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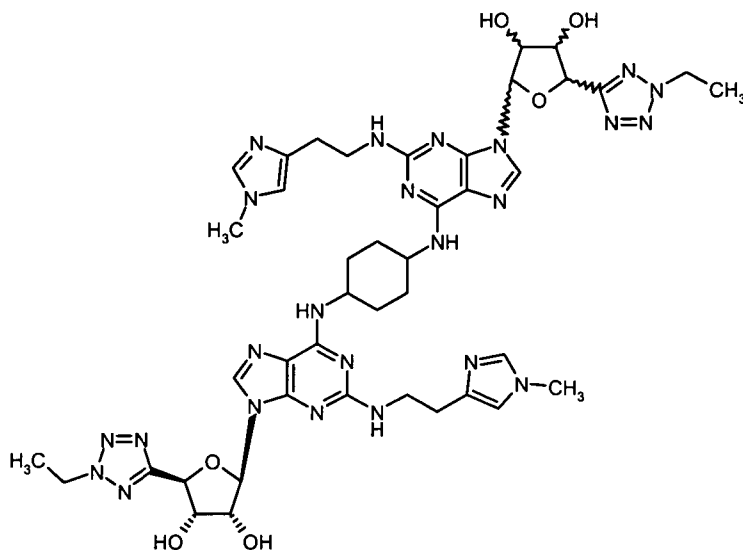
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(54) Title: NOVEL METHOD



(I)

(57) Abstract: The present invention provides a pharmaceutical dry powder composition comprising (i) a compound of formula (I); or a salt or solvate thereof in solid particulate form; (ii) one or more pharmaceutically acceptable particulate carriers; and (iii) one or more stabilising excipients of potential use in the treatment of inflammatory diseases such as asthma and chronic obstructive pulmonary disease.

Novel Method

This invention relates to novel pharmaceutical compositions comprising an adenosine A_{2A} agonist, a carrier and a stabilising excipient, methods of stabilising said agonist, processes for the preparation of said compositions, and to their use in therapy.

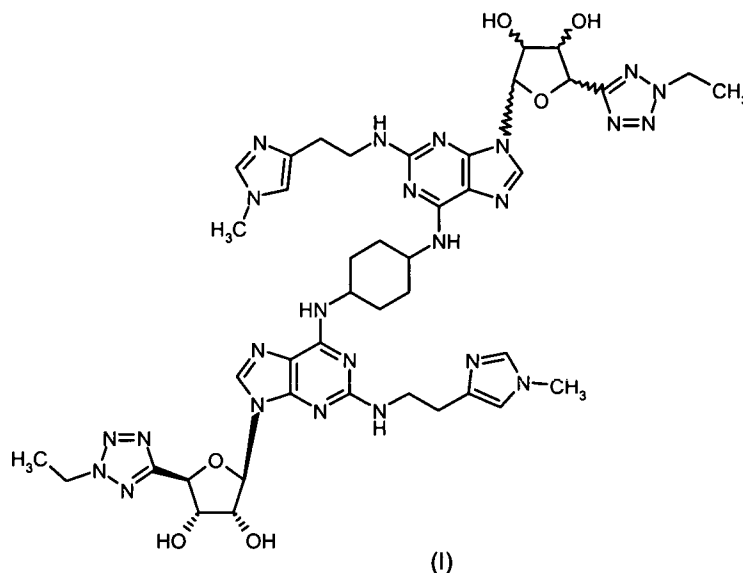
- 5 Inflammation is a primary response to tissue injury or microbial invasion and is characterised by leukocyte adhesion to the endothelium, diapedesis and activation within the tissue. Leukocyte activation can result in the generation of toxic oxygen species (such as superoxide anion), and the release of granule products (such as peroxidases and proteases). Circulating leukocytes include neutrophils, eosinophils, basophils, monocytes and lymphocytes.
- 10 Different forms of inflammation involve different types of infiltrating leukocytes, the particular profile being regulated by the profile of adhesion molecule, cytokine and chemotactic factor expression within the tissue.

- The primary function of leukocytes is to defend the host from invading organisms such as bacteria and parasites. Once a tissue is injured or infected a series of events occurs which
- 15 causes the local recruitment of leukocytes from the circulation into the affected tissue. Leukocyte recruitment is controlled to allow for the orderly destruction and phagocytosis of foreign or dead cells, followed by tissue repair and resolution of the inflammatory infiltrate. However, in chronic inflammatory states, recruitment is often inappropriate, resolution is not adequately controlled and the inflammatory reaction causes tissue destruction.

- 20 There is evidence from both *in vitro* and *in vivo* studies to suggest that compounds active at the adenosine A_{2A} receptor will have anti-inflammatory actions, for a review of the area see Cronstein BN *J. Appl. Physiol.* 1994 76:5-13. Studies on isolated neutrophils show an A₂ receptor-mediated inhibition of superoxide generation, degranulation, aggregation and adherence (Cronstein BN et al. *Trans. Assoc. Am. Physician* 1983 96:384-391; Cronstein BN
- 25 et al. *Ann. N.Y. Acad. Sci.* 1985 451:291-301; Burkey TH et al. *Biochim. Biophys. Acta* 1993 1175:312-318; Richter J J. *Leukocyte Biol.* 1992 51:270-275; Skubitz KM et al. *Blood* 1988 72:29-33). When agents selective for the A_{2A} receptor over the A_{2B} receptor (e.g. CGS21680) have been used, the profile of inhibition appears consistent with an action on the A_{2A} receptor subtype (Dianzani C et al. *Eur. J. Pharmacol.* 1994 263:223-226). Adenosine agonists may
- 30 also down-regulate other classes of leucocytes (Elliot KRF et al. *FEBS Lett.* 1989 254:94-98; Peachell PT et al. *Biochem. Pharmacol.* 1989 38:1717-1725). Studies on whole animals

have shown the anti-inflammatory effects of methotrexate to be mediated through adenosine and A₂ receptor activation (Asako H et al. *Gastroenterology* 1993 104:31-37; Cronstein BN et al. *J. Clin. Invest.* 1993 92:2675-2682; Cronstein BN et al. *Adv. Exp. Med. Biol.* 1994 370:411-416). Adenosine itself, and compounds that raise circulating levels of adenosine also show anti-inflammatory effects *in vivo* (Green PG et al. *Proc. Natl. Acad Sci.* 1991 88:4162-4165; Rosengren S et al. *J. Immunol.* 1995 154:5444-5451). In addition, raised levels of circulating adenosine in man (as a result of adenosine deaminase deficiency) results in immunosuppression (Hirschorn R *Pediatr. Res.* 1993 33:S35-S41).

International patent application PCT/EP2005/005651 describes compounds having the general formula (I):



which inhibit leukocyte recruitment and activation and which are potent agonists of the adenosine A_{2A} (hereinafter A_{2A}) receptor. The compounds are therefore expected to be of therapeutic benefit in providing protection from leukocyte-induced tissue damage in diseases where leukocytes are implicated at the site of inflammation. The compounds of formula (I) may also represent a safer alternative to corticosteroids in the treatment of inflammatory diseases, whose uses may be limited by their side-effect profiles.

Further, the compounds of formula (I) may show an improved profile over known A_{2A}-selective agonists in that they may possess one or more of the following properties:

- (i) approximately 100 fold more selective for A_{2A} over the human A₃ receptor;
- (ii) approximately 100 fold more selective for A_{2A} over the human A_{2B} receptor;
- (iii) approximately 100 fold more selective for A_{2A} over the human A₁ receptor;

- (iv) greater than approximately 90% binding to human serum albumin; and
- (v) less pronounced cardiovascular effects, in particular reduced tachycardia.

This profile can be considered of benefit as A_3 receptors are also found on leucocytes (e.g. eosinophils) and other inflammatory cells (e.g. mast cells) and activation of these receptors may have pro-inflammatory effects (Kohno Y et al. *Blood* 1996 88:3569-3574; Van Schaick EA et al. *Eur. J. Pharmacol.* 1996 308:311-314). It is even considered that the bronchoconstrictor effects of adenosine in asthmatics may be mediated via the adenosine A_3 receptor (Kohno Y et al. *Blood* 1996 88:3569-3574). A_{2B} receptors are also found on mast cells and may thus be implicated in mast cell activation. A_1 receptors have a wide tissue distribution and can be found on *inter alia* heart, adipocytes, respiratory smooth muscle, neutrophils, kidney, hippocampus and cortex. A_1 receptor activation may thus cause decreased lipolysis, diuresis and CNS activation (Fozard JR et al. *Curr. Opin. Invest. Drugs* 2002 3:69-77). A compound that exhibits greater than approximately 90% binding to human serum albumin, such as about 95% binding or more, may be expected to have an improved side effect profile, for example, such compounds may be expected to have less pronounced cardiac effects, such as tachycardia.

An important requirement of pharmaceutical formulations, such as formulations comprising a compound of formula (I), is that they should be stable on storage in a range of different conditions. It is known that drug substances can demonstrate instability to one or more of heat, light or moisture and various precautions must be taken in formulating and storing such substances to ensure that the pharmaceutical products remain in an acceptable condition for use over a reasonable period of time (i.e. such that they have an adequate shelf-life).

Instability of a drug substance may also arise from contact with one or more other components present in a formulation, for example a component present as an excipient. It is usual practice in the pharmaceutical art to formulate active ingredient substances with substances known as excipients which may be required as carriers, diluents, fillers, bulking agents, binders, lubricating agents etc. Such excipients are often used to give bulk to a pharmaceutical formulation where the active ingredient substance is present in very small quantities, and are generally chemically inert. Over prolonged storage times, or under conditions of extreme heat or humidity, and in the presence of other materials, such 'inert' substances can, however, undergo or participate in chemical degradation reactions.

Carrier substances that are commonly utilised in solid pharmaceutical formulations include reducing sugars, for example lactose, maltose and glucose. Lactose is particularly commonly used and is generally regarded as an inert excipient. However, it has been observed that certain active ingredient substances may undergo a chemical reaction in the presence of lactose and other reducing sugars. For example, it has been reported that fluoxetine hydrochloride (sold under the tradename Prozac®) undergoes degradation when present in solid tablets with a lactose excipient (Wirth DD et al. *J. Pharm. Sci.* 1998 87:31-39). The degradation was postulated to occur by formation of adducts via the Maillard reaction and a number of early Maillard reaction intermediates were identified. The authors concluded that drug substances which are secondary or primary amines may undergo the Maillard reaction with lactose under pharmaceutically relevant conditions.

Dry powder inhalers (DPIs) are well known devices for administering pharmaceutically active agents to the respiratory tract. Consequently, they are particularly suitable when used for the administration of active agents in the treatment of diseases such as asthma, bronchitis, chronic obstructive pulmonary disease (COPD), emphysema, rhinitis etc. Since the drug acts directly on the target organ much smaller quantities of the active ingredient may be used, thereby minimising any potential side effects.

Dry powder compositions for use as inhalable medicaments in dry powder inhalers (DPIs) typically comprise a pharmaceutically active agent intimately admixed with an excess of one or more pharmaceutically acceptable carriers. Such carriers serve not only to dilute the quantity of active agent administered in each dose but also to establish acceptable manufacture of the powder mixture and aid in the aerosolisation of the drug. Such a high proportion of carrier will essentially determine the properties of the powder formulation, particularly the manufacturing characteristics. A problem associated with the use of dry powder pharmaceutical compositions of this type is that they can be susceptible to poor stability performance due to moisture ingress. For example, significant deterioration in the fine particle fraction (FPF), namely that which has the potential to penetrate into the lower airways of the lung, is often observed upon protracted exposure of such compositions to conditions of elevated temperature and humidity.

In WO00/28979 (SkyePharma AG), for example, the presence of moisture was found to interfere with the physical interaction between a carrier and a drug substance and thus with

the effectiveness of drug delivery. Such interference with physical interactions between a carrier and a drug substance is distinct from chemical instability resulting from degradation.

WO00/28979 (SkyePharma AG) describes the use of magnesium stearate in dry powder formulations for inhalation to improve resistance to moisture and to reduce the effect of penetrating moisture on the fine particle fraction (FPF) of an inhaled formulation.

International patent application WO2005/004845 (Glaxo Group Limited) discloses the use of magnesium stearate to inhibit or reduce chemical reaction or degradation of an active ingredient substance in the presence of certain carriers.

International patent application WO2003/088943 (Glaxo Group Limited) discloses the enhanced physical stability (e.g. as manifested by increased fine particle fraction) of dry powder pharmaceutical compositions containing derivatised carbohydrates.

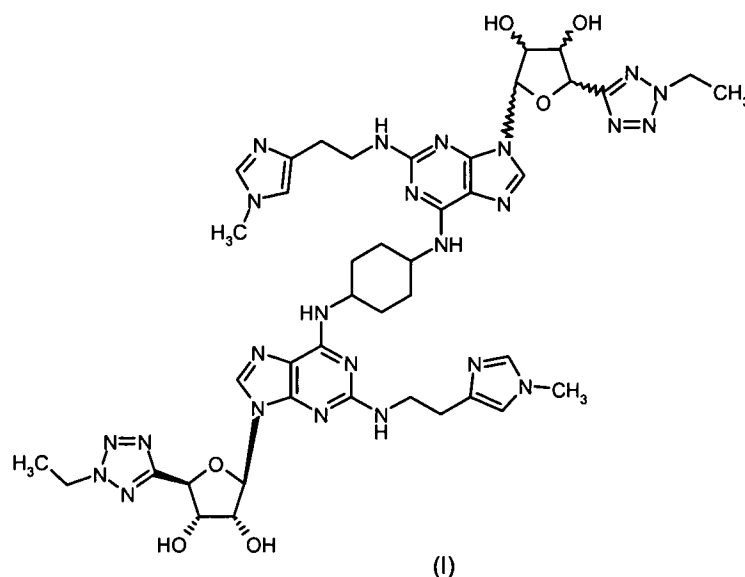
International patent application WO2005/004841 (Glaxo Group Limited) discloses the enhanced chemical stability of dry powder pharmaceutical compositions containing derivatised carbohydrates.

International patent application WO2005/004852 (Glaxo Group Limited) discloses the enhanced chemical stability of dry powder pharmaceutical compositions containing calcium stearate.

International patent application WO2005/041921 (Glaxo Group Limited) discloses the enhanced physical stability (e.g. as manifested by increased fine particle fraction) of dry powder pharmaceutical compositions containing calcium stearate.

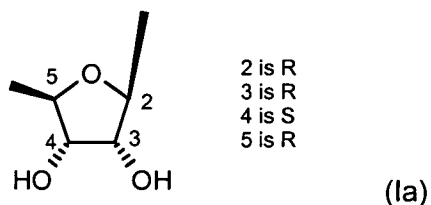
The present invention relates to the finding by the inventors that dry powder compositions comprising a compound of formula (I), and salts or solvates thereof, may be stabilised by the addition of certain excipients. Such stabilising excipients are beneficial in that they may increase the physical and/or chemical stability of the composition.

In a first aspect of the present invention there is provided a pharmaceutical dry powder composition comprising (i) a compound of formula (I):



or a salt or solvate thereof in solid particulate form; (ii) one or more pharmaceutically acceptable particulate carriers; and (iii) one or more stabilising excipients ("compositions of the invention").

- 5 Compounds of formula (I) require absolute stereochemistry about one of the tetrahydrofuran rings such that the stereochemistry about each stereocentre in the tetrahydrofuran ring is as follows:



- 10 However, the stereochemistry about each stereocentre in the other tetrahydrofuran ring need not be fixed. Within this requirement the invention encompasses all stereoisomers of the compounds of formula (I) (i.e. diastereoisomers), whether as individual stereoisomers isolated such as to be substantially free of the other stereoisomer (i.e. pure) or as mixtures thereof. An individual stereoisomer isolated such as to be substantially free of the other stereoisomer (i.e. pure) will be isolated such that less than about 10%, for example less than
- 15 about 1% or less than about 0.1% of the other stereoisomer is present.

Compounds of formula (I) in which the stereochemistry about both of the tetrahydrofuran rings is such that each stereocentre in the tetrahydrofuran rings is fixed as shown above in formula (Ia) are preferred (i.e. the stereochemistry of both tetrahydrofuran rings is the same).

Also included within the scope of formula (I) are all geometric isomers of the compound,
5 whether as individual isomers or mixtures thereof, for example, the compound of formula (I) in the *trans* or *cis* configurations about the cyclohexyl ring, in particular the *trans* configuration.

The use of salts of the compounds of formula (I) is also encompassed within the scope of the present invention. Because of their potential use in medicine, the salts of the compound of formula (I) are preferably pharmaceutically acceptable salts. Pharmaceutically acceptable
10 salts can include acid addition salts. 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, formic, sulfuric, nitric, phosphoric, succinic, maleic, terephthalic, phthalic, acetic, fumaric, citric, tartaric, benzoic, *p*-toluenesulfonic, methanesulfonic or naphthalenesulfonic acid), optionally in a suitable solvent such as an
15 organic solvent, to give the salt which is usually isolated for example by crystallisation and filtration. Thus, a pharmaceutically acceptable acid addition salt of a compound of formula (I) can be for example a hydrobromide, hydrochloride, formate, sulfate, nitrate, phosphate, succinate, maleate, phthalate, terephthalate, acetate, fumarate, citrate, tartrate, benzoate, *p*-toluenesulfonate, methanesulfonate or naphthalenesulfonate salt. All possible stoichiometric
20 and non-stoichiometric forms of the salts of the compounds of formula (I) are envisaged in the present invention.

In one embodiment of the invention the compound of formula (I) is in the form of the free base. In a second embodiment of the invention the compound of formula (I) is in the form of the mono maleate salt. In a third embodiment of the invention the compound of formula (I) is
25 in the form of the mono biphenylsulfonate salt. In a fourth embodiment of the invention the compound of formula (I) is in the form of the mono camphorsulfonate salt. In a fifth embodiment of the invention the compound of formula (I) is in the form of the mono terephthalate salt.

Also included within the scope of the invention are all solvates (e.g. hydrates), complexes and
30 polymorphic forms of the compounds of formula (I) and salts thereof.

