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A physical properties based approach for the exploration of a 4-hydroxybenzothiazolone series of β_2-adrenoceptor agonists as inhaled long-acting bronchodilators

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ABSTRACT

The chiral synthesis of a 4-hydroxybenzothiazolone based series of β_2-adrenoceptor agonists is described. Using this methodology a library of N-substituted analogues were prepared for the rapid identification of leads with the potential to be fast onset and long-acting inhaled bronchodilators with improved therapeutic margins. The design of the library to achieve the targeted profile was based upon lipophilicity and metabolism based hypotheses. This approach identified β-phenethyl, α-substituted cyclopentyl and monoterpene N-substituents to be of particular interest for further evaluation, as exemplified by structures **19**, **29** and **33**, respectively.

Long-acting inhaled β_2-adrenoceptor agonists when used in combination with inhaled corticosteroids currently represent the primary approach for the treatment of the moderate and severe forms of asthma. More recently, this drug combination has also been found to be increasingly effective for the treatment of the second major obstructive airway condition: chronic obstructive pulmonary disease (COPD).[1] However, one factor which has limited the effectiveness of these agents is the relatively poor compliance associated with drugs delivered by the inhaled route and in particular when multiple treatments are involved.[2] To overcome this issue, one approach which has been demonstrated to be successful in improving patient compliance is the co-formulation of the long-acting β_2-adrenoceptor agonist and the corticosteroid within a single inhalation device. Examples of this approach include the combination of salmeterol with fluticasone and formoterol with budesonide which have been shown to be more effective treatments when compared to the equivalent mono therapies used in free combination.[3] However, the opportunity still exists to further improve patient compliance with these types of inhaled therapies. Presently the inhaled long-acting β_2-adrenoceptor agonists in clinical use as single agents and in combination products are suitable for twice-daily administration in the majority of patient populations. As part of a strategy to further improve patient compliance, the next generation of inhaled products for the treatment of respiratory diseases are anticipated to be fixed dose combination products which are suitable for once-daily dosing. To address this opportunity research is ongoing in a number of groups to identify both once-daily inhaled bronchodilator and anti-inflammatory agents.[4] Included in these activities is indacaterol, Figure 1, which is the lead candidate from the Novartis long-acting β_2-adrenoceptor agonist project, and which has been shown to be an effective once-daily bronchodilator in both asthma and COPD.[5] In this letter we describe a biophysical properties based approach for the rapid identification of leads within a 4-hydroxybenzothiazolone series as part of an effort to identify back up long-acting β_2-adrenoceptor agonist candidates to indacaterol.

Although delivering effective 24 h bronchodilation was the primary objective for the project additional opportunities were also targeted to further improve upon the two long-acting β_2-adrenoceptor agonists currently in clinical use: formoterol and salmeterol. These opportunities are described below. As shown in Figure 1, both of the compounds presently in clinical use are employed as racemic mixtures and a single stereoisomer was targeted to avoid complications due to potential off-target activities from eutomeric stereoisomers.[6] In addition to delivering a sustained duration of effect, an agent providing a rapid onset of action was also targeted because for certain applications this has proven to be a positive discriminating attribute for formoterol over salmeterol.[7] The inhaled route of administration is employed for this class of compound to achieve the targeted efficacy whilst minimising unwanted side-effects arising from the activation of systemic

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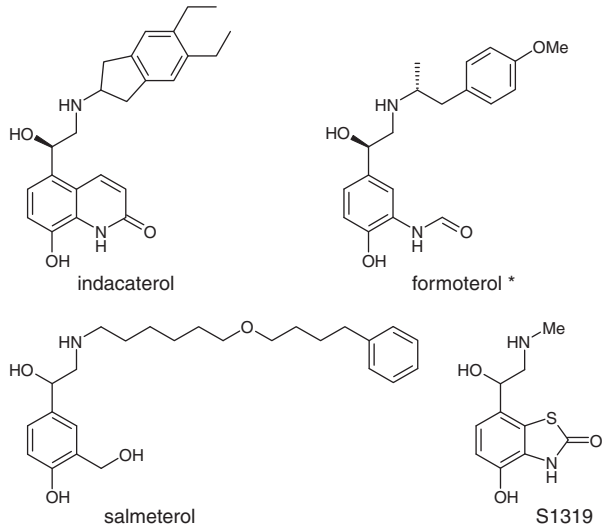


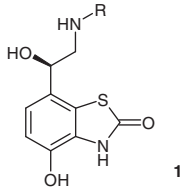
Figure 1. Structures of the β_2-adrenoceptor agonists indacaterol formoterol, salmeterol and S1319. *Racemic mixture of the *l*-diastereoisomer.

