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A multivalent approach to the discovery of long-acting β_2-adrenoceptor agonists for the treatment of asthma and COPD

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ABSTRACT

A multivalent approach was applied to the design of long-acting inhaled β_2-adrenoceptor agonists. A series of dimeric arylethanolamines based on the short acting β_2-adrenoceptor agonist albuterol were prepared, varying the nature and length of the linker between the basic nitrogens. None of the C_2-symmetric dimers demonstrated increased potency, however dimer **5j**, derived from 4-phenethylamine, was found to have increased binding potency in vitro relative to the parent monomer. Optimization of this structure led to the identification of **22** (milveterol) which demonstrates high potency in vitro and long duration of action in a guinea pig model of bronchoprotection.

Inhaled β_2-adrenoceptor agonists are highly effective bronchodilators with significant utility in the treatment of both asthma and chronic obstructive pulmonary disease (COPD).[1] The introduction 40 years ago of short-acting agents such as albuterol has led to the common use of this therapy for as-needed relief of acute bronchoconstriction. The more recently discovered long-acting β_2-adrenoceptor agonists (LABAs) salmeterol (**1**) and formoterol (**2**) (Fig. 1) have found broad utility when administered twice-daily for the management of more severe disease, often in combination with inhaled corticosteroids (commonly in a single inhaler device for both asthma and COPD)[1,2] or long-acting muscarinic antagonists (for COPD).[2,3] Longer lasting agents suitable for once-daily dosing have been sought to improve disease control and patient compliance[4] and a large number of once-daily LABAs have been recently reported.[5–18]

The physiological basis for the observed duration of action of LABAs is somewhat controversial and remains an active area of research.[19,20] In vitro studies have demonstrated that salmeterol and formoterol behave differently despite their similar duration of action in man. The long duration of formoterol is explained by the 'diffusion microkinetic model' which asserts that the cellular membrane acts as a reservoir for the lipophilic drug molecule, maintaining high local concentrations of drug long after concentrations decline in the surrounding medium. This hypothesis does not fully explain the in vitro properties of salmeterol, and interaction with an 'exosite'[21,22] has been proposed for this drug. The key structural elements of the lipophilic 'tail' of salmeterol responsible for potency and duration of action have been explored,[23] and Alikhani et al. have extended this exploration to derivatives of formoterol and identified potent and long-acting compounds containing phenylalkyl 'tails'.[24]

The dimeric β_2-agonist hexoprenaline (Fig. 2) has demonstrated efficacy as a bronchodilator in humans, but has limited duration of action.[25] Like hexoprenaline, salmeterol displays two aryl groups joined by a linear linker, although one of these is an unsubstituted phenyl group. We became interested in the possibility that a second terminal β_2-agonist head group might make an energetically favorable interaction with the exosite or other recognition site within the β_2-receptor and afford compounds with long intrinsic duration of action. Based on this hypothesis, we took a multivalent approach[26,27] to the identification of novel β_2-agonists. We anticipated that by optimization of the primary binding group (β_2-agonist head group) as well as 'linker' length and composition we could generate highly potent compounds. This approach has been used successfully in the identification of agonists for the 5-HT_4 receptor[28] (a member of the same G-protein coupled receptor family as the β_2-adrenoceptor).

The catechol 'head group' found in isoprenaline and hexoprenaline is known to be rapidly metabolized and has not been successfully employed in the design of long-acting β_2-agonists. We therefore focused initially on the saligenin head group (found in salmeterol) as a primary binding group. Based on the known

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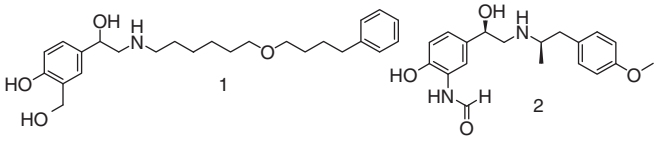


Figure 1. Structures of the b.i.d. LABAs salmeterol (**1**), and formoterol (**2**). Formoterol is a racemic mixture of the (*R*,*R*) (shown above) and (*S*,*S*) isomers.

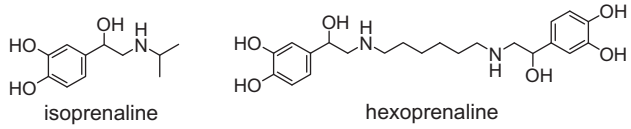


Figure 2. Structures of isoprenaline hexoprenaline.

structure–activity relationships of β_2-agonists, we selected the basic nitrogen as the 'point of attachment' for dimer formation (as in hexaprenaline), maintaining the secondary amine (a key element of the β_2-agonist pharmacophore). We explored dimeric structures displaying two β_2-agonist 'head groups' tethered with a variety of diamine linkers (Scheme 1).

