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The discovery of indole-derived long acting β_2-adrenoceptor agonists for the treatment of asthma and COPD

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Abstract—The design and profile of a series of indole containing long acting β_2-adrenoceptor agonists is described. Evaluation of these analogues using an in vitro guinea pig trachea tissue model demonstrates that analogues within this series have salmeterol-like duration of action with potential for long duration of action in humans.

Long acting β_2-adrenoceptor agonists are a highly precedented drug class used for the treatment of asthma and chronic obstructive pulmonary disease (COPD) and as such there is high confidence in the need for these agents.[1,2] There are currently two marketed long acting β_2-adrenoceptor agonists, salmeterol[3] and formoterol,[4,5] neither of which provide a once daily dosing regimen, Figure 1. It is believed that there is an opportunity for a once a day agent that in the future could become the therapy of choice over salmeterol or formoterol.

There has been considerable interest in the discovery of a once daily β_2-adrenoceptor agonist with a recent flurry of presentations, patent applications, and licensing agreements from a number of institutions.[6] There are numerous publications around how structurally differing long acting β_2-adrenoceptor agonists achieve their duration of action, including our recent article in BMCL.[7] The majority of papers focus on the ability of salmeterol to bind to an exo-site on the β_2-adrenoceptor near to the agonist binding site, termed the exo-site hypothesis.[8] However, formoterol would be unable to access this exo-site and an alternative diffusion microkinetic hypothesis suggests that the lipophilic, basic nature of these compounds allows them to partition effectively into the lipid bilayers of smooth muscle following inhalation.[4,9] This partitioning allows an effective concentration of agonist to be present over time, delivering sustained duration of action. Regardless of the exact molecular mechanism of sustaining duration of action, an elegant QSAR analysis of data within an inhaled long acting dual D_2-receptor/β_2-adrenoceptor agonists programme shows that lipophilicity and pK_a are key parameters within this project.[10]

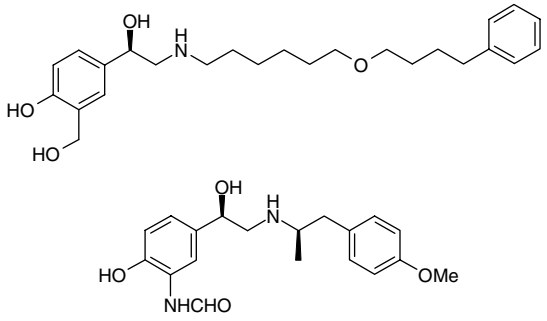

Figure 1. Structures of salmeterol (top) and formoterol (bottom).

We describe the design and profile of a novel series of indole containing long acting β_2-adrenoceptor agonists.[11] This template was initially discovered following a high throughput screen and subsequent hit follow-up that identified indole **1** as a potent β_2-adrenoceptor agonist, Table 1.

Keywords: Indole; Beta-2 adrenoceptor agonist; Long acting; Asthma; COPD.
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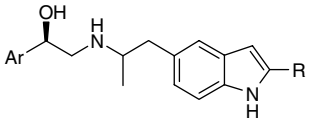
Table 1. Potency for initial lead indole analogue (Ar, R are not disclosed)



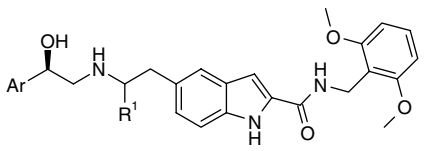
Compound	β_2 EC_{50}[a] (nM)
1	0.74
Salmeterol	0.070

[a] Potency and efficacy at human recombinant β_2-adrenoceptors expressed in CHO cells assessed as elevations in cyclic AMP. In this assay, all compounds appeared to be full agonists.

With these data in hand, we sought to further optimise the β_2-adrenoceptor potency of this indole template. Initial analogues synthesised as a mixture of diastereoisomers proved to be equi-potent with initial lead **1**. The potent di-methoxybenzyl amide analogue **2** was separated to provide single diastereoisomers **3** and **4** that were profiled to assess the effect of α-methyl stereochemistry on their potency at the β_2-adrenoceptor, Table 2. Data clearly demonstrated that the (R)-configuration of the α-methyl stereocentre (**3**) conferred approximately 100-fold greater activity than the (S)-configuration (**4**). Interestingly, it was also noted that the β_1-adrenoceptor potency was similar for both **3** and **4**, suggesting that the β_1-adrenoceptor was less sensitive to this change in stereochemistry. These data also established that **3** was clearly the most selective compound, and it became preferable that subsequent analogues were synthesised as (R,R)-stereoisomers. A similar preference has been observed for the single stereoisomers of formoterol.[12]

Whilst the undisclosed head group of **3** conferred good potency in this indole series, we were also keen to explore the profile of our indole linker with the precedented saligenin head group found in salmeterol. Therefore, we retained these key structural motifs whilst seeking to explore the effect of (R)-alkylation adjacent to the basic nitrogen, indole N-alkylation and amide substitution at the indole 2-position, on β_2-adrenoceptor potency. Initially, saligenin analogues **5** to **9** were synthesised to assess the effect of different α-alkyl and indole N-substitution on β_2-adrenoceptor potency and selectivity in this single stereoisomer saligenin template. The amide expression selected for these examples was also 2,6-dimethoxybenzyl, Table 3.[6]