β_2-adrenoceptors, such as tremor, hyperhidrosis, hypokalemia and tachycardia.[8] Because the systemic side-effects associated with these agents have been shown to limit compliance for some groups of patients our goal was to identify compounds delivering the desired efficacy with a reduced level of systemic β_2-adrenoceptor activation. Finally, a viable inhalation product requires a formulation with good long term stability and a high degree of reproducibility with respect to the delivered dose. The technical challenge to satisfy both of these properties having been shown to be increased for low-dose highly potent compounds such as formoterol.[9] Hence, a compound with an intermediate potency was targeted to minimise the risk of technical delays in producing a formulation suitable for a final drug product. This requirement being of increased importance because the ultimate goal for any β_2-adrenoceptor agonist selected from the project would be to co-formulate as part of fixed dose combination products with other classes of inhaled drug molecules.

To satisfy the above targeted profile for a once-daily long-acting β_2-adrenoceptor agonist we adopted the following strategy to identify potential candidates. Based upon our previous experience we sought to achieve the targeted duration of effect through the regulation of the overall lipophilicity of the molecules selected for synthesis.[10] In vitro studies with a superfused guinea-pig tracheal-strip assay had shown that a log $D_{7.4}$ value greater than 2.4 consistently produced compounds with a longer duration of effect compared to the twice-daily agent formoterol.[11] Our observations being consistent with those made by others using basic amine containing pharmacophores and closely related assay systems.[12] A proposed rationale for these observations being that this level of lipophilicity in combination with the basic secondary amine, a key element of the β_2-adrenoceptor pharmacophore, gives rise to a strong interaction with phospholipids. As a consequence of the strong phospholipid interaction it is hypothesised that a proportion of the dose is extensively distributed into lung tissue following inhaled delivery. This highly retained lung fraction then provides a local reservoir of compound to maintain the bronchodilating effect as it slowly redistributes into other compartments.[13] However, we had also observed that high levels of lipohilicity, greater than a log $D_{7.4}$ value of 3.5, produced profiles that were delayed with respect to their time to maximal effect (T_{max}) using the same guinea-pig tracheal-strip assay.[10] Such delayed T_{max} profiles, as seen with salmeterol, have been assigned as being consistent with a slowed onset of action in man. Hence, to provide the optimal opportunity to satisfy our selected profile for duration and onset of action we initially chose to focus on compounds which would fall within a log $D_{7.4}$ window between 2.4 and 3.5.

To minimise the potential for systemic side-effects we looked to identify compounds with high systemic clearance. More specifically, we sought to target compounds for which the primary metabolic pathway led to the production of metabolites with greatly reduced β_2-adrenoceptor activity. The benefit of this approach was anticipated to be twofold: firstly, the β_2-adrenoceptor activity associated with the compound leaving the lung and entering the systemic circulation would be cleared as rapidly as possible. Secondly, the swallowed fraction of an inhaled dose can constitute the largest percentage of the total compound delivered.[14] With a high metabolic clearance rate the fraction of the swallowed material being absorbed from the gut would then be anticipated to be susceptible to an extensive level of first pass clearance. The consequence of this is to limit the potential for the gut-absorbed material to contribute to the overall systemic side-effects. To generate metabolites with greatly reduced β_2-adrenoceptor activity we targeted agonists bearing a phenolic residue with a high susceptibility to glucuronidation. The formation of phenolic glucuronides in the case of formoterol and indacaterol having previously been shown to result in inactive metabolites.[10a,15] Applying this hypothesis, from an analysis of our earlier studies we had observed a trend for a greater in vitro susceptibility to glucuronidation for β_2-adrenoceptor agonists with phenol-containing catechol mimetics derived from bicyclic moieties, such as the 8-hydroxyquinolinone of indacaterol. This was when compared to catechol mimetics derived from monocyclic groups, such as the *ortho*-*N*-formylated phenolic residue of formoterol. At this early stage of the project only a small number of direct comparisons with the same amino substitution patterns could be made between monocyclic and bicyclic catechol mimetics. However, it was decided that to provide the highest chance of achieving the targeted profile β_2-adrenoceptor agonist series with phenol-containing catechol mimetics derived from bicyclic moieties would be prioritised.

