Drugs of the Future 2006, 31(11): 944-952

CCC: 0377-8282/2006
DOI: 10.1358/dof.2006.031.11.1047743

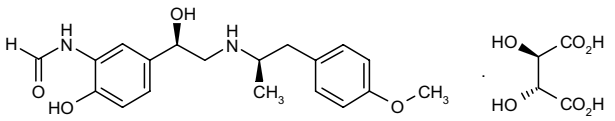
Arformoterol Tartrate

Prop INNM; USAN

β_2-Adrenoceptor Agonist
Bronchodilator
Treatment of Chronic Obstructive Pulmonary Disease

(*R,R*)-Formoterol tartrate
Brovana™

(–)-*N*-[2-Hydroxy-5-[1(*R*)-hydroxy-2-[2-(4-methoxyphenyl)-1(*R*)-methylethylamino]ethyl]phenyl]formamide tartrate



$C_{23}H_{30}N_2O_{10}$
Mol wt: 494.4918

CAS: 200815-49-2

CAS: 067346-49-0 (as free base)

EN: 236034

Abstract

β_2-Adrenoceptor agonists are widely used as adjuncts to corticosteroids for the treatment of chronic obstructive pulmonary disease (COPD) and asthma, providing relief of symptoms by dilating bronchial airways and vasculature. High doses and long-term use of β_2-adrenoceptor agonists, including the currently marketed racemic formoterol, have been associated with a small but significant increase in the risk of asthma-related deaths. The (*R,R*)-enantiomer of formoterol has been shown to be the active component with respect to bronchodilatation and has additional antiinflammatory properties. The (*S,S*)-enantiomer, on the other hand, has no bronchodilatory activity, is proinflammatory and exacerbates bronchial hypersensitivity to allergens in animal models of asthma. Arformoterol tartrate is an enantiomerically pure preparation of (*R,R*)-formoterol under development for the treatment of bronchoconstriction in patients with COPD. The FDA just recently approved the NDA for arformoterol tartrate.

Synthesis

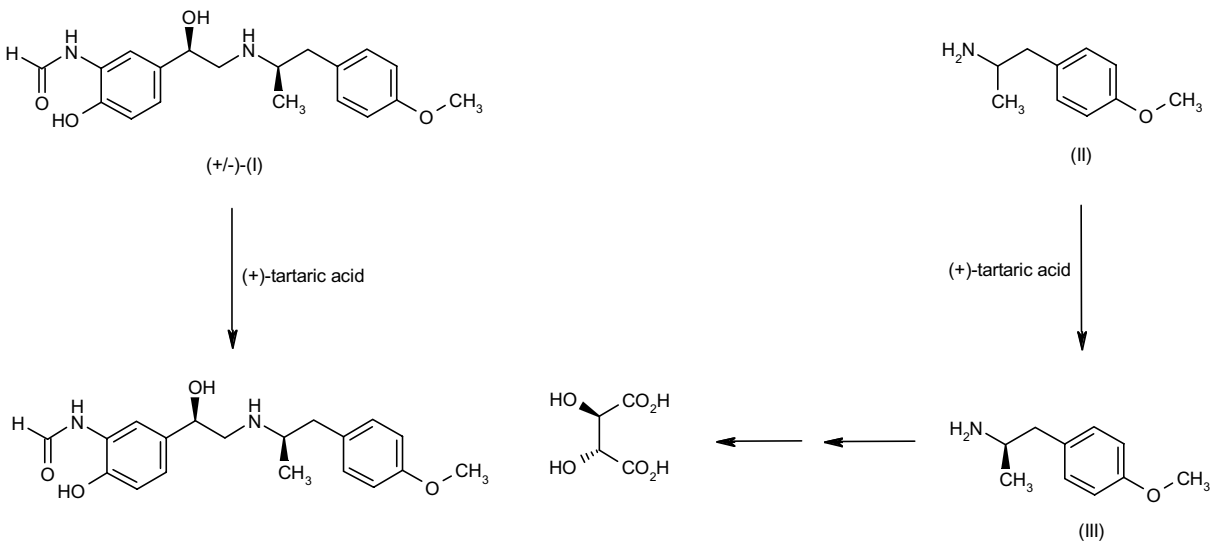
Arformoterol tartrate can be obtained by resolution of racemic formoterol (I) with D-(+)-tartaric acid. Analogously, racemic *p*-methoxy-α-methylphenethylamine (II) is resolved by means of D-(+)-tartaric acid to provide the (*R*)-amine (III), which is used as the starting material for the asymmetric synthesis of arformoterol tartrate (1). Scheme 1.

In an alternative method, (*p*-methoxyphenyl)acetone (IV) is condensed with 1(*R*)-phenylethylamine (V), followed by diastereoselective hydrogenation of the intermediate imine over Raney nickel to afford the (*R,R*)-amine (VI). Ring opening of the racemic epoxide (VII) with the chiral amine (VI) produces the amino alcohol adduct (VIII) as an epimeric mixture. Subsequent nitro group reduction and formylation in the presence of formic acid and Raney nickel leads to the corresponding mixture of epimeric formamides (IX) and (X), which are separated utilizing semi-preparative chromatography. The target isomer (X) is finally deprotected by hydrogenation over Pd/C (2). Scheme 2.

A different strategy involves the coupling between two enantiopure building blocks, the amine (XII) and epoxide (XVII). Reductive amination of (*p*-methoxyphenyl)acetone (IV) with benzylamine using H_2 and Pt/C yields the racemic secondary amine (XI), which is resolved by using (*S*)-mandelic acid to give the optically pure (*R*)-amine (XII) (3). The chiral epoxide (XVII) is in turn prepared by the following procedure. Enantioselective reduction of 2-bromo-4'-benzyloxy-3'-nitroacetophenone (XIII) with borane in the presence of the chiral oxazaborolidines (XVa,b) affords the (*R*)-bromohydrin (XVI), which is converted to the epoxide (XVII) in the presence of an aqueous base. The chiral oxazaborolidine catalysts (XVa) and (XVb) are prepared by reaction of 1(*R*)-amino-2(*S*)-indanol (XIV) with trimethylboroxine and borane, respectively (3-7). Condensation of epoxide (XVII) with amine (XII) affords the desired (*R,R*)-amino alcohol (XVIII) which after nitro group reduction and subsequent formylation of the resulting amine (XIX) leads to the formamide (XX). This is then converted to arformoterol by catalytic hydrogenolysis of its benzyl protecting groups (3, 5). Scheme 3.

