BENZODIAZEPINE BROMODOMAIN INHIBITOR

Field of the Invention

The present invention relates to a benzodiazepine compound, processes for its preparation, pharmaceutical compositions containing such a compound and to its use in therapy.

Background of the Invention

The genomes of eukaryotic organisms are highly organised within the nucleus of the cell. The long strands of duplex DNA are wrapped around an octomer of histone proteins (most usually comprising two copies of histones H2A, H2B H3 and H4) to form a nuclesome. This basic unit is then further compressed by the aggregation and folding of nucleosomes to form a highly condensed chromatin structure. A range of different states of condensation are possible, and the tightness of this structure varies during the cell cycle, being most compact during the process of cell division. Chromatin structure plays a critical role in regulating gene transcription, which cannot occur efficiently from highly condensed chromatin. The chromatin structure is controlled by a series of post translational modifications to histone proteins, notably histones H3 and H4, and most commonly within the histone tails which extend beyond the core nucleosome structure. These modifications include acetylation, methylation, phosphorylation, ubiquitinylation and SUMOylation. These epigenetic marks are written and erased by specific enzymes, which place the tags on specific residues within the histone tail, thereby forming an epigenetic code, which is then interpreted by the cell to allow gene specific regulation of chromatin structure and thereby transcription.

Histone acetylation is most usually associated with the activation of gene transcription, as the modification loosens the interaction of the DNA and the histone octomer by changing the electrostatics. In addition to this physical change, specific proteins bind to acetylated lysine residues within histones to read the epigenetic code. Bromodomains are small (~110 amino acid) distinct domains within proteins that bind to acetylated lysine resides commonly but not exclusively in the context of histones. There is a family of around 50 proteins known to contain bromodomains, and they have a range of functions within the cell.

The Bet family of bromodomain containing proteins comprises 4 proteins (BRD2, BRD3, BRD4 and BRD-t) which contain tandem bromodomains capable of binding to two acetylated lysine resides in close proximity, increasing the specificity of the interaction. BRD2 and BRD3 are reported to associate with histones along actively transcribed genes and may be involved in facilitating transcriptional elongation (Leroy et al, Mol. Cell. 2008 30(1):51-60), while BRD4 appears to be involved in the recruitment of the pTEF-B complex to inducible genes, resulting in phosphorylation of RNA polymerase and increased transcriptional output (Hargreaves et al, Cell, 2009 138(1): 129-145). It has also been reported that BRD4 and BRD3 fuse with NUT (nuclear protein in testis) forming a novel fusion oncogene, BRD4-NUT, in a highly malignant form of epithelial neoplasia (French *et al*. Cancer Research, 2003, 63, 304-307 and French *et al.* Journal of Clinical Oncology, 2004, 22 (20), 4135-4139). Data suggests that BRD-NUT fusion proteins contribute to carcinogensesis (Oncogene, 2008, 27, 2237-2242). BRD-t is uniquely expressed in the testes and ovary. All family members have been reported to have some function in controlling or executing aspects of the cell cycle, and have been shown to remain in complex with chromosomes during cell division – suggesting a role in the maintenance of epigenetic memory. In addition some viruses make use of these proteins to tether their genomes to the host cell chromatin, as part of the process of viral replication (You et al Cell, 2004 117(3):349-60).

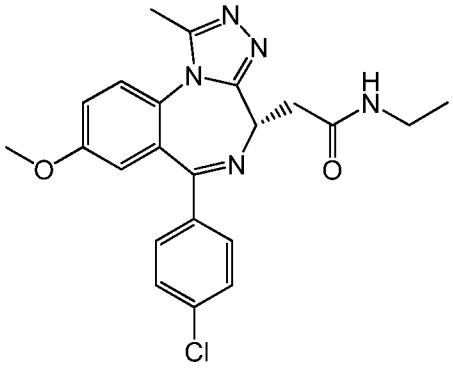
Japanese patent application JP2008-156311 discloses a benzimidazole derivative which is said to be a BRD2 bromodomain binding agent which has utility with respect to virus infection / proliferation.

Patent application WO2009/084693A1 discloses a series of thienotriazolodiazepiene derivatives that are said to inhibit the binding between an acetylated histone and a bromodomain containg protein which are said to be useful as anti-cancer agents.

A compound has been found which inhibits the binding of bromodomains with its cognate acetylated proteins, more particularly that inhibits the binding of Bet family bromodomains to acetylated lysine residues. Such a compound will hereafter be referred to as a "bromodomain inhibitor".

Summary of the Invention

In a first aspect of the present invention, there is provided a compound of formula (I) or a salt thereof



(I)

In a second aspect of the present invention, there is provided a pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof and one or more pharmaceutically acceptable carriers, diluents or excipients.

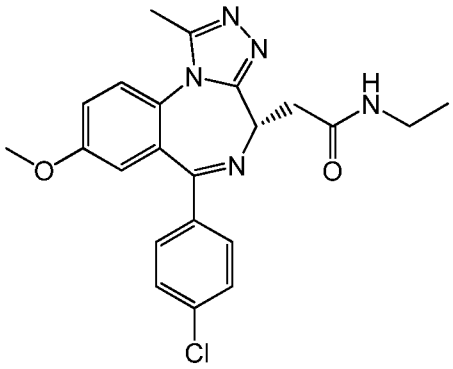
In a third aspect of the present invention, there is provided a compound of formula (I), or a pharmaceutically acceptable salt thereof for use in therapy, in particular in the treatment of diseases or conditions for which a bromodomain inhibitor is indicated.

In a fourth aspect of the present invention, there is provided a method of treating diseases or conditions for which a bromodomain inhibitor is indicated in a subject in need thereof which comprises administering a therapeutically effective amount of compound of formula (I) or a pharmaceutically acceptable salt thereof.

In a fifth aspect of the present invention, there is provided the use of a compound of formula (I), or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the treatment of diseases or conditions for which a bromodomain inhibitor is indicated.

Detailed Description of the Invention

The present invention relates to a compound of formula (I) which is 2-[(4*S*)-6-(4-Chlorophenyl)-1-methyl-8-(methyloxy)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl]-*N*-ethylacetamide



(I)

or a salt thereof.

It will be appreciated that the present invention covers compounds of formula (I) as the free base and as salts thereof, for example as a pharmaceutically acceptable salt thereof.

In one embodiment there is provided a compound which is 2-[(4*S*)-6-(4-Chlorophenyl)-1-methyl-8-(methyloxy)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl]-*N*-ethylacetamide.

Because of their potential use in medicine, salts of the compounds of formula (I) are desirably pharmaceutically acceptable. In another embodiment there is provided a compound which is 2-[(4*S*)-6-(4-Chlorophenyl)-1-methyl-8-(methyloxy)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl]-*N*-ethylacetamide or a pharmaceutically acceptable salt thereof.

Suitable pharmaceutically acceptable salts can include acid or base addition salts. For a review on suitable salts see Berge *et al.*, J. Pharm. Sci., 66:1-19, (1977). Typically, a pharmaceutically acceptable salt may be readily prepared by using a desired acid or base as appropriate. The resultant salt may precipitate from solution and be collected by filtration or may be recovered by evaporation of the solvent.

A pharmaceutically acceptable base addition salt can be formed by reaction of a compound of formula (I) with a suitable inorganic or organic base, (e.g. triethylamine, ethanolamine, triethanolamine, choline, arginine, lysine or histidine), optionally in a suitable solvent, to give the base addition salt which is usually isolated, for example, by crystallisation and filtration. Pharmaceutically acceptable base salts include ammonium salts, alkali metal salts such as those of sodium and potassium, alkaline earth metal salts

such as those of calcium and magnesium and salts with organic bases, including salts of primary, secondary and tertiary amines, such as isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexyl amine and N-methyl-D-glucamine.

A pharmaceutically acceptable acid addition salt can be formed by reaction of a compound of formula (I) with a suitable inorganic or organic acid (such as hydrobromic, hydrochloric, sulphuric, nitric, phosphoric, succinic, maleic, acetic, propionic, fumaric, citric, tartaric, lactic, benzoic, salicylic, glutamaic, aspartic, p-toluenesulfonic, benzenesulfonic, methanesulfonic, ethanesulfonic, naphthalenesulfonic such as 2-naphthalenesulfonic, or hexanoic acid), optionally in a suitable solvent such as an organic solvent, to give the salt which is usually isolated, for example, by crystallisation and filtration. A pharmaceutically acceptable acid addition salt of a compound of formula (I) can comprise or be, for example, a hydrobromide, hydrochloride, sulfate, nitrate, phosphate, succinate, maleate, acetate, propionate, fumarate, citrate, tartrate, lactate, benzoate, salicylate, glutamate, aspartate, p-toluenesulfonate, benzenesulfonate, methanesulfonate, ethanesulfonate, naphthalenesulfonate (e.g. 2-naphthalenesulfonate) or hexanoate salt.

Other non-pharmaceutically acceptable salts, e.g. formates, oxalates or trifluoroacetates, may be used, for example, in the isolation of the compound of formula (I), and are included within the scope of this invention.

The invention includes within its scope all possible stoichiometric and non-stoichiometric forms of the salts of the compound of formula (I).

It will be appreciated that many organic compounds can form complexes with solvents in which they are reacted or from which they are precipitated or crystallized. These complexes are known as “solvates”. For example, a complex with water is known as a “hydrate”. Solvents with high boiling points and/or capable of forming hydrogen bonds such as water, xylene, *N*-methyl pyrrolidinone, methanol and ethanol may be used to form solvates. Methods for identification of solvates include, but are not limited to, NMR and microanalysis. Solvates of the compound of formula (I) are within the scope of the invention.

The invention includes within its scope all possible stoichiometric and non-stoichiometric forms of the solvates of the compound of formula (I).

The invention encompasses all prodrugs, of the compound of formula (I) and pharmaceutically acceptable salts thereof, which upon administration to the recipient are capable of providing (directly or indirectly) a compound of formula (I) or a pharmaceutically acceptable salt thereof, or an active metabolite or residue thereof. Such derivatives are recognizable to those skilled in the art, without undue experimentation. Nevertheless, reference is made to the teaching of Burger's Medicinal Chemistry and Drug Discovery, 5th Edition, Vol 1: Principles and Practice, which is incorporated herein by reference to the extent of teaching such derivatives.

The compound of formula (I) may be in crystalline or amorphous form. Furthermore, some of the crystalline forms of the compound of formula (I) may exist as polymorphs, which are included within the scope of the present invention. Polymorphic forms of the compound of formula (I) may be characterized and differentiated using a number of conventional analytical techniques, including, but not limited to, X-ray powder diffraction (XRPD) patterns, infrared (IR) spectra, Raman spectra, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and solid state nuclear magnetic resonance (SSNMR).

The compound of formula (I) is an individual isomer isolated such as to be substantially free of the other isomer (i.e. enantionerically pure) such that less than 10%, particularly less than about 1%, for example less than about 0.1% of the other enantiomer is present.

Separation of isomers may be achieved by conventional techniques known to those skilled in the art, e.g. by fractional crystallisation, chromatography or HPLC.

The compound of formula (I) may exist in one of several tautomeric forms. It will be understood that the present invention encompasses all tautomers of the compound of formula (I) whether as individual tautomers or as mixtures thereof.

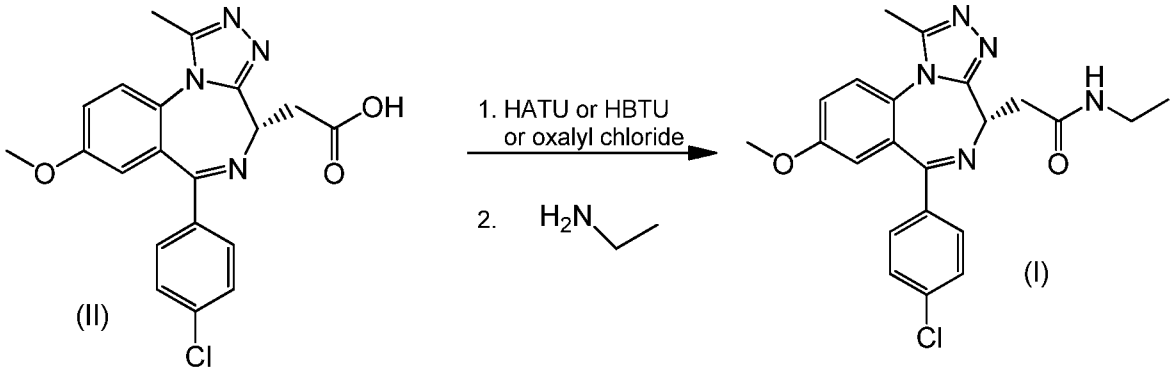
It will be appreciated from the foregoing that included within the scope of the invention are solvates, isomers and polymorphic forms of the compound of formula (I) and salts thereof.

