

## TETRAHYDROQUINOLINE DERIVATIVES USEFUL AS BROMODOMAIN INHIBITORS

### Field of the Invention

The present invention relates to tetrahydroquinoline derivatives, pharmaceutical  
5 compositions containing such compounds and to their 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 octamer of histone proteins  
10 (most usually comprising two copies of histones H2A, H2B H3 and H4) to form a nucleosome. 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  
15 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,  
20 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.

25 Histone acetylation is most usually associated with the activation of gene transcription, as the modification loosens the interaction of the DNA and the histone octamer 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 residues  
30 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,  
35 BRD4 and BRD-t) which contain tandem bromodomains capable of binding to two

acetylated lysine residues 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- $\beta$  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 or BRD3 may fuse with NUT (nuclear protein in testis) forming novel fusion oncogenes, BRD4-NUT or BRD3-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 carcinogenesis (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.

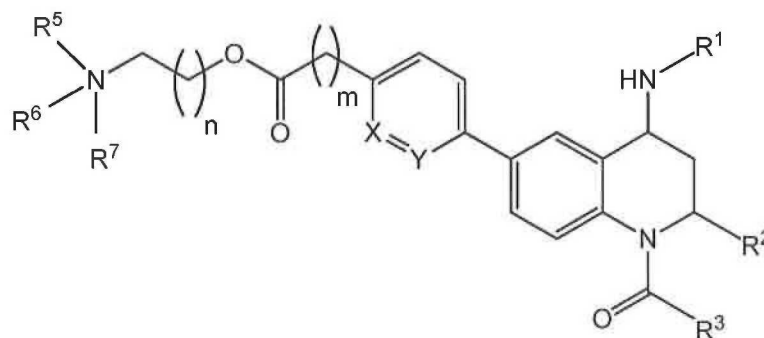
Patent application WO2009084693 discloses a series of thienotriazolodiazepine derivatives that are said to inhibit the binding between an acetylated histone and a bromodomain containing protein which are said to be useful as anti-cancer agents.

PCT patent applications PCT/EP2010/06693 and PCT/EP2010/066701 both disclose a series of tetrahydroquinoline derivatives that inhibit the binding of BET family bromodomains with acetylated lysine residues.

A novel class of compounds have been found which inhibit the binding of bromodomains with its cognate acetylated proteins, more particularly a class of compounds that inhibit the binding of BET family bromodomains to acetylated lysine residues. Such compounds will hereafter be referred to as “bromodomain inhibitors”.

Summary of the Invention

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



(I)

- 5 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.

10 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.

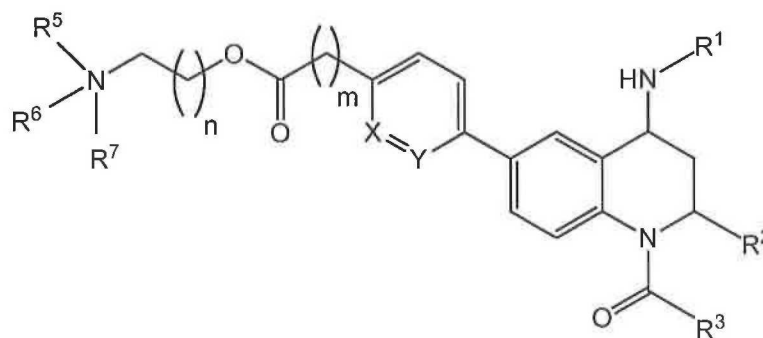
15 In a fourth aspect of the present invention, there is provided a method of treating diseases or conditions for which a bromodomain inhibitor is indicated in a subject in need thereof which comprises administering a therapeutically effective amount of a compound of formula (I) or a pharmaceutically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

20 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 compounds of formula (I) or a salt thereof

25



(I)

in which

X and Y are independently CH or N provided that at least one of X and Y must be CH;

5 R<sup>1</sup> is a group C(O)OR<sup>4</sup> in which R<sup>4</sup> is C<sub>1-4</sub>alkyl or C<sub>3-7</sub>cycloalkyl; or

R<sup>1</sup> is a group selected from phenyl, pyridyl, pyrazinyl and pyrimidinyl said groups being optionally substituted by one or two substituents selected from halogen, C<sub>1-4</sub>alkyl and CN;

R<sup>2</sup> is C<sub>1-4</sub>alkyl;

R<sup>3</sup> is C<sub>1-4</sub>alkyl;

10 R<sup>5</sup> and R<sup>6</sup> are independently C<sub>1-4</sub>alkyl; or

R<sup>5</sup> and R<sup>6</sup> combine together with the N to which they are attached form a 5 or 6 membered heterocyclyl;

R<sup>7</sup> is absent or is C<sub>1-4</sub>alkyl;

m is 0, 1 or 2;

15 n is 1 or 2.

In one embodiment the invention provides compounds of formula (I) with *cis* relative stereochemistry across the tetrahydroquinoline ring in respect of the substituents in the 2 and 4 position on the ring. In one embodiment the compound of formula (I) or a salt thereof is the (2*S*, 4*R*) enantiomer.

In one embodiment X and Y are both CH. In a further embodiment X is CH and Y is N.

In one embodiment R<sup>1</sup> is a group C(O)OR<sup>4</sup> in which R<sup>4</sup> is isopropyl.

25

In a further embodiment R<sup>1</sup> is phenyl or pyridyl optionally substituted by one or two substituents selected from halogen, C<sub>1-4</sub>alkyl and CN. In a yet further embodiment R<sup>1</sup> is 4-chlorophenyl or R<sup>1</sup> is 5-cyanopyridin-2-yl.

30

In one embodiment R<sup>2</sup> is methyl.



In one embodiment  $R^3$  is methyl.

In one embodiment  $m$  is 0.

5

In one embodiment  $n$  is 0. In a further embodiment  $n$  is 1.

In one embodiment  $R^5$  and  $R^6$  are both methyl.

10 It will be appreciated that when  $R^7$  is  $C_{1-4}$ alkyl a quaternised ammonium moiety will be formed. In one embodiment  $R^7$  is absent.

While the embodiments for each variable have generally been listed above separately for each variable, this invention is intended to include all combinations of embodiments  
15 described hereinabove including salts thereof.

Particular compounds according to the invention are:

2-(Dimethylamino)ethyl 4-((2*S*,4*R*)-1-acetyl-4-((4-chlorophenyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate;

20 2-((4-((2*S*,4*R*)-1-Acetyl-4-((4-chlorophenyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoyl)oxy)-*N,N,N*-trimethylethanaminium;

3-((4-((2*S*,4*R*)-1-Acetyl-4-((4-chlorophenyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoyl)oxy)-*N,N,N*-trimethylpropan-1-aminium;

3-(Dimethylamino)propyl 4-((2*S*,4*R*)-1-acetyl-4-((4-chlorophenyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate;

25 3-(Dimethylamino)propyl 6-((2*S*,4*R*)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)nicotinate;

2-(Dimethylamino)ethyl 6-((2*R*,4*R*)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)nicotinate;

30 3-(Dimethylamino)propyl 4-((2*S*,4*R*)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate; and

2-(Dimethylamino)ethyl 4-((2*S*,4*R*)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate

or a salt thereof.

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Throughout the present specification, unless otherwise stated:

- the term "halogen" is used to describe a group selected from fluorine, chlorine or bromine;
- the terms "C<sub>1-4</sub>alkyl" and "C<sub>1-6</sub>alkyl" are used to describe a group or a part of the group comprising a linear or branched alkyl group containing from 1 to 4 or 1 to 6 carbon atoms respectively. Suitable examples of such groups include methyl, ethyl, propyl, isopropyl, n-butyl, isobutyl, t-butyl, pentyl and hexyl;
- the term "C<sub>3-7</sub>cycloalkyl" is used to describe a non-aromatic carbocyclic ring containing at least three and at most seven carbon atoms. Examples of C<sub>3-7</sub>cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl.
- the term 5 or 6 membered heterocyclyl refers to a non-aromatic, saturated ring comprising 1, 2, or 3 heteroatoms selected from O, N and S. Examples of such groups include pyrrolidinyl, morpholinyl, piperidinyl and piperazinyl.

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 the invention relates to compounds of formula (I) or a pharmaceutically acceptable salt thereof.

Because of their potential use in medicine, salts of the compounds of formula (I) are desirably pharmaceutically acceptable. Suitable pharmaceutically acceptable salts can include acid or base addition salts. As used herein, the term 'pharmaceutically acceptable salt' means any pharmaceutically acceptable salt or solvate of a compound of the invention, which upon administration to the recipient is capable of providing (directly or indirectly). 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.

5 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, glutamic, aspartic, *p*-toluenesulfonic, benzenesulfonic, methanesulfonic, ethanesulfonic, naphthalenesulfonic such as 2-naphthalenesulfonic, or hexanoic acid), optionally in a suitable solvent such as an organic  
10 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,  
15 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 compounds of formula (I), and are  
20 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 compounds of formula (I).

25 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  
30 solvates. Methods for identification of solvates include, but are not limited to, NMR and microanalysis. Solvates of the compounds of formula (I) are within the scope of the invention.

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

The compounds of formula (I) may be in crystalline or amorphous form. Furthermore, some of the crystalline forms of the compounds of formula (I) may exist as polymorphs, which are included within the scope of the present invention. Polymorphic forms of compounds 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).

Compounds described herein contain chiral atoms so that optical isomers, e.g. enantiomers or diastereoisomers may be formed. Accordingly, the present invention encompasses all isomers of the compounds of formula (I) whether as individual isomers isolated such as to be substantially free of the other isomer (i.e. pure) or as mixtures (i.e. racemates and racemic mixtures). An individual isomer isolated such as to be substantially free of the other isomer (i.e. pure) may be isolated such that less than 10%, particularly less than about 1%, for example less than about 0.1% of the other isomer 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.

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

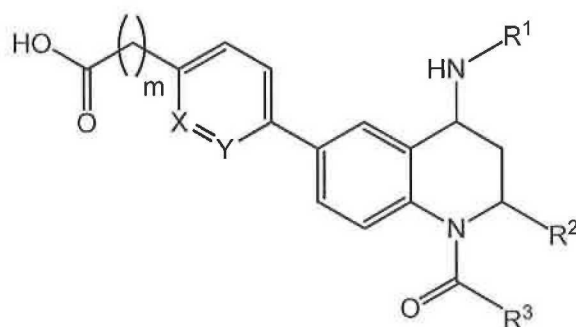
It will be appreciated from the foregoing that included within the scope of the invention are solvates, isomers and polymorphic forms of the compounds of formula (I) and salts thereof.

The compounds of formula (I) and salts thereof may be made by a variety of methods, including standard chemistry. Any previously defined variable will continue to have the previously defined meaning unless otherwise indicated. Illustrative general synthetic

methods are set out below and then specific compounds of formula (I) or salts thereof are prepared in the working Examples.

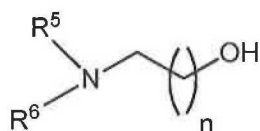
The present invention further provides a process for the preparation of a compound of formula (I) or a salt thereof which comprises a process selected from (a) and (b) in which:

(a) comprises reacting a compound of formula (II)



(II)

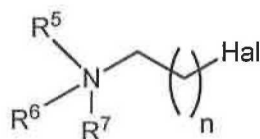
in which R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, X, Y and m as defined in formula (I) with a compound of formula (III)



(III)

in which R<sup>5</sup>, R<sup>6</sup> and n as defined in formula (I).

(b) comprises reacting a compound of formula (II) or a salt thereof with a compound of formula (IV)



(IV)

in which R<sup>5</sup>, R<sup>6</sup>, R<sup>7</sup> and n are as defined in formula (I) and Hal is halogen.

#### Process (a)

The reaction between the compounds of formula (II) and (III) may be carried out in the presence of a suitable activating agent agent (such as dicyclohexylcarbodiimide (DCC) or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)) and a suitable acyl transfer

catalyst (such as 4-dimethylaminopyridine) in a suitable solvent (such as dichloromethane or DMF).

Compounds of formula (II) can be prepared by methods described herein or analogous procedures thereto. Compounds of formula (III) are commercially available.

#### Process (b)

For process (b) a suitable Hal group is bromo. The reaction between the compounds of formula (II) and formula (IV) are typically carried out in a suitable solvent (such as DMF) in the presence of a suitable base (such as potassium carbonate).

Compounds of formula (II) can be prepared by methods described herein or analogous procedures thereto. Compounds of formula (IV) are commercially available.

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. 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<sub>3</sub>) which may be removed by base catalysed hydrolysis.

It will be appreciated that in any of the synthetic methods described herein, 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 of formula (II) are believed to be novel and therefore form a yet further aspect of the invention.

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

5 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 used in the treatment of diseases or conditions for which a bromodomain inhibitor is indicated.

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

Also provided is the use of a compound of formula (I) or a pharmaceutically acceptable  
15 salt thereof in the manufacture of a medicament for the treatment of diseases or conditions for which a bromodomain inhibitor is indicated.

Also provided is a method of treating diseases or conditions for which a bromodomain inhibitor is indicated in a subject in need thereof which comprises administering a  
20 therapeutically effective amount of compound of formula (I) or a pharmaceutically acceptable salt thereof.

Suitably the subject in need thereof is a mammal, particularly a human.

25 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  
30 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  
35 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.

5 Bromodomain inhibitors may be useful in the treatment of a wide variety of chronic autoimmune and/or 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  
10 airways disease, pneumonitis, myocarditis, pericarditis, myositis, eczema, dermatitis (such as atopic dermatitis), alopecia, vitiligo, bullous skin diseases, nephritis, vasculitis, atherosclerosis, Alzheimer's disease, depression, Sjögren's syndrome, sialoadenitis, central retinal vein occlusion, branched retinal vein occlusion, Irvine-Gass syndrome (post cataract and post-surgical), retinitis pigmentosa, pars planitis, birdshot  
15 retinopathology, epiretinal membrane, cystic macular edema, parafoveal telangiectasis, tractional maculopathies, vitreomacular traction syndromes, retinal detachment, neuroretinitis, idiopathic macular edema, retinitis, dry eye  
(keratoconjunctivitis Sicca), vernal keratoconjunctivitis, atopic keratoconjunctivitis, anterior uveitis, pan uveitis, posterior uveitis, uveitis-associated macula edema. scleritis, diabetic retinopathy, diabetic macula edema, age-related macula dystrophy, hepatitis, pancreatitis, primary biliary cirrhosis, sclerosing cholangitis, Addison's disease,  
20 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  
25 including giant cell arteritis, Wegener's granulomatosis, Polyarteritis nodosa, Behcet's disease, Kawasaki disease, Takayasu's Arteritis, pyoderma gangrenosum, vasculitis with organ involvement and acute rejection of transplanted organs.