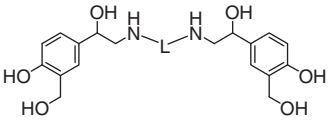
Compounds **5a–k** were derived from the corresponding diamines **4a–k** by condensation with arylglyoxal **3** followed by reduction of the intermediate α-iminoketones with borane-dimethylsulfide (Scheme 1).

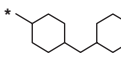
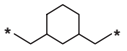
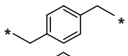
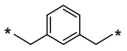
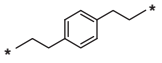
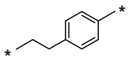
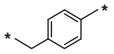
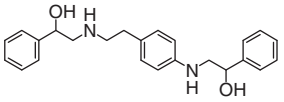
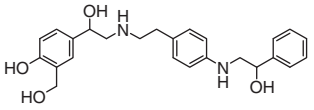
Linker length was varied from 3 to 9 atoms between basic nitrogen atoms, corresponding to a range of 9–15 atoms between the terminal aryl groups (compare to 14 atoms found between the aryl groups of salmeterol). Many linkers contained branching (alkyl or aryl) β- or γ- to the basic nitrogens. This substitution is known to confer selectivity for the β_2 receptor over β_1. As shown in Table 1, all of the C_2-symmetrical dimers tested in a radioligand displacement assay afforded lower binding potency for the β_2-adrenoceptor than albuterol. Intriguingly, the asymmetrically substituted **5k** matched the binding potency of albuterol and **5j** showed approximately 10-fold higher potency at the receptor. To investigate this scaffold further, we prepared derivatives **7** and **8** in which the hydroxy- and hydroxymethyl- substituents were removed from one or both of the 'head-groups' (Scheme 2).

Compound **8** afforded a further enhancement in potency. Despite approximately 10-fold lower binding potency than salmeterol for the receptor, compound **8** was found to be equipotent with salmeterol in a functional assay for β_2-adrenoceptor agonist activity (Table 2). For further optimization of this scaffold we adopted this functional agonist assay in cells heterologously expressing the human recombinant β_2-adrenoceptor as the primary screen.

We explored a number of modifications to the linker portion of compound **8** (Schemes 3–6). Substitution of the aniline nitrogen for oxygen (**10**) did not impact potency nor did the addition of *gem* -dimethyl substitution adjacent to the secondary amine (**11**). Methylation of the aniline (**12**) resulted in decreased potency and tying the phenethyl linkage into an indane ring (**13**) (comparable

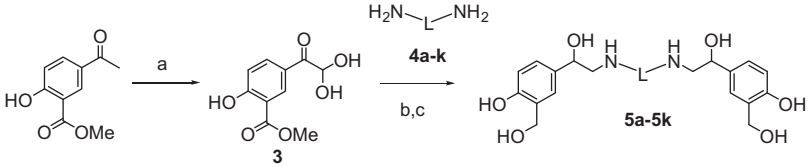
Table 1
β_2-Adrenoceptor binding potency of dimeric ligands[29]



Compound	L	β_2 pIC_{50} ± SD (*n*)
1 (salmeterol)	—	8.3 ± 0.2 (39)
Albuterol	—	5.7 ± 0.2 (12)
5a	*—$(CH_2)_4$—*	5.0 (1)
5b	*—$(CH_2)_6$—*	5.1 (1)
5c		3.6 (1)
5d		4.7 (1)
5e		5.3 (1)
5f		5.0 (1)
5g		4.3 (1)
5h		4.7 (1)
5i		4.0 (1)
5j		6.6 ± 0.3 (12)
5k		5.7 (1)
	Structure	
7		6.6 ± 0.6 (2)
8		7.4 ± 0.4 (34)

to the structure of indacaterol) decreased potency by more than 10-fold relative to **8**. A survey of aryl group substitutions (**15a–j**, Table 3, prepared as described Scheme 7) failed to identify any compounds with improved potency over the unsubstituted parent compound **8**, and most substitutions resulted in substantially decreased potency.

To further assess compound **8** as a lead, we focused on the synthesis of single diastereomers. Based on the well-established stereochemical preference of the β_2-adrenoceptor, we focused exclusively on compounds possessing the (*R*)-configuration at the α-position of the β_2-agonist head group. Compounds **17**-(*R*) and **17**-(*S*) were prepared by alkylation of bis-benzyl protected phenethylamine with (*R*)- and (*S*)-styrene oxide, respectively, under strongly basic conditions (Scheme 8). Reaction of **17**-(*R*) and **17**-(*S*) with epoxide



Scheme 1. Reagents and conditions: (a) 48% HBr/DMSO, 16 h, 59%; (b) diamines **4a–4k**, THF or CH_2Cl_2, rt, 6 h then concentrate under vacuum; (c) $BH_3{\cdot}Me_2S$, THF, CH_2Cl_2, 0–50 °C followed by MeOH.