The compound of formula (I) and pharmaceutically acceptable salts thereof may be made by a variety of methods, including standard chemistry. Illustrative general synthetic

methods are set out below and then the specific compound of formula (I) is prepared in the working Examples. These processes form further aspects of the present invention.

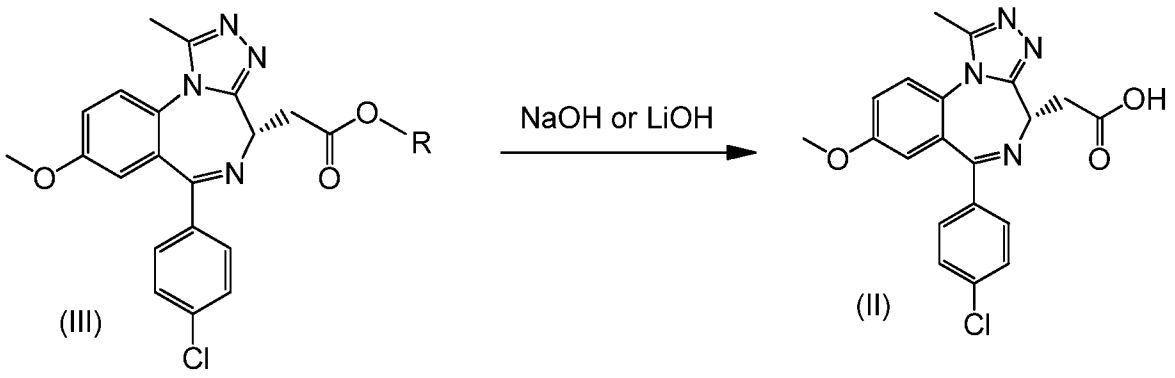
The compound of formula (I) may be prepared according to reaction scheme 1 by reaction of a compound of formula (II) with $EtNH_2$ in the presence of HATU or HBTU and DIEA at room temperature. Alternatively compounds of formula (I) may be prepared by reacting the compound of formula (II) with oxalyl chloride followed by addition of $EtNH_2$ in the presence of triethylamine.

Scheme 1



The compound of formula (II) may be prepared according to reaction Scheme 2. Suitable reaction conditions comprise reacting a compound of formula (III) with alkaline hydroxide preferably sodium hydroxide or lithium hydroxide.

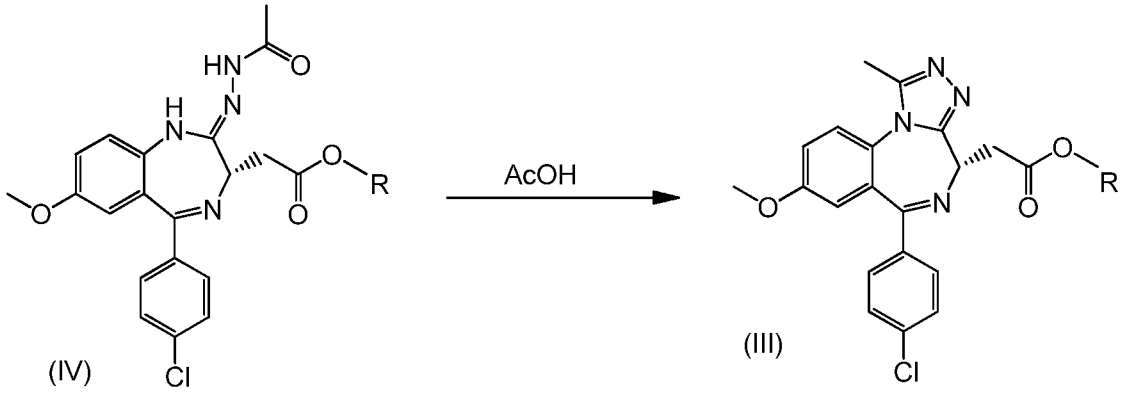
Scheme 2



wherein R represents C_{1-6}alkyl such as methyl.

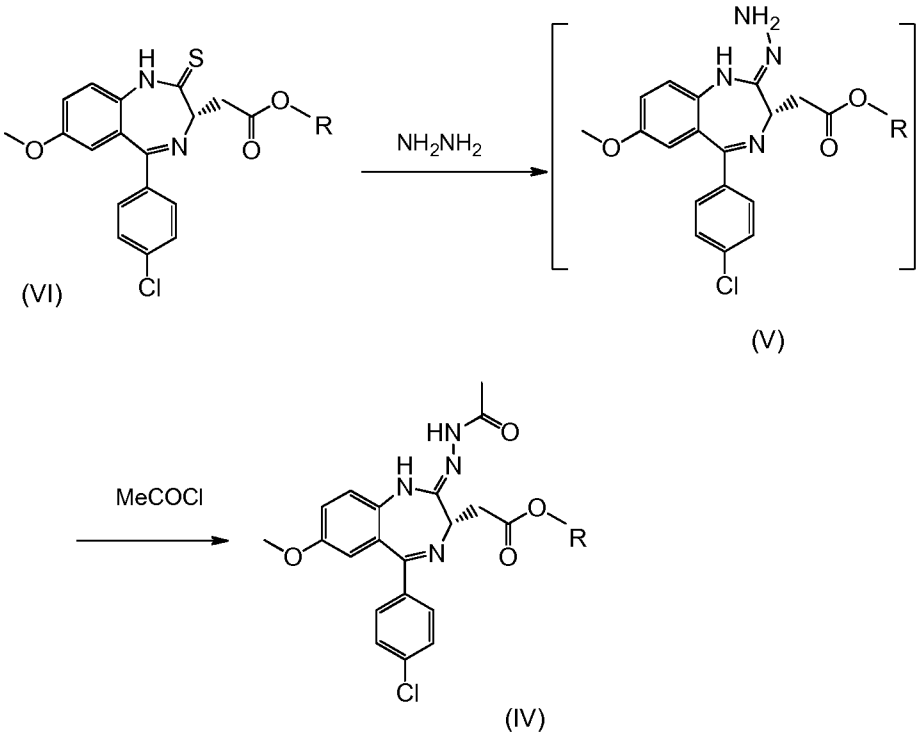
Compounds of formula (III), may be prepared according to reaction scheme 3 by reacting compounds of formula (IV) with AcOH.

Scheme 3



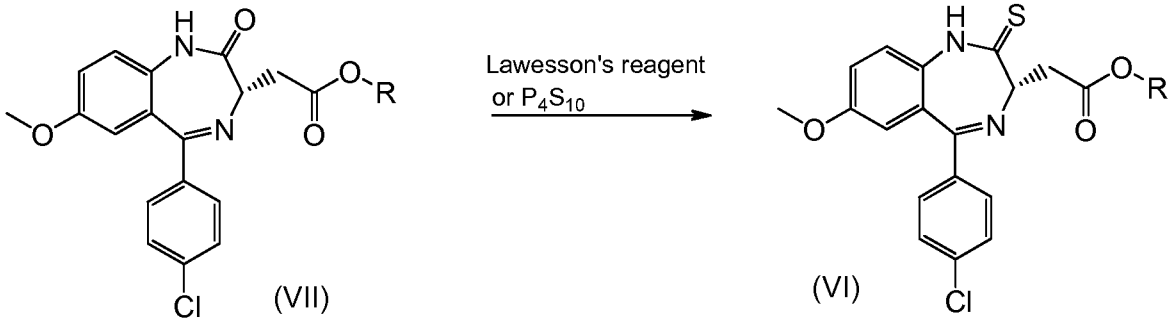
Compounds of formula (IV) may be prepared according to reaction scheme 4 by reacting compounds of formula (VI) with hydrazine below 15 °C followed by reaction of the resulting hydrazone (V) with MeCOCl at 0°C. Generally hydrazone (V) is used without further purification and is reacted with MeCOCl at , for example 0 °C.

Scheme 4



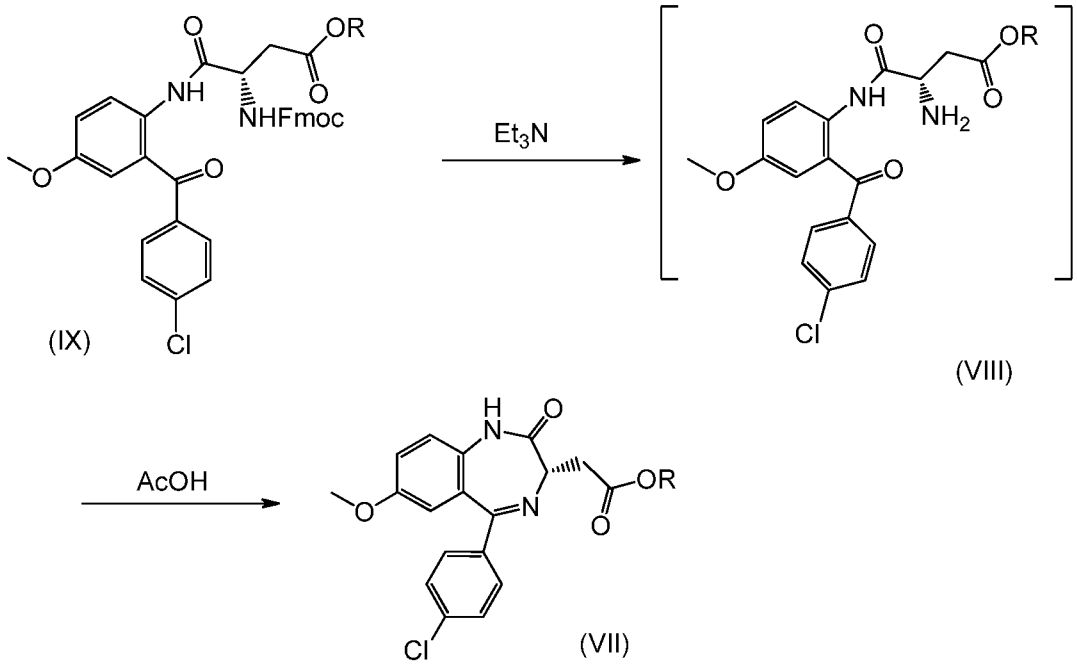
Compounds of formula (VI) in which R is C_{1-6}alkyl (such as methyl) may be prepared according to reaction scheme 5 from compounds of formula (VII) by treatment with Lawesson's reagent or P_4S_{10}. Suitable reaction conditions comprise reacting compounds of formula (VIII) with P_4S_{10} in 1,2-dichloroethane at, for example 70 °C.

Scheme 5



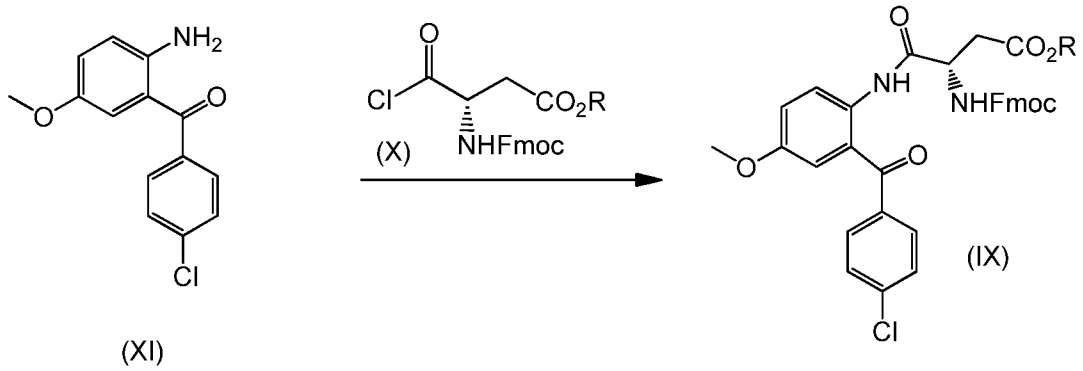
Compounds of formula (VII) may be prepared according to reaction scheme 6, by reacting compounds of formula (IX) with an organic base such as triethylamine followed by reaction of the resulting amine (VIII) with acetic acid. Generally, amine (VIII) is used without further purification and is reacted with AcOH at, for example 60 °C.

Scheme 6



Compounds of formula (IX) may be prepared according to reaction scheme 7, by reacting compounds of formula (XI) with the acylchloride (X) derived from protected aspartic acid.

