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- as to the identity of the inventor (Rule 4.17(i))
- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))
- of inventorship (Rule 4.17(iv))

Published:
- with international search report (Art. 21(3))
Hydrochloride salt of Biphenyl-2-yl-carbamic acid 1-f9-f(3-fluoro-4-hydroxy-benzoyl)-methyl-amino1-nonyl)-piperidin-4-yl ester

This invention relates to the hydrochloride salt of biphenyl-2-yl-carbamic acid 1-{9-[(3-fluoro-4-hydroxy-benzoyl)-methyl-amino]-nonyl}-piperidin-4-yl ester and to processes for the preparation of, intermediates used in the preparation of, compositions containing and the uses of, said compound. The invention also relates to the hydrates, solvates and polymorphs of the hydrochloride salt of biphenyl-2-yl-carbamic acid 1-{9-[(3-fluoro-4-hydroxy-benzoyl)-methyl-amino]-nonyl}-piperidin-4-yl ester.

Cholinergic muscarinic receptors are members of the G-protein coupled receptor super-family and are further divided into 5 subtypes, M₁ to M₅. Muscarinic receptor sub-types are widely and differentially expressed in the body. Genes have been cloned for all 5 sub-types and of these, M₁, M₂ and M₃ receptors have been extensively pharmacologically characterized in animal and human tissue. M₁ receptors are expressed in the brain (cortex and hippocampus), glands and in the ganglia of sympathetic and parasympathetic nerves. M₂ receptors are expressed in the heart, hindbrain, smooth muscle and in the synapses of the autonomic nervous system. M₃ receptors are expressed in the brain, glands and smooth muscle. In the airways, stimulation of M₂ receptors evokes contraction of airway smooth muscle leading to bronchoconstriction, while in the salivary gland M₂ receptor stimulation increases fluid and mucus secretion leading to increased salivation. M₂ receptors expressed on smooth muscle are understood to be pro-contractile while pre-synaptic M₂ receptors modulate acetylcholine release from parasympathetic nerves. Stimulation of M₂ receptors expressed in the heart produces bradycardia.

Short and long-acting muscarinic antagonists are used in the management of asthma and COPD; these include the short acting agents Atrovent® (ipratropium bromide) and Oxivent® (oxitropium bromide) and the long acting agent Spiriva® (tiotropium bromide). These compounds produce bronchodilation following inhaled administration. In addition to improvements in spirometric values, anti-muscarinic use in chronic obstructive pulmonary disease (COPD) is associated with improvements in health status and quality of life scores. As a consequence of the wide distribution of muscarinic receptors in the body, significant systemic exposure to muscarinic
antagonists is associated with effects such as dry mouth, constipation, mydriasis, urinary retention (all predominantly mediated via blockade of M₃ receptors) and tachycardia (mediated by blockade of M₂ receptors). A commonly reported side-effect following inhaled administration of therapeutic dose of the current, clinically used non-selective muscarinic antagonists is dry-mouth and while this is reported as only mild in intensity it does limit the dose of inhaled agent given.

Accordingly, there is still a need for improved M₃ receptor antagonists that would have an appropriate pharmacological profile, for example in term of potency, pharmacokinetics or duration of action and that would be particularly suitable for an administration by the inhalation route.

In this context, the present invention relates to a novel M₃ receptor antagonist which is the hydrochloride salt of biphenyl-2-yl-carbamic acid 1-{9-[(3-fluoro-4-hydroxy-benzoyl)-methyl-amino]-nonyl}-piperidin-4-yl ester of formula:

Preferably the invention relates to a substantially crystalline form of the hydrochloride salt of biphenyl-2-yl-carbamic acid 1-{9-[(3-fluoro-4-hydroxy-benzoyl)-methyl-amino]-nonyl}-piperidin-4-yl ester.

Preferably, the hydrochloride salt of the invention has an X-ray diffraction pattern characterized by the following 4 unique X-ray diffraction pattern peaks expressed in terms of 2-theta angle (± 0.1 ° 2θ) when measured using Cu Kα₁ radiation (Wavelength = 1.5406A):

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<thead>
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<th>Angle (°2θ)</th>
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<tr>
<td>10.5</td>
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<td>14.8</td>
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<td>18.6</td>
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The hydrochloride salt of the invention has an X-ray diffraction pattern which may also be characterized by the following 7 unique X-ray diffraction pattern peaks expressed in terms of 2-theta angle (± 0.1° 2θ) when measured using Cu Kα1 radiation (Wavelength = 1.5406A):

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<tr>
<th>Angle (°2Θ)</th>
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<td>10.5</td>
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<td>24.5</td>
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or further by the following 10 unique X-ray diffraction pattern peaks expressed in terms of 2-theta angle (± 0.1° 2θ) when measured using Cu Kα1 radiation (Wavelength = 1.5406A):

<table>
<thead>
<tr>
<th>Angle (°2Θ)</th>
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<td>8.2</td>
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<td>10.5</td>
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<td>12.2</td>
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<td>18.6</td>
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<tr>
<td>20.1</td>
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<tr>
<td>24.5</td>
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</table>

According to another embodiment, the hydrochloride salt of the invention may also be characterized by a solid state 13C NMR pattern having the following principal carbon chemical shifts referenced to external sample of solid phase adamantane at 29.5 ppm:
According to another embodiment, the hydrochloride salt of the invention may also be characterized by a solid state $^{19}$F NMR pattern having the following principal fluorine chemical shifts referenced to an external standard of trifluoroacetic acid, 50% V/V in water, at -76.54 ppm:

<table>
<thead>
<tr>
<th>$^{19}$F Chemical Shifts [ppm ± 0.4 ppm]</th>
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<tbody>
<tr>
<td>-133.5</td>
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It has now been found that the hydrochloride salt of the invention is an antagonist of the $M_3$ receptor, that is particularly useful for the treatment of $M_3$-mediated diseases and/or conditions, and shows good potency. Moreover, the hydrochloride salt of the invention is substantially crystalline and thus exhibits properties including those of solid state stability and compatibility with certain drug product excipients, such as e.g. lactose, in particular $\alpha$-lactose monohydrate, that render it superior to its corresponding free base which is not crystalline. The hydrochloride salt of the invention is thus particularly suitable for an administration by the inhalation route e.g. using a dry powder inhaler.

For avoidance of doubt, "substantially crystalline" according to the present invention means that the hydrochloride salt of the present invention is at least 70% crystalline, more preferably at least 80% crystalline, still more preferably at least 85% crystalline, still more preferably at least 90% crystalline and even more preferably at least 95% crystalline.

The hydrochloride salt of the invention may be prepared from biphenyl-2-yI-carbamic acid 1-[(3-fluoro-4-hydroxy-benzoyl)-methyl-amino]-nonyI]-piperidin-4-yI ester according to conventional processes for the preparation of salts such as those disclosed in "Handbook of Pharmaceutical Salts, Properties, Selection and Use. Published by Wiley-VCH, 2002. Edited by P. Heinrich Stahl, Camille G Wermuth."
ISBN 3-906390-26-8". As a matter of example, the hydrochloride salt of the present invention may be prepared by addition of aqueous hydrochloric acid to biphenyl-2-yl-carbamic acid 1-[(3-fluoro-4-hydroxy-benzoyl)-methyl-amino]-nonyl]-piperidin-4-yl ester as a free base in a suitable solvent.

