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From libraries to candidate: The discovery of new ultra long-acting dibasic β_2-adrenoceptor agonists

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ABSTRACT

Libraries of dibasic compounds designed around the molecular scaffold of the DA_2/β_2 dual agonist sibenadet (Viozan™) have yielded a number of promising starting points that have been further optimised into novel potent and selective target molecules with required pharmacokinetic properties. From a short-list, **31** was discovered as a novel, high potency, and highly efficacious β_2-agonist with high selectivity and a duration of action commensurable with once daily dosing.

Inhaled bronchodilators are the most commonly used drugs for the treatment of bronchial asthma and chronic obstructive pulmonary disease (COPD).[1] The ability of bronchodilators to relax smooth muscle tissue benefits patients with improvements in symptom control, dyspnea, exacerbations,[2] physical activity and quality of life.[3] Short-acting bronchodilators are the recommended first-line therapy for mild asthma and COPD, with long-acting inhaled bronchodilators being used in more severe, uncontrolled respiratory diseases.[4,5] Different long acting agents are available in the form of β_2-agonist[6] and anticholinergic compounds.[7] To provide effective disease control the long-acting β_2 adrenoceptor agonists (LABAs) formoterol and salmeterol require twice daily dosing (Fig. 1). The muscarinic receptor antagonist, tiotropium, however is used once daily to provide 24 h disease control. Recent development activity has been directed towards obtaining ultra-long acting beta agonists (uLABAs) with 24 h duration of action that are expected to improve patient compliance and enhance clinical outcomes.[4,8] In terms of safety, tolerability and lung function improvement, favourable clinical results have been demonstrated for indacaterol, now accepted in Europe,[9] with other uLABA compounds in development such as vilanterol and olodaterol awaiting investigation.[8] To achieve greater efficacy it is likely that future approaches will involve combinations of agents with enhanced and matched duration of action to prolong disease control and improve patient outcomes. The number of recent publications describing the discovery of novel series of compounds as potential once-a-day β_2-agonists clearly highlights the level of activity and interest in this field of research.[10] Here, we describe a novel series of compounds which combine extended duration of action with potential for reduced systemic exposure and rapid onset of effect.[11]

In parallel with other activities in this area within our laboratories,[12] and in an attempt to build on our experience gained during the research program that led to the discovery of the dual DA_2/β_2-agonist sibenadet (Viozan™),[13] a focused library of compounds that contained the benzothiazolone head group, a lipophilic portion and a second basic group was designed. In addition to potential increase in affinity and efficacy, it was postulated that the introduction of a second basic group would give rise to a set of physicochemical properties that could be advantageous in terms of lung residency, due to the expected increase in the terminal volume of distribution (Vz).[12b,14] Based around this concept, a compound library of generic structure **1**, which encompassed a set of nine scaffolds, was prepared and tested in a cAMP β_2 functional assay (Table 1).

