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The discovery of long-acting saligenin β_2 adrenergic receptor agonists incorporating hydantoin or uracil rings

Panayiotis A. Procopiou [a,*], Victoria J. Barrett [b], Nicola J. Bevan [b], Peter R. Butchers [b], Richard Conroy [a], Amanda Emmons [b], Alison J. Ford [b], Séverine Jeulin [a], Brian E. Looker [a], Gillian E. Lunniss [a], Valerie S. Morrison [b], Peter J. Mutch [c], Rossana Perciaccante [a], Mark Ruston [a], Claire E. Smith [c], Graham Somers [c]

[a] Department of Medicinal Chemistry, GlaxoSmithKline Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY, United Kingdom
[b] Department of Respiratory Biology, GlaxoSmithKline Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY, United Kingdom
[c] Department of Drug Metabolism and Pharmacokinetics, GlaxoSmithKline Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY, United Kingdom

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ABSTRACT

A series of novel, potent and selective human β_2 adrenoceptor agonists incorporating a hydantoin or a uracil ring on the right-hand side phenyl ring of (*R*)-salmeterol is presented. Hydantoin **12a** had long duration of action in vitro on guinea pig trachea, and 12 h in guinea pigs in vivo at its EC_{90} 25 μM. It had lower oral absorption than salmeterol in rats, and lower bioavailability than salmeterol in vivo in both rats and dogs (2% and 5%, respectively). An improved method for measuring the absorbed fraction of analogues dosed to rats, which considers the glucuronidated fraction is presented. Compound 12a was metabolised in human liver microsomes and hepatocytes to the active hydantoic acid **12m**.

1. Introduction

Asthma is a chronic disease affecting 300 million people worldwide, characterised by an increase in inflammatory cell populations in the epithelium and submucosa of the airways.[1] There are two main components of asthma pathophysiology, airway inflammation and smooth muscle dysfunction, which are treated by two major categories of medicines: anti-inflammatory drugs and bronchodilators. Inhaled corticosteroids are used to treat the inflammatory component of asthma. 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 in the US, affecting 5% of its population. 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. There are two classes of β_2-agonists: the short-acting (first generation) agonists, such as salbutamol (**1**) (Chart 1), which are used in rescue therapy, and the long-acting (second generation) agonists which are used in maintenance therapy. The short-acting agonists 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**). Salmeterol has lower intrinsic activity than salbutamol, a delayed onset of action, and 12 h duration, which is independent of dose.[4,5] In contrast, formoterol has high intrinsic efficacy, onset time similar to salbutamol, 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 third generation, once daily β_2 adrenoceptor agonist to be used in new combination therapies for the treatment of asthma and COPD. Novartis have recently published data on their clinical candidate, indacaterol (**4**),[7] which has now been approved for the treatment of COPD in Europe. A review by Glossop and Price summarises the progress up to 2006 made by pharmaceutical companies in identifying inhaled β_2-agonists with extended duration of action.[8] The Pfizer group have published three papers[9–11] describing their earlier candidates, and more recently on their clinical candidate PF-610355 (**5**).[12] Our group has published two papers one on sulfonamides, including our first candidate **6**,[13] and the second on antedrugs, including the clinical candidate vilanterol (**7**).[14] Finally the Boehringer-Ingelheim group have published three papers including their clinical candidate olodaterol (**8**).[15–17]

* Corresponding author. Tel.: +44 1438 762883; fax: +44 1438 768302.
E-mail address: pan.a.procopiou@gsk.com (P.A. Procopiou).

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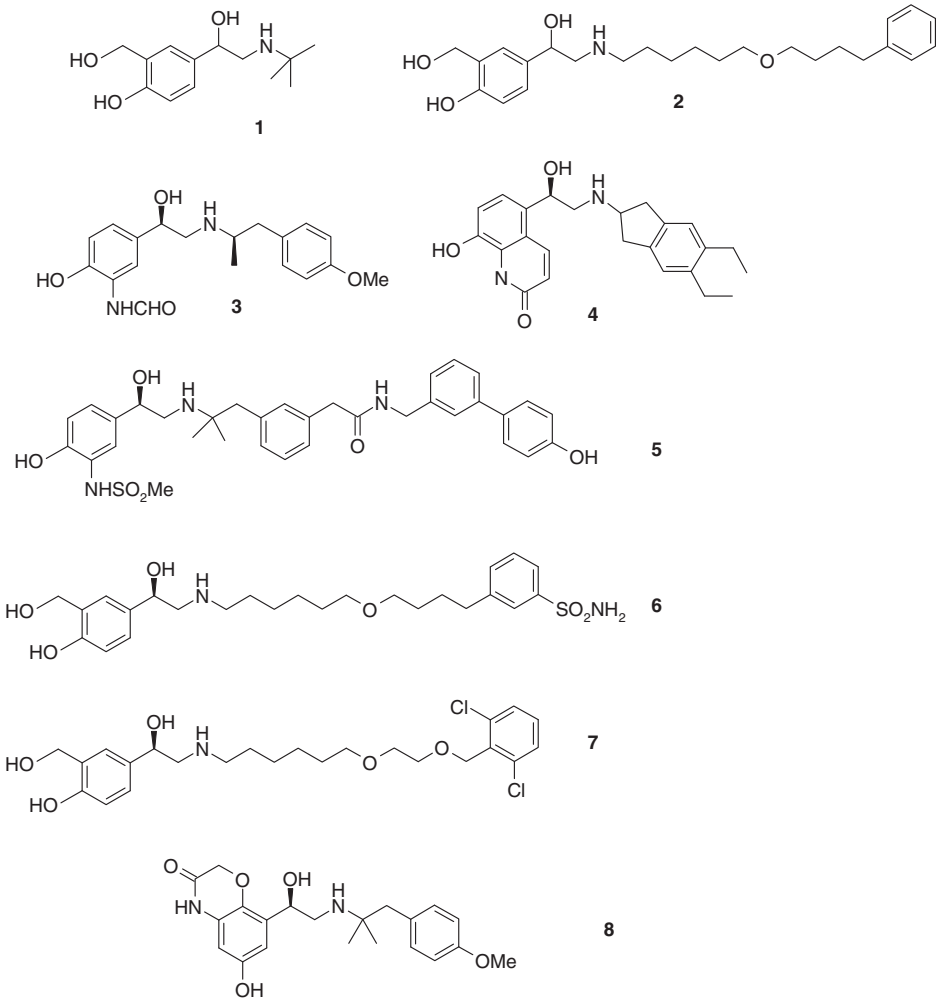


Chart 1. Clinically used β_2 agonists, and recent development candidates.

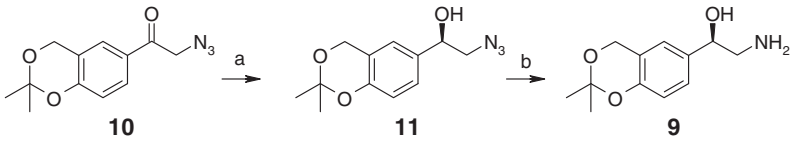
A major fraction of the dose (80–90%) of an inhaled drug is swallowed and liable to be absorbed from the gastro-intestinal tract.[18] Thus, one approach to improve the therapeutic index could be to alter the physicochemical properties of the drug and make it less prone to absorption.[13] A second approach (the antedrug approach) could be to introduce metabolic instability to the molecule to facilitate its conversion to inactive metabolites following systemic absorption from either the GI tract or the lung.[14] Better still, a combination of the two approaches may potentially deal with both the inhaled and swallowed fractions of each dose. In this report we present our studies in identifying alternative, non-sulfonamide, β_2 adrenoceptor 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.[19] Substitution on the right-hand side phenyl ring of salmeterol with the polar sulfonamide group was found to enhance β_2 agonist activity.[13] It was hypothesised that an alternative polar substituent, such as a heterocycle, with increased number of hydrogen-bond donors and acceptors which contravened the Lipinski rules,[20] might be expected to show reduced oral bioavailability. Hydantoin and uracil rings, which possess two hydrogen-bond acceptor groups and two hydrogen-bond donors, were considered as good starting points for investigation. We have demonstrated that introduction of the polar sulfonamide group brings about longer duration of action.[13] It was therefore hypothesised that introduction of the hydantoin or uracil ring might also bind in a similar way to the sulfonamide group of **6** and hence have similarly long duration of action.