In one embodiment of the present invention the compounds of formula (I) and salts thereof are provided in solvated form, for example as a hydrate (e.g. the hydrate of the mono maleate salt). In a further embodiment of the invention the compounds of formula (I) and salts thereof are unsolvated.

- 5 The compound of formula (I), or salt or solvate thereof, will be in the form of a particulate solid. Since the stabilised compositions according to the present invention have particular application in pharmaceutical formulations for administration by inhalation, in such applications the compound of formula (I), or salt or solvate thereof, will be in the form of a particulate solid which is suitable to be administered by inhalation.
- 10 In the field of inhalation therapy, the term "suitable to be administered by inhalation" for a therapeutic molecule is generally taken to mean particles having a mass median aerodynamic diameter (MMAD) in the range of 0.1-10 μm , and more particularly 1-5 μm . Particles of the desired size for inhalation are conventionally prepared by micronisation. Other methods of producing such particles are also known in the art. Therefore, such
- 15 particles can also be prepared using controlled precipitation methods (e.g. methods described in patent applications WO00/38811 and WO01/32125 (Glaxo Group Limited)), using supercritical fluid methodology or by spray drying techniques. The present invention provides no limitation on the method by which the compound of formula (I), or salt or solvate thereof, is made suitable to be administered by inhalation.
- 20 Aerodynamic diameter is the diameter of a unit-density sphere having the same terminal settling velocity as the particle in question. Mass median aerodynamic diameter (MMAD) is the geometric mean aerodynamic diameter (i.e. fifty per cent of the particles by weight will be smaller than the MMAD and fifty per cent of the particles by weight will be larger). Aerodynamic diameter may be measured by a range of methods known to those skilled in
- 25 the art (e.g. laser light diffraction techniques).

The compound of formula (I), or a salt or solvate thereof, is typically present in an amount of from 0.01% to 10% w/w based on the total weight of the composition, preferably from 0.02% to 10% w/w, particularly 0.03 to 5% w/w (such as 0.05% to 1% w/w, for example 0.1% w/w). The compound of formula (I) or a salt or solvate thereof will typically be formulated with a

30 view to a unit dose in the range 10-1000 μg , in particular 50-500 μg (for example 50-200 μg).

In compositions of the invention, the carrier may be composed of particles of any pharmacologically inert material or combination of materials which is/are suitable for inhalation. Preferred carriers include mono-saccharides, such as mannitol, arabinose, xylitol and dextrose and monohydrates thereof, disaccharides, such as lactose, maltose and sucrose, and polysaccharides such as starches, dextrans (e.g. maltodextrin) or dextrans. More preferred carriers comprise particulate crystalline sugars such as glucose, fructose, mannitol, sucrose and lactose, particularly lactose. Especially preferred carriers are anhydrous lactose and lactose monohydrate.

Generally, the particle size of the carrier particles will be greater than that of the inhaled active agent and as a result, do not penetrate into the respiratory tract. Thus, carrier particles for inhalable compositions may typically have particle sizes (MMAD) greater than 20 μm , more preferably in the range 20-150 μm . If desired, the inhalable compositions may also contain two or more carrier particle size ranges. For example, in order to control the proportion of inhaled medicament, while retaining a good accuracy for metering, it is often desirable to use one component of the carrier that has a particle size of less than 15 μm (the fine carrier component) and another component of the carrier that has a particle size of greater than 20 μm but lower than 150 μm , preferably lower than 80 μm (the coarse carrier component). The carrier may be commercially available in the desired particle size range or may be separated by air classification, sieving or any other method of size classification known in the art. Preferably the weight ratio of the fine and coarser carrier components will range from 1:99 to 50:50. Fine and coarse additional carrier components may consist of chemically identical or chemically different substances. The additional carrier mixtures may, for example, contain one chemical substance as the fine carrier and a different substance as the coarser carrier. However, the fine and coarser carriers in question may themselves constitute mixtures of different substances. Preferably the fine and coarser carriers will both be lactose.

The proportion of carrier material to be used in the inhalable dry powder formulations of this invention may vary depending upon the powder inhaler for administration etc. The proportion may, for example, be about 75% to 99.8% by weight of the composition as a whole.

It will be appreciated that such inhalable dry powder formulations may also contain minor amounts of other additives e.g. taste masking agents or sweeteners. Where such additives

are present, they will generally not exceed 10% by weight of the total weight of the composition.

Generally the stabilising excipient will be in particulate form.

5 The stabilising excipient may suitably be a stearate (for example calcium stearate or magnesium stearate) or alternatively may be a derivatised carbohydrate (for example celobiose octaacetate). In one embodiment of the invention the stabilising excipient is magnesium stearate. In a second embodiment of the invention the stabilising excipient is calcium stearate. In a third embodiment of the invention the stabilising excipient is celobiose octaacetate. Combinations of stabilising excipients are envisaged, but preferably a single
10 stabilising excipient will be used.

Where the stabilising excipient is magnesium stearate or calcium stearate, the optimal amount of magnesium stearate or calcium stearate present in a particular composition varies depending on the sizes of the various particles and other factors. In general, magnesium stearate or calcium stearate is preferably present in an amount of from about 0.01 to 20%
15 w/w based on the total weight of the composition. More preferably the magnesium stearate or calcium stearate is present in an amount of from 0.05 to 10% w/w based on the total weight of the composition. In certain embodiments of the invention the magnesium stearate or calcium stearate is present in an amount of from 0.3 to 6% w/w, for example from 0.5 to 4% w/w.

20 Due to their physical properties (waxy and poorly flowing), micronisation of stearates is generally not desirable and is not a required feature of the present invention. Typical stearate particle sizes will be in the range of 1-20 μm .

The term 'derivatised carbohydrates' is used herein to describe a class of carbohydrate molecules in which at least one hydroxyl group of the carbohydrate group is substituted with a
25 hydrophobic moiety via either ester or ether linkages. All isomers (both pure and mixtures thereof) are included within the scope of this term. Mixtures of chemically distinct derivatised carbohydrates may also be utilised. Suitably, the hydroxyl groups of the carbohydrate may be substituted by a straight or branched hydrocarbon chain comprising up to 20 carbon atoms, more typically up to 6 carbon atoms. The derivatised carbohydrates can be formed by
30 derivatisation of monosaccharides (e.g. mannitol, fructose and glucose) or of disaccharides

(e.g. maltose, trehalose, cellobiose, lactose and sucrose). Derivatised carbohydrates are either commercially available or can be prepared according to procedures readily apparent to those skilled in the art. Non-limiting examples of derivatised carbohydrates include cellobiose octaacetate, sucrose octaacetate, lactose octaacetate, glucose pentaacetate, mannitol
5 hexaacetate and trehalose octaacetate. Further suitable examples include those specifically disclosed in patent application WO99/33853 (Quadrant Holdings), particularly trehalose diisobutyrate hexaacetate. A particularly preferred derivatised carbohydrate is cellobiose octaacetate, most preferably α -D-cellobiose octaacetate.

10 A range of derivatised carbohydrates are commercially available, for example cellobiose octaacetate is available from Borregaard Synthesis, Norway. Other derivatised carbohydrates may be prepared by conventional means known to those skilled in the art.

Where the stabilising excipient is a derivatised carbohydrate in particulate form, typically, the MMAD of the derivatised carbohydrates will be between 0.1-50 μ m, particularly 0.1-20 μ m, especially 0.5-10 μ m and preferably 1-3 μ m. Derivatised carbohydrates for use in the
15 preparation of compositions in accordance with the present invention are typically micronised, although controlled precipitation, supercritical fluid methodology and spray drying techniques familiar to those skilled in the art may also be utilised. Suitably the derivatised carbohydrate is present in a concentration of 0.01-50% by weight of the total composition, particularly 0.1-25%, especially 0.25-15% (for example, 0.25-4% or 4-10%). The derivatised carbohydrate
20 may be in crystalline or amorphous form, preferably crystalline form.

Compositions of the invention containing a stabilising excipient may be expected to be of particular application in formulations which comprise a carrier which is a reducing sugar, for example lactose (for example lactose monohydrate or lactose anhydrate), maltose, maltodextrin or glucose (for example glucose monohydrate or glucose anhydrate).

25 It is to be understood that the compositions according to the present invention include not only those in which the components (including the stabilising excipient(s)) are incorporated as individual particles but also those including matrix particles of more than one component. For example, matrix particles of a compound of formula (I), or a salt or solvate thereof, and stabilising excipient or, where appropriate, matrix particles of stabilising excipient and another
30 carrier or further excipient as may be present can be utilised. Such matrix particles can be prepared by solid dispersion technology e.g. co-precipitation and particle coating methods

which are familiar to those skilled in the art. Suitably, the components are incorporated as individual particles. Thus, in one embodiment the stabilising excipient(s) together with the carrier (e.g. lactose) forms a matrix particle. In another embodiment the stabilising excipient(s) together with an active ingredient (such as the compound of formula (I), salt or solvate thereof) forms a matrix particle.

By the term 'stabilise' in connection with 'stabilising excipient' is meant to improve chemical and/or physical stability (i.e. to reduce or to inhibit chemical and/or physical degradation and/or deterioration of physical properties) of the compound of formula (I), or a salt or solvate thereof. In one embodiment of the invention the term 'stabilise' refers to an improvement in physical stability (i.e. reduction or inhibition of degradation of physical properties) of the compound of formula (I), or a salt or solvate thereof. In another embodiment of the invention the term 'stabilise' refers to an improvement in chemical stability (i.e. reduction or inhibition of chemical degradation) of the compound of formula (I), or a salt or solvate thereof. In another embodiment of the invention the term 'stabilise' refers to an improvement in physical stability (i.e. reduction or inhibition of degradation of physical properties) and chemical stability (i.e. reduction or inhibition of chemical degradation) of the compound of formula (I), or a salt or solvate thereof.

Improvement in physical stability may include, in particular, stabilisation of the fine particle fraction of a composition when this has a tendency to reduce with age or under exposure to moisture.

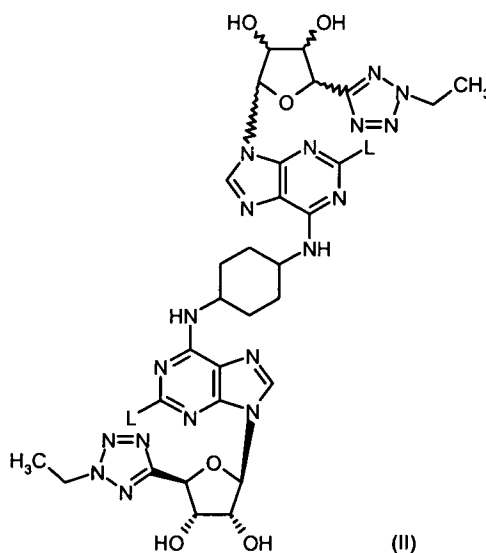
Improvement in chemical stability may include, in particular, reduction in the generation of any impurities caused by reaction of the compound of formula (I) with any of the carriers through the Maillard reaction.

To determine the improved physical stability associated with compositions prepared according to this invention, the blends thus formed can be placed on accelerated stability screen (e.g. 40°C / 75% relative humidity) and the fine particle fraction reduction (i.e. comparison of pre and post stability FPF data) measured as an analytical parameter using a Next Generation Impactor (NGI), Anderson Cascade Impactor (CI) or Twin Stage Impinger (TSI), for example an Anderson Cascade Impactor (CI) or Twin Stage Impinger (TSI).

Using an Andersen Cascade Impactor the fine particle mass may be defined as the mass of drug (i.e. compound of formula (I)) which is captured in certain stages of the instrument (for example stages 1-5 cover particles in the range 1.1-9 μm , while stages 3-5 cover particles in the range 1.1-4.7 μm). The fine particle fraction is the mass of drug which is captured in stages 1-5 (or 3-5 as appropriate) as a fraction of the total mass of drug which is captured. Such procedures are familiar to those skilled in the art.

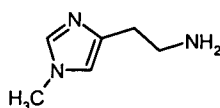
To determine the improved chemical stability associated with compositions prepared according to this invention, the blends thus formed can be placed on accelerated stability screen (e.g. 40°C / 75% relative humidity) and the chemical composition, in particular the generation of impurities, monitored using a conventional technique such as HPLC. Such procedures are familiar to those skilled in the art.

The compounds of formula (I), or protected derivatives thereof, may be prepared according to a first process (A) by reacting a compound of formula (II):



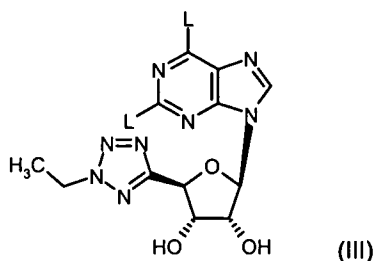
wherein L represents a leaving group, for example halogen (particularly chlorine), or a protected derivative thereof

with [2-(1-methyl-1H-imidazol-4-yl)ethyl]amine:



Said reaction will generally involve heating the reagents to a temperature of 50°C to 150°C, such as 100°C to 130°C (particularly about 110°C to 120°C) in the presence of an inert solvent such as DMSO. Alternatively, the reaction can be performed at a lower temperature, for example at approximately 100°C, for an extended period such as 18 to 24 hours.

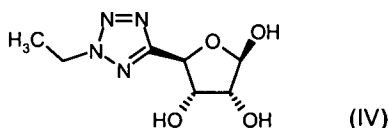
- 5 A compound of formula (II), or a protected derivative thereof, may be prepared by reacting a compound of formula (III):



wherein L represents a leaving group as defined above, or a protected derivative thereof

- 10 with 1,4-diaminocyclohexane, such as *trans*-1,4-diaminocyclohexane. This reaction will generally be performed in the presence of a base, such as an amine base (e.g. diisopropylethylamine), in a suitable solvent, such as an alcohol (e.g. isopropanol), at an elevated temperature (e.g. 50°C to 60°C).

- 15 A compound of formula (III), or a protected derivative thereof, and methods for its preparation are disclosed in WO98/28319 (Intermediate 7 therein). Briefly, a compound of formula (III) may be prepared by reacting a compound of formula (IV):

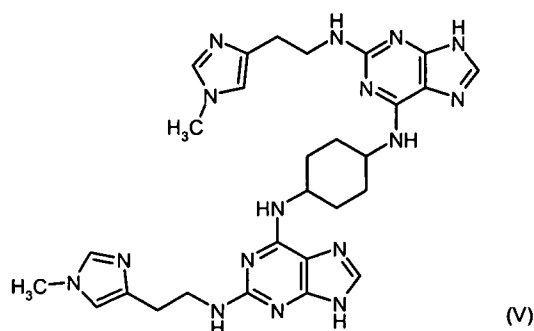


or a protected derivative thereof, with an agent such as 2,6-dichloropurine in a suitable solvent under inert conditions in the presence of a Lewis acid, such as trimethylsilyl triflate.

- 20 Compounds of formula (IV) may be prepared by the methods disclosed in WO98/28319 (Intermediate 6 therein) or by analogous methods.

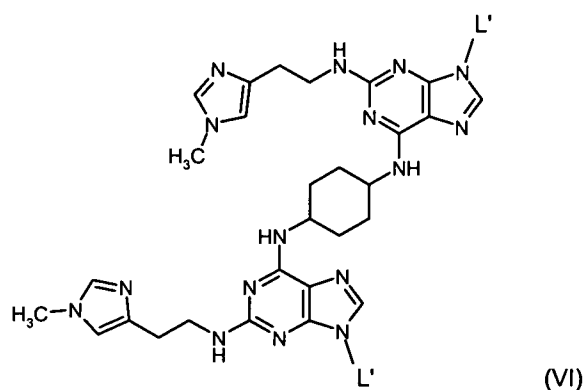
Compounds of formula (II), (III) and (IV) may be used in a form in which the hydroxyl groups are protected with suitable protecting groups, e.g. with acetonide or acetyl groups, particularly acetyl groups.

- 5 According to a second process (B) a compound of formula (I), or a protected derivative thereof, may be prepared by reacting a compound of formula (V):



with a compound of formula (IV) as defined above, or a protected derivative thereof. This reaction may be carried out in the presence of a hindered base, such as DBU, and a Lewis acid, such as trimethylsilyl triflate.

- 10 A compound of formula (V) may be prepared by deprotecting a compound of formula (VI)

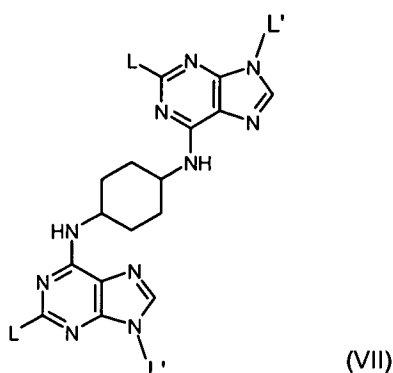


wherein L' is a suitable amine protecting group, such as 2-tetrahydropyran.

Deprotection may typically be achieved by acid hydrolysis with a suitable acid, such as HCl, at ambient temperature.

- 15 A compound of formula (VI) may be prepared by reacting a compound of formula (VII):

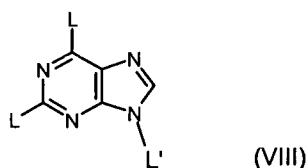
16



wherein L and L' are as defined above

with 2-(1-methyl-1*H*-imidazolyl-4-yl)ethyl amine. Said reaction will generally involve heating the reagents to a temperature of 50°C to 150°C, such as 100°C to 130°C (particularly about 110°C to 120°C), in the presence of an inert solvent, such as DMSO or ethylene glycol. An external base, such as dipotassium hydrogen phosphate, can also be used to enhance the reactivity.

A compound of formula (VII) may be prepared by reacting a compound of formula (VIII):



wherein L and L' are leaving groups as defined above

with 1,4-diaminocyclohexane, such as *trans*-1,4-diaminocyclohexane. This reaction will generally be performed in the presence of a base, such as an amine base (e.g. diisopropyl ethylamine), in a suitable solvent, such as an alcohol (e.g. isopropanol or n-butanol), at an elevated temperature (e.g. 60°C to 80°C).