In light of the above observations; the disclosure of the marine natural product S1319 as a β_2-adrenoceptor agonist with equivalent activity to the clinically prescribed compound formoterol had attracted our interest, Figure 1.[16] This was not only as a consequence of the high level of activity associated with this naturally occurring compound, but also due to the presence of the 4-hydroxybenzothiazolone as a phenol-containing bicyclic catechol mimetic. Further support for selecting to work with a 4-hydroxybenzothiazolone based series came from the reported use of this structural element as a catechol mimetic in active series of dopaminergic agonists and dual dopaminergic/β_2-adrenoceptor agonists.[17] The strategy we chose to explore such a 4-hydroxybenzothiazolone based series of β_2-adrenoceptor agonists involved the elaboration of the *N*-methyl residue of the natural product S1319 to generate derivatives of the general structure **1**. Selection of the *N*-sustituents (R) being restricted to analogues that would fall within the targeted lipophilicity window.



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To explore this opportunity in the most efficient manner a library approach was selected with no structural bias in the selection of the N-substituents other than to satisfy the above log $D_{7.4}$ window (2.4–3.5). Such an approach was anticipated to

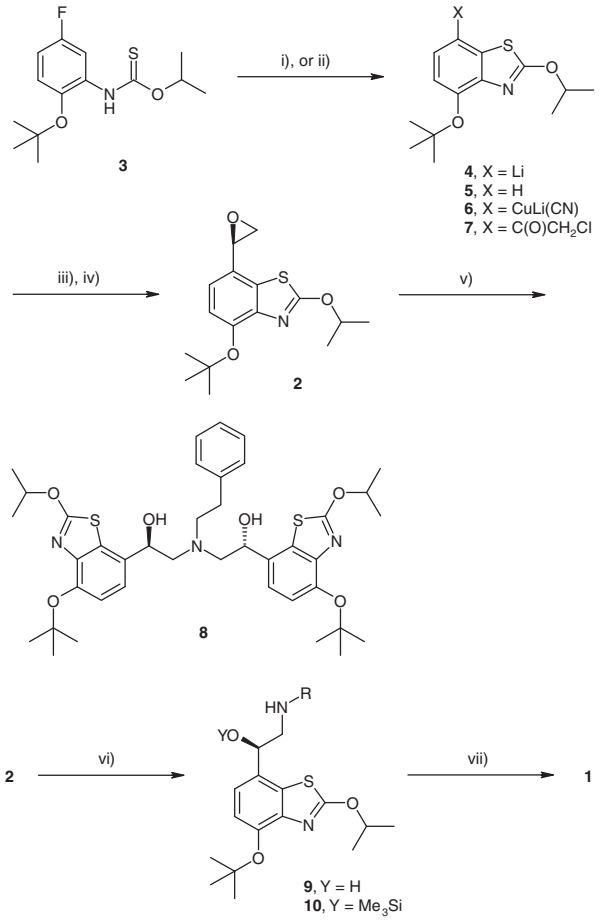
allow the opportunity to identify a candidate directly, or to generate as structurally broad a set of leads as possible for further optimisation that would have a high probability of satisfying our targeted onset and duration of action profile as a starting point. Following an initial screening for β_2-adrenoceptor activity and susceptibility towards glucuronidation it was then anticipated that it would be possible to rapidly prioritise the preferred leads for further progression based upon an understanding of what we believed to be some of the key parameters for satisfying the targeted profile.

To prepare the desired analogues of the general structure **1** the protected chiral epoxide **2** was targeted as the key intermediate to undergo a series of S_N2 addition reactions with a library of amines. The (*R*)-configuration at the benzylic alcohol of **1** was selected for initial screening to provide the most active stereoisomers based upon data from a number of β_2-adrenoceptor agonist series.[18] An efficient synthesis of the (*R*)-epoxide **2** we reasoned would be readily achieved from an extension of the route we had described earlier for the preparation of racemic S1319.[19]