2. Chemistry

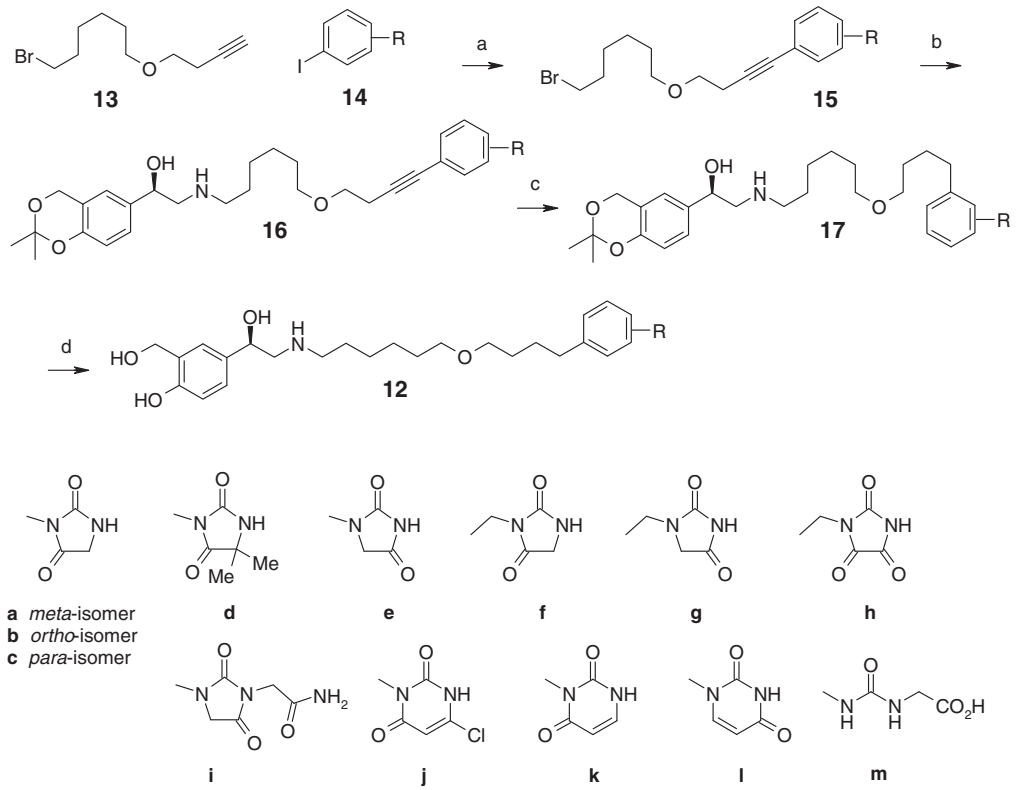
The aminoalcohol **9** with the *R* configuration was prepared by reduction of the ketone **10**[21] with borane-dimethylsulfide and (*R*)-2-methyl-CBS-oxazaborolidine as catalyst, followed by catalytic hydrogenation of the resulting azido alcohol **11** (Scheme 1). The enantiomeric ratio of **11** was determined by ^{1}H NMR spectroscopy using Pirkle's alcohol[22] at 750 MHz and found to be 9:1.

The β_2-adrenoceptor agonists **12** were prepared using the synthetic route outlined in Scheme 2, which involved Sonogashira coupling of acetylene **13**[13] with aryl iodides **14**, reaction of the resulting bromide **15** with 2 equiv of amino alcohol **9** in order to minimise bis-alkylation,[21] catalytic hydrogenation of the mono-alkylated product **16**, and finally deprotection of **17** with aqueous acetic acid.

The substituted aryl iodides **14** were obtained by the methods shown in Scheme 3.[23,24] Hydantoins **14a–c** were prepared from the appropriate regioisomeric iodoaniline and ethyl isocyanatoacetate, base-hydrolysis of the resulting ester **18**, followed by cyclisation under acidic conditions. The analogue **14d** was prepared from BOC-α-methyl-alanine and 3-iodoaniline, followed by cyclisation with sodium hydride in DMF. The regioisomeric hydantoin **14e**



Scheme 1. Synthesis of (*R*)-enantiomer of aminoalcohol **9**. Reagents: (a) $BH_3{\cdot}SMe_2$, (*R*)-2-methyl-CBS-oxazaborolidine, 68%; (b) H_2, 10% Pd/C, EtOH, 78%.

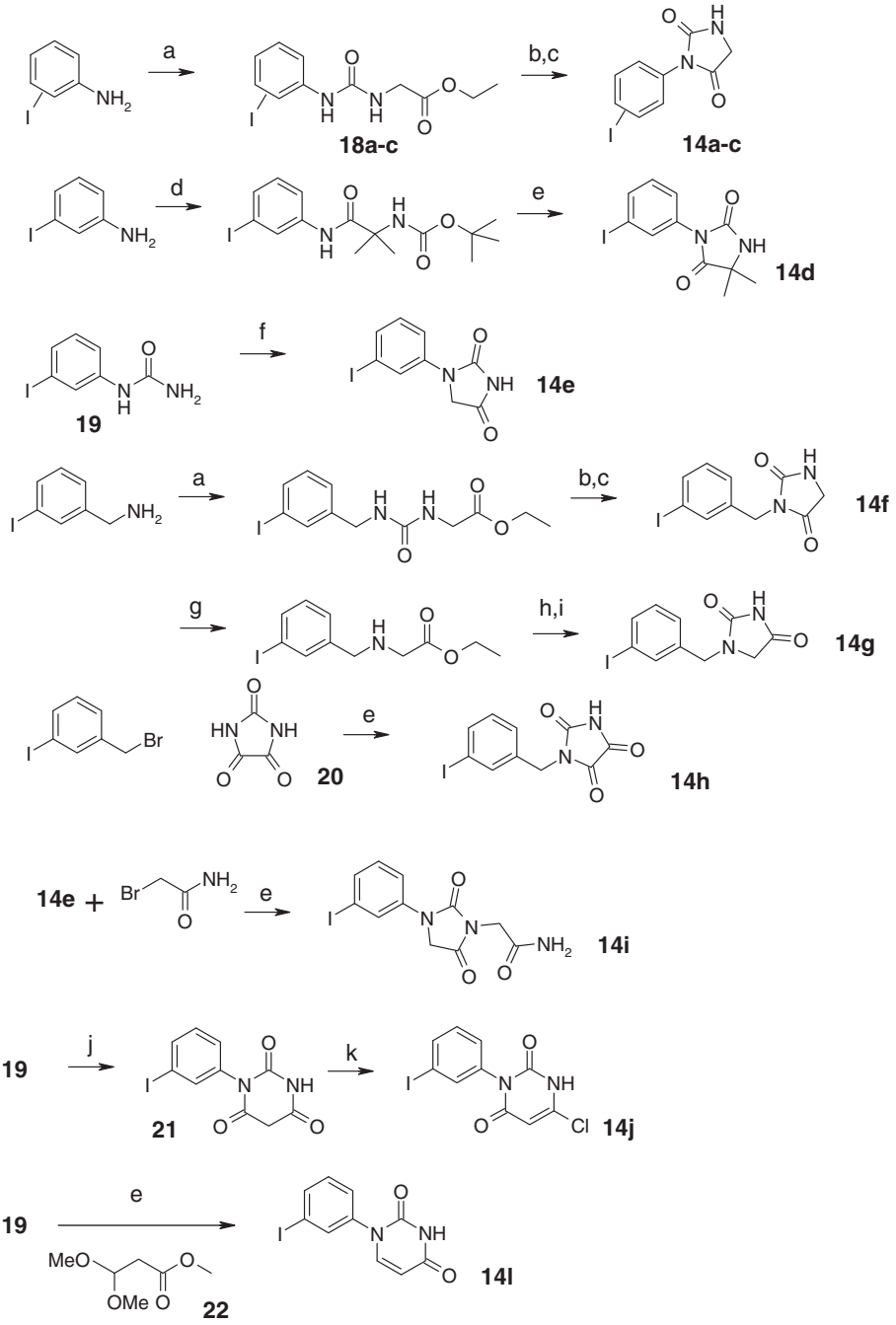


Scheme 2. Synthetic route to analogues **12** and list of substituents **R** present in compounds **12** and **14**–**17**. Reagents: (a) $Pd(PPh_3)_2Cl_2$, CuI, *i*-Pr_2EtN, DMF; (b) **9** (2 equiv), DMF; (c) H_2, PtO_2, EtOAc; (d) AcOH–H_2O (3:1), 75 °C.