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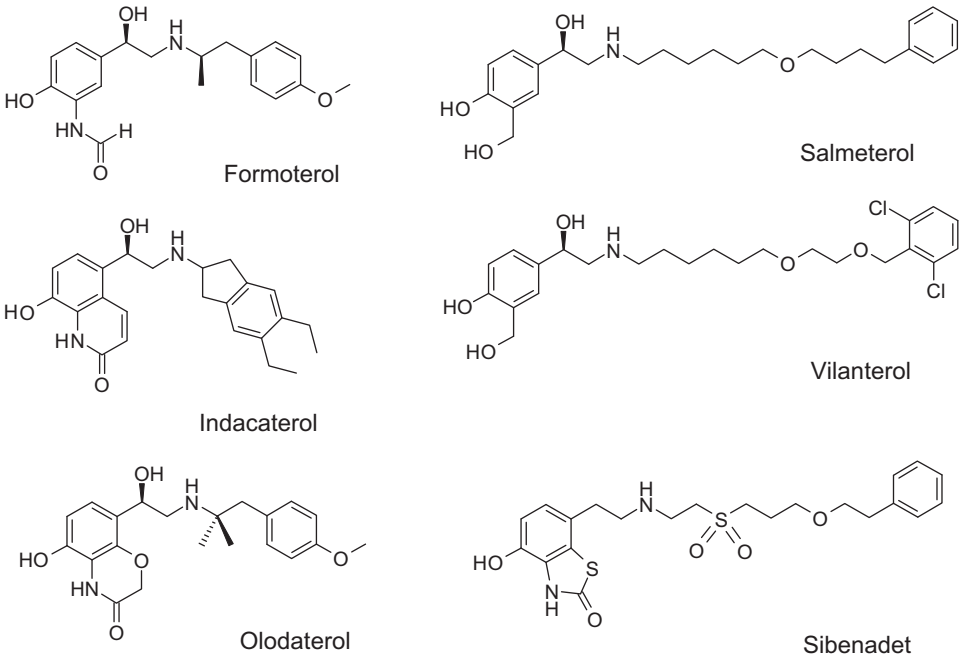
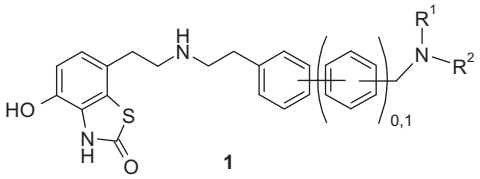
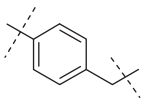
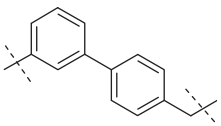
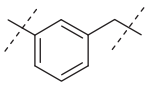
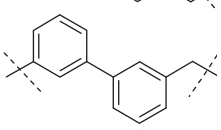
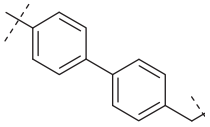
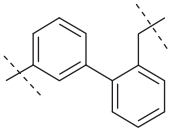
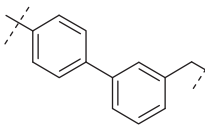
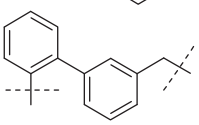
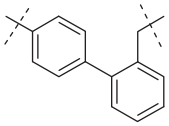


Figure 1. Examples of β_2-agonists.

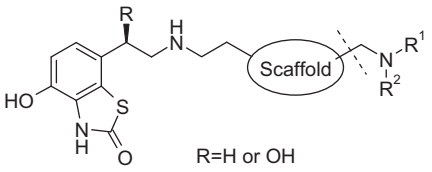
Table 1
Generic structures and spot test results for libraries 1–9

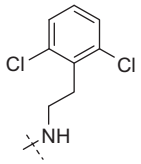
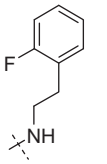
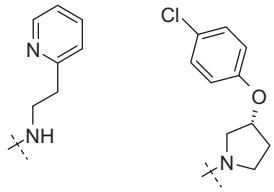
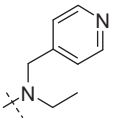
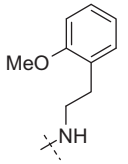
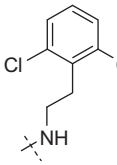
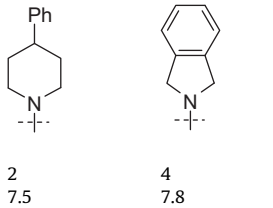
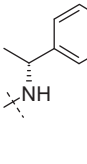
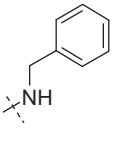


Library	Scaffold	No. of compds	No. of actives @1 μM[a]	Library	Scaffold	No. of compds	No. of actives @1 μM[a]
1		112	13	6		35	1
2		25	4	7		73	0
3		59	1	8		56	1
4		76	2	9		24	0
5		62	0				

[a] Stimulation of cAMP accumulation in H292 cells expressing human β_2. Cut off for hits: >20% activation at 1 μM compared to formoterol standard assigned 100% activation.

