Synthesis and Structure−Activity Relationships of Long-acting β_2 Adrenergic Receptor Agonists Incorporating Arylsulfonamide Groups

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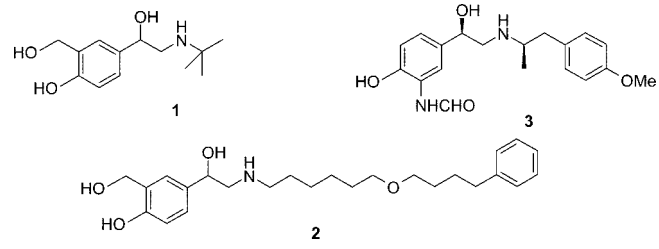
A series of saligenin alkoxyalkylphenylsulfonamide β_2 adrenoceptor agonists were prepared by reacting a protected saligenin oxazolidinone with alkynyloxyalkyl bromides, followed by Sonogashira reaction, hydrogenation, and deprotection. The *meta*-substituted primary sulfonamide was more potent than the *para*- and the *ortho*-analogues. Primary sulfonamides were more potent than the secondary and tertiary analogues. The onset and duration of action in vitro of selected compounds was assessed on isolated superfused guinea pig trachea. Sulfonamide **29b** had the best profile of potency, selectivity, onset, and duration of action on both guinea pig trachea and human bronchus. Furthermore, **29b** was found to have low oral bioavailability in rat and dog and also to have long duration of action in an in vivo model of bronchodilation. Crystalline salts of **29b** were identified that had suitable properties for inhaled administration. A proposed binding mode for **29b** to the β_2-receptor is presented.

Introduction

Asthma is a common and chronic disease characterized by an increase in inflammatory cell population in the epithelium and submucosa of the airways.[1] There are two major components of asthma pathophysiology, airway inflammation and smooth muscle dysfunction. Bronchoscopy studies of patients with asthma have shown that airway inflammation may lead to long-term structural changes in airway wall components.[2] The smooth-muscle dysfunction is evidenced by exaggerated bronchoconstriction, bronchial hyperresponsiveness, excessive proliferation (hyperplasia), and excessive growth (hypertrophy) of the airway smooth-muscle cells[3] and their release of pro-inflammatory mediators.[4] Increased vascularity in the subepithelial layers contributes to the thickening of the airway wall, leading to airway narrowing as the smooth muscle contracts. Airway inflammation is the result of the recruitment and activation of a range of inflammatory cells, including mast cells, eosinophils, and T-lymphocytes and the release of mediators that perpetuate the inflammatory cycle causing edema formation and epithelial damage.[5] Airway remodelling processes are thought to be mediated by angiogenic (blood vessel development) mediators such as vascular endothelial growth factor and basic fibroblast growth factor.[2,6] The bronchial hyper-responsiveness and constriction lead to the symptoms of the disease, which are cough, wheezing, breathlessness, and chest tightness.

There are two major categories of medicines used in asthma treatment: bronchodilators and anti-inflammatories. Bronchodilators may be β_2 adrenoceptor agonists or in some cases theophylline. Anti-inflammatories may be corticosteroids or nonsteroidal anti-inflammatories. Inhaled β_2-agonists are the most effective bronchodilators, and inhaled corticosteroids are the mainstay anti-inflammatory treatment for asthma, offering proven benefits in reducing the burden of this disease.[7,8] There are two classes of β_2-agonists: the short-acting (first-generation) and the long-acting (second-generation) agonists. The short-acting agonists, typified by salbutamol (**1**), have a rapid onset of action and relieve symptoms for 3−6 h. The two currently prescribed inhaled long-acting β_2-agonists are salmeterol (**2**) and formoterol (**3**). A racemic mixture of salmeterol xinafoate (1-hydroxy-2-naphthoic acid) salt marketed by GlaxoSmithKline has lower intrinsic activity than salbutamol and a delayed onset of action, but a duration of action of 12 h, which is independent of dose.[9,10] In contrast formoterol fumarate salt marketed by Novartis is a single racemic diastereoisomer (*RR*, *SS*), high intrinsic efficacy agonist with onset of action similar to salbutamol, and a dose-dependent duration of action.[10,11] We are interested in identifying inhaled β_2-agonists with extended duration of action that are suitable for once-daily dosing as the third-generation products from this therapeutic class. Although salmeterol and formoterol show quite different profiles of



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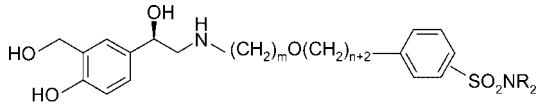


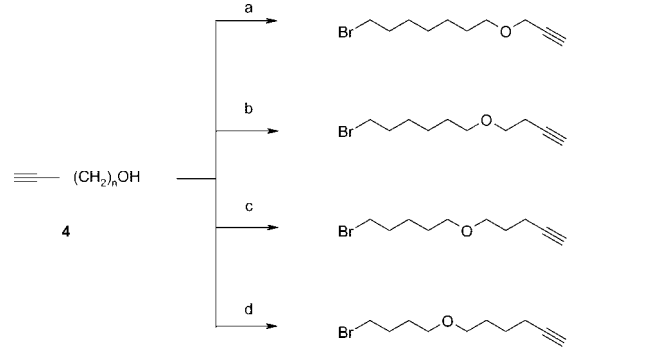
Figure 1

relaxation of both guinea pig trachea and human bronchus, both are used successfully as twice-daily bronchodilators in the clinic. In the search for a once-daily β_2 agonist, candidates have been sought, showing reduced systemic exposure/improved therapeutic index and/or an inherently longer duration of action at the β_2 adrenoceptor. Receptor desensitization is an autoregulatory process associated with β-adrenoceptor activation, which operates as a safety device to prevent overstimulation of receptors on excessive β-agonist exposure. As desensitization results from agonist occupancy and can be inhibited by antagonists, it follows that a lower intrinsic efficacy agonist would be less prone to induce receptor desensitization than a high intrinsic efficacy agonist.[12] Indeed, this has been demonstrated clinically with β_2-agonists, where a degree of bronchodilator tolerance was observed with the high-efficacy agonist formoterol on chronic exposure[13] but not with the lower intrinsic efficacy agonist salmeterol.[14] Recently, the mechanism of the difference in desensitization between salmeterol and formoterol has been reported.[15] Following phosphorylation, the formoterol-stimulated receptor binds β-arrestin and internalizes, whereas this does not occur with salmeterol. In addition to desensitization processes, β-agonists, acting through the cyclic AMP pathway, also dramatically modulate β_2-receptor gene expression. Cells treated with salmeterol, however, had no such down-regulating effect on β_2-receptor gene expression.[16] It was therefore decided to use our own salmeterol arylethanolamine (saligenin) pharmacophore as the starting point for this project. It was reported that a significant contribution to the cardiovascular effects of inhaled salmeterol seen at doses higher than those used clinically results from the major fraction of the dose (80−90%) that is swallowed and is available for absorption.[17] Therefore, reducing the oral bioavailability of our target compounds was considered critical to achieving a once-daily β_2 agonist with an improved safety profile. Substitution on the right-hand side phenyl ring of salmeterol was known to retain β_2 agonist affinity.[18] It was hypothesized that a polar substituent, such as urea, sulfonamide, or heterocycle, which contravened the Lipinski rules,[19] might be expected to show reduced oral bioavailability. Herein we describe our efforts in identifying a novel class of sulfonamide containing β_2-agonists (Figure 1). The profile of the target molecule was a potent, homochiral, and selective β_2-agonist with intrinsic activity similar to or better than that of salmeterol, improved therapeutic index over salmeterol, which might offer safe once daily bronchodilation in the clinic. The (*R*)-enantiomer of salmeterol was reported to be the more potent isomer,[9] so from the outset it was decided to prepare homochiral compounds with the (*R*)-configuration.

Chemistry. The intermediate alkynyl bromides[18] **5**−**8** were prepared by phase transfer catalyzed alkylation of alkynols **4** (n = 1−4) with excess of the appropriate dibromoalkanes (3 equiv) in order to minimize the bis-alkylation products (Scheme 1).

The intermediate iodo- or bromo-arenesulfonamides (**9** and **10**, respectively) were prepared by reaction of the respective sulfonyl chlorides with the appropriate amine or ammonia. The sulfonamide **9j** was prepared from 3-iodobenzyl bromide by displacement of bromide with sodium sulfite, followed by conversion to the sulfonyl chloride and then reaction with aqueous ammonia (Scheme 2).

Scheme 1[a]

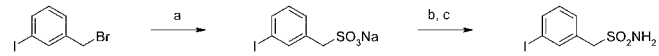


[a] Reagents and conditions: 50% NaOH, Bu_4NHSO_4, (a) 1,7-dibromoheptane; (b) 1,6-dibromohexane; (c) 1,5-dibromopentane; (d) 1,4-dibromobutane.

Scheme 2[a]

R =

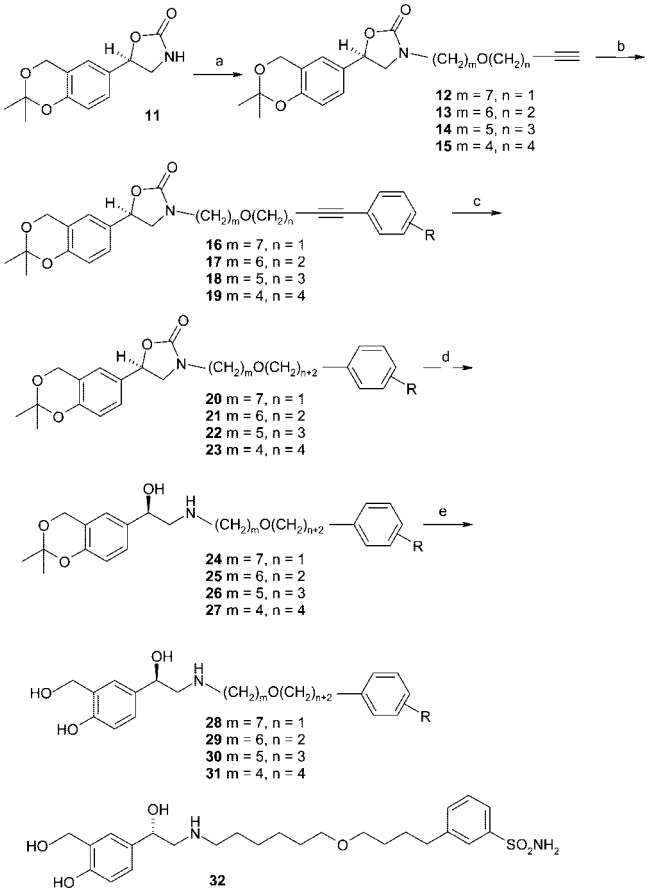
a	2-SO_2NH_2	**f**	3-SO_2NHPr^i
b	3-SO_2NH_2	**g**	3-SO_2NHcyclohexyl
c	4-SO_2NH_2	**h**	3-SO_2piperidinyl
d	3-SO_2NHMe	**i**	3-SO_2morpholinyl
e	3-SO_2NMe_2	**j**	3-$CH_2SO_2NH_2$



[a] Reagents and conditions: (a) sodium sulfite, acetone, water, 71 °C, 3 h; (b) $POCl_3$, sulfolane, MeCN, 80 °C, 2 h; (c) aq NH_3, THF.