was prepared by reaction of 3-iodophenylurea **19** and ethyl chloroacetate in the presence of sodium hydride. The homologues of **14a** and **14e** (**14f** and **14g**, respectively) were prepared by similar methods starting from 3-iodobenzylamine, whereas the analogue **14h** was prepared from 3-iodobenzyl bromide and parabanic acid (**20**). The hydantoin **14i** was prepared from **14e** by alkylation with 2-bromoacetamide in the presence of sodium hydride. The pyrimidinedione **14j** was prepared from urea **19** and diethyl malonate, followed by reaction of the resulting **21** with phosphorus oxychloride, whereas the regioisomeric pyrimidinedione **14l** was obtained by reacting **19** with methyl 3,3-dimethoxypropionate (**22**) in the presence of sodium hydride. The dechlorination of **14j** was not attempted as hydrogenolysis would have concurrently removed the iodo group. The chloro group was therefore removed at a later stage, at the hydrogenation of **16j** to give **17k**. Finally hydantoic acid **12m** was made by hydrolysis of **17a**, followed by aqueous acetic acid deprotection.

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. The relative instability of the compounds following oral absorption and first-pass metabolism in the liver was assessed by incubating test compounds 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 (**2**) which had a slightly higher turnover ratio (1.2) was also included as a standard. All the compounds tested showed high β_2 receptor agonist activity (substantially more potent than the standard, isoprenaline). The *meta*-hydantoin **12a** was more potent agonist than the *ortho*- (**12b**) and *para*- (**12c**) analogues, as was previously discovered in the sulfonamide series.[13] Furthermore **12a** had higher selectivity for β_2 over β_1 and β_3 receptors than the regioisomers **12b** and **12c**, and also had a higher turnover ratio, and hence all other analogues reported herein are in the *meta*- series. Substitution in the



Scheme 3. Synthesis of substituted aryl iodides **14**. Reagents: (a) EtO_2CCH_2NCO, DCM; (b) NaOH, H_2O, EtOH; (c) 2 M HCl; (d) tetramethylfluoroformamidinium hexafluorophosphate, *i*-Pr_2EtN, DCM; (e) NaH, DMF; (f) $ClCH_2CO_2Et$, NaH, DMF; (g) $BrCH_2CO_2Et$, *i*-Pr_2EtN, DMSO; (h) NaNCO, 1 M HCl; (i) concd HCl; (j) $EtO_2CCH_2CO_2Et$, NaH, MeOH; (k) $POCl_3$.

C5-position of the hydantoin ring (**12d**) was tolerated, although the IA was reduced slightly, and in addition its turnover ratio was lower than that of **12a**. The regioisomeric hydantoin **12e** was less potent and more stable in microsomes than **12a**, whereas the homologous hydantoins **12f** and **12g**, although they were more rapidly turned over in microsomes, had lower intrinsic activities than their respective parents **12a** and **12e**. Introduction of an additional carbonyl group as in the parabanic acid derivative **12h** was well tolerated; however, the intrinsic activity and the selectivity for β_2 were slightly reduced. Introduction of the acetamide moiety (**12i**) improved the β_2 potency, selectivity and turnover ratio; however, it lowered its intrinsic activity. The uracil derivative **12k** possessed excellent potency, intrinsic activity and selectivity, whereas the isomeric analogue **12l** had lower intrinsic activity and selectivity. Finally, the hydantoic acid **12m** had good potency, intrinsic activity and selectivity, but it was metabolically more stable than **12a** with a turnover ratio of 0.55.

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.[25] 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

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

	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	**12a**·AcOH	9.2 ± 0.1 (6)	0.40	7.1 ± 0.2 (4)	2.1	7.0 ± 0.1 (4)	2.1	0.86
2	**12b**	8.6 ± 0.1 (3)	0.49	6.7 ± 0.1 (6)	1.9	6.7 ± 0.1 (8)	1.9	0.46
3	**12c**·AcOH	8.7 ± 0.1 (2)	0.55	7.1 ± 0.1 (2)	1.6	7.5 ± 0.1 (2)	1.2	0.30
4	**12d**·AcOH	9.1 ± 0.2 (4)	0.32	6.7 ± 0.1 (4)	2.4	7.2 ± 0.1 (4)	1.9	0.48
5	**12e**·AcOH	8.6 ± 0.1 (7)	0.50	6.7 ± 0.1 (6)	1.9	6.5 ± 0.1 (6)	2.1	0.40
6	**12f**·AcOH	8.9 ± 0.2 (2)	0.33	6.9 ± 0.2 (6)	2.0	7.4 ± 0.1 (4)	1.5	1.62
7	**12g**·AcOH	9.2 ± 0.0 (2)	0.42	7.1 ± 0.0 (3)	2.1	7.2 ± 0.0 (4)	2.0	0.75
8	**12h**·HCO_2H	9.1 ± 0.1 (4)	0.32	7.3 ± 0.1 (4)	1.8	7.6 ± 0.0 (4)	1.5	ND[e]
9	**12i**·AcOH	9.6 ± 0.2 (4)	0.36	6.8 ± 0.1 (2)	2.8	7.1 ± 0.1 (2)	2.5	0.91
10	**12k**	9.1 ± 0.1 (4)	0.70	6.6 ± 0.1 (8)	2.5	6.4 ± 0.0 (8)	2.7	ND[e]
11	**12l**·AcOH	8.9 ± 0.1 (2)	0.49	7.3 ± 0.1 (8)	1.6	7.3 ± 0.0 (8)	1.6	ND[e]
12	**12m**	9.0 ± 0.2 (2)	0.52	6.9 ± 0.6 (2)	2.1	7.0 ± 0.4 (2)	2.0	0.55
13	Isoprenaline	7.4 ± 0.0 (767)	1.0	8.1 ± 0.0 (641)	−0.7	7.4 ± 0.0 (659)	0.0	ND[e]
14	**2**	9.6 ± 0.0 (929)	0.37	6.1 ± 0.0 (656)	3.5	5.9 ± 0.0 (849)	3.7	1.2
15	(*R*,*R*)-3·fumarate	9.3 ± 0.0 (791)	0.97	7.4 ± 0.0 (670)	1.9	7.6 ± 0.0 (653)	1.7	ND[e]

[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 relative to verapamil, where verapamil has a turnover ratio of 1.
[e] ND, not determined.