P. Revill, N. Serradell, J. Bolós, M. Bayés. Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

Scheme 1: Synthesis of Arformoterol Tartrate



The enantiomerically pure building blocks (III) and (XVII) have also been obtained by using a chemoenzymatic approach. Bromoketone (XIII), prepared by bromination of 4'-benzyloxy-3'-nitroacetophenone (XXI), is reduced with borane in cold THF to give the racemic bromohydrin (XXII). Treatment of (XXII) with vinyl acetate in the presence of lipase PS Amano produces the (*S*)-acetate ester (XXIII), while leaving unreacted the desired (*R*)-alcohol (XVI). Cyclization of bromohydrin (XVI) under alkaline conditions gives rise to the target chiral epoxide (XVII). Condensation of anisaldehyde (XXIV) with nitroethane, followed by hydride reduction of the obtained nitrostyrene derivative (XXV), leads to the racemic amine (XXVI). Subsequent treatment of (XXVI) with *Candida antarctica* lipase B (CALB) in the presence of ethyl acetate produces a mixture of unchanged (*S*)-amine (XXVII) along with the desired (*R*)-acetamide (XXVIII). This is then hydrolyzed under alkaline conditions to 1(*R*)-methyl-2-(*p*-methoxyphenyl)ethylamine (III). Coupling between amine (III) and epoxide (XVII) is carried out in the presence of *N,O*-bis-(trimethylsilyl)acetamide (BSA) to avoid the formation of dialkylated products. The obtained amino alcohol adduct (XXIX) is then subjected to nitro group reduction with Fe and HCl, followed by selective primary amino group formylation of diamine (XXX) and finally debenzylation of the *O*-protected arformoterol (XXXI) by hydrogenation over Pd/C (8). Scheme 4.

In a related strategy, the enantiopure bromohydrin (XVI) is first reduced at the nitro group by catalytic hydrogenation, and the resulting amine (XXXII) is then converted to formamide (XXXIII). Condensation between bromohydrin (XXXIII) and amine (XII) affords amino alcohol (XX), which is finally deprotected by hydrogenation over Pd/C (4, 9). Scheme 5.

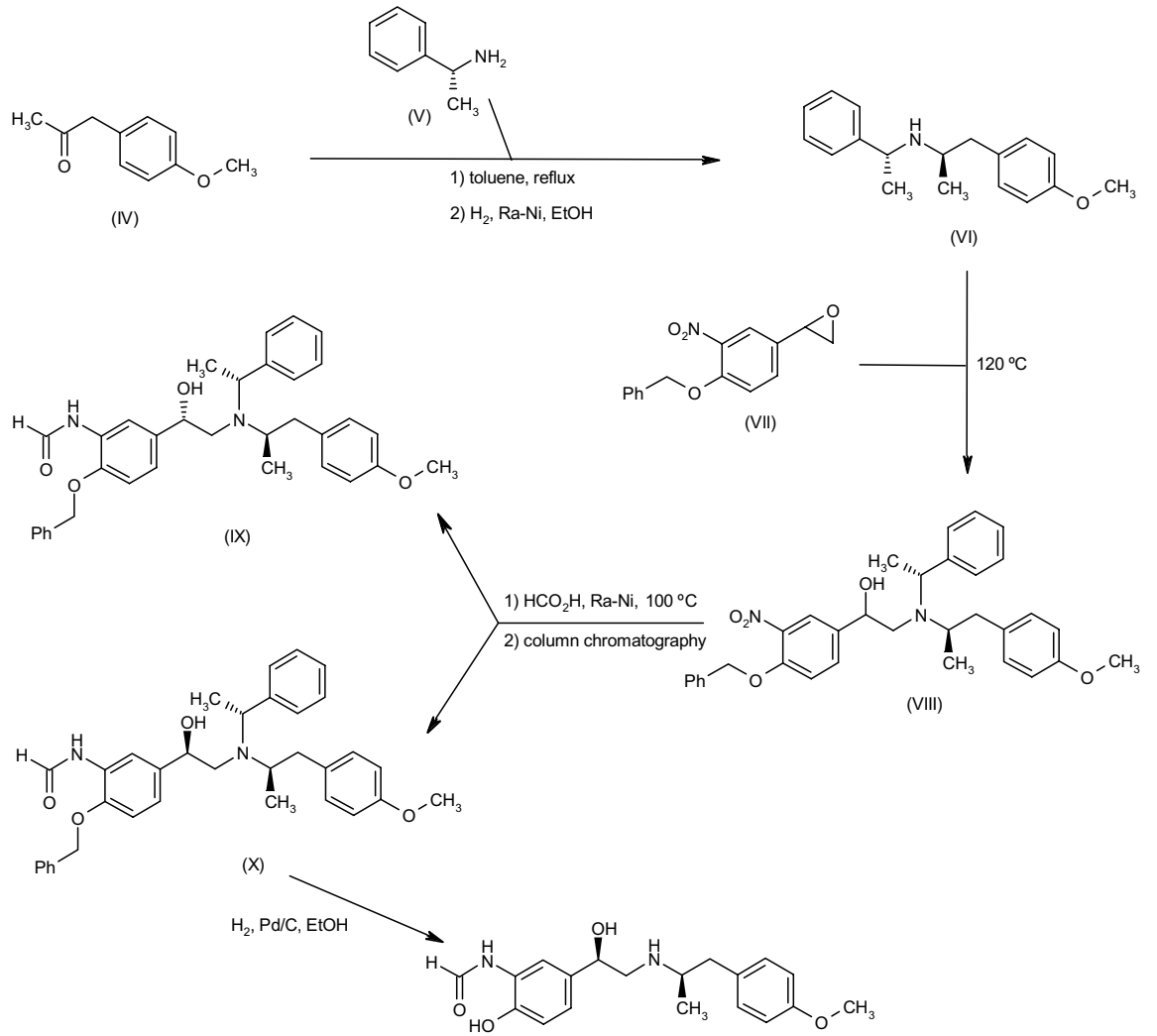
Introduction

Chronic obstructive pulmonary disease (COPD), which includes chronic bronchitis and emphysema, is characterized by an irreversible obstruction of airflow in the lungs and inflammation, and is associated with significant morbidity and mortality. A large epidemiological study indicated that some 24 million people in the U.S. were estimated to have COPD in 2000, and even this figure may be low; the death rate was > 50/100,000 and continues to rise (10-12).