Thus, as previously described deprotonation of the *meta*-fluorothiocarbamate **3** resulted in a benzyne-mediated cyclisation to generate the aryllithium **4**. However, following the attempted acylation of **4** with a range of two-carbon electrophiles, that would be anticipated to be readily converted to the targeted epoxide, it became apparent that **4** is a particularly hard anion. The major product from these reactions being derived from the protonation of **4** at the 7-position to give **5**. To overcome this problem, moderation of the reactivity of the lithiated species **4** was achieved through a transmetalation to produce the lower-order cyanocuprate intermediate **6** following the addition of 1 equiv of copper cyanide solubilised in tetrahydrofuran with lithium chloride.[20] The softer anion **6** could then be efficiently acylated with chloroacetylchloride to give the chloromethylketone **7**, as outlined in Scheme 1. Asymmetric reduction of **7** via the CBS protocol using (1*R*,2*S*)-aminoindanol as the catalyst gave the (*R*)-chlorohydrin in >95% ee.[21] The base catalysed closure of the isolated chlorohydrin then cleanly delivered the (*R*)-epoxide **2** in good overall yield for the two step sequence.

With the epoxide **2** in hand as the key common intermediate, the preferred reaction partners for varying the nature of the N-substituent within the library were primary amines based upon their ready availability from both commercial and internal sources. Our previous experience with the preparation of structurally related β_2-adrenoceptor agonists had shown that for certain combinations the addition of primary amines to epoxides could be an efficient process. However, for other examples the further reaction of the desired product with a second equivalent of the epoxide, leading to tertiary amine formation, had proven to be a problematic competing reaction. To avoid this unwanted double addition reaction the use of the corresponding N-benzylated secondary amine derivatives as the nucleophilic component had been shown to be an effective solution.[10] Initial exploratory reactions with the epoxide **2** and β-phenylethylamine showed tertiary amine formation to be particularly favoured in this case, with **8** being formed as essentially the sole product, even when a large excesses of amine were employed, as exemplified in Scheme 1.

Not wishing to be restricted in the choice of building blocks by the requirement for N-benzylated secondary amines as nucleophiles, and the associated additional requirement for a debenzylation step, an alternative strategy was sought to enable the direct reaction of epoxide **2** with primary amines. The in situ mono-silylation of primary amines has been reported as an effective way to regulate their reactivity in favour of the monoaddition product in a parallel synthesis setting.[22] Applying this protocol: primary amines were pretreated with 0.5 equiv of bistrimethylsilylacetamide for 30 min at room temperature prior to the addition of the epoxide **2**. Following the addition of the epoxide and heating for 18 h at



Scheme 1. Preparation of the 4-hydroxybenzothiazolone β_2-adrenoceptor agonists of the general structure **1**. Reagents and conditions: (i) 2.8 equiv *t*-BuLi, THF, −78 °C to −20 °C, then electrophile; (ii) 2.8 equiv *t*-BuLi, THF, −78 °C to −20 °C, then 1.2 equiv of CuCN, 1.2 equiv of LiCl, THF, 15 min −20 °C, then $ClCH_2C(O)Cl$, 1 h −20 °C to 0 °C (45–72%); (iii) 0.1 equiv (1*R*,2*S*)-(+)-*cis*-1-amino-2-indanol, 1.0 equiv 1 M borane in THF, THF, 15 min, room temperature; (iv) 2 equiv K_2CO_3, acetone, 60 °C, 18 h, (90–95%, two steps); (v) β-phenylethylamine, *n*BuOH, 100 °C, 18 h (50%); (vi) 1.2 equiv primary amine, 0.6 equiv *N*,*O*-bis-(trimethylsilyl)acetamide, DMF, 30 min, room temperature, then **2**, 80 °C, 18 h; (vii) HCO_2H, room temperature, 24 h.

80 °C the targeted aminoalcohol regioisomers were generated cleanly in these reactions and could be isolated either as the alcohol or the trimethylsilyl ether derivatives **9** and **10**, respectively, as shown in Scheme 1. The ratio of **9** and **10** being dependant on the nature of the N-substituent. Treatment of either **9** or **10** with formic acid overnight at room temperature then efficiently cleaved the two ether residues, and silyl ether when present, to generate the targeted products **1**. For parallel synthesis the overall efficiency could be improved by the direct reaction of the intermediate aminoalcohol derivatives **9** and **10** following evaporation. Similar overall yields were achieved when these crude intermediates were directly deprotected with formic acid in this manner.

