be felt as "gritty." The nanoparticulate active agent compositions of the invention, formulated into a liquid dosage form, can be poured out of or extracted from a container as easily as water, whereas a liquid dosage form of a non-nanoparticulate or solubilized composition of the same active agent is expected to exhibit notably more "sluggish" characteristics.

The liquid formulations of this invention can be formulated for dosages in any volume but preferably equivalent or smaller volumes than a liquid dosage form of a non-nanoparticulate composition of the same active agent.

G. Sterile Filtered Nanoparticulate Active Agent Compositions

The nanoparticulate active agent compositions of the invention can be sterile filtered. This obviates the need for heat sterilization, which can harm or degrade an active agent, as well as result in crystal growth and particle aggregation of the active agent.

Sterile filtration can be difficult because of the required small particle size of the composition. Filtration is an effective method for sterilizing homogeneous solutions when the membrane filter pore size is less than or equal to about 0.2 microns (200 nm) because a 0.2 micron filter is sufficient to remove essentially all bacteria. Sterile filtration is normally not used to sterilize suspensions of micron-sized active agents because the active agent particles are too large to pass through the membrane pores.

A sterile nanoparticulate active agent dosage form is particularly useful in treating immunocompromised patients, infants or juvenile patients, and the elderly, as these patient groups are the most susceptible to infection caused by a non-sterile liquid dosage form.

Because the nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer and formulated into a liquid dosage form, can be sterile filtered, and because the compositions can have a very small active agent effective average particle size, the compositions are suitable for parenteral administration.

H. Combination Pharmacokinetic Profile Compositions

In yet another embodiment of the invention, a first nanoparticulate active agent composition providing a desired pharmacokinetic profile is co-administered, sequentially administered, or combined with at least one other active agent composition that generates a desired different pharmacokinetic profile. More than two active agent compositions can be co-administered, sequentially administered, or combined. While the first active agent composition has a nanoparticulate particle size, the additional one or more active agent compositions can be nanoparticulate, solubilized, or have a microparticulate particle size.

The second, third, fourth, *etc.*, active agent compositions can differ from the first, and from each other, for example: (1) in the identity of the active agent; (2) in the effective average particle sizes of the active agent; or (3) in the dosage of the active agent. Such a combination composition can reduce the dose frequency required.

For example, a first active agent composition can have a nanoparticulate particle size, conferring a short T_{max} and typically a higher C_{max}. This first active agent composition can be combined, co-administered, or sequentially administered with a second composition comprising: (1) the same active agent having a larger (but still nanoparticulate as defined herein) particle size, and therefore exhibiting slower absorption, a longer T_{max}, and typically a lower C_{max}; or (2) a microparticulate or solubilized composition of the same active agent, exhibiting a longer T_{max}, and typically a lower C_{max}.

If the second active agent composition has a nanoparticulate particle size, then preferably the active agent particles of the second composition have at least one

surface stabilizer associated with the surface of the active agent particles. The one or more surface stabilizers can be the same as or different from the surface stabilizer(s) present in the first active agent 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.

I. Miscellaneous Benefits of the Nanoparticulate Active Agent Compositions of the Invention

The nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, preferably exhibit an increased rate of dissolution as compared to microcrystalline or non-nanoparticulate forms of the same active agent. In addition, the nanoparticulate active agent compositions preferably exhibit improved performance characteristics for oral, intravenous, subcutaneous, or intramuscular injection, such as higher dose loading and smaller tablet or liquid dose volumes. Moreover, the nanoparticulate active agent compositions of the invention do not require organic solvents or pH extremes.

II. Compositions

The compositions of the invention comprise a nanoparticulate active agent and at least one peptide as a surface stabilizer adsorbed to or associated with the surface of the active agent. In addition, the compositions can comprise one or more secondary surface stabilizers. Surface stabilizers useful herein physically adhere to or associate with the surface of the nanoparticulate active agent but do not chemically react with the active agent or itself. Individual molecules of the surface stabilizer are essentially free of intermolecular cross-linkages.

The present invention also includes nanoparticulate active agent compositions, having at least one peptide as a surface stabilizer, formulated into compositions together with one or more non-toxic physiologically acceptable carriers, adjuvants, or vehicles, collectively referred to as carriers.

A. Peptide Surface Stabilizer

The choice of a surface stabilizer is non-trivial and usually requires extensive experimentation to realize a desirable formulation. Accordingly, the present invention is directed to the surprising discovery that a peptide, used as a nanoparticulate surface stabilizer, yields stable nanoparticulate active agent compositions that exhibit low degrees of aggregation.

A "peptide" is defined as any compound consisting of two or more amino acids, which are the basic structural units or "building blocks" of peptides. All peptides in all species, from bacteria to humans, are constructed from the same set of twenty commonly occurring, genetically encoded amino acids, as shown in the table below.

Each amino acid contains an "amine" group (NH_3), a "carboxy" group (COOH), a hydrogen atom, and a distinctive R group, or sidechain, bonded to a carbon atom. The amino acids vary in their sidechains, with variations in size, shape, charge, hydrogen-bonding capacity, and chemical reactivity. *See e.g.*, L. Stryer, *Biochemistry,* 3rd Edition, 1-40 (W.H. Freeman & Co., NY, 1988).

Amino Acid	3 Letter Abbreviation	1 Letter Abbreviation
alanine	ALA	A
asparagine	ASN	N
aspartic acid	ASP	D
arginine	ARG	R
cysteine	CYS	C
glutamic acid	GLU	E
glutamine	GLN	Q
glycine	GLY	G
histidine	HIS	H
isoleucine	ILE	I
leucine	LEU	L

Amino Acid	3 Letter Abbreviation	1 Letter Abbreviation
lysine	LYS	K
methionine	MET	M
phenylalanine	PHE	F
proline	PRO	P
serine	SER	S
threonine	THR	T
tryptophan	TRP	W
tyrosine	TYR	Y
valine	VAL	V
aspartic acid or asparagines	ASX	
glutamic acid or glutamine	GLX	
Unknown or other	Xaa	X

Peptides useful in the present invention can also comprise substituents other than amino acids. There are also naturally occurring chemical modifications of these twenty genetically encoded amino acids, such as hydroxylation of proline, addition of carbohydrates and lipids, and phosphorylation of serine and tyrosine. In addition, D-isomers of the amino acids, as opposed to the L-isomers found in naturally-occuring peptides and proteins, have been synthesized.

The amino acids of a peptide are connected by a amide, covalent linkage between the alpha carboxyl group of one amino acid and the alpha amino group of another amino acid. Many amino acids are joined by peptide bonds to form a polypeptide chain, which is unbranched. A polypeptide chain is a long peptide chain, consisting of a regularly repeating part, called the main chain, and a variable part, comprising the distinctive sidechains. Disulfide cross-links can be formed by cysteine residues in polypeptides. Most natural polypeptide chains contain between 50 and

2000 amino acids residues. The mean molecular weight of an amino acid residue is about 110 daltons, and so the molecular weights of most polypeptide chains are between 5500 and 220,000. *See e.g.*, L. Stryer, *Biochemistry,* 3rd Edition, p. 22 (W.H. Freeman & Co., NY, 1988).

A protein is a large macromolecule composed of one or more polypeptide chains. In the context of the present invention, a "peptide" refers to a peptide or a polypeptide, but not a protein.

Preferably, the peptide surface stabilizers of the invention are water soluble. By "water soluble," it is meant that the peptide has a water solubility of greater than about 1 mg/mL, greater than about 10 mg/mL, greater than about 20 mg/mL, or greater than about 30 mg/mL. This is in contrast to prior art compositions teaching the use of a peptide as an active agent in a nanoparticulate active agent composition. *See e.g.*, U.S. Patent Nos. 6,270,806; 6,592,903; 6,428,814; and 6,375,986. In such prior art references, when a peptide is utilized as an active agent in a nanoparticulate composition, the peptide is *poorly water soluble.*

There is an extensive catalog of commercially available peptides that can be used in the compositions of the invention. For example, the on-line peptide catalog http://www.peptide-catalog.com/PC/Peptides provides a list of hundreds of commercially available peptides, along with their structure and molecular weight. In addition, to the many commercially available peptides, custom peptides can be made and utilized in the compositions of the invention.

A preferred peptide surface stabilizer is poly(Lysine, Tryptophan)) 4:1 hydrobromide.

B. Secondary or Auxiliary Surface Stabilizers

The compositions of the invention can also include one or more auxiliary non-peptide surface stabilizers in addition to the at least one peptide surface stabilizer.

The auxiliary surface stabilizers of the invention are preferably adsorbed on, or associated with, the surface of the active agent particles. The auxiliary surface

stabilizers especially useful herein preferably do not chemically react with the active agent particles or itself. Preferably, individual molecules of the auxiliary surface stabilizer are essentially free of intermolecular cross-linkages.

Two or more auxiliary surface stabilizers can be employed in the compositions and methods of the invention.

Suitable surface stabilizers can preferably be selected from known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products, and surfactants. Preferred auxiliary surface stabilizers include nonionic, anionic, cationic, zwitterionic, and ionic surfactants.

Representative examples of secondary surface stabilizers include 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, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropyl celluloses (e.g., HPC, HPC-SL, and HPC-L), hydroxypropyl methylcellulose (HPMC), hydroxypropylmethyl-cellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), 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), dialkylesters of sodium sulfosuccinic acid (*e.g.*, Aerosol OT®, which is a dioctyl ester of sodium sulfosuccinic acid (DOSS) (American Cyanamid)); Duponol P®, which is a sodium lauryl sulfate (DuPont); 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 $C_{18}H_{37}CH_2C(O)N(CH_3)$-$CH_2(CHOH)_4(CH_2OH)_2$ (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; lysozyme, PEG-derivatized phospholipid, PEG-derivatized cholesterol, PEG- derivatized cholesterol derivative, PEG-derivatized vitamin A, PEG-derivatized vitamin E, 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, a charged phospholipid such as dimyristoyl phophatidyl glycerol, 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, dodecyl trimethyl ammonium 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).

Particularly preferred nonpolymeric primary 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 immonium 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, 1986), specifically incorporated by reference. The surface stabilizers are commercially available and/or can be prepared by techniques known in the art.

C. Active Agents

The nanoparticles of the invention comprise at least one active, therapeutic, or diagnostic agent, collectively referred to as a "drug." A therapeutic agent can be a pharmaceutical agent, including biologics such as proteins, peptides, and nucleotides, or a diagnostic agent, such as a contrast agent, including x-ray contrast agents.

The active agent exists as a crystalline phase, an amorphous phase, a semi-amorphous phase, a semi-crystalline phase, or mixtures thereof. The crystalline phase differs from a non-crystalline or amorphous phase which results from precipitation techniques, such as those described in EP Patent No. 275,796.

The invention can be practiced with a wide variety of active agents. The active agent is preferably present in an essentially pure form, is poorly soluble, and is dispersible in at least one liquid dispersion media. By "poorly soluble" it is meant that the active agent has a solubility in a liquid dispersion media of less than about 30 mg/mL, less than about 20 mg/mL, less than about 10 mg/mL, or less than about 1 mg/mL. Useful liquid dispersion medias include, but are not limited to, water, aqueous salt solutions, safflower oil, and solvents such as ethanol, t-butanol, hexane, and glycol. A preferred liquid dispersion media is water.

Two or more active agents can be used in combination.

1. Active Agents Generally

The active agent can be selected from a variety of known classes of drugs, including, for example, nutraceuticals, COX-2 inhibitors, retinoids, anticancer agents, NSAIDS, proteins, peptides, nucleotides, anti-obesity drugs, nutraceuticals, dietary supplements, carotenoids, corticosteroids, elastase inhibitors, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, 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, 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 representative active agents useful in this invention include, but are not limited to, acyclovir, alprazolam, altretamine, amiloride, amiodarone, benztropine mesylate, bupropion, cabergoline, candesartan, cerivastatin, chlorpromazine, ciprofloxacin, cisapride, clarithromycin, clonidine, clopidogrel, cyclobenzaprine, cyproheptadine, delavirdine, desmopressin, diltiazem, dipyridamole, dolasetron, enalapril maleate, enalaprilat, famotidine, felodipine, furazolidone, glipizide, irbesartan, ketoconazole, lansoprazole, loratadine, loxapine, mebendazole, mercaptopurine, milrinone lactate, minocycline, mitoxantrone, nelfinavir mesylate, nimodipine, norfloxacin, olanzapine, omeprazole, penciclovir, pimozide, tacolimus, quazepam, raloxifene, rifabutin, rifampin, risperidone, rizatriptan, saquinavir, sertraline, sildenafil, acetyl-sulfisoxazole, temazepam, thiabendazole, thioguanine, trandolapril, triamterene, trimetrexate, troglitazone, trovafloxacin, verapamil, vinblastine sulfate, mycophenolate, atovaquone, atovaquone, proguanil, ceftazidime, cefuroxime, etoposide, terbinafine, thalidomide, fluconazole, amsacrine, dacarbazine, teniposide, and acetylsalicylate.

Exemplary nutraceuticals and dietary supplements are disclosed, for example, in Roberts et al., *Nutraceuticals: The Complete Encyclopedia of Supplements, Herbs, Vitamins, and Healing Foods* (American Nutraceutical Association, 2001), which is specifically incorporated by reference. A nutraceutical or dietary supplement, also known as a phytochemical or functional food, is generally any one of a class of dietary supplements, vitamins, minerals, herbs, or healing foods that have medical or

pharmaceutical effects on the body. Exemplary nutraceuticals or dietary supplements include, but are not limited to, lutein, folic acid, fatty acids (*e.g.*, DHA and ARA), fruit and vegetable extracts, vitamin and mineral supplements, phosphatidylserine, lipoic acid, melatonin, glucosamine/chondroitin, Aloe Vera, Guggul, glutamine, amino acids (*e.g.*, iso-leucine, leucine, lysine, methionine, phenylanine, threonine, tryptophan, and valine), green tea, lycopene, whole foods, food additives, herbs, phytonutrients, antioxidants, flavonoid constituents of fruits, evening primrose oil, flax seeds, fish and marine animal oils, and probiotics. Nutraceuticals and dietary supplements also include bio-engineered foods genetically engineered to have a desired property, also known as "pharmafoods."

Active agents to be administered in an aerosol formulation are preferably selected from the group consisting of proteins, peptide, bronchodilators, corticosteroids, elastase inhibitors, analgesics, anti-fungals, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, fungal infection therapies, respiratory illness therapies associated with acquired immune deficiency syndrome, an oncology drug, an anti-emetic, an analgesic, and a cardiovascular agent.

2. Anticancer Active Agents

Useful anticancer agents are preferably selected from alkylating agents, antimetabolites, natural products, hormones and antagonists, and miscellaneous agents, such as radiosensitizers.

Examples of alkylating agents include: (1) alkylating agents having the bis-(2-chloroethyl)-amine group such as, for example, chlormethine, chlorambucile, melphalan, uramustine, mannomustine, extramustinephoshate, mechlore-thaminoxide, cyclophosphamide, ifosfamide, and trifosfamide; (2) alkylating agents having a substituted aziridine group such as, for example, tretamine, thiotepa, triaziquone, and mitomycine; (3) alkylating agents of the alkyl sulfonate type, such as, for example,

busulfan, piposulfan, and piposulfam; (4) alkylating N-alkyl-N-nitrosourea derivatives, such as, for example, carmustine, lomustine, semustine, or streptozotocine; and (5) alkylating agents of the mitobronitole, dacarbazine and procarbazine type.

Examples of antimetabolites include: (1) folic acid analogs, such as, for example, methotrexate; (2) pyrimidine analogs such as, for example, fluorouracil, floxuridine, tegafur, cytarabine, idoxuridine, and flucytosine; and (3) purine derivatives such as, for example, mercaptopurine, thioguanine, azathioprine, tiamiprine, vidarabine, pentostatin, and puromycine.

Examples of natural products include: (1) vinca alkaloids, such as, for example, vinblastine and vincristine; (2) epipodophylotoxins, such as, for example, etoposide and teniposide; (3) antibiotics, such as, for example, adriamycine, daunomycine, doctinomycin, daunorubicin, doxorubicin, mithramycin, bleomycin, and mitomycin; (4) enzymes, such as, for example, L-asparaginase; (5) biological response modifiers, such as, for example, alpha-interferon; (6) camptothecin; (7) taxol; and (8) retinoids, such as retinoic acid.

Examples of hormones and antagonists include: (1) adrenocorticosteroids, such as, for example, prednisone; (2) progestins, such as, for example, hydroxyprogesterone caproate, medroxyprogesterone acetate, and megestrol acetate; (3) estrogens, such as, for example, diethylstilbestrol and ethinyl estradiol; (4) antiestrogens, such as, for example, tamoxifen; (5) androgens, such as, for example, testosterone propionate and fluoxymesterone; (6) antiandrogens, such as, for example, flutamide; and (7) gonadotropin-releasing hormone analogs, such as, for example, leuprolide.

Examples of miscellaneous agents include: (1) radiosensitizers, such as, for example, 1,2,4-benzotriazin-3-amine 1,4-dioxide (SR 4889) and 1,2,4-benzotriazine-7-amine 1,4-dioxide (WIN 59075); (2) platinum coordination complexes such as cisplatin and carboplatin; (3) anthracenediones, such as, for example, mitoxantrone;

(4) substituted ureas, such as, for example, hydroxyurea; and (5) adrenocortical suppressants, such as, for example, mitotane and aminoglutethimide.

In addition, the anticancer agent can be an immunosuppressive drug, such as, for example, cyclosporine, azathioprine, sulfasalazine, methoxsalen, and thalidomide.

The anticancer agent can also be a COX-2 inhibitor.

3. Analgesic Active Agents

An analgesic can be, for example, an NSAID or a COX-2 inhibitor.

Exemplary NSAIDS that can be formulated in compositions of the invention include, but are not limited to, suitable nonacidic and acidic compounds. Suitable nonacidic compounds include, for example, nabumetone, tiaramide, proquazone, bufexamac, flumizole, epirazole, tinoridine, timegadine, and dapsone. Suitable acidic compounds include, for example, carboxylic acids and enolic acids. Suitable carboxylic acid NSAIDs include, for example: (1) salicylic acids and esters thereof, such as aspirin, diflunisal, benorylate, and fosfosal; (2) acetic acids, such as phenylacetic acids, including diclofenac, alclofenac, and fenclofenac; (3) carbo- and heterocyclic acetic acids such as etodolac, indomethacin, sulindac, tolmetin, fentiazac, and tilomisole; (4) propionic acids, such as carprofen, fenbufen, flurbiprofen, ketoprofen, oxaprozin, suprofen, tiaprofenic acid, ibuprofen, naproxen, fenoprofen, indoprofen, and pirprofen; and (5) fenamic acids, such as flufenamic, mefenamic, meclofenamic, and niflumic. Suitable enolic acid NSAIDs include, for example: (1) pyrazolones such as oxyphenbutazone, phenylbutazone, apazone, and feprazone; and (2) oxicams such as piroxicam, sudoxicam, isoxicam, and tenoxicam.

Exemplary COX-2 inhibitors that can be formulated in combination with the nanoparticulate nimesulide composition of the invention include, but are not limited to, celecoxib (SC-58635, CELEBREX®, Pharmacia/Searle & Co.), rofecoxib (MK-966, L-748731, VIOXX®, Merck & Co.), meloxicam (MOBIC®, co-marketed by Abbott Laboratories, Chicago, IL, and Boehringer Ingelheim Pharmaceuticals), valdecoxib (BEXTRA®, G.D. Searle & Co.), parecoxib (G.D. Searle & Co.),

etoricoxib (MK-663; Merck), SC-236 (chemical name of 4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)] benzenesulfonamide; G.D. Searle & Co., Skokie, IL); NS-398 (N-(2-cyclohexyloxy-4-nitrophenyl)methane sulfonamide; Taisho Pharmaceutical Co., Ltd., Japan); SC-58125 (methyl sulfone spiro(2.4)hept-5-ene I; Pharmacia/Searle & Co.); SC-57666 (Pharmacia/Searle & Co.); SC-558 (Pharmacia/Searle & Co.); SC-560 (Pharmacia/Searle & Co.); etodolac (Lodine®, Wyeth-Ayerst Laboratories, Inc.); DFU (5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl 2(5H)-furanone); monteleukast (MK-476), L-745337 ((5-methanesulphonamide-6-(2,4-difluorothio-phenyl)-1-indanone), L-761066, L-761000, L-748780 (all Merck & Co.); DUP-697 (5-Bromo-2-(4-fluorophenyl)-3-(4-(methylsulfonyl)phenyl; DuPont Merck Pharmaceutical Co.); PGV 20229 (1-(7-tert.-butyl-2,3-dihydro-3,3-dimethylbenzo(b)furan-5-yl)-4-cyclopropylbutan-1-one; Procter & Gamble Pharmaceuticals); iguratimod (T-614; 3-formylamino-7-methylsulfonylamino-6-phenoxy-4H-1- benzopyran-4-one; Toyama Corp., Japan); BF 389 (Biofor, USA); CL 1004 (PD 136095), PD 136005, PD 142893, PD 138387, and PD 145065 (all Parke-Davis/Warner-Lambert Co.); flurbiprofen (ANSAID®; Pharmacia & Upjohn); nabumetone (FELAFEN®; SmithKline Beecham, plc); flosulide (CGP 28238; Novartis/Ciba Geigy); piroxicam (FELDANE®; Pfizer); diclofenac (VOLTAREN® and CATAFLAM®, Novartis); lumiracoxib (COX-189; Novartis); D 1367 (Celltech Chiroscience, plc); R 807 (3 benzoyldifluoromethane sulfonanilide, diflumidone); JTE-522 (Japan Tobacco, Japan); FK-3311 (4'-Acetyl-2'-(2,4-difluorophenoxy)methanesulfonanilide), FK 867, FR 140423, and FR 115068 (all Fujisawa, Japan); GR 253035 (Glaxo Wellcome); RWJ 63556 (Johnson & Johnson); RWJ 20485 (Johnson & Johnson); ZK 38997 (Schering); S 2474 ((E)-(5)-(3,5-di-tert-butyl-4-hydroxybenzylidene)-2-ethyl-1,2-isothiazolidine-1,1-dioxide indomethacin; Shionogi & Co., Ltd., Japan); zomepirac analogs, such as RS 57067 and RS 104897 (Hoffmann La Roche); RS 104894 (Hoffmann La Roche); SC 41930 (Monsanto); pranlukast (SB 205312, Ono-1078, ONON®, ULTAIR®; SmithKline Beecham); SB 209670 (SmithKline Beecham); and APHS (heptinylsulfide).

D. Nanoparticulate Active Agent Particle Size

The compositions of the invention contain nanoparticulate active agent particles which have an effective average particle size of less than about 2000 nm (*i.e.*, 2 microns). In other embodiments of the invention, the active agent particles have a 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 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 100 nm, less than about 75 nm, or less than about 50 nm, as measured by light-scattering methods, microscopy, or other appropriate methods.

By "an effective average particle size of less than about 2000 nm" it is meant that at least 50% by weight of the active agent particles have a particle size less than the effective average, *i.e.*, less than about 2000 nm, 1900 nm, 1800 nm, *etc.*, 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 active agent particles have a particle size less than the effective average, *i.e.*, less than about 2000 nm, 1900 nm, 1800 nm, *etc.*

If the nanoparticulate active agent composition is combined with a conventional active agent composition, then such a composition is either solubilized or has an effective average particle size greater than about 2 microns. By "an effective average particle size of greater than about 2 microns" it is meant that at least 50% of the microparticulate active agent particles have a particle size greater than about 2 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 active agent particles have a particle size greater than about 2 microns.

In the present invention, the value for D50 of a nanoparticulate active agent composition is the particle size below which 50% of the active agent particles fall, by weight. Similarly, D90 and D99 are the particle sizes below which 90% and 99%, respectively, of the active agent particles fall, by weight.

5. Concentration of Nanoparticulate Active Agent and Peptide Stabilizer

The relative amounts of active agent and peptide surface stabilizer, and optionally one or more secondary surface stabilizers, can vary widely. The optimal amount of the individual components can depend, for example, upon the particular active agent selected, the hydrophilic lipophilic balance (HLB), melting point, and the surface tension of water solutions of the stabilizer, *etc.*

The concentration of the peptide 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 the at least one active agent and at least one peptide surface stabilizer, not including other excipients.

The concentration of the at least one active agent 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 dry weight of the active agent and at least one peptide surface stabilizer, not including other excipients.

B. Methods of Making Nanoparticulate Active Agent Formulations

The nanoparticulate active agent compositions of the invention, comprising at least one peptide as a surface stabilizer, can be made using, for example, milling, homogenization, or precipitation techniques. Exemplary methods of making nanoparticulate compositions are described in the '684 patent. Methods of making nanoparticulate active agent 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.

The resultant nanoparticulate active agent compositions can be utilized in any desired dosage form.

1. Milling to obtain Nanoparticulate Active Agent Dispersions

Milling the active agent to obtain a nanoparticulate dispersion comprises dispersing active agent particles in a liquid dispersion media in which the active agent is poorly soluble, followed by applying mechanical means in the presence of grinding media to reduce the particle size of the active agent to the desired effective average particle size. The dispersion media can be, for example, water, safflower oil, ethanol, t-butanol, glycerin, polyethylene glycol (PEG), hexane, or glycol. Water is a preferred dispersion media.

The active agent particles are preferably reduced in size in the presence of at least one peptide surface stabilizer. Alternatively, the active agent particles can be contacted with at least one peptide surface stabilizer either during or after attrition. One or more secondary surface stabilizers may also be added before, during, or after attrition. Other compounds, such as a diluent, can be added to the active agent/peptide surface stabilizer composition before, during, or after the size reduction process. Dispersions can be manufactured continuously or in a batch mode.