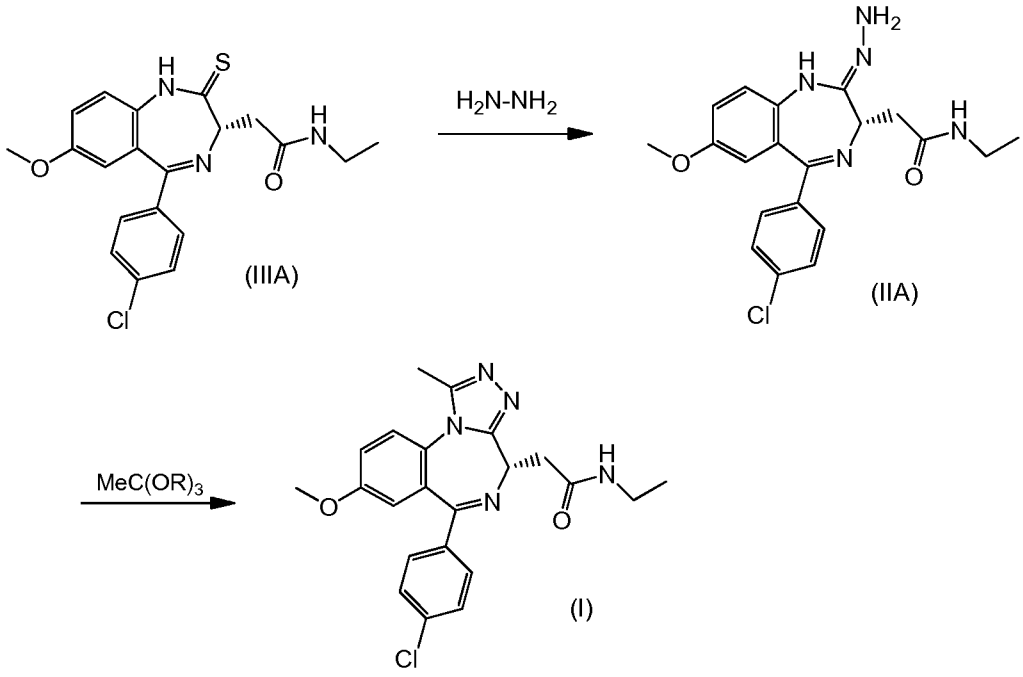
Scheme 7



Compounds of formula (XI) may be prepared according to procedures described in *Synthesis* **1980**, 677-688. Acyl chlorides of formula (X) may be prepared according to procedures described in J. Org. Chem., **1990**, *55*, 3068-3074 and J. Chem. Soc. Perkin Trans. 1, **2001**, 1673-1695.

Alternatively the compound of formula (I) may be prepared according to reaction scheme 8.

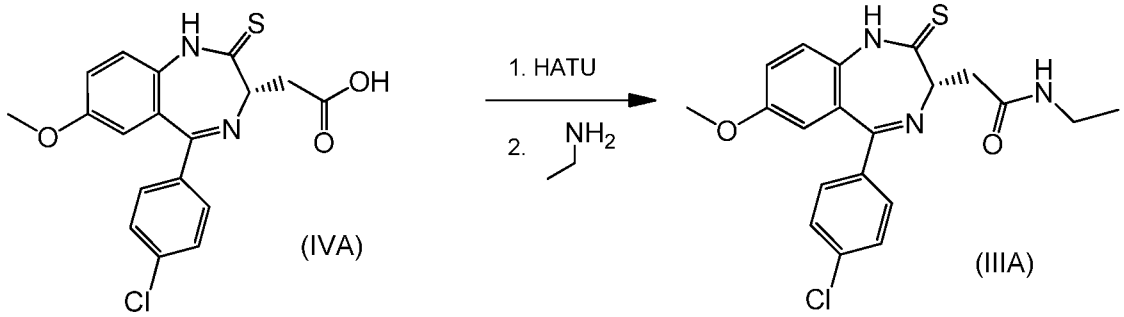
Scheme 8



wherein R represents C_{1-4}alkyl such as methyl.

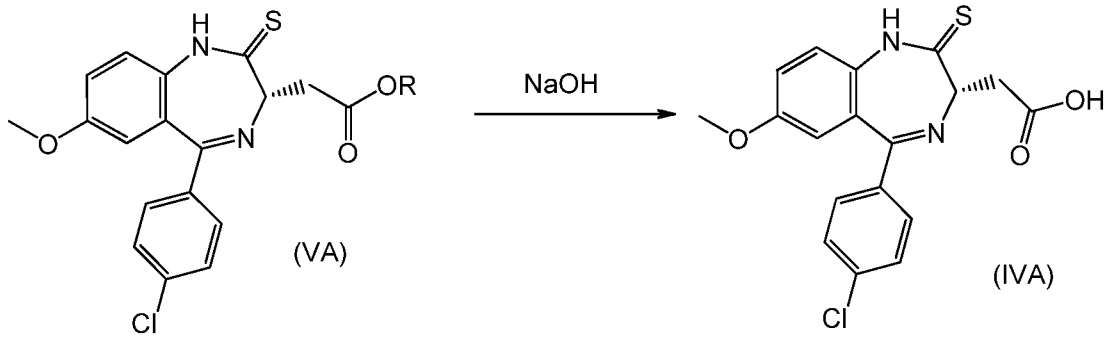
The compound of formula (IIIA) may be prepared according to reaction scheme 9 by reacting compounds of formula (IVA) with $EtNH_2$ in the presence of HATU and DIEA at, for example room temperature.

Scheme 9



The compound of formula (IVA) may be prepared according to reaction scheme 10. Suitable reaction conditions comprise reacting compounds of formula (VI) with alkaline hydroxide such as sodium hydroxide.

Scheme 10



It will be appreciated by those skilled in the art that it may be advantageous to protect one or more functional groups of the compounds described in the above processes. Examples of protecting groups and the means for their removal can be found in T. W. Greene 'Protective Groups in Organic Synthesis' (4th edition, J. Wiley and Sons, 2006). Suitable amine protecting groups include acyl (e.g. acetyl, carbamate (e.g. 2',2',2'-

trichloroethoxycarbonyl, benzyloxycarbonyl or t-butoxycarbonyl) and arylalkyl (e.g. benzyl), which may be removed by hydrolysis (e.g. using an acid such as hydrochloric acid in dioxane or trifluoroacetic acid in dichloromethane) or reductively (e.g. hydrogenolysis of a benzyl or benzyloxycarbonyl group or reductive removal of a 2',2',2'-trichloroethoxycarbonyl group using zinc in acetic acid) as appropriate. Other suitable amine protecting groups include trifluoroacetyl (-$COCF_3$) which may be removed by base catalysed hydrolysis.

It will be appreciated that in any of the routes described above, the precise order of the synthetic steps by which the various groups and moieties are introduced into the molecule may be varied. It will be within the skill of the practitioner in the art to ensure that groups or moieties introduced at one stage of the process will not be affected by subsequent transformations and reactions, and to select the order of synthetic steps accordingly.

Certain intermediate compounds described above are believed to be novel and therefore form a yet further aspect of the invention.

The compound of formula (I) and salts thereof is a bromodomain inhibitor, and thus is believed to have potential utility in the treatment of diseases or conditions for which a bromodomain is indicated.

The present invention thus provides a compound of formula (I) or a pharmaceutically acceptable salt thereof for use in therapy. The compound of formula (I) or pharmaceutically salt thereof can be for use in the treatment of diseases or conditions for which a bromodomain inhibitor indicated.

In one embodiment there is provided a compound of formula (I) or a pharmaceutically acceptable salt thereof for use in the treatment of diseases or conditions for which a bromodomain is indicated. In another embodiment, there is provided a compound or a pharmaceutically acceptable salt thereof for use in the treatment of a chronic autoimmune and/or inflammatory condition. In a further embodiment, there is provided a compound or a pharmaceutically acceptable salt thereof for use in the treatment of cancer, such as midline carcinoma.

In one embodiment there is provided the use of a compound of formula (I) or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the

treatment of diseases or conditions for which a bromodomain inhibitor is indicated. In another embodiment, there is provided the use of a compound of formula (I) or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the treatment of a chronic autoimmune and/or inflammatory condition. In a further embodiment, there is provided the use of a compound of formula (I) or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the treatment of cancer, such as midline carcinoma.

In one embodiment there is provided a method for treatment of a disease or condition, for which a bromodomain inhibitor is indicated, in a subject in need thereof which comprises administering a therapeutically effective amount of compound of formula (I) or a pharmaceutically acceptable salt thereof. In another embodiment there is provided a method for treatment of a chronic autoimmune and/or inflammatory condition, in a subject in need thereof which comprises administering a therapeutically effective amount of compound of formula (I) or a pharmaceutically acceptable salt thereof. In a further embodiment there is provided a method for treatment of cancer, such as midline carcinoma, in a subject in need thereof which comprises administering a therapeutically effective amount of compound of formula (I) or a pharmaceutically acceptable salt thereof.

In one embodiment the subject in need thereof is a mammal, particularly a human.

As used herein, the term "effective amount" means that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system, animal or human that is being sought, for instance, by a researcher or clinician. Furthermore, the term “therapeutically effective amount” means any amount which, as compared to a corresponding subject who has not received such amount, results in improved treatment, healing, prevention, or amelioration of a disease, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder. The term also includes within its scope amounts effective to enhance normal physiological function.

Bromodomain inhibitors are believed to be useful in the treatment of a variety of diseases or conditions related to systemic or tissue inflammation, inflammatory responses to infection or hypoxia, cellular activation and proliferation, lipid metabolism, fibrosis and in the prevention and treatment of viral infections.

Bromodomain inhibitors may be useful in the treatment of a wide variety of chronic autoimmune and inflammatory conditions such as rheumatoid arthritis, osteoarthritis, acute gout, psoriasis, systemic lupus erythematosus, multiple sclerosis, inflammatory bowel disease (Crohn's disease and Ulcerative colitis), asthma, chronic obstructive airways disease, pneumonitis, myocarditis, pericarditis, myositis, eczema, dermatitis, alopecia, vitiligo, bullous skin diseases, nephritis, vasculitis, atherosclerosis, Alzheimer's disease, depression, retinitis, uveitis, scleritis, hepatitis, pancreatitis, primary biliary cirrhosis, sclerosing cholangitis, Addison's disease, hypophysitis, thyroiditis, type I diabetes and acute rejection of transplanted organs.

Bromodomain inhibitors may be useful in the treatment of a wide variety of acute inflammatory conditions such as acute gout, giant cell arteritis, nephritis including lupus nephritis, vasculitis with organ involvement such as glomerulonephritis, vasculitis including giant cell arteritis, Wegener's granulomatosis, Polyarteritis nodosa, Behcet's disease, Kawasaki disease, Takayasu's Arteritis and acute rejection of transplanted organs.

Bromodomain inhibitors may be useful in the prevention or treatment of diseases or conditions which involve inflammatory responses to infections with bacteria, viruses, fungi, parasites or their toxins, such as sepsis, sepsis syndrome, septic shock, endotoxaemia, systemic inflammatory response syndrome (SIRS), multi-organ dysfunction syndrome, toxic shock syndrome, acute lung injury, ARDS (adult respiratory distress syndrome), acute renal failure, fulminant hepatitis, burns, acute pancreatitis, post-surgical syndromes, sarcoidosis, Herxheimer reactions, encephalitis, myelitis, meningitis, malaria, SIRS associated with viral infections such as influenza, herpes zoster, herpes simplex and coronavirus.

Bromodomain inhibitors may be useful in the prevention or treatment of conditions associated with ischaemia-reperfusion injury such as myocardial infarction, cerebro-vascular ischaemia (stroke), acute coronary syndromes, renal reperfusion injury, organ transplantation, coronary artery bypass grafting, cardio-pulmonary bypass procedures and pulmonary, renal, hepatic, gastro-intestinal or peripheral limb embolism.

Bromodomain inhibitors may be useful in the treatment of disorders of lipid metabolism via the regulation of APO-A1 such as hypercholesterolemia, atherosclerosis and Alzheimer's disease.

Bromodomain inhibitors may be useful in the treatment of fibrotic conditions such as idiopathic pulmonary fibrosis, renal fibrosis, post-operative stricture, keloid formation, scleroderma and cardiac fibrosis.

Bromodomain inhibitors may be useful in the prevention and treatment of viral infections such as herpes virus, human papilloma virus, adenovirus, poxvirus and other DNA viruses.

Bromodomain inhibitors may be useful in the treatment of cancer, including hematological (such as leukaemia), epithelial including lung, breast and colon carcinomas, midline carcinomas, mesenchymal, hepatic, renal and neurological tumours.

