

Respiratory Disease Treatment

This invention relates to the use of the substantially pure 5R enantiomers of the known glitazone drug class, such as the known pharmaceutical products pioglitazone and rosiglitazone, for pulmonary administration by inhalation, for treatment of inflammatory respiratory diseases.

Background to the invention

A broad spectrum of respiratory diseases and disorders has been recognized, many of which have overlapping and interacting etiologies. Two of the most widespread and prevalent of these diseases are chronic obstructive pulmonary disease (COPD) and asthma. Respiratory diseases have a significant inflammatory component. For example, current therapy for COPD and severe asthma focuses mainly on the reduction of symptoms using short and long acting bronchodilators either as monotherapies or combinations of long acting β_2 agonist bronchodilators with inhaled corticosteroids (ICS). The disappointing anti-inflammatory data for ICS either alone or in combination with β_2 agonists has intensified the search for an effective anti-inflammatory drug for COPD. COPD is clearly a chronic inflammatory disorder that involves complex interactions between cells of the innate and acquired immune response both in the lung and potentially also systemically. One hypothesis under intense investigation is whether novel, demonstrably anti-inflammatory agents can halt or slow the functional decline characteristic of COPD. Reducing the frequency and severity of exacerbations has become an increasingly important target for COPD therapy as the prognosis for patients following exacerbations is poor. Anti-inflammatory therapy in COPD, and in asthma, is expected to reduce the frequency and severity of exacerbations. It is also desirable that decline in lung function and quality of life are also ameliorated with treatment.

Hence, new treatments for inflammatory respiratory diseases, including asthma, COPD, allergic airway syndrome, bronchitis, cystic fibrosis, emphysema and pulmonary fibrosis (including idiopathic pulmonary fibrosis), are constantly sought.

Peroxisome Proliferation Receptor gamma receptor (PPAR γ) agonists are a class of drugs which increase sensitivity to glucose in diabetic patients. Physiological activation of PPAR γ is believed to increase the sensitivity of peripheral tissues to insulin, thus facilitating the clearance of glucose from the blood and producing the desired anti-diabetic effect.

Many PPAR γ agonists are known from the patent and other literature, but currently only two are approved for clinical use in diabetes; Rosiglitazone and Pioglitazone. See Campbell *et al.*, *Curr Mol Med*. 2005 May; 5(3):349-63. Both of these compounds are thiazolidinediones ("TZDs" or "glitazones"), and are in practice administered by the oral route for systemic delivery.

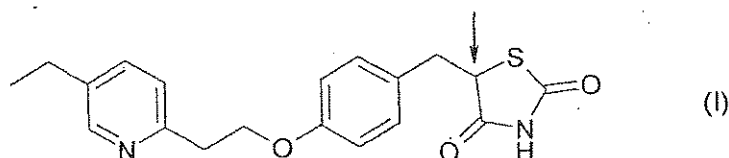
In addition to its effect on glucose metabolism, a variety of reports have been published which demonstrate that rosiglitazone also exerts anti-inflammatory effects. For instance, (i) rosiglitazone has been reported to exert effects in diabetic patients consistent with an anti-inflammatory effect (Haffner *et al.*, *Circulation*. 2002 Aug 6;106(6):679-84, Marx *et al.*, *Arterioscler. Thromb. Vasc. Biol*. 2003 Feb 1;23(2):283-8); (ii) Rosiglitazone has been reported to exert anti-inflammatory effects in a range of animal models of inflammation, including: carageenan-induced paw oedema (Cuzzocrea *et al.*, *Eur. J. Pharmacol*. 2004 Jan 1;483(1):79-93), TNBS-induced colitis (Desreumanux *et al.*, *J. Exp. Med*. 2001 Apr 2;193(7):827-38, Sanchez-Hidalgo *et al.*, *Biochem. Pharmacol*. 2005 Jun 15;69(12):1733-44), experimental encephalomyelitis (Feinstein *et al.*, *Ann. Neurol*. 2002 Jun;51(6):694-702) collagen-induced (Cuzzocrea *et al.*, *Arthritis Rheum*. 2003 Dec;48(12):3544-56) and adjuvant-induced arthritis (Shiojiri *et al.*, *Eur. J. Pharmacol*. 2002 Jul 19;448(2-3):231-8), carageenan-induced pleurisy (Cuzzocrea *et al.*, *Eur. J. Pharmacol*. 2004 Jan 1;483(1):79-93), ovalbumin-induced lung inflammation (Lee *et al.*, *FASEB J*. 2005 Jun;19(8):1033-5) and LPS-induced lung tissue neutrophilia (Birrell *et al.*, *Eur. Respir. J*. 2004 Jul;24(1):18-23) and (iii) rosiglitazone has been reported to exert anti-inflammatory effects in isolated cells, including iNOS expression in murine macrophages (Reddy *et al.*, *Am. J. Physiol. Lung Cell. Mol. Physiol*. 2004 Mar;286(3):L613-9), TNF α -induced MMP-9 activity in human bronchial epithelial cells (Hetzel *et al.*, *Thorax*. 2003 Sep;58(9):778-83), human airway smooth muscle cell proliferation (Ward *et al.*, *Br. J. Pharmacol*. 2004 Feb;141(3):517-25) and MMP-9 release by neutrophils (WO 0062766 – is this for year 2000?). PPAR γ agonists have also been shown to be effective in models of pulmonary fibrosis (Milam *et al.*, *Am. J. Physiol. Lung Cell. Mol. Physiol*, 2008, 294(5):L891-901) and pulmonary arterial hypertension (Crossno *et al.*, *Am. J. Physiol. Lung Cell. Mol. Physiol*, 2007, 292(4):L885-897).

Based on observations of anti-inflammatory activity in cells relevant to the lung, the utility of other PPAR γ agonists has been suggested for the treatment of inflammatory respiratory disorders including asthma, COPD, cystic fibrosis and pulmonary fibrosis. See WO0053601, WO0213812 and WO0062766. These suggestions include administration by both the systemic oral and pulmonary inhalation routes.

Unfortunately, PPAR γ agonists also have unwanted cardiovascular effects, including haemodilution, peripheral and pulmonary oedema and congestive heart failure (CHF). These effects are also believed to result from activation of PPAR γ . In particular, a significant effort has been devoted to investigating the hypothesis that PPAR γ agonists disturb the normal maintenance of fluid balance via binding to the PPAR γ receptor in the kidney. See Guan *et al*, *Nat. Med.* 2005;11(8):861-6 and Zhang *et. al.*, *Proc Natl. Acad. Sci. USA.* 2005 28;102(26):9406-11. Treatment with PPAR γ agonists by the oral route for systemic delivery is also associated with an unwanted increase in body weight.

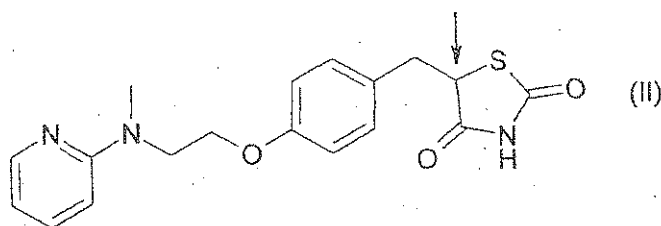
COPD patients are known to be at a higher risk than other clinical populations from congestive heart failure (CHF) (Curkendall *et al*, *Ann Epidemiol*, 2006;16: 63-70, Padeletti M *et al*, *Int J Cardiol.* 2008;125(2):209-15) and so it is important that systemic activation of the PPAR γ receptors is kept to a minimum in these patients to avoid increasing the likelihood of CHF being observed. Administering respiratory drugs by the inhaled route is one approach to target the lung with an anti-inflammatory agent whilst keeping systemic exposure of the drug low, thus reducing the likelihood of systemic activity and observation of side effects.

Pioglitazone has structural formula (I)



and can be named as 5-[4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl]-1,3-thiazolidine-2,4-dione. The carbon atom in the 5-position of the thiazolidine-dione ring of pioglitazone, indicated by an arrow in formula (I) above, is asymmetric, so pioglitazone has two enantiomers, the 5R and 5S enantiomers.

Rosiglitazone has the structural formula (II) and can be named as 5-[4-[2-[methyl (pyridin-2-yl)amino]ethoxy]benzyl]-1,3-thiazolidine-2,4-dione. The carbon atom in the 5-position of the thiazolidine-dione ring of rosiglitazone, indicated by an arrow in formula (II) below, is also asymmetric, so rosiglitazone also has two enantiomers, the 5R and 5S enantiomers.



The 5S enantiomer of rosiglitazone has a higher binding affinity for the PPAR γ receptor than the 5R enantiomer (30nM vs 2 μ M, Parks *et al.*, 1998, *Bioorg. Med. Chem. Lett.* 8(24):3657-8). For another member of the glitazone class, Rivoglitazone, the 5S enantiomer also has higher receptor binding affinity than the 5R enantiomer (see page 13 of WO2007100027).

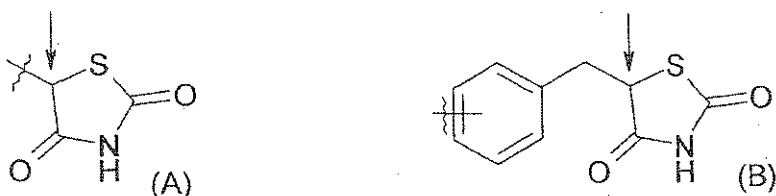
In practice, pioglitazone and rosiglitazone are administered for treatment of diabetes as a mixture of 5R and 5S enantiomers (a 1:1 racemic mixture) by the oral route for systemic delivery. The individual enantiomers of these compounds, and members of the glitazone family generally, are known to equilibrate rapidly *in vivo* after oral administration (see for example *J.Clin. Pharmacol.* 2007, 47, 323-33; *Rapid Commun. Mass Spectrom.* 2005, 19, 1125-9; *J. Chromatography*, 835 (2006), 40-46; *Biopharmaceutics and Drug Disposition* 1997, 18 (4), 305-24; *Chem. Pharm. Bull* 1984, 32, (11) 4460-65; *T. J. Med. Chem.* 1991, 34, 319-25) so there is no difference in practice between oral administration of either substantially pure isomer and oral administration of the racemic mixture. Specifically in relation to pioglitazone, it has been stated in a submission to the Federal Drug Administration (FDA) that there was no difference in activity following oral administration either of the racemate or the individual enantiomers in a rodent diabetes model

(www.fda.gov/medwatch/SAFETY/2007/Sep_PI/Actoplus_Met_PI.pdf):

"(Pioglitazone) contains one asymmetric carbon, and the compound is synthesized and used as the racemic mixture. The two enantiomers of pioglitazone interconvert in vivo. No differences were found in the pharmacologic activity between the two enantiomers".

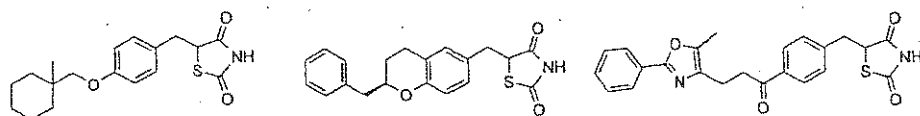
The effects of pulmonary inhalation of rosiglitazone or pioglitazone (or indeed any other glitazone) in either racemic or single enantiomer form do not appear to have been studied. It appears that nothing has been published concerning the potential equilibration of the 5R and 5S enantiomers of either compound, or any other glitazone, when contacted directly with lung tissue.