β_2-Adrenoceptor agonists are widely used in the management of COPD and asthma due to their bronchodilating effect (10, 13). Whereas the short-acting inhaled β_2-agonists are very useful in relieving exacerbations, the longer acting agents, such as formoterol, are useful for improving lung function and preventing exacerbations when given together with inhaled corticosteroids (13, 14). However, long-term or high-dose treatment with long-acting β_2-agonists has also been associated with an increased risk of asthma-related death or life-threatening events (13, 15-18). It was eventually discovered that the bronchodilating activity of most currently available β_2-agonists, which are racemic mixtures of (*R*)- and (*S*)-enantiomers, resides mainly in the (*R*)-isomers, while the deleterious effects are mainly attributed to the (*S*)-isomers (19).

Formoterol fumarate (eformoterol fumarate; commercialized as Foradil® by Novartis and as Oxis® by AstraZeneca), a rapid-acting β_2-adrenoceptor agonist that has a long duration of action of up to 12 h, available outside the U.S. to alleviate the symptoms of bronchoconstriction in patients with asthma or COPD whose symptoms do not improve with corticosteroids alone. Formoterol has

Scheme 2: Synthesis of Arformoterol

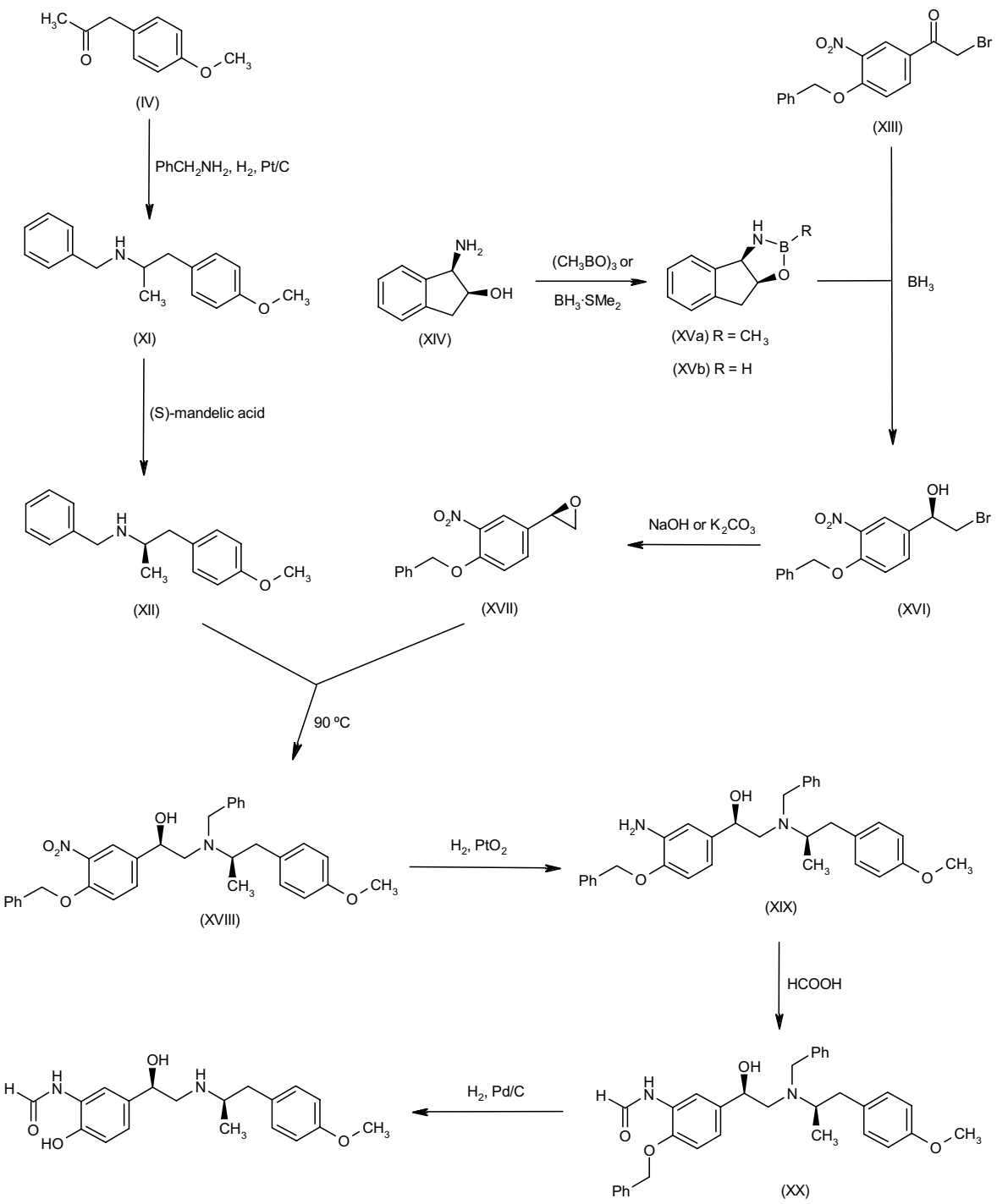


two stereocenters, making four stereoisomers possible: (*R,R*)-, (*R,S*)-, (*S,R*)- and (*S,S*)-formoterol. The commercially available formoterol is a 50:50 mixture of the (*R,R*)- and (*S,S*)-enantiomers. Preclinical studies have demonstrated that (*R,R*)-formoterol (arformoterol) is a full agonist at the β_2-adrenoceptor and is responsible for bronchodilatation. Arformoterol also has antiinflammatory properties. On the other hand, (*S,S*)-formoterol is proinflammatory and may antagonize the pharmacological properties of arformoterol. Therefore, arformoterol was selected for development as a long-acting agent for use in the treatment of bronchoconstriction symptoms in patients with COPD.