Table 2
β_2 binding and functional data for representative examples from libraries 1, 2 and 4 and representative pairs of des-hydro/hydroxy examples

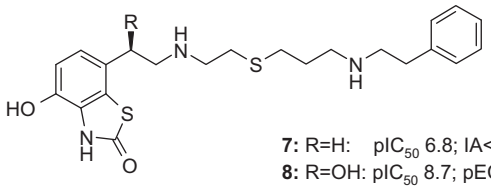


Compound		**2**	**3**	4	**5**	**6**	7
							
Scaffold entry		1	1	1	1	2	4
R = H	pEC_{50}[a] (IA)	7.0 (0.4)	7.1 (0.3)	6.8 (0.3)	6.8 (0.3)	8.4 (0.3)	6.5 (0.2)
R = H	pIC_{50}[b]	6.3	5.9	7.0	6.9	7.9	6.1
Compound		**9**	**10**	**11**	**12**	**13**	**14**
							
Scaffold entry		1	2	2	4	5	7
R = H	pIC_{50}[b]	6.7	9.2	7.5	7.8	9.1	7.5
R = OH	pIC_{50}[b]	8.7	9.7	8.4	7.2	8.7	8.0
	pEC_{50}[a] (IA)	9.5 (1.0)	9.3 (0.8)	9.0 (0.6)	8.0 (0.8)	8.4 (0.6)	9.2 (0.9)

[a] Stimulation of cAMP accumulation in H292 cells expressing human β_2, pEC_{50} is the negative logarithm of the molar drug concentration that produces a cAMP response equal to 50% of its maximal response and IA is the intrinsic activity measured relative to formoterol (IA = 1).
[b] Compound affinities were measured by competition with a radioligand for binding to membranes expressing the β_2 recombinant receptor using radiolabelled iodocyanopindolol. pIC_{50} is the negative logarithm of the molar drug concentration that causes 50% reduction in specific binding of the radioligand to the receptor.

The hit-rate in the spot test cAMP β_2-agonist assay was relatively low with the majority of the hits obtained from two scaffolds (entries 1 and 2; 11% and 16% hit-rate, respectively) and only a few compounds were worth progressing to a full dose response curve. These results are however consistent with the fact that designing compounds with high level of agonism (intrinsic activity (IA) > 0.6, compared to formoterol (IA = 1.0)) within the des-hydroxy benzothiazolone head group series had historically been challenging.[13] Typical results have been summarised in Table 2 (compounds 2–**7**). Generally, apart from compound **6**, compounds displayed moderate potency and were weak partial agonists with a maximum intrinsic activity (IA) of 0.4.

Whilst this work was underway, in a related series,[12b] it was found that the introduction of the benzylic hydroxyl group to a compound of low efficacy such as **7** could, in some cases, turn an antagonist into a potent agonist **8** (cAMP pEC_{50} 8.8, IA 0.9).