The hydrochloride salt of biphenyl-2-yl-carbamic acid 1-[(3-fluoro-4-hydroxy-benzoyl)-methyl-amino]-nonyl]-piperidin-4-yl ester that is thus obtained may further be re-crystallised from other solvents such as e.g. acetone, to give higher crystallinity material.

The hydrochloride salt of biphenyl-2-yl-carbamic acid 1-[(3-fluoro-4-hydroxy-benzoyl)-methyl-amino]-nonyl]-piperidin-4-yl ester according to the present invention may exist in both unsolvated and solvated forms. The term 'solvate' is used herein to describe a molecular complex comprising the hydrochloride salt of the invention and a stoichiometric amount of one or more pharmaceutically acceptable solvent molecules, for example, ethanol. The term 'hydrate' is employed when said solvent is water. These unsolvated/unhydrated and solvated/hydrated forms are included within the scope of the present invention.

Also included within the scope of the invention are complexes such as clathrates, drug-host inclusion complexes wherein, in contrast to the aforementioned solvates, the drug and host are present in stoichiometric or non-stoichiometric amounts. Also included are complexes of the drug containing two or more organic and/or inorganic components which may be in stoichiometric or non-stoichiometric amounts. The resulting complexes may be ionised, partially ionised, or non-ionised. For a review of such complexes, see J Pharm Sci, 64 (8), 1269-1288 by Haleblian (August 1975).

Polymorphs and crystal morphologies/habits of the hydrochloride salt of the invention are also included within the scope of the invention.

The term "hydrochloride salt of the invention" includes the hydrochloride salt of biphenyl-2-yl-carbamic acid 1-[(3-fluoro-4-hydroxy-benzoyl)-methyl-amino]-nonyl]-piperidin-4-yl ester as well as solvates and/or polymorphs thereof.
The hydrochloride salt of the invention is a valuable pharmaceutically active compound, which is suitable for the therapy and prophylaxis of numerous disorders in which the muscarinic receptor M3 is involved or in which antagonism of this receptor may induce benefit. The hydrochloride salt of the invention has the ability to interact with the M3 receptor and thereby have a wide range of therapeutic applications, as described further below, because of the essential role which the hydrochloride salt plays in the physiology of all mammals. For the avoidance of doubt, references herein to "treatment" include references to curative, palliative and prophylactic treatment.

The hydrochloride salt of the invention is thus particularly useful in the therapy and prophylaxis of the allergic and non-allergic airways diseases (e.g. asthma, COPD...) but also in the treatment of other diseases such as Inflammatory Bowel Disease, Irritable Bowel Disease, diverticular disease, motion sickness, gastric ulcers, radiological examination of the bowel, symptomatic treatment of BPH (benign prostatic hyperplasia), NSAID induced gastric ulceration, urinary incontinence (including urgency, frequency, urge incontinence, overactive bladder, nocturia and lower urinary tract symptoms), cycloplegia, mydriatics and parkinsons disease.

According to a preferred aspect of the present invention, the hydrochloride salt of the invention is suitable for use in the treatment of diseases, disorders, and conditions in which the M3 receptor is involved. More specifically, the present invention also concerns the hydrochloride salt of the invention for use in the treatment of diseases, disorders, and conditions selected from the group consisting of:

- chronic or acute bronchoconstriction, chronic bronchitis, small airways obstruction, and emphysema;
- obstructive or inflammatory airways diseases, in particular chronic eosinophilic pneumonia, chronic obstructive pulmonary disease (COPD), COPD that includes chronic bronchitis, pulmonary emphysema or dyspnea associated or not associated with COPD, COPD that is characterized by irreversible, progressive airways obstruction, adult respiratory distress syndrome (ARDS), exacerbation of airways hyper-reactivity consequent to other drug therapy and airways disease that is associated with pulmonary hypertension;
- bronchitis, in particular acute bronchitis, acute laryngotracheal bronchitis, arachidic bronchitis, catarrhal bronchitis, croupus bronchitis, dry bronchitis,
infectious asthmatic bronchitis, productive bronchitis, staphylococcus or streptococcal bronchitis and vesicular bronchitis;

• asthma, in particular atopic asthma, non-atopic asthma, allergic asthma, atopic bronchial IgE-mediated asthma, bronchial asthma, essential asthma, true asthma, intrinsic asthma caused by pathophysiologic disturbances, extrinsic asthma caused by environmental factors, essential asthma of unknown or inapparent cause, non-atopic asthma, bronchitic asthma, emphysematous asthma, exercise-induced asthma, allergen induced asthma, cold air induced asthma, occupational asthma, infective asthma caused by bacterial, fungal, protozoal, or viral infection, non-allergic asthma, incipient asthma, wheezy infant syndrome and bronchiolytis;

• acute lung injury;

• bronchiectasis, in particular cylindric bronchiectasis, sacculated bronchiectasis, fusiform bronchiectasis, capillary bronchiectasis, cystic bronchiectasis, dry bronchiectasis and follicular bronchiectasis.

Still more specifically, the present invention also concerns the hydrochloride salt of the invention for use in the treatment of diseases, disorders, and conditions selected from the group consisting of chronic or acute bronchoconstriction, emphysema, chronic obstructive pulmonary disease (COPD), adult respiratory distress syndrome (ARDS), bronchitis, asthma, acute lung injury and bronchiolytis.

A still further aspect of the present invention also relates to the use of the hydrochloride salt of the invention for the manufacture of a medicament having a M₃ antagonist activity. In particular, the present inventions concerns the use of the hydrochloride salt of the invention, for the manufacture of a medicament for the treatment of M₃ receptor-mediated diseases and/or conditions, in particular the diseases and/or conditions listed above.

As a consequence, the present invention provides a particularly interesting method to treat a mammal, including a human being, with an effective amount of the hydrochloride salt of the invention. More precisely, the present invention provides a particularly interesting method for the treatment of a M₃ receptor-mediated diseases and/or conditions in a mammal, including a human being, in particular the diseases
and/or conditions listed above, comprising administering said mammal with an effective amount of the hydrochloride salt of the invention.

The hydrochloride salt of the invention can be administered according to the invention to animals, preferably to mammals, and in particular to humans, as pharmaceutical for therapy and/or prophylaxis. It can be administered per se, in mixtures with one another or in the form of pharmaceutical preparations which as active constituent contain an efficacious dose of the hydrochloride salt of the invention, in addition to customary pharmaceutically innocuous excipients and/or additives.

The hydrochloride salt of the invention may be freeze-dried, spray-dried, or evaporatively dried to provide a solid plug, powder, or film of crystalline or amorphous material. Microwave or radio frequency drying may be used for this purpose.

The hydrochloride salt of the invention may be administered alone or in combination with other drugs and will generally 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 hydrochloride salt of the invention. The choice of excipient will to a large extent depend on the particular mode of administration.

The hydrochloride salt of the invention may 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.

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 the hydrochloride salt 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-poly(c-/lactic-coglycolic)acid (PGLA) microspheres.

The hydrochloride salt 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 hydrochloride salt 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 hydrochloride salt 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 a crosslinked 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 hydrochloride salt 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 91/1172, WO 94/02518 and WO 98/55148.
Finally, the hydrochloride salt of the invention can also be administered intranasally or by inhalation, typically in the form of a dry powder (either alone, as a mixture, for example, in a dry blend with lactose, preferably α-lactose monohydrate, or as a mixed component particle, for example, mixed with phospholipids, such as phosphatidylcholine) from a dry powder inhaler or as an aerosol spray from a pressurised container, pump, spray, atomiser (preferably an atomiser using electrohydrodynamics to produce a fine mist), or nebuliser, with or without the use of a suitable propellant, such as 1,1,1,2-tetrafluoroethane or 1,1,1,2,3,3,3-heptafluoropropane. For intranasal use, the powder may comprise a bioadhesive agent, for example, chitosan or cyclodextrin.