Preclinical Pharmacology

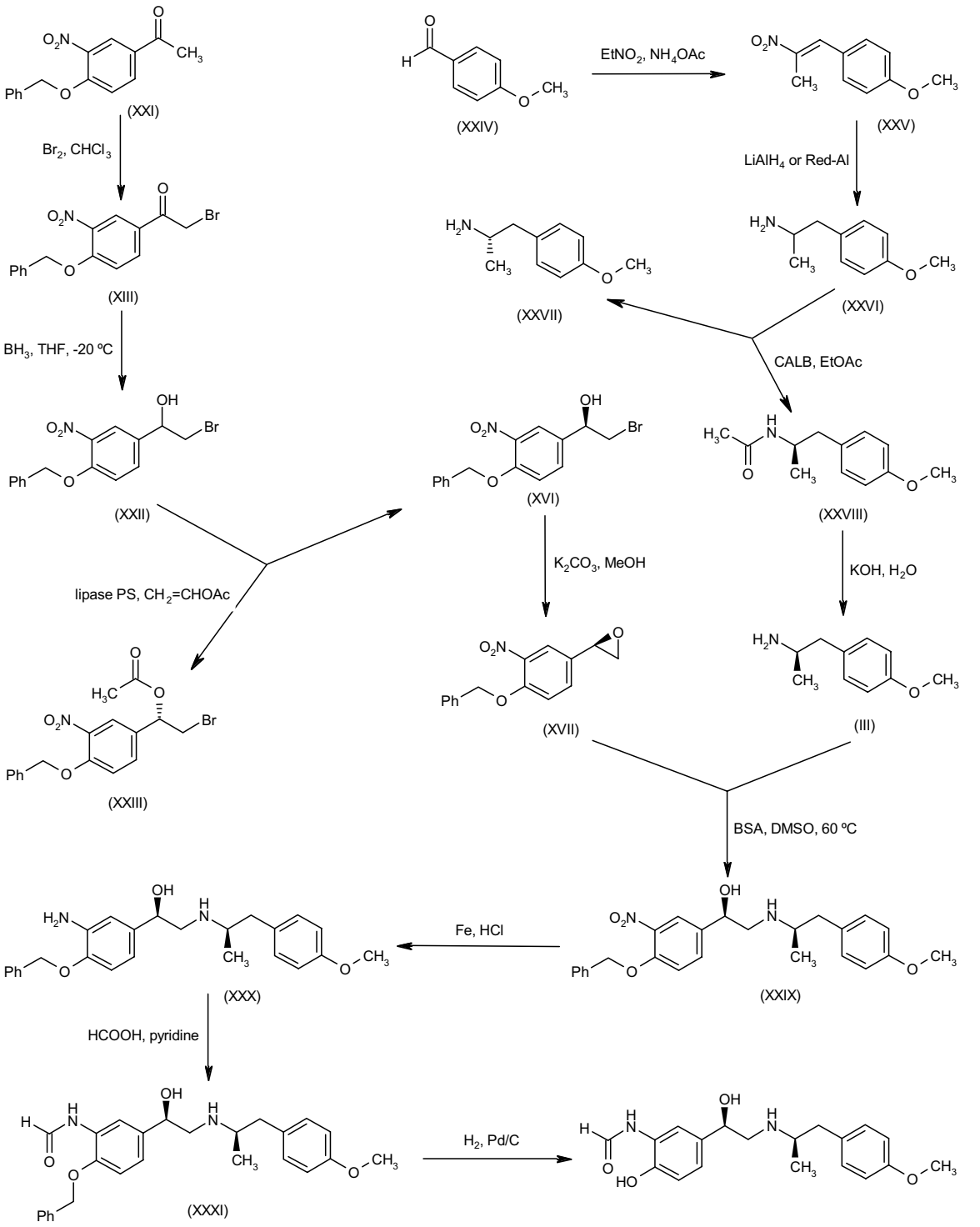
Arformoterol has high binding affinity (K_d = 2.9 nM) for the cloned human β_2-adrenoceptor and much lower affinity for the β_1-adrenoceptor (K_d = 113 nM); the respective K_d values for racemic formoterol are 5.2 and 192 nM, and for (*S,S*)-formoterol 3100 and 6800 nM. Arformoterol therefore has over 1,000 times higher affinity for the β_2-adrenoceptor than (*S,S*)-formoterol. The same order of potency, *i.e.*, arformoterol > racemic- >> (*S,S*)-formoterol, was confirmed in the human BEAS-2B bronchial epithelial cell line, where both arformoterol and racemic formoterol induced cAMP levels equivalent to those seen with the positive control agent isoproterenol. (*S,S*)-Formoterol, on the other hand, induced cAMP to just 18% of levels seen for isoproterenol. No effect was observed on muscarinic receptors (20, 21). Further studies demonstrated that arformoterol acts as a full or nearly full agonist at the β_2-adrenoceptor, while the (*S,S*)-enantiomer acts as an inverse agonist (22).

Scheme 3: Synthesis of Arformoterol



The antiinflammatory effects of arformoterol were demonstrated in several assays. It significantly induced nitric oxide (NO) release and slightly reduced levels of IL-8 and RANTES in stimulated small airways epithelial cells, whereas (*S,S*)-formoterol had no effect (23). In human peripheral blood mononuclear cells (PBMCs), it was also shown to inhibit the production of lipopolysaccharide (LPS)-stimulated TNF-α and enhance the release of IL-10, whereas the (*S*)- and (*S,S*)-enantiomers exhibited no significant effect at up to 10 μM (24). Moreover, in

