Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 4012–4015
Bioorganic & Medicinal Chemistry Letters

# The discovery of long acting $\beta_2$-adrenoreceptor agonists

Alan D. Brown, Mark E. Bunnage, Paul A. Glossop,* Kim James, Rhys Jones, Charlotte A. L. Lane, Russell A. Lewthwaite, Simon Mantell, Christelle Perros-Huguet, David A. Price,* Mike Trevethick and Rob Webster

*Pfizer Global Research and Development, Sandwich Laboratories, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK*

Received 12 March 2007; revised 19 April 2007; accepted 25 April 2007
Available online 29 April 2007

**Abstract**—The design and profile of a series of saligenin containing long acting $\beta_2$-adrenoreceptor agonists is described. Evaluation of these analogues using a guinea-pig tissue model demonstrates that analogues within this series have significantly longer durations of action than salmeterol and have the potential for a once daily profile in human.


Long acting $\beta_2$-adrenoreceptor 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 of these agents.[1,2] There are currently two marketed long acting $\beta_2$-adrenoreceptor agonists, salmeterol[3] and formoterol,[4,5] neither of which provides a once daily dosing regimen (Fig. 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.

The desire for a once daily $\beta_2$-adrenoreceptor agonist (while retaining the known efficacy of this class) would be increased convenience and so potentially compliance within the patient population. Asthma is a chronic inflammatory disorder of the airways causing recurrent episodes of wheezing, breathlessness, chest tightness and coughing. These symptoms often occur at night or in the early morning, impacting on sleep patterns and so reducing overall quality of life. A once daily profile would therefore be a valued addition to the treatment of asthmatics.

COPD is the fourth leading cause of death in the US and is characterized by airflow obstruction due to chronic bronchitis or emphysema and symptoms are typically breathing-related (e.g., chronic cough, exertional dyspnoea, expectoration and wheeze). A quality long acting $\beta_2$-adrenoreceptor agonist would again be of tremendous value in the treatment of this condition.

There has been considerable interest in the discovery of a once daily $\beta_2$-adrenoreceptor agonist with a recent flurry of presentations, patent applications and licencing agreements from a number of institutions.[6] There are numerous publications around how structurally differing long acting $\beta_2$-adrenoreceptor agonists achieve their duration of action including discussions around slow offset phenomena, however, this argument appears limited with relatively few publications.[7] The majority of papers focus on the ability of salmeterol to bind to an exosite on the $\beta_2$-adrenoreceptor near the agonists' binding site, termed the exosite theory.[8] However, formoterol would be unable to access this exosite and an alternative hypothesis suggests that the lipophilic, basic nature of these compounds allows them to partition effectively into the lipid bilayers of smooth muscle

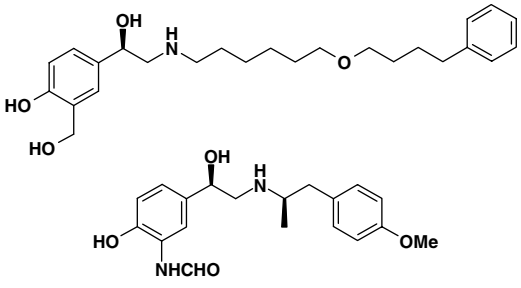



**Figure 1.** Structures of salmeterol and formoterol.

*Keywords*: $\beta_2$; Asthma; COPD; Duration of action; Salmeterol; Formoterol; Bioavailability.
* Corresponding authors. E-mail: david.a.price@pfizer.com

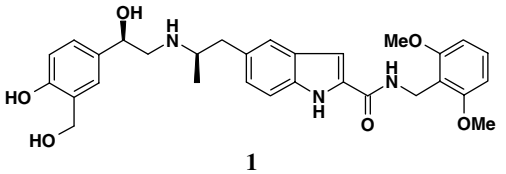
0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.04.081

following inhalation. This partitioning then allows an effective concentration of agonist to be present over time and delivers duration of action, this is known as the diffusion microkinetic theory.[9] 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/$\beta_2$-adrenoreceptor agonists' programme shows that lipophilicity and p$K_a$ are key parameters within this project.[10]

We have previously reported the design and profile of a novel series of $\beta_2$-adrenoreceptor agonists exemplified by **1** using an indole derived template discovered from a high throughput screen.[6] From the structural overlay it is clear that even though there is significantly less conformational flexibility in the indole series compared to salmeterol these analogues can still potentially access the exosite binding region to drive duration of action (Fig. 2). These compounds are also isolipophilic with salmeterol so that the diffusion microkinetic theory would also suggest an extended duration of action to be present in these compounds.