The pressurised container, pump, spray, atomizer, or nebuliser contains a solution or suspension of the compound(s) of the invention comprising, for example, ethanol, aqueous ethanol, or a suitable alternative agent for dispersing, solubilising, or extending release of the active, a propellant(s) as solvent and an optional surfactant, such as sorbitan trioleate, oleic acid, or an oligolactic acid.

Prior to use in a dry powder or suspension formulation, the drug product is micronised to a size suitable for delivery by inhalation (typically less than 5 microns). This may be achieved by any appropriate comminuting method, such as for example spiral jet milling, fluid bed jet milling, supercritical fluid processing to form nanoparticles, high pressure homogenisation, or spray drying.

Capsules (made, for example, from gelatin or hydroxypropylmethylcellulose), blisters and cartridges for use in an inhaler or insufflator may be formulated to contain a powder mix of the hydrochloride salt of the invention, a suitable powder base such as lactose or starch and a performance modifier such as α-leucine, mannitol, or magnesium stearate. The lactose may be anhydrous or in the form of the monohydrate, preferably the latter. Other suitable excipients include dextran, glucose, maltose, sorbitol, xylitol, fructose, sucrose and trehalose.

Suitable flavours, such as menthol and levomenthol, or sweeteners, such as saccharin or saccharin sodium, may be added to those formulations of the invention intended for inhaled/intranasal administration.
Formulations for inhaled/intranasal administration may be formulated to be immediate and/or modified release using, for example, PGLA. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release.

For administration to human patients, the total daily dose of the hydrochloride salt of the invention is typically in the range 0.001 mg to 5000 mg depending, of course, on the mode of administration. Thus the present invention also relates to pharmaceutical compositions comprising from 0.001 mg to 5000 mg of the hydrochloride salt of the present invention together with one or more pharmaceutically acceptable excipients. Optionally, these pharmaceutical compositions may further comprise one or more other therapeutic agent(s).

For example, an intravenous daily dose may only require from 0.001 mg to 40mg. The total daily dose may be administered in single or divided doses and may, at the physician's discretion, fall outside of the typical range given herein.

A suitable solution formulation for use in an atomiser using electrohydrodynamics to produce a fine mist may contain from 1 µg to 20 mg of the hydrochloride salt of the invention per actuation and the actuation volume may vary from 1 µl to 100 µl. A typical formulation may comprise the hydrochloride salt of the invention, propylene glycol, sterile water, ethanol and sodium chloride. Alternative solvents which may be used instead of propylene glycol include glycerol and polyethylene glycol.

In the case of dry powder inhalers and aerosols, the dosage unit is determined by a prefilled capsule, blister or pocket or by a system that utilises gravimetrically fed dosing chamber. Units in accordance with the invention are typically arranged to administer a metered dose or "puff" containing from 0.001 mg to 10 mg of the hydrochloride salt of the invention. The overall daily dose will typically be in the range 0.001 mg to 40 mg which may be administered in a single dose or, more usually, as divided doses throughout the day.

The hydrochloride salt of the invention is particularly suitable for an administration by inhalation. In particular, the hydrochloride salt of the invention is suitable for a
formulation with lactose, preferably lactose monohydrate, as a dry powder and can thus be administered using a dry powder inhaler, e.g. the dry powder inhaler described in WO 2005/002654. Thus the present invention also relates to pharmaceutical dry powders comprising 0.001 mg to 40 mg of the hydrochloride salt of biphenyl-2-yl-caraamic acid 1-{9-[(3-fluoro-4-hydroxy-benzoyl)-methyl-amino]-nonyl}-piperidin-4-yl ester blended with lactose monohydrate. Optionally, these pharmaceutical formulations may further comprise one or more other therapeutic agent(s).

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

According to another embodiment of the present invention, the hydrochloride salt of the invention or compositions thereof, can also be used as a combination with one or more additional therapeutic agents to be co-administered to a patient to obtain some particular desired therapeutic end result such as the treatment of pathophysiologically-relevant disease processes including, but not limited to (i) bronchoconstriction, (ii) inflammation, (iii) allergy, (iv) tissue destruction, (v) signs and symptoms such as breathlessness, cough.

As used herein, the terms "co-administration", "co-administered" and "in combination with", referring to the hydrochloride salt of the invention and one or more other therapeutic agents, is intended to mean, and does refer to and include the following:

- simultaneous administration of such combination of hydrochloride salt of the invention and therapeutic agent(s) to a patient in need of treatment, when such components are formulated together into a single dosage form which releases said components at substantially the same time to said patient,

- substantially simultaneous administration of such combination of hydrochloride salt of the invention and therapeutic agent(s) to a patient in need of treatment, when such components are formulated apart from each other into separate dosage forms which are taken at substantially the same time by said patient, whereupon said components are released at substantially the same time to said patient,
• sequential administration of such combination of hydrochloride salt of the invention and therapeutic agent(s) to a patient in need of treatment, when such components are formulated apart from each other into separate dosage forms which are taken at consecutive times by said patient with a significant time interval between each administration, whereupon said components are released at substantially different times to said patient; and
• sequential administration of such combination of hydrochloride salt of the invention and therapeutic agent(s) to a patient in need of treatment, when such components are formulated together into a single dosage form which releases said components in a controlled manner whereupon they are concurrently, consecutively, and/or overlapingly administered at the same and/or different times by said patient, where each part may be administered by either the same or different route.

Suitable examples of other therapeutic agents which may be used in combination with hydrochloride salt of the invention, include, but are by no means limited to:
(a) 5-Lipoxygenase (5-LO) inhibitors or 5-lipoxygenase activating protein (FLAP) antagonists;
(b) Leukotriene antagonists (LTRA) including antagonists of LTB₄, LTC₄, LTD₄, and LTE₄;
(c) Histamine receptor antagonists including H₁ and H₃ antagonists;
(d) α₁-adrenoceptor agonist vasoconstrictor sympathomimetic agents for decongestant use;
(e) PDE inhibitors including PDE3, PDE4 and PDE5 inhibitors;
(f) Beta 2 receptor agonists;
(g) Theophylline;
(h) Sodium cromoglycate;
(i) COX inhibitors both non-selective and selective COX-1 or COX-2 inhibitors (NSAIDs);
(j) Prostaglandin receptor antagonists and inhibitors of prostaglandin synthase;
(k) Oral and inhaled glucocorticosteroids;
(l) Dissociated agonists of the corticoid receptor (DAGR);
(m) Monoclonal antibodies active against endogenous inflammatory entities;
(n) Anti-tumor necrosis factor (anti-TNF-a) agents;
(o) Adhesion molecule inhibitors including VLA-4 antagonists;
(p) Kinin-B₁- and B₂-receptor antagonists;
(q) Immunosuppressive agents including inhibitors of the IgE pathway and cyclosporine;
(r) Inhibitors of matrix metalloproteases (MMPs);
(s) Tachykinin NK₁, NK₂ and NK₃ receptor antagonists;
(t) Protease inhibitors such as elastase inhibitors;
(u) Adenosine A2a receptor agonists and A2b antagonists;
(v) Inhibitors of urokinase;
(w) Compounds that act on dopamine receptors such as D2 agonists;
(x) Modulators of the NFκB pathway such as IKK inhibitors;
(y) Modulators of cytokine signalling pathways such as p38 MAP kinase, PI3 kinase, JAK kinase, syk kinase, EGFR or MK-2;
(z) Agents that can be classed as mucolytics or anti-tussive;
(aa) Agents which enhance responses to inhaled corticosteroids;
(bb) Antibiotics and antiviral agents effective against micro-organisms which can colonise the respiratory tract;
(cc) HDAC inhibitors;
(dd) CXCR2 antagonists;
(ee) Integrin antagonists;
(ff) Chemokines;
(gg) Epithelial sodium channel (ENaC) blockers or Epithelial sodium channel (ENaC) inhibitors;
(hh) P2Y2 Agonists and other Nucleotide receptor agonists;
(ii) Inhibitors of thromboxane;
(jj) Inhibitors of PGD₂ synthesis and PGD₂ receptors (DP1 and DP2/CRTH2);
(kk) Niacin; and
(ll) Adhesion factors including VLAM, ICAM, and ELAM.

