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The identification of 7-[(*R*)-2-((1*S*,2*S*)-2-benzyloxycyclopentylamino)-1-hydroxyethyl]-4-hydroxybenzothiazolone as an inhaled long-acting β_2-adrenoceptor agonist

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ABSTRACT

The optimisation of two series of 4-hydroxybenzothiazolone derived β_2-adrenoceptor agonists, bearing α-substituted cyclopentyl and β-phenethyl amino-substituents, as inhaled long-acting bronchodilators is described. Analogues were selected for synthesis using a lipophilicity based hypothesis to achieve the targeted rapid onset of action in combination with a long duration of action. The profiling of the two series led to identification of the α-substituted cyclopentyl analogue **2** as the optimal compound with a comparable profile to the inhaled once-daily long-acting β_2-adrenoceptor agonist indacaterol. On the basis of these data **2** was promoted as the backup development candidate to indacaterol from the Novartis LABA project.

Following the efforts of several groups over the last 15 years a number of inhaled long-acting β_2-adrenoceptor agonists (LABAs) have entered into development. The most advanced of these LABAs have now received regulatory approval and are set to become the basis for the next generation of once-daily treatments for asthma and chronic obstructive pulmonary disease (COPD).[1–3] The successful LABAs from this group are anticipated to fulfil this role, either alone, or in fixed-dose combination-products with other once-daily inhaled therapies.[4] Of these LABAs indacaterol was the first to be approved as a single agent in 2008 for the treatment of COPD in Europe (brand name: Onbrez), and is now approved for this indication in several countries including the United States (Arcapta).[5] More recently the fixed-dose combination-product of indacaterol with the muscarinic receptor antagonist glycopyrronium bromide has been approved in Europe and Japan for the treatment of COPD (Ultibro).[6] To manage risk following the identification of indacaterol, and to ensure the highest probability of success for the Novartis LABA project, activities were initiated at an early stage to find a backup compound to indacaterol. As no issues were apparent with indacaterol during the early stages of development a backup compound had been sought with an equivalent preclinical profile.[7] In addition, to be as divergent as possible from indacaterol a compound from a different structural series to the 8-hydroxyquinolinone of the lead candidate was preferred. In this letter we describe the identification of a backup inhaled-LABA development candidate to indacaterol from a 4-hydroxybenzothiazolone β_2-adrenoceptor (β_2AR) agonist series.

Previously we have described a lipophilicity-based library approach to generate LABA leads within a 4-hydroxybenzothiazolone series which was inspired by the natural product S1319.[8] More recently others have also described LABA development candidates from the same 4-hydroxybenzothiazolone series, including the analogue **1**.[3] The Novartis library approach had identified interesting amino-substituents in the 4-hydroxybenzothiazolone series including the α-substituted cyclopentyl analogues **2**, **3** and **4**, as shown in Figure 1.

One optimisation strategy explored from this starting point was to investigate the structure activity relationships (SAR) surrounding the α-substituent and the role of the cyclopentyl stereochemistry,

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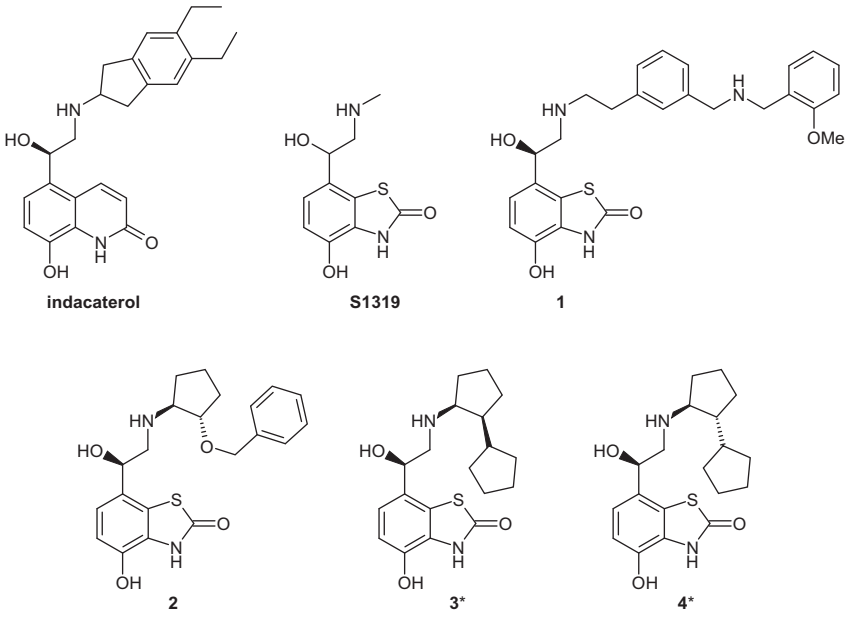


Figure 1. Structures of the β_2AR agonists indacaterol, S1319, **1**, **2**, **3** and **4**. *1:1 Mixtures of diastereoisomers containing the racemic *cis*- and *trans*-cyclopentyl amino moieties respectively.

leading to the targets of the general structure **5**. In addition, β-phenethyl amino-substituents had provided library members with favourable profiles, as well as being established in a number of previous β_2AR agonist structures, such as formoterol and **1**, leading to the targets of the general structure 6, as shown in Figure 2. Additionally, the possibility to combine these two structural features, by introducing an α-phenyl substituent into the cyclopentyl moiety, was also seen as an interesting possibility (**5**, R = phenyl).