Following on from the indole series the remit of the project was to retain the excellent pharmacological profile present in **1** and utilize common synthetic intermediates while delivering a structurally differentiated series. We decided to remain within the saligenin series as we had experience with this motif and we were also keen to initially use benzylamines for the amide coupling partner. This was due to the large number of commercially available benzylamines that we could use readily to investigate structure–activity relationships/physiochemical space without requiring bespoke synthesis. The amide expression was also an attractive feature for reasons beyond its synthetic utility. It has been documented that after inhalation of salmeterol a significant contribution to the systemic effects results from the oral bioavailability of the swallowed fraction of the inhaled dose.[11] Reducing the oral bioavailability of our compounds was a key goal by simultaneously limiting absorption across the gut wall and ensuring high hepatic turnover of any fraction absorbed.[12] There have been numerous analysis's investigating molecular properties that influence oral bioavailability.[13,14] Some of the properties that have an effect include molecular weight, lipophilicity, conformational flexibility, basicity as well as many others. Retaining the secondary amide present in **1** and its propensity for hydrogen bonding to potentially limit its ability to cross the gut wall was a desirable feature.[15] With this project strategy we turned to replacing the indole linker using structural overlays to guide design and prepared phenyl acetic alternatives to the indole, Table 1.

Pleasingly, initial analogues were in the same potency range as salmeterol and highly selective over the $\beta_1$-adrenoreceptor when assessed using human recombinant β-adrenoreceptors expressed in a CHO cell line. The effect of **2**–**4** and salmeterol on airway smooth muscle was investigated using tracheal strips taken from the guinea-pig. The pharmacology of $\beta_2$ agonists in this model correlates well with clinical data and gives a measurement of potency, efficacy and duration of action.[16] Isolated tracheal strips were contracted via stimulating the release of endogenous acetylcholine with electrical field stimulation and test agents were assessed for their ability to oppose this through functional antagonism. A duration of action was defined as the time taken for the muscle tone at a just Emax concentration of the compound to recover by 50% of the inhibition induced where the Emax is the maximum inhibition achievable by that compound. In this study all the compounds were essentially equipotent with salmeterol and also equivalent in the magnitude of Emax. Compared to salmeterol they were all significantly shorter in their durations of action, with compound **2** being the shortest. With the relatively low lipophilicity of these compounds ($\log D \leqslant 1$) we were not surprised to find that their durations of action when assessed using guinea-pig trachea were significantly shorter than that of salmeterol. Compounds **3** and **4** were assessed for their human liver microsomal (HLM) stability and we were delighted to find that even with their relatively low lipophilicity they were both rapidly metabolised (half-life <20 min). The cell permeability as measured by apical to basal flux rate through a




**1**

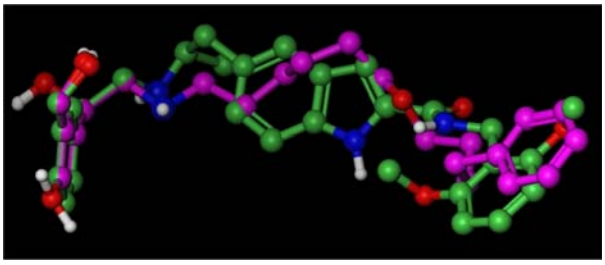
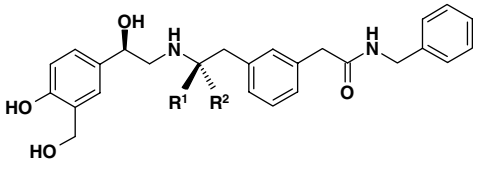


**Figure 2.** Structural overlay of salmeterol (purple) with **1**.

**Table 1.** Potency, selectivity and log $D$ for initial analogues




| Compound | $\beta_2$ $EC_{50}$[a] (nM) | $\beta_1/\beta_2$[b] | log $D$ |
|---|---|---|---|
| **2** $R^1 = R^2 = H$ | 0.096 | 8597 | 0.4 |
| **3** $R^1 = R^2 = Me$ | 0.020 | 51418 | 1.0 |
| **4** $R^1 = Me$, $R^2 = H$ | 0.009 | 10054 | 0.8 |
| Salmeterol | 0.070 | 7885 | 2.5 |

[a] Potency and efficacy at human recombinant $\beta_2$ and $\beta_1$-adrenoreceptors expressed in CHO cells assessed as elevations in cyclic AMP. In this assay, all compounds appeared to be full agonists.