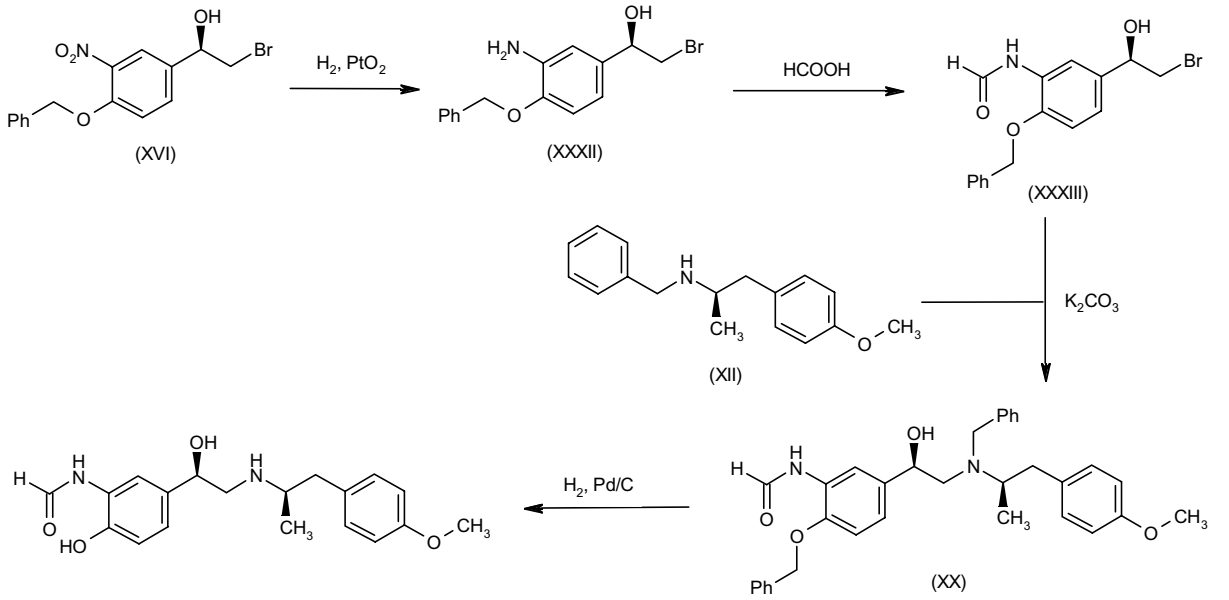
Scheme 4: Synthesis of Arformoterol



contrast to the (*S,S*)-enantiomer, arformoterol was not associated with enhanced production of proinflammatory mediators (IL-4, histamine, PGD_2) in stimulated human mast cells (25).

In human airways smooth muscle cells, the dexamethasone-induced reduction in granulocyte-macrophage colony-stimulating factor (GM-CSF) production was further decreased by the addition of arformoterol or

Scheme 5: Synthesis of Arformoterol



(*R*)-albuterol, whereas (*S*)-albuterol increased GM-CSF production in the presence of dexamethasone (26).

Incubation of equine parasympathetic prejunctional nerves pretreated with atropine to block muscarinic receptors with the racemate (1 μM), arformoterol (1 μM) or (*S,S*)-formoterol (10 μM) augmented the release of acetylcholine upon electrical stimulation of the nerves. Spasmolytic activity was seen for arformoterol and the racemate, but not the (*S*)-enantiomer, in acetylcholine-precontracted equine tracheal smooth muscle. It was suggested that the cholinergic effect of the (*S*)-enantiomer may potentially contribute to the bronchial hyperresponsiveness that is seen with the continuous use of the racemate (27).

Arformoterol was 1,000-fold more potent than (*S,S*)-formoterol in relaxing human bronchial rings and in protecting guinea pig tracheal strips and human bronchial rings against carbachol- and histamine-induced contractions (20, 28, 29). Moreover, in tissues preincubated with (*S,S*)-formoterol, the contractile response to high concentrations of carbachol was enhanced, an effect not seen with arformoterol (20).

In murine asthma models, arformoterol reduced the early and late responses to ovalbumin challenge, and it reduced airways hyperresponsiveness to methacholine. Arformoterol was also associated with reduced numbers of mononuclear cells and eosinophils infiltrating the lungs compared to controls. (*S,S*)-Formoterol had no affect on the allergic responses to ovalbumin or the responsiveness to methacholine, but it was associated with an increase in airways IL-4 levels and basal airways tone, and enhanced peribronchial and perivascular inflammation. These data provide further evidence that arformoterol is antiinflammatory, while (*S,S*)-formoterol is proinflammatory and may enhance the bronchoconstrictor response in asthma (25, 30).

In a guinea pig model of ovalbumin-induced asthma, the ID_{50} values for racemate and arformoterol, given by intragastric gavage, for reducing lung resistance over the first 30 min of treatment were 0.96 and 0.52 mg/kg, respectively; they also dose-dependently increased dynamic lung compliance with respective ID_{50} values of 1.59 and 0.43 mg/kg. These improvements correlated with reduced levels of inflammatory cells in the bronchoalveolar lavage fluid (BALF; ID_{50} = 1.48 and 0.8 mg/kg, respectively) (31). In further studies of histamine- or allergen-induced bronchoconstriction in guinea pigs, the ED_{50} values for arformoterol were 0.5 and 20 μg/kg i.v., respectively. By contrast, the ED_{50} values for (*S,S*)-formoterol were 20-200-fold higher (32). In another study, arformoterol and the racemate gave ED_{50} values of 1 and 1.6 nmole/kg i.v., respectively, for inhibition of histamine-induced bronchoconstriction in guinea pigs, whereas (*S,S*)-formoterol had no significant effect at up to 200 nmole/kg. Similar results were obtained against ovalbumin-induced allergic bronchospasm (20).

In a model of methacholine-induced bronchoconstriction in rhesus monkeys, racemic formoterol inhibited bronchoconstriction in a dose-dependent manner by up to 76% at the highest dose tested of 1.15 μg/kg. An equivalent response was seen with arformoterol at 0.56 μg/kg, but no response was seen with 0.54 μg/kg (*S,S*)-formoterol.

Racemic formoterol and arformoterol, but not (*S,S*)-formoterol, also increased heart rate (up to 24%) (33).

Arformoterol is unstable in aqueous solution. In order to find a more stable form for storage of the drug, arformoterol was lyophilized in various excipients. Arformoterol was found to be optimally stable in lactose over a range of different temperatures and at low humidity (34).