Bromodomain inhibitors may be useful in the prevention or treatment of diseases or  
30 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,  
35 sarcoidosis, Herxheimer reactions, encephalitis, myelitis, meningitis, malaria and SIRS



associated with viral infections such as influenza, herpes zoster, herpes simplex and coronavirus.

5 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, pulmonary, renal, hepatic, gastro-intestinal or peripheral limb embolism.

10 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.

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

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

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

30 Bromodomain inhibitors may be useful in the treatment of dermal pathology such as non-malignant melanoma (actinic keratosis and basal cell), *in-situ* melanoma, squamous cell carcinoma and cutaneous T-cell lymphoma.

35 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 (multiple organ dysfunction syndrome). In a particular embodiment the disease or condition for which a bromodomain inhibitor is indicated is sepsis, sepsis syndrome, septic shock 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, human immunodeficiency virus (HIV), cervical neoplasia, adenovirus infections, including acute respiratory disease, 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 of or all of the above disease states.

In one embodiment, there is provided a method for inhibiting a bromodomain which comprises contacting the bromodomain with a compound of formula (I) or a pharmaceutically acceptable salt thereof.

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 and one or more pharmaceutically acceptable carriers, diluents or excipients. The compounds of the formula (I) and pharmaceutically acceptable salts, are as described above. The carrier(s), diluent(s) or excipient(s) 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.

- 5 Since the compounds 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).

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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  
15 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.

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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), ocular (including topical, intraocular, subconjunctival, episcleral or sub-Tenon), 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  
25 active ingredient with the carrier(s) or excipient(s).

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

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

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

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A preferred dosage form that results in occlusion and modification of skin permeation either to increase or decrease the systemic exposure of bromodomain compounds,

including but not limited to the pharmaceutically acceptable forms of carboxymethylcellulose, an algininate, gelatin or polyvinyl pyrrolidone.

5 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  
10 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.

15 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.

20 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  
25 as an edible carbohydrate, 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  
30 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.

35 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 formula (I) and pharmaceutically acceptable salts thereof 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.

5

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.

10

Pharmaceutical compositions adapted for topical administration may be formulated as ointments, creams, suspensions, emulsions, lotions, powders, solutions, pastes, gels, sprays, foams, aerosols or oils. Such pharmaceutical compositions may include conventional additives which include, but are not limited to, preservatives, solvents to assist drug penetration, co-solvents, emollients, propellants, viscosity modifying agents (gelling agents), surfactants and carriers. In one embodiment there is provided a pharmaceutical composition adapted for topical administration which comprises between 0.01 – 10%, or between 0.01 – 1% of the compound of formula (I), or a pharmaceutically acceptable salt thereof, by weight of the composition.

20

For treatments of the eye or other external tissues, for example mouth and skin, the compositions are preferably applied as a topical solution, suspension, emulsion, ointment, cream, gel, spray or foam. 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. When formulated in a foam, the active agent may be formulated with propellants, surfactants, solvents, co-solvents and viscosity modifying agents.

25

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. Compositions to be administered to the eye will have ophthalmically compatible pH and osmolality. One or more ophthalmically acceptable pH adjusting agents and/or buffering agents can be included in a composition of the invention, including acids such as acetic, boric, citric, lactic, phosphoric and hydrochloric acids; bases such as sodium hydroxide, sodium phosphate, sodium borate, sodium citrate, sodium acetate, and sodium lactate; and buffers such as citrate/dextrose, sodium

35

bicarbonate and ammonium chloride. Such acids, bases, and buffers can be included in an amount required to maintain pH of the composition in an ophthalmically acceptable range. One or more ophthalmically acceptable salts can be included in the composition in an amount sufficient to bring osmolality of the composition into an ophthalmically acceptable range. Such salts include those having sodium, potassium or ammonium cations and chloride, citrate, ascorbate, borate, phosphate, bicarbonate, sulfate, thiosulfate or bisulfite anions.

An ocular delivery device may be designed for the controlled release of one or more therapeutic agents with multiple defined release rates and sustained dose kinetics and permeability. Controlled release may be obtained through the design of polymeric matrices incorporating different choices and properties of biodegradable/bioerodable polymers (e.g. poly(ethylene vinyl) acetate (EVA), superhydrolyzed PVA), hydroxyalkyl cellulose (HPC), methylcellulose (MC), hydroxypropyl methyl cellulose (HPMC), polycaprolactone, poly(glycolic) acid, poly(lactic) acid, polyanhydride, of polymer molecular weights, polymer crystallinity, copolymer ratios, processing conditions, surface finish, geometry, excipient addition and polymeric coatings that will enhance drug diffusion, erosion, dissolution and osmosis.

Pharmaceutical compositions for ocular delivery also include in situ gellable aqueous composition. Such a composition comprises a gelling agent in a concentration effective to promote gelling upon contact with the eye or with lacrimal fluid. Suitable gelling agents include but are not limited to thermosetting polymers. The term "in situ gellable" as used herein includes not only liquids of low viscosity that form gels upon contact with the eye or with lacrimal fluid, but also includes more viscous liquids such as semi-fluid and thixotropic gels that exhibit substantially increased viscosity or gel stiffness upon administration to the eye. See, for example, Ludwig (2005) Adv. Drug Deliv. Rev. 3;57:1595-639, herein incorporated by reference for purposes of its teachings of examples of polymers for use in ocular drug delivery.

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) and pharmaceutically acceptable salts thereof are 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 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 compounds of formula (I) and pharmaceutically acceptable salts 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 WO-A-2005/044354.

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

The compounds 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 compound(s) of 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  
5 administered separately this may occur simultaneously or sequentially in any order. The amounts of the compound(s) 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 pharmaceutical product comprising a  
10 compound of formula (I) or a pharmaceutically acceptable salt thereof and at least one other pharmaceutically active agent.

Thus in one aspect, the compound of formula (I) or a pharmaceutically acceptable salt thereof and pharmaceutical compositions comprising a compound of formula (I) or a  
15 pharmaceutically acceptable salt thereof may be used in combination with or include one or more other therapeutic agents, for example selected from antibiotics, anti-virals, glucocorticosteroids, muscarinic antagonists, beta-2 agonists and vitamin D3 analogues. In a further aspect a compound of formula (I) or a pharmaceutically acceptable salt thereof, according to the invention may be used in combination with a further therapeutic  
20 agent which is suitable for the treatment of cancer, .

It will be appreciated that when the compound of formula (I) or a pharmaceutically acceptable salt thereof is administered in combination with other therapeutic agents normally administered by the inhaled, intravenous, oral or intranasal route, that the  
25 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  
30 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  
35 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.

5 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.

10 The compounds of formula (I) and pharmaceutically acceptable salts thereof 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 compounds of formula (I) and pharmaceutically acceptable salts thereof, and are not to be considered as limiting the scope of the invention in any way.

## 15 General Experimental Details

All temperatures referred to are in °C.

20 The names of the following compounds have been obtained using the compound naming programme "ACD Name Pro 6.02" or Chem Draw Ultra 12.0.

### Abbreviations

AcOH refers to acetic acid

BINAP refers to 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl

25 BOC refers to tert-butoxycarbonyl

CV refers to column volumes

DCM refers to dichloromethane

1,2-DCE refers to 1,2-dichloroethane

DCC refers to dicyclohexylcarbodiimide

30 DIPEA refers to diisopropylethylamine

DMAP refers to 4-dimethylaminopyridine

DMSO refers to dimethylsulfoxide.

DMF refers to *N,N*-dimethylformamide

Ether refers to diethyl ether

35 Et<sub>2</sub>O refers to diethyl ether

EtOAc refers to ethyl acetate

Fmoc refers to 9-fluorenylmethoxycarbonyl

HATU refers to O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate

HPLC refers to high performance liquid chromatography

5 IPA refers to propan-2-ol

i-Pr<sub>2</sub>O refers to di-isopropyl ether

LiAlH<sub>4</sub> refers to lithium aluminium hydride

MDAP refers to Mass directed autoprep refers preparative mass directed HPLC

MeCN refers to acetonitrile

10 MeOH refers to methanol

MgSO<sub>4</sub> refers to magnesium sulfate

Mp refers to melting point

r.t. refers to room temperature

Rt refers to retention time

15 Na<sub>2</sub>SO<sub>4</sub> refers to sodium sulphate

TMEDA refers to tetramethylethylenediamine

TFA refers to trifluoroacetic acid

THF refers to tetrahydrofuran

TLC refers to thin layer chromatography

20

#### **LC/MS Methodology (Used for Certain Intermediates and reference Compounds)**

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

25 LC/MS (Method A) was conducted on an Acquity UPLC BEH C18 column (50mm x 2.1mm i.d. 1.7µm packing diameter) at 40 degrees centigrade, eluting with 10 mM Ammonium Bicarbonate in water adjusted to pH 10 with Ammonia solution (Solvent A) and Acetonitrile (Solvent B) using the following elution gradient 0-1.5min 1 – 97% B, 1.5-1.9min 97% B, 1.9 – 2.0min 100% B at a flow rate of 1ml/min. The UV detection was a  
30 summed signal from wavelength of 210nm to 350nm. The mass spectra were recorded on a Waters ZQ Mass Spectrometer using Alternate-scan Positive and Negative Electrospray. Ionisation data was rounded to the nearest integer.

LC/MS (Method B) was conducted on an Acquity UPLC BEH C18 column (50mm x  
35 2.1mm i.d. 1.7µm packing diameter) at 40 degrees centigrade, eluting with 0.1% v/v solution of Formic Acid in Water (Solvent A) and 0.1% v/v solution of Formic Acid in

Acetonitrile (Solvent B) using the following elution gradient 0-1.5min 3 – 100% B, 1.5-1.9min 100% B, 1.9 – 2.0min 3% B at a flow rate of 1ml/min. The UV detection was a summed signal from wavelength of 210nm to 350nm. The mass spectra were recorded on a Waters ZQ Mass Spectrometer using Alternate-scan Positive and Negative Electropray. Ionisation data was rounded to the nearest integer.

LC/MS (Method C) was conducted on an Acquity UPLC BEH C18 column (50mm x 2.1mm i.d. 1.7µm packing diameter) at 40 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 0-1.5min 3 – 100% B, 1.5-1.9min 100% B, 1.9 – 2.0min 3% B at a flow rate of 1ml/min. The UV detection was a summed signal from wavelength of 210nm to 350nm. The mass spectra were recorded on a Waters ZQ Mass Spectrometer using Positive Electropray. Ionisation data was rounded to the nearest integer.

LC/MS (Method D) was conducted on a Supelcosil LCABZ+PLUS column (3µm, 3.3cm x 4.6mm ID) eluting with 0.1% HCO<sub>2</sub>H and 0.01 M ammonium acetate in water (solvent A), and 95% acetonitrile and 0.05% HCO<sub>2</sub>H 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]<sup>+</sup> and [M+NH<sub>4</sub>]<sup>+</sup> molecular ions] or electrospray negative ionisation [(ES-ve to give [M-H]<sup>-</sup> molecular ion] modes. Analytical data from this apparatus are given with the following format : [M+H]<sup>+</sup> or [M-H]<sup>-</sup>.

LC/MS (Method E) was conducted 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<sup>+</sup> molecular ions] or atmospheric pressure chemical negative ionisation [AP-ve to give (M-H)<sup>-</sup> molecular ions] modes. Analytical data from this apparatus are given with the following format: [M+H]<sup>+</sup> or [M-H]<sup>-</sup>.

LC/MS (Method F) 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/MS (Method G) was conducted on an Acquity UPLC BEH C18 column (50mm x 2.1mm i.d. 1.7µm packing diameter) at 40 degrees centigrade, eluting with 0.1% v/v solution of Formic Acid in Water (Solvent A) and 0.1% v/v solution of Formic Acid in Acetonitrile (Solvent B) using the following elution gradient 0-1.5min 1 – 97% B, 1.5-1.9min 97% B, 1.9 – 2.0min 97 to 100% B at a flow rate of 1ml/min. The UV detection was a summed signal from wavelength of 210nm to 350nm. The mass spectra were recorded on a Waters ZQ Mass Spectrometer using Alternate-scan Positive and Negative Electrospray. 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.

“Mass directed autoprep” / “preparative mass directed HPLC” was conducted on a system such as; a Waters FractionLynx system comprising of a Waters 600 Gradient pump, a Waters 2767 inject / collector, a Waters Reagent manager, a Gilson Aspec - waste collector, a Gilson 115 post-fraction UV detector and a Computer System. The column used is typically a Supelco LCABZ++ column whose dimensions are 20mm internal diameter by 100mm in length. The stationary phase particle size is 5µm. A flow rate was used of 20mL/min with either 0.1% formic acid or trifluoroacetic acid in water (solvent A)

and 0.1% formic or trifluoroacetic acid in acetonitrile (solvent B) using the appropriate elution gradient. Mass spectra were recorded on Micromass ZQ mass spectrometer using electrospray positive and negative mode, alternate scans. The software used was MassLynx 4.0 or using equivalent alternative systems.

5

## **LCMS methodology**

### **Method Formate (Formic acid modifier)**

#### 10 **LC conditions**

The UPLC analysis was conducted on an Acquity UPLC BEH C18 column (50mm x 2.1mm, i.d. 1.7µm packing diameter) at 40°C.

