Abstract:
The invention provides a pharmaceutical product, kit or composition comprising a first active ingredient which is a selected muscarinic receptor antagonist selected, and a second active ingredient which is a β₂-adrenoceptor agonist, of use in the treatment of respiratory diseases such as chronic obstructive pulmonary disease and asthma.
PHARMACEUTICAL PRODUCT COMPRISING A MUSCARINIC RECEPTOR ANTAGONIST AND A β₂-ADRENOCEPTOR AGONIST

The present invention relates to combinations of pharmaceutically active substances for use in the treatment of respiratory diseases, especially chronic obstructive pulmonary disease (COPD) and asthma.

The essential function of the lungs requires a fragile structure with enormous exposure to the environment, including pollutants, microbes, allergens, and carcinogens. Host factors, resulting from interactions of lifestyle choices and genetic composition, influence the response to this exposure. Damage or infection to the lungs can give rise to a wide range of diseases of the respiratory system (or respiratory diseases). A number of these diseases are of great public health importance. Respiratory diseases include Acute Lung Injury, Acute Respiratory Distress Syndrome (ARDS), occupational lung disease, lung cancer, tuberculosis, fibrosis, pneumoconiosis, pneumonia, emphysema, Chronic Obstructive Pulmonary Disease (COPD) and asthma.

Among the most common of the respiratory diseases is asthma. Asthma is generally defined as an inflammatory disorder of the airways with clinical symptoms arising from intermittent airflow obstruction. It is characterised clinically by paroxysms of wheezing, dyspnea and cough. It is a chronic disabling disorder that appears to be increasing in prevalence and severity. It is estimated that 15% of children and 5% of adults in the population of developed countries suffer from asthma. Therapy should therefore be aimed at controlling symptoms so that normal life is possible and at the same time provide basis for treating the underlying inflammation.

COPD is a term which refers to a large group of lung diseases which can interfere with normal breathing. Current clinical guidelines define COPD as a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to
noxious particles and gases. The most important contributory source of such particles and
gases, at least in the western world, is tobacco smoke. COPD patients have a variety of
symptoms, including cough, shortness of breath, and excessive production of sputum; such
symptoms arise from dysfunction of a number of cellular compartments, including
neutrophils, macrophages, and epithelial cells. The two most important conditions covered
by COPD are chronic bronchitis and emphysema.

Chronic bronchitis is a long-standing inflammation of the bronchi which causes increased
production of mucous and other changes. The patients’ symptoms are cough and
expectoration of sputum. Chronic bronchitis can lead to more frequent and severe
respiratory infections, narrowing and plugging of the bronchi, difficult breathing and
disability.

Emphysema is a chronic lung disease which affects the alveoli and/or the ends of the
smallest bronchi. The lung loses its elasticity and therefore these areas of the lungs become
enlarged. These enlarged areas trap stale air and do not effectively exchange it with fresh
air. This results in difficult breathing and may result in insufficient oxygen being delivered
to the blood. The predominant symptom in patients with emphysema is shortness of breath.

Therapeutic agents used in the treatment of respiratory diseases include β₂-adrenoceptor
agonists. These agents (also known as beta2 (β₂) - agonists) may be used to alleviate
symptoms of respiratory diseases by relaxing the bronchial smooth muscles, reducing
airway obstruction, reducing lung hyperinflation and decreasing shortness of breath.
Compounds currently under evaluation as once-daily β₂ agonists are described in Expert

A further class of therapeutic agent used in the treatment of respiratory diseases are
muscarinic antagonists. Muscarinic receptors are a G-protein coupled receptor (GPCR)
family having five family members M₁, M₂, M₃, M₄ and M₅. Of the five muscarinic
subtypes, three (M₁, M₂ and M₃) are known to exert physiological effects on human lung
tissue. Parasympathetic nerves are the main pathway for reflex bronchoconstriction in human airways and mediate airway tone by releasing acetylcholine onto muscarinic receptors. Airway tone is increased in patients with respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD), and for this reason muscarinic receptor antagonists have been developed for use in treating airway diseases. Muscarinic receptor antagonists, often called anticholinergics in clinical practice, have gained widespread acceptance as a first-line therapy for individuals with COPD, and their use has been extensively reviewed in the literature (e.g. Lee et al, Current Opinion in Pharmacology 2001,1, 223-229).

Whilst treatment with a β2-adrenoceptor agonist or a muscarinic antagonist can yield important benefits, the efficacy of these agents is often far from satisfactory. Moreover, in view of the complexity of respiratory diseases such as asthma and COPD, it is unlikely that any one mediator can satisfactorily treat the disease alone. Hence there is a pressing medical need for new therapies against respiratory diseases such as COPD and asthma, in particular for therapies with disease modifying potential.

The present invention provides a pharmaceutical product comprising, in combination, a first active ingredient which is a muscarinic antagonist selected from:

(R)-1-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane X; and

(R)-1-[3-(2-Methyl-pyridin-4-yl)-propyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane X;

wherein X represents a pharmaceutically acceptable anion of a mono or polyvalent acid, and a second active ingredient which is a β2-adrenoceptor agonist, with the proviso that the second active ingredient is not N-Cyclohexyl-\(\Lambda^3\)-[2-(3-fluorophenyl)ethyl]-N-(2-[[2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl]amino] ethyl)-β-alaninamide or a salt thereof.
A beneficial therapeutic effect may be observed in the treatment of respiratory diseases if a muscarinic antagonist according to the present invention is used in combination with a β₂-adrenoreceptor agonist. The beneficial effect may be observed when the two active substances are administered simultaneously (either in a single pharmaceutical preparation or via separate preparations), or sequentially or separately via separate pharmaceutical preparations.

The pharmaceutical product of the present invention may, for example, be a pharmaceutical composition comprising the first and second active ingredients in admixture. Alternatively, the pharmaceutical product may, for example, be a kit comprising a preparation of the first active ingredient and a preparation of the second active ingredient and, optionally, instructions for the simultaneous, sequential or separate administration of the preparations to a patient in need thereof.

The first active ingredient in the combination of the present invention is a muscarinic antagonist selected from:

(R)-1-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-l-azonia-bicyclo[2.2.2]octane X; and

(R)-1-[3-(2-Methyl-pyridin-4-yl)-propyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-l-azonia-bicyclo[2.2.2]octane X;

wherein X represents a pharmaceutically acceptable anion of a mono or polyvalent acid.

The muscarinic antagonists of the invention are selected members of a novel class of compound described in co-pending application PCT/GB2007/004817 (WO2008/075005), which display high potency to the M3 receptor. The names of the muscarinic antagonists are IUPAC names generated by the Beilstein Autonom 2000 naming package, as supplied by MDL Information Systems Inc., based on the structures depicted in the examples, and stereochemistry assigned according to the Cahn-Ingold-Prelog system. For example, the name (i?)-l-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-l-azonia-bicyclo[2.2.2]octane, was generated from the structure:
The muscarinic antagonists of the present invention comprise an anion X associated with the positive charge on the quaternary nitrogen atom. The anion X may be any pharmaceutically acceptable anion of a mono or polyvalent (e.g. bivalent) acid. In an embodiment of the invention X may be an anion of a mineral acid, for example chloride, bromide, iodide, sulfate, toluenesulfonate (tosylate or 4-methylbenzenesulphonate), edisylate (ethane-1,2-disulfonate), isethionate (2-hydroxyethylsulfonate), nitrate or phosphate; or an anion of a suitable organic acid, for example acetate, maleate, fumarate, citrate, lactate, oxalate, oleic, succinate, tartrate, methanesulphonate (mesylate), p-toluenesulphonate, benzenesulphonate, napadisylate (naphthalene-1,5-disulphonate) (e.g. a heminapadisylate), maleate ((Z)-3-carboxy-acrylate), succinate (3-carboxy-propionate), malate ((5)-3-carboxy -2-hydroxy-propionate), p-acetamidobenzoate, 2,5-dichlorobenzenesulphonate, 1-hydroxy-2-naphthoate (xinafoate) or 1-hydroxynaphthalene-2-sulphonate.

In an embodiment of the invention, the first active ingredient is a muscarinic antagonist which is in the form of a bromide, benzenesulphonate or naphthalene-1,5-disulphonate salt.

In an embodiment of the invention, the first active ingredient is a muscarinic antagonist which is in the form of a bromide salt.
In an embodiment of the invention, the first active ingredient is a muscarinic antagonist which is in the form of a benzenesulphonate salt.

In an embodiment of the invention, the first active ingredient is a muscarinic antagonist which is in the form of a 4-methylbenzenesulphonate salt.

In an embodiment of the invention, the muscarinic receptor antagonist is selected from:

- (R)-1-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane bromide;
- (i?)-l-[3-(2-Methyl-pyridin-4-yl)-propyl]-3-((5)-2-phenyl-2-piperidin-l-yl-propionyloxy)-1-azonia-bicyclo [2.2.2]octane bromide.

The second active ingredient in the combination of the present invention is a \( \beta_2 \)-adrenoceptor agonist. The \( \beta_2 \)-adrenoceptor agonist of the present invention may be any compound or substance capable of stimulating the \( \beta_2 \)-receptors and acting as a bronchodilator. In the context of the present specification, unless otherwise stated, any reference to a \( \beta_2 \)-adrenoceptor agonist includes active salts, solvates or derivatives that may be formed from said \( \beta_2 \)-adrenoceptor agonist and any enantiomers and mixtures thereof. Examples of possible salts or derivatives of \( \beta_2 \)-adrenoceptor agonist are acid addition salts such as the salts of hydrochloric acid, hydrobromic acid, sulphuric acid, phosphoric acid, methanesulphonic acid, acetic acid, fumaric acid, succinic acid, lactic acid, citric acid, tartaric acid, 1-hydroxy-2-naphthalene-carboxylic acid, maleic acid, and pharmaceutically acceptable esters (e.g. C<sub>1</sub>-C<sub>6</sub> alkyl esters). The \( \beta_2 \)-agonists may also be in the form of solvates, e.g. hydrates.

Examples of a \( \beta_2 \)-adrenoceptor agonist that may be used in the pharmaceutical product according to this embodiment include metaproterenol, isoproterenol, isoprenaline, albuterol, salbutamol (e.g. as sulphate), formoterol (e.g. as fumarate), salmeterol (e.g. as xinafoate), terbutaline, orciprenaline, bitolterol (e.g. as mesylate), pirbuterol or indacaterol.

The \( \beta_2 \)-adrenoceptor agonist of this embodiment may be a long-acting \( \beta_2 \)-agonist (i.e. a \( \beta_2 \)-
agonist with activity that persists for more than 24 hours), for example salmeterol (e.g. as xinafoate), formoterol (e.g. as fumarate), bambuterol (e.g. as hydrochloride), carmoterol (TA 2005, chemically identified as 2(1H)-Quinolone, 8-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxy-phenyl)-1-methylethyl]-amino]ethyl]-monohydrochloride, [R-(R*,R*)] also identified by Chemical Abstract Service Registry Number 137888-1 1-0 and disclosed in U.S. Patent No 4,579,854), indacaterol (CAS no 312753-06-3; QAB-149), formanilide derivatives e.g. 3-(4-[[6-((2R)-2-[3-(formylamino)-4-hydroxyphenyl]-2-hydroxyethyl]amino)hexyl]oxy]-butyl)-benzenesulfonamide as disclosed in WO 2002/76933, benzenesulfonamide derivatives e.g. 3-(4-[[6-((2R)-2-hydroxy-2-[4-hydroxy-3-(hydroxy-methyl)phenyl]ethyl] amino-hexyl]oxy]butyl)benzenesulfonamide as disclosed in WO 2002/88167, aryl aniline receptor agonists as disclosed in WO 2003/042164 and WO 2005/025555, indole derivatives as disclosed in WO 2004/032921, in US 2005/222144, compounds GSK 159797, GSK 159802, GSK 597901, GSK 642444 and GSK 678007.

The β2-adrenoceptor agonist of the present invention is not N-Cyclohexyl-N3-[2-(3-fluorophenyl)ethyl]-N-[[2-(4-hydroxy-2-oxo-2,3-dihydro-l,3-benzothiazol-7-yl)ethyl]amino]ethyl]-β-alaninamide or a salt thereof. N-Cyclohexyl-N3-[2-(3-fluorophenyl)ethyl]-N-[[2-(4-hydroxy-2-oxo-2,3-dihydro-l,3-benzothiazol-7-yl)ethyl]amino]ethyl]-β-alaninamide and its di-D-mandelate, dihydrobromide and bis-trifluoroacetate salts are described in PCT/GB2007/004861.

In an embodiment of the present invention, the β2-adrenoceptor agonist is formoterol. The chemical name for formoterol is 7V-[2-hydroxy-5-[[[(1)l]-l-hydroxy-2-[[[(1)l]-2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]-formamide. The preparation of formoterol is described, for example, in WO 92/05147. In one aspect of this embodiment, the β2-adrenoceptor agonist is formoterol fumarate. It will be understood that the invention encompasses the use of all optical isomers of formoterol and mixtures thereof including racemates. Thus for example, the term formoterol encompasses N-[2-hydroxy-5-[[[(IR)-l-hydroxy-2-[[[(IR)-2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]-formamide, N-[2-hydroxy-5-[[[(IS)-l-hydroxy-2-[[[(IS)-2-(4-methoxyphenyl)-1-
methylethylaminoethylphenyl-formamide and a mixture of such enantiomers, including
a racemate.

In an embodiment of the invention, the $\beta_2$-adrenoceptor agonist is selected from:

$N$-[2-(Diethylamino)ethyl]-$N$-{(2-[(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-
yl)ethyl]amino)ethyl]-3-[2-(1-naphthyl)ethoxy]propanamide,

$N$-[2-(Diethylamino)ethyl]-$N$-{(2-[(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-
yl)ethyl]amino)ethyl]-3-[2-(3-chlorophenyl)ethoxy]propanamide, and

7-$(\mathcal{R})$-2-((2-[(3-[(2-Chlorophenyl)ethyl]amino)propyl]thio)ethyl] amino)-1-
hydroxyethyl]-4-hydroxy-1,3-benzothiazol-2(3$H$)-one, or a pharmaceutically acceptable salt thereof. The $\beta_2$-adrenoceptor agonists according to this embodiment may be prepared as described in the experimental preparation section of the present application. The names of the $\beta_2$-adrenoceptor agonists of this embodiment are IUPAC names generated by the IUPAC NAME, ACD Labs Version 8 naming package.

In a further embodiment of the invention, the $\beta_2$-adrenoceptor agonist is selected from:

$N$-[2-(Diethylamino)ethyl]-$N$-{(2-[(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-
yl)ethyl]amino)ethyl]-3-[2-(1-naphthyl)ethoxy]propanamide dihydrobromide,

$N$-[2-(Diethylamino)ethyl]-$N$-{(2-[(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-
yl)ethyl]amino)ethyl]-3-[2-(3-chlorophenyl)ethoxy]propanamide dihydrobromide, and

7-$(\mathcal{R})$-2-((2-[(3-[(2-Chlorophenyl)ethyl]amino)propyl]thio)ethyl] amino)-1-
hydroxyethyl]-4-hydroxy-1,3-benzothiazol-2(3$H$)-one dihydrobromide.

In an embodiment of the invention, the muscarinic receptor antagonist is (i?)-1-[2-(4-
Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-
bicyclo[2.2.2]octane X wherein X represents a pharmaceutically acceptable anion of a
mono or polyvalent acid, and the $\beta_2$-adrenoceptor agonist is formoterol (e.g. as fumarate).

In one aspect of this embodiment, the muscarinic receptor antagonist is (i?)-1-[2-(4-Fluoro-
phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-
bicyclo[2.2.2]octane bromide.
In an embodiment of the invention, the muscarinic receptor antagonist is (i?)-l-[3-(2-Methyl-pyridin-4-yl)-propyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane X, and the ß2-adrenoceptor agonist is formoterol (e.g. as fumarate).

In one aspect of this embodiment, the muscarinic receptor antagonist is (7?)-l-[3-(2-Methyl-pyridin-4-yl)-propyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane bromide.

In an embodiment of the invention, the muscarinic receptor antagonist is (i?)-l-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane X wherein X represents a pharmaceutically acceptable anion of a mono or polyvalent acid, and the ß2-adrenoceptor agonist is N-[2-(Diethylamino)ethyl]-7V-(2-[(2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl]amino)ethyl]-3-[2-(1-naphthyl)ethoxy]propanamide or a pharmaceutically acceptable salt thereof (e.g. dihydrobromide). In one aspect of this embodiment, the muscarinic receptor antagonist is (R)-1-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane bromide. In a second aspect of this embodiment, the muscarinic receptor antagonist is (i?)-l-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane 4-methylbenzenesulphonate.

In an embodiment of the invention, the muscarinic receptor antagonist is (i?)-l-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane bromide and the ß2-adrenoceptor agonist is N-[2-(Diethylamino)ethyl]-N-[2-[(2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl]amino]ethyl]-3-[2-(1-naphthyl)ethoxy]propanamide dihydrobromide.