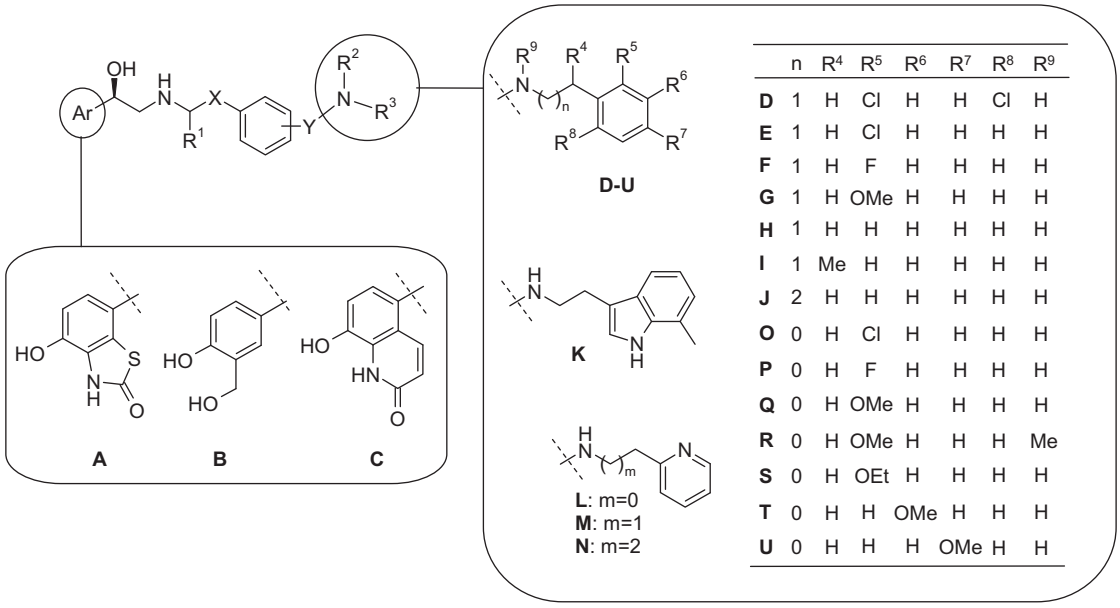


7: R=H: pIC_{50} 6.8; IA<0.1 @ 10μM
8: R=OH: pIC_{50} 8.7; pEC_{50} 8.8 IA 0.9

In the light of these results, the libraries were screened in a β_2 binding assay to identify high affinity antagonists. From the screening campaign, a range of potent binders with varied structures were identified and, from these, the corresponding hydroxyl analogues were prepared. In most cases, a substantial gain in both potency and efficacy over the parent des-hydroxy compounds was observed.[15] Some representative examples can be found in Table 2 (compounds **9**–**14**). Generally, it was found that both the level of affinity or efficacy of the corresponding hydroxy compounds could not be predicted from the binding activity of the corresponding des-hydroxy analogues and that potency in the series was not driven by lipophilicity. In addition, probably due to their more structurally rigid nature, the limited numbers of biphenyl-linked analogues made, with the exception of **14**, were found to be less potent and/or less efficacious (compounds **12** and **13**). The same was true for bulky tertiary amines on the *meta*-phenyl scaffold **11**. As a consequence, it was decided to focus lead optimisation around the mono-phenyl lead compounds **9** and **10**. A range of analogues was prepared and key in vitro results are summarised in Table 3.

The first part of our screening cascade was designed to identify potent and efficacious compounds displaying good level of selectivity against the closely related α_1-adrenergic, β_1-adrenergic, and D_2 dopamine receptors with a minimum target of 30- to 50-fold selectivity for the β_2-receptor. Balancing the overall profile of the compounds, while maintaining a good selectivity profile has been one of the main challenges of this program, although selectivity against the D_2 receptor was generally good in this series. Early examples, as exemplified by compounds **15** and **16**, were relatively lipophilic and, although displaying both high potency and efficacy as well as promising selectivity, were found to inhibit cytochrome P450 enzymes 2D6 and 3A4 as well as the hERG channel at sub-micromolar levels. A range of replacements for the 2,6-dichlorophenyl portion was explored leading to selective sub-nanomolar analogues both in the *para*- and *meta*-positions (compounds **17**–**26**), whereas the *ortho*-isomer appear to reduce β_2 potency whilst

Table 3
Summary of in vitro biological data for compounds **15**–**50**



	n	R^4	R^5	R^6	R^7	R^8	R^9
D	1	H	Cl	H	H	Cl	H
E	1	H	Cl	H	H	H	H
F	1	H	F	H	H	H	H
G	1	H	OMe	H	H	H	H
H	1	H	H	H	H	H	H
I	1	Me	H	H	H	H	H
J	2	H	H	H	H	H	H
O	0	H	Cl	H	H	H	H
P	0	H	F	H	H	H	H
Q	0	H	OMe	H	H	H	H
R	0	H	OMe	H	H	H	Me
S	0	H	OEt	H	H	H	H
T	0	H	H	OMe	H	H	H
U	0	H	H	H	OMe	H	H