Bromodomain inhibitors may be useful in the treatment of treatment of ophthamological indications such as dry eye.

In one embodiment the disease or condition for which a bromodomain inhibitor is indicated is selected from diseases associated with systemic inflammatory response syndrome, such as sepsis, burns, pancreatitis, major trauma, haemorrhage and ischaemia. In this embodiment the bromodomain inhibitor would be administered at the point of diagnosis to reduce the incidence of: SIRS, the onset of shock, multi-organ dysfunction syndrome, which includes the onset of acute lung injury, ARDS, acute renal, hepatic, cardiac and gastro-intestinal injury and mortality. In another embodiment the bromodomain inhibitor would be administered prior to surgical or other procedures associated with a high risk of sepsis, haemorrhage, extensive tissue damage, SIRS or MODS. In a particular embodiment the disease or condition for which a bromodomain inhibitor is indicated is sepsis, sepsis syndrome, septic shock and/or endotoxaemia. In another embodiment, the bromodomain inhibitor is indicated for the treatment of acute or chronic pancreatitis. In another embodiment the bromodomain is indicated for the treatment of burns.

In one embodiment the disease or condition for which a bromodomain inhibitor is indicated is selected from herpes simplex infections and reactivations, cold sores, herpes zoster infections and reactivations, chickenpox, shingles, human papilloma virus, cervical neoplasia, adenovirus infections, including acute respiratory disease, and poxvirus infections such as cowpox and smallpox and African swine fever virus. In one particular

embodiment a bromodomain inhibitor is indicated for the treatment of Human papilloma virus infections of skin or cervical epithelia.

The term “diseases or conditions for which a bromodomain inhibitor is indicated”, is intended to include any or all of the above disease states.

While it is possible that for use in therapy, a compound of formula (I) as well as pharmaceutically acceptable salts thereof may be administered as the raw chemical, it is common to present the active ingredient as a pharmaceutical composition.

The present invention therefore provides in a further aspect a pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof and one or more pharmaceutically acceptable carriers, diluents or excipients.

Thus there is provided a pharmaceutical composition for the treatment of diseases or conditions in which a bromodomain inhibitor is indicated comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof.

The carrier(s), diluent(s) or excipient(s) used in such pharmaceutical compositions must be acceptable in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof. In accordance with another aspect of the invention there is also provided a process for the preparation of a pharmaceutical composition including admixing a compound of formula (I), or a pharmaceutically acceptable salt thereof, with one or more pharmaceutically acceptable carriers, diluents or excipients. The pharmaceutical composition can be for use in the treatment of any of the conditions described herein.

Since the compound of formula (I) and pharmaceutically acceptable salts thereof are intended for use in pharmaceutical compositions it will be readily understood that they are each preferably provided in substantially pure form, for example, at least 60% pure, more suitably at least 75% pure and preferably at least 85% pure, especially at least 98% pure (% in a weight for weight basis).

Pharmaceutical compositions may be presented in unit dose forms containing a predetermined amount of active ingredient per unit dose. Preferred unit dosage compositions are those containing a daily dose or sub-dose, or an appropriate fraction

thereof, of an active ingredient. Such unit doses may therefore be administered more than once a day. Preferred unit dosage compositions are those containing a daily dose or sub-dose (for administration more than once a day), as herein above recited, or an appropriate fraction thereof, of an active ingredient.

Pharmaceutical compositions may be adapted for administration by any appropriate route, for example by the oral (including buccal or sublingual), rectal, inhaled, intranasal, topical (including buccal, sublingual or transdermal), vaginal or parenteral (including subcutaneous, intramuscular, intravenous or intradermal) route. Such compositions may be prepared by any method known in the art of pharmacy, for example by bringing into association the active ingredient with the carrier(s) or excipient(s).

In one embodiment the pharmaceutical composition is adapted for oral administration.

In one embodiment the pharmaceutical composition is adapted for parenteral administration, particularly intravenous administration.

Pharmaceutical compositions adapted for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the composition isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The compositions may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

Pharmaceutical compositions adapted for oral administration may be presented as discrete units such as capsules or tablets; powders or granules; solutions or suspensions in aqueous or non-aqueous liquids; edible foams or whips; or oil-in-water liquid emulsions or water-in-oil liquid emulsions.

For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Powders suitable for incorporating

into tablets or capsules may be prepared by reducing the compound to a suitable fine size (e.g. by micronisation) and mixing with a similarly prepared pharmaceutical carrier such as an edible carbohydrate, as, for example, starch or mannitol. Flavoring, preservative, dispersing and coloring agent can also be present.

Capsules may be made by preparing a powder mixture, as described above, and filling formed gelatin sheaths. Glidants and lubricants such as colloidal silica, talc, magnesium stearate, calcium stearate or solid polyethylene glycol can be added to the powder mixture before the filling operation. A disintegrating or solubilizing agent such as agar-agar, calcium carbonate or sodium carbonate can also be added to improve the availability of the medicament when the capsule is ingested.

Moreover, when desired or necessary, suitable binders, glidants, lubricants, sweetening agents, flavours, disintegrating agents and coloring agents can also be incorporated into the mixture. Suitable binders include starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum and the like. Tablets are formulated, for example, by preparing a powder mixture, granulating or slugging, adding a lubricant and disintegrant and pressing into tablets. A powder mixture is prepared by mixing the compound, suitably comminuted, with a diluent or base as described above, and optionally, with a binder such as carboxymethylcellulose, an aliginate, gelatin, or polyvinyl pyrrolidone, a solution retardant such as paraffin, a resorption accelerator such as a quaternary salt and/or an absorption agent such as bentonite, kaolin or dicalcium phosphate. The powder mixture can be granulated by wetting with a binder such as syrup, starch paste, acadia mucilage or solutions of cellulosic or polymeric materials and forcing through a screen. As an alternative to granulating, the powder mixture can be run through the tablet machine and the result is imperfectly formed slugs broken into granules. The granules can be lubricated to prevent sticking to the tablet forming dies by means of the addition of stearic acid, a stearate salt, talc or mineral oil. The lubricated mixture is then compressed into tablets. The compounds of the present invention can also be combined with a free flowing inert carrier and compressed into tablets directly without going through the granulating or slugging steps. A clear or opaque protective coating consisting of a sealing coat of shellac, a

coating of sugar or polymeric material and a polish coating of wax can be provided. Dyestuffs can be added to these coatings to distinguish different unit dosages.

Oral fluids such as solution, syrups and elixirs can be prepared in dosage unit form so that a given quantity contains a predetermined amount of the compound. Syrups can be prepared by dissolving the compound in a suitably flavored aqueous solution, while elixirs are prepared through the use of a non-toxic alcoholic vehicle. Suspensions can be formulated by dispersing the compound in a non-toxic vehicle. Solubilizers and emulsifiers such as ethoxylated isostearyl alcohols and polyoxy ethylene sorbitol ethers, preservatives, flavor additive such as peppermint oil or natural sweeteners or saccharin or other artificial sweeteners, and the like can also be added.

Where appropriate, dosage unit compositions for oral administration can be microencapsulated. The formulation can also be prepared to prolong or sustain the release as for example by coating or embedding particulate material in polymers, wax or the like.

The compounds of formula (I) and pharmaceutically acceptable salts thereof can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

Pharmaceutical compositions adapted for topical administration may be formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols or oils.

For treatments of the eye or other external tissues, for example mouth and skin, the compositions are preferably applied as a topical ointment or cream. When formulated in an ointment, the active ingredient may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredient may be formulated in a cream with an oil-in-water cream base or a water-in-oil base.

Pharmaceutical compositions adapted for topical administrations to the eye include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent.

Dosage forms for nasal or inhaled administration may conveniently be formulated as aerosols, solutions, suspensions, gels or dry powders.

For compositions suitable and/or adapted for inhaled administration, it is preferred that the compound of formula (I) or pharmaceutically acceptable salt thereof is in a particle-size-reduced form e.g. obtained by micronisation. The preferable particle size of the size-reduced (e.g. micronised) compound or salt is defined by a D50 value of about 0.5 to about 10 microns (for example as measured using laser diffraction).

Aerosol formulations, *e.g.* for inhaled administration, can comprise a solution or fine suspension of the active substance in a pharmaceutically acceptable aqueous or non-aqueous solvent. Aerosol formulations can be presented in single or multidose quantities in sterile form in a sealed container, which can take the form of a cartridge or refill for use with an atomising device or inhaler. Alternatively the sealed container may be a unitary dispensing device such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve (metered dose inhaler) which is intended for disposal once the contents of the container have been exhausted.

Where the dosage form comprises an aerosol dispenser, it preferably contains a suitable propellant under pressure such as compressed air, carbon dioxide or an organic propellant such as a hydrofluorocarbon (HFC). Suitable HFC propellants include 1,1,1,2,3,3,3-heptafluoropropane and 1,1,1,2-tetrafluoroethane. The aerosol dosage forms can also take the form of a pump-atomiser. The pressurised aerosol may contain a solution or a suspension of the active compound. This may require the incorporation of additional excipients e.g. co-solvents and/or surfactants to improve the dispersion characteristics and homogeneity of suspension formulations. Solution formulations may also require the addition of co-solvents such as ethanol.

For pharmaceutical compositions suitable and/or adapted for inhaled administration, the pharmaceutical composition may be a dry powder inhalable composition. Such a composition can comprise a powder base such as lactose, glucose, trehalose, mannitol or starch, the compound of formula (I) or a pharmaceutically acceptable salt thereof (preferably in particle-size-reduced form, e.g. in micronised form), and optionally a performance modifier such as L-leucine or another amino acid and/or metals salts of stearic acid such as magnesium or calcium stearate. Preferably, the dry powder inhalable composition comprises a dry powder blend of lactose e.g. lactose monohydrate and the

compound of formula (I) or a pharmaceutically acceptable salt thereof. Such compositions can be administered to the patient using a suitable device such as the DISKUS® device, marketed by GlaxoSmithKline which is for example described in GB 2242134 A.

The compound of formula (I) and pharmaceutically acceptable salts thereof thereof may be formulated as a fluid formulation for delivery from a fluid dispenser, for example a fluid dispenser having a dispensing nozzle or dispensing orifice through which a metered dose of the fluid formulation is dispensed upon the application of a user-applied force to a pump mechanism of the fluid dispenser. Such fluid dispensers are generally provided with a reservoir of multiple metered doses of the fluid formulation, the doses being dispensable upon sequential pump actuations. The dispensing nozzle or orifice may be configured for insertion into the nostrils of the user for spray dispensing of the fluid formulation into the nasal cavity. A fluid dispenser of the aforementioned type is described and illustrated in WO2005/044354 A1.

A therapeutically effective amount of a compound of formula (I) or pharmaceutically acceptable salt thereof will depend upon a number of factors including, for example, the age and weight of the animal, the precise condition requiring treatment and its severity, the nature of the formulation, and the route of administration, and will ultimately be at the discretion of the attendant physician or veterinarian. In the pharmaceutical composition, each dosage unit for oral or parenteral administration preferably contains from 0.01 to 3000 mg, more preferably 0.5 to 1000 mg, of a compound of formula (I) or pharmaceutically acceptable salt thereof calculated as the free base. Each dosage unit for nasal or inhaled administration preferably contains from 0.001 to 50 mg, more preferably 0.01 to 5 mg, of a compound of the formula (I) or a pharmaceutically acceptable salt thereof, calculated as the free base.

The compound of formula (I) and pharmaceutically acceptable salts thereof can be administered in a daily dose (for an adult patient) of, for example, an oral or parenteral dose of 0.01 mg to 3000 mg per day or 0.5 to 1000 mg per day, or a nasal or inhaled dose of 0.001 to 50 mg per day or 0.01 to 5 mg per day, of the compound of formula (I) or a pharmaceutically acceptable salt thereof, calculated as the free base. This amount may be given in a single dose per day or more usually in a number (such as two, three, four, five or six) of sub-doses per day such that the total daily dose is the same. An effective

amount of a pharmaceutically acceptable salt thereof, may be determined as a proportion of the effective amount of the compound of formula (I) *per se*.

Thus there is provided a pharmaceutical composition comprising a) 0.01 to 3000mg of a compound of formula (I) or a pharmaceutically acceptable salt thereof, and (b) 0.1 to 2g of one or more pharamceutically acceptable carriers, diluents and/or excipients.