2. Precipitation to Obtain Nanoparticulate Active Agent Compositions

Another method of forming the desired nanoparticulate active agent composition is by microprecipitation. This is a method of preparing stable dispersions of poorly soluble active agents in the presence of one or more peptide 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 poorly soluble active agent in a suitable solvent; (2) adding the formulation from step (1) to a solution comprising at least one peptide surface stabilizer and optionally one or more secondary surface stabilizers, to form a clear solution; and (3) precipitating the formulation from step (2) using an appropriate non-solvent. 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.

3. Homogenization to Obtain Nanoparticulate Active Agent Compositions

Exemplary homogenization methods of preparing active agent nanoparticulate compositions are described in U.S. Patent No. 5,510,118, for "Process of Preparing Therapeutic Compositions Containing Nanoparticles."

Such a method comprises dispersing active agent particles in a liquid dispersion media in which the active agent is poorly soluble, followed by subjecting the dispersion to homogenization to reduce the particle size of the active agent to the desired effective average particle size. The active agent particles can be reduced in size in the presence of at least one peptide surface stabilizer and, if desired, one or more additional surface stabilizers. Alternatively, the active agent particles can be contacted with at least one peptide surface stabilizer and, if desired, one or more additional surface stabilizers, either during or after attrition. Other compounds, such as a diluent, can be added to the active agent/peptide surface stabilizer composition either before, during, or after the size reduction process. Dispersions can be manufactured continuously or in a batch mode.

C. Methods of Using Nanoparticulate Active Agent Formulations

The nanoparticulate active agent compositions of the present invention can be administered to humans and animals via any conventional means including, but not limited to, orally, rectally, ocularly, parenterally (intravenous, intramuscular, or subcutaneous), intracisternally, pulmonary, intravaginally, intraperitoneally, locally (powders, ointments or drops), or as a buccal or nasal spray.

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 active agent 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.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active agent is admixed with at least one of the following: (a) one or more inert excipients (or carrier), such as sodium citrate or dicalcium phosphate; (b) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; (c) binders, such as carboxymethylcellulose, alignates, gelatin, polyvinylpyrrolidone, sucrose and acacia; (d) humectants, such as glycerol; (e) disintegrating agents, such as agar-agar, calcium

carbonate, potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate; (f) solution retarders, such as paraffin; (g) absorption accelerators, such as quaternary ammonium compounds; (h) wetting agents, such as cetyl alcohol and glycerol monostearate; (i) adsorbents, such as kaolin and bentonite; and (j) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or mixtures thereof. For capsules, tablets, and pills, the dosage forms may also comprise buffering agents.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs. In addition to the active agent, the liquid dosage forms may comprise inert diluents commonly used in the art, such as water or other solvents, solubilizing agents, and emulsifiers. Exemplary emulsifiers are ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propyleneglycol, 1,3-butyleneglycol, dimethylformamide, oils, such as cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, and sesame oil, glycerol, tetrahydrofurfuryl alcohol, polyethyleneglycols, fatty acid esters of sorbitan, or mixtures of these substances, and the like.

Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Actual dosage levels of active agent in the nanoparticulate compositions of the invention may be varied to obtain an amount of active agent 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 active agent, 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

including the body weight, general health, sex, diet, time and route of administration, potency of the administered active agent, rates of absorption and excretion, combination with other active agents, and the severity of the particular disease being treated.

* * * * *

The following examples are given to illustrate the present invention. It should be understood, however, that the invention is not to be limited to the specific conditions or details described in these examples. Throughout the specification, any and all references to a publicly available document, including a U.S. patent, are specifically incorporated by reference.

The formulations in the examples that follow were also investigated using a light microscope. Here, "stable" nanoparticulate dispersions (uniform Brownian motion) were readily distinguishable from "aggregated" dispersions (relatively large, nonuniform particles without motion).

Example 1

The purpose of this example was to prepare a nanoparticulate nystatin composition having a peptide surface stabilizer.

Nystatin is a poorly water-soluble antimycotic polyene antibiotic obtained from *Streptomyces noursei.* It is an antifungal agent indicated for oral, gastrointestinal, and vaginal candidiasis. Oral candidiasis, in particular, is a common affliction of immunocompromised patients. Nystatin is indicated in the therapy of all infections caused by susceptible microorganisms in those patients in whom candidal (monilial) infections are most likely to complicate therapy.

A slurry of 2% (w/w) nystatin (Sigma-Aldrich Co.) and 1% (w/w) poly(Lysine, Tryptophan) 4:1 hydrobromide ("Poly(Lys,Trp)") (Sigma; St. Louris, MO), which is a cationic random co-polyamino acid having a molecular weight of 38,000, in water was

milled for 1 day using low energy (ball milling) techniques in the presence of ceramic YTZ grinding media.

The mean size of the nystatin particles following milling was 149 nm, with a D90 of 270 nm, as determined by static light scattering using a Horiba LA-910 light-scattering particle size analyzer (Horiba Instruments, Irvine, CA). The composition had a zeta potential of 47.7 mV, as measured by electrophoresis in $5x10^{-4}$ M NaCl (Malvern ZetaSizer). Dispersibility was verified by phase contrast microscopy.

Figure 1 shows representative photomicrographs of the nystatin crystals before (Fig. 1A) and after (Fig. 1B) milling.

Particle size stability under controlled conditions was monitored over time. Figure 2 shows the results of monitoring the nystatin particle size stability over time at 5°C (solid line), 25°C (dashed line), and 40°C (dotted line) for the nanoparticulate nystatin/peptide composition.

These results demonstrate that a peptide surface stabilizer can be successfully used to stabilize an active agent at a nanoparticulate particle size. Moreover, such a peptide surface stabilizer may confer additional therapeutic advantages to the final formulation. For example, the peptide surface stabilizer Poly(Lys,Trp) is cationic and, therefore, nanoparticulate active agent compositions utilizing this surface stabilizer will be bioadhesive.

The resultant composition exhibited a mean particle sizes of 149 nm and were free of agglomeration. Moreover, the nanoparticulate nystatin/peptide composition exhibited virtually no particle size growth at all three temperatures tested.

Example 2

The purpose of this example was to determine whether a cationic surface charge, such as that obtained with the use of a cationic peptide surface stabilizer, enhances the adhesion of small particles to cells.

Cell-binding experiments were performed with polystyrene latex microspheres as a model. A positive surface charge would be expected to enhance the interaction of particles with cell-surface macromolecules, which have a net negative charge.

Cationic microspheres with a mean zeta-potential (51.5 mV) comparable to the nanoparticulate nystatin/peptide composition of Example 1 were tested against anionic microspheres (mean zeta-potential = -50.9 mV). The microspheres were incubated with NIH/3T3 fibroblasts, washed thoroughly, fixed, and subjected to SEM analysis.

Figure 3 shows representative micrographs of cells with anionic particles (Fig. 3A) and cationic particles (Fig. 3B).

The results indicate that positively-charged particles interact more strongly with the cell surface than negatively-charged particles, and it is believed that nanoparticulate active agent compositions having a cationic peptide as a surface stabilizer with comparable zeta potentials will follow the same trend.

Example 3

The purpose of this example was to determine if milling of an active agent, such as nystatin, having a peptide surface stabilizer affects the active agent's activity.

The minimum inhibitory concentration (MIC) of a milled nystatin composition having as a peptide surface stabilizer Poly(Lys, Trp) was compared to the MIC of two unmilled nystatin compositions. Nystatin for the milled nanoparticulate composition was obtained from Sigma-Aldrich Co. and the two unmilled nystatin compositions were obtained from Sigma-Aldrich Co. and Paddock Laboratories, Inc. Details regarding the milled and unmilled nystatin compositions are given in Table 1 below, including particle size of the milled nanoparticulate nystatin/Poly(Lys, Trp) composition and the potency (USP U/ml) and MIC for each nystatin composition.

TABLE 1				
Nystatin Concentration	**Surface Stabilizer and Concentration**	**Mean Particle Size (nm)**	**Potency (USP U/ml)**	**MIC**
2% (Sigma)	1% Poly(Lys, Trp)[1]	129	101,200	1:10,000
5% (Sigma)	N/A – unmilled	N/A	253,000	1:10,000
4% (Paddock)	N/A – unmilled	N/A	253,000	1:100,000

[1]Poly(Lysine, Tryptophan) is a cationic random co-polyamino acid.

The nanoparticulate sample was ball milled for 26 hours with ceramic YTZ milling media.

The minimum inhibitory concentration (MIC) of the milled nystatin/peptide composition and the two unmilled samples were determined in cultures of *C. albicans*. MIC as reported here is the maximum dilution of formulation in culture broth which inhibits growth of *C albicans*. As shown in Table 1, above, the milled nystatin/peptide composition did not exhibit any significant differences in MIC, and surprisingly, was more active than at least one of the unmilled nystatin samples. These data confirm that the milling process does not decrease the activity of nystatin.

Example 4

The purpose of this example was to prepare a nanoparticulate composition of a diuretic, Compound A, utilizing a peptide surface stabilizer. Diuretics can be used to reduce the swelling and fluid retention caused by various medical problems, including heart or liver disease. They are also is used to treat high blood pressure.

A slurry of 2% (w/w) Compound A and 1% (w/w) poly(Lysine, Tryptophan) 4:1 hydrobromide as a peptide surface stabilizer in water was milled for 3 days in an aqueous environment in a low energy mill, in the presence of 0.8 mm yttrium-stabilized ceramic media.

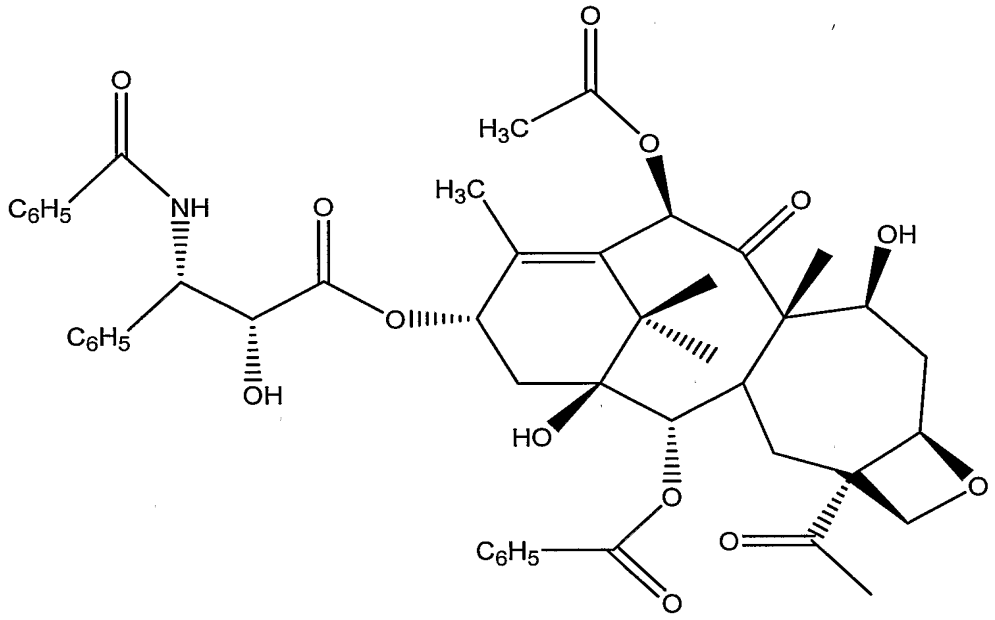
Particle size analysis of the resulting Compound A dispersion was conducted via laser light diffraction using the Horiba LA 910 particle size analyzer (Horiba Instruments, Irvine, CA) and water as a diluent. The mean particle size of the milled

Compound A dispersion was 99 nm, with a D90 of 138 nm. The composition was stable.

Example 5

The purpose of this example was to prepare a nanoparticulate composition of paclitaxel utilizing a peptide surface stabilizer. Paclitaxel belongs to the group of medicines called antineoplastics. It is used to treat cancer of the ovaries, breast, certain types of lung cancer, and a cancer of the skin and mucous membranes more commonly found in patients with acquired immunodeficiency syndrome (AIDS). It may also be used to treat other kinds of cancer.

Paclitaxel has the following chemical structure:



A slurry of 2% (w/w) paclitaxel and 1% (w/w) poly(Lysine, Tryptophan) 4:1 hydrobromide as a peptide surface stabilizer in water was milled for 3 days in an aqueous environment in a low energy mill, in the presence of 0.8 mm yttrium-stabilized ceramic media.

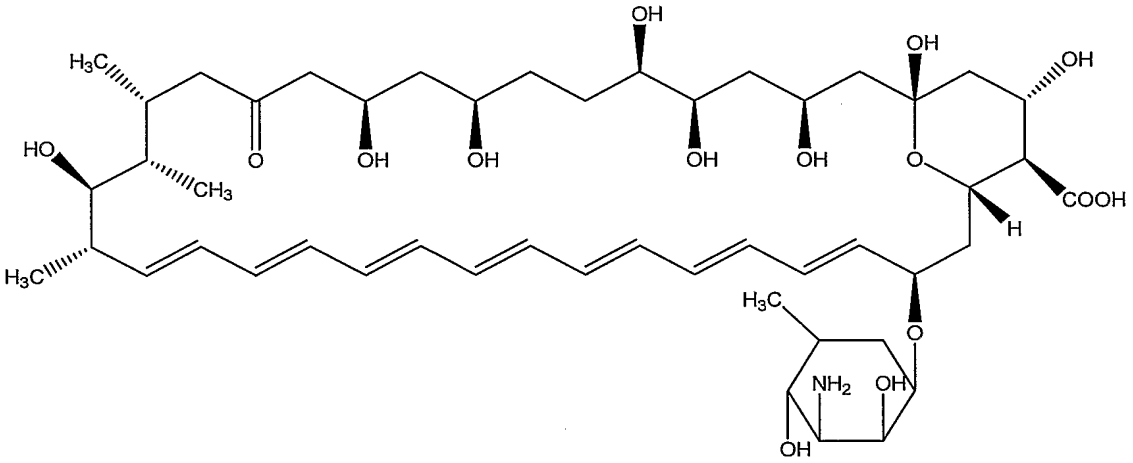
Particle size analysis of the resulting paclitaxel dispersion was conducted via laser light diffraction using the Horiba LA 910 particle size analyzer (Horiba Instruments, Irvine, CA) and water as a diluent. The mean particle size of the milled

paclitaxel dispersion was 139 nm, with a D90 of 185 nm. The composition was stable.

Example 6

The purpose of this example was to prepare a nanoparticulate composition of amphotericin B utilizing a peptide surface stabilizer. Amphotericin B is a poorly water soluble antifungal agent. Topically, it is used to treat skin yeast infections; intravenously, it is used to treat a variety of life-threatening fungal infections.

Amphotericin B has the following chemical structure:



In this experiment, amphotericin B was milled with Poly (Lys, Trp) 4:1 Hydrobromide as a peptide surface stabilizer. A 2% (w/w) slurry of amphotericin B (Sigma) in water was prepared with 1% (w/w) poly (Lys, Trp) (Sigma). The composition was ball-milled for 24 hours with 0.8 mm ceramic YTZ milling media. The particle size of the resulting amphotericin B dispersion was characterized by static laser light scattering on a Horiba LA-910 particle size distribution analyzer. The results are shown in Table 2, below.

TABLE 2

Drug and Concentration	Surface Stabilizer and Concentration	Mean Particle Size (nm)	D50 (nm)	D90 (nm)
2% Amphotericin B	1% Poly(Lys, Trp)	121	96	230

These results demonstrate that amphotericin B dispersions can be successfully stabilized by a peptide surface stabilizer, such as the random copolypeptide poly (Lys, Trp) 4:1 Hydrobromide.

* * * *

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.

We claim:

1. A composition comprising:
   (a) particles of at least one active agent having an effective average particle size of less than about 2000 nm; and
   (b) at least one water soluble peptide surface stabilizer.

2. The composition of claim 1, wherein the peptide surface stabilizer is poly(Lysine, Tryptophan) 4:1 hydrobromide.

3. The composition of claim 1 or claim 2, further comprising at least one secondary surface stabilizer.

4. The composition of any one of claims 1 to 3, wherein the secondary surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a zwitterionic surface stabilizer, and an ionic surface stabilizer.

5. The composition of claim 3 or claim 4, wherein the secondary 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, and random copolymers of vinyl acetate and vinyl pyrrolidone, a cationic polymer, a cationic biopolymer, a cationic polysaccharide, a cationic cellulosic, a cationic alginate, a cationic nonpolymeric compound, a 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.

6. The composition of any one of claims 1 to 5, wherein the composition is formulated for administration selected from the group consisting of oral, pulmonary, rectal, opthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration.

7. The composition of any one of claims 1 to 6 formulated into a dosage form selected from the group consisting of liquid dispersions, oral suspensions, gels, aerosols, ointments, creams, tablets, capsules, sachets, lozenges, powders, pills, granules, controlled release formulations, fast melt formulations, lyophilized

formulations, delayed release formulations, extended release formulations, pulsatile release formulations, and mixed immediate release and controlled release formulations.

8. The composition of any one of claims 1 to 7, wherein:

(a) the active agent 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%, and from about 90% to about 0.5%, by weight, based on the total combined dry weight of the active agent and at least one peptide surface stabilizer, not including other excipients; or

(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, and from about 10% to about 99.5% by weight, based on the total combined dry weight of the active agent and at least one peptide surface stabilizer, not including other excipients.

9. The composition of any one of claims 1 to 8, wherein the active agent is selected from the group consisting of a crystalline phase, an amorphous phase, a semi-crystalline phase, a semi-amorphous phase, and mixtures thereof.

10. The composition of any one of claims 1 to 10, wherein the effective average particle size of the active agent particles is 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 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 100 nm, less than about 75 nm, and less than about 50 nm.

11. The composition of any one of claims 1 to 10, wherein at least about 70%, at least about 90%, at least about 95%, or at least about 99% of the active agent particles have a particle size less than the effective average particle size.

12. The composition of any one of claims 1 to 11, further comprising at least one additional active agent composition having an effective average particle size which is different that the effective average particle size of the active agent composition of claim 1.

13. The composition of any one of claims 1 to 12, wherein the active agent is selected from the group consisting of nystatin, paclitaxel, amphotericin B, a diuretic, a dermal agent, nutraceuticals, COX-2 inhibitors, retinoids, anticancer agents, NSAIDS, proteins, peptides, nucleotides, anti-obesity drugs, dietary supplements, carotenoids, corticosteroids, elastase inhibitors, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytics, sedatives, astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio-pharmaceuticals, sex hormones, anti-allergic agents, stimulants, anoretics, sympathomimetics, thyroid agents, vasodilators, xanthines, acyclovir, alprazolam, altretamine, amiloride, amiodarone, benztropine mesylate, bupropion, cabergoline, candesartan, cerivastatin, chlorpromazine, ciprofloxacin, cisapride, clarithromycin, clonidine, clopidogrel, cyclobenzaprine, cyproheptadine, delavirdine, desmopressin, diltiazem, dipyridamole, dolasetron, enalapril maleate, enalaprilat, famotidine, felodipine, furazolidone, glipizide, irbesartan, ketoconazole, lansoprazole,

loratadine, loxapine, mebendazole, mercaptopurine, milrinone lactate, minocycline, mitoxantrone, nelfinavir mesylate, nimodipine, norfloxacin, olanzapine, omeprazole, penciclovir, pimozide, tacolimus, quazepam, raloxifene, rifabutin, rifampin, risperidone, rizatriptan, saquinavir, sertraline, sildenafil, acetyl-sulfisoxazole, temazepam, thiabendazole, thioguanine, trandolapril, triamterene, trimetrexate, troglitazone, trovafloxacin, verapamil, vinblastine sulfate, mycophenolate, atovaquone, atovaquone, proguanil, ceftazidime, cefuroxime, etoposide, terbinafine, thalidomide, fluconazole, amsacrine, dacarbazine, teniposide, and acetylsalicylate.

14. The composition of claim 13, wherein the nutraceutical is selected from the group consisting of dietary supplements, vitamins, minerals, herbs, lutein, folic acid, fatty acids, fruit extracts, vegetable extracts, phosphatidylserine, lipoic acid, melatonin, glucosamine/chondroitin, Aloe Vera, Guggul, glutamine, amino acids, green tea, lycopene, whole foods, food additives, herbs, phytonutrients, antioxidants, flavonoid constituents of fruits, evening primrose oil, flax seeds, fish oils, marine animal oils, and probiotics.

15. The composition of claim 13, wherein the anticancer agent is selected from the group consisting of alkylating agents, antimetabolites, anthracenediones, natural products, hormones, antagonists, radiosensitizers, platinum coordination complexes, adrenocortical suppressants, immunosuppressive agent, substituted ureas, and COX-2 inhibitors.

16. The composition of claim 15, wherein:

(a) the alkylating agent is selected from the group consisting of chlormethine, chlorambucile, melphalan, uramustine, mannomustine, extramustinephoshate, mechlore-thaminoxide, cyclophosphamide, ifosfamide, trifosfamide, tretamine, thiotepa, triaziquone, mitomycine, busulfan, piposulfan, piposulfam, carmustine, lomustine, semustine, streptozotocine, mitobronitole, dacarbazine and procarbazine; or

(b) the antimetabolite is selected from the group consisting of methotrexate, fluorouracil, floxuridine, tegafur, cytarabine, idoxuridine, flucytosine, mercaptopurine, thioguanine, azathioprine, tiamiprine, vidarabine, pentostatin, and puromycine; or

(c) the natural product is selected from the group consisting of vinblastine, vincristine, etoposide, teniposide, adriamycine, daunomycine, doctinomycin, daunorubicin, doxorubicin, mithramycin, bleomycin, mitomycin, L-asparaginase, alpha-interferon, camptothecin, taxol, and retinoic acid; or

(d) the hormone or antagonist is selected from the group consisting of prednisone, hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate, diethylstilbestrol, ethinyl estradiol, tamoxifen, testosterone propionate, fluoxymesterone, flutamide, leuprolide; or

(e) the anticancer agent is selected from the group consisting of cisplatin, carboplatin, mitoxantrone, hydroxyurea, mitotane, aminoglutethimide, cyclosporine, azathioprine, sulfasalazine, methoxsalen, and thalidomide.

17. The composition of claim 13, wherein the NSAID is selected from the group consisting of nabumetone, tiaramide, proquazone, bufexamac, flumizole, epirazole, tinoridine, timegadine, dapsone, aspirin, diflunisal, benorylate, fosfosal, diclofenac, alclofenac, fenclofenac, etodolac, indomethacin, sulindac, tolmetin, fentiazac, tilomisole, carprofen, fenbufen, flurbiprofen, ketoprofen, oxaprozin, suprofen, tiaprofenic acid, ibuprofen, naproxen, fenoprofen, indoprofen, pirprofen, flufenamic, mefenamic, meclofenamic, niflumic, oxyphenbutazone, phenylbutazone, apazone, feprazone, piroxicam, sudoxicam, isoxicam, and tenoxicam.

18. The composition of claim 13, wherein the COX-2 inhibitor is selected from the group consisting of nimesulide, celecoxib, rofecoxib, meloxicam, valdecoxib, parecoxib, etoricoxib, flurbiprofen, nabumetone, etodolac, iguratimod, flosulide, piroxicam, diclofenac, lumiracoxib, monteleukast, pranlukast, heptinylsulfide, SC-236, SC-58125, SC-57666, SC-558, SC-560, SC 41930, NS-398, DFU, L-745337, L-

761066, L-761000, L-748780, DUP-697, PGV 20229, BF 389, CL 1004, PD 136005, PD 142893, PD 138387, PD 145065, D 1367, R 807, JTE-522, FK-3311, FK 867, FR 140423, FR 115068, GR 253035, RWJ 63556, RWJ 20485, ZK 38997, S 2474, RS 57067, RS 104897, RS 104894, and SB 209670.

19. The composition of any one of claims 1 to 18, wherein upon administration to a mammal the active agent particles redisperse such that the particles have an effective average particle size selected from the group consisting of less than about 2 microns, 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 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 100 nm, less than about 75 nm, and less than about 50 nm.

20. The composition of any one of claims 1 to 19, wherein the composition redisperses in a biorelevant media such that the active agent particles have an effective average particle size selected from the group consisting of less than about 2 microns, 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 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 100 nm, less than about 75 nm, and less than about 50 nm.

21. The composition of claim 20, wherein the biorelevant media is selected from the group consisting of water, aqueous electrolyte solutions, aqueous solutions of a

salt, aqueous solutions of an acid, aqueous solutions of a base, and combinations thereof.

22. The composition of any one of claims 1 to 21, wherein:

(a) the T_{max} of the active agent, when assayed in the plasma of a mammalian subject following administration, is less than the T_{max} for a non-nanoparticulate composition of the same active agent, administered at the same dosage; or

(b) the C_{max} of the active agent, when assayed in the plasma of a mammalian subject following administration, is greater than the C_{max} for a non-nanoparticulate composition of the same active agent, administered at the same dosage; or

(c) the AUC of the active agent, when assayed in the plasma of a mammalian subject following administration, is greater than the AUC for a non-nanoparticulate composition of the same active agent, administered at the same dosage.

23. The composition of claim 22, wherein the T_{max} is selected from the group consisting of 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%, and not greater than about 5% of the T_{max} exhibited by a non-nanoparticulate composition of the same active agent, administered at the same dosage.

24. The composition of claim 22, wherein the C_{max} is selected from the group consisting of 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 a non-nanoparticulate composition of the same active agent, administered at the same dosage.

