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The discovery of long-acting saligenin β_2 adrenergic receptor agonists incorporating a urea group

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ABSTRACT

A series of novel, potent and selective human β_2 adrenoceptor agonists incorporating a urea moiety on the terminal right-hand side phenyl ring of (*R*)-salmeterol is presented. Urea **9j** had long duration of action in vitro on guinea pig trachea, and also in vivo similar to that of salmeterol. It had lower oral absorption and bioavailability than salmeterol in both rat and dog. It had a turnover ratio similar to salmeterol, with no evidence for formation of any aniline metabolites in human liver microsomes and hepatocytes. However no crystalline salts suitable for inhaled delivery were identified.

1. Introduction

Asthma is a chronic disease affecting 300 million people worldwide, characterised by an increase in inflammatory cell population in the epithelium and submucosa of the airways.[1] There are two main components of asthma pathophysiology, airway inflammation and smooth muscle dysfunction leading to two major categories of medicines used in asthma treatment: anti-inflammatory drugs and bronchodilators. Inhaled corticosteroids are used to treat the inflammatory component of asthma, whereas inhaled β_2-agonists are the most effective bronchodilators, offering proven benefits in reducing the burden of this disease.[2,3] Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death and is projected to rise to third place by 2020. Bronchodilators such as inhaled β_2-agonists and muscarinic antagonists are currently the mainstay of treatment for COPD and combinations with inhaled corticosteroids are known to reduce the incidence of exacerbations. The two twice-daily prescribed inhaled β_2-agonists for the treatment of asthma are salmeterol (**1**) and formoterol (**2**) (Chart 1). Salmeterol has intrinsic activity of 0.37 versus isoprenaline, a delayed onset of action, and a dose-independent duration of action,[4,5] whereas formoterol is a high intrinsic activity agonist (0.97) with a short onset of action, and a dose-dependent duration of action.[5,6] In the last 10 years there has been great interest within the pharmaceutical industry in the discovery of a once-daily β_2 adrenoceptor agonist to be used in new combination therapies for the treatment of asthma and COPD. There are currently at least five candidates in clinical development, the two more advanced candidates are Novartis' indacaterol (**3**),[7] which has already been approved for use in COPD in some markets, and GlaxoSmithKline's vilanterol (**4**),[8] which is in late phase 3 clinical trials. The remaining three development candidates are Boehringer-Ingelheim's olodaterol (**5**),[9] Pfizer's candidate PF-610355 (**6**),[10] and Chiesi's carmoterol (**7**).[11] Our group published three papers, one on sulfonamides including GlaxoSmithKline's first candidate **8**,[12] on our clinical candidate vilanterol (**4**),[8] and on hydantoins.[13]

A major fraction of the dose (up to 90%) of an inhaled drug is swallowed and liable to be absorbed from the gastro-intestinal tract.[14] Thus, one approach to improve the therapeutic index could be to alter the physicochemical properties of the drug and make it less prone to absorption. Our group has published two papers using this approach, one on sulfonamides including our first candidate **8**,[12] and on hydantoins.[13] A second approach (the antedrug approach) could be to introduce metabolic instability to the molecule to facilitate its conversion into inactive metabolites following systemic absorption from either the GI tract or the lung. Better still, a combination of the two approaches may potentially deal with both the inhaled and swallowed fractions of each dose.[8] In this report we present our studies in identifying urea β_2 adrenoceptor

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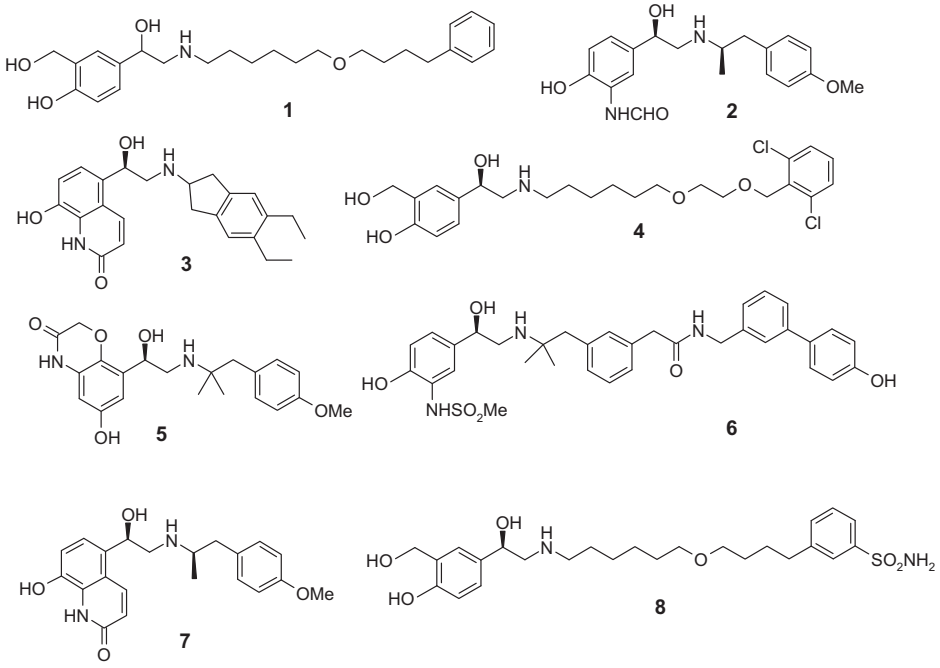