The compound of formula (I) and pharmaceutically acceptable salts thereof may be employed alone or in combination with other therapeutic agents. Combination therapies according to the present invention thus comprise the administration of at least one compound of formula (I) or a pharmaceutically acceptable salt thereof, and the use of at least one other pharmaceutically active agent. Preferably, combination therapies according to the present invention comprise the administration of at least one compound of formula (I) or a pharmaceutically acceptable salt thereof, and at least one other pharmaceutically active agent. The compoundof formula (I) and pharmaceutically acceptable salts thereof, and the other pharmaceutically active agent(s) may be administered together in a single pharmaceutical composition or separately and, when administered separately this may occur simultaneously or sequentially in any order. The amounts of the compound of formula (I) and pharmaceutically acceptable salts thereof, and the other pharmaceutically active agent(s) and the relative timings of administration will be selected in order to achieve the desired combined therapeutic effect. Thus in a further aspect, there is provided a combination comprising a compound of formula (I) or pharmaceutically acceptable salt thereof and at least one other pharmaceutically active agent. In one embodiment there is provided a combination pharmaceutical product comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof together with one or more other therapeutically active agents.

Thus in one aspect, the compound of formula (I) and pharmaceutical compositions according to the invention may be used in combination with or include one or more other therapeutic agents, for example selected from antibiotics, anti-virals, glucocorticosteroids, muscarinic antagonists and beta-2 agonists.

It will be appreciated that when the compound of formula (I) and pharmaceutically acceptable sdalt thereof the are administered in combination with other therapeutic agents normally administered by the inhaled, intravenous, oral or intranasal route, that the

resultant pharmaceutical composition may be administered by the same routes. Alternatively the individual components of the composition may be administered by different routes.

One embodiment of the invention encompasses combinations comprising one or two other therapeutic agents.

It will be clear to a person skilled in the art that, where appropriate, the other therapeutic ingredient(s) may be used in the form of salts, for example as alkali metal or amine salts or as acid addition salts, or prodrugs, or as esters, for example lower alkyl esters, or as solvates, for example hydrates, to optimise the activity and/or stability and/or physical characteristics, such as solubility, of the therapeutic ingredient. It will be clear also that, where appropriate, the therapeutic ingredients may be used in optically pure form.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical composition and thus pharmaceutical compositions comprising a combination as defined above together with a pharmaceutically acceptable diluent or carrier represent a further aspect of the invention.

The compound of formula (I) may be prepared by the methods described below or by similar methods. Thus the following Intermediates and Examples serve to illustrate the preparation of the compound of formula (I) and is not to be considered as limiting the scope of the invention in any way.

General Experimental Details

All temperatures referred to are in °C.

Abbreviations

TLC	- thin layer chromatography
AcOH	- acetic acid
AcCl	- acetyl chloride
PPTS	- pyridinium *p*-toluenesulfonate
DCM	- dichloromethane

1,2-DCE	- 1,2-dichloroethane
DIC	- Diisopropylcarbodiimide
DIEA	- *N*,*N*-diisopropylethylamine
DMF	- N,N-dimethylformamide
DMAP	- 4-dimethylaminopyridine
Fmoc	- 9*H*-fluoren-9-ylmethyl)oxy]carbonyl
HATU	-*O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate
HBTU	- *O*-(Benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate
Et_2O	- diethyl ether
EtOAc	- ethyl acetate
i-Pr_2O	- di-isopropyl ether
Config.	- absolute configuration
Lawesson's Reagent	- 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulphide
MeCN	- acetonitrile
MeOH	- methanol
Rt	- retention time
THF	- tetrahydrofuran
RT	- room temperature
Pd/C	- palladium on carbon

LC/MS refers to analyses by analytical HPLC which were conducted on two kinds of apparatus:

a) On a Supelcosil LCABZ+PLUS column (3µm, 3.3cm x 4.6mm ID) eluting with 0.1% HCO_2H and 0.01 M ammonium acetate in water (solvent A), and 95% acetonitrile and 0.05% HCO_2H in water (solvent B), using the following elution gradient 0-0.7 minutes 0%B, 0.7-4.2 minutes 0→100%B, 4.2-5.3 minutes 100%B, 5.3-5.5 minutes 100→0%B at a flow rate of 3 mL/minute. The mass spectra (MS) were recorded on a Fisons VG Platform mass spectrometer using electrospray positive ionisation [(ES+ve to give $[M+H]^+$ and $[M+NH_4]^+$ molecular ions] or electrospray negative ionisation [(ES-ve to give [M-H]- molecular ion] modes. Analytical data from this apparatus are given with the following format : $[M+H]^+$ or $[M-H]^-$.

b) On a Chromolith Performance RP 18 column (100 x 4.6 mm id) eluting with 0.01M ammonium acetate in water (solvent A) and 100% acetonitrile (solvent B), using the following elution gradient 0-4 minutes 0 →100% B, 4-5 minutes 100% B at a flow rate of 5 mL/minute. The mass spectra (MS) were recorded on a micromass Platform-LC mass spectrometer using atmospheric pressure chemical positive ionisation [AP+ve to give MH^+ molecular ions] or atmospheric pressure chemical negative ionisation [AP-ve to give $(M-H)^-$ molecular ions] modes. Analytical data from this apparatus are given with the following format: [M+H]+ or [M-H]- preceded by the acronym APCI to specify between both mass spectrometry analyses sources.

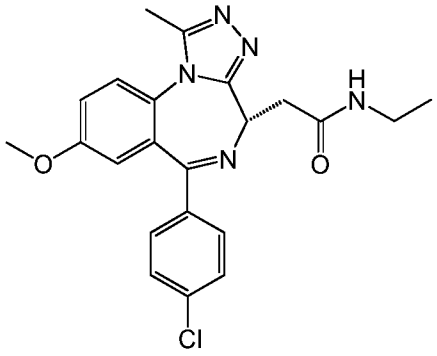
LC/HRMS: Analytical HPLC was conducted on a Uptisphere-hsc column (3µm 33 x 3 mm id) eluting with 0.01M ammonium acetate in water (solvent A) and 100% acetonitrile (solvent B), using the following elution gradient 0-0.5 minutes 5% B, 0.5-3.75 minutes 5→100% B, 3.75-4.5 100% B, 4.5-5 100→5% B, 5-5.5 5% B at a flow rate of 1.3 mL/minute. The mass spectra (MS) were recorded on a micromass LCT mass spectrometer using electrospray positive ionisation [ES+ve to give MH^+ molecular ions] or electrospray negative ionisation [ES-ve to give (M-H)- molecular ions] modes.

Mass directed auto-prep HPLC refers to the method where the material was purified by high performance liquid chromatography on a HPLCABZ+ 5µm column (5cm x 10mm i.d.) with 0.1% HCO_2H in water and 95% MeCN, 5% water (0.5% HCO_2H) utilising the following gradient elution conditions: 0-1.0 minutes 5%B, 1.0-8.0 minutes 5→30%B, 8.0-8.9 minutes 30%B, 8.9-9.0 minutes 30→95%B, 9.0-9.9 minutes 95%B, 9.9-10 minutes 95→0%B at a flow rate of 8mL/minute. The Gilson 202-fraction collector was triggered by a VG Platform Mass Spectrometer on detecting the mass of interest.

Proton NMR (^1H NMR) spectra were recorded at ambient temperature on a Bruker Avance 300 DPX spectrometer using solvant as internal standard and proton chemical shifts are expressed in ppm in the indicated solvent. The following abbreviations are used for multiplicity of NMR signals: s = singlet, d = doublet, t = triplet, q = quadruplet, dd = double doublet, m = multiplet.

TLC (thin layer chromatography) refers to the use of TLC plates sold by Merck coated with silica gel 60 F254.

Example 1: 2-[(4*S*)-6-(4-Chlorophenyl)-1-methyl-8-(methyloxy)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl]-*N*-ethylacetamide

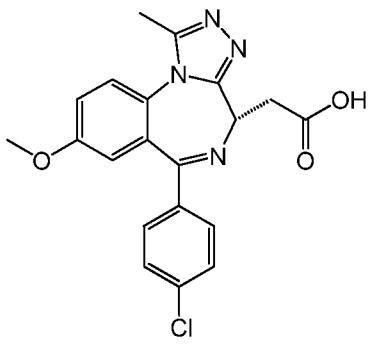


To a solution of [(4S)-6-(4-Chlorophenyl)-1-methyl-8-(methyloxy)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl]acetic acid (for a preparation see Intermediate 1)(16.0 g, 40 mmol) in THF at RT was added DIEA (14 mL, 80 mmol) followed by HATU (30.4 g, 80 mmol). The reaction mixture was stirred for 3h at this temperature and ethylamine (40 mL, 2M in THF, 80 mmol) was added. The mixture was stirred for 48h before being concentrated under reduced pressure. The crude material was suspended in water and extracted with DCM. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude solid was purified by chromatography on SiO_2 (DCM/MeOH 95/5) and the resulting solid recrystallised in MeCN. The solid was then dissolved in DCM and precipited with *i*-Pr_2O to give the title compound (8 g, 47% yield) as a white solid.

R_f = 0.48 (DCM/MeOH : 90/10). Mp >140 °C (becomes gummy). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53-7.47 (m, 2H), 7.39 (d, *J* = 8.9 Hz, 1H), 7.37-7.31 (m, 2H), 7.20 (dd, *J* = 2.9 and 8.9 Hz, 1H), 6.86 (d, *J* = 2.9 Hz, 1H), 6.40 (m, 1H), 4.62 (m, 1H), 3.80 (s, 3H), 3.51 (dd, *J* = 7.3 and 14.1 Hz, 1H), 3.46-3.21 (m, 3H), 2.62 (s, 3H), 1.19 (t, *J* = 7.3 Hz, 3H).

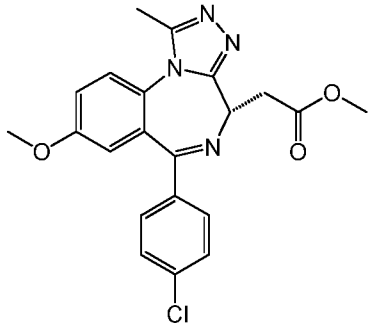
LC/MS : m/z 424 $[M(^{35}Cl)+H]^+$, Rt 2.33 min.

Intermediate 1: [(4S)-6-(4-Chlorophenyl)-1-methyl-8-(methyloxy)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl]acetic acid



To a solution of methyl [(4*S*)-6-(4-chlorophenyl)-1-methyl-8-(methyloxy)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl]acetate (for a preparation see Intermediate 2)(28 g, 68 mmol) in THF (450 mL) at RT was added 1N NaOH (136 mL, 136 mmol). The reaction mixture was stirred at this temperature for 5h before being cooled down and quenched with 1N HCl (136 mL). THF was removed under reduced pressure and the aqueous layer was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude solid was recrystallised in CH_3CN to give the title compound (23.9 g, 89% yield) as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55-7.48 (m, 2H), 7.41 (d, J = 8.9 Hz, 1H), 7.38-7.31 (m, 2H), 7.22 (dd, *J* = 2.9 and 8.9 Hz, 1H), 6.90 (d, *J* = 2.9 Hz, 1H), 4.59 (dd, *J* = 6.9 and 6.9 Hz, 1H), 3.81 (s, 3H), 3.70 (dd, *J* = 6.9 and 25.7 Hz, 1H), 3.61 (dd, *J* = 6.9 and 25.7 Hz, 1H), 2.63 (s, 3H). LC/MS: m/z 397 [M(^{35}Cl)+H]$^+$, Rt 2.11 min.

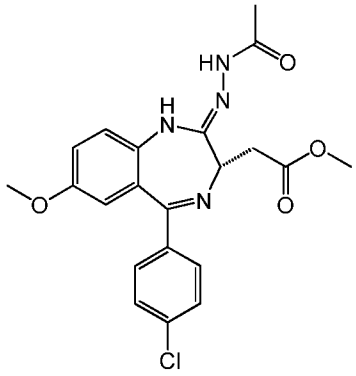
Intermediate 2: Methyl [(4*S*)-6-(4-chlorophenyl)-1-methyl-8-(methyloxy)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl]acetate



To crude methyl [(3*S*)-2-[(1*Z*)-2-acetylhydrazino]-5-(4-chlorophenyl)-7-(methyloxy)-3*H*-1,4-benzodiazepin-3-yl]acetate (for a preparation see Intermediate 3) (34 g, 79 mmol) was suspended in THF (200 mL) and AcOH (200 mL) was added at RT. The reaction mixture was stirred at this temperature overnight before being concentrated to dryness. The residue was suspended in saturated $NaHCO_3$ and extracted with DCM. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude solid was purified by chromatography on SiO_2 (DCM/MeOH : 90/10) to give the title compound (28 g, 86% yield) as a yellow powder.