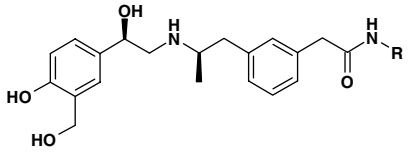
[b] Ratio of $EC_{50}$'s generated at human recombinant $\beta_2$ and $\beta_1$-adrenoreceptors expressed in CHO cells assessed as elevations in cyclic AMP.

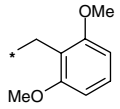
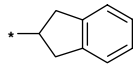
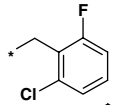
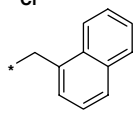
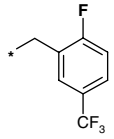
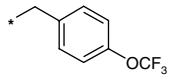
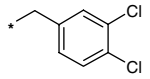
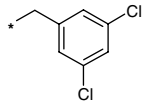
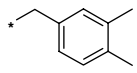
monolayer of CaCo-2 cell was also low, predicting for poor oral absorption in human.[17] These in vitro pharmacokinetic data suggested the profile we desired of poor gut wall permeability coupled with high first pass metabolism was achievable in this expression (Table 2).

To increase the duration of action we decided to prepare further analogues in the series exemplified by **4** and to investigate the structure–activity relationship of the benzylamine group. A key design feature was to increase the overall lipophilicity of the compounds to a log *D* range >1.5. If we could achieve this initial goal then the compounds would be progressed to in vitro pharmacokinetic studies to prioritise for rat in vivo work to assess oral bioavailability. A concern was that as we increased the lipophilicity we may also increase the cell permeability of the analogues and this was to be closely monitored.

In terms of potency all the analogues prepared were in the same range as that of salmeterol when tested in the guinea-pig trachea model. Assuming that compounds **5**–**13** have a similar binding mode to that of salmeterol it would suggest that the $\beta_2$-adrenoreceptor is tolerant of differing substituents of this right-hand side phenyl ring. The durations of action in the trachea model do appear to correlate generally to the lipophilicity of the compounds with a log *D* > 2 having a longer duration of action, except for compound **13**. Compounds **9**–**12**

**Table 2.** Tissue potency, duration and log *D* for analogues




| Compound | | log *D* | CaCo-2 flux[c], Papp × $10^{-6}$ cm/s | HLM (min)[d] | Potency[a] | Duration of action (h)[b] |
|---|---|---|---|---|---|---|
|  | **5** | 0.8 | Not done | Not done | 0.30 | 1.5 ($n$ = 2) |
|  | **6** | 1.6 | 1 | Not done | 0.10 | 5.4 ($n$ = 2) |
|  | **7** | 1.5 | 1 | Not done | 0.07 | 5.2 ($n$ = 2) |
|  | **8** | 1.5 | Not done | 2 | 1.00 | 3.9 ($n$ = 3) |
|  | **9** | 2.2 | 2 | 9 | 0.90 | 6.6 ($n$ = 4) |
|  | **10** | 2.4 | Not done | 15 | 1.00 | 8.1 ($n$ = 2) |
|  | **11** | 2.6 | 1 | 4 | 0.40 | 9.0 ($n$ = 4) |
|  | **12** | 2.3 | 1 | 8 | 0.50 | 7.2 ($n$ = 2) |
|  | **13** | 2.2 | Not done | 10 | 0.50 | 4.6 ($n$ = 2) |
| Salmeterol | — | 2.1 | — | — | 1.00 | 6.9 ($n$ = 5) |

[a] All potencies in the guinea-pig trachea model are quoted relative to salmeterol so that a compound that is twice as potent will be quoted as 0.5.
[b] A duration of action is measured as the time taken for the muscle tone at an Emax concentration of the compound to recover by 50% of the inhibition induced where the Emax is the maximum inhibition achievable by that compound.
[c] Apical to basal flux rate of compound through a monolayer of CaCo-2 cells at 25 μM, pH 7.4.
[d] Half-life of compound in microsomal preparation when assayed at 1 μM.

where the log $D > 2$ have durations of action in the same region as salmeterol or even significantly longer as for compound **11**. For compound **11** there was still incomplete tissue recovery after 14h in this model which could be reversed using the selective $\beta_2$-adrenoreceptor antagonist ICI-118551 thus demonstrating the effects were $\beta_2$ mediated. It is interesting to compare compounds **11** and **13** which are structurally similar and also essentially isolipophilic yet have profoundly differing durations of action in the guinea-pig trachea. The difference in pharmacology is hard to rationalise particularly as they are also equipotent, however, if the exosite model is invoked it could suggest that there are differing structure–activity relationships regarding potency and duration in this region of the $\beta_2$-adrenoreceptor.

