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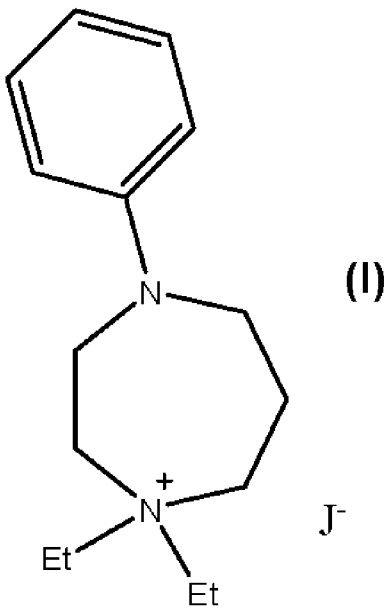

(I)

(57) Abstract: The present disclosure relates to novel combination comprising a homopiperazinium compound having the formula (I): wherein J is a counter ion,; and one or more agents for treating or preventing the pulmonary diseases, for use and method for the treatment or prevention of respiratory diseases. Also provided are novel compounds and intermediates as well as processes for preparing same.

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COMBINATION THERAPY AND METHODS FOR THE TREATMENT OF RESPIRATORY DISEASES

FIELD OF THE DISCLOSURE

The present disclosure relates to novel combination, use and method for the treatment or prevention of respiratory diseases. The disclosure also relates to novel compounds and intermediates as well as processes for preparing same.

BACKGROUND OF THE DISCLOSURE

For patients with severe persistent asthma, the Global Initiative for Asthma 2005 guidelines recommend the use of high-dose inhaled corticosteroids (ICS) in combination with a long-acting beta2-agonist (LABA), with one or more additional controller medications if required. Recent studies have shown that asthma remains inadequately controlled in many patients with asthma, despite treatment in accordance with guidelines. It is now recognized that asthma is a heterogeneous disease with some patients non responsive to conventional therapy (Bateman ED et al. Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J 2008, 31: 143-78). There is clearly an unmet need for the effective and safe treatment of patients with asthma who remain symptomatic despite optimized standard treatment. A strong association exists between asthma control/hospitalization and asthma-related mortality. While all patients are susceptible to exacerbations, uncontrolled asthma increases the risk of an exacerbation being life-threatening or fatal.

The GOAL study (Bateman ED et al.. Can guideline-defined asthma control be achieved? The Gaining Optimal Asthma Control study. Am J Respir Crit Care Med 2004, 170: 836-44) demonstrated that better guideline-derived asthma control is achievable in a majority of patients and that inhaled corticosteroid (ICS) in combination with a long-acting beta agonist (LABA) is more effective than ICS alone. However, asthma was not well controlled in 38% of patients with the most severe asthma despite optimized ICS/LABA combination therapy. Furthermore, total asthma control was only achieved in 30% of patients with the most severe asthma. Adding oral corticosteroids had little effect on asthma control, with asthma remaining inadequately controlled in 31% of patients with the most severe asthma and the proportion of patients with total control rising only slightly from 30% to 35%. This and other recent studies indicate the need for additional, novel controller therapies for patients with persistent asthma.

Chronic treatment of asthma often requires increasingly higher doses of the inhaled corticosteroids (ICS) and long-acting beta-agonists (LABAs), however this has caused concerns to attending physicians. Chronic use of certain LABAs can increase airway hyperresponsiveness (AHR) and reduce response to short-acting beta-agonists when needed for acute use (Deborah H et al. Effect of regular salmeterol treatment on albuterol-induced bronchoprotection in mild asthma. Am J Respir Crit Care Med 1997, 156: 988-991). Also,

maintenance treatment with LABAs (with or without ICS) has been shown to induce tolerance to their bronchoprotective effects and cross-tolerance to the reliever effects of the short-acting beta-agonists (Cheung D et al. Long-term effects of a LABA agonist, salmeterol, on airway AHR in patients with mild asthma. N Engl J Med. 1992;327: 1198-203 ; Haney S and Hancox RJ. Rapid onset of tolerance to beta-agonist bronchodilation. Respir Med. 2005, 99: 566-71. Epub 2004 Dec 2 ; van der Woude HJ et al. Decreased bronchodilating effect of salbutamol in relieving methacholine induced moderate to severe bronchoconstriction during high dose treatment with long acting beta2 agonists, Thorax. 2001;56: 529-35; van Veen A et al. A comparison of salmeterol and formoterol in attenuating airway responses to short-acting beta2-agonists. Pulm Pharmacol Ther. 2003;16: 153-61). The approved dosing levels for the long-acting muscarinic antagonist (LAMA) tiotropium (Spiriva), were selected not for maximal bronchodilation but to limit cholinergic side effects (Littner MR et al. Long-acting bronchodilation with once-daily dosing of tiotropium (Spiriva) in stable chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2000, 151: 1136-1142).

Chronic obstructive pulmonary disease (COPD) is common world-wide. The prevalence of the disease continues to grow in most industrialized nations. It is mostly associated with cigarette smoking. Although COPD is a leading cause of illness and death, its recognition as a public health problem has been slow to evolve despite the rising mortality rate for COPD. Additionally, COPD imparts substantial economic burden on individuals and society.

Corticosteroids are potent anti-inflammatory drugs used, for example, in the treatment of asthma and COPD. Their systemic use at high doses causes major side effects that preclude their long-term uses whenever possible. Inhaled poorly absorbed steroids are useful to treat airway inflammation. At low doses these drugs have little or no side effects. However, higher doses increase the risks for oral candidasis, vocal cords paralysis, cataracts and osteoporosis. Inhaled steroids have no effects on lung interstitium and have no anti-fibrotic properties (Boulet LP et al. Airway hyperresponsiveness, inflammation, and subepithelial collagen deposition in recently diagnosed versus long-standing mild asthma. Influence of inhaled corticosteroids. Am J Respir Crit Care Med 2000, 162: 1308-1313).

More recent drugs, such as anti-leukotrienes, are useful in some asthmatics but have no effects in COPD and other lung diseases. These drugs have anti-inflammatory properties limited to the components of inflammation caused by leukotrienes.

The treatment of interstitial lung disease such as IPF, Sarcoidosis, HP, and BOOP basically rests on the use of systemic corticosteroids. This treatment is effective in controlling some of the inflammation but induces serious side effects and does not reverse underlying fibrotic changes. Immunosupressive agents such as cyclophosphamide and azathioprine are sometimes tried in severe IPF but their therapeutic values are unproven and at most, very limited (Zisman D et al. Cyclophosphamide in the treatment of idiopathic pulmonary fibrosis: a prospective study in patients who failed to respond to corticosteroids. Chest 2000, 117: 1619-

1626). In essence, lung fibrosis is usually progressive and untreatable, with most IPF patients dying of this condition.

Despite advances in the treatment of inflammatory illnesses, including pulmonary inflammatory diseases, treatment using available drugs or agents frequently results in undesirable side effects.

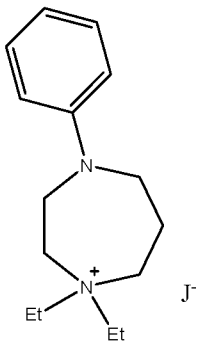
The above suggest there is a need for a combinations and therapy that offers an adjunctive benefit to current treatments diseases such as asthma and COPD.

It would be desirable to offer patients, for example suffering from pulmonary inflammatory diseases such as asthma and COPD, a drug acting through a mechanism of action different from clinically used drugs

SUMMARY

In one aspect, there is provided a method for treating or preventing diseases selected from the group consisting of asthma, chronic obstructive pulmonary disease (COPD), interstitial pulmonary fibrosis (IPF), sarcoidosis, hypersensitivity pneumonitis (HP) and bronchiolitis obliterans with organizing pneumonitis (BOOP), comprising administering an effective amount of :

i) a homopiperazinium compound having the formula:



wherein J is a counter ion; and

ii) one or more agents for treating or preventing the pulmonary diseases: beta2 agonists, muscarinic antagonists, leukotriene modulators and phosphodiesterase (PDE) inhibitors or a combination thereof.

In one aspect, there is provided a combination for treating or preventing disease selected from the group consisting of asthma, chronic obstructive pulmonary disease (COPD), interstitial pulmonary fibrosis (IPF), sarcoidosis, hypersensitivity pneumonitis (HP) and

bronchiolitis obliterans with organizing pneumonitis (BOOP), comprising an effective amount of i) a homopiperazinium compound as defined herein and ii) one or more agents for treating or preventing the pulmonary diseases: beta2 agonists, muscarinic antagonists, leukotriene modulators and phosphodiesterase (PDE) inhibitors or a combination thereof.

In one aspect, there is provided a method for treating or preventing pulmonary inflammation, inducing airway smooth muscle relaxation and improving airway hyperresponsiveness in an asthma or COPD patient which comprises administering an effective amount of i) a homopiperazinium compound as defined herein and ii) one or more agents for treating or preventing the pulmonary diseases: beta2 agonists, muscarinic antagonists, leukotriene modulators and phosphodiesterase (PDE) inhibitors or a combination thereof.

In one aspect, there is provided a method of decreasing the risk of recurrence of symptoms associated with a disease selected from the group consisting of asthma, chronic obstructive pulmonary disease (COPD), interstitial pulmonary fibrosis (IPF), sarcoidosis, hypersensitivity pneumonitis (HP) and bronchiolitis obliterans with organizing pneumonitis (BOOP), comprising administering an effective amount of i) a homopiperazinium compound as defined herein and ii) one or more agents for treating or preventing the pulmonary diseases: beta2 agonists, muscarinic antagonists, leukotriene modulators and phosphodiesterase (PDE) inhibitors or a combination thereof.

In one aspect, there is provided a method for reducing the side effects associated with one or more agents for treating or preventing the pulmonary diseases: beta2 agonists, muscarinic antagonists, leukotriene modulators and phosphodiesterase (PDE) inhibitors or a combination thereof in a patient suffering from a disease selected from the group consisting of asthma, chronic obstructive pulmonary disease (COPD), interstitial pulmonary fibrosis (IPF), sarcoidosis, hypersensitivity pneumonitis (HP) and bronchiolitis obliterans with organizing pneumonitis (BOOP), said patient, the method comprising administering a homopiperazinium compound as defined herein.

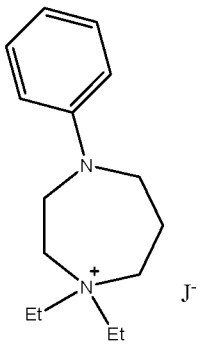
In one aspect, there is provided a method for treating a patient suffering from a disease selected from the group consisting of asthma, chronic obstructive pulmonary disease (COPD), interstitial pulmonary fibrosis (IPF), sarcoidosis, hypersensitivity pneumonitis (HP) and bronchiolitis obliterans with organizing pneumonitis (BOOP), said patient being desensitized to a treatment with one or more of the following agents for treating or preventing the pulmonary diseases: beta2 agonists, muscarinic antagonists, leukotriene modulators and phosphodiesterase (PDE) inhibitors or a combination thereof, the method comprising administering a homopiperazinium compound as defined herein.

In one aspect, there is provided a method for reducing airflow obstruction, preventing bronchospasm and reducing exacerbations in patients with asthma and COPD, comprising administering an effective amount of i) a homopiperazinium compound as defined herein and ii) one or more agents for treating or preventing the pulmonary diseases: beta2 agonists, muscarinic antagonists, leukotriene modulators and phosphodiesterase (PDE) inhibitors or a combination thereof.

In one aspect, there is provided a pharmaceutical composition comprising an effective amount of i) a homopiperazinium compound as defined herein and ii) one or more agents for treating or preventing the pulmonary diseases: beta2 agonists, muscarinic antagonists, leukotriene modulators and phosphodiesterase (PDE) inhibitors or a combination thereof.

In one aspect, there is provided a pharmaceutical composition comprising an effective amount of a homopiperazinium compound as defined herein and a pharmaceutically acceptable carrier, said composition is for use in the treatment or prevention of pulmonary disease together with one or more agents for treating or preventing the pulmonary diseases: beta2 agonists, muscarinic antagonists, leukotriene modulators and phosphodiesterase (PDE) inhibitors or a combination thereof.

In further aspects, there is provided useful intermediates as well as processes for the synthesis of a compound having the formula:



wherein J^- is a counter ion; as well as the synthesis of intermediates useful in the synthesis of same.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows the effect of ASM-024 in *in vitro* relaxation response to maximally effective concentration of short–acting beta-2 agonists in dog bronchi;

Fig. 2 shows the effect of ASM-024 in *in vitro* relaxation response to maximally effective concentration of short–acting beta-2 agonists in human bronchi;

Fig. 3 is a bar graph representing guinea pig trachea unresponsiveness to salbutamol following adrenoreceptor desensitization;
Fig. 4 shows the effect of ASM-024 on guinea pig trachea responsiveness following salbutamol-induced beta2-adrenoreceptor desensitization;
Fig. 5 is a bar graph representing guinea pig trachea unresponsiveness to salbutamol following salmeterol -induced beta2-adrenoreceptor desensitization;
Fig. 6 shows the effect of ASM-024 on guinea pig trachea responsiveness following salmeterol-induced beta2-adrenoreceptor desensitization;
Fig. 7 is a bar graph representing guinea pig trachea unresponsiveness to salbutamol following formoterol -induced beta2-adrenoreceptor desensitization;
Fig. 8 shows the effect of ASM-024 on guinea pig trachea responsiveness following formoterol-induced beta2-adrenoreceptor desensitization;
Fig. 9 shows the effect of ASM-024 over ipratropium resistance to histamine-induced contraction;
Fig. 10 shows the effect of ASM-024 over tiotropium resistance to histamine-induced contraction;
Fig. 11 shows the additive effect of ASM-024 and ipratropium on the relaxation response to methacholine;
Fig. 12 shows the additive effect of ASM-024 and tiotropium on the relaxation response to methacholine; and
Fig. 13 is a XRD diffraction of ASM-024.

DESCRIPTION OF THE EMBODIMENTS

In the methods or combinations described herein, one or more of the following agents can be used: beta2 agonists, muscarinic antagonists, leukotriene modulators and phosphodiesterase (PDE) inhibitors.

Beta2 agonists can be separated in three classes: ultra-long-acting beta2 agonist (such as indacaterol, carmoterol, vilanterol, olodaterol, milveterol, Abediterol, PF-610355; AZD-3199 and phenethanolamine derivatives), long acting beta2 agonist (LABA) (such as formoterol, salmeterol, bambuterol and bedoradrine) and short acting beta2-agonist (SABA) (such as salbutamol, carbuterol, clenbuterol, fenoterol, metaproterenol, terbutaline, pirbuterol, albuterol, levalbuterol, reprotenol and bitolterol.

Muscarinic antagonists may be separated in two classes: long acting muscarinic antagonist (LAMA) (such as tiotropium, aclidinium, glycopyrronium, GSK656398, umeclidinium, vilanterol, CHF5407, QAT370, EP-101, TD4208 and glycopyrrolate) and short acting muscarinic antagonist (SAMA) (such as ipratropium,oxitropium and atropine). A subclass includes also muscarinic antagonist/beta2 agonist (MABA) such as GSK961081, THRX-200495, AZD2115, PF3429281, PF4348235 and LAS190792.

Leukotriene modulators may be separated in two classes: leukotriene antagonists (such as zafirlukast, montelukast and pranlukast) and Inhibitors of leukotriene synthesis (such as zileuton, GSK2190915 and Setileuton).

Selective Phosphodiesterase (PDE) inhibitors may be separated in two classes: PDE4 inhibitors (such as roflumilast, cilomilast, rolipram, GSK256066, apremilast and CHF6001) and PDE4/PDE3 inhibitors (such as RPL554).

In one embodiment, the methods or combinations use a homopiperazinium compound as defined herein and one or more of beta2 agonists. In one embodiment, the methods or combinations use a homopiperazinium compound as defined herein and one or more of muscarinic antagonists. In one embodiment, the methods or combinations use a homopiperazinium compound as defined herein and one or more of leukotriene modulators. In one embodiment, the methods or combinations use a homopiperazinium compound as defined herein and one or more phosphodiesterase (PDE) inhibitors.

The combinations and methods as defined herein may be useful for diseases selected from the group consisting of asthma, chronic obstructive pulmonary disease (COPD), interstitial pulmonary fibrosis (IPF), sarcoidosis, hypersensitivity pneumonitis (HP) and bronchiolitis obliterans with organizing pneumonitis (BOOP).

In one embodiment, the disease is asthma. In one embodiment, the disease is COPD. In one embodiment, the disease is IPF. In one embodiment, the disease is sarcoidosis. In one embodiment, the disease is HP. In one embodiment, the disease is BOOP.