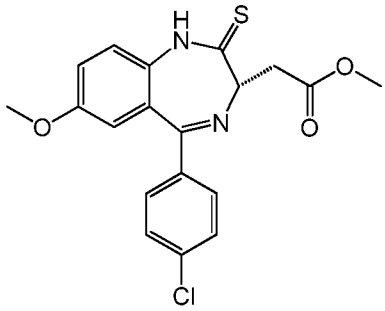
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54-7.47 (m, 2H), 7.40 (d, *J* = 8.8 Hz, 1H), 7.37-7.31 (m, 2H), 7.22 (dd, *J* = 2.8 and 8.8 Hz, 1H), 6.89 (d, *J* = 2.8 Hz, 1H), 4.61 (dd, *J* = 6.4 and 7.8 Hz, 1H), 3.82 (s, 3H), 3.78 (s, 3H), 3.66 (dd, *J* = 7.8 and 16.9 Hz, 1H), 3.60 (dd, *J* = 6.4 and 16.9 Hz, 1H), 2.62 (s, 3H). LC/MS m/z 411 [M(^{35}Cl)+H]$^+$, Rt 2.88 min.

Intermediate 3: Methyl [(3*S*)-2-[2-acetylhydrazino]-5-(4-chlorophenyl)-7-(methyloxy)-3*H*-1,4-benzodiazepin-3-yl]acetate



To a suspension of methyl [(3*S*)-5-(4-chlorophenyl)-7-(methyloxy)-2-thioxo-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl]acetate (for a preparation see Intermediate 4)(30.2 g, 77.7 mmol) in THF (800 mL) at 0°C was added hydrazine monohydrate (11.3 mL, 233 mmol) dropwise. The reaction mixture was stirred for 4h between 0°C and 15°C before being cooled at 0°C. Et_3N (32.4 mL, 230 mmol) was then added slowly and AcCl (16.3 mL, 230 mmol) was added dropwise. The mixture was allowed to warm to RT and stir for 1h then quenched with water and concentrated under reduced pressure. The resulting aqueous layer was then extracted with DCM and the organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to give the crude title compound (34 g, 100% yield) which was used without further purification. LC/MS: m/z 429 $[M(^{35}Cl)+H]^+$, Rt 2.83 min.

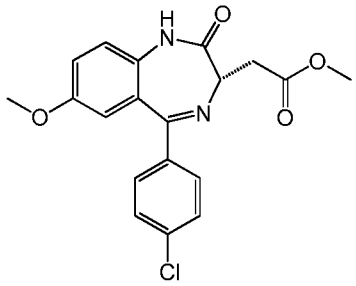
Intermediate 4: Methyl [(3*S*)-5-(4-chlorophenyl)-7-(methyloxy)-2-thioxo-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl]acetate



A suspension of P_4S_{10} (85.8 g, 190 mmol) and Na_2CO_3 (20.5 g, 190 mmol) in 1,2-DCE (1.5 L) at RT was stirred for 1 h before methyl [(3*S*)-5-(4-chlorophenyl)-7-(methyloxy)-2-oxo-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl]acetate (for a preparation see Intermediate 5) (40 g, 107 mmol) was added. The resulting mixture was stirred at 65°C for 4 h before being cooled and filtered. The solid was washed with DCM and the filtrate washed with sat. $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The title compound was precipitated from a DCM/*i*-Pr_2O mixture and

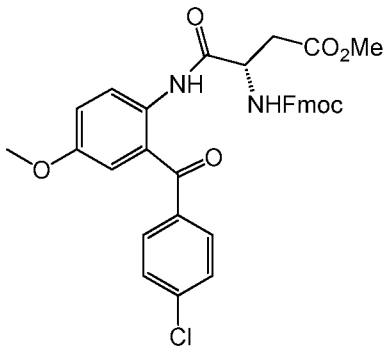
filtered. The filtrate was then concentrated and purified by flash chromatography (DCM/MeOH : 98/2) to afford another batch of product. The title compound was obtained combining the two fractions (30.2 g, 73%) as a yellow powder. LC/MS: m/z 389 $[M(^{35}Cl)+H]^+$, Rt 3.29 min.

Intermediate 5: Methyl [(3*S*)-5-(4-chlorophenyl)-7-(methyloxy)-2-oxo-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl]acetate



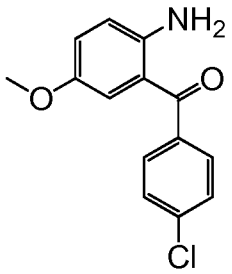
To a solution of the crude methyl N^1-[2-[(4-chlorophenyl)carbonyl]-4-(methyloxy)phenyl]-N^2-{[(9*H*-fluoren-9-ylmethyl)oxy]carbonyl}-L-α-asparaginate (for a preparation see Intermediate 6) (assumed 0.2 mol) in DCM (500 mL) was added Et_3N (500 mL, 3.65 mol) and the resulting mixture was refluxed for 24h before being concentrated. The resulting crude amine was dissolved in 1,2-DCE (1.5 L) and AcOH (104 mL, 1.8 mol) was added carefully. The reaction mixture was then stirred at 60°C for 2h before being concentrated *in vacuo* and dissolved in DCM. The organic layer was washed with 1N HCl and the aqueous layer was extracted with DCM (x3). The combined organic layers were washed twice with water, and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude solid was recrystallised in MeCN leading to the title compound (51 g) as a pale yellow solid. The filtrate could be concentrated and recrystallised in MeCN to give another 10 g of Intermediate 9 (total: 61 g, 69% yield based on recovered Intermediate 12). R_f = 0.34 (DCM/MeOH : 95/5). LC/MS m/z 373 $[M(^{35}Cl)+H]^+$, Rt 2.76 min.

Intermediate 6: Methyl N^1-[2-[(4-chlorophenyl)carbonyl]-4-(methyloxy)phenyl]-N^2-{[(9*H*-fluoren-9-ylmethyl)oxy]carbonyl}-L-α-asparaginate



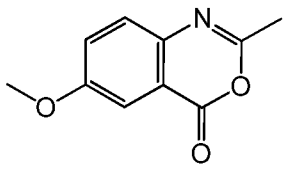
A mixture of Methyl *N*-{[(9*H*-fluoren-9-ylmethyl)oxy]carbonyl}-L-α-aspartyl chloride (prepared from *J. Org. Chem.* **1990**, *55*, 3068-3074 and *J. Chem. Soc. Perkin Trans. 1* **2001**, 1673-1695) (221 g, 0.57 mol) and [2-amino-5-(methyloxy)phenyl](4-chlorophenyl)methanone (for a preparation see Intermediate 7) (133 g, 0.5 mol) in $CHCl_3$ (410 mL) was stirred at 60°C for 1.5h before being cooled and concentrated under reduced pressure and used without further purification. LC/MS: m/z 613 $[M(^{35}Cl)+H]^+$, Rt = 3.89 min.

Intermediate 7: [2-amino-5-(methyloxy)phenyl](4-chlorophenyl)methanone



To a solution of 2-methyl-6-(methyloxy)-4*H*-3,1-benzoxazin-4-one (for a preparation see Intermediate 8)(40.0 g, 0.21 mol) in a toluene (560 mL)/ether (200 mL) mixture at 0°C was added dropwise a solution of 4-chlorophenylmagnesium bromide (170 mL, 1M in Et_2O, 0.17 mol). The reaction mixture was allowed to warm to RT and stirred for 1h before being quenched with 1N HCl. The aqueous layer was extracted with EtOAc (3 x) and the combined organics were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude compound was then dissolved in EtOH (400 mL) and 6N HCl (160 mL) was added. The reaction mixture was refluxed for 2 h before being concentrated under reduced pressure. The resulting solid was filtered and washed twice with ether before being suspended in EtOAc and neutralised with 1N NaOH. The aqueous layer was extracted with EtOAc (3 x) and the combined organics were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The title compound was obtained as a yellow solid (39 g, 88 % yield) which was used without further purification.

Intermediate 8 : 2-methyl-6-(methyloxy)-4*H*-3,1-benzoxazin-4-one



A solution of 5-methoxyanthranilic acid (7.8 g, 46.5 mmol) was refluxed in acetic anhydride (60 mL) for 2h15 before being cooled and concentrated under reduced pressure. The crude residue was then concentrated twice in the presence of toluene before being filtered and washed with ether to yield to the title compound (6.8 g, 77% yield) as a beige solid; LC/MS: m/z 192 $[M+H]^+$, Rt 1.69 min.

Preparation of reference compound for use in biological assays

Experimental details of LC-MS methods A and B as referred to herein are as follows:

LC/MS (Method A) was conducted on a Supelcosil LCABZ+PLUS column (3µm, 3.3cm x 4.6mm ID) eluting with 0.1% HCO_2H and 0.01 M ammonium acetate in water (solvent A), and 95% acetonitrile and 0.05% HCO_2H in water (solvent B), using the following elution gradient 0-0.7 minutes 0%B, 0.7-4.2 minutes 0→100%B, 4.2-5.3 minutes 100%B, 5.3-5.5 minutes 100→0%B at a flow rate of 3 mL/minute. The mass spectra (MS) were recorded on a Fisons VG Platform mass spectrometer using electrospray positive ionisation [(ES+ve to give $[M+H]^+$ and $[M+NH_4]^+$ molecular ions] or electrospray negative ionisation [(ES-ve to give [M-H]- molecular ion] modes. Analytical data from this apparatus are given with the following format : $[M+H]^+$ or $[M-H]^-$.

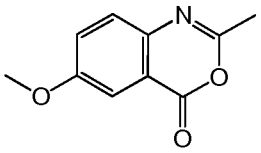
LC/MS (Method B) was conducted on an Sunfire C18 column (30mm x 4.6mm i.d. 3.5µm packing diameter) at 30 degrees centigrade, eluting with 0.1% v/v solution of Trifluoroacetic Acid in Water (Solvent A) and 0.1% v/v solution of Trifluoroacetic Acid in Acetonitrile (Solvent B) using the following elution gradient 0-0.1min 3%B, 0.1- 4.2min 3 – 100% B, 4.2-4.8min 100% B, 4.8-4.9min 100-3%B, 4.9 – 5.0min 3% B at a flow rate of 3ml/min. The UV detection was an averaged signal from wavelength of 210nm to 350nm and mass spectra were recorded on a mass spectrometer using positive electrospray ionization. Ionisation data was rounded to the nearest integer.

LC/HRMS: Analytical HPLC was conducted on a Uptisphere-hsc column (3µm 33 x 3 mm id) eluting with 0.01M ammonium acetate in water (solvent A) and 100% acetonitrile (solvent B), using the following elution gradient 0-0.5 minutes 5% B, 0.5-3.75 minutes 5→100% B, 3.75-4.5 100% B, 4.5-5 100→5% B, 5-5.5 5% B at a flow rate of 1.3 mL/minute. The mass spectra (MS) were recorded on a micromass LCT mass spectrometer using electrospray positive ionisation [ES+ve to give MH^+ molecular ions] or electrospray negative ionisation [ES-ve to give (M-H)- molecular ions] modes.

TLC (thin layer chromatography) refers to the use of TLC plates sold by Merck coated with silica gel 60 F254.

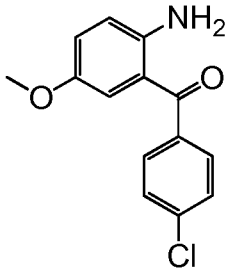
Silica chromatography techniques include either automated (Flashmaster or Biotage SP4) techniques or manual chromatography on pre-packed cartridges (SPE) or manually-packed flash columns.

Reference compound A : 2-methyl-6-(methyloxy)-4*H*-3,1-benzoxazin-4-one



A solution of 5-methoxyanthranilic acid (Lancaster) (41.8 g, 0.25 mol) was refluxed in acetic anhydride (230 mL) for 3.5 h before being concentrated under reduced pressure. The crude compound was then concentrated twice in the presence of toluene before being filtered and washed twice with ether to yield to the title compound (33.7 g, 71% yield) as a brown solid; LC/MS (Method A): m/z 192 $[M+H]^+$, Rt 1.69 min.