The solvents employed were:

A = 0.1% v/v solution of formic acid in water

15

B = 0.1% v/v solution of formic acid in acetonitrile

The gradient employed was:

| Time (min) | Flow rate (ml/min) | %A | %B  |
|------------|--------------------|----|-----|
| 0          | 1                  | 99 | 1   |
| 1.5        | 1                  | 3  | 97  |
| 1.9        | 1                  | 3  | 97  |
| 2.0        | 1                  | 0  | 100 |

The UV detection was a summed signal from wavelength of 210nm to 350nm.

20

#### **MS conditions**

MS : Waters ZQ

Ionisation mode : Alternate-scan positive and negative electrospray

Scan range : 100 to 1000 AMU

25

Scan time : 0.27sec

Inter scan delay : 0.10sec

### **Method HpH (ammonium bicarbonate modifier)**

30

#### **LC conditions**

The UPLC analysis was conducted on an Acquity UPLC BEH C18 column (50mm x 2.1mm, i.d. 1.7µm packing diameter) at 40°C.

The solvents employed were:

- 5           A = 10mM ammonium hydrogen carbonate in water adjusted to pH10 with ammonia solution  
            B = acetonitrile

The gradient employed was:

| Time (min) | Flow rate (ml/min) | %A | %B  |
|------------|--------------------|----|-----|
| 0          | 1                  | 99 | 1   |
| 1.5        | 1                  | 3  | 97  |
| 1.9        | 1                  | 3  | 97  |
| 2.0        | 1                  | 0  | 100 |

- 10    The UV detection was a summed signal from wavelength of 210nm to 350nm.

MS conditions

- MS                       :     Waters ZQ  
Ionisation mode       :     Alternate-scan positive and negative electrospray  
15   Scan range         :     100 to 1000 AMU  
Scan time             :     0.27sec  
Inter scan delay      :     0.10sec

MDAP methodology

20

**Method Formate (Formic acid Modifier)**

LC conditions

- 25    The HPLC analysis was conducted on either a Sunfire C18 column (100mm x 19mm, i.d. 5µm packing diameter) or a Sunfire C18 column (150mm x 30mm, i.d. 5µm packing diameter) at ambient temperature.

The solvents employed were:

- A = 0.1% v/v solution of formic acid in water  
          B = 0.1% v/v solution of formic acid in acetonitrile  
30



Run as a gradient over either 15 or 25min (extended run) with a flow rate of 20ml/min (100mm x 19mm, i.d 5µm packing diameter) or 40ml/min (150mm x 30mm, i.d. 5µm packing diameter).

- 5 The UV detection was a summed signal from wavelength of 210nm to 350nm.

MS conditions

|    |                  |   |                                                   |
|----|------------------|---|---------------------------------------------------|
|    | MS               | : | Waters ZQ                                         |
|    | Ionisation mode  | : | Alternate-scan positive and negative electrospray |
| 10 | Scan range       | : | 100 to 1000 AMU                                   |
|    | Scan time        | : | 0.50sec                                           |
|    | Inter scan delay | : | 0.20sec                                           |

**Method HpH (Ammonium bicarbonate modifier)**

15

LC conditions

The HPLC analysis was conducted on either an Xbridge C18 column (100mm x 19mm, i.d 5µm packing diameter) or a Xbridge C18 column (100mm x 30mm, i.d. 5µm packing diameter) at ambient temperature.

- 20 The solvents employed were:

A = 10mM ammonium bicarbonate in water, adjusted to pH10 with ammonia solution

B = acetonitrile

- 25 Run as a gradient over either 15 or 25min (extended run) with a flow rate of 20ml/min (100mm x 19mm, i.d 5µm packing diameter) or 40ml/min (100mm x 30mm, i.d 5µm packing diameter).

The UV detection was a summed signal from wavelength of 210nm to 350nm.

30

MS conditions

|    |                  |   |                                                   |
|----|------------------|---|---------------------------------------------------|
|    | MS               | : | Waters ZQ                                         |
|    | Ionisation mode  | : | Alternate-scan positive and negative electrospray |
|    | Scan range       | : | 100 to 1000 AMU                                   |
| 35 | Scan time        | : | 0.50sec                                           |
|    | Inter scan delay | : | 0.20sec                                           |

## Intermediate 1

### 1-((2S,4R)-4-Amino-6-bromo-2-methyl-3,4-dihydroquinolin-1(2H)-yl)ethanone



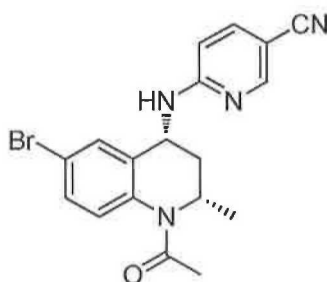
5

A suspension of aluminium chloride (41.2 g, 309 mmol) in DCM (480 mL) at 0 °C under nitrogen was treated with a solution of isopropyl ((2S,4R)-1-acetyl-6-bromo-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (30 g, 81 mmol) in DCM (80 ml) *via* cannula and the resulting mixture was stirred at this temperature for 30 minutes. The reaction mixture was then slowly treated with a mixture of triethylamine (136 mL, 975 mmol) and methanol (48 mL) *via* cannula. The resulting cake formed was stirred in ethyl acetate (800 mL), isolated by filtration and subsequently partitioned between DCM (800 mL) and saturated aqueous NaHCO<sub>3</sub> solution (800 mL). Sodium potassium tartrate (300 g) was added and the resulting mixture was stirred vigorously for 2 h. The layers were separated and the DCM layer was filtered through a sinter funnel. The filtrate was dried (MgSO<sub>4</sub>) and concentrated *in vacuo* to give a first crop of 1-((2S,4R)-4-amino-6-bromo-2-methyl-3,4-dihydroquinolin-1(2H)-yl)ethanone (19.6 g, 69.2 mmol, 85 % yield) as a yellow foam. The aqueous phase was treated further with DCM (800 mL) and the biphasic mixture stirred overnight. The layers were then separated and the organic layer was dried over (MgSO<sub>4</sub>) and concentrated *in vacuo* to give a second crop of 1-((2S,4R)-4-amino-6-bromo-2-methyl-3,4-dihydroquinolin-1(2H)-yl)ethanone (3.4 g, 12.01 mmol, 14.78 % yield). LCMS (HpH, 2min), Rt=0.77min, MH<sup>+</sup> = 283 (1 Br).

25

## Intermediate 2

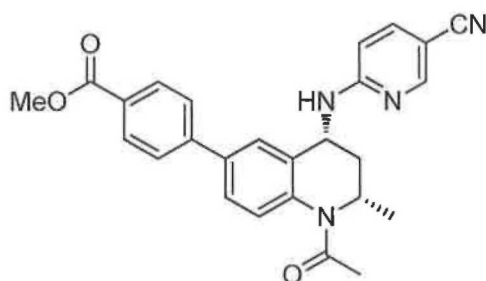
### 6-(((2S,4R)-1-Acetyl-6-bromo-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)amino)nicotinonitrile



To a mixture of 1-((2*S*,4*R*)-4-amino-6-bromo-2-methyl-3,4-dihydroquinolin-1(2*H*)-yl)ethanone (for a preparation see Intermediate 1)(2.28 g, 8.05 mmol) and 6-chloronicotinonitrile (2.231 g, 16.10 mmol) was added NMP (20 mL) and the mixture treated with DIPEA (4.22 mL, 24.16 mmol). The mixture was split between 2 flasks and  
 5 each flask was flushed with nitrogen, sealed and stirred under microwave irradiation at 200 °C for 2 h. The reaction mixtures were combined and partitioned between water and EtOAc. The aqueous layer was extracted with EtOAc (x4) and the combined organic layers were washed with water (x3), then brine and were dried (MgSO<sub>4</sub>) filtered and concentrated *in vacuo*. The brown solid residue was purified by chromatography (EtOAc  
 10 in Hexanes gradient) to give 6-(((2*S*,4*R*)-1-acetyl-6-bromo-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)amino)nicotinonitrile (1.940 g, 5.04 mmol, 62.5 % yield) as a pale yellow foam. LCMS (HpH, 2min), Rt=1.02min, MH<sup>+</sup> = 386 (1 Br).

### Intermediate 3

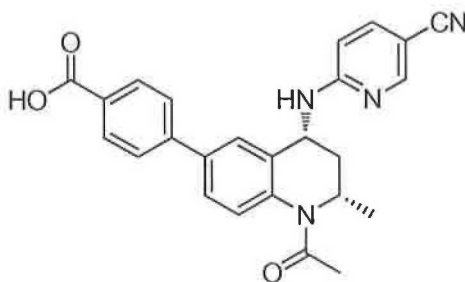
15 **Methyl 4-((2*S*,4*R*)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate**



To a flask charged with 6-(((2*S*,4*R*)-1-acetyl-6-bromo-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)amino)nicotinonitrile (for a preparation see Intermediate 2)(1000 mg, 2.60 mmol), (4-(methoxycarbonyl)phenyl)boronic acid (561 mg, 3.11 mmol), tetrakis(triphenylphosphine)palladium(0) (300 mg, 0.260 mmol) and potassium carbonate (1076 mg, 7.79 mmol) was added DME (20 mL) and water (4.0 mL). The resulting mixture was stirred at 100 °C under nitrogen for 1 h, then cooled to room temperature and  
 25 concentrated *in vacuo*. The residue was partitioned between EtOAc and water and the layers were separated. The organic phase was dried (MgSO<sub>4</sub>), concentrated *in vacuo* and the residue was purified by chromatography (10 g column, MeOH / DCM gradient) to give methyl  
 30 4-((2*S*,4*R*)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate (1002 mg, 2.275 mmol, 88 % yield) as an orange foam. LCMS (HpH, 2min), Rt=1.09min, MH<sup>+</sup> = 441.

### Intermediate 4

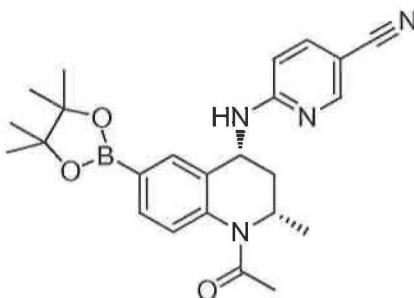
**4-((2S,4R)-1-Acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoic acid**



- 5 A solution of methyl 4-((2S,4R)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate (for a preparation see Intermediate 3) (1.0 g, 2.270 mmol) in methanol (15 mL) at room temperature was treated with aqueous sodium hydroxide solution (2N, 2.27 mL, 4.54 mmol) and the resulting mixture was stirred at this temperature for 24 h. The bulk of MeOH was evaporated *in vacuo* and the aqueous  
10 residue was treated with acetic acid (0.39 mL, 6.81 mmol) giving precipitate which was isolated by filtration and dried under vacuum at 40 °C to afford 4-((2S,4R)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoic acid (820 mg, 1.923 mmol, 85 % yield) as a yellow solid. LCMS (HpH, 2min), Rt=0.64min, MH<sup>+</sup> = 427.

**15 Intermediate 5**

**6-(((2S,4R)-1-Acetyl-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,4-tetrahydroquinolin-4-yl)amino)nicotinonitrile**

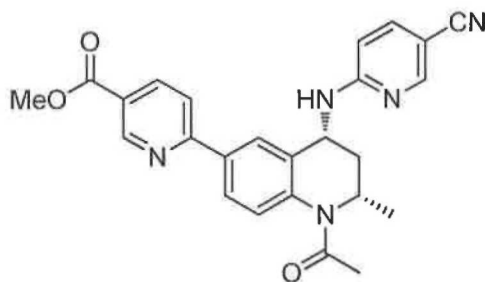


- 20 To a flask charged with 6-(((2S,4R)-1-acetyl-6-bromo-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)amino)nicotinonitrile (for a preparation see Intermediate 2) (847 mg, 2.199 mmol), bis(pinacolato)diboron (1228 mg, 4.84 mmol), PdCl<sub>2</sub>(dppf) (161 mg, 0.220 mmol) and potassium acetate (324 mg, 3.30 mmol) was added DMSO (7 mL), the mixture degassed under nitrogen and stirred under nitrogen for 1 h at 80 °C. Further portions of  
25 bis(pinacolato)diboron (1 g), PdCl<sub>2</sub>(dppf) (100 mg) and potassium acetate (150 mg) were added and the mixture was stirred for 45 min. Further portions of bis(pinacolato)diboron (1 g), PdCl<sub>2</sub>(dppf) (100 mg) and potassium acetate (150 mg) was added and the mixture

was stirred for 45 min. The reaction mixture was cooled to room temperature and treated with EtOAc and water. The biphasic mixture was filtered through a pad of Celite™ (10 g) and the layers were separated. The aqueous layer was extracted with EtOAc (x2) and the combined organic layers were washed with water (x4), then brine, dried (MgSO<sub>4</sub>) and concentrated *in vacuo*. Purification of the residue by chromatography [50 g column, EtOAc/Hexanes gradient] afforded 6-(((2*S*,4*R*)-1-acetyl-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,4-tetrahydroquinolin-4-yl)amino)nicotinonitrile (800 mg, 1.850 mmol, 84 % yield) as a red gum. LCMS (Formate, 2min), Rt=1.08min, MH<sup>+</sup> = 433.