25. The composition of claim 22, 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 composition of the same active agent, administered at the same dosage.

26. The composition of any one of claims 1 to 25 which does not produce significantly different absorption levels when administered under fed as compared to fasting conditions.

27. The composition of claim 26, wherein the difference in absorption of the active agent composition of the invention, when administered in the fed versus the fasted state, is selected from the group consisting of 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 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, and less than about 3%.

28. The composition of any one of claims 1 to 27, wherein administration of the composition to a human in a fasted state is bioequivalent to administration of the composition to a subject in a fed state.

29. The composition of claim 28, wherein "bioequivalency" is established by:

(a) a 90% Confidence Interval of between 0.80 and 1.25 for both C_{max} and AUC; or

(b) a 90% Confidence Interval of between 0.80 and 1.25 for AUC and a 90% Confidence Interval of between 0.70 to 1.43 for C_{max}.

30. The composition of any one of claims 1 to 29, formulated into a liquid dosage form and having a viscosity at a shear rate of 0.1 (1/s), measured at 20°C, selected from the group consisting of less than about 2000 mPa·s, from about 2000 mPa·s to about 1 mPa·s, from about 1900 mPa·s to about 1 mPa·s, from about 1800 mPa·s to about 1 mPa·s, from about 1700 mPa·s to about 1 mPa·s, from about 1600 mPa·s to about 1 mPa·s, from about 1500 mPa·s to about 1 mPa·s, from about 1400 mPa·s to about 1 mPa·s, from about 1300 mPa·s to about 1 mPa·s, from about 1200 mPa·s to about 1 mPa·s, from about 1100 mPa·s to about 1 mPa·s, from about 1000 mPa·s to about 1 mPa·s, from about 900 mPa·s to about 1 mPa·s, from about 800 mPa·s to about 1 mPa·s, from about 700 mPa·s to about 1 mPa·s, from about 600 mPa·s to about 1 mPa·s, from about 500 mPa·s to about 1 mPa·s, from about 400 mPa·s to about 1 mPa·s, from about 300 mPa·s to about 1 mPa·s, from about 200 mPa·s to about 1 mPa·s, from about 175 mPa·s to about 1 mPa·s, from about 150 mPa·s to about 1 mPa·s, from about 125 mPa·s to about 1 mPa·s, from about 100 mPa·s to about 1 mPa·s, from about 75 mPa·s to about 1 mPa·s, from about 50 mPa·s to about 1 mPa·s, from about 25 mPa·s to about 1 mPa·s, from about 15 mPa·s to about 1 mPa·s, from about 10 mPa·s to about 1 mPa·s, and from about 5 mPa·s to about 1 mPa·s.

31. The composition of claim 30, wherein the viscosity of the dosage form is:

(a) selected from the group consisting of less than about 1/200, less than

about 1/100, less than about 1/50, less than about 1/25, and less than about 1/10 of the viscosity of a liquid dosage form of a non-nanoparticulate composition of the same active agent, at about the same concentration per ml of active agent; or

(b) selected from the group consisting of less than about 5%, less than about 10%, less than about 15%, less than about 20%, less than about 25%, less than about 30%, less than about 35%, less than about 40%, less than about 45%, less than about 50%, less than about 55%, less than about 60%, less than about 65%, less than about 70%, less than about 75%, less than about 80%, less than about 85%, and less than about 90% of the viscosity of a liquid dosage form of a non-nanoparticulate composition of the same active agent, at about the same concentration per ml of active agent.

32. The composition of any one of claims 1 to 31, further comprising one or more pharmaceutically acceptable excipients, carriers, or a combination thereof.

33. The composition according to any one of claims 1 to 32, wherein the composition is bioadhesive.

34. The use of a composition according to any one of claims 1 to 33 for the manufacture of a pharmaceutical medicament.

35. A method of making a composition according to any one of claims 1 to 33, comprising contacting particles of at least one active agent with at least one water-soluble peptide surface stabilizer for a time and under conditions sufficient to provide an active agent composition having an effective average particle size of less than about 2000 nm.

FIGURE 1

A B

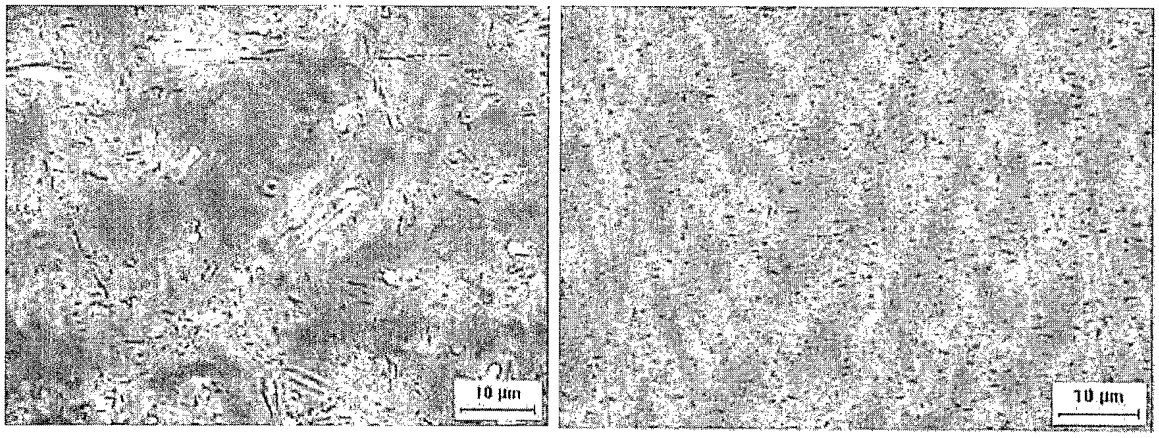


FIGURE 2

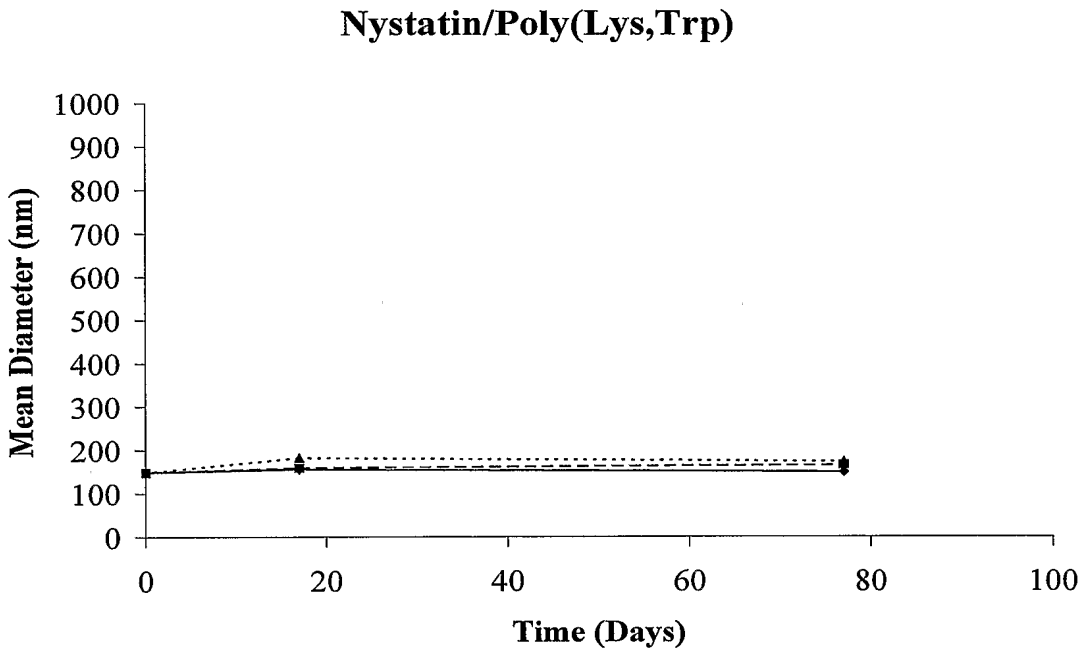
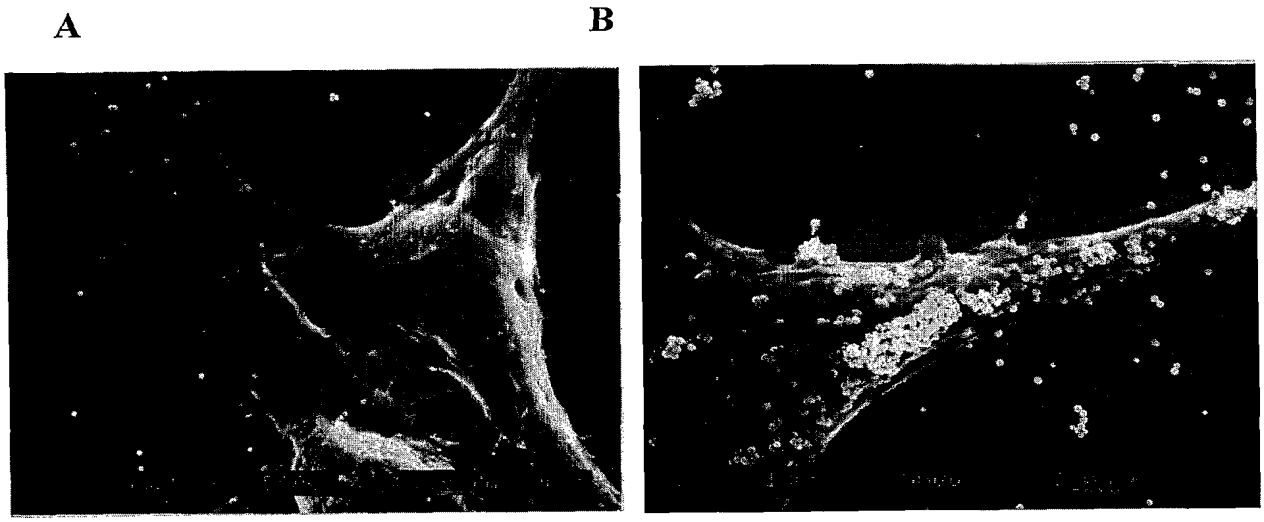

Nystatin/Poly(Lys,Trp)
Mean Diameter (nm)
1000
900
800
700
600
500
400
300
200
100
0
0
20
40
60
80
100
Time (Days)

FIGURE 3



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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 September 2005 (29.09.2005)

PCT

(10) International Publication Number
WO 2005/089768 A1

(51) International Patent Classification[7]: A61K 31/55, A61L 9/14

(21) International Application Number: PCT/US2005/008090

(22) International Filing Date: 11 March 2005 (11.03.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/803,521 17 March 2004 (17.03.2004) US

(71) Applicant *(for all designated States except US)*: UNIVERSITY OF KENTUCKY RESEARCH FOUNDATION [US/US]; A148, ASTeCC Building, Lexington, KY 40506-0286 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: WERMELING, Daniel, P. [US/US]; 4889 Wyndhurst Road, Lexington, KY 40515 (US).

(74) Agent: MAYER BROWN ROWE & MAW LLP; P.O.Box 2828, Chicago, Illinois 60609-2828 (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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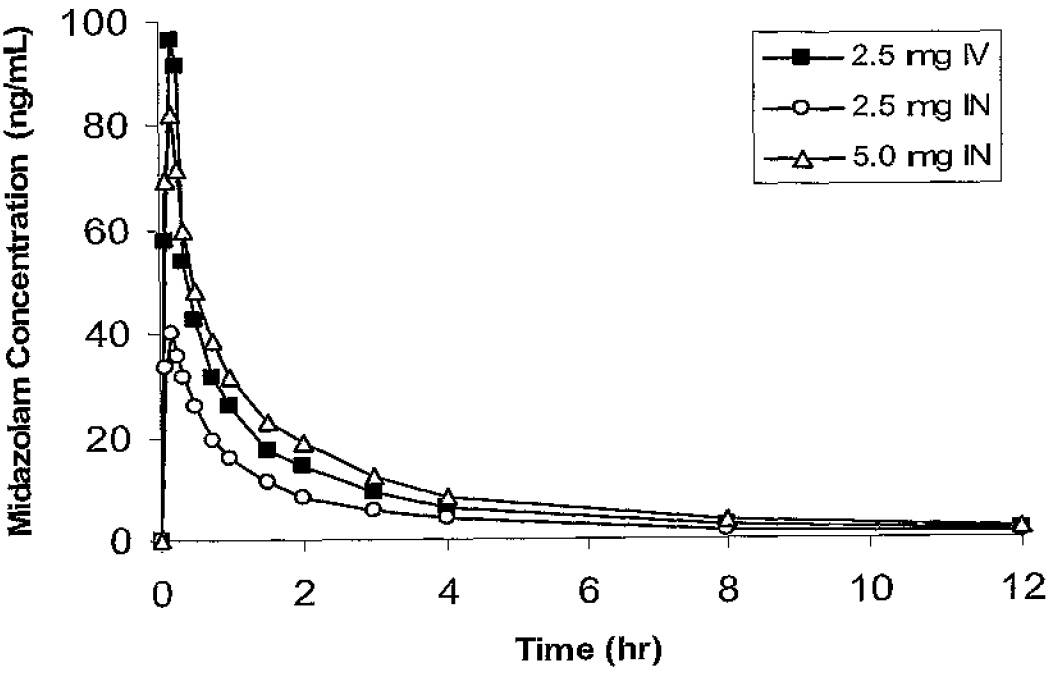
(54) Title: INTRANASAL BENZODIAZEPINE COMPOSITIONS



(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, or 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

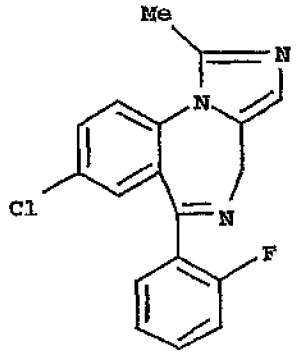
Various embodiments will now be described. These embodiments are presented to aid in an understanding of the claims and are not intended to, and should not be construed to, limit the claims in any way. All alternatives, modifications and equivalents that may become obvious to those of ordinary skill 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 $Dose/(\lambda_z * AUC_{0-\infty})$. 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.

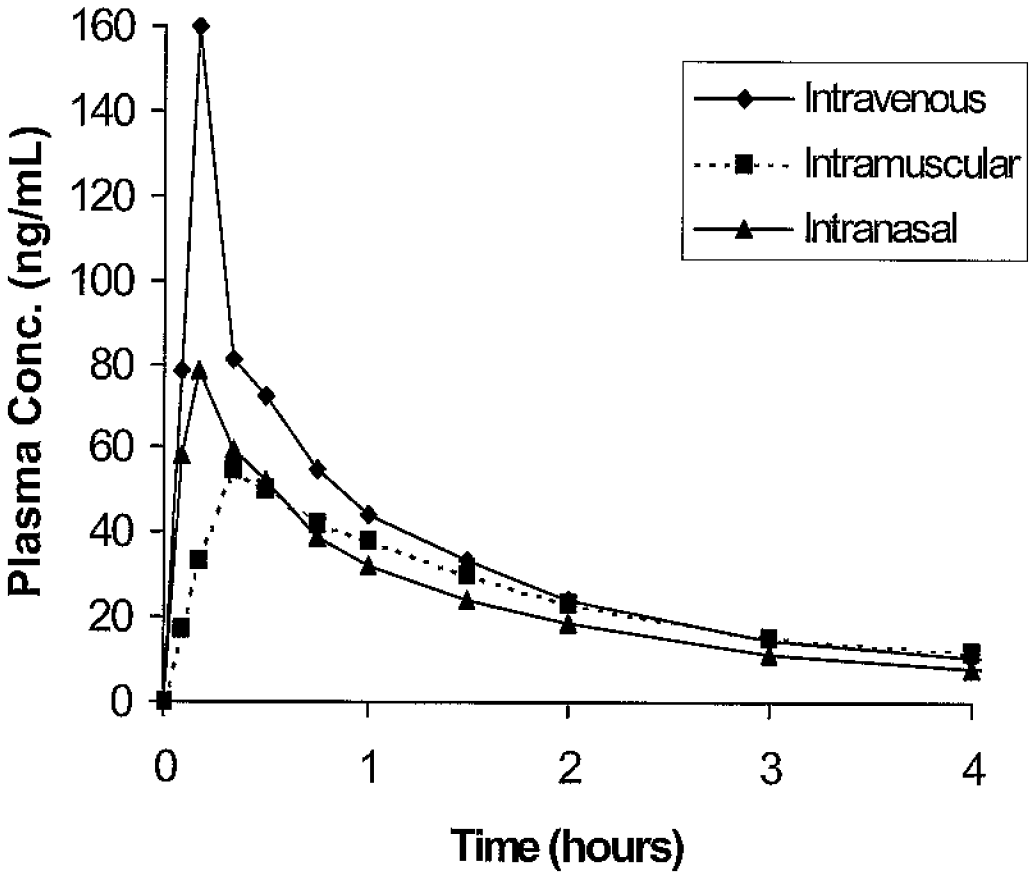


Figure 1. Mean (n=12) plasma midazolam concentration versus time profiles following 5.0 mg midazolam treatments A, B and C (IV, IM and IN, respectively).

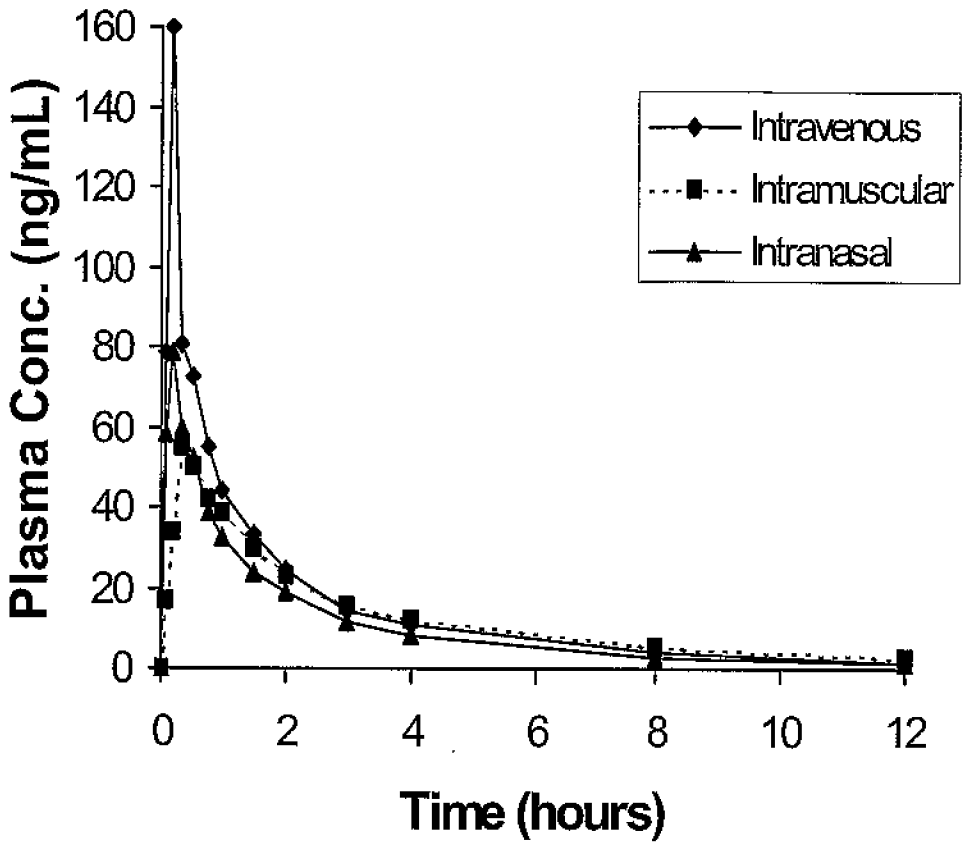


Figure 2. Mean (n=12) plasma midazolam concentration versus time profiles following 5.0 mg midazolam treatments A, B and C (IV, IM and IN, respectively).

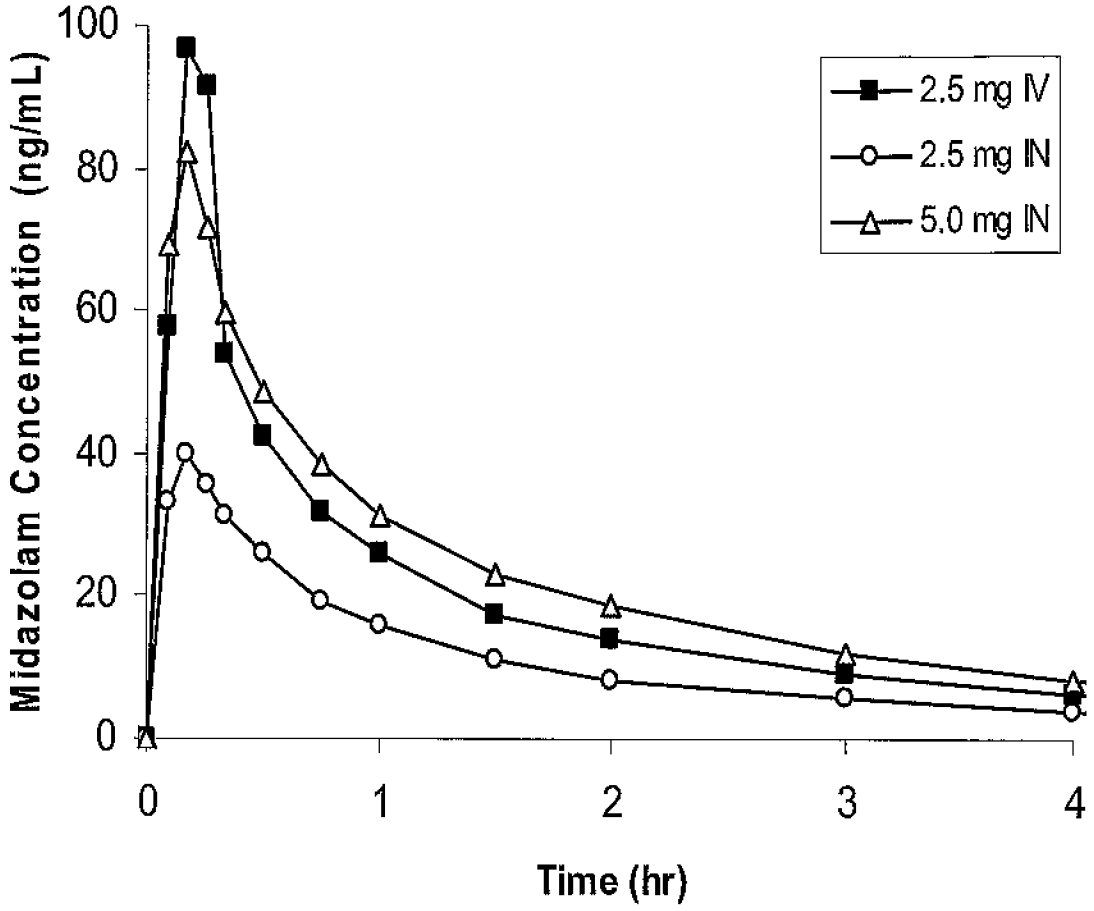


Figure 3. Mean (n=17) plasma midazolam concentration versus time profiles following treatments A, B and C (IV, IM and IN, respectively).

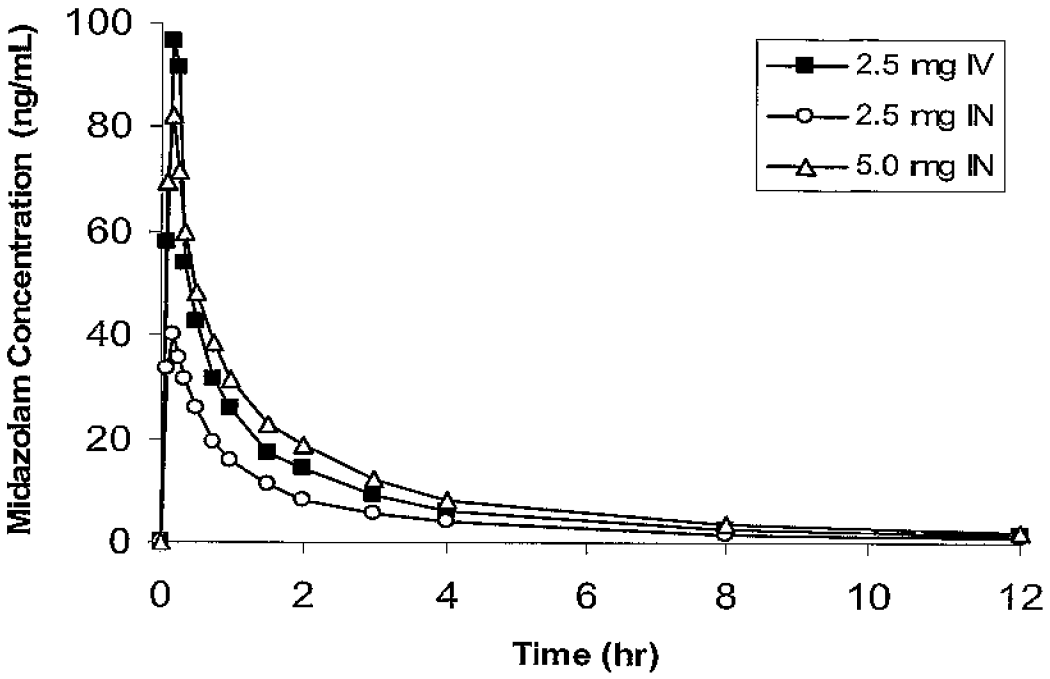


Figure 4. Mean (n=17) plasma midazolam concentration versus time profiles following treatments A, B and C

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.	9, 22, 21, 26
Y	US 5,474,759 (FASSBERG et al.), 12 December 1995 (12.12.1995), column 3, line 1 - column 4, line 50.	1-4, 8, 20-, 21, 23-26
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.	