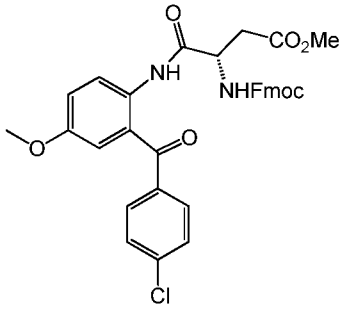
Reference compound B: [2-amino-5-(methyloxy)phenyl](4-chlorophenyl)methanone



To a solution of 2-methyl-6-(methyloxy)-4H-3,1-benzoxazin-4-one (for a preparation see Reference compound A) (40.0 g, 0.21 mol) in a toluene/ether (2/1) mixture (760 mL) at 0°C was added dropwise a solution of 4-chlorophenylmagnesium bromide (170 mL, 1M in Et_2O, 0.17 mol). The reaction mixture was allowed to warm to room temperature and

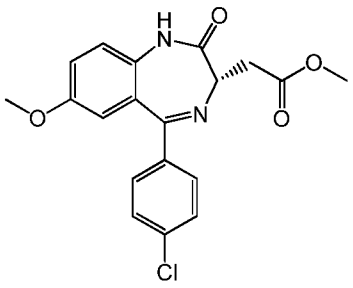
stirred for 1h before being quenched with 1N HCl (200 mL). The aqueous layer was extracted with EtOAc (3 x 150 mL) and the combined organics were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude compound was then dissolved in EtOH (400 mL) and 6N HCl (160 mL) was added. The reaction mixture was refluxed for 2 h before being concentrated to one-third in volume. The resulting solid was filtered and washed twice with ether before being suspended in EtOAc and neutralised with 1N NaOH. The aqueous layer was extracted with EtOAc (3 x 150 mL) and the combined organics were washed with brine (150 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The title compound was obtained as a yellow solid (39 g, 88 % yield); LC/MS (Method A): m/z 262 [M+H]+, Rt 2.57 min.

Reference Compound C: Methyl *N*^{1}-[2-[(4-chlorophenyl)carbonyl]-4-(methyloxy)phenyl]-*N*^{2}-{[(9*H*-fluoren-9-ylmethyl)oxy]carbonyl}-L-α-asparaginate



Methyl *N*-{[(9*H*-fluoren-9-ylmethyl)oxy]carbonyl}-L-α-aspartyl chloride (*Int. J. Peptide Protein Res.* **1992**, *40*, 13-18) (93 g, 0.24 mol) was dissolved in $CHCl_3$ (270 mL) and [2-amino-5-(methyloxy)phenyl](4-chlorophenyl)methanone (for a preparation see Reference compound B) (53 g, 0.2 mol) was added. The resulting mixture was stirred at 60°C for 1h before being cooled and concentrated at 60% in volume. Ether was added at 0°C and the resulting precipitate was filtered and discarded. The filtrate was concentrated under reduced pressure and used without further purification.

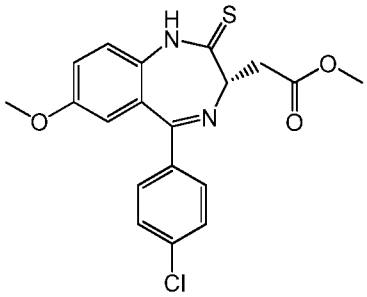
Reference compound D: Methyl [(3*S*)-5-(4-chlorophenyl)-7-(methyloxy)-2-oxo-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl]acetate



To a solution of Methyl N1-[2-[(4-chlorophenyl)carbonyl]-4-(methyloxy)phenyl]-N2-{[(9H-fluoren-9-ylmethyl)oxy]carbonyl}-L-α-asparaginate (for a preparation see Reference compound C) (assumed 0.2 mol) in DCM (500 mL) was added Et_3N (500 mL, 3.65 mol) and the resulting mixture was refluxed for 24h before being concentrated. The resulting crude amine was dissolved in 1,2-DCE (1.5 L) and AcOH (104 mL, 1.8 mol) was added carefully. The reaction mixture was then stirred at 60°C for 2h before being concentrated *in vacuo* and dissolved in DCM. The organic layer was washed with 1N HCl and the aqueous layer was extracted with DCM (x3). The combined organic layers were washed twice with water, and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude solid was recrystallised in MeCN leading to the title compound (51 g) as a pale yellow solid. The filtrate could be concentrated and recrystallised in MeCN to give to another 10 g of the desired product R_f = 0.34 (DCM/MeOH : 95/5).

HRMS $(M+H)^+$ calculated for $C_{19}H_{18}{}^{35}ClN_2O_4$ 373.0955; found 373.0957.

Reference compound E: Methyl [(3S)-5-(4-chlorophenyl)-7-(methyloxy)-2-thioxo-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]acetate

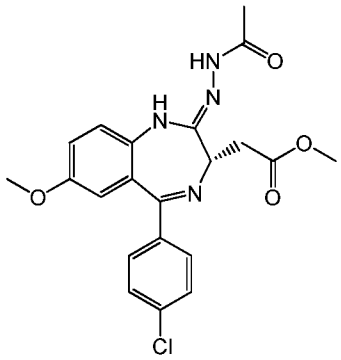


A suspension of P_4S_{10} (36.1 g, 81.1 mmol) and Na_2CO_3 (8.6 g, 81.1 mmol) in 1,2-DCE (700 mL) at room temperature was stirred for 2 h before Methyl [(3*S*)-5-(4-chlorophenyl)-7-(methyloxy)-2-oxo-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl]acetate (for a preparation see Reference compound D) (16.8 g, 45.1 mmol) was added. The resulting mixture was stirred at 70°C for 2 h before being cooled and filtered. The solid was washed twice with DCM and the filtrate washed with sat. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by flash-chromatography on silica gel (DCM/MeOH : 99/1) to afford the title

compound (17.2 g, 98% yield) as a yellowish solid. LC/MS (Method A): m/z 389 $[M(^{35}Cl)+H]^+$, Rt 2.64 min

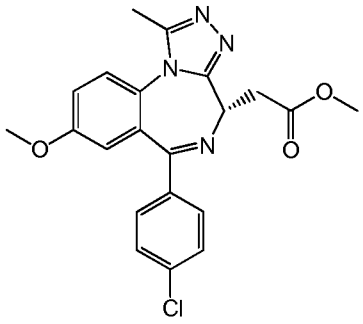
HRMS $(M+H)^+$ calculated for $C_{19}H_{18}{}^{35}ClN_2O_3S$ 389.0727; found 389.0714.

Reference compound F: Methyl [(3*S*)-2-[2-acetylhydrazino]-5-(4-chlorophenyl)-7-(methyloxy)-3*H*-1,4-benzodiazepin-3-yl]acetate



To a suspension of Methyl [(3S)-5-(4-chlorophenyl)-7-(methyloxy)-2-thioxo-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]acetate (for a preparation see Reference compound E (9.0 g, 23.2 mmol) in THF (300 mL) at 0°C was added hydrazine monohydrate (3.4 mL, 69.6 mmol) dropwise. The reaction mixture was stirred for 5h between 5°C and 15°C before being cooled at 0°C. Et_3N (9.7 mL, 69.6 mmol) was then added slowly and acetyl chloride (7.95 mL, 69.6 mmol) was added dropwise. The mixture was then allowed to warm to room temperature for 16h before being concentrated under reduced pressure. The crude product was dissolved in DCM and washed with water. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to give the crude title compound (9.7 g, 98% yield) which was used without further purification. R_f = 0.49 (DCM/MeOH : 90/10).

Reference compound G: Methyl [(4*S*)-6-(4-chlorophenyl)-1-methyl-8-(methyloxy)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl]acetate

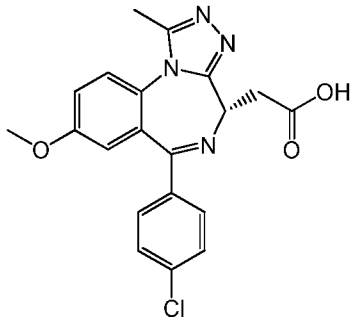


The crude Methyl [(3S)-2-[(1Z)-2-acetylhydrazino]-5-(4-chlorophenyl)-7-(methyloxy)-3H-1,4-benzodiazepin-3-yl]acetate (for a preparation see Reference compound F) (assumed

9.7 g) was suspended in THF (100 ml) and AcOH (60 mL) was added at room temperature. The reaction mixture was stirred at this temperature for 2 days before being concentrated under reduced pressure. The crude solid was triturated in *i*-Pr_2O and filtered to give the title compound (8.7 g, 91% over 3 steps) as an off-white solid.

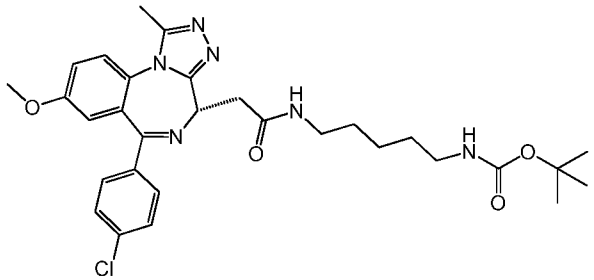
HRMS $(M+H)^+$ calculated for $C_{21}H_{20}ClN_4O_3$ 411.1229; found 411.1245.

Reference compound H: [(4S)-6-(4-Chlorophenyl)-1-methyl-8-(methyloxy)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl]acetic acid



To a solution of Methyl [(4*S*)-6-(4-chlorophenyl)-1-methyl-8-(methyloxy)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl]acetate (for a preparation see Reference compound G)(7.4 g, 18.1 mmol) in THF (130 mL) at room temperature was added 1N NaOH (36.2 mL, 36.2 mmol). The reaction mixture was stirred at this temperature for 5h before being quenched with 1N HCl (36.2 mL) and concentrated *in vacuo*. Water is then added and the aqueous layer was extracted with DCM (x3) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give the title compound (7 g, 98% yield) as a pale yellow solid.

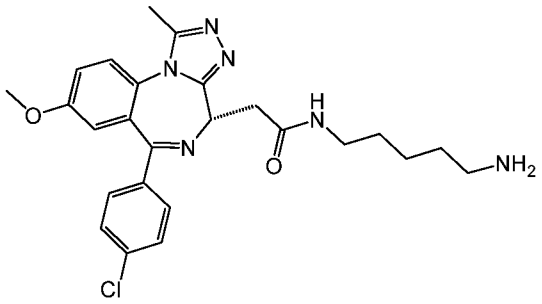
Reference compound I: 1,1-dimethylethyl [5-({[(4S)-6-(4-chlorophenyl)-1-methyl-8-(methyloxy)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl]acetyl}amino)pentyl]carbamate



A mixture of [(4*S*)-6-(4-chlorophenyl)-1-methyl-8-(methyloxy)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl]acetic acid (for a preparation see Reference compound H) (1.0g, 2.5mmol), HATU (1.9g, 5mmol) and DIPEA (0.88ml, 5mmol) was stirred for 80

minutes at room temperature, to this was added 1,1-dimethylethyl (4-aminobutyl)carbamate (1.05ml, 5.0mmol, available from Aldrich). The reaction mixture was stirred at room temperature for 2h before it was concentrated. The residue was taken up in dichloromethane and washed with 1N HCl. The aqueous layer was extracted with dichloromethane twice. Organic layer was washed with 1N sodium hydroxide, followed by a saturated solution of sodium chloride, dried over sodium sulphate and concentrated. The residue was purified by flash-chromatography on silica using dichloromethane/ methanol 95/5 to give the title compound as a yellow solid (1.2g). LC/MS (Method A): rt = 3.04 min.

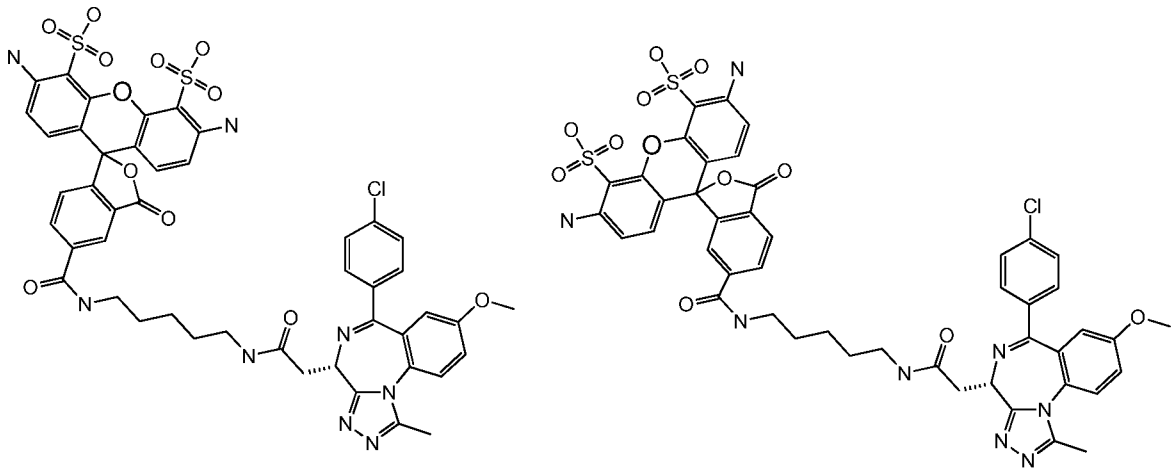
Reference compound J: N-(5-aminopentyl)-2-[(4S)-6-(4-chlorophenyl)-1-methyl-8-(methyloxy)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl]acetamide trifluoroacetate



.TFA

To a solution of 1,1-dimethylethyl [5-({[(4S)-6-(4-chlorophenyl)-1-methyl-8-(methyloxy)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl]acetyl}amino)pentyl]carbamate (for a preparation see Reference compound H) (0.2 g, 0.34 mmol) in dichloromethane (3 ml) was added trifluoroacetic acid (0.053 ml, 0.68 mmol) dropwise at 0°C. The reaction mixture was stirred for 3h from 0°C to room temperature. The reaction mixture was concentrated to dryness to afford the title compound as a hygroscopic yellow oil (200mg)
LC/MS (Method A): rt = 2.33min.
HRMS $(M+H)^+$ calculated for $C_{25}H_{29}ClN_6O_2$ 481.2119; found 481.2162.