The glitazone class of PPAR γ agonists as a whole is characterised by the presence in the molecule of a thiazolidin-2,4-dione radical (A), often as part of a (thiazolidin-2,4,dione-5-yl)methylphenyl radical (B):



and the ring carbon atom indicated by the arrow is numbered as the 5-position of the thiazolidinone ring. The term "glitazone" as used herein refers to a PPAR γ agonist compound whose structure includes a thiazolidin-2,4-dione radical (A), or a (thiazolidin-2,4,dione-5-yl)methylphenyl radical (B):

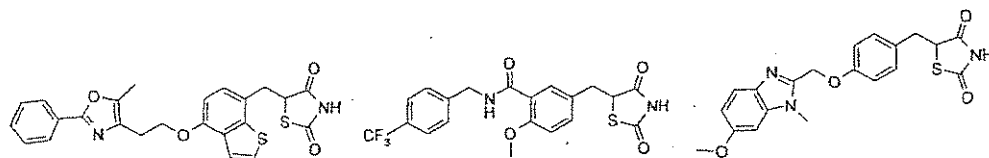
Besides the approved and marketed rosiglitazone and pioglitazone, there is a multitude of glitazones known from the patent and scientific literature. Known examples include the following:



Ciglitazone

Englitazone

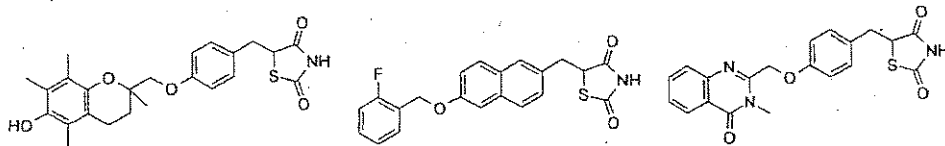
Darglitazone



Edaglitazone

MK-0767

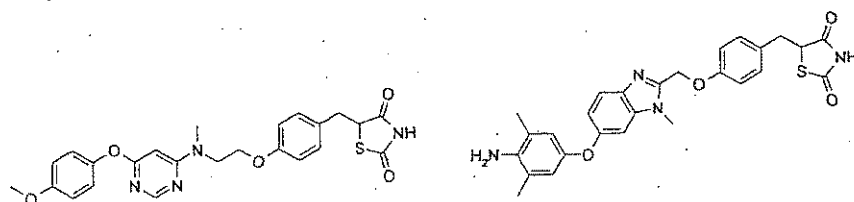
Rivoglitazone



Troglitazone

Netoglitazone

Balaglitazone



Lobeglitazone

Inoglitazone

Brief summary of the invention

This invention is based on the finding that, for treatment of inflammatory respiratory disease by inhalation, the 5R-enantiomer of a glitazone is more effective than the 5S enantiomer. Proof of principle derives from an animal model of treatment of inflammatory respiratory disease by inhalation, in which the 5R-enantiomers of pioglitazone and rosiglitazone have been shown to be active, whereas the 5S enantiomers were essentially inactive. This finding leads to the conclusion that inhaled pulmonary administration of the 5R enantiomer of a glitazone, in particular the 5R-enantiomer of pioglitazone or rosiglitazone, allows the anti-inflammatory effect of the compound to be achieved more efficiently than by similar administration of the racemate, with all the concomitant reduced side effect benefits of lower systemic exposure than oral administration.

In one aspect, there is provided a glitazone for use in the treatment of inflammatory respiratory disease by pulmonary administration by inhalation, wherein the glitazone inhaled consists of at least 95% by weight of the 5R configuration and less than 5% by weight of the 5S configuration, and wherein the said glitazone is pioglitazone or rosiglitazone.

In another aspect, there is provided the use of a glitazone in the preparation of a medicament for the treatment of inflammatory respiratory disease by pulmonary administration by inhalation wherein the glitazone content of the medicament consists of at least 95% by weight of the 5R configuration and less than 5% by weight of the 5S configuration, and wherein the said glitazone is pioglitazone or rosiglitazone.

In another aspect, there is provided a pharmaceutical composition adapted for pulmonary administration by inhalation, which composition comprises a glitazone and one or more pharmaceutically acceptable carriers and/or excipients, and wherein the glitazone content of the composition consists of at least 95% by weight of the 5R configuration and less than 5% by weight of the 5S configuration, and wherein the said glitazone is pioglitazone or rosiglitazone.

In another aspect, there is provided a kit for treatment of respiratory disorders in a subject, the kit comprising one dosage form comprising a composition as defined above and a second dosage form comprising another therapeutic agent selected from anti-inflammatory agents, bronchodilators, mucolytic agents, antitussive agents, leukotriene inhibitors, and antibiotics.

Brief description of the figures

Figure. 1 is a bar graph that illustrates the effect of intranasal administration to laboratory mice with vehicle (0.2% tween 80 in saline), 5S-pioglitazone (1 or 3 $\mu\text{g/kg}$), 5R-pioglitazone (1 or 3 $\mu\text{g/kg}$), or racemic pioglitazone (3 $\mu\text{g/kg}$) on the number of BAL cells induced by tobacco smoke 24 hours post the final exposure.

Figure. 2 is a bar graph that illustrates the effect of intranasal administration to laboratory mice with vehicle (0.2% tween 80 in saline), 5S-pioglitazone (1 or 3 $\mu\text{g/kg}$), 5R-pioglitazone (1 or 3 $\mu\text{g/kg}$), or racemic pioglitazone (3 $\mu\text{g/kg}$) on the number of BAL neutrophils induced by tobacco smoke 24 hours post the final exposure.

Figure. 3 is a bar graph that illustrates the effect of intranasal administration to laboratory mice with vehicle (0.2% tween 80 in saline), 5S-Rosiglitazone (3 or 10 $\mu\text{g/kg}$), 5R-Rosiglitazone (3 or 10 $\mu\text{g/kg}$), or racemic Rosiglitazone (10 $\mu\text{g/kg}$) on the number of BAL cells induced by tobacco smoke 24 hours post the final exposure.

Figure. 4 is a bar graph that illustrates the effect of intranasal administration to laboratory mice with vehicle (0.2% tween 80 in saline), 5S-Rosiglitazone (3 or 10 $\mu\text{g/kg}$), 5R-Rosiglitazone (3 or 10 $\mu\text{g/kg}$), or racemic Rosiglitazone (10 $\mu\text{g/kg}$) on the number of BAL neutrophils induced by tobacco smoke 24 hours post the final exposure.

Detailed description of the invention

As used herein, the term "glitazone" has the meaning ascribed to it above, i.e., a PPAR γ agonist compound whose structure includes a thiazolidin-2,4-dione radical (A), or a (thiazolidin-2,4-dione-5-yl)methylphenyl radical (B):

As used herein the term "pioglitazone" or "pioglitazone component" means the compound 5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione of formula (I) above, or a pharmaceutically acceptable salt thereof.

As used herein the term "rosiglitazone" or "rosiglitazone component" means the compound 5-{4-[2-[methyl (pyridin-2-yl)amino]ethoxy]benzyl}-1,3-thiazolidine-2,4-dione of formula (II) above, or a pharmaceutically acceptable salt thereof.

As used herein, the term "enantiomeric excess" or its abbreviation "e.e." is defined as the percentage:

$$((R-S)/(R+S)) \times 100 \text{ percent}$$

where R and S are the respective weight fractions of the R and S enantiomers in a sample. Thus for a glitazone sample containing 95% by weight of the 5R enantiomer and 5% of the 5S enantiomer, the enantiomeric excess of R over S enantiomer is $((95-5)/(95+5)) \times 100 = 90\%$.

In one aspect, the present invention provides a pharmaceutical composition adapted for pulmonary administration by inhalation, which composition comprises a glitazone, particularly pioglitazone or rosiglitazone, and one or more pharmaceutically acceptable carriers and/or excipients, wherein the glitazone content of the composition consists of at least 95% by weight of the 5R enantiomer and less than 5% of the 5S enantiomer.

In another aspect, the invention provides a glitazone, for example pioglitazone or rosiglitazone, for use in the treatment of inflammatory respiratory disease by pulmonary administration by inhalation, wherein the glitazone inhaled consists of at least 95% by weight of the 5R enantiomer and less than 5% of the 5S enantiomer.

In another aspect, the invention provides the use of a glitazone, for example pioglitazone or rosiglitazone, in the preparation of a medicament for the treatment of inflammatory respiratory disease by pulmonary administration by inhalation, wherein the glitazone content of the medicament consists of at least 95% by weight of the 5R enantiomer and less than 5% of the 5S enantiomer.

In another aspect, the invention provides a method of treatment of inflammatory respiratory disease comprising pulmonary administration of a therapeutically effective amount of a glitazone, for example pioglitazone or rosiglitazone, to a subject suffering such disease by inhalation, wherein the glitazone inhaled consists of at least 95% by weight of the 5R enantiomer and less than 5% of the 5S enantiomer.

In all aspects of the invention, the glitazone component, for example the pioglitazone or rosiglitazone component, may be inhaled via the nose or the mouth. Preferably it is inhaled via the mouth.

In all aspects of the invention, the glitazone component, for example pioglitazone or rosiglitazone, should preferably contain as little of the 5S enantiomer as possible. For example, the 5R enantiomer may constitute at least 97%, or at least 98%, or at least 99% by weight of the glitazone component.

In all aspects of the invention, the glitazone component, for example the pioglitazone or rosiglitazone component, may be accompanied by, or administered sequentially or concurrently with, one or more other therapeutic agents useful for the purpose of preventing and treating respiratory disorders, other than a PPAR γ agonist.

In all aspects of the invention, currently the most preferred glitazone component is pioglitazone.

In all aspects of the invention, the wherein the inflammatory respiratory disease may be selected from, for example, mild asthma, moderate asthma, severe asthma, steroid resistant asthma, bronchitis, chronic obstructive pulmonary disease (COPD), cystic fibrosis, pulmonary edema, pulmonary embolism, pneumonia, pulmonary sarcoidosis, silicosis, pulmonary fibrosis, respiratory failure, acute respiratory distress syndrome, emphysema, chronic bronchitis, tuberculosis, and lung cancer.

The glitazone component can be in the form of a pharmaceutically acceptable salt. The term "pharmaceutically acceptable salt" refers to salts prepared from pharmaceutically acceptable inorganic and organic acids and bases.

Pharmaceutically acceptable inorganic bases include metallic ions. More preferred metallic ions include, but are not limited to, appropriate alkali metal salts, alkaline earth metal salts and

other physiological acceptable metal ions. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, zinc, and the like and in their usual valences. Exemplary salts include aluminum, calcium, lithium, magnesium, potassium, sodium and zinc. Particularly preferred are the ammonium, calcium, magnesium, potassium, and sodium salts.

Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines; including in part, trimethylamine, diethylamine, N, N'-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine; substituted amines including naturally occurring substituted amines; cyclic amines; and quaternary ammonium cations. Examples of such bases include arginine, betaine, caffeine, choline, N,N-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine and the like.

Illustrative pharmaceutically acceptable acid addition salts of the compounds of the present invention can be prepared from the following acids, including, without limitation formic, acetic, propionic, benzoic, succinic, glycolic, gluconic, lactic, maleic, malic, tartaric, citric, nitric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, hydrochloric, hydrobromic, hydroiodic, isocitric, xinafoic, tartaric, trifluoroacetic, pamoic, propionic, anthranilic, mesylic, napadisylate, oxalacetic, oleic, stearic, salicylic, p-hydroxybenzoic, nicotinic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, phosphoric, phosphonic, ethanesulfonic, benzenesulfonic, pantothenic, toluenesulfonic, 2-hydroxyethanesulfonic, sulfanilic, sulfuric, salicylic, cyclohexylaminosulfonic, algenic, β -hydroxybutyric, galactaric and galacturonic acids. Exemplary pharmaceutically acceptable salts include the salts of hydrochloric acid and hydrobromic acid.

Compositions of the invention are useful for treatment of inflammatory respiratory disorders, for example asthma (mild, moderate or severe), e.g., bronchial, allergic, intrinsic, extrinsic, exercise-induced, drug-induced (including aspirin and NSAID-induced) and dust-induced asthma, steroid resistant asthma, bronchitis including infectious and eosinophilic bronchitis, chronic obstructive pulmonary disease (COPD), cystic fibrosis, pulmonary fibrosis including cryptogenic fibrosing alveolitis, idiopathic pulmonary fibrosis, idiopathic interstitial pneumonias, fibrosis complicating anti-neoplastic therapy and chronic infection, including

tuberculosis and aspergillosis and other fungal infections; complications of lung transplantation; vasculitic and thrombotic disorders of the lung vasculature, and pulmonary hypertension (including pulmonary arterial hypertension); antitussive activity including treatment of chronic cough associated with inflammatory and secretory conditions of the airways, and iatrogenic cough; acute and chronic rhinitis including rhinitis medicamentosa, and vasomotor rhinitis; perennial and seasonal allergic rhinitis including rhinitis nervosa (hay fever); nasal polyposis; acute viral infection including the common cold, and infection due to respiratory syncytial virus, influenza, coronavirus (including SARS) and adenovirus, pulmonary edema, pulmonary embolism, pneumonia, pulmonary sarcoidosis, silicosis, farmer's lung and related diseases; hypersensitivity pneumonitis, respiratory failure, acute respiratory distress syndrome, emphysema, chronic bronchitis, tuberculosis, and lung cancer. In particular, the methods and compositions of the present invention encompass the prevention and treatment of the respiratory disorder, COPD.

As used herein, the term "chronic obstructive pulmonary disease" or "COPD" refers to a set of physiological symptoms including chronic bronchitis, chronic cough, expectoration, exertional dyspnea and a significant, progressive reduction in airflow that may or may not be partly reversible. Emphysema may also be present in the lungs. COPD is a disease characterized by a progressive airflow limitation caused by an abnormal inflammatory reaction to the chronic inhalation of particles.

In subjects with the disorder, poor gas exchange in the lungs leads to decreased oxygen levels in the blood, increased levels of carbon dioxide and shortness of breath. Chronic airflow obstruction in COPD is complicated by the loss of lung elasticity resulting from enzymatic destruction of the lung parenchyma. Rather than a single pathologic condition, COPD is an umbrella term encompassing chronic obstructive bronchitis and emphysema.

Compositions suitable for administration by inhalation via the mouth or the nose are known, and may include carriers and/or diluents that are known for use in such compositions. The composition may contain 0.01-99% by weight of the pioglitazone or rosiglitazone component. Preferably, a unit dose comprises the pioglitazone or rosiglitazone component in an amount of 1µg to 50 mg.

The most suitable dosage level may be determined by any suitable method known to one skilled in the art. It will be understood, however, that the specific amount for any particular patient will depend upon a variety of factors, including the activity of the specific compound that is used, the age, body weight, diet, general health and sex of the patient, time of

administration, the route of administration, the rate of excretion, the use of any other drugs, and the severity of the disease undergoing treatment. Optimum dosages will be determined by clinical trial, as is required in the art.

Compositions of the invention may be used in combination with other therapeutic agents that are used in the treatment/prevention/suppression or amelioration of the diseases or conditions for which present compounds are useful. Such other therapeutic agents may be administered, by a route and in an amount commonly used therefore, contemporaneously or sequentially with the glitazone component, particularly the pioglitazone or rosiglitazone component. When a compound of the invention is used contemporaneously with one or more other therapeutic agents, a pharmaceutical composition containing such other therapeutic agents in addition to the pioglitazone component is preferred. Accordingly, the pharmaceutical compositions of the present invention include those that also contain one or more other active ingredients, in addition to the glitazone component, particularly the pioglitazone or rosiglitazone component.

Suitable therapeutic agents for a combination therapy with the glitazone compositions, particularly the pioglitazone or rosiglitazone compositions, of the invention include one or more other therapeutic agents selected from anti-inflammatory agents, bronchodilators, mucolytic agents, antitussive agents, leukotriene inhibitors, and antibiotics.

Suitable therapeutic agents for a combination therapy with the glitazone compositions, particularly the pioglitazone or rosiglitazone compositions, of the invention include: (1) a steroid drug such as a corticosteroid, for example beclomethasone, (e.g., as the mono or the dipropionate ester), flunisolide, fluticasone (e.g., as the propionate or furoate ester), ciclesonide, mometasone (e.g., as the furoate ester), mometasone desonide, rofleponide, hydrocortisone, prednisone, prednisolone, methyl prednisolone, naflocort, deflazacort, halopredone acetate, fluocinolone acetonide, fluocinonide, clocortolone, tipredane, prednicarbate, alclometasone dipropionate, halometasone, rimexolone, deprodone propionate, triamcinolone, betamethasone, fludrocortisone, desoxycorticosterone, etiprendnol dicloacetate and the like. Steroid drugs can additionally include steroids in clinical or pre-clinical development for respiratory diseases such as GW-685698, GW-799943, GSK 870086, QAE397, NCX-1010, NCX-1020, NO-dexamethasone, PL-2146, NS-126 (formerly ST-126) and compounds referred to in international patent applications WO0212265, WO0212266, WO02100879, WO03062259, WO03048181 and WO03042229. Steroid drugs can also additionally include next generation molecules in development with reduced side effect profiles such as selective glucocorticoid receptor agonists (SEGRAs), including ZK-216348

and compounds referred to in international patent applications WO-00032585, WO-000210143, WO-2005034939, WO-2005003098, WO-2005035518 and WO-2005035502 and functional equivalents and functional derivatives thereof; (2) a β 2-adrenoreceptor agonist, such as albuterol, bambuterol, terbutaline, fenoterol, formoterol, formoterol fumarate, salmeterol, salmeterol xinafoate, arformoterol, arformoterol tartrate, indacaterol (QAB-149), carmoterol, picumeterol, BI 1744 CL, GSK159797, GSK59790, GSK159802, GSK642444, GSK678007, GSK96108, clenbuterol, procaterol, bitolterol, and broxaterol, TA-2005 and also compounds of EP1440966, JP05025045, WO93/18007, WO99/64035, US2002/0055651, US2005/0133417, US2005/5159448, WO00/075114, WO01/42193, WO01/83462, WO02/66422, WO02/70490, WO02/76933, WO03/24439, WO03/42160, WO03/42164, WO03/72539, WO03/91204, WO03/99764, WO04/16578, WO04/016601, WO04/22547, WO04/32921, WO04/33412, WO04/37768, WO04/37773, WO04/37807, WO04/39762, WO04/39766, WO04/45618, WO04/46083, WO04/71388, WO04/80964, EP1460064, WO04/087142, WO04/89892, EP01477167, US2004/0242622, US2004/0229904, WO04/108675, WO04/108676, WO05/033121, WO05/040103, WO05/044787, WO04/071388, WO05/058299, WO05/058867, WO05/065650, WO05/066140, WO05/070908, WO05/092840, WO05/092841, WO05/092860, WO05/092887, WO05/092861, WO05/090288, WO05/092087, WO05/080324, WO05/080313, US20050182091, US20050171147, WO05/092870, WO05/077361, DE10258695, WO05/111002, WO05/111005, WO05/110990, US2005/0272769 WO05/110359, WO05/121065, US2006/0019991, WO06/016245, WO06/014704, WO06/031556, WO06/032627, US2006/0106075, US2006/0106213, WO06/051373, WO06/056471, WO08/096112, WO08/104790, WO08/096119, WO08/096112; (3) a leukotriene modulator, for example, montelukast or pranlukast; (4) anticholinergic agents, for example, selective muscarinic-3 (M3) receptor antagonists such as ipratropium bromide, tiotropium, tiotropium bromide (Spiriva®), glycopyrrolate, NVA237, LAS34273, GSK656398, GSK233705, GSK 573719, LAS35201, QAT370 and oxytropium bromide; (5) phosphodiesterase-IV (PDE-IV) inhibitors, for example, roflumilast or cilomilast; (6) an antitussive agent, such as codeine or dexamorphan; (7) a non-steroidal anti-inflammatory agent (NSAID), for example, ibuprofen or ketoprofen; (8) a mucolytic, for example, N acetyl cysteine or fudostein; (9) a expectorant/mucokinetic modulator, for example, ambroxol, hypertonic solutions (e.g., saline or mannitol) or surfactant; (10) a peptide mucolytic, for example, recombinant human deoxyribonuclease I (dornase-alfa and rhDNase) or helcidin; (11) antibiotics, for example, azithromycin, tobramycin and aztreonam; and (12) p38 MAP kinase inhibitors such as GSK 856553 and GSK 681323.

In one aspect, the invention provides for the use of inhaled administration of the glitazone compositions, particularly the pioglitazone or rosiglitazone compositions, of the invention in combination with other anti-inflammatory drugs and bronchodilator drug combinations (i.e. triple combination product), including but not limited to salmeterol xinafoate/fluticasone propionate (Advair/Seretide®), formoterol fumarate/budesonide (Symbicort®), formoterol fumarate/mometasone furoate, formoterol fumarate/beclometasone dipropionate (Foster®), formoterol fumarate/fluticasone propionate (FlutiForm®), Indacaterol/mometasone furoate, Indacaterol/QAE-397, GSK159797/GSK 685698, GSK159802/GSK 685698, GSK642444/GSK 685698, formoterol fumarate/ciclesonide, arformoterol tartrate/ciclesonide.

In another aspect, the invention provides for the use of inhaled administration of the glitazone compositions, particularly the pioglitazone or rosiglitazone compositions, of the invention in combination with other bronchodilator drug combinations, particularly B2 agonist/M3 antagonist combinations (i.e. triple combination product), including but not limited to salmeterol xinafoate/tiotropium bromide, formoterol fumarate/tiotropium bromide, BI 1744 CL/tiotropium bromide, indacaterol/NVA237, indacaterol/QAT-370, formoterol/ LAS34273, GSK159797/GSK 573719, GSK159802/GSK 573719, GSK642444/GSK 573719, GSK159797/GSK 233705, GSK159802/GSK 233705, GSK642444/GSK 233705, and compounds which possess both $\beta 2$ agonist and M3 antagonist activity in the same molecule (dual functionality) such as GSK 961081.

Thus in another aspect, the invention provides a kit for treatment of respiratory disorders in a subject, the kit comprising one dosage form comprising a composition adapted for pulmonary administration by inhalation, which composition comprises a glitazone, particularly pioglitazone or rosiglitazone, and one or more pharmaceutically acceptable carriers and/or excipients, wherein the glitazone content of the composition consists of at least 95% by weight of the 5R enantiomer and less than 5% of the 5S enantiomer, and a second dosage form comprising another therapeutic agent, for example as discussed above, selected from anti-inflammatory agents, bronchodilators, mucolytic agents, antitussive agents, leukotriene inhibitors, and antibiotics.

For delivery by inhalation, the active compound is preferably in the form of microparticles. These may be prepared by a variety of techniques, including spray-drying, freeze-drying and micronisation. Following size reduction to produce microparticles, particle size distribution (PSD) of the compound is examined and generally described in the art by specifying d10, d50 and d90 values. The average particle size, i.e. the average equivalent diameter, is defined as the diameter where 50 mass-% (of the particles) of the powder have a larger equivalent

diameter, and the other 50 mass-% have a smaller equivalent diameter. Hence the average particle size is denoted as equivalent d50. For inhaled use a d50 of less than 10 microns, preferably less than 5 microns is desired.

By way of example, a composition of the invention may be prepared as a suspension for delivery from a nebuliser or as an aerosol in a liquid propellant, for example for use in a pressurised metered dose inhaler (PMDI). Propellants suitable for use in a PMDI are known to the skilled person, and include CFC-12, HFA-134a, HFA-227, HCFC-22 (CCl_2F_2) and HFA-152 (CH_4F_2 and isobutane).

In a preferred embodiment of the invention, a composition of the invention is in dry powder form, for delivery using a dry powder inhaler (DPI). Many types of DPI are known.

Microparticles for delivery by inhalation may be formulated with excipients that aid delivery and release. For example, in a dry powder formulation, microparticles may be formulated with large carrier particles that aid flow from the DPI into the lung. Suitable carrier particles are known, and include lactose particles; they may have a mass median aerodynamic diameter of greater than 90 μm .

Aerosol generation can be carried out using, for example, pressure-driven jet atomizers or ultrasonic atomizers, preferably using propellant-driven metered aerosols or propellant-free administration of micronized active compounds from, for example, inhalation capsules or other "dry powder" delivery systems.

The compositions may be dosed as described depending on the inhaler system used. In addition to the active compounds, the administration forms may additionally contain excipients, such as, for example, propellants (e.g., Frigen in the case of metered aerosols), surface-active substances, emulsifiers, stabilizers, preservatives, flavourings, fillers (e.g. lactose in the case of powder inhalers) or, if appropriate, further active compounds.

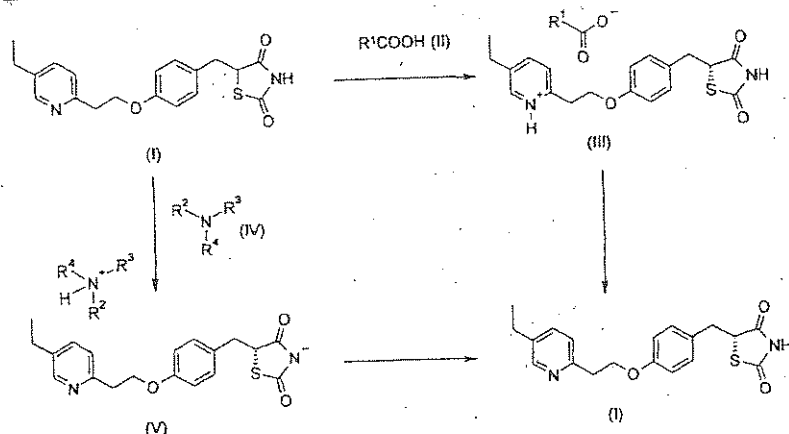
For the purposes of inhalation, a large number of systems are available with which aerosols of optimum particle size can be generated and administered, using an inhalation technique which is appropriate for the patient. In addition to the use of adaptors (spacers, expanders) and pear-shaped containers (e.g. Nebulator®, Volumatic®), and automatic devices emitting a puffer spray (Autohaler®), for metered aerosols, in particular in the case of powder inhalers, a number of technical solutions are available (e.g. Diskhaler®, Rotadisk®, Turbohaler® or the inhalers for example as described in EP-A-0505321).

Methods of preparation of Glitazone Enantiomers

Glitazones can be separated using chiral HPLC (see for example Methods 1-3 and 5-7 below). Chiral columns include CHIRALPAK AD, AD-H, AS-V, 50801, IA, IC, OD, OF, OG, OJ, OK, and OZ. Preferred chiral columns for HPLC are CHIRALPAK AD-H and CHIRALPAK IA using elution with ethanol and varying portions of TFA, preferably 0.05-0.2% TFA.

An alternative method of resolution, using pioglitazone as an example, is shown in Scheme 2 (see also Example 10).

Scheme 2



Recent review articles on optical resolution methods: E. Fogassy *et. al.*, *Tetrahedron: asymmetry*, **2008**, 19, 519-536; S.H.Wilen, *Topics in Stereochemistry*, Wiley-Interscience: NY, 1972, 6, 107 Eds. E.L. Eliel, N.L. Allinger; P.Newman, *Optical resolution Procedures of Chiral Compounds* 1-3, Resolution Information Center, NY, 1978-1984; J.Jaques, S.H.Wilen, A. Collett, *Enantiomers Racemates and Resolutions*, Wiley-Interscience: NY, 1991; R.A. Sheldon, *Chirotechnology*, Marcel Dekker, NY, 1993; *Optical Resolutions via diastereomeric salt formation*, CRC Press, 2002, Ed. David Kozma.

Commonly used chiral acid resolving agents include, without limitation, dibenzoyl tartaric acid, dianisoyl tartaric acid, ditoluyl tartaric acid, tartaric acid, phencyphos, chlocyphos, anicyphos, 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate, camphorsulfonic acid, bromo camphorsulfonic acid, camphoric acid, phenylethylsulfonic acid, malic acid, mandelic acid, 4-bromomandelic acid, 4-methylmandelic acid, lactic acid and chalcone sulfonic acids.

A more preferred set of chiral acid resolving agents include (-)-O,O'-dibenzoyl-L-tartaric acid (anhydrous), (-)-O,O'-dibenzoyl-L-tartaric acid monohydrate, (-)-di-O-p-toluoyl-L-tartaric acid, L-(+)-tartaric acid and (-)-di-p-anisoyl-L-tartaric acid.

The most preferred chiral acid resolving agent is (-)-O,O'-dibenzoyl-L-tartaric acid (anhydrous).

Chiral acid resolving agents can be used in different stoichiometries to effect resolutions. The above resolving agents can be used in a ratio of 1 part (I) to 10 parts of chiral acids (II), more preferably in a ratio of 1 part (I) to 5 parts of chiral acids (II), and most preferably in a ratio of 1 part (I) to 2 part of chiral acids (II). Even more preferred is a ratio of 1 part (I) to 1 part of chiral acids (II).

A variety of solvents may be useful to form diastereomeric salts (III) with chiral acids (II). Preferred solvents will include ethyl acetate, dichloromethane, tetrahydrofuran, 1,4-dioxane, acetone, acetonitrile, MeOH, EtOH, IPA, 1-propanol, 1,2-dimethoxyethane, diethyl ether, dichloroethane, *tert*-butylmethyl ether, 1-butanol, 2-butanol, *t*-butanol, 2-butanone, toluene, cyclohexane, heptane, hexane, H₂O, DMF, petroleum ethers and CHCl₃.

The most preferred solvent is acetonitrile.

Solvents may be used in combination with each other and preferred combinations include 10% HCl in H₂O, 10% H₂O in DMF, 10% H₂O in EtOH, 10% H₂O in IPA, methanol and water in varying portions, methanol and water in varying portions with HCl, CHCl₃ and ethyl acetate in varying portions, EtOH and water in varying portions, IPA and water in varying portions, 1-propanol and water in varying portions and CHCl₃ and dioxane in varying portions.

More preferred solvent combinations include CHCl₃ and dioxane in varying portions, CHCl₃ and ethyl acetate in varying portions, methanol and water in varying portions with HCl and methanol and water in varying portions.

Commonly used chiral amine resolving agents include, without limitation, 1-phenylethyl amine, cinchonidine, cinchonine, quinidine, codeine, morphine, strychnine, brucine, quinine, ephedrine, amino butanol, dehydroabiethylamine, 2-phenyl glycinol, 1,2-cyclohexyldiamine, 1-naphthylethylamine, 2-amino-1-phenylpropane-1,3-diol, 4-chloro-1-phenylamine, N-(4-methoxybenzyl)-1-phenylethyl amine, fenchylamine, N-benzyl-1-phenylethyl amine, N-(4-

dimethylaminobenzyl)-1-phenylethyl amine, 3-amino-2,2-dimethyl-3-phenylpropan-1-ol, sparteine, proline, serine, phenylalanine, lysine, threonine, valine, histidine, alanine, glutamic acid and glutamine, arginine, homo-arginine, N- α -acetyl lysine, N- ϵ -acetyllysine and ornithine.

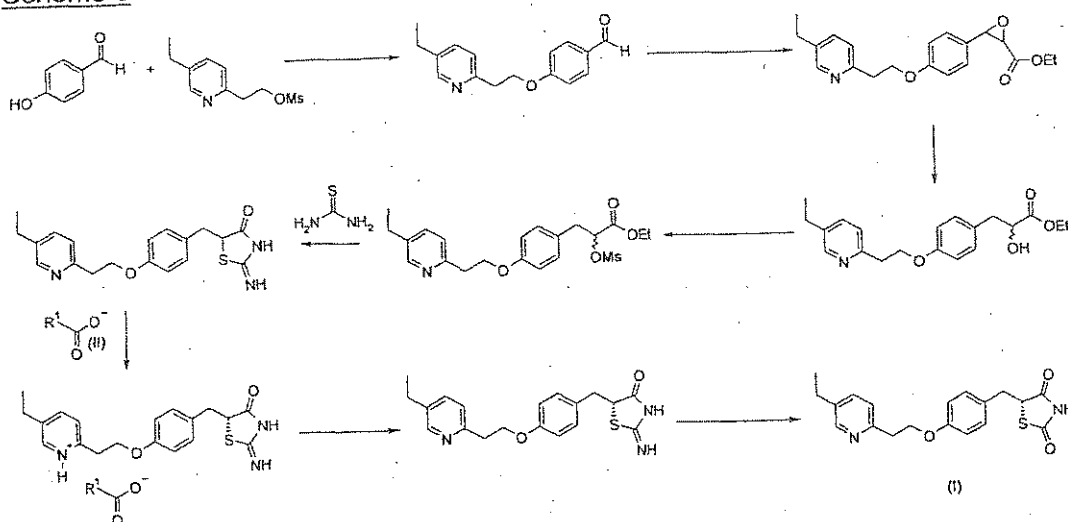
The most preferred chiral amine resolving agent is L-lysine.

Chiral amine resolving agents can be used in different stoichiometries to effect resolutions. The above resolving agents can be used in a ratio of 1 part (I) to 10 parts of chiral amine (IV), more preferably in a ratio of 1 part (I) to 5 parts of chiral amine (IV), and most preferably in a ratio of 1 part (I) to 1 part of chiral amine (IV).

A variety of solvents may be useful to form diastereomeric salts (V) with chiral amines (IV). Preferred solvents will include ethyl acetate, dichloromethane, tetrahydrofuran, 1,4-dioxane, acetone, acetonitrile, MeOH, EtOH, IPA, 1-propanol, 1,2-dimethoxyethane, diethyl ether, dichloroethane, *tert*-butylmethyl ether, 1-butanol, 2-butanol, *t*-butanol, 2-butanone, toluene, cyclohexane, heptane, hexane, H₂O, DMF, petroleum ethers and CHCl₃.

References K. Akiba *et. al. Bull. Chem. Soc. Jpn.*, 1974, 47(4), 935-937; R.A. Bartsch *et. al., JACS*, 2001, 123(31), 7479 describe the decomposition of heterocyclic nitrosimines and may be applicable to the synthesis and resolution of glitazones. Therefore, an alternative method of synthesis/resolution, using pioglitazone as an example may be as shown in Scheme 3:

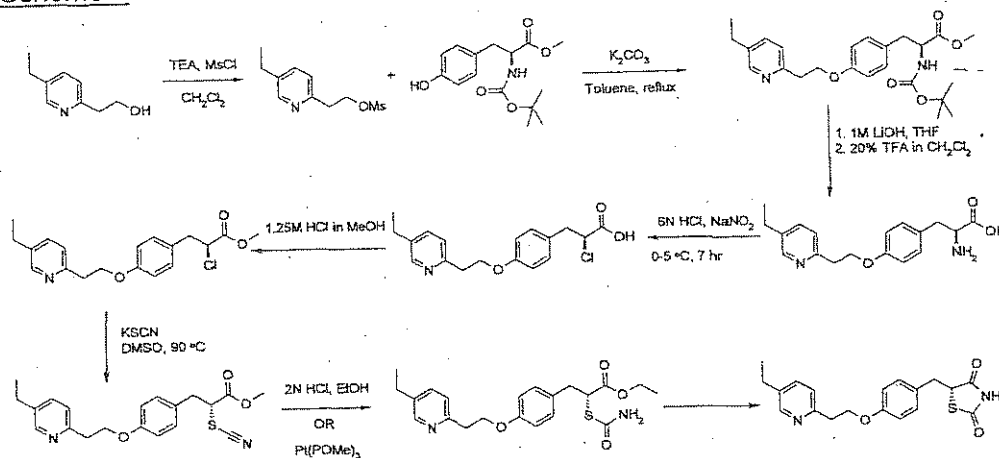
Scheme 3



The final product may of course be recrystallised to increase enantiomeric purity.

Reference T. Sohda *et al.*, *Chem. Pharm. Bull.*, **1984**, 32(11), 4460-5 describes the chiral synthesis of related compounds and may be applicable to the synthesis of other glitazones. Therefore, an alternative method of synthesis, using pioglitazone as an example may be as shown in Scheme 4:

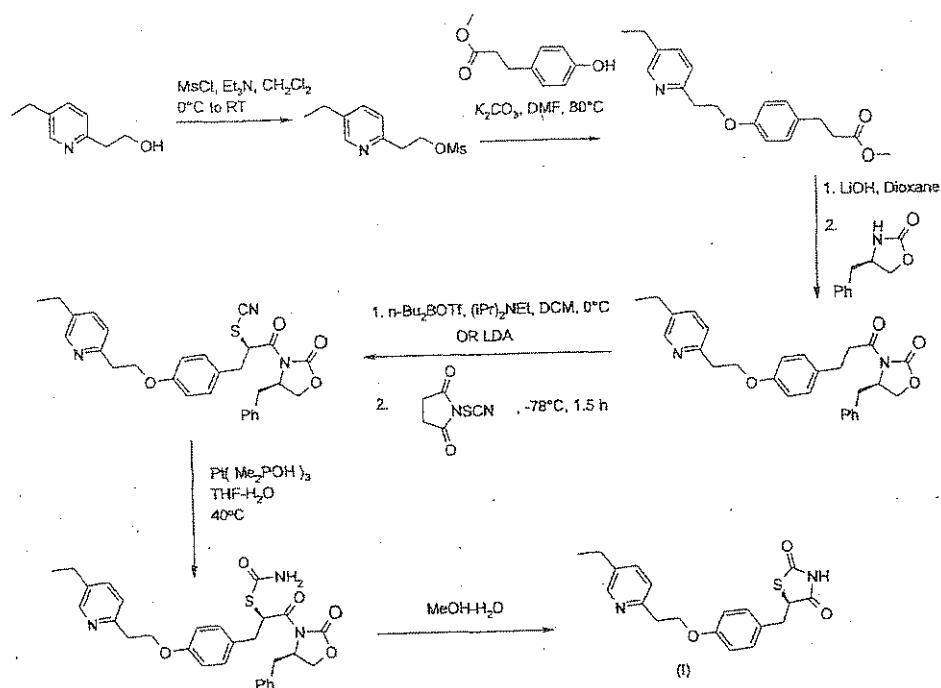
Scheme 4



The final product may of course be recrystallised to increase enantiomeric purity.

Reference J.R. Falck *et al.*, *Bioorg. Med. Chem. Lett.*, **2008**, 18, 1768-1771 describes the chiral synthesis of related compounds and may be applicable to the synthesis of glitazones. Therefore, an alternative method of synthesis, using pioglitazone as an example is as shown in scheme 5 (see also Example 13).

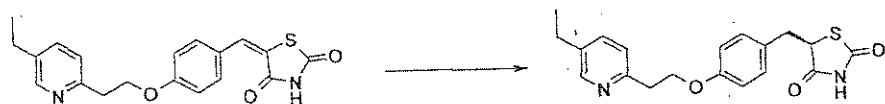
Scheme 5



The final product may of course be recrystallised to increase enantiomeric purity.

In the case of rosiglitazone, reference *J. Chem. Soc. Perkin Trans. I*, **1994**, 3319-3324 shows that 5-[1-{4-[3-(methyl-pyridin-2-yl-amino)-propoxy]-phenyl}-meth-(E)-ylidene]-thiazolidine-2,4-dione can be reduced using bio-catalysis. Yeast strains suggested to be useful for the purpose of biocatalysis are *Rhodotorula rubra* (or *mucilaginosa*) and *Rhodotorula glutinis*. Therefore an alternative preparation of glitazones may be, using pioglitazone as the example, as in Scheme 6:

Scheme 6



Alternatively, asymmetric hydrogenolysis of 5-[1-{4-[2-(5-ethyl-pyridin-2-yl)-ethoxy]-phenyl}-meth-(E)-ylidene]-thiazolidine-2,4-dione using Rhodium and Iridium catalysts in the presence of chiral ligands is a method known to those skilled in the art (See also Example 12 below).

The following Examples illustrate the preparation of pioglitazone and rosiglitazone enantiomers, and the biological results on which the present invention is based:

CHEMICAL EXAMPLES

General Experimental Details

Abbreviations used in the experimental section:

c = concentration; h = hour; H₂O = distilled water; HPLC = high performance liquid chromatography; LCMS = liquid chromatography mass spectrometry; MeOH = methanol; TFA = trifluoroacetic acid; DMSO = dimethyl sulphoxide; HCl = hydrogen chloride; EtOH = ethanol; IPA = isopropyl alcohol; EtOAc = ethyl acetate; THF = tetrahydrofuran; NH₄Cl = ammonium chloride; LDA = lithium diisopropylamide; min = minutes; RT = room temperature; Rt = retention time; e.e. = enantiomeric excess; MP-Carbonate = macroporous triethylammonium methylpolystyrene carbonate (0.5% inorganic antistatic agent). d.e. = diastereomeric excess; SL-W003-2 = (S)-1-[(S)-2-(2i-diphenylphosphinophenyl)ferrocenyl]ethylidicyclohexyl phosphine; Rh(COD)₂BF₄ = bis(1,5-cyclooctadiene) Rhodium I tetrafluoroborate; pioglitazone = 5-[4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl]-1,3-thiazolidine-2,4-dione; L-DBTA = L-dibenzoyl tartaric acid

The nomenclature of structures was assigned using ACD Labs version 10.

NMR spectra were obtained on a Varian Unity Inova 400 spectrometer with a 5mm inverse detection triple resonance probe operating at 400MHz or on a Bruker Avance DRX 400 spectrometer with a 5mm inverse detection triple resonance TXI probe operating at 400MHz or on a Bruker Avance DPX 300 spectrometer with a standard 5mm dual frequency probe operating at 300MHz. Shifts are given in ppm relative to tetramethylsilane. Optical rotations were measured using an AA-10R automatic polarimeter with 5x25 mm jacketed sample cell. Asymmetric hydrogenolysis experiments were performed using Biotage Endeavor hydrogenation equipment.

All solvents and commercial reagents were used as received.

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

Method 1

CHIRALPAK AD-H (250 x 30 mm, 5 μ m), elution with EtOH +0.05%TFA – flow rate 30 ml/min.
Detection –In-line UV detection set at 250 nM wavelength

Method 2

CHIRALPAK 1A (250 x 4.6 mm, 5 μ M), elution with EtOH +0.05%TFA – flow rate 0.7 ml/min.
Detection – In-line DAD set at 280 nM wavelength

Method 3

CHIRALCEL OD-RH (150 X 4.6 mm), elution with 90% MeOH + 10% H₂O - flow rate 0.5 ml/min. Detection – In-line UV detection set at 254 nM wavelength

Method 4

Waters Micromass ZQ2000 with a C18-reverse-phase column (100 x 3.0 mm Higgins Clipeus with 5 μ m particle size), elution with A: water + 0.1% formic acid; B: acetonitrile + 0.1 % formic acid. Gradient:

Gradient - Time	flow mL/min	%A	%B
0.00	1.0	95	5
1.00	1.0	95	5
15.00	1.0	5	95
20.00	1.0	5	95
22.00	1.0	95	5
25.00	1.0	95	5

Detection - MS, ELS, UV (100 μ l split to MS with in-line UV detector). MS ionisation method - Electrospray (positive ion)

Method 5

CHIRALPAK IA (250 x 21 mm, 5 μ m), elution with ethanol +0.2%TFA – flow rate 13 ml/min.
Detection – In-line UV detection set at 220 nM wavelength

Method 6

Chiral-AGP (150 x 4.0 mm, 5 μ M), elution with A: 86% 10 mM potassium dihydrogen phosphate buffer pH 7.0; B: 14% acetonitrile + 0.1% formic acid – flow rate 0.8 ml/min.
Detection – In-line DAD set at 254 nM wavelength

Method 7

CHIRALPAK 1A (250 x 4.6 mm, 5 μ M), elution with A, 0.05% TFA in EtOH; B, heptane; D, IPA (A:B:D = 40:30:30), – flow rate 0.7 ml/min. Detection – In-line DAD set at 225 nM wavelength

Detection - MS, ELS, UV PDA. MS ionisation method - Electrospray (positive/negative ion)

Method 8

Waters Micromass ZQ2000 with a Acquity BEH or Acquity BEH Shield RP18 1.7 μ M 100 x 2.1mm C18-reverse-phase column, elution with A: water + 0.1% formic acid; B: acetonitrile + 0.1 % formic acid. Gradient:

Gradient - Time	flow mL/min	%A	%B
0.00	0.4	95	5
0.4	0.4	95	5
6.00	0.4	5	95
6.80	0.4	5	95
7.00	0.4	95	5
8.00	0.4	95	5

Detection - MS, ELS, UV PDA. MS ionisation method - Electrospray (positive/negative ion)

Method 9

Agilent 1100 series with a CHIRALPAK 1A (150 x 4.6 mm, 5 μ m), elution with A: heptane, B: ethanol + 0.05%TFA – flow rate 0.5 ml/min. Detection – In-line polarimeter and UV detection set at 270 nM wavelength. Gradient:

Gradient - Time	flow mL/min	%A	%B
0.00	0.5	40	60
20.0	0.5	40	60
30.0	0.5	0	100
45.0	0.5	0	100

Method 10

Phenomenex Gemini C18-reverse-phase column (250 × 21.20 mm 5 µm particle size), elution with A: water + 0.1% formic acid; B: methanol + 0.1 % formic acid. Gradient - 50% A/50% B to 5% A/95% B over 15 min - flow rate 18 mL/min. Detection - In-line UV detector set at 254 nm wavelength.

Method 11

Phenomenex Luna 3 micron C18(2) 30 × 4.6mm, elution with A: water + 0.1% formic acid; B: acetonitrile + 0.1 % formic acid. Gradient:

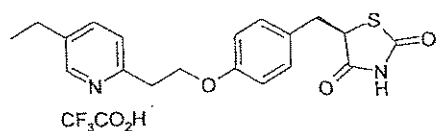
Gradient - Time	flow mL/min	%A	%B
0.00	2.0	95	5
.50	2.0	95	5
4.50	2.0	5	95
5.50	2.0	5	95
6.00	2.0	95	5

Detection - MS, ELS, UV (200 µl/min split to MS with in-line HP1100 DAD detection). MS ionisation method - Electrospray (positive and negative ion)

All reactions were carried out under an atmosphere of nitrogen unless specified otherwise. Racemic rosiglitazone was used as a free base and racemic pioglitazone was used as a free base or HCl salt as indicated.

Example 1

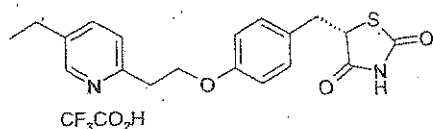
(5R)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione trifluoroacetate



The title compound (480 mg) was isolated using method 1. LCMS (Method 4): Rt 6.00 min, m/z 357 [M-CF₃CO₂H⁺]. [α]_D²⁵ +104° (c 1.0, MeOH). e.e. (Method 2) ≥ 98%, Rt 4.69 min. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.02-11.88 (1H, bs), 8.68-8.60 (1 H, d, *J* 1.7), 8.32-8.23 (1 H, d, *J* 7.7), 7.90-7.82 (1 H, d, *J* 8.4), 7.14-7.06 (2 H, d, *J* 8.7), 6.85-6.78 (2 H, d, *J* 8.7), 4.85-4.78 (1 H, dd, *J* 4.4, 8.9), 4.35-4.27 (2 H, t, *J* 6.2), 3.40-3.34 (2 H, t, *J* 6.1), 3.28-3.21 (1 H, dd, *J* 4.3, 14.3), 3.05-2.97 (1 H, dd, *J* 9.0, 14.3), 2.77-2.67 (2 H, q, *J* 7.6), 1.22-1.14 (3 H, q, *J* 7.5).

Example 2

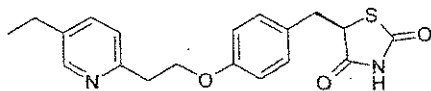
(5S)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione trifluoroacetate



The *title compound* (674 mg) was isolated using method 1. LCMS (Method 4): Rt 6.01 min, m/z 357 [M-CF₃CO₂H⁺]. [α]_D²⁵ -76° (c 1.0, MeOH). e.e. (Method 2) $\geq$ 98%, Rt 7.00 min. ¹H NMR (400 MHz, DMSO-d₆): δ 12.02-11.88 (1H, bs), 8.68-8.60 (1H, d, J 1.7), 8.32-8.23 (1H, d, J 7.7), 7.90-7.82 (1H, d, J 8.4), 7.14-7.06 (2H, d, J 8.7), 6.85-6.78 (2H, d, J 8.7), 4.85-4.78 (1H, dd, J 4.4, 8.9), 4.35-4.27 (2H, t, J 6.2), 3.40-3.34 (2H, t, J 6.1), 3.28-3.21 (1H, dd, J 4.3, 14.3), 3.05-2.97 (1H, dd, J 9.0, 14.3), 2.77-2.67 (2H, q, J 7.6), 1.22-1.14 (3H, q, J 7.5).

Example 3

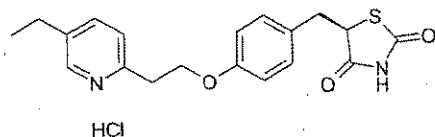
(5R)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione



MP-Carbonate (389 mg, 1.06 mmol) was added to a solution of (5R)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione trifluoroacetate (100 mg, 0.21 mmol) in MeOH (100 mL) and stirred at RT for 2 h, filtered and the resin washed with MeOH (3 X 10 mL). The filtrate was concentrated in vacuo to afford the *title compound* (35 mg, 47%). e.e. (Method 2) 92.90%, Rt 6.27 min. ¹H NMR (400 MHz, DMSO-d₆): δ 12.44-11.11 (1H, bs), 8.34-8.29 (1H, d, J 1.9), 7.55-7.49 (1H, dd, J 2.2, 7.9), 7.24-7.20 (1H, d, J 7.8), 7.12-7.05 (2H, d, J 8.6), 6.84-6.77 (2H, d, J 8.6), 4.78-4.71 (1H, dd, J 4.3, 9.1), 4.30-4.19 (1H, d, J 4.3), 3.24-3.18 (2H, d), 3.11-3.03 (2H, t, J 6.6), 3.00-2.92 (1H, dd, J 9.2, 14.2), 2.59-2.50 (2H, q, J 7.6), 1.17-1.09 (3H, t, J 7.7).

Example 4

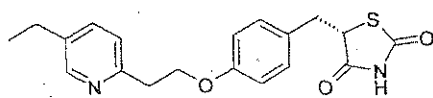
(5R)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione hydrochloride



~1.25 M HCl in MeOH (0.33 mL, 0.33 mmol) was added to a suspension of (5R)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione (from Example 3) (30 mg, 0.084 mmol) in MeOH (5 mL) and stirred at RT for 1 h. The solvent was removed in vacuo to afford the *title compound* (32.4 mg, 100%). LCMS (Method 4): Rt 5.95 min, m/z 357 [M-HCl]⁺. e.e. (Method 3) 93.2%, Rt 12.10 min. Stereochemistry at C-5 was assigned (R) configuration by single crystal X-ray diffraction analysis. $[\alpha]_D^{24} +108^\circ$ (c 1.0, MeOH). ¹H NMR (400 MHz, DMSO-d₆): δ 12.03-11.88 (1 H, bs), 8.68-8.62 (1 H, d, J 1.7), 8.34-8.25 (1 H, d, J 7.9), 7.91-7.83 (1 H, d, J 8.3), 7.14-7.05 (2 H, d, J 8.7), 6.86-6.77 (2 H, d, J 8.7), 4.85-4.77 (1 H, dd, J 4.3, 8.9), 4.38-4.28 (2 H, t, J 6.0), 3.42-3.36 (2 H, t, J 6.2), 3.28-3.20 (1 H, dd, J 9.0, 14.2), 3.06-2.96 (1 H, dd, J 9.0, 14.2), 2.77-2.67 (2 H, q, J 7.7), 1.23-1.15 (3 H, t, J 7.7). Subsequent recrystallisations using MeOH-EtOAc or MeOH-Et₂O gave the *title compound* with an e.e. >97%.

Example 5

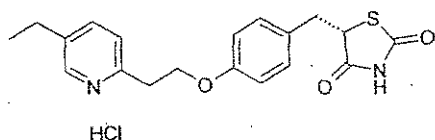
(5S)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione



The *title compound* (28 mg, 37%) was prepared using a method analogous to that outlined in Example 3 starting from (5S)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione trifluoroacetate. e.e. (Method 2) 90.9%, Rt 9.21 min. ¹H NMR (400 MHz, DMSO-d₆): δ 12.44-11.11 (1H, bs), 8.34-8.29 (1 H, d, J 1.9), 7.55-7.49 (1 H, dd, J 2.2, 7.9), 7.24-7.20 (1 H, d, J 7.8), 7.12-7.05 (2 H, d, J 8.6), 6.84-6.77 (2 H, d, J 8.6), 4.78-4.71 (1 H, dd, J 4.3, 9.1), 4.30-4.19 (1 H, d, J 4.3), 3.24-3.18 (2 H, d), 3.11-3.03 (2 H, t, J 6.6), 3.00-2.92 (1 H, dd, J 9.2, 14.2), 2.59-2.50 (2H, q, J 7.6), 1.17-1.09 (3 H, t, J 7.7).

Example 6

(5S)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione hydrochloride

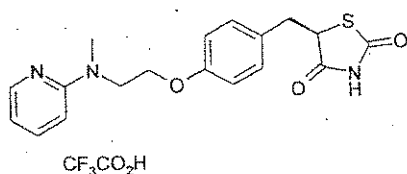


The *title compound* (25.7 mg, 100%) was prepared using a method analogous to that outlined in Example 4 starting from (5S)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione (from Example 2). LCMS (Method 4): Rt 5.94 min, m/z 357 [M-HCl]⁺. e.e. (Method 3) 92.7%, Rt 13.25 min. Stereochemistry at C-5 was assigned (S) configuration by single crystal

X-ray diffraction analysis. $[\alpha]_D^{23} -104^\circ$ (c 1.0, MeOH). ^1H NMR (400 MHz, DMSO- d_6): δ 12.03-11.88 (1 H, bs), 8.68-8.62 (1 H, d, J 1.7), 8.34-8.25 (1 H, d, J 7.9), 7.91-7.83 (1 H, d, J 8.3), 7.14-7.05 (2 H, d, J 8.7), 6.86-6.77 (2 H, d, J 8.7), 4.85-4.77 (1 H, dd, J 4.3, 8.9), 4.38-4.28 (2 H, t, J 6.0), 3.42-3.36 (2 H, t, J 6.2), 3.28-3.20 (1 H, dd, J 9.0, 14.2), 3.06-2.96 (1 H, dd, J 9.0, 14.2), 2.77-2.67 (2 H, q, J 7.7), 1.23-1.15 (3 H, t, J 7.7). Subsequent recrystallisations using MeOH-EtOAc or MeOH-Et₂O gave the *title compound* with an e.e. >97%.

Example 7

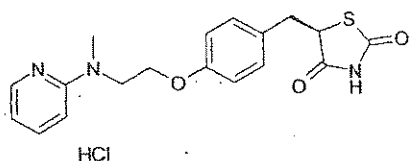
(5R)-5-{4-[2-[methyl(pyridin-2-yl)amino]ethoxy]benzyl}-1,3-thiazolidine-2,4-dione trifluoroacetate



The *title compound* (149 mg) was isolated using method 5. Rt 7.14 min. ^1H NMR (400 MHz, DMSO- d_6): δ 12.04-11.86 (1 H, s), 7.99-7.94 (1 H, dd, J 1.1, 6.2), 7.92-7.83 (1 H, t, J 6.6), 7.27-7.15 (1 H, d, J 8.7), 7.13-7.05 (2 H, d, J 8.6), 6.88-6.82 (1 H, t, J 6.6), 6.81-6.76 (2 H, d, J 8.7), 4.83-4.78 (1 H, dd, J 4.4, 8.8), 4.18-4.12 (2 H, t, J 5.3), 3.99-3.94 (2 H, t, J 5.3), 3.27-3.20 (1 H, dd, J 4.2, 14.4), 3.18 (3 H, s), 3.05-2.97 (1 H, dd, J 8.9, 14.6).

Example 8

(5R)-5-{4-[2-[methyl(pyridin-2-yl)amino]ethoxy]benzyl}-1,3-thiazolidine-2,4-dione hydrochloride

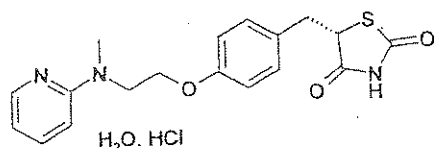


The *title compound* (38 mg, 64%) was prepared using method analogous to that outlined in Example 3 and 4 starting from (5R)-5-{4-[2-methyl-2-pyridylamino]ethoxy]benzyl}-1,3-thiazolidine-2,4-dione trifluoroacetate. $[\alpha]_D^{26} +100^\circ$ (c 1.0, MeOH). LCMS (Method 4): Rt 5.41 min, m/z 358 $[\text{M}-\text{HCl}]^+$. e.e. (Method 6) 85.7%, Rt 8.03 min. ^1H NMR (400 MHz, DMSO- d_6): δ 12.09-11.81 (1 H, s), 7.98-7.94 (1 H, dd, J 1.1, 6.3), 7.93-7.82 (1 H, m), 7.31-7.14 (1 H, bs), 7.13-7.05 (2 H, d, J 8.5), 6.90-6.82 (1 H, t, J 6.7), 6.80-6.76 (2 H, d, J 8.5), 4.84-4.78 (1 H, dd, J 4.4, 8.9), 4.19-4.12 (2 H, t, J 5.2), 4.02-3.94 (2 H, t, J 5.2), 3.27-3.21 (1 H, dd, J 4.4, 10.1), 3.20 (3 H, s), 3.05-2.97 (1 H, dd, J 9.0, 14.2).

R-enantiomer with greater than 90% e.e. can be obtained using literature procedures, *J. Chem. Soc. Perkin Trans. 1*, **1994**, 3319-3324.

Example 9

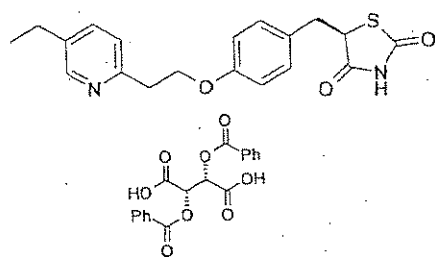
(5S)-5-{4-[2-[methyl(pyridin-2-yl)amino]ethoxy]benzyl}-1,3-thiazolidine-2,4-dione hydrochloride monohydrate



The title compound (123 mg) was prepared using literature procedures, *J. Chem. Soc. Perkin Trans. 1*, **1994**, 3319-3324. $[\alpha]_D^{23}$ -100° (c 1.0, MeOH). LCMS (Method 4): Rt 5.44 min, m/z 358 [M-HCl]⁺. e.e. (Method 6) 92.7%, Rt 8.99 min. Stereochemistry at C-5 was assigned (S) configuration by single crystal X-ray diffraction analysis. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.01-11.88 (1 H, s), 7.98-7.94 (1 H, dd, *J* 1.4, 6.1), 7.93-7.86 (1 H, t, *J* 7.7), 7.31-7.18 (1 H, m), 7.12-7.05 (2 H, d, *J* 8.7), 6.90-6.83 (1 H, t, *J* 6.3), 6.81-6.75 (2 H, d, *J* 8.7), 4.83-4.78 (1 H, dd, *J* 4.5, 8.8), 4.19-4.13 (2 H, t, *J* 5.1), 4.02-3.96 (2 H, t, *J* 5.1), 3.26-3.22 (1 H, m), 3.21 (3 H, s), 3.05-2.97 (1 H, dd, *J* 8.8, 14.0).

Example 10

(5R)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione (-)-O,O'-dibenzoyl-L-tartrate



10a. To a mixture of (5R)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione hydrochloride (50mg) (example 4) and (-)-dibenzoyl-L-tartaric acid (50mg) was added MeOH (1.5 mL). The clear solution was rapidly stirred whilst adding H₂O dropwise until a cloudiness persisted. The reaction was allowed to stand at ambient temperature over 48h and the solid collected by filtration to give the title compound (43mg). (Method 7) 99.01% Rt 10.83 min, 0.98% Rt 15.83 min; d.e. 98.03%

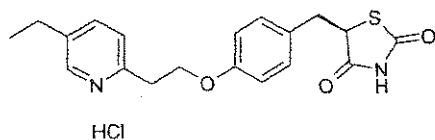
10b. A slurry of (-)-dibenzoyl-L-tartaric acid (1.0g, 2.79 mmol) in H₂O (20mL) was stirred at ambient temperature and a solution of 5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione hydrochloride (1.01g, 2.57mmol) in MeOH (20mL) was added over 5 min. When addition was complete, the product from Example 10a (5mg) was added and the reaction allowed to stir for 93h. The reaction was filtered and the solid dried to give the *title compound* (0.863g). (Method 7) 79.72%, Rt 10.82 min.; 20.27%, Rt 15.14 min.; d.e. 59.45%.

10c. The product from Example 10b (0.863g) was dissolved in MeOH (8.5 mL) containing 1M HCl (1.21 mL) and H₂O (5 mL) added dropwise. The product from Example 10a (1mg) was added followed by dropwise addition of H₂O (2.3 mL). The reaction was allowed to stir for 22h, filtered, the solid washed with H₂O-MeOH (2:1, 3 mL) and dried at 40°C under high vacuum to give the *title compound* (0.582g). (Method 7) 93.2%, Rt 10.82 min.; 6.8%, Rt 15.14 min.; d.e. 86.4%.

10d. The product from Example 10c (0.582g) was dissolved in MeOH (5.5 mL) containing 1M HCl (0.795 mL) and H₂O (2 mL) added dropwise. The product from Example 10a (1mg) was added followed by dropwise addition of H₂O (3.5 mL). The reaction was allowed to stir for 22h, filtered, the solid washed with H₂O-MeOH (2:1, 3 mL) and dried at 40°C under high vacuum to give the *title compound* (0.453g). (Method 7) 97.3%, Rt 10.65 min.; 2.7%, Rt 14.83 min.; d.e. 94.6%. ¹H NMR (400 MHz, DMSO-*d*₆): 14.25-13.60 (bs, 1H, D₂O exchangeable), 12.05-12.00 (bs, 1H, D₂O exchangeable), 8.37 (d, *J* = 2.0 Hz, 1H), 8.02 (d, *J* = 7.6 Hz, 4H), 7.73 (t, *J* = 7.6 Hz, 2H), 7.60 (t, *J* = 7.6 Hz, 5H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.13 (d, *J* = 8.8 Hz, 2H), 6.86 (d, *J* = 8.8 Hz, 2H), 5.88 (s, 2H), 4.86 (q, *J* = 4.4 Hz, 1H), 4.30 (t, *J* = 6.8 Hz, 2H), 3.30 (dd, *J* = 4.0 & 10.0 Hz, 1H), 3.13 (t, *J* = 6.8 Hz, 2H), 3.04 (dd, *J* = 5.2 & 9.2 Hz, 1H), 2.60 (q, *J* = 7.6 Hz, 2H), 1.17 (t, *J* = 7.6 Hz, 3H).

Example 11

(5R)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione hydrochloride



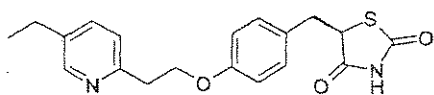
11a. The product from Example 10d was dissolved in MeOH (2.25 mL) containing 37% HCl (0.134 mL) at 35 °C. The solution was filtered, EtOAc (9 mL) was poured into the stirred solution and the mixture stirred for 20 min. The white solid was collected by filtration, washed with EtOAc and dried at 30 °C under high vacuum to give the *title compound* (0.181 g).

(Method 7) 98.3%, Rt 10.65 min.; 1.7%, Rt 14.83 min e.e. 96.6%. LCMS (Method 8): Rt 2.90 min 99.39%, m/z 357 [MH⁺-HCl]. LCMS (Method 11) Rt 2.91 min, m/z 357 [MH⁺-HCl]. ¹H NMR (400 MHz, DMSO-d₆): δ 12.0 (1 H, s), 8.70 (1 H, d, J 1.7 Hz), 8.36 (1 H, bd, J 8.3 Hz), 7.93 (1 H, d, J 8.2 Hz), 7.15, 6.87 (4 H, A2B2q, J 8.7 Hz), 4.86 (1 H, dd, J 4.4, 8.9 Hz), 4.38 (2 H, t, J 6.3 Hz), 3.44 (2 H, t, J 6.2 Hz), 3.29 (1 H, dd, J 4.3, 14.2 Hz), 3.06 (1 H, dd, J 9.0, 14.3 Hz), 2.78 (2 H, q, J 7.6 Hz), 1.23 (3 H, t, J 7.6 Hz).

11b. The product from Example 10c (1g) could also be reacted as described in Example 11a to give the *title compound* (473mg). (Method 7) 95.6%, Rt 10.65 min.; 4.3%, Rt 14.83 min e.e. 91.3%. All other characterisation data was the same as Example 11a.

Example 12

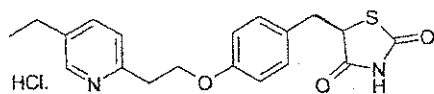
(5R)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione



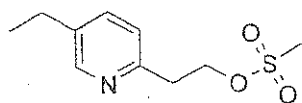
A stock solution of the Rh(COD)₂BF₄ (10.6 mg, 26.1 μmol) in DCM (9.5 mL) was made in a glove box. Ligand SL-W003-2 (8.4 mg, 12.5 μmol), was weighted into a vial and 500 μL of the Rh(COD)₂BF₄ stock solution was added. A solution of 5-[1-{4-[2-(5-ethyl-pyridin-2-yl)-ethoxy]-phenyl}-meth-(E)-ylidene]-thiazolidine-2,4-dione (4.5 mL of a stock solution containing 78.2 mg in 35 mL MeOH) was added to the reaction vial containing the catalyst solution. The vial was purged with N₂ (5 x) and H₂ (5 x) and finally charged with 25 bar of H₂. After 18h the H₂ was released and the reaction vial was purged with N₂ (3 x). A sample of the reaction vial was taken and diluted with an equal volume of EtOH containing 1% formic acid. e.e. (Method 9) 75%, Rt 36.11 min.

Example 13

(5R)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione Hydrochloride

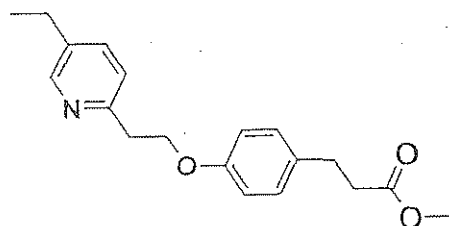


13a. Methanesulfonic acid 2-(5-ethyl-pyridin-2-yl)-ethyl ester



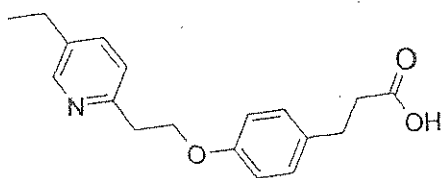
To a solution of 2-(5-ethyl-pyridine-2-yl)-ethanol (25.34 g) and triethylamine (46.7 mL) in DCM (130 mL) at 0 °C under nitrogen was added methane sulfonyl chloride (15.56 mL), then the reaction mixture was allowed to warm to room temperature and stirred overnight. A further portion of methane sulfonyl chloride (3.88 mL) was added and the reaction stirred for 30 min. The reaction was diluted with DCM and washed with H₂O and brine. The organic layer was dried (MgSO₄) and the solution concentrated to give the title compound as a red oil (38.24 g). ¹H NMR (400 MHz, CDCl₃): δ 8.39 (d, *J* = 2.2 Hz, 1H), 7.47 (dd, *J* = 7.7, 2.3 Hz, 1H), 7.14 (d, *J* = 7.7 Hz, 1H), 4.64 (t, *J* = 6.5 Hz, 2H), 3.19 (t, *J* = 6.5 Hz, 2H), 2.90 (s, 3H), 2.64 (q, *J* = 7.6 Hz, 2H), 1.25 (t, *J* = 7.6 Hz, 3H).

13b. 3-{4-[2-(5-Ethyl-pyridin-2-yl)-ethoxy]-phenyl}-propionic acid methyl ester



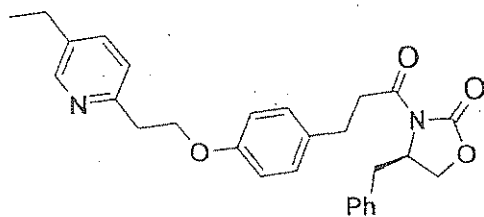
The product from Example 13a (12.72 g) in toluene (50 mL) was added dropwise to a stirred mixture of methyl-3-(4-hydroxyphenyl)propionate (10 g) and potassium carbonate (23.01 g) in toluene (180 mL). The reaction was heated to reflux for 23 h, then left at room temperature for 90 h. The reaction mixture was treated with H₂O and extracted with three portions of diethyl ether. The combined extracts were washed twice with H₂O, once with brine, dried and evaporated. The residue was purified by chromatography eluting with 20-40% EtOAc in petroleum ether (bp = 40-60 °C) to give the title product as a yellow solid (10.73 g). ¹H NMR (400 MHz, CDCl₃): δ 8.39 (d, *J* = 2.1 Hz, 1H), 7.44 (dd, *J* = 7.8, 2.4 Hz, 1H), 7.18 (d, *J* = 7.8 Hz, 1H), 7.08, 6.83 (A₂B₂q, *J* = 8.6 Hz, 4H), 4.31 (t, *J* = 6.7 Hz, 2H), 3.66 (s, 3H), 3.22 (t, *J* = 6.7 Hz, 2H), 2.87 (t, *J* = 7.6 Hz, 2H), 2.66-2.55 (m, 4H), 1.24 (t, *J* = 7.6 Hz, 3H).

13c. 3-{4-[2-(5-Ethyl-pyridin-2-yl)-ethoxy]-phenyl}-propionic acid



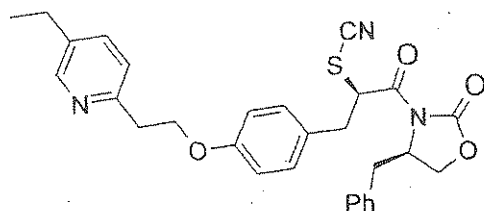
The product from Example 13b (10.73 g) was dissolved in 1,4-dioxan/ water (350/100 mL), lithium hydroxide monohydrate (4.3 g) added, and the reaction stirred overnight. The dioxan was removed by evaporation under reduced pressure, the suspension was diluted with H₂O and treated with 2.0 M HCl until the pH was 6-7. The solid was filtered and dried to give the title compound (9.57 g). ¹H NMR (400 MHz, CDCl₃): δ 8.41 (d, *J* = 2.3 Hz, 1H), 7.52 (dd, *J* = 7.9, 2.3 Hz, 1H), 7.25 (d, *J* = 7.9 Hz, 1H), 7.11, 6.81 (A₂B₂q, *J* = 8.6 Hz, 4H), 4.27 (t, *J* = 6.6 Hz, 2H), 4.0 (bs, 2H, COOH+water) 3.24 (t, *J* = 6.6 Hz, 2H), 2.90 (t, *J* = 7.6 Hz, 2H), 2.68-2.59 (m, 4H), 1.24 (t, *J* = 7.6 Hz, 3H).

13d. (R)-4-Benzyl-3-(3-(4-[2-(5-ethyl-pyridin-2-yl)-ethoxy]-phenyl)-propionyl)-oxazolidin-2-one



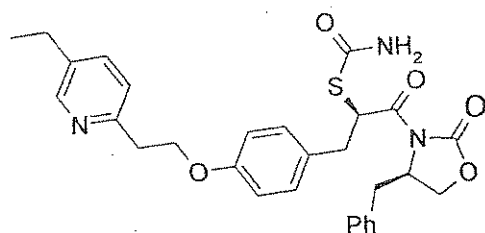
A suspension of the product from Example 13c (9.0 g) and (R)-(+)-4-benzyloxazolidinone (2.67 g) in triethylamine (8.38 mL) and toluene (90 mL) was heated to 80 °C. Pivaloyl chloride (3.71 mL) was added dropwise maintaining the temperature between 80 and 85 °C. The reaction was then heated to reflux for 22.5 h. The reaction was allowed to cool to room temperature, the reaction partitioned between H₂O-EtOAc and the aqueous layer was extracted twice with EtOAc and the combined organics dried (MgSO₄) and concentrated. The crude product was purified by chromatography on silica gel eluting with 0-40% EtOAc-cyclohexane to give the title product as a white solid (2.06 g). ¹H NMR (300 MHz, CDCl₃): δ 8.39 (d, *J* = 2.2 Hz, 1H), 7.45 (dd, *J* = 7.9, 2.2 Hz, 1H), 7.36-7.24 (m, 3H), 7.21-7.12 (m, 5H), 6.84 (d, *J* = 8.6 Hz, 2H), 4.65 (m, 1H), 4.32 (t, *J* = 6.6 Hz, 2H), 4.16 (m, 2H), 3.33-3.12 (m, 5H), 3.04-2.87 (m, 2H), 2.74 (dd, *J* = 9.5, 13.4 Hz, 1H), 2.63 (q, *J* = 7.6 Hz, 2H), 1.24 (t, *J* = 7.6 Hz, 3H).

13e. (R)-4-Benzyl-3-((R)-3-{4-[2-(5-ethyl-pyridin-2-yl)-ethoxy]-phenyl}-2-thiocyanato-propionyl)-oxazolidin-2-one



To LDA (0.528 mmol) in THF/hexanes (3/0.3 mL) at -78°C under argon was added dropwise the product from Example 13d (0.20 g) in THF (4 mL). After 30 min N-thiocyanatosuccinimide (JACS, 2004, 126, 10216-7) (0.137 g) in THF (2 mL) was added dropwise. After a further 110 min at -78°C the reaction was quenched with sat aqueous NH_4Cl (5 mL) and allowed to warm to room temperature. The mixture was extracted with three 15 mL portions of EtOAc, the combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure to low volume. Toluene (20 mL) was added and evaporated under reduced pressure. The residue was dissolved in DCM (3 mL) and stored at -20°C for 16 h. The soluble material was purified by chromatography on silica gel eluting with 20-50% EtOAc in cyclohexane, repurifying impure fractions by the same method, to give the title product and succinimide in 1:2.4 molar ratio as a colourless semi solid (0.121 g). ^1H NMR (300 MHz, CDCl_3): δ 8.60 (bs, 1H succinimide) 8.40 (d, $J = 2.3$ Hz, 1H), 7.47 (dd, $J = 7.8, 2.3$ Hz, 1H), 7.39-7.12 (m, 8H), 6.85 (d, $J = 8.7$ Hz, 2H), 5.07 (dd, $J = 8.1, 7.2$ Hz, 1H), 4.63 (m, 1H), 4.32 (t, $J = 6.7$ Hz, 2H), 4.24-4.11 (m, 2H), 3.47 (dd, $J = 14.0, 8.0$ Hz, 1H), 3.33 (dd, $J = 13.4, 3.2$ Hz, 1H), 3.28-3.16 (m, 3H), 2.84 (dd, $J = 9.3, 13.4$ Hz, 1H), 2.76 (s, 4H succinimide) 2.63 (q, $J = 7.6$ Hz, 2H), 1.24 (t, $J = 7.6$ Hz, 3H).

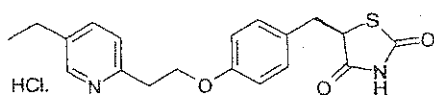
13f. Thiocarbamic acid S-((R)-2-((R)-4-benzyl-2-oxo-oxazolidin-3-yl)-1-{4-[2-(5-ethyl-pyridin-2-yl)-ethoxy]-benzyl}-2-oxo-ethyl) ester



The product from Example 13e (51 mg) was dissolved in THF/water (2/1 mL) under argon and treated with platinum tris(dimethylphosphine oxide) (*Tet. Lett.*, 2002, 43, 8121) (4 mg). The reaction was warmed to 40°C for 2 h then allowed to cool to room temperature. EtOAc (10 mL) was added, the organic phase dried (Na_2SO_4), concentrated and purified by chromatography on silica gel (2 g) eluting with 40-70% EtOAc in cyclohexane to give the title product and succinimide in 1:3.3 molar ratio as a colourless semi solid (0.029 g). ^1H NMR

(400 MHz, CDCl_3): δ 8.66 (bs, 1H succinimide) 8.39 (d, $J = 2.0$ Hz, 1H), 7.47 (dd, $J = 7.9, 2.2$ Hz, 1H), 7.37-7.17 (m, 6H), 7.17, 6.81 (A_2B_2q $J = 8.5$ Hz, 4H), 5.74 (t, $J = 7.9$ Hz, 1H), 5.63 (bs, 2H), 4.53 (m, 1H), 4.30 (t, $J = 6.7$ Hz, 2H), 4.08 (dd, $J = 8.9, 2.5$ Hz, 1H), 3.95 (dd, $J = 8.9, 7.8$ Hz, 1H), 3.31 (dd, $J = 13.5, 3.1$ Hz, 1H), 3.26-3.18 (m, 3H), 2.96 (dd, $J = 13.5, 8.1$ Hz, 1H), 2.74 (s, 4H succinimide), 2.72 (dd, $J = 13.5, 9.8$ Hz, 1H), 2.63 (q, $J = 7.6$ Hz, 2H), 1.24 (t, $J = 7.6$ Hz, 3H).

13g. (5R)-5-[4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl]-1,3-thiazolidine-2,4-dione hydrochloride



The product from Example 13f was dissolved in MeOH (10 mL) and H_2O (10 mL) added to give a cloudy solution. This was left at room temperature for 2.5h then 1M HCl (0.15 mL) added. The solution was evaporated to dryness and the residue taken up in MeOH (0.1 mL) containing concentrated HCl (0.0015 mL). EtOAc (5 mL) was added and the solution concentrated slightly under reduced pressure, causing a white solid to precipitate. The solution was removed and the solid (7 mg) washed with EtOAc. This material was purified by preparative HPLC (Method 10), and the fractions containing the first eluting component combined and treated with 1M HCl (1 mL) and evaporated to dryness to give the *title product* and succinimide in a 1:0:0.19 molar ratio as a colourless gum (0.002 g). (Method 7) 99.175%, Rt 10.65 min.; 0.825%, Rt 14.83 min.; e.e. 98.35%. LCMS (Method 11) Rt 2.9 min, m/z 357 [MH^+]. ^1H NMR (300 MHz, d_4 -MeOH): 8.62 (bd, 1H), 8.42 (dd, $J = 8.2, 2.0$ Hz, 1H), 7.98 (d, $J = 8.2$ Hz, 1H), 7.16, 6.85 (A_2B_2q , $J = 8.7$ Hz, 4H), 4.68 dd, $J = 8.7, 4.2$ Hz, 1H), 4.38 (t, $J = 5.8$ Hz, 2H), 3.34 (m obscured, 1H), 3.11 (dd, $J = 14.3, 8.8$ Hz, 1H), 2.87 (q, $J = 7.6$ Hz, 2H), 2.68 (s, 4H succinimide), 1.33 (t, $J = 7.6$ Hz, 3H).

Biological Results:

Example 14

Pre-clinical mouse model of COPD inflammation – Tobacco smoke induced pulmonary inflammation.

Previous studies have established that the number of inflammatory cells recovered in the bronchoalveolar lavage (BAL) is significantly elevated 24 h following the final Tobacco Smoke

(TS) exposure of 4 or 11 consecutive daily TS exposures, this time point was used in the studies reported here.

Protocols for the exposure of mice to TS, obtaining bronchoalveolar lavage (BAL), preparation of cytospin slides for differential cell counts are as outlined below.

Exposure of mice to TS daily for 4 or 11 consecutive days

In this exposure protocol, mice were exposed in groups of 5 in individual clear polycarbonate chambers (27 cm x 16 cm x 12 cm). The TS from the cigarettes was allowed to enter the exposure chambers at a flow rate of 100 ml/min. In order to minimise any potential problems caused by repeated exposure to a high level of TS (6 cigarettes), the exposure of the mice to TS was increased gradually over the exposure period to a maximum of 6 cigarettes. The exposure schedule used for 4 days was as follows:

Day 1: 4 cigarettes	(approximately 32 min exposure)
Day 2: 4 cigarettes	(approximately 32 min exposure)
Day 3: 6 cigarettes	(approximately 48 min exposure)
Day 4: 6 cigarettes	(approximately 48 min exposure)

The exposure schedule used for 11 days exposure was as follows:

Day 1: 2 cigarettes	(approximately 16 min exposure)
Day 2: 3 cigarettes	(approximately 24 min exposure)
Day 3: 4 cigarettes	(approximately 32 min exposure)
Day 4: 5 cigarettes	(approximately 40 min exposure)
Day 5 to 11: 6 cigarettes	(approximately 48 min exposure)

A further group of mice were exposed to air on a daily basis for equivalent lengths of time as controls (no TS exposure).

Bronchoalveolar lavage (BAL) analysis

Bronchoalveolar lavage was performed as follows: the trachea was cannulated using a Portex nylon intravenous cannula (pink luer fitting) shortened to approximately 8 mm. Phosphate buffered saline (PBS) was used as the lavage fluid. A volume of 0.4 ml was gently instilled and withdrawn 3 times using a 1ml syringe and then placed in an Eppendorf tube and kept on ice prior to subsequent determinations.

Cell counts:

Lavage fluid was separated from cells by centrifugation and the supernatant decanted and frozen for subsequent analysis. The cell pellet was re-suspended in a known volume of PBS

and total cell numbers calculated by counting a stained (Turks stain) aliquot under a microscope using a haemocytometer.

Differential cell counts were performed as follows:

The residual cell pellet was diluted to approximately 10^5 cells per ml. A volume of 500 μ l was placed in the funnel of a cytopsin slide and centrifuged for 8 min at 800 rpm. The slide was air dried and stained using 'Kwik-Diff' solutions (Shandon) as per the proprietary instructions. When dried and cover-slipped, differential cells were counted using light microscopy. Up to 400 cells were counted by unbiased operator using light microscopy. Cells were differentiated using standard morphometric techniques.

Drug Treatment

Rodents such as mice and rats are obligate nasal breathers thus oral delivery of test materials (such as therapeutic agents) for inhalation will not produce good lung exposure. As a consequence, delivery of therapeutic agents to the lungs in rodents is generally achieved by intra-nasal, intra-tracheal or inhalation by whole body aerosol exposure in a chamber.

The chamber method utilises large amounts of test material and is generally reserved for inhalation toxicology studies rather than pharmacological efficacy studies. Intra-tracheal administration is a very efficient delivery method as almost all of the test material is delivered to the lungs, but this is quite an invasive technique. For studies in the mouse particularly, it is also quite technically demanding as the diameter of the trachea is quite small. The intranasal route is less invasive than the intra-tracheal route and so is particularly suitable for repeat dosing studies such as the 4-11 day mouse model described below. Following intranasal administration ~50% of the dose administered is delivered to the lungs (Eyles JE, Williamson ED and Alpar HO. 1999, *Int J Pharm*, 189(1):75-9).

As a surrogate route for oral inhalation, mice were dosed intra-nasally with vehicle (0.2% tween 80 in saline), 5S-pioglitazone (as prepared in Example 6) (3 μ g/kg), 5S-pioglitazone (as prepared in Example 6) (1 μ g/kg), 5R-pioglitazone (as prepared in Example 4) (3 μ g/kg), 5R-pioglitazone (as prepared in Example 4) (1 μ g/kg), or racemic pioglitazone (3 μ g/kg). The control group of mice received vehicle 1 hr prior to being exposed to air daily for a maximum of 50 minutes per day. BAL was performed 24 h following the final TS exposure. All compounds were dosed as the HCl salt with doses corrected as base.

In a second experiment, mice were dosed intra-nasally with vehicle (0.2% tween 80 in saline), 5S-Rosiglitazone (as prepared in Example 9) (3 µg/kg), 5S-Rosiglitazone (as prepared in Example 9) (10 µg/kg), 5R-Rosiglitazone (as prepared in Example 8) (3 µg/kg), 5R-Rosiglitazone (as prepared in Example 8) (10 µg/kg), or racemic Rosiglitazone (10 µg/kg). The control group of mice received vehicle 1 hr prior to being exposed to air daily for a maximum of 50 minutes per day. BAL was performed 24 h following the final TS exposure. All compounds were dosed as the HCl salt with doses corrected as free base.

Data management and statistical analysis

All results are presented as individual data points for each animal and the mean value was calculated for each group. Since tests for normality were positive, the data were subjected to a one way analysis of variance test (ANOVA), followed by a Bonferroni correction for multiple comparisons in order to test for significance between treatment groups. A "p" value of < 0.05 was considered to be statistically significant. Percentage inhibitions were automatically calculated within the Excel spreadsheets for the cell data using the formula below:

$$\% \text{ Inhibition} = 1 - \left(\frac{\text{Treatment group result} - \text{sham group result}}{\text{TS vehicle group result} - \text{sham group result}} \right) \times 100$$

Inhibition data for other parameters were calculated manually using the above formula.

As illustrated in Figure 1, there was a clear difference in activity between the two enantiomers of pioglitazone on total cell BAL numbers following exposure to TS. The 5R enantiomer (e.e. 97.8%) of Pioglitazone significantly inhibited the BAL cell influx induced by TS at both 1 and 3 µg/kg when administered by the intranasal route. In contrast, the 5S-enantiomer (e.e. 97.5%) failed to inhibit the BAL cell inflammation at either dose examined.

Following examination of the BAL cell cytopins, BAL neutrophil numbers were determined. In concert with the activity on total BAL cells, the 5R enantiomer of pioglitazone significantly inhibited BAL neutrophil numbers induced by TS exposure at both doses whereas the 5S enantiomer of pioglitazone was ineffective (Figure 2).

Racemic pioglitazone (which contains 50% 5R enantiomer of pioglitazone) at the 3 µg/kg dose also significantly inhibited total and neutrophil BAL cells induced by TS.

As illustrated in Figure 3, there was a clear difference in activity between the two enantiomers of Rosiglitazone on total cell BAL numbers following exposure to TS. The 5R enantiomer (e.e. 85.7%) of Rosiglitazone significantly inhibited the BAL cell influx induced by TS at both 3 and 10 µg/kg when administered by the intranasal route. In contrast, the 5S-enantiomer (e.e. 92.7%) failed to inhibit the BAL cell inflammation at either dose examined.

Following examination of the BAL cell cytopins, BAL neutrophil numbers were determined. In concert with the activity on total BAL cells, the 5R enantiomer of Rosiglitazone significantly inhibited BAL neutrophil numbers induced by TS exposure at both doses whereas the 5S enantiomer of Rosiglitazone was ineffective (Figure 2).

Racemic rosiglitazone (which contains 50% 5R enantiomer of Rosiglitazone) at the 10 µg/kg dose also significantly inhibited total and neutrophil BAL cells induced by TS.

Taken together, the results of the two studies identify the 5R enantiomer of both Pioglitazone and Rosiglitazone as possessing the anti-inflammatory activity required for inhibition of BAL cell influx whilst the 5S enantiomer does not.

Whilst the e.e. of the preparation of the 5R enantiomer of Rosiglitazone was lower than optimal (i.e., 85.7% rather than > 90%) differential activity of the two enantiomers was still observed. This suggests that an optimal preparation of 5R enantiomer of Rosiglitazone would show similar or even improved activity compared with the data presented herein. Therefore the data presented herein is indicative of what would be achieved with a preparation that contains by weight at least 95% 5R enantiomer of Rosiglitazone.

Claims

1. A glitazone for use in the treatment of inflammatory respiratory disease by pulmonary administration by inhalation, wherein the glitazone inhaled consists of at least 95% by weight of the 5R configuration and less than 5% by weight of the 5S configuration, and wherein the said glitazone is pioglitazone or rosiglitazone.
2. A glitazone for use as claimed in claim 1, wherein the said inflammatory respiratory disease is selected from mild asthma, moderate asthma, severe asthma, steroid resistant asthma, bronchitis, chronic obstructive pulmonary disease (COPD), cystic fibrosis, pulmonary edema, pulmonary embolism, pneumonia, pulmonary sarcoidosis, silicosis, pulmonary fibrosis, respiratory failure, acute respiratory distress syndrome, emphysema, chronic bronchitis, tuberculosis, and lung cancer.
3. A glitazone for use as claimed in claim 1, wherein the said inflammatory respiratory disease is chronic obstructive pulmonary disease
4. The use of a glitazone in the preparation of a medicament for the treatment of inflammatory respiratory disease by pulmonary administration by inhalation wherein the glitazone content of the medicament consists of at least 95% by weight of the 5R configuration and less than 5% by weight of the 5S configuration, and wherein the said glitazone is pioglitazone or rosiglitazone.
5. The use as claimed in claim 4, wherein the said inflammatory respiratory disease is selected from mild asthma, moderate asthma, severe asthma, steroid resistant asthma, bronchitis, chronic obstructive pulmonary disease (COPD), cystic fibrosis, pulmonary edema, pulmonary embolism, pneumonia, pulmonary sarcoidosis, silicosis, pulmonary fibrosis, respiratory failure, acute respiratory distress syndrome, emphysema, chronic bronchitis, tuberculosis, and lung cancer.
6. The use as claimed in claim 4, wherein the said inflammatory respiratory disease is chronic obstructive pulmonary disease

7. A pharmaceutical composition adapted for pulmonary administration by inhalation, which composition comprises a glitazone and one or more pharmaceutically acceptable carriers and/or excipients, and wherein the glitazone content of the composition consists of at least 95% by weight of the 5R configuration and less than 5% by weight of the 5S configuration, and wherein the said glitazone is pioglitazone or rosiglitazone.

8. A pharmaceutical composition as claimed in claim 7 which additionally comprises one or more other therapeutic agents selected from anti-inflammatory agents, bronchodilators, mucolytic agents, antitussive agents, leukotriene inhibitors, and antibiotics.

9. A kit for treatment of respiratory disorders in a subject, the kit comprising one dosage form comprising a composition as claimed in claim 7 and a second dosage form comprising another therapeutic agent selected from anti-inflammatory agents, bronchodilators, mucolytic agents, antitussive agents, leukotriene inhibitors, and antibiotics.