According to the present invention, pharmaceutical composition comprising the hydrochloride salt of the invention in combination with H3 antagonists, β₂ agonists including long-acting β₂ agonists, PDE4 inhibitors, steroids including inhaled glucocorticosteroids, adenosine A2a receptor agonists, modulators of cytokine signalling pathways including p38 MAP kinase or syk kinase, and/or leukotriene
antagonists (LTRAs) including antagonists of $\text{LTB}_4$, $\text{LTC}_4$, $\text{LTD}_4$, and $\text{LTE}_4$, are preferred.

According to the present invention, pharmaceutical compositions comprising the hydrochloride salt of the invention in combination with a glucocorticosteroid, in particular an inhaled glucocorticosteroid with reduced systemic side effects and/or a $\beta_2$ agonist, are further preferred.

Examples of suitable glucocorticosteroids include, but is not limited to, prednisone, prednisolone, flunisolide, triamcinolone acetonide, beclomethasone, budesonide, fluticasone, ciclesonide, mometasone and their salts.

Examples of suitable $\beta_2$ agonists include, but is not limited to, salbutamol, terbutaline, bambuterol, fenoterol, salmeterol, formoterol, tulobuterol and their salts.

According to another preferred aspect, the pharmaceutical compositions of the present invention comprise the hydrochloride salt of the invention in combination with a glucocorticosteroid, e.g. those listed above, and a $\beta_2$ agonist, e.g. those listed above, so as to form a 'triple combination' together with one or more pharmaceutically acceptable excipient.

According to a further preferred aspect, the pharmaceutical compositions of the present invention include the dry powders comprising the hydrochloride salt of the invention, a glucocorticosteroid and a $\beta_2$ agonists, including long-acting $\beta_2$ agonists, together with lactose monohydrate.

Inasmuch as it may desirable to administer a combination of active compounds, for example, for the purpose of treating a particular disease or condition, it is within the scope of the present invention that two or more pharmaceutical compositions, at least one of which contains the hydrochloride salt of the invention, may conveniently be combined in the form of a kit suitable for coadministration of the compositions.

Thus the kit of the invention comprises two or more separate pharmaceutical compositions, at least one of which contains the hydrochloride salt of the invention in
accordance with the invention, and means for separately retaining said compositions, such as a container, divided bottle, or divided foil pockets. 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 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.

The following experimental details illustrate specifically how the hydrochloride salt of the invention may be prepared.

**FIGURES**

Figure 1/4: DSC thermogram of examples 1 and 2.
Figure 2/4: PXRD pattern of example 1.
Figure 3/4: Carbon CPMAS spectrum of example 2.
Figure 4/4: Fluorine MAS spectrum of example 2.

**EXAMPLES**

**Preparation 1: 9-Methylamino-nonan-1-ol**

To 9-bromononanol (25g) was added methylamine (33% solution in ethanol, 200ml) and the solution stirred for 18 hours at room temperature under nitrogen. The solvent was removed under vacuum, the resulting colourless solid was dissolved in dichloromethane (200ml), washed with aqueous sodium hydroxide solution (2M, 100ml), water (100 ml), dried (sodium sulphate) and concentrated *in vacuo* to give the title compound as a yellow oil that solidified on standing, 14.95g. Said compound was used as such in preparation 2.

**Preparation 2: (9-Hydroxy-nonyl)-methyl-carbamic acid tert-butyl ester**
9-Methylamino-nonan-1-ol (Preparation 1, 14.95g) was suspended in a mixture of dichloromethane (250ml) and triethylamine (17.6g) and cooled in an ice-bath with stirring. Boc anhydride (18.8g) was added in portions over 5 minutes and the reaction stirred in the ice bath for 1 hour and then at room temperature for 4 hours. The reaction was washed with water (150ml), 10% aqueous citric acid solution (50ml) and saturated brine (50ml), then dried (sodium sulphate) and concentrated in vacuo to give the title compound as a yellow liquid, 22.95 g, 97%.

$^1$H NMR (400MHz, CDCl$_3$) $\delta$ = 1.20-1.38 (m, 10H), 1.47 (s, 9H), 1.47-1.60 (m, 4H), 2.80 (s, 3H), 3.10-3.22 (t, 2H), 3.78-3.83 (t, 2H) ppm.

Preparation 3: Methanesulfonic acid 9-(tert-butoxycarbonyl-methyl-amino)-nonyl ester

To a solution of (9-hydroxy-nonyl)-methyl-carbamic acid tert-butyl ester (Preparation 2, 22.95g) in dichloromethane (230 ml.) and triethylamine (18 ml.) at 5°C, was added methane sulphonyl chloride (7.2 ml.) dropwise and the viscous, cloudy solution stirred at room temperature for 1 hour. The mixture was washed with water, saturated aqueous sodium bicarbonate solution and the organic layer dried (magnesium sulphate) and evaporated in vacuo to give the title compound as a light yellow oil, 29.30g.

$^1$H NMR (400MHz, CDCl$_3$) $\delta$ = 1.20-1.45 (m, 10H), 1.44 (s, 9H), 1.44-1.52 (m, 2H), 1.70-1.79 (m, 2H), 2.82 (s, 3H), 2.98 (s, 3H), 3.14-3.24 (m, 2H), 4.20-4.4.24 (t, 2H) ppm.

Preparation 4: Biphenyl-2-yl-carbamic acid 1-[9-(tert-butoxycarbonyl-methyl-amino)-nonyl]-piperidin-4-yl ester
4-Piperidinyl-N-(2-biphenylyl)-carbamate hydrochloride (US 2006/205779, 29.3g) was stirred with potassium carbonate (46g) in dimethylformamide (250ml) for 0.5 hour. Methanesulfonic acid 9-(tert-butoxycarbonyl-methyl-amino)-nonyl ester (Preparation 3, 27.7g) and potassium iodide (277mg) were then added. The reaction mixture was stirred at 65°C for 24 hours, then additional dimethylformamide (100 ml) was added to aid stirring at 65°C for a further 24 hours. The solvent was removed in vacuo and the residue partitioned between water and ethyl acetate (500ml each). The aqueous layer was separated and extracted with further ethyl acetate (200ml). The combined organic layers were washed with saturated brine, dried (sodium sulphate) and concentrated in vacuo. The crude residue (46.46g) was purified by normal phase silica gel column chromatography using ethyl acetate:heptane:880 ammonia (80:20:0.5, by volume) as eluant to give the title compound as a colourless oil which crystallised on standing, 30g, 65%.