In an embodiment of the invention, the muscarinic receptor antagonist is (i?)-l-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane 4-methylbenzene sulphonate and the ß2-adrenoceptor agonist is N-[2-
In an embodiment of the invention, the muscarinic receptor antagonist is (i?)-l-[2-(4-
Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-
bicyclo[2.2.2]octane X wherein X represents a pharmaceutically acceptable anion of a mono or polyvalent acid, and the β2-adrenoceptor agonist is N-[2-(Diethylamino)ethyl]-7V-
(2-[2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl]amino)ethyl]-3-[2-(1-
naphthyl)ethoxy]propanamide or a pharmaceutically acceptable salt thereof (e.g.
dihydrobromide). In one aspect of this embodiment, the muscarinic receptor antagonist is
(R)-1-[3-(2-Methyl-pyridin-4-yl)-propyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-
azonia-bicyclo[2.2.2]octane bromide.

In an embodiment of the invention, the muscarinic receptor antagonist is (i?)-l-[2-(4-
Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-
bicyclo[2.2.2]octane X wherein X represents a pharmaceutically acceptable anion of a mono or polyvalent acid, and the β2-adrenoceptor agonist is 7-[l(i?)-2-((2-[3-((2-
Chlorophenyl)ethyl]amino)propyl)thio)ethyl] amino]-1-hydroxyethyl]-4-hydroxy-1,3-
benzothiazol-2(3 H)-one or a pharmaceutically acceptable salt thereof (e.g.
dihydrobromide). In one aspect of this embodiment, the muscarinic receptor antagonist is
(R)-1-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-
azonia-bicyclo[2.2.2]octane bromide.

In an embodiment of the invention, the muscarinic receptor antagonist is (i?)-l-[2-(4-
Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-
bicyclo[2.2.2]octane X wherein X represents a pharmaceutically acceptable anion of a mono or polyvalent acid, and the β2-adrenoceptor agonist is 7-[li?]2-((2-[3-((2-
Chlorophenyl)ethyl]amino)propyl)thio)ethyl] amino)-1-hydroxyethyl]-4-hydroxy-1,3-
benzothiazol-2(3 H)-one or a pharmaceutically acceptable salt thereof (e.g.
dihydrobromide). In one aspect of this embodiment, the muscarinic receptor antagonist is
(R)-1-[3-(2-Methyl-pyridin-4-yl)-propyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane bromide.

The combination of the present invention may provide a beneficial therapeutic effect in the treatment of respiratory diseases. Examples of such possible effects include improvements in one or more of the following parameters: reducing inflammatory cell influx into the lung, mild and severe exacerbations, FEVi (forced expiratory volume in one second), vital capacity (VC), peak expiratory flow (PEF), symptom scores and Quality of Life.

The muscarinic antagonist (first active ingredient) and β₂-adrenoceptor agonist (second active ingredient) of the present invention may be administered simultaneously, sequentially or separately to treat respiratory diseases. By sequential it is meant that the active ingredients are administered, in any order, one immediately after the other. They may still have the desired effect if they are administered separately, but when administered in this manner they will generally be administered less than 4 hours apart, more conveniently less than two hours apart, more conveniently less than 30 minutes apart and most conveniently less than 10 minutes apart.

The active ingredients of the present invention may be administered by oral or parenteral (e.g. intravenous, subcutaneous, intramuscular or intraarticular) administration using conventional systemic dosage forms, such as tablets, capsules, pills, powders, aqueous or oily solutions or suspensions, emulsions and sterile injectable aqueous or oily solutions or suspensions. The active ingredients may also be administered topically (to the lung and/or airways) in the form of solutions, suspensions, aerosols and dry powder formulations. These dosage forms will usually include one or more pharmaceutically acceptable ingredients which may be selected, for example, from adjuvants, carriers, binders, lubricants, diluents, stabilising agents, buffering agents, emulsifying agents, viscosity-regulating agents, surfactants, preservatives, flavourings and colorants. As will be understood by those skilled in the art, the most appropriate method of administering the active ingredients is dependent on a number of factors.
In one embodiment of the present invention the active ingredients are administered via separate pharmaceutical preparations. Therefore, in one aspect, the present invention provides a kit comprising a preparation of a first active ingredient which is a muscarinic antagonist according to the present invention, and a preparation of a second active ingredient which is a $\beta_2$-adrenoceptor agonist, and optionally instructions for the simultaneous, sequential or separate administration of the preparations to a patient in need thereof.

In another embodiment the active ingredients may be administered via a single pharmaceutical composition. Therefore, the present invention further provides a pharmaceutical composition comprising, in admixture, a first active ingredient, which is a muscarinic antagonist according to the present invention, and a second active ingredient, which is a $\beta_2$-adrenoceptor agonist.

The pharmaceutical compositions of the present invention may be prepared by mixing the muscarinic antagonist (first active ingredient) with a $\beta_2$-adrenoceptor agonist (second active ingredient) and a pharmaceutically acceptable adjuvant, diluent or carrier. Therefore, in a further aspect of the present invention there is provided a process for the preparation of a pharmaceutical composition, which comprises mixing a muscarinic antagonist according to the present invention with a $\beta_2$-adrenoceptor agonist and a pharmaceutically acceptable adjuvant, diluent or carrier.

It will be understood that the therapeutic dose of each active ingredient administered in accordance with the present invention will vary depending upon the particular active ingredient employed, the mode by which the active ingredient is to be administered, and the condition or disorder to be treated.

In one embodiment of the present invention, the muscarinic antagonist according to the present invention is administered via inhalation. When administered via inhalation the dose
of the muscarinic antagonist according to the present invention will generally be in the range of from 0.1 microgram (µg) to 5000 µg, 0.1 to 1000 µg, 0.1 to 500 µg, 0.1 to 100 µg, 0.1 to 50 µg, 0.1 to 5 µg, 5 to 5000 µg, 5 to 1000 µg, 5 to 500 µg, 5 to 100 µg, 5 to 50 µg, 5 to 10 µg, 10 to 5000 µg, 10 to 1000 µg, 10 to 500 µg, 10 to 100 µg, 10 to 50 µg, 20 to 5000 µg, 20 to 1000 µg, 20 to 500 µg, 20 to 100 µg, 20 to 50 µg, 50 to 5000 µg, 50 to 1000 µg, 50 to 500 µg, 50 to 100 µg, 50 to 50 µg, 100 to 5000 µg, 100 to 1000 µg or 100 to 500 µg. The dose will generally be administered from 1 to 4 times a day, conveniently once or twice a day, and most conveniently once a day.

In one embodiment of the present invention the β₂-adrenoceptor agonist may conveniently be administered by inhalation. When administered via inhalation the dose of the β₂-agonist will generally be in the range of from 0.1 to 50 µg, 0.1 to 40 µg, 0.1 to 30 µg, 0.1 to 20 µg, 0.1 to 10 µg, 5 to 10 µg, 5 to 50 µg, 5 to 40 µg, 5 to 30 µg, 5 to 20 µg, 5 to 10 µg, 10 to 50 µg, 10 to 40 µg, 10 to 30 µg, or 10 to 20 µg. The dose will generally be administered from 1 to 4 times a day, conveniently once or twice a day, and most conveniently once a day.

In one embodiment, the present invention provides a pharmaceutical product comprising, in combination, a first active ingredient which is a muscarinic antagonist according to the present invention, and a second active ingredient which is a β₂-adrenoceptor agonist, wherein each active ingredient is formulated for inhaled administration.

In yet a further embodiment of the present invention, the first active ingredient, which is a muscarinic antagonist, and the second active ingredient(s), as defined herein above, wherein each active ingredient is formulated for oral administration.

In one embodiment, the pharmaceutical preparations of active ingredients may be administered simultaneously.

In one embodiment, the different pharmaceutical preparations of active ingredients may be administered sequentially.
In one embodiment, the different pharmaceutical preparations of active ingredients may be administered separately.

The active ingredients of the present invention are conveniently administered via inhalation (e.g. topically to the lung and/or airways) in the form of solutions, suspensions, aerosols and dry powder formulations. For example metered dose inhaler devices may be used to administer the active ingredients, dispersed in a suitable propellant and with or without additional excipients such as ethanol, surfactants, lubricants or stabilising agents. Suitable propellants include hydrocarbon, chlorofluorocarbon and hydrofluoroalkane (e.g. heptafluoroalkane) propellants, or mixtures of any such propellants. Preferred propellants are P134a and P227, each of which may be used alone or in combination with other propellants and/or surfactant and/or other excipients. Nebulised aqueous suspensions or, preferably, solutions may also be employed, with or without a suitable pH and/or tonicity adjustment, either as a unit-dose or multi-dose formulations.

Dry powder formulations and pressurized HFA aerosols of the active ingredients may be administered by oral or nasal inhalation. For inhalation, the compound is desirably finely divided. The finely divided compound preferably has a mass median diameter of less than 10 µm, and may be suspended in a propellant mixture with the assistance of a dispersant, such as a Cs-C₂₈ fatty acid or salt thereof, (for example, oleic acid), a bile salt, a phospholipid, an alkyl saccharide, a perfluorinated or polyethoxylated surfactant, or other pharmaceutically acceptable dispersant.

One possibility is to mix the finely divided compound of the invention with a carrier substance, for example, a mono-, di- or polysaccharide, a sugar alcohol, or another polyol. Suitable carriers are sugars, for example, lactose, glucose, raffinose, melezitose, lactitol, maltitol, trehalose, sucrose, mannitol, and starch. Alternatively the finely divided compound may be coated by another substance. The powder mixture may also be dispensed into hard gelatine capsules, each containing the desired dose of the active compound.
Another possibility is to process the finely divided powder into spheres which break up during the inhalation procedure. This spheronized powder may be filled into the drug reservoir of a multidose inhaler, for example, that known as the Turbuhaler® in which a dosing unit meters the desired dose which is then inhaled by the patient. With this system the active ingredient, with or without a carrier substance, is delivered to the patient.

The combination of the present invention is useful in the treatment or prevention of respiratory-tract disorders such as chronic obstructive pulmonary disease (COPD), chronic bronchitis of all types (including dyspnoea associated therewith), asthma (allergic and non-allergic; 'wheezy-infant syndrome'), adult/acute respiratory distress syndrome (ARDS), chronic respiratory obstruction, bronchial hyperactivity, pulmonary fibrosis, pulmonary emphysema, and allergic rhinitis, exacerbation of airway hyperreactivity consequent to other drug therapy, particularly other inhaled drug therapy or pneumoconiosis (for example aluminosis, anthracosis, asbestosis, chalicosis, ptilosis, siderosis, silicosis, tabacosis and byssinosis).

Dry powder inhalers may be used to administer the active ingredients, alone or in combination with a pharmaceutically acceptable carrier, in the later case either as a finely divided powder or as an ordered mixture. The dry powder inhaler may be single dose or multi-dose and may utilise a dry powder or a powder-containing capsule.

Metered dose inhaler, nebuliser and dry powder inhaler devices are well known and a variety of such devices are available.

The present invention further provides a pharmaceutical product, kit or pharmaceutical composition according to the invention for simultaneous, sequential or separate use in therapy.
The present invention further provides the use of a pharmaceutical product, kit or pharmaceutical composition according to the invention in the treatment of a respiratory disease, in particular chronic obstructive pulmonary disease or asthma.

The present invention further provides the use of a pharmaceutical product, kit or pharmaceutical composition according to the invention in the manufacture of a medicament for the treatment of a respiratory disease, in particular chronic obstructive pulmonary disease or asthma.

The present invention still further provides a method of treating a respiratory disease which comprises simultaneously, sequentially or separately administering:

(a) a (therapeutically effective) dose of a first active ingredient which is a muscarinic antagonist according to the present invention; and
(b) a (therapeutically effective) dose of a second active ingredient which is a $\beta_2$-adrenoceptor agonist according to the present invention;

to a patient in need thereof.

In the context of the present specification, the term "therapy" also includes "prophylaxis" unless there are specific indications to the contrary. The terms "therapeutic" and "therapeutically" should be construed accordingly. Prophylaxis is expected to be particularly relevant to the treatment of persons who have suffered a previous episode of, or are otherwise considered to be at increased risk of, the condition or disorder in question. Persons at risk of developing a particular condition or disorder generally include those having a family history of the condition or disorder, or those who have been identified by genetic testing or screening to be particularly susceptible to developing the condition or disorder.

The pharmaceutical product, kit or composition of the present invention may optionally comprise a third active ingredient which third active ingredient is a substance suitable for
use in the treatment of respiratory diseases. Examples of a third active ingredient that may be incorporated into the present invention include

- a phosphodiesterase inhibitor,
- a modulator of chemokine receptor function,
- an inhibitor of kinase function,
- a protease inhibitor,
- a steroidal glucocorticoid receptor agonist, and a
- a non-steroidal glucocorticoid receptor agonist.

Examples of a phosphodiesterase inhibitor that may be used as a third active ingredient according to this embodiment include a PDE4 inhibitor such as an inhibitor of the isoform PDE4D, a PDE3 inhibitor and a PDE5 inhibitor. Examples include the compounds

(Z)-3-(3,5-dichloro-4-pyridyl)-2-[4-(2-indanyloxy-5-methoxy-2-pyridyl]propenenitrile,

JV-[9-amino-4-oxo-1-phenyl-3,4,6,7-tetrahydropyrrolo[3,2, 1-jk] [1,4]benzodiazepin-3(7?)-yl]pyridine-3-carboxamide (CI-1044),

3-(benzyloxy)-1-(4-fluorobenzyl)- N-[3-[(methylsulphonyl)phenyl]-7 H-indole-2-carboxamide,

(75'-exo)-5-[3-(bicyclo[2.2.1]hept-2-yloxy)-4-methoxyphenyl]tetrahydro-2(i H)-pyrimidinone (Atizoram),

N-(3,5,dichloro-4-pyridinyl)-2-[l-(4-fluorobenzyl)-5-hydroxy-i H-indol-3-yl]-2-oxoacetamide (AWD-12-281),

β-[3-(cyclopentloxy)-4-methoxyphenyl]-l,3-dihydro-1,3-dioxo-2/f-isoindole-2-propanamide (CDC-801),

JV-[9-methyl-4-oxo-1-phenyl-3,4,6,7-tetrahydropyrrolo[3,2, 1-jk] [1,4]benzodiazepin-3(i?)-yl]pyridine-4-carboxamide (CI-1018),

Cis-[4-cyano-4-(3-cyclopentloxy-4-methoxyphenyl)cyclohexane-1-carboxylic acid (Cilomilast),

8-amino-1,3-bis(cyclopropymethyl)xanthine (Cipamfylline),

N-(2,5-dichloro-3-pyridinyl)-8-methoxy-5-quinolinecarboxamide (D-4418),

5-(3,5-di-tert-butyl-4-hydroxybenzylidene)-2-iminothiazolidin-4-one (Darbufelone),
2-methyl-1-[2-[(1-methylethyl)pyrazolo[1,5-a]pyridin-3-yl]-1-propanone (Ibudilast),
2-(2,4-dichlorophenylcarbonyl)-3-ureidobenzofuran-6-yl methanesulphonate (Lirimilast),
(-)-(iR)-5-(4-methoxy-3-propoxyphenyl)-5-methyloxazolidin-2-one (Mesopram),
(-)-cis-9-ethoxy-8-methoxy-2-methyl-1,2,3,4,4a,10b-hexahydro-6-(4-
 diisopropylaminocarbonylethyl)benzo[c][1,6]naphthyridine (Pumafentrine),
3-[(3-cyclopropyloxy)-4-methoxyphenyl]-methyl]-N-ethyl-8-(1-methylethyl)-1H-purine-
6-amine (V-11294A).

Examples of a modulator of chemokine receptor function that may be used as a third active
ingredient according to this embodiment include a CCR3 receptor antagonist, a CCR4
receptor antagonist, a CCR5 receptor antagonist and a CCR8 receptor antagonist.

Examples of an inhibitor of kinase function that may be used as a third active ingredient
according to this embodiment include a p38 kinase inhibitor and an IKK inhibitor.

Examples of a protease inhibitor that may be used as a third active ingredient according to
this embodiment include an inhibitor of neutrophil elastase or an inhibitor of MMP12.