Compd	Ar	R^1	X	Subst.	Y	R^2, R^3	β_2[a] pEC_{50} (IA)	β_2[b] pIC_{50}	α_{1D}[b] pIC_{50}	β_1[b] pIC_{50}	D_2[b] pIC_{50}	Log D^{16}
15	A	H	CH_2	Meta	CH_2	D	9.3 (0.8)	9.7	7.2	7.9	6.6	3.2
16	A	H	CH_2	Para	CH_2	D	9.5 (0.9)	8.4	7.1	6.4	6.0	2.3
17	A	H	CH_2	Meta	CH_2	E	9.6 (1.0)	9.2	7.3	6.8	6.2	1.6
18	A	H	CH_2	Meta	CH_2	F	9.8 (0.9)	9.3	6.9	7.2	6.4	1.1
19	A	H	CH_2	Para	CH_2	F	9.4 (0.9)	8.7	7.3	6.4	5.7	1.3
20	A	H	CH_2	Meta	CH_2	G	9.5 (1.0)	9.1	7.1	6.6	<6.1	0.8
21	A	H	CH_2	Para	CH_2	G	9.5 (1.0)	8.7	8.1	6.5	6.2	0.8
22	A	H	CH_2	Para	CH_2	H	9.6 (0.8)	8.7	7.9	6.4	5.7	1.1
23	A	H	CH_2	Meta	CH_2	K	9.5 (0.7)	9.2	6.4	7.1	6.4	1.1
24	A	H	CH_2	Para	CH_2	K	9.6 (0.8)	8.7	7.0	6.5	<6.1	0.4[c]
25	A	H	CH_2	Meta	CH_2	M	9.3 (0.9)	8.6	6.7	6.8	5.8	−0.1
26	A	H	CH_2	Para	CH_2	M	9.7 (1.0)	8.5	7.6	<6.1	5.6	−0.3
27	A	H	CH_2	Ortho	CH_2	F	8.8 (0.8)	7.9	6.7	8.7	6.1	1.6
28	A	H	CH_2	Para	CH_2	I	9.4 (0.9)	9.7	7.5	6.3	5.6	1.2
29	A	H	CH_2	Meta	CH_2	J	9.3 (0.6)	9.1	6.8	7.7	6.5	1.1
30	A	H	CH_2	Meta	CH_2	N	9.0 (0.4)	9.3	6.6	7.2	<5.6	−1.2[c]
31	A	H	CH_2	Meta	CH_2	Q	9.2 (0.8)	8.2	6.7	7.3	6.0	1.1
32	A	H	CH_2	Para	CH_2	Q	8.5 (1.0)	6.9	7.0	5.9	5.6	0.2
33	A	H	CH_2	Meta	CH_2	O	9.5 (0.9)	8.7	6.8	8.0	6.6	1.9
34	A	H	CH_2	Meta	CH_2	P	9.2 (0.9)	9.0	7.1	8.2	6.2	0.7
35	A	H	CH_2	Meta	CH_2	L	9.2 (0.9)	7.8	6.5	7.1	5.3	0.3[c]
36	A	H	CH_2	Meta	CH_2	T	9.0 (1.0)	8.2	7.0	7.2	6.1	0.9
37	A	H	CH_2	Meta	CH_2	U	9.1 (0.8)	8.3	6.8	8.0	6.1	0.9
38	A	H	CH_2	Meta	CH_2	S	8.5 (0.8)	7.6	7.5	7.0	5.9	1.1
39	A	H	CH_2	Meta	CH_2	R	8.5 (0.6)	7.6	6.6	6.7	6.0	1.5
40	A	H	CH_2	Meta	CH_2	NH–$(CH_2)_2$–OMe	8.7 (0.8)	7.6	6.3	6.8	6.1	0.0
41	A	H	CH_2	Para	CH_2	NH–$(CH_2)_2$–OMe	7.8 (1.0)	6.5	6.2	6.1	<5	−0.5
42	A	**H**	CH_2	Meta	CH_2	Pyrolidine	9.2 (0.7)	8.4	6.0	6.5	5.5	0.8
43	A	H	CH_2	Para	CH_2	Pyrolidine	7.4 (0.8)	ND	ND	ND	ND	−0.4[c]
44	A	H	$(CH_2)_2$	Meta	CH_2	D	8.9 (1.0)	8.2	7.4	8.1	7.1	1.7[c]
45	A	Me	CH_2	Meta	CH_2	D	9.1 (0.8)	ND	7.4	7.7	6.9	2.4
46	A	H	CH_2O	Meta	CH_2	F	8.6 (0.8)	8.1	7.6	6.7	6.4	1.9
47	A	H	CH_2	Meta	$(CH_2)_2$	Q	9.4 (1.0)	8.8	7.6	7.2	6.0	0.9
48	B	H	CH_2	Para	CH_2	H	7.5 (0.5)	6.2	6.3	<5.5	<5.1	−1.1[c]
49	C	H	CH_2	Meta	CH_2	M	8.6 (0.8)	7.2	6.4	5.5	<5.0	−0.1[c]
50	(*S*)–A	H	CH_2	Para	CH_2	Q	8.1 (0.9)	6.6	7.1	5.9	5.7	0.7[c]

ND = Not Determined.

[a] Stimulation of cAMP accumulation in H292 cells expressing human β_2, pEC_{50} is the negative logarithm of the molar drug concentration that produces a cAMP response equal to 50% of its maximal response and IA is the intrinsic activity measured relative to formoterol (IA = 1).