#### 10 Intermediate 6

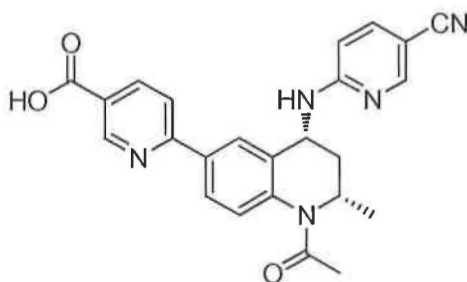
**Methyl 6-((2*S*,4*R*)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)nicotinate**



To a flask charged with 6-(((2*S*,4*R*)-1-acetyl-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,4-tetrahydroquinolin-4-yl)amino)nicotinonitrile (for a preparation see Intermediate 5) (800 mg, 1.850 mmol), methyl 6-bromonicotinate (440 mg, 2.036 mmol), potassium carbonate (767 mg, 5.55 mmol) and tetrakis(triphenylphosphine)palladium(0) (214 mg, 0.185 mmol) were added toluene (8 mL) and ethanol (8 mL). The resulting mixture was stirred at 90 °C under nitrogen for 3 h at which point portions of potassium carbonate (384 mg) tetrakis(triphenylphosphine)palladium(0) (107 mg) and methyl 6-bromonicotinate (220 mg) were added and the reaction mixture stirring continued for 5 h. The mixture was then cooled to room temperature and concentrated *in vacuo*. The residue was partitioned between EtOAc and water. The phases were separated and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried (MgSO<sub>4</sub>) and concentrated *in vacuo*. The residue was purified by chromatography [25 g column, EtOAc / Hexanes gradient] to give ethyl 6-((2*S*,4*R*)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)nicotinate (600 mg, 1.317 mmol, 71.2 % yield) as a white foam. LCMS (HpH, 2min), Rt=1.07min, MH<sup>+</sup> = 456.

#### Intermediate 7

**6-((2S,4R)-1-Acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)nicotinic acid**

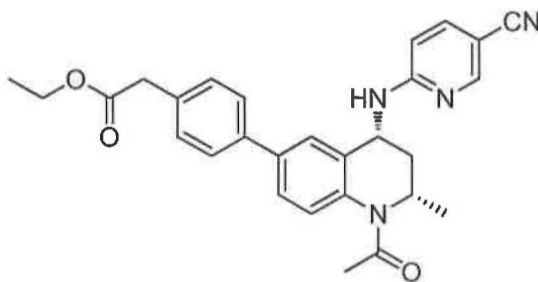


- 5 A solution of methyl 6-((2S,4R)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)nicotinate (for a preparation see Intermediate 6) (580 mg, 1.273 mmol) in ethanol (10 mL) was treated with a aqueous lithium hydroxide solution (1N, 2.55 mL, 2.55 mmol) and the resulting mixture was stirred for 2 h. The bulk of ethanol was evaporated *in vacuo* and the residue was diluted with water (*ca.* 5 mL). The cloudy mixture was treated with acetic acid (0.146 mL, 2.55 mmol) and the precipitate formed was isolated by filtration, washed with Et<sub>2</sub>O and dried at 60 °C for 16 h to give 6-((2S,4R)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)nicotinic acid (410 mg, 0.959 mmol, 75 % yield) as a pale yellow solid which was used in the next step without further purification. LCMS (HpH, 2min), Rt=0.63min, MH<sup>+</sup> = 428.

15

**Intermediate 8**

**Ethyl 2-(4-((2S,4R)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)acetate**



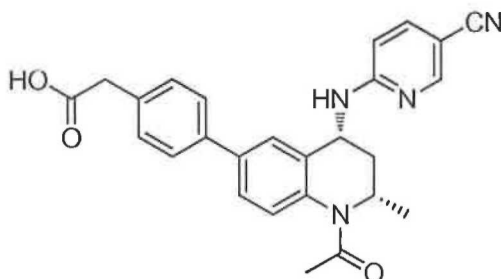
20

- To a solution of 6-(((2S,4R)-1-acetyl-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,4-tetrahydroquinolin-4-yl)amino)nicotinonitrile (for a preparation see Intermediate 5) (1.67 g, 3.86 mmol), ethyl 2-(4-bromophenyl)acetate (1.127 g, 4.64 mmol) and potassium carbonate (1.602 g, 11.59 mmol) in toluene (10 mL) and ethanol (10.0 mL) was added tetrakis(triphenylphosphine)palladium(0) (0.446 g, 0.386 mmol) under nitrogen. The reaction mixture heated at 100 °C for 1 h, then partitioned between EtOAc and water. The layers were separated and the aqueous phase was extracted with EtOAc (x3). The

combined organic layers were washed with brine, dried (MgSO<sub>4</sub>), filtered and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (25g), eluting with an EtOAc / cyclohexane gradient (10 to 80%). The appropriate fractions were combined and concentrated *in vacuo* to give ethyl 2-(4-((2*S*,4*R*)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)acetate (752 mg, 42%) as a viscous colourless oil. LCMS (Formate, 2min), Rt=1.10min, MH<sup>+</sup> = 469.

#### Intermediate 9

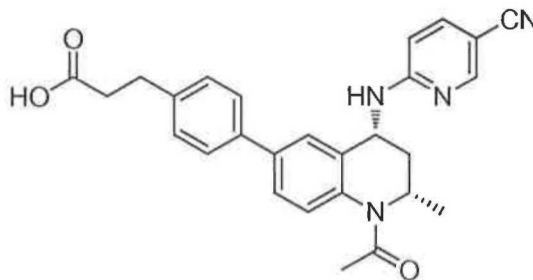
**2-(4-((2*S*,4*R*)-1-Acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)acetic acid, lithium salt**



To a solution of ethyl 2-(4-((2*S*,4*R*)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)acetate (for a preparation see Intermediate 8) (300 mg, 0.640 mmol) in methanol (5 mL) was added lithium hydroxide solution (0.768 mL, 0.768 mmol). The resulting mixture was stirred at 40 °C for 2 h, whereupon it was concentrated under vacuum to give the crude lithium carboxylate of 2-(4-((2*S*,4*R*)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)acetic acid (286 mg, 100%) as a white solid which was used in the next step without further purification. LCMS (Formate, 2min), Rt=1.10min, MH<sup>+</sup> = 433.

#### Intermediate 10

**3-(4-((2*S*,4*R*)-1-Acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)propanoic acid**

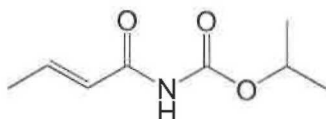


To a solution of 6-(((2*S*,4*R*)-1-acetyl-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,4-tetrahydroquinolin-4-yl)amino)nicotinonitrile (for a preparation see Intermediate 5) (382 mg, 0.884 mmol) and 3-(4-bromophenyl)propanoic acid (243 mg, 1.060 mmol) in

toluene (6 mL) and ethanol (6 mL) were successively added tetrakis(triphenylphosphine)palladium(0) (102 mg, 0.088 mmol) and potassium carbonate (366 mg, 2.65 mmol). The resulting mixture was stirred at 80 °C for 2 h, then partitioned between water and EtOAc. The layers were separated and the aqueous phase was  
5 extracted with EtOAc (x3). The combined organic layers were washed with brine, dried (MgSO<sub>4</sub>), filtered and concentrated *in vacuo*. The crude residue was purified by flash chromatography on silica gel (25g), eluting with EtOAc in cyclohexane (10-70%). The appropriate fractions were combined and concentrated under reduced pressure to give 3-(4-((2*S*,4*R*)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)propanoic acid (209 mg, 52%) as a white solid. LCMS (Formate, 2min),  
10 Rt=0.91min, MH<sup>+</sup> = 455.

### Intermediate 11

#### 1-Methylethyl (2*E*)-2-butenoylcarbamate

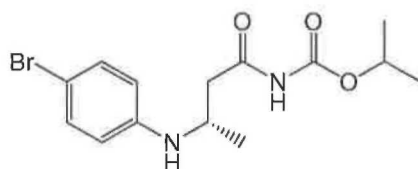


15 Isopropyl carbamate (30 g, 291 mmol, available from TCI) was charged to a 3L Lara vessel and dry tetrahydrofuran (THF) (150 ml) added. (2*E*)-2-Butenoyl chloride (31.2 ml, 326 mmol, available from Aldrich) was added under nitrogen and the jacket cooled to -30 °C. When the solution temperature reached -17 °C Lithium tert-butoxide (1M, 655 mL,  
20 655 mmol) was added by peristaltic pump over 2 h, keeping the reaction temperature between -10 °C and -18 °C. Once the addition was complete the mixture was stirred for 30 min and brought to 0 °C. Diethyl ether (450ml) and hydrochloric acid (1M, 375 mL) were added and the mixture brought to 20 °C with vigorous stirring. The stirring was stopped, the layers allowed to separate and the aqueous layer run off. Brine (375 mL)  
25 was added and the mixture stirred vigorously. The stirring was stopped, the layers allowed to separate and the aqueous layer run off. The organic layer was dried (MgSO<sub>4</sub>), filtered and evaporated to a brown oil (60g). The was applied to a silica column (40+M Biotage) and eluted with DCM / ethyl acetate (1:1 to 0:1, 10CV). The product containing fractions were evaporated to dryness and loaded on to a Redisep Isco silica column  
30 (1500g) and eluted with a gradient ethyl acetate in cyclohexane (0-40%). The clean, product containing fractions were evaporated to an off-white solid (15.41g). LCMS (Method C): Rt = 0.68, MH<sup>+</sup> = 172

### Intermediate 12



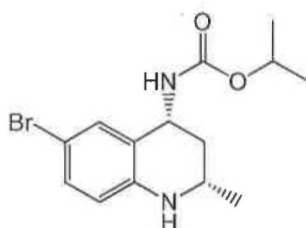
### 1-Methylethyl {(3S)-3-[(4-bromophenyl)amino]butanoyl}carbamate



1-Methylethyl (2*E*)-2-butenoylcarbamate (for a preparation see Intermediate 11) (9.38 g, 54.8 mmol) was stirred in toluene (281 mL) under nitrogen and (R-BINAP)ditriflatebis(acetonitrile)palladium(II) (Intermediate 24, 3.35 g, 3.01 mmol) added. The catalyst formed a gummy ball, the solution turned to an opaque yellow mixture and was stirred for 20 min. 4-Bromoaniline (14.14 g, 82 mmol) was added, the solution turned to a clear light brown and the gummy catalyst dissolved further. The mixture was stirred for 16 h. Similarly a second batch of 1-methylethyl (2*E*)-2-butenoylcarbamate (Intermediate 11, 8.51 g, 49.7 mmol) was stirred in toluene (255 mL) under nitrogen and (R-BINAP)ditriflatebis(acetonitrile)palladium(II) (3.04 g, 2.73 mmol) added. The catalyst formed a gummy ball, the solution turned to an opaque yellow mixture and was stirred for 20 min. 4-Bromoaniline (12.83 g, 74.6 mmol) was added, the solution turned to a clear light brown and the gummy catalyst dissolved further. The mixture was stirred for 16 h. The two reaction mixtures were combined and loaded on to a 1.5 kg Isco silica Redisep column. The column was eluted with DCM / MeOH (0%→0.5%, 19 CV). The clean, product containing fractions were evaporated to a pale brown oil. The mixture was dried in a vacuum oven overnight at 40 °C to give a white solid (24.2 g, 67% overall). LCMS (Method C): *R*<sub>t</sub> = 0.91, *M*H<sup>+</sup> = 343. ee = 92%.

### Intermediate 13

#### 1-Methylethyl [(2*S*,4*R*)-6-bromo-2-methyl-1,2,3,4-tetrahydro-4-quinoliny]carbamate



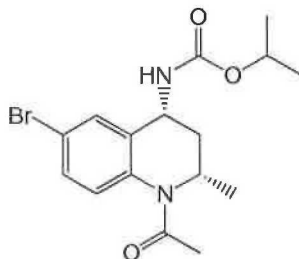
1-Methylethyl {(3*S*)-3-[(4-bromophenyl)amino]butanoyl}carbamate (for a preparation see Intermediate 12) (17.9 g, 52.2 mmol) was taken up in ethanol (150 mL) and cooled to below -10 °C (internal temperature) in a CO<sub>2</sub>/acetone bath. NaBH<sub>4</sub> (1.381 g, 36.5 mmol) was added followed by magnesium chloride hexahydrate (11.35 g, 55.8 mmol) in water (25 mL) keeping the temperature below -5 °C. The mixture was allowed to stir at < 0 °C for 1 h then warmed to room temperature and stirred for 1 h. The resulting thick suspension was poured into a mixture of citric acid (25.05 g, 130 mmol), HCl (1M in water,

205 mL, 205 mmol) and DCM (205 mL). The biphasic mixture was stirred at room temperature for 1 h. The layers were separated and the organic layer dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated to yield the product as a light brown solid (14.1 g). LCMS (Method B): Rt = 1.13, MH<sup>+</sup> = 327

5

#### Intermediate 14

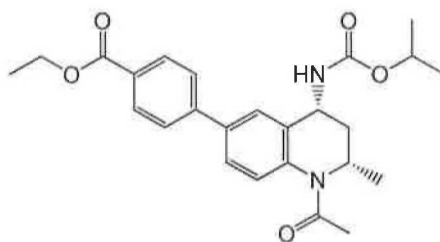
**1-Methylethyl [(2S,4R)-1-acetyl-6-bromo-2-methyl-1,2,3,4-tetrahydro-4-quinolinyl]carbamate**



- 10 1-Methylethyl [(2S,4R)-6-bromo-2-methyl-1,2,3,4-tetrahydro-4-quinolinyl]carbamate (for a preparation see Intermediate 13) (14.1 g, 43.1 mmol) was taken up in DCM (400 mL) under nitrogen at room temperature. Pyridine (10.46 mL, 129 mmol), then acetyl chloride (4.60 mL, 64.6 mmol), were added and the reaction stirred at room temperature for 16 h, then partitioned between EtOAc (2000 mL) and a saturated NaHCO<sub>3</sub> aqueous solution
- 15 (800 mL). The layers were separated and the organic phase was washed with water then brine (1500 mL each) and then dried with Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo* to yield a purple solid. The crude product was taken up in the minimum of DCM and applied to a 330g Companion XL column and eluted with a gradient of 12-63% Ethyl Acetate in cyclohexane to give the product as an off-white solid (12.37 g).
- 20 LCMS (Method B): Rt = 1.03, MH<sup>+</sup> = 369  
[α]<sub>D</sub> = +281.1025° (T = 20.7°C, 10mm cell, c=0.508g/100ml, ethanol).

