Bioorganic & Medicinal Chemistry Letters 21 (2011) 4027–4031

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Design driven HtL: The discovery and synthesis of new high efficacy $\beta_2$-agonists

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ARTICLE INFO

*Article history:*
Received 30 March 2011
Revised 27 April 2011
Accepted 28 April 2011
Available online 20 May 2011

*Keywords:*
LABA
$\beta_2$-Agonists
Adrenoreceptor agonist
Asthma
COPD
Inhaled

ABSTRACT

The design and synthesis of a new series of high efficacy $\beta_2$-agonists devoid of the key benzylic alcohol present in previously described highly efficacious $\beta_2$-agonists is reported. A hypothesis for the unprecedented level of efficacy is proposed based on considerations of $\beta_2$-adrenoceptor crystal structure, other biophysical data and modeling studies.



The $\beta_2$-adrenoceptor ($\beta_2$AR) is a member of the class A, G-protein coupled receptor (GPCR) family and is widely distributed in the respiratory tract and particularly in airway smooth muscle. Agonists of this receptor are effective bronchodilators[1] and their use has been pivotal in the treatment of asthma[2] and chronic obstructive pulmonary disease COPD.[3] The 'first generation' $\beta_2$-agonists (e.g., salbutamol ($pEC_{50}$ 6.7, intrinsic activity (IA–relative to formoterol IA = 1) 0.8), terbutaline ($pEC_{50}$ 5.5, IA 0.7) and isoprenaline ($pEC_{50}$ 7.4, IA 1.0) were classed as short duration agonists requiring multiple daily dosing. The subsequent 'second generation' compounds (e.g., salmeterol or formoterol) exhibited longer duration of action amenable to twice-daily dosing. Formoterol is characterized as a high efficacy agonist ($pEC_{50}$ 9.3, IA 1.0) with a rapid onset of action,[4] whereas salmeterol is reported as a low efficacy $\beta_2$-agonist ($pEC_{50}$ 9.1, IA 0.6) with a slower onset of action.[5] More recently, 'third generation' long acting $\beta_2$-agonists[6] designed to have once a day duration of action have been described and a number (e.g., olodaterol ($pEC_{50}$ 9.9, IA 0.9))[7] are in late stage clinical development.[8] Notably, indacaterol ($pEC_{50}$ 7.9, IA 0.8)[9] has recently been approved for treatment of COPD in Europe (Fig. 1).

A screening of betamimetic compounds from AstraZeneca's previous sibenadet (Viozan™-a dual $DA_2/\beta_2$-agonist) program[10] revealed an interesting set of lead compounds amenable to further study (Table 1).

Interestingly, **1** and **2** were shown to be low-efficacy $\beta_2$-agonists, whereas **3** was shown to be a high-efficacy $\beta_2$-agonist, highlighting the need of the benzylic hydroxyl function in promoting $\beta_2$AR agonism. A medicinal chemistry program was established to search for high efficacy agonists in the class of both des-hydroxyl and hydroxy $\beta_2$-agonists.[11] Our initial SAR demonstrated that it is advantageous to have the requirement for both the phenol and secondary phenethylamine to achieve high-efficacy $\beta_2$-agonists (Table 2).

Compound **7** was shown to be a weak but high-efficacy (IA 1.4) $\beta_2$-agonist and it was postulated that substitution of the amide nitrogen may well lead to enhancement in both the potency and efficacy of the series. Indeed, exploration through the substitution of the amide group rapidly highlighted a new series of highly efficacious $\beta_2$-agonists similar in potency and intrinsic activity to the corresponding C-1 hydroxy series (Table 3).

Increasing the size of the alkyl group on the amide (**1, 2, 8, 9,** 10, and **11**) showed an increase in both potency and efficacy. Comparing the activities of the hydroxyl and des-hydroxy series showed comparable levels of potency and efficacy (**11,** 12).