Chart 1. Clinically used β_2 agonists, and recent development candidates.

agonists with reduced oral absorption. Some of the physicochemical parameters that influence oral absorption are molecular weight, lipophilicity, membrane permeability, the number of hydrogen-bond donors and acceptors, conformational flexibility and solubility.[15] Substitution on the right-hand side phenyl ring of salmeterol with the polar sulfonamide group was found to enhance β_2 agonist affinity.[12] It was hypothesised that a polar substituent, such as a urea, with increased number of hydrogen-bond donors and acceptors which contravened the Lipinski rules,[16] might be expected to show reduced oral bioavailability. We have demonstrated that introduction of either a sulfonamide group,[12] or a heterocyclic ring such as hydantoin[13] brings about longer duration of action. It was therefore hypothesised that introduction of the urea group might also bind in a similar way to the sulfonamide group of **8** and hence have similarly long duration of action.

2. Chemistry

The target compounds **9** were synthesised by two general routes, the first one was a linear synthetic route outlined in Scheme 1. This involved Sonogashira coupling of the acetylene **10**[12] with aryl iodides/bromides **11**, reaction of the resulting bromide **12** with 2 equiv of the amino alcohol **13**[13] in order to minimise bis-alkylation,[17] catalytic hydrogenation of the mono-alkylated product **14**, and finally hydrolysis of the acetonide protecting group with aqueous acetic acid provided **9a–d,i**.

The second route, outlined in Scheme 2, used (*R*)-2-oxazolidinone protected saligenin **15**[18] and bypassed the requirement of using greater than stoichiometric quantities of the valuable chiral amino-alcohol moiety, unlike the previous route where excess **13** was needed to minimise bis-alkylation on nitrogen. Thus, **15** was alkylated with the bromoacetylene **10**[12] in the presence of sodium hydride in DMF to give the intermediate **16**, which was coupled with the appropriate aryl iodide/bromide **11** using Sonogashira conditions to provide acetylene intermediates **17**. The latter were reduced by catalytic hydrogenation over platinum oxide, the oxazolidinone ring of the resulting saturated intermediates **18** were hydrolysed by our previously reported KOTMS method[18] to provide ethanolamines **19**. A final aqueous acetic acid hydrolysis of the acetonide protecting group gave the required target compounds **9e,f**.

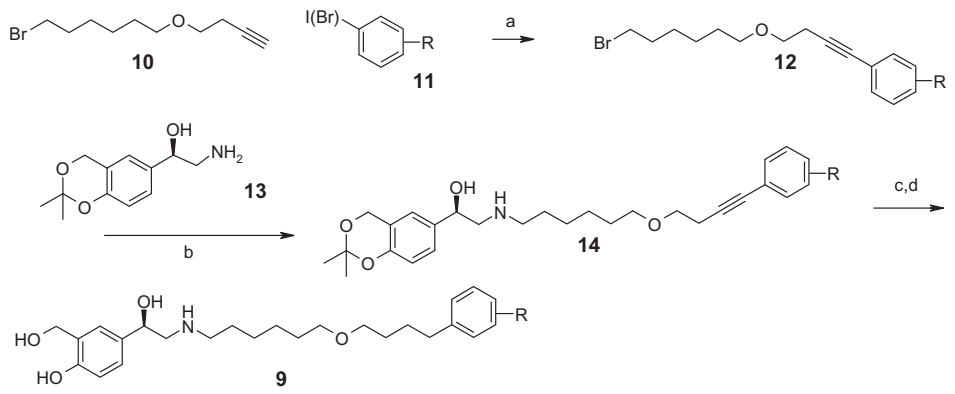
The substituted aryl iodides/bromides **11** were obtained by reaction of the appropriate iodo/bromo-aniline/benzylamines with the corresponding isocyanate/sodium cyanate.[19] The synthesis of the intermediate aryl bromide **22** used in the synthesis of **9j** is shown in Scheme 3. Nitroaniline **20** was brominated in acetic acid with bromine to provide 2-bromo-4-methyl-6-nitroaniline (**21**),[20] which was diazotised and reduced to give **22**[21] in 62% yield over the two steps.

Completion of the synthesis of **9j** is shown in Scheme 4. Reaction of acetylene **16** with bromide **22** provided **23**, which was converted to **24** by simultaneous hydrogenation of the acetylene and reduction of the nitro group, formation of the primary urea **18j** with sodium cyanate, cleavage of the oxazolidinone ring with potassium trimethylsilanolate to **19j**, and finally hydrolysis of the acetonide group as before, gave **9j**.