With an efficient synthetic route developed a set of primary amines were identified by in silico calculation using the Available Chemicals Directory and corporate Novartis Chemical Archive to give analogues of the general structure **1** satisfying the lipophilicity based hypothesis described above.[23] This initial selection was then refined manually to: remove amines with incompatible reactivity; additionally, some amines with lower lipophilicity and molecular weight were added which were anticipated to be readily derivatised to satisfy the targeted lipophilicity criteria. This approach provided a set of 105 primary amines for construction of the

library. To prepare the library the epoxide **2** was reacted individually with each amine following mono N-silylation. Final purification, after removal of the formic acid, was then carried out by preparative reversed phase chromatography to give the targeted products **1** as triflouroacetate salts. Following this approach material of >85% purity, as determined by ^{1}H NMR and HPLC/MS, was isolated for >90% of the selected primary amines to provide the material for the initial screening.

The library of analogues corresponding to the general structure **1** were first screened to determine their affinities for the human β_2-adrenoceptor in a radioligand binding assay versus the β-adrenoceptor antagonist CGP12171.[24] Figure 2 shows the range of K_i values obtained for the whole library. Table 1 and Figure 3 show selected β_2- and β_1-adrenoceptor binding affinities and calculated log $D_{7.4}$ values for the representative library members **11**–**41**, corresponding to the general structure **1**. The examples being grouped according to the nature of their N-substituents to assist with highlighting some of the common themes encountered in the structure–activity relationships from the overall library.

Assessing the human β_2-adrenoceptor binding data in Figure 2 shows the 4-hydroxybenzothiazolone moiety to be a very effective catechol mimetic with a large proportion of the library members exhibiting high affinities for the human β_2-adrenoceptor. More than half of the analogues **1** (56%) exhibited a K_i value below 10 nM, of which one quarter had a K_i value lower than formoterol. These examples with increased β_2-adrenoceptor binding affinities relative to formoterol were considered too potent for further evaluation due to an increased potential for encountering difficulties in producing an inhaled formulation within the required technical specifications. Confidence in this extrapolation, which would deprioritise a quarter of the library members, was based upon our previous experience which had shown that K_i values generated in the human β_2-adrenoceptor binding assay had correlated well with cellular functional activity and also, when measured, with in vivo potency. For example, the benzyloxycyclopentyl analogue **26** was more potent when compared to formoterol in the above binding assay: the compounds exhibiting K_i values of 0.5 and 2.6 nM, respectively. Similarly in a cAMP assay with the human β_2-adrenoceptor expressed in A431 cells compound **26** exhibited greater potency compared to formoterol with pEC_{50} values of 10.5 and 8.1 and relative intrinsic efficacies of 140% and 100%, respectively.[25] The high intrinsic efficacy observed in activating the human β_2-adrenoceptor with the derivative **26** being typical of the majority of the library members corresponding to the general structure **1**. These data demonstrate the key 4-hydroxybenzothiazolone structural element of **1** to be a particularly effective catechol mimetic not only for binding to, but also for activating the β_2-adrenoceptor. Similarly very high levels of intrinsic efficacy have also recently been reported, for examples, from a series of long-acting β_2-adrenoceptor agonists based upon a 5-hydroxy-4*H*-benzo[1,4]oxazin-3-one bicyclic catechol mimetic.[4b]

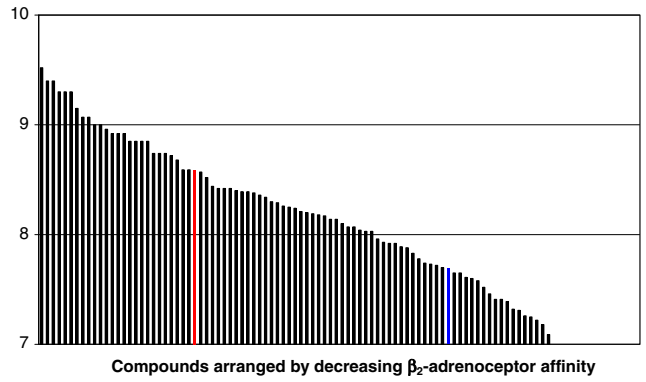


Figure 2. Human β_2-adrenoceptor pK_i values (*y*-axis) for formoterol (■), indacaterol (■) and the 4-hydroxybenzothiazolone library members **1** (■). Fifteen library members exhibited pK_i values <7.

