Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 1280–1283
Bioorganic & Medicinal Chemistry Letters

The discovery of adamantyl-derived, inhaled, long acting β_2-adrenoreceptor agonists

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

[a]*Pfizer Global Research and Development, Sandwich Laboratories, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK*
[b]*Pfizer Global Research and Development, Groton/New London Laboratories, Pfizer Inc., Eastern Point Road, Groton, CT 06340, USA*

Received 2 December 2007; revised 7 January 2008; accepted 9 January 2008
Available online 12 January 2008

Abstract—The design and profile of a series of adamantyl-containing long acting β_2-adrenoreceptor agonists are described. An optimal pharmacokinetic profile of low oral bioavailability was combined with a strong pharmacology profile when assessed using a guinea pig trachea tissue model. A focus was then placed on developing a robust synthetic route to ensure rapid delivery of material for clinical trials.

Long acting β_2-adrenoreceptor agonists are a highly precedented drug class used for the treatment of asthma and chronic obstructive pulmonary disease (COPD).[1,2] There are currently two marketed long acting β_2-adrenoreceptor agonists, salmeterol[3] and formoterol,[4,5] which are approved for twice daily dosing, (Fig. 1).

There has been considerable interest in the discovery of a once daily β_2-adrenoreceptor agonist with a recent flurry of presentations, patent applications and licensing agreements from a number of institutions. This activity prompted our initial disclosure[6] of **1** as a long acting β_2-adrenoreceptor agonist with the potential for an inhaled, once daily dosing regime. We described our medicinal chemistry strategy in the design of **1** to ensure that any oral fraction following inhalation does not contribute to the overall systemic exposure of **1**.[7] This was achieved by ensuring the compound had low cell permeability as measured using a CaCo-2 cell line to predict for poor absorption across the gut wall.[8] Combined with high microsomal instability to ensure high first pass metabolism in the liver of any absorbed fraction this ensured low oral bioavailability. Coupled with this optimal pharmacokinetic profile the compound possessed a strong pharmacology profile, it was equipotent with salmeterol and displayed a longer duration of action than salmeterol when profiled in a guinea pig trachea model.[9] Considering the fully optimised preclinical

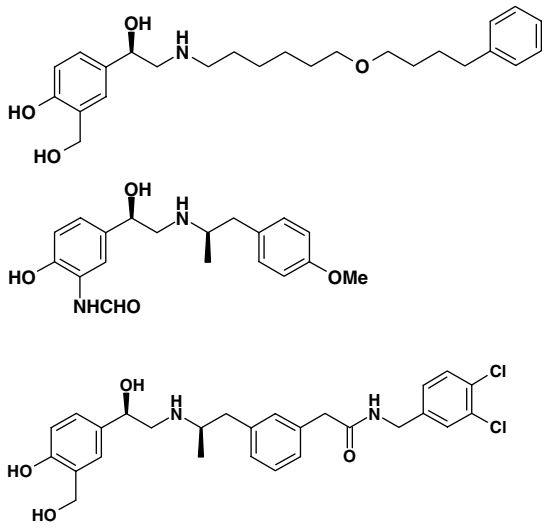


Figure 1. Structures of salmeterol, formoterol and **1**.

Keywords: β_2; Adrenoreceptor; Adamantyl; Saligenin; Asthma; Crystalline.
* Corresponding author. Tel.: +44 1034 644690; e-mail: david.a.price@pfizer.com

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.01.034

pharmacokinetic/pharmacology package that we had achieved with **1** it was going to be a significant challenge to deliver a compound with a superior profile; indeed we decided that a compound with an equivalent pharmacology/pharmacokinetic profile but which addressed potential risks with **1** would be of value to the portfolio as a back up strategy.