Pharmacokinetics and Metabolism

In vitro studies using human liver microsomes demonstrated that the rate of sulfation of (*S,S*)-formoterol or the racemate was 2-fold faster than for arformoterol, indicating that the clearance of the latter may be slower and the half-life longer (20, 32).

In an analysis of the exposure-response relationship for single doses of nebulized arformoterol in 479 subjects with COPD (data from 1 phase II and 2 phase III studies), a pharmacokinetic/pharmacodynamic (PK/PD) model demonstrated a clear relationship between the peak percent change in forced expiratory volume in 1 s (FEV_1) and plasma concentrations of arformoterol. The maximal % change in FEV_1 from the predose baseline was 38%, and the estimated EC_{50} was 0.61 pg/ml (plasma). The estimated first-order distribution rate constant (k_{eo}) was 1.49 h^{-1}, which accounts for the rapid action of the drug (35). The data were further used to develop a 2-compartment population pharmacokinetic model with first-order absorption and elimination. This model predicts a linear AUC:dose-response relationship over the doses used in these studies and should be useful in estimating AUC in further clinical studies (36).

Safety

In rats, the i.v. LD_{50} of arformoterol was 100 mg/kg and that for (*S,S*)-formoterol was 50 mg/kg; the LD_{50} value for oral administration was > 1000 mg/kg. Both isomers were associated with abnormal stance/gait, decreased activity, decreased muscle tone and labored breathing (20, 37).

Clinical Studies

In a double-blind, crossover study, 31 adults with asthma were randomized to receive single nebulized doses of arformoterol of 12, 24, 48 or 72 μg, placebo or Ventolin® (salbutamol sulfate; 2.5 μg) on separate days. The peak % change in FEV_1 was superior to placebo for all doses of arformoterol, and not different from that of Ventolin®. At 24 h postdose, the FEV_1 for the 24- and 72-μg doses of arformoterol was significantly greater than for placebo or Ventolin®. The onset of action was comparable to that for Ventolin® and shorter than for placebo. The use of rescue medication and exacerbation of asthma attacks were lower in all arformoterol groups than on placebo or Ventolin®. Safety parameters of ventricular heart rate, glucose, potassium and Q-T_c interval were similar for all groups (38).

In a randomized, double-blind, double-dummy study, single nebulized doses of the racemate (4.5 and 36 μg), arformoterol (2.25 and 18 μg) or (*S,S*)-formoterol (18 μg) or placebo were administered to 46 asthma patients. Single high doses of both racemate and arformoterol caused bronchodilatation even up to 24 h after administration (average FEV_1 = 8% and 11% above placebo, respectively), although these doses were also associated with an initial increase in heart rate. (*S,S*)-Formoterol showed no significant effects on FEV_1 or heart rate (39).

Arformoterol was compared to the long-acting β_2-adrenoceptor agonist salmeterol in a dose-ranging study in patients with COPD. Sixty-two patients received nebulized arformoterol at doses of 9.6 μg once daily, 24 μg b.i.d., 48 μg once daily or 96 μg once daily, or placebo and then received open-label salmeterol 42 μg b.i.d. All doses of arformoterol, except the lowest dose, led to significant improvements in the $AUC_{0\text{-}24h}$ for % change in FEV_1 *versus* placebo and salmeterol. Improvements in lung function were comparable at the two higher doses of arformoterol (40). The results from this and one of the following phase III trials are summarized in Table I.

In two identical multicenter, double-blind, double-dummy phase III studies, 717 and 739 COPD patients were randomized to either nebulized arformoterol (15 μg

Table I: Clinical studies of arformoterol tartrate (from Prous Science Integrity®).

Drug	Design	Treatments	n	Conclusions	Ref.
Chronic obstructive pulmonary disease	Randomized Crossover	Arformoterol, 9.6 μg o.d. → Salmeterol, 42 μg b.i.d. Arformoterol, 24 μg b.i.d. → Salmeterol, 42 μg b.i.d. Arformoterol, 48 μg o.d. → Salmeterol, 42 μg b.i.d. Arformoterol, 96 μg o.d. → Salmeterol, 42 μg b.i.d. Placebo → Salmeterol, 42 μg b.i.d.	62	Long-lasting bronchodilatation was seen in chronic obstructive pulmonary disease patients treated with arformoterol at doses above 9.6 μg o.d.	40
Chronic obstructive pulmonary disease	Randomized Double-blind Multicenter	Arformoterol, 15 μg b.i.d. x 12 wks Arformoterol, 25 μg b.i.d. x 12 wks Arformoterol, 50 μg b.i.d. x 12 wks Salmeterol, 42 μg b.i.d. x 12 wks Placebo b.i.d.	717	Arformoterol significantly improved airways function in patients with chronic obstructive pulmonary disease	41

b.i.d., 25 μg b.i.d. or 50 μg once daily), placebo or salmeterol (42 μg b.i.d. by metered-dose inhaler) for 12 weeks. Using data from both studies, the primary endpoint, % change in morning trough FEV_1 (*i.e.*, 12 h after the first dose in the b.i.d groups or 24 h after the first dose in the once-daily group), was significantly improved in all arformoterol groups by 22.1%, 25.9% and 20.3%, respectively, and in the salmeterol group by 21.5% *versus* 6.9% for placebo. After 12 weeks of treatment, % change in FEV_1 values for all active treatment groups was significantly greater than for placebo (14.6-17.8% *versus* 4.7%). The secondary endpoint of mean % change in FEV_1 AUC_{0-12h} was also significantly improved in the arformoterol treatment groups *versus* placebo throughout the study, whereas the improvement for salmeterol at the end of the study was not significantly different from placebo. The median time to achieve a response of at least 10% in FEV_1 at week 12 was 3-14 min for patients treated with arformoterol and 132 min for those treated with salmeterol. The percentage of patients who achieved at least 10% improvement in FEV_1 at week 0 was 88.3-95.7% on arformoterol and 85.1% on salmeterol *versus* 55% on placebo; at 12 weeks, the respective percentages were 77.1-87.5%, 58.4% and 40.7-47.6%. Arformoterol was well tolerated in both studies, with exacerbations of COPD symptoms being the same for all active and placebo treatment groups (41-46). A 6-month phase III safety and efficacy study of arformoterol in patients with COPD is ongoing (47).