Examples of a steroidal glucocorticoid receptor agonist that may be used as a third active
ingredient according to this embodiment include budesonide, fluticasone (e.g. as
propionate ester), mometasone (e.g. as furoate ester), beclometasone (e.g. as 17-
propionate or 17,21-dipropionate esters), ciclesonide, loteprednol (as e.g. etabonate),
etiprednol (as e.g. dicloacetate), triamcinolone (e.g. as acetonide), flunisolide, zoticasone,
flumoxonide, rolfeponide, butixocort (e.g. as propionate ester), prednisolone, prednisone,
tipredane, steroid esters e.g. 6α,9α-difluoro-17α-[(2-furanylcarbonyl)oxy]-1 1β-hydroxy-
16α-methyl-3-oxo-androsta-1,4-diene-17 β-carbothioic acid S-fluoromethyl ester, 6α,9α-
difluoro-11\beta\text{-}hydroxy-16\alpha\text{-}methyl-3\text{-}oxo-17\alpha\text{-}propionyloxy-androsta-1,4\text{-}diene-17\beta\text{-}carbothioic acid S^-\text{oxy-tetrahydro-furan-SS-yl) ester and 6\alpha,9\alpha\text{-}difluoro-11\beta\text{-}hydroxy-16\alpha\text{-}methyl-17\alpha\text{-}[(4-methyl-1,3-thiazole-5-carbonyl)oxy]-3\text{-}oxo-androsta-1,4\text{-}diene-17\beta\text{-}carbothioic acid S-fluoromethyl ester, steroid esters according to DE 4129535, steroids according to WO 2002/00679, WO 2005/041980, or steroids GSK 870086, GSK 685698 and GSK 799943.

Examples of a modulator of a non-steroidal glucocorticoid receptor agonist that may be used as a third active ingredient according to this embodiment include those described in WO2006/046916.

The invention is illustrated by the following non-limiting Examples. In the Examples the following Figures are presented:

Figure 1: X-ray powder diffraction (XRPD) pattern of muscarinic antagonist (7\text{-})-l-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane bromide Form A.

Figure 2: X-ray powder diffraction (XRPD) pattern of muscarinic antagonist (i\text{-})-l-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane bromide Form C.

Figure 3: X-ray powder diffraction (XRPD) pattern of muscarinic antagonist (i\text{-})-l-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane 4-methylbenzene sulphonate.

Figure 4: Percentage relaxation to indacaterol (10nM). (i\text{-})-l-[2-(4-Fluoro-phenyl)-ethyl]-3-((S)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane bromide (InM) and the combination of indacaterol (10nM) and (i\text{-})-l-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane bromide (InM) in guinea pig trachea in vitro.

Figure 5: Percentage relaxation to 77\text{-}[2-(Diethylamino)ethyl]-N-2-[(2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl]amino]ethyl)-3-[2-(1-naphthylethoxy)propanamide dihydrobromide (3nM), (i\text{-})-l-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-
Preparation of Muscarinic Antagonists

Muscarinic antagonists according to the present invention may be prepared as follows. Alternative salts to those described herein may be prepared by conventional chemistry using methods analogous to those described.

General Experimental Details for Preparation of Muscarinic Antagonists

Unless otherwise stated the following general conditions were used in the preparation of the Muscarinic Antagonists.

All reactions were carried out under an atmosphere of nitrogen unless specified otherwise.

In the examples the NMR spectra were measured on a Varian Unity Inova spectrometer at a proton frequency of either 300, 400 or 500 MHz. The MS spectra were measured on either an Agilent 1100 MSD G1946D spectrometer or a Hewlett Packard HP1 100 MSD G1946A spectrometer. Preparative HPLC separations were performed using a Waters Symmetry® or Xterra® column using 0.1% aqueous trifluoroacetic acid: acetonitrile, 0.1% aqueous ammonia: acetonitrile or 0.1% ammonium acetate: acetonitrile as the eluent.

Preparative Chiral HPLC separations were performed by the systems listed in the examples and chiral purity of the resulting fragments confirmed to be >95% enantiomeric excess by analytical Chiral HPLC. SCX and NH₂ resin were obtained from Varian Incorporated. IUPAC names were generated using Beilstein Autonom 2000. Stereochemistry was assigned according to the Cahn-Ingold-Prelog system. Absolute configuration at the 3 position is assigned on the basis of the (i?)-quinuclidin-3-ol intermediate; (i?)-quinulcidin-3-ol is commercially available from Acros Organics. Absolute configuration at the 2' position of Example 2 is assigned on the basis of the (5)-2-phenyl-2-piperidin-l-yl-
propionic acid methyl ester intermediate (Intermediate A - Isomer 1) used in its preparation. The absolute configuration of Intermediate A - Isomer 1 is assigned on the basis of the absolute configuration of Example 1, which was assigned by single crystal X-ray diffraction. The preparation of Intermediate A - Isomer 1, its use in the preparation of Example 1, and the assignment of the absolute configuration of Example 1 by single crystal X-ray diffraction is described below.

Abbreviations used in the experimental section:

Aq = aqueous
DCE = 1,2-dichloroethane
DCM = dichloromethane
DMF = dimethylformamide
DMSO = Dimethylsulfoxide
EtOAc = ethyl acetate
EtOH = ethanol
DSC = Differential Scanning Calorimeter
GVS = Gravimetric vapour sorption
TGA = Thermogravimetric analysis
XRPD = X-Ray Powder Diffraction
HATU = O-(7-Azabenzotriazol-1-yl)-Λ,N,Λ',N'-tetramethyluronium hexafluorophosphohte
MeCN - Acetonitrile
MeOH = methanol
RT = Room Temperature
Rt = retention time
THF = tetrahydrofuran
Satd = saturated

Instrument Details for Solid State Analysis

- X-Ray Powder Diffraction (XRPD) - PANalytical X'Pert machine in 20 - O configuration or a PANalytical Cubix machine in O - O configuration over the scan range 2° to 40° 20 with 100-second exposure per 0.02° increment. The X-rays
were generated by a copper long-fine focus tube operated at 45kV and 40mA. The wavelength of the copper X-rays was 1.5418 Å. The data was collected on zero background holders on which ~2mg of the compound was placed. The holder was made from a single crystal of silicon, which had been cut along a non-diffracting plane and then polished on an optically flat finish. The X-rays incident upon this surface were negated by Bragg extinction.

Differential Scanning Calorimetry (DSC) thermograms were measured using a TA Q1000 Differential Scanning Calorimeter, with aluminium pans and pierced lids. The sample weights varied between 0.5 to 5mg. The procedure was carried out under a flow of nitrogen gas (50mL/min) and the temperature studied from 30 to 230°C at a constant rate of temperature increase of 10°C per minute.

Gravimetric Vapour Sorption (GVS) profiles were measured using a Surface Measurements Systems Dynamic Vapour Sorption DVS-I or a DVS Advantage instrument. The solid sample ca. 1-5mg was placed into a glass vessel and the weight of the sample was recorded during a dual cycle step method (40 to 90 to 0 to 90 to 0% relative humidity (RH), in steps of 10% RH).

Preparation of Intermediates

**Intermediate A** (Isomers 1 & 2): 2-Phenyl-2-piperidin-1-yl-propionic acid methyl ester

![Chemical Structure](image)

A solution of methyl 2-bromo-2-phenylpropanoate (1 g) in acetonitrile (30 mL) was treated with piperidine (1 mL). The solution was stirred and heated under reflux for 3 h then concentrated to dryness. The residue was purified by flash column chromatography on
silica gel using ether/isohexane (3:7) to afford the racemic sub-titled compound as a colourless oil (0.8 g). The mixture of enantiomers was separated by chiral HPLC using a chiracel OJ-H column using an isocratic system of 80% isohexane/ethanol to afford the two enantiomers, which were defined as Isomer 1 and Isomer 2 in order of elution.

2-Phenyl-2-piperidin-1-yl-propionic acid methyl ester (Isomer 1)

Chiral HPLC 80:20 isohexane:ethanol (isocratic). Chiracel OJ-H 4.6mm x 50mm
Retention time 1.09min.

1H NMR (400 MHz, CDCl3) δ 7.56 - 7.49 (2H, m), 7.35 - 7.20 (3H, m), 3.68 (3H, s), 2.54 - 2.45 (2H, m), 2.41 - 2.32 (2H, m), 1.64 - 1.54 (7H, m), 1.50 - 1.42 (2H, m).

2-Phenyl-2-piperidin-1-yl-propionic acid methyl ester (Isomer 2)

Chiral HPLC 80:20 isohexane:ethanol (isocratic). Chiracel OJ-H 4.6mm x 50mm
Retention time 2.52min.

1H NMR (400 MHz, CDCl3) δ 7.56 - 7.49 (2H, m), 7.35 - 7.20 (3H, m), 3.68 (3H, s), 2.54 - 2.45 (2H, m), 2.41 - 2.32 (2H, m), 1.64 - 1.54 (7H, m), 1.50 - 1.42 (2H, m).

Example 1 (Form A): (R)-1-[2-(4-Fluoro-phenyl)-ethyl]-3-((S)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane bromide (Form A)

Intermediate B: 2-Phenyl-2-piperidin-1-yl-propionic acid (i?)-(1-aza-bicyclo[2.2.2]oct-3-yl) ester (Isomer 1)
A mixture of 2-phenyl-2-piperidin-1-yl-propionic acid methyl ester (Intermediate A, Isomer 1) (0.9 g), (i?)-quinuclidin-3-ol (1.157 g) and sodium hydride (60% in mineral oil, 0.335 g) in dry toluene (20 mL) was heated at 120°C under an atmosphere of nitrogen for 8 h. The cooled reaction mixture was diluted with water (100 mL) and extracted with diethyl ether (2 x 150 mL). The combined extracts were dried (MgSO₄) and concentrated to give an oil. The crude product was purified by flash column chromatography on silica eluting with (ethyl acetate / methanol 9:1) to afford the titled compound (0.500 g).

¹H NMR (400 MHz, DMSO) δ 7.59 - 7.51 (2H, m), 7.40 - 7.21 (3H, m), 4.72 - 4.62 (IH, m), 3.34 - 3.26 (IH, m), 3.04 - 2.92 (IH, m), 2.75 - 2.13 (7H, m), 1.89 - 1.75 (IH, m), 1.71 - 1.20 (14H, m).

(R)-l-[2-(4-Fluoro-phenyl)-ethyl]-3-((S)-2-phenyl-2-piperidin-1-yl-propionyloxy)-l-azonia-bicyclo [2.2.2]octane bromide (Form A)

2-Phenyl-2-piperidin-1-yl-propionic acid (i?)-(l-aza-bicyclo[2.2.2]oct-3-yl) ester (Intermediate B, Isomer 1) (3 g) in acetonitrile (25 mL) was treated with l-(2-bromoethyl)-4-fluorobenzene (2.384 g) and the mixture stirred at RT for 24 h. The mixture was concentrated to dryness, and the residue purified on silica gel eluting with 10% methanol in dichloromethane. The product containing fractions were combined, concentrated to dryness and the foam residue re-dissolved in acetonitrile (20 mL). To the solution was
added diethyl ether (40 mL) and the resulting solid collected by filtration. The solid was dissolved in hot acetone (75 mL) and then allowed to cool overnight. The resulting solid was collected by filtration and dried at 50°C to afford the titled compound (3.70 g).

m/e 465 [M]+

$^1$H NMR (400 MHz, DMSO) $\delta$ 7.58 - 7.54 (2H, m), 7.40 - 7.32 (4H, m), 7.31 - 7.26 (1H, m), 7.23 - 7.16 (2H, m), 5.14 - 5.09 (1H, m), 3.95 - 3.85 (1H, m), 3.62 - 3.51 (1H, m), 3.50 - 3.36 (4H, m), 3.25 - 3.16 (2H, m), 2.95 (2H, t), 2.48 - 2.31 (4H, m), 2.24 - 2.18 (1H, m), 2.02 - 1.69 (4H, m), 1.57 (3H, s), 1.56 - 1.48 (4H, m), 1.47 - 1.40 (2H, m).

Single crystal X-ray diffraction data obtained for Example 1 proved the structure to be (R)-1-[2-(4-Fluoro-phenyl)-ethyl]-3-(S)-2-phenyl-2-piperidin-1-yl-propionyloxy-1-azonia-bicyclo[2.2.2]octane bromide. The data set was collected at RT with graphite monochromatized MoK(α) radiation on a KappaCCD Single-Crystal X-Ray diffractometer equipped with an k-axis goniometer and a CCD area detector (Nonius, 1998). The diffraction raw data were processed within the Denzo-SMN program package (Otwinowski & Minor, 1998) converting the information from the digital image frame to a file containing h, k, l indices, background and Lp corrected intensities of the diffraction spots, along with estimate of errors.

On the basis of the crystal structure determined for Example 1, the absolute configuration of Intermediate A - Isomer 1 used in the preparation of Example 2 has been assigned as (S)-2-Phenyl-2-piperidin-1-yl-propionic acid methyl ester.

On the basis of the crystal structure determined for Example 1, the absolute configuration of Intermediate B - Isomer 1 used in the preparation of Example 2 has been assigned as (S)-2-Phenyl-2-piperidin-1-yl-propionic acid (S)-1-aza-bicyclo[2.2.2]oct-3-yl ester.

Analysis of Example 1 Form A: (R)-1-[2-(4-Fluoro-phenyl)-ethyl]-3-(S)-2-phenyl-2-piperidin-1-yl-propionyloxyj-l-azonia-bicyclo[2.2.2]octane bromide (Form A)
A sample of crystalline Example 1 bromide Form A obtained by the procedure described above was analysed by XRPD (PANalytical X'Pert or Cubix system), GVS, DSC and TGA.

The melting temperature of Example 1 bromide Form A as determined by DSC gave found a double endothermic events occurring at 170°C (1st onset) and 183.0°C (2nd onset) (±2°C). Weight loss observed prior to melting by TGA was negligible. GVS determination gave 0.1% weight increase (%w/w) at 80% RH (±0.2%).

An XRPD spectrum of Example 1 bromide Form A is presented in Figure 1.

**Preparation of Example 1 Form C**: (R)-1-[2-(4-Fluoro-phenyl)-ethyl]-3-((S)-2-phenyl-2-piperidin-1-yl-propionyloxy)-l-azonia-bicyclo[2.2.2]octane bromide (Form C)

(R)-1-[2-(4-Fluoro-phenyl)-ethyl]-3-((S)-2-phenyl-2-piperidin-1-yl-propionyloxy)-l-azonia-bicyclo[2.2.2]octane bromide (Example 1) (1g) was dissolved in methanol (5 mL) and the mixture warmed to 60°C. The mixture was allowed to cool to 40°C whereupon solid started to form and the mixture was then re-heated to 50°C. Three 10 mL aliquots of methyl acetate were added to the mixture which was then allowed to slowly cool to room temperature and stirred for 18 h. The resulting solid was collected by filtration and then dried under reduced pressure at 50°C to afford the titled compound (50 mg).

**1H NMR** (400 MHz, DMSO) δ 7.51 - 7.60 (2H, m), 7.31 - 7.41 (4H, m), 7.25 - 7.31 (IH, m), 7.13 - 7.21 (2H, m), 5.08 - 5.15 (IH, m), 3.88 - 3.97 (IH, m), 3.53 - 3.63 (IH, m), 3.38 - 3.52 (4H, m), 3.15 - 3.26 (2H, m), 2.92 - 3.01 (2H, m), 2.31 - 2.48 (4H, m), 2.20 - 2.25 (IH, m), 1.72 - 2.04 (4H, m), 1.58 (3H, s), 1.48 - 1.56 (4H, m), 1.39 - 1.48 (2H, m).

**Analysis of Example 1 Form C**: (R)-1-[2-(4-Fluoro-phenyl)-ethyl]-3-((S)-2-phenyl-2-piperidin-1-yl-propionyloxy)-l-azonia-bicyclo[2.2.2]octane bromide (Form C)
A sample of crystalline Example 1 Crystalline Form C obtained by the procedure described above was analysed by XRPD (PANalytical X’Pert or Cubix system), GVS, DSC and TGA.

The melting temperature of Example 1 bromide Form C as determined by DSC was found to be 184°C (onset) (±2°C). Weight loss observed prior to melting by TGA was 4%. GVS determination gave 4% weight increase (%w/w) at 80% RH (±0.2%).

An XRPD spectrum of Example 1 bromide Form C is presented in Figure 2.

Example 2: \((R)-1-[3-(2-Methyl-pyridin-4-yl)-propyl]-3-((S)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo [2.2.2] octane bromide

\begin{align*}
\text{a) 4-(3-Bromo-propyl)-2-methyl-pyridine hydrobromide} \\
\begin{array}{c}
\text{Br} \\
\end{array}
\end{align*}

3-(2-Methyl-pyridin-4-yl)-propan-1-ol (0.5 g) in hydrobromic acid (4 mL, 73.66 mmol) was heated at 105°C for 18h. The mixture was evaporated under reduced pressure and azeotroped with isopropanol (x6) to yield the sub-titled compound as an oil (0.53 g).