To prepare the desired α-substituted cyclopentyl targets **5** an improved version of our original synthesis was developed, as shown in Scheme 1.[8,9] As previously, a benzyne-mediated cyclisation-reaction of the 3-fluorophenyl thiocarbamate derivative **7** gave the 7-lithiated benzothiazole intermediate **8**, which could be acylated directly at −40 °C with the Weinreb amide of chloroacetic acid **9** to give **10**. In contrast to the earlier route, under these conditions no transmetalation step was required to modulate the basicity of **8**, and this transformation could be run reliably on a multi-kilogram scale. Conversion of the chloroketone **10** to the (*R*)-epoxide **11** followed the original sequence using a CBS reduction to generate the desired chirality.[8] The α-substituted cyclopentylamines **12** were then monosilylated prior to reaction with the epoxide **11**. Epoxide opening occurred by addition to the least hindered position to give the protected target-compounds **13**. Acidic deprotection then provided the targeted 4-hydroxybenzothiazolones **5**.

The chiral α-substituted cyclopentylamines **12** were prepared based upon a previously described procedure, as shown in Scheme 2.[10] In the key step, reductive amination with α-phenylethylamine as the chiral auxiliary generated the *cis*-isomers with a high level of facial selectivity. Absolute stereochemistry was assigned based upon the reported observation that *like generates like* between the α-phenylethylamine centre and the induced aminocyclopentyl centre.[10]

To prepare the desired substituted β-phenethyl targets **6** an alternative synthesis was employed in which tertiary amines were used in the epoxide opening step, as shown in Scheme 3.[11] For the alkyl-substituted analogues: starting from either 3- or 4-bromophenylacetic acid, conversion to the corresponding benzyl amide was followed by the introduction of the desired alkyl groups through Negishi couplings to give **14**. Borane reduction then gave the desire *N*-benyl β-phenylethylamines which cleanly opened the epoxide **11**, at the least-hindered position, to give the protected target-compounds **15**. Transfer hydrogenation under acidic conditions then removed all three protecting groups to provide the targeted 4-hydroxybenzothiazolones **6**. The amines to prepare the ether-substituted β-phenethyl containing analogues were prepared from the corresponding hydroxyphenylacetic acids in a closely related manner via the amides **16**.[11,12]

Following the above routes the analogues **17** to **35**, shown in Table 1, were prepared as single stereoisomers of the (*R*)-configuration at the benzylic alcohol centre of the ethanolamine moiety. We have previously described in detail the targeted LABA preclinical profile that was put in place for the identification of indacaterol, and the goal for the backup project was also to fulfil

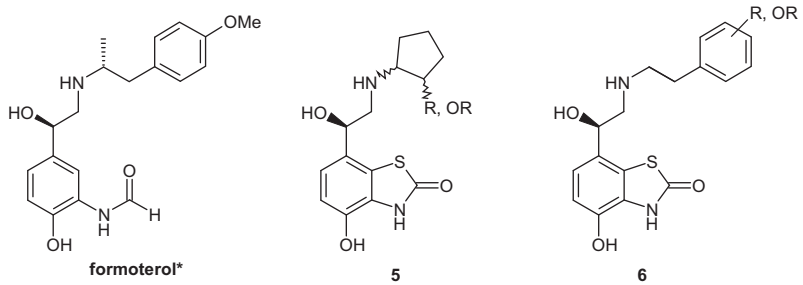
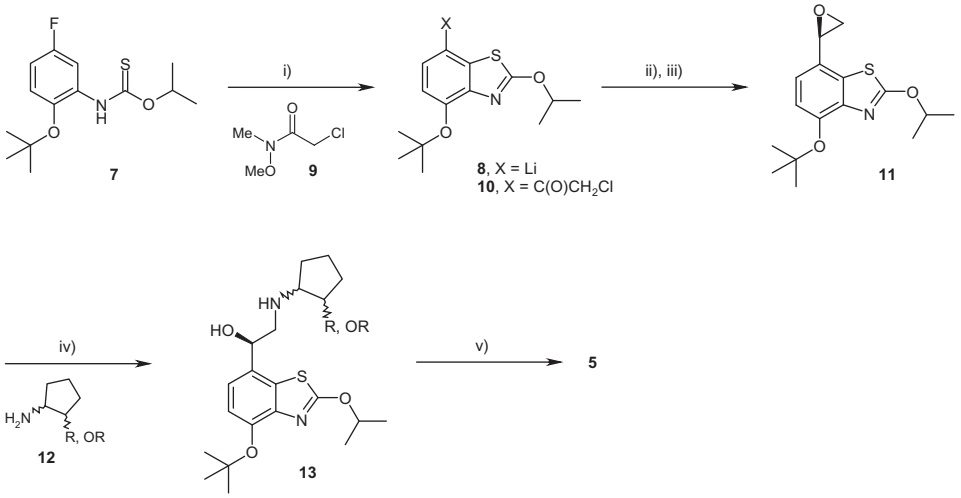
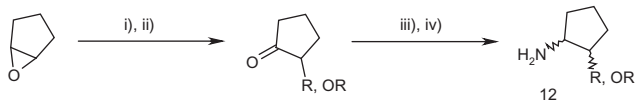