#### Intermediate 15

- Ethyl 4-[(2S,4R)-1-acetyl-2-methyl-4-({[(1-methylethyl)oxy]carbonyl}amino)-1,2,3,4-tetrahydro-6-quinolinyl]benzoate**
- 25

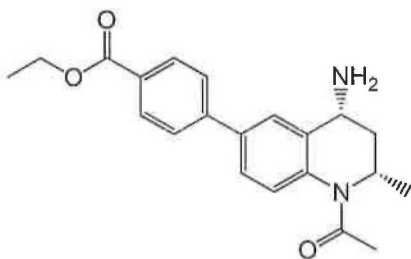


1-Methylethyl [(2S,4R)-1-acetyl-6-bromo-2-methyl-1,2,3,4-tetrahydro-4-quinolinyl]carbamate (for a preparation see Intermediate 14), (39.0 g, 106 mmol), {4-

[(ethyloxy)carbonyl]phenyl}boronic acid (22.5 g, 116 mmol) and tetrakis(triphenylphosphine)palladium(0) (1.83 g, 1.58 mmol) were mixed in DME (430 mL) and the resulting mixture was treated with aqueous Na<sub>2</sub>CO<sub>3</sub> (2N, 210 mL, 420 mmol). The mixture was degassed under house vacuum with several quenches with nitrogen and then stirred at 105 °C under nitrogen for approximately 6 h before being allowed to cool to room temperature. The mixture was partitioned between EtOAc and water and the layers were separated. The aqueous phase was extracted with EtOAc and the combined organic phases were washed with brine. The organic phase was then filtered through a 70 g silica cartridge, washing the cartridge with EtOAc. The combined filtrate and washings were concentrated *in vacuo*. The residue was triturated with Et<sub>2</sub>O then filtered off. The solid obtained was air-dried to give ethyl 4-[(2*S*,4*R*)-1-acetyl-2-methyl-4-({[(1-methylethyl)oxy]carbonyl}amino)-1,2,3,4-tetrahydro-6-quinolinyl]benzoate (35.2 g, 80.2 mmol, 76%) as a grey solid. The filtrate was concentrated *in vacuo* and the residue obtained triturated with Et<sub>2</sub>O (approximately 30 mL). The solid formed was isolated by filtration and air-dried, to give ethyl 4-[(2*S*,4*R*)-1-acetyl-2-methyl-4-({[(1-methylethyl)oxy]carbonyl}amino)-1,2,3,4-tetrahydro-6-quinolinyl]benzoate as a grey solid (5.96 g, 13.5 mmol, 13%). LCMS (formate, 2min), Retention time 1.16 min, MH<sup>+</sup> = 439

#### Intermediate 16

#### Ethyl 4-[(2*S*,4*R*)-1-acetyl-4-amino-2-methyl-1,2,3,4-tetrahydro-6-quinolinyl]benzoate



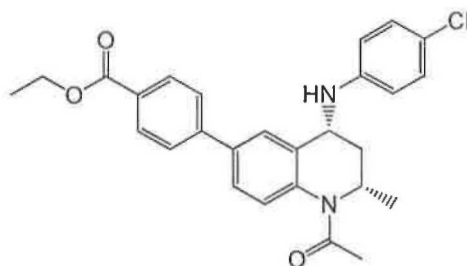
Ethyl 4-[(2*S*,4*R*)-1-acetyl-2-methyl-4-({[(1-methylethyl)oxy]carbonyl}amino)-1,2,3,4-tetrahydro-6-quinolinyl]benzoate (for a preparation see intermediate 15) (8.90 g, 20.30 mmol) was added to a suspension of aluminium chloride (10.3 g, 77 mmol) in DCM (160 mL) cooled with an ice/water bath. The temperature rose from 0 °C to approximately 6 °C after the addition. The resulting mixture was stirred at approximately 0 °C for 20 min, and then treated with a solution of methanol (18 mL) and triethylamine (34 mL, 245 mmol) over ~30 sec. The resulting mixture was stirred at 0 °C for ~30 min, and then partitioned between EtOAc and a saturated NaHCO<sub>3</sub> aqueous solution.

The same reaction was done in parallel, using of ethyl 4-[(2*S*,4*R*)-1-acetyl-2-methyl-4-({[(1-methylethyl)oxy]carbonyl}amino)-1,2,3,4-tetrahydro-6-quinolinyl]benzoate (for a preparation see Intermediate 15) (0.89 g 2.030 mmol), aluminium chloride (1.03 g, 7.72

mmol), triethylamine (3.4 mL, 24.53 mmol), DCM (16 mL) and MeOH (1.3 mL). The products of both reactions were combined at this stage and the resulting mixture was stirred at room temperature for approximately 10 min (total volume: approximately 1 L). The mixture was filtered through Celite™, the insoluble residue was washed with EtOAc and a saturated NaHCO<sub>3</sub> aqueous solution and the layers were separated. The aqueous phase was extracted with EtOAc and the combined organic phases were washed with brine, dried (hydrophobic frit) and concentrated *in vacuo* to give ethyl 4-[(2*S*,4*R*)-1-acetyl-4-amino-2-methyl-1,2,3,4-tetrahydro-6-quinolinyl]benzoate (6.6 g, 84% - allowing for the addition of the parallel experiment) as a cream solid. LCMS (formate, 2 min), Retention time 0.73 min, [M-NH<sub>2</sub>]<sup>+</sup> = 336

### Intermediate 17

**Ethyl 4-[(2*S*,4*R*)-1-acetyl-4-[(4-chlorophenyl)amino]-2-methyl-1,2,3,4-tetrahydro-6-quinolinyl]benzoate**

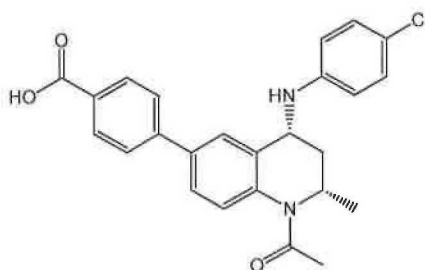


Ethyl 4-[(2*S*,4*R*)-1-acetyl-4-amino-2-methyl-1,2,3,4-tetrahydro-6-quinolinyl]benzoate (for a preparation see Intermediate 16) (6.6 g, 18.73 mmol), 1-bromo-4-chlorobenzene (3.94 g, 20.60 mmol), bis(dibenzylideneacetone)palladium (0) (690 mg, 1.2 mmol) and [2'-(dicyclohexylphosphanyl)-2-biphenyl]dimethylamine (Dave-phos) (590 mg, 1.499 mmol) were mixed in toluene (120 mL) and the resulting mixture was treated with sodium *t*-butoxide (2.52 g, 26.2 mmol). The reaction was degassed under house vacuum with several quenches with nitrogen, heated at 70 °C under nitrogen for 16 h, then was allowed to cool to room temperature and filtered. The insoluble residue was washed with toluene and then Et<sub>2</sub>O. The combined filtrate and washings were washed with water (x2) then extracted with hydrochloric acid (2N, x20, resulting in the precipitation of an orange oil which was collected with the aqueous acidic phases. The acidic extracts were washed with Et<sub>2</sub>O and the combined organic phases were washed with brine, dried (hydrophobic frit) and concentrated *in vacuo*. The residue was purified by chromatography on a silica cartridge (330 g), eluting with an EtOAc / cyclohexane gradient (5-45%). The appropriate fractions were combined and reduced to dryness *in vacuo* to give a pale yellow foam. This foam was dissolved in EtOAc (50 mL) and treated with functional thiourea silica (0.56 g,

palladium scavenger). The mixture was stirred at room temperature (air atmosphere) for ~20 min and then left at room temperature for 16 h. The mixture was filtered and the insoluble residues washed with EtOAc. The combined filtrate and washings were concentrated *in vacuo* to give ethyl 4-((2S,4R)-1-acetyl-4-[(4-chlorophenyl)amino]-2-methyl-1,2,3,4-tetrahydro-6-quinolinyl)benzoate (3.7 g, 8.0 mmol, 32%) as a yellow oil.

#### Intermediate 18

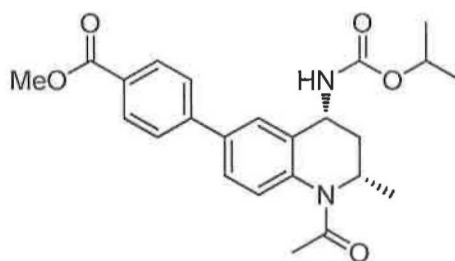
#### 4-((2S,4R)-1-Acetyl-4-[(4-chlorophenyl)amino]-2-methyl-1,2,3,4-tetrahydro-6-quinolinyl)benzoic acid



Ethyl 4-((2S,4R)-1-acetyl-4-[(4-chlorophenyl)amino]-2-methyl-1,2,3,4-tetrahydro-6-quinolinyl)benzoate (for a preparation see Intermediate 17) (5.41 g, 11.69 mmol) was dissolved in ethanol (100 mL) and the solution was treated with aqueous NaOH solution (2M, 50 mL, 100 mmol). The resulting mixture was stirred at room temperature (air atmosphere) for approximately 2 h then most of the ethanol was removed *in vacuo*. The resulting yellow solution was diluted with water (resulting in the formation of an oily yellow precipitate). The aqueous phase was washed twice with DCM (which didn't dissolve the precipitate previously formed) then was acidified with hydrochloric acid (2N) to pH 1 and extracted with EtOAc (x2). The combined EtOAc phases were washed with brine, dried (hydrophobic frit) and concentrated *in vacuo*. The residual yellow foam was triturated with Et<sub>2</sub>O over approximately 1 h. The resulting solid was isolated by filtration, washed with Et<sub>2</sub>O and air-dried to give 4-((2S,4R)-1-acetyl-4-[(4-chlorophenyl)amino]-2-methyl-1,2,3,4-tetrahydro-6-quinolinyl)benzoic acid (4.41 g, 10.1 mmol, 87%) as a cream solid. LCMS (HpH), Retention time 1.08 min, [M-H]<sup>-</sup> = 433

#### Intermediate 19

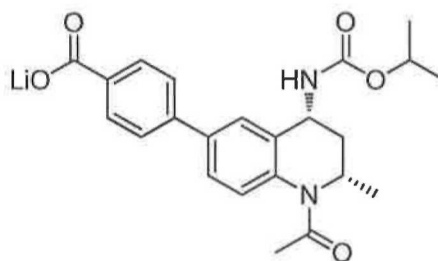
#### Methyl 4-((2S,4R)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate



To a solution of isopropyl ((2S,4R)-1-acetyl-6-bromo-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (5 g, 13.54 mmol) and methyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (3.90 g, 14.89 mmol) in DME (50 mL) and water (10.0 mL) were successively added palladium tetrakis(triphenylphosphine) (1.565 g, 1.354 mmol) and potassium carbonate (5.61 g, 40.6 mmol). The resulting mixture was stirred at 100 °C for 1 h, whereupon it was allowed to cool down to room temperature and was filtered through Celite™. The filtrate was concentrated *in vacuo* and the residue was partitioned between EtOAc and water. The aqueous phase was extracted with EtOAc (x3) and the combined organic layers were washed with brine, dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo*. The crude compound was purified by flash chromatography on a silica gel cartridge (50g) eluting with EtOAc in cyclohexane (5-60%). The appropriate fractions were combined and concentrated under reduced pressure to give methyl 4-((2S,4R)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate (4.64 g, 81%) as a white gum. LCMS (Formate, 2min), Rt=1.09min, MH<sup>+</sup> = 425.

#### Intermediate 20

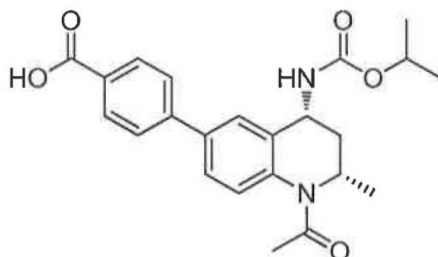
**Lithium 4-((2S,4R)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate**



To a solution of methyl 4-((2S,4R)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate (for a preparation see Intermediate 19) (1.63 g, 3.84 mmol) in methanol (20 mL) was added lithium hydroxide (4.61 mL, 4.61 mmol). The resulting mixture was stirred at 40 °C for 6 h, whereupon it was concentrated under reduced pressure to give lithium 4-((2S,4R)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate (1.65 g, 100%) which was not purified but directly used in the subsequent step. LCMS (Formate, 2min), Rt=0.87, MH<sup>+</sup> = 411.

### Intermediate 21

#### 4-((2S,4R)-1-Acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoic acid



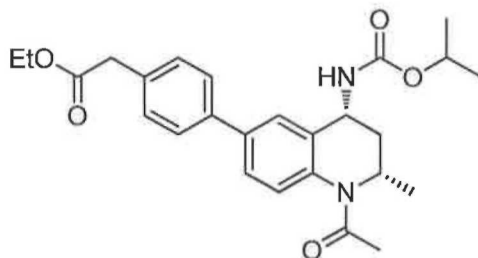
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Lithium 4-((2S,4R)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate (1.05 g, 2.52 mmol) (for a preparation see Intermediate 20) was partitioned between EtOAc and hydrochloric acid (2M). The phases were separated and the aqueous phase was extracted with EtOAc (x3). The combined organic layers were washed with brine, dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to give 4-((2S,4R)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoic acid (898 mg, 87%) as a white solid. LCMS (Formate, 2min), Rt=0.87, MH<sup>+</sup> = 411.

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### Intermediate 22

#### Ethyl 2-(4-((2S,4R)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)acetate



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To a flask was charged with ethyl (4-bromophenyl)acetate (0.174 mL, 1.000 mmol), 1-methylethyl [(2S,4R)-1-acetyl-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,4-tetrahydro-4-quinoliny]carbamate (416 mg, 1 mmol), potassium carbonate (415 mg, 3.00 mmol) and PdCl<sub>2</sub>(dppf) (73.2 mg, 0.100 mmol) was added 1,4-dioxane (6 mL) and water (2.0 mL) and the flask flushed with nitrogen. The resulting mixture was stirred under microwave irradiation at 120 °C for 30 min then cooled to room temperature. The bulk of dioxane was removed *in vacuo* and the residue partitioned between EtOAc and water. The layers were separated and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with brine, dried over MgSO<sub>4</sub> and concentrated *in*

25

*vacuo*. The residue was purified by chromatography [(25g column, MeOH / DCM)] to give ethyl 2-(4-((2*S*,4*R*)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)acetate (270 mg, 59.7 % yield). This compound was used in the next step without further purification. LCMS (HpH, 2min), *R*<sub>t</sub>=1.16, *M*H<sup>+</sup> = 453.