$^1$H NMR (400MHz, CDCl$_3$) $\delta$ = 1.22-1.38 (m, 12H), 1.44 (s, 9H), 1.44-1.56 (m, 2H), 1.61-1.73 (m, 2H), 1.88-1.97 (m, 2H), 2.12-2.24 (t, 2H), 2.23-2.30 (t, 2H), 2.64-2.72 (m, 2H), 2.82 (s, 3H), 3.16-3.24 (m, 2H), 4.63-4.78 (m, 1H), 6.60 (s, 1H), 7.08-7.56 (m, 8H), 8.03-8.15 (d, 1H) ppm.

Preparation 5: Biphenyl-2-yl-carbamic acid 1-[9-(methylamino-nonyl)-piperidin-4-yl ester; dihydrochloride salt

Biphenyl-2-yl-carbamic acid 1-[9-(tert-butoxycarbonyl-methyl-amino)-nonyl]-piperidin-4-yl ester (Preparation 4, 18.5g) was stirred in a solution of hydrochloric acid in dioxane (85ml, 4M) at room temperature for 18 hours. The solvent and excess acid were removed in vacuo and the residue azeotroped twice with dichloromethane (100ml) to give the title compound as a white solid, 18.0g.
\textbf{Preparation 6: Biphenyl-2-yl-carbamic acid 1-\{(3-fluoro-4-hydroxy-benzoyl)-methyl-aminol-nonyl\)-piperidin-4-yl ester}

To a solution of biphenyl-2-yl-carbamic acid 1-(9-methylamino-nonyl)-piperidin-4-yl ester dihydrochloride salt (Preparation 5, 4.47g, 9.90mmol) in tetrahydrofuran (150ml), was added 3-fluoro-4-hydroxybenzoic acid (1.85g, 11.9mmol), triethylamine (2.07ml, 14.8 mmol), N,N-dimethylaminopyridine (484mg, 3.96mmol) and (3-(dimethylamino)propyl)ethyl carbodiimide hydrochloride (2.66g, 13.9mmol). The mixture was stirred at room temperature for 15 minutes and then at 60°C for 18 hours. Further 3-fluoro-4-hydroxybenzoic acid (308mg, 2.0mmol) was added and the reaction heated at 60°C for a further 18 hours. The solvent was removed \textit{in vacuo} and residue partitioned between ethyl acetate (200ml) and water (150ml). The aqueous layer was further extracted with ethyl acetate (200ml) and the combined organic layers dried over magnesium sulphate and concentrated \textit{in vacuo}. The residue was dissolved in methanol/water (115ml/23ml), treated with potassium carbonate (12.9g, 93.2mmol) and heated at 50°C for 18 hours. The solvent was removed \textit{in vacuo} and residue partitioned between dichloromethane (200ml) and water (200ml). The organic layer was washed with brine (100ml) and concentrated \textit{in vacuo}. The residue was purified by column chromatography on silica gel eluting with dichloromethane:methanol:880 ammonia (100:0:0 to 95:5:0.5, by volume), to furnish the title compound as an oily foam, in 52% yield, 3.01g.

LCMS: APCI ESI m/z 590 [M+H]^+

$^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ = 1.12-1.40 (m, 10H), 1.45-1.54 (m, 2H), 1.56-1.68 (m, 4H), 1.81-1.91 (m, 2H), 2.29-2.40 (m, 4H), 2.64-2.75 (m, 2H), 3.01 (s, 3H), 3.34-3.53 (m, 2H), 4.58-4.65 (m, 1H), 6.92-6.96 (m, 1H), 7.05 (d, 1H), 7.12 (d, 1H), 7.23-7.44 (m, 8H), 7.55 (d, 1H) ppm.
Example 1: hydrochloride salt of biphenyl-2-yl-carbamic acid 1-f9-f(3-fluoro-4-hydroxy-benzoyl)-methyl-amino1-nonyl)-piperidin-4-yl ester

To a solution of biphenyl-2-yl-carbamic acid 1-{9-[(3-fluoro-4-hydroxy-benzoyl)-methyl-amino]-nonyl}-piperidin-4-yl ester (preparation 6, 118mg, 0.2mmol) in ethyl alcohol (1 ml) was added aqueous hydrochloric acid (0.1 ml at 2M, 0.2mmol) and the solution was allowed to evaporate to low bulk overnight. To the residue was added ie/f-butyl methyl ether (4ml) and the mixture was stirred at room temperature in the presence of a previously prepared seed crystal. The resultant colourless crystalline solid was filtered and dried in vacuo to furnish the title compound in 55% yield, 65 mg.

\[ \text{H NMR (DMSO-d6)} \delta = 1.0-2.05 \text{ (m, 18H), 2.74-3.00 (m, 4H), 2.84 (s, 3H), 3.21-3.43 (m, 4H), 4.56-4.68 (m), 4.70-4.74 (m) (1H, rotamers), 6.94-7.04 (m, 2H), 7.10-7.20 (d, 1H), 7.25-7.44 (m, 9H), 8.74 (s), 8.82 (s) (1H, rotamers), 10.05-10.30 (m, 1H), 10.27 (s, 1H) ppm.} \]

The seed crystal was prepared as follows:
To a solution of biphenyl-2-yl-carbamic acid 1-{9-[(3-fluoro-4-hydroxy-benzoyl)-methyl-amino]-nonyl}-piperidin-4-yl ester free base (preparation 6, 15mg, 0.025mmol) in ethanol (0.5ml) was added a solution of fumaric acid (1.5mg, 0.0125mmol) in ethanol (0.1ml) and aqueous hydrochloric acid (0.012ml at 2M, 0.024mmol) and the solution allowed to evaporate to a gum at room temperature exposed to the air. The gum was treated with methyl tert-butyl ether (1ml) and allowed to stand for 6 weeks. One crystal was present and on manipulation with a spatula the remaining gum crystallised over 2 hours, and was filtered and dried in vacuo to furnish the required seed crystal in 60% yield, 9 mg.

DSC Data
The melting point of the material obtained in example 1 was determined by Differential Scanning Calorimetry (DSC) using a TA instruments Q1000 differential scanning
The sample was heated at 20°C/minute, from 10 °C to 250° C, in a standard aluminium pan. The DSC thermogram obtained trace is shown in Figure 1 with flat baselines and sharp endotherms corresponding to the melt. The melting point for 2.072 mg of the material obtained in example 1 was evidenced by a strong endotherm with onset temperature at 111.2 °C.

CHN Data
Analysis for C_{35}H_{45}N_{3}O_{4}FCl: C 67.13, H 7.24, N 6.71. Found: C 66.79, H 7.23, N 6.62.

Powder X-Ray Diffraction Data
The powder X-ray diffraction pattern of example 1 was determined using a Bruker-AXS Ltd. D4 ENDEAVOR powder X-ray diffractometer fitted with an automatic sample changer, a theta-theta goniometer geometry, automatic beam divergence slit and a PSD Vantec-1 detector. The sample was prepared for analysis by mounting onto a low background silicon wafer specimen mount with a 0.5mm cavity. The specimen was rotated whilst being irradiated with copper κα1 X-rays (wavelength = 1.5406 Angstroms) with the X-ray tube operated at 35kV/40mA. The analysis was in continuous mode set for data acquisition at 0.2 second count per 0.018° step size over a two theta range of 2° to 55° at room temperature. Peak search was carried out using the threshold and width parameters set to 1 and 0.3, respectively, within the Eva software released by Bruker-AXS. The instrument calibration was verified using a corundum reference standard (NIST: SRM 1976 XRD flat plate intensity standard).