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doi:10.1016/j.bmcl.2011.04.135

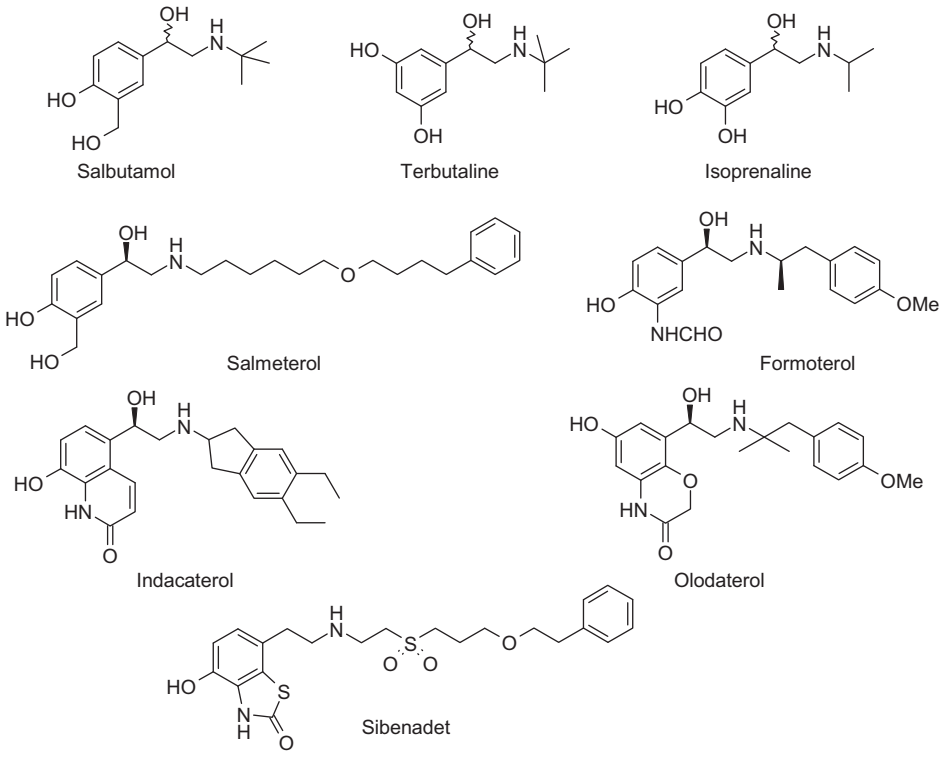
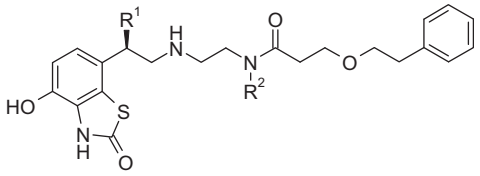



**Figure 1.** Examples of first, second and third generation $\beta_2$-agonists.

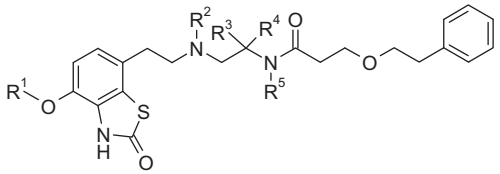
**Table 1**
Potency and intrinsic activity at the human $\beta_2$AR



| Entry | $R^1$ | $R^2$ | $\beta_2$ potency[#] ($pEC_{50}$) | Intrinsic activity (IA)[*] |
|---|---|---|---|---|
| 1 | H | H | 8.8 | 0.3 |
| 2 | H | Me | 9.3 | 0.7 |
| 3 | OH | Me | 9.7 | 1.0 |

[#] $\beta_2$ Receptor agonism was performed in H292 cells (bronchial epithelial cell line) expressing the human $\beta_2$ adrenergic receptor. Functional activity was determined by measuring accumulation of intracellular cAMP using AlphaScreen™. The compounds were incubated for 1 h at 22 °C and the data expressed as intrinsic activity relative to formoterol. $pEC_{50}$ is the negative logarithm of the molar drug concentration that produces a cAMP response equal to 50% of its maximal response.
[*] Intrinsic activity measured relative to formoterol (IA = 1).