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### Intermediate 23

#### 2-(4-((2*S*,4*R*)-1-Acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)acetic acid



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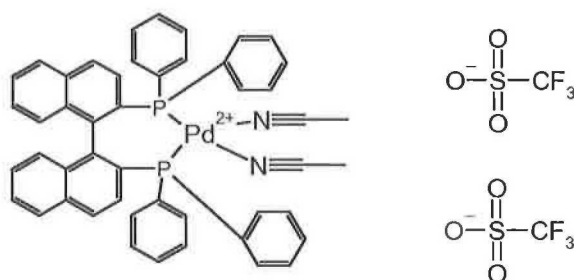
To a solution of ethyl {4-[(2*S*,4*R*)-1-acetyl-2-methyl-4-({[(1-methylethyl)oxy]carbonyl}amino)-1,2,3,4-tetrahydro-6-quinoliny]]phenyl}acetate (for a preparation see Intermediate 22)(270 mg, 0.597 mmol) in methanol (6 mL) and water (2.0 mL) was added aqueous sodium hydroxide (2*N*, 0.597 mL, 1.193 mmol) at room temperature and the resulting mixture was stirred for 6 h. Aqueous sodium hydroxide (2*N*, 0.5 mL) was added and the mixture was left standing overnight. The bulk of methanol was removed *in vacuo* and the resulting residue was partitioned between water and Et<sub>2</sub>O and the layers were separated. The aqueous layer was acidified with hydrochloric acid (2*N*, 2 mL) and extracted twice with EtOAc. The combined organic phases were dried over MgSO<sub>4</sub> and concentrated *in vacuo* to give {4-[(2*S*,4*R*)-1-acetyl-2-methyl-4-({[(1-methylethyl)oxy]carbonyl}amino)-1,2,3,4-tetrahydro-6-quinoliny]]phenyl}acetic acid (200 mg, 0.471 mmol, 79 % yield) as a brown foam. This compound was used in the next step without further purification. LCMS (HpH, 2min), *R*<sub>t</sub>=0.65, *M*H<sup>+</sup> = 425.

25

### Intermediate 24

#### (*R*-BINAP)ditriflatebis(acetonitrile)palladium(II)



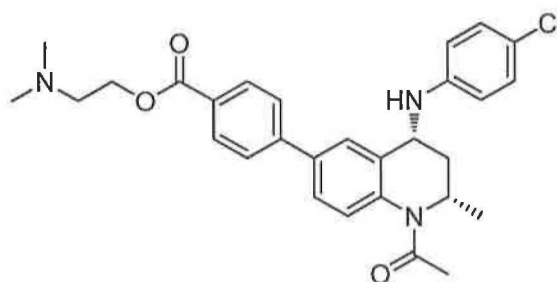


R-(+)-BINAP (6.08 g, 9.76 mmol, available from Avocado) was stirred in DCM (626 ml) and dichlorobis(acetonitrile)palladium (II) (2.5g, 9.64 mmol, available from Aldrich) added. The mixture was stirred under nitrogen for 30min, the suspension had not become a solution and more DCM (100ml) was added. The mixture was stirred for a further 30 min and silver triflate (5.00 g, 19.47 mmol, available from Aldrich) dissolved in acetonitrile (250 ml) was added. The mixture changed from an orange cloudy suspension to a yellow suspension. The mixture was stirred for 1 h, filtered through Celite™ and evaporated to an orange solid. The residue was dried under vacuum (at approximately 14mbar) at room temperature over the weekend to give the desired product (10.69g).

<sup>1</sup>H NMR (400 MHz, MeCN-*d*<sub>3</sub>) δ ppm 2.0 (s, 6 H), 6.7 (d, 2H), 6.9 (br m, 4H), 7.1 (br t, 2H), 7.2 (t, 2H), 7.5 – 7.9 (m, 22H)

### Example 1

#### 2-(Dimethylamino)ethyl 4-((2*S*,4*R*)-1-acetyl-4-((4-chlorophenyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate hydrochloride



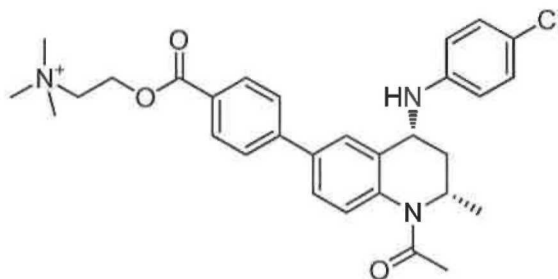
4-((2*S*,4*R*)-1-Acetyl-4-[(4-chlorophenyl)amino]-2-methyl-1,2,3,4-tetrahydro-6-quinoliny]benzoic acid (for a preparation see Intermediate 18 (100 mg, 0.230 mmol) was suspended in DCM (1 mL). DMAP (33.7 mg, 0.276 mmol) and DCC (52.2 mg, 0.253 mmol) were added and the mixture was stirred for 5 min producing a clear yellow solution. 2-(Dimethylamino)ethanol (20.5 mg, 0.230 mmol) in DCM (0.5 mL) was subsequently added and the reaction stirred at room temperature overnight. The reaction was diluted with DCM (8.5ml) and washed with NaOH (2N), water and brine (10ml each) then dried

with Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated *in vacuo* to yield an off-white semi-solid. The crude product dissolved in a minimal volume of DCM (with a few drops of MeOH to aid solubility) and applied to a 25g cartridge. The cartridge was dried under vacuum at 40 °C for 1 h then eluted with 1% 2M NH<sub>3</sub> in methanol / DCM for 2CV then 1-5% 2M NH<sub>3</sub> in methanol in DCM over 10CV then held at 5% for 5CV. The appropriate fractions were concentrated *in vacuo* to yield the free amine product (18.8 mg) as a clear oil. The latter was taken up in the minimum of DCM and HCl in Et<sub>2</sub>O (1N, 0.19 mL, 0.190 mmol) added and the solvent evaporated under a stream of nitrogen. A small amount of Et<sub>2</sub>O was added (~1mL) and evaporated under nitrogen to yield 2-(dimethylamino)ethyl 4-((2S,4R)-1-acetyl-4-[(4-chlorophenyl)amino]-2-methyl-1,2,3,4-tetrahydro-6-quinolinyl)benzoate hydrochloride (76.7 mg, 0.135 mmol, 58.6 % yield) as a white solid. LCMS (Formate, 2min), Rt=0.91min, MH<sup>+</sup> = 506.

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>): δ 1.16 (3H, d), 1.30 (1H, m), 2.21 (3H, s), 2.65-2.74 (7H, m), 3.24 (2H, m), 4.36 (1H, m), 4.56 (2H, m), 4.76 (1H, m), 6.38 (1H, d), 6.80 (2H, d), 7.19 (2H, d), 7.52 (1H, s), 7.54 (1H, d), 7.72 (1H, dd), 7.77 (2H, d), 8.13 (2H, d), 9.92 (1H, bs).

## Example 2

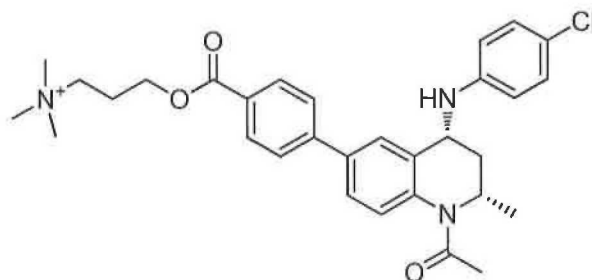
**2-((4-((2S,4R)-1-Acetyl-4-((4-chlorophenyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoyl)oxy)-N,N,N-trimethylethanaminium, formate**



To a solution of 4-((2S,4R)-1-acetyl-4-[(4-chlorophenyl)amino]-2-methyl-1,2,3,4-tetrahydro-6-quinolinyl)benzoic acid (for a preparation see Intermediate 18) (200 mg, 0.460 mmol) in DMF (5 mL) were successively added K<sub>2</sub>CO<sub>3</sub> (95 mg, 0.690 mmol) and (2-bromoethyl)trimethylammonium bromide (170 mg, 0.690 mmol). The resulting mixture was stirred at room temperature overnight, whereupon it was concentrated under reduced pressure. The residue was dissolved in a 1:1 MeOH/DMSO mixture and purified by MDAP (formate). The appropriate fractions were combined and concentrated *in vacuo* to give 2-((4-((2S,4R)-1-acetyl-4-((4-chlorophenyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoyl)oxy)-N,N,N-trimethylethanaminium, formate (133 mg, 51%) as an off-white solid. LCMS (Formate, 2min), Rt=0.89min, MH<sup>+</sup> = 520.

### Example 3

**3-((4-((2S,4R)-1-Acetyl-4-((4-chlorophenyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoyl)oxy)-N,N,N-trimethylpropan-1-aminium, formate**



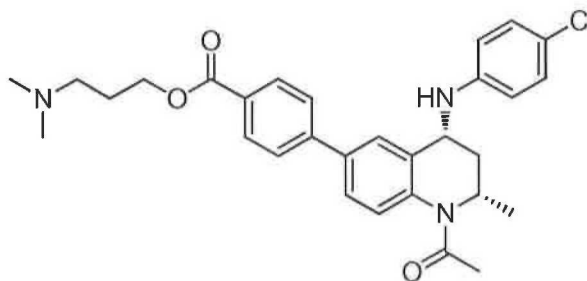
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4-((2S,4R)-1-Acetyl-4-((4-chlorophenyl)amino)-2-methyl-1,2,3,4-tetrahydro-6-quinoliny]benzoic acid (for a preparation see Intermediate 18)(150 mg, 0.345 mmol) was dissolved in DMF (3 mL) and K<sub>2</sub>CO<sub>3</sub> (47.7 mg, 0.345 mmol) then 3-bromo-N,N,N-trimethyl-1-propanaminium, bromide (90 mg, 0.345 mmol) were added. The resulting mixture was stirred at room temperature overnight, whereupon the reaction mixture was concentrated *in vacuo* and purified by MDAP (formate) to give 3-[[4-((2S,4R)-1-acetyl-4-[(4-chlorophenyl)amino]-2-methyl-1,2,3,4-tetrahydro-6-quinoliny]phenyl)carbonyl]oxy]-N,N,N-trimethyl-1-propanaminium, formate (41 mg, 0.064 mmol, 19 % yield) as a yellow oil. LCMS (Formate, 2min), Rt=0.86min, MH<sup>+</sup> = 534.

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### Example 4

**3-(Dimethylamino)propyl 4-((2S,4R)-1-acetyl-4-((4-chlorophenyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate**



20

To a solution of 4-((2S,4R)-1-acetyl-4-[(4-chlorophenyl)amino]-2-methyl-1,2,3,4-tetrahydro-6-quinoliny]benzoic acid (for a preparation see Intermediate 18)(250 mg, 0.575 mmol) in DMF (10 mL) were successively added 3-(dimethylamino)-1-propanol (71.2 mg, 0.690 mmol), EDC (220 mg, 1.150 mmol) and DMAP (7.0 mg, 0.057 mmol). The resulting mixture was stirred at room temperature overnight, then concentrated *in vacuo*, dissolved in a 1:1 MeOH/DMSO mixture and purified by MDAP (HpH). The

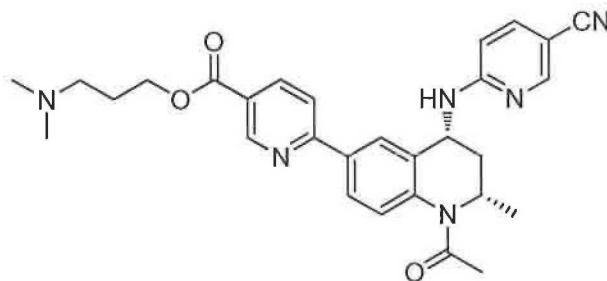
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appropriate fractions were combined and concentrated under reduced pressure to give 3-(dimethylamino)propyl 4-((2*S*,4*R*)-1-acetyl-4-((4-chlorophenyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate (41 mg, 17%) as a viscous colourless oil. LCMS (Formate, 2min),  $R_t$ =0.94min,  $MH^+ = 520$ .

5

#### Example 5

##### 3-(Dimethylamino)propyl 6-((2*S*,4*R*)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)nicotinate



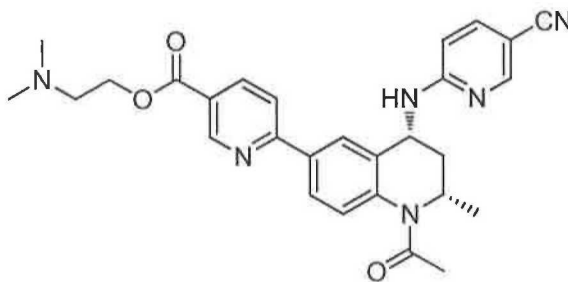
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To a solution of 6-((2*S*,4*R*)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)nicotinic acid (for a preparation see Intermediate 7)(100 mg, 0.234 mmol) in DMF (5 mL) were added 3-(dimethylamino)-1-propanol (121 mg, 1.170 mmol), EDC (90 mg, 0.468 mmol) and DMAP (2.86 mg, 0.023 mmol). The resulting mixture was stirred at room temperature overnight, the reaction mixture was concentrated *in vacuo*, dissolved in a 1:1 MeOH/DMSO mixture and purified by MDAP (HpH). The appropriate fractions were combined and concentrated under reduced pressure to give 3-(dimethylamino)propyl 6-((2*S*,4*R*)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)nicotinate (23 mg, 19%) as a colourless oil. LCMS (Formate, 2min),  $R_t$ =0.70min,  $MH^+ = 513$ .