\(1^H\) NMR (400 MHz, DMSO): \(\delta\) 8.73 (IH, d), 7.87 (IH, s), 7.81 (IH, dd), 3.57 (2H, t), 2.97 (2H, t), 2.70 (3H, s), 2.50 (2H, quint.).
Example 2: \((R)-\text{L}-[3-(2-\text{Methyl-pyridin-4-yl})-\text{propyl}]-3-((\text{S})-2-\text{phenyl-2-piperidin-1-yl-propionyloxy})-\text{L-azonia-bicycle [2.2.2]octane bromide}\)

\[
\begin{align*}
\text{Br}^- & \quad \text{O} \quad \text{N} \\
\text{Br}^- & \quad \text{O} \quad \text{N} \\
\text{Br}^- & \quad \text{O} \quad \text{N} \\
\end{align*}
\]

4-(3-Bromo-propyl)-2-methyl-pyridine hydrobromide (Example 2a) (~200mg wet) was treated with 10% sodium hydroxide solution (2 mL) and then extracted with diethyl ether (x3). The organic layer was washed with brine, dried (MgSO\(_4\)) and the solvent was evaporated to yield a colourless oil. The residual free base (114 mg) was added to a solution of 2-phenyl-2-piperidin-1-yl-propionic acid (i?)-(1-aza-bicyclo[2.2.2]oct-3-yl) ester (Intermediate B, Isomer 1) (122 mg) in acetonitrile (0.5 mL) and the mixture was stirred at room temperature for 18h. The volume of solvent was reduced under reduced pressure and diethyl ether was added. The solvents were decanted and the product crystallised by addition of acetone and diethyl ether. The resulting white solid was collected by filtration, washed with diethyl ether and dried to afford the titled compound (122 mg).

m/e 476 [M]+

\(^1\)H NMR (400 MHz, DMSO) \(\delta\) 8.37 (IH, d), 7.54 (2H, d), 7.33 (2H, t), 7.22 (IH, t), 7.14 (s, IH), 7.07 (IH, d), 5.09 - 5.03 (IH, m), 3.84 - 3.76 (IH, m), 3.50 - 3.00 (7H, m), 2.58 - 2.47 (2H, m), 2.45 (3H, s), 2.47 - 2.38 (2H, m), 2.38 - 2.30 (2H, m), 2.18 (IH, s), 1.96 - 1.82 (4H, m), 1.81 - 1.73 (2H, m), 1.55 (3H, s), 1.57 - 1.47 (4H, m), 1.47 - 1.37 (2H, m).
Example 3: (R)-1-(4-fluorophenethyl)-3-v2-phenyl-2-(piperidin-1-yl)propanoyloxy)-1-azoniabicclo[2.2.2]octane 4-methylbenzenesulphonate

General Experimental Details for Preparations 1 and 2

Unless otherwise stated all reactions were carried out under an inert atmosphere: reagents and solvents were obtained commercially and used as received; reagent grade solvents were used. NMR spectra were measured on a Varian Unity Inova spectrometer at a proton frequency of 400 MHz. The MS spectra were measured on an Agilent 1100 MSD G1946D spectrometer.

Preparation 1

(R)-1-(4-Fluorophenethyl)-3-((5)-2-phenyl-2-(piperidin-1-yl)propanoyloxy)-1-azoniabicclo[2.2.2]octane bromide salt (0.6 g) prepared as described in WO2008/075005 (Example 44) was dissolved in dichloromethane (50 mL) and shaken with a solution of sodium 4-methylbenzenesulphonate (3.1 g) in water (100 mL), in three equal portions (~33 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The white foam was dissolved in hot acetonitrile (~5 mL) and allowed to cool to RT with stirring for 3 days. A white solid formed which was collected by filtration, washed with cold acetonitrile (~2 mL) and dried in vacuo at 60°C for 2 days to yield the product (0.490 g).

m/e M+ 465
$^1$H NMR (400 MHz, $d_6$-DMSO) δ 7.55 (2H, d), 7.47 (2H, d), 7.40-7.31 (4H, m), 7.28 (IH, t), 7.19 (2H, t), 7.11 (2H, d), 5.1-5.08 (IH, m), 3.9 -3.83 (IH, m), 3.59-3.50 (IH, m), 3.47-3.35 (4H, m), 3.25-3.14 (2H, m), 2.99-2.90 (2H, m), 2.47-2.31 (4H, m), 2.28 (3H, s), 2.24-2.18 (IH, m), 2.02-1.70 (4H, m), 1.57 (3H, s), 1.56-1.48 (4H, m), 1.48-1.38 (2H, m).

**Preparation 2**

Analytical HPLC and GC Conditions used in Steps a) - g)

Step a) was monitored by HPLC using an Ace phenyl column with standard aqueous/acetonitrile/TFA mobile phase on a gradient, with UV detection at 230 nm.

Steps b), c) and d) were monitored by GC using DB-5 capillary column with FID detection and standard oven gradient from 40°C to 300°C, with split injection.

Steps e), f), g) and h) are monitored by HPLC using C18 phase with standard aqueous/acetonitrile/TFA mobile phase on a gradient, with UV detection at 220 nm.

Step e) solvent composition was monitored by GC using a DB-624 capillary column with FID detection and oven gradient from 40°C to 250°C, with split injection.

Step e) was monitored for levels of quinuclidinol by GC using an HP-I capillary column with FID detection and oven gradient from 40°C to 300°C, with split injection.

a) Methyl 2-phenylpropanoate

(+/-)-2-Phenylpropionic acid (20.5g) was dissolved in methanol (62mL) in a reaction vessel. Sulfuric acid (98%, 0.82mL) was then charged followed by methanol (20.5mL) as a line rinse. The reaction was then heated to 63°C (±3°C) and stirred at this temperature for up to 4hrs. The reaction was monitored by HPLC analyzing the methyl 2-phenylpropanoate: (+/-)-2-phenylpropionic acid ratio (specification >97:3). Upon completion the reaction mixture was cooled to 23°C (±3°C). Cyclohexane (102mL) was added followed by Na$_2$CO$_3$ (aq) (3.7% wt/wt, 61.5mL). Layers were allowed to separate and the lower aqueous phase discarded. Water (61.5mL) was then charged and the mixture
stirred for 10 mins before the layers were separated discarding the lower aqueous phase. Cyclohexane (205mL) was then charged to the organic phase. The reaction mixture was then distilled under reduced pressure at 45°C, 150-240mbar removing 180mL solvent. The reaction mixture was then cooled to 23°C (±3°C) yielding methyl 2-phenylpropanoate in a solution in cyclohexane.

b) Methyl 2-bromo-2-phenylpropanoate

Methyl 2-phenylpropanoate in a solution in cyclohexane (prepared in step a) (22.42g; based on 100% yield from step a) was charged to a reaction vessel. Hydrobromic acid (48%, 0.62mL) was then charged followed by cyclohexane (22.4mL) as a line wash. Dibenzoyl peroxide (75%, 2.21g) and JV-bromosuccinimide (31.61g) were then charged to the vessel and the reaction heated to 50°C (±3°C) and stirred at this temperature for at least 4hrs. The reaction was monitored by GC analyzing the methyl 2-bromo-2-phenylpropanoate :methyl 2-phenylpropanoate ratio (specification >96:4). Upon completion the reaction mixture was cooled to 20°C (±3°C). The reaction mixture was filtered to remove the solid succinimide by-product, washing the filter cake twice with cyclohexane (22.4mL). The solid by-product was discarded. NaHSO₃ (aq) (10% w/w, 81.9mL) was then charged and stirred for 15 mins before allowing the phases to separate discarding the lower aqueous phase. Water (81.9mL) was then charged and stirred for 15 mins before allowing the phases to separate discarding the lower aqueous phase. 3-Pentanone (201.9mL) was then charged and the mixture was distilled at 45°C, 150-280mbar removing 210mL of solvent. The reaction mixture was cooled to 23°C (±3°C). 3-Pentanone (101mL) was then charged and the solvent composition analyzed by GC (specification <30% cyclohexane) to yield methyl 2-bromo-2-phenylpropanoate in a solution of 3-pentanone.

c) Methyl 2-phenyl-2-piperidin-1-ylpropanoate

Methyl 2-bromo-2-phenylpropanoate in a solution of 3-pentanone (prepared in step b) (33.21g; based on 100% yield from step b) was charged to a reaction vessel followed by piperidine (40.5mL). The reaction was heated to 40°C (±3°C) and held for at least 4hrs.
The reaction was monitored by GC analyzing the methyl 2-phenyl-2-piperidin-1-ylpropanoate : methyl 2-bromo-2-phenylpropanoate ratio (specification >97:3). The reaction mixture was then cooled to 23°C (±3°C) and then filtered to remove the piperidine hydrobromide salt by-product, and the filter cake washed with methyl 'butyl ether (66.4mL). The filter cake was discarded. Methyl 'butyl ether (133mL) and hydrogen chloride (2.74M, 172.6mL) were then added and the reaction mixture stirred for 15mins before taking a pH reading to ensure pH <4. The layers were then allowed to separate retaining the lower aqueous phase. Hydrogen chloride (2.74M, 60.4mL) was then added to the organic phase and the mixture stirred for at least 15mins before allowing the phases to separate retaining the lower aqueous phase. The two aqueous phases were then combined, sampled and analyzed by GC to ensure all impurities were <0.5 % with the exception of methyl 2-phenyl-3-(piperidin-1-yl)propanoate impurity. The aqueous phase was then charged to a mixture of Na₂CO₃ (32.29g), water (232mL) and methyl 'butyl ether (332mL). The mixture was stirred for at least 15mins before taking a pH reading to ensure pH >6. The layers were then allowed to separate discarding the lower aqueous phase. Water (66.4mL) was then charged and stirred for 15mins before allowing the phases to separate discarding the lower aqueous phase. Citric acid (0.8wt%, 66.4mL) was then charged to the organic phase and the mixture stirred for 15mins before allowing the phases to separate discarding the lower aqueous phase. A second charge of citric acid (0.8wt%, 66.4mL) was then added to the organic phase and the mixture stirred for 15mins before allowing the phases to separate discarding the lower aqueous phase. The organic phase was sampled and analyzed by GC to ensure methyl 2-phenyl-3-(piperidin-1-yl)propanoate impurity was less than 0.5%. The mixture was then distilled at 45°C, 80-220mbar removing 265mLsolvent. Methanol (332mL) was then charged to the vessel and the mixture again distilled at 45°C, 80-220mbar removing 332mL solvent. The reaction mixture was cooled to 23°C (±3°C) to yield methyl 2-phenyl-2-piperidin-1-ylpropanoate in a solution of methanol. The product was then analyzed by NMR assay and HPLC for purity. 23.8g (at 100w/w%) 70.5% yield, >99.5% HPLC purity. d) (S)-methyl 2-phenyl-2-(piperidin-1-yl)propanoate
Racemic methyl 2-phenyl-2-piperidin-1-ylpropanoate (prepared in step c) was purified by Simulated Moving Bed (SMB) chromatography to yield methyl (5)-2-phenyl-2-piperidin-1-ylpropanoate. (5)-methyl 2-phenyl-2-(piperidin-1-yl)propanoate was isolated as a 40w/w% solution in toluene. Typical conditions for the SMB purification were as follows:

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<th>Stationary Phase</th>
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<tr>
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</tr>
<tr>
<td>Wavelength (nm)</td>
<td>230</td>
</tr>
</tbody>
</table>

6) (S)-((R)-quinuclidin-3-yl) 2-phenyl-2-(piperidin-1-yl)propanoate

(S)-methyl 2-phenyl-2-(piperidin-1-yl)propanoate (prepared in step d) (17.6g as a 40w/w% solution in toluene) was charged to a reaction vessel followed by (i?)-(--)3-quinuclidinol (9.5g) and toluene (106mL). The mixture was distilled at 60°C, 180-450mbar removing 52mL solvent. A sample was taken and analyzed by HPLC assay (specification 180-220mg/mL (5)-methyl 2-phenyl-2-(piperidin-1-yl)propanoate. The reaction was then heated to 60°C (±5°C) and potassium tert-pentoxide (25w/w%, 43.12g) was added. The reaction mixture was stirred at 60°C (±5°C) for at least 2hrs and monitored by HPLC analyzing the methyl (5)-methyl 2-phenyl-2-(piperidin-1-yl)propanoate : (S)-((R)-quinuclidin-3-yl) 2-phenyl-2-(piperidin-1-yl)propanoate ratio (specification >95:5) followed by toluene (8.8 mL) as a line rinse. The reaction mixture was cooled to 20°C (±5°C). Butanenitrile (88mL) and water (88mL) were charged and the mixture stirred for 20mins before allowing the phases to separate discarding the lower aqueous phase. Water (88mL) was charged and the mixture stirred for 20mins before allowing the phases to
separate discarding the lower aqueous phase. The organic phase was analysed by GC to ensure residual (7?)-(−)-3-quinuclidinol levels were below 0.5%. The organic phase was distilled at 60°C, 100-430mbar removing 142mL of solvent. The reaction was then weighed and analysed by; NMR assay (w/w% of product) and GC (solvent composition) to determine the amount of product in solution and the solvent composition, toluene (18.5mL, 1.05vol) and butanenitrile (52.5mL, 3vol) was then added to the mixture to yield (S)-(R)-quinuclidin-3-yl) 2-phenyl-2-(piperidin-1-yl)propanoate (19.67g, 81% yield) in a 7:3 butanenitrile :toluene solvent composition at 140mg/mL concentration.

1f) (R)-l-(4-Fluorophenethyl)-3-((S)-2-phenyl-2-(piperidin-l-yl)propanoxyloxy)-l-azoniabicyclo [2.2.2]octane bromide

(S)-(R)-quinuclidin-3-yl) 2-phenyl-2-(piperidin-1-yl)propanoate (prepared in step e) (19.67g as a 140mg/mL solution in butanenitrile :toluene) was charged to a reaction vessel followed by 4-fluorophenethylbromide (13.99g) and butanenitrile (19.7mL). The reaction mixture was heated to 60°C (±5°C) and stirred at this temperature for at least 8hrs. The reaction was monitored by HPLC analyzing the (S)-((R)-quinuclidin-3-yl) 2-phenyl-2-(piperidin-1-yl)propanoate : product ratio (specification >96:4). The reaction mixture was cooled to 40°C over at least 40mins (0.5°C/min) and then cooled to -5°C over at least 6hrs (0.125°C/min). During the cool no crystallisation had occurred when at 20°C. Therefore the reaction was seeded with a sample of (i)-l-(4-fluorophenethyl)-3-((S)-2-phenyl-2-(piperidin-1-yl)propanoyleoxy)-l-azoniabicyclo[2.2.2]octane bromide (25mg - obtainable by methods described in WO 2008/075005 - Form A). After the reaction mixture reached -5°C toluene (39.3mL) was added and the slurry stirred at -5°C for at least 1hr. (R)-\((4-Fluorophenethyl)-3-((S)-2-phenyl-2-(piperidin-1-yl)propanoyleoxy)-l-azoniabicyclo[2.2.2]octane bromide product was then dried under vacuum at 45°C. The product was then analysed by HPLC purity and NMR assay. 30g, 96% yield, >99.5% HPLC purity, >99.5w/w% assay.
g) (R)-1-(4-Fluorophenethyl)-3-((S)-2-phenyl-2-(piperidin-l-yl)propanoyloxy)-l-azoniabicyclo [2.2.2]octane 4-methylbenzenesulphonate

A solution of sodium p-toluenesulphonate (26.97 g) in water (300 mL; 16.65 moles) was prepared. A 500 mL jacketed vessel was charged with (i)-l-(4-fluorophenethyl)-3-((5)-2-phenyl-2-(piperidin-l-yl)propanoyloxy)-l-azoniabicyclo[2.2.2]octane bromide (15.00 g). Butanenitrile (225 mL) and half of the sodium tosylate solution were added to the reaction vessel. The vessel was then stirred and heated to 35°C. When the vessel contents reached 35°C and were adequately mixed the stirring was stopped and the phases allowed to settle. The lower aqueous phase was removed and discarded. The second half of the sodium tosylate solution was added and the vessel contents heated to 35°C with stirring. When the vessel contents reached 35°C and were adequately mixed the stirring was stopped and the phases allowed to settle. The lower aqueous phase was removed and discarded. Water (75 mL) was added and the mixture heated to 70°C. When the vessel contents reached 70°C and were adequately mixed the stirring was stopped and the phases allowed to settle. The lower aqueous phase was removed and discarded. The hot organic phase was filtered into a clean vessel. The original vessel was washed with butanenitrile (30 mL) and this solvent was added to the filtrate via the filter into the clean vessel. The wet organic solution was distilled in order to azeodry it (120-150mbar - vessel jacket at 80°C). After ca. 60 mL of solvent had been distilled a precipitate was observed; contents were at 48°C. In total, 110 mL of solvent (10 mL water; 100 mL butanenitrile) was collected. At this point the vacuum was released and the vessel contents warmed to 75°C. Acetonitrile (45 mL) was added and the vessel contents re-heated to 75°C (not all material dissolved). More acetonitrile (45 mL) was added and the vessel contents re-heated to 75°C (all material dissolved). The solution was cooled to 5°C over 120 minutes (precipitation started at 65°C). With the vessel contents at 5°C the product was collected by filtration, washed with cold (5°C) butanenitrile (30 mL) and pulled as dry as possible on the filter to give 15.27 g of solid. This solid was left open in a fume cupboard overnight to give (i)-l-(4-fluorophenethyl)-3-((5)-2-phenyl-2-(piperidin-l-yl)propanoyloxy)-l-azoniabicyclo[2.2.2]octane 4-methylbenzenesulphonate (15.22 g). The ratio of quaternary species to tosylate was determined as 1:1.01 by 400MHz IHNMR using a 30s relaxation delay.
h) (R)-1-(4-Fluorophenethyl)-3-((S)-2-phenyl-2-(piperidin-1-yl)propanoyloxy)-1-azoniabicyclo [2.2.2] octane 4-methylbenzenesulphonate (Recrystalisation)

(R)-1-(4-Fluorophenethyl)-3-((S)-2-phenyl-2-(piperidin-1-yl)propanoyloxy)-1-azoniabicyclo[2.2.2]octane 4-methylbenzenesulphonate (7.50 g) and acetonitrile (90.00 mL) were charged to a vessel. The mixture was heated to 80°C and the resulting solution held at 80°C for 30 mins. The mixture was then cooled to 65°C over 20 minutes. The solution was seeded with seed crystals of (i?)-1-(4-fluorophenethyl)-3-((S)-2-phenyl-2-(piperidin-1-yl)propanoyloxy)-1-azoniabicyclo[2.2.2]octane 4-methylbenzenesulphonate (6 mg) and stirred at 65°C for 1 hour. The reaction was then cooled to 5°C over 10 hours and stirred at 5°C for 6 hours. The solid product was then isolated by filtration, washing the filter cake with acetonitrile (15.00 mL). The product was then dried under vacuum at 45°C to yield (i?)-1-(4-fluorophenethyl)-3-((S)-2-phenyl-2-(piperidin-1-yl)propanoyloxy)-1-azoniabicyclo[2.2.2]octane 4-methylbenzenesulphonate as a white solid (6.6 g).