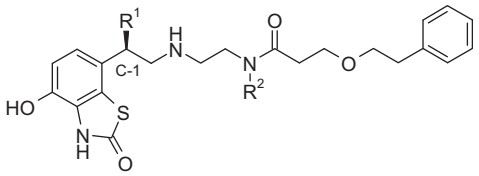
**Table 2**
Potency and intrinsic activity at the human $\beta_2$AR



| Entry | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | $\beta_2$ potency ($pEC_{50}$) | Intrinsic activity (IA) |
|---|---|---|---|---|---|---|---|
| 4 | Me | H | H | H | Me | n/a[#] | |
| 5 | H | Me | H | H | Me | n/a[#] | |
| 6 | H | H | Me | Me | H | 8.1 | 0.2 |
| 7 | H | Me | H | H | nBu | 6.9 | 1.4 |

[#] n/a reflects a compounds with a $\beta_2$ potency ($pEC_{50}$) of <6.0.

**Table 3**
Potency and intrinsic activity at the human $\beta_2$AR




| Entry | $R^1$ | $R^2$ | Function ($pEC_{50}$) | Intrinsic activity (IA) |
|---|---|---|---|---|
| 8 | H | Et | 9.0 | 0.7 |
| 9 | H | Pr | 8.1 | 0.9 |
| 10 | H | *i*Pr | 8.1 | 0.6 |
| 11 | H | *n*Bu | 8.8 | 0.9 |
| 12 | OH | *n*Bu | 9.1 | 1.0 |

Receptor activation is believed to involve a rearrangement of the helical bundle[12] causing relatively large conformational shifts remote to the ligand binding site. Closer to the binding site is a highly conserved tryptophan residue 286, suggesting it has a functional role,[13] and its reorientation from the indole plane being parallel with the bilayer normal to perpendicular has been implicated in receptor activation.[14] Mutagenesis studies have shown an aspartic acid residue 113, which is conserved in the cationic neurotransmitter family of receptors, is involved in binding[15] as well as two serine residues,[16] which are conserved in receptors binding catechols, with the para phenolic hydroxyl forming an interaction with serine 207 and the *meta* phenolic hydroxyl binding interacting with serine 204. This characterization of the binding site allowed modeling agonist ligands into the inactive conformation of the $\beta_2$ adrenergic receptor structure.[17] We propose a hypothesis that hydrogen bonds between the protein and the catechol hydroxyls, the β-hydroxl and the basic amine of agonists hold the ligand in place with its aromatic group in an orientation such that it induces a conformational shift in the tryptophan that leads to receptor activation. Conversely antagonists or inverse agonists (which generally have a longer chain length between the basic amine and the aromatic group) stabilize the inactive conformation of the tryptophan. Modeling studies suggested the movement of the tryptophan opens up a pocket (christened the 'agonist pocket') that accommodates the amide substituents $R^2$ from Tables 1 and 3, and broadly explains the structure activity relationship since this pocket is generally lipophilic (see Fig. 2). Thus, it can be postulated that the high efficacy agonism in the des-hydroxyl compounds can be explained by the group $R^2$ occupying the agonist pocket requiring an induced

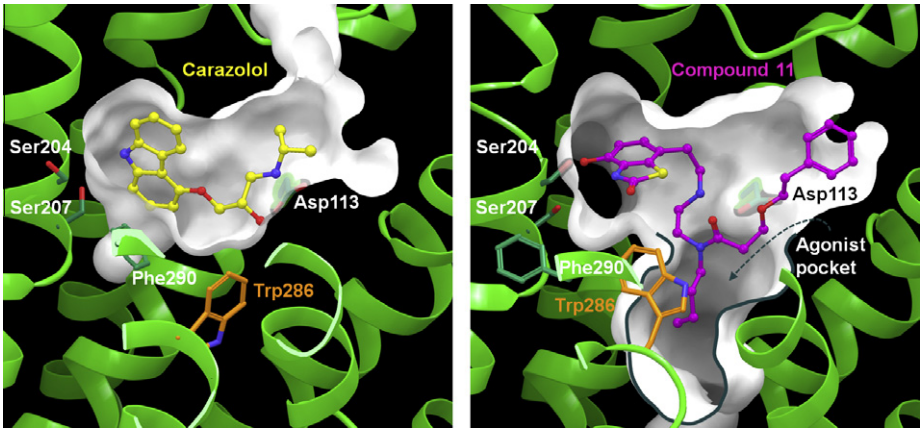



**Figure 2.** Shows on the left the X-ray structure of $\beta_2$AR bound with a partial agonist carazolol (yellow) and on the right the model of the $\beta_2$AR complexed with Compound **11** (magenta). The surface of the active sites are displayed in gray. The key aspartic acid and serine residues are displayed along with tryptophan 286 in orange and showing the different orientations between the X-ray structure and the model. It is proposed that movement of the tryptophan opens up the 'agonist pocket' which is occupied by the $R^2$ substituents—a butyl side chain in the case of compound **11.**