☐ Further documents are listed in the continuation of Box C. ☐ See patent family 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
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

received by the International Bureau on 30 August 2005 (30.08.05): original claims 1-26 have been replaced by amended claims 1-20.

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 December 2005 (15.12.2005)

PCT

(10) International Publication Number
WO 2005/117830 A1

(51) International Patent Classification[7]: A61K 9/10, 9/06, 9/12

(21) International Application Number: PCT/GB2005/002217

(22) International Filing Date: 6 June 2005 (06.06.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

0412530.8	4 June 2004 (04.06.2004)	GB
0500807.3	14 January 2005 (14.01.2005)	GB
0507811.8	18 April 2005 (18.04.2005)	GB

(71) Applicant *(for all designated States except US)*: **CAMURUS AB** [SE/SE]; Ideon, Gamma 1, Sölvegatan 41, S-223 70 Lund (SE).

(71) Applicant *(for GB only)*: **GODDARD, Chris** [GB/GB]; Frank B. Dehn & Co., 179 Queen Victoria Street, London EC4V 4EL (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **THURESSON, Krister** [SE/SE]; Camurus AB, Ideon, Gamma 1, Sölvegatan 41, S-223 70 Lund (SE). **TIBERG, Fredrik** [SE/SE]; Camurus AB, Ideon, Gamma 1, Sölvegatan 41, S-223 70 Lund (SE). **JOHANSSON, Markus** [SE/SE]; Camurus AB, Ideon, Gamma 1, Sölvegatan 41, S-223 70 Lund (SE). **HARWIGSSON, Ian** [SE/SE]; Camurus AB, Ideon, Gamma 1, Sölvegatan 41, S-223 70 Lund (SE). **JOABSSON, Fredrik** [SE/SE]; Camurus AB, Ideon, Gamma 1, Sölvegatan 41, S-223 70 Lund (SE). **JOHNSSON, Markus** [SE/SE]; Camurus AB, Ideon, Gamma 1, Sölvegatan 41, S-223 70 Lund (SE).

(74) Agent: **FRANK B. DEHN & CO.**; 179 Queen Victoria Street, London EC4V 4EL (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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: LIQUID DEPOT FORMULATIONS

(57) Abstract: The present invention relates to pre-formulations comprising low viscosity, non-liquid crystalline, mixtures of: a) at least one neutral diacyl lipid and/or at least one tocopherol; b) at least one phospholipid; c) at least one biocompatible, oxygen containing, low viscosity organic solvent; wherein at least one bioactive agent is dissolved or dispersed in the low viscosity mixture and wherein the pre-formulation forms, or is capable of forming, at least one liquid crystalline phase structure upon contact with an aqueous fluid. The preformulations are suitable for generating parenteral, non-parenteral and topical depot compositions for sustained release of active agents. The invention additionally relates to a method of delivery of an active agent comprising administration of a preformulation of the invention, a method of treatment comprising administration of a preformulation of the invention and the use of a preformulation of the invention in a method for the manufacture of a medicament.

WO 2005/117830 A1

Lipid Depot Formulations

The present invention relates to formulation precursors (pre-formulations) for the *in situ* generation of controlled release lipid compositions. In particular, the invention relates to pre-formulations in the form of low viscosity mixtures (such as molecular solutions) of amphiphilic components and at least one bioactive agent which undergo at least one phase transition upon exposure to aqueous fluids, such as body fluids, thereby forming a controlled release matrix which optionally is bioadhesive.

Many bioactive agents including pharmaceuticals, nutrients, vitamins and so forth have a "functional window". That is to say that there is a range of concentrations over which these agents can be observed to provide some biological effect. Where the concentration in the appropriate part of the body (e.g. locally or as demonstrated by serum concentration) falls below a certain level, no beneficial effect can be attributed to the agent. Similarly, there is generally an upper concentration level above which no further benefit is derived by increasing the concentration. In some cases increasing the concentration above a particular level results in undesirable or even dangerous effects.

Some bioactive agents have a long biological half-life and/or a wide functional window and thus may be administered occasionally, maintaining a functional biological concentration over a substantial period of time (e.g. 6 hours to several days). In other cases the rate of clearance is high and/or the functional window is narrow and thus to maintain a biological concentration within this window regular (or even continuous) doses of a small amount are required. This can be particularly difficult where non-oral routes of administration (e.g. parenteral administration) are desirable. Furthermore, in some circumstances, such as in the fitting of implants (e.g. joint replacements or oral implants) the area of desired action may not remain accessible for repeated administration. In such cases a single administration must provide active agent at a therapeutic level over the whole period during which activity is needed.

Various method have been used and proposed for the sustained release of biologically active agents. Such methods include slow-release, orally administered compositions, such as coated tablets, formulations designed for gradual absorption,

such as transdermal patches, and slow-release implants such as "sticks" implanted under the skin.

One method by which the gradual release of a bioactive agent has been proposed is a so-called "depot" injection. In this method, a bioactive agent is formulated with carriers providing a gradual release of active agent over a period of a number of hours or days. These are often based upon a degrading matrix which gradually disperses in the body to release the active agent.

The most common of the established methods of depot injection relies upon a polymeric depot system. This is typically a biodegradable polymer such poly (lactic acid) (PLA) and/or poly (lactic-co-glycolic acid) (PLGA) and may be in the form of a solution in an organic solvent, a pre-polymer mixed with an initiator, encapsulated polymer particles or polymer microspheres. The polymer or polymer particles entrap the active agent and are gradually degraded releasing the agent by slow diffusion and/or as the matrix is absorbed. Examples of such systems include those described in US 4938763, US 5480656 and US 6113943 and can result in delivery of active agents over a period of up to several months. These systems do, however, have a number of limitations including the complexity of manufacturing and difficulty in sterilising (especially the microspheres). The local irritation caused by the lactic and/or glycolic acid which is released at the injection site is also a noticeable drawback. There is also often quite a complex procedure to prepare the injection dose from the powder precursor

From a drug delivery point of view, polymer depot compositions also have the disadvantage of accepting only relatively low drug loads and having a "burst/lag" release profile. The nature of the polymeric matrix, especially when applied as a solution or pre-polymer, causes an initial burst of drug release when the composition is first administered. This is followed by a period of low release, while the degradation of the matrix begins, followed finally by an increase in the release rate to the desired sustained profile. This burst/lag release profile can cause the *in vivo* concentration of active agent to burst above the functional window immediately following administration, then drop back through the bottom of the functional window during the lag period before reaching a sustained functional concentration. Evidently, from a functional and toxicological point of view this burst/lag release profile is undesirable and could be dangerous. It may also limit the equilibrium

concentration which can be provided due to the danger of adverse effects at the "peak" point.

Previous depot systems have been sought to address the problem of burst release. In particular, the use of hydrolysed polylactic acid and the inclusion of poly lactic acid-polyethylene glycol block copolymers have been proposed to provide the "low burst" polymeric system described in US 6113943 and US 6630115. These systems provide improved profiles but the burst/lag effect remains and they do not address other issues such as the irritation caused by the use of polymers producing acidic degradation products.

One alternative to the more established, polymer based, depot systems was proposed in US 5807573. This proposes a lipid based system of a diacylglycerol, a phospolipid and optionally water, glycerol, ethylene glycol or propylene glycol to provide an administration system in the reversed micellar "L2" phase or a cubic liquid crystalline phase. Since this depot system is formed from physiologically well tolerated diacyl glycerols and phospholipids, and does not produce the lactic acid or glycolic acid degradation products of the polymeric systems, there is less tendency for this system to produce inflammation at the injection site. The liquid crystalline phases are, however, of high viscosity and the L2 phase may also be too viscous for ease of application. The authors of US 5807573 also do not provide any *in vivo* assessment of the release profile of the formulation and thus it is uncertain whether or not a "burst" profile is provided.

The use of non-lamellar phase structures (such as liquid crystalline phases) in the delivery of bioactive agents is now relatively well established. Such structures form when an amphiphilic compound is exposed to a solvent because the amphiphile has both polar and apolar groups which cluster to form polar and apolar regions. These regions can effectively solubilise both polar and apolar compounds. In addition, many of the structures formed by amphiphiles in polar and/or apolar solvents have a very considerable area of polar/apolar boundary at which other amphiphilic compounds can be adsorbed and stabilised. Amphiphiles can also be formulated to protect active agents, to at least some extent, from aggressive biological environments, including enzymes, and thereby provide advantageous control over active agent stability and release..

The formation of non-lamellar regions in the amphiphile/water, amphiphile/oil and amphiphile/oil/water phase diagrams is a well known phenomenon. Such phases include liquid crystalline phases such as the cubic P, cubic D, cubic G and hexagonal phases, which are fluid at the molecular level but show significant long-range order, and the L3 phase which comprises a multiply interconnected bi-continuous network of bilayer sheets which are non-lamellar but lack the long-range order of the liquid crystalline phases. Depending upon their curvature of the amphiphile sheets, these phases may be described as normal (mean curvature towards the apolar region) or reversed (mean curvature towards the polar region).

The non-lamellar liquid crystalline and L3 phases are thermodynamically stable systems. That is to say, they are not simply a meta-stable state that will separate and/or reform into layers, lamellar phases or the like, but are the stable thermodynamic form of the lipid/solvent mixture.

While the effectiveness of known lipid depot formulations is high, there are certain aspects in which the performance of these is less than ideal. In particular, cubic liquid crystalline phases proposed are relatively viscous in nature. This makes application with a standard syringe difficult, and possibly painful to the patient, and makes sterilisation by filtration impossible because the composition cannot be passed through the necessary fine-pored membrane. As a result, the compositions must be prepared under highly sterile conditions, which adds to the complexity of manufacturing. Where L2 phases are used, these are generally of lower viscosity but these may still cause difficulty in application and allow access to only a small region of the phase diagram. Specifically, the solvents used in known lipid formulations have only a limited effect in reducing the viscosity of the mixture. Water, for example, will induce the formation of a highly viscous liquid crystalline phase and solvents such as glycerol and glycols have a high viscosity and do not provide any greatly advantageous decrease in the viscosity of the composition. Glycols are also typically toxic or poorly tolerated *in vivo* and can cause irritation when applied topically.

Furthermore, the known lipid compositions in the low-solvent L2 phase may support only a relatively low level of many bioactive agents because of their limited solubility in the components of the mixture in the absence of water. In the presence of water, however, the formulations adopt a highly viscous cubic liquid crystalline

phase. It would be a clear advantage to provide a depot system that could be injected at low viscosity and allowed release of the required concentration of bioactive with a smaller depot composition volume.

The known lipid depot compositions also have practical access to only certain phase structures and compositions because other mixtures are either too highly viscous for administration (such as those with high phospholipid concentrations) or run the risk of separation into two or more separate phases (such as an L2 phase in equilibrium with a phase rich in phospholipid). In particular, phospholipid concentrations above 50% are not reachable by known methods and from the phase diagram shown in US 5807573 it appears that the desired cubic phase is stable at no higher than 40% phospholipid. As a result, it has not been possible in practice to provide depot compositions of high phospholipid concentration or having a hexagonal liquid crystalline phase structure.

The present inventors have now established that by providing a pre-formulation comprising certain amphiphilic components, at least one bioactive agent and a biologically tolerable solvent, especially in a low viscosity phase such as molecular solution, the pre-formulation may be generated addressing many of the shortfalls of previous depot formulations. In particular, the pre-formulation is easy to manufacture, may be sterile-filtered, it has low viscosity (allowing easy and less painful administration), allows a high level of bioactive agent to be incorporated (thus allowing a smaller amount of composition to be used) and/or forms a desired non-lamellar depot composition *in vivo* having a controllable "burst" or "non-burst" release profile. The compositions are also formed from materials that are non-toxic, biotolerable and biodegradable. Furthermore, the pre-formulation is suitable for the formation of depot compositions following parenteral administration and also following non-parenteral (e.g. topical) administration to body cavities and/or surfaces of the body or elsewhere.

In a first aspect, the present invention thus provides a pre-formulation comprising a low viscosity mixture of:

a) at least one neutral diacyl lipid and/or a tocopherol;

b) at least one phospholipid;

c) at least one biocompatible, (preferably oxygen containing) organic solvent;

wherein at least one bioactive agent is dissolved or dispersed in the low viscosity mixture and wherein the pre-formulation forms, or is capable of forming, at least one liquid crystalline phase structure upon contact with an aqueous fluid.

Generally, the aqueous fluid will be a body fluid such as fluid from a mucosal surface, tears, sweat, saliva, gastro-intestinal fluid, extra-vascular fluid, extracellular fluid, interstitial fluid or plasma, and the pre-formulation will form a liquid crystalline phase structure when contacted with a body surface, area or cavity (e.g. *in vivo*) upon contact with the aqueous body fluid. The pre-formulation of the invention will generally not contain any significant quantity of water prior to administration.

In a second aspect of the invention, there is also provided a method of delivery of a bioactive agent to a human or non-human animal (preferably mammalian) body, this method comprising administering (preferably parenterally) a pre-formulation comprising a low viscosity mixture of:

a) at least one neutral diacyl lipid and/or a tocopherol;

b) at least one phospholipid;

c) at least one biocompatible, (preferably oxygen containing) organic solvent;

and at least one bioactive agent is dissolved or dispersed in the low viscosity mixture, whereby to form at least one liquid crystalline phase structure upon contact with an aqueous fluid *in vivo* following administration. Preferably, the pre-formulation administered in such a method is a pre-formulation of the invention as described herein.

The method of administration suitable for the above method of the invention will be a method appropriate for the condition to be treated and the bioactive agent used. A parenteral depot will thus be formed by parenteral (e.g. subcutaneous or intramuscular) administration while a bioadhesive non-parenteral (e.g. topical) depot composition may be formed by administration to the surface of skin, mucous membranes and/or nails, to opthalmological, nasal, oral or internal surfaces or to cavities such as nasal, rectal, vaginal or buccal cavities, the periodontal pocket or cavities formed following extraction of a natural or implanted structure or prior to insertion of an implant (e.g a joint, stent, cosmetic implant, tooth, tooth filling or other implant).

In a further aspect, the present invention also provides a method for the preparation of a liquid crystalline composition (especially a depot composition) comprising exposing a pre-formulation comprising a low viscosity mixture of:

a) at least one neutral diacyl lipid and/or a tocopherol;

b) at least one phospholipid;

c) at least one biocompatible (preferably oxygen containing), organic solvent;

and at least one bioactive agent dissolved or dispersed in the low viscosity mixture, to an aqueous fluid (particularly *in vivo and/or particularly a body fluid as indicated herein*). Preferably the pre-formulation administered is a pre-formulation of the present invention as described herein. The exposure to a fluid "in vivo" may evidently be internally within the body or a body cavity, or may be at a body surface such as a skin surface, depending upon the nature of the composition.

The liquid crystalline composition formed in this method is preferably bioadhesive as described herein.

In a still further aspect the present invention provides a process for the formation of a pre-formulation suitable for the administration of a bioactive agent to a (preferably mammalian) subject, said process comprising forming a low viscosity mixture of

a) at least one neutral diacyl lipid and/or a tocopherol;

b) at least one phospholipid;

c) at least one biocompatible (preferably oxygen containing), organic solvent;

and dissolving or dispersing at least one bioactive agent in the low viscosity mixture, or in at least one of components a, b or c prior to forming the low viscosity mixture. Preferably the pre-formulation so-formed is a formulation of the invention as described herein.

In a yet still further aspect the present invention provides the use of a low viscosity mixture of:

a) at least one neutral diacyl lipid and/or a tocopherol;

b) at least one phospholipid;

c) at least one biocompatible (preferably oxygen containing), organic solvent;

wherein at least one bioactive agent is dissolved or dispersed in the low viscosity mixture in the manufacture of a pre-formulation for use in the sustained administration of said active agent, wherein said pre-formulation is capable of

forming at least one liquid crystalline phase structure upon contact with an aqueous fluid.

As used herein, the term "low viscosity mixture" is used to indicate a mixture which may be readily administered to a subject and in particular readily administered by means of a standard syringe and needle arrangement. This may be indicated, for example by the ability to be dispensed from a 1 ml disposable syringe through a 22 awg (or a 23 gauge) needle by manual pressure. In a particularly preferred embodiment, the low viscosity mixture should be a mixture capable of passing through a standard sterile filtration membrane such as a 0.22 μm syringe filter. In other preferred embodiments, a similar functional definition of a suitable viscosity can be defined as the viscosity of a pre-formulation that can be sprayed using a compression pump or pressurized spray device using conventional spray equipment. A typical range of suitable viscosities would be, for example, 0.1 to 5000 mPas, preferably 1 to 1000 mPas at 20°C.

It has been observed that by the addition of small amounts of low viscosity solvent, as indicated herein, a very significant change in viscosity can be provided. As indicated in Figure 2, for example, the addition of only 5% solvent can reduce viscosity 100-fold and addition of 10% may reduce the viscosity up to 10,000 fold. In order to achieve this non-linear, synergistic effect, in lowering viscosity it is important that a solvent of appropriately low viscosity and suitable polarity be employed. Such solvents include those described herein infra.

Particularly preferred examples of low viscosity mixtures are molecular solutions and/or isotropic phases such as L2 and/or L3 phases. As describe above, the L3 is a non-lamellar phase of interconnected sheets which has some phase structure but lacks the long-range order of a liquid crystalline phase. Unlike liquid crystalline phases, which are generally highly viscous, L3 phases are of lower viscosity. Obviously, mixtures of L3 phase and molecular solution and/or particles of L3 phase suspended in a bulk molecular solution of one or more components are also suitable. The L2 phase is the so-called "reversed micellar" phase or microemulsion. Most preferred low viscosity mixtures are molecular solutions, L3 phases and mixtures thereof. L2 phases are less preferred, except in the case of swollen L_2 phases as described below.

The present invention provides a pre-formulation comprising components a, b, c and at least one bioactive agent as indicated herein. One of the considerable advantages of the pre-formulations of the invention is that components a and b may be formulated in a wide range of proportions. In particular, it is possible to prepare and use pre-formulations of the present invention having a much greater proportion of phospholipid to neutral, diacyl lipid and/or tocopherol than was previously achievable without risking phase separation and/or unacceptably high viscosities in the pre-formulation. The weight ratios of components a:b may thus be anything from 5:95 right up to 95:5. Preferred ratios would generally be from 90:10 to 20:80 and more preferably from 85:15 to 30:70. In one preferred embodiment of the invention, there is a greater proportion of component b than component a. That is, the weight ratio a:b is below 50:50, e.g. 48:52 to 2:98, preferably, 40:60 to 10:90 and more preferably 35:65 to 20:80.

The amount of component c in the pre-formulations of the invention will be at least sufficient to provide a low viscosity mixture (e.g. a molecular solution, see above) of components a, b and c and will be easily determined for any particular combination of components by standard methods. The phase behaviour itself may be analysed by techniques such as visual observation in combination with polarized light microscopy, nuclear magnetic resonance, and cryo-transmission electron microscopy (cryo-TEM) to look for solutions, L2 or L3phases, or liquid crystalline phases. Viscosity may be measured directly by standard means. As described above, an appropriate practical viscosity is that which can effectively be syringed and particularly sterile filtered. This will be assessed easily as indicated herein. The maximum amount of component c to be included will depend upon the exact application of the pre-formulation but generally the desired properties will be provided by any amount forming a low viscosity mixture (e.g. a molecular solution, see above) and/or a solution with sufficiently low viscosity. Since the administration of unnecessarily large amounts of solvent to a subject is generally undesirable the amount of component c will typically be limited to no more than ten times (e.g. three times) the minimum amount required to form a low viscosity mixture, preferably no more than five times and most preferably no more than twice this amount. The composition of the present invention may, however, contain a greater quantity of solvent than would be acceptable in an immediate dosage composition. This is because the process by which the active agents are slowly released (e.g. formation of shells of liquid crystalline phase se described herein) also

serve to retard the passage of solvent from the composition. As a result, the solvent is released over some time (e.g. minutes or hours) rather than instantaneously and so can be better tolerated by the body.

Higher proportions of solvent may also be used for non-parenteral (e.g. topical) applications, especially to body surfaces, where the solvent will be lost by evaporation rather than absorbed into the body. For such applications up to 100 times the minimum amount of solvent may be used (e.g. up to 95% by weight of the composition, preferably up to 80% by weight and more preferably up to 50% by weight), especially where a very thin layer of the resulting non-parenteral depot is desired.

Where the compositions of the invention are formulated as (non-parenteral) aerosol spray compositions (e.g. for topical or systemic delivery of an active), the composition may also comprise a propellant. Such compositions may also include a high proportion of solvent component c), as considered above, since much of the solvent will evaporate when the composition is dispensed.

Suitable propellants are volatile compounds which will mix with the composition of the invention under the pressure of the spray dispenser, without generating high viscosity mixtures. They should evidently have acceptable biocompatibility. Suitable propellants will readily be identified by simple testing and examples include hydrocarbons (especially C_1 to C_4 hydrocarbons) , carbon dioxide and nitrogen. Volatile hydrofluorocarbons such as HFCs 134, 134a, 227ea and/or 152a may also be suitable.

As a general guide, the weight of component c will typically be around 0.5 to 50% of the total weight of the a-b-c solution. This proportion is preferably (especially for injectable depots) 2 to 30% and more preferably 5 to 20% by weight.

Component "a" as indicated herein is a neutral lipid component comprising a polar "head" group and also non-polar "tail" groups. Generally the head and tail portions of the lipid will be joined by an ester moiety but this attachment may be by means of an ether, an amide, a carbon-carbon bond or other attachment. Preferred polar head groups are non-ionic and include polyols such as glycerol, diglycerol and sugar moieties (such as inositol and glucosyl based moieties); and esters of polyols, such

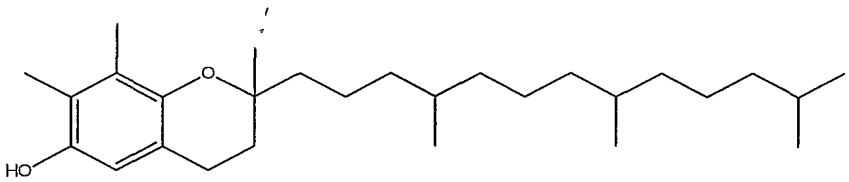
as acetate or succinate esters. Preferred polar groups are glycerol and diglycerol, especially glycerol.

In one preferred aspect, component a is a diacyl lipid in that it has two non-polar "tail" groups. This is generally preferable to the use of mono-acyl ("lyso") lipids because these are typically less well tolerated *in vivo*. The two non-polar groups may have the same or a differing number of carbon atoms and may each independently be saturated or unsaturated. Examples of non-polar groups include C_6-C_{32} alkyl and alkenyl groups, which are typically present as the esters of long chain carboxylic acids. These are often described by reference to the number of carbon atoms and the number of unsaturations in the carbon chain. Thus, CX:Z indicates a hydrocarbon chain having X carbon atoms and Z unsaturations. Examples particularly include caproyl (C6:0), capryloyl (C8:0), capryl (C10:0), lauroyl (C12:0), myristoyl (C14:0), palmitoyl (C16:0), phytanoly (C16:0), palmitoleoyl (C16:1), stearoyl (C18:0), oleoyl (C18:1), elaidoyl (C18:1), linoleoyl (C18:2), linolenoyl (C18:3), arachidonoyl (C20:4), behenoyl (C22:0) and lignoceroyl (C24:9) groups. Thus, typical non-polar chains are based on the fatty acids of natural ester lipids, including caproic, caprylic, capric, lauric, myristic, palmitic, phytanic, palmitolic, stearic, oleic, elaidic, linoleic, linolenic, arachidonic, behenic or lignoceric acids, or the corresponding alcohols. Preferable non-polar chains are palmitic, stearic, oleic and linoleic acids, particularly oleic acid.

The diacyl lipid, when used as all or part of component "a", may be synthetic or may be derived from a purified and/or chemically modified natural sources such as vegetable oils. Mixtures of any number of diacyl lipids may be used as component a. Most preferably this component will include at least a portion of diacyl glycerol (DAG), especially glycerol dioleate (GDO). In one favoured embodiment, component a consists of DAGs. These may be a single DAG or a mixture of DAGs. A highly preferred example is DAG comprising at least 50%, preferably at least 80% and even comprising substantially 100% GDO.

An alternative or additional highly preferred class of compounds for use as all or part of component a are tocopherols. As used herein, the term "a tocopherol" is used to indicate the non-ionic lipid tocopherol, often known as vitamin E, and/or any suitable salts and/or analogues thereof. Suitable analogues will be those providing the phase-behaviour, lack of toxicity, and phase change upon exposure to aqueous

fluids, which characterise the compositions of the present invention. Such analogues will generally not form liquid crystalline phase structures as a pure compound in water. The most preferred of the tocopherols is tocopherol itself, having the structure below. Evidently, particularly where this is purified from a natural source, there may be a small proportion of non-tocopherol "contaminant" but this will not be sufficient to alter the advantageous phase-behaviour or lack of toxicity. Typically, a tocopherol will contain no more than 10% of non-tocopherol - analogue compounds, preferably no more than 5% and most preferably no more than 2% by weight.



Tocopherol

In a further advantageous embodiment of the invention, component a) consists essentially of tocopherols, in particular tocopherol as shown above.