20

#### Example 6

##### 2-(Dimethylamino)ethyl 6-((2*S*,4*R*)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)nicotinate

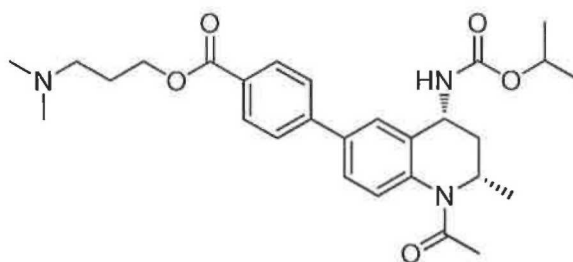


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To a solution of 6-((2*S*,4*R*)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)nicotinic acid (for a preparation see Intermediate 7)(54 mg, 0.126 mmol) in DMF (5 mL) were added K<sub>2</sub>CO<sub>3</sub> (26.2 mg, 0.189 mmol) and 2-bromo-*N,N*-dimethylethanamine (28.8 mg, 0.189 mmol). The resulting mixture was stirred at room temperature for 3 h, the reaction mixture was concentrated under reduced pressure and the residue was dissolved in a 1:1 MeOH/DMSO mixture and purified by MDAP (formate). The appropriate fractions were combined and concentrated *in vacuo* to give 2-(dimethylamino)ethyl 6-((2*R*,4*R*)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)nicotinate (16.5 mg, 24%) as a viscous colourless oil. LCMS (Formate, 2min), Rt=0.69min, MH<sup>+</sup> = 499.

### Example 7

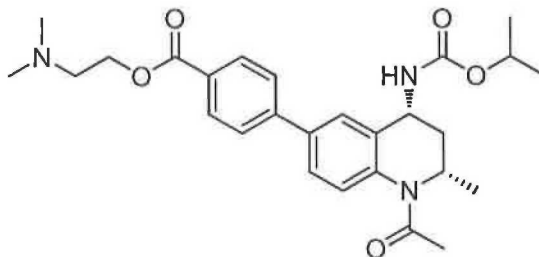
#### 3-(Dimethylamino)propyl 4-((2*S*,4*R*)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate



To a solution of 4-((2*S*,4*R*)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoic acid (for a preparation see Intermediate 21)(164 mg, 0.400 mmol) and 3-(dimethylamino)propan-1-ol (206 mg, 1.998 mmol) in DMF (3 mL) were added *N*1-((ethylimino)methylene)-*N*3,*N*3-dimethylpropane-1,3-diamine hydrochloride (153 mg, 0.799 mmol) and *N,N*-dimethylpyridin-4-amine (4.88 mg, 0.040 mmol). The resulting mixture was stirred at room temperature for 2 h, the reaction mixture was diluted with water and EtOAc was added. The layers were separated and the aqueous phase was extracted with EtOAc (x3). The combined organic layers were washed with brine, dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo*. The residue was dissolved in a 1:1 MeOH/DMSO mixture and was purified by MDAP (HpH). The appropriate fractions were combined and concentrated under reduced pressure to give 3-(dimethylamino)propyl 4-((2*S*,4*R*)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate (29.3 mg, 15%) as a viscous colourless oil. LCMS (Formate, 2min), Rt=0.75min, MH<sup>+</sup> = 476.

### Example 8

**2-(Dimethylamino)ethyl 4-((2S,4R)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate**

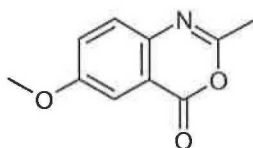


- 5 To a solution of lithium 4-((2S,4R)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate (for a preparation see Intermediate 20)(246 mg, 0.591 mmol) and 2-bromo-N,N-dimethylethanamine (135 mg, 0.886 mmol) in DMF (5 mL) was added potassium carbonate (122 mg, 0.886 mmol). The resulting mixture was stirred at room temperature for 2 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in a 1:1 MeOH/DMSO mixture and purified by MDAP (formate). The appropriate fractions were combined and concentrated *in vacuo* to give 2-(dimethylamino)ethyl 4-((2S,4R)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate (14.5 mg, 5%) as a viscous colourless oil. LCMS (Formate, 2min),  $R_t=0.72\text{min}$ ,  $MH^+ = 482$ .

15

**REFERENCE COMPOUNDS**

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

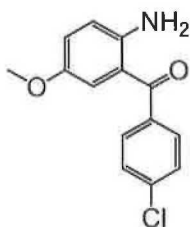


20

- 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 D):  $m/z$  192  $[M+H]^+$ ,  $R_t$  1.69 min.

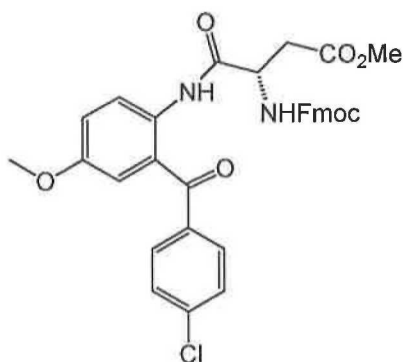
25

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



To a solution of 2-methyl-6-(methoxy)-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<sub>2</sub>O, 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<sub>2</sub>SO<sub>4</sub>, 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<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure. The title compound was obtained as a yellow solid (39 g, 88 % yield); LC/MS (Method D): m/z 262 [M+H]<sup>+</sup>, Rt 2.57 min.

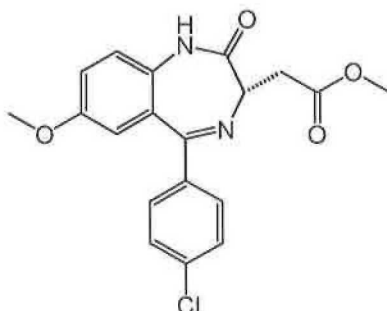
**Reference Compound C: Methyl N<sup>1</sup>-[2-[(4-chlorophenyl)carbonyl]-4-(methoxy)phenyl]-N<sup>2</sup>-{[(9H-fluoren-9-ylmethyl)oxy]carbonyl}-L-α-asparaginate**



Methyl N-[[[(9H-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<sub>3</sub> (270 mL) and [2-amino-5-(methoxy)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 to 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.

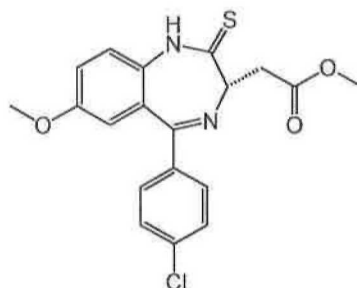
**Reference compound D: Methyl [(3S)-5-(4-chlorophenyl)-7-(methoxy)-2-oxo-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]acetate**



To a solution of methyl N1-[2-[(4-chlorophenyl)carbonyl]-4-(methoxy)phenyl]-N2-[(9H-fluoren-9-ylmethyl)oxy]carbonyl]-L- $\alpha$ -asparaginate (for a preparation see Reference compound C) (assumed 0.2 mol) in DCM (500 mL) was added Et<sub>3</sub>N (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<sub>2</sub>SO<sub>4</sub>, 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)<sup>+</sup> calculated for C<sub>19</sub>H<sub>18</sub><sup>35</sup>ClN<sub>2</sub>O<sub>4</sub> 373.0955; found 373.0957.

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



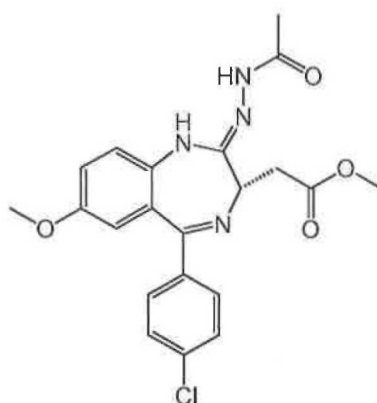
A suspension of P<sub>4</sub>S<sub>10</sub> (36.1 g, 81.1 mmol) and Na<sub>2</sub>CO<sub>3</sub> (8.6 g, 81.1 mmol) in 1,2-DCE (700 mL) at room temperature was stirred for 2 h before Methyl [(3S)-5-(4-chlorophenyl)-



7-(methyloxy)-2-oxo-2,3-dihydro-1H-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<sub>3</sub> and brine. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, 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 D): m/z 389 [M(<sup>35</sup>Cl)+H]<sup>+</sup>, Rt 2.64 min

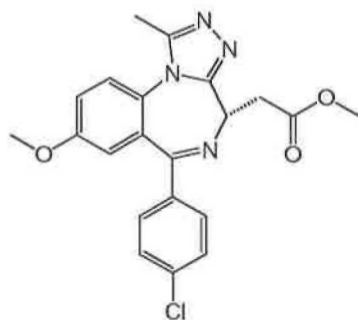
HRMS (M+H)<sup>+</sup> calculated for C<sub>19</sub>H<sub>18</sub><sup>35</sup>ClN<sub>2</sub>O<sub>3</sub>S 389.0727; found 389.0714.

**Reference compound F: Methyl [(3S)-2-[(1Z)-2-acetylhydrazino]-5-(4-chlorophenyl)-7-(methyloxy)-3H-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<sub>3</sub>N (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<sub>2</sub>SO<sub>4</sub>, filtered and concentrated *in vacuo* to give the crude title compound (9.7 g, 98% yield) which was used without further purification. R<sub>f</sub> = 0.49 (DCM/MeOH : 90/10).

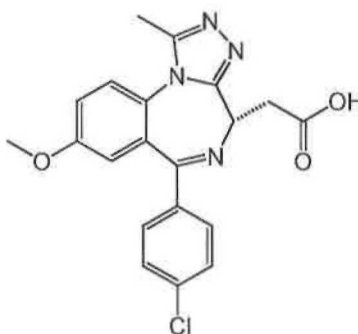
**Reference compound G: Methyl [(4S)-6-(4-chlorophenyl)-1-methyl-8-(methyloxy)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl]acetate**



The crude methyl [(3S)-2-[(1Z)-2-acetylhydrazino]-5-(4-chlorophenyl)-7-(methoxy)-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<sub>2</sub>O and filtered to give the title compound (8.7 g, 91% over 3 steps) as an off-white solid.

HRMS (M+H)<sup>+</sup> calculated for C<sub>21</sub>H<sub>20</sub>ClN<sub>4</sub>O<sub>3</sub> 411.1229; found 411.1245.

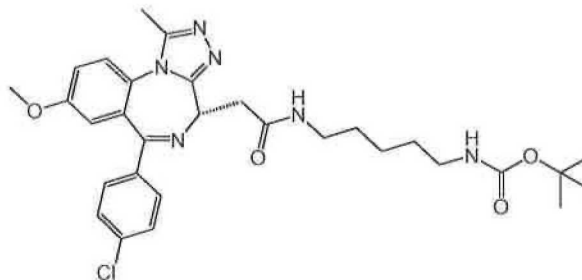
10 **Reference compound H: [(4S)-6-(4-Chlorophenyl)-1-methyl-8-(methoxy)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl]acetic acid**



To a solution of methyl [(4S)-6-(4-chlorophenyl)-1-methyl-8-(methoxy)-4H-[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<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give the title compound (7 g, 98% yield) as a pale yellow solid.

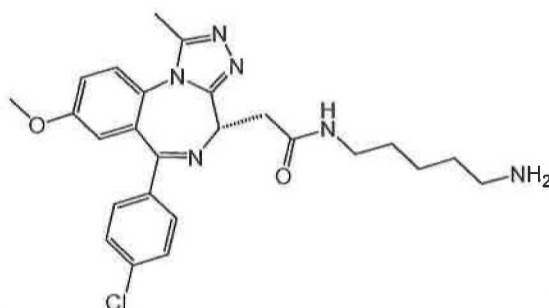
LC/MS (Method D): m/z 397 [M+H]<sup>+</sup>

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



5 A mixture of [(4S)-6-(4-chlorophenyl)-1-methyl-8-(methoxy)-4H-[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  
10 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/  
15 methanol 95/5 to give the title compound as a yellow solid (1.2g). LC/MS (Method D): *rt* = 3.04 min.

**Reference compound J: N-(5-aminopentyl)-2-[(4S)-6-(4-chlorophenyl)-1-methyl-8-(methoxy)-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-(methoxy)-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-4-yl]acetyl}amino)pentyl]carbamate (for a preparation see Reference compound I) (0.2 g, 0.34 mmol) in dichloromethane (3 ml) was  
25 added trifluoroacetic acid (0.053 ml, 0.68 mmol) dropwise at 0°C. The reaction mixture

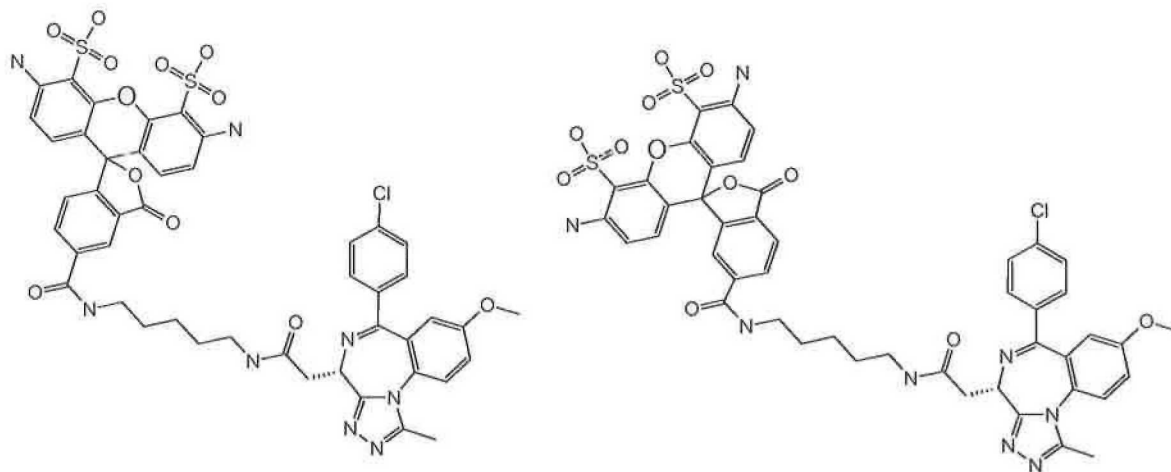
was stirred for 3h from 0°C to room temperature. The reaction mixture was concentrated to dryness to afford the title compound as a hygroscopic yellow oil (200mg)

LC/MS (Method D): rt = 2.33min.