Lastly the ureido-amides **9g** and **9h** were obtained by reaction of the hydantoin **25**[13] with ammonia or cyclopentylamine, respectively (Scheme 5).

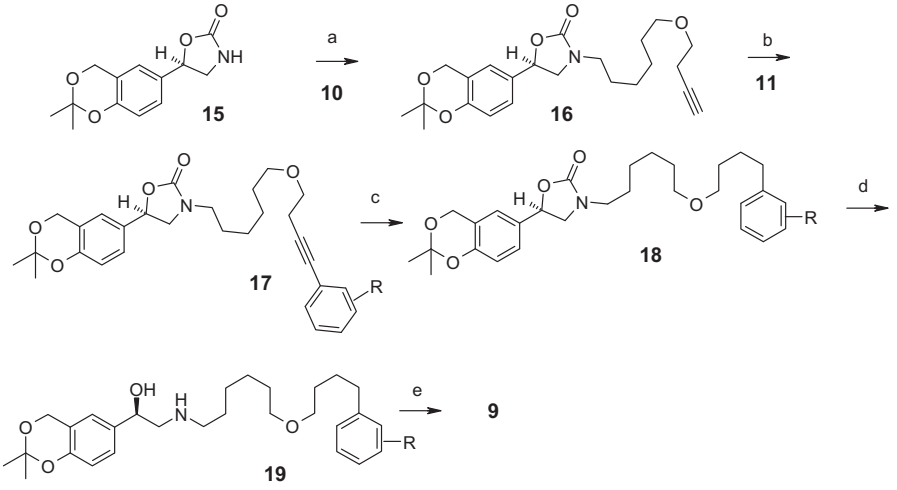
3. Results and discussion

Compounds in Table 1 were tested for their ability to cause cyclic AMP accumulation in Chinese hamster ovary (CHO) 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 as pEC_{50} values (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. Required selectivity for β_2 over β_1 and β_3 adrenoceptors was set as better than that of (*R*,*R*)-formoterol. Microsomal instability of the compounds towards high

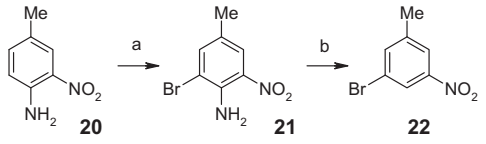


a	3-$NHCONH_2$	**f**	3-$CH_2NHCONHEt$
b	3-NHCONHEt	**g**	3-$NHCONHCH_2CONH_2$
c	3-NHCONH-cC_6H_{11}	**h**	3-$NHCONHCH_2CONH$-cC_5H_9
d	4-NHCONHEt	**i**	3-$NHCONH_2$-5-CF_3
e	3-$CH_2NHCONH_2$	**j**	3-$NHCONH_2$-5-CH_3

Scheme 1. Synthetic route to analogues **9** via **13** and list of substituents **R** present in compounds **9**, **11**, **12**, **14**, **17**, **18** and **19**. Reagents: (a) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF; (b) **13** (2 equiv), DMF; (c) H_2, PtO_2, EtOAc; (d) AcOH–H_2O (3:1), 75 °C.

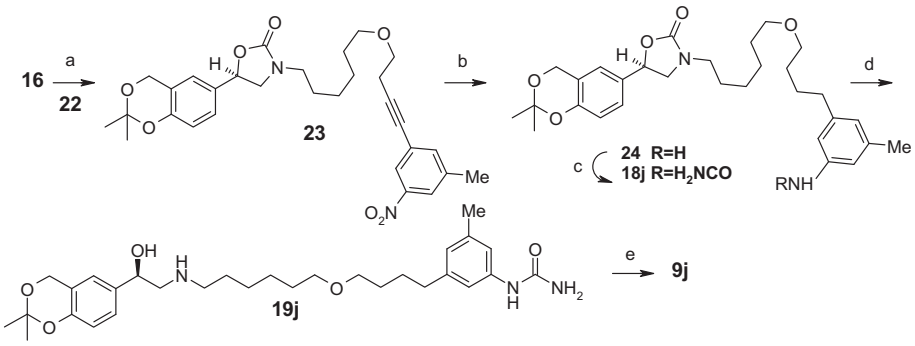


Scheme 2. Synthetic route to analogues **9** via **15**. Reagents: (a) NaH, DMF; (b) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, MeCN; (c) H_2, PtO_2, EtOH-EtOAc; (d) KOTMS, THF, 65 °C; (e) AcOH–H_2O (3:1), 75 °C.