In one embodiment, the combinations and methods as defined herein can be useful for treating one or more of asthma, including bronchial, allergic, intrinsic, extrinsic, exercise-induced, drug-induced (including aspirin and NSAID-induced) and occupational asthma, both intermittent and persistent and of all severities, and other causes of airway hyper-responsiveness; chronic obstructive pulmonary disease (COPD); bronchitis, including infectious and eosinophilic bronchitis; emphysema; bronchiectasis; cystic fibrosis; sarcoidosis; hypersensitivity pneumonitis; lung fibrosis, including cryptogenic fibrosing alveolitis, idiopathic interstitial pneumonias, fibrosis complicating anti-neoplastic therapy.

Asthma can be classified according to severity and/or control of the condition.

In one embodiment, the combinations and methods as defined herein can be useful for treating one or more of Intermittent asthma, Persistent mild asthma, Persistent moderate asthma and Persistent severe asthma. In one embodiment, the combinations and methods

as defined herein can be useful for treating Persistent moderate asthma and/or Persistent severe asthma.

In one embodiment, the combinations and methods as defined herein can be useful for treating one or more of Controlled asthma, Partly controlled asthma, Uncontrolled asthma and asthma Exacerbation. In one embodiment, the combinations and methods as defined herein can be useful for treating Partly controlled asthma, Uncontrolled asthma and/or asthma Exacerbation.

In one embodiment, the homopiperazinium compound is a compound wherein J^- is a halogen, a sulphate, acetate or a sulfonate.

In one embodiment, the homopiperazinium compound is a compound wherein J^- is a halogen or a sulfonate.

In one embodiment, the homopiperazinium compound is a compound wherein J^- is a halogen. In one embodiment, the halogen is iodide, chloride or bromide. In one embodiment, the halogen is iodide. In one embodiment, the halogen is chloride. In one embodiment, the halogen is bromide.

In one embodiment, the homopiperazinium compound is a compound wherein J^- is a sulfonate. In one embodiment, the sulfonate is 4-toluenesulfonate, phenylsulfonate or methanesulfonate. In one embodiment, the sulfonate is 4-toluenesulfonate. In one embodiment, the sulfonate is phenylsulfonate. In one embodiment, the sulfonate is methanesulfonate.

In one embodiment, the methods or combinations are useful for asthma or COPD, the homopiperazinium compound is as defined herein wherein J^- is a sulfonate and one or more agents is, beta2 agonists, muscarinic antagonists, leukotriene modulators, phosphodiesterase (PDE) inhibitors. In one embodiment, the methods or combinations are useful for asthma. In one embodiment, the methods or combinations are useful for COPD. In one embodiment, the homopiperazinium compound is as defined herein wherein J^- is a 4-toluenesulfonate. In one embodiment, the one or more agents is beta2 agonists, muscarinic antagonists, leukotriene modulators, phosphodiesterase (PDE) inhibitors.

Without being bound to theory, it is believed that the homopiperazine compound of the invention exerts a dual nicotinic and muscarinic activity. In addition, ASM-024 mechanism of action appears to involve a direct action on airway smooth muscle cells (SMC) by a mechanism independent of cGMP or cAMP pathways. Moreover, ASM-024 had no relaxant effect on LTD4-induced contraction suggesting a mechanism of action independent from the leukotriene pathway.

As used herein, “treatment” or “treating” refers to at least i) controlling or ameliorating at least one disease described herein or associated symptoms, at least for the duration of said treatment, ii) reduce one or more side effects caused by agents used in the combination or iii) reduce the amount or dosage regimen of other agents of a patient.

Although not limited to such patients, “prevention” or “prophylaxis” treatment (which may be used interchangeably) is expected to be particularly useful to the treatment of patients who have suffered a previous episode associated with diseases described herein, or are otherwise considered to be at increased risk of said diseases. A successful preventive treatment would normally be expected to i) reduce the occurrences of a further episode, ii) reduce its severity or iii) prevent occurrences of further episodes, at least for the duration of the therapy.
It will be clear to a person of ordinary skill that the amounts and/or ratios of therapeutic agents will be readily adjusted. It will be understood that the scope of combinations described herein is not particularly limited, but includes in principle any therapeutic agent useful for preventing or treating the diseases described herein.

It will also be appreciated that the amounts and/or ratios of therapeutic agents for use in treatment will vary not only with the particular agent selected but also with the route of administration, the nature of the condition for which treatment is required and the age and condition of the patient and will be ultimately at the discretion of the attendant physician.

The homopiperazinium compounds defined herein can be administered concurrently to the one or more agents used herein in the methods and combinations. The desired doses may conveniently be presented in a single dose or as divided dose administered at appropriate intervals, for example as two, three, four or more doses per day. The compound can be administered on a dosage regimen distinct to the one or more agents used herein in the methods and combinations. Alternatively, the compound can be administered sequentially or concurrently in distinct formulations or in a common formulation.

While it is possible that, for use in therapy, therapeutic agents or combination of the invention may be administered as the raw chemical it may be preferable to present the active ingredient as a pharmaceutical composition.

Pharmaceutical compositions may comprise pharmaceutically acceptable carriers. Compositions include those suitable for oral, nasal, topical (including buccal and sub-lingual), transdermal, or parenteral (including intramuscular, sub-cutaneous and intravenous) administration or in a form suitable for administration by inhalation. The formulations may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active compound with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired formulation.

Pharmaceutical compositions suitable for oral administration may conveniently be presented as discrete units such as capsules, cachets or tablets each containing a predetermined

amount of the active ingredient; as a powder or granules; as a solution, a suspension or as an emulsion. The active ingredient may also be presented as a bolus, electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives.

The compounds and combinations according to the invention may also be formulated for parenteral administration (e.g. by injection, for example bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing an/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilisation from solution, for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use.

Compositions suitable for topical administration in the mouth include lozenges comprising active ingredient in a flavoured base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

For administration by inhalation the compounds and combinations according to the invention are conveniently delivered from an insufflator, nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation, the compounds and combinations according to the invention may take the form of a dry powder composition, for example a powder mix of the compound and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges or e.g. gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflator.

For preparation and development of pharmaceutical products, an efficient and cost effective synthetic route is desirable. It is therefore often desirable that the synthesis have the smallest number of steps but it is also necessary that the route be suitable to carry out the synthesis on sufficiently large scale.

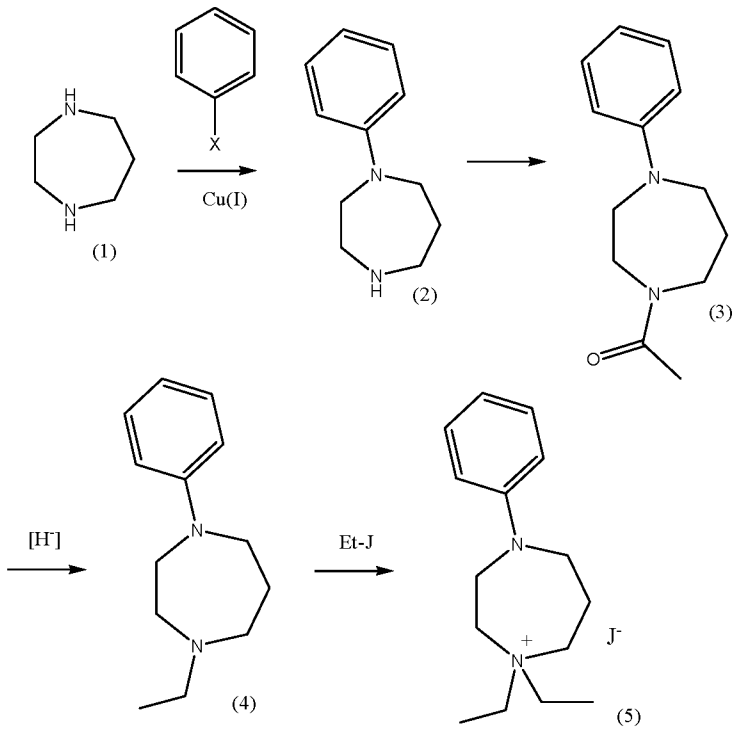
It may be advantageous that certain “advanced” building blocks be commercially available. Certain products such as N-BOC-homopiperazine or N-formyl-homopiperazine may be obtained from commercial sources but have the drawback of either being more expensive than homopiperazine or do not present the right protective groups. One option is to prepare the “protected” compounds mentioned. The drawback is that the synthesis is likely to require one or more extra steps of protection and deprotection.

Other homopiperazine derivatives such as N-methyl-homopiperazine and N-isopropyl-homopiperazine may also be available however these do not have the required chemical functions for the synthesis of the compounds defined herein.

Homopiperazine is available from various commercial sources at a reasonable price however one concern with homopiperazine is that the molecule has two reactive NH groups. A skilled organic chemist will recognize that these groups can both get involved in successive and/or simultaneous chemical reactions. In case a single NH must react with a given reagent, a classical method to reduce undesired reactions of the NH groups is to use a smaller molar amount of the reagent however this is susceptible to unacceptably reduce the yield of the desired monoreacted product and nevertheless still lead to a mixture of unreacted, mono as well as bis-reacted products.

Applicant has designed a practical and suitable synthetic method for preparing the compounds defined herein starting from simplest suitable reagent homopiperazine without the need to modify substituents or the use of protecting groups. Scheme 1 provides a summary of the approach.

Scheme 1



As seen from scheme 1, homopiperazine is reacted with a Ph-X reagent to introduce a phenyl residue on one of the NH in the presence of a Cu catalyst, preferably a Cu(I), to produce compound (2). "Ph" is phenyl and X is a suitable atom or group for effecting a Cu catalyzed coupling. An example of such catalyst is CuI however other Copper catalyst such as CuBr may also be suitable. A suitable reagent for introducing the phenyl ring is phenyl iodide, however other agents such asphenyl bromide alone or in the presence of sodium or potassium iodide in order to exchange the bromide on phenyl bromide to form phenyl iodide *in situ* may also be used. Compound (2) is acylated, using for example acetic anhydride or an acyl halide to produce compound (3). Compound (3) is reduced to compound of formula (4) using a suitable reducing agent, for example a hydride reducing agent such as $LiAlH_4$ or a borane reagent like BH_3 or a complexed borane reagent like BH_3-Me_2S. Advantageously, the steps from homopiperazine (1) to crude compound (4) can be conducted without purifying intermediate compounds (2) and (3). From a commercial standpoint, avoiding purification of intermediates while maintaining an acceptable yield is an advantage when conducting large scale synthesis. Compound (4) is in turn reacted with a suitable reagent of general formula Et-J, wherein Et is ethyl and J is a leaving group that will provide a counterion J^-, for introducing the second ethyl group to produce compound (5) having a quaternary ammonium functionality wherein J^- is a counterion. Preferably, J is I, Br, Cl, tosyl, mesyl, bezyl; most preferably a tosyl.

Compounds 5 may also be formed using a reagent Et-J wherein J is other than I, Br, Cl, tosyl, mesyl, bezyl, for example to form a synthetically suitable salt for the purpose of isolation or

purification. In the context of this disclosure, a "synthetically suitable salt" is a salt that may or may not be a pharmaceutically acceptable salt and that is used in the process to obtain a desired intermediate. When J^- is other than I^-, Br^-, Cl^-, tosylate, mesylate, bezylate, the desired salt is obtained by methods known in the art such as by ion exchanged. Compound 5 can optionally be purified using techniques known in the art such as crystallization or chromatography.

In one embodiment, there is provided a process for preparing a compound of formula (5) wherein J^- is a counterion, preferably, J^- is I^-, Br^-, Cl^-, tosylate, mesylate, bezylate; most preferably a tosylate comprising the steps as defined in scheme 1.

In one embodiment, there is provided a process for preparing a compound of formula 5 wherein J^- is tosylate comprising conducting one or more steps defined in scheme 1 above.

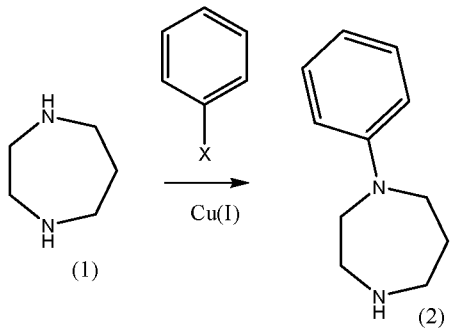
In embodiments:

- compound of formula 5 is prepared from transforming compound (4) to compound (5);

- compound of formula 5 is prepared from transforming compound (3) to compound (4) and to compound (5); or

- compound of formula 5 is prepared from transforming compound (2) to compound (3) to compound (4) and to compound (5).

In one embodiment, there is provided a process for preparing a compound of formula (2) comprising conducting the step defined in the following scheme:



(1) (2)

wherein X is as defined herein.

In further embodiments:

- there is provided a process for preparing a compound of formula (2), from transforming compound (1) to compound (2);

- there is provided a process for preparing a compound of formula (3), from transforming compound (1) to compound (2) to compound (3);

- there is provided a process for preparing a compound of formula (4), from transforming compound (1) to compound (2) to compound (3) to compound (4).

In one embodiment, there is provided the compound of formula (2).

In one embodiment, there is provided the compound of formula (3).

In one embodiment, there is provided the compound of formula (4).

In one embodiment, there is provided the compound of formula (5), wherein J^- is a counterion, preferably, J is I, Br, Cl, tosyl, mesyl, bezyl; preferably tosyl, mesyl, bezyl, most preferably a tosyl.

In one embodiment, there is provided a crystalline compound of formula (5) wherein J^- tosyl, mesyl, bezyl, or preferably a tosyl.

The following examples are provided to further illustrate details for the preparation and use of the compounds of the present invention. They are not intended to be limitations on the scope of the instant invention in any way, and they should not be so construed. Furthermore, the compounds described in the following examples are not to be construed as forming the only genus that is considered as the invention, and any combination of the compounds or their moieties may itself form a genus.

General Experimental Methods

Reactions were performed under argon atmosphere. Melting points are uncorrected. ^{1}H NMR spectra were recorded at 400 MHz and were referenced to the peak for residual solvent. ^{13}C NMR spectra were recorded at 100 MHz (^{13}C NMR at 75 MHz for Preparative Example) and were referenced to the peak for residual solvent. Chemical shifts in ^{1}H and ^{13}C NMR spectra are reported in ppm. All reagents were obtained from Aldrich Co as ''reagent'' grade. Usual solvents and chemicals were obtained from VWR, A&C or Fisher and were also ''reagent'' grade. Chromatography was performed using Silica Gel 60 (Merck; 230-400 mesh). Accurate mass measurements were performed on a LC-MSD-Tof instrument from Agilent technologies in positive electrospray mode. Protonated molecular ions $(M+H)^+$ was used for empirical formula confirmation.

Preparative Example: 1-Phenyl-4-ethyl-homopiperazine

RP-HPLC conditions (Preparative example):

HPLC analysis were performed on a Waters C18 reversed-phase analytical column (5µm, Atlantis, 100 x 3.9 mm) using a flow rate of 1 mL/min and a gradient of 0% to 95% A/B over 15 min, where A = 0.1% aqueous Formic Acid and B = CH_3CN + 0.1% FA

A solution of homopiperazine (50 g, 499.1 mmol, 1.2 eq), iodobenzene (84.86 g, 416 mmol, 1 eq), ethylene glycol (46.4 mL, 832 mmol, 2 eq), CuI (3.96 g, 20.8 mmol, 5% mol) and K_3PO_4 (88.3 g, 416 mmol, 1 eq) and isopropanol (416 mL) was stirred at reflux for 46 h. The resulting mixture was cooled down to room temperature and isopropanol was evaporated. Water (200 mL), containing NH_4OH (1%), and EtOAc (250 mL) were added to the mixture. The aqueous layer was extracted with EtOAc (4 × 200 mL), and the combined organic layers were washed with brine (2 × 200 mL), dried over Na_2SO_4, filtered and evaporated. The crude product (52.2 g) was obtained as a brown oil and was used in the next step without any purification.

To a solution of 1-phenylhomopiperazine (52.2 g, 292.6 mmol, 1 eq) in dichloromethane (300 mL) were added at 0°C Et_3N (90 mL, 890 mmol, 3 eq) and Ac_2O (112.15 mL, 1186 mmol, 4 eq). The mixture was stirred at room temperature for 2 h. A 4N NaOH solution (200mL) was added and the resulting mixture was extracted with CH_2Cl_2 (3 × 150 mL). The combined organic layers were washed with brine (2 × 150 mL), dried over Na_2SO_4 and evaporated. The resulting oil was coevaporated with EtOH (3 ×), EtOAc (3 ×) and Et_2O (3 ×), to give 68.03 g of crude product which was used in the next step without any purification.