A preferred combination of constituents for component a) is a mixture of at least one DAG (e.g. GDO) with at least one tocopherol. Such mixtures include 2:98 to 98:2 by weight tocopherol:GDO, e.g.10:90 to 90:10 tocopherol:GDO and especially 20:80 to 80:20 of these compounds. Similar mixtures of tocopherol with other DAGs are also suitable.

Component "b" in the present invention is at least one phospholipid. As with component a, this component comprises a polar head group and at least one non-polar tail group. The difference between components a and b lies principally in the polar group. The non-polar portions may thus suitably be derived from the fatty acids or corresponding alcohols considered above for component a. It will typically be the case that the phospholipid will contain two non-polar groups, although one or more constituents of this component may have one non-polar moiety. Where more than one non-polar group is present these may be the same or different.

Preferred phospholipid polar "head" groups include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol. Most preferred is phosphatidylcholine (PC). In a preferred embodiment, component b)

thus consists of at least 50% PC, preferably at least 70% PC and most preferably at least 80% PC. Component b) may consist essentially of PC.

The phospholipid portion, even more suitably than any diacyl lipid portion, may be derived from a natural source. Suitable sources of phospholipids include egg, heart (e.g. bovine), brain, liver (e.g. bovine) and plant sources including soybean. Such sources may provide one or more constituents of component b, which may comprise any mixture of phospholipids.

Since the pre-formulations of the invention are to be administered to a subject for the controlled release of an active agent, it is preferable that the components a and b are biocompatible. In this regard, it is preferable to use, for example, diacyl lipids and phospholipids rather than mono-acyl (lyso) compounds. A notable exception to this is tocopherol, as described above. Although having only one alkyl chain, this is not a "lyso" lipid in the convention sense. The nature of tocopherol as a well tolerated essential vitamin evidently makes it highly suitable in biocompatibility.

It is furthermore most preferable that the lipids and phospholipids of components a and b are naturally occurring (whether they are derived from a natural source or are of synthetic origin). Naturally occurring lipids tend to cause lesser amounts of inflammation and reaction from the body of the subject. Not only is this more comfortable for the subject but it may increase the residence time of the resulting depot composition, especially for parenteral depots, since less immune system activity is recruited to the administration site. In certain cases it may, however, be desirable to include a portion of a non-naturally-occurring lipid in components a and/or b. This might be, for example an "ether lipid" in which the head and tail groups are joined by an ether bond rather than an ester. Such non-naturally-occurring lipids may be used, for example, to alter the rate of degradation of the resulting depot-composition by having a greater or lesser solubility or vulnerability to breakdown mechanisms present at the site of active agent release. Although all proportions fall within the scope of the present invention, generally, at least 50% of each of components a and b will be naturally occurring lipids. This will preferably be at least 75% and may be up to substantially 100%.

Two particularly preferred combinations of components a and b are GDO with PC and tocopherol with PC, especially in the region 30-90wt% GDO/tocopherol, 10-60 wt% PC and 1-30% solvent (especially ethanol, NMP and/or ispropanol).

In addition to amphiphilic components a and b, the pre-formulations of the invention may also contain additional amphiphilic components at relatively low levels. In one embodiment of the invention, the pre-formulation contains up to 10% (by weight of components a and b) of a charged amphiphile, particularly an anionic amphiphile such as a fatty acid. Preferred fatty acids for this purpose include caproic, caprylic, capric, lauric, myristic, palmitic, phytanic, palmitolic, stearic, oleic, elaidic, linoleic, linolenic, arachidonic, behenic or lignoceric acids, or the corresponding alcohols. Preferable fatty acids are palmitic, stearic, oleic and linoleic acids, particularly oleic acid. It is particularly advantageous that this component be used in combination with a cationic peptide active agent (see below). The combination of an anionic lipid and a cationic peptide is believed to provide a sustained release composition of particular value. This may in part be due to increased protection of the peptide from the degradative enzymes present *in vivo*.

Component "c" of the pre-formulations of the invention is an oxygen containing organic solvent. Since the pre-formulation is to generate a depot composition following administration (e.g. *in vivo),* upon contact with an aqueous fluid, it is desirable that this solvent be tolerable to the subject and be capable of mixing with the aqueous fluid, and/or diffusing or dissolving out of the pre-formulation into the aqueous fluid. Solvents having at least moderate water solubility are thus preferred.

In a preferred version, the solvent is such that a relatively small addition to the composition comprising a and b, i.e. below 20%, or more preferably below 10%, give a large viscosity reductions of one order of magnitude or more. As described herein, the addition of 10% solvent can give a reduction of two, three or even four orders of magnitude in viscosity over the solvent-free composition, even if that composition is a solution or L_2 phase containing no solvent, or an unsuitable solvent such as water (subject to the special case considered below), or glycerol.

Typical solvents suitable for use as component c include at least one solvent selected from alcohols, ketones, esters (including lactones), ethers, amides and sulphoxides. Examples of suitable alcohols include ethanol, isopropanol and glycerol formal.

Monools are preferred to diols and polyols. Where diols or polyols are used, this is preferably in combination with an at least equal amount of monool or other preferred solvent. Examples of ketones include acetone, n-methyl pyrrolidone (NMP), 2-pyrrolidone, and propylene carbonate. Suitable ethers include diethylether, glycofurol, diethylene glycol monoethyl ether, dimethylisobarbide, and polyethylene glycols. Suitable esters include ethyl acetate and isopropyl acetate and dimethyl sulphide is as suitable sulphide solvent. Suitable amides and sulphoxides include dimethylacetamide (DMA) and dimethylsulphoxide (DMSO), respectively. Less preferred solvents include dimethyl isosorbide, tetrahydrofurfuryl alcohol, diglyme and ethyl lactate.

Since the pre-formulations are to be administered to a living subject, it is necessary that the solvent component c is sufficiently biocompatible. The degree of this biocompatibility will depend upon the application method and since component c may be any mixture of solvents, a certain amount of a solvent that would not be acceptable in large quantities may evidently be present. Overall, however, the solvent or mixture forming component c must not provoke unacceptable reactions from the subject upon administration. Generally such solvents will be hydrocarbons or preferably oxygen containing hydrocarbons, both optionally with other substituents such as nitrogen containing groups. It is preferable that little or none of component c contains halogen substituted hydrocarbons since these tend to have lower biocompatibility. Where a portion of halogenated solvent such as dichloromethane or chloroform is necessary, this proportion will generally be minimised. Where the depot composition is to be formed non-parenterally a greater range of solvents may evidently be used than where the depot is to be parenteral.

Component c as used herein may be a single solvent or a mixture of suitable solvents but will generally be of low viscosity. This is important because one of the key aspects of the present invention is that it provides preformulations that are of low viscosity and a primary role of a suitable solvent is to reduce this viscosity. This reduction will be a combination of the effect of the lower viscosity of the solvent and the effect of the molecular interactions between solvent and lipid composition. One observation of the present inventors is that the oxygen-containing solvents of low viscosity described herein have highly advantageous and unexpected molecular interactions with the lipid parts of the composition, thereby providing a non-linear reduction in viscosity with the addition of a small volume of solvent.

The viscosity of the "low viscosity" solvent component c (single solvent or mixture) should typically be no more than 18 mPas at 20°C. This is preferably no more than 15 mPas, more preferably no more than 10 mPas and most preferably no more than 7 mPas at 20°C.

The solvent component c will generally be at least partially lost upon *in vivo* formation of the depot composition, or diluted by absorption of water from the surrounding air and/or tissue. It is preferable, therefore, that component c be at least to some extent water miscible and/or dispersible and at least should not repel water to the extent that water absorption is prevented. In this respect also, oxygen containing solvents with relatively small numbers of carbon atoms (for example up to 10 carbons, preferably up to 8 carbons) are preferred. Obviously, where more oxygens are present a solvent will tend to remain soluble in water with a larger number of carbon atoms. The carbon to heteroatom (e.g. N, O, preferably oxygen) ratio will thus often be around 1:1 to 6:1, preferably 2:1 to 4:1. Where a solvent with a ratio outside one of these preferred ranges is used then this will preferably be no more than 75%, preferably no more than 50%, in combination with a preferred solvent (such as ethanol). This may be used, for example to decrease the rate of evaporation of the solvent from the pre-formulation in order to control the rate of liquid crystalline depot formation.

A further advantage of the present pre-formulations is that a higher level of bioactive agent may be incorporated into the system. In particular, by appropriate choice of components a-c (especially c), high levels of active agent may be dissolved or suspended in the pre-formulations. Generally, the lipid components in the absence of water are relatively poorly solubilising but in the presence of water form phases too viscous to administer easily. Higher proportions of bioactive agent may be included by use of appropriate solvents as component c and this level will either dissolve in the depot composition as it forms *in situ* or may form microdrops or microcrystals which will gradually dissolve and release active agent. A suitable choice of solvent will be possible by routine experimentation within the guidelines presented herein.

The pre-formulations of the present invention typically do not contain significant amounts of water. Since it is essentially impossible to remove every trace of water

from a lipid composition, this is to be taken as indicating that only such minimal trace of water exists as cannot readily be removed. Such an amount will generally be less than 1% by weight, preferably less that 0.5% by the weight of the pre-formulation. In one preferred aspect, the pre-formulations of the invention do not contain glycerol, ethylene glycol or propylene glycol and contain no more than a trace of water, as just described.

There is, however, a certain embodiment of the present invention in which higher proportions of water may be tolerated. This is where water is present as a part of the solvent component in combination with an additional water-miscible component c (single solvent or mixture). In this embodiment, up to 10 wt% water may be present providing that at least 3 wt%, preferably at least 5% and more preferably at least 7 wt% component c is also present, that component c is water miscible, and that the resulting preformulation remains non-viscous and thus does not form a liquid crystalline phase. Generally there will be a greater amount of component c) by weight than the weight of water included in the preformulation. Most suitable solvents of use with water in this aspect of the invention include ethanol, isopropyl alcohol, NMP, acetone and ethyl acetate.

The pre-formulations of the present invention contain one or more bioactive agents (described equivalently as "active agents" herein). Active agents may be any compound having a desired biological or physiological effect, such as a protein, drug, antigen, nutrient, cosmetic, fragrance, flavouring, diagnostic, pharmaceutical, vitamin, or dietary agent and will be formulated at a level sufficient to provide an *in vivo* concentration at a functional level (including local concentrations for topical compositions). Under some circumstances one or more of components a, b and/or c may also be an active agent, although it is preferred that the active agent should not be one of these components. Most preferred active agents are pharmaceutical agents including drugs, vaccines, and diagnostic agents.

Drug agents that may be delivered by the present invention include drugs which act on cells and receptors, peripheral nerves, adrenergic receptors, cholinergic receptors, the skeletal muscles, the cardiovascular system, smooth muscles, the blood circulation system, endocrine and hormone system, blood circulatory system, synoptic sites, neuroeffector junctional sites, the immunological system, the

reproductive system, the skeletal system, autacoid system, the alimentary and excretory systems, the histamine system, and the central nervous system.

Examples of drugs which may be delivered by the composition of the present invention include, but are not limited to, antibacterial agents such as β-lactams or macrocyclic peptide antibiotics, anti fungal agents such as polyene macrolides (e.g. amphotericin B) or azole antifungals, anticancer and/or anti viral drugs such as nucleoside analogues, paclitaxel and derivatives thereof, anti inflammatorys, such as non-steroidal anti inflammatory drugs and corticosteroids, cardiovascular drugs including cholesterol lowering and blood-pressure lowing agents, analgesics, antipsychotics and antidepressants including seritonin uptake inhibitors, prostaglandins and derivatives, vaccines, and bone modulators. Diagnostic agents include radionuclide labelled compounds and contrast agents including X-ray, ultrasound and MRI contrast enhancing agents. Nutrients include vitamins, coenzymes, dietary supplements etc.

Particularly suitable active agents include those which would normally have a short residence time in the body due to rapid breakdown or excretion and those with poor oral bioavailability. These include peptide, protein and nucleic acid based active agents, hormones and other naturally occurring agents in their native or modified forms. By administering such agents in the form of a depot composition formed from the pre-formulation of the present invention, the agents are provided at a sustained level for a length of time which may stretch to days, weeks or even several months in spite of having rapid clearance rates. This offers obvious advantages in terms of stability and patient compliance over dosing multiple times each day for the same period. In one preferred embodiment, the active agent thus has a biological half life (upon entry into the blood stream) of less than 1 day, preferably less than 12 hours and more preferably less than 6 hours. In some cases this may be as low as 1-3 hours or less. Suitable agents are also those with poor oral bioavailability relative to that achieved by injection, for where the active agent also or alternatively has a bioavailability of below 0.1%, especially below 0.05% in oral formulations.

Peptide and protein based active agents include human and veterinary drugs selected from the group consisting of adrenocorticotropic hormone (ACTH) and its fragments, angiotensin and its related peptides, antibodies and their fragments, antigens and their fragments, atrial natriuretic peptides, bioadhesive peptides,

Bradykinins and their related peptides, calcitonins and their related peptides, cell surface receptor protein fragments, chemotactic peptides, cyclosporins, cytokines, Dynorphins and their related peptides, endorphins and P-lidotropin fragments, enkephalin and their related proteins, enzyme inhibitors, immunostimulating peptides and polyaminoacids, fibronectin fragments and their related peptides, gastrointestinal peptides, gonadotrophin-releasing hormone (GnRH) agonists and antagonist, glucagons like peptides, growth hormone releasing peptides, immunostimulating peptides, insulins and insulin-like growth factors, interleukins, luthenizing hormone releasing hormones (LHRH) and their related peptides, melanocyte stimulating hormones and their related peptides, nuclear localization signal related peptides, neurotensins and their related peptides, neurotransmitter peptides, opioid peptides, oxytocins, vasopressins and their related peptides, parathyroid hormone and its fragments, protein kinases and their related peptides, somatostatins and their related peptides, substance P and its related peptides, transforming growth factors (TGF) and their related peptides, tumor necrosis factor fragments, toxins and toxoids and functional peptides such as anticancer peptides including angiostatins, antihypertension peptides, anti-blood clotting peptides, and antimicrobial peptides; selected from the group consisting of proteins such as immunoglobulins, angiogenins, bone morphogenic proteins, chemokines, colony stimulating factors (CSF), cytokines, growth factors, interferons (Type I and II), interleukins, leptins, leukaemia inhibitory factors, stem cell factors, transforming growth factors and tumor necrosis factors.

A further considerable advantage of the depot compositions of the present invention is that active agents are released gradually over long periods without the need for repeated dosing. The composition are thus highly suitable for situations where patient compliance is difficult, unreliable or where a level dosage is highly important, such as mood-altering actives, those actives with a narrow therapeutic window, and those administered to children or to people who's lifestyle is incompatible with a reliable dosing regime. Also for "lifestyle" actives where the inconvenience of repeated dosing might outweigh the benefit of the active. Particular classes of actives for which this aspect offers a particular advantage include contraceptives, hormones including contraceptive hormones, and particularly hormones used in children such as growth hormone, anti-addictive agents, supplements such as vitamin or mineral supplements, anti-depressants and anticonvulsants

Cationic peptides are particularly suitable for use where a portion of the pre-formulation comprises an anionic amphiphile such as a fatty acid. In this embodiment, preferred peptides include octreotide, lanreotide, calcitonin, oxytocin, interferon-beta and -gamma, interleukins 4, 5, 7 and 8 and other peptides having an isoelectric point above pH 7, especially above pH 8.

In one preferred aspect of the present invention, the composition of the invention is such that an I_2 phase, or a mixed phase including I_2 phase is formed upon exposure to aqueous fluids and a polar active agent is included in the composition. Particularly suitable polar active agents include peptide and protein actives, oligo nucleotides, and small water soluble actives, including those listed above. Of particular interest in this aspect are the peptide octreotide and other somatostatin related peptides, interferons alpha and beta, glucagon-like peptides 1 and 2, luprorelin and other GnRH agonist, abarelix and other GnRH antagonists, interferon alpha and beta, zolendronate and ibandronate and other bisphosponates, and polar active chlorhexidine (e.g. chlorhexidine digluconate or chlorhexidine dihydrochloride).

A particular advantage of the present invention when used in combination with protein / peptide active agents is that aggregation of the active agent is suppressed. In one preferred embodiment, the present invention thus provides a depot precursor and particularly a depot composition as described herein comprising at least one peptide (e.g. antibody) or protein active agent wherein no more than 5% of the active agent is in aggregated form. Preferably no more than 3% is aggregated and most preferably no more than 2% (especially less than 2%) is in aggregated form. This stabilisation of non-aggregated protein is highly advantageous from the point of view of high effectiveness, low side effects and predictable absorption profile. Furthermore, it is increasingly expected that protein / peptide therapeutics will have low levels of protein aggregation in order to secure regulatory approval.

The amount of bioactive agent to be formulated with the pre-formulations of the present invention will depend upon the functional dose and the period during which the depot composition formed upon administration is to provide sustained release. Typically, the dose formulated for a particular agent will be around the equivalent of the normal daily dose multiplied by the number of days the formulation is to provide release. Evidently this amount will need to be tailored to take into account any adverse effects of a large dose at the beginning of treatment and so this will

generally be the maximum dose used. The precise amount suitable in any case will readily be determined by suitable experimentation.

In one embodiment, the pre-formulations of the present invention will generally be administered parenterally. This administration will generally not be an intravascular method but will preferably be subcutaneous intracavitary or intramuscular. Typically the administration will be by injection, which term is used herein to indicate any method in which the formulation is passed through the skin, such as by needle, catheter or needle-less injector.

In parenteral (especially sub cutaneous) depot precursors, preferred active agents are those suitable for systemic administration including antibacterials (including amicacin, monocycline anddoxycycline), local and systemic anagesics (including bupivacain, tramadol, fentanyl, morphine, hydromorphone, methadone, oxycodone, codeine, asperine, acetaminophen), NSAIDS (such as ibuprofene, naproxene, keteprofene, indomethansine, sulindac, tolmethin, salysylic acids such as salisylamide, diflunisal), Cox1 or Cox2 inhibitors (such as celecoxib, rofecoxib, valdecoxib) anticancer agents (including octreotide, lanreotide, buserelin, luprorelin, goserelin, triptorelin, avorelin, deslorein, abarelix, degarelix, fulvestrant, interferon alpha, interferon beta, darbepoetin alpha, epoetin alpha, beta, delta, and paclitaxel), antipsychotics (like bromperidol, risperidone, olanzapine, iloperidone, paliperadone, pipotiazine and zuclopenthixol), antivirals, anticonvulsants (for instance tiagabine topiramate or gabapentin) or nicotine, hormones (such as testosterone, and testosterone undecanoate, medroxyprogesterone, estradiol) growth hormones (like human growth hormone), and growth factors (like granulocyte macrophage colony-stimulating factor)

In an alternative embodiment, the formulations of the present invention may form non-parenteral depots where the active agent is slowly released at a body surface. It is especially important in this embodiment that the pre-formulations of the invention and/or the liquid crystalline depot compositions formed therefrom should preferably be bioadhesive. That is to say that the compositions should coat the surface to which they are applied and/or upon which they form as appropriate and should remain even when this surface is subject to a flow of air or liquid and/or rubbing. It is particularly preferable that the liquid crystalline depot compositions formed should be stable to rinsing with water. For example, a small volume of depot

precursor may be applied to a body surface and be exposed to a flow of five hundred times its own volume of water per minute for 5 minutes. After this treatment, the composition can be considered bioadhesive if less than 50% of the bioactive agent has been lost. Preferably this level of loss will be matched when water equalling 1000 times and more preferably 10 000 times the volume of the composition is flowed past per minute for five, or preferably 10, minutes.

Although the non-parenteral depot compositions of the present invention may absorb some or all of the water needed to form a liquid crystalline phase structure from the biological surfaces with which they are contacted, some additional water may also be absorbed from the surrounding air. In particular, where a thin layer of high surface area is formed then the affinity of the composition for water may be sufficient for it to form a liquid crystalline phase structure by contact with the water in the air. The "aqueous fluid" are referred to herein is thus, at least partially, air containing some moisture in this embodiment.

Non-parenteral depot compositions will typically be generated by applying the pre-formulation topically to a body surface or to a natural or artificially generated body cavity and/or to the surface of an implant. This application may be by direct application of liquid such as by spraying, dipping, rinsing, application from a pad or ball roller, intra-cavity injection (e.g to an open cavity with or without the use of a needle), painting, dropping (especially into the eyes) and similar methods. A highly effective method is aerosol or pump spraying and evidently this requires that the viscosity of the pre-formulation be as low as possible and is thus highly suited to the compositions of the invention. Non-parenteral depots may, however, be used to administer systemic agents e.g. transmucosally or transdermally.

Non-parenteral depots may also be used for application to surfaces, particularly of implants and materials which will be in contact with the body or a body part or fluid. Devices such as implants, catheters etc. may thus be treated e.g. by dipping or spraying with the preformulations of the invention, which will form a robust layer to reduce the introduction of infection. Anti-infective actives are particularly suited to this aspect.

Conditions particularly suitable for causative or symptomatic treatment by topical bioadhesive depot compositions of the present invention include skin conditions

(such as soreness resulting from any cause including chapping, scratching and skin conditions including eczema and herpes) eye conditions, genital soreness (including that due to genital infection such as genital herpes), infections and conditions for the finger and/or toe nails (such as bacterial or fungal infections of the nails such as onychomycosis or poronychia). Topical-type bioadhesive formulations may also be used to administer systemic active agents (e.g. medication), particularly by skin adsorption, oral, transdermal or rectal routes. Travel sickness medication is a preferred example, as is nicotine (e.g. in anti-smoking aids). Where context permits, "topical application" as referred to herein includes systemic agents applied non-parenterally to a specific region of the body.

Periodontal infections are particularly suitable for treatment by the compositions of the present invention. In particular, known compositions for treating periodontal infection are difficult to apply or are generally ineffective. The most widely used periodontal depot composition comprises insertion of a collagen "chip" into the periodontal space, from which an anti-infective agent is released. This chip is difficult to insert and does not form to match the shape and volume of the periodontal space, so that pockets of infection may remain untreated. In contrast to this, the compositions of the present invention, applied as a low viscosity preformulation, can be easily and quickly injected into the periodontal space and will flow to conform exactly to that space and fill the available volume. The compositions then quickly absorb water to form a robust gel which is resistant to aqueous conditions of the mouth. The only known previous attempt at such an injectible periodontal treatment relied on dispersions of relatively high viscosity which were difficult to apply and were subject to undesirable phase separation. All of these drawbacks are now addressed in the compositions of the present invention as described herein. Highly suitable actives for periodontal administration are antiinfectives, especially benzydamine, tramadol and chlorhexidine.

Non-parenteral depot compositions are also of significant benefit in combination with non-pharmaceutical active agents, such as cosmetic actives, fragrances, essential oils etc. Such non-pharmaceutical depots will maintain the important aspects of bioadhesion and sustained release to provide prolonged cosmetic effects, but may easily be applied by spraying or wiping. This additionally applies to agents which have both cosmetic and medical (especially prophylactic) benefits such as sun-protective agents. Since the topical depot compositions provide robust, water

resistant barriers which can solubilise high levels of actives, they are especially suitable for sunscreens and sunblocks in combination with ultra violet light (UV, e.g. UVa, UVb and/or UVc) absorbing and/or scattering agents, particularly where high levels of protection is desirable. The compositions are furthermore highly biocompatible and may act to moisten and soothe the skin during sun exposure. Compositions of the invention containing soothing agents such as aloe vera are also highly suitable for soothing and moistening application after exposure to sunlight, or to skin which is dry, inflamed or damaged due to, for example irritation, burning or abrasion.

Active agents particularly suited to non-parenteral (e.g. topical) depot administration, which comprises intra oral, buccal, nasal, ophthalmic, dermal, vaginal delivery routes, include antibacterials such as chlorhexidine, chloramphenicol, triclosan, tetracycline, terbinafine, tobramycin, fusidate sodium, butenafine, metronidazole (the latter particularly for the (e.g. symtomatic) treatment of acne rosacea - adult acne or some vaginal infections), antiviral, including acyclovir, anti infectives such as bibrocathol, ciprofloxacin, levofloxacin, local analgesics such as benzydamine, lidocaine, prilocaine, xylocaine, bupivacaine, analgesics such as tramadol, fentanyl, morphine, hydromorphone, methadone, oxycodone, codeine, asperine, acetaminophen, NSAIDS such as ibuprofen, flurbiprofen, naproxene, ketoprofen, fenoprofen, diclofenac, etodalac, diflunisal, oxaproxin, piroxicam, piroxicam, indomethansine, sulindac, tolmethin, salysylic acids such as salisylamide and diflunisal, Cox1 or Cox2 inhibitors such as celecoxib, rofecoxib or valdecoxib, corticosteroids, anticancer and immuno stimulating agents (for instance ,metylaminolevulinat hydrocloride, interferon alpha and beta), anticonvulsants (for instance tiagabine topiramate or gabapentin), hormones (such as testosterone, and testosterone undecanoate, medroxyprogesterone, estradiol) growth hormones (like human growth hormone), and growth factors (like granulocyte macrophage colony-stimulating factor), immuno suppressants (cyclosporine, sirolimus, tacrolimus), nicotine and antivirals (e.g. acyclovir).