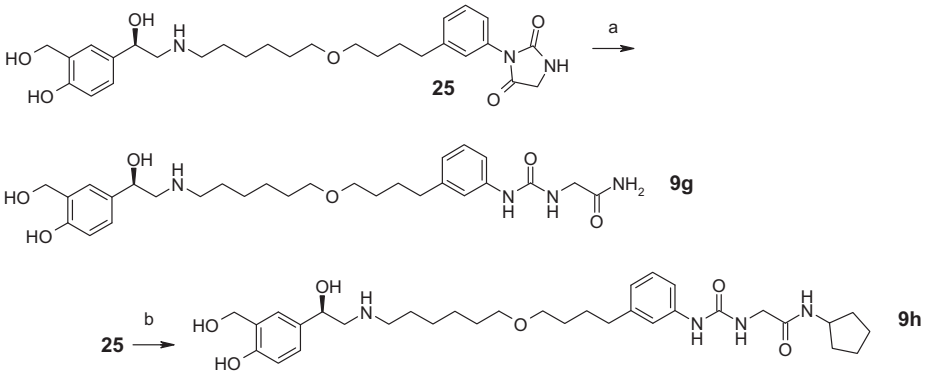


Scheme 3. Synthetic route to compound **22**. Reagents: (a) Br_2, AcOH, 94%; (b) $NaNO_2$, H_2SO_4, EtOH, 66%.

first-pass metabolism in the liver was assessed by incubating samples with human liver microsomes (P450 content = 125 pmol/mL) at a compound concentration of 5 μM for 30 min at 37 °C. Compound turnover was expressed as a ratio relative to the assay standard, verapamil. Salmeterol which had a slightly higher turnover ratio (1.2) was also included as a standard. All the compounds tested were potent β_2 receptor agonists (substantially more potent than the standard, isoprenaline), and some were more potent than salmeterol and formoterol. The primary urea and two secondary ureas **9a–c** had similar β_2 receptor agonist activity. However, **9c** had lower intrinsic activity, inferior β_1 and β_3 selectivity, and was rejected despite its high metabolic turnover ratio. The *para*-ethylurea **9d** had lower IA than the *meta*-ethylurea **9b** and hence all other analogues reported herein are in the *meta*-series. This observation confirms our previous findings within other salmeterol series.[12,13] The homologous urea **9e** was equipotent to its parent **9a** and had a high turnover ratio, however, it had a lower intrinsic activity and was therefore not investigated further. The homologous urea **9f** was equipotent to its parent **9b** and was very selective for β_2 over β_1 and β_3. Its intrinsic activity was lower than that of salmeterol and therefore not investigated any further. Introduction of an additional amide group as in the derivatives **9g** and **9h** caused a



Scheme 4. Synthetic route to analogues **9j**. Reagents: (a) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, 43%; (b) H_2, PtO_2, EtOAc; EtOH, 99%; (c) NaNCO, AcOH, H_2O, 75%; (d) KOTMS, THF, 65 °C, 85%; (e) AcOH–H_2O (3:1), 75 °C, 77%.



Scheme 5. Synthetic route to analogues **9g** and **9h**. Reagents: (a) 2 M NH_3 in MeOH, 10%; (b) *c*-$C_5H_9NH_2$, EtOH, 48%.

Table 1
Stimulation of cAMP Accumulation in CHO Cells Expressing Human β_2, β_1 and β_3 adrenoceptors, and human liver microsomal turnover ratio against verapamil

Entry	Compound	β_2[a] pEC_{50} (n)	IA	β_1[a] pEC_{50} (n)	β_2–β_1[b]	β_3[a] pEC_{50} (n)	β_2–β_3[c]	Turnover ratio[d]
1	**9a**.**AcOH**	9.1 ± 0.1 (4)	0.41	7.2 ± 1.0 (2)	1.9	8.1 ± 0.0 (2)	1.0	0.97
2	**9b**.**AcOH**	9.0 ± 0.0(3)	0.46	7.5 ± 0.3 (2)	1.5	6.8 ± 0.1 (2)	2.2	0.81
3	**9c**.**AcOH**	9.0 ± 0.2 (2)	0.35	8.1 ± 0.0 (2)	0.9	8.6 ± 0.1 (2)	0.4	2.18
4	**9d**.**AcOH**	9.3 ± 0.3 (6)	0.37	7.5 ± 0.1 (11)	1.7	7.9 ± 0.1 (12)	1.4	1.37
5	**9e**.**AcOH**	9.3 ± 0.2 (4)	0.36	8.1 ± 0.2 (5)	1.2	7.8 ± 0.1 (8)	1.5	1.64
6	**9f**.**AcOH**	9.2 ± 0.2 (4)	0.32	7.5 ± 0.1 (4)	1.7	7.5 ± 0.1 (4)	1.7	0.72
7	**9g**.**HCO_2H**	9.6 ± 0.1 (5)	0.39	7.5 ± 0.1 (4)	2.1	7.0 ± 0.1 (4)	2.6	ND[e]
8	**9h**.**AcOH**	9.5 ± 0.1 (2)	0.30	8.0 ± 0.1 (4)	1.5	7.9 ± 0.0 (4)	1.6	ND[e]
9	**9i**.**AcOH**	9.0 ± 0.3 (6)	0.34	7.3 ± 0.1 (3)	1.7	7.6 ± 0.1 (4)	1.4	1.34
10	**9j**.**AcOH**	9.8 ± 0.1(17)	0.72	7.4 ± ± 0.1 (24)	2.4	7.4 ± 0.1 (24)	2.5	1.17
11	**Isoprenaline**	7.4 ± 0.0 (767)	1.0	8.1 ± 0.0 (641)	−0.7	7.4 ± 0.0 (659)	0.0	ND[e]
12	**1**	9.6 ± 0.0 (929)	0.37	6.1 ± 0.0 (656)	3.5	5.9 ± 0.0 (849)	3.7	1.2
13	(***R***,***R***)-**2**.**fumarate**	9.3 ± 0.0 (791)	0.97	7.4 ± 0.0 (670)	1.9	7.6 ± 0.0 (653)	1.7	ND[e]
14	**4**.**AcOH**	9.4 ± 0.0 (8)	0.69	6.4 ± 0.1 (8)	3.0	6.1 ± 0.2 (8)	3.3	1.65