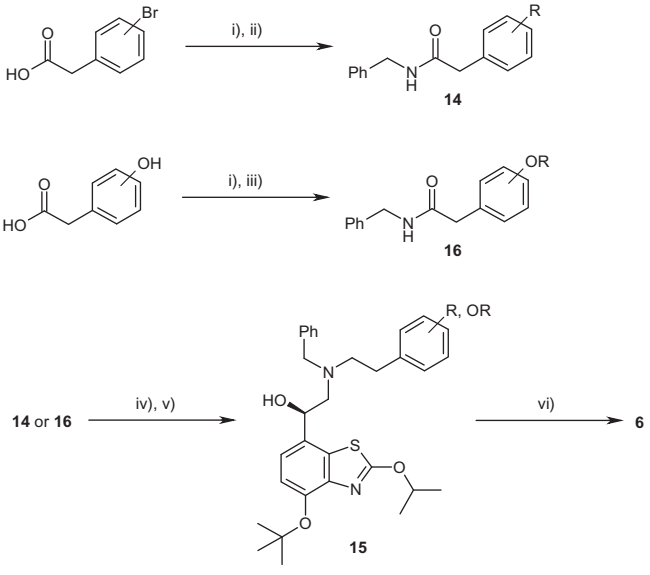
Figure 2. Structures of the β_2AR agonists formoterol and the targeted series **5** and **6**. *Racemic mixture of the *l*-diastereoisomer.



Scheme 1. Preparation of the 4-hydroxybenzothiazolone β_2-adrenoceptor agonists of the general structure **5**. Reagents and conditions: (i) 1.1 equiv *t*-BuLi (15% in pentanes), THF (0.35 M), −70 °C, then warmed to −30 °C, 1.4 equiv *t*-BuLi (15% in pentanes), 1.5 h, then cooled −70 °C, 1.3 equiv **9** in THF (6.8 M), 3 h, then quenched at −30 °C (60%); (ii) 0.1 equiv (1*R*,2*S*)-(+)-*cis*-1-amino-2-indanol, 1.0 equiv borane (1 M in THF), THF, 15 min, room temperature, then recrystallized from heptane (98% ee, 77%); (iii) 2.0 equiv K_2CO_3, 9:1 acetone/water, 50 °C, 24 h, (99% ee, 99%); (iv) 1.2 equiv **12**, 0.6 equiv *N*,*O*-bis-(trimethylsilyl)acetamide, DMF, 30 min, room temperature, then **11**, 80 °C, 18 h (43–89%); (v) HCO_2H, room temperature, 24–48 h, or 2:1 *i*PrOH/1N $HCl_{(aq)}$, 80 °C, 24 h (43–83%).



Scheme 2. Preparation of the alkyl and aryl α-substituted cyclopentylamines of the general structure **12**. Reagents and conditions: (i) 1.0 equiv R-MgX (2.0 M in diethylether, where X = Cl, Br), 0.1 equiv CuI, 10 min room temperature, then drop wise cyclopentene oxide (exothermic), room temperature, 4 h (46–70%); (ii) 3.0 equiv $C_6H_6N^+ClCrO_3^-$ (PCC, 20% on neutral alumina), toluene, room temperature, 18 h (27–66%); (iii) 1.2 equiv α-phenylethylamine (either (*R*)- or (*S*)-enantiomer: see text), 1.5 equiv AcOH, 1.5 equiv $NaBH(OAc)_3$, 1,2-dichloroethane, room temperature, 48 h (33–70%); (iv) 5 atm H_2, 3 equiv AcOH, MeOH, room temperature, 18 h (81–93%).