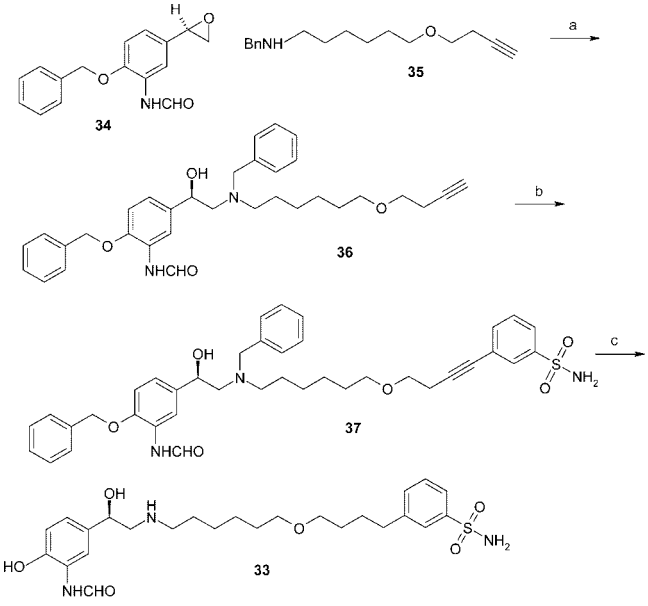
Recently, we have published a versatile and enantioselective route to (*R*)-salmeterol, which utilizes the oxazolidinone moiety as a protecting group for both the amino and hydroxyl groups present in β_2-agonists in order to block further reaction (dialkylation on nitrogen) and concurrently moderate the reactivity of the amine nitrogen toward electrophiles.[20] The general synthetic route to the target sulfonamides (Figure 1) is shown in Scheme 3. Alkylation of the homochiral (*R*)-oxazolidinone **11**[20] with the alkynyl bromides **5**−**8** provided the corresponding alkynes **12**−**15**. Sonogashira coupling[21] of alkynes **12**−**15** with iodo- (**9**) or bromo-arenesulfonamides (**10**) gave alkynyl arenesulfonamides **16**−**19**, which were saturated by catalytic hydrogenation to alkyl arenesulfonamides **20**−**23**. The oxazolidinone ring of **20**−**23** was cleaved using our mild, anhydrous conditions of potassium trimethylsilanolate in THF[20] to provide the amino alcohols **24**−**27**, which were then hydrolyzed with aqueous acetic acid to give the saligenin sulfonamides **28**−**31** as their acetate salts. The compounds were initially assayed as the acetate salts, and subsequently the most promising compounds were converted into crystalline salts suitable for inhalation. The (*S*)-enantiomer of the *meta*-primary sulfonamide **29b** (**32**) was obtained from resolution by chiral HPLC of a mixture of (*R*)- and (*S*)- isomers of **25b**, followed by hydrolysis of the acetonide protecting group by heating in aqueous acetic acid. Alternatively, (*S*)-**29b** could be prepared via the (*S*)-enantiomer of oxazolidinone **11**, which was prepared from the parent (*S*)-amino alcohol[22] and 1,1-carbonyldiimidazole in THF. The synthesis of sulfonamide **33**, the formoterol analogue of **29b**, is shown in Scheme 4. Treatment of the (*R*)-epoxide **34**[23] with the benzylamine **35** (prepared by reaction of

Scheme 3[a]



[a] Reagents and conditions: (a) NaH (1.4 equiv), **5**−**8** (1.5 equiv), DMF, 2 h; (b) **9** or **10** (1.1 equiv), CuI (0.1 equiv), $(PPh_3)_2PdCl_2$ (0.06 equiv), MeCN-Et_3N (1:1), 17 h; (c) H_2, PtO_2, THF, 2 h; (d) $KOSiMe_3$ (4 equiv), THF, 70 °C, 2.5 h; (e) aqueous AcOH (1:2), 70 °C, 0.5 h.

Scheme 4[a]



[a] Reagents and conditions: (a) 120 °C; (b) **9b**, CuI (0.16 equiv), $(PPh_3)_2PdCl_2$ (0.12 equiv), MeCN-Et_3N (1:1), 2 h; (c) H_2, 10% Pd/C, $Pd(OH)_2$, EtOH, 100 psi.

benzylamine with the alkyne bromide **6**) gave **36**, which was then coupled with **9b** to provide the alkyne **37**. Hydrogenation over a mixture of 10% Pd/C and Pearlman's catalyst and under pressure gave **33**, isolated as its fumarate salt. Finally, (*R*,*R*)-formoterol was prepared as a standard according to Hett's published procedure.[23]

Results and Discussion

All compounds in Table 1 were tested for their ability to cause cyclic AMP accumulation in Chinese hamster ovary (CHO[a]) cells transfected with human β_1, β_2, or β_3 adrenoceptors. Agonist activity was assessed by measuring changes in intracellular cyclic AMP, and the potency is reported in terms of pEC_{50} (negative $\log_{10}$ molar concentration for half-maximal response ± SEM). The efficacy of the test compounds was expressed as intrinsic activity (IA), which is defined as the maximal response of the test compound, relative to the maximum effect of the high intrinsic efficacy agonist isoprenaline. By definition, isoprenaline's intrinsic activity is 1. The IA for formoterol was 0.97, whereas that of salmeterol was 0.37. Preferred compounds had IA > 0.37. Test compounds were considered as selective if the difference between β_2 and β_1 pEC_{50} value or β_2 and β_3 pEC_{50} value was better than that of (*R*,*R*)-formoterol, that is, values greater than 2.

The primary sulfonamide analogues **29a**−**c** were very potent agonists, with the *meta*-analogue (**29b**) being significantly more potent than the *para*-(**29c**) and the *ortho*-(**29a**) analogues. The monomethyl sulfonamide **29d** appears slightly more potent than the dimethyl sulfonamide **29e**, but both analogues were less potent than **29b**. Similarly, the more lipophilic *iso*-propyl secondary sulfonamide **29f** was equipotent to the monomethyl analogue **29d** but still more potent than the dimethyl analogue **29e**. The cyclohexylsulfonamide **29g**, however, was found to be nearly as potent as **29b**. They both possessed similar IA, however **29b** was more selective against both β_1 and β_3 adrenoceptors. The cyclic tertiary sulfonamides derived from piperidine (**29h**) and morpholine (**29i**) possessed similar potency to that of the dimethyl analogue **29e**. The (*S*)-enantiomer of **29b** (**32**), in common with other known β_2 agonists, was found to be significantly less potent than the corresponding (*R*)-enantiomer.[9,24−27] The homologous primary sulfonamide **29j** was considerably less potent than its parent **29b**. All saligenin sulfonamides were found to have IA < 0.77. The *para*-sulfonamide **29c** possessed the highest IA, whereas the tertiary sulfonamides **29e**, **29i**, and **32** possessed the lowest IA. Finally, the sulfonamide **33** with the formoterol arylethanolamine pharmacophore was as potent as **29b** and as expected had a higher IA, however, **29b** had better selectivity against β_1 and β_3 receptors.

The optimal position of the ether oxygen in the chain was found to be six carbons from the basic nitrogen, as is the case for salmeterol. The analogue with seven carbon atoms in the chain (**28b**) was less potent, and the analogue with five (**30b**) even less potent. The analogue with four carbon atoms in the chain (**31b**) was almost devoid of any activity in CHO cells. The hexyl side chain does allow for enormous conformational freedom, which makes difficult any accurate prediction of the active spatial orientation in this region of the molecule. The six atom distance between the basic nitrogen and ether oxygen is present, however, in a diverse number of other β_2 agonists including salmeterol, formoterol, picumeterol, salmefamol, carmoterol, and sibenadet (Figure 2). This observation has been noted and commented upon by other groups.[28,29] A notable exception is indacaterol, which lacks the ether oxygen at the

[a] Abbreviations: CHO, Chinese hamster ovary; cAMP, adenosine 3′,5′-monophosphate; COPD, chronic obstuctive pulmonary disease; DMA, dimethyl acetamide; MDCK, Madin−Darby canine kidney cells.

Table 1. Stimulation of cAMP Accumulation in CHO Cells Expressing Human β_2, β_1, and β_3 Adrenoceptors

	reg. no.	β_2[a]pEC$_{50}$	IA (L95%conf−U95% conf)[b]	*n*	β_1[a] pEC$_{50}$	*n*	$\beta_2-\beta_1$	β_3[a]pEC$_{50}$	*n*	$\beta_2-\beta_3$
1	**29a.AcOH**	9.1 ± 0.1	0.35 (0.34−0.39)	14	6.6 ± 0.1	16	2.5	6.3 ± 0.1	14	2.8
2	**29b.cinnamate**	9.8 ± 0.2	0.54 (0.50−0.59)	4	6.5 ± 0.1	4	3.3	5.8 ± 0.2	4	4.0
3	**29c.AcOH**	9.3 ± 0.2	0.77 (0.66−0.90)	10	6.3 ± 0.2	11	3.0	5.9 ± 0.1	9	3.4
4	**29d.AcOH**	9.2 ± 0.1	0.35 (0.29−0.42)	11	6.3 ± 0.1	11	2.9	6.1 ± 0.1	14	3.1
5	**29e.AcOH**	9.0 ± 0.1	0.32 (0.26−0.39)	10	6.2 ± 0.1	12	2.8	6.2 ± 0.1	14	2.8
6	**29f.AcOH**	9.3 ± 0.1	0.36 (0.32−0.41)	18	6.0 ± 0.1	11	3.3	6.5 ± 0.1	16	2.8
7	**29 g.AcOH**	9.7 ± 0.1	0.57 (0.53−0.61)	16	7.0 ± 0.1	13	2.7	7.3 ± 0.1	14	2.4
8	**29 h.AcOH**	9.0 ± 0.1	0.45 (0.42−0.49)	18	6.9 ± 0.1	15	2.1	6.8 ± 0.1	16	2.2
9	**29i.AcOH**	8.9 ± 0.1	0.32 (0.27−0.39)	14	6.2 ± 0.1	14	2.7	6.2 ± 0.1	14	2.7
10	**32.AcOH**	8.0 ± 0.1	0.28 (0.24−0.32)	7	5.3 ± 0.1	7	2.7	4.7 ± 0.1	6	3.3
11	**29j.free base**	8.8 ± 0.1	0.32 (0.29−0.36)	9	6.0 ± 0.1	9	2.8	5.6 ± 0.1	10	3.2
12	**28b.sulfamate**	8.6 ± 0.1	0.63 (0.58−0.69)	4	5.9 ± 0.0	4	2.7	6.5 ± 0.0	4	2.1
13	**30b.AcOH**	8.1 ± 0.1	0.25 (0.22−0.28)	4	5.9 ± 0.0	4	2.2	5.6 ± 0.1	4	2.5
14	**31b.AcOH**	<4.6	ND	12	<4.1	12	ND	5.7 ± 0.1	10	ND
15	**33.fumarate**	9.8 ± 0.2	0.8 (0.75−0.85)	6	7.6 ± 0.0	6	2.2	6.7 ± 0.1	6	3.1
16	**isoprenaline**	7.4 ± 0.0	1.0	767	8.1 ± 0.0	641	−0.7	7.4 ± 0.0	659	0.0
17	**2**	9.6 ± 0.0	0.37 (0.36−0.38)	929	6.1 ± 0.0	656	3.5	5.9 ± 0.0	849	3.7
18	(*R*,*R*)-**3.fumarate**	9.3 ± 0.0	0.97 (0.97−0.98)	791	7.4 ± 0.0	670	1.9	7.6 ± 0.0	653	1.7