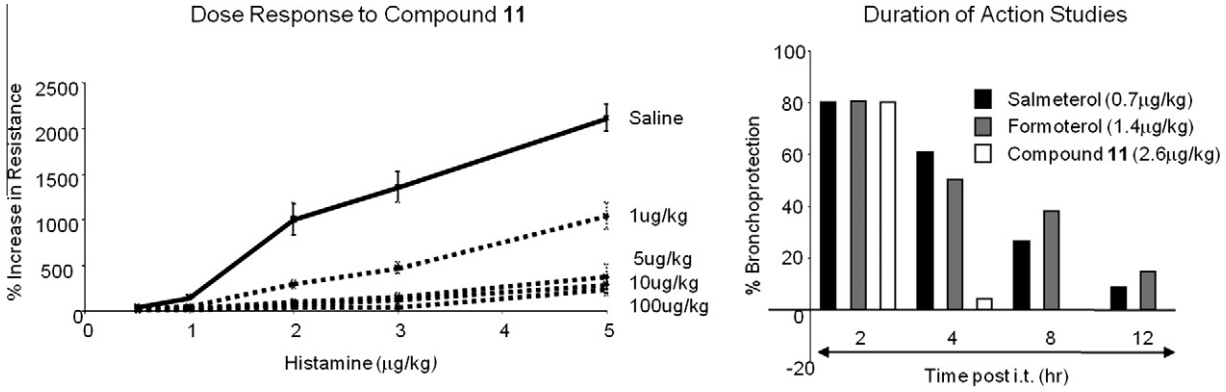
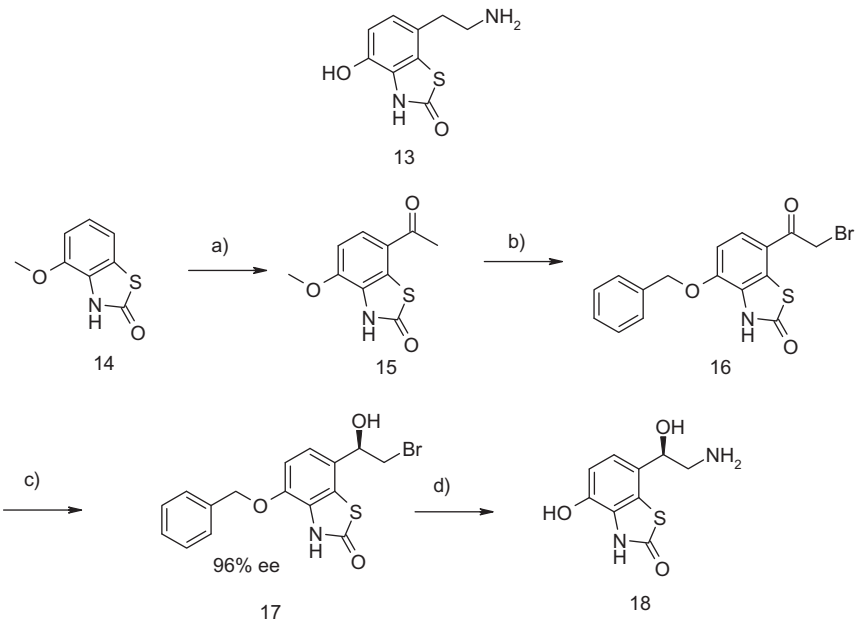



**Figure 3.** Dose response of **11** in guinea pig histamine-induced bronchoconstriction model. The potency was measured in vivo using intratracheal (i.t.) administration. Guinea pigs were dosed with compounds and 2 h later anaesthetised and their respiratory resistance was measured after administering intravenous histamine. The dose of compound that inhibited 80% of the bronchoconstriction induced by histamine ($ED_{80}$) was calculated from the dose-response curve and was used in the resulting duration of action studies.