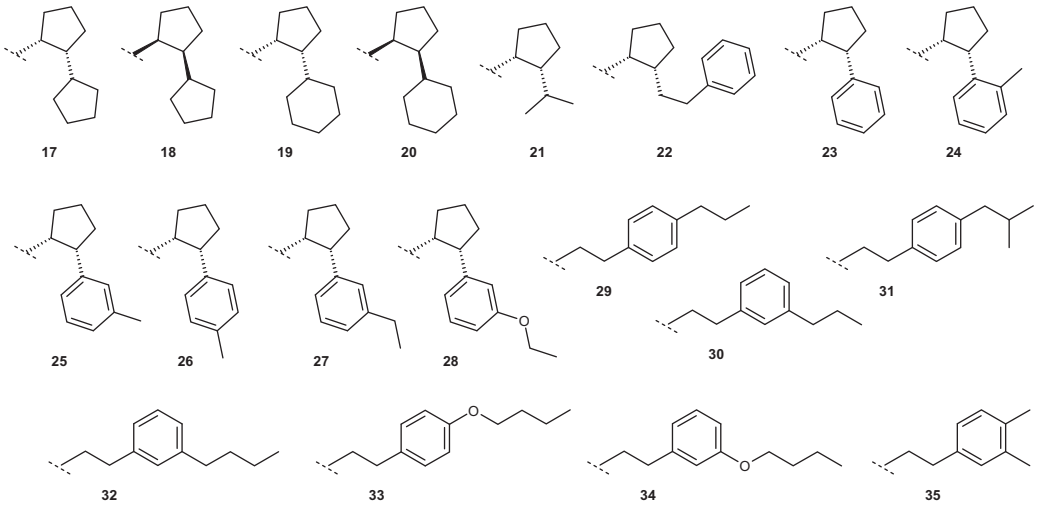


Scheme 3. Preparation of the 4-hydroxybenzothiazolone β_2AR agonists of the general structure **6**. Reagents and conditions: (i) 1.0 equiv $BnNH_2$, 1.2 equiv EDCI, 1.2 equiv HOBt, CH_2Cl_2, water, room temperature, 18–24 h (35–75%); (ii) 1.5 equiv R-ZnBr, 0.1 equiv $Pd(dppf)Cl_2$, THF, room temperature, 24–48 h (68–93%); (iii); 1.2 equiv nBuBr, 2.0 equiv Cs_2CO_3, 0.3 equiv KI, AcCN, 16 h, reflux (86–87%); (iv) 2 equiv DIBALH, toluene, 50 °C, 4 h (39–54%); (v) 0.9 equiv **11**, nBuOH, 110 °C, 24 h (56–67%); (vi) Pd black, HCO_2H, room temperature, 24 h (38–46%).

these criteria as closely as possible.[7,13] To achieve the desired onset and duration of action profiles the lipophilicity of the potential targets were estimated prior to synthesis. To select suitable R-substituents, to introduce into the series **5** and **6**, calculated $\log D_{7.4}$ values were used to make this assessment. As described previously maintaining the lipophilicity close to that which had been targeted during the lead-generation phase was implemented (within the $c\log D_{7.4}$ window of 2.4 to 3.5).[8,12,13] To assess the biological profiles of the compounds: affinities for the human β_1AR and β_2AR were initially assessed using radio-ligand binding assays. In terms of the functional selectivity for the β_2AR over the β_1AR the binding assays, which measure the interaction with the low-affinity state of the receptors, provided a good selectivity ranking, but were found to underestimate the functional selectivity.[14] The 4-hydroxybenzothiazolone catechol mimetic was first described in a series of dopamine receptor (DR) agonists,[15] and DR activity was identified to be the most significant off-target activity for the series **5** and **6** following screening versus an internal panel of enzymes and receptors.[16] For the most interesting analogues functional β_2AR potency and efficacy, onset of action (OoA) and duration of action (DoA) were then assessed in an electrically stimulated superfused guinea-pig tracheal-strip assay as described previously.[14] These data for the reference compounds and analogues **2**, **3**, **4** and **17**–**35** are shown in Table 1.

For the α-substituted cyclopentyl series **5** the observation from the library members was for little difference between the *cis*- and *trans*-substituted analogues **3** and **4** in terms of β_2AR activity and selectivity. However, the ready access to the optically pure *cis*-α-substituted cyclopentylamines described above and the trend for greater activity and selectivity for the cyclopentyl *cis*-analogues **3**, versus the *trans*-analogues **4**, led to focusing exclusively on the *cis*-analogues going forward. In terms of the cyclopentyl stereochemistry: comparing **17** and **19** with **18** and **20** indicated the (*R*,*R*)-diastereoisomers to be more active than the (*S*,*S*)-diastereoisomers. This relationship was evident throughout the series, when both diastereoisomers were prepared, and the emphasis was directed towards the more active (*R*,*R*)-diastereoisomers. However, the likelihood of an increased level of technical complexity being required to produce low dose formulations for inhalation resulted in an upper limit on potency. As discussed previously, compounds with β_2AR K_i values less than formoterol were deprioritised to