[a] Human β_1, β_2 and β_3 receptors expressed in CHO cells. pEC$_{50}$ is the negative logarithm of the molar drug concentration that produces a cAMP response equal to 50% of its maximal response. [b] L95%conf = lower 95% confidence limit. U95%conf = upper 95% confidence limit.

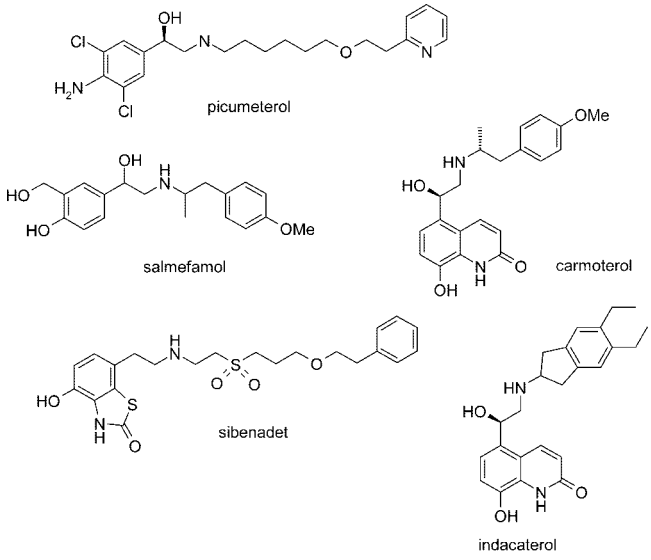


Figure 2

end of the six-atom chain and displays lower binding affinity for the human β_2 receptor.[25]

The pharmacology of salmeterol assessed on isolated superfused guinea pig trachea strips correlates well with clinical data and gives a measurement of potency, efficacy, onset time, and duration of action.[30] Test compounds were investigated for their ability to inhibit the contraction of guinea pig trachea strips expressed as a measure of the functional response at the β_2 adrenoceptor. Tissues were contracted electrically, agonist was perfused over the tissue until maximum relaxation was achieved, and onset of action determined. Perfusion of the agonist was then ceased, tissue continued to be perfused with buffer, and duration of action determined by the time taken for the contractile response to re-establish. In Table 2, the potency, the onset time, and the duration after 1 and 3 h is presented. Potency was expressed in absolute terms (concentration required to induce 50% inhibition, EC$_{50}$). Onset of action was calculated as the time taken for an EC$_{50}$ concentration to achieve 50% maximum relaxant effect. Duration of action was determined by measuring the recovery of electrically induced contraction following washout of agonist. This was expressed as the rightward shift in the agonist concentration−effect curve following 1 and 3 h of washout (EC$_{50}$ for test compound after

Table 2. Onset and Duration of Action of Selected Compounds on Isolated Superfused Guinea Pig Trachea (Minimum $n = 2$)

entry	compd	pEC$_{50}$	onset time (min)	shift (1 h)	shift (3 h)
1	**29a.AcOH**	8.6	6.6	48	75
2	**29b.AcOH**	9.1	10.8	7.0	8.3
3	**29c.AcOH**	8.7	7.0	7.1	8.4
4	**28b.AcOH**	8.6	8.9	9.8	10
5	**29f.AcOH**	8.0	6.0	3.0	3.7
6	**33.fumarate**	10.4	18.9	3.1	4.2
7	**salmeterol**	8.3	27.6	1.0	1.1
8	(*R*,*R*)-**3.fumarate**	9.5	10	20	>1940

60 or 180 min of washout/EC$_{50}$ at equilibrium, time 0 min). With this analysis, the greater the shift values, the greater the recovery. Shift values of 1 (after 1 h) and 1 (after 3 h) indicate no washout (a salmeterol-like profile). Shift values of about 20 and >300 indicate slow continuous washout (formoterol-like profile). Shift values of infinity indicate rapid and complete washout (isoprenaline-like profile). All the test compounds in Table 2 exhibited onset times faster than salmeterol and similar to formoterol. The isopropyl analogue **29f** was the only compound with a lower potency than salmeterol. The *ortho*-sulfonamide had a formoterol-like profile; the remaining sulfonamides, however, had a unique profile, intermediate between that of salmeterol and that of formoterol. These compounds showed a biphasic washout profile on guinea pig isolated superfused trachea. On removal of the agonist from the superfusing fluid, the tissue showed a degree of recovery, followed by no further recovery over 3 h. In contrast, tissues showed little or no recovery to salmeterol upon washout. The effects of formoterol were progressively reversed upon washing, whereas the effects of salbutamol and isoprenaline were rapidly and fully reversed.

On the basis of the data in Tables 1 and 2, sulfonamide **29b**, which had high potency, fast onset of action, long duration of action, and selectivity for β_2 over β_1 and β_3 receptors, was progressed to pharmacokinetic studies. In Table 3, selected physicochemical and structural parameters for **29b** are shown and contrasted to those for salmeterol. log *D* (at pH 6.4) for sulfonamide **29b** was found to be 0.52 compared with 1.06 for salmeterol, its polar surface area (PSA) was calculated to be 142 Å, the total number of hydrogen bond donors and acceptors was 13, its molecular weight was 494.7, the number of rotatable bonds was 15, and the non-hydrogen atom count was 34. The passive membrane permeability (P_{exact}) of **29b** across MDCKII-

Table 3. Physicochemical, Structural Parameters, and MDCKII Permeability for **29b** and Salmeterol

compd	MW	log $D_{6.4}$	PSA (Å^2)	H-bond acceptors	H-bond donors	rotatable bonds	heavy atoms	P_{exact} (nm s^{-1})
29b	494.7	−0.52	142	8	5	15	34	4.5
salmeterol	415.6	1.06	82	5	4	16	30	119

Table 4. Rat and Dog Pharmacokinetic Data for **29b** Acetate

species	route	dose (mg/kg)	Clp (mL/min/kg)	V_{dss} (L/kg)	$T_{1/2}$ (h)	C_{max} (ng/mL)	F%
rat	intravenous	2	79	16	2.3		
	oral	5				<1	<1
dog	intravenous	0.05	20	1.9	2.1		
	oral	0.25				6	12

MDR1 cells in the presence of a potent P-glycoprotein inhibitor was measured as 4.5 nm s^{-1}, whereas salmeterol was 119 nm s^{-1}. On the basis of all these data, **29b** was expected to have lower absorption than salmeterol, hence the swallowed fraction of the inhaled dose was not expected to have a major contribution to the overall systemic exposure of **29b**. In vitro metabolic stability was measured as a ratio vs verapamil in human microsomes, and **29b** (ratio 0.9) was found to have moderate turnover, similar to that of salmeterol (ratio 1.2). Pharmacokinetic studies in vivo with **29b** were initiated and compared with salmeterol. The acetate salt of **29b** was dosed as a solution orally and intravenously to rats and dogs, and the data are presented in Table 4. High plasma clearance and a moderately long half-life was observed in both species, and oral bioavailability was low in the dog (mean 12%) and very low in the rat (<1%). This oral bioavailability was much lower than that previously reported for salmeterol (66−78% in dog, and 10−12% in rat)[31] and strengthened the confidence that the swallowed fraction of the inhaled dose in humans would be unlikely to contribute to the systemic exposure of **29b.**

As mentioned above, the washout profile of sulfonamide β_2 agonists on guinea pig isolated airway preparations was biphasic in nature. When the β_2-adrenoceptor antagonist, sotalol, was perfused over the trachea during the washout of **29b**, there was a rapid and full reversal of β_2 agonist relaxation. However, upon withdrawl of sotalol, reassertion of the relaxant effects was seen, suggesting retention of **29b** in the vicinity of the β_2 adrenoceptor. Bradshaw et al. suggested that the mechanism by which salmeterol exhibits long activity was due to the anchoring of the agonist to an "exosite" present in the cell membrane adjacent to the β_2-adrenoceptor[32] but subsequently shown to be in TM4 of the receptor.[33] This hypothesis was supported by the Nials et al. studies.[30] In these studies, the residual effects of salmeterol (after washout) were reversed by sotalol. On withdrawal of sotalol, full relaxant effect was reasserted. This suggested that salmeterol was bound in an area where it could associate with the receptor causing relaxation but not readily be washed off, i.e., "exosite" bound. In the current study, the effect of sotalol on **29b** showed a similar profile. This suggests that the second phase of **29b** activity is by a mechanism similar to that of salmeterol in that it is bound somewhere in the cell membane where it can readily associate with the receptor but is not easily washed out.