Some specific actives found by the inventors to form highly effective depots of the present invention include the following:

For long acting injectable depot products of hydrophilic active agents;

i. octreotide (or other somatostatin analogues such as lanreotide for treatment of carcoid and VIP producing tumours and acromegali). Subcutaneous depots formable, especially with GDO and PC having a sustained release duration of more than one month and showing less than 20% octreotide degraded in one month in water-swollen depot at 37°C. Surprisingly good stability was observed and found to be better than octreotide formulated in microspheres. Depot showed less than 5% degradation in product preformulation over eight weeks at 4°C.

ii. human growth hormone. For treatment of growth disorders and growth hormone deficiencies. Subcutaneous depot formable, especially with GDO and PC having a sustained release duration of more than two weeks

iii. interferon alpha, for treatment of cancer and viral infections. Subcutaneous depots formable, especially with GDO and PC, having a sustained release duration of more than one month

iv. leuprolide. Depots formable having continuous delivery (preferably continuous delivery inside therapeutic window) for minimum of one month.

For long acting injectable depots of lipophilic/amphiphilic actives;

i. risperidone

ii. olanzapine

iii. testosterone undecanoate

Depots i to iii formable having continuous delivery (preferably continuous delivery inside therapeutic window) for minimum of two weeks.

For topical bioadhesive, controlled release products for intraoral (including buccal & periodontal) administration;

i. benzydamine (local analgesic, anti inflammatory,) or other local analgesic, analgesic, anti inflammatory, anti bacterial, anti fungal or combination thereof. Composition provides sustained effect at intraoral mucosa, in particular damaged, sensitised, infected mucosa e.g. in patients suffering from oral mucositis (induced by e.g. chemo- and radiotherapy). In particular for treatment of oral mucositis.

ii. tramadol (analgesic). Provides a composition with sustained systemic analgesic effect.

iii. chlorhexidine gluconate (antibacterial) for treatment of periodontal and topical infections. Particularly for long acting effect in periodontal pocket. Compositions result in depots releasing chlorhexidine over more than 1h, preferably more than 6h, most preferably more than 24 h when applied as a liquid, forming a bioadhesive gel *in situ*. Surface gel formation time observed to be between 1 second. and 5 min.

Depots i to iii formable having high level of active agent incorporation and high degree of resistance to washing away. Preformulations in the form of a liquid administered as spray or liquid wash/rinse for i and ii and gel-forming liquid for iii, *wherein liquid is applied to periodontal pocket, e.g. by injection.*

For non-parenteral (e.g. topical or systemic) bioadhesive, controlled release products for nasal administration;

i. fentanyl (analgesic) provides rapid onset and sustained duration analgesia when administered as spray

ii. diazepam (anti anxiety) provides non-parenteral, nasal depot with systemic effect giving rapid onset and sustained duration. Administered as a spray

For topical bioadhesive, controlled release products for ophthalmic administration;

i. diclofenac (NSAID) with sustained duration. Administered as in situ phase forming liquid

ii. pilocarpine (parasymptomimetic, cholinergic agonist) for treatment of glaucoma.

iii levocabastine hydrochloride, ketotifen fumarate providing liquid for eye-*dropping to give long lasting relief from allergic conjunctivitis with long* period between reapplication.

iv Pilocarpine hydrochloride for the treatment of Sjögrens syndrome.

v dexamethasone, (corticosteroid)

vi chloramphenicol (primarily bacteriostatic antiinfective)

vii indomethacin (NSAID)

Depots i to vii formulated as liquid spray or more preferably drops for direct application to eye surface and provide *in situ* depot formation with high resistance to washing out by tears and wear from blinking/eye rubbing.

Other actives suitable for ophthalmic compositions include Antihistamines, Mast cell stabilizers, Nonsteroidal anti-inflammatory drugs (NSAIDs), Corticosteroids (e.g. to treat allergic conjunctivitis), Anti-Glaucoma actives including inflow suppressing/inhibiting agents (beta blocking agents: timolol, betaxolol, carteolol, levobunolol, etc., topical carbonic anhydrase inhibitors: dorzolamide, brinzolamide, sympathomimetics: epinephrine, dipivefrin, clonidine, apraclonidine, brimonidine), outflow facilitating agents (parasympathomimetics (cholinergic agonists): pilocarpine prostaglandin analogues and related compounds: atanoprost, travoprost, bimatoprost, unoprostone)

For non-parenteral (e.g. topical or systemic) bioadhesive, controlled release products for dermatological administration;

i. acyclovir (antiviral). Composition generates a bioadhesive, film forming product with sustained duration. Applied as spray or liquid

ii. testosterone undecanoate (hormone deficiency). bioadhesive, film forming composition with sustained duration. May be applied as aerosol- or pump-spray, or as liquid.

Particularly suitable applications of dermatological formulations are anti-infective dermatological bioadhesive depots for protection in environments where contact with infective agents likely (e.g. human or veterinary surgery, abattoir work, certain types of cleaning etc.). Bioadhesive depots generated from composition of the invention provide robust and sustained protection for the wearer. The compositions with antiinfective agents may also be used in situations where skin sterility of the wearer is important for the health of others, such as for nurses or doctors visiting multiple patients in hospital, where cross-infection must be avoided. A prior coating with a composition of the present invention may serve to provide resistance against picking up of infectives from one area and thus prevent transmission to another.

The pre-formulations of the present invention provide non-lamellar liquid crystalline depot compositions upon exposure to aqueous fluids, especially *in* vivo and in contact with body surfaces. As used herein, the term "non-lamellar" is used to indicate a normal or reversed liquid crystalline phase (such as a cubic or hexagonal phase) or the L3 phase or any combination thereof. The term liquid crystalline indicates all hexagonal, all cubic liquid crystalline phases and/or all mixtures

thereof. Hexagonal as used herein indicates "normal" or "reversed" hexagonal (preferably reversed) and "cubic" indicates any cubic liquid crystalline phase unless specified otherwise. By use of the pre-formulations of the present invention it is possible to generate any phase structure present in the phase-diagram of components a and b with water. This is because the pre-formulations can be generated with a wider range of relative component concentrations than previous lipid depot systems without risking phase separation or resulting in highly viscous solutions for injection. In particular, the present invention provides for the use of phospholipid concentrations above 50% relative to the total amphiphile content. This allows access to phases only seen at high phospholipid concentrations, particularly the hexagonal liquid crystalline phases.

For many combinations of lipids, only certain non-lamellar phases exist, or exist in any stable state. It is a surprising feature of the present invention that compositions as described herein frequently exhibit non-lamellar phases which are not present with many other combinations of components. In one particularly advantageous embodiment, therefore, the present invention relates to compositions having a combination of components for which an I_2 and/or L_2 phase region exists when diluted with aqueous solvent. The presence or absence of such regions can be tested easily for any particular combination by simple dilution of the composition with aqueous solvent and study of the resulting phase structures by the methods described herein.

In a highly advantageous embodiment, the compositions of the invention may form an I_2 phase, or a mixed phase including I_2 phase upon contact with water. The I_2 phase is a reversed cubic liquid crystalline phase having discontinuous aqueous regions. This phase is of particular advantage in the controlled release of active agents and especially in combination with polar active agents, such as water soluble actives because the discontinuous polar domains prevent rapid diffusion of the actives. Depot precursors in the L_2 are highly effective in combination with an I_2 phase depot formation. This is because the L_2 phase is a so-called "reversed micellar" phase having a continuous hydrophobic region surrounding discrete polar cores. L_2 thus has similar advantages with hydrophilic actives.
In transient stages after contact with body fluid the composition can comprise multiple phases since the formation of an initial surface phase will retard the passage of solvent into the core of the depot, especially with substantial sized

administrations of internal depots. Without being bound by theory, it is believed that this transient formation of a surface phase, especially a liquid crystalline surface phase, serves to dramatically reduce the "burst/lag" profile of the present compositions by immediately restricting the rate of exchange between the composition and the surroundings. Transient phases may include (generally in order from the outside towards the centre of the depot): H_{II} or L_α, I_2, L_2, and liquid (solution). It is highly preferred that the composition of the invention is capable forming at least two and more preferably at least three of these phases simultaneously at transient stages after contact with water at physiological temperatures. In particular, it is highly preferred that one of the phases formed, at least transiently, is the I_2 phase.

It is important to appreciate that the preformulations of the present invention are of low viscosity. As a result, these preformulations must not be in any bulk liquid crystalline phase since all liquid crystalline phases have a viscosity significantly higher than could be administered by syringe or spray dispenser. The preformulations of the present invention will thus be in a non-liquid crystalline state, such as a solution, L_2 or L_3 phase, particularly solution or L_2. The L_2 phase as used herein throughout is preferably a "swollen" L_2 phase containing greater than 10 wt% of solvent (component c) having a viscosity reducing effect. This is in contrast to a "concentrated" or "unswollen" L_2 phase containing no solvent, or a lesser amount of solvent, or containing a solvent (or mixture) which does not provide the decrease in viscosity associated with the oxygen-containing, low viscosity solvents specified herein.

Upon administration, the pre-formulations of the present invention undergo a phase structure transition from a low viscosity mixture to a high viscosity (generally tissue adherent) depot composition. Generally this will be a transition from a molecular mixture, swollen L_2 and/or L3 phase to one or more (high viscosity) liquid crystalline phases such as normal or reversed hexagonal or cubic liquid crystalline phases or mixtures thereof. As indicated above, further phase transitions may also take place following administration. Obviously, complete phase transition is not necessary for the functioning of the invention but at least a surface layer of the administered mixture will form a liquid crystalline structure. Generally this transition will be rapid for at least the surface region of the administered formulation (that part in direct contact with air, body surfaces and/or body fluids). This will

most preferably be over a few seconds or minutes (e.g. up to 30 minutes, preferably up to 10 minutes, more preferably 5 minutes of less). The remainder of the composition may change phase to a liquid crystalline phase more slowly by diffusion and/or as the surface region disperses.

In one preferred embodiment, the present invention thus provides a pre-formulation as described herein of which at least a portion forms a hexagonal liquid crystalline phase upon contact with an aqueous fluid. The thus-formed hexagonal phase may gradually disperse, releasing the active agent, or may subsequently convert to a cubic liquid crystalline phase, which in turn then gradually disperses. It is believed that the hexagonal phase will provide a more rapid release of active agent, in particular of hydrophilic active agent, than the cubic phase structure, especially the I_2 and L_2 phase. Thus, where the hexagonal phase forms prior to the cubic phase, this will result in an initial release of active agent to bring the concentration up to an effective level rapidly, followed by the gradual release of a "maintenance dose" as the cubic phase degrades. In this way, the release profile may be controlled.

Without being bound by theory, it is believed that upon exposure (e.g. to body fluids), the pre-formulations of the invention lose some or all of the organic solvent included therein (e.g. by diffusion and/or evaporation) and take in aqueous fluid from the bodily environment (e.g. moist air close to the body or the *in vivo* environment) such that at least a part of the formulation generates a non-lamellar, particularly liquid crystalline phase structure. In most cases these non-lamellar structures are highly viscous and are not easily dissolved or dispersed into the *in vivo* environment and are bioadhesive and thus not easily rinsed or washed away. Furthermore, because the non-lamellar structure has large polar, apolar and boundary regions, it is highly effective in solubilising and stabilising many types of active agents and protecting these from degradation mechanisms. As the depot composition formed from the pre-formulation gradually degrades over a period of days, weeks or months, the active agent is gradually released and/or diffuses out from the composition. Since the environment within the depot composition is relatively protected, the pre-formulations of the invention are highly suitable for active agents with a relatively low biological half-life (see above).

It is an unexpected finding of the present inventors that the pre-formulations result in a depot composition that have very little "burst" effect in the active agent release

profile. This is unexpected because it might be expected that the low viscosity mixture (especially if this is a solution) of the pre-composition would rapidly lose active agent upon exposure to water. In fact, pre-formulations of the invention have shown considerably less of an initial "burst" than previously known polymer-base depot compositions. This is illustrated in the Examples below and Figures attached hereto. In one embodiment, the invention thus provides injectable preformulations and resulting depot compositions wherein the highest plasma concentration of active after administration is no more than 5 times the average concentration between 24 hours and 5 days of administration. This ratio is preferably no more than 4 times and most preferably no more than 3 times the average concentration.

In an additional aspect of the invention, the topical compositions may be used to provide a physical barrier on body surfaces, in the absence of any active agent. In particular, because of the very high bioadherance of the compositions, "barrier" coatings formed by spraying or application of liquid may be formed from the present compositions so as to reduce contact with potential infective or irritant agents or to reduce soiling of the body surfaces. The robust nature of the compositions and resistance to washing provide advantageous characteristics for such barriers, which could conveniently be applied as a liquid or by spraying.

The Invention will now be further illustrated by reference to the following non-limiting Examples and the attached Figures, in which;

Figure 1 shows the cumulative release of methylene blue (MB) from a depot formulation comprising PC/GDO/EtOH (45/45/10 wt%) when injected into excess water;

Figure 2 demonstrates the non-linear decrease of pre-formulation viscosity upon addition of N-methyl pyrolidinone (NMP) and EtOH;

Figure 3 shows the plasma concentration (in rats) of salmon calcitonin (sCT) after subcutaneous injection of various PC/GDO/EtOH depot precursors containing 500 μg sCT / g of formulation;

Figure 4 shows the initial *in vivo* release (up to 48 hours) to plasma (in rats) of sCT from two different depot formulations following subcutaneous injection;

Figure 5 shows the plasma concentration (in rats) of octreotide (OCT) following subcutaneous injection of a depot formulation comprising PC/GDO/EtOH (36/54/10 wt%) containing 5 mg OCT / g formulation, corresponding to 0.5% drug load.

Figure 6 shows the plasma concentration (in rats) of octreotide (OCT) following subcutaneous injection of a depot formulation comprising PC/GDO/EtOH (47.5/47.5/5.0 wt%) containing 30 mg OCT / g formulation, corresponding to 3% drug load.

Figure 7 displays the *in vitro* release in excess aqueous phase of chlorhexidine from a depot formulation comprising PC/GDO/EtOH (36/54/10 wt%) containing 50 mg chlorhexidine / g of formulation, corresponding to 5% drug load.

Examples:

Example 1

Availability of various liquid crystalline phases in the depot by choice of composition

Injectable formulations containing different proportions of phosphatidyl choline ("PC" - Epikuron 200) and glycerol dioleate (GDO) and with EtOH as solvent were prepared to illustrate that various liquid crystalline phases can be accessed after equilibrating the depot precursor formulation with excess water.

Appropriate amounts of PC and EtOH were weighed in glass vials and the mixture was placed on a shaker until the PC completely dissolved to form a clear liquid solution. GDO was then added to form an injectable homogenous solution.

Each formulation was injected in a vial and equilibrated with excess water. The phase behaviour was evaluated visually and between crossed polarizes at 25°C. Results are presented in Table 1.

TABLE 1

Formulation	PC (wt%)	GDO (wt%)	EtOH (wt%)	Phase in H_2O
A	22.5	67.5	10.0	L_2
B	28.8	61.2	10.0	I_2
C	45.0	45.0	10.0	H_{II}
D	63.0	27.0	10.0	H_{II}/L_α

L_2 = reversed micellar phase

I_2 = reversed cubic liquid crystalline phase

H_{II} = reversed hexagonal liquid crystalline phase

L_α = lamellar phase

Example 2

In vitro release of a water-soluble substance

A water-soluble colorant, methylene blue (MB) was dispersed in formulation C (see Example 1) to a concentration of 11 mg/g formulation. When 0.5 g of the formulation was injected in 100 ml water a stiff reversed hexagonal H_{II} phase was formed. The absorbency of MB released to the aqueous phase was followed at 664 nm over a period of 10 days. The release study was performed in an Erlenmeyer flask at 37°C and with low magnetic stirring.

The release profile of MB (see Figure 1) from the hexagonal phase indicates that this (and similar) formulations are promising depot systems. Furthermore, the formulation seems to give a low initial burst, and the release profile indicates that the substance can be released for several weeks; only about 50% of MB is released after 10 days.

Example 3

Viscosity in PC/GDO (6:4) or PC/GDO (3:7) on addition of solvent (EtOH, PG and NMP)

A mixture of PC/GDO/EtOH was manufactured according to the method in Example 1. All, or nearly all, of the EtOH was removed from the mixture with a

rotary evaporator (vacuum, 40°C, 1h) and the resulting solid mixture were weighed in glass vial after which 2, 5, 10 or 20% of a solvent (EtOH, propylene glycol (PG) or n-methyl pyrrolidone (NMP)) was added. The samples were allowed to equilibrate several days before the viscosity was measured at a shear rate of $0.1s^{-1}$ with a Physica UDS 200 rheometer at 25°C.

This example clearly illustrates the need for solvent with certain depot precursors in order to obtain an injectable formulation (see Figure 2). The viscosity of solvent-free PC/GDO mixtures increases with increasing ratio of PC. Systems with low PC/GDO ratio (more GDO) are injectable with a lower concentration of solvent.

Example 4

Composition and in vitro phase study

The formulations were manufactured according to the method described in Example 1 with compositions according to Table 2. An active substance (peptide), salmon calcitonin (sCT), was added to each formulation to a concentration of 500 µg sCT/g formulation. The formulations were designed as homogenous suspensions for parenteral administration (mixing required shortly prior to use since the drug is not completely dissolved in the PC/GDO/EtOH system).

The phase study in this example is performed in excess of rat serum at 37°C in order to simulate an *in vivo* situation. Table 2 shows that the same phases as those in water are formed (compare Table 1).

TABLE 2

Formulation	PC (wt%)	GDO (wt%)	OA (wt%)	EtOH (wt%)	Phase in rat serum
E	18	72	-	10	L_2
F	36	54	-	10	I_2
G	34	51	5	10	I_2
H	54	36	-	10	H_{II}
I	72	18	-	10	H_{II}/L_α

OA = Oleic Acid

Example 5

Sterile filtration of formulations with reduced viscosity

To lower the viscosity with various solvents is sometimes necessary in order to obtain an injectable formulation and to be able to administrate the system with a regular syringe (see Example 3). Another important effect from the viscosity-lowering solvent is that the formulations can be sterile filtrated.

Formulations E to I in Example 4 were studied in a sterile filtration test by using a 0.22µm filter (before addition of the active substance). Formulations E to H were successfully filtrated, but formulation I failed since the viscosity was too high. An aseptic manufacturing procedure was therefore needed for this formulation.

Example 6

***In vivo* release study from depot formulations subcutaneously administered**

Formulations E to I in Example 4 were used in an *in vivo* drug release study in rat. The formulations were administrated subcutaneously between the scapulae by using a syringe (21G, 0.6mm x 30mm) and the dose of sCT was 500 µg/ kg body weight. The release profile was monitored for a period of 13 days. The sCT concentration in the rat plasma samples was analysed with sandwich-type immunoassay using a commercial kit from DSLabs.

Figure 3 shows the results (n=4). A pure triglyceride vehicle based on sesame oil was selected as a lipid reference system.

Example 7

***In vivo* release study in the initial phase**

Formulations F and G as in Example 6 were used in an *in vivo* study in rat designed to investigate the initial "burst effect". From Figure 4 (n=8) it appears that none of the investigated formulations has a severe burst effect.

Example 8: Preparation of depot precursor compositions with various solvents.

Depending on composition of the formulation and the nature and concentration of active substance certain solvents may be preferable.

Depot precursor formulations (PC/GDO/solvent (36/54/10)) were prepared by with various solvents; NMP, PG, PEG400, glycerol/EtOH (90/10) by the method of Example 1. All depot precursor compositions were homogeneous one phase solutions with a viscosity that enabled injection through a syringe (23G - i.e. 23 gauge needle; 0.6mm x 30mm). After injecting formulation precursors into excess water a liquid crystalline phase in the form of a high viscous monolith rapidly formed with NMP and PG containing precursors. The liquid crystalline phase had a reversed cubic micellar (I_2) structure. With PEG400, glycerol/EtOH (90/10) the viscosification/solidification process was much slower and initially the liquid precursor transformed to a soft somewhat sticky piece. The difference in appearance probably reflects the slower dissolution of PEG400 and glycerol towards the excess aqueous phase as compared to that of EtOH, NMP and PG.

Example 9: Preparation of depot composition containing human growth hormone (HGH).

Human growth hormone (hGH) plays a critical role in stimulating body growth and development, and is involved in the production of muscle protein and in the breakdown of fats. A deficiency of the hormone adversely affects numerous body processes such as lipid profile, insulin status, physical performance, bone-mineral density and quality of life. A targeted dose every 2 weeks is estimated at 0.10 to 0.24 mg/kg of body weight.
1ml of a 2 weeks depot formulation precursor was formed by sequentially mixing 10mg hGH and 360mg PC in 0.1ml NMP. 540mg GDO was added to the mixture to obtain a low viscosity depot formulation precursor. Injecting the formulation precursor into excess water (syringe 23G; 0.6mm x 30mm) resulted in a monolithic liquid crystalline phase (I_2 structure).

Example 10: Preparation of depot composition containing a sparingly soluble active substance.

Risperidone is an antipsychotic medication agent belonging to the chemical class of benzisoxazole derivatives. It is a very strong dopamine blocker (antagonist); ie, it inhibits functioning of dopamine receptors, it is practically insoluble in water, and it has log(P)= 3.49.

1g of a depot formulation containing 50mg of risperidone was prepared by dissolving the active substance in 0.7g of a mixture 95%wt in EtOH (99.5%) and 5%wt in acetic acid. 0.34g PC and 0.51g GDO were subsequently dissolved in this solution followed by solvent reduction to remaining 0.15g solvent (0.55g was evaporated under vacuum). The composition of the final homogenous and clear depot formulation with 50mg risperidone was PC/GDO/solvent/risperidone (32/49/14/5). Injecting the formulation precursor into excess water (syringe 23G; 0.6mm x 30mm) resulted in a monolithic liquid crystalline phase (I_2 structure). I.e. the amount of active substance (5%) did not change monolith formation and phase behavior after exposure to an aqueous environment.

Example 11: Alternate preparation of depot composition containing risperidone.

A risperidone depot precursor formulation could also be prepared by using a solvent mixture composed of 90%wt EtOH (99.5%) and 10%wt in acetic acid.

50mg of risperidone was dissolved in 0.7g of the solvent mixture, after which 0.36g PC and 0.54g GDO were subsequently dissolved in this solution. 0.60g of the solvent mixture was evaporated under vacuum to a homogenous and clear depot formulation precursor with 50mg risperidone (PC/GDO/solvent/risperidone (34/51/10/5)). Injecting the formulation precursor into excess water (syringe 23G; 0.6mm x 30mm) resulted in a monolithic liquid crystalline phase (I_2 structure). I.e. the amount of active substance (5%) did not change monolith formation and phase behavior after exposure to an aqueous environment.

Example 12: Temperature stability of depot composition containing a sparingly soluble active substance.

The risperdone depot precursor formulations in examples 10 and 11 were tested for stability against crystallization during storage. Each formulation was stable at 25°C for at least two weeks and at +8°C for at least one week.

Example 13: Preparation of depot composition containing benzydamine.

Benzydamine is a non-steroidal antiinflammatory drug and is extensively used as a topical drug in inflammatory conditions.

1g of a depot formulation containing 1.5mg benzydamine was prepared by dissolving the active substance in a mixture of PC/GDO/EtOH (36/54/10) prepared as described in Example 1. The depot composition was stable against crystallization during storage at 25°C for at least two weeks. Equilibration of the formulation precursor with excess water resulted in a high viscous monolithic liquid crystalline phase (I_2 structure).

Example 14: Robustness of the behaviour of the formulation against variations in the excipient quality.

Depot precursor formulations were prepared with several different GDO qualities (supplied by Danisco, Dk), Table 3, using the method of Example 1. The final depot precursors contained 36%wt PC, 54%wt GDO, and 10%wt EtOH. The appearance of the depot precursors was insensitive to variation in the quality used, and after contact with excess water a monolith was formed with a reversed micellar cubic phase behaviour (I_2 structure).

Table 3. Tested qualities of GDO.

GDO quality	Monoglyceride (%wt)	Diglyceride (%wt)	Triglyceride (%wt)
A	10.9	87.5	1.6
B	4.8	93.6	1.6
C	1.0	97.3	1.7
D	10.1	80.8	10.1
E	2.9	88.9	8.2
F	0.9	89.0	10.1

Example 15: Preparation of depot composition containing saturated PC (Epikuron 200SH).

Depot precursor formulations were prepared with various amounts PC comprising saturated hydrocarbon chains by addition of Epikuron 200SH directly to a mixture of PC/GDO/EtOH, prepared as for Example 1. The formulations are shown in Table 4. All precursor formulations were homogenous one phase samples in RT, while they became more viscous with increasing amount Epikuron 200SH. Injecting the depot precursor into excess water gave a monolith comprising a reversed miceller cubic (I_2) structure. Monoliths formed from samples containing higher amounts of Epikuron 200SH became turbid, possibly indicating segregation between Epikuron 200SH and the other components upon exposure to water and formation of the I2 phase.

Table 4. Depot composition containing saturated PC

Formulation	Saturated PC, Epikuron 200SH (%wt)	PC (%wt)	GDO (%wt)	EtOH (%wt)
G1	3.9	34.6	51.9	9.6
G2	7.0	33.5	50.2	9.3
G3	14.3	30.8	46.3	8.6

Example 16: Preparation of depot precursor being a dispersion or solution of the peptide salmon calcitonin.

By adding 500µg sCT/g formulation to a solution of PC/GDO/EtOH (36/54/10), obtained as in Example 1, a dispersion of sCT was formed.

In an alternative method, 500µg sCT was dissolved in excess of EtOH followed by addition of PC and GDO. The solvent concentration was then reduced (EtOH evaporation) to form a homogenous (active drug in solution) formulation. This latter technique can be used to obtain higher drug loads. Precursor compositions corresponding to at least 1500µg dissolved sCT per gram of the final depot precursor composition could be obtained by this method.

Example 17: In vivo release study from depot formulation subcutaneously administered.