[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] Selectivity for β_2 over β_1 expressed as pEC_{50} at β_2 receptor—pEC_{50} at β_1.
[c] Selectivity for β_2 over β_3 expressed as pEC_{50} at β_2 receptor—pEC_{50} at β_3.
[d] Compound turnover in human liver microsomes expressed as a ratio ralative to verapamil, where verapamil has a turnover ratio of 1.
[e] ND not determined.

significant increase in the β_2 receptor agonist activity by comparison to **9a**. Furthermore the selectivity of both compounds was significantly increased. In particular **9g** possessed one of the highest selectivity ratios of all the compounds in Table 1. The intrinsic activity of **9h** was lower than salmeterol, therefore this compound was rejected too. The intrinsic activity of **9g** was marginally higher than salmeterol and the compound was retained for further investigation. Introduction of a trifluoromethyl substituent to the phenyl ring bearing the urea moiety (**9i**) increased the metabolic turnover ratio, but reduced the IA, and did not offer any advantage. The methyl substituted primary urea **9j** possessed the highest β_2 potency of all the compounds in Table 1, highest β_2 selectivity over β_1 and β_3, similar turnover ratio to salmeterol, and a higher intrinsic activity than salmeterol. Furthermore it compared favourably with vilanterol (Table 1). Urea **9j** improved on all criteria of potency, IA, β_2 selectivity and turnover ratio, and therefore presented a very attractive profile.

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

Table 2
The potency, onset time and duration of action of selected compounds on isolated superfused guinea pig trachea (minimum $n = 2$), and lipophilicity measurements

Entry	Compound	pEC_{50}	Onset time (min)	Shift (1 h)	Shift (3 h)	$c\log P$	$\log D_{6.4}$[a]
1	**9a**.**AcOH**	8.6	13.5	5	7	1.8	−0.28
2	**9b**.**AcOH**	8.9	15	11	19	2.6	0.5
3	**9g**.**HCO_2H**	9.6	9	78	149	0.8	−0.78
4	**9j**.**AcOH**	8.8	10	1	2	2.3	0.2
5	**Salmeterol**	8.3	27.6	1.0	1.1	3.0	1.06
6	**(*R*,*R*)-2**.**fumarate**	9.5	10	20	>1940	1.3	ND[b]

[a] $\log D_{6.4}$ measured $\log D$ at pH 6.4.
[b] ND not determined.

Table 3
Han Wistar rat and Beagle dog pharmacokinetic data for **9j** acetate

Species	Route	Dose (mg/kg)	Cl_p (mL/min/kg)	V_{dss} (L/kg)	$T_{½}$ (h)	*F*%
Rat	Intravenous	0.25	83	2.0	0.9	
	Oral	2.0				<5
Dog	Intravenous	0.05	6–19	0.55	1–3	
	Oral	0.25				6