Modeling Studies. A homology model was prepared to assist in the understanding of the SAR for the sulfonamides. The model was built using the bovine rhodopsin structure as the template[34] and applying the methods of Blaney.[35] This model was build several years ago, when this research work was underway, and well before the recent publication of the structure of the human β_2 receptor, which was crystallized in a lipid environment when bound to the inverse agonist carazolol.[36−38] Computational docking of compounds to the homology model were carried out using the FLO+ docking algorithm[39] and incorporating flexibility in the side chains interacting with the ligand. Wherever possible, the "interacting" residues identified by receptor mutagenesis and molecular modeling studies were utilized to guide the docking. These are the catechol mimic with the serine residues 204 and 207 on the fifth transmembrane spanning domain (TM5),[40] the benzylic alcohol with the chirally discriminating Asn-293 on TM6,[41] and the protonated amine with Asp-113 on TM3.[42] Tyr-308 on TM7 was identified as the major amino acid interacting with the methylene groups near the protonated amine of salmeterol conferring the β_2 selectivity.[43] In addition, Tyr-308 has been identified as the residue defining the interactions of the amine substituents of formoterol and carmoterol.[44] The ether oxygen in the side chain of salmeterol interacts with Tyr-316.[43] The 4-phenylbutyl ether moiety of salmeterol consequently orients itself into an interaction with the amino acids around positions 149−158 on TM4, which define the key residues of the exosite binding pocket.[33] Sulfonamide **29b** was docked in the above model, and for most residues, the interactions were clearly observed in the docking. Ser-207 was found to H-bond to the phenolic hydroxyl, while Ser-204 and Ser-203 were positioned to interact with the primary benzylic hydroxyl. A potential H-bonding interaction was identified between Asn-293 and the asymmetric benzylic hydroxyl, while Asp-113 and the amine were in proximity, allowing a strong charge interaction. The ether oxygen in the chain was seen to be positioned within H-bonding range of Asn-318 (TM6), while the *meta*-sulfonamido group was seen to be ideally situated to form H-bonds to both Ser-120 (TM3) and Asn-322 (TM6) (Figure 3). These latter interactions are important as they might provide a rational explanation for the biphasic behavior of **29b** on guinea pig isolated airway preparations. In this model, direct interactions were neither seen for Tyr308 or Tyr318. The sulfonamide tail was found to project down toward the "exosite binding pocket" near the base of TM4, but again no direct interaction was possible. The model was superimposed and compared with the recent crystal structure

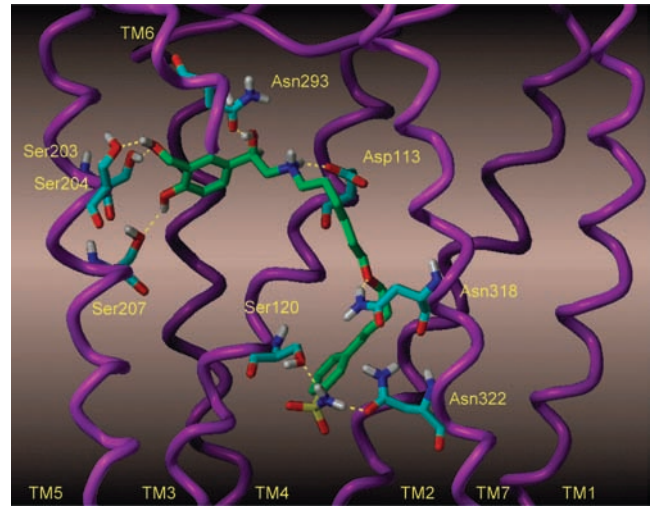


Figure 3. Postulated interactions of the *meta*-sulfonamide **29b** with the β_2 receptor. Portions of TM6 are omitted for clarity.

for the β_2 receptor with carazolol bound (PDB ID 2RH1).[36−38] All the proposed interacting residues were similarly placed in the model and the crystal structure.

A variety of salts of **29b** were prepared and examined for crystallinity, and two such salts were identified, the cinnamate (mp 127−128 °C) and triphenylacetate (mp 99−102 °C).[45] Cinnamic and triphenylacetic acids are not precedented in marketed inhaled products and therefore warrant further toxicological investigation. The cinnamate salt (solubility 0.382 mg/mL at pH 7.4) was screened for stability in a range of humidity and thermal conditions, for lactose compatibility, and for particle size after micronization and was found to be suitable for inhaled administration. The cinnamate salt of **29b** was also investigated in vivo in histamine-induced bronchospasm in the guinea pig in a plethysmograph chamber (Buxco) and found to be about 7-fold more potent than salmeterol (EC_{90} 8 and 55 μM, respectively). At an equi-effective (EC_{90}) dose, the duration of action of **29b**-cinnamate (time to 50% recovery) was longer than that of salmeterol (18 h vs 12 h) when administered as a nebulized solution in a DMA/saline vehicle using micronized material.[46] In conscious guinea pigs, **29b**-cinnamate demonstrated a wider (>600) therapeutic index compared with salmeterol (>10) when comparing nebulized concentrations effective at preventing histamine-induced bronchoconstriction with those inducing significant decreases in mean arterial pressure. Furthermore **29b**-cinnamate reversed spasmogen-induced contraction of human isolated bronchial preparations with an onset time of 3 min and potency similar to that seen on guinea pig trachea (EC_{50} = 2.4 nM), whereas salmeterol had an onset time of 36 min and potency of 5 nM. The sulfonamide **29b**-cinnamate was therefore selected for further development.

Conclusion

Incorporation of the sulfonamide functionality on the right-hand side phenyl ring of (*R*)-salmeterol has given a series of very potent human β_2 adrenoceptor agonists. The primary sulfonamide at the *meta*-position (**29b**) was identified as the analogue that fulfilled all the criteria of potency, selectivity, rapid onset, and long duration of action in vivo, low oral bioavailability, and higher than salmeterol therapeutic index. The cinnamate salt of **29b** was found to have suitable properties for inhaled administration and was therefore selected as a candidate for further development. The interest in the pharmaceutical industry for the development of the third-generation β_2 agonists for the treatment of asthma and COPD is continuing[25,47−51] and will intensify as data from clinical trials become available in the next few years.

Experimental Section

Organic solutions were dried over anhydrous $MgSO_4$. TLC was performed on Merck 0.25 mm Kieselgel 60 F_{254} plates. Products were visualized under UV light and/or by staining with aqueous $KMnO_4$ solution. LCMS analysis was conducted on a Supelcosil LCABZ+PLUS column (3.3 cm × 4.6 mm) eluting with 0.1% formic acid and 0.01 M ammonium acetate in water (solvent A) and 0.05% formic acid and 5% water in acetonitrile (solvent B), using the following elution gradient 0−0.7 min 0% B, 0.7−4.2 min 100% B, 4.2−5.3 min 0% B, 5.3−5.5 min 0% B at a flow rate of 3 mL/min. The mass spectra were recorded on a Fisons VG Platform spectrometer using electrospray positive and negative mode (ES +ve and ES −ve). Column chromatography was performed on Merck Kieselgel 60 (article 9385) or Biotage prepacked silica gel cartridges containing KP-Sil run on a flash 12i chromatography module. ^{1}H NMR spectra were recorded at 400 MHz unless otherwise stated and ^{13}C NMR at 100 MHz. The chemical shifts are expressed in ppm relative to tetramethylsilane. High resolution positive ion mass spectra were acquired on a Micromass Q-Tof 2 hybrid quadrupole time-of-flight mass spectrometer. The elemental composition was calculated using MassLynx v4.0 for the $[M + H]^+$.

All separations for HRMS were achieved using a Phenomenex Luna C18(2) reversed phase column (150 mm × 2.1 mm, 3 μm particle size). Gradient elution was carried out with the mobile phases as (A) water containing 0.1% (v/v) formic acid and (B) MeCN containing 0.1% (v/v) formic acid. The conditions for the gradient elution were initially 0% B for 2 min, increasing linearly to 100% B over 25 min, remaining at 100% B for 2 min and then decreasing linearly to 0% B over 0.5 min, followed by an equilibration period of 2.5 min prior to the next injection. The flow rate was 0.4 mL/min, temperature controlled at 25 °C with an injection volume of 5 μL.

General Procedure for the Alkylation of Oxazolidinone 11. (5*R*)-3-[6-(But-3-ynyloxy)hexyl]-5-(2,2-dimethyl-4*H*-1,3-benzodioxin-6-yl)-1,3-oxazolidin-2-one (13). (5*R*)-5-(2,2-dimethyl-4*H*-1,3-benzodioxin-6-yl)-1,3-oxazolidin-2-one[20] (**11**) (10 g, 40 mmol) in DMF (100 mL) was added dropwise to a stirred suspension of sodium hydride (60% oil dispersion, 2.33 g, 58.2 mmol) in DMF (50 mL) with stirring under nitrogen and maintaining the internal temperature at 0 °C. Stirring was continued at 0−5 °C for 1 h. The mixture was recooled to 0 °C, and a solution of 6-bromohexyl but-3-ynyl ether (**6**) (14.7 g, 63.0 mmol) in DMF (50 mL) was added over 1 min. The mixture was then stirred at 20−30 °C for 2 h. Then 2 M HCl (9 mL) was added and the mixture was partitioned between water and Et_2O. The aqueous layer was extracted with more Et_2O, and the combined organic layers were washed twice with brine. After drying, the solution was concentrated and the residue was purified by column chromatography on silica gel (600 g) eluting with Et_2O-petroleum ether (bp 40−60 °C) (1:2), (1:1) and then Et_2O to give **13** (13.88 g, 86%) as a waxy solid: ES +ve *m*/*z* 402 $(M + H)^+$. ^{1}H NMR δ ($CDCl_3$) 7.12 (1H, dd, *J* 8 and 1 Hz), 7.00 (1H, d, *J* 1 Hz), 6.83 (1H, d, *J* 8 Hz), 5.39 (1H, t, *J* 8 Hz), 4.83 (2H, s), 3.85 (1H, t, *J* 9 Hz), 3.54 (2H, t, *J* 7 Hz), 3.45 (2H, t, *J* 7 Hz), 3.40 (1H, t, *J* 9 Hz), 3.38−3.20 (2H, m), 2.45 (2H, dt, 3 and 7 Hz), 1.98 (1H, t, *J* 3 Hz), 1.62−1.52 (4H, m), 1.54 (6H, s), 1.42−1.30 (4H, m). Anal. C, H, N.

General Procedure for the Sonogashira Coupling of Alkynes 12−15 with Aryl Iodides 9 or Aryl Bromides 10. Reactions with iodides were carried out at room temperature; however, reactions with bromides were performed at 80 °C.