The two sCT compositions described in Example 16 were administered in an *in vivo* rat model by subcutaneous injection (between the scapulae). The first depot precursor having dispersed sCT was found to give somewhat unstable initial plasma concentrations, while the second depot precursor, having sCT dissolved therein, gave much more stable initial plasma levels (see Table 5).

Table 5

Formulations	Coefficient of variation (%CV)
Dispersed: 500μg sCT/g PC/GDO/EtOH (36/54/10)	32-127
Dissolved: 500μg sCT/g PC/GDO/EtOH (36/54/10)	20-37

Example 18: Preparation of depot composition containing the peptide octreotide.

Octreotide is an acetate salt of a synthetic octa-peptide and is similar to the hormone somatostatin. Octreotide decreases production of substances such as growth hormone, insulin and glucagons. It is used in treatment of acromegaly, and to reduce flushing and watery diarrhoea caused by metastatic cancerous tumors (carcinoid syndrome) or tumors called vasoactive intestinal peptide tumors (VIPomas).

24mg or 60mg octreotide was dissolved in 0.1g EtOH. 0.36g PC and 0.54g GDO were subsequently dissolved in this solution and a depot formulation precursor was obtained. Injecting the formulation precursor into excess aqueous phase (syringe 23G; 0.6mm x 30mm) resulted in a monolithic liquid crystalline phase (I_2 structure). I.e. octreotide (2.4% or 6.0%) did not change monolith formation and phase behaviour after exposure to an aqueous environment.

The octreotide depot precursor formulations in this Example were tested for stability against crystallization during storage. Each formulation was stable at 4-8°C for at least two weeks.

Example 19: In vivo release study from depot formulation containing octreotide subcutaneously administered.

In an in vivo rat model the drug release of octreotide was followed during 28 days. The formulations were administered subcutaneously between the scapulae by using a syringe (23G, 0.6mm x 25mm). The octreotide concentration in the rat plasma was followed for a period of 28 days (see Figure 5). The dose was 5 mg/kg and volume 1 ml/kg corresponding to a drug load of 0.5% octreotide in the depot formulation precursor (PC/GDO/EtOH (36/54/10)).
From Figure 5 (n=3) it appears that the investigated formulation gives a release profile essentially without a burst effect.

Figure 5 shows Octreotide plasma levels in the rat model following administration of octreotide formulation precursor (0.5% in octreotide).

Example 20: Degradation of depot formulation in the rat.

Various volumes (1, 2, 6 ml/kg) of the depot precursor (36%wt PC, 54%wt GDO, and 10%wt EtOH) were injected in the rat and were removed again after a period of 14 days. It was found that substantial amounts of the formulations were still present subcutaneously in the rat after this time, see Table 6.

Table 6. Mean diameter of depot monolith.

Dose (ml/kg)	Mean diameter day 3 (mm)	Mean diameter day 14 (mm)
1 (n=3)	15.8	12.5
2 (n=3)	18.5	15.3
6 (n=3)	23.3	19.3

Example 21: In vitro study of formation of depot monolith after injection of depot formulation precursor between the bone and periostium.

A precursor (36%wt PC, 54%wt GDO, and 10%wt EtOH prepared as described in Example 1) was injected by syringe between the bone and periostium. The composition was observed to spread to fill voids and after uptake of aqueous fluids formed a monolith that was bioadhesive to both the bone and periostium.

Example 22: Bioadhesive spray of depot precursor formulation.

A pump spray bottle was found to be a convenient way to apply the formulation topically, e.g. to the skin or the oral mucosa.

A depot precursor formulation prepared as in Example 1 (36%wt PC, 54%wt GDO, and 10%wt EtOH) was sprayed with a pump spray bottle onto the skin and oral mucosa. A film with solid mechanical properties formed shortly after application.

Example 23: Robustness of a topical film.

After applying the depot precursor formulation, as described in Example 22, (36%wt PC, 54%wt GDO, and 10%wt EtOH) to the skin, the applied formulation was exposed to flushing water (10L/min) for 10 minutes. The formulation showed excellent bioadhesive properties and resistance against rinsing and no loss of the formulation could be discerned.

Example 24: Formation of cubic phase with solid properties after exposure of depot precursor formulation to air.

After exposing a depot precursor formulation prepared as described in Example 1 (36%wt PC, 54%wt GDO, and 10%wt EtOH) to air (RT, relative humidity 40%) for at least 3 hours, a solid cubic phase was formed. This formation of a cubic phase structure demonstrates that a topical film will acquire bulk non-lamellar depot properties after application without the need for direct exposure to excess aqueous fluid.

Example 25: Formulation to treat periodontitis or perimplantitis.

In order to treat periodontitis or perimplantitis an antibacterial formulation is injected in the periodontal pocket, and a prolonged effect of the formulation is normally desired.

100μL of a formulation as prepared in Example 1, with the addition of the antibiotic chlorohexidine (PC/GDO/EtOH/chlorhexidine (35/53/10/2)), is injected via a syringe into a rat peridontal pocket. The injected composition is observed to

transform from the low viscous formulation, and which initially spreads out to fill voids, to form a solid mass by uptake of gingival fluids. An antibacterial depot system is thus provided.

Chlorhexidine remains at clinically effective levels (MIC 125µg/ml) in the GCF of the periodontal pockets for over 1 week. The depot system is completely degraded by enzymes within 7 to 10 days and does not need to be removed.

Example 26: Alternate antibacterial formulation to treat periodontitis or perimplantitis.

An alternate antibacterial formulation was provided by a formulation prepared as described in Example 1 and containing the antibacterial detergent Gardol (Glycine, N-methyl-N-(1-oxododecyl)-, sodium salt) (PC/GDO/EtOH/Gardol (34/51/10/5)). This formulation is injected into the rat periodontal pocket.

Gardol is observed to remain at clinically effective levels in the GCF of the periodontal pockets for a prolonged period (several days). The depot system is completely degraded by enzymes within 7 to 10 days and did not need to be removed.

Example 27: Adhesion of the formulation to high energy surfaces.

In order to treat perimplantitis, adhesion not only to biological surfaces but also to high energy surfaces such as a gold or titanium implant is important. It is also important that the formulation adheres to ceramic and plastic surfaces.

A formulation (PC/GDO/EtOH (36/54/10)) as prepared in Example 1 was applied to various surfaces in the oral cavity. The composition showed excellent adhesion to ceramic, plastic, gold, as well as to a normal tooth surface and could not be rinsed away by excess aqueous fluid. The depot resulting from the composition stayed at the site in the oral cavity where it was applied for at least 6h.

Example 28: Bioadhesive sustained release formulation of sodium fluoride for use on the teeth.

Fluoride containing compounds are often needed to oppose caries attack and a bioadhesive formulation precursor with depot effect was prepared as indicated in Example 1 from a mixture of PC/GDO/EtOH/sodium fluoride (35/53/10/2). The formulation was a dispersion of sodium fluoride since it could not be dissolved in the precursor. The liquid formulation was applied to the teeth with the aid of a brush. By uptake of saliva the formulation solidified and formed a depot providing sustained release of sodium fluoride for an extended period (several hours).

Example 29: Oral Cavity Spray Depot Composition

To be suitable as a topical depot system in the oral cavity the mechanical properties of the system was adjusted by decreasing the PC/GDO ratio.

A mixture containing PC/GDO/EtOH (27/63/10) was prepared according to Example 1. A drop of patent blue was added to visualize the formulation after application. About 300µl of the formulation was sprayed into the oral cavity with pump spray bottle. Shortly after application the formulation viscosified/solidified since it underwent a phase transformation by uptake of aqueous fluid (saliva) and loss of solvent (EtOH). The formulation had excellent bioadhesion to keritinized surfaces such as the hard palate and the gum. Here the film lasted for several hours despite saliva secretion and mechanical wear by the tongue. At soft mucosal surfaces the duration was much shorter (minutes).

Example 30: Oral Cavity Liquid Depot Composition

To be suitable for application with a pipette to the oral cavity the solidification/ viscosification of the formulation has to be delayed relative to the spray formulation. This is to allow the formulation to be conveniently distributed with the tongue to a thin film in the oral cavity after application.

Propylene glycol (PG) and EtOH were added to a formulation prepared as in Example 1, to the final composition PC/GDO/EtOH/PG (24/56/10/10). 300µl of the formulation was conveniently applied with a pipette to the oral cavity and distributed with the tongue to a thin film in the oral cavity. After about 20 seconds the viscosification of the formulation started since it underwent a phase transformation by uptake of aqueous fluid (saliva) and loss of solvent (EtOH and PG). After about one minute the solidification/viscosification appeared to be

finished. The formulation had excellent bioadhesion to keritinized surfaces such as the hard palate and the gum. Here the film lasted for several hours despite saliva secretion and mechanical wear by the tongue. At soft mucosal surfaces the duration was much shorter (minutes).

Example 31 - Bioadhesive depot for nails

The mixture in Example 29 was sprayed to the nail bed and in between the toes. The formulation solidifies/viscosifies slowly by uptake of aqueous fluids (cf. sweat). The solidification can be speeded up by adding water after spray application. The formulation had excellent bioadhesive properties and had a duration for several hours.

Eample 32: Loading capacity of the bioactive agent benzydamine in the formulation precursors.

Formulations with compositions as specified in Table 7 were prepared using the method in Example 1. An excess amount of benzydamine (50mg) was added to 0.5 g of the formulations. The vials were placed on a shaker at 15 °C for three days after which the solutions were filtered through a filter (0.45μm) to get rid of crystals of undissolved benzydamine. The benzydamine concentration in each formulation was determined with reversed phase gradient HPLC and UV detection at 306nm and the results are given in Table 7.

Table 7

Composition GDO/PC(Lipoid S100)/EtOH	Benzydamine concentration in formulation
67.5/22.5/10	3.4%
63/27/10	3.2%
58.5/31.5/10	3.3%
60/20/20	4.0%
56/24/20	4.5%
52/28/20	4.3%

Example 33: Compositions containing PC and tocopherol

Depot precursor formulations were prepared with several different PC/α-tocopherol compositions using the method of Example 1 (PC was first dissolved in the appropriate amount of EtOH and thereafter α-tocopherol was added to give clear homogenous solutions).

Each formulation was injected in a vial and equilibrated with excess water. The phase behaviour was evaluated visually and between crossed polarizes at 25°C. Results are presented in Table 8.

Table 8

α-tocopherol	PC	Ethanol	Phase in excess H_2O
2.25g	2.25g	0.5g	H_{II}
2.7g	1.8g	0.5g	H_{II}/I_2
3.15g	1.35g	0.5g	I_2
3.6g	0.9g	0.5g	I_2/L_2

Example 34: Composition containing octreotide

60mg octreotide was dissolved in 0.1g EtOH. 0.25g PC and 0.59g α-tocopherol were subsequently dissolved in this solution and a depot formulation precursor was obtained. Injecting the formulation precursor into excess aqueous solution (phosphate buffered saline - PBS) resulted in a monolithic liquid crystalline phase (I_2 structure) i.e. octreotide (6.0%) did not change monolith formation and phase behaviour after exposure to an aqueous environment.

The octreotide depot precursor formulation in this Example was tested for stability against crystallization during storage. The formulation was stable at 4-8°C for at least two weeks.

Example 35: In vitro release of water-soluble disodium fluorescein

A water-soluble colorant, disodium fluorescein (Fluo), was dissolved in a formulation containing PC/α-tocopherol/Ethanol (27/63/10 wt%) to a concentration

of 5 mg Fluo/g formulation. When 0.1 g of the formulation was injected in 2 ml of phosphate buffered saline (PBS) a reversed micellar (I_2) phase was formed. The absorbency of Fluo released to the aqueous phase was followed at 490 nm over a period of 3 days. The release study was performed in a 3 mL vial capped with an aluminium fully tear off cap at 37°C. The vial was placed on a shaking table at 150 rpm.

The release of Fluo from the PC/α-tocopherol formulation (see Table 9) indicates that this (and similar) formulations are promising depot systems. Furthermore, the absence of a burst effect is noteworthy, and the release indicates that the substance can be released for several weeks to months; only about 0.4% of Fluo is released after 3 days.

Table 9

Formulation	% release (37°C)	
	24 h	72 h
PC/α-tocopherol/EtOH: 27/63/10 wt%	< 0.1*	0.43

* Release below detection limit of the absorbance assay

Example 36: **Formulations of the analgesic/antiinflammatory benzydamine**

Formulations were prepared as in Example 1 by mixing benzydamine with a mixture of GDO, PC, ethanol and optionally PG/AP in the following proportions.

Formulation	BZD	GDO	PC	EtOH	PG	AP
1	3.0	53.3	28.7	10.0	5.0	0.01
2	3.0	53.3	28.7	15.0	0	0.01
3	3.0	57.4	24.6	10.0	5.0	0.01
4	3.0	49.2	32.8	10.0	5.0	0.01

where BZD is benzydamine, EtOH is ethanol, PC is LIPOID S100 soybean phosphatidylcholine, GDO is glycerol dioleate, PG is propylene glycol, and AP is ascorbyl palmitate.

All formulations are low viscosity liquids which generate liquid crystalline phase compositions upon exposure to aqueous conditions.

Example 37: **Fentanyl nasal formulation**

Formulations were prepared as in Example 1 by mixing the narcotic analgesic fentanyl with a mixture of GDO, PC, ethanol and optionally PG in the following proportions.

Formulation	Fentanyl	PC	GDO	EtOH	PG
1	0.05	34	51	10	5
2	0.05	36	54	10	-
3	0.05	42	43	10	5
4	0.05	45	45	10	-
5	0.15	34	51	10	5
6	0.15	36	54	10	-
7	0.05	30	45	15	10
8	0.15	30	45	15	10

where EtOH is ethanol, PC is LIPOID S100 soybean phosphatidylcholine, GDO is glycerol dioleate, and PG is propylene glycol

All formulations are low viscosity liquids suitable for administration by nasal spray, which generate liquid crystalline phase compositions upon exposure to aqueous conditions.

Example 38: **Diazepam nasal formulation**

Formulations were prepared as in previous examples by mixing the benzodiazepine antianxiety agent diazepam with a mixture of GDO, PC, ethanol and optionally PG in the following proportions.

Formulation	Diazepam	PC	GDO	EtOH	PG
1	5	32	48	10	5
2	5	34	51	10	-
3	10	37	38	10	5
4	10	40	40	10	-
5	10	30	45	10	5
6	10	32	48	10	-
7	10	26	39	15	10
8	10	30	45	15	-

where EtOH is ethanol, PC is LIPOID S100 soybean phosphatidylcholine, GDO is glycerol dioleate, and PG is propylene glycol

All formulations are low viscosity liquids suitable for administration by nasal spray, which generate liquid crystalline phase compositions upon exposure to aqueous conditions.

Example 39: **Interferon Alpha-2a**

Interferons (IFNs) are used as a treatment for many types of systemic cancer, often in combination with chemotherapy or radiation. Recent data suggest that IFN Alpha is a multifunctional immunomodulatory cytokine with profound effects on the cytokine cascade including several anti-inflammatory properties. These newly identified immunoregulatory and anti-inflammatory functions may also be of importance in treatment of diseases such as chronic viral hepatitis and help to explain some of the IFN mechanisms.

A non-aqueous precursor formulation was formed by dissolving PC (360 mg) and GDO (540 mg) in EtOH (100 mg). Interferon Alpha-2a (4 mg) was dissolved in water (76 mg) and this solution was thereafter added to the non-aqueous precursor formulation to form a depot formulation precursor of low viscosity.

Injecting the depot precursor into excess water (syringe 23 G; 0.6mm x 30 mm) resulted in a monolithic liquid crystalline phase (I_2 structure).

Example 40 **Leuprorelin (Leuprolide)**

Leuprorelin acetate (or leuprolide acetate) is a synthetic nonapeptide analogue of naturally occurring gonadotropin releasing hormone (GnRH or LH-RH) that, when given continuously (e.g. as a depot formulation), inhibits pituitary gonadotropin secretion and suppresses testicular and ovarion steroidogenesis. Leuprorelin is used for the treatment of advanced prostate cancer.

A depot formulation precursor was formed by sequentially dissolving 22.5 mg leuprorelin acetate and 360 mg PC in 100 mg of NMP. 540 mg of GDO was added to the mixture yielding a molecular solution depot formulation precursor of low viscosity. Injecting the formulation precursor into excess water (syringe 23 G; 0.6mm x 30 mm) resulted in a monolithic liquid crystalline phase (I_2 structure).

Example 41: **Alendronate**

Bisphosphonates are structural analogues of pyrophosphates and have pharmacologic activity specific for bone due to the strong affinity of bisphosphonates for hydroxyapatite, a major inorganic component of bone. The compounds are used to treat postmenopausal osteoporosis, hypercalcemia of malignancy and metastatic bone disease (MBD).

A non-aqueous precursor formulation was formed by dissolving PC (360 mg) and GDO (540 mg) in EtOH (100 mg). Alendronate (12 mg) was dissolved in water (80 mg) and this solution was thereafter added to the non-aqueous precursor formulation to form a depot formulation precursor of low viscosity. Injecting the depot precursor into excess water (syringe 23 G; 0.6mm x 30 mm) resulted in a monolithic liquid crystalline phase (I_2 structure).

Example 42: **Olanzapine**

Olanzapine is a low molecular weight drug used for the treatment of patients with schizophrenia.

A depot formulation precursor was formed by sequentially mixing 50 mg olanzapine, 360 mg PC and 100 mg of EtOH. 540 mg of GDO was added to the mixture resulting in the final depot formulation precursor.

Injecting the formulation precursor into excess water (syringe 23 G; 0.6mm x 30 mm) resulted in a monolithic liquid crystalline phase (I_2 structure).

Example 43: **Acne formulations with Clindamycin**

Formulations were prepared as in previous examples by mixing the semisynthetic antibiotic clindamycin (free base or salt) with a mixture of GDO, PC, ethanol and PG in the following proportions (by weight).

Formulation	Clindamycin HCl	PC	GDO	EtOH	PG
1	1	30	54	10	5
2	2	29	54	10	5
3	1	34	50	10	5
4	2	33	50	10	5

Formulation	Clindamycin base	PC	GDO	EtOH	PG
5	1	30	54	10	5
6	2	29	54	10	5
7	1	33	54	2	10
8	2	32	54	2	10

The resulting preformulations are low viscosity liquids which, after application resistant to water, sweat, etc. The formulation are applied locally on the skin as a gel or by spraying and are bioadhesive with good film-forming properties.

Example 44: **Further examples of viscosity in PC/GDO mixtures on addition of co-solvent**

Mixtures of PC/GDO and co-solvent were prepared according to the methods of Example 1 and Example 3 in the proportions indicated in the table below. The samples were allowed to equilibrate for several days before viscosity measurements were performed using a Physica UDS 200 rheometer at 25 °C.

Sample	PC/GDO (wt/wt)	EtOH / wt%	Glycerol / wt%	H_2O / wt%	Viscosity / mPas
1	50/50	3	-	-	1900
2	50/50	5	-	-	780
3	50/50	7	-	-	430
4	50/50	8	-	-	300
5	50/50	10	-	-	210
6	50/50	15	-	-	100
7	45/55	3	-	-	1350
8	45/55	5	-	-	540
9	45/55	7	-	-	320
10	45/55	8	-	-	250
11	45/55	10	-	-	150
12	45/55	15	-	-	85
13	40/60	3	-	-	740
14	40/60	5	-	-	400
15	40/60	7	-	-	240
16	40/60	8	-	-	200
17	40/60	10	-	-	130
18	40/60	15	-	-	57
19	40/60	-	10	-	$8*10^6$
20	40/60	-	-	3	$2.5*10^8$
21	40/60	-	-	5	$4*10^7$

This example further illustrates the need for a solvent with viscosity lowering properties in order to obtain injectable formulations. The mixtures containing glycerol (sample 19) or water (samples 20 and 21) are too viscous to be injectable at solvent concentrations equivalent to the samples containing EtOH (compare with samples 13, 14 and 17).

Example 45: **Occtreotide Formulation compositions**

Formulations were prepared as in Example 1 by mixing the peptide active octreotide with a mixture of GDO (at one of several purity levels) or tocopherol, PC, ethanol and optionally dioleoyl PG in the following proportions (by weight)

Formulation	OCT	EtOH	PC	GDO1	GDO2	GDO3	TP	DOPG
E	2	10	35.2	-	-	52.8	-	-
F	2	10	35.2	52.8	-	-	-	-
G	2	10	35.2	-	52.8	-	-	-
H	2	10	26.4	-	-	-	61.6	-
I	1	10	35.6	53.4	-	-	-	-
J	2	5	37.2	-	-	55.8	-	-
K	3	5	36.8	-	-	55.2	-	-
L	6	5	35.6	-	-	53.5	-	-
M	3	5	35.8	-	-	55.2	-	1
N	3	5	33.8	-	-	55.2	-	3
O	3	5	30.8	-	-	55.2	-	6
P	3	5	46	-	-	46	-	-
Q	3	10	43.5	-	-	43.5	-	-
R	6	10	42	-	-	42	-	-
S	3	7	45	-	-	45	-	-
T	6	7	43.5	-	-	43.5	-	-

where OCT is octreotide, EtOH is ethanol, PC is LIPOID S100 soybean phosphatidylcholine, GDO is glycerol dioleate, TP is α-tocopherol, DOPG is dioleoyl phosphatidylglycerol

GDO quality (according to AC)

	Monoglycerides	Diglycerides	Triglycerides
GDO1	10.9%	87.5%	1.4%
GDO2	4.2%	92.1%	3.5%
GDO3	0.5%	95.3%	4.0%

Formulation P (for composition see above) was administered by s.c.injection in the rat at a level of 1 ml formulation per kg body weight, corresponding to 30 mg/kg of octreotide.

Octreotide plasma levels after administration were monitored for 5 days to examine any burst profile. It was observed that the highest plasma concentration was less than three fold greater than the average plasa concentration over the first 5 days.

The results of the study are shown in Figure 6

Example 46: ***Sunscreen formulations***

Formulations were prepared as in Example 1 by mixing each of several UV absorbing/scattering agents with a mixture of GDO, PC, and ethanol in the following proportions (by weight)

Formulation	PC	GDO	EtOH	Tioveil CM	Spectraveil FIN	Solaveil CT-100	Tioveil 50 MOTG
1	38	42	5	-	-	-	15
2	38	42	5	-	-	15	-
3	37	38	5	15	5	-	-

Where TIOVEIL CM (Uniqema) comprises Cyclomethicone (and) Titanium Dioxide (and) Dimethicone Copolyol (and) Aluminium Stearate (and) Alumina, SPECTRAVEIL FIN(Uniqema) comprises Zinc Oxide (and) C12-15 Alkyl Benzoate (and) Polyhydroxystearic Acid, SOLAVEIL CT-100 (Uniqema) comprises C12-15 Alkyl Benzoate (and) Titanium Dioxide (and) Polyhydroxystearic Acid (and) Aluminum Stearate (and) Alumina, and TIOVEIL 50 MOTG (Uniqema) comprises Titanium Dioxide (and) Caprylic/Capric Triglyceride (and) Mineral Oil (and) Polyhydroxystearic Acid (and) Aluminum Stearate (and) Alumina.

The resulting formulation precursors show low viscosity upon formulation and are readily applied by pump spray. Upon contact with body surfaces a resilient UV protective layer is formed.

Example 47: **Chlorhexidine periodontal depots.**

Formulations were prepared as in Example 1 by mixing the antiinfective agent chlorhexidine digluconate with a mixture of GDO, PC, and ethanol in the following proportions (by weight)

Table. Chlorhexidine digluconate depot formulation compositions.

Formulation	Chlorhexidine digluconate	PC	GDO	EtOH
A	5	34	51	10
B	5	36	54	5
C	7	33	50	10
D	10	32	48	10
E	15	30	45	10

The chlorhexidine depot preformulations have low viscosity and are easily administered to the periodontal pocket. The compositions provide better distribution and spreading of the active substance throughout the periodontal pocket when compared to current products, such as Periochip®.

The depot formed after application gives protection against re-infection of the pocket. The depot also has excellent bioadhesive properties and sticks to mucosal, teeth and bone surfaces.

Release of chlorhexidine digluconate from 250 mg Formulation A (see above) in 0.9% aqueous NaCl (500 ml) was studdied. The formulation was held in a cylindrical metal cup which was placed in a teflon holder at the bottom of a standard

USP release bath. The contact area between the formulation and surrounding saline solution was 2.4 cm^2, and the solution was stirred by paddle at 100 rpm.

The release curve shown in Figure 7 demonstrates the sustained and essentially uniform release of chlorhexidine from the formulation over a period of 24 hours.

Claims:

1) A pre-formulation comprising a low viscosity, non-liquid crystalline, mixture of:

a) at least one neutral diacyl lipid and/or at least one tocopherol;

b) at least one phospholipid;

c) at least one biocompatible, oxygen containing, low viscosity organic solvent;

wherein at least one bioactive agent is dissolved or dispersed in the low viscosity mixture and wherein the pre-formulation forms, or is capable of forming, at least one liquid crystalline phase structure upon contact with an aqueous fluid.

2) A pre-formulation as claimed in claim 1 wherein said liquid crystalline phase structure is bioadhesive.

3) A pre-formulation as claimed in claim 1 or claim 2 wherein component a) consists essentially of diacyl glycerols, especially glycerol dioleate.

4) A pre-formulation as claimed in claim 1 or claim 2 wherein component a) consists essentially of at least one tocopherol.

5) A pre-formulation as claimed in claim 1 or claim 2 wherein component a) consists essentially of a mixture of GDO and tocopherol.