duration of action.[22] 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 of the more promising compounds (**9a,b,g,j**) is presented and contrasted to salmeterol. 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 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 compounds in Table 2 had potency at the guinea pig trachea higher than that of salmeterol. Onset times were faster than salmeterol and closer to that of formoterol. Clearly the urea **9g** despite its very high potency on both CHO cells and guinea pig trachea, had a significantly shorter duration of action. The shorter duration of action was related to the lower lipophilicity of **9g** ($\log D_{6.4}$–0.78, $c\log P$ 0.82, Table 2) as we have previously discovered,[8,12] and others have recently disclosed.[23,24] From the remaining three compounds **9j** had shift values of 1 and 2, a duration in vitro very similar to salmeterol. Furthermore **9j** had a better overall profile of potency on both CHO cells and guinea pig trachea, β_2 selectivity, faster onset time, and higher metabolic turnover ratio. For these reasons **9j** was progressed to pharmacokinetic studies in rats and dogs and the data is shown in Table 3. The compound had lower oral absorption (AUC 4811 ng min/mL) than salmeterol in rats (deconjugated HPV model).[13] In the male Han Wistar rat **9j** had a moderate volume of distribution (2 L/kg) and a high plasma clearance (83 mL/min/kg, 100% liver blood flow) resulting in a short half-life of less than 1 h. In the female beagle dog **9j** had a low volume of distribution (0.55 L/kg) and a low to moderate plasma clearance (<67% liver blood flow). Low oral bioavailability was observed in both the rat (<5%) and the dog (6%) (Table 3). This oral bioavailability was much lower than that previously reported for salmeterol (66–78% in dog, and 10–12% in rat)[25] and strengthened the confidence that low systemic exposure would be expected from the swallowed fraction of the inhaled dose of **9j** in humans. In addition to the low oral absorption of **9j** in the rat, first pass metabolism in the gastrointestinal tract may also contribute to the resultant low oral bioavailability. Deconjugated rat plasma samples, following intravenous or oral administration were analysed in an attempt to identify possible metabolites of **9j**, such as the aniline derived from cleavage of the primary urea moiety. However no aniline metabolite of **9j** was detected.

The acetate salt of **9j** was also investigated in vivo in histamine-induced bronchospasm in the guinea pig in a plethysmograph chamber (Buxco) and found to have similar potency to salmeterol (EC_{90} 10 and 50 μM respectively, nebuliser concentration). At an equi-effective (EC_{90}) dose, the duration of action of **9j** acetate (time to 50% recovery) was similar to that of salmeterol (7 vs 8 h) when administered as a nebulised solution in a dimethyl acetamide-saline vehicle. Furthermore the duration of **9j** was extended to 18 h when the dose was increased to 10-fold the EC_{90}. Urea **9j** was progressed towards a salt selection screen to identify a stable, non-hygroscopic and crystalline salt, suitable for inhaled delivery. Unfortunately **9j** acetic acid salt was a gum, and no developable crystalline salts were identified, whereas the free base was an amorphous solid.

4. Conclusion

Incorporation of a urea group on the right-hand side phenyl ring of (*R*)-salmeterol has provided a series of potent and selective human β_2 adrenoceptor agonists. Urea **9j** had duration of action on guinea pig trachea, and also in vivo similar to that of salmeterol and vilanterol. It had lower oral absorption and bioavailability than salmeterol in both rat and dog. It had a turnover ratio similar to salmeterol, with no evidence for formation of any aniline metabolites. No crystalline salts suitable for inhaled delivery were identified; for this reason coupled with the steady progress of vilanterol in clinical trials, compound **9j** was not progressed any further.

5. Experimental

Organic solutions were dried over anhydrous $MgSO_4$. TLC was performed on Merck 0.25 mm Kieselgel 60 F_{254} plates. Products were visualised 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 (art. 9385), or Biotage pre-packed 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. The chemical shifts are expressed in ppm relative to tetramethylsilane. All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.

5.1. 2-Bromo-4-methyl-6-nitroaniline (21)[20]

4-Methyl-2-nitroaniline (131.2 g, 862 mmol) was suspended in glacial acetic acid (1.25 L) and bromine (54 mL, 1.05 mol) added over 1 h at ambient temperature. The reaction mixture was stirred for 1 h, poured into water (7.5 L) and the suspension stirred for 30 min. The solid was filtered, washed with water (5 × 1 L) and dried to give **21** (187.2 g, 94%) as an orange solid: ^{1}H NMR δ ($CDCl_3$) 7.94 (1H, s), 7.56 (1H, s), 6.47 (2H, br s), 2.27 (3H, s).

5.2. 3-Bromo-5-nitrotoluene (22)[21]

2-Bromo-4-methyl-6-nitroaniline (20.5 g, 88.7 mmol) was suspended in ethanol (105 mL) and sulfuric acid (s.g. 1.84, 14 mL) added portionwise. The solution was heated to 73 °C and sodium nitrite (13.7 g, 198.5 mmol) added over 25 min, maintaining the temperature at 73–78 °C for 30 min. The reaction mixture was cooled and then poured into water (700 mL). The solid was collected by filtration, washed with water and the product purified by steam distillation to give **22** (12.6 g, 66%) as a yellow solid: ^{1}H NMR δ ($CDCl_3$) 8.19 (1H, br s) 7.99 (1H, br s), 7.66 (1H, br s), 2.47 (3H, s); ^{13}C NMR δ ($CDCl_3$) 148.7, 141.6, 138.1, 123.9, 122.7, 122.5, 21.1; IR ν_{max} 1533, 1342, 740 cm^{-1}.