Table 2. Potency and selectivity for initial analogues (Ar is not disclosed, but is the same for **2**, **3**, and **4**)



Compound	β_2 EC_{50}[a] (nM)	β_1/β_2[b]
2 R^1 = Me	0.628	—
3 R^1 = (*R*)-Me	0.060	2960
4 R^1 = (*S*)-Me	5.64	87
Salmeterol	0.070	7885

[a] Potency and efficacy at human recombinant β_2-adrenoceptors expressed in CHO cells assessed as elevations in cyclic AMP. In this assay, all compounds appeared to be full agonists.

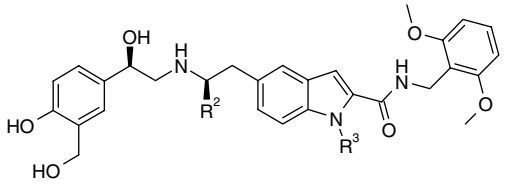
[b] Ratio of EC_{50}s generated at human recombinant β_1- and β_2-adrenoceptors expressed in CHO cells assessed as elevations in cyclic AMP.

Excellent potency and selectivity was retained through incorporation of the saligenin motif **5**, confirming the need to fully explore this novel template. The α-ethyl analogue **6** retained good potency and selectivity, being 5-fold weaker than its α-methyl counterpart **5**, but did not represent a significant advantage given the additional synthetic complexity involved. The effect of *N*-indole substitution also led to respectable, lower potency (~12-fold) in the methyl analogue **7** and benzyl analogue **9**. The *N*-ethyl analogue **8** displayed the largest drop in potency (~44-fold). From these data it was thus clear that for optimum potency the saligenin template required R,R-stereochemistry, α-methyl substitution and an N–H indole bearing amide substitution at the 2-position. Once we had established this we investigated the structure–activity relationships of the amide portion through amide coupling reactions with a variety of amines, Table 4.

Compounds **10** to **21** demonstrated excellent β_2-adrenoceptor potency that was generally salmeterol-like or better (e.g. **11**, **19**) and tolerated a wide range of benzylic substitution, heterocycles **16**, **17** and cyclo-alkyl groups **21**. Selectivity over the β_1-adrenoceptor was generally good to excellent with only the more polar benzylic analogues **14**, **15** and phenethyl compound **20** showing reduced selectivity, as a consequence of their lower β_2-adrenoceptor potency.

The effect of **5**, **11**, **18**, **19** and salmeterol on airway smooth muscle was investigated using tracheal strips

Table 3. Potency and selectivity for initial saligenin analogues

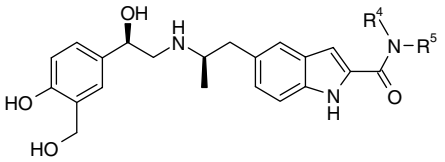


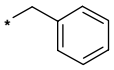
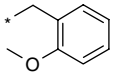
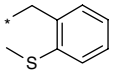
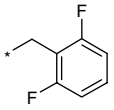
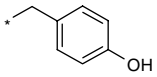
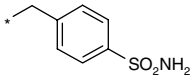
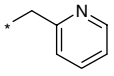
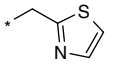
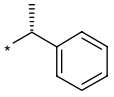
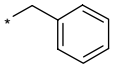
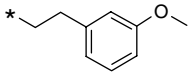
Compound			β_2 EC_{50}[a] (nM)	β_1/β_2[b]
R^2	R^3			
Me	H	**5**	0.058	4035
Et	H	**6**	0.258	5736
Me	Me	**7**	0.706	1084
Me	Et	**8**	2.550	254
Me	Bn	**9**	0.687	237
Salmeterol			0.070	7885

[a] Potency and efficacy at human recombinant β_2-adrenoceptors expressed in CHO cells assessed as elevations in cyclic AMP. In this assay, all compounds appeared to be full agonists.

[b] Ratio of EC_{50}s generated at human recombinant β_1- and β_2-adrenoceptors expressed in CHO cells assessed as elevations in cyclic AMP.

Table 4. Potency, selectivity, log D and in vitro guinea pig trachea tissue data for saligenin analogues



Compound			β_2 EC_{50}[a] (nM)	β_1/β_2[b]	log D	Guinea pig trachea potency[c]	Guinea pig trachea duration of action[d] (h)
R^4	R^5						
H		**10**	0.049	480	—	—	—
H		**11**	0.020	1235	2.2	0.21	4.6 (n = 2)
H		**12**	0.038	332	—	—	—
H		**13**	0.053	296	—	—	—
H		**14**	0.164	75	—	—	—
H		**15**	0.122	42	—	—	—
H		**16**	0.038	1526	—	—	—
H		**17**	0.031	1336	0.8	—	—
H		**18**	0.058	585	2.2	3.81	5.16 (n = 2)
Me		**19**	0.022	882	2.1	1.00	6.97 (n = 2)
H		**20**	0.254	35	2.0	—	—
H		**21**	0.051	410	1.9	—	—
		5	0.058	4035	2.2	0.43	7.39 (n = 5)
		Salmeterol	0.070	7885	2.1	1.00	6.9 (n = 5)

[a] Potency and efficacy at human recombinant β_2-adrenoceptors expressed in CHO cells assessed as elevations in cyclic AMP. In this assay, all compounds appeared to be full agonists.