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. The more potent analogues with the highest IA, selectivity, and highest turnover ratio (**12a, e, k, m**) were screened on isolated electrically-stimulated superfused guinea pig trachea and their potency, onset-time and in vitro duration after 1 and 3 h is presented and contrasted to salmeterol and (*R*,*R*)-formoterol (Table 2). Potency was expressed in absolute terms (concentration required to induce 50% inhibition, EC_{50}). Onset of action was calculated as the time taken for 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 h 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. The hydantoins **12a** and **12e** had low shift values indicating a salmeterol-like duration of action, whereas uracil **12k** and hydantoic acid **12m** had a different profile, intermediate between that of salmeterol and that of formoterol. Hydantoin **12a** was more potent on both CHO cells and guinea pig trachea, had a significantly shorter onset time (26 min vs 44 min), and was metabolised more rapidly (0.86 vs 0.40) than **12e**. For these reasons **12a** was progressed to pharmacokinetic studies in rats and dogs and the data is shown in Table 3. The gut appears to contribute significantly to phase II first-pass metabolism of salmeterol in the rat, potentially limiting the discrimination window for compounds in this model. Salmeterol is subject to conjugation as it is absorbed (90% glucuronidated), thus the measurement of free salmeterol in the hepatic portal vein is a large underestimate of compound actually absorbed. Up to 30 times higher levels of salmeterol were detected in hepatic portal and systemic blood after deconjugation with β-glucuronidase. Deconjugation of hepatic portal blood samples with β-glucuronidase and measurement of total parent compound was therefore used to increase the resolution between well and poorly absorbed compounds. Compounds were classified as less absorbed than salmeterol if the levels of HPV—CP AUC was <7000 ng min/mL following a 2 mg/kg oral dose, salmeterol-like if the levels were 7000–23,000 ng min/mL, and better absorbed than salmeterol if AUC was >23,000 ng min/mL. Deconjugated hepatic portal vein samples from rats dosed with salmeterol and **12a** indicated that **12a** had lower absorption than salmeterol (AUC <2340 ng min/mL). Furthermore **12a** had a half-life of 0.3 h and high plasma clearance of 120 mL/min/kg (140% liver blood flow) in rats. Its half-life in dogs was 2 h, and its clearance was moderate (42% liver blood flow). Low oral bioavailability of **12a** was observed in vivo in both rats (2%) and dogs (5%) (Table 3). This oral bioavailability was much lower than that previously reported for salmeterol (66–78% in dog, and 10–12% in rat)[26] and strengthened the confidence that the swallowed fraction of the inhaled dose in humans would be unlikely to contribute to the systemic exposure of **12a**.

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

Entry	Compound	pEC_{50}	Onset time (min)	Shift (1 h)	Shift (3 h)
1	**12a**·AcOH	9.1	26.4	3.7	3.9
2	**12e**·AcOH	8.6	44	2.9	3.3
3	**12k**·AcOH	9.7	9.0	15	77
4	**12m**·AcOH	9.0	14	19	35
5	Salmeterol	8.3	27.6	1.0	1.1
6	(*R*,*R*)-3·fumarate	9.5	10	20	>1940

The acetate salt of **12a** was also investigated in vivo in the guinea pig in a whole animal plethysmograph chamber (Buxco) using histamine-induced bronchospasm and found to have similar potency to salmeterol (EC_{90} 25 μM and 50 μM, respectively, nebulizer concentration). At an equi-effective (EC_{90}) dose, the duration of action of **12a** acetate (time to 50% recovery) was similar or slightly longer than that of salmeterol (12 h vs 10 h) when administered as a nebulised solution in a dimethyl acetamide-saline vehicle. Furthermore the duration of **12a** was extended to 18 h when the dose was increased to 10-fold the EC_{90}. Hydantoin **12a** was then progressed towards a salt selection screen to identify a stable, non-hygroscopic and crystalline salt, suitable for inhaled delivery.

Table 3
Rat and dog pharmacokinetic data for **12a** acetate

Species	Route	Dose (mg/kg)	Cl_p (mL/min/kg)	V_{dss} (L/kg)	$T_{½}$ (h)	C_{max} (ng/mL)	*F*%
Rat	Intravenous	0.25	120	2	0.3		
	Oral	2.0					2
Dog	Intravenous	0.052	13	0.8	2.0		
	Oral	0.25				12	5

Unfortunately **12a** free base was found to be unstable in the solid state to storage at 4 °C over 18 days [13% dimerisation with loss of water; LCMS ES+ve *m/z* 1009 $(2M-18)^+$]. Furthermore the hydantoin ring was unstable to nucleophilic solvents, such as methanol, forming slowly the methyl ester of the hydantoic acid **12m**. The acetic acid salt of **12a,** which was amorphous, appeared to be stable; however, no other salts were found to be crystalline. Finally, during drug metabolite identification studies it was discovered that the potent hydantoic acid **12m** was a minor metabolite of **12a** in human liver microsomes and hepatocytes. Further progression of **12a** was halted as we were interested only in analogues that rapidly metabolised to inactive metabolites.

4. Conclusion

Incorporation of a hydantoin or a uracil ring on the right-hand side phenyl ring of (*R*)-salmeterol has provided a series of novel, very potent and selective human β_2 adrenoceptor agonists. Hydantoin **12a** had long duration of action in vitro on guinea pig trachea, and 12 h in guinea pigs in vivo at its EC_{90} 25 μM. It had lower oral absorption than salmeterol in rats, and lower bioavailability than salmeterol in vivo in both rats and dogs (2% and 5%, respectively). However **12a** was metabolised in human liver microsomes and hepatocytes to the active hydantoic acid **12m**, and no crystalline salts suitable for inhaled delivery were identified.

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-Azido-1-(2,2-dimethyl-4*H*-1,3-benzodioxin-6-yl)ethanone (10)

2-Bromo-1-(2,2-dimethyl-4*H*-1,3-benzodioxin-6-yl)ethanone[27] (370 g, 1.29 mol) in DMF (2.3 L) was treated portionwise with sodium azide (87.06 g, 1.33 mol) over 30 min. The temperature rose to 30 °C towards the end of the addition and was cooled in ice-water bath to maintain the temperature at 20 °C. The mixture was stirred for 2 h at 20 °C and turned orange colour while a white precipitate formed. The reaction mixture was divided into two equal portions and each portion was diluted with EtOAc (1.2 L) and water (3 L), the phases were separated and the aqueous phase extracted with more EtOAc (1 L). The combined organic solutions were washed with saturated sodium bicarbonate (2 L), brine (2 L) and dried ($MgSO_4$). The solvent was removed under reduced pressure, the resulting orange solid was dissolved in dichloromethane, and filtered through silica (1 kg) eluting with dichloromethane. Appropriate fractions were combined and evaporated under reduced pressure to give **10** (310 g, 97%) as a pale yellow solid: MS TSP+ve *m/z* 248 $(M+H)^+$; ^{1}H NMR δ ($CDCl_3$) 7.72 (1H, dd, *J* 8.6, 2 Hz), 7.63 (1H, d, *J* 2 Hz), 6.88 (1H, d, *J* 8.6 Hz), 4.89 (2H, s), 4.48 (2H, s), 1.56 (6H, s).

5.2. (1*R*)-2-Azido-1-(2,2-dimethyl-4*H*-1,3-benzodioxin-6-yl)ethanol (11)

(*R*)-Tetrahydro-1-methyl-3,3-diphenyl-1*H*,3*H*-pyrrolo[1,2-*c*][1,3,2]oxazaborole solution in toluene (1 M, 7.5 mL) was added to THF (75 mL) and the solution was cooled to 0 °C. Borane–THF complex (1 M solution in THF, 125 mL) was added and the mixture was stirred under nitrogen for 15 min. A solution of **10** (24.7 g, 0.1 mol) in THF (250 mL) was added dropwise over 1.5 h at 5 °C. The mixture was stirred for a further 1 h and then cautiously treated with 2 M HCl (100 mL). The reaction mixture was extracted with ether and the organic layer was washed with 2 M HCl, saturated aq $NaHCO_3$, brine and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was chromatographed on a Biotage column eluting with diethyl ether–petroleum ether (40–60 °C) (1:9; 1:1) to give **11** (16.99 g, 68%) as a white solid: MS ES+ve *m/z* 250 $(M+H)^+$; ^{1}H NMR δ ($CDCl_3$) 7.10 (1H, dd, *J* 8, 2 Hz), 6.95 (1H, d, *J* 2 Hz), 6.78 (1H, d, *J* 8 Hz), 4.78 (2H, s), 4.75 (1H, m), 3.42 (1H, dd, *J* 13, 8 Hz), 3.33 (1H, dd, *J* 13, 4 Hz), 2.90 (1H, d, *J* 4 Hz), 1.52 (6H, s). $C_{12}H_{15}N_3O_3$ requires C, 57.8; H, 6.1; N, 16.9. Found: C, 57.9; H, 6.0; N, 16.5.