Solid State Analysis of (R)-1-(4-Fluorophenethyl)-3-((S)-2-phenyl-2-(piperidin-1-yl)propanoyloxy)-1-azoniabicyclo[2.2.2] octane 4-methylbenzenesulphonate

A sample of material obtained by Procedure 1 as described herein above was analysed by XRPD (PANalytical X’Pert or Cubix system), GVS and DSC. The melting temperature as determined by DSC was found to be 189°C (onset) (±2°C). GVS determination gave 0.1% weight increase (%w/w) at 80% Relative Humidity (±0.2%).

An XRPD spectrum of (i?)-1-(4-Fluorophenethyl)-3-((S)-2-phenyl-2-(piperidin-1-yl)propanoyloxy)-1-azoniabicyclo[2.2.2]octane 4-methylbenzenesulphonate prepared according to Preparation 1 is presented in Figure 3.

Pharmacological Analysis

The affinity (pIC50) of compounds to the M3 receptor was determined by competition binding of [3H]N-methyl scopolamine (NMS) to CHO-K1 (Chinese Hamster Ovary) cell
membranes expressing the human muscarinic acetylcholine M<sub>3</sub> receptor (M<sub>3</sub>-ACh) in a scintillation proximity assay (SPA) format.

SPA beads were precoated with membranes and then incubated at 2mg of beads per well with serial dilutions of the compounds of the invention, [³H]NMS at 0.2nM, half Kd (experimentally determined dissociation constant) and assay buffer (20 mM HEPES pH 7.4 containing 5 mM MgCl<sub>2</sub>). The assay was conducted in a final volume of 200 µL, in the presence of 1% (v/v) dimethyl sulphoxide (DMSO). Total binding of [³H]NMS was determined in the absence of competing compound and non-specific binding of [³H]NMS was determined in the presence of 1 µM atropine. The plates were incubated for 16 hours at room temperature and then read on Wallac Microbeta™ using a normalised ³H protocol. The PIC50, defined as the negative logarithm of the concentration of compound required for 50% reduction in specific [³H]-NMS binding, was determined. Using the procedure given above pICso values determined for Examples 1 and 2, were 9.9 and 9.9 respectively.

The standard deviation of the M<sub>3</sub> receptor assay described above, is between 0.2 and 0.3 log units. The pIC50 values quoted above are means of replicate determinations which were within 2 x SD (95% confidence) of each other.

**Measurement of Plasma Protein Binding**

The extent of plasma protein binding was determined via equilibrium dialysis of a compound between human plasma and aqueous buffer at 37°C and determination of the concentration of compound in the plasma and buffer by HPLC-MS/MS.

**Method**

Dialysis cells (molecular weight cut-off 5000) were prepared by rinsing with water followed by soaking in the dialysis buffer for a minimum of 1 hour. The dialysis buffer was isotonic buffered saline pH 7.4. Stock solutions of compound in dimethyl sulphoxide were prepared at a concentration of 0.5mM. Frozen pooled Human plasma was obtained from volunteers.
The stock DMSO solution of a compound was added to the plasma at a ratio of 10 µl of DMSO to each ml of plasma. This gave a 1% DMSO in plasma solution with each compound at a concentration of 5 µM.

Dialysis cells were then prepared and one half of the cell filled with 750 µl of dialysis buffer and the other half of the cell with 750 µl of plasma solution of compound. Once prepared the cells were sealed and placed in an incubator box at 37°C. These cells were then rotated for a minimum of 4 hours to equilibrate.

After equilibration 500 µl of the buffer samples were removed and added to HPLC vials along with 100 µl of plasma (sample in 6-fold diluted plasma), and 100 µl of the plasma samples were removed and added to HPLC vials along with 500 µl of dialysis buffer (sample in 6-fold diluted plasma).

The samples were then analysed using HPLC-MS/MS. A four point calibration curve was obtained by dilutions of the stock solutions with 6-fold diluted plasma at concentrations of 0.013 µM, 0.05 µM, 0.25 µM and 1.25 µM which were injected in this order followed by the buffer sample and then the plasma sample.

**Calculation**

The concentration of compound in the samples were determined using MassLynx version 4.1 software (produced by Waters/Micromass) that automatically calculated a calibration curve and the concentration of compound in the cells. Plasma protein binding was determined from the calibration curve as the percentage of compound bound in human plasma (% bound) using the following equation:

\[
\% \text{ bound} = 100 - 100 \left( \frac{\text{buffer peak area}}{\text{buffer injection volume}} \div \frac{\text{plasma peak area}}{\text{plasma injection volume}} \right)
\]

For Example 57 the measured human plasma protein binding figure using the procedure described above was 98% bound.
Methacholine Induced Bronchoconstriction in vivo

Dunkin-Hartley guinea-pigs (300 - 600g) were supplied by a designated breeding establishment. Animals were dosed with test compound or vehicle either by inhalation in conscious guinea-pigs or by intratracheal instillation (0.5ml/kg) under recoverable gaseous anaesthesia (5% halothane). Animals were allowed to recover from the anaesthesia prior to the measurement of bronchoconstriction. Up to 48 hours post-dosing guinea-pigs were terminally anaesthetized with sodium pentobarbitone (60 mg/kg), the trachea cannulated for artificial ventilation and the jugular vein was cannulated for intravenous administration of methacholine. The guinea-pigs were ventilated using a constant volume respiratory pump (Harvard Rodent Ventilator model 683) at a rate of 60 breath/min and a tidal volume of 5 ml/kg during surgical preparation. Lung function (lung resistance and compliance) was measured in anaesthetised and ventilated guinea-pigs using a pulmonary measurement Flexivent system (SCIREQ, Montreal, Canada) connected to the tracheal cannulae. The animals were ventilated (quasi-sinusoidal ventilation pattern) at 60 breaths/min at a tidal volume of 5 ml/kg. A positive end expiratory pressure of 2-3 cmH₂O was applied. Respiratory resistance was measured using the Flexivent "snapshot" facility (1 second duration, 1 Hz frequency). Lung resistance and compliance was measured before and after intravenous administration of methacholine (3, 10 and 30 ìg/kg). The peak increase in resistance following methacholine challenge was calculated and the effect of the test compound on methacholine-induced lung function changes was calculated.

Percentage inhibition of bronchoconstriction was calculated at each dose of methacholine as follows:

\[
\text{Percentage inhibition} = \left( \frac{\text{Change in resistance in vehicle treated group} - \text{Change in resistance in compound treated group}}{\text{Change in resistance in vehicle treated group}} \right) \times 100
\]

Inhibition of pilocarpine induced salivation by i.n. administered compounds.

Guinea pigs (450-550g) supplied by Harlan UK or David Hall, Staffs UK and acclimatised to the in-house facilities for a minimum of three days before use. Guinea pigs were randomly assigned into treatment groups and weighed. Each animal was lightly anaesthetised (4% Halothane) and administered compound or vehicle intranasally
(0.5ml/kg) at up to 24 hours before challenge with pilocarpine. At the test time point, guinea pigs were terminally anaesthetised with urethane (25% solution in H₂O, 1.5g/kg). Once sufficient anaesthesia had developed (absence of toe pinch reflex) each animal had an absorbent pad placed in the mouth for 5 minutes to dry residual saliva, this pad was removed and replaced with a new pre-weighed pad for 5 minutes to establish a reading of baseline saliva production. At the end of this 5 minute period the pad was removed and weighed. A new pre-weighed pad was inserted into the mouth before each animal received s.c. pilocarpine administered under the skin at the back of the neck (0.6mg/kg @ 2ml/kg). The pad was removed, weighed and replaced with a new pre-weighed pad every 5 minutes up to 15 minutes.

Saliva production was calculated by subtracting the pre-weighed weight of the pad from each 5 minute period post weighed pad and these numbers added together to produce an accumulation of saliva over 15 minutes. Each 5 minute period could be analysed in addition to the whole 15 minute recording period. Baseline production of saliva was assumed to be constant and multiplied by three to produce a reading for baseline saliva production over 15 minutes.

Inhibition of saliva produced by the compound could be calculated by using the following equation: \((1-(\text{Test}-\text{baseline})/(\text{Veh}-\text{baseline}))\times100\).

**Preparation of β2-adrenoceptor agonists**

The following β₂-adrenoceptor agonists that may be employed in the combination of the present invention may be prepared as follows.

**General Experimental Details for Preparation of β₂-adrenoceptor Agonists**

¹H NMR spectra were recorded on a Varian *Inova* 400 MHz or a Varian *Mercury-VX* 300 MHz instrument. The central peaks of chloroform-<i>δ</i> (δ<sub>H</sub> 7.27 ppm), dimethylsulfoxide-<i>d₆</i> (δ<sub>H</sub> 2.50 ppm), acetonitrile-<i>δ</i> (δ<sub>H</sub> 1.95 ppm) or methanol-<i>δ</i> (δ<sub>H</sub> 3.31 ppm) were used as internal references. Column chromatography was carried out using silica gel (0.040-0.063
mm, Merck). Unless stated otherwise, starting materials were commercially available. All solvents and commercial reagents were of laboratory grade and were used as received.

The following method was used for LC/MS analysis:

Instrument Agilent 1100; Column Waters Symmetry 2.1 x 30 mm; Mass APCI; Flow rate 0.7 ml/min; Wavelength 254 nm; Solvent A: water + 0.1% TFA; Solvent B: acetonitrile + 0.1% TFA; Gradient 15-95%/B 8 min, 95% B 1 min.

Analytical chromatography was run on a Symmetry Cis-column, 2.1 x 30 mm with 3.5 µm particle size, with acetonitrile/water/0.1% trifluoroacetic acid as mobile phase in a gradient from 5% to 95% acetonitrile over 8 minutes at a flow of 0.7 ml/min.

The abbreviations or terms used in the examples have the following meanings:

SCX: Solid phase extraction with a sulfonic acid sorbent

HPLC: High performance liquid chromatography

DMF: N,N-Dimethylformamide

The β₂-adrenoceptor agonists and the intermediates used in their preparation are herein named, based upon the structures depicted, using the IUPAC NAME, ACD Labs Version 8 naming package.

**32-Adrenoceptor Agonist 1: (BAI): Preparation 1**

\[\text{tv-r2-(Diethylaminokthyll} \quad \text{-NAl-(r2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethylamino)ethyl)-3-[2-fl-naphthyl]ethoxypropanamide dihydrobromide}\]

![Chemical Structure](image)

a) tert-B\textit{xy} 3-[2-(1-naphthyl)ethoxy]propanoate
1-Naphthalene ethanol (10 g) was treated with benzyltrimethylammonium hydroxide (Triton B®; 0.9 mL of a 40% solution in methanol) and the resulting mixture stirred in vacuo for 30 minutes. The mixture was then cooled to 0°C and treated with tert-butyl acrylate (8.19 g). The resulting mixture was slowly warmed to room temperature and stirred overnight. The crude mixture was subsequently absorbed onto aluminium oxide (30 g) and eluted with diethylether (200 mL). The organics were concentrated to give a crude material (16.6 g) which was purified by flash silica chromatography eluting with 1:8 diethylether : hexane to give the subtitled compound (12.83 g).

$^1$H NMR (CDCl$_3$) δ 8.05 (dd, IH), 7.84 (dd, IH), 7.72 (dd, IH), 7.54-7.34 (m, 4H), 3.81-3.69 (m, 4H), 3.35 (t, 2H), 2.52-2.47 (m, 2H), 1.45 (s, 9H).

b) 3-[2-(1-Naphthyl)ethoxy]propanoic acid

tert-Buty 3-[2-(1-naphthyl)ethoxy]propanoate (6.19 g) was taken up in dichloromethane (30 mL) and treated with trifluoroacetic acid (5 mL). The resulting solution was stirred at room temperature for 2 hours, an additional 1 mL of trifluoroacetic acid was added and the solution stirred overnight. The mixture was concentrated, taken up in 2M sodium hydroxide solution (30 mL) and washed with ether (2 x 20 mL). The aqueous layer was subsequently acidified (using 1M hydrochloric acid) and extracted with ether (2 x 30 mL). The combined organics were washed with brine (20 mL), dried over anhydrous magnesium sulphate, filtered and concentrated in vacuo to give the sub-titled compound (5.66 g) as a clear oil.

$^1$H NMR (CDCl$_3$) δ 8.05 (bs, IH), 7.85 (bs, IH), 7.74 (bs, IH), 7.50-7.38 (m, 4H), 3.84-3.75 (bm, 4H), 3.39 (bs, 2H), 2.65 (bs, 2H).

c) 7V-(2-Diethylaminoethyl)-7V-(2-hydroxyethyl)-3-[2-(1-naphthyl)ethoxy]-propanamide

Oxalyl chloride (0.33 g) was added dropwise to a solution of 3-[2-(1-naphthyl)ethoxy]propanoic acid (0.53 g) in dichloromethane (10 mL), dimethylformamide (1 drop) was added and stirring continued at room temperature for 1 hour. The mixture was subsequently concentrated, re-dissolved in dichloromethane (10 mL) and added dropwise to a solution of 2-(2-diethylaminoethylamino)ethanol (0.35 g) and diisopropylethylamine (0.56 g) in dichloromethane (10 mL). The resulting mixture was stirred at room
temperature for 1 hour, diluted (dichloromethane, 50 mL), washed with water (2 x 20 mL), brine (20 mL), dried over magnesium sulfate and concentrated to give the crude product (0.91 g) which was purified by flash column chromatography (eluting with 5-7% methanol in dichloromethane) to give 0.63 g of the sub-titled compound.

\[ ^1H \text{NMR (CDCl}_3 \delta 8.05 \text{ (d, IH)}, 7.85 \text{ (d, IH)}, 7.73 \text{ (d, IH)}, 7.52-7.47 \text{ (m, 2H)}, 7.42-7.35 \text{ (m, 1H+1/2H)}, 3.84-3.78 \text{ (m, 6H)}, 3.72-3.70 \text{ (m, 1/2H)}, 3.45-3.35 \text{ (m, 6H)}, 2.79-2.77 \text{ (m, 1H)}, 2.62-2.58 \text{ (m, 2H)}, 2.54-2.49 \text{ (m, 4H)}, 1.04-1.01 \text{ (m, 6H)}. \]

d) 7V-[2-(Diethylamino)ethyl]-7V-(2-[(2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl]amino)ethyl]-3-[(2-(1-naphthyl)ethoxy]propanamide

A solution of dimethyl sulfoxide (0.097 g) in dichloromethane (1 mL) was added to a solution of oxaly chloride (0.079 g) in dichloromethane (10 mL) at -78°C. The reaction was stirred for 15 minutes and then a solution of 1-(2-diethylaminoethyl)-N-(2-hydroxyethyl)-3-{2-(1-naphthyl)ethoxy]propanamide (0.22 g) in dichloromethane (1 mL + 1 mL wash) was added and the reaction mixture stirred for another 15 minutes. Triethylamine (0.29 g) was added and the reaction allowed to warm to room temperature over 1 hour, the mixture was subsequently diluted (dichloromethane 30 mL), the organics washed with sodium bicarbonate (20 mL), brine (20 mL), dried over anhydrous magnesium sulphate, filtered and concentrated in vacuo to give the sub-titled compound (0.21 g).