HRMS (M+H)<sup>+</sup> calculated for C<sub>25</sub>H<sub>29</sub>ClN<sub>6</sub>O<sub>2</sub> 481.2119; found 481.2162.

5

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



10 N-(5-Aminopentyl)-2-[(4S)-6-(4-chlorophenyl)-1-methyl-8-(methoxy)-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 F):, MH<sup>+</sup> = 999, rt = 1.88min.

25

### Biological Test Methods

### Fluorescence anisotropy binding assay

The binding of the compounds of formula (I) to Bromodomains BRD2, BRD3 and BRD4 can be assessed using a Fluorescence Anisotropy Binding Assay.

- 5 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  
10 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:

15 
$$y = a + ((b - a) / (1 + (10^x / 10^c)^d))$$

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

- Recombinant Human Bromodomains (BRD2 (1-473), BRD3 (1-435) and BRD4 (1-477))  
20 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.  
25 Purified protein was stored at -80C in 20mM HEPES pH 7.5 and 100mM NaCl.

- Protocol for Bromodomain BRD2: All components were dissolved in buffer composition of 50 mM HEPES pH7.4, 150mM NaCl and 0.5mM CHAPS with final concentrations of BRD2, 75nM, fluorescent ligand 5nM. 10 µl of this reaction mixture was added using a  
30 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 (λ<sub>ex</sub>= 485nm, λ<sub>EM</sub> = 530nm; Dichroic -505nm).

- 35 Protocol for Bromodomain BRD3: All components were dissolved in buffer of composition 50 mM HEPES pH7.4, 150mM NaCl and 0.5mM CHAPS with final concentrations of

BRD3 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  
5 Envision ( $\lambda_{ex}$ = 485nm,  $\lambda_{EM}$  = 530nm; Dichroic -505nm).

Protocol for Bromodomain BRD4: All components were dissolved in buffer of composition 50 mM HEPES pH7.4, 150mM NaCl and 0.5mM CHAPS with final concentrations of BRD4 75nM, fluorescent ligand 5nM. 10 µl of this reaction mixture was  
10 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 ( $\lambda_{ex}$ = 485nm,  $\lambda_{EM}$  = 530nm; Dichroic -505nm).

15

**Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET) assay**

The binding of the compounds of formula (I) to Bromodomains BRD2, BRD3 and BRD4 was assessed using a time resolved fluorescent resonance energy transfer binding assay, that measures the binding of an acetylated histone peptide to the bromodomain protein.

20

The bromodomain protein, histone peptide and a variable concentration of test compound are incubated together to reach thermodynamic equilibrium. The assay is configured such that in the absence of test compound the bromodomain and peptide are significantly bound (~30%) and in the presence of a sufficient concentration of a potent inhibitor this  
25 interaction is disrupted leading to a measurable drop in fluorescent resonance energy transfer.

Histone Peptide:

30 H-Ser-Gly-Arg-Gly-Lys(Ac)-Gly-Gly-Lys(Ac)-Gly-Leu-Gly-Lys(Ac)-Gly-Gly-Ala-Lys(Ac)-Arg-His-Gly-Ser-Gly-Ser-Lys(Biotin)-OH. 3TFA

The protected peptide was assembled on a solid-phase synthesiser using preloaded Wang resin and utilising standard Fmoc synthesis protocols. The C-terminal lysine was  
35 protected by a hyper acid-labile group allowing for its selective removal at the end of the

assembly and attachment of the biotin. The crude peptide was obtained after cleavage from the resin with a mixture of trifluoroacetic acid (TFA), triisopropylsilane and water (95:2.5:2.5) for 3h at room temperature and was then purified using a C18 reverse-phase column utilising a 0.1%TFA-buffered water/acetonitrile gradient. The resulting fractions were analysed and fractions which were >95% pure by analytical HPLC and giving the correct mw (by MALDI-TOF mass spectroscopy) were pooled and freeze dried. The final material was analysed by HPLC to confirm purity.

Protein production: Recombinant Human Bromodomains (BRD2 (1-473), BRD3 (1-435) and BRD4 (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 sonication and purified using a nickel sepharose 6FF column, the proteins were washed and then eluted with 50mM Tris-HCl pH8.0, 300mM NaCl, 1mM  $\beta$ -mercaptoethanol and 20mM Imidazole. Further purification was performed by affinity chromatography on a HisTRAP HP column, eluting with a linear 0-500mM sodium chloride gradient, over 20 column volumes. Final 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. Protein identity was confirmed by peptide mass fingerprinting and predicted molecular weight confirmed by mass spectrometry.

Protocol for Bromodomain BRD2, 3 and 4 assays: All assay components were dissolved in buffer composition of 50 mM HEPES pH7.4, 50mM NaCl and 0.5mM CHAPS. The final concentration of bromodomain proteins were 100nM and the histone peptide was 300nM, these components are premixed and allowed to equilibrate for 1 hour in the dark. 8  $\mu$ l of this reaction mixture was added to all wells containing 50nl of various concentrations of test compound or DMSO vehicle (0.5% final) in Greiner 384 well black low volume microtitre plates and incubated in dark for 60 mins at room temperature. 2 $\mu$ l of detection mixture containing anti-6his XL665 labeled antibody and streptavidin labeled with europium cryptate was added to all wells and a further dark incubation of at least 30mins was performed. Plates were then read on the Envision platereader, ( $\lambda_{ex}$ = 317nm, donor  $\lambda_{EM}$  = 615nm; acceptor  $\lambda_{EM}$  = 665nm; Dichroic LANCE dual). Time resolved fluorescent intensity measurements were made at both emission wavelengths and the ratio of acceptor/donor was calculated and used for data analysis. 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:

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

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

5

Examples 1 - 8 were tested in each of the above assays and were found to have a pIC50 in the range 6.3 – 7.2.

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#### **Measurement of LPS induced IL-6 secretion from whole blood**

Activation of monocytic cells by agonists of toll-like receptors such as bacterial lipopolysaccharide (LPS) results in production of key inflammatory mediators including IL-6. Such pathways are widely considered to be central to the pathophysiology of a range of auto-immune and inflammatory disorders.

15

Compounds to be tested are diluted to give a range of appropriate concentrations of which 1µl of the diluted stocks is added to a 96 well plate. Following addition of whole blood (130µl) the plates are incubated at 37 degrees (5% CO2) for 30 min before the addition of 10µl 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, 140µl of PBS are added to each well. The plates are sealed, shaken for 10 minutes and then centrifuged (2500rpm x 10 min). 100µl of the supernatant are removed and IL-6 levels assayed by immunoassay (typically by MesoScale Discovery technology) either immediately or following storage at -20 degrees. Concentration response curves for each compound was generated from the data and an IC50 value was calculated.

20

25

Examples 1, 2, 3, 5, 6, 7 and 8 were tested in the above assay and were found to have a pIC50 in the range 5.5 – 6.7.

30

These data demonstrate that bromodomain inhibitors tested in the above whole blood assays inhibited the production of key inflammatory mediator IL-6.

#### **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

35



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.

- 5 To test the compounds for use in the invention groups of eight Balb/c male mice are 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 is monitored at 4 days.

10 **Oncology Cell Growth Assay**

Human cell lines (n = 33 comprising 15 heme cell lines, 14 breast cell lines and 4 other cell lines) were cultured in RPMI-1640 containing 10% fetal bovine serum, 1000 viable cells per well were plated in 384-well black flat bottom polystyrene plates (Greiner #781086) in 48  $\mu$ l of culture media. All plates were placed at 5% CO<sub>2</sub>, 37°C overnight. The following day one plate was harvested with CellTiter-Glo (CTG, Promega #G7573) for a time equal to 0 (T0) measurement and compound (20 point titration from 14.7  $\mu$ M to 7 pM) was added to the remaining plates. The final concentration of DMSO in all wells was 0.15%. Cells were incubated for 72 hours or the indicated time and each plate was developed with CellTiter-Glo reagent using a volume equivalent to the cell culture volume in the wells. Plates were shaken for approximately 2 minutes and chemiluminescent signal was read on the Analyst GT (Molecular Devices) or EnVision Plate Reader (Perkin Elmer).

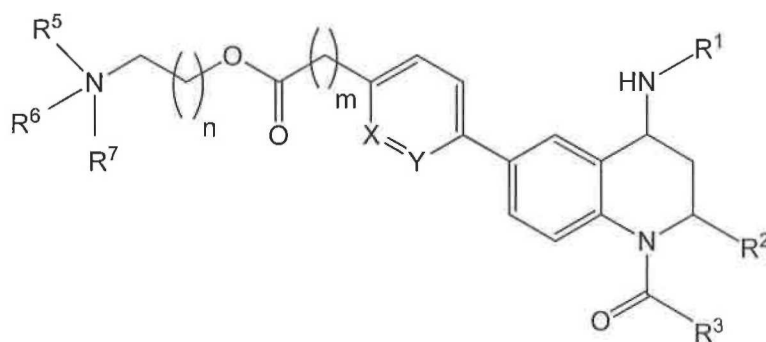
Results are expressed as a percent of the T0 and plotted against the compound concentration. The T0 value was normalized to 100% and represents the number of cells at time of compound addition and the concentration response data were fit with a 4 parameter curve fit using XLfit software (model 205). The concentration that inhibited cell growth by 50% (gIC<sub>50</sub>) is the midpoint of the 'growth window' (between the T0 and DMSO control). The Ymin - T0 value is determined by subtracting the T0 value (100%) from the Ymin value (%) determined from the fit of the concentration response curve. Values from the wells with no cells were subtracted from all samples for background correction.

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) or a salt thereof



(I)

in which

X and Y are independently CH or N provided that at least one of X and Y must be CH;

R¹ is a group C(O)OR⁴ in which R⁴ is C₁-₄alkyl or C₃-₇cycloalkyl; or

R¹ is a group selected from phenyl, pyridyl, pyrazinyl and pyrimidinyl said groups being optionally substituted by one or two substituents selected from halogen, C₁-₄alkyl and CN;

R² is C₁-₄alkyl;

R³ is C₁-₄alkyl;

R⁵ and R⁶ are independently C₁-₄alkyl; or

R⁵ and R⁶ combine together with the N to which they are attached form a 5 or 6 membered heterocyclyl;

R⁷ is absent or is C₁-₄alkyl;

m is 0, 1 or 2;

n is 1 or 2.

2. A compound or a salt thereof according to claim 1 in which X and Y are both CH.
3. A compound or a salt thereof according to claim 1 in which X is CH and Y is N.
4. A compound or a salt thereof according to any one of claims 1 – 3 in which R¹ is a group C(O)OR⁴ in which R⁴ is isopropyl.
5. A compound or a salt thereof according to any one of claims 1 – 3 in which R¹ is phenyl or pyridyl optionally substituted by one or two substituents selected from halogen, C₁-₄alkyl and CN.

6. A compound or a salt thereof according to claim 5 in which R<sup>1</sup> is 4-chlorophenyl or 5-cyanopyridin-2-yl.
7. A compound or a salt thereof according to any one of claims 1 - 6 in which R<sup>2</sup> is methyl.
8. A compound or a salt thereof according to any one of claims 1 - 7 in which R<sup>3</sup> is methyl.
9. A compound or salt thereof according to any one of claims 1 – 8 in which m is 0.
10. A compound or salt thereof according to any one of claims 1 – 9 in which R<sup>5</sup> and R<sup>6</sup> are both methyl.
11. A compound or a salt thereof according to any one of claims 1 – 10 in which R<sup>7</sup> is absent.
12. A compound or a salt thereof according to any one of claims 1 – 11 in which the compound of formula (I) is the (2S, 4R) enantiomer.
13. A compound according to claim 1 which is 2-(dimethylamino)ethyl 4-((2S,4R)-1-acetyl-4-((4-chlorophenyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate or a salt thereof.
14. A compound according to claim 1 which is selected from
  - 2-((4-((2S,4R)-1-Acetyl-4-((4-chlorophenyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoyl)oxy)-N,N,N-trimethylethanaminium;
  - 3-((4-((2S,4R)-1-Acetyl-4-((4-chlorophenyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoyl)oxy)-N,N,N-trimethylpropan-1-aminium;
  - 3-(Dimethylamino)propyl 4-((2S,4R)-1-acetyl-4-((4-chlorophenyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate;
  - 3-(Dimethylamino)propyl 6-((2S,4R)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)nicotinate;
  - 2-(Dimethylamino)ethyl 6-((2R,4R)-1-acetyl-4-((5-cyanopyridin-2-yl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)nicotinate;
  - 3-(Dimethylamino)propyl 4-((2S,4R)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate; and

2-(Dimethylamino)ethyl 4-((2*S*,4*R*)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)benzoate  
or a salt thereof.

15. A compound according to any one of claims 1 – 14 or a pharmaceutically acceptable salt thereof.

16. A pharmaceutical composition which comprises a compound or a pharmaceutically acceptable salt thereof as defined in claim 15 and one or more pharmaceutically acceptable carriers, diluents or excipients.

17. A combination pharmaceutical product comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof as defined in 15 together with one or more other therapeutically active agents.

18. A compound or a pharmaceutically acceptable salt thereof as defined in claim 15 for use in therapy.

19. A compound or a pharmaceutically acceptable salt thereof as defined in claim 15 for use in the treatment of a chronic autoimmune and/or inflammatory condition.

20. A compound or a pharmaceutically acceptable salt thereof according to claim 15 for use in the treatment of cancer.

21. The use of a compound or a pharmaceutically acceptable salt thereof according to claim 15 in the manufacture of a medicament for the treatment of a chronic autoimmune and/or inflammatory condition.

22. The use of a compound or a pharmaceutically acceptable salt thereof according to claim 15 in the manufacture of a medicament for the treatment of cancer.