[b] Compound affinities were measured by competition with radioligand for binding to membranes expressing various recombinant receptors: Adrenergic β_1-receptor with radiolabelled iodocyanopindolol, adrenergic α_{1D} with radiolabelled Prazosin and dopamine D_2 (*S*-isomer) using radiolabelled spiperone. pIC_{50} is the negative logarithm of the molar drug concentration that causes 50% reduction in specific binding of the radioligand to the receptor.

[c] ACD Log *D*.

dramatically increasing β_1 affinity (compound **27** compared to **18** and **19**). Removal of one of the chlorines led to a compound of similar β_1 potency but of lower β_1 affinity (compound **17** compared to **15**). Replacement of the chlorine atom for fluorine (compounds **18** and **19**), methoxy (compounds **20** and **21**), proton (compound **22**), or replacement of the terminal phenyl ring with heterocyclic systems such as indole (compounds **23** and **24**) or pyridine (compounds **25** and **26**) all provided high potency and more polar analogues. Their selectivity was generally good, except for two *para*-isomers (compounds **21** and **22**) that have increased affinity for the α_1-receptor. Addition of a methyl group on the phenethyl linker (compound **28** compared to **22**) did not improve the compound's overall profile, whereas its extension by one carbon (compounds **29**, and **30** compared **25**) led to a decrease in efficacy. Less basic *N*-benzylic analogues were explored (compounds **31**–**39**). In this sub-series, it was rapidly found that the *para*-substituted analogues were generally less potent at the β_2-receptor and, as a consequence, analogues were prepared with the *meta*-substitution pattern (compound **31** compared to **32**). These findings are analogous to that observed by researchers at Almirall in their urea series.[10a] *meta*-Substituted *N*-benzyl-linked compounds were generally of similar potencies and efficacies to that of the corresponding *N*-phenethyl analogues (compounds **31** compared to **20**, **33**–**15**, **34**–**18** and **35**–**25**). The 2- and 3-methoxy analogues had similar in vitro profiles (compound **31** compared to **36**) whereas the 4-methoxy compound had reduced β_1 selectivity (compound **37** compared to **31**). Increased steric bulk on the *ortho*-ether substituent (compound **38** compared to **31**) or alkylation of the benzylic nitrogen (compound **39** compared to **31**) led to loss of potency with an additional decrease in efficacy in the case of the tertiary amine. Some alkyl analogues were also prepared (compounds **40** to **43**). In these cases, a marked difference in β_2 potency in favour of the *meta*-substituted analogues was also observed (compounds **40** compared to **41** and **42** to **43**) and the pyrrolidine analogue had an excellent selectivity profile (compound **42**). Additional SAR around the central phenyl ring was generated (compounds **44**–**47**). One atom chain extension on the left hand side through addition of a methylene group led to slight decrease in primary potency and increase of D_2 affinity, whilst not affecting either α_1 or β_1 affinities (compound **44** compared to **15**), whereas primary β_2 potency was reduced when using an oxygen linker (compound **46** compared to **18**). Introduction of an *alpha*-methyl group next to the amine nitrogen did not affect the profile of the compound (compound **45** compared to **15**) and the use of phenethyl linker on the right hand side increased α_1 affinity (compound **47** compared to **31**). Finally, changes in the β_2 headgroup amine for the saligenin or 8-hydroxycarbostril moiety, or reversal of chirality of the α-hydroxyl group generally decreased potency and/or efficacy of the compounds (compounds **48** compared to **22**, **49** to **26** and **50** to **31**).

Based on encouraging in vitro profiles as well as good level of potency and intrinsic activity in guinea pig tissues (GPT, tracheal rings contracted with methacholine in an organ bath), selected compounds were progressed to in vivo rat intra-venous (iv) pharmacokinetic (PK) profiling in order to determine their terminal half-lives (Table 4). Indeed, in the course of this project it was found that in vivo rat plasma iv $T_{1/2}$ can be used as a predictor of in vivo duration in a bronchoprotection guinea pig model following intra-tracheal (i.t.) dosing (cf. plasma $T_{1/2} > 10$ h for 24 h duration).[12b] As it can be seen from Table 4, compounds present similar long terminal half-lives that are achieved through a combination of moderate clearances (Cl) and high terminal volumes of distribution (Vz). The exception being **18**, for which the long half-life is a result of a relatively low clearance and moderate volume of distribution.