Compounds of formula (VIII) may be prepared according to the methods described in WO03/080613 (Intermediate 1 therein) or by analogous methods.

Compounds of formula (I) may further be prepared according to a third process (C) by deprotecting a protected derivative of a compound of formula (I), for example where the hydroxyl groups on the sugar moiety are protected by acetyl groups.

As described above, protected derivatives of compounds of formula (I) or intermediates for preparing compounds of formula (I) may be used. Examples of protecting groups and the means for their removal can be found in TW Greene and PGM Wuts "Protective Groups in Organic Synthesis" (J Wiley and Sons, 1991). Suitable hydroxyl protecting groups include
5 alkyl (e.g. methyl), acetal (e.g. acetonide) and acyl (e.g. acetyl or benzoyl) which may be removed by hydrolysis, and arylalkyl (e.g. benzyl) which may be removed by catalytic hydrogenolysis. Suitable amine protecting groups include sulphonyl (e.g. tosyl), acyl e.g. benzyloxycarbonyl or t-butoxycarbonyl) and arylalkyl (e.g. benzyl) which may be removed by hydrolysis or hydrogenolysis as appropriate.

- 10 The potential for compounds of formula (I) and salts or solvates thereof to inhibit leukocyte function may be demonstrated, for example, by their ability to inhibit superoxide (O_2^-) generation from neutrophils stimulated with chemoattractants such as N-formylmethionyl-leucyl-phenylalanine (fMLP). Accordingly, compounds of formula (I) may be of potential therapeutic benefit in providing protection from leukocyte-induced tissue damage in diseases
15 where leukocytes are implicated at the site of inflammation.

Examples of disease states in which compounds that inhibit leukocyte function may have potentially beneficial anti-inflammatory effects include diseases of the respiratory tract such as adult respiratory distress syndrome (ARDS), bronchitis (including chronic bronchitis), cystic fibrosis, asthma (including allergen-induced asthmatic reactions), emphysema, rhinitis
20 and septic shock. Other relevant disease states include diseases of the gastrointestinal tract, such as intestinal inflammatory diseases, including inflammatory bowel disease (e.g. Crohn's disease or ulcerative colitis), *Helicobacter pylori* induced gastritis and intestinal inflammatory diseases secondary to radiation exposure or allergen exposure, and non-steroidal anti-inflammatory drug-induced gastropathy. Further diseases may include skin diseases such as
25 psoriasis, allergic dermatitis and hypersensitivity reactions and diseases of the central nervous system which have an inflammatory component e.g. Alzheimer's disease and multiple sclerosis.

Further examples of disease states in which such compounds may have potentially beneficial effects include cardiac conditions such as peripheral vascular disease, post-ischaemic
30 reperfusion injury and idiopathic hypereosinophilic syndrome.

Yet further, compounds which inhibit lymphocyte function may be useful as immunosuppressive agents and so have use in the treatment of auto-immune diseases such as rheumatoid arthritis and diabetes, and may be useful in inhibiting metastasis.

5 It will be appreciated by those skilled in the art that reference herein to treatment extends to prophylaxis as well as the treatment of established conditions.

Of particular interest is the treatment and/or prophylaxis of asthma, chronic pulmonary obstructive disease (COPD), chronic bronchitis and emphysema in a mammal (e.g. human) especially asthma and COPD.

10 As mentioned above, compositions of the invention may be useful in human or veterinary medicine, in particular as anti-inflammatory agents.

15 In another aspect of the present invention there is provided a pharmaceutical dry powder composition comprising (i) a compound of formula (I), or a salt or solvate thereof in solid particulate form, (ii) one or more pharmaceutically acceptable particulate carriers; and (iii) one or more stabilising excipients for use in human or veterinary medicine, particularly in the treatment of patients with inflammatory conditions who are susceptible to leukocyte-induced tissue damage.

20 In another aspect of the present invention there is provided the use of a pharmaceutical dry powder composition comprising (i) a compound of formula (I), or a salt or solvate thereof in solid particulate form, (ii) one or more pharmaceutically acceptable particulate carriers; and (iii) one or more stabilising excipients in the manufacture of a medicament for the treatment of patients with inflammatory conditions who are susceptible to leukocyte-induced tissue damage.

25 In another aspect of the present invention there is provided a method for the treatment of a human or animal subject with an inflammatory condition and/or allergic condition who is susceptible to leukocyte-induced tissue damage, which method comprises administering to said human or animal subject a safe and effective amount of a pharmaceutical dry powder composition comprising (i) a compound of formula (I), or a salt or solvate thereof in solid particulate form, (ii) one or more pharmaceutically acceptable particulate carriers; and (iii) one or more stabilising excipients.

In another aspect there is provided a method of stabilising a pharmaceutical dry powder composition comprising (i) a compound of formula (I), or a salt or solvate thereof, and (ii) one or more pharmaceutically acceptable particulate carriers, by the addition of one or more stabilising excipients.

5

In another aspect of the present invention there is provided the use of a stabilising excipient to stabilise a pharmaceutical dry powder composition comprising (i) a compound of formula (I), or a salt or solvate thereof and (ii) one or more pharmaceutically acceptable particulate carriers.

- 10 Pharmaceutical dry powder compositions comprising (i) a compound of formula (I), or a salt or solvate thereof in solid particulate form, (ii) one or more pharmaceutically acceptable particulate carriers; and (iii) one or more stabilising excipients may be administered, by topical delivery to the lung or nose. Routes of administration of particular interest include inhaled and intra-nasal. Inhaled administration involves topical administration to the lung,
- 15 typically as a dry powder composition.

Preferably the composition of the present invention is formulated for topical administration to the lung by inhalation.

- Preferred embodiments recited in respect of an element or feature in one particular aspect of the invention will be understood to apply equally to the same element or feature in respect of
- 20 other aspects of the invention.

- In another aspect there is provided a process for the preparation of a pharmaceutical dry powder composition comprising (i) a compound of formula (I), or a salt or solvate thereof in solid particulate form, (ii) one or more pharmaceutically acceptable particulate carriers; and (iii) one or more stabilising excipients which process comprises intimately mixing a compound
- 25 of formula (I), or a salt or solvate thereof, and one or more pharmaceutically acceptable carriers and one or more stabilising excipients.

- The dry powder pharmaceutical formulations in accordance with this invention can be prepared using standard methods. For example, the pharmaceutically active agent, stabilising excipient and additional excipients can be intimately mixed using any suitable
- 30 blending apparatus, such as high shear blenders. The particular components of the

formulation can generally be admixed in any order. Pre-mixing of particular components may be found to be advantageous in certain circumstances. The progress of the blending process can be monitored by carrying out content uniformity determinations. For example, the blending apparatus may be stopped, materials removed using a sample thief and then
5 analysed for homogeneity by High Performance Liquid Chromatography (HPLC).

According to the invention, the inhalable dry powder formulations can be delivered by any suitable inhalation device that is adapted to administer a controlled amount of such a pharmaceutical formulation to a patient. Suitable inhalation devices may rely upon the aerosolisation energy of the patient's own breath to expel and disperse the dry powder dose.
10 Alternatively, this energy may be provided by an energy source independent of the patient's inhalation effort, such as by impellers, patient/device created pressurised gas sources or physically (e.g. compressed gas) or chemically stored energy sources. Suitable inhalation devices can also be of the reservoir type i.e. where the dose is withdrawn from a storage vessel using a suitably designed dosing device or alternatively, inhalation devices that
15 release drug from pre-metered units e.g. blisters, cartridges or capsules.

Packaging of the inhalable dry powder formulation may be suitable for unit dose or multi-dose delivery. In the case of multi-dose delivery, the dry powder formulation can be pre-metered (e.g. Diskhaler® as described in US4811731 and US5035237) or metered in use (e.g. Turbuhaler® as described in US4668218). An example of a unit-dose device is Rotahaler®
20 (as described in US4353365).

Optionally the dry powder inhalable pharmaceutical formulations can be incorporated into a plurality of sealed dose containers (e.g. containing the dry powder formulation) mounted longitudinally in a strip or ribbon inside a suitable inhalation device. The container is rupturable or peel-openable on demand and the dose of e.g. the dry powder formulation can
25 be administered by inhalation via a device such as the Diskus® device, marketed by GlaxoSmithKline. The Diskus® inhalation device, for example, has at least one container for the pharmaceutical composition in powder form (the container or containers preferably being a plurality of sealed dose containers mounted longitudinally in a strip or ribbon) which is defined between two members peelably secured to one another; the device comprises: a
30 means of defining an opening station for the said container or containers; a means for peeling the members apart at the opening station to open the container; and an outlet, communicating with the opened container, through which a user can inhale the

pharmaceutical composition in powder form from the opened container. A particularly preferred inhalation device for dry powder pharmaceutical formulations of this invention is the Diskus® inhaler (described in US patents 5590645 and 5860149) which may be charged with blister (medicament) packs as described in US5873360. The drawings of said United States patents are specifically incorporated by reference.

In another aspect of the present invention there is provided an dry powder inhaler comprising a pharmaceutical formulation according to the present invention. A dry powder inhaler according to the present invention will typically comprise (or be adapted to receive) one or more reservoirs containing of the inventive composition in a dry powder formulation. The inhaler may provide (or be adapted to receive) one or more pre-metered doses or may provide for metering in use.

The dry powder inhaler, or at least the reservoir therefore, may suitably be overwrapped to resist moisture ingress during storage. The overwrap may optionally include a desiccant material.

The pharmaceutical compositions according to the invention may be used in combination with or include one or more other therapeutic agents, for example selected from anti-inflammatory agents, anticholinergic agents (particularly an M_1 , M_2 , M_1/M_2 or M_3 receptor antagonist), β_2 -adrenoreceptor agonists, antiinfective agents (e.g. antibiotics, antivirals), or antihistamines. The invention thus provides, in a further aspect, (a) a pharmaceutical composition of the invention combination comprising and (b) one or more other therapeutically active agents, for example selected from an anti-inflammatory agent (for example a corticosteroid or an NSAID), an anticholinergic agent, a β_2 -adrenoreceptor agonist, an antiinfective agent (e.g. an antibiotic or an antiviral), or an antihistamine. Particular combinations include a composition of the invention with a steroid, a β_2 -adrenoreceptor agonist, an anticholinergic, and/or a PDE-4 inhibitor. Preferred combinations are those comprising one or two other therapeutic agents.

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

The invention thus provides, in a further aspect, a pharmaceutical composition according to the invention further comprising one or more other therapeutically active agents, for example, a β_2 -adrenoreceptor agonist, an anti-histamine, an anti-allergic agent, an anti-inflammatory agent (including a steroid or a PDE-4 inhibitor), an anticholinergic agent or an antiinfective agent (e.g. antibiotics or antivirals). Further therapeutically active agents are suitably provided in particulate (e.g. micronised) form.

Examples of β_2 -adrenoreceptor agonists include salmeterol (which may be a racemate or a single enantiomer, such as the *R*-enantiomer), salbutamol, formoterol, salmefamol, fenoterol or terbutaline and salts thereof, for example the xinafoate salt of salmeterol, the sulphate salt or free base of salbutamol or the fumarate salt of formoterol. Long-acting β_2 -adrenoreceptor agonists such as salmeterol or formoterol may be preferred.

Other long acting β_2 -adrenoreceptor agonists include those described in WO02/66422A, WO02/270490, WO02/076933, WO03/024439, WO03/072539, WO 03/091204, WO04/016578, WO04/022547, WO04/037807, WO04/037773, WO04/037768, WO04/039762, WO04/039766, WO01/42193 and WO03/042160.

Particular long-acting β_2 -adrenoreceptor agonists are:

3-(4-{{6-(((2R)-2-hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl)amino)hexyl}oxy}butyl)benzenesulfonamide;

3-(3-{{7-(((2R)-2-hydroxy-2-[4-hydroxy-3-hydroxymethyl)phenyl]ethyl)-amino)heptyl}oxy}propyl)benzenesulfonamide;

4-{{(1R)-2-[(6-{2-[(2,6-dichlorobenzyl)oxy]ethoxy}hexyl)amino]-1-hydroxyethyl}-2-(hydroxymethyl)phenol};

4-{{(1R)-2-[(6-{4-[3-(cyclopentylsulfonyl)phenyl]butoxy}hexyl)amino]-1-hydroxyethyl}-2-(hydroxymethyl)phenol};

N-[2-hydroxyl-5-[(1R)-1-hydroxy-2-[[2-4-[(2R)-2-hydroxy-2-phenylethyl]amino]phenyl]ethyl]amino]ethyl]phenyl]formamide, and

N-2{2-[4-(3-phenyl-4-methoxyphenyl)aminophenyl]ethyl}-2-hydroxy-2-(8-hydroxy-2(1*H*)-quinolinon-5-yl)ethylamine.

Anti-inflammatory agents that may be incorporated in a combination include corticosteroids particularly inhaled corticosteroids and their pro-drugs which have anti-inflammatory activity. Examples of corticosteroids include methyl prednisolone, prednisolone, dexamethasone,

fluticasone propionate, 6 α ,9 α -difluoro-17 α -[(2-furanylcarbonyl)oxy]-11 β -hydroxy-16 α -methyl-3-oxo-androsta-1,4-diene-17 β -carbothioic acid S-fluoromethyl ester, 6 α ,9 α -difluoro-11 β -hydroxy-16 α -methyl-3-oxo-17 α -propionyloxy-androsta-1,4-diene-17 β -carbothioic acid S-(2-oxo-tetrahydro-furan-3S-yl) ester, 6 α ,9 α -difluoro-11 β -hydroxy-16 α -methyl-17 α -(1-methylcyclopropylcarbonyl)oxy-3-oxo-androsta-1,4-diene-17 β -carbothioic acid S-fluoromethyl ester, 6 α ,9 α -difluoro-11 β -hydroxy-16 α -methyl-3-oxo-17 α -(2,2,3,3-tetramethylcyclopropylcarbonyl)oxy-androsta-1,4-diene-17 β -carboxylic acid cyanomethyl ester, beclomethasone esters (such as the 17-propionate ester or the 17,21-dipropionate ester), budesonide, flunisolide, mometasone esters (such as the furoate ester), triamcinolone acetonide, rofleponide, ciclesonide, (16 α ,17-[[*(R)*-cyclohexylmethylene]bis(oxy)]-11 β ,21-dihydroxy-pregna-1,4-diene-3,20-dione), butixocort propionate, RPR-106541, and ST-126. Preferred corticosteroids include fluticasone propionate, 6 α ,9 α -difluoro-11 β -hydroxy-16 α -methyl-17 α -[(4-methyl-1,3-thiazole-5-carbonyl)oxy]-3-oxo-androsta-1,4-diene-17 β -carbothioic acid S-fluoromethyl ester and 6 α ,9 α -difluoro-17 α -[(2-furanylcarbonyl)oxy]-11 β -hydroxy-16 α -methyl-3-oxo-androsta-1,4-diene-17 β -carbothioic acid S-fluoromethyl ester, more preferably 6 α ,9 α -difluoro-17 α -[(2-furanylcarbonyl)oxy]-11 β -hydroxy-16 α -methyl-3-oxo-androsta-1,4-diene-17 β -carbothioic acid S-fluoromethyl ester.

Non-steroidal compounds that may have glucocorticoid activity include those covered in the following patent applications WO03/082827, WO01/10143, WO98/54159, WO04/005229, WO04/009016, WO04/009017, WO04/018429, WO03/104195, WO03/082787, WO03/082280, WO03/059899, WO03/101932, WO02/02565, WO01/16128, WO00/66590, WO03/086294, WO04/026248, WO03/061651, WO03/08277.

Anti-inflammatory agents include non-steroidal anti-inflammatory drugs (NSAID's).

Possible NSAID's that may be used in a combination include sodium cromoglycate, nedocromil sodium, phosphodiesterase (PDE) inhibitors (for example, theophylline, PDE4 inhibitors or mixed PDE3/PDE4 inhibitors), leukotriene antagonists, inhibitors of leukotriene synthesis (for example, montelukast), iNOS inhibitors, tryptase and elastase inhibitors, beta-2 integrin antagonists and adenosine receptor agonists or antagonists (for example, adenosine 2a agonists), cytokine antagonists (for example, chemokine antagonists, such as a CCR3 antagonist) or inhibitors of cytokine synthesis, or 5-lipoxygenase inhibitors. An iNOS (inducible nitric oxide synthase inhibitor) is preferably for oral administration. Other iNOS

inhibitors include those disclosed in WO93/13055, WO98/30537, WO02/50021, WO95/34534 and WO99/62875. Suitable CCR3 inhibitors include those disclosed in WO02/26722.

Phosphodiesterase 4 (PDE4) inhibitors that may be used in a combination include any compound that is known to inhibit the PDE4 enzyme or which is discovered to act as a PDE4 inhibitor, and which are only PDE4 inhibitors, not compounds which inhibit other members of the PDE family, such as PDE3 and PDE5, as well as PDE4.

Compounds include *cis*-4-cyano-4-(3-cyclopentyloxy-4-methoxyphenyl)cyclohexan-1-carboxylic acid, 2-carbomethoxy-4-cyano-4-(3-cyclopropylmethoxy-4-difluoromethoxyphenyl)cyclohexan-1-one and *cis*-[4-cyano-4-(3-cyclopropylmethoxy-4-difluoromethoxyphenyl)cyclohexan-1-ol]. Another compound of interest is *cis*-4-cyano-4-[3-(cyclopentyloxy)-4-methoxyphenyl]cyclohexane-1-carboxylic acid (also known as cilomilast) and its salts, esters, pro-drugs or physical forms, which is described in U.S. patent 5,552,438 issued 03 September, 1996; this patent and the compounds it discloses are incorporated herein in full by reference.