The measured pattern is shown in Figure 2. Resultant powder X-ray diffraction pattern with intensities and peaks location (angle 2θ error is +/- 0.1 degrees) are shown in the table 1:

<table>
<thead>
<tr>
<th>Angle (°2θ)</th>
<th>Intensity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.2</td>
<td>22.3</td>
</tr>
<tr>
<td>10.5</td>
<td>16.4</td>
</tr>
<tr>
<td>12.2</td>
<td>15.7</td>
</tr>
<tr>
<td>13.6</td>
<td>21.4</td>
</tr>
<tr>
<td>14.0</td>
<td>15.5</td>
</tr>
<tr>
<td>14.8</td>
<td>39.3</td>
</tr>
<tr>
<td>16.4</td>
<td>23.4</td>
</tr>
<tr>
<td>17.1</td>
<td>55.7</td>
</tr>
</tbody>
</table>
Example 2: re-crystallisation of the compound of example 1 from acetone

The hydrochloride salt of biphenyl-2-yl-carbamic acid 1-{9-[(3-fluoro-4-hydroxy-benzoyl)-methyl-amino]-nonyl}-piperidin-4-yl ester (example 1, 100 mg) was dissolved in dry acetone (approximatively 1 ml) at reflux. The solution was allowed to cool overnight and the crystalline product isolated by filtration and dried in vacuo to give the hydrochloride salt of biphenyl-2-yl-carbamic acid 1-{9-[(3-fluoro-4-hydroxy-benzoyl)-methyl-amino]-nonyl}-piperidin-4-yl ester with higher crystallinity in 60% yield, 60 mg.

DSC Data
The melting point of 2.027 mg of the material obtained in example 2 was determined using the same method as described for example 1 and was evidenced by a strong endotherm with onset temperature at 118.1°C. The DSC thermogram obtained trace is shown in Figure 2 with flat baselines and sharp endotherms corresponding to the melt. This higher melting point of acetone produced material can be attributed to a higher level of crystallinity and larger particle size.

Powder X-Ray Diffraction Data
The same anhydrous polymorphic form was obtained with the material of example 2 when characterised by PXRD using the same method as described for example 1.
Solid state NMR data

Approximately 80 mg of a 2 g sample of the material obtained in the same way as described above in example 2 (said sample having the same form and crystallinity by PXRD) were tightly packed into a 4 mm ZrO₂ rotor. Spectra were collected at ambient temperature and pressure on a Bruker-Biospin 4 mm BL CPMAS probe positioned into a wide-bore Bruker-Biospin DSX 500 MHz (¹H frequency) NMR spectrometer. The packed rotor was oriented at the magic angle and spun at 15.0 kHz. The ¹³C solid state spectrum was collected using a proton decoupled cross-polarization magic angle spinning (CPMAS). The cross-polarization contact time was set to 2.0 ms. A proton decoupling field of approximately 85 kHz was applied. 4096 scans were collected with a 8.5 second recycle delay. The carbon spectrum was referenced using an external standard of crystalline adamantane, setting its upfield resonance to 29.5 ppm. The fluorine solid state spectrum was collected using a proton decoupled magic angle spinning experiment (MAS). A proton decoupling field of approximately 85 kHz was applied. 8 scans were collected with recycle delay of 420 seconds. The fluorine spectrum was referenced using an external standard of trifluoroacetic acid (50% VA in H₂O), setting its resonance to -76.54 ppm.

The carbon chemical shifts observed are as follows:

<table>
<thead>
<tr>
<th>¹³C Chemical Shifts [ppm ± 0.2 ppm] a</th>
<th>Intensity b</th>
</tr>
</thead>
<tbody>
<tr>
<td>168.8</td>
<td>2.5</td>
</tr>
<tr>
<td>155.3</td>
<td>1.9</td>
</tr>
<tr>
<td>155.0</td>
<td>1.6</td>
</tr>
<tr>
<td>148.3</td>
<td>3.8</td>
</tr>
<tr>
<td>140.0</td>
<td>4.0</td>
</tr>
<tr>
<td>135.8</td>
<td>3.6</td>
</tr>
<tr>
<td>135.2</td>
<td>2.4</td>
</tr>
<tr>
<td>129.9</td>
<td>9.1</td>
</tr>
<tr>
<td>129.6</td>
<td>8.2</td>
</tr>
<tr>
<td>128.5</td>
<td>7.0</td>
</tr>
<tr>
<td>128.2</td>
<td>8.2</td>
</tr>
<tr>
<td>127.9</td>
<td>8.6</td>
</tr>
<tr>
<td>127.3</td>
<td>7.2</td>
</tr>
<tr>
<td>124.5</td>
<td>8.4</td>
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<tr>
<td>123.8</td>
<td>4.0</td>
</tr>
<tr>
<td>118.1</td>
<td>2.4</td>
</tr>
<tr>
<td>115.4</td>
<td>3.1</td>
</tr>
<tr>
<td>70.7</td>
<td>5.7</td>
</tr>
</tbody>
</table>
The 4 unique carbon chemical shifts observed are as follows:

<table>
<thead>
<tr>
<th>(^{13}\text{C} \text{ Chemical Shifts [ppm} \pm 0.2 \text{ ppm} )</th>
<th>Intensity (^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>140.0</td>
<td>4.0</td>
</tr>
<tr>
<td>124.5</td>
<td>8.4</td>
</tr>
<tr>
<td>49.1</td>
<td>6.0</td>
</tr>
<tr>
<td>30.9</td>
<td>12.0</td>
</tr>
</tbody>
</table>

The corresponding carbon CPMAS spectrum is illustrated in Figure 3.

The fluorine chemical shifts observed are as follows:

<table>
<thead>
<tr>
<th>(^{19}\text{F} \text{ Chemical Shifts [ppm} \pm 0.4 \text{ ppm} )</th>
<th>Intensity (^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>-133.5</td>
<td>12.0</td>
</tr>
</tbody>
</table>

\(^{a}\) Referred to external standard of trifluoroacetic acid (50% V/V in water) at -76.54 ppm.

\(^{b}\) Defined as peak heights.
Example 3: Binding affinity assessment at the human recombinant M₃ muscarinic receptor

Membrane preparation

Cell Pellets from CHO (Chinese Hamster Ovary) cells recombinantly expressing the human muscarinic M₃ receptor were homogenised in 20mM HEPES (pH 7.4) and centrifuged at 48000 x g for 20min at 4°C. The pellet was re-suspended in buffer and the homogenisation and centrifugation steps repeated. The resulting pellet was re-suspended in 1ml buffer per 1ml original packed cell volume and the homogenisation step repeated. Protein estimation was carried out on the suspension and 1ml aliquots of about 1mg/ml frozen at -80°C.

hM₃ competition binding Assay Protocol

Membranes (5Dg/well) were incubated with [³²H]-NMS (at a concentration 5 x Kᵰ) plus/minus test compound for 24hr at RT (room temperature) in a 1ml polystyrene 96-well deep well block. The final assay volume was 200µl, comprising of: 20µl plus/minus test compound; 20µl [³²H]-NMS (Perkin Elmer NEN 636) and 160µl membrane solution. Total Binding was defined with 0.1% DMSO; Non-Specific Binding was defined with 1µM Atropine. Assay buffer was 20mM Hepes (pH 7.4). Once all assay components were added, plates were covered and incubated at room temperature for 24 hrs with shaking. The assay was terminated by rapidly filtering through GF/B Unifilter plates pre-soaked with 0.5% polyethylenimine, using a Packard filtermate harvester, the filter plate was then washed with 3x1 ml 4°C assay buffer. The filter plates were dried at 45°C for 1hour. The bottoms of the filter plates were sealed and 50µl/well of Microscint Ò’ added, the top of the plates were sealed with a Topseal. Following 90 minutes, the plates were read on an NXT Topcount (1 minute read time per well).