Table 4
iv PK profiles of selected examples

Compound	GPT pEC_{50} (IA)	Cl (ml/min/kg)	Vz (l/kg)	$T_{1/2}$ (h)	Log D^{15}
13	9.6 (0.9)	19	22	15	−0.3
14	9.3 (0.9)	23	29	14	0.8
18	9.6 (0.9)	6	8.2	16	1.1
24	9.7 (0.8)	18	29	18	0.4[a]
25	9.5 (0.9)	23	41	20	−0.1
31	9.3 (0.9)	20	22	14	1.1
40	8.5 (0.8)	18	28	18	0.0

[a] ACD predicted.

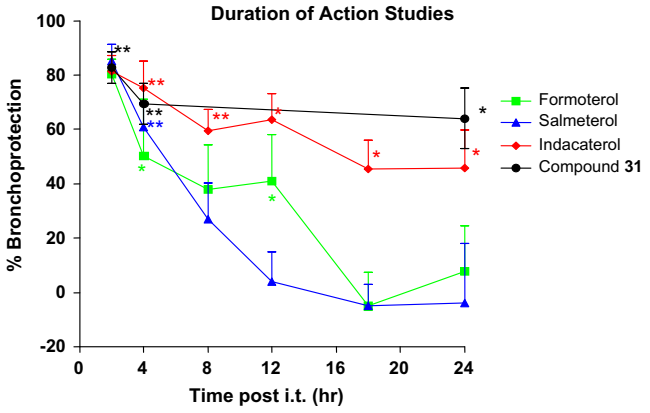
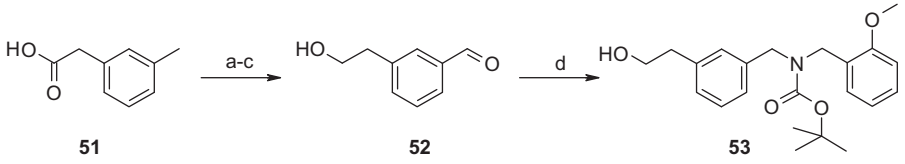


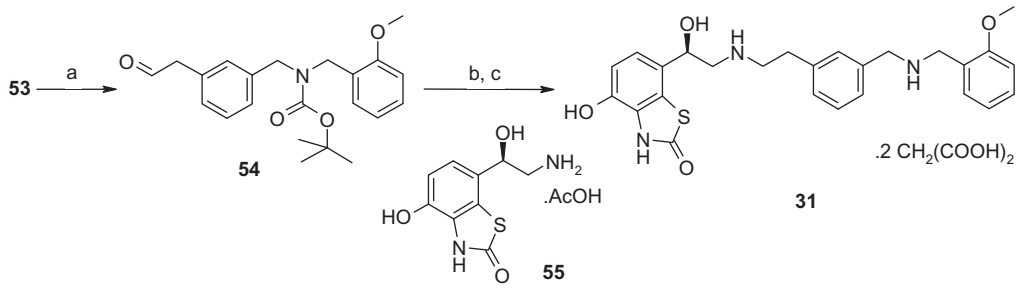
Figure 2. Duration of action of compound **31** versus standards dosed i.t. at ED_{80} in guinea pigs (formoterol 1 μg/kg, salmeterol 1 μg/kg, indacaterol 14 μg/kg and **31** 7 μg/kg).

The fact that all compounds have a similar pharmacokinetic (PK) profile regardless of their lipophilicity implies that the dibasic nature dictates the disposition of the compounds. The PK for these compounds can be described by a three-compartmental PK model formed of blood, tissues and a 'deep compartment'. We define this 'deep compartment' as specific tissues that have higher affinity for these dibasic compounds compared to other tissues and speculate that the 'deep compartment' is significantly comprised of lysosomes. It is therefore hypothesised that the terminal half-lives are largely controlled by the rate of diffusion of the compounds from this compartment back to the blood.