To a suspension of $AlLiH_4$ (28.46 g, 750 mmol, 1.5 eq) in THF (400 mL) at 0°C was added dropwise a solution of 1-phenyl-4-acyl-homopiperazine (109 g, 500 mmol, 1 eq) in THF (500 mL). The mixture was warmed up to room temperature and stirred for 24 h. The mixture was then cooled down to 0°C and H_2O (350 mL) was added dropwise. THF was evaporated, TBME (400 mL) was added and the mixture was filtered on Celite ®. The layers were separated, and the aqueous phase was extracted with TBME (3 × 150 mL). The organic layers were combined and washed with brine (2 x 150 mL), dried over Na_2SO_4, filtered and evaporated. The crude product was purified by chromatography on silica gel using 100% hexanes and a gradient of 0% to 20% MeOH in CH_2Cl_2. The desired compound 1-Phenyl-4-ethyl-homopiperazine was obtained as a orange oil (38.1 g, 19 % overall yield): ^1H NMR $CDCl_3$ (ppm): 7.26 (dd, 2H), 6.73 (m, 3H), 3.61(t, 2H), 3.53 (t, 2H), 2.81 (m, 2H), 2.62 (m, 4H), 2.03 (m, 2H), 1.12 (t, 3H); ^{13}C NMR $CDCl_3$ (ppm): 148.8, 128.9, 115.4, 111.2, 54.7, 53.8, 51.3, 48.3, 47.6, 27.4, 12.1.

RP-HPLC conditions (examples):

HPLC analysis were performed on a Waters C18 reversed-phase analytical column (5µm, Atlantis, 100 x 3.9 mm) using a flow rate of 1 mL/min and a gradient of 0% to 95% A/B over 15 min (condition A) or on a Phenomenex CN reversed-phase analytical column (5µm, Luna

CN, 150 x 4.6 mm) with a flow rate of 0.5 mL/min and a gradient of 15% to 95% A/B over 30 min (condition B), where A = CH_3CN + 0.1% formic acid + 0.1% triethylamine and B = 0.1% aqueous formic acid + 0.1% triethylamine.

Example 1: 1,1-Diethyl-4-phenyl-homopiperazinium Iodide **(ASM-009)**

To a solution of 1-phenyl-4-ethylhomopiperazine (14.87 g, 73 mmol) in acetone (75 mL) was added ethyliodide (22.74 g, 145.8 mmol). The mixture was heated to reflux for 22 h, cooled down to room temperature, and the resulting white solid was filtered under vacuum to afford 23.5 g (89% yield) of the **ASM-009** : mp = 190.5-192.5 °C; ^{1}H NMR D_2O (ppm): 7.31 (dd, 2H), 6.87 (m, 3H), 3.74(br s, 2H), 3.52 (m, 4H), 3.34 (m, 6H), 2.20 (br s, 2H), 1.26 (t, 6H); ^{13}C NMR D_2O (ppm): 147.6, 129.4, 118.1, 113.1, 60.1, 59.6, 54.6, 46.5, 42.9, 21.5, 6.6; MS ES(+) : (M – I^-) = 233.2; 100% homogeneity (RT = 13.94 min) by LC-MS using CN column with ACN-H_2O(0.1% formic acid) as eluent and UV detection at 240 nm.

Example 2: 1,1-Diethyl-4-phenyl-homopiperazinium Bromide **(ASM-021)**

The resin Amberlite® IRA-400(Cl) (100 mL) was treated with 2N KBr (250 mL), and then washed with 200 mL H_2O. The compound **ASM-009** (FG1-62, 2.286 g, 6.35 mmol) was dissolved in H_2O (50 mL) by heating slightly and put down on the resin. The product was eluted with water (500 mL) and the solvent was evaporated. The residue was analyzed by MS and a signal at m/e 127 was present. So, the resin was retreated with 2N KBr and the residue dissolved in 100 mL H_2O. By MS, always one signal at m/e 127. The resin was washed with deionized water and treated with 2N HBr (500mL), washed with deionized water (350 mL), the residue dissolved in deionized water (100 mL) was passed through the resin. But by MS, 12.5% of I^- were still present. So, a new resin was used: Amberlite® IRA-410(Cl) (100 mL), treated with 2N HBr (2 × 250 mL), washed with deionized water (250 mL). The residue was dissolved in deionized water (50 mL) and eluted from the resin with deionized water. After evaporation of the water, an oil was obtained and coevaporated with Et_2O/acetone to afford **ASM-021** as a solid (1.616 g). The compound was dried by heating at 40°C under vacuum for 24 h, but traces of acetone (5%) were detected by ^{1}H NMR at 2.22 ppm. The product was analyzed by ES negative ion mode MS and signals at m/e 125 and 127 were present. But, these were adducts Br^- + HCO_2H (79 + 46 = 125; 81 + 46 = 127), because when acetic acid was used instead of formic acid, just two signals were present at m/e 79 and 81: ^{1}H NMR D_2O (ppm): 7.38 (dd, 2H), 6.97 (m, 3H), 3.81 (br s, 2H), 3.56 (m, 4H), 3.41 (m, 6H), 2.24 (br s, 2H), 1.29 (t, 6H); MS ES(+) : (M – Br^-) = 233.2; 100% homogeneity (RT = 14.21 min) by LC-MS using CN column with ACN-H_2O(0.1% formic acid) as eluent and UV detection at 240 nm.

Example 3: 1,1-Diethyl-4-phenyl-homopiperazinium Chloride **(ASM-022)**

The resin Amberlite® IRA-410(Cl) (100 mL) was treated with 2N HCl (250 mL), washed with 200 mL of distilled water. The compound **ASM-009** (FG1-62, 2.84 g, 7.89 mmol) was dissolved in H_2O (60 mL) by heating slightly and put down on the column of resin. The product was eluted with water (500 mL) and the solvent was evaporated. The residue was

triturated and coevaporated with Et_2O/acetone to afford **ASM-022** as a solid (1.755 g). The compound was dried by heating at 40°C under vacuum for 24 h, but traces of acetone (1%) were detected by ^1H NMR at 2.22 ppm : mp = 163.3-164.6°C; ^1H NMR D_2O (ppm): 7.34 (dd, 2H), 6.91 (m, 3H), 3.79 (br s, 2H), 3.56 (m, 4H), 3.46 (m, 6H), 2.24 (br s, 2H), 1.28 (t, 6H). By negative ES mode MS, Cl^- formed adducts with formic acid: m/e 35 + 46 = 81 and m/e 37 + 46 = 83; MS ES(+) : (M – Cl^-) = 233.2; 100% homogeneity (RT = 14.12 min) by LC-MS using CN column with ACN-H_2O(0.1% formic acid) as eluent and UV detection at 240 nm.

Example 4: 1,1-Diethyl-4-phenyl-homopiperazinium Acetate **(ASM-023)**

The resin Amberlite® IRA-410(Cl) (100 mL) was treated with 2N HBr (250 mL), washed with 250 mL of distilled water, treated with 2N NaOH (50 mL) in an ultrasonic bath for 10 minutes, washed with 2N NaOH (200 mL), treated with 2N AcOH (50 mL) in an ultrasonic bath for 20 minutes, washed with 2N AcOH (200 mL), and finally washed with water (250 mL). Compound **ASM-009** (1.68 g, 4.67 mmol) was dissolved in H_2O (60 mL) by heating slightly and eluted through the column of resin. By ES negative ion mode MS, the two signals corresponding to Br^- were present. Then the resin was retreated: 2N NaOH (100 mL) in an ultrasonic bath for 15 minutes, washed with 2 N NaOH (300 mL), washed with H_2O (250 mL), treated with 2N AcOH (100 mL) in an ultrasonic bath for 25 minutes, washed with 2N AcOH (100 mL) and H_2O (250 mL). The residue was dissolved in water (30 mL) and eluted through the column of resin with water. After evaporation, **ASM-023** was obtained as an oil (1.40 g): ES negative mode MS: no signals for Br^- were detected; ^1H NMR D_2O (ppm): 7.21 (dd, 2H), 6.7 (m, 3H), 3.50 (br s, 2H), 3.30 (m, 4H), 3.15 (m, 6H), 2.01 (br s, 2H), 1.83 (s, 3H), 1.13 (t, 6H); MS ES(+) : (M - AcO^-) = 233.2; 100% homogeneity (RT = 11.95 min) by LC-MS using CN column with ACN-H_2O(0.1% formic acid) as eluent and UV detection at 240 nm.

Example 5: 1,1-Diethyl-4-phenyl-homopiperazinium Tosylate **(ASM-024)**

A 5 L three-necked flask, equipped with a mechanical stirrer and a condenser, is charged with 189.55 g (0.93 mol) of 1-phenyl-4-ethyl-homopiperazine and then 1 L of acetone. Ethyl *p*-toluenesulfonate (371.6 g, 1.86 mol, 2 equiv) plus 200 mL of acetone for wash then added and the mixture was heated gently to reflux temperature. After 4 h, crystals had started to form and 350 ml of acetone were added to facilitate the stirring. After 24 h, HPLC analysis indicated that there was some starting material left. Consequently, 1 additional equivalent of TsOEt was added (186 g) and the mixture was further heated to reflux. After a total of 94 h, HPLC analysis indicated that the reaction had not progress much further and therefore, the heating was stopped and after 1 h, *t*-butyl methyl ether (1 L) was added. The mixture was stirred 15 min, then the crystals were filtered and washed with 5 portions of 500 mL of *t*-butyl methyl ether. The fine white needles were dried at room temperature under vacuum for 24 hours to afford 336.86 g (90% yield) of **ASM-024**: mp 167.5°-168.8°C; LC-UV-MS analysis: 100% homogeneity (RT = 13.4 min) using UV detection at 240 nm and CN column with ACN-H_2O (0.1% formic acid) as gradient eluent; ES(+) m/z 233.2 (M – TsO^-); ES(-) m/z 171 (TsO^-).

^{1}H NMR (D_2O) δ 7.74 (d, 2H), 7.40 (m, 4H), 6.93 (m, 3H), 3.75 (br s, 2H), 3.54 (m, 4H), 3.37 (m, 6H), 2.42 (s, 3H), 2.23 (br s, 2H), 1.32 (t, 6H); ^{13}C NMR (D_2O) δ 6.7, 20.3, 21.5, 42.6, 46.2, 54.5, 59.5, 60.3, 112.8, 117.6, 125.2, 129.0, 129.4, 140.4, 141.2, 147.9. The crude product **ASM-024** (222.11 g) was dissolved in hot CH_2Cl_2 (750 mL). Then, *t*BuOMe (160 mL) was added slowly in order to create a mild milky vein and until the appearance of the first crystal, and the mixture was left at room temperature for 3 h. Then, the white solid was filtered, washed with *t*BuOMe (500 mL) and dried under vacuum. For the second and third recrystallization, the same procedure was used with CH_2Cl_2 (750 mL)/ *t*BuOMe (110 mL) and CH_2Cl_2 (720 mL)/ *t*BuOMe (120 mL) respectively, to afford 211.7 g of **ASM-024** (99% recovery).

Example 6: 1,1-Diethyl-4-phenyl-homopiperazinium Mesylate **(ASM-025)**

The resin Amberlite® IRA-400(Cl) (150-170 mL) was treated with 2N HCl (250 mL), and then washed with 250 mL of distilled water. Then the resin was washed with 2N NaOH (250 mL), treated with 2N NaOH (50 mL) in an ultrasonic bath for 20 minutes, washed with 2N NaOH (200 mL). It was washed with a 2N solution of methane sulfonic acid (250 mL), treated with this 2N solution of methane sulfonic acid (50mL) in an ultrasonic bath for 30 minutes and washed again with this 2N of methane sulfonic acid (200 mL). Finally the resin washed with water (250 mL) and the compound **ASM-009** (FG1-60, 2.02 g, 5.61 mmol) was dissolved in H_2O (50 mL) by heating slightly and put down on the resin. The product was eluted with water (500 mL) and the solvent was evaporated and coevaporated with EtOH (3 ×) to afford **ASM-025** as a white solid (1.707 g): mp = 92.8-94.3°C; ^{1}H NMR D_2O (ppm): 7.34 (m, 2H), 6.90 (m, 3H), 3.78 (br s, 2H), 3.55 (m, 4H), 3.39 (m, 6H), 2.78 (s, 3H), 2.31 (br s, 2H), 1.28 (t, 6H); MS ES(+) : (M – MsO^-) = 233.2; 100% homogeneity (RT = 13.66 min) by LC-MS using CN column with ACN-H_2O(0.1% formic acid) as eluent and UV detection at 240 nm; MS ES(-) : MsO^- = 95.2.

Example 7: 1,1-Diethyl-4-phenyl-homopiperazinium Bezylate **(ASM-033)**

To a solution of 1-phenyl-4-ethyl-homopiperazine (6 g, 29.41 mmol) in acetone (30 mL) was added ethyl benzenesulfonate (10.94 g, 58.82 mmol). The mixture was heated gently to reflux temperature for 25 h. The mixture was cooled to room temperature and filtered. The white solid was dried at room temperature under vacuum to afford 10.58 g (92% yield) of **ASM-033**: LC-UV-MS analysis: 100% homogeneity (RT = 10.45 min) using UV detection at 240 nm and CN column with ACN-H_2O (0.1% formic acid) as gradient eluent; ES(+) m/z 233.2 (M – $C_6H_5SO_3^-$); ES(-) m/z 157 ($C_6H_5SO_3^-$); ^{1}H NMR D_2O (ppm): 7.79 (dd, 2H), 7.53 (m, 3H), 7.33 (m, 2H), 6.87 (m, 3H), 3.74 (br s, 2H), 3.51 (m, 4H), 3.35 (m, 6H), 2.20 (brs, 2H), 1.26 (t, 6H).

Example 8: 1,1-Diethyl-4-(2-pyridyl)-homopiperazinium Tosylate **(ASM-037)**

To a solution of 1-ethyl-4-(2-pyridyl)homopiperazine (1.99 g, 9.7 mmol) in acetone (15 mL) was added ethyl *p*-toluenesulfonate (3.9 g, 19.4 mmol). The mixture was first stirred at room

temperature. After 18 h, the reaction was not complete, then the mixture was heated gently to reflux temperature for 7 h. There was some starting material left, ethyl *p*-toluenesulfonate (3.9 g, 19.4 mmol) was added and the reaction was stirred for 4 additional days. The mixture was cooled to room temperature and filtered. The white solid was dried at room temperature under vacuum to afford 2.46 g (63% yield) of **ASM-037**: LC-UV-MS analysis: 100% homogeneity (RT = 13.70 min) using UV detection at 240 nm and CN column with ACN-H_2O (0.1% formic acid) as gradient eluent; ES(+) m/z 234.2 (M – TsO^-); ES(-) m/z 171 (TsO^-); ^1H NMR D_2O (ppm): 8.03 (d, 1H), 7.63 (d, 3H), 7.29 (d, 2H), 6.73 (m, 2H), 3.87 (br s, 2H), 3.60 (t, 2H), 3.50 (m, 2H), 3.32 (m, 6H), 2.32 (s, 3H), 2.20 (br s, 2H), 1.26 (t, 6H).

Example 9: 1,1-Diethyl-4-(phenyl-4-hydroxy)-homopiperazinium tosylate **(ASM-073)**

step 1

To a solution of benzyl 1-homopiperazine carboxylate **1** (5.27 g, 22.5 mmol) in *t*-BuOH (30 mL) were added EtI (4.2 g, 27 mmol) and Na_2CO_3 (4.8 g, 45 mmol) at 0°C. Then, the mixture was stirred at reflux temperature for 2.5 h. The volatile was evaporated and the residue was dissolved in H_2O and extracted 3 times with Et_2O. The organic layer was washed with brine, dried under Na_2SO_4, filtered and evaporated to afford crude product 1-(Benzylcarboxy)-4-ethyl-homopiperazine (4.95 g, 84% yield) as a orange oil: $(M+H)^+$: 263.6.

step 2:

To a solution of 1-(benzylcarboxy)-4-ethyl-homopiperazine **14** (4.95 g, 18.89 mmol) in EtOAc (110 mL) was added 10% Pd/C (1 g). The mixture was filled, vented, and filled 3 times with hydrogen and stirred at room temperature under H_2 atmosphere for 17 h. The catalyst was filtered on Celite®, washed with EtOAc, and the filtrate and washings were combined and evaporated to provide crude product 1-Ethyl-homopiperazine (2 g, 83% yield) as an oil: $(M+H)^+$: 129.5.

step 3:

To a solution of 1-ethyl-homopiperazine (740 mg, 5.78 mmol) in toluene (7 mL) were added 4-benzyloxybromobenzene (2 g, 7.51 mmol), KO*t*Bu (8.7 mL, 8.7 mmol), $Pd_2(dba)_3$ (264.6 mg, 0.289 mmol) and BINAP (540 mg, 0.867 mmol). The mixture was stirred at 90°C for 18 h, then cooled down to room temperature. The mixture was diluted with EtOAc (50 mL) and H_20 (25 mL). After the separation of the layers, the organic phase was washed once with H_20 (25 mL). The combined aqueous phases were back extracted with EtOAc (25 mL). The resulting combined organic phases were extracted with a 2N HCl solution (3 x 20 mL). The aqueous phases were combined, cooled down with an ice bath and basified up to pH 10 with a 5N NaOH solution. The resulting aqueous phase was extracted with DCM (3 x 20 mL), washed with brine (25 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford product 1-(phenyl-4-benzyloxy)-4-ethyl-homopiperazine as a brown oil (1.52 g, 85% yield) : ^1H NMR (400 MHz, $CDCl_3$) δ : ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 (m, 5H), 6.92 (m,

2H), 6.67 (m, 2H), 5.03 (s, 2H), 3.54 (t, 2H), 3.46 (t, 2H), 2.78 (m, 2H), 2.61 (m, 4H), 2.01 (m, 2H), 1.10 (t, 3H); $(M+H)^+$: 311.2.

Step 4:

To a solution of 1-(phenyl-4-benzyloxy)-4-ethyl-homopiperazine (4.51 g, 14.55 mmol) in acetone (20 mL) was added EtOTs (8.73 g, 43.65 mmol). The mixture was stirred at reflux for 41 h, and after cooling down, MTBE (30 mL) was added. After stirring 15 min, the precipitate was filtered and washed with MTBE, dried under vacuum to afford product 1,1-Diethyl-4-(phenyl-4-benzyloxy)-homopiperazinium tosylate (6.88 g, 93%) as a beige solid: ^{1}H NMR (400 MHz, CD_3OD) δ 7.72 (d, 2H), 7.36 (m, 5H), 7.24 (d, 2H), 6.93 (m, 2H), 6.82 (m, 2H), 5.03 (s, 2H), 3.67 (br s, 2H), 3.61 (m, 2H), 3.46 (m, 8H), 2.38 (s, 3H), 2.23 (br s, 2H), 1.33 (t, 6H); $(M)^+$: 339.3.

Step 5: (ASM-073)

To a solution of 1,1-diethyl-4-(phenyl-4-benzyloxy)-homopiperazinium tosylate (6.87 g, 13.47 mmol) in DCM (90 mL) was added 10% Pd/C (700 mg). The mixture was filled, vented, and filled 3 times with hydrogen and stirred at 40°C under H_2 atmosphere for 21 h. The reaction was incomplete by MS analysis, the catalyst was filtered on Celite®, washed with MeOH, and the filtrate and washings were evaporated. The residue was coevaporated with DCM, and dissolved in DCM (90 mL), 10% Pd/C (700 mg) was added, the mixture was filled, vented, and filled 3 times with hydrogen and was heated at 40°C for an additional 7 h. However, there was some material left, the catalyst was filtered on Celite®, washed with MeOH, and the filtrate and washings were evaporated. The residue was coevaporated with DCM, and dissolved in DCM (90 mL), 10% Pd/C (700 mg) was added. The mixture was filled, vented, and filled 3 times with hydrogen and stirred at 40°C under H_2 atmosphere for an additional 15 h. The catalyst was filtered on Celite®, washed with MeOH, and the filtrate and washings were evaporated. The crude solid was triturated with MTBE and filtered, then triturated 3 times with DCM to afford after drying under vacuum **ASM-073** as a beige solid (4.25 g, 75%): mp = 144.0-145.5°C; ^{1}H NMR (400 MHz, CD_3OD) δ 7.72 (d, 2H), 7.24 (d, 2H), 6.75 (m, 4H), 3.60 (br s, 4H), 3.45 (m, 8H), 2.38 (s, 3H), 2.20 (br s, 2H), 1.32 (t, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 7.5, 20.7 23.4, 46.0, 49.9, 55.7, 60.9, 62.6, 116.4, 117.1, 126.4, 129.3, 141.1, 143.1, 143.7, 151.2; HPLC: condition B, RT = 5.92 min, 100% homogeneity; ES-MS [*p*-TsO^-] = 171.0; Exact Mass Calcd for $[M^+]$ $C_{15}H_{25}N_2O$ 249.19614, found 249.19575.

Example 10: EC_{50} TNF (Inflammation) and the EC_{50} isometric studies of selected compounds.

Inflammation

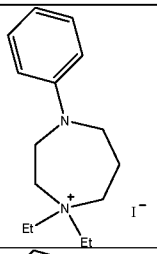
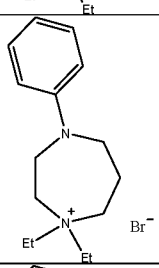
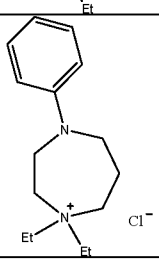
Human mononuclear cells were isolated on Lymphocyte Separation Media (Wisent, Canada) gradient. Cells were let to adhere on tissue culture plates for 2 hours at 37C in culture medium. Plates were washed and adherent monocytes were stimulated with LPS (100 ng/mL)

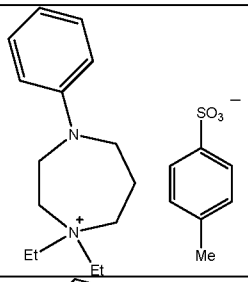
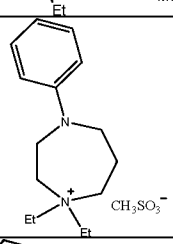
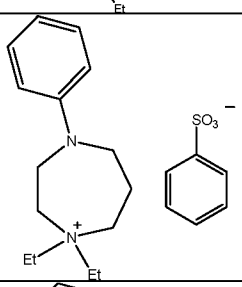
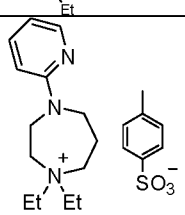
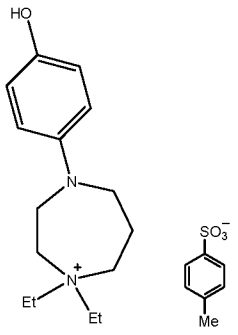
(E.coli 026:B6) for 18 hours at 37ºC with or without increasing concentrations of the various compounds. The production of TNF in the supernatant was measured by ELISA (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

Isometric studies

Mouse tracheas were isolated, adherent connective tissue removed and tracheal preparations were suspended in organ baths containing Krebs bicarbonate solution at 37ºC, and bubbled with 95% O_2-5% CO_2. A passive tension of 0.5g was applied followed by a 30-60 min equilibration period. Tissues were then contracted with methacholine (10-5M) which was maintained during addition of cumulative concentrations of the relaxant compounds and changes of tension recorded. The results are expressed as a percentage of maximal methacholine or histamine-induced contraction.

The table below provides for illustrative examples for the EC50 for TNF production, a marker of inflammation) and the EC50 for the relaxant responses in mouse isolated tracheas for several claimed analogs.

Entry	Compound	EC_{50} TNF (μM)	EC_{50} relaxant response(μM)
ASM-009		2.03	70.87
ASM-021		33.04	37.02
ASM-022		32.38	73.12

ASM-024		55.68	68.84
ASM-025		37.64	55.98
ASM-033		235	56
ASM-037		NI ‡	59.89
ASM-073		TX	2177

NI: No Inhibition at the tested concentration and

TX: effect on cell viability, no conclusion were drawn on the EC50's because the tested concentration was detrimental to the cultured cells.

Example 11: Characterization of compound of Example 5 (ASM-024)

i) Simultaneous Thermal Analysis (STA) Testing

Using a Perkin-Elmer TGA2000, approximately 20mg of ASM-024 was loaded into a tared ceramic crucible, and the sample was heated to 300°C at a rate of 10°C/minute. A 20cc/min

purge flow of nitrogen was used to prevent any oxidative side reactions. Sample weight and heat flow response was monitored and recorded.. A sharp endotherm was seen at onset 164°C. This was assigned as a melt. No other thermal events or weight losses were observed. This indicated that ASM-024 as tested is in the anhydrous crystalline form.

ii) Differential Scanning Calorimetry (DSC) Testing

DSC testing was performed using a Perkin Elmer Jade DSC. Approximately 2mg of ASM-024 was loaded into an aluminum sample pan and non-hermetically sealed. The sample was heated to 300°C at a rate of 10°C/minute, and heat flow response was monitored and recorded. The DSC thermogram supports the STA result above, only showing a melt at onset 166°C.

iii) XRPD

XRPD testing was performed using a PANalytical X'pert PRO XRPD. Approximately 20mg of sample was placed onto a low background XRPD sample holder. The sample was then loaded into the instrument and diffraction patterns recorded using the following experimental conditions:

- Tube anode: Cu
- Generator tension: 40 kV
- Generator current: 40 mA
- Wavelength alpha1: 1.5406 Å
- Wavelength alpha2: 1.5444 Å
- Start angle [2 theta]: 5
- End angle [2 theta]: 50
- Scan time: 11 minutes

Fig. 13 shows a typical diffractogram of dry ASM-024 obtained from example 5. The API was seen to be crystalline with sharp peaks. The crystalline form is designated anhydrous Form I for the purpose of the description herein. Table 1 summarizes the list of peaks of the crystalline ASM-024.

Table 1

No.	Pos. [°2Th.]	FWHM [°2Th.]	Area [cts*°2Th.]	d-spacing [Å]	Height [cts]	Rel. Int. [%]
1	8.2026	0.1004	77.31	10.77926	780.89	28.07
2	8.9818	0.1338	237.85	9.84585	1801.74	86.35
3	9.1732	0.1004	138.61	9.64079	1400.02	50.32
4	10.4746	0.2007	44.92	8.44576	226.86	16.31
5	13.8222	0.8029	53.21	6.4069	67.17	19.32
6	15.278	0.1338	48.97	5.79951	370.98	17.78

7	16.1464	0.1004	72.08	5.48953	727.97	26.17
8	16.3634	0.1004	115.82	5.41721	1169.84	42.05
9	17.5626	0.1004	112.25	5.04991	1133.72	40.75
10	18.0752	0.1673	135.5	4.90786	821.12	49.19
11	18.2573	0.1004	76.73	4.85931	774.99	27.86
12	18.856	0.1004	197.76	4.70633	1997.46	71.79
13	19.0333	0.1004	169.69	4.66291	1713.94	61.6
14	19.8906	0.1004	32.74	4.46382	330.66	11.88
15	20.2545	0.1004	54.62	4.38443	551.7	19.83
16	20.5537	0.2676	275.46	4.32128	1043.32	100
17	20.9122	0.1004	137.27	4.24801	1386.45	49.83
18	21.339	0.1004	23.85	4.164	240.84	8.66
19	21.736	0.3346	104.72	4.08883	317.3	38.02
20	22.4503	0.1004	97.67	3.96033	986.52	35.46
21	22.6217	0.1004	141.6	3.9307	1430.14	51.4
22	23.8494	0.1338	45.08	3.73107	341.52	16.37
23	25.0483	0.1004	97.66	3.55515	986.41	35.45
24	26.222	0.1338	71.01	3.39861	537.89	25.78
25	26.431	0.1338	99.6	3.37221	754.52	36.16
26	27.7048	0.1004	54.36	3.22	549.09	19.74
27	27.9132	0.1338	102.7	3.19644	777.97	37.28
28	29.0577	0.1171	63.85	3.07308	552.8	23.18
29	29.428	0.1004	46.71	3.03525	471.74	16.96
30	30.1912	0.2007	39.53	2.96024	199.64	14.35
31	30.7341	0.1673	39.17	2.90917	237.35	14.22
32	32.7089	0.3346	65.76	2.73791	199.26	23.87
33	34.9665	0.2676	46.97	2.56614	177.92	17.05
34	35.6949	0.1338	35.1	2.51543	265.9	12.74
35	36.1958	0.1338	35.26	2.48176	267.11	12.8
36	37.2009	0.2007	43.95	2.41698	221.95	15.96
37	37.867	0.1004	24.62	2.37599	248.69	8.94
38	39.4215	0.1673	42.44	2.2858	257.2	15.41
39	40.0555	0.1004	47.77	2.25107	482.49	17.34
40	40.6543	0.2007	47.58	2.21929	240.27	17.27
41	41.1541	0.2007	54.42	2.19349	274.85	19.76
42	42.3166	0.3346	69.33	2.13588	210.06	25.17
43	43.3031	0.1338	44.75	2.08948	338.96	16.24
44	44.7201	0.2007	40.93	2.02651	206.71	14.86
45	48.4688	0.3264	70.01	1.87662	160.88	25.42

The XRD pattern of ASM-024 Form I is characterized by 16 peaks. Table 2 represents the positions of these peaks according to the solid state form.

Table 2

Peak #	Form I	
	2θ	d spacing (Å)
1	8.2	10.770
2	9.2	9.647
3	10.4	8.494
4	15.3	5.783
5	16.3	5.433
6	17.7	5.006
7	18.2	4.876
8	18.8	4.722
9	20.4	4.353
10	20.8	4.272
11	21.8	4.073
12	22.5	3.945
13	23.7	3.743
14	25.0	3.557
15	26.3	3.382
16	27.8	3.207

The XRP pattern of ASM-024 Form I could be further characterised and classified by the intensity of the peaks relative to each other. Two % of peaks relative intensity patterns were determined according the highest value of the peak # 7 or 8 (Table 3 and 4).

Table 3 : % of Peaks Intensity Relative to Peak #7 of ASM-024 FORM I.

Peak #	d-spacing (A)	Position (2θ)	% of Intensity relative to peak #7)				
			Ave.	σ	RSD(%)	Min.	Max.
1	88.271	8.203	11.7	1.6	13.6	10.4	14.4
2	44.137	9.159	55.2	19.9	36.1	34.1	82.9
3	29.427	10.407	7.9	1.8	22.5	6.2	11.1
4	22.072	15.310	3.6	2.1	58.8	0.0	5.8
5	17.660	16.301	40.2	12.1	30.2	25.9	60.0
6	14.718	17.702	20.3	6.8	33.2	14.6	30.6
7	12.618	18.180	100.0	0.0	0.0	100.0	100.0
8	11.043	18.778	18.5	11.8	63.5	7.5	35.9
9	9.818	20.384	8.8	4.7	53.5	3.2	13.7
10	8.838	20.777	44.5	21.9	49.3	24.4	81.9
11	8.037	21.802	8.7	3.2	36.7	5.2	13.7
12	7.369	22.519	17.8	7.6	42.7	8.2	27.2
13	6.805	23.750	7.2	3.5	48.3	3.5	12.8
14	6.321	25.014	9.2	2.5	27.5	6.4	12.4
15	5.901	26.329	16.9	3.0	17.9	13.4	21.6
16	5.535	27.799	13.4	1.6	11.8	11.4	15.3

Table 4 : % of Peaks Intensity Relative to Peak #8 of ASM-024 FORM I

Peak #	d-spacing (A)	Position (2θ)	% of Intensity relative to peak #8)				
			Ave.	σ	RSD(%)	Min.	Max.
1	88.271	8.203	27.8	13.9	49.9	15.6	49.7
2	44.137	9.159	57.4	24.4	42.6	33.8	95.8
3	29.427	10.407	13.3	4.5	33.9	9.9	21.7
4	22.072	15.310	15.5	4.0	25.6	10.9	21.7
5	17.660	16.301	73.5	23.7	32.3	52.6	113.3
6	14.718	17.702	33.4	17.5	52.4	15.2	56.8
7	12.618	18.180	54.9	26.8	48.8	33.8	98.6
8	11.043	18.778	100.0	0.0	0.0	100.0	100.0
9	9.818	20.384	43.1	5.1	11.9	39.8	52.2
10	8.838	20.777	81.0	29.7	36.7	56.0	121.0
11	8.037	21.802	17.2	5.3	30.7	10.5	24.4
12	7.369	22.519	70.9	13.6	19.2	45.5	82.8
13	6.805	23.750	14.3	4.5	31.2	9.7	21.0
14	6.321	25.014	31.9	9.6	30.1	21.8	49.4
15	5.901	26.329	29.4	9.4	32.1	17.8	42.7
16	5.535	27.799	38.9	14.4	37.1	27.1	65.5

It has been found that the 1,1-diethyl-4-phenyl-homopiperazinium compounds of this description, especially those having a counterion which is I, Br, Cl, tosyl, mesyl or bezyl, and especially ASM-024, have surprisingly advantageous features.

For example, as can be seen from the table of EC50 (TNF) and EC50 relaxant responses above, the compound having a 4-(2-piridyl) (i.e.ASM-037) showed no Inhibition at the tested concentration in the TNF assay compared EC50 of less than 100uM for most compounds in the table having a 4-phenyl residue. In the table, ASM-009 is reported to have an EC50 (TNF) as low as about 2uM. ASM-073 bearing a 4-hydroxyphenyl residue, which may be considered an analogue closely related to the 4-phenyl substituent, showed a negative effect on cell viability in the EC50 (TNF) assay and no conclusion were drawn on the EC50's because the tested concentration was detrimental to the cultured cells. The EC50 relaxant responses of ASM-073 was higher than 2000uM which is from about 29-57 times higher than most 4-phenyl compounds in the table.

It is often desired that compounds used to treat respiratory diseases do not cross the blood-brain barrier (BBB) in order to prevent unwanted central effects. The main function of the blood-brain barrier (BBB) is to protect the brain from changes in the levels in the blood of ions, amino acids, peptides, and other substances. The BBB prevent water-soluble substances in the blood from freely entering the fluid environment of the brain cells.

In addition to the biological properties referred to above, Applicant has surprisingly identified a combination of substituents to the homopiperazine core providing desirable physico-chemical properties as defined herein.

For example, the solubility (in deionized water) of certain compounds is provided below:

- 1,1-diethyl-4-(2-naphthyl)-homopiperazinium tosylate (ASM-067): about 12mg/mL;

- 1,1-diethyl-4-(phenyl-4-trifluoromethyl)-homopiperazinium iodide (ASM-071): about 13 mg/mL;

- 1,1-diethyl-4-(phenyl)-homopiperazinium iodide (ASM-002): about 33 mg/mL

- 1,1-diethyl-4-(phenyl)-homopiperazinium tosylate (ASM-024): greater than 1000mg /mL.

As can be seen from the above results, a more lipophilic 2-naphthyl group on the homopiperazinium of ASM-067 provides much lower water solubility than that obtained for ASM-024 despite the fact that they both have a tosyl counterion. Also, the trifluoromethyl phenyl homopiperazinium compounds ASM-071 has a water solubility of almost 3 times less than the phenyl homopiperazinium ASM-002, both compounds having the same counterion.

Another desirable feature of a compound from a development and/or commercial standpoint is the ability to obtain a solid. It has been found that 1,1-diethyl-4-(phenyl)-homopiperazinium compounds having a counterion which is I, Br, Cl, tosyl, mesyl or bezyl are obtained as solid.

Another desirable feature of a compound to be developed is the avoidance of a chiral center in the molecule, which at the same time avoids the formation of enantiomers. Since it is desired that active pharmaceutical ingredients be preferably provided as a single enantiomer, the development of chiral compounds will require either the development of asymmetric synthetic processes or enantiomeric purifications. The compounds described herein avoid this unnecessary burden since they are symmetrical.

Example 12: *In vitro* evaluation of ASM-024 mediated smooth muscle relaxation.

In vitro evaluation of ASM-024 mediated smooth muscle relaxation was done by isometric tension measurement using isolated guinea pig tracheal rings, dog and human bronchial segments (Duringer C et al. Agonist-specific patterns of beta2-adrenoreceptor responses in human airway cells during prolonged exposure. British J Pharm 2009: 158: 169-179.). Adherent connective tissue was removed and tracheal or bronchial preparations were suspended in organ baths containing Krebs bicarbonate solution at 37ºC, and bubbled with 95% O_2-5% CO_2. A passive tension of 0.5 g was applied to mouse tracheas and dog bronchi and 1.0 g to human bronchi, followed by a 30-60 min equilibration period. Tissues were then contracted with methacholine (10^{-5}M) or histamine (10^{-5}M) which was maintained during addition of cumulative concentrations of the relaxant compounds and changes of tension

recorded. The results are expressed as a percentage of maximal methacholine or histamine-induced contraction.

Additive effects of ASM-024 on beta2-agonists airway smooth muscle relaxation in dog and human bronchi.

i) ASM-024 increased *in vitro* relaxation response to maximally effective concentration of short–acting beta-2 agonists in dog bronchi.

Dog bronchial preparations were contracted with 10^{-5}M methacholine and cumulative concentrations of ASM-024 or salbutamol added. Although ASM-024-induced bronchial relaxation requires higher concentrations than the β2-agonist salbutamol (EC_{50}=45.21 µM ± 13.74 for ASM-024 vs EC_{50}=1.75 µM ± 1.83 for salbutamol), ASM-024 elicits a rapid and complete relaxation at 10^{-4}M and 10^{-3}M whereas the highest salbutamol concentration utilised decreases the tension of contracted bronchi by a maximum of about 50%; the addition of ASM-024 (10^{-3}M) to the maximal effective concentration of salbutamol elicited the same extent of relaxation as ASM-024 (n=4). (see Fig. 1)

ii) ASM-024 increased *in vitro* relaxation response to maximally effective concentration of short–acting beta-2 agonists in human bronchi.

Human bronchi preparations were contracted with 10^{-5}M methacholine and relaxation induced by addition of cumulative concentrations of salbutamol (10^{-6}M to 10^{-4}M). When the maximal relaxation response was obtained, vehicle or ASM-024 (10^{-5}M to 10^{-3}M) was added to the tissue baths and tension recorded. Salbutamol decreases the tension of contracted human bronchi by a maximum of 67%; the addition of 10^{-4}M and 10^{-3}M ASM-024 to the maximal effective concentration of salbutamol increased relaxation to below the applied basal tension (preliminary data, n=1) (see Fig. 2).

Relaxant Effects of ASM-024, on beta2-Agonist Desensitized Guinea-Pig Tracheas

Chronic use of beta2-agonists bronchodilators, widely used in the respiratory diseases of asthma and COPD, induces functional desensitization of the beta2-adrenoreceptors (Davies AO et al. Regulation of beta-adrenergic receptors by steroid hormones. Annu Rev Physiol 1984, 46: 119-130), potentially leading to reduced responsiveness to to the short-acting β-agonist rescue medication, salbutamol, when used acutely (Lipworth BJ. Airway subsensitivity with long-acting beta2-agonists. Is there cause for concern? Drug Saf 1997, 16: 295-308). The efficacy of ASM-024 to promote relaxation of guinea-pig tracheas unresponsive to beta2-agonists was assessed.

i) Guinea pig trachea unresponsiveness to salbutamol following adrenoreceptor desensitization.

To induce desensitization, tracheal preparations were exposed to submaximal concentrations of the short-acting beta agonist (SABA) salbutamol ($5X10^{-8}$M) for 4 hours. Relaxation

response to salbutamol was measured before and after prolonged exposure to the drug. Reduced relaxation response is observed after a 4 hour exposure to salbutamol (6% relaxation response as measured 10 minutes after application of the drug compared to 57% initial relaxation) (see Fig. 3).

ii) Guinea pig trachea responsiveness to ASM-024 following salbutamol-induced beta2-adrenoreceptor desensitization.

Cumulative concentrations of ASM-024 (10^{-7}M to 10^{-3}M) were tested on intact or beta2-adrenoreceptor desensitized tracheal preparations by salbutamol. Relaxation response of ASM-024 on intact or adrenoreceptor desensitized tracheas was similar (EC_{50} = 37.1 ± 8.1 µM, n=3, and 24.5 ± 11.2 µM, n=4 respectively)(see Fig. 4).

iii) Guinea pig trachea unresponsiveness to salbutamol following salmeterol-induced β2-adrenoreceptor desensitization.

To induce desensitization, tracheal preparations were exposed to submaximal concentrations of the long-acting β agonist (LABA) salmeterol ($5X10^{-8}$M) for 4 hours. Initial relaxant response to salmeterol was measured before exposure to the drug, relaxant response following the desensitization process was measured using salbutamol. Reduced relaxation response is observed after 4 hour exposure to salmeterol (1% relaxation compared to 66% relaxation response as measured 10 minutes after application of salbutamol) (see Fig. 5).

iv) Guinea pig trachea responsiveness to ASM-024 following salmeterol-induced beta2-adrenoreceptor desensitization.

Cumulative concentrations of ASM-024 (10^{-7}M to 10^{-3}M) were tested on intact or β2-adrenoreceptor desensitized tracheal preparations by salmeterol. Relaxation response of ASM-024 on intact or adrenoreceptor desensitized tracheas was similar (EC_{50} = 38.9 ± 7.4µM, n=3 and 17.7 ± 7.5 µM, n=4 respectively) (see Fig. 6).

v) Guinea pig trachea unresponsiveness to salbutamol following formoterol-induced β2-adrenoreceptor desensitization

To induce desensitization, tracheal preparations were exposed to submaximal concentrations of the lond-acting β2 agonist (LABA) formoterol ($5X10^{-8}$M) for 18 hours. Initial relaxant response to formoterol was measured before exposure to the drug, relaxant response following the desensitization process was measured using salbutamol (2% initial relaxation compared to 95% relaxation response as measured 10 minutes after application of salbutamol) (see Fig. 7).

vi) Guinea pig trachea responsiveness to ASM-024 following formoterol-induced β2-adrenoreceptor desensitization.

Cumulative concentrations of ASM-024 (10^{-7}M to 10^{-3}M) were tested on intact or β2-adrenoreceptor desensitized tracheal preparations by formoterol. Relaxation response of

ASM-024 on intact or adrenoreceptor desensitized tracheas was similar (EC50 = 35.15 ± 6.68 µM, n= 2, and 25.29 ± 12.91 µM, n=2, respectively) (see Fig 8).

Overall the data show that ASM-024 was able to maintain its relaxation capacity on beta2-adrenoreceptor desensitized tracheal preparations. These findings suggest that ASM-024 mediates smooth muscle relaxation through a different target and signaling pathway than currently used beta2-adrenergic receptor agonists.

Beneficial Effects of ASM-024 on Muscarinic Receptor Antagonists Relaxation of Airway Smooth Muscle

Muscarinic acetylcholine receptors (mAChR) antagonists are bronchodilators drugs used in COPD and in acute asthma exacerbations to reduce the enhanced cholinergic activity. These pharmaceutical agents are effective for their indicated uses but have limitations. They are however less effective on histamine-induced bronchoconstriction. Moreover the maximal approved therapeutic dose of these compounds, 18 µg, was selected to limit systemic side effects rather than to induce maximal bronchodilation at these tolerable doses. A series of experiments were designed to explore and differentiate the pharmacological functionality of ASM-024 to the muscarinic antagonists and examine the potential additive benefits of ASM-024 to this class of compounds.

The effect on histamine and methacholine-induced contraction was used to differentiate between the functional efficacy of ASM-024 and the currently used short acting (ipratropium) and long-acting (tiotropium) cholinergic antagonists and to assess the potential use of ASM-024 as an add-on therapy.

<u>i) ASM-024 overcomes ipratropium resistance to histamine-induced contraction.</u>

Guinea pig tracheal preparations were contracted *in vitro* with histamine (10^{-5}M), cumulative concentrations of ipratropium (10^{-8}M to 10^{-4}M) were added, and changes of tension recorded. Once equilibration was reached after addition of the highest concentration of the cholinergic antagonist, ASM-024 (10^{-4}M to 10^{-3}M) was applied to the organ baths.

Ipratropium was ineffective on histamine-induced contraction whereas complete relaxation even below the applied tension was observed upon addition of ASM-024, n= 4 (see Fig. 9).

<u>ii) ASM-024 overcomes tiotropium resistance to histamine-induced contraction.</u>

Guinea pig tracheal preparations were contracted *in vitro* with histamine (10^{-5}M), cumulative concentrations of tiotropium (10^{-8}M to 10^{-4}M) were added, and changes of tension recorded. Once equilibration was reached after addition of the highest concentration of the cholinergic antagonist, ASM-024 (10^{-4}M to 10^{-3}M) was applied to the organ baths.

Tiotropium was ineffective on histamine-induced contraction whereas complete relaxation even below the applied tension was observed upon addition of ASM-024, n= 3 (see Fig. 10).

These data provide further evidence of the differentiated pharmacological functionality of ASM-024 to the long-acting muscarinic antagonist, tiotropium.

Additive Effects of ASM-024 and Ipratropium on *In Vitro* Relaxation of Airway Smooth Muscle

Ipratropium is an effective and potent relaxant compound on contraction induced by muscarinic agonists such as methacholine or acetylcholine. The approved therapeutic dose for ipratropium is 18 µg, yet this dose level is well below the maximal bronchodilation dose. A lower therapeutic dose was chosen for regulatory approval due to the increase in anticholinergic side effects associated with the dose level required for maximal bronchodilatory effect. The potential for a greater bronchodilator effect when given in combination with ASM-024 was assessed.

Tiotropium is structurally related to ipratropium but has a significantly higher affinity for muscarinic receptors. The affinity of tiotropium for all muscarinic receptor subtypes is similar but tiotropium dissociates from the M2 receptors 10 times faster than it does from the M3 receptors making it more selective for the M3 receptors than ipratropium. As with ipratropium, the submaximal dose 18 µg was selected to limit systemic side effects and not to induce maximal bronchodilation, (Littner et al., 2000) and there is also a potential for underdosing with tiotropium.

Additive effect of ASM-024 and ipratropium on the relaxation response to methacholine.

Guinea-pig tracheal rings were exposed to a suboptimal concentration of ipratropium ($5x10^{-9}$M) for 10 minutes, giving about 10-20% relaxation, followed by cumulative additions of ASM-024 (10^{-6}M to 10^{-3}M). The combination of ASM-024 and a suboptimal dose of ipratropium improved the relaxation response, n=6, EC_{50} = 43.3 ± 13 µM for ASM-024 alone vs 18.7 ± 18.2µM for ipratropium + ASM-024 , p= 0.03 (see Fig. 11).

Additive effect of ASM-024 and tiotropium on the relaxation response to methacholine.

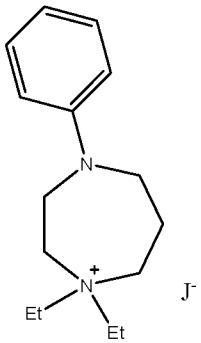
Guinea-pig tracheal rings were exposed to a suboptimal concentration of tiotropium ($9x10^{-7}$M) for 10 minutes, giving about 30% relaxation, followed by cumulative additions of ASM-024 (10^{-6}M to 10^{-3}M). The combination of ASM-024 and a suboptimal dose of tiotropium improved the relaxation response.

n=4, EC50 = 37 ± 25 µM for ASM-024 alone vs 45 ± 24 µM for tiotropium + ASM-024 (see Fig. 12)

Claims

1. A method for treating or preventing diseases selected from the group consisting of asthma, chronic obstructive pulmonary disease (COPD), interstitial pulmonary fibrosis (IPF), sarcoidosis, hypersensitivity pneumonitis (HP) and bronchiolitis obliterans with organizing pneumonitis (BOOP), comprising administering an effective amount of :

i) a homopiperazinium compound having the formula:



wherein J is a counter ion; and

ii) one or more agents for treating or preventing the pulmonary diseases: beta2 agonists, muscarinic antagonists, leukotriene modulators and phosphodiesterase (PDE) inhibitors or a combination thereof.

2. The method of claim 1, wherein said disease is asthma or chronic obstructive pulmonary disease (COPD).

3. The method of claim 1, wherein said disease is asthma.

4. The method of claim 1, wherein said disease is chronic obstructive pulmonary disease (COPD).

5. The method of any one of claims 1 to 4, wherein J^- is a halogen, a sulphate, acetate or a sulfonate.

6. The method of any one of claims 1 to 4, wherein J^- is a halogen or a sulfonate.

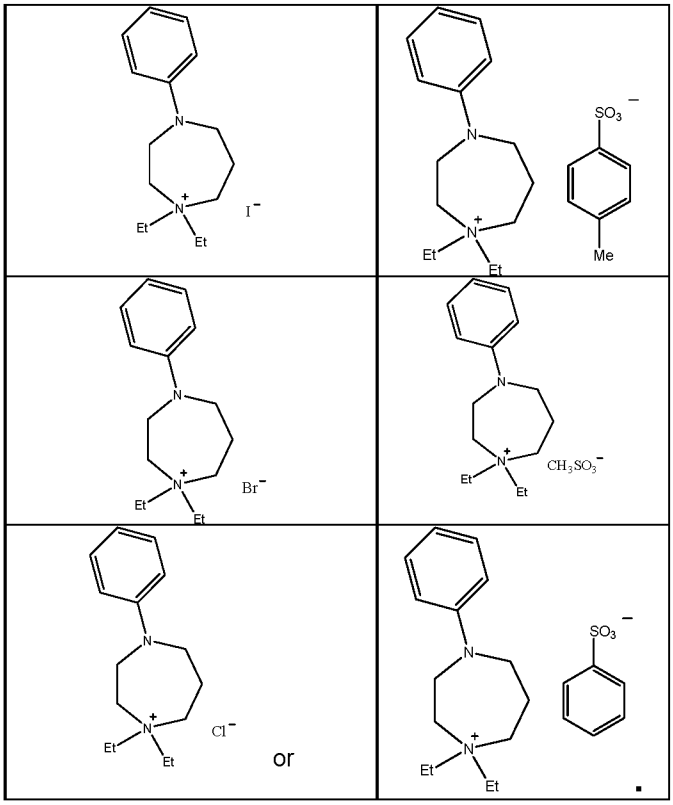
7. The method of any one of claims 1 to 6 wherein said one or more agents for treating or preventing the pulmonary diseases is a beta2 agonist.

8. The method of any one of claims 1 to 6 wherein said one or more agents is a muscarinic antagonist.

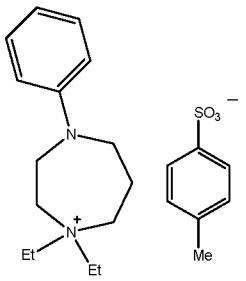
9. The method of any one of claims 1 to 6 wherein said one or more agents is a leukotriene modulator.

10. The method of any one of claims 1 to 6 wherein said one or more agents is a phosphodiesterase (PDE) inhibitor.

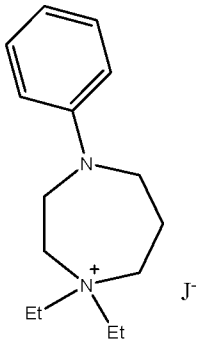
11. The method of any one of claims 1 to 10, wherein the homopiperazinium compound is



12. The method of any one of claims 1 to 10, wherein the homopiperazinium compound is of formula:



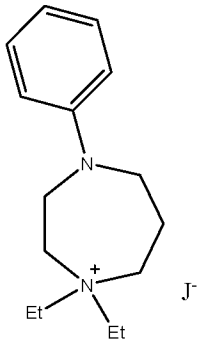
13. A pharmaceutical composition comprising an effective amount of a homopiperazinium compound of formula



wherein J is a counter ion, and a pharmaceutically acceptable carrier, and wherein said composition is for use in the treatment or prevention of pulmonary disease together with one or more agents for treating or preventing the pulmonary diseases: beta2 agonists, muscarinic antagonists, leukotriene modulators and phosphodiesterase (PDE) inhibitors or a combination thereof.

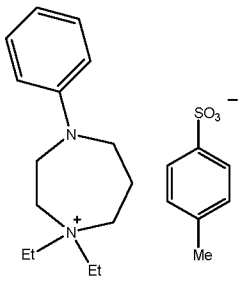
14. A pharmaceutical composition comprising an effective amount of i) a homopiperazinium compound as defined in claim 1 and ii) one or more agents for treating or preventing the pulmonary diseases: beta2 agonists, muscarinic antagonists, leukotriene modulators and phosphodiesterase (PDE) inhibitors or a combination thereof.

15. A compound of formula:



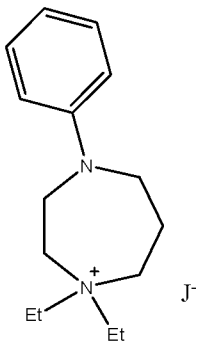
wherein J^- is I^-, Br^-, Cl^-, tosylate, mesylate or bezylate.

16. A compound of formula:



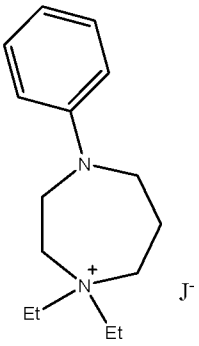
or a crystalline form thereof.

17. A method for treating a patient suffering from a disease selected from the group consisting of asthma, chronic obstructive pulmonary disease (COPD), interstitial pulmonary fibrosis (IPF), sarcoidosis, hypersensitivity pneumonitis (HP) and bronchiolitis obliterans with organizing pneumonitis (BOOP), said patient being desensitized to a treatment with one or more beta2 agonists, the method comprising administering a homopiperazinium having the formula:



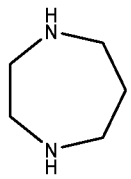
wherein J^- is a counter ion.

18. A process for preparing a homopiperazinium compound having the formula:



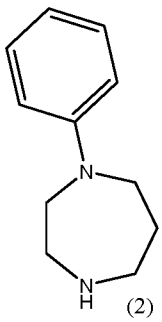
wherein J^- is a counter ion, said process comprising:

reacting homopiperazine of formula (1)



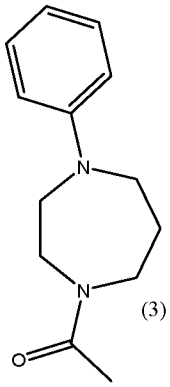
(1)

with a reagent of formula Ph-X in the presence of a Cu catalyst, wherein Ph is phenyl and X is an atom or group suitable for effecting a Cu catalyzed coupling, to produce compound (2)



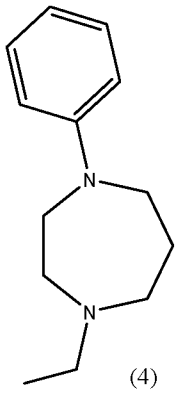
(2)

acylating compound (2) to produce compound (3)



(3)

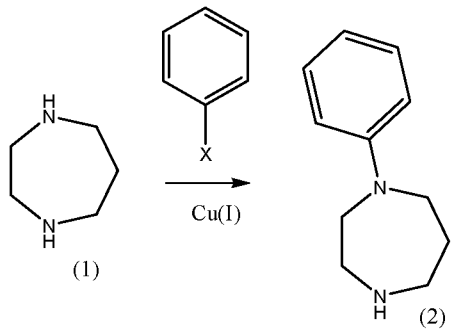
reducing compound (3) to compound of formula (4)



(4)

reacting compound (4) with a reagent of formula Et-J, wherein Et is ethyl and J is leaving group, to produce compound (5).

19. A process for preparing a compound of formula (2) comprising conducting the step defined in the following scheme:



(1) (2)

wherein Ph is phenyl and X is an atom or group suitable for effecting a Cu catalyzed coupling.

20. The compound of formula (2) as defined in claim 18.

21. The compound of formula (3) as defined in claim 18.

22. The compound of formula (4) as defined in claim 18.

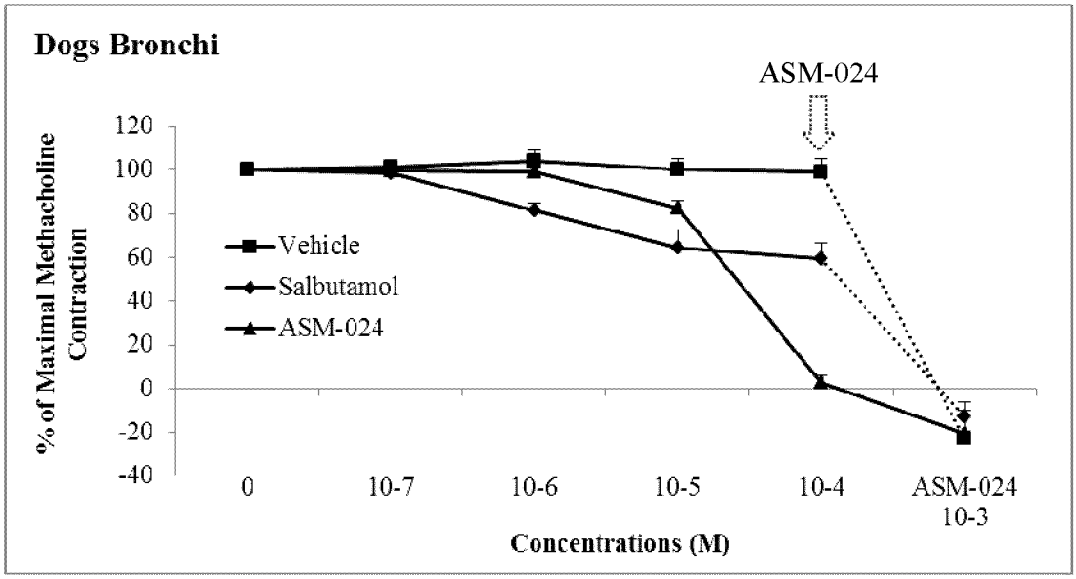

Dogs Bronchi
ASM-024
% of Maximal Methacholine Contraction
120
100
80
60
40
20
0
-20
-40
Vehicle
Salbutamol
ASM-024
0
10-7
10-6
10-5
10-4
ASM-024 10-3
Concentrations (M)

Fig. 1.

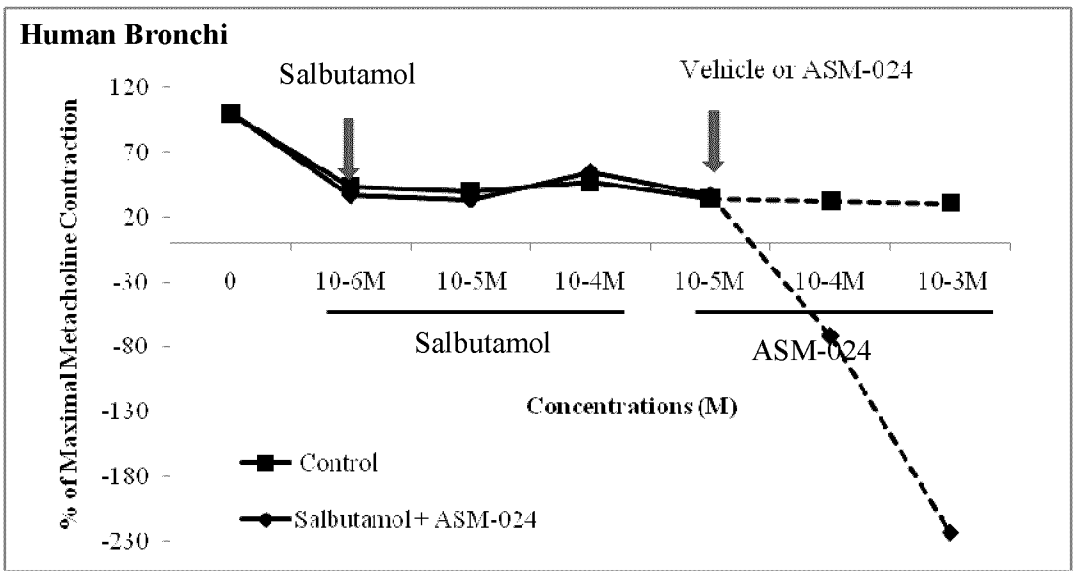

Human Bronchi
Salbutamol
Vehicle or ASM-024
% of Maximal Metacholine Contraction
120
70
20
-30
-80
-130
-180
-230
0
10-6M
10-5M
10-4M
10-5M
10-4M
10-3M
Salbutamol
ASM-024
Concentrations (M)
Control
Salbutamol+ ASM-024

Fig. 2

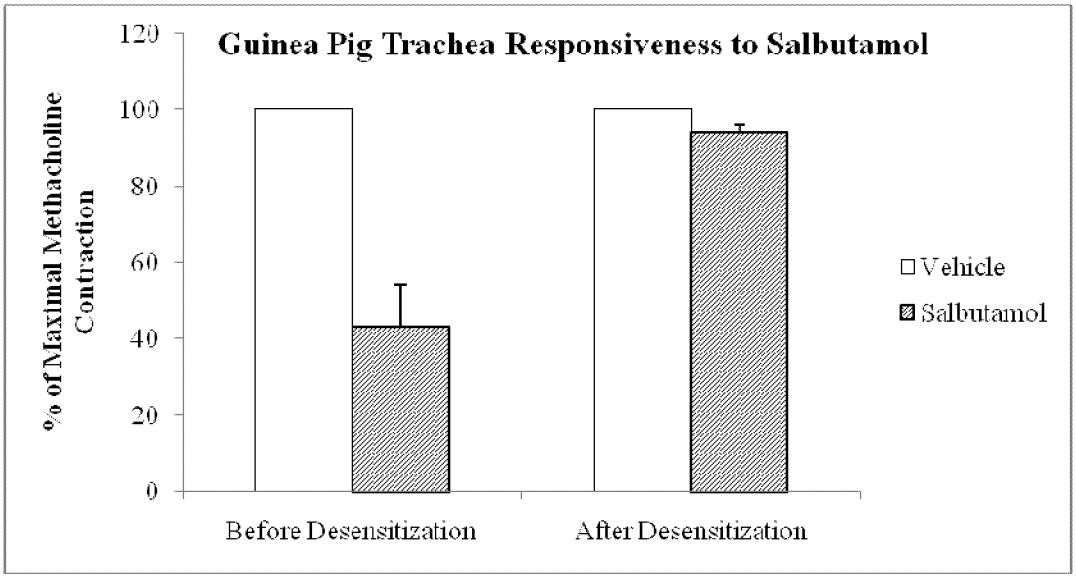

Guinea Pig Trachea Responsiveness to Salbutamol
% of Maximal Methacholine Contraction
120
100
80
60
40
20
0
Before Desensitization
After Desensitization
Vehicle
Salbutamol

Fig. 3

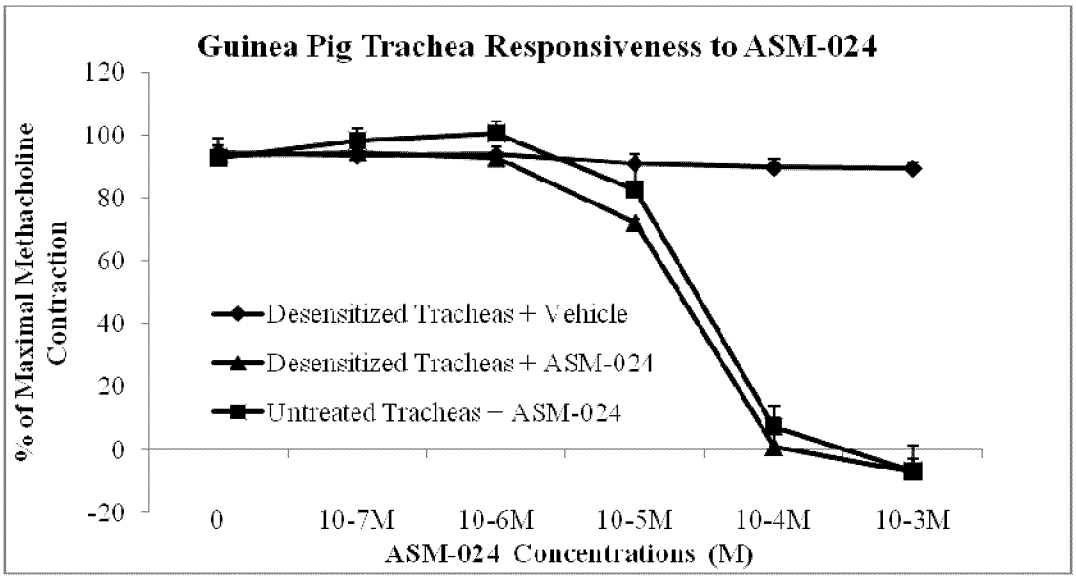

Guinea Pig Trachea Responsiveness to ASM-024
% of Maximal Methacholine Contraction
120
100
80
60
40
20
0
-20
Desensitized Tracheas + Vehicle
Desensitized Tracheas + ASM-024
Untreated Tracheas – ASM-024
0
10-7M
10-6M
10-5M
10-4M
10-3M
ASM-024 Concentrations (M)

Fig. 4

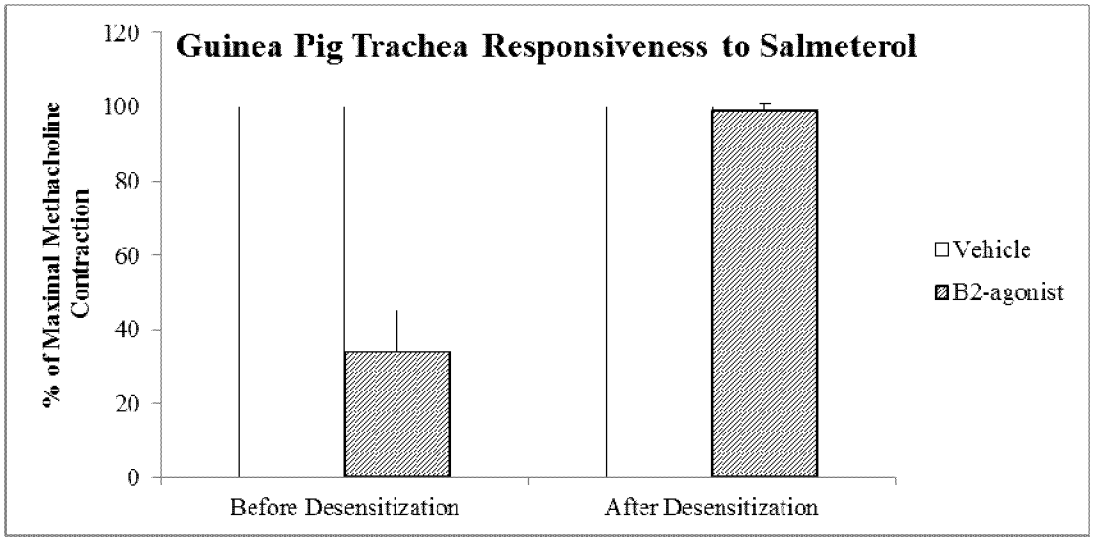

Guinea Pig Trachea Responsiveness to Salmeterol
% of Maximal Methacholine Contraction
120
100
80
60
40
20
0
Before Desensitization
After Desensitization
Vehicle
B2-agonist

Fig. 5

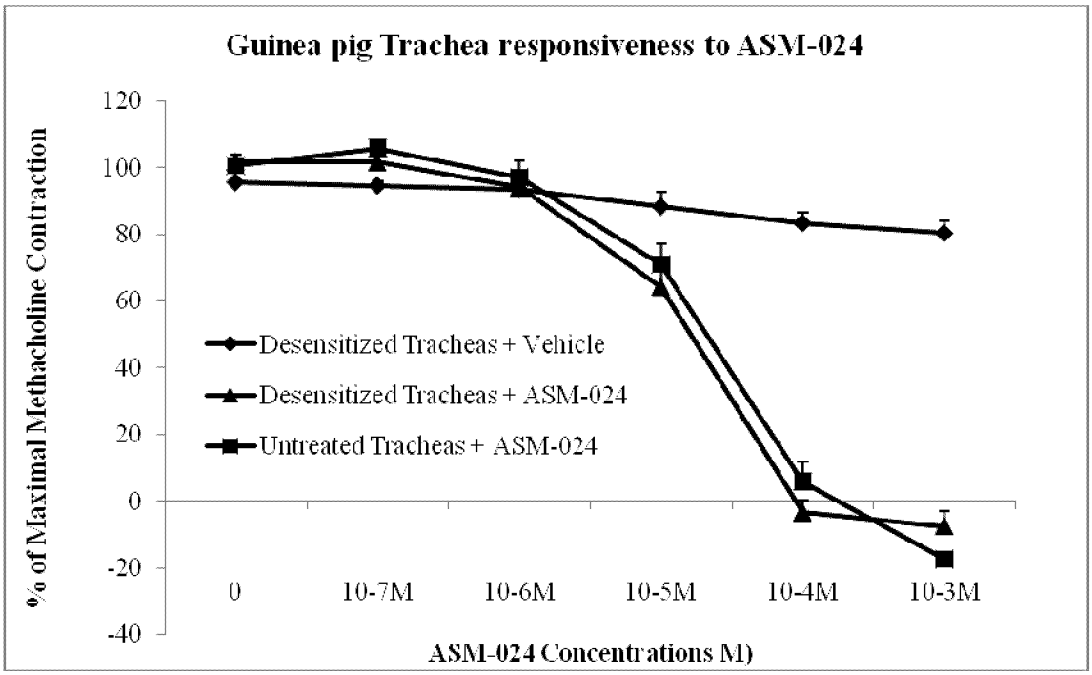

Guinea pig Trachea responsiveness to ASM-024
% of Maximal Methacholine Contraction
120
100
80
60
40
20
0
-20
-40
Desensitized Tracheas + Vehicle
Desensitized Tracheas + ASM-024
Untreated Tracheas + ASM-024
0
10-7M
10-6M
10-5M
10-4M
10-3M
ASM-024 Concentrations M)

Fig. 6

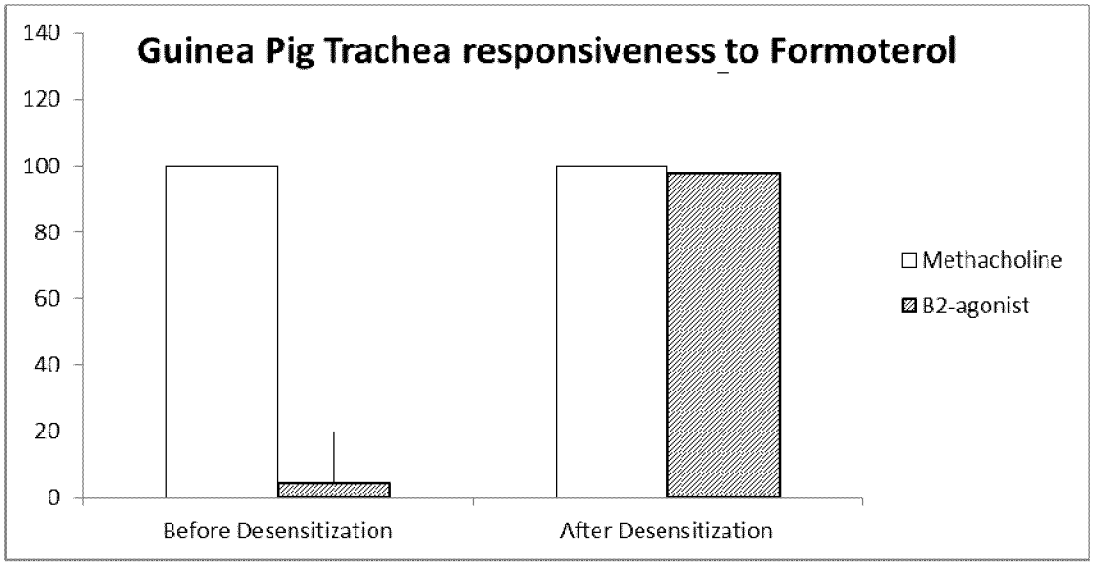

Guinea Pig Trachea responsiveness to Formoterol
140
120
100
80
60
40
20
0
Methacholine
B2-agonist
Before Desensitization
After Desensitization

Fig 7

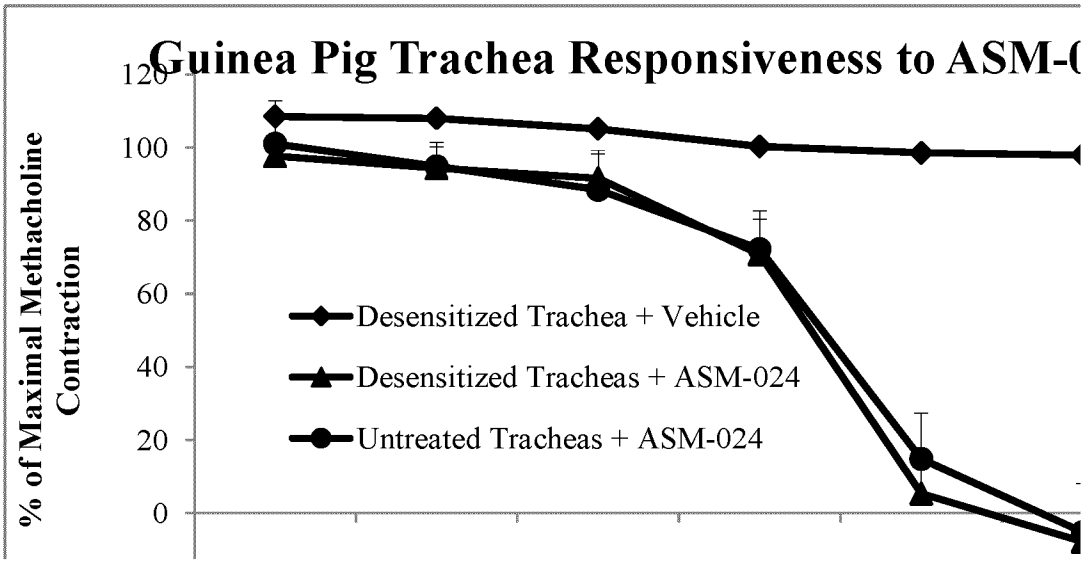

Guinea Pig Trachea Responsiveness to ASM-(
% of Maximal Methacholine Contraction
120
100
80
60
40
20
0
Desensitized Trachea + Vehicle
Desensitized Tracheas + ASM-024
Untreated Tracheas + ASM-024

Fig. 8

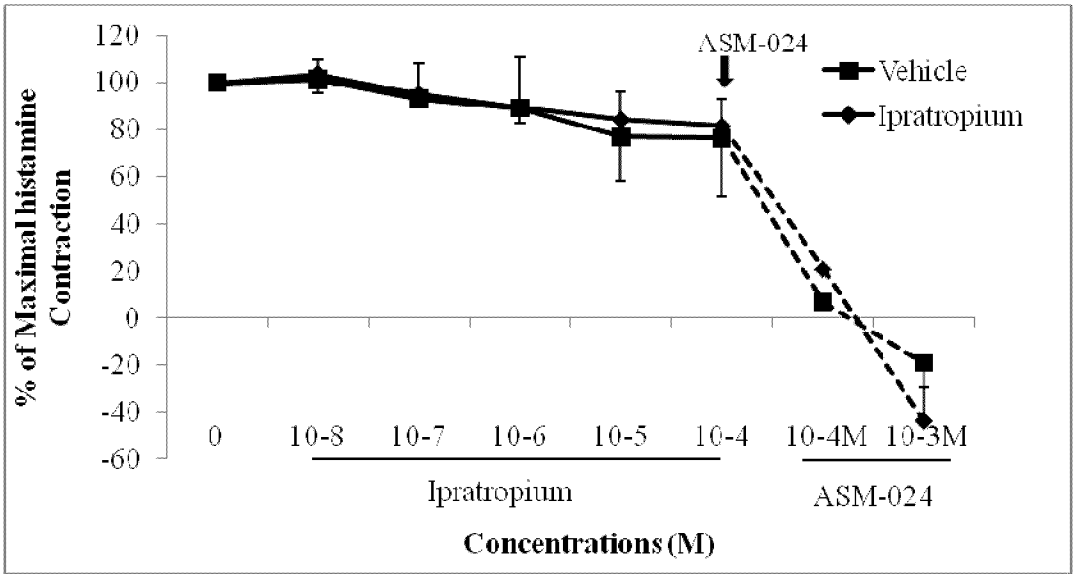

120
100
80
60
40
20
0
-20
-40
-60
% of Maximal histamine Contraction
ASM-024
Vehicle
Ipratropium
0
10-8
10-7
10-6
10-5
10-4
10-4M
10-3M
Ipratropium
ASM-024
Concentrations (M)

Fig. 9

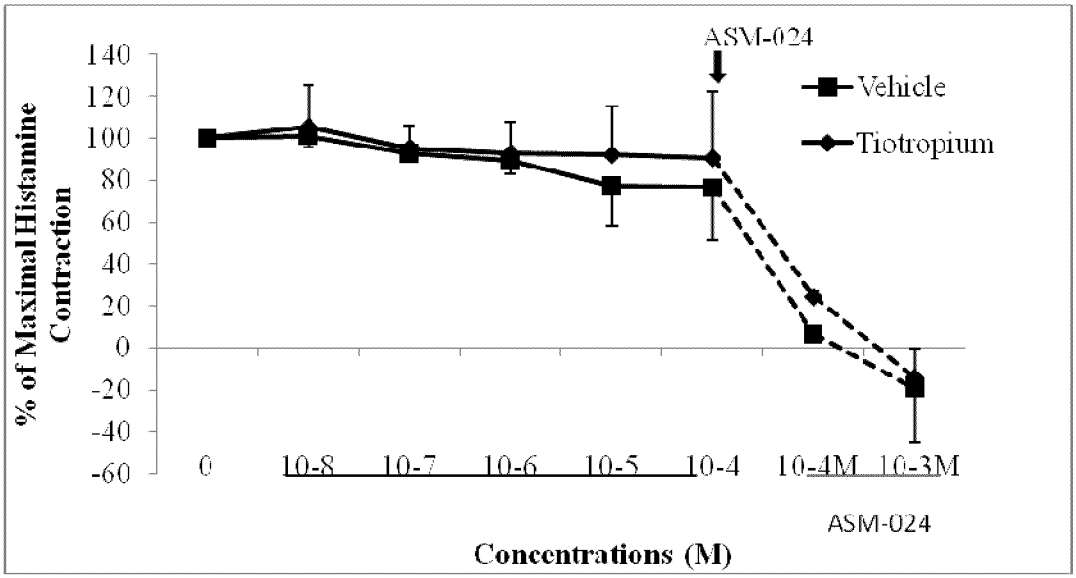

ASM-024
Vehicle
Tiotropium
% of Maximal Histamine Contraction
140
120
100
80
60
40
20
0
-20
-40
-60
0
10-8
10-7
10-6
10-5
10-4
10-4M
10-3M
ASM-024
Concentrations (M)

Fig. 10

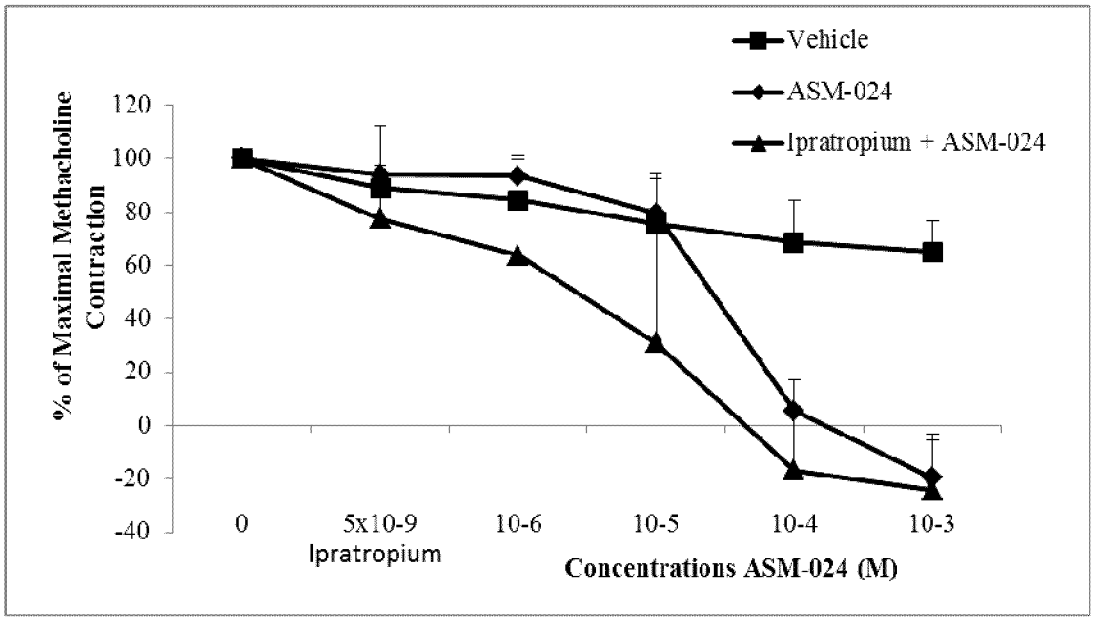

Vehicle
ASM-024
Ipratropium + ASM-024
% of Maximal Methacholine Contraction
120
100
80
60
40
20
0
-20
-40
0
5x10-9
Ipratropium
10-6
10-5
10-4
10-3
Concentrations ASM-024 (M)