Reference compound K: Mixture of 5- and 6- isomers of Alexa Fluor 488-N-(5-aminopentyl)-2-[(4S)-6-(4-chlorophenyl)-1-methyl-8-(methyloxy)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl]acetamide



N-(5-aminopentyl)-2-[(4S)-6-(4-chlorophenyl)-1-methyl-8-(methyloxy)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl]acetamide trifluoroacetate (for a preparation see Reference compound J)(7.65 mg, 0.013 mmol) was dissolved in N,N-Dimethylformamide (DMF) (300 µl) and added to Alexa Fluor 488 carboxylic acid succinimidyl ester (5 mg, 7.77 µmol, mixture of 5 and 6 isomers, available from Invitrogen, product number A-20100) in an Eppendorf centrifuge tube. Hunig's base (7.0 µl, 0.040 mmol) was added and the mixture vortex mixed overnight. After 18h the reaction mixture was evaporated to dryness and the residue redissolved in DMSO/water (50%, <1ml total), applied to a preparative Phenomenex Jupiter C18 column and eluted with a gradient of 95% A: 5% B to 100% B (A = 0.1% trifluoroacetic acid in water, B= 0.1% TFA/90% acetonitrile/10% water) at a flow rate of 10ml/min over 150 minutes. Impure fractions were combined and re-purified using the same system. Fractions were combined and evaporated to yield the title product (2.8mg) as a mixture of the 2 regioisomers shown. LC/MS (Method B):, MH+ = 999, rt = 1.88min.

Biological Test Methods

Fluorescence anisotropy binding assay

The binding of the compound of formula (I) to Bromodomain 2, 3 and 4 was assessed using a Fluorescence Anisotropy Binding Assay.

The Bromodomain protein, fluorescent ligand (Reference compound K see above) and a variable concentration of test compound are incubated together to reach thermodynamic

equilibrium under conditions such that in the absence of test compound the fluorescent ligand is significantly (>50%) bound and in the presence of a sufficient concentration of a potent inhibitor the anisotropy of the unbound fluorescent ligand is measurably different from the bound value.

All data was normalized to the mean of 16 high and 16 low control wells on each plate. A four parameter curve fit of the following form was then applied:

$$y = a + ((b - a) / (1 + (10 \wedge x / 10 \wedge c) \wedge d)$$

Where '*a*' is the minimum, '*b*' is the Hill slope, '*c*' is the pIC50 and '*d*' is the maximum.

Recombinant Human Bromodomains (Bromodomain 2 (1-473), Bromodomain 3 (1-435) and Bromodomain 4 (1-477)) were expressed in *E.coli* cells (in pET15b vector) with a six-His tag at the N-terminal. The His-tagged Bromodomain was extracted from *E.coli* cells using 0.1mg/ml lysozyme and sonication. The Bromodomain was then purified by affinity chromatography on a HisTRAP HP column, eluting with a linear 10-500mM Imidazole gradient, over 20 Cv. Further purification was completed by Superdex 200 prep grade size exclusion column. Purified protein was stored at -80C in 20mM HEPES pH 7.5 and 100mM NaCl.

Protocol for Bromodomain 2: All components were dissolved in buffer composition of 50 mM HEPES pH7.4, 150mm NaCl and 0.5mM CHAPS with final concentrations of Bromodomain 2, 75nM, fluorescent ligand 5nM.10 μl of this reaction mixture was added using a micro multidrop to wells containing 100nl of various concentrations of test compound or DMSO vehicle (1% final) in Greiner 384 well Black low volume microtitre plate and equilibrated in dark 60 mins at room temperature. Fluorescence anisotropy was read in Envision (λex= 485nm, λEM = 530nm; Dichroic -505nM).

Protocol for Bromodoamin 3: All components were dissolved in buffer of composition 50 mM HEPES pH7.4, 150mm NaCl and 0.5mM CHAPS with final concentrations of Bromodomains 3 75nM, fluorescent ligand 5nM. 10 μl of this reaction mixture was added using a micro multidrop to wells containing 100nl of various concentrations of test compound or DMSO vehicle (1% final) in Greiner 384 well Black low volume microtitre

plate and equilibrated in dark 60 mins at room temperature. Fluorescence anisotropy was read in Envision (λex= 485nm, λEM = 530nm; Dichroic -505nM).

Protocol for Bromodomain 4: All components were dissolved in buffer of composition 50 mM HEPES pH7.4, 150mm NaCl and 0.5mM CHAPS with final concentrations of Bromodomain 4 75nM, fluorescent ligand 5nM. 10 μl of this reaction mixture was added using a micro multidrop to wells containing 100nl of various concentrations of test compound or DMSO vehicle (1% final) in Greiner 384 well Black low volume microtitre plate and equilibrated in dark 60 mins at room temperature. Fluorescence anisotropy was read in Envision (λex= 485nm, λEM = 530nm; Dichroic -505nM).

Example 1 had a pIC50 ≥ 6.0 in each of the BRD2, BRD3 and BRD4 assays described above.

LPS stimulated Whole Blood measuring TNFα levels assay

Activation of monocytic cells by agonists of toll-like receptors such as bacterial lipopolysaccharide (LPS) results in production of key inflammatory mediators including TNFα. Such pathways are widely considered to be central to the pathophysiology of a range of auto-immune and inflammatory disorders.

Compounds to be tested are diluted to give a range of appropriate concentrations and 1ul of the dilution stocks is added to wells of a 96 plate. Following addition of whole blood (130ul) the plates are incubated at 37 degrees (5% CO2) for 30 min before the addition of 10ul of 2.8ug/ml LPS, diluted in complete RPMI 1640 (final concentration =200ng/ml), to give a total volume of 140ul per well. After further incubation for 24 hours at 37 degrees, 140ul of PBS are added to each well. The plates are sealed, shaken for 10 minutes and then centrifuged (2500rpm x 10 min). 100ul of the supernatant are removed and TNFα levels assayed by immunoassay (typically by MesoScale Discovery technology) either immediately or following storage at -20 degrees. Dose response curves for each compound was generated from the data and an IC50 value was calculated.

Example 1 was found to have a pIC50 > 6.0 in the above assay.

These data demonstrate that Example 1 tested in the above assay inhibited the production of the key inflammatory mediator TNFα. This suggests that such a compound

has a strong anti-inflammatory profile, which is likely to translate into clinical benefit in inflammatory disorders.

In Vivo Mouse Endotoxemia Model

High doses of Endotoxin (bacterial lipopolysaccharide) administered to animals produce a profound shock syndrome including a strong inflammatory response, dysregulation of cardiovascular function, organ failure and ultimately mortality. This pattern of response is very similar to human sepsis and septic shock, where the body's response to a significant bacterial infection can be similarly life threatening.

To test the compound of formula (I) and pharmaceutically acceptable salts thereof groups of eight Balb/c male mice were given a lethal dose of 15 mg/kg LPS by intraperitoneal injection. Ninety minutes later, animals were dosed intravenously with vehicle (20% cyclodextrin 1% ethanol in apyrogen water) or compound (10 mg/kg). The survival of animals was monitored at 4 days.

Numbers of animals surviving at 4 days (summed across multiple repeat experiments)

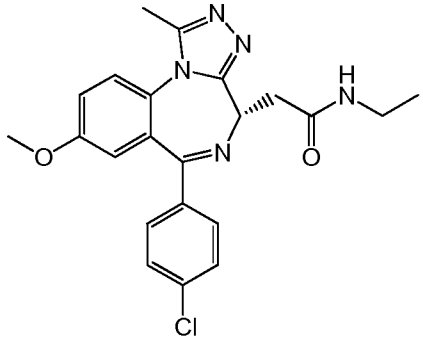
Vehicle	4/66	(6%)
Example 1	24/56	(52%)

These data demonstrate that Example 1 tested in the above model gave rise to a significant animal survival effect following intravenous administration. This suggests that the compound of formula (I) has the potential for a profound effect on inflammatory responses in humans.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

CLAIMS

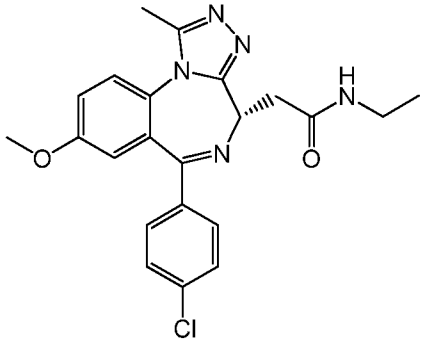
1. A compound of formula (I) which is 2-[(4*S*)-6-(4-Chlorophenyl)-1-methyl-8-(methyloxy)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl]-*N*-ethylacetamide



(I)

or a salt thereof.

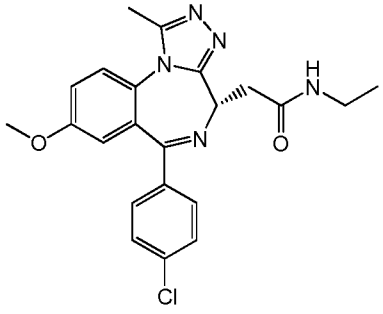
2. A compound of formula (I) which is 2-[(4*S*)-6-(4-Chlorophenyl)-1-methyl-8-(methyloxy)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl]-*N*-ethylacetamide



(I)

or a pharmaceutically acceptable salt thereof.

3. A compound of formula (I) which is 2-[(4*S*)-6-(4-Chlorophenyl)-1-methyl-8-(methyloxy)-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl]-*N*-ethylacetamide



(I)

4. A pharmaceutical composition which comprises a compound of formula (I) or a pharmaceutically acceptable salt thereof as defined in claim 2 and one or more pharmaceutically acceptable carriers, diluents or excipients.

5. A pharmaceutical composition which comprises a compound of formula (I) as defined in claim 3 and one or more pharmaceutically acceptable carriers, diluents or excipients.

6. A combination pharmaceutical product comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof as defined in claim 2 together with one or more other therapeutically active agents.

7. A compound of formula (I) or a pharmaceutically acceptable salt thereof as defined in claim 2 for use in therapy.

8. A compound of formula (I) as defined in claim 3 for use in therapy.

9. A compound of formula (I) or a pharmaceutically acceptable salt thereof as defined in claim 2, for use in the treatment of diseases or conditions for which a bromodomain inhibitor is indicated.

10. A compound of formula (I) or a pharmaceutically acceptable salt thereof for use according to claim 9, wherein the disease or condition is a chronic autoimmune and/or inflammatory condition.

11. A compound of formula (I) or a pharmaceutically acceptable salt thereof for use according to claim 9, wherein the disease or condition is cancer.

12. The use of a compound of formula (I) or a pharmaceutically acceptable salt thereof as defined in claim 2, in the manufacture of a medicament for the treatment of diseases or conditions for which a bromodomain inhibitor is indicated.

13. A method for treatment of a disease or condition, for which a bromodomain inhibitor is indicated, in a subject in need thereof which comprises administering a therapeutically

effective amount of compound of formula (I) or a pharmaceutically acceptable salt thereof as defined in claim 2.

14. A method for treatment according to claim 13, wherein the disease or condition is a chronic autoimmune and/or inflammatory condition.

15. A method for treatment according to claim 13, wherein the disease or condition is cancer.

16. A method for treatment according to any one of claims 13 to 15, wherein the subject is a human.

17. A method for inhibiting a bromodomain which comprises contacting the bromodomain with a compound of formula (I) or a pharmaceutically acceptable salt thereof as defined in claim 2.