Based on its overall profile, **31** was selected for further progression. First, the lung half-life ($T_{1/2}$ 10 h) obtained from i.t. dosing was predicted from the rat iv dosing. The extended i.t. half-life of **31** was measured in dog ($T_{1/2}$ 48 h) giving confidence of potential for u.i.d. dosing. The low oral availability of **31** in both rat and dog ($F < 2\%$) will limit any systemic exposure due to swallowed fraction. The potency of **31** hydrochloride salt was measured



Scheme 1. Reagents and conditions: (a) NBS, benzoyl peroxide, $CHCl_3$, reflux, (66%); (b) BH_3, THF, 0 °C, (66%); (c) NaOEt, 2-nitropropane, EtOH, rt, (50%); (d) (1) 2-methoxybenzylamine, cat. TsOH, toluene, Dean–Stark, reflux; (2) $NaBH_4$, EtOH, rt; (3) BOC_2O, CH_2Cl_2 (75% over the three steps).



Scheme 2. Reagents and conditions: (a) Dess–Martin, DCM, rt; (b) 1 equiv HCl, $NaCNBH_3$, MeOH, rt (50%); (c) (i) TFA, rt (ii) 2 equiv malonic acid, MeCN/EtOH, stirred at rt for 5 days (94%).

in vivo using intratracheal (i.t.) administration in a well established histamine-induced bronchonstriction guinea pig model.[17,18] The duration of action of **31** and reference compounds (formoterol, salmeterol and indacaterol) were measured in the guinea pig by the i.t. route (Fig. 2).[19] Compound **31**, when dosed at 7 μg/kg (ED_{80}, free base equivalent), retained 64% bronchoprotection at 24 h post dose. This bronchoprotection was significantly different to that of control animals, clearly longer than the duration of action of both formoterol and salmeterol, and was similar to that of indacaterol. The good selectivity observed in the binding assays (Table 3) was confirmed in relevant functional assays (β_1 pEC_{50} 7.2 IA 0.9 (CHO cells, cAMP), α_{1D} pEC_{50} 6.9 IA 0.3 (HEK cells, Calcium flux), D_2 pEC_{50} 6.6 IA 0.4 (HEK cells, GTPγS)), further profiling through a panel of more than 140 assays did not reveal any activities inferior to 100 nM, and activities at hERG (patch clamp) and cytochrome P450s (5 isoforms, binding) were all inferior to 10 μM.

Since the hydrochloride salt of **31** did not possess optimal properties, extensive work was carried out in order to identify alternative crystalline salts. From these studies, the di-malonate salt emerged as having good solid state properties and was selected for further progression.[20]

Compounds **2**–**50** have been synthesised using similar procedures to that employed for the preparation of compound **31**. The side chain was prepared in five steps from the commercially available 3-methylphenyl acetic acid **5** as outlined in Scheme 1. Bromination of **51** with *N*-bromo succinimide was followed by reduction of the acid to the corresponding alcohol. The resulting benzyl bromide was then converted into the benzaldehyde **52** using the Hass–Bender procedure.[21] Finally, reduction of the imine formed from aldehyde **52** and 2-methoxybenzylamine followed by BOC protection yielded **53**.

Intermediate **53** was converted through to **31** as shown in Scheme 2. Dess–Martin oxidation in dichloromethane was found to give a smooth conversion to aldehyde **54** that was used immediately after work up in the next step. It is worth noting that oxidation with other reagents such as PCC also gave a good yields of aldehyde **54** however, an inseparable side product resulting from oxidative cleavage of the enol form of the resulting phenacetaldehyde giving benzaldehyde[22] proved problematic. The coupling through reductive amination of aldehyde **54** and amine **55**[12] was carried out using sodium cyanoborohydride in methanol followed by removal of the BOC protecting group using a 50% solution of trifluoroacetic acid in dichloromethane for 20 min at room temperature to avoid racemisation of the hydroxyl group. The 'free base' of **31** was precipitated from an aqueous solution by treatment with aqueous ammonia and compound **31** crystallised as its *bis*-malonate salt which was found to be the most suitable for pharmaceutical development and was selected for progression.

The discovery and development of a new series of potent and efficacious dibasic β_2-agonists have been described. This work led to the identification of the **31** which was shown to be potent and efficacious at the β_2-receptor, to have good selectivity over closely related receptors and to have a duration of action, in a bronchoconstriction guinea pig model, that is superior to that of both formoterol and salmeterol, and similar to that of indacaterol. Based on the promising profile, compound **31** was progressed into development for further studies as the *bis*-malonate salt.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.049.

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