3-[4-({6-[(5*R*)-5-(2,2-Dimethyl-4*H*-1,3-benzodioxin-6-yl)-2-*oxo*-1,3-oxazolidin-3-yl]hexyl}oxy)but-1-ynyl]benzenesulfonamide (17b). Compound **13** (1.79 g, 4.46 mmol) was stirred with **9b** (1.4 g, 5.1 mmol) in MeCN-Et_3N (1:1, 42 mL) under nitrogen for 10 min. Cuprous iodide (83 mg, 0.43 mmol) and dichlorobis-(triphenylphosphine)palladium(II) (192 mg, 0.27 mmol) were added, and the mixture was stirred for 17 h under nitrogen at 21 °C. The mixture was evaporated to dryness, and the residue was purified by chromatography on silica gel (250 g) eluting with EtOAc-petroleum ether (bp 40−60 °C) (3:7), then (1:1), then (3:1), and finally EtOAc to give **17b** (2.35 g, 95%) as a colorless glass: ES +ve *m*/*z* 557 $(M + H)^+$. ^{1}H NMR δ ($CDCl_3$) 7.95 (1H, t, *J* 1 Hz), 7.80 (1H, dt, *J* 8 and 1 Hz), 7.55 (1H, dt, *J* 8, 1 Hz), 7.40 (1H, t, *J* 8 Hz), 7.12 (1H, dd, *J* 8, 2 Hz), 7.01 (1H, d, *J* 2 Hz), 6.83 (1H, d, *J* 8 Hz), 5.39 (1H, t, *J* 8 Hz), 5.29 (2H, br s), 4.84 (2H,s), 3.84 (1H, t, *J* 8 Hz), 3.62 (2H, t, *J* 6 Hz), 3.50 (2H, t, *J* 6 Hz), 3.40 (1H, dd, *J* 8, 7.5 Hz), 3.35−3.18 (2H, m), 2.68 (2H, t, 6 Hz), 1.63−1.50 (4H, m), 1.53 (6H, s), 1.50−1.30 (4H, m). Anal. C, H, N, S.

General Procedure for Hydrogenation of Alkynes 16−19. 3-[4-({6-[(5*R*)-5-(2,2-Dimethyl-4*H*-1,3-benzodioxin-6-yl)-2-*oxo*-1,3-oxazolidin-3-yl]hexyl}oxy)butyl]benzenesulfonamide (21b). Compound **17b** (2.35 g, 4.22 mmol) was stirred with platinum oxide (0.3 g) in THF (30 mL) under hydrogen for 2 h. The catalyst was removed by filtration using a filter aid, and the filter cake was leached with EtOAc. The combined filtrates were passed through silica gel (200 g) in EtOAc, and the eluate was evaporated to give **21b** (2.32 g, 98%) as a colorless gum: ES +ve *m*/*z* 561 $(M + H)^+$. ^{1}H NMR δ ($CDCl_3$) 7.76 (1H, s), 7.71 (1H, dt, *J* 6, 2 Hz),

7.41−7.35 (2H, m), 7.11 (1H, dd, *J* 8, 2 Hz), 7.00 (1H, d, *J* 2 Hz), 6.81 (1H, d, *J* 8 Hz), 5.39 (1H, t, *J* 8 Hz), 5.27 (2H, s), 4.83 (2H,s), 3.85 (1H, t, *J* 9 Hz), 3.45−3.35 (5H, m), 3.35−3.20 (2H, m), 2.68 (2H, t, 8 Hz), 1.74−1.63 (2H, m), 1.62−1.48 (8H, m), 1.53 (6H, s), 1.42−1.29 (4H, m).

General Procedure for the Cleavage of Oxazolidinone Rings with $KOSiMe_3$. 3-{4-[(6-{[(2*R*)-2-(2,2-Dimethyl-4*H*-1,3-benzodioxin-6-yl)-2-hydroxyethyl]amino}hexyl)oxy]butyl}benzenesulfonamide (25b). A solution of **21b** (0.43 g, 0.76 mmol) in THF (10 mL) was treated with potassium trimethylsilanolate (90% pure, 0.43 g, 3 mmol), and the mixture was stirred at 70 °C under nitrogen for 2.5 h. The mixture was partitioned between CH_2Cl_2 and pH 6.4 phosphate buffer, and the aqueous layer was extracted with more CH_2Cl_2. The combined organic layers were washed with water, dried, and concentrated. The residue was purified by column chromatography on silica gel (60 g), eluting successively with EtOAc-petroleum ether (bp 40−60 °C) (1:1), EtOAc, MeOH-EtOAc (1:9 to 1:4) to give **25b** (0.286 g, 70%): ES +ve *m*/*z* 535 $(M + H)^+$. ^{1}H NMR δ ($CDCl_3$) 7.77−7.72 (2H, m), 7.43−7.35 (2H, m), 7.11 (1H, br d, *J* 8 Hz), 7.00 (1H, br s), 6.78 (1H, d, *J* 8 Hz), 4.83 (2H, s), 4.70 (1H, dd, *J* 9, 3 Hz), 3.42 (2H, t, *J* 6 Hz), 3.40 (2H, t, *J* 6 Hz), 2.87 (1H, dd, *J* 12, 4 Hz), 2.75−2.60 (5H, m), 1.76−1.66 (2H, m), 1.65−1.46 (6H, m), 1.53 (6H, s), 1.40−1.30 (4H, m).

3-{4-[(6-{[(2*S*)-2-(2,2-Dimethyl-4*H*-1,3-benzodioxin-6-yl)-2-hydroxyethyl]amino}hexyl)oxy]butyl}benzenesulfonamide (ent 25b). Resolution of 3-{4-[(6-{[2-(2,2-dimethyl-4*H*-1,3-benzodioxin-6-yl)-2-hydroxyethyl]amino}hexyl)oxy]butyl}benzenesulfonamide (403 mg) on an HPLC Chiralcel OJ column using 40% ethanol-heptane afforded **ent 25b** (96 mg). Analytical HPLC Chiralcel OJ column (25 cm × 4.6 mm) RT for **ent 25b** = 22.9 min, (40% EtOH-heptane, flow rate 1 mL/min, detected at 215 nm). RT for **25b** = 15.3 min.

General Procedure for Acetal Hydrolysis. 3-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl}amino)-hexyl]oxy}butyl)benzenesulfonamide (29b) Acetate Salt. A solution of **25b** (283 mg, 0.53 mmol) in acetic acid (8 mL) and water (4 mL) was heated at 70 °C for 35 min before evaporating to dryness. The residue was re-evaporated twice with toluene to give **29b**-acetate salt (318 mg) as a gum: ES +ve *m*/*z* 495 $(M + H)^+$. ^{1}H NMR δ (DMSO-d_6) 7.65−7.63 (2H, m), 7.47 (1H, t, *J* 8 Hz), 7.43 (1H, t, *J* 8 Hz), 7.26 (1H, d, *J* 2 Hz), 7.18 (1H, d, *J* 8 Hz), 7.00 (1H, dd, *J* 8, 2 Hz), 6.70 (1H, d, *J* 8 Hz), 4.59 (1H, dd, *J* 9, 4 Hz), 4.46 (2H, s), 3.36 (2H, t, *J* 6 Hz), 3.33 (2H, t, *J* 6 Hz), 2.72−2.58 (6H, m), 1.90 (3H, s), 1.65−1.55 (2H, m), 1.55−1.35 (6H, m), 1.35−1.30 (4H, m). Analytical chiral HPLC RT = 21.3 min, 99.5% (Chirobiotic column 25 cm × 4.6 mm, flow rate 0.8 mL/min, MeOH−AcOH−triethylamine (1000:0.05:0.2) at 10 °C, detected at 210 nm).

3-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)-phenyl]ethyl}amino)hexyl]oxy}butyl)benzenesulfonamide (29b Free Base). The acetate salt was converted to the free base by passing through an SCX-2 ion exchange cartridge, eluting with MeOH and then with 0.67 M NH_3 in MeOH. The ammoniacal fractions were concentrated under reduced pressure to give **29b**-free base as a colorless gum. ^{1}H NMR δ (CD_3OD) 7.73 (1H, s), 7.70 (1H, dt, *J* 6, 2 Hz), 7.45−7.40 (2H, m), 7.28 (1H, d, *J* 2 Hz), 7.10 (1H, dd, *J* 8, 2 Hz), 6.75 (1H, d, *J* 8 Hz), 4.69 (1H, dd, *J* 9, 4 Hz), 4.64 (2H, s), 3.44 (2H, t, *J* 6 Hz), 3.40 (2H, t, *J* 6 Hz), 2.78 91H, dd, *J* 12, 9 Hz), 2.74−2.68 (3H, m), 2.67−2.56 (2H, m), 1.75−1.65 (2H, m), 1.63−1.45 (6H, m), 1.40−1.29 (4H, m).

29b-Triphenylacetate Salt. Triphenylacetic acid (115 mg, 0.4 mmol) was added to a solution of **29b**-free base (198 mg, 0.4 mmol) in EtOH (3 mL), and the suspension was warmed to effect dissolution. The solvent was removed under reduced pressure to give a gum, which was dissolved in EtOAc (3 mL) and allowed to stand at 20 °C for 3 d. The crystals were collected by filtration and recrystallized from EtOAc to give **29b**-triphenylacetate salt (91 mg) as a white solid: mp 99−102 °C. ^{1}H NMR δ (CD_3OD) 7.75−7.68 (2H, m), 7.44−7.40 (2H, m), 7.33 (1H, br s), 7.29−7.26 (6H, m), 7.22−7.09 (10H, m), 6.77 (1H, d, *J* 8 Hz), 4.65 (2H, s), 3.45 (2H, t, *J* 6 Hz), 3.41 (2H, t, *J* 6 Hz), 3.08−2.89 (4H, m), 2.72 (2H, t, *J* 7 Hz), 1.76−1.52 (8H, m), 1.43−1.33 (4H, m). Anal. C, H, N, S.

29b-Cinnamate Salt. Cinnamic acid (0.3 g, 2 mmol) was added to a solution of **28b** free base (1.0 g, 2 mmol) in MeOH (5 mL) at room temperature. The solution was stirred for 5 min before being concentrated under reduced pressure. Water (10 mL) was added to the residue, and the resulting suspension was stirred at room temperature for 24 h. The solid was collected by filtration and recrystallized from EtOH (5 mL) to give **29b**-cinnamate (0.54 g, 42%) as a white solid: mp 127−128 °C. ^{1}H NMR δ (500 MHz; CD_3OD) 7.74 (1H, s), 7.71 (1H, d, *J* 7.5 Hz), 7.50 (2H, d, *J* 7 Hz), 7.41 (3H, m), 7.36−7.28 (4H, m), 7.17 (1H, dd, *J* 2, 8 Hz), 6.79 (1H, d, *J* 8 Hz), 6.51 (1H, d, *J* 16 Hz), 4.89 (1H, dd, *J* 4, 9.5 Hz), 4.66 (2H, s), 3.43 (2H, t, *J* 6 Hz), 3.39 (2H, t, *J* 6 Hz), 3.10 (2H, m), 3.00 (2H, m), 2.70 (2H, t, *J* 7 Hz), 1.68 (4H, m), 1.55 (4H, m), 1.40 (4H, m). ^{13}C NMR δ (125 MHz; CD_3OD) 175.3, 156.3, 145.1, 144.9, 141.2, 137.2, 133.3, 133.0, 130.1, 130.0, 129.8, 128.9, 128.5, 127.1, 127.0, 126.8, 126.2, 124.6, 116.0, 71.6, 71.5, 70.2, 60.9, 55.4, 49.0, 36.3, 30.5, 30.1, 29.0, 27.5, 27.2, 26.8. Anal. C, H, N, S.