Table 1
Structures of the amino substituents of the analogues **17**–**35**, human β_2AR, β_1AR and D_3R K_i values, calculated log$D_{7.4}$ values and guinea-pig electrically stimulated superfused tracheal strip data for the reference compounds and the 4-hydroxybenzothiazolone analogues **2**, **3**, **4** and **17**–**35**



Compound	β_2 K_i (nM)	β_1 K_i (nM)	D_3 K_i (nM)	*c*log$D_{7.4}$	Guinea-pig tracheal strip		
					IC_{50} (nM)	OoA (min)[a]	DoA (h)[b]
Indacaterol	20.6 ± 2.8	91 ± 4	1048	3.0	7.9	35	>10
Formoterol	2.6 ± 0.1	315 ± 162	1626	1.3	0.4	28	1.2
Salmeterol	3.2 ± 1.0	784 ± 452	1049	3.1	3.5	120	>10
2	2.5 ± 0.1	70 ± 27	895	2.4	2.2	36	>10
3	0.8	6.9	782	3.0	—	—	—
4	0.6	18	1141	3.0	—	—	—
17	0.8 ± 0.3	17 ± 3.3	842	3.0	0.3	87	>10
18	6.7 ± 3.1	66 ± 19	745	3.0	23	66	>10
19	2.0 ± 1.6	40 ± 16	1100	3.6	5.7	61	>10
20	97 ± 51	391 ± 271	64	3.6	—	—	—
21	4.2 ± 2.6	62 ± 26	512	2.5	<0.3	47	>10
22	0.4 ± 0.1	0.8 ± 0.3	120	3.5	—	—	—
23	5.5 ± 2.9	30 ± 8.6	512	2.6	12	33	5.5
24	7.1 ± 1.8	58 ± 24	575	3.0	—	—	—
25	15 ± 1.4	77 ± 35	279	3.1	22	32	3.7
26	149 ± 19	343 ± 138	502	3.1	—	—	—
27	12 ± 1.7	33 ± 10	477	3.6	20	68	8.6
28	266 ± 63	438 ± 74	—	3.0	—	—	—
29	19 ± 5.4	28 ± 13	149	3.2	14	36	6.4
30	3.2 ± 1.1	5.5 ± 4.0	152	3.2	12	32	8.9
31	6.8 ± 4.3	6.1 ± 1.7	115	3.6	—	—	—
32	1.4 ± 0.6	7.9 ± 1.8	73	3.7	6.4	54	9.0
33	3.9 ± 2.0	13 ± 0.8	129	3.1	—	—	—
34	1.7 ± 0.7	4.3 ± 0	144	3.1	8.5	33	9.6
35	32 ± 18	21 ± 8.0	—	2.6	—	—	—

[a] Onset of action: time to maximal effect at the IC_{50} dose level.
[b] Duration of action: time for response to fall to 50% of maximal response during washout phase at the IC_{50} dose level.

facilitate formulation for inhalation.[8,13] Based upon the targeted profile, the most interesting α-substituents with respect to the targeted β_2AR potency were the (*S*,*S*)-cyclopentyl **18**, (*R*,*R*)-cyclohexyl **19**, (*R*,*R*)-isopropyl **21** and (*R*,*R*)-phenyl **23**. The analogues **17** and **22** were considered to be too highly potent based upon the above criteria and analogue **20** was deemed not to be sufficiently potent. Exploring the possibility for substitution in the α-phenyl substituent of **23** to further increase lipophilicity within the targeted range: methyl and ethyl groups in the *ortho* and *meta*-positions were well tolerated as exemplified by **24**, **25** and **27**. In contrast introducing a methyl substituent into the *para*-position, **26**, led to a 27-fold decrease in potency, and as a result substituents in this position were not pursued further. Similarly *meta*-alkoxy substituents led to an even greater decrease in potency as exemplified by the ethoxy analogue **28**.

For the β-phenethyl series **6**: propyl, butyl and *n*-butoxy residues in the *meta*- and *para*-positions, as exemplified by compounds **29**–**34**, were well tolerated and provided analogues in the desired lipophilicity range. These analogues exhibited β_2AR potencies at the higher potency limit of the targeted profile, and remained within 4-fold of the previously reported unsubstituted parent compound.[8] However, introducing two substituents at both the *meta*- and *para*-positions, as exemplified by the 3,4-dimethyl analogue **35**, led to 27-fold decrease in potency relative to the unsubstituted parent compound.