In October, the FDA approved arformoterol tartrate (Brovana™) inhalation solution (nebulizer) as long-term twice-daily maintenance treatment for bronchoconstriction in patients with COPD (48).

Source

Sepracor, Inc. (US).

References

1. Murase, K., Mase, T., Ida, H., Takahashi, K., Murakami, M. *Absolute configurations of four isomers of 3-formamido-4-hydroxy-α-[[N-(p-methoxy-α-methylphenethyl)amino]methyl]benzyl alcohol, a potent β-adrenoreceptor stimulant*. Chem Pharm Bull 1978, 26(4): 1123-9.

2. Trofast, J., Österberg, K., Källström, B.-L., Waldeck, B. *Steric aspects of agonism and antagonism at β-adrenoceptors: Synthesis of and pharmacological experiments with the enantiomers of formoterol and their diastereomers*. Chirality 1991, 3(6): 443-50.

3. Hett, R., Fang, Q.K., Gao, Y., Hong, Y., Butler, H.T., Nie, X., Wald, S.A. *Enantio- and diastereoselective synthesis of all four stereoisomers of formoterol*. Tetrahedron Lett 1997, 38(7): 1125-8.

4. Hett, R. et al. *Large-scale synthesis of enantio- and diastereomerically pure (R,R)-formoterol*. Org Process Res Dev 1998, 2(2): 96-9.

5. Wilkinson, H.S. et al. *Modulation of catalyst reactivity for the chemoselective hydrogenation of functionalized nitroarene: Preparation of a key intermediate in the synthesis of (R,R)-formoterol tartrate*. Org Process Res Dev 2000, 4(6): 567-70.

6. Wilkinson, S.C., Tanoury, G.J., Wald, S.A., Senanayake, C.H. *Diethylanilineborane: A practical, safe, and consistent-quality borane source for the large-scale enantioselective reduction of a ketone intermediate in the synthesis of (R,R)-formoterol*. Org Process Res Dev 2002, 6(2): 146-8.

7. Hett, R., Senanayake, C.H., Wald, S.A. *Conformational toolbox of oxazaborolidine catalysts in the enantioselective reduction of α-bromo-ketone for the synthesis of (R,R)-formoterol*. Tetrahedron Lett 1998, 39(13): 1705-8.

8. Campos, F., Bosch, M.P., Guerrero, A. *An efficient enantioselective synthesis of (R,R)-formoterol, a potent bronchodilator, using lipases*. Tetrahedron: Asymmetry 2000, 11(13): 2705-17.

9. Tanoury, G.J., Hett, R., Kessler, D.W., Wald, S.A., Senanayake, C.H. *Taking advantage of polymorphism to effect an impurity removal: Development of a thermodynamic crystal form of (R,R)-formoterol tartrate*. Org Process Res Dev 2002, 6(6): 855-62.

10. Prous Science Disease Briefings: Chronic Obstructive Pulmonary Disease (Chronic Bronchitis and Emphysema) (online publication).

11. Spurzem, J.R., Rennard, S.I. *Pathogenesis of COPD*. Semin Respir Crit Care Med 2005, 26(2): 142.

12. Mannino, D.M., Homa, D.M., Akinbami, L.J., Ford, E.S., Redd, S.C. *Chronic obstructive pulmonary disease surveillance—United States, 1971-2000*. Respir Care 2002, 47(10): 1184-99.

13. Prous Science Disease Briefings: Asthma (online publication).

14. Blaiss, M.S., Heinly, T. *Pharmacologic agents for the long-term control of asthma*. P T 1999, 24(9): 416.

15. Spitzer, W.O., Suissa, S., Ernst, P. et al. *The use of beta-agonists and the risk of death and near death from asthma*. New Engl J Med 1992, 326(8): 501-6.

16. Suissa, S., Ernst, P., Boivin, J.F. et al. *A cohort analysis of excess mortality in asthma and the use of inhaled beta-agonists*. Am J Respir Crit Care Med 1994, 149(3): 604-10.

17. Salpeter, S.R., Buckley, N.S., Ormiston, T.M., Salpeter, E.E. *Meta-analysis: Effect of long-acting beta-agonists on severe asthma exacerbations and asthma-related deaths*. Ann Intern Med 2006, 144(12): 904-12.

18. Donohue, J.F., Fromer, L. *Long-acting beta-agonists role in asthma management*. J Fam Pract 2006, Suppl.: 1.

19. Handley, D.A., Anderson, A.J., Koester, J., Snider, M.E. *New millennium bronchodilators for asthma: Single-isomer beta agonists*. Curr Opin Pulm Med 2000, 6(1): 43-9.

20. Handley, D.A., Senanayake, C.H., Dutczak, W. et al. *Biological actions of formoterol isomers*. Pulm Pharmacol Ther 2002, 15(2): 135-45.

21. Handley, D.A., Walle, T., Fang, K.Q., Hett, R., Gao, Y. *Receptor binding profiles of (RR)-eformoterol*. Am J Respir Crit Care Med 2000, 161(3, Part 2): A436.

22. Moore, R.H., Knoll, B.J., Dickey, B.F. *High efficacy of (R,R)-formoterol in β_2-adrenoceptor internalization*. Am J Respir Crit Care Med 2001, 163(5, Suppl.): A144.

23. Dominguez, P.J., Pergolizzi, R., Millan, C., Frieri, M. *Cytokine, chemokine, and nitric oxide (NO) release in stimulated small airway epithelial cells (SAEC) treated with β_2-agonist enantiomers of formoterol*. J Allergy Clin Immunol 2000, 105(1, Part 2): Abst 857.

24. Calhoun, W.J., Ameredes, B.T., Neely, C., Dixon-McCarthy, B. *Production of IL-10 relative to TNF-α by blood mononuclear cells is enhanced by R-enantiomers of beta-receptor agonists*. J Allergy Clin Immunol 2002, 109(1, Part 2): Abst 3.