The most interesting compounds were all progressed to in vitro pharmacokinetic assays and all analogues were rapidly metabolised in a human microsomal assay with short half lives (<20 min). With these data compound **11** was further progressed to assess cell permeability as measured by apical to basal flux rate through a monolayer of CaCo-2 cells. This assay suggested that not only did compound **11** possess intrinsically poor cell permeability but it was also highly effluxed by transporter proteins with an A to B:B to A ratio of 1:15. These in vitro data confirmed that **11** should have low oral bioavailability due to poor absorption through the gut wall and high first pass metabolism, this warranted progression of **11** into rat in vivo studies. Following iv administration the volume of distribution was high ($V_d = 16.6$ L/kg), however, with a clearance of greater than liver blood flow (123 ml/min/kg) the measured half-life was 1.5 h. An oral leg was not performed as the iv data and the in vitro CaCo-2 data suggested that the oral bioavailability would be very low. Compound **11** was also screened for off target pharmacology and showed no significant affinity (<100 nM) for other receptors, enzymes or ion channels.

In conclusion, we have described our efforts to deliver a $\beta_2$-adrenoreceptor agonist that has a longer duration of action than salmeterol in the well-validated guinea-pig trachea model. Additionally a key design feature was to ensure that compounds would have low oral bioavailability compared to salmeterol to reduce systemic effects through the swallowed fraction after inhalation. This was achieved through introducing amide functionality with high hydrogen bonding potential to limit absorption while also ensuring high first pass metabolism. Compound **11** delivers this pharmacology profile with improved potency and duration of action compared to salmeterol in the guinea-pig trachea model. Since salmeterol is reported to have a duration of action of up to 18 h in human this warranted further progression of **11** into further in vivo studies where its superior duration of action was confirmed.

Furthermore **11** possesses the required in vivo pharmacokinetics with clearance greater than liver blood flow in the rat and consequently short half-life. Further work and efforts in this series will be described in future publications.

## References and notes


1. Waldeck, B. *Eur. J. Pharmacol.* **2002**, *445*, 1.
2. Siafakas, N. M.; Vermeire, P.; Price, N. B. *Eur. Respir. J.* **1995**, *8*, 1398.
3. Johnson, M. *Med. Res. Rev.* **1995**, *15*(3), 225.
4. Anderson, G. P. *Life Sci.* **1993**, *52*, 2145.
5. Palmqvist, M.; Persson, G.; Lazer, L.; Rosenborg, J.; Larsson, P.; Lotvall, J. *Eur. Respir. J.* **1997**, *10*, 2484.
6. Glossop, P. A.; Price, D. A. *Annu. Rep. Med. Chem.* **2006**, *41*, 237.
7. Voss, H.; Donnell, D.; Bast, A. *Eur. J. Pharmacol., Mol. Pharacol. Sect.* **1992**, *227*, 403.
8. Bradshaw, J.; Brittain, R. T.; Coleman, R. A.; Jack, D.; Kennedy, I. *Br. J. Pharmacol.* **1993**, *108*, 507.
9. Anderson, G. P.; Linden, A.; Rabe, K. F. *Eur. Respir. J.* **1994**, *7*, 569.
10. Austin, R. P.; Barton, P.; Bonnert, R. V.; Brown, R. C.; Cage, P. A.; Cheshire, D. R.; Davis, A. M.; Dougall, I. G.; Ince, F.; Pairaudeau, G.; Young, A. *J. Med. Chem.* **2003**, *46*, 3210.
11. Bennett, J. A.; Harrison, T. W.; Tattersfield, A. E. *Eur. Respir. J.* **1999**, *13*, 445.
12. Taburet, A. M.; Schmit, B. *Clin. Phamacokinetics* **1994**, *26*(5), 396.
13. Veber, D. F.; Johnson, S. R.; Cheng, H. Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615.
14. Smith, A. B., III; Hirschmann, R.; Pasternak, A.; Yao, W.; Sprengler, P. A.; Halloway, M. K.; Kuo, L. C.; Chen, Z.; Darke, P. L.; Schleif, W. A. *J. Med. Chem.* **1997**, *40*, 2440.
15. Abraham, M. H.; Duce, P. P.; Prior, D. V.; Barratt, D. G.; Morris, J. J.; Taylor, P. J. *J. Chem. Soc., Perkin Trans. 2* **1989**, 1355.
16. Nials, A. T.; Sumner, M. J.; Johnson, M.; Coleman, R. A. *Br. J. Pharmacol.* **1993**, *108*, 507.
17. Artursson, P.; Palm, K.; Luthman, K. *Adv. Drug Delivery Rev.* **1996**, *12*, 67.