5.3. (5*R*)-5-(2,2-Dimethyl-4*H*-1,3-benzodioxin-6-yl)-3-(6-{[4-(3-methyl-5-nitrophenyl)-3-butyn-1-yl]oxy}hexyl)-1,3-oxazolidin-2-one (23)

A degassed solution of anhydrous tetrahydrofuran (80 mL) and triethylamine (10 mL) was treated with 1-bromo-3-methyl-5-nitrobenzene (2.69 g, 12.4 mmol), dichloro bis(triphenylphosphine) palladium(II) (613 mg, 0.87 mmol) and cuprous iodide (308 mg, 1.6 mmol). The resultant mixture was then purged with nitrogen and heated to 70 °C. After 10 min, a solution of (5*R*)-3-[6-(but-3-ynyloxy)hexyl]-5-(2,2-dimethyl-4*H*-1,3-benzodioxin-6-yl)-1,3-oxazolidin-2-one (5 g, 12.4 mmol) in anhydrous degassed THF (20 mL) was added and the reaction mixture stirred at 70 °C for 3 h. The cooled reaction mixture was evaporated to dryness and the residue was suspended in diethyl ether and filtered through celite. The filtrate was concentrated under reduced pressure and the residue was purified by chromatography on a Biotage cartridge (90 g), eluting with 20% ethyl acetate in cyclohexane (1 L), 25% ethyl acetate in cyclohexane (0.5 L), 30% ethyl acetate in cyclohexane (2 L), to give **23** (2.86 g, 43%): MS ES+ve *m/z* 537 $(M+H)^+$; ^{1}H NMR δ ($CDCl_3$) 8.04 (1H, br s), 7.93 (1H, br s), 7.51 (1H, br s), 7.12 (1H, br d, *J* 8 Hz), 7.00 (1H, br s), 6.84 (1H, d, *J* 8 Hz), 5.39 (1H, t, *J* 8 Hz), 4.84 (2H, s), 3.85 (1H, t, *J* 8 Hz), 3.63 (2H, t, *J* 7 Hz), 3.49 (2H, t, *J* 6 Hz), 3.43–3.20 (3H, m), 2.69 (2H, t, *J* 7 Hz), 2.42 (3H, s), 1.65–1.51 (4H, m), 1.54 (6H, s), 1.47–1.31 (4H, m).

5.4. (5*R*)-3-(6-{[4-(3-Amino-5-methylphenyl)butyl]oxy}hexyl)-5-(2,2-dimethyl-4*H*-1,3-benzodioxin-6-yl)-1,3-oxazolidin-2-one (24)

(5*R*)-5-(2,2-Dimethyl-4*H*-1,3-benzodioxin-6-yl)-3-(6-{[4-(3-methyl-5-nitrophenyl)but-3-ynyl]oxy}hexyl)-1,3-oxazolidin-2-one (3.18 g, 5.92 mmol) was stirred with platinum oxide (515 mg) in ethanol (50 mL) and EtOAc (10 mL) under hydrogen for 2.5 h. The catalyst was removed by filtration through a pad of celite. The filtrate was evaporated to dryness to give **24** (2.99 g, 99%): MS ES+ve *m/z* 511 $(M+H)^+$; ^{1}H NMR δ ($CDCl_3$) 7.12 (1H, br d, *J* 8.5 Hz), 7.00 (1H, br s), 6.84 (1H, d, *J* 8.5 Hz), 6.41 (1H, br s), 6.34 (2H, br s), 5.39 (1H, t, *J* 8 Hz), 4.84 (2H, s), 3.85 (1H, t, *J* 9 Hz), 3.70–3.45 (2H, br), 3.44–3.35 (6H, m), 3.35–3.20 (1H, m), 2.49 (2H, t, *J* 7 Hz), 2.22 (3H, s), 1.71–1.46 (14 H, m), 1.43–1.30 (4H, m).

5.5. *N*-{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]-5-methylphenyl}urea (18j)