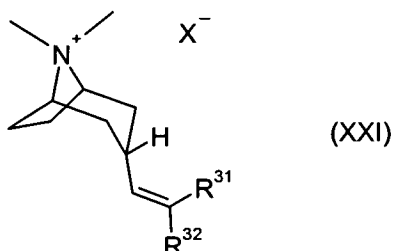
Other PDE4 inhibitors include AWD-12-281 from Elbion (Hofgen, N. *et al.* 15th EFMC Int Symp Med Chem (Sept 6-10, Edinburgh) 1998, Abst P.98; CAS reference No. 247584020-9); a 9-benzyladenine derivative nominated NCS-613 (INSERM); D-4418 from Chiroscience and Schering-Plough; a benzodiazepine PDE4 inhibitor identified as CI-1018 (PD-168787) and attributed to Pfizer; a benzodioxole derivative disclosed by Kyowa Hakko in WO99/16766; K-34 from Kyowa Hakko; V-11294A from Napp (Landells, L.J. *et al.* Eur Resp J [Annu Cong Eur Resp Soc (Sept 19-23, Geneva) 1998] 1998, 12 (Suppl. 28): Abst P2393); roflumilast (CAS reference No 162401-32-3) and a pthalazinone (WO99/47505, the disclosure of which is hereby incorporated by reference) from Byk-Gulden; arofylline under development by Almirall-Prodesfarma; VM554/UM565 from Vernalis; or T-440 (Tanabe Seiyaku; Fuji, K. *et al.* J Pharmacol Exp Ther, 1998, 284(1): 162), and T2585.

Further compounds are disclosed in the published international patent application WO04/024728 (Glaxo Group Ltd), PCT/EP2003/014867 (Glaxo Group Ltd) and PCT/EP2004/005494 (Glaxo Group Ltd).

Anticholinergic agents are those compounds that act as antagonists at the muscarinic receptors, in particular those compounds which are antagonists of the M₁ or M₃ receptors,

dual antagonists of the M_1/M_3 or M_2/M_3 , receptors or pan-antagonists of the $M_1/M_2/M_3$ receptors. Exemplary compounds for administration via inhalation include ipratropium (for example, as the bromide, CAS 22254-24-6, sold under the name Atrovent), oxitropium (for example, as the bromide, CAS 30286-75-0) and tiotropium (for example, as the bromide, CAS 136310-93-5, sold under the name Spiriva). Also of interest are revatropate (for example, as the hydrobromide, CAS 262586-79-8) and LAS-34273 which is disclosed in WO01/04118. Exemplary compounds for oral administration include pirenzepine (for example, CAS 28797-61-7), darifenacin (for example, CAS 133099-04-4, or CAS 133099-07-7 for the hydrobromide sold under the name Enablex), oxybutynin (for example, CAS 5633-20-5, sold under the name Ditropan), terodiline (for example, CAS 15793-40-5), tolterodine (for example, CAS 124937-51-5, or CAS 124937-52-6 for the tartrate, sold under the name Detrol), otilonium (for example, as the bromide, CAS 26095-59-0, sold under the name Spasmomen), trospium chloride (for example, CAS 10405-02-4) and solifenacin (for example, CAS 242478-37-1, or CAS 242478-38-2, or the succinate also known as YM-905 and sold under the name Vesicare).

Other anticholinergic agents include compounds of formula (XXI), which are disclosed in US patent application 60/487981:



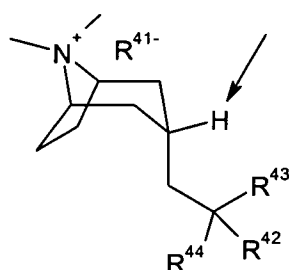
in which the preferred orientation of the alkyl chain attached to the tropane ring is endo;

R^{31} and R^{32} are, independently, selected from the group consisting of straight or branched chain lower alkyl groups having preferably from 1 to 6 carbon atoms, cycloalkyl groups having from 5 to 6 carbon atoms, cycloalkyl-alkyl having 6 to 10 carbon atoms, 2-thienyl, 2-pyridyl, phenyl, phenyl substituted with an alkyl group having not in excess of 4 carbon atoms and phenyl substituted with an alkoxy group having not in excess of 4 carbon atoms;

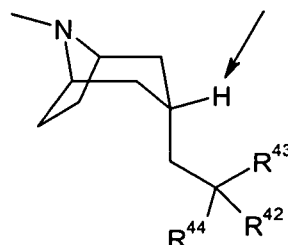
X⁻ represents an anion associated with the positive charge of the N atom. X⁻ may be but is not limited to chloride, bromide, iodide, sulfate, benzene sulfonate, and toluene sulfonate, including, for example:

- (3-*endo*)-3-(2,2-di-2-thienylethenyl)-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane bromide;
 5 (3-*endo*)-3-(2,2-diphenylethenyl)-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane bromide;
 (3-*endo*)-3-(2,2-diphenylethenyl)-8,8-dimethyl-8-azoniabicyclo[3.2.1]octane 4-methylbenzenesulfonate;
 (3-*endo*)-8,8-dimethyl-3-[2-phenyl-2-(2-thienyl)ethenyl]-8-azoniabicyclo[3.2.1]octane bromide;
 and/or
 10 (3-*endo*)-8,8-dimethyl-3-[2-phenyl-2-(2-pyridinyl)ethenyl]-8-azoniabicyclo[3.2.1]octane bromide.

Further anticholinergic agents include compounds of formula (XXII) or (XXIII), which are disclosed in US patent application 60/511009:



(XXII)



(XXIII)

15 wherein:

the H atom indicated is in the exo position;

R⁴¹⁻ represents an anion associated with the positive charge of the N atom. R⁴¹⁻ may be but is not limited to chloride, bromide, iodide, sulfate, benzene sulfonate and toluene sulfonate;

R⁴² and R⁴³ are independently selected from the group consisting of straight or branched chain lower alkyl groups (having preferably from 1 to 6 carbon atoms), cycloalkyl groups (having from 5 to 6 carbon atoms), cycloalkyl-alkyl (having 6 to 10 carbon atoms), heterocycloalkyl (having 5 to 6 carbon atoms) and N or O as the heteroatom, heterocycloalkyl-alkyl (having 6 to 10 carbon atoms) and N or O as the heteroatom, aryl, optionally substituted aryl, heteroaryl, and optionally substituted heteroaryl;

25 R⁴⁴ is selected from the group consisting of (C₁-C₆)alkyl, (C₃-C₁₂)cycloalkyl, (C₃-C₇)heterocycloalkyl, (C₁-C₆)alkyl(C₃-C₁₂)cycloalkyl, (C₁-C₆)alkyl(C₃-C₇)heterocycloalkyl, aryl, heteroaryl, (C₁-C₆)alkyl-aryl, (C₁-C₆)alkyl-heteroaryl, -OR⁴⁵, -CH₂OR⁴⁵, -CH₂OH, -CN, -CF₃,

$-\text{CH}_2\text{O}(\text{CO})\text{R}^{46}$, $-\text{CO}_2\text{R}^{47}$, $-\text{CH}_2\text{NH}_2$, $-\text{CH}_2\text{N}(\text{R}^{47})\text{SO}_2\text{R}^{45}$, $-\text{SO}_2\text{N}(\text{R}^{47})(\text{R}^{48})$, $-\text{CON}(\text{R}^{47})(\text{R}^{48})$,
 $-\text{CH}_2\text{N}(\text{R}^{48})\text{CO}(\text{R}^{46})$, $-\text{CH}_2\text{N}(\text{R}^{48})\text{SO}_2(\text{R}^{46})$, $-\text{CH}_2\text{N}(\text{R}^{48})\text{CO}_2(\text{R}^{45})$, $-\text{CH}_2\text{N}(\text{R}^{48})\text{CONH}(\text{R}^{47})$;

R^{45} is selected from the group consisting of $(\text{C}_1\text{-C}_6)\text{alkyl}$, $(\text{C}_1\text{-C}_6)\text{alkyl}(\text{C}_3\text{-C}_{12})\text{cycloalkyl}$, $(\text{C}_1\text{-C}_6)\text{alkyl}(\text{C}_3\text{-C}_7)\text{heterocycloalkyl}$, $(\text{C}_1\text{-C}_6)\text{alkyl-aryl}$, $(\text{C}_1\text{-C}_6)\text{alkyl-heteroaryl}$;

- 5 R^{46} is selected from the group consisting of $(\text{C}_1\text{-C}_6)\text{alkyl}$, $(\text{C}_3\text{-C}_{12})\text{cycloalkyl}$, $(\text{C}_3\text{-C}_7)\text{heterocycloalkyl}$, $(\text{C}_1\text{-C}_6)\text{alkyl}(\text{C}_3\text{-C}_{12})\text{cycloalkyl}$, $(\text{C}_1\text{-C}_6)\text{alkyl}(\text{C}_3\text{-C}_7)\text{heterocycloalkyl}$, *aryl*, *heteroaryl*, $(\text{C}_1\text{-C}_6)\text{alkyl-aryl}$, $(\text{C}_1\text{-C}_6)\text{alkyl-heteroaryl}$;

R^{47} and R^{48} are, independently, selected from the group consisting of *H*, $(\text{C}_1\text{-C}_6)\text{alkyl}$, $(\text{C}_3\text{-C}_{12})\text{cycloalkyl}$, $(\text{C}_3\text{-C}_7)\text{heterocycloalkyl}$, $(\text{C}_1\text{-C}_6)\text{alkyl}(\text{C}_3\text{-C}_{12})\text{cycloalkyl}$, $(\text{C}_1\text{-C}_6)\text{alkyl}(\text{C}_3\text{-C}_7)\text{heterocycloalkyl}$, $(\text{C}_1\text{-C}_6)\text{alkyl-aryl}$, and $(\text{C}_1\text{-C}_6)\text{alkyl-heteroaryl}$, including, for example:

- 10 (Endo)-3-(2-methoxy-2,2-di-thiophen-2-yl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide;

3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionitrile;

(Endo)-8-methyl-3-(2,2,2-triphenyl-ethyl)-8-aza-bicyclo[3.2.1]octane;

- 15 3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionamide;

3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionic acid;

(Endo)-3-(2-cyano-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide;

(Endo)-3-(2-cyano-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane bromide;

3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propan-1-ol;

- 20 *N*-Benzyl-3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propionamide;

(Endo)-3-(2-carbamoyl-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide;

1-Benzyl-3-[3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-urea;

1-Ethyl-3-[3-((endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-urea;

N-[3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-acetamide;

- 25 *N*-[3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-benzamide;

3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-di-thiophen-2-yl-propionitrile;

(Endo)-3-(2-cyano-2,2-di-thiophen-2-yl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide;

N-[3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-benzenesulfonamide;

- 30 [3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-urea;

N-[3-((Endo)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-2,2-diphenyl-propyl]-methanesulfonamide;

and/or

(Endo)-3-{2,2-diphenyl-3-[(1-phenyl-methanoyl)-amino]-propyl}-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane bromide.

More preferred compounds useful in the present invention include:

(Endo)-3-(2-methoxy-2,2-di-thiophen-2-yl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide;

(Endo)-3-(2-cyano-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide;

5 (Endo)-3-(2-cyano-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane bromide;

(Endo)-3-(2-carbamoyl-2,2-diphenyl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide;

(Endo)-3-(2-cyano-2,2-di-thiophen-2-yl-ethyl)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane iodide; and/or

10 (Endo)-3-{2,2-diphenyl-3-[(1-phenyl-methanoyl)-amino]-propyl}-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane bromide.

Antihistamines (also referred to as H1-receptor antagonists) include any one or more of the numerous antagonists known which inhibit H1-receptors, and are safe for human use. First generation antagonists, include derivatives of ethanolamines, ethylenediamines, and alkylamines, such as diphenylhydramine, pyrilamine, clemastine, chlorpheniramine. Second
15 generation antagonists, which are non-sedating, include loratidine, desloratidine, terfenadine, astemizole, acrivastine, azelastine, levocetirizine fexofenadine, cetirizine and efletirizine.

The invention thus provides, in a further aspect, a pharmaceutical composition according to the invention further comprising a PDE4 inhibitor.

20 The invention thus provides, in a further aspect, a pharmaceutical composition according to the invention further comprising a β_2 -adrenoreceptor agonist.

The invention thus provides, in a further aspect, a pharmaceutical composition according to the invention further comprising an anticholinergic.

The invention thus provides, in a further aspect, a pharmaceutical composition according to the invention further comprising an antihistamine.

25 The individual compounds/compositions of such drug combinations may be administered separately, sequentially or simultaneously in individual pharmaceutical formulations. Preferably, the individual compounds/compositions will be administered simultaneously in a combined pharmaceutical formulation. Appropriate doses of known therapeutic agents will be readily appreciated by those skilled in the art.

The compositions of the invention may have one or more of the following advantageous properties: more efficacious; show greater selectivity; have fewer side effects; have a longer duration of action; be more bioavailable by the preferred route; show less systemic activity when administered by inhalation; and/or have other more desirable properties than

5 compositions comprising similar known compounds.

Compositions of the invention may be tested for *in vitro* and *in vivo* biological activity in accordance with the following or similar assays/models.

1) *In Vitro*: Agonist activity against adenosine A₁, A_{2A}, A_{2B} and A₃ receptors.

10 The agonist potency and selectivity of compounds against human adenosine receptors is determined using Chinese hamster ovary (CHO) cells or yeast cells transfected with the gene for the relevant receptor.

(a) CHO cells

Two methods may be used in the CHO cells. (i) For the SPAP assay, cells are also transfected with cyclic AMP (cAMP) response elements promoting the gene for secreted placental alkaline phosphatase (SPAP). Changes in cAMP are measured as changes in the levels of SPAP. (ii) The DiscoverX assay is an enzyme complementation assay that involves two fragments of β -galactosidase, enzyme acceptor (EA) and enzyme donor (ED). Following the production of cAMP EA binds to ED, active enzyme is produced and a luminescent product is formed following the addition of substrate. For both methods the effect of test compounds is determined by their effects on basal levels of cAMP (A_{2A} and A_{2B}) or on forskolin enhanced cAMP (A₁ and A₃).

(b) Yeast cells

For the yeast assay, receptor stimulation causes activation of a reporter gene, namely FUS1-HIS3, resulting in histidine production which is essential for cell growth. Yeast cells are cultured in growth medium lacking histidine, and addition of a test compound causes histidine production which in turn stimulates cell growth. This response is measured from the production of the exoglucanase, an enzyme secreted constitutively by yeast cells.

In all of the *in vitro* assays the activity of test compounds is expressed as a ratio to that of the non-selective adenosine receptor agonist, N-ethyl carboxamide adenosine (NECA).

In these or similar assays the formate salt of the compound of formula (I) was shown to be highly selective - being greater than 100 fold more selective for A_{2A} than A₁, A_{2B} and A₃.

- 5 Potency at A_{2A} was <0.5 (EMR vs NECA), and generally about 0.02 (EMR vs NECA).

In Vivo anti-inflammatory agonist activity

LPS model:

Test compound was administered to male CD albino rats prior to exposure to LPS.

- 10 Compound (or vehicle) was injected in a 200ul volume into the trachea, via a cannula placed trans-orally, whilst the animals were under isoflurane anaesthesia. After a recovery period of 30 min, rats were placed in a chamber and exposed to an aerosol of *E. Coli*-derived LPS for 15 min. Four hours after LPS challenge the rats were killed, the lungs lavaged, and both total and differential cell counts determined. The dose of test compound giving a 50% reduction in neutrophil accumulation (ED50) was determined.

- 15 In this or a similar assay the formate salt of the compound of formula (I) gave greater than 50% reduction in neutrophil accumulation at a dose of 30µg/kg or less.

3) Therapeutic index (TI)

Cardiovascular model:

- 20 Male Wistar rats were anaesthetised with chloralose/pentobarbitone and the jugular vein, left carotid artery and trachea were cannulated. The arterial cannula was connected to a transducer for the continuous measurement of blood pressure and heart rate. Compound (or vehicle) was administered into the trachea in a 100ul volume, and the dose of test compound giving a 20% increase in blood pressure and heart rate (ED20) was determined.

- 25 The TI for a test compound is calculated as the ratio of the ED20 in the cardiovascular model compared with the ED50 in the LPS model.

The dose so determined for the formate salt of the compound of formula (I) in this or a similar model was about 7µg/kg.

(4) HSA binding

Instrument: Agilent HP1100 HPLC instruments were used throughout.

- 5 *HPLC columns:* Chromtech Immobilised HSA HPLC column 50 x 3 mm was purchased from Chromtech (Cheshire, UK).

Mobile phase and detection: The mobile phase A was 50 mM pH 7.4 ammonium acetate solution, while mobile phase B was 2-Propanol (HPLC grade, Runcorn, UK). The mobile phase flow rate was 1.8 ml/min. The column temperature was kept at 30 °C. The gradient profile and run time were the same with each column, the linear gradient from 0 to 30% 2-propanol was applied from 0 to 3 minutes. From 3 to 10 minutes, the mobile phase composition was constant 30% 2-propanol and 70% 50-mM ammonium acetate. From 10 min to 10.5 min the mobile phase composition was change to 100% ammonium acetate buffer only and remained the same until the end of the run. Each separation was stopped after 15 minutes.

Detection: Chromatograms were recorded at 230 and 254 nm by a diode array UV absorption detector at room temperature.

Calibration of the protein columns: The column performance check and the calibration have been performed before the analysis of every 96 well plate. The compounds used for the column calibrations were dissolved separately in 0.5 mg/ml concentration in 50% 2-propanol and 50% pH 7.4 ammonium acetate solution mixtures. The calibration set of compounds their literature % plasma protein binding and its linear conversion value (logK_{lit}), as well as typical retention times, their logarithmic values, log K derived from the calibration curve and % binding data are listed in Table 1.

Table 1. Calibration set of compounds with their literature and typical measured chromatographic data obtained with the HSA column. (Literature data were obtained from ref. 16.)