[b] Ratio of EC_{50}s generated at human recombinant β_1- and β_2-adrenoceptors expressed in CHO cells assessed as elevations in cyclic AMP.

[c] Potencies in the in vitro guinea pig trachea model are quoted relative to salmeterol so that a compound that is twice as potent will be quoted as 0.5.

[d] Duration of action is measured as the time taken for the muscle tone at an E_{max} concentration of the compound to recover by 50% of the inhibition induced, where the E_{max} is the maximum inhibition achievable by that compound.

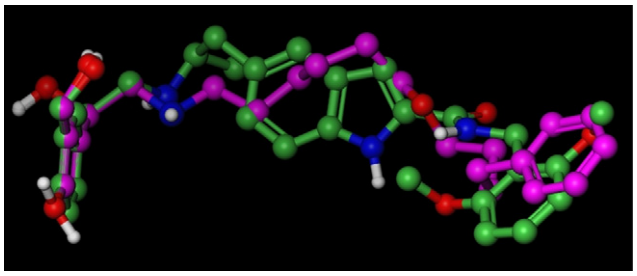


Figure 2. Structural overlay of salmeterol (purple) with **5**.

taken from the guinea pig. The pharmacology of β_2 agonists in this model correlates well with clinical data and gives a measurement of potency, efficacy and duration of action.[8] Isolated tracheal strips were contracted via electrical field stimulation and subsequent release of endogenous acetylcholine. Test agents were then assessed for their ability to oppose this contraction through functional antagonism. Duration of action was defined as the time taken for the muscle tone at an E_{max} concentration of the compound to recover by 50% of the inhibition induced, where the E_{max} is the maximum inhibition achievable by that compound. In this study all the compounds tested were essentially equipotent with salmeterol and also equivalent in the magnitude of their E_{max}. The durations of action of the compounds tested were generally salmeterol-like, including *N*-methyl benzyl amide **19**and di-methoxybenzyl amide **5** that also appeared to have the best balance of potency and duration of action.[6]

Given these data and their similarity to salmeterol, it is intriguing to postulate what factors may be responsible for the duration of action of these indole derivatives. The compounds tested are iso-lipophilic with salmeterol, so it is possible that their lipophilicity drives their duration of action as proposed for both formoterol and salmeterol by the diffusion microkinetic hypothesis.[4,9] The receptor exo-site hypothesis has also been proposed to rationalise the long duration of action of salmeterol.[8,13,14] High affinity binding of the long side chain deep into a hydrophobic core domain of the receptor effectively anchors the molecule and allows the saligenin head group to continuously activate the β_2-adrenoceptor. Although there is significantly less conformational flexibility in the indole series compared to salmeterol, it is clear from the structural overlay of salmeterol and **5** that these indole analogues retain the potential to access the receptor exo-site to drive duration of action, Figure 2.

With encouraging data in hand, **5** was profiled through a variety of in vitro pharmacokinetic assays. We were delighted to observe rapid metabolism in a human microsomal assay ($t^{1/2} = 4$ min) and only moderate cell permeability with high transporter mediated efflux ($P_{app}A - B < 1 \times 10^{-6}$ cm/s, $B - A = 14 \times 10^{-6}$ cm/s) as measured by apical to basal flux rate through a monolayer of CaCo-2 cells. These in vitro data suggested that **5** would have low oral bioavailability due to poor absorption through the gut wall and high first pass metabolism,[15,16] and warranted further progression of **5** to rat in vivo pharmacokinetic studies. Following i.v. administration the volume of distribution was high (18 L/kg), however, with total clearance greater than liver blood flow (130 ml/min/kg) the measured half life was 1.6 h. Plasma protein binding in rat of 90% also gave very high unbound clearance (1200 ml/min/kg). Following p.o. administration in rat, the oral bioavailability was found to be <5%, which was consistent with predictions from the in vitro data. This pharmacokinetic profile suggests that relative to salmeterol, compound **5** should demonstrate reduced systemically mediated adverse events from the swallowed fraction of the dose following inhalation in humans.[17]

In summary, we have described the discovery of a novel indole containing series of potent, long acting β_2-adrenoceptor agonists with equivalent potency and duration of action to salmeterol in an in vitro guinea pig trachea assay. Data obtained from in vitro and in vivo pharmacokinetic studies have also demonstrated that compounds from this series have limited permeability, high clearance and low oral bioavailability. This profile suggests that **5** has potential for long duration of action in humans with reduced systemically mediated adverse events relative to salmeterol.

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