Table 1
Human β_2- and β_1-adrenoceptor binding and calculated log $D_{7.4}$ values for formoterol, indacaterol, salmeterol, S1319 and the representative library members **11**–**41**

Compound	*c* log $D_{7.4}$[a]	β_2 K_i (nM)	β_1 K_i (nM)
Formoterol	1.3	2.6	319
Indacaterol	3.0	20.6	91.4
Salmeterol	3.1	3.2	801
S1319	−0.5	5.0	60.6
Alkyl			
11	2.1	24.7	169
12	2.5	1.0	1.5
13	2.5	25.1	39.3
14	2.6	1.9	9.2
15	3.9	26.5	35.4
Aryl-substituted alkyl			
16	1.6	1.8	3.8
17	1.7	19.8	902
18	2.0	1.2	2.0
19	2.3	0.4	1.8
20	2.4	9.4	112
21	2.4	30.2	58.2
22	2.5	1.8	15.0
23	2.9	2.7	1.4
24	2.9	48.9	33.7
Cycloalkyl			
25	2.1	11.8	78.8
26	2.4	0.5	0.9
27	2.4	6.1	107
28	3.0	0.6	17.8
29	3.0	0.8	6.9
30	3.0	7.2	40.4
31	3.0	362	1059
32	3.4	40.4	1511
33	3.4	3.8	450
34	3.6	4.0	123
35	4.1	151	662
Ether, thioether and amino-substituted alkyl			
36	0.7	6.3	187
37	1.6	4.4	17.8
38	2.3	0.3	8.4
39	2.5	>1000	>1000
40	2.9	3.0	1.7
41	3.5	18.3	31.3

[a] The *c* log $D_{7.4}$ values were generated from pK_a values calculated with a proprietary method and *c* log *P*.[26]

Assessing the selectivity of the series **1**: initially a counter screen was carried out with the closely related β_1-adrenoceptor using an equivalent binding assay format, as shown in Table 1.[24] For the majority of the examples this produced relatively small differences in K_i values between the β_1- and β_2-adrenoceptors. However, assessment of the functional activities at the β_1-adrenoceptor showed for the analogues **1**, that high affinity did not translate into a potent or efficacious agonist activity for the examples tested. This discrepancy is consistent with our previous observations with other β_2-adrenoceptor agonist series.[10] For example, compounds **12**, **17** and **30** exhibited K_i values of 1.5, 902 and 40.4 nM, respectively, in the human β_1-adrenoceptor binding assay. In contrast using a functional β_1-adrenoceptor assay, in the form of an electrically stimulated guinea-pig left-atria preparation, these analogues at a concentration of 1 μM only produced increases in contraction of 69%, 19% and 13%, respectively.[27] All three compounds **12**, **17** and **30** having been shown to be competent β_2-adrenoceptor agonists in the complimentary electrically stimulated guinea-pig tracheal-strip assay where they were all determined to be full agonists relative to formoterol exhibiting IC_{50} values of <1.0, 14.0