The resulting data was expressed as a percentage of the specific binding (Specific binding = Total binding - Non-Specific Binding). % specific binding versus test compound concentration was plotted to determine an IC₅₀ from a sigmoid curve using an in-house data analysis programme. IC₅₀ values corrected to Ki values by applying the Cheng-Prussoff equation:

\[
K_i = \frac{IC_{50}}{1 + [L]/K_D}
\]
where IC<sub>50</sub> is the concentration of unlabelled drug which inhibits by 50% the specific radioligand binding. [L] is the free radioligand concentrations and K<sub>D</sub> and K<sub>i</sub> are the equilibrium dissociation constants of the radioligand and unlabelled drug respectively.

It has thus been found that the hydrochloride salt of biphenyl-2-yl-carbamic acid 1-{(9-[(3-fluoro-4-hydroxy-benzoyl)-methyl-amino]-nonyl)-piperidin-4-yl ester that has been tested in the above assay show hM<sub>3</sub> receptor antagonist activity of 0.615 nM.

**Example 4: Guinea Pig Trachea assay**

Male, Dunkin-Hartley guinea-pigs weighing 350-450g are culled in a rising concentration of CO<sub>2</sub>, followed by exsanguinations of the vena cava. Tracheas are dissected from the larynx to the entry point into the chest cavity and then placed in fresh, oxygenated, modified Krebs buffer solution (Krebs containing 10µM propranolol, 10µM guanethidine and 3µM indomethacin) at room temperature. The tracheas are opened by cutting through the cartilage opposite the trachealis muscle. Strips approximately 3-5 cartilage rings wide are cut. A cotton thread is attached to the cartilage at one end of the strip for attachment to the force transducer and a cotton loop made at the other end to anchor the tissue in the organ bath. The strips are mounted in 5ml organ baths filled with warm (37°C) aerated modified Krebs. The pump flow rate is set to 1.0 ml/min and the tissues washed continuously. Tissues are placed under an initial tension of 1000mg. Tissues are re-tensioned after 15 and 30 minutes, then allowed to equilibrate for a further 30-45 minutes.

Tissues are subjected to electrical field stimulation (EFS) of the following parameters: 10 secondes trains every 2 minutes, 0.1 ms pulse width, 10Hz and 10-30V. The voltage is raised 5V every 10 minutes within the stated range until a maximum contractile response for each tissue is observed. This just maximum voltage for each tissue is then used throughout the remainder of the experiment. Following equilibration to EFS for 20 minutes, the pump is stopped and after 15 minutes control readings are taken over a 8-1 0 minutes period (4-5 responses). The compound is then added to each tissue as a bolus dose at 30xKi (determined at the human M<sub>3</sub> receptor expressed in CHO cells in a filtration binding assay), and left to incubate for 2 hours. The compound is then washed from tissues using a rapid wash with modified Krebs for 1 minutes and flow is restored to 1ml/min for the remainder of the experiment. At the end of the experiment tissues are challenged with histamine (1µM)
to determine viability. Readings taken during the experiment are automatically collected using Notocord ® software. The raw data are converted into percent response taking into account measurements of inhibition of the EFS response. After starting washout, the times taken for the tissue to recover by 25% from the inhibition induced are recorded and used as a measure of compound duration of action. Tissue viability limits the duration of the experiment to 16 hours post-compound washout. Compounds are typically tested at n=2 to 5 to estimate duration of action.

Alternatively the following Guinea Pig Trachea assay can also be used: 
Trachea are removed from male Dunkin-Hartley guinea-pigs (wt 350-450g) and following removal of adherent connective tissue, an incision is made through the cartilage opposite the trachealis muscle and tracheal strips 3-5 cartilage rings wide prepared. The tracheal strips are suspended between an isometric strain gauge and a fixed tissue hook with the muscle in the horizontal plane in 5ml tissue baths under an initial tension of 1g and bathed in warmed (37°C) aerated (95%O₂/5%CO₂) Krebs solution containing 3µM indomethacin and 10µM guanethidine. The tissues are positioned between parallel platinum wire electrodes (~1cm gap). A constant 1ml/min flow of fresh Krebs solution (of the above composition) is maintained through the tissue baths using peristaltic pumps. The tissues are allowed to equilibrate for an hour with re-tensioning to 1g at 15 minutes and 30 minutes from the start of the equilibration period. At the end of the equilibration, tissues are electrically field stimulated (EFS) using the following parameters: 10V, 10Hz 0.1 ms pulse width with 10 seconds trains every 2 minutes. In each tissue a voltage response curve is constructed over the range 10v - 30V (keeping all other stimulation parameters constant) to determine a just maximal stimulation. Using these stimulation parameters EFS responses are 100% nerve mediated and 100% cholinergic as confirmed by blockade by 1µM tetrodotoxin or 1µM atropine. Tissues are then repeatedly stimulated at 2 minutes intervals until the responses were reproducible. The peristaltic pump is stopped 20 minutes prior to the addition of the study compound and the average twitch contraction over the last 10 minutes recorded as the control response. The study compound is added to the tissue baths, with each tissue receiving a single concentration of compound and allowed to equilibrate for 2 hours. At 2 hours post addition the inhibition of the EFS response is recorded and IC₅₀ curves generated using a range of compound concentrations over tracheal strips from the same animal.
The tissues are then rapidly washed and the 1ml/minute perfusion with Krebs solution re-established. Tissues are stimulated for a further 16 hours and recovery of the EFS response recorded. At the end of the 16 hours, 10\(\mu\)M histamine is added to the baths to confirm tissue viability. The just max concentration (tested concentration giving a response > 70% inhibition but less than 100%) of antagonist is identified from the IC\(_{50}\) curve and the time to 25% recovery of the induced inhibition (\(T_{25}\)) calculated in tissues receiving this concentration. Compounds are typically tested at \(n=2\) to 5 to estimate duration of action.
CLAIMS

1. A compound which is the hydrochloride salt of biphenyl-2-yl-carbamic acid 1-{9-[(3-fluoro-4-hydroxy-benzoyl)-methyl-amino]-nonyl}-piperidin-4-yl ester of formula:

![Chemical Structure]

2. The compound according to claim 1 characterized in that it is substantially crystalline.

3. The compound according to claim 2 having an X-ray diffraction pattern characterized by the following principal X-ray diffraction pattern peaks expressed in terms of 2-theta angle (± 0.1° 2θ) when measured using Cu Kα₁ radiation (Wavelength = 1.5406A):

<table>
<thead>
<tr>
<th>Angle (°2θ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.5</td>
</tr>
<tr>
<td>13.6</td>
</tr>
<tr>
<td>14.8</td>
</tr>
<tr>
<td>18.6</td>
</tr>
</tbody>
</table>

4. The compound according to claim 2 having a solid state $^{13}$C nuclear magnetic resonance characterized by the following principal chemical shifts expressed in parts per million (± 0.2 ppm) referenced to an external standard of solid phase adamantane at 29.5 ppm: 30.9, 49.1, 124.5 and 140.0.