25. Abraha, D., Cho, S.H., Agrawal, D.K., Park, J.M., Oh, C.K. *(S,S)-Formoterol increases the production of IL-4 in mast cells and the airways of a murine asthma model*. Int Arch Allergy Immunol 2004, 133(4): 380-8.

26. Ameredes, B.T., Gligonic, A.L., Calhoun, W.J. *Enantiomers of beta-agonists exhibit differential effects in combination with dexamethasone on GM-CSF production in human airway smooth muscle cells*. J Allergy Clin Immunol 2004, 113(2, Suppl. 1): Abst 659.

27. Zhang, X.Y., Zhu, F.X., Olszewski, M.A., Robinson, N.E. *Effects of enantiomers of β_2-agonists on ACh release and smooth muscle contraction in the trachea*. Am J Physiol 1998, 274(1, Part 1): L32-8.

28. Schmidt, D., Källström, B.L., Waldeck, B., Branscheid, D., Magnussen, H., Rabe, K.F. *The effect of the enantiomers of formoterol on inherent and induced tone in guinea-pig trachea and human bronchus*. Naunyn-Schmied Arch Pharmacol 2000, 361(4): 405-9.

29. Schmidt, D., Waldeck, B., Magnussen, H., Rabe, K.F. *Effect of formoterol enantiomers on inherent induced tone in human airway smooth muscle*. Am J Respir Crit Care Med 1999, 159(3, Part 2): A113.

30. Agrawal, D.K. *Protective effect of (RR)-formoterol in early and late allergic response and airway hyperresponsiveness in a mouse model of adthma*. Am J Respir Crit Care Med 2001, 163(5, Suppl.): A590.

31. Xie, Q.M., Chen, J.Q., Shen, W.H., Yang, Q.H., Bian, R.L. *Comparison of bronchodilating and antiinflammatory activities of oral formoterol and its (R,R)-enantiomers*. Acta Pharmacol Sin 2003, 24(3): 277-82.

32. Handley, D.A., Walle, T., Fang, K.Q., Gao, Y. *In vivo pharmacological properties of (R,R)-eformoterol*. Am J Respir Crit Care Med 2000, 161(3, Part 2): A106.

33. Fozard, J.R., Buescher, H. *Comparison of the anti-bronchoconstrictor activities of inhaled formoterol, its (R,R)- and (S,S)-enantiomers and salmeterol in the rhesus monkey*. Pulm Pharmacol Ther 2001, 14(4): 289-95.

34. Kirsch, L., Zhang, S., Muangsiri, W., Redmon, M., Luner, P., Wurster, D. *Development of a lyophilized formulation for (R,R)-formoterol (L)-tartrate*. Drug Dev Ind Pharm 2001, 27(1): 89-96.

35. Maier, G., Kharidia, J., Hanrahan, J.P. et al. *Exposure-response relationship for nebulized arformoterol in subjects with COPD*. 16th Annu Congr Eur Respir Soc (ERS) (Sept 2-6, Munich) 2006, Abst P2498.

36. Maier, G., Kharidia, J., Hanrahan, J.P. et al. *Population pharmacokinetics of nebulized arformoterol in subjects with COPD*. 16th Annu Congr Eur Respir Soc (ERS) (Sept 2-6, Munich) 2006, Abst P2499.

37. Dutczak, W., Kern, T., Handley, D.A. *Acute IV and oral toxicity of (R,R)- and (S,S)-eformoterol in rats*. Am J Respir Crit Care Med 2000, 161(3, Part 2): A106.

38. Vaickus, L., Claus, R. *(R,R)-Formoterol - Rapid onset and 24 hour duration of response after a single dose*. Am J Respir Crit Care Med 2000, 161(3, Part 2): A191.

39. Lötvall, J., Palmqvist, M., Ankerst, J. et al. *The effect of formoterol over 24 h in patients with asthma: The role of enantiomers*. Pulm Pharmacol Ther 2005, 18(2): 109-13.

40. Baumgartner, R.A., Razzetti, A., Claus, R., McVicar, W., Hanrahan, J.P. *A crossover dose-ranging study of arformoterol in patients with COPD*. 102nd Int Conf Am Thorac Soc (May 19-24, San Diego) 2006, Abst A847.

41. Hanrahan, J.P., Sahn, S.A., Fogarty, C.M., Sciarappa, K., Baumgartner, R.A. *Efficacy and safety of arformoterol in COPD: A prospective phase 3 clinical trial*. 102nd Int Conf Am Thorac Soc (May 19-24, San Diego) 2006, Abst A847.

42. Hanrahan, J.P., Smith, W.B., Sciarappa, K., McVicar, W.K., Baumgartner, R.A. *Efficacy and safety of nebulized arformoterol in COPD: A prospective, phase III clinical trial*. Chest 2006, 130(4, Suppl.): 181S.

43. Hanrahan, J.P., Sahn, S.A., Busse, W.W., Sciarappa, K., Baumgartner, R.A. *Efficacy of nebulized arformoterol, a long-acting β_2-adrenergic bronchodilator, in patients with COPD*. 16th Annu Congr Eur Respir Soc (ERS) (Sept 2-6, Munich) 2006, Abst P1276.

44. Hanrahan, J.P., Kerwin, E., Cheng, H., Grogan, D.R., Baumgartner, R.A. *Arformoterol in COPD: safety results from two pooled phase 3 trials*. 16th Annu Congr Eur Respir Soc (ERS) (Sept 2-6, Munich) 2006, Abst P2500.

45. *Sepracor presents arformoterol data at American Thoracic Society International Conference*. Sepracor Press Release May 24 2006.

46. *Brovana™ (arformoterol tartrate) inhalation solution study data presented at American College of Chest Physicians Annual Conference*. Sepracor Press Release October 23, 2006.

47. *Safety and efficacy study of arformoterol tartrate inhalation solution in the treatment of subjects with chronic obstructive pulmonary disease (NCT00250679)*. ClinicalTrials.gov Web site 2006.

48. *FDA approves Sepracor's Branova(TM) (arformoterol tartrate) inhalation solution for chronic obstructive pulmonary disease*. Sepracor Press Release October 6, 2006.