Compound	Literature % PPB	tR	logtR	lit logK	logK measured	%HSA measured
Warfarin2	98	4.393	0.64	1.51	1.53	98.1
Nizatidine	35	0.6	-0.22	-0.28	-0.35	31.1
Bromazepam	60	1.299	0.11	0.17	0.38	71.2
Carbamazepine	75	1.48	0.17	0.46	0.50	76.8
Budesonide	88	1.826	0.26	0.83	0.70	84.2
Piroxicam	94.5	2.787	0.45	1.16	1.10	93.6
Nicardipine	95	3.768	0.58	1.20	1.38	97.0
Ketoprofen	98.7	3.916	0.59	1.63	1.42	97.3
Indomethacin	99	6.023	0.78	1.69	1.83	99.5
Diclofenac	99.8	5.94	0.77	1.92	1.81	99.5

- 5 The literature % PPB (bound in plasma) values were converted to the linear free energy related logK values (logarithm of apparent affinity constant) using the following equation.

$$\text{Log K} = \log \left[\frac{\% \text{ PPB}}{(101 - \% \text{ PPB})} \right] - [\text{Plasma Protein}]$$

- 10 The formate salt of the compound of formula (I) in this or a similar assay showed greater than about 90% binding to HSA.

The various aspects of the invention will now be described by reference to the following Examples. These Examples are merely illustrative and are not to be construed as a limitation of the scope of the present invention.

15 Brief description of the figures

Figure 1 shows the XRPD trace of (2*R*,3*R*,4*S*,5*R*,2'*R*,3'*R*,4'*S*,5'*R*)-2,2'-{*trans*-1,4-cyclohexanedylbis[imino(2-{[2-(1-methyl-1*H*-imidazol-4-yl)ethyl]amino}-9*H*-purine-6,9-diyl)]}bis[5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydro-3,4-furandiol] mono maleate hydrate.

Figure 2 shows the XRPD trace of (2*R*,3*R*,4*S*,5*R*,2'*R*,3'*R*,4'*S*,5'*R*)-2,2'-{*trans*-1,4-cyclohexanediylbis[imino(2-[[2-(1-methyl-1*H*-imidazol-4-yl)ethyl]amino]-9*H*-purine-6,9-diyl)]}bis[5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydro-3,4-furandiol] mono terephthalate.

5

Figure 3 shows the XRPD trace of (2*R*,3*R*,4*S*,5*R*,2'*R*,3'*R*,4'*S*,5'*R*)-2,2'-{*trans*-1,4-cyclohexanediylbis[imino(2-[[2-(1-methyl-1*H*-imidazol-4-yl)ethyl]amino]-9*H*-purine-6,9-diyl)]}bis[5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydro-3,4-furandiol] mono phthalate.

10 Examples

General Experimental Details

All reactions were carried out under an atmosphere of nitrogen unless specified otherwise All temperatures are given in degrees centigrade.

Where products were purified by column chromatography, 'flash silica' refers to silica gel for chromatography, 0.035 to 0.070mm (220 to 440mesh) (e.g. Fluka silica gel 60), where column elution was accelerated by an applied pressure of nitrogen at up to 10 p.s.i. Where thin layer chromatography (TLC) has been used, it refers to silica gel TLC using plates typically 4 x 10 cm silica gel on aluminium foil plates with a fluorescent indicator (254nm), (e.g. Fluka 60778). Biotage refers to prepacked silica gel caRTridges containing KP-Sil run on flash 12i chromatography module. Solid Phase Extraction (SPE) columns are pre-packed caRTridges used in parallel purifications, normally under vacuum. These are commercially available from Varian. SCX caRTridges are Ion Exchange SPE columns where the stationary phase is polymeric benzene sulfonic acid. These are used to isolate amines.

The ¹H-NMR spectra were recorded on a Bruker AV400 operating at 400MHz or a Bruker DPX-250 operating at 250MHz. D₆-DMSO was used as solvent unless stated otherwise. Tetramethylsilane was used as internal standard.

LC/MS Systems

The Liquid Chromatography Mass Spectroscopy (LC/MS) systems used:

LCMS System : LCMS was conducted on a Supelcosil LCABZ+PLUS column (3.3cm x 4.6mm ID) eluting with 0.1 % HCO₂H and 0.01M ammonium acetate in water (solvent A) and 0.05% HCO₂H 5% water in acetonitrile (solvent B), using the following elution gradient 0.0-7 min 0%B, 0.7-4.2 min 100%B, 4.2-5.3 min 100%B, 5.3-5.5min 0%B at a flow rate of 3mL/min.

- 5 The mass spectra were recorded on a Fisons VG Platform spectrometer using electro spray positive and negative mode (ES+ve and ES-ve).

Preparative HPLC conditions

- Where products were purified by preparative HPLC, this was carried out on a C18-reverse-phase column (10 x 2.1cm i.d. Genesis column with 7µm particle size), eluting first
10 isocratically with 10% acetonitrile phase then with a gradient of acetonitrile (containing 0.1% trifluoroacetic acid) in water (containing 0.1% trifluoroacetic acid) at a flow rate of 5ml/min. The gradient was started at 10% acetonitrile and was increased at a rate of 1% per minute. UV detection at 230nm was used unless otherwise stated.

Mass Directed Auto Prep (MDAP) HPLC conditions

- 15 Preparative mass directed HPLC was conducted on a Waters FractionLynx system comprising of a Waters 600 pump with extended pump heads, Waters 2700 autosampler, Waters 996 diode array and Gilson 202 fraction collector on a 10 cm X 2.54 cm ill ABZ+ column, eluting with 0.1 % formic acid in water (solvent A) and 0.1 % formic acid in acetonitrile (solvent B), using the following elution gradient: 0.0-1.0 min 15%B, 1.0-10.0 min
20 55%B, 10.0-14.5 min 99%B, 14.5-14.9 min 99%B, 14.9-15.0 min 15%B at a flow rate of 20 ml/min and detecting at 200-320 nm at room temperature. Mass spectra were recorded on Micromass ZMD mass spectrometer using electro spray positive and negative mode, alternate scans. The software used was *MassLyn.x* 3.5 with *OpenLynx* and *FractionLynx* options.
- 25 XRPD analysis of certain salts was carried out according to the following or similar methodology.

Manufacturer	PANalytical - The Netherlands
Instrument	X'Pert Pro
Diffractometer Type	DY1850
Tube anode	Cu
K-Alpha1 wavelength (Å)	1.54056
K-Alpha2 wavelength (Å)	1.54439
Ration Alpha 1:2	0.50000
Divergence slit	Prog.Div.Slit
Receiving slit	Prog.Rec.Slit
Generator voltage (kV)	40
Tube Current (mA)	45
Detector	X'celerator
Data Angle range ($^{\circ}2\theta$)	2.000-40.000
Scan type	Continuous
Scan step size	0.0167
Scan step time (seconds)	31.75
Sample preparation	Flush Silicon wafer

15 XRPD analysis was performed on a PANalytical X'Pert Pro X-ray powder diffractometer, model X' Pert Pro PW3040/60, serial number DY1850 using an X'Celerator detector. The acquisition conditions were: radiation: Cu K, generator tension: 40 kV, generator current: 45 mA, start angle: $2.000^{\circ}2\theta$, end angle: $40.000^{\circ}2\theta$, step size: 0.0167 , time per step: 31.75 seconds. The sample was prepared using flush Silicon wafer.

Abbreviations used in the experimental section

IPA = isopropanol
 DCM = dichloromethane
 20 THF = tetrahydrofuran
 MeOH = methanol
 DMF = dimethylformamide
 DIPEA = di-isopropylethylamine
 EtOAc = ethyl acetate

ACN = acetonitrile

CHC = cyclohexane

DMSO = dimethylsulphoxide

RT = room temperature

5 DMAP = 4-dimethylaminopyridine

HATU = O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate. NBS = N-bromosuccinimide

IMS = industrial methylated spirit

TF A = trifluoroacetic acid

10 Boc = tert-butyl oxycarbonyl

R_t = retention time

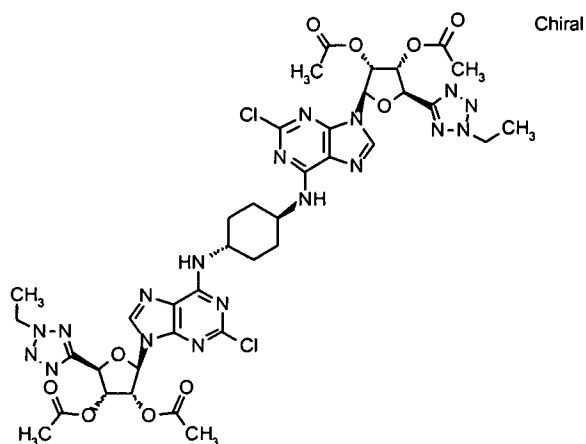
h: hour(s)

min: minute(s)

Flash silica gel refers to Merck ART No. 9385; silica gel refers to Merck ART No. 7734

15 Intermediate 1

***Trans*-1,4-cyclohexanediylbis[imino(2-chloro-9*H*-purine-6,9-diyl)(2*R*,3*R*,4*R*,5*R*)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-2,3,4-triyl]tetraacetate**



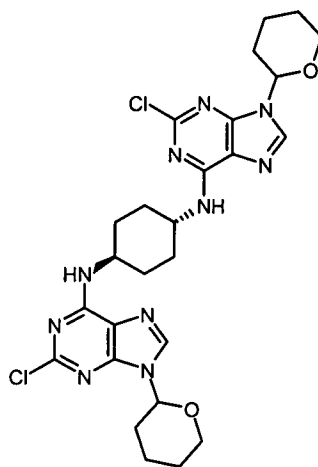
20 A mixture of (2*R*,3*R*,4*R*,5*R*)-2-(2,6-dichloro-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-3,4-diyl diacetate (Intermediate 7 in WO98/28319), (2.36g, 5mmol), *trans*-1,4-diaminocyclohexane (630mg, 5.5mmol) and DIPEA (0.960mL) in IPA (30mL) was stirred and heated at 60°C for 19.5h. After cooling solvent was removed in vacuo. Crude reaction mixture was purified on Flash Silica Isolute cartridge (50g) eluted in a gradient from cyclohexane through to cyclohexane/ethyl acetate (1:3 to 1:1) to neat ethylacetate.

Appropriate fractions were combined and upon removal of solvent in vacuo, the **title compound** was obtained as a white solid (1.46g).

LC/MS R_t 3.58min m/z 983 $[MH]^+$

Intermediate 2

5 ***Trans-N,N'*-bis[2-chloro-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-yl]-1,4-cyclohexanediamine**



10 A stirred suspension of 2,6-dichloro-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (Intermediate 1 in WO03/080613) (17g; 62.3 mmol) in *iso*-propanol (500ml) was treated with *trans*-1,4-diaminocyclohexane (3.6g; 31.5mmol) and diisopropylethylamine (15ml) was heated at 75°C for 6 hours.

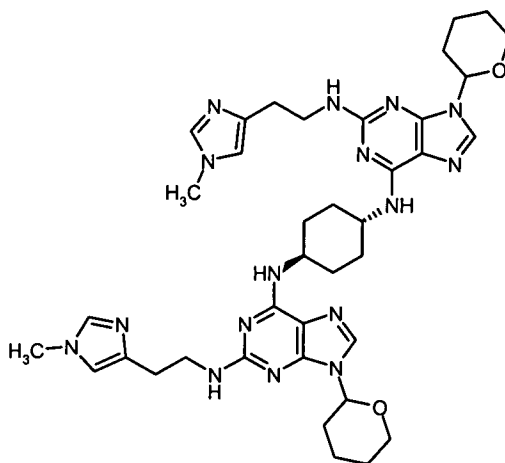
15 Additional *trans*-1,4-diaminocyclohexane (0.9g; 7.9mmol) was added and heating was continued for a further 16 hours. A further portion of *trans*-1,4-diaminocyclohexane (0.9g; 7.9mmol) was added and heating was continued for a further 7 hours at 85°C. The suspension was allowed to cool to ambient temperature and was allowed to stand. The solid was filtered off and was washed with *iso*-propanol followed by ether then was sucked dry. The solid (18.5g) was partitioned between ethyl acetate and saturated aqueous sodium bicarbonate solution. The aqueous phase and solid was separated and was extracted with ethylacetate. Undissolved solid was filtered and partitioned between chloroform and
20 saturated sodium bicarbonate solution. The combined organic extracts were dried over sodium sulphate and the solvent was evaporated. The resultant foam was suspended in ethyl acetate to give a solid. The solvent was evaporated and the residue was triturated with

ether to give a solid which was filtered off, was washed with ether and was dried to provide the **title compound** as a white solid (16.3g).

LC/MS R_t 3.33min m/z 587, 589[MH]⁺

Intermediate 3

- 5 ***N*⁶,*N*^{6'}-trans-1,4-cyclohexanediylbis[*N*²-[2-(1-methyl-1*H*-imidazol-4-yl)ethyl]-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine-2,6-diamine]**



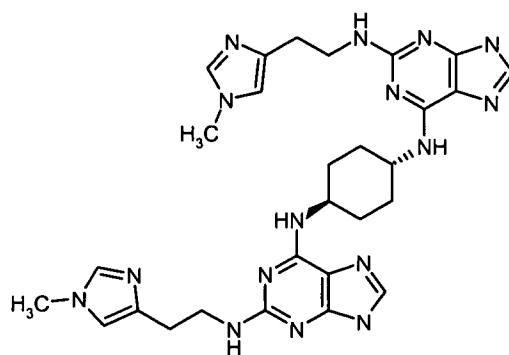
- 10 A mixture of Intermediate 2 (10g; 17.0mmoles) and N-methylhistamine (25.4g; from 100g bistosylate, 203mmoles) in anhydrous dimethylsulphoxide (20ml) was heated at 115°C for 24hours. The dark solution was allowed to cool to ambient temperature then was diluted slowly with water (400ml) followed by ethyl acetate (75ml). The mixture was stirred vigorously until the sticky lumps had solidified and broken up. The solid was filtered off, was washed with water and was dried to give the **title compound** (12.2g).

LC/MS R_t 2.08min m/z 383[(M+2H)/2]⁺, 765[MH]⁺

- 15 Intermediate 4

***N*⁶,*N*^{6'}-trans-1,4-cyclohexanediylbis{*N*²-[2-(1-methyl-1*H*-imidazol-4-yl)ethyl]-3*H*-purine-2,6-diamine}**

39

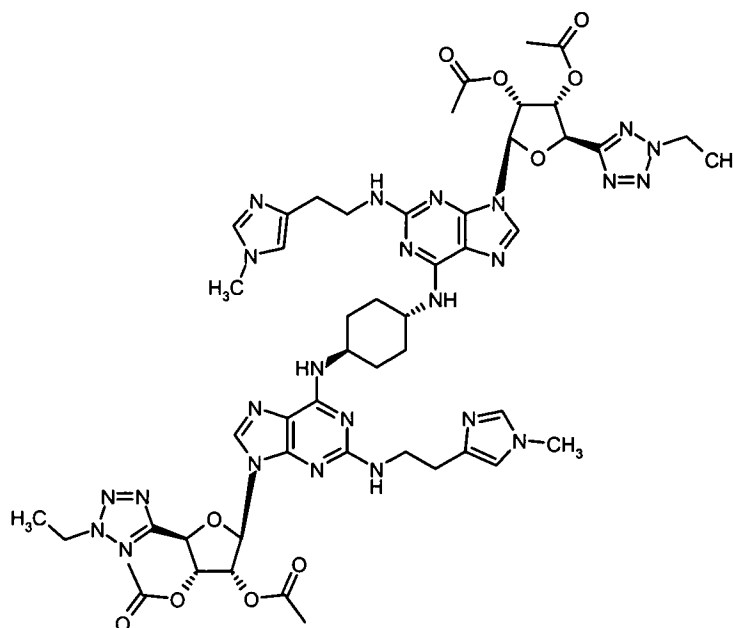


A solution of Intermediate 3 (11.8g; 15.4mmoles) in methanol (100ml) was treated with 2M hydrochloric acid (25ml) and was stirred at ambient temperature for 4hours. The mixture was concentrated to remove methanol then was diluted with water (50ml). Saturated aqueous sodium bicarbonate solution (70ml) was added and the mixture was stirred for 1 hour. The solid was filtered off and was washed with water and dried. The solid was stirred with water for 15mins. Suspension was mixed with chloroform and water. Methanol was added and the mixture was shaken. The mixture was concentrated in vacuo, methanol was added and evaporated. This was done three times to furnish a solid. The solid was titrated with ether filtered off and dried in vacuo at 35°C to give the **title compound** (8.2g).

LC/MS R_t 1.73min m/z 299 $[(M+2H)/2]^+$, 597 $[MH]^+$

Intermediate 5

***Trans*-1,4-cyclohexanediylbis[imino(2-{[2-(1-methyl-1*H*-imidazol-4-yl)ethyl]amino}-9*H*-purine-6,9-diyl)(2*R*,3*R*,4*R*,5*R*)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-2,3,4-triyl] tetraacetate**



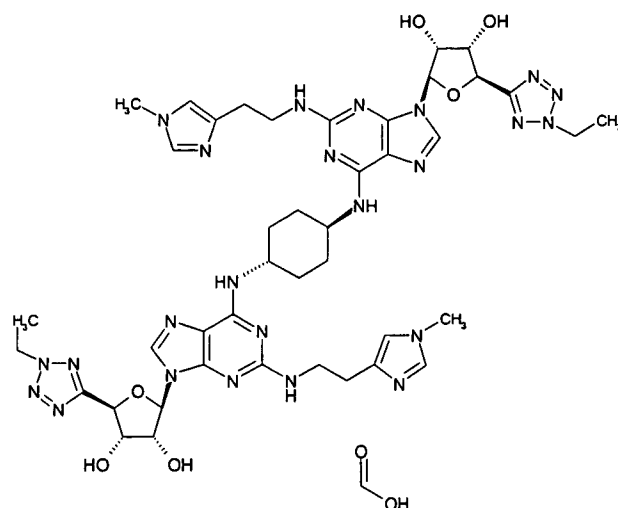
A suspension of Intermediate 4 (5.5g; 9.22mmoles) in ethyl acetate (50ml) was treated with a solution of rel-Acetic acid 4R,5-diacetoxy-2R-(2-ethyl-2H-tetrazol-5-yl)-tetrahydro-furan-3R-yl ester (Intermediate 6 of WO98/28319), (11.4g, 33.2mmoles) in ethyl acetate (50ml) and was cooled to 0°C. DBU (3.54ml; 23.6mmoles) was added followed by trimethylsilyl triflate (15.4ml; 92.9mmoles). The reaction was stirred at ambient temperature for 4.5hours then was heated at 50°C for 3hours. The reaction was allowed to cool then was left standing overnight. Water was added followed by saturated aqueous sodium bicarbonate solution. After stirring three phases were obtained. The aqueous phase and oil were separated and were extracted with chloroform (x3). The combined organic extracts (chloroform and ethylacetate) were dried over sodium sulphate and the solvent was evaporated. The residue was chromatographed down a column of silica (Merck ART 9385 600ml) eluted with chloroform (400ml) followed by chloroform/methanol/0.88 aqueous ammonia solution (95:5:0.4). Appropriate fractions were combined and the solvent was evaporated to give the **title compound** (9.5g, 4.2g of 90% pure product approximately).