The crude product was dissolved in methanol (10 mL) and 7-(2-aminoethyl)-4-hydroxy-1,3-benzthiazol-2(3H)-one hydrochloride (prepared according to the procedure outlined in Organic Process Research & Development 2004, 8(4), 628-642; 0.131 g) was added along with acetic acid (0.1 mL) and water (0.1 mL). After stirring at room temperature for 30 minutes, sodium cyanoborohydride (0.020 g) was added and the reaction mixture was stirred overnight. Ammonia (7N in methanol, 1 mL) was added and the mixture was concentrated. The crude residue was purified by flash column chromatography eluting with 1% ammonia; 5%-7% methanol in dichloromethane. The crude product was used directly in the next step.
e) 7V-[2-(Diethylamino)ethyl]-7V-(2-[2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl]amino)ethyl]-3-[2-(1-naphthyl)ethoxy]propanamide dihydrobromide

\[
\text{N-[2-(Diethylamino)ethyl]-N-[2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl]amino} \ \text{ethyl}-3-[2-(1-naphthyl)ethoxy]propanamide} \quad (0.052 \text{ g}) \text{ was dissolved in ethanol (1.5 mL) and treated with 48\% hydrobromic acid (21 \mu L). The white solid dihydrobromide salt (0.058 g) was collected by filtration.}
\]

\[\text{MS: APCI(+ve) 579 (M+)}\]

\[\text{H NMR (DMSO)} \quad 11.78-1.17 \text{ (m, IH), 10.11-10.06 (m, IH), 9.51-9.43 (m, 0.33H), 9.21-9.13 (m, 0.66H), 8.75-8.66 (m, IH), 8.59-8.51 (m, IH), 8.06 (d, IH), 7.95-7.90 (m, IH), 7.79 (d, IH), 7.60-7.48 (m, 2H), 7.47-7.39 (m, 2H), 6.87 (t, IH), 6.76 (dd, IH), 3.78-3.53 (m, 10H), 3.25-3.09 (m, 10H), 2.91-2.80 (m, 2H), 2.73-2.61 (m, 2H), 1.26-1.15 (m, 6H). NMR indicates approximately 2:1 mixture of rotamers at 298K.}\]

β2-Adrenoceptor Agonist 1: (BA): Preparation 2

\[\text{r2-(Diethylaminokthyll-NAl-(r2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethylamino)ethyl)-3-[2-fl-naphthyl]ethoxy]propanamide dihydrobromide}\]
a) 7V-(2,2-Dimethoxyethyl)-7V,JV-diethyl-ethane-1,2-diamine.

\[
\text{MeO} \quad \text{N} \quad \text{MeO} \\
\text{OMe} \quad \text{N} \quad \text{MeO}
\]

A solution of N,N-diethyl-ethylenediamine (150 g) in methanol (500 mL) was treated dropwise rapidly with glyoxal dimethylacetal (60wt% soln. in water, 225 g) at 10-15°C. After the addition was complete the solution was warmed to 150°C, then to 220°C and left at this temperature for 16 hours. The reaction mixture was treated with 5% palladium on carbon (Johnson-Matthey type 38H paste, 15 g) and hydrogenated at 6 bar until the reaction was complete as judged by GC/MS. The catalyst was removed by filtration and the filtrate evaporated to dryness (toluene azeotrope, 2.5 L), affording 196.2 g of the sub-titled compound.

\[^{1}H\text{NMR (CDCl}_3\text{): 4.48 (t, 1H), 3.39 (s, 6H), 2.75 (d, 2H), 2.69 (t, 2H), 2.57-2.48 (m, 6H), 1.01 (ts, 6H).}\]

b) 7V-[2-(Diethylamino)ethyl]-7V-(2,2-dimethoxyethyl)-3-[2-(1-naphthyl)ethoxy] propanamide.

\[
\text{MeO} \quad \text{N} \quad \text{MeO} \\
\text{OMe} \quad \text{N} \quad \text{MeO}
\]

Oxalyl chloride (151 mL) was added dropwise over 45 minutes to a solution of 3-[2-(1-naphthyl)ethoxy]proanoic acid (389 g) (Example 7 step b)) in dichloromethane (2.1 L) and DMF (0.5 mL). The reaction mixture was stirred for a further 16 hours. The mixture was subsequently concentrated, redissolved in DCM (1.7 L) and added dropwise over 1.75 hours at 0°C to a solution of \(\text{N}^\prime\)-(2,2-dimethoxyethyl)-\(\text{N},\text{N}\)-diethylethane-1,2-diamine (325 g) and isopropylidethyamine (551 mL) in DCM (1.7 L). The resulting mixture was stirred at room temperature for 3 hours, washed with aqueous saturated sodium bicarbonate
solution (5x1 L), water (1.5 L) and dried over sodium sulphate and concentrated to give 650 g of the sub-titled compound.

\[ m/e \ 431 \ (M+H^+, \ 100\%) \]

c) 7V-[2-(Diethylamino)ethyl]-3-[2-(1-naphthyl)ethoxy]-7V-(2-oxoethyl)propanamide.

A solution of \( N_1-N_2[2-(diethylamino)ethyl]-N_1-(2,2-dimethoxyethyl)-3-[2-(1\text{-naphthyl})ethoxy]propanamide \) (93 g) in DCM (270 mL) was treated dropwise at 0°C with trifluoroacetic acid (270 mL) over 1.5 hours. After the addition the reaction mixture was allowed to warm to room temperature and stirred for a further 1 hour. The reaction mixture was concentrated and the residue poured into aqueous saturated sodium bicarbonate solution (1800 mL, caution). The aqueous mixture was extracted with DCM (4x400 mL) and the combined extracts were dried over magnesium sulphate and concentrated. The residue was used directly in the following reaction.

d) TV-[2-(Diethylamino)ethyl]-7V-[2-[2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl]amino]ethyl]-3-[2-(1-naphthyl)ethoxy]propanamide dihydrobromide.

A suspension of 7-(2-amino-ethyl)-4-hydroxy-3H-benzothiazol-2-one hydrochloride (53 g) in dry NMP (216 mL) was heated to 60°C and treated in one portion with a solution of
NaOH (8.2 g) in methanol (102 mL). The bright orange suspension was cooled to room temperature and treated dropwise with a solution of \(N\)-[2-(diethylamino)ethyl]-3-[2-[(1-naphthyl)ethoxy]-N-(2-oxoethyl)propanamide in dichloromethane (475 mL) over 20 minutes. The reaction was left to stir for 25 minutes. Sodium triacetoxyborohydride (91.5 g) was then added in portions over 20 minutes and the mixture stirred for a further 50 minutes. The reaction mixture was poured into water (1.8 L) and the acidic solution (pH5) was washed with tert. butyl methyl ether (TBME) (3x500 mL). The aqueous phase was basified to pH8 by the addition of solid potassium carbonate and extracted with dichloromethane (3x750 mL); the combined organic extracts were dried over magnesium sulphate and concentrated to give a dark oil. This was dissolved in ethanol (200 mL) and 48% aqueous hydrobromic acid (73 mL) was added. The solution was aged for 30 minutes then evaporated to dryness. The residue was triturated with ethanol (560 mL); the resultant solid was collected by filtration and dried in vacuo at 50°C. The sticky solid was suspended in boiling ethanol (100 mL) and filtered while hot. The collected solid was dried in vacuo at 50°C. This material was recrystallised from ethanol/water (3:1, 500 mL). After standing overnight the resultant solid was collected by filtration and washed with ice-cold ethanol (75 mL). Drying in vacuo at 50°C for 24hr afforded 57g of the title compound.

\(\beta_2\)-Adrenoceptor Agonist 2: (BA2):

\[
\text{TV-r2-(Diethylaminokthyll} -N\text{Al-(r2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-}
\text{vDethyll amino)ethyl}-3\text{-}(3-chlorophenyl)ethoxy propanamide dihydrobromide}
\]

\[\text{\textbackslash a) tert-Butyl 3-}(2\text{-}(3\text{-chlorophenyl})\text{ethoxy})\text{propanoate}\]

2-(3-chlorophenyl)ethanol (20 g) was treated with benzyltrimethylammonium hydroxide (Triton B\textsuperscript{®}) (2.67 mL) and the resultant mixture was stirred \textit{in vacuo} for 30 minutes. The mixture was then cooled to 0°C and treated with t-butyl acrylate (17.40 g). The reaction was warmed to room temperature and stirred for 16 hours. The mixture was filtered
through aluminium oxide (15 g) eluting with ether (75 mL). The collected filtrate was concentrated to give the sub-titled compound (34.40 g) as an oil.

\[ ^1H \text{NMR (CDCl}_3\text{)} \delta 7.26-7.07 \text{ (m, 4H), 3.69-3.59 (m, 4H), 2.86-2.81 (t, 2H), 2.50-2.45 (t, 2H), 1.43 (s, 9H) } \]

b) 3-[2-(3-chlorophenyl)ethoxy] propanoic acid
tert-Butyl 3-[2-(3-chlorophenyl)ethoxy]propanoate (example 1a), 34.40 g) was dissolved in dichloromethane (150 mL) and treated with trifluoroacetic acid (50 mL). The mixture was stirred at room temperature for 3 hours, then concentrated in vacuo and azeotroped with dichloromethane (2 x 10 mL). The residue was taken up in dichloromethane (300 mL) and extracted with saturated sodium hydrogen carbonate (200 mL). The basic layer was washed with dichloromethane (20 mL) then acidified with 2M hydrochloric acid. The acidic layer was extracted with dichloromethane (2 x 200 mL). The organic layers were combined, washed with brine, dried over anhydrous magnesium sulphate, filtered and concentrated to yield the sub-titled compound (24.50 g) as an oil.

\[ m/e 227 \text{ [M-H]} \]

c) 7V-(2,2-dimethoxyethyl)-7V,7V-diethylethane-1,2-diamine propanamide

Oxalyl chloride (9.50 mL) was added dropwise over 45 minutes to a solution of 3-[2-(3-chlorophenyl)ethoxy]propanoic acid (22.50 g) (example 1b) in dichloromethane (120 mL) and DMF (0.5 mL). The reaction mixture was stirred for a further 16 hours. The mixture was subsequently concentrated, redissolved in DCM (1.7 L) and added dropwise over 1.75 hours at O°C to a solution of 7V-(2,2-dimethoxyethyl)-7V,7V-diethylethane-l,2-diamine (20.20 g)(example 16a) and isopropyldiethylamine (34.43 mL) in DCM (200 mL).
resulting mixture was stirred at room temperature for 16 hours, washed with aqueous saturated sodium bicarbonate solution (3x1 L), water (1.5 L) and dried over sodium sulphate and concentrated to give 39.50 g of the sub-titled compound. m/e 415 (M+H+, 83%)

d) 7V-\{2-(Diethylamino)ethyl\} \cdot 3-\{2-(3-chlorophenyl)ethoxy\} \cdot 7V-(2-oxoethyl)propanamide

A solution of N-\{2-(Diethylamino)ethyl\} \cdot N-(2,2-dimethoxyethyl) 1)-3-\{2-(3-chlorophenyl)ethoxy\}propanamide (example Ic) (20 g) in DCM (500 mL) was treated dropwise at 0°C with trifluoroacetic acid (50 mL) over 30 minutes. After the addition the reaction mixture was allowed to warm to room temperature and stirred for a further 1 hour. The reaction mixture was concentrated and the residue poured into aqueous saturated sodium bicarbonate solution (1800 mL, caution). The aqueous mixture was extracted with DCM (3x400 mL) and the combined extracts were dried over magnesium sulphate and concentrated. The residue was used directly in the following reaction.

e) 7V-\{2-(Diethylamino)ethyl\} \cdot 7V-\{2-\{2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl\} amino\}ethyl\} \cdot 3-\{2-(3-chlorophenyl)ethoxy\} propanamide dihydrobromide

\[
\begin{align*}
\text{O} & \text{N} \\
\text{N} & \text{O} \\
\text{Cl} & \\
\end{align*}
\]

\[
\begin{align*}
\text{HO} & \text{N} \\
\text{S} & \text{N} \\
\text{O} & \\
\text{Cl} & 2\text{HBr}
\end{align*}
\]
A suspension of 7-(2-amino-ethyl)-4-hydroxy-3H-benzothiazol-2-one hydrochloride (11.77 g) in dry NMP (50 mL) was heated to 65°C and treated in one portion with a solution of NaOH (1.83 g) in methanol (23 mL). The bright orange suspension was cooled to room temperature and treated dropwise with a solution of 7V-[2-(diethylamino)ethyl]-3-[2-(3-chlorophenyl)ethoxy]-N-(2-oxoethyl)propanamide (example Id) in dichloromethane (50 mL) over 30 minutes. The reaction was left to stir for 30 minutes. Sodium triacetoxyborohydride (20.33 g) was then added in portions over 20 minutes and the mixture stirred for a further 16 hours. The reaction mixture was poured into water (1.8 L), basified to pH 8 by the addition of solid potassium carbonate and extracted with dichloromethane (2x500 mL); the combined organic extracts were dried over magnesium sulphate and concentrated to give a dark oil. The residue was purified by chromatography on silica with 10% (0.1% aqNH3/MeOH)/DCM as eluent to give the sub-title compound as a brown oil. Yield (6.58 g). This was dissolved in ethanol (150 mL) and 48% aqueous hydrobromic acid (10 mL) was added. The solution was aged for 30 minutes then evaporated to dryness. The residue was triturated with ethanol (100 mL); the resultant solid was collected by filtration and dried in vacuo at 50°C. This material was recrystallised from ethanol/water (6:1, 500 mL); after standing overnight the resultant solid was collected by filtration and washed with ice-cold ethanol (75 mL). Drying in vacuo at 50°C for 24 hr afforded 4.96 g of the title compound.

**MS:** APCI (+ve): 563 (M+1) 99.3% purity (T9505M).

**1H NMR** (DMSO, 90°C), δ 11.75-1.73 (m, IH), 10.08-10.06 (d, IH), 8.65 (bs, IH), 7.33-7.19 (m, 4H), 6.89-6.84 (t, IH), 6.77-6.74 (m, IH), 3.68-3.58 (m, 8H), 3.17-3.16 (m, 10H), 2.86-2.80 (m, 4H), 2.67-2.62 (m, 2H), 1.23-1.19 (t, 6H).

Elemental Analysis

CHNS C:46.54%(46.39);H:5.75%(5.70);N:7.94%(7.73);S:4.46%(4.42)
β2-Adrenoceptor Agonist 3: (BA3):

\[
\text{7-\(r\alpha\ R\)}\text{-2-\(\{2-r\text{-}(2\text{-Chlorophenyl})ethyllamino\}\text{propyl} \text{thioethyllamino}\text{-l-hydroxyethyl \text{-4-hydroxy-}1,3\text{-benzothiazol-2f} 3\text{H})-one} \text{ dihydrobromide}
\]

\[
\begin{align*}
\text{OH} & \text{N} & \text{S} \\
\text{H} & \text{S} & \text{N} \\
\text{Cl} & \text{Cl} \\
\end{align*}
\]

2 \text{-HBr}

\text{a) 1-Chloro-2-[}(\$)-2\text{-nitrovinyl]} \text{ benzene}

\[
\begin{align*}
\text{O}_2\text{N} & \text{\varepsilon-\varepsilon} \\
\text{Cl} & \\
\end{align*}
\]

2-Chlorobenzaldehyde (ex Aldrich) (10.0 g) was mixed with nitromethane (26.05 g) and ammonium acetate (21.92 g) in acetic acid (200 mL), and the mixture was heated at reflux for 40 minutes. The mixture was allowed to cool to room temperature, and the majority of the acetic acid was removed in vacuo. The residue was dissolved in dichloromethane and washed with water, then potassium carbonate solution (x2), then water again. The organics were dried over anhydrous magnesium sulfate, filtered and evaporated to give the desired material, as an orange oil (12.83 g).

\text{\(1H\) NMR} \delta (\text{CDCl}_3) 8.41 (d, IH), 7.62-7.57 (m, 2H), 7.52-7.48 (m, IH), 7.43 (dt, IH), 7.34 (ddd, IH)

\text{b) 2-(2-Chlorophenyl)ethanamine}

\[
\begin{align*}
\text{H} & \text{N} \\
\text{Cl} & \\
\end{align*}
\]
Aluminium hydride was prepared by the drop-wise addition of a solution of sulphuric acid (8.40 mL) in dry THF (60 mL) to a stirred solution of 1.0M lithium aluminium hydride in THF (314 mL), at 0-10°C, under a nitrogen atmosphere. After stirring at 5°C for 30 minutes, a solution of 1-chloro-2-[(±)-2-nitrovinyl]benzene (12.83 g) in dry THF (160 mL) was added dropwise maintaining the internal temperature between 0°C and 10°C. When the addition was complete the reaction was heated at reflux for 5 minutes. The mixture was allowed to cool to room temperature, then cooled to 0°C and isopropanol (22 mL) carefully added dropwise maintaining the temperature below 0°C. 2M Sodium hydroxide (35 mL) was carefully added dropwise maintaining the temperature below 0°C. The mixture was stirred at room temperature for 30 minutes, then filtered through a layer of celite, which was then washed with THF (x3). The filtrate was evaporated to dryness. The residue was purified using silica column chromatography, using ethyl acetate to load the material, then 10% triethylamine in ethyl acetate, followed by 10% triethylamine in 45% ethanol: 45% ethyl acetate as the eluents, to give the desired material (4.66 g).