6) A pre-formulation as claimed in any of claims 1 to 5 wherein component b) is selected from phosphatidylcholines, phosphatidylethanolamines, phosphatidylserines, phosphatidylinositols and mixtures thereof.

7) A preformulation as claimed in any of claims 1 to 6 having a viscosity of 0.1 to 5000 mPas.

8) A preformulation as claimed in any of claims 1 to 7 having a molecular solution, L_2 and/or L_3 phase structure.

9) A preformulation as claimed in any of claims 1 to 8 having a ratio of a) to b) of between 95:5 and 5:95 by weight.

10) A preformulation as claimed in any of claims 1 to 9 having 0.5 to 50% component c) by weight of components a) + b) + c).

11) A preformulation as claimed in any of claims 1 to 10 wherein component c) is selected from alcohols, ketones, esters , ethers, amides, sulphoxides and mixtures thereof.

12) A preformulation as claimed in any of claims 1 to 11 additionally comprising up to 10% by weight of a)+b) of a charged amphiphile.

13) A preformulation as claimed in any of claims 1 to 12 wherein said active agent is selected from drugs, antigens, nutrients, cosmetics, fragrances, flavourings, diagnostic agents, vitamins, dietary supplements and mixtures thereof.

14) A preformulation as claimed in calim 13 wherein said drus is selected from hydrophilic small molecule drugs, lipophilic small molecule drugs, amphiphilic small molecule drugs, peptides, proteins, oligonucleotids and mixtures thereof.

15) A preformulation as claimed in claim 13 wherein said drug is selected from somatostatin related peptides, interferons, glucagon-like peptides 1 and 2, GnRH agonists, GnRH antagonists, bisphosponates, chlorhexidine and mixtures thereof.

16) A preformulation as claimed in any of claims 1 to 15 which is administrable by injection.

17) A preformulation as claimed in any of claims 1 to 15 which is administrable by spraying, dipping, rinsing, application from a pad or ball roller, painting, dropping, aerosol spraying or pump spraying.

18) An injectable preformulation as claimed in any of claims 1 to 16 which forms a depot providing continuous release of active agent for at least two weeks, wherein said active agent comprises at least one selected from

i. octreotide
ii. human growth hormone
iii. interferon alpha
iv. leuprolide

19) An injectable preformulation as claimed in any of claims 1 to 16 which forms a depot providing continuous release of active agent for at least two weeks, wherein said active agent comprises at least one selected from

i. risperidone
ii. olanzapine
iii. testosterone undecanoate

20) A topical formulation as claimed in any of claims 1 to 15 for intraoral administration which forms a bioadhesive, controlled release product, wherein said active agent comprises at least one selected from

i. benzydamine
ii. tramadol

21) A topical preformulation as claimed in any of claims 1 to 15 suitable for intraoral administration for treatment of periodontal and topical infections, wherein the active agent is chlorhexidine gluconate, and where the preformulation is applied as a liquid product which forms a surface gel *in situ* between 1 second. and 5 min after application.

22) A non-parenteral formulation as claimed in any of claims 1 to 15 for intranasal spray administration which forms a bioadhesive, controlled release product, wherein said active agent comprises at least one selected from

i. fentanyl
ii. diazepam

23) A topical formulation as claimed in any of claims 1 to 15 suitable for ocular administration, wherein said active agent comprises at least one selected from diclofenac, pilocarpine, levocabastine hydrochloride, ketotifen fumarate, timolol, betaxolol, carteolol, levobunolol, dorzolamide, brinzolamide, epinephrine, dipivefrin, clonidine, apraclonidine, brimonidine, pilocarpine, atanoprost, travoprost, bimatoprost, unoprostone, pilocarpine hydrochloride, dexamethasone, chloramphenicol, and indomethacin.

24) A non-parenteral formulation as claimed in any of claims 1 to 15 for dermatological administration which forms a bioadhesive, controlled release product, wherein the active agent is selected from;

i. acyclovir

ii. testosterone undecanoate.

25) A topical formulation as claimed in any of claims 1 to 15 for dermatological administration which forms a bioadhesive, controlled release product, wherein the active agent is selected from cosmetic agents, fragrances, flavourings, essential oils UV absorbing agents and mixtures thereof.

26) A method of delivery of a bioactive agent to a human or non-human animal (preferably mammalian) body, this method comprising administering a pre-formulation comprising a non-liquid crystalline, low viscosity mixture of:

a) at least one neutral diacyl lipid and/or at least one tocopherol;

b) at least one phospholipid;

c) at least one biocompatible, oxygen containing, low viscosity organic solvent;

and at least one bioactive agent is dissolved or dispersed in the low viscosity mixture, whereby to form at least one liquid crystalline phase structure upon contact with an aqueous fluid *in vivo* following administration.

27) A method as claimed in claim 26 wherein said preformulation is a preformulation as claimed in any of claims 1 to 25.

28) The method as claimed in claim 26 or claim 27 wherein said pre-formulation is administered by a method selected from subcutaneous injection, intramuscular injection, intra-cavity injection through tissue, intra-cavity injection into an open cavity without tissue penetration, spraying, rolling, wiping, dabbing, painting, rinsing, or dropping.

29) A method for the preparation of a liquid crystalline composition comprising exposing a pre-formulation comprising a non-liquid crystalline, low viscosity mixture of:

a) at least one neutral diacyl lipid and/or at least one tocopherol;

b) at least one phospholipid;

c) at least one biocompatible, oxygen containing, low viscosity organic solvent;

and at least one bioactive agent dissolved or dispersed in the low viscosity mixture, to an aqueous fluid *in vivo*.

30) A method as claimed in claim 29 wherein said preformulation is a preformulation as claimed in any of claims 1 to 25.

31) A process for the formation of a pre-formulation suitable for the administration of a bioactive agent to a (preferably mammalian) subject, said process comprising forming a non-liquid crystalline, low viscosity mixture of

a) at least one neutral diacyl lipid and/or at least one tocopherol;

b) at least one phospholipid;

c) at least one biocompatible, oxygen containing low viscosity, organic solvent;

and dissolving or dispersing at least one bioactive agent in the low viscosity mixture, or in at least one of components a, b or c prior to forming the low viscosity mixture.

32) A process as claimed in claim 31 wherein said preformulation is a preformulation as claimed in any of claims 1 to 25.

33) The use of a non-liquid crystalline, low viscosity mixture of:

a) at least one neutral diacyl lipid and/or at least one tocopherol;

b) at least one phospholipid;

c) at least one biocompatible, oxygen containing, low viscosity organic solvent;

wherein at least one bioactive agent is dissolved or dispersed in the low viscosity mixture in the manufacture of a pre-formulation for use in the sustained administration of said active agent, wherein said pre-formulation is capable of forming at least one liquid crystalline phase structure upon contact with an aqueous fluid.

34) The use as claimed in claim 33 wherein said preformulation is a preformulation as claimed in any of claims 1 to 25.

35) A method of treatment or prophylaxis of a human or non-human animal subject comprising administration of a preformulation as claimed in any of claims 1 to 25.

36) The method of claim 35 for the treatment of a condition selected from bacterial infection, fungal infection, skin soreness, eye conditions, genital soreness, infections and conditions for the finger and/or toe nails, travel sickness, addiction including nicotine addiction, periodontal infection, conjunctivitis, glaucoma and hormone deficiency or imbalance.

37) The method of claim 35 for prophylaxis against at least one condition selected from infection during surgery, infection during implantation, sunburn, infection at the site of burns, cuts or abrasions, oral infections, genital infections and infections resulting from activities resulting in exposure to infective agents.

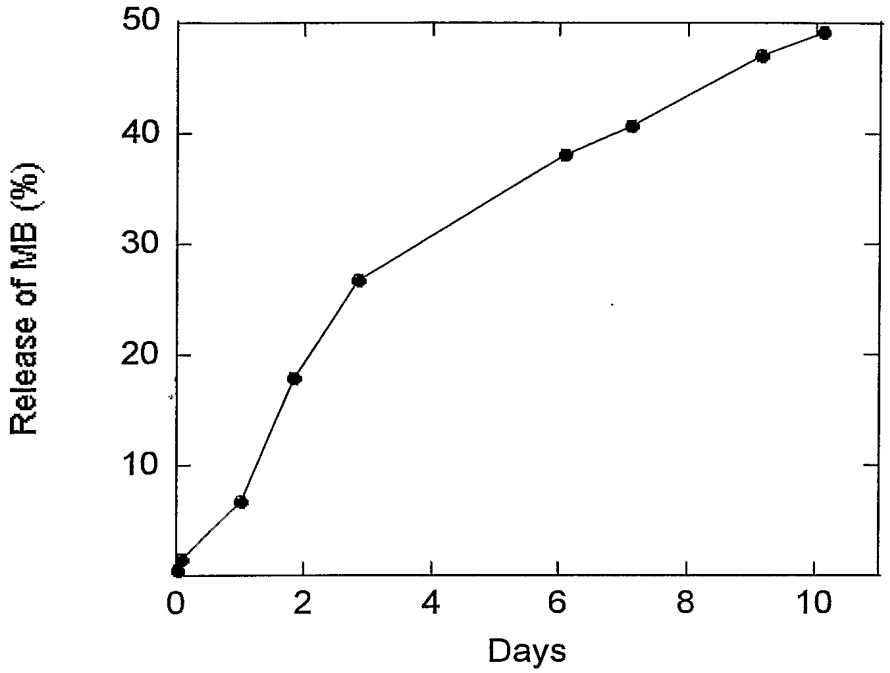


Figure 1. Cumulative release of MB from a depot forming a reversed hexagonal H_{II} phase.

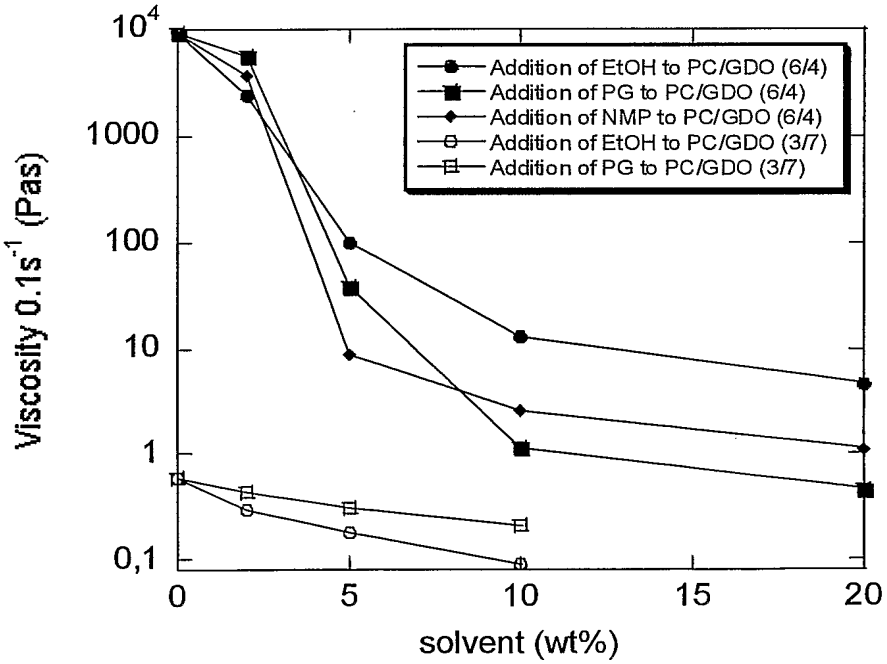


Figure 2. Decrease in viscosity of the depot precursor on addition of solvents. PC/GDO (6/4) is a precursor to a reversed hexagonal H_{II} phase and PC/GDO (3/7) is a precursor to a reversed cubic I_2 phase.

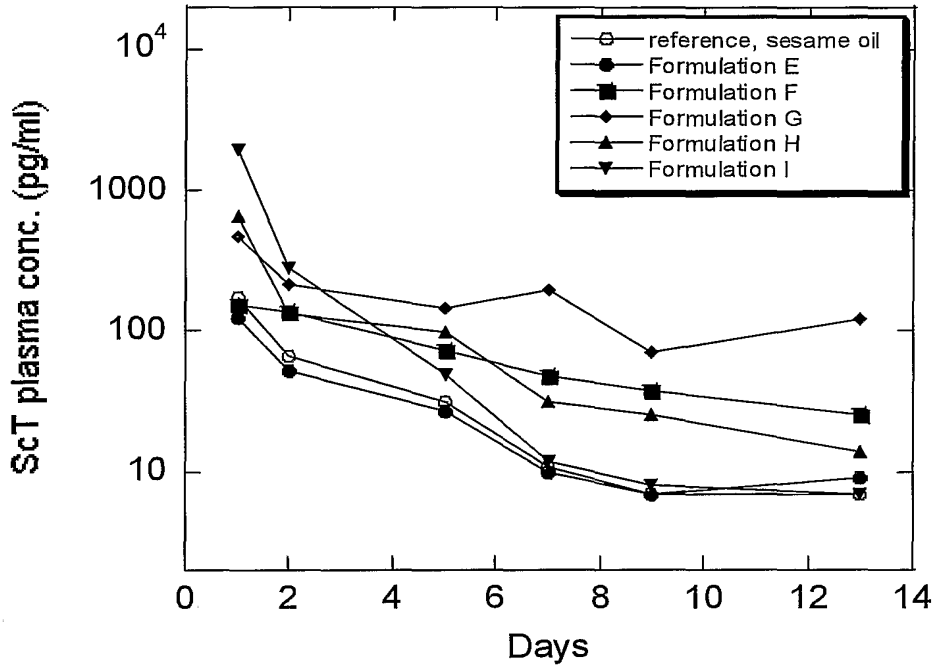


Figure 3. Plasma concentrations in the rat model after subcutaneous administration of formulations E to I. A depot based on sesame oil was used as reference.

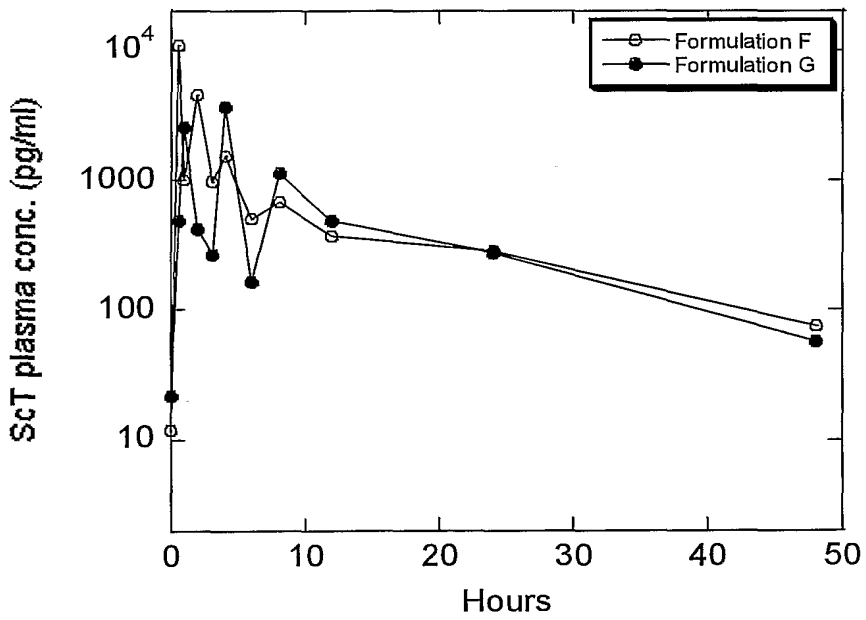


Figure 4. Plasma concentrations in the rat model after subcutaneous administration of formulations F and G.

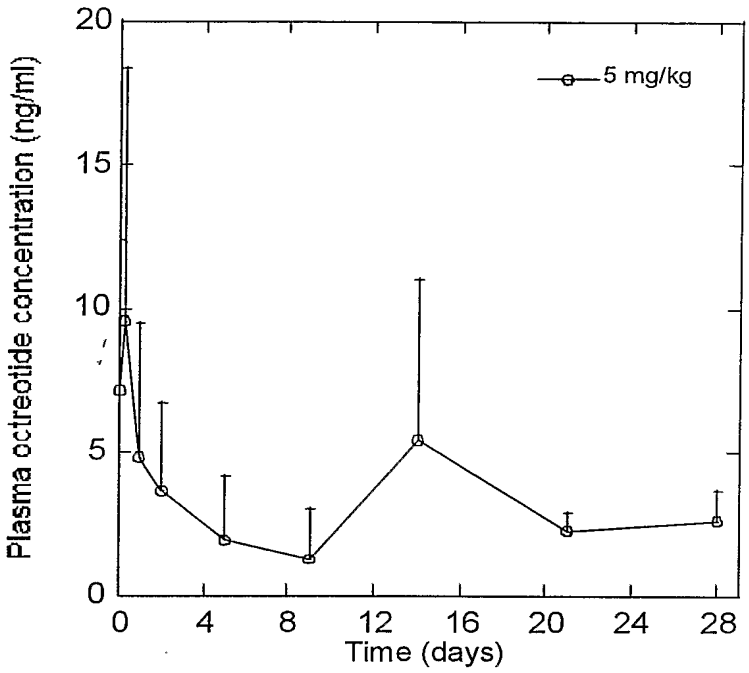


Figure 5: Octreotide plasma levels in the rat model following administration of octreotide formulation precursor (0.5% by weight octreotide).

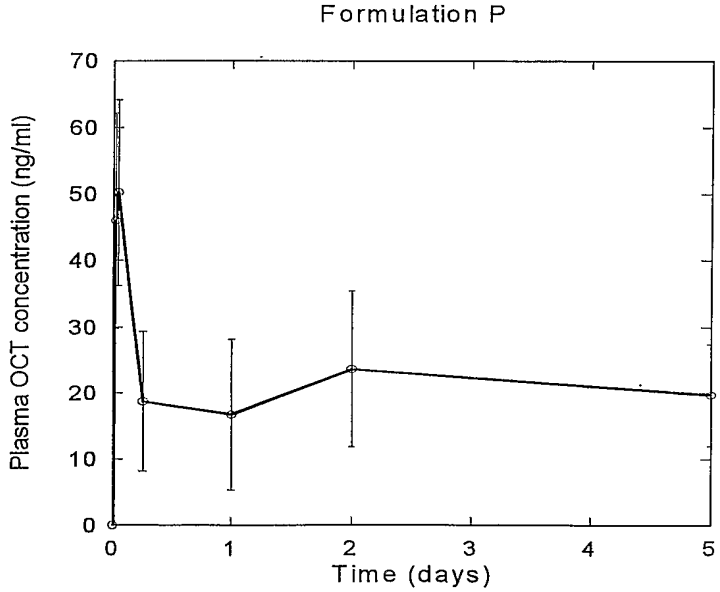


Figure 6: Octreotide plasma levels in the rat model following administration of octreotide formulation P, see Example 45.

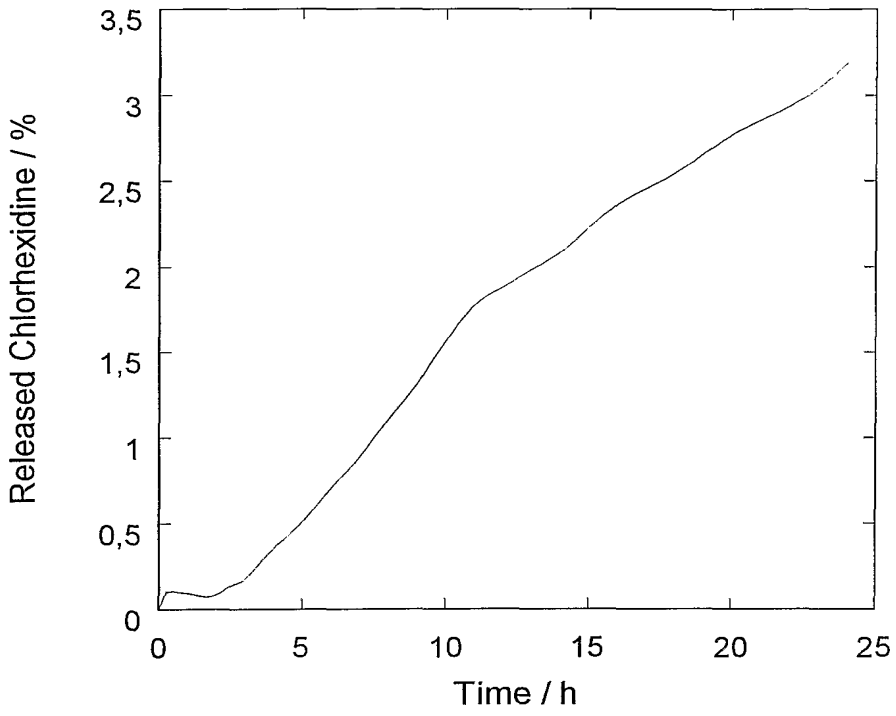


Figure 7: Release of Chlorhexidine from formulation A, see Example 47.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB2005/002217

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/10 A61K9/06 A61K9/12

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 7 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)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 2005/046642 A (CAMURUS AB; JOABSSON, FREDRIK; TIBERG, FREDRIK; GODDARD, CHRISTOPHER) 26 May 2005 (2005-05-26) page 13, last paragraph page 27, paragraph 3 - page 28, paragraph 3 examples 5,6	1-37
X	US 5 807 573 A (LJUSBERG-WAHREN ET AL) 15 September 1998 (1998-09-15) cited in the application column 2, line 60 - line 64 column 4, line 4 - line 62 examples 1,3	1-37

-/--

[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

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"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

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Date of the actual completion of the international search	Date of mailing of the international search report
7 October 2005	19/10/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Giménez Miralles, J

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB2005/002217

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97/13528 A (DUMEX-ALPHARMA A/S; NIELSEN, LISE, SYLVEST; HANSEN, JENS) 17 April 1997 (1997-04-17) page 2, paragraph 2 page 11, paragraph 4 page 58, line 25 - line 27 page 61, paragraph 2	1-37
Y	US 6 464 987 B1 (FANARA DOMENICO ET AL) 15 October 2002 (2002-10-15) column 4, line 52 - line 65 claim 10	1-37
Y	WO 02/068562 A (THE PROCTER & GAMBLE COMPANY) 6 September 2002 (2002-09-06) page 10, last paragraph	1-37
A	SHAH JAYMIN C ET AL: "Cubic phase gels as drug delivery systems" ADVANCED DRUG DELIVERY REVIEWS, AMSTERDAM, NL, vol. 47, no. 2-3, 25 April 2001 (2001-04-25), pages 229-250, XP002320651 ISSN: 0169-409X the whole document	1-37

Form PCT/ISA/210 (continuation of second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2005/002217

Box II Observations where certain claims were found unsearchable (Continuation of item 2 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.: 26-28, 35-37
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 26-28 and 35-37 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 III Observations where unity of invention is lacking (Continuation of item 3 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

International Application No
PCT/GB2005/002217

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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			MX	PA03007511 A	04-12-2003

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 May 2006 (26.05.2006)

PCT

(10) International Publication Number
WO 2006/055603 A2

(51) International Patent Classification: Not classified

(21) International Application Number:
PCT/US2005/041470

(22) International Filing Date:
16 November 2005 (16.11.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/628,748 16 November 2004 (16.11.2004) US

(71) Applicant *(for all designated States except US)*: **ELAN PHARMA INTERNATIONAL LTD.** [IE/IE]; Treasury Building, Lower Grand Canal Street, Dublin 2 (IE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **LIVERSIDGE, Gary** [US/US]; 258 Colwyn Terrace, West Chester, PA 19380 (US). **JENKINS, Scott** [US/US]; 774 Southwind Lane, Downingtown, PA 19335 (US).

(74) Agent: **SIMKIN, Michele, M.**; Foley & Lardner LLP, Washington Harbour, Suite 500, 3000 K Street, NW, Washington, DC 20007-5143 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

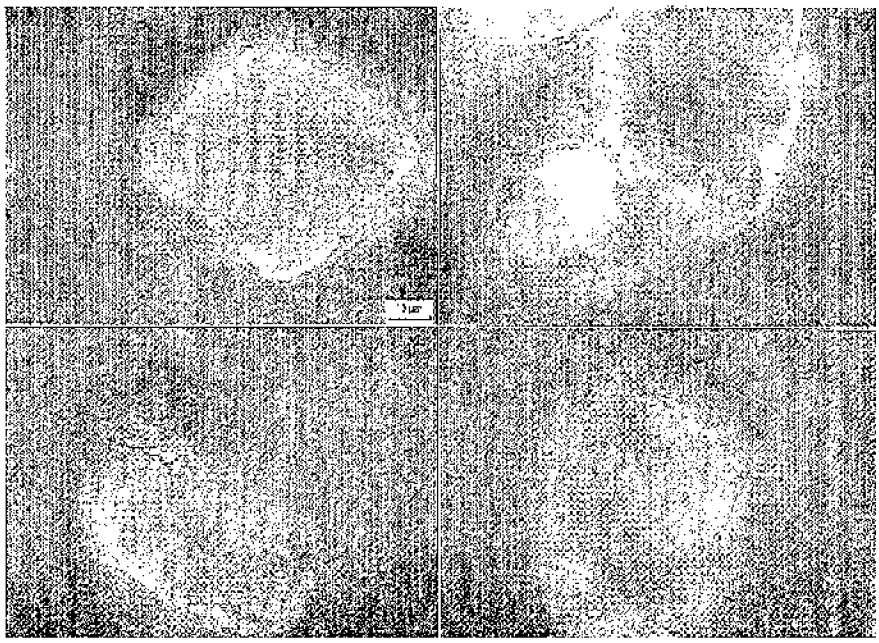
Published:

— *without international search report and to be republished upon receipt of that report*

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: