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(54) Title: COMBINATION FOR TREATING INFLAMMATORY DISEASES

(57) Abstract: The present invention relates to a pharmaceutical composition containing a combination of a leukotriene receptor antagonist and a histamine H4 receptor antagonist, wherein the leukotriene receptor antagonist is particularly selected from montelukast, pranlukast and zafirlukast. The composition can be used for treating an allergic and/or inflammatory condition, such as seasonal and perennial allergic rhinitis, non-allergic rhinitis, asthma, COPD, sinusitis, colds, dermatitis and uriticaria.

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# Combination for treating inflammatory diseases

The present invention relates to a pharmaceutical composition containing a combination of a leukotriene antagonist and a histamine $H_4$ receptor antagonist. The composition can be used in the treatment of inflammatory and/or allergic conditions.

Leukotrienes are mediators which belong to the group of eicosanoids. They are derivatives of arachidonic acid, a fatty acid which is a constituent of membrane phospholipids. The leukotrienes are formed from arachidonic acid via 5-lipoxygenase (5-LOX). At the present time, only the pathogenetically relevant role of the so-called cysteinyl-leukotrienes, to which $LTC_4$, $LTD_4$ and $LTE_4$ belong, has been confirmed. The leukotrienes are very potent substances, producing a variety of biological effects when present in the nanomolar or picomolar concentration range. They have been implicated in a variety of disease states, including allergic rhinitis and adult respiratory distress syndrome. Leukotriene receptor (LT) antagonists, such as montelukast, have been shown to antagonize the effects of cysteinyl-leukotrienes, particularly $LTD_4$.

The histamine $H_4$ receptors has recently been identified. It is a 390 amino-acid, seven-transmembrane G protein coupled receptor with approximately 40 % homology to the histamine $H_3$ receptor. In contrast to the $H_3$ receptor which is primarily located in the brain, the $H_4$ receptor is expressed at greater levels in neutrophils and mast cells, among other inflammatory cells.

The present invention provides a pharmaceutical composition containing a combination of a leukotriene antagonist and a histamine $H_4$ receptor antagonist.

It is believed that this combination gives a synergistic clinical effect in relief of the symptoms of allergic rhinitis, asthma and other diseases involving airway

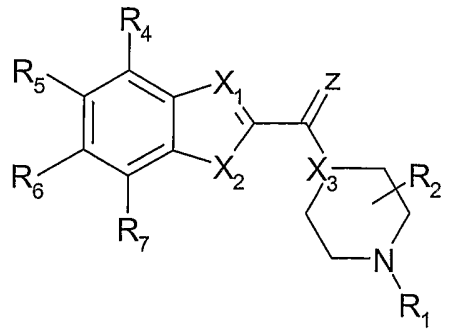
inflammation such as COPD, and avoids or reduces certain side effects associated with treatments in current use such as histamine $H_1$ receptor antagonists/pseudoephedrine combinations or steroids. Histamine and leukotrienes are major mediators in allergy manifest in diseases such as allergic rhinitis and potentially in asthma. Surprisingly and unexpectedly, histamine H4 receptor antagonists and leukotriene receptor antagonists produce synergistic relief of the signs and symptoms of allergic rhinitis, as assessed by methods such as nasal congestion, nasal and ocular itching and lachrymation, and also in other measures of airway inflammation such as allergic lung inflammation.

The suitability of the histamine $H_4$ receptor antagonist and the leukotriene antagonist can be readily determined by evaluation of their potency and selectivity followed by evaluation of their toxicity, pharmacokinetics (absorption, metabolism, distribution and elimination), etc in accordance with standard pharmaceutical practice. Suitable compounds are those that are potent and selective, have no significant toxic effect at the therapeutic dose, and preferably are bioavailable following administration.

Potency of the histamine $H_4$ receptor antagonist can be determined according to the assays known to the skilled person, Preferred histamine $H_4$ receptor ligands have a potency, expressed as one or other well-accepted measure of affinity for the human $H_4$ receptor e.g. $K_i$ of 15 μM or less. Suitable assays and references are described in the experimental section hereafter.

A background on histamine $H_4$ receptor antagonists can be found in *Expert Opin. Ther. Patents (2003)* ***13****(6):851-865*. Examples of histamine $H_4$ receptor antagonists for use in the present invention are described in WO02/072548 (Ortho Mc Neil) and *J. Med. Chem.,* 2003; 46(19), pp/ 3957-3960.

Thus, suitable $H_4$ antagonists for use in the present invention are compounds having formula (I):



(I)

wherein $R_1$ is $R_a$, $R_aR_b$-, $R_a$-O-$R_b$-, or $(R_c)(R_d)$N-$R_b$-, where $R_a$ is H, $C_1$-$C_{10}$ alkyl, $C_3$-$C_8$ alkenyl, $C_3$-$C_8$ cycloalkyl, $C_2$-$C_5$ heterocyclic radical, or phenyl;

where $R_b$ is $C_1$-$C_8$ alkylene, $C_3$-$C_8$ alkenylene, $C_3$-$C_8$ cycloalkylene, bivalent $C_3$-$C_8$ heterocyclic radical, or phenylene; and $R_c$ and $R_d$ are each independently H, $C_1$-$C_8$ alkyl, $C_2$-$C_8$ alkenyl, $C_3$-$C_8$ cycloalkyl, or phenyl;

$R_2$ is ortho or meta, and is methyl or H;

$X_1$ is $CR_3$;

$R_3$ is F, Cl, Br, $R_f$, $R_fR_g$-, $R_f$-O-$R_g$-, or $(R_h)(R_j)$N-$R_g$-, where $R_f$ is H, $C_1$-$C_6$ alkyl, $C_2$-$C_6$ alkenyl, $C_3$-$C_6$ cycloalkyl, $C_2$-$C_5$ heterocyclic radical, or phenyl; where $R_g$ is $C_1$-$C_6$ alkylene, $C_2$-$C_6$ alkenylene, $C_3$-$C_6$ cycloalkylene, bivalent $C_3$-$C_6$ heterocyclic radical, or phenylene; and $R_h$ and $R_j$ are each independently H, $C_1$-$C_6$ alkyl, $C_2$-$C_6$ alkenyl, $C_3$-$C_6$ cycloalkyl, or phenyl;

$X_2$ is $NR_e$ or O, provided that $X_2$ is $NR_e$ where $X_1$ is N; $R_e$ is H or $C_1$-$C_6$ alkyl;

$X_3$ is N;

Z is =O or =S;

each of $R_4$ and $R_6$ is independently H, F, Cl, Br, I, COOH, OH, nitro, amino, cyano, $C_1$-$C_4$ alkoxy or $C_1$-$C_4$ alkyl;

$R_5$ is H, F, Cl, Br, I, (C=O)$R_j$, OH, nitro, $NR_jR_k$, cyano, -$OCH_2$-Ph, $C_1$-$C_4$ alkoxy, or $C_1$-$C_4$ alkyl;

$R_7$ is H, F, Cl, Br, I, (C=O)$R_m$, OH, nitro, $NR_lR_m$, cyano, $C_1$-$C_4$ alkoxy, or $C_1$-$C_4$ alkyl;

wherein each of $R_j$, $R_k$, $R_l$ and $R_m$ is independently selected from H, $C_1$-$C_6$ alkyl, hydroxy, phenyl, benzyl, phenethyl and $C_1$-$C_6$ alkoxy;

each of the above hydrocarbyl or heterocyclic groups being independently and optionally substituted with between 1 and 3 substituents selected from $C_1$-$C_3$ alkyl, halo, hydroxy, amino and $C_1$-$C_3$ alkoxy;

provided at least one of $R_1$, $R_2$, $R_3$, $R_4$, $R_5$, $R_6$ and $R_7$ is other than H when Z is O;

or a pharmaceutically acceptable salt, ester or amide thereof. For methods of preparation of the above compounds WO02/072548 is referred to.

In the compound of formula (I) preferably

$R_1$ is H, methyl or ethyl;

where $R_1$ is H, $R_2$ is methyl or H, otherwise $R_2$ is H;

$X_1$ is $CR_3$, where $R_3$ is H, F, Cl or Br;

$X_2$ is NH or N($C_1$-$C_3$ alkyl);

Z is =O or =S;

each or $R_4$ and $R_6$ is independently H, OH, $C_1$-$C_4$ alkyl, $C_1$-$C_4$ alkoxy, cyano, or amino;

$R_5$ is H, F, Cl, Br, COOH, OH, amino, cyano, $C_1$-$C_4$ alkoxy or $C_1$-$C_4$ alkyl; and

$R_7$ is H, F, Cl, Br, $C_1$-$C_4$ alkyl, $C_1$-$C_4$ alkoxy, cyano or amino; provided that at least one of $R_5$ and $R_7$ is not H.

In the present description the following terms have the following meanings:

"Alkyl" includes straight chain and branched hydrocarbons. Alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, 1-methylpropyl, pentyl, isopentyl, sec-pentyl, hexyl, heptyl, octyl, and so on.

"Alkenyl" includes straight chain and branched hydrocarbon radicals as above with at least one carbon-carbon double bond (sp2). Alkenyls include ethenyl (or vinyl), prop-1-enyl, prop-2-enyl (or allyl), isopropenyl (or 1methylvinyl), but-1-enyl, but-2-enyl, butadienyls, pentenyl, hexa-2,4-dienyl, and so on.

"Alkynyl"include straight chain and branched hydrocarbon radicals as above with at least one carbon-carbon triple bond (sp). Alkynyls include ethynyl,

propynyls, butynyls, and pentynyls. Hydrocarbon radicals having a mixture of double bonds and triple bonds, such as 2-penten-4-ynyl, are grouped as alkynyls herein.

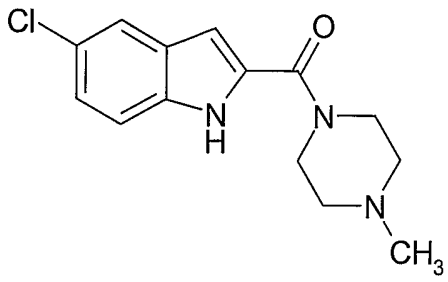
"Alkoxy" includes a straight chain or branched alkyl group with a terminal oxygen linking the alkyl group to the rest of the molecule. Alkoxy includes methoxy, ethoxy, propoxy, isopropoxy, butoxy, t-butoxy, pentoxy and so on.

"Cycloalkyl" includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, and so on.

"Heterocyclic radicals" include aromatic and nonaromatic rings having carbon atoms and at least one heteroatom (O, S, N) or heteroatom moiety ($SO_2$, CO, CONH, COO) in the ring. Unless otherwise indicated, a heterocyclic radical may have a valence connecting it to the rest of the molecule through a carbon atom, such as 3-furyl or 2-imidazolyl, or through a heteroatom, such as N-piperidyl or 1-pyrazolyl. Examples of heterocyclic radicals include thiazoylyl, furyl, pyranyl, isobenzofuranyl, pyrrolyl, imidazolyl, pyrazolyl, isothiazolyl, isoxazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolizinyl, isoindolyl, indolyl, indazolyl, purinyl, quinolyl, furazanyl, pyrrolidinyl, pyrrolinyl, imdazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperidyl, piperazinyl, indolinyl, and morpholinyl. For example, preferred heterocyclic radicals for Ra include morpholinyl, piperazinyl, pyrrolidinyl, pyridyl, cyclohexylimino, cycloheptylimino, and more preferably, piperidyl.

"Halo"includes fluoro, chloro, bromo, and iodo, and preferably fluoro or chloro.

A particular suitable compound to be used in the present invention is;



(5-chloro-1H-indol-2-yl)-(4-methyl-piperazin-1-yl)-methanone.

Preferred leukotriene receptor antagonists are:

montelukast (Singulair®, CAS 151767-02-1): 1-((R-(3-(2-(7-chloro-2-quinolinyl)ethenyl)phenyl)-3-(2-(2-hydroxy-2-propyl)phenyl)thio)methyl)cyclopropoaneacetate, and pharmaceutically acceptable salts thereof, in particular the sodium salt, which is described in US 5,565,473;

pranlukast (CAS 103177-37-3); N-[4-oxo-2-( 1 H-tetrazol-5 -yl)-4H- 1 -benzopyran-8-yl] -p-(4-phenylbutoxy) benzamide and pharmaceutically acceptable salts thereof, which is described in EP 173,516;

zafirlukast (CAS 107753-78-6) Cyclopentyl-3 -[2-methoxy4-[(o-tol ylsulfonyl)carbamoyl] - benzyl]- 1 -methylindole-5-carbamate and pharmaceutically acceptable salts thereof, which is described in EP 199,543.

Most preferably the histamine $H_4$ receptor antagonist is combined with montelukast, in particular montelukast sodium.

The ideal ratio of these therapeutic principles is that which delivers free concentrations of each compound which are active at their respective receptors i.e. typically in the range of 1-5 x their respective pA2 values (Smith D, Jones B and Walker D (1996) "Design of drugs involving the concepts and theories of drug metabolism and pharmacokinetics", *Medicinal Research Reviews*, 16(3), 243-266) or other functionally equivalent measures of potency (such as $pK_b$ or Ki).

The combination according to the invention can be used for the treatment of a patient (a mammal, in particular a human being) suffering from a $H_4$ mediated disease and/or leukotriene mediated disease. In particular the combination is used for treating a patient suffering from an allergic and/or inflammatory condition. Examples of such allergic and/or inflammatory conditions are seasonal and perennial allergic rhinits, non-allergic rhinitis, asthma, chronic obstructive pulmonary disease (COPD), sinusitis, colds, dermatitis and uriticaria.

It is to be appreciated that all references herein to treatment include curative, palliative and prophylactic treatment.

The histamine $H_4$ receptor antagonist and the leukotriene receptor antagonist according to the invention can be administered sequentially, separately or simultaneously. To this effect, the compounds can be formulated as a single dose, as separate doses or as a kit.

Thus, according to a further aspect of the invention, there is provided a kit containing a leukotriene antagonist and a histamine $H_4$ receptor antagonist for sequential, separate or simultaneous use in the treatment of inflammatory diseases.

The kit of the invention comprises two or more separate pharmaceutical compositions, and means for separately retaining said compositions, such as a container, divided bottle, or divided foil packet. An example of such a kit is the familiar blister pack used for the packaging of tablets, capsules and the like.

The kit of the invention is particularly suitable for administering different dosage forms, for example, oral and parenteral, for administering the separate compositions at different dosage intervals, or for titrating the separate compositions against one another. To assist compliance, the kit typically

comprises directions for administration and may be provided with a so-called memory aid.

Pharmaceutically acceptable salts of the leukotriene antagonists and the $H_4$ antagonists to be used in the combination of the invention include the acid addition and base salts thereof.

Suitable acid addition salts are formed from acids which form non-toxic salts. Examples include the acetate, aspartate, benzoate, besylate, bicarbonate/carbonate, bisulphate/sulphate, borate, camsylate, citrate, edisylate, esylate, formate, fumarate, gluceptate, gluconate, glucuronate, hexafluorophosphate, hibenzate, hydrochloride/chloride, hydrobromide/bromide, hydroiodide/iodide, isethionate, lactate, malate, maleate, malonate, mesylate, methylsulphate, naphthylate, 2-napsylate, nicotinate, nitrate, orotate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, saccharate, stearate, succinate, tartrate, tosylate and trifluoroacetate salts.

Suitable base salts are formed from bases which form non-toxic salts. Examples include the aluminium, arginine, benzathine, calcium, choline, diethylamine, diolamine, glycine, lysine, magnesium, meglumine, olamine, potassium, sodium, tromethamine and zinc salts.

For a review on suitable salts, see "Handbook of Pharmaceutical Salts: Properties, Selection, and Use" by Stahl and Wermuth (Wiley-VCH, Weinheim, Germany, 2002).

Compounds used in the combination of the invention intended for pharmaceutical use may be administered as crystalline or amorphous products. They may be obtained, for example, as solid plugs, powders, or films by methods such as precipitation, crystallization, freeze drying, spray drying, or evaporative drying. Microwave or radio frequency drying may be used for this purpose.

They may be administered as a formulation in association with one or more pharmaceutically acceptable excipients. The term "excipient" is used herein to describe any ingredient other than the compound(s) of the invention. The choice of excipient will to a large extent depend on factors such as the particular mode of administration, the effect of the excipient on solubility and stability, and the nature of the dosage form.

Pharmaceutical compositions suitable for the delivery of compounds of the present invention and methods for their preparation will be readily apparent to those skilled in the art. Such compositions and methods for their preparation may be found, for example, in 'Remington's Pharmaceutical Sciences', 19th Edition (Mack Publishing Company, 1995).

The compounds in the combination of the invention may be administered orally. Oral administration may involve swallowing, so that the compound enters the gastrointestinal tract, or buccal or sublingual administration may be employed by which the compound enters the blood stream directly from the mouth.

Formulations suitable for oral administration include solid formulations such as tablets, capsules containing particulates, liquids, or powders, lozenges (including liquid-filled), chews, multi- and nano-particulates, gels, solid solution, liposome, films (including muco-adhesive), ovules, sprays and liquid formulations.

Liquid formulations include suspensions, solutions, syrups and elixirs. Such formulations may be employed as fillers in soft or hard capsules and typically comprise a carrier, for example, water, ethanol, polyethylene glycol, propylene glycol, methylcellulose, or a suitable oil, and one or more emulsifying agents and/or suspending agents. Liquid formulations may also be prepared by the reconstitution of a solid, for example, from a sachet.

The compounds in the combination of the invention may also be used in fast-dissolving, fast-disintegrating dosage forms such as those described in Expert Opinion in Therapeutic Patents, 11 (6), 981-986 by Liang and Chen (2001).

For tablet dosage forms, depending on dose, the drug may make up from 1 wt% to 80 wt% of the dosage form, more typically from 5 wt% to 60 wt% of the dosage form. In addition to the drug, tablets generally contain a disintegrant. Examples of disintegrants include sodium starch glycolate, sodium carboxymethyl cellulose, calcium carboxymethyl cellulose, croscarmellose sodium, crospovidone, polyvinylpyrrolidone, methyl cellulose, microcrystalline cellulose, lower alkyl-substituted hydroxypropyl cellulose, starch, pregelatinised starch and sodium alginate. Generally, the disintegrant will comprise from 1 wt% to 25 wt%, preferably from 5 wt% to 20 wt% of the dosage form.

Binders are generally used to impart cohesive qualities to a tablet formulation. Suitable binders include microcrystalline cellulose, gelatin, sugars, polyethylene glycol, natural and synthetic gums, polyvinylpyrrolidone, pregelatinised starch, hydroxypropyl cellulose and hydroxypropyl methylcellulose. Tablets may also contain diluents, such as lactose (monohydrate, spray-dried monohydrate, anhydrous and the like), mannitol, xylitol, dextrose, sucrose, sorbitol, microcrystalline cellulose, starch and dibasic calcium phosphate dihydrate.

Tablets may also optionally comprise surface active agents, such as sodium lauryl sulfate and polysorbate 80, and glidants such as silicon dioxide and talc. When present, surface active agents may comprise from 0.2 wt% to 5 wt% of the tablet, and glidants may comprise from 0.2 wt% to 1 wt% of the tablet.

Tablets also generally contain lubricants such as magnesium stearate, calcium stearate, zinc stearate, sodium stearyl fumarate, and mixtures of magnesium stearate with sodium lauryl sulphate. Lubricants generally

comprise from 0.25 wt% to 10 wt%, preferably from 0.5 wt% to 3 wt% of the tablet.

Other possible ingredients include anti-oxidants, colourants, flavouring agents, preservatives and taste-masking agents.

Exemplary tablets contain up to about 80% drug, from about 10 wt% to about 90 wt% binder, from about 0 wt% to about 85 wt% diluent, from about 2 wt% to about 10 wt% disintegrant, and from about 0.25 wt% to about 10 wt% lubricant.

Tablet blends may be compressed directly or by roller to form tablets. Tablet blends or portions of blends may alternatively be wet-, dry-, or melt-granulated, melt congealed, or extruded before tabletting. The final formulation may comprise one or more layers and may be coated or uncoated; it may even be encapsulated.

The formulation of tablets is discussed in "Pharmaceutical Dosage Forms: Tablets, Vol. 1", by H. Lieberman and L. Lachman, Marcel Dekker, N.Y., N.Y., 1980 (ISBN 0-8247-6918-X).

Solid formulations for oral administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release.

Suitable modified release formulations for the purposes of the invention are described in US Patent No. 6,106,864. Details of other suitable release technologies such as high energy dispersions and osmotic and coated particles are to be found in Verma *et al*, Pharmaceutical Technology On-line, 25(2), 1-14 (2001). The use of chewing gum to achieve controlled release is described in WO 00/35298.

The compounds in the combination of the invention may also be administered directly into the blood stream, into muscle, or into an internal organ. Suitable means for parenteral administration include intravenous, intraarterial, intraperitoneal, intrathecal, intraventricular, intraurethral, intrasternal, intracranial, intramuscular and subcutaneous. Suitable devices for parenteral administration include needle (including microneedle) injectors, needle-free injectors and infusion techniques.

Parenteral formulations are typically aqueous solutions which may contain excipients such as salts, carbohydrates and buffering agents (preferably to a pH of from 3 to 9), but, for some applications, they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water.

The preparation of parenteral formulations under sterile conditions, for example, by lyophilisation, may readily be accomplished using standard pharmaceutical techniques well known to those skilled in the art.

The solubility of compounds used in the preparation of parenteral solutions may be increased by the use of appropriate formulation techniques, such as the incorporation of solubility-enhancing agents.

Formulations for parenteral administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release. Thus compounds of the invention may be formulated as a solid, semi-solid, or thixotropic liquid for administration as an implanted depot providing modified release of the active compound. Examples of such formulations include drug-coated stents and PGLA microspheres.

The compounds in the combination of the invention may also be administered topically to the skin or mucosa, that is, dermally or transdermally. Typical formulations for this purpose include gels, hydrogels, lotions, solutions,

creams, ointments, dusting powders, dressings, foams, films, skin patches, wafers, implants, sponges, fibres, bandages and microemulsions. Liposomes may also be used. Typical carriers include alcohol, water, mineral oil, liquid petrolatum, white petrolatum, glycerin, polyethylene glycol and propylene glycol. Penetration enhancers may be incorporated - see, for example, J Pharm Sci, 88 (10), 955-958 by Finnin and Morgan (October 1999).

Other means of topical administration include delivery by electroporation, iontophoresis, phonophoresis, sonophoresis and microneedle or needle-free (*e.g.* Powderject™, Bioject™, *etc.*) injection.

Formulations for topical administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release.

The compounds in the combination of the invention can also be administered intranasally or by inhalation.

The combinations of the invention may be administered rectally or vaginally, for example, in the form of a suppository, pessary, or enema. Cocoa butter is a traditional suppository base, but various alternatives may be used as appropriate.

Formulations for rectal/vaginal administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release.

The compounds in the combination of the invention may also be administered directly to the eye or ear, typically in the form of drops of a micronised suspension or solution in isotonic, pH-adjusted, sterile saline. Other formulations suitable for ocular and aural administration include ointments, biodegradable (*e.g.* absorbable gel sponges, collagen) and non-biodegradable (*e.g.* silicone) implants, wafers, lenses and particulate or

vesicular systems, such as niosomes or liposomes. A polymer such as crossed-linked polyacrylic acid, polyvinylalcohol, hyaluronic acid, a cellulosic polymer, for example, hydroxypropylmethylcellulose, hydroxyethylcellulose, or methyl cellulose, or a heteropolysaccharide polymer, for example, gelan gum, may be incorporated together with a preservative, such as benzalkonium chloride. Such formulations may also be delivered by iontophoresis.

Formulations for ocular/aural administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted, or programmed release.

The compounds in the combination of the invention may be combined with soluble macromolecular entities, such as cyclodextrin and suitable derivatives thereof or polyethylene glycol-containing polymers, in order to improve their solubility, dissolution rate, taste-masking, bioavailability and/or stability for use in any of the aforementioned modes of administration.

Drug-cyclodextrin complexes, for example, are found to be generally useful for most dosage forms and administration routes. Both inclusion and non-inclusion complexes may be used. As an alternative to direct complexation with the drug, the cyclodextrin may be used as an auxiliary additive, *i.e.* as a carrier, diluent, or solubiliser. Most commonly used for these purposes are alpha-, beta- and gamma-cyclodextrins, examples of which may be found in International Patent Applications Nos. WO 91/11172, WO 94/02518 and WO 98/55148.

For administration to human patients, the total daily dose of the histamine $H_4$ receptor antagonist is typically in the range 0.1mg to 100mg and the total daily dose of the leukotriene receptor antagonist is typically in the range of 1mg to 10mg, depending, of course, on the mode of administration. The total daily dose may be administered in single or divided doses.

These dosages are based on an average human subject having a weight of about 65kg to 70kg. The physician will readily be able to determine doses for subjects whose weight falls outside this range, such as infants and the elderly.

Assays

To determine the activity of the histamine $H_4$ receptor antagonists of the invention the following assay was used.

Binding to human $H_4$ receptors

Cell pellets from CHO cells expressing the histamine $H_4$ receptor were homogenised in ice-cold 50mM Tris-HCl/0.5mM $CaCl_2$ buffer containing a protease inhibitor cocktail (Roche®, United Kingdom) using a ground glass homogeniser. Homogenates were centrifuged at 48000g for 30min at 4°C. The membrane pellet was resuspended in fresh buffer and the centrifugation step was repeated as described above. The membrane pellet was resuspended in 50mM Tris-HCl in the same volume as the original cell pellet. Aliquots of membrane preparations were stored at -80°C and were used for [$^3$H]-Histamine binding experiments.

Cell membranes (20-35μg/well) were incubated for 90min shaking at room temperature with 3nM [$^3$H]-Histamine (23Ci/mmol) in 50mM Tris-HCl (pH 7.4), with or without competing $H_4$ ligands. The reaction was terminated by rapid filtration through 0.5% polyethylenimine-soaked Unifilter GF/B plates (Packard) followed by three washes with 1ml ice-cold 50mM Tris-HCl. Filters were dried for 45min at 45°C and bound radiolabel was determined using scintillation counting techniques. Non-specific binding was defined with 5μM clobenpropit. For competition binding studies, Ki values were calculated from the $IC_{50}$ value (concentration of test compound which displaces 50% of the specific binding) based on an experimentally determined ligand $K_d$ of 3.5 nM and a ligand concentration of 3 nM according to the Cheng-Prussoff equation (*Biochem. Pharmacol.* (1973), vol 22, p3099-3108) where; $K_i = (IC_{50})/(1+([L]/K_d))$

Animal study

The efficacy of the combinations of the invention can be demonstrated in an animal model of airway inflammation or nasal congestion.

Sephadex-induced pulmonary eosinophilia

Compounds were administered by various routes (e.g. sub-cutaneously) before or after a suspension of Sephadex G-200 superfine (BioChemika) was instilled directly into the lungs of rats to induce a pulmonary eosinophilia 24-72 hours later. Under brief Isoflurane anaesthesia 100µl of a suspension of Sephadex (20 mg/ml) was instilled into the trachea of male Sprague-Dawley rats (Charles River, 350-400 g) by the oro-laryngeal route from a microsyringe (Hamilton, 725RN fitted with a blunt 125 mm 22G needle). Twenty-four hours later the rats were overdosed with sodium pentobarbitone and bronchoalveolar lavage (BAL) was performed. The trachea was exposed by a mid-ventral incision caudal to the larynx followed by blunt dissection. A cut-down Portex leur-fitting cannula (2.0 mm outside diameter) was inserted into the trachea and tied-in securely. Using a 2.5 ml syringe, 2.5 ml of room temperature PBS (pH 7.4) containing 2.6 mM EDTA was slowly instilled into the lungs via the cannula and the BAL fluid (BALf) was immediately aspirated and stored on ice. This process was repeated a further three times until a total volume of 10 ml was instilled. The total leukocyte numbers in the BALf samples were evaluated using an $A^c$.T5Diff haematology analyser (Beckman Coulter). Aliquots of the BALf samples were then diluted in PBS to give approximately $0.5x10^6$ cells/ml. Cytospins prepared from these diluted aliquots (200µl per slide) by centrifugation at 2000 rpm for 5 min at room temperature were allowed to air-dry before staining using the DiffQuik stain system (Dade Behring). Differential leukocyte counts were determined from cytospins under light microscopy using standard morphological criteria and the numbers of eosinophils per ml of BALf were enumerated. Activities of $H_4$ receptor antagonists and leukotriene receptor antagonists, dosed alone or in combination, and given as pre- or post-treatments, were determined.

Antigen induced pulmonary eosinophilia

Lung inflammation can be induced and modified according to the method of Yeadon et al (1993, Agents Actions, 38, 8-18). Briefly, male Dunkin-Hartely guinea-pigs were actively sensitized to ovalbumin by a single 50mg i.p. and s.c dose and 14 days later exposed to an aerosol of ovalbumin in a cloud chamber. At various times afterwards, animals were euthanised with sagatal overdose, the lungs lavaged and total and differential cell counts made by microscopy. Compounds were given orally or s.c, alone or in combination, as pretreatments.

Nasal congestion in anaesthetized cats

Nasal congestion, as measured by rhinomanometry, was determined in anesthetized cats according to the method of McLeod et al 1999 (Am J Rhonil, 13, 391-99). Briefly, pentobarbitone anesthetized cats were mechanically ventilated with ambient air. One nostril was sealed externally and a cuffed endotracheal tube inserted in a retrograde manner via the oesophagus into the nasopharynx. Using a constant air flow through the tube, the pressure (and thus resistance) was determined and modulated by nasal exposure to the mast cell degranulating agent 48/80, and pre or post treatment with $H_4$ receptor antagonists or leukotriene receptor antagonists, or a combination of these agents.

Nasal congestion in guinea-pigs

Nasal congestion, as measured by acoustic rhinomanonmetry, was determined in anesthetized guinea-pigs, according to the method of Joynson et al 2003 (proceedings of the World Inflammation Congress, Vancouver). Briefly, male Dunkin-Hartley guinea-pigs were anaesthetized with urethane (50mg/kg i.p.) and placed in a supine position. The trachea was cannulated through which the animals breathed spontaneously. An acoustic rhinomter (GJ Electronik, Denmark) was used to measure cross-sectional area of the nasal cavity as a function of distance, and was modulated by various stimuli including nasal allergen (ovalbumin) in previously actively sensitized animals.

Test compounds were administered alone or in combination as pre- or post-treatments.

## Claims

1. A pharmaceutical composition containing a combination of a leukotriene receptor antagonist and a histamine $H_4$ receptor antagonist.

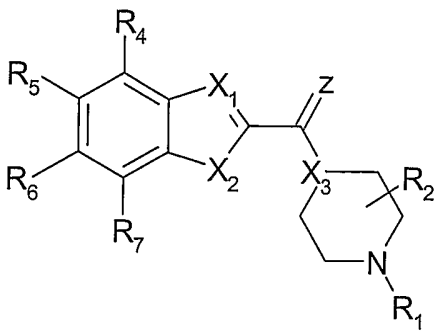
2. The pharmaceutical composition according to claim 1, wherein the leukotriene receptor antagonist is selected from montelukast, pranlukast and zafirlukast and pharmaceutically acceptable salts thereof.

3. The pharmaceutical composition according to claim 2, wherein the leukotriene receptor antagonist is montelukast sodium.

4. The pharmaceutical composition according to claim 1 or 2, wherein the histamine $H_4$ receptor antagonist has a $K_i$ of less than 15 μM.

5. The pharmaceutical composition according to any one of claims 1 to 4, wherein the histamine $H_4$ receptor antagonist is selected from compounds described in WO02/072548.

6. The pharmaceutical composition according to any one of claims 1 to 4, wherein the histamine $H_4$ receptor antagonist is selected from compounds having formula (I):




(I)

wherein $R_1$ is $R_a$, $R_aR_b$-, $R_a$-O-$R_b$-, or $(R_c)(R_d)$N-$R_b$-, where $R_a$ is H, $C_1$-$C_{10}$ alkyl, $C_3$-$C_8$ alkenyl, $C_3$-$C_8$ cycloalkyl, $C_2$-$C_5$ heterocyclic radical, or phenyl; where $R_b$ is $C_1$-$C_8$ alkylene, $C_3$-$C_8$ alkenylene, $C_3$-$C_8$ cycloalkylene, bivalent $C_3$-$C_8$ heterocyclic radical, or phenylene; and $R_c$ and $R_d$ are each independently H, $C_1$-$C_8$ alkyl, $C_2$-$C_8$ alkenyl, $C_3$-$C_8$ cycloalkyl, or phenyl;

$R_2$ is ortho or meta, and is methyl or H;

$X_1$ is $CR_3$;

$R_3$ is F, Cl, Br, $R_f$, $R_fR_g$-, $R_f$-O-$R_g$-, or $(R_h)(R_j)$N-$R_g$-, where $R_f$ is H, $C_1$-$C_6$ alkyl, $C_2$-$C_6$ alkenyl, $C_3$-$C_6$ cycloalkyl, $C_2$-$C_5$ heterocyclic radical, or phenyl; where $R_g$ is $C_1$-$C_6$ alkylene, $C_2$-$C_6$ alkenylene, $C_3$-$C_6$ cycloalkylene, bivalent $C_3$-$C_6$ heterocyclic radical, or phenylene; and $R_h$ and $R_j$ are each independently H, $C_1$-$C_6$ alkyl, $C_2$-$C_6$ alkenyl, $C_3$-$C_6$ cycloalkyl, or phenyl;

$X_2$ is $NR_e$ or O, provided that $X_2$ is $NR_e$ where $X_1$ is N; $R_e$ is H or $C_1$-$C_6$ alkyl;

$X_3$ is N;

Z is =O or =S;

each of $R_4$ and $R_6$ is independently H, F, Cl, Br, I, COOH, OH, nitro, amino, cyano, $C_1$-$C_4$ alkoxy or $C_1$-$C_4$ alkyl;

$R_5$ is H, F, Cl, Br, I, $(C=O)R_j$, OH, nitro, $NR_jR_k$, cyano, -$OCH_2$-Ph, $C_1$-$C_4$ alkoxy, or $C_1$-$C_4$ alkyl;

$R_7$ is H, F, Cl, Br, I, $(C=O)R_m$, OH, nitro, $NR_lR_m$, cyano, $C_1$-$C_4$ alkoxy, or $C_1$-$C_4$ alkyl;

wherein each of $R_j$, $R_k$, $R_l$ and $R_m$ is independently selected from H, $C_1$-$C_6$ alkyl, hydroxy, phenyl, benzyl, phenethyl and $C_1$-$C_6$ alkoxy;

each of the above hydrocarbyl or heterocyclic groups being independently and optionally substituted with between 1 and 3 substituents selected from $C_1$-$C_3$ alkyl, halo, hydroxy, amino and $C_1$-$C_3$ alkoxy;

provided at least one of $R_1$, $R_2$, $R_3$, $R_4$, $R_5$, $R_6$ and $R_7$ is other than H when Z is O;

or a pharmaceutically acceptable salt, ester or amide thereof.

7. The pharmaceutical composition according to any one of claims 1 to 6 for use as a medicament.

8. A method for treating a patient suffering from a $H_4$ mediated disease and/or leukotriene mediated disease, by administering to said patient an effective amount of a leukotriene antagonist and an effective amount of a histamine $H_4$ receptor antagonist.

9. The method according to claim 8, for treating a patient suffering from an allergic and/or inflammatory condition.

10. The method according to claim 9, wherein the allergic and/or inflammatory condition is selected from the group consisting of seasonal and perennial allergic rhinits, non-allergic rhinitis, asthma, COPD, sinusitis, colds, dermatitis and uriticaria,

11. Kit containing a leukotriene antagonist and a histamine $H_4$ receptor antagonist for sequential, separate or simultaneous use in the treatment of inflammatory diseases.

# INTERNATIONAL SEARCH REPORT

Int ›nal Application No
PCT/IB2005/000594

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/404 A61K31/47 A61P37/08

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, PAJ, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO 97/28797 A (MERCK & CO., INC; DAHLEN, SVEN-ERIK; SCOLNICK, EDWARD, M) 14 August 1997 (1997-08-14) claims 1,4,7 | 1-11 |
| Y | WO 02/072548 A (ORTHO-MCNEIL PHARMACEUTICAL, INC) 19 September 2002 (2002-09-19) cited in the application page 28, line 1 - line 8; claims 1-40 | 1-11 |

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

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 19 May 2005 | 01/06/2005 |

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

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# INTERNATIONAL SEARCH REPORT

Int ›nal Application No
PCT/IB2005/000594

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | TAKESHITA KEISUKE ET AL: "Critical role of histamine H4 receptor in leukotriene B4 production and mast cell-dependent neutrophil recruitment induced by zymosan in vivo." JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, vol. 307, no. 3, December 2003 (2003-12), pages 1072-1078, XP002328714 ISSN: 0022-3565 page 1074, column 2, paragraph 2 page 1077, column 2, paragraph 2 - page 1078, column 1, paragraph 1 ----- | 1-11 |

# INTERNATIONAL SEARCH REPORT

ıational application No.
PCT/IB2005/000594

**Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

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

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

Although claims 8-10 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

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

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

**Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

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

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

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

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

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

**Remark on Protest**

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

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

# INTERNATIONAL SEARCH REPORT

Int nal Application No
PCT/IB2005/000594

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| | | WO 02072548 A2 | 19-09-2002 |
| | | US 2003207893 A1 | 06-11-2003 |
| | | US 2005085487 A1 | 21-04-2005 |