In terms of selectivity for the β_2AR over the β_1AR, the α-substituted cyclopentyl analogues **5** exhibited a clear trend for greater selectivity in comparison to the β-phenethyl analogues **6** (mean selectivities of 11-fold versus 2.3-fold for **5** verses **6**). However, some overlap in selectivity was observed between the series. When

compared to the reference compounds the majority of the 4-hydroxybenzothiazolone analogues exhibited a greater selectivity than determined for indacaterol, and this was used as the basis for further progressing compounds. Employing these criteria the analogues **27**, **29**–**31**, **33** and **34** were not pursued further due to concerns over the increased potential for producing β_1AR mediated side effects.

In terms of selectivity for the β_2AR over the DR family, again the α-substituted cyclopentyl analogues **5** exhibited a clear trend for greater selectivity in comparison to the β-phenethyl analogues **6** (mean selectivities of 214-fold versus 40-fold for **5** vs **6**). When compared to the reference compounds the more selective examples from the 4-hydroxybenzothiazolone analogues such as **2**, **17**–**19**, **21**–**24**, **32** and **34** were comparable to, or better than, indacaterol in terms of relative affinities for the β_2AR verses the dopamine D_3 receptor (D_3R). To assess the functional consequences, a dopamine D_2 receptor (D_2R) cellular assay revealed some of the analogues **6** to be agonists of intermediate intrinsic efficacy at this receptor, as exemplified by **34**, for which an EC_{50} of 123 nM was determined with a maximal effect of 43% relative to the reference agonist dopamine.[17] In terms of applying criteria for progressing compounds, a cut off of greater than 30-fold selectivity was implemented. This lead to the analogues **20**, **25**, **26**, **29**, and **31** being deprioritised due to concerns over the potential for producing DR mediated side effects.

In the next stage of evaluation the most promising compounds were tested in an electrically-stimulated guinea-pig tracheal strip assay. A number of the α-substituted cyclopentyl analogues **5** exhibited a longer time to maximal effect which extended well beyond the end of the 30 min drug administration phase. As a result the analogues **17**–**19**, **21** and **27** were deprioritised due to the risk of a delayed onset of action. This differentiation was based upon the delayed OoA observed in man with the twice-daily LABA salmeterol and the corresponding delayed OoA profile measured in the above tracheal strip assay.[18] In contrast the α-phenyl cyclopentyl analogues **23** and **25** produced times to maximal effect consistent with a rapid OoA. However, the DoA for both these compounds were shorter than for indacaterol and suggested they would not satisfy the targeted once-daily profile. Therefore, the only analogue from the series **5** satisfying the targeted OoA and DoA profile was the original library member **2**, which showed a very similar profile to indacaterol. From the β-phenethyl analogues **6** the less lipophilic analogues **29**, **30** and **34** produced times to maximal effect consistent with a rapid OoA. In contrast the more lipophilic analogue **32** resulted in an extended time to maximal effect and was deprioritised due to OoA concerns. Of the series **6** with satisfactory OoA profiles, compounds **30** and **34** also showed DoA profiles approaching indacaterol and were selected as the most promising compounds. Although lipophilicity had proven to be a good predictor of OoA and DoA previously in the tracheal-strip assay, we have also discussed the role of potency and intrinsic efficacy on these parameters.[18] In that context all the analogues **5** and **6** tested in the tracheal-strip assay were able to function as full agonists at the highest concentrations tested and demonstrated high levels of potency. Thus, although a clear trend is evident for increased OoA and DoA with increased lipophilicity for the series **5** and **6**, the reason for the relatively low success rate in identifying compounds with an overlapping rapid OoA and suitably long DoA are unclear at the present time when compared to previous applications of this approach.

To achieve the tolerability component of the targeted profile, the 4-hydroxybenzothiazolone series had in part been initially selected due to exhibiting a high susceptibility towards glucuronidation. Such a clearance pathway was anticipated to provide a means to rapidly eliminate the systemic component of the dose in the form of a β_2AR inactive metabolite, so as to minimise undesirable β_2AR mediated systemic side-effects.[8] Measuring the in vitro glucuronidation rates for the series **5** and **6** showed high intrinsic clearance values for the majority of examples in incubations in microsomes with the uridine 5′-diphospho-glucuronic acid (UDPGA) cofactor. However, assessing in more detail the overall metabolic profiles of selected compounds revealed complex patterns, thus making prediction of the likely exposure to parent and β_2AR active metabolites more difficult. As a result the decision was made to assess the tolerability of the compounds in vivo at the earliest opportunity, rather than extensive early in vitro pharmacokinetic profiling, to select compounds with the most favourable side effect profiles.