2-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)-phenyl]ethyl}amino)hexyl]oxy}butyl)benzenesulfonamide (29a)-Acetate Salt. ES +ve *m*/*z* 495 $(M + H)^+$. ^{1}H NMR δ (CD_3OD) includes 7.97 (1H, dd, *J* 8, 1 Hz), 7.52 (1H, dt, *J* 8, 1 Hz), 7.42 (1H, d, *J* 8 Hz), 7.38 (1H, d, *J* 2 Hz), 7.33 (1H, dt, *J* 8, 1 Hz), 7.18 (1H, dd, *J* 8, 2 Hz), 6.82 (1H, d, *J* 8 Hz), 4.68 (2H, s), 3.52 (2H, t, *J* 6 Hz), 3.48 (2H, t, *J* 6 Hz), 3.17−3.12 (6H, m), 1.97 (6H, s), 1.82−1.68 (6H, m), 1.68−1.58 (2H, m), 1.48−1.42 (4H, m). HRMS found: 495.2531 $C_{25}H_{38}N_2O_6S$ requires 495.2529.

4-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)-phenyl]ethyl}amino)hexyl]oxy}butyl)benzenesulfonamide (29c)-Acetate Salt. ES +ve *m*/*z* 495 $(M + H)^+$. ^{1}H NMR δ (DMSO-d_6) includes 7.72 (2H, d, *J* 8 Hz), 7.38 (2H, d, *J* 8 Hz), 7.27 (1H, d, *J* 2 Hz), 6.99 (1H, dd, *J* 8, 2 Hz), 6.69(1H, d, *J* 8 Hz), 4.57 (1H, dd, *J* 9, 4.5 Hz), 3.38−3.28 (4H, m), 2.69−2.55 (6H, m), 1.90 (3H, s), 1.65−1.55 (2H, m), 1.53−1.35 (6H, m), 1.35−1.23 (4H, m). HRMS found: 495.2521 $C_{25}H_{38}N_2O_6S$ requires 495.2529.

3-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)-phenyl]ethyl}amino)hexyl]oxy}butyl)-*N*-methylbenzenesulfonamide (29d)-Acetate Salt. ES +ve *m*/*z* 509 $(M + H)^+$. ^{1}H NMR δ (CD_3OD) 7.68−7.60 (2H, s), 7.53−7.43 (2H, m), 7.34 (1H, br d, *J* 2 Hz), 7.13 (1H, dd, *J* 8, 2 Hz), 6.78 (1H, d, *J* 8 Hz), 4.64 (2H, s), 3.45 (2H, t, *J* 6 Hz), 3.41 (2H, t, *J* 6 Hz), 3.13−2.93 (4H, m), 2.73 (2H, t, *J* 8 Hz), 2.49 (3H, s), 1.92 (3H, s), 1.77−1.64 (4H, m), 1.63−1.53 (4H, m), 1.46−1.35 (4H, m). HRMS found: 509.2680 $C_{26}H_{40}N_2O_6S$ requires 509.2685.

3-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)-phenyl]ethyl}amino)hexyl]oxy}butyl)-*N*,*N*-dimethylbenzenesulfonamide (29e)-Acetate Salt. ES +ve *m*/*z* 523 $(M + H)^+$. ^{1}H NMR δ (CD_3OD) 7.64−7.56 (2H, m), 7.54−7.50 (2H, m), 7.34 (1H, br d, *J* 2 Hz), 7.16 (1H, dd, *J* 8, 2 Hz), 6.78 (1H, d, *J* 8 Hz), 4.64 (2H, s), 3.45 (2H, t, *J* 6 Hz), 3.41 (2H, t, *J* 6 Hz), 3.13−2.98 (2H, m), 2.75 (2H, t, *J* 8 Hz), 2.65 (6H, s), 1.95 (3H, s), 1.76−1.52 (8H, m), 1.45−1.36 (4H, m). HRMS found: 523.2840 $C_{27}H_{42}N_2O_6S$ requires 523.2842.

3-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)-phenyl]ethyl}amino)hexyl]oxy}butyl)-*N*-isopropylbenzenesulfonamide (29f)-Acetate Salt. ES +ve *m*/*z* 537 $(M + H)^+$. ^{1}H NMR δ (CD_3OD) 7.72−7.63 (2H, m), 7.48−7.40 (2H, m), 7.34 (1H, d, *J* 2 Hz), 7.16 (1H, dd, *J* 8, 2 Hz), 6.78 (1H, d, *J* 8 Hz), 4.64 (2H, s), 3.44 (2H, t, *J* 6 Hz), 3.40 (2H, t, *J* 6 Hz), 3.13−2.96 (4H, m), 2.73 (2H, t, *J* 8 Hz), 1.95(3H, s), 1.74−1.64 (4H, m), 1.60−1.50 (4H, m), 1.46−1.35 (4H, m), 0.99 (6H, d, *J* 6 Hz). HRMS found: 537.2999 $C_{28}H_{44}N_2O_6S$ requires 537.2998.

***N*-Cyclohexyl-3-(4-{[6-({(2*R*)-2-hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl}amino)hexyl]oxy}butyl)benzenesulfonamide (29g)-Acetate Salt.** ES +ve *m*/*z* 577 $(M + H)^+$. ^{1}H NMR δ (CD_3OD) 7.72−7.63 (2H, m), 7.47−7.40 (2H, m), 7.34 (1H, d, *J* 2 Hz), 7.16 (1H, dd, *J* 8, 2 Hz), 6.78 (1H, d, *J* 8 Hz), 4.65 (2H, s), 3.44 (2H, t, *J* 6 Hz), 3.42 (2H, t, *J* 6 Hz), 3.13−2.95 (5H, m),

2.73 (2H, t, *J* 8 Hz), 1.95 (3H, m), 1.76−1.61 (17H, m), 1.45−1.07 (5H, m). HRMS found: 577.3314 $C_{31}H_{48}N_2O_6S$ requires 577.3311.

2-(Hydroxymethyl)-4-{(1*R*)-1-hydroxy-2-[(6-{4-[3-(piperidin-1-ylsulfonyl)phenyl]butoxy}hexyl)amino]ethyl}phenol (29h)-Acetate Salt. ES +ve *m/z* 563 $(M + H)^+$. ^{1}H NMR δ (CD_3OD) 7.58−7.46 (4H, m), 7.33 (1H, br d, *J* 2 Hz), 7.15 (1H, dd, *J* 8, 2 Hz), 6.77 (1H, d, *J* 8 Hz), 4.64 (2H, s), 3.44 (2H, t, *J* 6 Hz), 3.40 (2H, t, *J* 6 Hz), 3.13−2.98 (4H, m), 2.94 (4H, t, *J* 6 Hz), 2.74 (2H, t, *J* 7 Hz), 1.95 (3H, s), 1.78−1.52 (12H, m), 1.45−1.35 (6H, m). HRMS found: 563.3151 $C_{30}H_{46}N_2O_6S$ requires 563.3155.

2-(Hydroxymethyl)-4-{(1*R*)-1-hydroxy-2-[(6-{4-[3-(morpholin-4-ylsulfonyl)phenyl]butoxy}hexyl)amino]ethyl}phenol (29i)-Acetate Salt. ES +ve *m/z* 565 $(M + H)^+$. ^{1}H NMR δ (CD_3OD) 7.60−7.52 (4H, m), 7.34 (1H, br d, *J* 2 Hz), 7.15 (1H, dd, *J* 8, 2 Hz), 6.78 (1H, d, *J* 8 Hz), 4.65 (2H, s), 3.66 (4H, t, *J* 5 Hz), 3.45 (2H, t, *J* 6 Hz), 3.41 (2H, t, *J* 6 Hz), 3.15−2.96 (4H, m), 2.92 (4H, t, *J* 5 Hz), 2.76 (2H, t, *J* 8 Hz), 1.92 (3H, s), 1.75−1.64 (4H, m), 1.63−1.51 (4H, m), 1.46−1.34 (4H, m). HRMS found: 565.2943 $C_{29}H_{44}N_2O_7S$ requires 565.2947.

[3-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)-phenyl]-ethyl}amino)hexyl]oxy}butyl)phenyl]methanesulfonamide (29j). ES +ve *m/z* 509 $(M + H)^+$. ^{1}H NMR δ (CD_3OD) 7.30−7.15 (5H, m), 7.10 (1H, dd, *J* 8, 2 Hz), 6.75 (1H, d, *J* 8 Hz), 4.69 (1H, dd, *J* 9, 4 Hz), 4.64 (2H, s), 4.29 (2H, s), 3.43 (2H, t, *J* 7 Hz), 3.42 (2H, t, *J* 7 Hz), 2.80 (1H, dd, *J* 12, 9 Hz), 2.72 (1H, dd, *J* 12, 4 Hz), 2.69−2.60 (4H, m), 1.72−1.62 (2H, m), 1.62−1.49 (6H, m), 1.42−1.30 (4H, m). HRMS found: 509.2690 $C_{26}H_{40}N_2O_6S$ requires 509.2685.

3-(4-{[6-({(2*S*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)-phenyl]ethyl}amino)hexyl]oxy}butyl)benzenesulfonamide (32)-Acetate Salt. ES +ve *m/z* 495 $(MH)^+$. ^{1}H NMR δ (CD_3OD) 7.76−7.68 (2H, m), 7.46−7.40 (2H, m), 7.34 (1H, d, *J* 2 Hz), 7.16 (1H, dd, *J* 8, 2 Hz), 6.78 (1H, d, *J* 8 Hz), 4.64 (2H, s), 3.45 (2H, t, *J* 6 Hz), 3.42 (2H, t, *J* 6 Hz), 3.23−2.97 (4H, m), 2.72 (2H, t, *J* 8 Hz), 1.94 (3H, s), 1.78−1.65 (4H, m), 1.64−1.52 (4H, m), 1.45−1.36 (4H, m). HRMS found: 495.2522 $C_{25}H_{38}N_2O_6S$ requires 495.2529. Analytical chiral HPLC RT = 23.0 min, 99.9% (Chirobiotic column 25 cm × 4.6 mm, flow rate 0.8 mL/min, MeOH−AcOH−triethylamine (1000:0.05:0.2) at 10 °C, detected at 210 nm).