5.3. (1*R*)-2-Amino-1-(2,2-dimethyl-4*H*-1,3-benzodioxin-6-yl)ethanol (9)

A solution of **11** (16.99 g, 68.2 mmol) was hydrogenated over 10% Pd–C (1 g) in EtOH (300 mL). The catalyst was collected by filtration, and washed with EtOH. The combined filtrate and washings were evaporated under reduced pressure and the residue was triturated in ether to give **9** (5.86 g, 38%) as a white solid. The mother liquors were chromatographed on a Biotage column eluting with toluene–EtOH–0.88 aqueous ammonia (85:14:1) to give a further batch of **9** (5.99 g, 39%) as a white solid: $[\alpha]_D^{20}$ 21.4 (*c* 1.26 in MeOH); MS ES+ve *m/z* 206 $(MH-H_2O)^+$; ^{1}H NMR δ ($CDCl_3$) 7.11 (1H, dd, *J* 8.2 Hz), 6.99 (1H, br s), 6.79 (1H, d, *J* 8 Hz), 4.82 (2H, s), 4.54 (1H, dd, *J* 8.4 Hz), 2.96 (1H, dd, *J* 13.4 Hz), 2.77 (1H, dd, *J* 13.8), 1.54 (6H, s), 1.90–1.20 (3H, br). The enantiomeric ratio was determined by ^{1}H NMR (750 MHz) using Pirkle's

alcohol [2,2,2-trifluoro-1-(9-anthracenyl)ethanol] (20 mg) on the crude product before chromatography, and was found to be (10:90) [4.30 ppm (minor isomer); 4.26 ppm (major isomer)]. $C_{12}H_{17}NO_3$ requires C, 64.6; H, 7.7; N, 6.3. Found: C, 64.8; H, 7.7; N, 6.2.

5.4. Ethyl *N*-{[(3-iodophenyl)amino]carbonyl}glycinate (18a)

A solution of 3-iodoaniline (9.39 g, 42.9 mmol) in dichloromethane (75 mL) was treated with ethyl isocyanatoacetate (7.21 mL, 64.3 mmol) at 0 °C and the mixture was stirred for 2 h and allowed to warm to 20 °C. EtOH (10 mL) was added and the mixture was stirred for 15 h. The solvents were evaporated under reduced pressure and the residue was triturated in Et_2O to give **18a** (12.9 g, 86%) as an off-white solid: MS ES+ve *m/z* 349 $(M+H)^+$; ^1H NMR δ ($CDCl_3$) 7.74 (1H, t, *J* 2 Hz), 7.35 (1H, br d, *J* 8 Hz), 7.29–7.24 (2H, m), 6.97 (1H, t, *J* 8 Hz), 5.72 (1H, br), 4.21 (2H, q, *J* 7 Hz), 4.04 (2H, br s), 1.30 (3H, t, *J* 7 Hz).

5.5. 3-(3-Iodophenyl)imidazolidine-2,4-dione (14a)

A solution of ethyl *N*-{[(3-iodophenyl)amino]carbonyl}glycinate (9.42 g, 27 mmol) in DMF (60 mL) was treated with sodium hydride (60% oil dispersion, 1.2 g, 30 mmol) at 20 °C. After 2 h the reaction mixture was treated with aqueous 2 M HCl (200 mL) and stirred overnight. The solid was collected by filtration, washed with water and dried to give **14a** (6.9 g, 84%) as a white solid: MS ES+ve *m/z* 320 $(M+NH_4)^+$; ^1H NMR δ (DMSO-d_6) 8.36 (1H, s), 7.76 (2H, m), 7.40 (1H, br d, *J* 8 Hz), 7.28 (1H, t, *J* 8 Hz), 4.05 (2H, s).

5.6. 3-(3-{4-[(6-Bromohexyl)oxy]but-1-ynyl}phenyl)imidazolidine-2,4-dione (15a)

A mixture of 3-(3-iodophenyl)imidazolidine-2,4-dione (0.75 g, 2.5 mmol), 6-bromohexyl but-3-ynyl ether[13] (1.3 g, 3.0 mmol), bis(triphenylphosphine)palladium dichloride (87 mg, 0.12 mmol) in DMF (10 mL) was treated with diisopropylethylamine (3 mL) and copper(I) iodide (23 mg, 0.12 mmol), and the mixture was stirred under nitrogen for 24 h. The solvents were removed under reduced pressure, the residue was diluted with EtOAc and washed with aqueous 2 M HCl, aqueous dilute ammonia, brine and dried ($MgSO_4$). The solvent was removed under reduced pressure, the residue was purified on two Bond Elut 10 g silica cartridges eluting with dichloromethane, Et_2O, and EtOAc to give a mixture of **15a** and the corresponding iodide, 3-(3-{4-[(6-iodohexyl)oxy]but-1-ynyl} phenyl)imidazolidine-2,4-dione, (760 mg, ratio 22:3) as a brown oil: ^1H NMR δ ($CDCl_3$) 7.46–7.31 (4H, m), 6.53 (1H, br s), 4.11 (2H, s), 3.62 (2H, t, *J* 7 Hz), 3.49 (2H, t, *J* 7 Hz), 3.40 (2H, t, *J* 7 Hz), 2.68 (2H, t, *J* 7 Hz), 1.90–1.80 (2H, m), 1.65–1.55 (2H, m), 1.51–1.35 (4H, m), and 3.18 (2H, t, *J* 7 Hz) for the iodide minor component.

5.7. 3-(3-{4-[(6-{[(2*R*)-2-(2,2-Dimethyl-4*H*-1,3-benzodioxin-6-yl)-2-hydroxyethyl]amino}hexyl)oxy]but-1-ynyl}phenyl)imidazolidine-2,4-dione (16a)

A mixture of **15a** and 3-(3-{4-[(6-iodohexyl)oxy]but-1-ynyl} phenyl)imidazolidine-2,4-dione (760 mg, 22:3, 1.8 mmol total alkylating agent), **9** (800 mg, 3.6 mmol, 2 equiv) in DMF (5 mL) was stirred at 20 °C for 22 h. The solvent was removed under reduced pressure and the residue was diluted with EtOAc and washed with water, brine and dried ($MgSO_4$). The solution was concentrated under reduced pressure and the residue was purified by chromatography on a Biotage cartridge (40 g) eluting with dichloromethane–MeOH–1 M ammonia in methanol (98:1:1–95:4:1) to give **16a** as a yellow gum (368 mg, 37%): MS ES+ve *m/z* 550 $(M+H)^+$; ^1H NMR δ ($CDCl_3$) 7.45 (1H, br s), 7.40–7.30 (3H, m), 7.12 (1H, dd, *J* 8, 2 Hz), 7.00 (1H, br s), 6.78 (1H, d, *J* 8 Hz), 4.83 (2H, s), 4.61 (1H, dd, *J* 9, 3 Hz), 4.09 (2H, s), 3.61 (2H, t, *J* 7 Hz), 3.49 (2H, t, *J* 6.5 Hz), 2.82 (1H, dd, *J* 12, 4 Hz), 2.70–2.55 (6H, m), 1.64–1.30 (10H, m), 1.53 (6H, s).

5.8. 3-(3-{4-[(6-{[(2*R*)-2-(2,2-Dimethyl-4*H*-1,3-benzodioxin-6-yl)-2-hydroxyethyl]amino}hexyl)oxy]butyl}imidazolidine-2,4-dione (17a)

A solution of **16a** (368 mg, 0.67 mmol) was hydrogenated over platinum oxide (100 mg) in EtOAc (100 mL) over 3 h. The catalyst was collected by filtration, washed with EtOAc and EtOH. The combined filtrate and washings were evaporated under reduced pressure to give **17a** as a colourless gum: MS ES+ve *m/z* 554 $(M+H)^+$; ^1H NMR δ ($CDCl_3$) 7.37 (1H, t, *J* 7 Hz), 7.23–7.16 (3H, m), 7.11 (1H, d, *J* 8 Hz), 7.00 (1H, br s), 6.78 (1H, br d, *J* 8 Hz), 4.83 (2H, s), 4.63 (1H, dd, *J* 9, 3.5 Hz), 4.07 (2H, s), 3.41 and 3.38 (2t, 2H each, *J* 6.5 Hz), 2.83 (1H, dd, *J* 12, 4 Hz), 2.71–2.56 (6H, m), 1.75–1.45 (10H, m), 1.53 (6H, s), 1.40–1.28 (4H, m).