**Scheme 1.** Reagents and conditions: (a) acetyl chloride, $AlCl_3$, $CH_2Cl_2$ (83%); (b) (i) 48% aq HBr reflux (83%); (ii) Hunig's base, BnBr, NMP, 65 °C (47%), (iii) phenyltrimethylammonium tribromide, THF, 65 °C (52%); (c) (1*R*,2*S*)-(+)-*cis*-1-amino-2-indanol, $BH_3$.THF (81%); (d) (i) $NaN_3$, NaI, DMSO, 65 °C (85%) (ii) $H_2$, Pd/C, ethanol; (iii) Pd black, formic acid (65%) or $H_2$, Pd/C, MeOH + 10% concd HCl (92%).

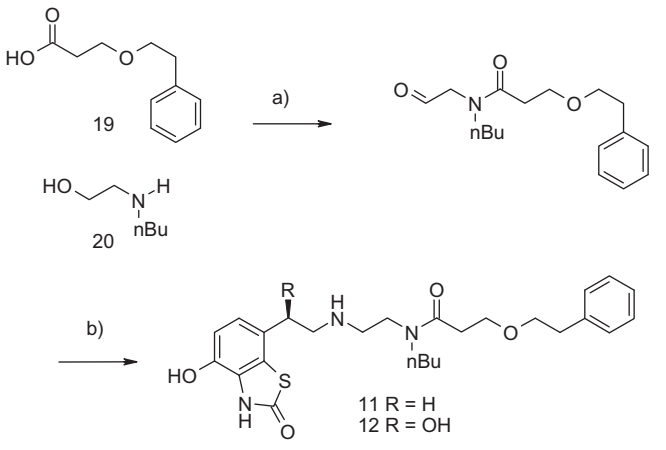
fit of the conserved tryptophan 286 resulting in receptor activation.

Compound **11** was progressed to the guinea pig histamine-induced bronchoconstriction model—a well characterized species for modeling human lung disease. Bronchodilators offering protection in this model have historically shown good efficacy in man with a comparable duration of action.[18] Compound **11** was tested at various concentrations and demonstrated a dose-dependent protection against histamine-induced brochoconstriction with an $ED_{80}$ of 2.6 μ/kg. The $ED_{80}$ dose was used to determine the duration of action and in this model formoterol ($ED_{80}$ of 1.4 μg/kg) and salmeterol ($ED_{80}$ of 0.7 μg/kg) gave protection up to 12 h, where as **11** only gave protection up to 2 h and, as a consequence, was not progressed further (Fig. 3).

The synthesis of compounds (**1–12**) required the synthesis of both the known compound (**13**)[19] and (**18**). Benzthiazolone (**14**)[20] was acylated with acetyl chloride to afford (**15**). A sequence of deprotection and re-protection followed by bromination afforded the bromo ketone (**16**) that was selectively reduced[21] to afford the chiral bromohydrin (**17**) in greater than 96% enantiomeric excess. Displacement with sodium azide followed by a 2-step hydrogenation procedure afforded (**18**) as a stable crystalline compound (Scheme 1).

The amino compounds (**13** and **18**) were then used in the synthesis of the final compounds where examples **11** and **12** are shown as representative examples (Scheme 2).[22]

A new series of potent and highly-efficacious $\beta_2$-agonists have been described that are devoid of the key 1-hydroxy group previously considered important to achieve full agonism. Consideration of the $\beta_2$AR crystal structure, other biophysical data and modeling studies has led to the proposal that the unprecedented level of efficacy within the described series is achieved by an induced fit where a lipophilic pocket is opened up after movement of a key tryptophan (286) in the $\beta_2$AR crystal structure. Compound **11** was shown to be highly efficacious, however when dosed in vivo in a guinea pig trachea model the compound showed limited duration of action. Work is on-going within our laboratories to build on this key observation to deliver new $\beta_2$-agonists to enable once a day dosing in man.




**Scheme 2.** Reagents and conditions: (a) (i) (**19**) + $(COCl)_2$, $CH_2Cl_2$, DMF (one drop), rt 2 h, (ii) add (**20**), $NEt_3$, $CH_2Cl_2$, (iii) $(COCl)_2$; DMSO, $Et_3N$, −70 °C to rt; (b) (**13**) or (**18**) + acetic acid (1 equiv), $NaCNBH_3$, MeOH 30–45%.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.04.135.

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