LC/MS R_t 2.33min m/z 581 $[(M+2H)/2]^+$

Example 1

Formic acid - (2R,3R,4S,5R,2'R,3'R,4'S,5'R)-2,2'-{trans-1,4-cyclohexanediylbis[imino(2-{[2-(1-methyl-1H-imidazol-4-yl)ethyl]amino}-9H-purine-6,9-diyl)]}bis[5-(2-ethyl-2H-tetrazol-5-yl)tetrahydro-3,4-furandiol] (4:1)

41



5 [2-(1-methyl-1*H*-imidazol-4-yl)ethyl]amine (763mg, 6.1mM) was added to Intermediate 1 (300mg, 0.31mM) dissolved in anhydrous DMSO (5 ml) and the solution was stirred under nitrogen at 120°C. for 21h. and then allowed to cool. Water (20ml) was added and precipitate was filtered off, which was purified by mass-directed autoprep HPLC. Appropriate fractions were combined and evaporated to afford the **title compound** (36mg) as an off-white solid.

MDAP LC/MS R_t 2.39min m/z 993.7[MH]⁺

¹HNMR: 250MHz⁺

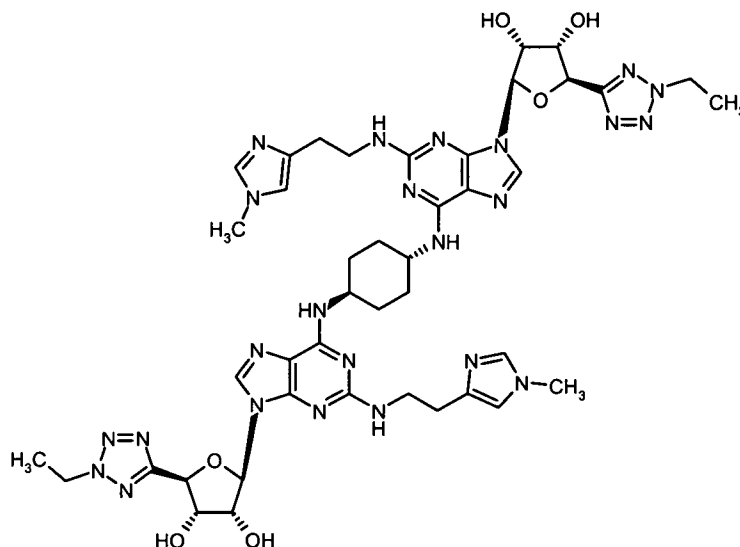
Shift (ppm)	Multiplicity	Integral
8.15	s	2H
7.80	s br	2H
7.40	s	2H
6.80	s	2H
6.30	s br	2H
6.00	d	2H
5.85	s br	2H
5.20	d	2H
4.82	m	4H
4.68	q	4H
4.18	s br	2H
3.60	s	6H

Shift (ppm)	Multiplicity	Integral
3.52	t br	4H
2.78	t	4H
2.08	d br	4H
1.54	t + m	10H

Example 2

(2*R*,3*R*,4*S*,5*R*,2'*R*,3'*R*,4'*S*,5'*R*)-2,2'-{*trans*-1,4-cyclohexanediylbis[imino(2-{[2-(1-methyl-1*H*-imidazol-4-yl)ethyl]amino}-9*H*-purine-6,9-diyl)]}bis[5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydro-3,4-furandiol]

5



A solution of Intermediate 5 (18.1g; 15.6mmoles) in methanol (150ml) was treated with sodium methoxide (1g; 18.5mmoles). After 30minutes Dowex 50 [H⁺] was added to neutralise the solution and additional methanol (100ml) was added. The resin was filtered off and the filtrate was evaporated to leave the **title compound** as a foam/gum (13.3g).

10

LC/MS R_t 2.06min *m/z* 497 [(M+2H)/2]⁺.

¹HNMR: 400MHz

Shift (ppm)	Multiplicity	Integral
8.31	s	2H

Shift (ppm)	Multiplicity	Integral
7.92	s br	2H
7.47	s	2H
7.08	s br	2H
6.90	s	2H
6.55	s br	2H
5.95	d	2H
5.70	s br	2H
5.11	d	2H
4.79	s br	2H
4.66	q	4H
4.04	s br	2H
3.58	s	6H
3.41	m	4H
2.72	t	4H
1.92	s br	4H
1.46	t + m	10H

Salt preparation

Mono maleate hydrate salt

Compound as free base (300mg) was dissolved in ethanol (4.4ml, 14.7vols) at 75°C. Maleic acid (35.9mg, 1.05 equivs) was dissolved in ethanol (1ml, 3.3vols) at room temperature. The maleic acid solution was added portion wise to the compound/ethanol solution with seeding with heating to 75°C as appropriate. The resulting suspension was aged at 75°C for 30 mins before cooling to room temperature over 2hours and then aged at room temperature for a further hour. The product was isolated by filtration, washed with ethanol (1ml) and dried at room temperature overnight under vacuum. The yield was 84.9%. The XRPD trace is shown in Figure 1.

Mono terephthalate salt

Compound as free base (300mg) and terephthalic acid (49.2mg, 1.05 equivs) were suspended in ethanol (5.4ml, 18vols). The suspension was heated to 75°C for 30 mins, then cooled to 40°C and left to temperature cycle between 0-40°C overnight with magnetic stirring.

- 5 The product was isolated by filtration, washed with ethanol (3 x 2ml) and dried at 60°C overnight under vacuum. The yield was 71.3%. The XRPD trace is shown in Figure 2.

In an alternative method the terephthalate salt was prepared as follows:

- 10 The compound as free base (1 g) was suspended in ethanol (80 ml, 80 vols) and was heated at 80 °C to form a solution. The terephthalic acid (123mg, 1.05 eq) was then added to the solution portion wise over a period of 7 hours. The suspension was then cooled to room temperature for two days with magnetic stirring and was then left at room temperature for 3 days without any magnetic stirrer. The product was isolated by filtration, washed with ethanol (3 x 5ml) and dried overnight under vacuum. The yield was 55%.

Mono phthalate salt

- 15 Compound as free base (300mg) and phthalic acid (49.2mg, 1.05 equivs) were suspended in ethanol (5.4ml, 18vols). The suspension was heated to 75°C for 30 mins, where it virtually formed a solution. The reaction mixture was cooled to 40°C and left to temperature cycle between 0-40°C overnight with magnetic stirring. The product was isolated by filtration, washed with ethanol (2 x 2ml) and dried at 60°C overnight under vacuum. The yield was
20 80.0%. The XRPD trace is shown in Figure 3.

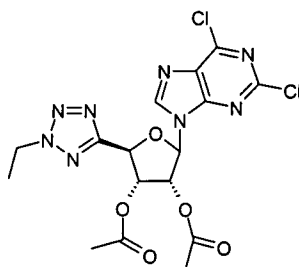
- Seeds may be produced by conventional methods using the desired acid (for example, maleic acid, hydrochloric acid, terephthalic acid, and phthalic acid) and by the methods described herein. The resultant seeds may then be used in subsequent salt preparations of typically the same salt but may also be used in the preparation of different salts, to improve
25 the crystallinity of the salt product.

Example 3

Further preparations of (2*R*,3*R*,4*S*,5*R*,2'*R*,3'*R*,4'*S*,5'*R*)-2,2'-{*trans*-1,4-cyclohexanediylbis[imino(2-{[2-(1-methyl-1*H*-imidazol-4-yl)ethyl]amino}-9*H*-purine-6,9-diyl)]}bis[5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydro-3,4-furandiol] by process A

5 *Stage 1:*

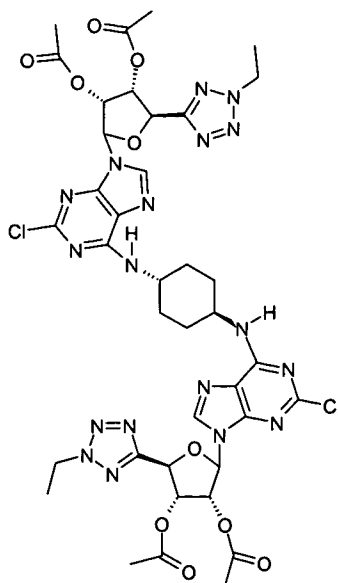
Acetic acid, 4*R*-acetoxy-2*R*-(2,6-dichloro-purin-9-yl)-5*R*-(2-ethyl-2*H*-tetrazol-5-yl)-tetrahydro-furan-3*R*-yl ester (Intermediate 7 of WO98/28319)



Trimethylsilyl trifluoromethanesulfonate (200 g, 900.9 mmol) was added to a suspension of
 10 2,6-dichloropurine (85.1 g, 450.5 mmol) in acetonitrile (850 ml) and stirred for 45 minutes. Then a solution of rel-Acetic acid 4*R*,5-diacetoxy-2*R*-(2-ethyl-2*H*-tetrazol-5-yl)-tetrahydro-furan-3*R*-yl ester (Intermediate 6 of WO98/28319) (123.2 g, 360.4 mmol) in acetonitrile (510 ml) was added over 55 minutes. The mixture was stirred at ambient temperature overnight then quenched with water (200 ml) for 10 minutes and then saturated aqueous sodium
 15 bicarbonate (1.6 L). The acetonitrile was evaporated and the resulting aqueous was extracted with dichloromethane (2 x 400 ml). The combined organic extracts was washed with saturated aqueous sodium bicarbonate (200 ml), water (200 ml), dried over anhydrous sodium sulphate and evaporated to an oil. The oil was dissolved in IPA (1L) at 50°C, cooled to about 42°C and seeded and sonicated. Cooling was continued to about 28°C and the
 20 resultant mixture was aged at 38 - 40°C for 30 mins. The mixture was cooled to about 25°C, filtered, washed with IPA (3x170 ml) and dried to give the **title compound** (111.5g).

Stage 2:

Trans-1,4-cyclohexanediylbis[imino(2-chloro-9*H*-purine-6,9-diyl)(2*R*,3*R*,4*R*,5*R*)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-2,3,4-triyl]tetraacetate (Intermediate 1)

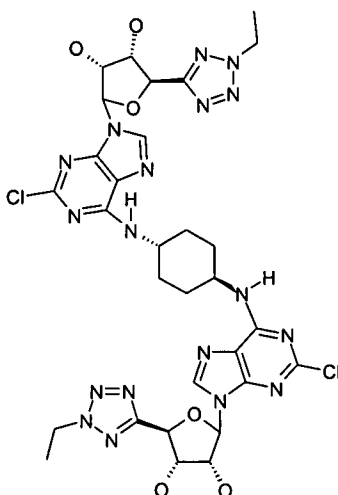


A mixture of Stage 1 (110.3 g, 234.2 mmol), 1,4-*trans*-diaminocyclohexane (16.02 g, 140.5 mmol), di-*isopropylethylamine* (122.2 ml, 702.6 mmol) and *iso*-propanol (550 ml) was heated at 82 °C for 20 hours. Extra 1,4-*trans*-diaminocyclohexane (0.8 g), di-*isopropylethylamine* (6.1 ml) was added and heating continued for another 24 hours. The mixture was cooled to 50 °C, diluted with ethyl alcohol (250 ml) and heated at 50 °C for 1 hour. The slurry was then cooled to ambient temperature, filtered and washed with ethyl alcohol (250 ml). The wet cake was reslurried with ethyl alcohol (550 ml) at 78 °C for 1 hour, cooled to ambient temperature (about 30°C), filtered and washed with ethyl alcohol (250 ml). The wet cake was reslurried with ethyl alcohol (550 ml) and water (110 ml) at 78 °C for 1 hour, cooled to ambient temperature, filtered, washed with 5:1 ethyl alcohol / water (240 ml) and ethyl alcohol (250 ml) then dried to give the **title compound** (77.9 g).

Stage 3:

(3*R*,4*S*,5*R*,3'*R*,4'*S*,5'*R*)-2,2'-{*trans*-cyclohexane-1,4-diylbis[imino(2-chloro-9H-purine-6,9-diyl)]}bis[5-(2-ethyl-2H-tetrazol-5-yl)tetrahydrofuran-3,4-diol]

47

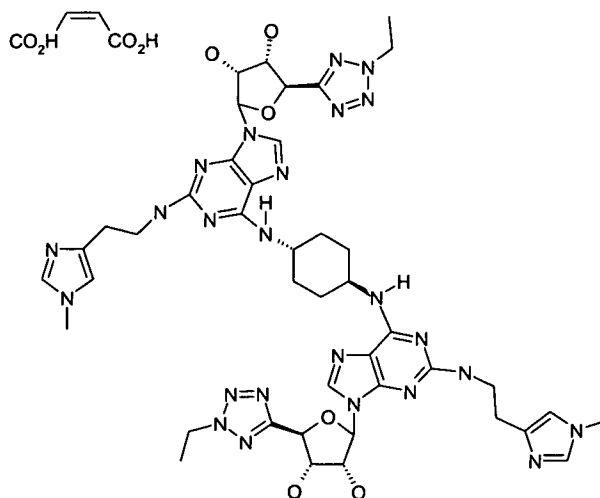


Sodium methoxide (0.63 g, 11.7 mmol) was added to a slurry of Stage 3 (77 g, 78.2 mmol) in methyl alcohol (770 ml) and stirred for 23 hours. The slurry was filtered, washed with methyl alcohol (380 ml) and dried to give the **title compound** (61.4 g).

- 5 ^1H NMR (d^6 - DMSO, 400 MHz) δ 1.45-1.68 (10 H, m), 1.83-2.07 (4 H, m), 4.03 (1.4 H, br s)^{*}, 4.54-4.59 (2 H, m), 4.63 (0.6 H, br s)^{*}, 4.69-4.82 (6 H, m), 5.22 (2 H, d), 5.86 (4 H, br s), 6.03-6.09 (2 H, m), 8.26-8.37 (2 H, m), 8.44 (1 H, s), 8.46 (1 H, s). * indicates non-whole integrals due to the presence of rotamers.

Stage 4:

- 10 (2*R*,3*R*,4*S*,5*R*,2'*R*,3'*R*,4'*S*,5'*R*)-2,2'-{*trans*-1,4-cyclohexanediylbis[imino(2-{[2-(1-methyl-1*H*-imidazol-4-yl)ethyl]amino}-9*H*-purine-6,9-diyl)]bis[5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydro-3,4-furandiol] maleate hydrate salt



A mixture of Stage 3 (55 g, 67.4 mmol), *N*-methyl histamine (84.7 g, 674 mmol) and anhydrous dimethyl sulfoxide (138 ml) was heated at 100 °C for 16.5 hours. The mixture was cooled to ambient temperature then added over 45 minutes to water (1.4 L). The slurry was stirred for 45 minutes, filtered, washed with water (2 x 700 ml) and dried. The crude product (60 g), maleic acid (7 g) and methyl alcohol (360 ml) was heated at 65 °C for 90 minutes, cooled to 30 °C and seeded, then cooled to ambient temperature (20-25 °C) and stirred for 90 minutes. The slurry was filtered, washed with methyl alcohol (110 ml) and dried to give the **title compound** (39.2 g).

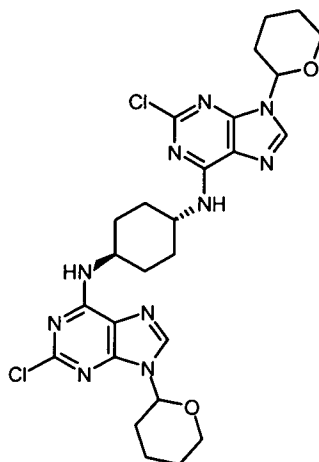
¹H NMR (d⁴-MeOH, 400 MHz) δ 1.39-1.53 (4 H, m), 1.6 (6 H, t), 2.10-2.21 (4 H, m), 2.92 (4 H, t), 3.64 (4 H, t), 3.71 (6 H, s), 4.06 (2 H, br s), 4.70 (4 H, q), 4.80 (2 H, br s), 4.89 (2 H, br s)*, 5.30 (2 H, d), 6.09 (2 H, d), 6.24 (2 H, s), 7.08 (2 H, s), 8.06 (2 H, s), 8.08 (2 H, s). * signal obscured by HOD

Example 4

Further preparation of (2*R*,3*R*,4*S*,5*R*,2'*R*,3'*R*,4'*S*,5'*R*)-2,2'-{*trans*-1,4-cyclohexanediylbis[imino(2-{[2-(1-methyl-1*H*-imidazol-4-yl)ethyl]amino}-9*H*-purine-6,9-diyl)]}bis[5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydro-3,4-furandiol] by process B

Stage 1:

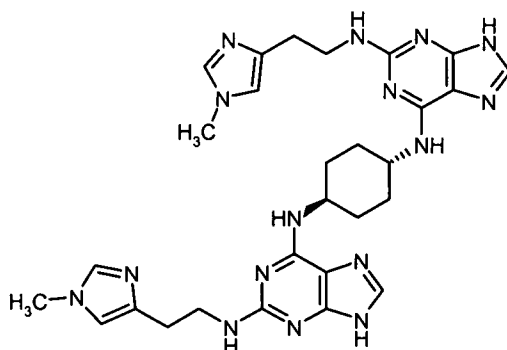
Trans-N,N-bis[2-chloro-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purin-6-yl]-1,4-cyclohexanediamine (Intermediate 2)



A stirred suspension of 2,6-dichloro-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (Intermediate 1 in WO03/080613) (1.14 kg) in *n*-butanol (1.7 L) was treated with *trans*-1,4-diaminocyclohexane (239.2g) and di-*iso*-propylethylamine (2.5 L), then heated at 75°C for 17 hours. The suspension was allowed to cool to ambient temperature, filtered, washed with *n*-butanol (2 x 2.3 L) and dried *in vacuo* at 60 °C to give the **title compound** (0.9 kg).

Stage 2:

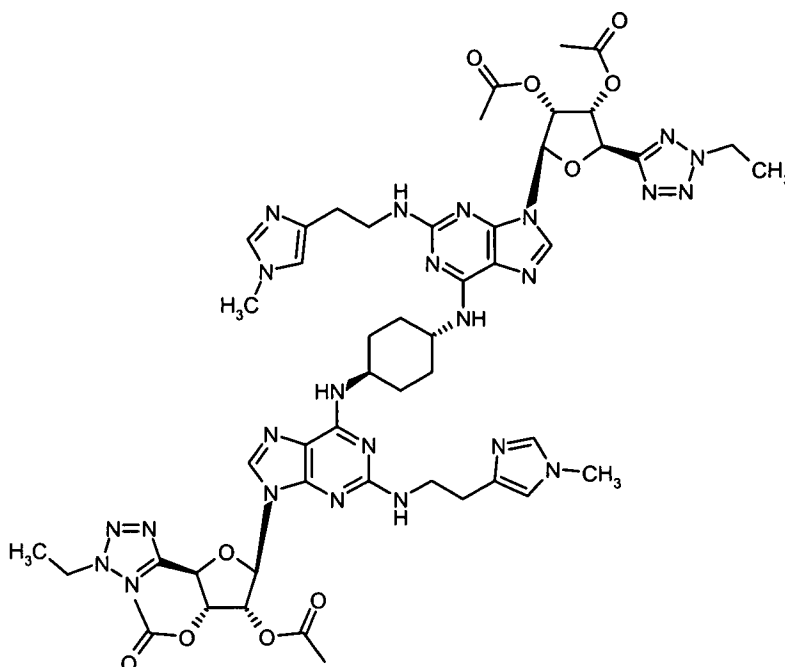
*N*⁶,*N*⁶-*trans*-1,4-cyclohexanediylbis{*N*²-[2-(1-methyl-1*H*-imidazol-4-yl)ethyl]-3*H*-purine-2,6-diamine} (Intermediate 4)



A mixture of Stage 1 (59.3 g; 100 mmol), *N*-methylhistamine (50.5 g; 400 mmoles), dipotassium hydrogen phosphate (35.1 g, 200 mmol) in ethylene glycol (60 ml) was heated at 120 °C for 8 days. The mixture was allowed to cool to ambient temperature then 5M aqueous hydrochloric acid (245 ml) is added with ice cooling for 50 minutes. Methanol (296 ml) was added followed by di-*iso*-propylethylamine (246 ml) added dropwise over 30 minutes and the solution was heated to 60 °C for 1 hour. Water (178 ml) was slowly added at 60 °C over 30 minutes then stirred overnight at 25 °C. The resulting slurry was heated to 60 °C and water (160 ml) was added dropwise. The slurry was cooled to ambient temperature, filtered, washed with water (120 ml), 1:2 methyl alcohol / water (120 ml), methyl alcohol (120 ml) and dried *in vacuo* at 40 °C to give a damp product (48.8 g). The damp product (40.8 g) was dried further *in vacuo* at 60 °C for 2 days to give the **title compound** (38.9 g).

Stage 3:

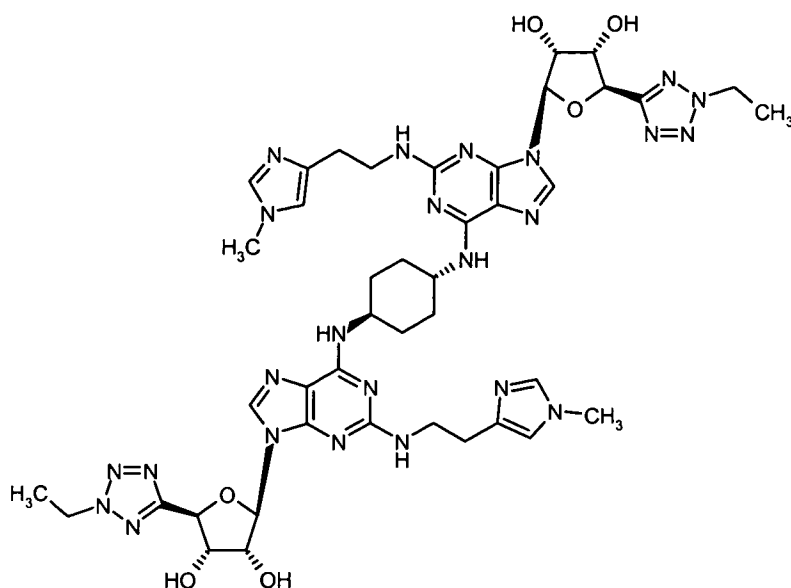
Trans-1,4-cyclohexanediylbis[imino(2-[[2-(1-methyl-1*H*-imidazol-4-yl)ethyl]amino]-9*H*-purine-6,9-diyl)(2*R*,3*R*,4*R*,5*R*)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-2,3,4-triyl] tetraacetate (Intermediate 5)



Trimethylsilyl trifluoromethanesulfonate (30.3 ml, 167 mmol) was added to a suspension of Stage 2 (20 g, 33.9 mmol) in acetonitrile (200 ml) then heated at 50 °C for 30 minutes. Then a solution of rel-Acetic acid 4R,5-diacetoxy-2R-(2-ethyl-2H-tetrazol-5-yl)-tetrahydro-furan-3R-yl ester (Intermediate 6 of WO98/28319)(28.7 g, 84 mmol) in acetonitrile (200 ml) was added over 30 minutes and stirred for 20 hours. The reaction mixture was cooled to ambient temperature and quenched with water (50 ml) for 35 minutes then 5M aqueous hydrochloric acid (2 x 50 ml) for 90 minutes. The mixture was partitioned between dichloromethane (250 ml) and aqueous saturated sodium bicarbonate (700 ml) and the dichloromethane layer was allowed to stand at ambient temperature overnight. The organic portion was then extracted with 1M aqueous hydrochloric acid (2 x 300 ml). The acidic extracts were neutralised with aqueous saturated sodium bicarbonate (750 ml) then extracted with dichloromethane (2 x 200 ml). The combined dichloromethane extracts was washed with brine (100 ml), dried over anhydrous magnesium sulphate and concentrated to give the **title compound** (28.9 g) that was used without purification.

Stage 4:

(2R,3R,4S,5R,2'R,3'R,4'S,5'R)-2,2'-{*trans*-1,4-cyclohexanediylbis[imino(2-([2-(1-methyl-1H-imidazol-4-yl)ethyl]amino)-9H-purine-6,9-diyl))]bis[5-(2-ethyl-2H-tetrazol-5-yl)tetrahydro-3,4-furandiol]



A solution of Stage 3 (28.6 g; assume 24.7 mmol) in methanol (515 ml) was treated with sodium methoxide (0.44 g, 8.13 mmol) for 90 minutes before being treated with a further portion of sodium methoxide (0.44 g, 8.13 mmol). After 30 minutes Dowex 50 [H⁺] was added to neutralise the solution. The resin was filtered off and the filtrate was evaporated to leave the **title Compound** as a foam (24 g).

Stage 5:

Mono maleate hydrate salt

A solution of maleic acid (0.25 g, 2.11 mmol) in methanol (2 ml) was added to a solution of (2*R*,3*R*,4*S*,5*R*,2'*R*,3'*R*,4'*S*,5'*R*)-2,2'-{*trans*-1,4-cyclohexanediylbis[imino(2-{[2-(1-methyl-1*H*-imidazol-4-yl)ethyl]amino}-9*H*-purine-6,9-diyl)]}bis[5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydro-3,4-furandiol] (2 g, 2.01 mmol) in methanol (8 ml) at 65 °C and stirred for 1 hour. The mixture was cooled to ambient temperature (seeded at 40 °C) then stirred for overnight, filtered, washed with methanol (2 x 2 ml) and dried *in vacuo* at 55°C. The solid was further recrystallised (twice) from methanol (2 x 5 volumes) to give the maleate salt (0.6 g).

Example 5

Formulations were prepared as follows:

(2*R*,3*R*,4*S*,5*R*,2'*R*,3'*R*,4'*S*,5'*R*)-2,2'-{*trans*-1,4-cyclohexanediylbis[imino(2-{[2-(1-methyl-1*H*-imidazol-4-yl)ethyl]amino}-9*H*-purine-6,9-diyl)]}bis[5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydro-3,4-furandiol] is referred to as "Compound A".

Cellobiose octaacetate is referred to as COA.

5

Formulation 5A

Component	Composition	Batch Quantity *
Compound A mono maleate salt hydrate (micronised)	0.08%w/w*	0.0800g*
Lactose Monohydrate, EP & USP & JP (Lactohale Inhalation grade)	To 100.0%w/w	To 100.00g

*stated as the free base. The weighed quantity was adjusted for the weighing factor for the batch of Compound A salt used.

10 Formulation 5B

Component	Composition	Batch Quantity *
Compound A mono maleate salt hydrate (micronised)	0.08%w/w*	0.0800g*
COA (Micronised)	2.0%w/w	2.000g
Lactose Monohydrate, EP & USP & JP (Lactohale Inhalation grade)	To 100.0%w/w	To 100.00g

*stated as the free base. The weighed quantity was adjusted for the weighing factor for the batch of Compound A salt used.

Formulation 5C

Component	Composition	Batch Quantity *
Compound A mono maleate salt hydrate (micronised)	0.08%w/w*	0.0800g*
COA (micronised)	10.0%w/w	10.000g

Component	Composition	Batch Quantity *
Lactose Monohydrate, EP & USP & JP (Lactohale Inhalation grade)	To 100.0%w/w	To 100.00g

*stated as the free base. The weighed quantity was adjusted for the weighing factor for the batch of Compound A salt used.

Formulation 5D

Component	Composition	Batch Quantity *
Compound A mono maleate salt hydrate (micronised)	4.0%w/w*	3.0000g*
Lactose Monohydrate, EP & USP & JP (Lactohale Inhalation grade)	To 100.0%w/w	To 75.00g

5 *stated as the free base. The weighed quantity was adjusted for the weighing factor for the batch of Compound A salt used.

Formulation 5E

Component	Composition	Batch Quantity *
Compound A mono maleate salt hydrate (micronised)	4.0%w/w*	3.0000g*
COA (micronised)	2.0%w/w	1.500g
Lactose Monohydrate, EP & USP & JP (Lactohale Inhalation grade)	To 100.0%w/w	To 75.000g

10 *stated as the free base. The weighed quantity was adjusted for the weighing factor for the batch of Compound A salt used.

Formulation 5F

Component	Composition	Batch Quantity *
Compound A mono maleate salt hydrate (micronised)	4.0%w/w*	3.0000g*
COA micronised	10.0%w/w	7.500g
Lactose Monohydrate, EP & USP & JP (Lactohale Inhalation grade)	To 100.0%w/w	To 75.000g

*stated as the free base. The weighed quantity was adjusted for the weighing factor for the batch of Compound A salt used.

- 5 The concentrations of Compound A mono maleate salt hydrate were chosen to be equivalent to dose of either 10 ug or 500 ug in a 12.5mg inhaled unit dose (Diskus®)

The method of manufacture was as follows:

10 Binary Mixtures

1. weigh the calculated quantities of micronised Compound A mono maleate salt ("drug substance") and lactose
2. transfer approximately half of the weighed lactose to a glass bottle
3. transfer the drug substance to the glass bottle
- 15 4. transfer the remaining lactose to the glass bottle
5. shake and tumble the glass bottle for approximately 2 minutes to form a pre-mix
6. transfer the pre-mix to the bowl of a high shear blender
7. blend the pre-mix for 10 minutes at 600rpm
8. sample the blend for homogeneity testing and transfer the remaining bulk to a glass
- 20 bottle ready for stability testing

Ternary Mixtures

1. weigh the calculated quantities of micronised drug substance, micronised COA, and lactose
- 25 2. transfer approximately half of the weighed lactose to a glass bottle
3. transfer the COA to the glass bottle
4. transfer the remaining lactose to the glass bottle

5. shake and tumble the glass bottle for approximately 2 minutes to form a pre-mix
6. transfer approximately half pre-mix to the bowl of a high shear blender
7. add the drug substance
8. transfer the remainder of the pre-mix to the bowl
- 5 9. blend for 10 minutes at 600rpm
10. sample the blend for homogeneity testing and transfer the remaining bulk to a glass bottle ready for stability testing

Each formulation was stored in a glass container and kept in an environmentally controlled
10 chamber.

Stability testing

For stability testing, samples of the formulations were stored under the following conditions:

- 25°C/60%RH ;
15 40°C/20%RH ;
40°C/75%RH ; and
50°C/Ambient Humidity

Testing of Chemical stability

- 20 Drug substance content and drug related impurities were analysed initially, and after 14 days and 28 days storage by HPLC using the following method:

Analytical Column: Zorbax SBC8 3.5 um 150mm x 4.6mm i.d.

Mobile Phase A: Water + 0.1% Trifluoroacetic acid (TFA)

- 25 Mobile Phase B: Acetonitrile + 0.1% TFA

Gradient:

Time	%A	%B
0	90	10
20	60	40
30	5	95
30.1	90	10
33	90	10

Flow Rate: 1.5ml/min

Temperature: 50°C

Detection: UV @ 260nm

Injection Volume: 50 ul of a 0.05 mg/ml solution

Run Time: 33 mins (data collection for 30mins)

5

Sample preparation:

Samples should contain approximately 0.05 mg/l Compound A mono maleate salt in 10% acetonitrile in water containing 0.025% TFA

The results are presented in Tables 1 and 2:

10

Table 1: Increase in drug related impurity content (%area/area) of binary and ternary blends containing 0.08%w/w Compound A (as mono maleate hydrate)

Storage Condition	14 day storage			28 day storage		
	0% COA (Form 5A)	2% COA (Form 5B)	10% COA (Form 5C)	0% COA (Form 5A)	2% COA (Form 5B)	10% COA (Form 5C)
25°C/60%RH	0.5	0.3	0.0	1.0	0.5	0.3
40°C/20%RH	0.4	0.1	0.0	0.7	0.6	0.4
50°C/ ambient H	0.8	0.4	0.4	1.1	0.8	1.2
40°C/75%RH (exposed)	7.4	6.2	3.9	11.9	8.8	7.0

Table 2: Increase in drug related impurity content (%area/area) of binary and ternary blends containing 4.0%w/w Compound A (as mono maleate hydrate)

15

Storage Condition	14 day storage			28 day storage		
	0% COA (Form 5D)	2% COA (Form 5E)	10% COA (Form 5F)	0% COA (Form 5D)	2% COA (Form 5E)	10% COA (Form 5F)
25°C/60%RH	0.1	0.1	0.0	0.2	0.1	0.1
40°C/20%RH	0.5	0.1	0.1	0.4	0.3	0.3
50°C ambient H	0.2	0.2	0.3	0.6	0.5	0.5

40°C/75%RH (exposed)	0.3	0.3	0.2	1.3	0.5	0.5
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There were increases in drug related impurities on storage under all conditions. Especially significant degradation was seen in blends containing 0.08%w/w Compound A stored at 40°C/75%RH for 28 days. The extent of degradation was reduced in each case by the inclusion of cellobiose octaacetate.

Testing of physical stability

Physical stability of formulations may be determined as follows:

10

The blends are added to blister packs, of the type described in patent US 5,873,360, using filling methods according to procedures outlined in WO 00/71419 (Glaxo Group Limited). Each blister contains approximately 12.5mg of the blend.

15

For rapid screening, the pockets in one portion of the blister packs are then pierced with a 0.75mm pin and the blister packs are loaded into a Diskus® device.

The loaded Diskus® devices containing blends are placed in accelerated stability test environment at 40°C / 75% relative humidity for a period of 48 or 72 hours.

20

For longer term screening, another set of blister packs are loaded into a Diskus® device without piercing. Those Diskus® devices containing blends are placed in accelerated stability test environment at 40°C / 75% relative humidity for period of one month.

25

Twin stage impinger analysis is performed (at 60 l/min) on the blends before storage and after storage by the method detailed in the British Pharmacopoeia (Method A) with the exception that a USP throat is substituted for the glass one and is sealed to the stage 1 jet tube using a rubber gasket. The devices are tested pre and post storage by discharging the contents of 14 blisters into the Twin Stage Impinger apparatus. The fine particle fraction can then be determined.

30

Similar testing may also be performed with an Andersen cascade impactor used at 60 l/min flow rate.

Example 6

- 5 Stearate containing formulations may be prepared as follows, using methods analogous to those presented above for ternary mixtures containing COA.

Formulation 6A

Component	Composition	Batch Quantity *
Compound A mono maleate salt hydrate (micronised)	0.08%w/w*	0.0800g*
Calcium Stearate	1.0%w/w	1.000g
Lactose Monohydrate, EP & USP & JP (Lactohale Inhalation grade)	To 100.0%w/w	To 100.00g

*stated as the free base. The weighed quantity is adjusted for the weighing factor for the batch of Compound A salt used.

10

Formulation 6B

Component	Composition	Batch Quantity *
Compound A mono maleate salt hydrate (micronised)	0.08%w/w*	0.0800g*
Calcium Stearate	2.0%w/w	2.000g
Lactose Monohydrate, EP & USP & JP (Lactohale Inhalation grade)	To 100.0%w/w	To 100.00g

*stated as the free base. The weighed quantity is adjusted for the weighing factor for the batch of Compound A salt used.

15

Formulation 6C

Component	Composition	Batch Quantity *
Compound A mono maleate salt hydrate (micronised)	0.08%w/w*	0.0800g*
Magnesium Stearate	1.0%w/w	1.000g
Lactose Monohydrate, EP & USP & JP (Lactohale Inhalation grade)	To 100.0%w/w	To 100.00g

*stated as the free base. The weighed quantity is adjusted for the weighing factor for the batch of Compound A salt used.

5 Formulation 6D

Component	Composition	Batch Quantity *
Compound A mono maleate salt hydrate (micronised)	0.08%w/w*	0.0800g*
Magnesium Stearate	2.0%w/w	2.000g
Lactose Monohydrate, EP & USP & JP (Lactohale Inhalation grade)	To 100.0%w/w	To 100.00g

*stated as the free base. The weighed quantity is adjusted for the weighing factor for the batch of Compound A salt used.

Formulation 6E

Component	Composition	Batch Quantity *
Compound A mono maleate salt hydrate (micronised)	4.0%w/w*	3.0000g*
Calcium Stearate	1.0%w/w	0.750g
Lactose Monohydrate, EP & USP & JP (Lactohale Inhalation grade)	To 100.0%w/w	To 75.000g

*stated as the free base. The weighed quantity is adjusted for the weighing factor for the batch of Compound A salt used.

Formulation 6F

Component	Composition	Batch Quantity *
Compound A mono maleate salt hydrate (micronised)	4.0%w/w*	3.0000g*
Calcium Stearate	2.0%w/w	1.500g
Lactose Monohydrate, EP & USP & JP (Lactohale Inhalation grade)	To 100.0%w/w	To 75.000g

- 5 *stated as the free base. The weighed quantity is adjusted for the weighing factor for the batch of Compound A salt used.

Formulation 6G

Component	Composition	Batch Quantity *
Compound A mono maleate salt hydrate (micronised)	4.0%w/w*	3.0000g*
Magnesium Stearate	1.0%w/w	0.750g
Lactose Monohydrate, EP & USP & JP (Lactohale Inhalation grade)	To 100.0%w/w	To 75.000g

- 10 *stated as the free base. The weighed quantity is adjusted for the weighing factor for the batch of Compound A salt used.

Formulation 6H

Component	Composition	Batch Quantity *
Compound A mono maleate salt hydrate (micronised)	4.0%w/w*	3.0000g*
Magnesium Stearate	2.0%w/w	1.500g

Component	Composition	Batch Quantity *
Lactose Monohydrate, EP & USP & JP (Lactohale Inhalation grade)	To 100.0%w/w	To 75.000g

*stated as the free base. The weighed quantity is adjusted for the weighing factor for the batch of Compound A salt used.

Example 7

5

Binary formulations were prepared in the range described in Example 5.

Physical stability of formulations was determined as follows:

10 The blends were filled to blister packs, of the type described in patent US 5,873,360, using filling methods according to procedures outlined in WO 00/71419 (Glaxo Group Limited). Each blister contains approximately 12.5mg of the blend.

Blister packs were stored under the following conditions:

5°C/Ambient RH
15 30°C/65% RH
40°C/75% RH

Initially and after 28 days, 2 months and 3 months, blister packs stored at the appropriate condition were loaded into a Diskus® device.

20

Particle Size Distribution by Inertial Impaction was performed by Next Generation Impactor (NGI) at 60 l/min flow rate technique by discharging the contents of an appropriate number of blisters into the apparatus. The fine particle fraction was then determined.

25 Example 8

Magnesium stearate formulations were prepared as follows:

Formulation 8A

Component	Composition	Batch Quantity *
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Compound A mono maleate salt hydrate (micronised)	0.4%w/w*	0.8000g*
Magnesium Stearate	0.5%w/w	1.000g
Lactose Monohydrate, EP & USP & JP (Lactohale Inhalation grade)	To 100.0%w/w	To 200.000g

*stated as the free base. The weighed quantity is adjusted for the weighing factor for the batch of Compound A salt used.

The concentrations of Compound A mono maleate salt hydrate were chosen to be equivalent to dose of 50 ug in a 12.5mg inhaled unit dose (Diskus®).

5

The method of manufacture was as follows:

1. weigh the required quantities of micronised drug substance, magnesium stearate and lactose
2. transfer approximately a third of the weighed lactose to the blender
- 10 3. transfer a third of the weighed lactose to the bag containing magnesium stearate container, and pre-blend manually
4. transfer the magnesium stearate/lactose pre-blend into the blender
5. transfer the remaining lactose to the blender
6. blend for 10 minutes at 600rpm
- 15 7. discharge the magnesium stearate/lactose blend into a stainless steel bowl
8. transfer approximately a third of the magnesium stearate/lactose blend into the blender
9. transfer a third of the magnesium stearate/lactose blend into the bag containing the drug substance, and pre-blend manually
- 20 10. transfer the magnesium stearate/lactose/drug substance blend to the blender
11. transfer the remaining magnesium stearate/lactose blend to the blender
12. blend for 10 minutes at 600rpm
13. discharge the blend into a stainless steel bowl before transferring to suitable size labelled amber glass container.

25

Stability testing

For stability testing, samples of the formulations were stored under the following conditions:
5°C/Ambient RH

30°C/65% RH

40°C/20% RH

40°C/75% RH

50°C/Ambient RH

5

Testing of chemical stability

Drug substance content and drug-related impurity content were analysed initially, and after 28 days, 2 months and 3 months storage by HPLC.

Table 1 Increase in drug-related impurity content (%area/area) of binary and ternary blends containing 0.4%w/w Compound A (as mono maleate hydrate)

10

	28-day storage	2-month storage	3-month storage
30°C/65% RH	0.2	0.3	-
40°C/20% RH	0.1	0.3	0.2
40°C/75% RH	0.4	0.6	0.2
50°C/Amb RH	0.4	0.3	0.5

Key:

- : Not tested

Data demonstrate an improvement in chemical stability in ternary blends containing magnesium stearate over binary formulations; reduction in the generation of impurities was observed. This was especially demonstrated in samples exposed to high relative humidities.

15

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.

20

Throughout the specification and the claims which follow, unless the context requires otherwise, the word 'comprise', and variations such as 'comprises' and 'comprising', will be understood to imply the inclusion of a stated integer or step or group of integers but not to the exclusion of any other integer or step or group of integers or steps.

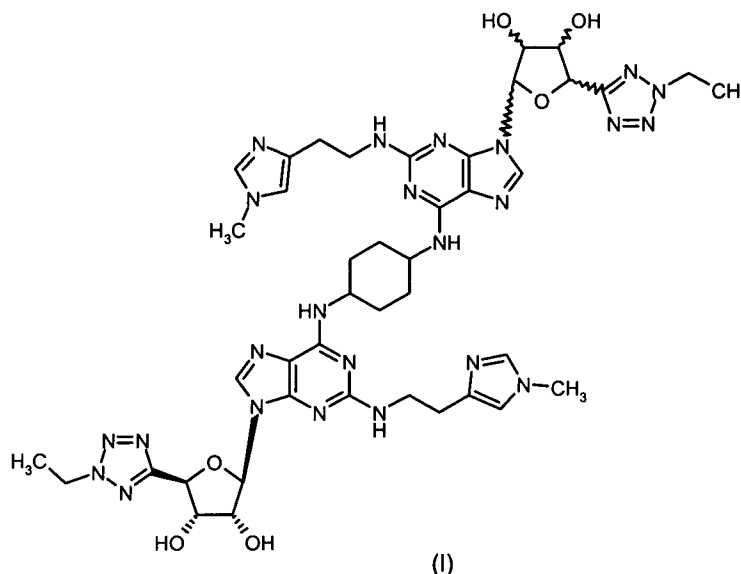
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The application of which this description and claims forms part may be used as a basis for priority in respect of any subsequent application. The claims of such subsequent application may be directed to any feature or combination of features described herein. They may take

the form of product, composition, process, or use claims and may include, by way of example and without limitation, the following claims:

Claims

1. A pharmaceutical dry powder composition comprising (i) a compound of formula (I):



or a salt or solvate thereof in solid particulate form, (ii) one or more pharmaceutically acceptable particulate carriers and (iii) one or more stabilising excipients.

2. A composition according to claim 1, wherein the stereochemistry of both tetrahydrofuran rings is the same.
3. A composition according to claim 1 or claim 2, wherein the cyclohexyl ring is in the *trans* configuration.
4. A composition according to claim 1 or claim 2, wherein the compound of formula (I) is (2*R*,3*R*,4*S*,5*R*,2'*R*,3'*R*,4'*S*,5'*R*)-2,2'-[*trans*-1,4-cyclohexanediyldiylbis[imino(2-{[2-(1-methyl-1*H*-imidazol-4-yl)ethyl]amino}-9*H*-purine-6,9-diyl)]]bis[5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydro-3,4-furandiol].
5. A composition according to any one of claims 1 to 4, wherein the compound of formula (I) is in the form of the free base.
6. A composition according to any one of claims 1 to 4, wherein the compound of formula (I) is in the form of the mono maleate salt.

7. A composition according to any one of claims 1 to 4, wherein the compound of formula (I) is in the form of the mono biphenylsulfonate salt
8. A composition according to any one of claims 1 to 4, wherein the compound of formula (I) is in the form of the mono camphorsulfonate salt.
- 5 9. A composition according to any one of claims 1 to 8 wherein the one or more pharmaceutically acceptable carriers comprises a reducing sugar.
10. A composition according to claim 9 wherein the reducing sugar is selected from lactose, maltose and glucose.
- 10 11. A composition according to claim 9 wherein the reducing sugar is lactose monohydrate.
12. A composition according to claim 9 wherein the reducing sugar is anhydrous lactose.
13. A composition according to any one of claims 1 to 12, wherein the stabilising excipient is a stearate.
14. A composition according to claim 13, wherein the stearate is magnesium stearate.
- 15 15. A composition according to claim 14, wherein the stearate is calcium stearate.
16. A composition according to any one of claims 1 to 12, wherein the stabilising excipient is a derivatised carbohydrate.
17. A composition according to claim 16, wherein derivatised carbohydrate is cellobiose octaacetate.
- 20 18. A composition according to any one of claims 1 to 17 wherein one or more stabilising excipients are in particulate form.
19. A composition according to any one of claims 1 to 18 further comprising one or more other therapeutic agents.
20. A dry powder inhaler comprising a formulation according to any one of claims 1 to 19.

21. A composition according to any one of claims 1 to 19 for use in the treatment of patients with inflammatory conditions who are susceptible to leukocyte-induced tissue damage.
- 5 22. Use of a composition according to any one of claims 1 to 19 in the manufacture of a medicament for the treatment of patients with inflammatory conditions who are susceptible to leukocyte-induced tissue damage.
- 10 23. A method for the treatment of a human or animal subject with an inflammatory condition and/or allergic condition who is susceptible to leukocyte-induced tissue damage, which method comprises administering to said human or animal subject a safe and effective amount of a composition according to any one of claims 1 to 19.
24. A process for the preparation of a composition according to any one of claims 1 to 18 comprising intimately mixing (i) a compound of formula (I), or a salt or solvate thereof, (ii) one or more pharmaceutically acceptable particulate carriers and (iii) one or more stabilising excipients.
- 15 25. A method of stabilising a pharmaceutical composition comprising a compound of formula (I), or a salt or solvate thereof and one or more pharmaceutically acceptable particulate carriers by the addition of one or more stabilising excipients.
- 20 26. Use of one or more stabilising excipients to stabilise a composition comprising a compound of formula (I), or a salt or solvate thereof and one or more pharmaceutically acceptable particulate carriers.

Figure 1

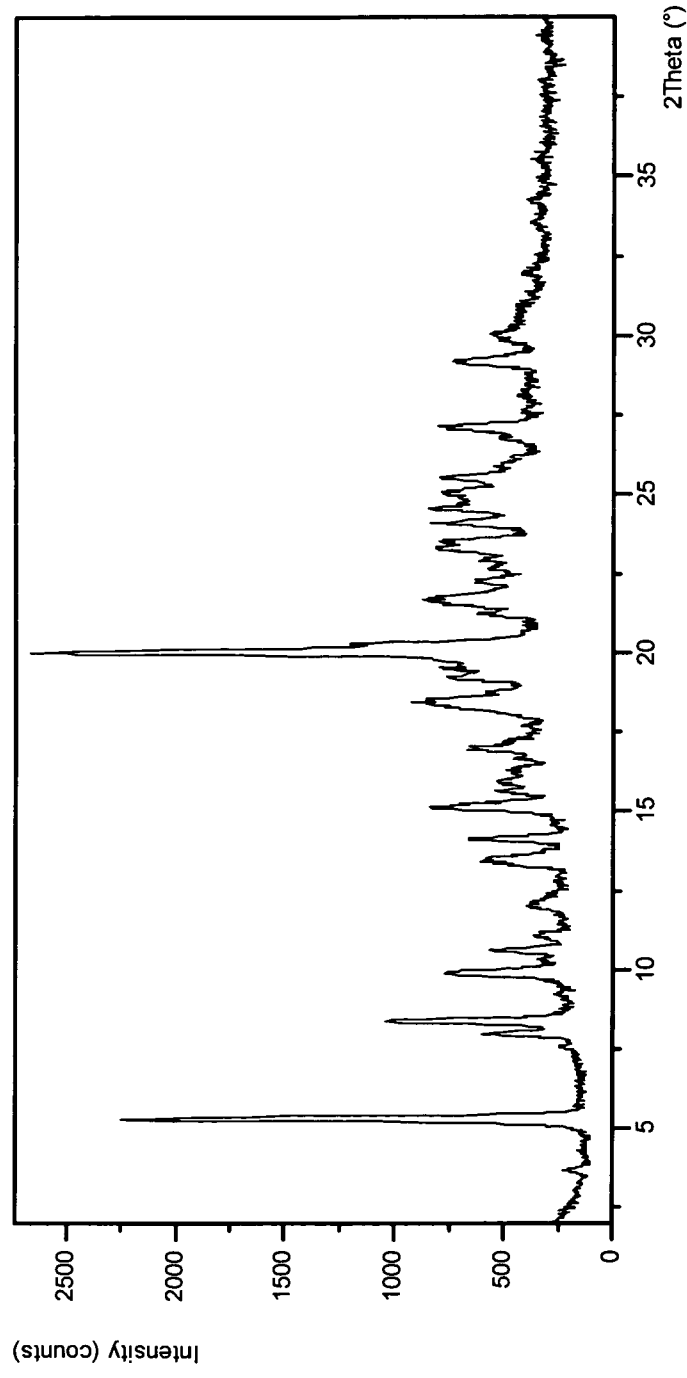


Figure 2

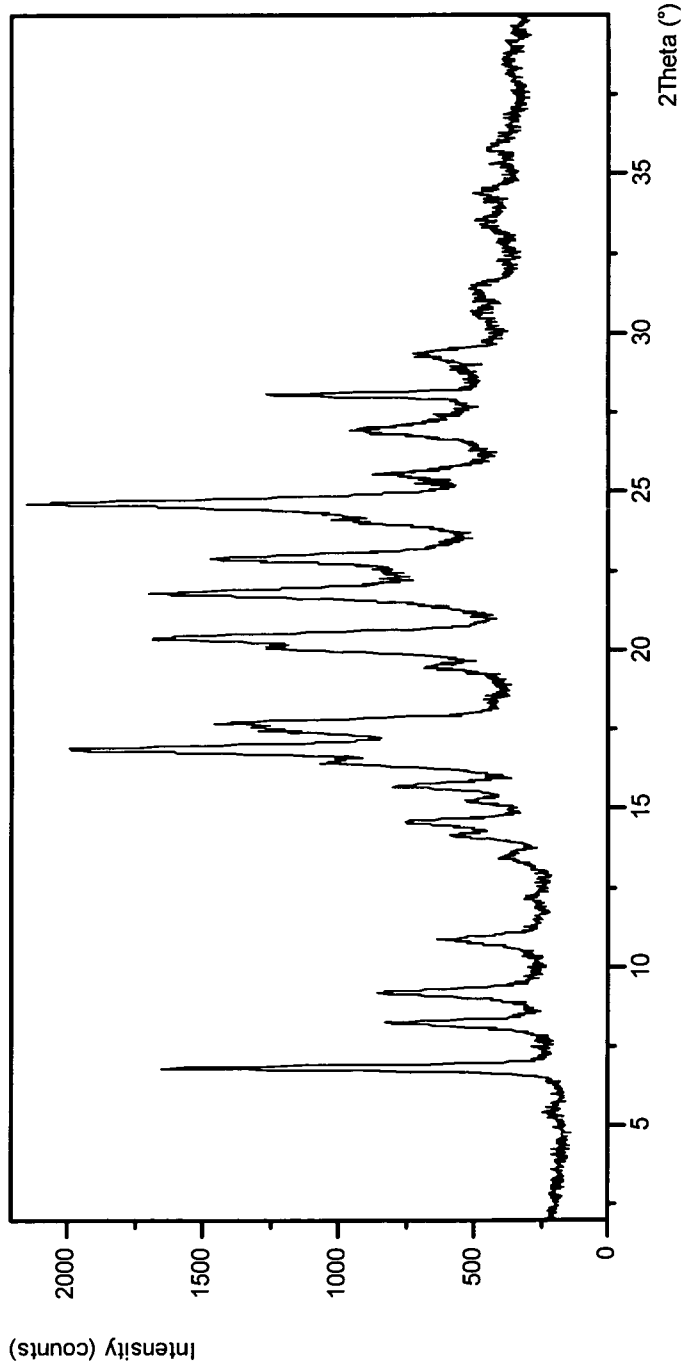


Figure 3