5.9. 3-[3-(4-{[6-([(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl}amino) hexyl]oxy}butyl)phenyl]imidazolidine-2,4-dione acetate (12a)

A solution of **17a** (417 mg, 0.75 mmol) in acetic acid (20 mL) and water (5 mL) was heated to 75 °C for 30 min before evaporating to dryness under reduced pressure. The residue was purified by chromatography on a Biotage cartridge (40 g) eluting with CH_2Cl_2–MeOH–2 M NH_3 in MeOH (85:10:5). Appropriate fractions were combined and evaporated to dryness. Acetic acid (4 mL) was added and the mixture was evaporated under reduced pressure. The residue was dissolved in EtOAc and re-evaporated under reduced pressure (twice). This process of dissolving and re-evaporating was repeated with diethyl ether, followed by acetone to give **12a** (290 mg, 67%) as a white foam: LCMS RT = 2.44 min; ES+ve *m/z* 514 $(M+H)^+$; ^1H NMR (DMSO-d_6+D_2O) 7.36 (1H, t, *J* 8 Hz), 7.28 (1H, br s), 7.20 (1H, d, *J* 8 Hz), 7.10 (1H, s), 7.09 (1H, d, *J* 8 Hz), 7.04(1H, dd, *J* 8, 2 Hz), 6.73 (1H, d, *J* 8 Hz), 4.73 (1H, dd, *J* 5, 8 Hz), 4.45 (2H, s), 4.08 (2H, s), 3.33 and 3.30 (2t, 2H each, *J* 7 Hz), 2.98–2.90 (2H, m), 2.86 (2H, t, *J* 7 Hz), 2.59 (2H, t, *J* 7 Hz), 1.85 (3H, s), 1.62–1.40 (8H, m), 1.30–1.20 (4H, m); HRMS ES+ve *m/z* 514.2917. $C_{28}H_{40}N_3O_6$ requires 514.2917.

5.10. 3-(2-Iodophenyl)-2,4-imidazolidinedione (14b)

MS ES+ve *m/z* 303 $(M+H)^+$; ^1H NMR δ (DMSO-d_6) 8.43 (1H, br s), 8.04 (1H, br d, *J* 8 Hz), 7.58 (1H, br t, *J* 8 Hz), 7.43 (1H, br d, *J* 8 Hz), 7.30 (1H, br t, *J* 8 Hz), 4.19 (2H, s).

5.11. 3-(4-Iodophenyl)-2,4-imidazolidinedione (14c)

MS ES-ve *m/z* 301 $(M-H)^-$; ^1H NMR δ (DMSO-d_6) 8.35 (1H, br s), 7.83 (2H, d, *J* 8.5 Hz), 7.18 (2H, d, *J* 8.5 Hz), 4.05 (2H, s).

5.12. 3-(3-Iodophenyl)-5,5-dimethyl-2,4-imidazolidinedione (14d)

MS ES+ve *m/z* 331 $(M+H)^+$; ^1H NMR δ (DMSO-d_6) 8.60 (1H, br s), 7.80 (1H, t, *J* 2 Hz), 7.77 (1H, dt, *J* 8, 2 Hz), 7.42 (1H, br d, *J* 8 Hz), 7.28 (1H, t, *J* 8 Hz), 1.40 (6H, s).

5.13. 1-(3-Iodophenyl)-2,4-imidazolidinedione (14e)

MS ES+ve *m/z* 303 $(M+H)^+$; ^1H NMR δ (DMSO-d_6) 11.26 (1H, br s), 8.06 (1H, br s), 7.55 (1H, br d, *J* 8 Hz), 7.46 (1H, br d, *J* 8 Hz), 7.16 (1H, t, *J* 8 Hz), 4.42 (2H, s).

5.14. 3-[(3-Iodophenyl)methyl]-2,4-imidazolidinedione (14f)

^{1}H NMR δ (DMSO-d_6) 8.17 (1H, s), 7.65 (2H, m), 7.28 (1H, br d, *J* 8 Hz), 7.14 (1H, t, *J* 8 Hz), 4.49 (2H, s), 3.99 (2H, s).

5.15. 1-[(3-Iodophenyl)methyl]-2,4-imidazolidinedione (14g)

^{1}H NMR δ(DMSO-d_6) 10.89 (1H, br s), 7.65 (2H, m), 7.30 (1H, br d, *J* 8 Hz), 7.16 (1H, t, *J* 8 Hz), 5.76 (2H, s), 4.40 (2H, s).

5.16. 1-[(3-Iodophenyl)methyl]-2,4,5-imidazolidinetrione (14h)

MS ES+ve *m/z* 348 $(M+NH_4)^+$; ^{1}H NMR δ (CD_3OD) 7.97 (1H, br s), 7.76 (1H, br s), 7.65 (1H, d, *J* 8 Hz), 7.38 (1H, d, *J* 8 Hz), 7.11 (1H, t, *J* 8 Hz), 4.68 (2H, s).

5.17. 2-[3-(3-Iodophenyl)-2,5-dioxo-1-imidazolidinyl]acetamide (14i)

MS ES+ve *m/z* 360 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 8.24 (1H, br s), 7.85 (1H, br s), 7.73 (1H, br d, *J* 8 Hz), 7.65 (1H, br d, *J* 8 Hz), 7.44 (1H, br s), 7.35 (1H, t, *J* 8 Hz), 4.69 (2H, s), 4.16 (2H, s).

5.18. 6-Chloro-3-phenyl-2,4(1*H*,3*H*)-pyrimidinedione (14j)

MS ES+ve *m/z* 349/351 $(M+H)^+$; ^{1}H NMR δ (DMSO-d_6) 12.5 (1H, br s), 7.77 (1H, br d, *J* 8 Hz), 7.69 (1H, br s), 7.31 (1H, br d, *J* 8 Hz), 7.26 (1H, t, *J* 8 Hz), 6.00 (1H, br s).

5.19. 1-(3-Iodophenyl)-2,4(1*H*,3*H*)-pyrimidinedione (14l)

MS ES+ve *m/z* 315 $(M+H)^+$; ^{1}H NMR δ (DMSO-d_6) 11.5 (1H, br s), 7.85 (1H, br s), 7.80 (1H, br d, *J* 8 Hz), 7.72 (1H, d, *J* 8 Hz), 7.46 (1H, br d, *J* 8 Hz), 7.29 (1H, t, *J* 8 Hz), 5.66 (1H, d, *J* 8 Hz).

5.20. 3-[2-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl}amino)hexyl]oxy}butyl)phenyl]-2,4-imidazolidinedione acetate salt (12b)

LCMS RT = 2.08 min; ES+ve *m/z* 514 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 7.40–7.32 (3H, m), 7.32–7.25 (1H, m), 7.15 (2H, d, *J* 8 Hz), 6.79 (1H, d, *J* 8 Hz), 4.84 (1H, dd, *J* 9, 4 Hz), 4.65 (2H, s), 4.17 (2H, s), 3.41 (4H, t, *J* 7 Hz), 3.19 (2H, m), 2.99 (2H, t, *J* 8 Hz), 2.53 (2H, t, *J* 7.5 Hz), 1.94 (3H, s), 1.76–1.51 (8H, m), 1.47–1.35 (4H, m).

5.21. 3-[4-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phen yl]ethyl}amino)hexyl]oxy}butyl)phenyl]-2,4-imidazolidinedione acetate salt (12c)

LCMS RT = 2.34 min; ES+ve *m/z* 514 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 7.37 (1H, br s), 7.34–7.26 (4H, m), 7.18 (1H, dd, *J* 8, 2 Hz), 6.81 (1H, d, *J* 8 Hz), 4.87 (1H, dd, *J* 9.4 Hz), 4.67 (2H, s), 4.12 (2H, s), 3.47 (2H, t, *J* 7 Hz), 3.45 (2H, t, *J* 7 Hz), 3.16–3.08 (2H, m), 3.02 (2H, t, *J* 8 Hz), 2.71 (2H, t, *J* 8 Hz), 1.99 (3H, s), 1.77–1.56 (8H, m), 1.47–1.39 (4H, m).