Assessing the data for series **5** and **6** up to this point led to compounds **2** and **34** being identified as the most promising examples, and prior to in vivo profiling both compounds were further screened more broadly for off-targets and to generate further comparative data with indacaterol. These data showed **2** to have an acceptable off-target profile when screened against the broad panel of assays and in follow up functional screens. In contrast, compound **34** produced a weakly positive result in a micronucleus screen which stopped the further progression of this compound.[19] Evaluating the remaining compound, **2** was further assessed in comparison with indacaterol in physicochemical and pharmacokinetic studies, selected data are shown in Table 2. Overall these data showed compound **2** to possess a very similar profile to indacaterol. In a functional cAMP assay in A431 cells, endogenously expressing the β_2AR, **2** was found to be 6-fold more potent than indacaterol and of slightly higher intrinsic efficacy. High selectivities in functional β_1AR and D_2R assays were observed for **2**, which were comparable to those observed with indacaterol. One property where a difference was seen for **2** was the 100-fold higher solubility when compared to indacaterol. This increased solubility was considered to be potentially beneficial, and seen as a way to accelerate dissolution to ensure a rapid OoA following dry-powder delivery. Log$D_{7.4}$ and affinity for immobilised artificial membrane (K_{IAM}) measurements showed **2** to be slightly less lipophilic than indacaterol. Comparison of the calculated with the measured log$D_{7.4}$ values shows the prediction to be greater than a log unit higher in both cases. Similar ionisation constants for both compounds also indicated **2** to be predominantly zwitterionic at physiological pH, a property which has been linked to a favourable colocalisation of indacaterol with the β_2AR in lipid raft micro domains.[20] In terms of the pharmacokinetic profiles, comparable plasma protein binding and rat clearance and volume of distribution data provided further confidence that **2** would exhibit a comparable LABA profile to indacaterol, based upon the previously hypothesised roles of these parameters in satisfying the targeted profile.[13]

The similar profile of **2** in comparison with indacaterol led to the compound entering into in vivo profiling to assess DoA and tolerability. Previously we have shown an anti-bronchoconstrictor model in the rhesus monkey to be a good predictor of intrinsic DoA and tolerability using clinically established β_2AR agonists, and also as a key model in the selection of indacaterol.[14,21] Compound **2** was initially tested in a dose response study from which an ED_{80} of 2.6 μg/kg was determined for the inhibition of a methacholine-challenge administered 5 min after the end of the drug administration period. To have the highest confidence in the key time course arm of the study, **2** was dosed head-to-head with indacaterol. Both compounds were administered at the ED_{80} dose level, the indacaterol dose being taken from the previously reported dose response study (12.4 μg/kg).[14] Data from the time course profiles for the inhibition of repeated methacholine challenges and for the changes in heart rate (HR) in the rhesus monkey are shown in Figure 3.

From this rhesus monkey study, comparison of the indacaterol data with the previously reported data for this compound shows

Table 2
Comparison of the data for **2** with indacaterol

Assay/measurement	Compound **2**	Indacaterol
Cellular β_2AR: A_{50} (nM), % efficacy[a]	0.25 ± 0.05, 103.4 ± 8.2%	1.5, 91%
Functional β_1AR: EC_{50} (μM)[b]	$2.2 \pm 1.3 \times 10^{-6}$	$3.5 \pm 2.2 \times 10^{-5}$
Functional D_2R: A_{50} (M)[17]	$>10^{-5}$	$>10^{-5}$
Solubility pH6.8 (μM)[c]	924	8
pK_a: phenol, amine[d]	8.5, 7.5	8.3, 6.7
Log $D_{7.4}$, log K_{IAM}[e]	1.25, 1.21	1.77, 1.90
Plasma protein binding[f]	96.1%	95.1–96.2%
Rat PK: Cl (l/kg), V_{ss} (ml/min/kg)[g]	79.8 ± 3.6, 6.2 ± 0.6	66.9 ± 7.5, 4.1 ± 1.0

[a] Elevation of cAMP measured in A431 cells endogenously expressing the β_2AR, % efficacy relative to maximum response to formoterol.[8]
[b] Inhibition of the electrically stimulated contraction of guinea-pig left atria.[14]
[c] Thermodynamic solubility was measured using the shake vial method.[14]
[d] pK_a values were determined by potentiometric titration.[14]
[e] Lipophilicity values were measured using chromatographic methods.[14]
[f] Human protein binding was measured for **2** using an ultrafiltration method, and for indacaterol as previously reported.[13]
[g] Pharmacokinetic data were generated in separate experiments following the i.v. administration of a 2.1 and 5.0 μmol/kg dose of **2** and indacaterol, respectively.