5. The compound according to claim 2 having a solid state $^{19}$F nuclear magnetic resonance characterized by the following principal chemical shift expressed in parts per million (± 0.4 ppm) referenced to an external standard of trifluoroacetic acid, 50% V/V in water, at -76.54 ppm: -133.5.
6. A pharmaceutical composition comprising from 0.001 mg to 5000 mg of the compound according to any one of claims 1 to 5 together with one or more pharmaceutically acceptable excipients.

7. A pharmaceutical dry powder comprising 0.001 mg to 40 mg of the compound according to any one of claims 1 to 5 and lactose monohydrate.

8. A compound according to any one of claims 1 to 5 for use as a medicament.

9. A compound according to any one of claims 1 to 5 for use in the treatment of diseases, disorders, and conditions selected from the group consisting of chronic or acute bronchoconstriction, chronic bronchitis, small airways obstruction, emphysema, chronic eosinophilic pneumonia, chronic obstructive pulmonary disease (COPD), COPD that includes chronic bronchitis, pulmonary emphysema or dyspnea associated or not associated with COPD, COPD that is characterized by irreversible, progressive airways obstruction, adult respiratory distress syndrome (ARDS), exacerbation of airways hyper-reactivity consequent to other drug therapy, airways disease that is associated with pulmonary hypertension, bronchitis, acute bronchitis, acute laryngotracheal bronchitis, arachidic bronchitis, catarrhal bronchitis, croupus bronchitis, dry bronchitis, infectious asthmatic bronchitis, productive bronchitis, staphylococcus or streptococcal bronchitis vesicular bronchitis, asthma, atopic asthma, non-atopic asthma, allergic asthma, atopic bronchial IgE-mediated asthma, bronchial asthma, essential asthma, true asthma, intrinsic asthma caused by pathophysiologic disturbances, extrinsic asthma caused by environmental factors, essential asthma of unknown or inapparent cause, non-atopic asthma, bronchitic asthma, emphysematous asthma, exercise-induced asthma, allergen induced asthma, cold air induced asthma, occupational asthma, infective asthma caused by bacterial, fungal, protozoal, or viral infection, non-allergic asthma, incipient asthma, wheezy infant syndrome, bronchiolytis, acute lung injury, bronchiectasis, cylindrical bronchiectasis, sacculated bronchiectasis, fusiform bronchiectasis, capillary bronchiectasis, cystic bronchiectasis, dry bronchiectasis and follicular bronchiectasis.

10. The combination of a compound according to any one of claims 1 to 5 with one or more therapeutic agents selected from:
(a) 5-Lipoxygenase (5-LO) inhibitors or 5-lipoxygenase activating protein (FLAP) antagonists;
(b) Leukotriene antagonists (LTRAs) including antagonists of LTB$_4$, LTC$_4$, LTD$_4$, and LTE$_4$;
(c) Histamine receptor antagonists including H$_1$ and H$_3$ antagonists;
(d) $\alpha_1$- and $\alpha_2$-adrenoceptor agonist vasoconstrictor sympathomimetic agents for decongestant use;
(e) PDE inhibitors including PDE3, PDE4 and PDE5 inhibitors;
(f) Beta 2 receptor agonists;
(g) Theophylline;
(h) Sodium cromoglycate;
(i) COX inhibitors both non-selective and selective COX-1 or COX-2 inhibitors (NSAIDs);
(j) Prostaglandin receptor antagonists and inhibitors of prostaglandin synthase;
(k) Oral and inhaled glucocorticosteroids;
(l) Dissociated agonists of the corticoid receptor (DAGR);
(m) Monoclonal antibodies active against endogenous inflammatory entities;
(n) Anti-tumor necrosis factor (anti-TNF-a) agents;
(o) Adhesion molecule inhibitors including VLA-4 antagonists;
(p) Kinin-B$_1$- and B$_2$-receptor antagonists;
(q) Immunosuppressive agents including inhibitors of the IgE pathway and cyclosporine;
(r) Inhibitors of matrix metalloproteases (MMPs);
(s) Tachykinin NK$_1$, NK$_2$ and NK$_3$ receptor antagonists;
(t) Protease inhibitors such as elastase inhibitors;
(u) Adenosine A2a receptor agonists and A2b antagonists;
(v) Inhibitors of urokinase;
(w) Compounds that act on dopamine receptors such as D2 agonists;
(x) Modulators of the NFKB pathway such as IKK inhibitors;
(y) modulators of cytokine signalling pathway such as p38 MAP kinase, PI3 kinase, JAK kinase, syk kinase, EGFR or MK-2;
(z) Agents that can be classed as mucolytics or anti-tussive;
(aa) Agents, which enhance responses to inhaled corticosteroids;
(bb) Antibiotics and antiviral agents effective against micro-organisms which can colonise the respiratory tract;
(cc) HDAC inhibitors;
(dd) CXCR2 antagonists;
(ee) Integrin antagonists;
(ff) Chemokines;
(gg) Epithelial sodium channel (ENaC) blockers or Epithelial sodium channel (ENaC) inhibitors;
(hh) P2Y2 Agonists and other Nucleotide receptor agonists;
(iii) Inhibitors of thromboxane;
(jj) Inhibitors of PGD<sub>2</sub> synthesis and PGD<sub>2</sub> receptors (DP1 and DP2/CRTH2);
(kk) Niacin; and
(II) Adhesion factors including VLAM, ICAM, and ELAM.
The peaks marked by asterisks are spinning sidebands.
The peaks marked by asterisks are spinning sidebands.
### A. CLASSIFICATION OF SUBJECT MATTER

**INV.** C07D211/46  A61K31/4465  A61P11/00

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

### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D  A61K  A61P

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal , WPI Data, BIOSIS, EMBASE, CHEM ABS Data

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>wo 2010/007552 AI (PFIZER LTD [GB]); GLOSSOP PAUL ALAN [GB]; LANE CHARLOTTE ALICE LOUISE [] 21 January 2010 (2010-01-21) the whole document in particular example 69, page 9, line 21 and claims 1-10</td>
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<td>wo 2006/099032 AI (THERAVANCE INC [US]; JI YU-HUA [US]; HUSFELD CRAIG [US]; LI LI [US]; M) 21 September 2006 (2006-09-21) * abstract; claims 1-29; examples 1-5, 10</td>
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<td>US 2005/182092 AI (CHAO ROBERT [US] ET AL) 18 August 2005 (2005-08-18) page 1; claims 1-21; examples 1-4</td>
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**X** Further documents are listed in the continuation of Box C.  
**X** See patent family annex.

* Special categories of cited documents :
  - **"A"** document defining the general state of the art which is not considered to be of particular relevance
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  **"A"** document member of the same patent family

Date of the actual completion of the international search  
28 March 2011

Date of mailing of the international search report  
05/04/2011

Name and mailing address of the ISA/  
European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Fax. (+31-70) 340-3016

Authorized officer  
Papathoma, Sofi a
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