5.22. 3-[3-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl}amino)hexyl]oxy}butyl)phenyl]-5,5-dimethyl-2,4-imidazolidinedione acetate salt (12d)

MS ES+ve *m/z* 542 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 7.39–7.34 (2H, m), 7.25–7.13 (4H, m), 6.78 (1H, d, *J* 8 Hz), 4.85 (1H, overlapping with CD_3OH), 4.65 (2H, s), 3.48–3.40 (4H, m), 3.12–2.97 (4H, m), 2.69 (2H, t, *J* 7 Hz), 1.93 (3H, s), 1.76–1.52 (8H, m), 1.49 (6H, s), 1.46–1.37 (4H, m).

5.23. 1-[3-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl}amino)hexyl]oxy}butyl)phenyl]-2,4-imidazolidinedione formate salt (12e)

LCMS RT = 2.47 min; ES+ve *m/z* 514 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 8.27 (1H, s), 7.47 (1H, br s), 7.37–7.32 (2H, m), 7.26 (1H, t, *J* 8 Hz), 7.14 (1H, dd, *J* 8.2 Hz), 6.97 (1H, d. *J* 8 Hz), 6.77 (1H, d, *J* 8 Hz), 4.83 (1H, overlapping with CD3OH), 4.64 (2H, s), 4.42 (2H, s), 3.44–3.42 (4H, m), 3.12–3.06 (2H, m), 3.02 (2H, t, *J* 8 Hz), 2.64 (4H, m), 1.77–1.63 (4H, m), 1.63–1.54 (4H, m), 1.47–1.37 (4H, m).

5.24. 3-{[3-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl}amino) hexyl]oxy}butyl)phenyl]methyl}-2,4-imidazolidinedione acetate salt (12f)

LCMS RT = 2.44 min; ES+ve *m/z* 528 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 7.36 (1H, br s), 7.25–7.09 (5H, m), 6.80 (1H, d, *J* 8 Hz), 4.87 (1H, overlapping with CD_3OH), 4.67 (2H, s), 4.61 (2H, s), 4.00 (2H, s), 3.46 (2H, t, *J* 7 Hz), 3.44 (2H, t, *J* 7 Hz), 3.17–3.08 (2H, m), 3.04 (2H, t, *J* 7 Hz), 2.63 (2H, t, *J* 7 Hz), 1.99 (3H, s), 1.78–1.54 (8H, m), 1.50–1.40 (4H, m).

5.25. 1-{[3-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl}amino) hexyl]oxy}butyl)phenyl]methyl}-2,4-imidazolidinedione acetate salt (12g)

LCMS RT = 2.42 min; ES+ve *m/z* 528 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 7.32 (1H, br s), 7.26 (1H, t, *J* 7.5 Hz), 7.17–7.07 (4H, m), 6.77 (1H, d, *J* 8 Hz), 4.83 (1H, dd, *J* 9, 4 Hz), 4.64 (2H, s), 4.47 (2H, s), 3.80 (2H, s), 3.43 (2H, t, *J* 7 Hz), 3.41 (2H, t, *J* 7 Hz), 3.13–3.05 (2H, m), 2.99 (2H, t, *J* 7 Hz), 2.63 (2H, t, *J* 7 Hz), 1.91 (3H, s), 1.74–1.52 (8H, m), 1.44–1.38 (4H, m).

5.26. 1-{[3-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl}amino)hexyl] oxy}butyl)phenyl]methyl}-2,4,5-imidazolidinetrione formate salt (12h)

LCMS RT = 2.56 min; ES+ve *m/z* 542 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 8.45 (1H, s), 7.34 (1H, br s), 7.25–7.08 (5H, m), 6.77 (1H, d, *J* 8 Hz), 6.67 (2H, s), 4.64 (2H, s), 3.45–3.37 (4H, m), 3.16–3.07 (2H, m), 3.02 (2H, t, *J* 8 Hz), 2.61 (2H, t, *J* 7.5 Hz), 1.76–1.52 (8H, m), 1.45–1.37 (4H, m).

5.27. 2-{3-[3-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl}amino)hexyl]oxy}butyl)phenyl]-2,5-dioxo-1-imidazolidinyl}acetamide acetate salt (12i)

MS ES+ve *m/z* 571 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 7.54 (1H, br s), 7.40 (1H, br d, *J* 8 Hz), 7.34 (1H, br s), 7.29 (1H, t, *J* 8 Hz), 7.15 (1H, dd, *J* 8, 2 Hz), 7.01 (1H, d, *J* 8 Hz), 6.78 (1H, d, *J* 8 Hz), 4.85 (1H, overlapping with CD_3OH), 4.65 (2H, s), 4.48 (2H, s), 4.24 (2H, s), 3.49–3.40 (4H, m), 3.15–3.05 (2H, m), 3.00 (2H, t, *J* 8 Hz), 2.67 (2H, t, *J* 7.5 Hz), 1.94 (3H, s), 1.76–1.53 (8H, m), 1.47–1.36 (4H, m).

5.28. 3-[3-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl}amino)hexyl]oxy}butyl)phenyl]-2,4(1*H*,3*H*)-pyrimidinedione acetate salt (12k)

LCMS RT = 2.21 min; ES+ve *m/z* 526 $(M+H)^+$; ^{1}H NMR δ (CD_3OD) 7.48 (1H, br d, *J* 8 Hz), 7.37 (1H, t, *J* 8 Hz), 7.33 (1H, br s), 7.25 (1H, br d, *J* 7 Hz), 7.14 (1H, d, *J* 7 Hz), 7.05 (1H, br s), 7.01 (1H, br d, *J* 7 Hz), 6.77 (1H, d, *J* 8 Hz), 5.78 (1H, d, *J* 7 Hz), 4.64 (2H, s), 3.47–3.38 (4H, m), 3.08–3.01 (2H, m), 2.95 (2H, t, *J* 8 Hz), 2.68 (2H, t, *J* 7 Hz), 1.92 (3H, s), 1.75–1.52 (8H, m), 1.45–1.33 (4H, m).

5.29. 1-[3-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl}amino)hexyl]oxy}butyl)phenyl]-2,4(1*H*,3*H*)-pyrimidinedione acetate salt (12l)

LCMS RT = 2.36 min; ES+ve *m/z* 526 $(M+H)^+$; ^1H NMR δ (CD_3OD) 7.63 (1H, d, *J* 8 Hz), 7.42 (1H, t, *J* 8 Hz), 7.37 (1H, d, *J* 2 Hz), 7.31 (1H, br d, *J* 8 Hz), 7.28 (1H, br s), 7.21 (1H, br d, *J* 8 Hz), 7.18 (1H, dd, *J* 8, 2 Hz), 6.80 (1H, d, *J* 8 Hz), 5.80 (1H, d, *J* 8 Hz), 4.88 (1H, dd, *J* 4, 9 Hz), 4.68 (2H, s), 3.47 (2H, t, *J* 7 Hz), 3.45 (2H, t, *J* 7 Hz), 3.15–3.07 (2H, m), 3.05–2.98 (2H, m), 2.72 (2H, t, *J* 7 Hz), 2.00 (3H, s), 1.78–1.55 (8H, m), 1.50–1.35 (4H, m).

5.30. *N*-({[3-(4-{[6-({(2*R*)-2-Hydroxy-2-[4-hydroxy-3-(hydroxymethyl)phenyl]ethyl}amino) hexyl]oxy}butyl)phenyl]amino} carbonyl)glycine acetate salt (12m)

LCMS RT = 2.46 min; ES+ve *m/z* 532 $(M+H)^+$; ^1H NMR δ (CD_3OD) 7.33 (1H, br s), 7.25 (1H, br s), 7.16–7.08 (3H, m), 6.80–6.75 (2H, m), 4.87 (1H, dd, *J* 9.5, 3.8 Hz), 4.63 (2H, s), 3.81 (2H, s), 3.45–3.35 (4H, m), 3.12–3.02 (2H, m), 2.98 (2H, t, *J* 8 Hz), 2.55 (2H, t, *J* 7.5 Hz), 1.96 (3H, s), 1.74–1.49 (8H, m), 1.42–1.32 (4H, m).