Fig. 11

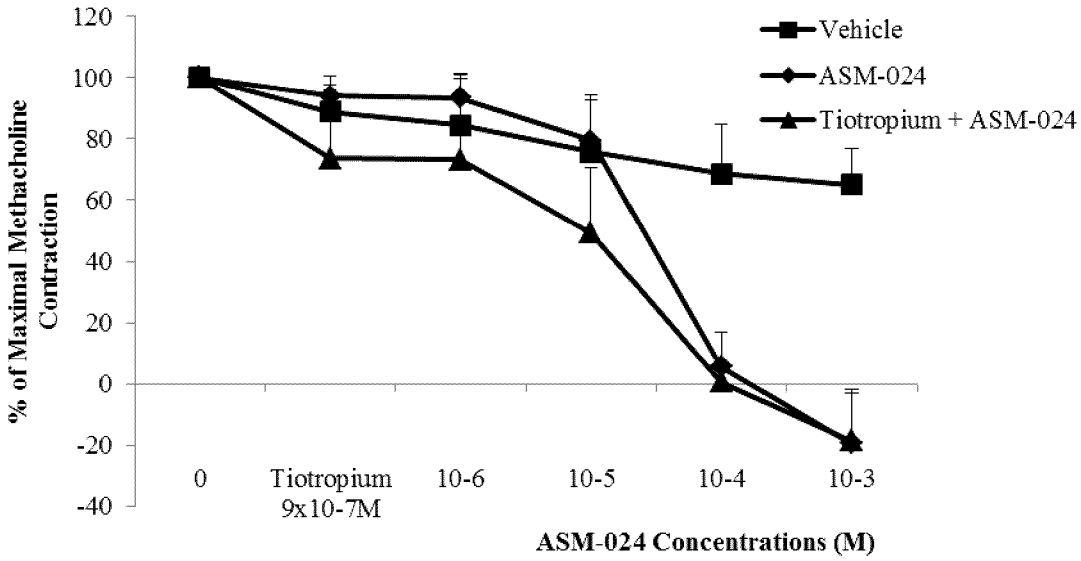
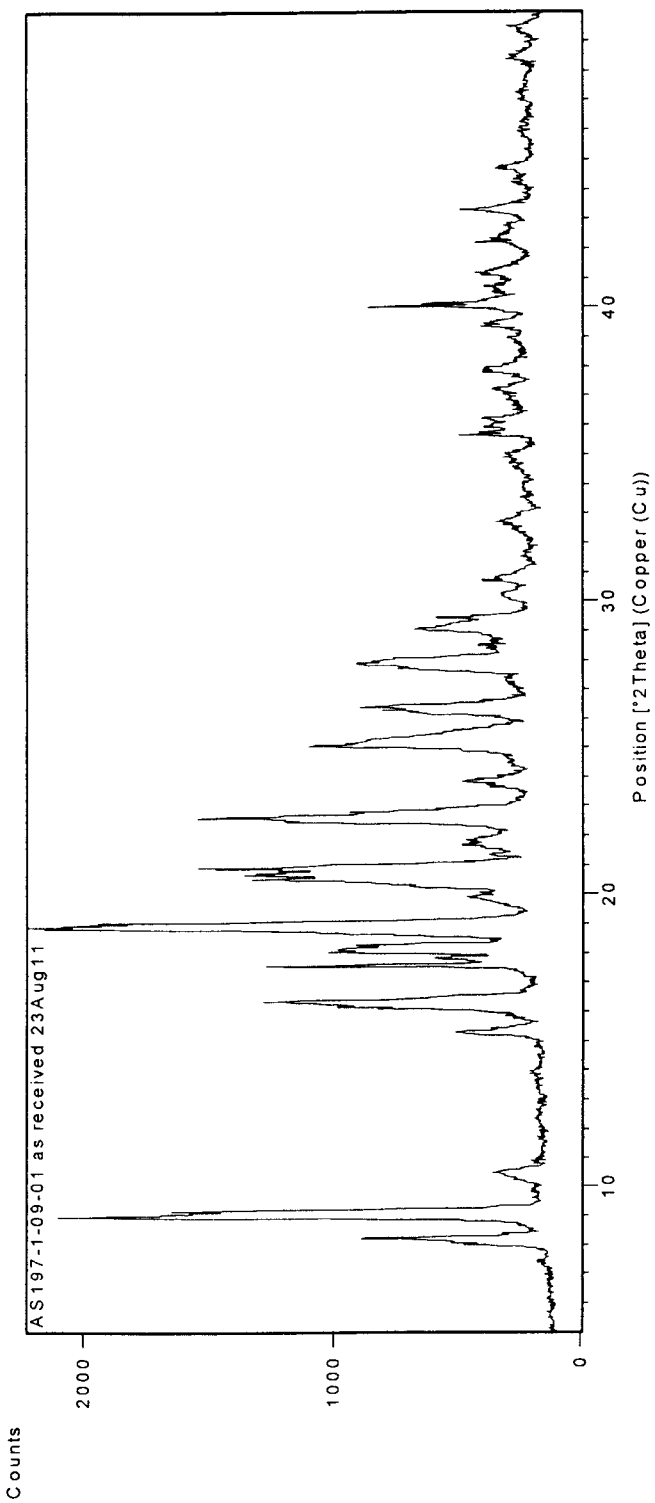

120
100
80
60
40
20
0
-20
-40
% of Maximal Methacholine Contraction
Vehicle
ASM-024
Tiotropium + ASM-024
0
Tiotropium 9x10-7M
10-6
10-5
10-4
10-3
ASM-024 Concentrations (M)

Fig. 12

Fig. 13


AS197-1-09-01 as received 23Aug11
Counts
2000
1000
0
10
20
30
40
Position [°2Theta] (Copper (Cu))

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2013/050559

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C07D 295/037*** (2006.01) , ***A61K 31/551*** (2006.01) , ***A61P 11/00*** (2006.01) , ***A61P 11/06*** (2006.01) , ***C07C 309/30*** (2006.01)

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: ***C07D 295/037*** (2006.01) , ***A61K 31/551*** (2006.01) , ***A61P 11/00*** (2006.01) , ***A61P 11/06*** (2006.01) , ***C07C 309/30*** (2006.01)

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

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

STN and Canadian Patent Database (keyword: piperazinium)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008/0221085 A1 (UNIVERSITE LAVAL) 11 September 2008 (11-09-2008) *see entire document, especially Paragraphs [87], [109], [140], [155], [239] and [240], claims 29, 33 and 46-50*	13-16 and 19
X	US 2005/0130990 A1 (UNIVERSITE LAVAL) 16 June 2005 (16-06-2005) *see entire document, especially Paragraphs [23] and [55], claims 3-7*	13-16
X	CA 2410237 C (SCHERING CO.) 06 December 2001 (06-12-2001) *see entire document, especially Preparation 5*	20
X	CA 2445159 C (MIT, USA) 31 October 2002 (31-10-2002) *see entire document, especially example 203 and claim 1*	19

[x] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 September 2013 (13-09-2013)	10 October 2013 (10-10-2013)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Ayub Reayi (819) 934-0481

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010/0331307 A1 (SALITURO ET AL.) 30 December 2010 (30-12-2010) *see entire document, especially Paragraph [0576]*	19

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 1-12 and 17

because they relate to subject matter not required to be searched by this Authority, namely :

Claims 1-12 and 17 are directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. However, this Authority has carried out a search based on the alleged effects or purposes/uses of the product defined in claims 15 and 16.

2. [ ] Claim Nos. :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

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

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

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

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

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.

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US2008221085A1	11 September 2008 (11-09-2008)	AT378051T	15 November 2007 (15-11-2007)
		AU2002249023B2	02 February 2006 (02-02-2006)
		AU2005262174A1	19 January 2006 (19-01-2006)
		AU2005262174A2	19 January 2006 (19-01-2006)
		AU2005262174B2	08 October 2009 (08-10-2009)
		AU2005262174C1	06 May 2010 (06-05-2010)
		BR0208305A	09 March 2004 (09-03-2004)
		BRPI0513305A	06 May 2008 (06-05-2008)
		CA2341952A1	23 September 2002 (23-09-2002)
		CA2441096A1	03 October 2002 (03-10-2002)
		CA2441096C	30 June 2009 (30-06-2009)
		CA2573977A1	19 January 2006 (19-01-2006)
		CA2573977C	10 April 2012 (10-04-2012)
		CN1498108A	19 May 2004 (19-05-2004)
		CN100528159C	19 August 2009 (19-08-2009)
		CN101001843A	18 July 2007 (18-07-2007)
		CN102872022A	16 January 2013 (16-01-2013)
		DE60223508D1	27 December 2007 (27-12-2007)
		DE60223508T2	25 September 2008 (25-09-2008)
		DK1370264T3	17 March 2008 (17-03-2008)
		EP1370264A2	17 December 2003 (17-12-2003)
		EP1370264B1	14 November 2007 (14-11-2007)
		EP1773779A1	18 April 2007 (18-04-2007)
		EP1773779A4	23 September 2009 (23-09-2009)
		ES2295331T3	16 April 2008 (16-04-2008)
		IL157757D0	28 March 2004 (28-03-2004)
		IL180721D0	03 June 2007 (03-06-2007)
		IL180721A	31 October 2012 (31-10-2012)
		JP2004523588A	05 August 2004 (05-08-2004)
		JP4377586B2	02 December 2009 (02-12-2009)
		JP2008505938A	28 February 2008 (28-02-2008)
		JP4783787B2	28 September 2011 (28-09-2011)
		KR20070085207A	27 August 2007 (27-08-2007)
		KR100860903B1	29 September 2008 (29-09-2008)
		MXPA03008423A	19 March 2004 (19-03-2004)
		MX2007000576A	25 June 2007 (25-06-2007)
		NO20034185D0	19 September 2003 (19-09-2003)
		NO20034185A	24 November 2003 (24-11-2003)
		NO331674B1	20 February 2012 (20-02-2012)
		NO20070789A	21 March 2007 (21-03-2007)
		NZ528042A	28 October 2005 (28-10-2005)
		NZ553211A	26 November 2010 (26-11-2010)
		PL365224A1	27 December 2004 (27-12-2004)
		PL207880B1	28 February 2011 (28-02-2011)
		RU2003131178A	10 April 2005 (10-04-2005)
		RU2299731C2	27 May 2007 (27-05-2007)
		RU2007105582A	20 August 2008 (20-08-2008)
		RU2414461C2	20 March 2011 (20-03-2011)
		US2004132737A1	08 July 2004 (08-07-2004)
		US7601720B2	13 October 2009 (13-10-2009)
		US8039459B2	18 October 2011 (18-10-2011)
		US2011245270A1	06 October 2011 (06-10-2011)
		US8377936B2	19 February 2013 (19-02-2013)
		US2005130990A1	16 June 2005 (16-06-2005)
		US2007249622A1	25 October 2007 (25-10-2007)
		US2010227871A1	09 September 2010 (09-09-2010)
		US2011301152A1	08 December 2011 (08-12-2011)
		US2012022049A1	26 January 2012 (26-01-2012)
		WO02076434A2	03 October 2002 (03-10-2002)
		WO02076434A3	17 July 2003 (17-07-2003)
		WO2006005195A1	19 January 2006 (19-01-2006)

*****Continued on next page*****

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US2005130990A1	16 June 2005 (16-06-2005)	AT378051T	15 November 2007 (15-11-2007)
		AU2002249023B2	02 February 2006 (02-02-2006)
		AU2005262174A1	19 January 2006 (19-01-2006)
		AU2005262174A2	19 January 2006 (19-01-2006)
		AU2005262174B2	08 October 2009 (08-10-2009)
		AU2005262174C1	06 May 2010 (06-05-2010)
		BR0208305A	09 March 2004 (09-03-2004)
		BRPI0513305A	06 May 2008 (06-05-2008)
		CA2341952A1	23 September 2002 (23-09-2002)
		CA2441096A1	03 October 2002 (03-10-2002)
		CA2441096C	30 June 2009 (30-06-2009)
		CA2573977A1	19 January 2006 (19-01-2006)
		CA2573977C	10 April 2012 (10-04-2012)
		CN1498108A	19 May 2004 (19-05-2004)
		CN100528159C	19 August 2009 (19-08-2009)
		CN101001843A	18 July 2007 (18-07-2007)
		CN102872022A	16 January 2013 (16-01-2013)
		DE60223508D1	27 December 2007 (27-12-2007)
		DE60223508T2	25 September 2008 (25-09-2008)
		DK1370264T3	17 March 2008 (17-03-2008)
		EP1370264A2	17 December 2003 (17-12-2003)
		EP1370264B1	14 November 2007 (14-11-2007)
		EP1773779A1	18 April 2007 (18-04-2007)
		EP1773779A4	23 September 2009 (23-09-2009)
		ES2295331T3	16 April 2008 (16-04-2008)
		IL157757D0	28 March 2004 (28-03-2004)
		IL180721D0	03 June 2007 (03-06-2007)
		IL180721A	31 October 2012 (31-10-2012)
		JP2004523588A	05 August 2004 (05-08-2004)
		JP4377586B2	02 December 2009 (02-12-2009)
		JP2008505938A	28 February 2008 (28-02-2008)
		JP4783787B2	28 September 2011 (28-09-2011)
		KR20070085207A	27 August 2007 (27-08-2007)
		KR100860903B1	29 September 2008 (29-09-2008)
		MXPA03008423A	19 March 2004 (19-03-2004)
		MX2007000576A	25 June 2007 (25-06-2007)
		NO20034185D0	19 September 2003 (19-09-2003)
		NO20034185A	24 November 2003 (24-11-2003)
		NO331674B1	20 February 2012 (20-02-2012)
		NO20070789A	21 March 2007 (21-03-2007)
		NZ528042A	28 October 2005 (28-10-2005)
		NZ553211A	26 November 2010 (26-11-2010)
		PL365224A1	27 December 2004 (27-12-2004)
		PL207880B1	28 February 2011 (28-02-2011)
		RU2003131178A	10 April 2005 (10-04-2005)
		RU2299731C2	27 May 2007 (27-05-2007)
		RU2007105582A	20 August 2008 (20-08-2008)
		RU2414461C2	20 March 2011 (20-03-2011)
		US2004132737A1	08 July 2004 (08-07-2004)
		US7601720B2	13 October 2009 (13-10-2009)
		US2008221085A1	11 September 2008 (11-09-2008)
		US8039459B2	18 October 2011 (18-10-2011)
		US2011245270A1	06 October 2011 (06-10-2011)
		US8377936B2	19 February 2013 (19-02-2013)
		US2007249622A1	25 October 2007 (25-10-2007)
		US2010227871A1	09 September 2010 (09-09-2010)
		US2011301152A1	08 December 2011 (08-12-2011)
		US2012022049A1	26 January 2012 (26-01-2012)
		WO02076434A2	03 October 2002 (03-10-2002)
		WO02076434A3	17 July 2003 (17-07-2003)
		WO2006005195A1	19 January 2006 (19-01-2006)

*****Continued on next page*****

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
CA2410237C	06 December 2001 (06-12-2001)	AR028621A1	14 May 2003 (14-05-2003)
		AT293627T	15 May 2005 (15-05-2005)
		AU6808901A	11 December 2001 (11-12-2001)
		AU2001268089B2	28 April 2005 (28-04-2005)
		AU2001268089C1	13 April 2006 (13-04-2006)
		BR0111015A	11 January 2005 (11-01-2005)
		CA2410237C	08 January 2008 (08-01-2008)
		CN1451007A	22 October 2003 (22-10-2003)
		CN1247588C	29 March 2006 (29-03-2006)
		CN1800186A	12 July 2006 (12-07-2006)
		CN100384847C	30 April 2008 (30-04-2008)
		CZ20023886A3	12 February 2003 (12-02-2003)
		CZ303790B6	09 May 2013 (09-05-2013)
		DE60110219D1	25 May 2005 (25-05-2005)
		DE60110219T2	09 March 2006 (09-03-2006)
		DK1283839T3	25 July 2005 (25-07-2005)
		ECSP024364A	31 March 2003 (31-03-2003)
		EP1283839A1	19 February 2003 (19-02-2003)
		EP1283839B1	20 April 2005 (20-04-2005)
		ES2237576T3	01 August 2005 (01-08-2005)
		HK1049007A1	16 September 2005 (16-09-2005)
		HU0600239A2	28 July 2006 (28-07-2006)
		HU0600239A3	30 June 2008 (30-06-2008)
		IL152726A	31 July 2012 (31-07-2012)
		IL152726D0	24 June 2003 (24-06-2003)
		IL220174D0	31 July 2012 (31-07-2012)
		JP2003535094A	25 November 2003 (25-11-2003)
		JP4574112B2	04 November 2010 (04-11-2010)
		JP2006219497A	24 August 2006 (24-08-2006)
		JP4938348B2	23 May 2012 (23-05-2012)
		JP2007145875A	14 June 2007 (14-06-2007)
		MXPA02011625A	27 March 2003 (27-03-2003)
		MY132006A	28 September 2007 (28-09-2007)
		NO20025651D0	25 November 2002 (25-11-2002)
		NO20025651A	23 January 2003 (23-01-2003)
		NO325008B1	14 January 2008 (14-01-2008)
		NZ522326A	31 March 2006 (31-03-2006)
		PE00622002A1	02 February 2002 (02-02-2002)
		PL360472A1	06 September 2004 (06-09-2004)
		PT1283839E	31 August 2005 (31-08-2005)
		RU2315053C2	20 January 2008 (20-01-2008)
		SI1283839T1	31 August 2005 (31-08-2005)
		SK16712002A3	05 August 2003 (05-08-2003)
		SK287748B6	04 August 2011 (04-08-2011)
		TWI288137B	11 October 2007 (11-10-2007)
		USRE44205E1	07 May 2013 (07-05-2013)
		US2002099061A1	25 July 2002 (25-07-2002)
		US6630475B2	07 October 2003 (07-10-2003)
		US2004023997A1	05 February 2004 (05-02-2004)
		US6897216B2	24 May 2005 (24-05-2005)
		US2005026932A1	03 February 2005 (03-02-2005)
		US7067655B2	27 June 2006 (27-06-2006)
		WO0192264A1	06 December 2001 (06-12-2001)
		ZA200208898A	01 March 2004 (01-03-2004)
CA2445159C	31 October 2002 (31-10-2002)	CA2445159A1	31 October 2002 (31-10-2002)
		CA2445159C	11 September 2012 (11-09-2012)
		CN1518534A	04 August 2004 (04-08-2004)
		CN1266112C	26 July 2006 (26-07-2006)
		CN1854128A	01 November 2006 (01-11-2006)
		CN1854128B	16 May 2012 (16-05-2012)
		EP1390340A1	25 February 2004 (25-02-2004)
		EP1390340A4	25 July 2007 (25-07-2007)
		EP2272813A2	12 January 2011 (12-01-2011)
		EP2272813A3	25 January 2012 (25-01-2012)
		EP2275395A2	19 January 2011 (19-01-2011)
		EP2275395A3	25 January 2012 (25-01-2012)
		JP2004536798A	09 December 2004 (09-12-2004)
		JP2009149606A	09 July 2009 (09-07-2009)