3-(3-{[7-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)-phenyl]ethyl}amino)heptyl]oxy}propyl)benzenesulfonamide (28b)-Acetate Salt. ES +ve 495 $(M + H)^+$. ^{1}H NMR δ (DMSO-d_6) 7.67−7.63 (2H, m), 7.47 (1H, t, *J* 8 Hz), 7.43 (1H, br d, *J* 8 Hz), 7.27 (1H, d, *J* 2 Hz), 7.00 (1H, dd, *J* 8, 2 Hz), 6.70 (1H, d, *J* 8 Hz), 4.61 (1H, dd, *J* 9, 4 Hz), 4.46 (2H, s), 3.35 (2H, t, *J* 7 Hz), 3.34 (2H, t, *J* 7 Hz), 2.73−2.60 (5H, m), 1.89 (3H, s), 1.85−1.77 (2H, m), 1.54−1.40 (4H, m), 1.35−1.24 (6H, m).

28b-Sulfamic Acid Salt. A solution of **28b**-free base (15 g, 30 mmol) in MeOH (250 mL) was treated with sulfamic acid (2.94 g, 30 mmol) at room temperature and the mixture was stirred for 1 h. The solvent was removed under reduced pressure, and the residue was heated to reflux in EtOH (90 mL) for 30 min and then allowed to cool to room temperature with gentle stirring for 48 h. The resulting white solid was collected by filtration, washed with EtOH, and dried to give **28b**-sulfamic acid salt (10.0 g, 57%) as a white crystalline solid: mp 108−115 °C; ES +ve 495 $(M + H)^+$. ^{1}H NMR δ (DMSO-d_6) 9.40 (1H, br s), 8.45 (1H, br s), 7.65 (2H, m), 7.45 (2H, m), 7.32 (3H, br d), 7.06 (1H, dd, *J* 8, 2 Hz), 6.75 (1H, d, *J* 8 Hz), 5.95 (1H, br s), 5.02 (1H, t, *J* 5 Hz), 4.80 (1H, d, *J* 10 Hz), 4.48 (2H, d, *J* 4.5 Hz), 3.35 (6H, m), 3.04 (1H, dd, *J* 12, 2.5 Hz), 2.92 (3H, m), 2.69 (2H, t, *J* 7.5 Hz), 1.80 (2H, m), 1.62 (2H, m), 1.50 (2H, m), 1.28 (6H, br s). HRMS ES +ve found: 495.2523 $C_{25}H_{38}N_2O_6S$ requires 495.2523.

3-(5-{[5-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)-phenyl]ethyl}amino)pentyl]oxy}pentyl)benzenesulfonamide (30b)-Acetate Salt. ES +ve *m/z* 495 $(M + H)^+$. ^{1}H NMR δ (DMSO-d_6) 7.65−7.62 (2H, m), 7.49−7.41 (2H, m), 6.99 (1H, dd, *J* 8, 2 Hz), 6.70 (1H, d, *J* 8 Hz), 4.55 (1H, t, *J* 6 Hz), 4.46 (2H, s), 3.37−3.30 (4H, m), 2.68−2.54 (6H, m), 1.89 (3H, s), 1.63−1.55 (2H, m), 1.55−1.38 (6H, m), 1.37−1.23 (4H, m). HRMS found: 495.2529 $C_{25}H_{38}N_2O_6S$ requires 495.2529.

3-{6-[4-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)-phenyl]ethyl}amino)butoxy]hexyl}benzenesulfonamide (31b)-Acetate Salt. ES +ve 495 *m/z* $(M + H)^+$. ^{1}H NMR δ (DMSO-d_6) 7.66−7.61 (2H, m), 7.49−7.40 (2H, m), 7.26 (1H, d, *J* 2 Hz), 6.98 (1H, dd, *J* 8, 2 Hz), 6.69 (1H, d, *J* 8 Hz), 4.53 (1H, t, *J* 6 Hz), 4.46 (2H, s), 3.33 (2H, t, *J* 6 Hz), 2.68−2.53 (8H, m), 1.89 (3H, s), 1.63−1.53 (2H, m), 1.53−1.38 (6H, m), 1.35−1.25 (4H, m). HRMS found: 495.2529 $C_{25}H_{38}N_2O_6S$ requires 495.2529.

3-(4-{[6-({(2*R*)-2-[3-(Formylamino)-4-hydroxyphenyl]-2-hydroxyethyl}amino)hexyl]oxy}butyl)benzenesulfonamide compound with (2*E*)-But-2-enedioic Acid (2:1) (33). A solution of **37** (320 mg, 0.47 mmol) in EtOH (15 mL) was hydrogenated in the presence of 10% palladium on carbon (50 mg) and palladium hydroxide on carbon (100 mg) at 100 psi for 18 h. The catalyst was removed by filtration over celite, and the filtrate was evaporated to dryness. The residual oil was purified by chromatography on a Biotage (8 g) cartridge eluting with CH_2Cl_2-EtOH-0.88 aqueous ammonia (25:8:1) to give **33**-free base (118 mg, 49%), which was dissolved in MeOH (20 mL) and treated with fumaric acid (13.3 mg, 0.11 mmol). The mixture crystallized to give **33**-fumarate salt (80 mg, 56%): ES +ve *m/z* 508 $(M + H)^+$. ^{1}H NMR δ (DMSO-d_6) 9.54 (1H, s), 8.25 (1H, s), 8.03 (1H, d, *J* 2 Hz), 7.63 (1H, s), 7.62 (1H, d, *J* 8 Hz), 7.44 (1H, t, *J* 8 Hz), 7.40 (1H, d, *J* 8 Hz), 6.88 (1H, dd, *J* 8, 2 Hz), 6.81 (1H, d, *J* 8 Hz), 6.40 (2H, s), 4.60 (1H, dd, *J* 9, 4 Hz), 3.34 (2H, t, *J* 6.5 Hz), 3.31 (2H, t, *J* 6.5 Hz), 2.78−2.61 (6H, m), 1.66−1.54 (2H, m), 1.54−1.40 (6H, m), 1.29−1.18 (4H, m). HRMS found: 508.2478 $C_{25}H_{38}N_3O_6S$ requires 508.2481.

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Supporting Information Available: NMR spectral data for **5**, **7**, **8**, **9d**, **9i**, **9j**, **10e**−**10h**, **12**, **13**, **14**, **15**, **16b**, **17a**−**17j**, **18b**, **19b**, **20b**, **21a**−**21j**, **22b**, **23b**, **24b**, **25a**−**25j**, **26b**, **27b**, **29b**, **29b**−cinnamate, **35**, **36**, **37**, biological screens, pharmacokinetic studies, cell permeability, HPLC retention times and purity of target compounds, microanalytical data for **13**, **17b**, **29b**−triphenylacetate and **29b**−cinnamate, LCMS traces for **28b**, **29c**, **29g**, **29j**, and **33**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Barnes, P. J. Immunology of asthma and chronic obstructive pulmonary disease. *Nat. Rev. Immunol.* **2008**, *8*, 183–192.
(2) Orsida, B. E.; Ward, C.; Li, X.; Bish, R.; Wilson, J. W.; Thien, F.; Walters, E. H. Effect of a long-acting β_2-agonist over three months on airway wall vascular remodelling in asthma. *Am. J. Respir. Crit. Care Med.* **2001**, *164*, 117–121.
(3) Carrol, N.; Elliot, J.; Morton, A.; James, A. The structure of large and small airways in nonfatal and fatal asthma. *Am. Rev. Respir. Dis.* **1993**, *47*, 405–410.
(4) John, N.; Au, B. T.; Jose, P. J.; Lim, S.; Barnes, P. J.; Mitchell, J. A.; Belvisi, M. G.; Chung, K. F. Expression and release of interleukin by HASM cells: Inhibition by Th-2 cytokines and corticosteroids. *Am. J. Respir. Crit. Care Med.* **1998**, *18*, 84–90.
(5) Bousquet, J.; Jeffrey, P. K.; Busse, W.; Johnson, M.; Vignola, A. M. Asthma: from bronchoconstriction to airway inflammation and remodelling. *Am. J. Respir. Crit. Care Med.* **2000**, *161*, 1720–1745.
(6) Bonner, J. C.; Badgett, A.; Lindroos, P. M.; Coin, P. G. Basic fibroblast growth factor induces expression of the PDGF receptor-alpha on human bronchial smooth muscle cells. *Am. J. Physiol.* **1996**, *271*, 880–888.
(7) Barnes, P. J. Efficacy of inhaled corticosteroids in asthma. *J. Allergy Clin. Immunol.* **1998**, *102*, 531–538.
(8) Boushey, H. A. Effects of inhaled corticosteroids on the consequences of asthma. *J. Allergy Clin. Immunol.* **1998**, *102*, S5−S16.
(9) Johnson, M. Salmeterol. *Med. Res. Rev.* **1995**, *15*, 225–257.
(10) Palmqvist, M.; Persson, G.; Lazer, L.; Rosenborg, J.; Larsson, P.; Lötvall, J. Inhaled dry-powder formoterol and salmeterol in asthmatic patients: onset of action, duration of effect and potency. *Eur. Respir. J.* **1997**, *10*, 2484–2489.