5.31. Pharmacokinetic studies in rats

Male Han Wistar rats were fasted for 18 h prior to dose administration. **12a** acetate salt was formulated as a solution in DMSO-PEG 200-distilled water (5:30:65, v/v/v) for oral dosing, and DMSO-PEG 200-saline (10:45:45, v/v/v) for intravenous dosing. Rats were dosed orally by gavage tube or intravenously via a tail vein at nominal dose levels of 2.0 mg **12a** base/kg or 0.25 mg **12a** base/kg, respectively. Blood samples were taken at 0.03 (intravenous only), 0.08, 0.25, 0.5, 0.75, 1, 2, 4 and 8 h post dose (n = 2 animals/time-point). Plasma was prepared from blood by centrifugation, and analysed for **12a** content by LC–MS/MS. Non-compartmental methods were used to calculate pharmacokinetic parameters from plasma concentration versus time profiles.

5.32. Oral absorption study in rats

Male Han Wistar rats were dosed discretely via the oral route at a nominal dose of 2 mg base/kg. Animals were provided with food and water ad libitum. Terminal blood samples were taken from two animals per time point at intervals up to 2 h. Samples were taken from the hepatic portal vein (HPV) and by cardiac puncture (CP) from each animal and centrifuged immediately to yield plasma for analysis. The plasma samples were store at −20 °C until prepared for analysis. Prior to analysis, samples were defrosted and vortex mixed. Sample (200 μL) was mixed with pH 5 sodium acetate buffer (50 mM, 200 μL) and β-glucuronidase (5 mg/mL, 100 μL). Samples were incubated overnight in a shaking water bath heated to 37 °C. Following incubation, samples were extracted by protein precipitation and analysed by LC–MSMS.

5.33. Pharmacokinetic studies in dogs

Female beagle dogs (n = 2), were fasted for 18 h prior to dose administration. Compound **12a** acetate salt was formulated as a solution in DMSO-PEG 200-distilled water (5:30:65, v/v/v) for oral dosing, and DMSO-PEG 200-saline (10:45:45, v/v/v) for intravenous dosing. Dogs were dosed orally by gavage tube or intravenously via the cephalic vein at nominal dose levels of 0.25 mg **12a** base/kg or 0.052 mg **12a** base/kg, respectively in a crossover design. Blood samples were taken from the jugular vein at 0.03 (intravenous only), 0.08, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6 and 8 h post dose. Plasma was prepared from blood by centrifugation, and analysed for **12a** content by LC–MS/MS. Non-compartmental methods were used to calculate pharmacokinetic parameters from plasma concentration versus time profiles.

5.34. Metabolite identification study

Compound **12a** at 25 μM was incubated with human liver microsomes and hepatocytes and analysed by LCMS. Samples were mixed with an equal volume of acetonitrile, centrifuged, and the supernatant transferred to auto-sampler vials for analysis. Aliquots (15 μL) were injected into a reverse phase gradient LCMS system (10 cm × 2.1 mm i.d. Luna C_{18} 3 μm column) and eluted components detected by electrospray mass spectrometry using a Quattro LC mass spectrometer. Two metabolites were detected: **12m** (ES+ve *m/z* 532 $(M+H)^+$ (parent + 18), and a hydroxylation metabolite (ES+ve *m/z* 530 $(M+H)^+$ (parent + 16). There was no evidence for the presence of any phase II metabolites.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.05.064. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Barnes, P. J. *Nat. Rev. Immunol.* **2008**, *8*, 183.
2. Barnes, P. J. *J. Allergy Clin. Immunol.* **1998**, *102*, 531.
3. Boushey, H. A. *J. Allergy Clin. Immunol.* **1998**, *102*, S5.
4. Johnson, M. *Med. Res. Rev.* **1995**, *15*, 225.
5. Matthys, H. *Respiration* **2001**, *68*, 432.
6. Johnson, M. *Am. J. Respir. Crit. Care Med.* **1998**, *158*, S146.
7. Baur, F.; Beattie, D.; Beer, D.; Bentley, D.; Bradley, M.; Bruce, I.; Charlton, S. J.; Cuenoud, B.; Ernst, R.; Fairhurst, R. A.; Faller, B.; Farr, D.; Keller, T.; Fozard, J. R.; Fullerton, J.; Garman, S.; Hatto, J.; Hayden, C.; He, H.; Howes, C.; Janus, D.; Jiang, Z.; Lewis, C.; Loeuillet-Ritzler, F.; Moser, H.; Reilly, J.; Steward, A.; Sykes, D.; Tedaldi, L.; Trifilieff, A.; Tweed, M.; Watson, S.; Wissler, E.; Wyss, D. *J. Med. Chem.* **2010**, *53*, 3675.
8. Glossop, P. A.; Price, D. A. *Ann. Rep. Med. Chem.* **2006**, *41*, 237.
9. 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. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4012.
10. 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. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6188.
11. 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. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1280.
12. Glossop, P. A.; Lane, C. A. L.; Price, D. A.; Bunnage, M. E.; Lewthwaite, R. A.; James, K.; Brown, A. D.; Yeadon, M.; Perros-Huguet, C.; Trevethick, M. A.; Clarke, N. P.; Webster, R.; Jones, R. M.; Burrows, J. L.; Feeder, N.; Taylor, S. C. J.; Spence, F. J. *J. Med. Chem.* **2010**, *53*, 6640.
13. Procopiou, P. A.; Barrett, V. J.; Bevan, N. J.; Biggadike, K.; Butchers, P. R.; Coe, D. M.; Conroy, R.; Edney, D. D.; Field, R. N.; Ford, A. J.; Guntrip, S. B.; Looker, B. E.; McLay, I. M.; Monteith, M. J.; Morrison, V. S.; Mutch, P. J.; Stephen, A.; Richards, S. A.; Sasse, R.; Smith, C. E. *J. Med. Chem.* **2009**, *52*, 2280.
14. Procopiou, P. A.; Barrett, V. J.; Bevan, N. J.; Biggadike, K.; Box, P. C.; Butchers, P. R.; Coe, D. M.; Conroy, R.; Emmons, A.; Ford, A. J.; Holmes, D. S.; Horsley, H.; Kerr, F.; Li-Kwai-Cheung, A.-M.; Looker, B. E.; Mann, I. S.; McLay, I. M.; Morrison, V. S.; Mutch, P. J.; Smith, C. E.; Tomlin, P. *J. Med. Chem.* **2010**, *53*, 4522.
15. Bouyssou, T.; Rudolf, K.; Hoenke, C.; Lustenberger, P.; Schnapp, A.; Konetzki, I. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5237.
16. Hoenke, C.; Bouyssou, T.; Tautermann, C. S.; Rudolf, K.; Schnapp, A.; Konetzki, I. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6640.
17. Bouyssou, T.; Hoenke, C.; Rudolf, K.; Lustenberger, P.; Pestel, S.; Sieger, P.; Lotz, R.; Heine, C.; Büttner, F. H.; Schnapp, A.; Konetzki, I. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1410.
18. Bennett, J. A.; Harrison, T. W.; Tattersfield, A. E. *Eur. Respir. J.* **1999**, *13*, 445.
19. Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615.

20. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3.
21. Procopiou, P. A.; Morton, G. E.; Todd, M.; Webb, G. *Tetrahedron: Asymmetry* **2001**, *12*, 2005.
22. Pirkle, W. H.; Sikkenga, D. L.; Pavlin, M. S. *J. Org. Chem.* **1977**, *42*, 384.
23. Coe, D. M.; Monteith, M. J.; Procopiou, P. A. WO2002070490A1.
24. Guntrip, S. B.; Procopiou, P. A. WO2004022547A1.
25. Nials, A. T.; Sumner, M. J.; Johnson, M.; Coleman, R. A. *Br. J. Pharmacol.* **1993**, *108*, 507.
26. Manchee, G. R.; Barrow, A.; Kulkarni, S.; Palmer, E.; Oxford, J.; Colthup, P. V.; Maconochie, J. G.; Tarbit, M. H. *Drug Metab. Dispos.* **1993**, *21*, 1022.
27. Skidmore, I. F.; Lunts, L. H. C.; Finch, H.; Naylor, A.; Campbell, I. B. DE3513885, 1985.