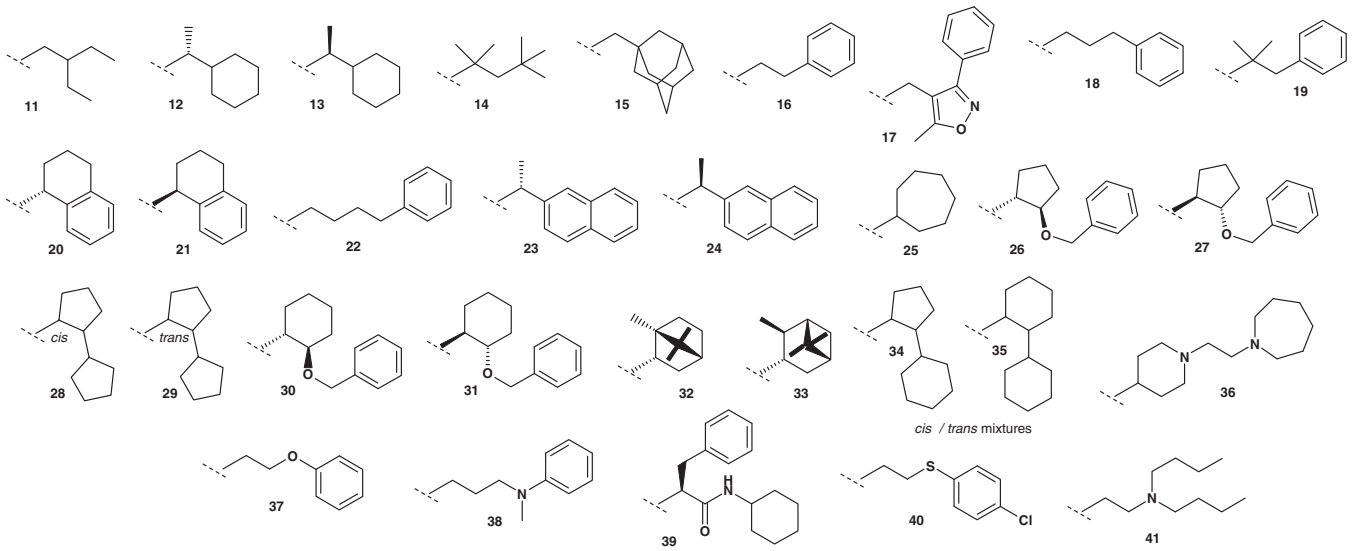


Figure 3. Structures of the N-substituents R for the representative library members **11**–**41**.

and 5.6 nM, respectively.[11] Thus using CGP12171 as a radio-labelled antagonist, competing primarily for the low affinity binding site, proved to be a good measure of agonist functional activity at the β_2-adrenoceptor, but a relatively poor measure of agonist activity at the β_1-adrenoceptor. Therefore, to characterise this 4-hydroxybenzothiazolone containing series a functional readout was found to be necessary for most effectively assessing the β_1-adrenoceptor off-target liabilities.

In terms of the impact of the structural changes within the N-substituent: Table 1 and Figure 3 show that a diverse range of substituents were well tolerated within the targeted lipophilicity window with many examples exhibiting an increased affinity for the β_2-adrenoceptor relative to the *N*-methyl residue of the racemic natural product S1319. For both the *N*-alkyl and *N*-aryl-substituted alkyl derivatives potency was only moderately reduced with the introduction of bulky substituents at the α-position to the secondary amine, for example, derivatives **11** and **15**. However, for the examples where α-substitution generated an asymmetric centre the (*R*)-configuration still retained a respectable level of potency, for example, comparing derivatives **12**, **20** and **23** with their epimers **13**, **21** and **24**. In contrast, as reported previously the introduction of two *a*-methyl substituents is well tolerated and results in a trend for an increased level of activity at the β_2-adrenoceptor, for example, **14** and **19**.[28] In addition to the well reported β-phenylethyl residue, example **16**, the introduction of a terminal aryl residue into alkyl substituents at the δ- or γ-positions also resulted in highly potent analogues, for examples, derivatives **18** and **22**. In the *N*-cycloalkyl series a ring size of cyclohexyl and larger produced analogues with a trend for relatively lower levels of activity, but as described previously, an increased level of activity was possible with the smaller cyclopentyl analogues, for example, comparing derivatives **25**, **30**, **31** and **35** with **26**–**29** and **34**.[29] In particular, the 2-substituted cyclopentyl derivatives **26**–**29** appeared to offer both *cis*- and *trans*-substituted analogues of very high potency with the possibility to moderate the activity through the stereochemistry at both the α- and β-positions within the cyclopentylamino ring. Interestingly, for the analogues bearing monoterpene derived N-substituents, **32** and **33**, relatively high binding selectivities for the β_2- over the β_1-adrenoceptor of 37- and 90-fold, respectively, were determined. For the heteroatom substituted *N*-alkyl series: both β-arylether and β-arylthioethers provided high affinity analogues, derivatives **37** and **40**. Several structurally varied amino-substituted analogues were also included in the library, derivatives **36**, **38** and **41** which delivered a range of potencies, including highly active examples. These amino-containing N-substituents provided the opportunity to further explore the effect of a second basic group on the amphiphilic interaction with phospholipid membranes.[30] Although the majority of the library members demonstrated K_i values below 100 nM some weakly active examples were encountered. For example, a number of N-substituents derived from the amide derivatives of α-amino acids exhibited no significant affinity for the β_2-adrenoceptor at concentrations up to 1 μM, as exemplified by derivative **39**.