(11) Matthys, H. Is There a Need for Another Inhalative β_2-Agonist besides Formoterol in Patients with Asthma? *Respiration* **2001**, *68*, 432–437.
(12) Johnson, M. The β-adrenoceptor. *Am. J. Respir. Crit. Care Med.* **1998**, *158*, S146–S153.
(13) Pauwels, R. A.; Lofdahl, C. G.; Postma, D. S.; Tattersfield, A. E.; O'Byrne, P.; Barnes, P. J.; Ullman, A. Effect of inhaled formoterol and budesonide on exacerbations of asthma. *N. Engl. J. Med.* **1997**, *337*, 1405–1411.
(14) Ullman, A.; Hedner, J.; Svedmyr, N. Inhaled salmeterol in asthmatic patients: an evaluation of asthma symptoms and the possible development of tachyphylaxis. *Am. Rev. Respir. Dis.* **1990**, *142*, 571–575.
(15) Moore, R. H.; Millman, E. E.; Godines, V.; Hanania, N. A.; Tran, T. M.; Peng, H.; Dickey, B. F.; Knoll, B. J.; Clark, R. B. Salmeterol stimulation dissociates β_2-adrenergic receptor phosphorylation and internalization. *Am. J. Respir. Cell Mol. Biol.* **2007**, *36*, 254–261.
(16) Wang, S.; Collins, S. Regulation of the β_2-adrenergic receptor by the long-acting agonist salmeterol in human bronchial epithelial cells. *Am. J. Respir. Crit. Care Med.* **1996**, *153*, A730.
(17) Bennett, J. A.; Harrison, T. W.; Tattersfield, A. E. The contribution of the swallowed fraction of an inhaled dose of salmeterol to its systemic effects. *Eur. Respir. J.* **1999**, *13*, 445–448.
(18) Skidmore, I. F.; Lunts, L. H. C.; Finch, H.; Naylor, A.; Campbell, I. B. Optically active nitro alcohol derivatives, optically active amino alcohol derivatives, and process for preparing the same. German Patent DE3513885, 1985.
(19) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **1997**, *23*, 3–25.
(20) Coe, D. M.; Perciaccante, R.; Procopiou, P. A. Potassium trimethylsilanolate induced cleavage of 1,3-oxazolidin-2- and 5-ones, and application to the synthesis of (*R*)-salmeterol. *Org. Biomol. Chem.* **2003**, *1*, 1106–1111.
(21) Sonogashira, K.; Tohda, Y.; Hagihara, N. A convenient synthesis of acetylenes: catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes and bromopyridines. *Tetrahedron Lett.* **1975**, *16*, 4467–4470.
(22) Procopiou, P. A.; Morton, G. E.; Todd, M.; Webb, G. Enantioselective synthesis of (*S*)-salmeterol via asymmetric reduction of azidoketone by *Pichia angusta*. *Tetrahedron: Asymmetry* **2001**, *12*, 2005–2008.
(23) Hett, R.; Fang, Q. K.; Gao, Y.; Wald, S. A.; Senanayake, C. H. Large-scale synthesis of enantio- and diastereomerically pure (*R*,*R*)-formoterol. *Org. Process Res. Dev.* **1998**, *2*, 96–99.
(24) 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-methylphenyl)amino]methyl]benzyl alcohol, a potent β-adrenoreceptor stimulant. *Chem. Pharm. Bull.* **1978**, *26*, 1123–1129.
(25) Battram, C.; Charlton, S. J.; Cuenoud, B.; Dowling, M. R.; Fairhurst, R. A.; Farr, D.; Fozard, J. R.; Leighton-Davies, J. R.; Lewis, C. A.; McEvoy, L.; Turner, R. J.; Trifilieff, A. In vitro and in vivo pharmacological characterization of 5-[(*R*)-2-(5,6-diethyl-indan-2-ylamino)-1-hydroxy-ethyl]-8-hydroxy-1*H*-quinolin-2-one (Indacaterol), a novel inhaled β2 adrenoceptor agonist with a 24 h duration of action. *J. Pharmacol. Exp. Ther.* **2006**, *317*, 762–770.
(26) Jozwiak, K.; Khalid, C.; Tanga, M. J.; Berzetei-Gurske, I.; Jimenez, L.; Kozocas, J. A.; Woo, A.; Zhu, W.; Xiao, R.-P.; Abernethy, D. R.; Wainer, I. W. Comparative molecular field analysis of the binding of the stereoisomers of Fenoterol and Fenoterol derivatives to the β_2 adrenergic receptor. *J. Med. Chem.* **2007**, *50*, 2903–2915.
(27) Weersink, E. J. M.; Postma, D. S.; Koester, G. H.; Man, Y.; Nials, A. T.; Coleman, R. A. Picumeterol: dissociation of improvement in lung function and reduction of airways hyperresponsiveness in asthmatics. *Br. J. Clin. Pharmacol.* **1997**, *43*, 169–176.
(28) Ince, F. Dual D2-receptor and beta2-adrenoceptor agonists for the treatment of airways diseases; Special Publication—Royal Society of Chemistry, 264 (Medicinal Chemistry into the Millenium); Royal Society of Chemistry: London, 2001; pp 120−131.
(29) Alikhani, V.; Beer, D.; Bentley, D.; Bruce, I.; Cuenoud, B. M.; Fairhurst, R. A.; Gedeck, P.; Haberthuer, S.; Hayden, C.; Janus, D.; Jordan, L.; Lewis, C.; Smithies, K.; Wissler, E. Long-chain formoterol analogues: an investigation into the effect of increasing amino-substituent chain length on the β_2-adrenoceptor activity. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4705–4710.
(30) Nials, A. T.; Sumner, M. J.; Johnson, M.; Coleman, R. A. Investigations into factors determining the duration of action of the β_2 adrenoceptor agonist salmeterol. *Br. J. Pharmacol.* **1993**, *108*, 507–515.
(31) Manchee, G. R.; Barrow, A.; Kulkarni, S.; Palmer, E.; Oxford, J.; Colthup, P. V.; Maconochie, J. G.; Tarbit, M. H. Disposition of salmeterol xinafoate in laboratory animals and humans. *Drug Metab. Dispos.* **1993**, *21*, 1022–1028.
(32) Bradshaw, J.; Brittain, R. T.; Coleman, R. A.; Jack, D.; Kennedy, I.; Lunts, L. H. C.; Skidmore, I. F. The design of salmeterol, a long-acting selective β_2-adrenoceptor agonist. *Br. J. Pharmacol.* **1987**, *92*, 590P.
(33) Green, S. A.; Spasoff, A. P.; Coleman, R. R.; Johnson, M.; Liggett, S. B. Sustained activation of a G-protein-coupled receptor via "anchored" agonist binding. Molecular localization of the salmeterol exosite within the β_2-adrenergic receptor. *J. Biol. Chem.* **1996**, *271*, 24029–24035.
(34) Polczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Le Trong, I.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. Crystal structure of rhodopsin: A G protein-coupled receptor. *Science* **2000**, *289*, 739–745.
(35) Blaney, F. E.; Tennant, M. Computational tools and results in the construction of G protein-coupled receptor models. In *Membrane Protein Models*; Findlay, J. B. C., Ed.; Bios Scientific Publishers, Ltd: Oxford, 1996; pp 161−176.
(36) Cherezov, V.; Rosenbaum, D. M.; Hanson, M. A.; Rasmussen, S. G. F.; Thian, F. S.; Kobilka, T. S.; Choi, H.-J.; Kuhn, P.; Weis, W. I.; Kobilka, B. K.; Stevens, R. C. High-Resolution Crystal Structure of an Engineered Human β_2-Adrenergic G Protein-Coupled Receptor. *Science* **2007**, *318*, 1258–1265.
(37) Rosenbaum, D. M.; Cherezov, V.; Hanson, M. A.; Rasmussen, S. G. F.; Thian, F. S.; Kobilka, T. S.; Choi, H.-J.; Yao, X.-J.; Weis, W. I.; Stevens, R. C.; Kobilka, B. K. GPCR Engineering Yields High-Resolution Structural Insights into β_2-Adrenergic Receptor Function. *Science* **2007**, *318*, 1266–1273.
(38) Rasmussen, S. G. F.; Choi, H.-J.; Rosenbaum, D. M.; Kobilka, T. S.; Thian, F. S.; Edwards, P. C.; Burghammer, M.; Ratnala, V. R. P.; Sanishvili, R.; Fischetti, R. F.; Schertler, G. F. X.; Weis, W. I.; Kobilka, B. K. Crystal structure of the human β_2 adrenergic G-protein-coupled receptor. *Nature (London)* **2007**, *450*, 383–388.
(39) *FLO+, version Feb02, mcdock+ facility*; Colin McMartin, Thistlesoft: Colebrook, CT; PO Box 227, Colebrook, CT 06021.
(40) Strader, C. D.; Candelore, M. R.; Rands, E.; Hill, W. S.; Dixon, R. A. F. Conserved aspartic acid residues 79 and 113 of the beta-adrenergic receptor have different roles in receptor function. *J. Biol. Chem.* **1988**, *263*, 10267–10271.
(41) Wieland, K.; Zuurmond, H. M.; Krasel, C.; Ijzerman, A. P.; Lohse, M. J. Involvement of Asn-293 in stereospecific agonist recognition and in activation of the β_2-adrenergic receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9276–9281.
(42) Strader, C. D.; Candelore, M. R.; Hill, W. S.; Sigal, I. S.; Dixon, R. A. F. Identification of two serine residues involved in agonist activation of the beta-adrenergic receptor. *J. Biol. Chem.* **1989**, *264*, 13572–13578.
(43) Isogaya, M.; Yamagiwa, Y.; Fujita, S.; Sugimoto, Y.; Nagao, T.; Kurose, H. Identification of a Key Amino Acid of the β_2-Adrenergic Receptor for High Affinity Binding of Salmeterol. *Mol. Pharmacol.* **1998**, *54*, 616–622.
(44) Isogaya, M.; Sugimoto, Y.; Tanimura, R.; Tanaka, R.; Kikkawa, H.; Nagao, T.; Kurose, H. Binding pockets of the β_1 and β_2 adrenergic receptors for sub-type selective agonists. *Mol. Pharmacol.* **1999**, *56*, 875–885.
(45) Biggadike, K.; Coe, D. M.; Edney, D. D.; Halton, A.; Looker, B. E.; Montheith, M. J.; Moore, R. M.; Patel, R.; Procopiou, P. A. Patent WO 200266422 A1, 29 Aug 2002.
(46) Ford, A. J. et al. Forthcoming publication.
(47) Glossop, P. A.; Price, D. A. Progress in the Development of Inhaled, Long-Acting β_2-Adrenoceptor Agonists. *Ann. Rep. Med. Chem.* **2006**, *41*, 237–248.
(48) Brown, A. D.; Bunnage, M. E.; Glossop, P. A.; James, K.; Jones, R.; Lane, C. A. L.; Lewthwaite, R. A.; Mantell, S.; Perros-Huguet, C.; Price, D. A.; Trevethick, M.; Webster, R. The discovery of long acting β_2-adrenoreceptor agonists. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4012–4015.
(49) Brown, A. D.; Bunnage, M. E.; Glossop, P. A.; Holbrook, M.; Jones, R. D.; Lane, C. A. L.; Lewthwaite, R. A.; Mantell, S.; Perros-Huguet, C.; Price, D. A.; Webster, R. The discovery of indole-derived long acting β_2-adrenoceptor agonists for the treatment of asthma and COPD. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6188–6191.
(50) Brown, A. D.; Bunnage, M. E.; Glossop, P. A.; James, K.; Jones, R.; Lane, C. A. L.; Lewthwaite, R. A.; Mantell, S.; Perros-Huguet, C.; Price, D. A.; Trevethick, M.; Webster, R. The discovery of adamantyl-derived, inhaled, long acting β_2-adrenoreceptor agonists. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1280–1283.
(51) Noronha, O. P. D. Preparation of *N*-3-iodo(131I)benzenesulfonyl *N*′-propylurea and its tissue distribution. *J. Labelled Compd.* **1973**, *9*, 261–268.

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