1H NMR δ (CDCl3) 7.36 (dd, 1H), 7.25-7.13 (m, 3H), 2.98 (dt, 2H), 2.91-2.87 (m, 2H)

c) tert-Butyl [2-(2-chlorophenyl)ethyl] carbamate

To a stirred solution of 2-(2-chlorophenyl)ethanamine (25.57 g) and triethylamine (22.87 mL) in dry THF (300 mL) was added a solution of di-tert-butyl dicarbonate (35.85 g) in dry THF (50 mL) over 10 minutes, at ambient temperature, under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 3 hours. The solvents were removed in vacuo to give the desired material, as a yellow oil (42.0 g).

1H NMR δ (CDCl3) 7.35 (d, 1H), 7.25-7.14 (m, 3H), 4.57 (s, 1H), 3.43-3.35 (m, 2H), 2.95 (t, 2H), 1.43 (d, 9H)
d) tert-Butyl[2-(2-chlorophenyl)ethyl] carbamate

\[
\begin{align*}
\text{O} & \quad \text{N} \\
\text{Cl} & \quad \text{O} \\
\end{align*}
\]

To a suspension of sodium hydride (60% in mineral oil) (7.23 g), which had been washed with ether (x3), in dry DMF (200 mL) was added a solution of tert-butyl[2-(2-chlorophenyl)ethyl] carbamate (42.0 g) in dry DMF (50 mL), over a 15 minute period, at 35°C, under a nitrogen atmosphere. When the addition was complete, the mixture was stirred at 50°C for 90 minutes. The mixture was allowed to cool to room temperature, then allyl bromide (15.63 mL) was added slowly, keeping the temperature at 25°C, using external cooling. The mixture was stirred at room temperature for 2 hours, then diluted with water and extracted with ethyl acetate (x3). The organics were combined, washed with water, dried over anhydrous magnesium sulfate, filtered and evaporated. The residue was purified using silica column chromatography, loading with 1% ethyl acetate in isohexane, then using isohexane with ethyl acetate (0%, 1%, 2%, %5) as the eluents to give the desired material (27.0 g). There were several mixed fractions, so these were combined, and re-purified using silica column chromatography, as above, to give a further 4g of desired material. Both crops of product were combined to give 31.0 g in total.

\[\text{H NMR } \delta (\text{CDCl}_3) 7.36-7.31 \text{ (m, IH), 7.21-7.12 \text{ (m, 3H), 5.83-5.68 \text{ (m, IH), 5.17-5.05 \text{ (m, 2H), 3.86-3.66 \text{ (m, 2H), 3.41 \text{ (t, 2H), 3.03-2.90 \text{ (m, 2H), 1.43 \text{ (s, 9H)}}}}}}\]

HPLC: 95.90% @ 220nm [M+H-Boc]+ = 196.1 (Calc = 295.1339) (multimode+)
e) tert-Butyl [2-(2-chlorophenyl)ethyl] {3-[2-(hydroxyethyl)thio]propyl} carbamate

\[
\text{HO-S-N=O}
\]

tert-Butyl allyl[2-(2-chlorophenyl)ethyl]carbamate (3.10 g) was mixed with 2-mercaptoethanol (7.37 mL), and AIBN (1.15 g), and stirred at 65°C for 45 minutes. The mixture was cooled and more mercaptoethanol (1 mL) and AIBN (200 mg) added. The mixture was then heated at 65°C for a further 30 minutes. The material was purified by silica column chromatography, loading the material in 20% ethyl acetate in isohexane, then eluting with 20% ethyl acetate in isohexane, changing to 50%, to give the desired material (31.94 g).

\[\delta (\text{CDCl}_3) 7.38-7.32 (m, 1H), 7.22-7.13 (m, 3H), 3.75-3.68 (m, 2H), 3.41 (t, 2H), 3.32-3.14 (m, 2H), 3.03-2.91 (m, 2H), 2.72 (t, 2H), 2.54-2.36 (m, 2H), 1.85-1.71 (m, 2H), 1.42 (s, 9H)\]

HPLC: 92.31% @ 220 nm [M+H-Boc]+ = 274.1 (Calc = 373.1478) (multimode+)

f) tert-Butyl [2-(2-chlorophenyl)ethyl] {3-[(2-oxoethyl)thio]propyl}carbamate

\[
\text{O=S-N=O}
\]

Sulfur trioxide:pyridine complex (30.52 g) was dissolved in DMSO (200 mL) and stirred at room temperature, under a nitrogen atmosphere, for 15 minutes. DCM (100 mL) was added, followed by a solution of tert-butyli [2-(2-chlorophenyl)ethyl][3-[(2-hydroxyethyl)thio]propyl]carbamate (23.9 g) and Hunigs base (63.5 mL) in DCM (160 mL), which was added in one portion (exotherm). The resulting mixture was stirred at
ambient temperature for 15 minutes. The reaction mixture was diluted with ethyl acetate, washed with water, then IN HCl, then saturated sodium bicarbonate solution, dried over anhydrous magnesium sulfate, filtered and the solvents removed in vacuo. The material was purified by silica column chromatography eluting with 20% ethyl acetate in isohexane to give the desired material (12.43 g).

$^1$H NMR $\delta$ (CDCl$_3$) 9.46 (t, 1H), 7.36-7.32 (m, 1H), 7.21-7.13 (m, 3H), 3.40 (t, 2H), 3.29-3.13 (m, 4H), 3.02-2.90 (m, 2H), 2.45-2.34 (m, 2H), 1.82-1.69 (m, 2H), 1.49-1.36 (m, 9H)

g) tert-Butyl[(2-(2-chlorophenyl)ethyl){3-[(2-oxoethyl)thio]propyl}carbamate

The tert-butyl [2-(2-chlorophenyl)ethyl]{3-[(2-oxoethyl)thio]propyl} carbamate (11.32 g) was dissolved in a mixture of methanol (200 mL) and acetic acid (1.74 ml). 7-[(li?)]-2-amino-l-hydroxyethyl]-4-hydroxy-1,3-benzothiazol-2(3 $H$)-one hydrochloride (8.0 g) was added to the solution, and the mixture stirred at room temperature, under a nitrogen atmosphere, for 1 hour. Sodium cyanoborohydride (1.92 g) was added and the mixture stirred for a further 2 hours. The solvents were removed in vacuo, and the residue diluted with water, basified with 0.880 aqueous ammonia, and extracted with ethyl acetate (x3) (filtered through celite during extraction). The organics were combined, washed with brine, dried over anhydrous sodium sulfate, filtered and evaporated to give a brown residue (15.5 g). The material was purified using silica column chromatography, using DCM with MeOH (2%, 5%, 10%, 20% and 30%, all with 1% 0.880 aq NH$_3$) as the eluent, to give the desired material (6.67 g) (38% yield)
\( \text{\`U NMR } \delta (\text{DMSO}) \ 7.43-7.38 \ (\text{m, IH}), \ 7.30-7.21 \ (\text{m, 3H}), \ 6.86 \ (\text{d, IH}), \ 6.69 \ (\text{d, IH}), \ 4.56 \ (\text{dd, IH}), \ 3.23-3.10 \ (\text{m, 2H}), \ 2.88 \ (\text{t, 2H}), \ 2.71-2.48 \ (\text{m, 8H}), \ 2.46-2.39 \ (\text{m, 2H}), \ 1.72-1.62 \ (\text{m, 9H}) \)

\( \text{HPLC: } 97.46\% \ @ \ 220\text{nm} \ [\text{M+H}+]=582.1 \ (\text{Calc} = 582.1863) \ (\text{multimode+}) \)

\( \text{h) 7-[(1R)-2-[[2-[(2-Chlorophenyl)ethyl]amino]propyl]thio[ethyl]amino]-1-hydroxyethyl]-4-hydroxy-1,3-benzothiazol-2(3H)-one dihydrobromide} \)

To a stirred suspension of the Boc compound from part g) (5.93 g) in DCM (20 mL) was added trifluoroacetic acid (20 mL) at 0°C, and the resulting mixture was stirred under nitrogen for 30 minutes. The mixture was diluted with toluene, and solvents removed, then azeotroped with toluene (x2). The residue was dissolved in acetonitrile, acidified with 48% aq HBr and concentrated in vacuo (not to dryness). The mixture was further diluted with acetonitrile and the precipitated solid collected by filtration, washed with acetonitrile and dried under vacuum to give 6.35 g. A 3.8% impurity was present (isomer from part e)), so the material was redissolved in a 1:1 mixture of acetonitrile:water and purified using prep HPLC (Sunfire 30x80mm C8 column; NH\textsubscript{4}OAc buffer; acetonitrile 5-50% over 10 minutes). The resultant material was dried overnight in a dessicator at 10 mbar over KOH and H\textsubscript{2}SO\textsubscript{4}. The resulting di-acetate salt was dissolved in water and basified with 0.880 aq ammonia. A white gum formed, so the aqueous was decanted off, and the gum dried in vacuo to give the free base (4.11 g). This was dissolved in hot ethanol, and the solution was filtered, then allowed to cool to room temperature. The solution was acidified with 48% aq. HBr and left to crystallize. The white solid was collected by filtration, washed with ethanol and dried in vacuo to give 3.81 g Crop 1.
\[ ^1\text{H NMR} \ \text{O(DMSO)} 11.67 \text{ (s, IH)}, \ 10.15 \text{ (s, IH)}, \ 8.70 \text{ (s, 4H)}, \ 7.50\text{-}7.30 \text{ (m, 4H)}, \ 6.94 \text{ (d, IH)}, \ 6.78 \text{ (d, IH)}, \ 6.45 \text{ (s, IH)}, \ 4.96\text{-}4.90 \text{ (m, IH)}, \ 3.22\text{-}3.02 \text{ (m, 10H)}, \ 2.86\text{-}2.76 \text{ (m, 2H)}, \ 2.66 \text{ (t, 2H)}, \ 1.91 \text{ (quintet, 2H)} \]

HPLC: 99.63\% @ 220nm \hspace{1em} [M+H]+=482 \hspace{1em} (calc=482.1339) \hspace{1em} (MultiMode+)

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The mother liquors were evaporated to dryness then triturated with acetonitrile. The solid was collected by filtration to give 719 mg Crop 2 (4.53 g total).

\[ ^1\text{H NMR} \ \text{O(DMSO)} 11.67 \text{ (s, IH)}, \ 10.15 \text{ (s, IH)}, \ 8.80\text{-}8.60 \text{ (m, 4H)}, \ 7.50\text{-}7.29 \text{ (m, 4H)}, \ 6.94 \text{ (d, IH)}, \ 6.78 \text{ (d, IH)}, \ 6.45 \text{ (s, IH)}, \ 4.96\text{-}4.89 \text{ (m, IH)}, \ 3.22\text{-}3.00 \text{ (m, 10H)}, \ 2.85\text{-}2.76 \text{ (m, 2H)}, \ 2.66 \text{ (t, 2H)}, \ 1.90 \text{ (quintet, 2H)} \]

HPLC: 99.20\% @ 220nm \hspace{1em} [M+H]+=482 \hspace{1em} (calc=482.1339) \hspace{1em} (MultiMode+)

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**Biological Activity of 32-Adrenoceptor Agonists**

**Adrenergic 32 mediated cAMP production**

Cell preparation

H292 cells were grown in 225cm2 flasks incubator at 37°C, 5% CO₂ in RPMI medium containing, 10% (v/v) FBS (foetal bovine serum) and 2 mM L-glutamine.
Experimental Method

Adherent H292 cells were removed from tissue culture flasks by treatment with Accutase™ cell detachment solution for 15 minutes. Flasks were incubated for 15 minutes in a humidified incubator at 37°C, 5% CO₂. Detached cells were re-suspended in RPMI media (containing 10% (v/v) FBS and 2 mM L-glutamine) at 0.05 x 10⁶ cells per mL. 5000 cells in 100 µL were added to each well of a tissue-culture-treated 96-well plate and the cells incubated overnight in a humidified incubator at 37°C, 5% CO₂. The culture media was removed and cells were washed twice with 100 µL assay buffer and replaced with 50 µL assay buffer (HBSS solution containing 10mM HEPES pH7.4 and 5 mM glucose). Cells were rested at room temperature for 20 minutes after which time 25 µL of rolipram (1.2 mM made up in assay buffer containing 2.4% (v/v) dimethylsulphoxide) was added. Cells were incubated with rolipram for 10 minutes after which time Compound A was added and the cells were incubated for 60 minutes at room temperature. The final rolipram concentration in the assay was 300 µM and final vehicle concentration was 1.6% (v/v) dimethylsulphoxide. The reaction was stopped by removing supernatants, washing once with 100 µL assay buffer and replacing with 50 µL lysis buffer. The cell monolayer was frozen at -80°C for 30 minutes (or overnight).

AlphaScreen™ cAMP detection

The concentration of cAMP (cyclic adenosine monophosphate) in the cell lysate was determined using AlphaScreen™ methodology. The frozen cell plate was thawed for 20 minutes on a plate shaker then 10 µL of the cell lysate was transferred to a 96-well white plate. 40 µL of mixed AlphaScreen™ detection beads pre-incubated with biotinylated cAMP, was added to each well and the plate incubated at room temperature for 10 hours in the dark. The AlphaScreen™ signal was measured using an EnVision spectrophotometer (Perkin-Elmer Inc.) with the recommended manufacturer’s settings. cAMP concentrations were determined by reference to a calibration curve determined in the same experiment using standard cAMP concentrations. A concentration response curve for Compound A was constructed and data was fitted to a four parameter logistic equation to determine both the PEC50 and Intrinsic Activity. Intrinsic Activity was expressed as a fraction relative to
the maximum activity determined for formoterol in each experiment. Result are in Table 1.

Selectivity Assays

**Adrenergic α1D**

**Membrane Preparation**
Membranes were prepared from human embryonic kidney 293 (HEK293) cells expressing recombinant human α1D receptor. These were diluted in Assay Buffer (50mM HEPES, ImM EDTA, 0.1% gelatin, pH 7.4) to provide a final concentration of membranes that gave a clear window between maximum and minimum specific binding.

**Experimental Method**
Assays were performed in U-bottomed 96-well polypropylene plates. 10 μL [³H]-prazosin (0.3 nM final concentration) and 10 μL of Compound A (10x final concentration) were added to each test well. For each assay plate 8 replicates were obtained for [³H]-prazosin binding in the presence of 10 μL vehicle (10% (v/v) DMSO in Assay Buffer; defining maximum binding) or 10μL BMY7378 (10 μM final concentration; defining non-specific binding (NSB)). Membranes were then added to achieve a final volume of 100 μL. The plates were incubated for 2 hours at room temperature and then filtered onto PEI coated GF/B filter plates, pre-soaked for 1 hour in Assay Buffer, using a 96-well plate Tomtec cell harvester. Five washes with 250 μL wash buffer (50mM HEPES, ImM EDTA, pH 7.4) were performed at 4°C to remove unbound radioactivity. The plates were dried then sealed from underneath using Packard plate sealers and MicroScint-0 (50 μL) was added to each well. The plates were sealed (TopSeal A) and filter-bound radioactivity was measured with a scintillation counter (TopCount, Packard BioScience) using a 3-minute counting protocol.

Total specific binding (Bo) was determined by subtracting the mean NSB from the mean maximum binding. NSB values were also subtracted from values from all other wells.
These data were expressed as percent of B₀. Compound concentration-effect curves (inhibition of [³H]-prazosin binding) were determined using serial dilutions typically in the range 0.1 nM to 10 µM. Data was fitted to a four parameter logistic equation to determine the compound potency, which was expressed as pIC₅₀ (negative log molar concentration inducing 50% inhibition of [³H]-prazosin binding). Results are shown in Table 1 below.

### Adrenergic 31

#### Membrane Preparation

Membranes containing recombinant human adrenergic beta 1 receptors were obtained from Euroscreen. These were diluted in Assay Buffer (50mM HEPES, 1mM EDTA, 120mM NaCl, 0.1% gelatin, pH 7.4) to provide a final concentration of membranes that gave a clear window between maximum and minimum specific binding.

#### Experimental Method

Assays were performed in U-bottomed 96-well polypropylene plates. 10 µL [¹²⁵I]-Iodocyanopindolol (0.036 nM final concentration) and 10 µL of Compound A (10x final concentration) were added to each test well. For each assay plate 8 replicates were obtained for [¹²⁵I]-Iodocyanopindolol binding in the presence of 10 µL vehicle (10% (v/v) DMSO in Assay Buffer; defining maximum binding) or 10 µL Propranolol (10 µM final concentration; defining non-specific binding (NSB)). Membranes were then added to achieve a final volume of 100 µL. The plates were incubated for 2 hours at room temperature and then filtered onto PEI coated GF/B filter plates, pre-soaked for 1 hour in Assay Buffer, using a 96-well plate Tomtec cell harvester. Five washes with 250 µL wash buffer (50mM HEPES, 1mM EDTA, 120mM NaCl, pH 7.4) were performed at 4°C to remove unbound radioactivity. The plates were dried then sealed from underneath using Packard plate sealers and MicroScint-0 (50 µL) was added to each well. The plates were sealed (TopSeal A) and filter-bound radioactivity was measured with a scintillation counter (TopCount, Packard BioScience) using a 3-minute counting protocol.
Total specific binding ($B_0$) was determined by subtracting the mean NSB from the mean maximum binding. NSB values were also subtracted from values from all other wells. These data were expressed as percent of Bo. Compound concentration-effect curves (inhibition of $[^{125}I]$-Iodocyanopindolol binding) were determined using serial dilutions typically in the range 0.1 nM to 10 µM. Data was fitted to a four parameter logistic equation to determine the compound potency, which was expressed as pICso (negative log molar concentration inducing 50% inhibition of $[^{125}I]$-Iodocyanopindolol binding). Results are shown in Table 1 below.

**Dopamine D2**

**Membrane Preparation**

Membranes containing recombinant human Dopamine Subtype D2s receptors were obtained from Perkin Elmer. These were diluted in Assay Buffer (50mM HEPES, 1mM EDTA, 120mM NaCl, 0.1% gelatin, pH 7.4) to provide a final concentration of membranes that gave a clear window between maximum and minimum specific binding.

**Experimental Method**

Assays were performed in U-bottomed 96-well polypropylene plates. 30 µL $[^3H]$-spiperone (0.16 nM final concentration) and 30 µL of Compound A (10x final concentration) were added to each test well. For each assay plate 8 replicates were obtained for $[^3H]$-spiperone binding in the presence of 30 µL vehicle (10% (v/v) DMSO in Assay Buffer; defining maximum binding) or 30 µL Haloperidol (10 µM final concentration; defining non-specific binding (NSB)). Membranes were then added to achieve a final volume of 300 µL. The plates were incubated for 2 hours at room temperature and then filtered onto PEI coated GF/B filter plates, pre-soaked for 1 hour in Assay Buffer, using a 96-well plate Tomtec cell harvester. Five washes with 250 µL wash buffer (50mM HEPES, 1mM EDTA, 120mM NaCl, pH 7.4) were performed at 4°C to remove unbound radioactivity. The plates were dried then sealed from underneath using Packard plate sealers and MicroScint-0 (50 µL) was added to each well. The plates were
sealed (TopSeal A) and filter-bound radioactivity was measured with a scintillation counter (TopCount, Packard BioScience) using a 3-minute counting protocol.

Total specific binding (Bo) was determined by subtracting the mean NSB from the mean maximum binding. NSB values were also subtracted from values from all other wells. These data were expressed as percent of Bo. Compound concentration-effect curves (inhibition of [3H]-spiperone binding) were determined using serial dilutions typically in the range 0.1 nM to 10 µM. Data was fitted to a four parameter logistic equation to determine the compound potency, which was expressed as pICso (negative log molar concentration inducing 50% inhibition of [3H]-spiperone binding). Results are shown in Table 3.

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**Combination Data**

**Evaluation of bronchodilator activity in the guinea pig isolated tracheal ring preparation.**

Guinea pigs (300-500g) were killed by cervical dislocation and the trachea was isolated. The trachea was cut into segments 2-3 cartilage rings in width and suspended in 10ml organ baths in modified Krebs' solution (mM; NaCl, 90; NaHCO₃, 45; KCl, 5; MgSO₄·7H₂O, 0.5; Na₂HPO₄·2H₂O, 1; CaCl₂, 2.25; glucose, 10; pH 7.4 gassed with 5% CO₂, 95% O₂ at 37°C). The tracheal rings were attached to an isometric force transducer for the measurement of isometric tension. The tissues were washed and a force of 1g was applied to each tissue. The rings were contracted with methacholine (1 µM). Once the
contraction had reached a plateau, vehicle (0.01% DMSO in distilled H₂O), indacaterol (1OnM), \( N\)-[2-(Diethylamino)ethyl]-N'-[2-\{2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl\} amino]ethyl]-3-[2-(1-naphthyl)ethoxy]propanamide dihydrobromide (3nM), (\(R\))-1-[2-(4-Fluoro-phenyl)-ethyl]-3-\((S)\)-2-phenyl-2-piperidin-1-yl-propionyloxy)-l-azonia-bicyclo[2.2.2]octane bromide, (InM), a combination of \((i?)\)-l-[2-(4-Fluoro-phenyl)-ethyl]-3-\((S)\)-2-phenyl-2-piperidin-1-yl-propionyloxy)-l-azonia-bicyclo[2.2.2]octane bromide (InM) and \( N\)-[2-(Diethylamino)ethyl]-N'-[2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl]amino]ethyl]-3-[2-(1-naphthyl)ethoxy]propanamide dihydrobromide (3nM) was added and the tissue left for 60 min. The tension was measured in each ring at 60 min following compound addition and was expressed as a % relaxation of the constriction to methacholine (1µM) (mean ± s.e.mean). Data were collected using the Chart 4 software (ADInstruments, Charlgrove, UK).

Muscarinic antagonist \((i?)\)-l-[2-(4-Fluoro-phenyl)-ethyl]-3-\((S)\)-2-phenyl-2-piperidin-1-yl-propionyloxy)-l-azonia-bicyclo[2.2.2]octane bromide was prepared as in Example 1 (Form A) described herein above. \( \beta_2 \)-Adrenoceptor agonist \( N\)-[2-(Diethylamino)ethyl]-iV-(2-\{2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl]amino)ethyl]-3-[2-(1-naphthyl)ethoxy]propanamide dihydrobromide was prepared as in preparation (BAI) described herein above.

Assessment of the combination of indacaterol and \((i?)\)-l-[2-(4-Fluoro-phenyl)-ethyl]-3-\((S)\)-2-phenyl-2-piperidin-1-yl-propionyloxy)-l-azonia-bicyclo[2.2.2]octane bromide: The relaxation (expressed as a percentage of the maximum response to methacholine (1µM)) to indacaterol (1OnM) was 11±1=2.5, the percentage relaxation to \((i?)\)-l-[2-(4-Fluoro-phenyl)-ethyl]-3-\((S)\)-2-phenyl-2-piperidin-1-yl-propionyloxy)-l-azonia-bicyclo[2.2.2]octane bromide (InM) was 23±1=10.1 and the percentage relaxation to a combination of indacaterol (1OnM) and \((R)\)-1-[2-(4-Fluoro-phenyl)-ethyl]-3-\((S)\)-2-phenyl-2-piperidin-1-yl-propionyloxy)-l-azonia-bicyclo[2.2.2]octane bromide (InM) was 41±6.2. The percentage relaxation to vehicle was 7±2.5 (n = 5; see Figure 4 where compound Y is \((i?)\)-l-[2-(4-}

Assessment of the combination of N-[2-(Diethylamino)ethyl]-N-(2-[[2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl]amino]ethyl)-3-[2-(1-naphthyl)ethoxy]propanamide dihydrobromide and (i?)-l-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane bromide: The relaxation (expressed as a percentage of the maximum response to methacholine (1 µM)) to N-[2-(Diethylamino)ethyl]-N-(2-[[2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl]amino]ethyl)-3-[2-(1-naphthyl)ethoxy]propanamide dihydrobromide (3nM) was 21±1=5.1, the percentage relaxation to (i?)-l-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane bromide (InM) was 12±4.2 and the percentage relaxation to a combination of N-[2-(Diethylamino)ethyl]-iV-(2-[[2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl]amino]ethyl)-3-[2-(1-naphthyl)ethoxy]propanamide dihydrobromide (3nM) and (i?)-l-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane bromide (InM) was 40±9.7. The percentage relaxation to vehicle was 12±8.0 (n = 4; see Figure 5 where compound V is N-[2-(Diethylamino)ethyl]-N-(2-[[2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl]amino]ethyl)-3-[2-(1-naphthyl)ethoxy]propanamide dihydrobromide and compound Y is (i?)-l-[2-(4-Fluoro-phenyl)-ethyl]-3-((5)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane bromide).

In Vivo Combination Experimental Protocol

The following protocol is a prophetic description of an experimental model that might be used to evaluate the effects of muscarinic receptor antagonists according to the present invention in combination with β₂-adrenoceptor agonists.

Evaluation of lung function in anaesthetised guinea pigs.

Male Dunkin-Hartley guinea pigs (300-600g) are weighed and dosed with vehicle (0.05M phosphate, 0.1% Tween 80, 0.6% saline, pH 6) or compound via the intratracheal route.
under recoverable gaseous anaesthesia (5% halothane in oxygen). Animals are dosed with compound or vehicle two hours prior to the administration of methacholine. Guinea pigs are anaesthetised with pentobarbitone (1 mL/kg of 60 mg/mL solution i.p.) approximately 30 minutes prior to the first bronchoconstrictor administration. The trachea is cannulated and the animal ventilated using a constant volume respiratory pump (Harvard Rodent Ventilator model 683) at a rate of 60 breath/min and a tidal volume of 5 mL/kg. A jugular vein is cannulated for the administration of methacholine or maintenance anaesthetic (0.1 mL of pentobarbitone solution, 60 mg/mL, as required).

The animals are transferred to a Flexivent System (SCIREQ, Montreal, Canada) in order to measure airway resistance. The animals are ventilated (quasi-sinusoidal ventilation pattern) at 60 breaths/min at a tidal volume of 5 mL/kg. A positive end expiratory pressure of 2-3 cm H₂O was applied. Respiratory resistance is measured using the Flexivent "snapshot" facility (1 second duration, 1 Hz frequency). Once a stable baseline resistance value has been obtained the animals are given methacholine in ascending doses (0.5, 1, 2, 3 and 5µg/kg, i.v) at approximately 4-minute intervals via the jugular catheter. After each administration of bronchoconstrictor the peak resistance value is recorded. Guinea pigs are euthanised with approximately 1.0mL pentobarbitone sodium (Euthatal) intravenously after the completion of the lung function measurements.

Percentage bronchoprotection produced by the compound is calculated at each dose of bronchoconstrictor as follows:

\[
\text{% bronchoprotection} = \frac{\% \text{change} R_{veh} - \% \text{change} R_{comp}}{\% \text{change} R_{veh}}
\]

Where \% change \text{R}_{veh} is the mean of the maximum percentage change in airway resistance in the vehicle treated group.
CLAIMS

1. A pharmaceutical product comprising, in combination, a first active ingredient which is a muscarinic antagonist selected from:
(i?)-l-[2-(4-Fluoro-phenyl)-ethyl]-3-((S)-2-phenyl-2-piperidin-l-yl-propionyloxy)-l-azonia-bicyclo[2.2.2]octane X; and
(i?)-l-[3-(2-Methyl-pyridin-4-yl)-propyl]-3-((S)-2-phenyl-2-piperidin-l-yl-propionyloxy)-l-azonia-bicyclo[2.2.2]octane X;
wherein X represents a pharmaceutically acceptable anion of a mono or polyvalent acid, and a second active ingredient which is a $\beta_2$-adrenoceptor agonist, with the proviso that the second active ingredient is not $\Lambda$-Cyclohexyl-$\Lambda^3$-[2-((3-fluorophenyl)ethyl]-$\Lambda$-(2-{[2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl]amino}ethyl)-$\beta$-alaninamide or a salt thereof.

2. A product according to claim 1 wherein the first active ingredient is a muscarinic antagonist which is a bromide, benzenesulphonate or naphthalene-1,5-disulphonate salt.

3. A product according to claim 1 wherein the first active ingredient is a muscarinic antagonist which is a bromide salt.

4. A product according to claim 1 wherein the first active ingredient is a muscarinic antagonist which is a 4-methylbenzenesulphonate salt.

5. A product according to any one of claims 1 to 4, wherein the $\beta_2$-adrenoceptor agonist is formoterol.

6. A product according to any one of claims 1 to 4, wherein the $\beta_2$-adrenoceptor agonist is selected from:
7. A product according to any one of claims 1 to 4, wherein the β2-adrenoceptor agonist is indacaterol.

8. A pharmaceutical product comprising, in combination, a first active ingredient which is (R)-1-[2-(4-Fluoro-phenyl)-ethyl]-3-((S)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane X wherein X represents a pharmaceutically acceptable anion of a mono or polyvalent acid, and a second active ingredient which is indacaterol.

9. A pharmaceutical product comprising, in combination, a first active ingredient which is (i?)-1-[2-(4-Fluoro-phenyl)-ethyl]-3-((S)-2-phenyl-2-piperidin-1-yl-propionyloxy)-1-azonia-bicyclo[2.2.2]octane X wherein X represents a pharmaceutically acceptable anion of a mono or polyvalent acid and a second active ingredient which is N-[2-(Diethylamino)ethyl]-N-2-[(2-(4-hydroxy-2-oxo-2,3-dihydro-1,3-benzothiazol-7-yl)ethyl)amino]ethyl]-3-[2-(1-naphthyl)ethoxy]propanamide or a pharmaceutically acceptable salt thereof.

10. Use of a product according to any one of claims 1 to 9 in the manufacture of a medicament for the treatment of a respiratory disease.

11. Use according to claim 10, wherein the respiratory disease is chronic obstructive pulmonary disease.
12. A method of treating a respiratory disease, which method comprises simultaneously, sequentially or separately administering:

(a) a (therapeutically effective) dose of a first active ingredient which is a muscarinic receptor antagonist as defined as defined in any one of claims 1 to 4; and

(b) a (therapeutically effective) dose of a second active ingredient which is a β₂-adrenoceptor agonist as defined in claim 1; to a patient in need thereof,

13. A kit comprising a preparation of a first active ingredient which is a muscarinic receptor antagonist as defined in any one of claims 1 to 4, and a preparation of a second active ingredient which is a β₂-adrenoceptor agonist as defined in claim 1 and optionally instructions for the simultaneous, sequential or separate administration of the preparations to a patient in need thereof.

14. A pharmaceutical composition comprising, in admixture, a first active ingredient is a muscarinic receptor antagonist as defined in any one of claims 1 to 4 and a second active ingredient which is a β₂-adrenoceptor agonist as defined in claim 1.
Figure 4

Substitute Sheet (Rule 26)
INTERNATIONAL SEARCH REPORT

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2009/050743

A. CLASSIFICATION OF SUBJECT MATTER

IPC: see extra sheet
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: A61K, C07D

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

SE, DK, FI, NO classes as above

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

EPO-INTERNAL; WPI DATA, PAJ, CHEM.ABS DATA, MEDLINE, EMBASE, BIOSIS, BEILSTEIN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<td>WO 2008075005 A1 (ASTRAZENECA AB), 26 June 2008 (26.06.2008), claim 16, abstract, example 112</td>
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<td>Y</td>
<td>WO 2006048225 A1 (NOVARTIS AG), 11 May 2006 (11.05.2006), claims 1-12</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

Date of the actual completion of the international search
28 August 2009

Date of mailing of the international search report
01-09-2009

Name and mailing address of the ISA/
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Form PCT/ISA/210 (second sheet) (July 2008)
**INTERNATIONAL SEARCH REPORT**

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<th>Box No. II</th>
<th>Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)</th>
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<td>This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:</td>
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<td>1.</td>
<td>☒ Claims Nos.: 1 2 because they relate to subject matter not required to be searched by this Authority, namely:</td>
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<td>Claim 12 relates to a method for treatment of the human or animal body by surgery or by therapy, as well as diagnostic .../...</td>
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<td>2.</td>
<td>☐ 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:</td>
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<td>3.</td>
<td>☐ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).</td>
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<th>Box No. III</th>
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<td>This International Searching Authority found multiple inventions in this international application, as follows:</td>
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<td>1.</td>
<td>☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.</td>
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<td>☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.</td>
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<td>☐ 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.:</td>
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<td>4.</td>
<td>☒ 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.:</td>
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**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.
methods, see PCT rule 39.1(iv). Nevertheless, a search has been made for this claim. The search has been directed to the technical content of the claim.
<table>
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<th>Category</th>
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<td>WO 2004096800 A2 (NOVARTIS AG), 11 November 2004 (11.11.2004), claims 1-18</td>
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International patent classification (IPC)

A61K 31/439 (2006.01)
A61K 31/428 (2006.01)
A61K 31/444 (2006.01)
A61P 11/06 (2006.01)
A61P 11/08 (2006.01)

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Use the application number as username. The password is TNIYNOJDZ.

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Cited literature, if any, will be enclosed in paper form.
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