At the onset of this work one hypothesis which had in part motivated our interest in investigating the 4-hydroxybenzothiazolone series **1** was the observation of the potential for an increased susceptibility for bicyclic catechol mimetics to undergo glucuronidation. To explore the validity of this hypothesis the most interesting examples from the library in terms of their β_2-adrenoceptor potency were assessed in a rat liver microsome assay to determine their rates of glucuronidation in the presence of the cofactor uridine 5′-diphospho-glucuronic acid (UDPGA).[31] This resulted in the screening of 31 examples, and these data are shown in Figure 4 with indacaterol and formoterol included as reference compounds. Analysis of these data indicates that the nature of the N-substituent has a significant impact on the rates of glucuronidation for the series **1**. However, there is a clear trend towards higher intrinsic clearance values for the majority of the screened examples, with 84% being more rapidly metabolised than formo-

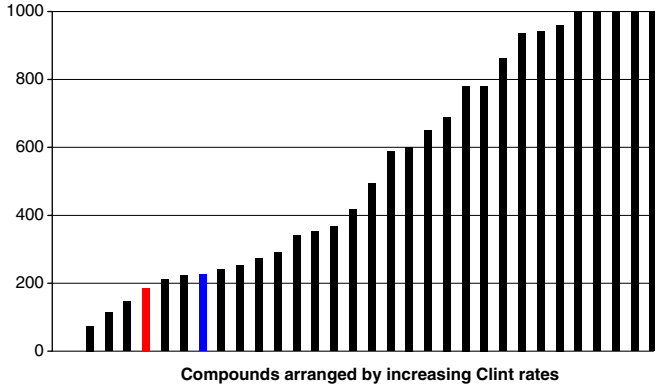


Figure 4. Glucuronidation rates (*y*-axis, μl/min/mg) for formoterol (■), indacaterol (■) and the 4-hydroxybenzothiazolone library member **1** (■).

terol (Clint 186 μl/min/mg) and 77% being more rapidly metabolised than indacaterol (Clint 226 μl/min/mg). In terms of the original hypothesis, the approach had satisfied the initial objective by providing a large proportion of the analogues with high susceptibilities for undergoing glucuronidation. However, when a diverse set of amino substituents are considered it is clear that changes to this portion of the molecule also make a significant contribution to modulating the rates of glucuronidation observed within the 4-hydroxybenzothiazolone series **1**. Analysis of the overall data identified groups of analogues of particular interest consistently exhibiting increased glucuronidation rates compared to formoterol and indacaterol, for example: β-phenylethylamines, as represented by the analogue **19** which exhibited a Clint of 291 μl/min/mg; the α-substituted cyclopentylamines, as represented by the analogue **29** which exhibited a Clint value 781 μl/min/mg; and the monoterpene analogues, as represented by **33** which exhibited a Clint value 589 μl/min/mg. Therefore, these series of N-substituents were identified as being of particular interest for further follow-up activities based upon the proposed hypothesis. In contrast, the analogues bearing additional basic groups in the amino substituent exhibited lower glucuronidation rates, for example, the tribasic analogue **36** proved to be resistant to metabolism in the in vitro rat microsome assay in the presence of the UDPGA cofactor.

In summary, as part of a project with the overall goal to identify once-daily inhaled bronchodilators with improved profiles we have explored a 4-hydroxybenzothiazolone series of β_2-adrenoceptor agonists **1** based upon the marine natural product S1319. An efficient synthetic route to the series **1**, as single stereoisomers, has been described which makes use of a benzyne-mediated cyclisation as the key transformation. This route supported a parallel synthetic approach for the introduction of a diverse range of N-substituents, within a defined lipophilicity window, of which a high proportion was shown to be highly potent and efficacious agonists of the human β_2-adrenoceptor. An in vitro glucuronidation screen further identified analogues with the potential for improved side-effect profiles based upon the proposed hypothesis for minimising the systemic impact of the agonists following inhaled delivery. This approach identified the β-phenethyl, α-substituted cyclopentyl and monoterpene amino-substituted analogues of **1** as particularly interesting for more extensive evaluation having satisfied the hypothesised potency and clearance profiles. Further studies exploring the potential of these lead 4-hydroxybenzothiazolone series as once-daily bronchodilators will be the subject of future publications.

References and notes

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31. The susceptibility of the test compounds to undergo glucuronidation was assessed in vitro using pooled rat hepatic microsome preparations; microsomes (protein concentration 1 mg/ml) were incubated with alamethicin and the test compounds (1 μM) at 37 °C for 15 min prior to the addition of the co-factor UDPGA. Aliquots were removed at 0, 5, 15 and 30 min. Reactions were stopped by addition of acetonitrile and samples were subsequently analysed by LC–MS/MS after protein precipitation. The data were analysed as percentage disappearance of parent relative to the zero time sample and are expressed as intrinsic clearance rates.