A suspension of potassium cyanate (952 mg, 11.7 mmol) in water (23 mL) was slowly added to a solution of (5*R*)-3-{6-[4-(3-amino-5-methylphenyl)butoxy]hexyl}-5-(2,2-dimethyl-4*H*-1,3-benzodioxin-6-yl)-1,3-oxazolidin-2-one (2.99 g, 5.86 mmol) in glacial acetic acid (23 mL) containing water (11 mL) at ~0 °C under nitrogen. The mixture was warmed to room temperature over ~2 h and then stirred at room temperature for 20 min. The reaction mixture was evaporated to dryness and the residue purified by chromatography on a Biotage (90 g) cartridge eluting with 20% ethyl acetate–cyclohexane (1 L), 25% ethyl acetate–cyclohexane (0.5 L), 50% ethyl acetate–cyclohexane (1 L), 60% ethyl acetate–cyclohexane (1 L), 70% ethyl acetate–cyclohexane (1 L), and finally ethyl acetate (1 L). Appropriate fractions were combined and purified further by chromatography on a Biotage cartridge (90 g) eluting with a gradient of 50–80% ethyl acetate–cyclohexane to give **18j** (2.45 g, 75%): MS ES+ve *m/z* 554 $(M+H)^+$; ^{1}H NMR δ ($CDCl_3$) 7.15-7.05 (2H, m), 7.00 (1H, br s), 6.84 (1H, d, *J* 8 Hz), 6.76 (1H, br s), 6.71 (1H, br s), 5.41 (1H, t, *J* 8 Hz), 4.84 (2H, s), 4.80 (2H, br), 3.87 (1H, t, *J* 9 Hz), 3.48–3.23 (7H, m), 2.55 (2H, t, J 8 Hz), 2.29 (3H, s), 1.73–1.49 (15H, m), 1.48–1.32 (4H, m); ^{13}C NMR δ ($CDCl_3$) 158.7, 151.5, 143.9, 139.2, 138.9, 132.7, 126.1, 124.9, 122.8, 120.0, 118.8, 117.6, 100.0, 70.4, 69.6, 61.2, 55.6, 49.0, 36.0, 29.6, 28.7, 26.7, 26.3, 25.2, 25.0, 21.8.

5.6. *N*-(3-{4-[(6-{[(2*R*)-2-(2,2-Dimethyl-4*H*-1,3-benzodioxin-6-yl)-2-hydroxyethyl]amino}hexyl)oxy]butyl}-5-methylphenyl)-urea (19j)

To a solution of *N*-{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]-5-methylphenyl}urea (2.45 g, 4.42 mmol) in anhydrous THF (100 mL) was added potassium trimethylsilanolate (2.27 g, 17.7 mmol). The reaction was stirred under nitrogen at 65 °C for 45 min. The cooled reaction mixture was then diluted with water and extracted into ethyl acetate, the organic layer was dried ($MgSO_4$) and filtered. The filtrate was evaporated to dryness and the residue purified by chromatography on a Biotage (90 g) cartridge, eluting with CH_2Cl_2, 0–100% ethyl acetate in cyclohexane

gradient, followed by 0–8% MeOH (containing trace of aqueous ammonia) in dichloromethane gradient to give **19j** (1.97 g, 85%): MS ES+ve *m/z* 528 $(M+H)^+$; ^1H NMR δ ($CDCl_3$) 7.16 (1H, br), 7.11 (1H, br d, *J* 8 Hz), 7.00 (1H, br s), 6.96 (1H, br s), 6.88 (1H, br s), 6.78 (1H, d, *J* 8 Hz), 6.74 (1H, br s), 4.99 (2H, s), 4.82 (2H, s), 4.64 (1H, dd, *J* 9, 3 Hz), 3.44–3.35 (4H, m), 2.82 (1H, dd, *J* 12, 3 Hz), 2.73–2.57 (4H, m), 2.55 (2H, t, *J* 8 Hz), 2.27 (3H, s), 1.71–1.44 (15 H, m), 1.40–1.30 (4H, m).

5.7. *N*-[3-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl}amino)hexyl]oxy}butyl)-5-methylphenyl]urea (9j) acetate salt

N-(3-{4-[(6-{[(2*R*)-2-(2,2-Dimethyl-4*H*-1,3-benzodioxin-6-yl)-2-hydroxyethyl]amino}hexyl)oxy]butyl}-5-methylphenyl)urea (1.972 g, 3.73 mmol) was stirred with glacial acetic acid (50 mL) and water (25 mL) at 80 °C for 45 min. The resultant reaction mixture was cooled, evaporated to dryness and the residue was dissolved in MeOH and a minimum amount of 2 M aqueous ammonia in methanol. The solution was applied to a Biotage cartridge (90 g) and eluted with a gradient of 0–15% [(10% concentrated aqueous ammonia in methanol)–dichloromethane]. Appropriate fractions were combined and evaporated and the residue was dissolved in acetic acid and then re-evaporated under reduced pressure to give **9j.AcOH** (1.4 g, 77%): LC–MS RT = 2.36 min, ES+ve *m/z* 488 $(M+H)^+$; ^1H NMR δ (CD_3OD) 7.35 (1H, br s), 7.16 (1H, br d, *J* 8 Hz), 7.06 (1H, br s), 6.97 (1H, br s), 6.80 (1H, d, *J* 8 Hz), 6.68 (1H, br s), 4.83 (1H, t, *J* 6.5 Hz), 4.67 (2H, s), 3.46 (2H, t, *J* 6.5 Hz), 3.44 (2H, t, *J* 6.5 Hz), 3.02 (2H, d, *J* 6.5 Hz), 2.92 (2H, t, *J* 7.5 Hz), 2.56 (2H, t, *J* 7.5 Hz), 2.27 (3H, s), 1.92 (3H, s), 1.73–1.54 (8H, m), 1.47–1.37 (4H, m).

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