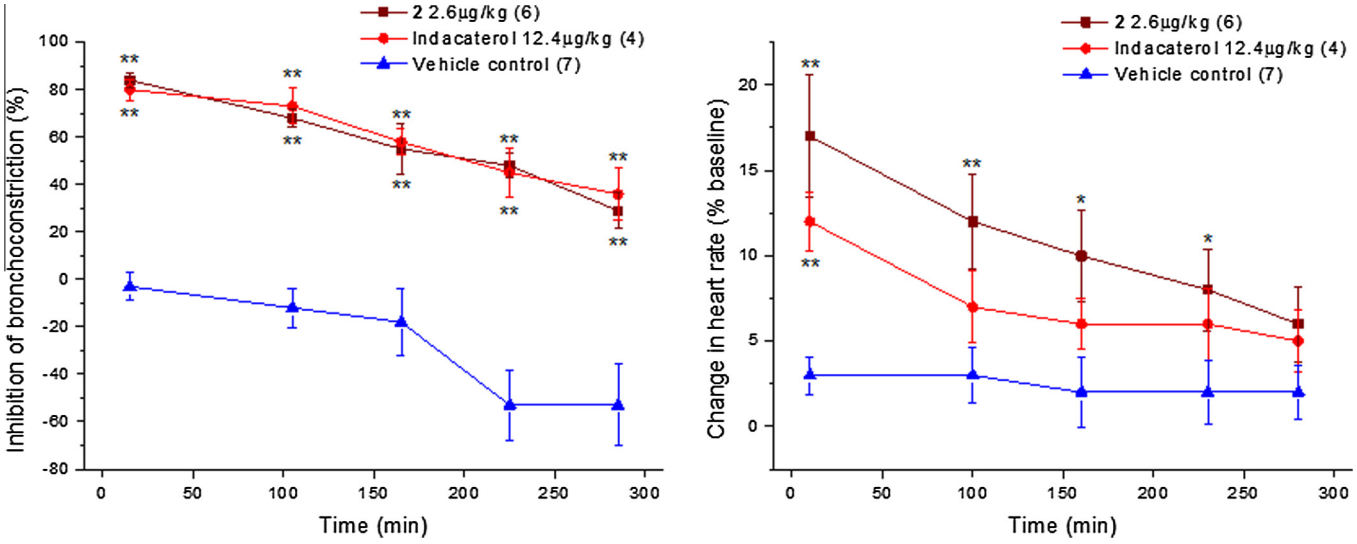


Figure 3. Comparison of **2** and indacaterol at the ED_{80} dose level in the methacholine-induced bronchoconstriction model in the rhesus monkey and the associated changes in heart rate. Results are expressed as mean ± standard error of the mean of the number of animals shown in parentheses. $^{*}P$ <0.05 and $^{**}P$ <0.001 indicates that the value differs significantly from the equivalent value in the vehicle treated animals. The following parameters were also measured with no significant differences observed versus the vehicle treated controls at each time point: systolic and diastolic arterial blood pressure; serum potassium.

an excellent level of reproducibility with this model.[14] When administered at equipotent bronchodilating doses **2** and indacaterol showed very similar levels of antibronchoconstrictor activity at all time points, indicating both compounds to have very similar intrinsic DoA and for **2** to be 5-fold more potent. In contrast, the associated β_2AR systemic side effects, as assessed by increased HR, remained significantly higher for **2** than for indacaterol. However, when compared to the historical data generated in the rhesus monkey with the well-tolerated twice-daily β_2AR formoterol at the same dose level (ED_{80}):[20] **2** shows an improved side effect profile and greater intrinsic duration of action. Based upon these data **2** was considered to be capable of delivering an equivalent DoA to indacaterol and to have a good tolerability profile with respect to the associated systemic β_2AR mediated side effects.

Taking the above data set into consideration **2** was considered to possess a very similar profile to indacaterol and to satisfy all aspects of the targeted product profile. As a result **2** was promoted as the second compound to progress into development from the project, as the backup candidate to indacaterol. To prepare the material to support the early development activities the synthesis described in Scheme 1 was used with the commercially available primary amine (1*S*,2*S*)-2-benzyloxycyclopentylamine.[22]

In summary, by following up on the two most promising leads from a 4-hydroxybenzothiazolone β_2AR agonist series a number of analogues satisfying the activity and selectivity criteria, whilst falling within the targeted physical property space, have been identified. Upon further profiling 7-[(*R*)-2-((1*S*,2*S*)-2-benzyloxycyclopentylamino)-1-hydroxyethyl]-4-hydroxybenzothiazolone, compound **2**, a member of the original N-substituent screening library, was identified as the optimal compound from the series. The data supported **2** to be: 5- to 6-fold more potent than indacaterol; to possess a good selectivity profile; to combine a rapid OoA, with a comparable intrinsic DoA to indacaterol; and to have a favourable tolerability profile with respect to the systemic β_2AR mediated side effects that are associated with the targeted levels of bronchodilation. On the basis of these results **2** was progressed into development as the backup LABA candidate to indacaterol.

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