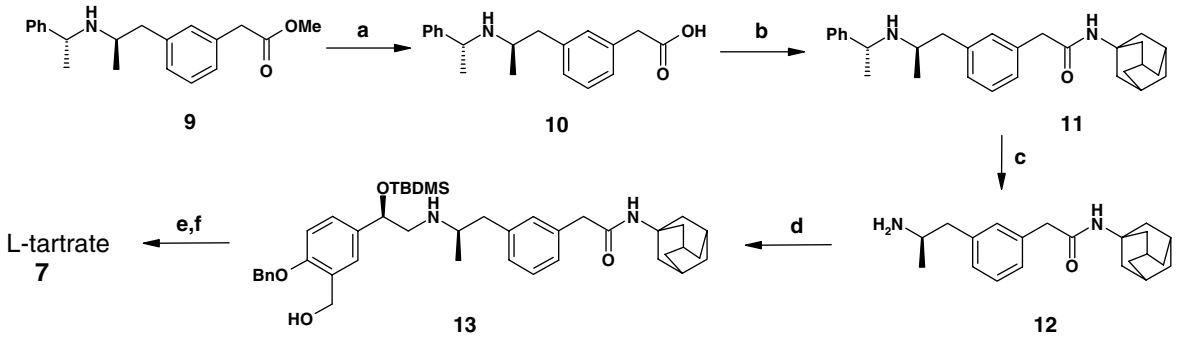
The inhaled β_2 agonist market is a highly competitive arena and it was imperative that we obtained clinical data as rapidly as possible to validate our screening cascade and biological models. A key goal to expedite progression to the clinic is ensuring the synthesis of the clinical candidate is as short as possible to maximise synthetic accessibility of bulk quantities of the active pharmaceutical ingredient (API). A parameter often used in the measurement of synthetic complexity is the number of steps contained in the longest linear sequence. With the intrinsic structural complexity of many β_2-adrenoreceptor agonists it was accepted that the synthesis of these compounds would be relatively complex and require numerous steps. However, to try and counter this it was decided to ensure as large a number of crystalline intermediates as possible and a crystalline active pharmaceutical ingredient (API) so that purification of bulk material was as simple as possible. While compound **1** itself was crystalline as the L-tartrate salt, in the route used there were no crystalline intermediates and this presented challenges in the efficiency of scaling up the chemistry to deliver bulk quantities for any clinical studies (Scheme 1).

After our early extensive efforts using benzylamines as coupling partners in this template it was proving difficult to deliver a compound with the required pharmacological/pharmacokinetic properties that offered differentiation over **1**. Attention now turned to examining whether alternative amines could be used and in particular cyclic saturated amines. Initial replacement of the dichlorophenyl expression in **1** with a cyclohexyl group generated **2** and we were delighted to find that this compound retained many of the attractive features of the lead series. The compound was essentially equipotent at the recombinant human β_2 receptor expressed in CHO cells and still retained excellent selectivity over β_1. Even though the compound was significantly less lipophilic than **1** it was still rapidly turned over in a human microsomal preparation and possessed poor cell permeability as measured in the CaCo-2 cell line. This in vitro pharmacokinetic data suggested the profile we desired of poor gut wall permeability coupled with high first pass metabolism was achievable in this alternative expression. The duration of action as measured in a guinea pig trachea model was shorter than the lead **1** which was attributed to the reduction in lipophilicity. In previous work we had found a correlation between lipophilicity and duration of action in this tissue based model and this has also been observed by other groups.[6,10]

Previous experience in the project showed an excellent correlation between the potencies of compounds using the cell based assay and potency in guinea pig tissue and to reduce ex vivo tissue work potencies were only run on compounds that were of sufficient interest to the project.[6] A duration of action was 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. The E_{max} concentration was estimated from the potency measured in the cell based assay. The compounds were described as long (L) if they possessed a duration of action similar to that of lead **1** (>6 h) and medium (M) if they possessed a duration of action shorter than **1** (<6 h). After this period of time tissue fade would begin and further measure of duration of action would be unreliable. This contracted tissue screening paradigm was essential for the rapid prosecution of the project and selection of a candidate for clinical studies.

To increase the lipophilicity of the cyclohexyl lead **2** the homologated analogue cycloheptane analogue **3** was prepared which gave a compound that was less potent than **1** but showed a long duration of action in the guinea pig tissue model. This gave us confidence that within these cycloalkyl expressions we should be able to deliver the required pharmacological profile.

Truncation and concomitant ring expansion to generate the isolipophilic **4** gave the required duration of action in a compound that was still highly potent in its effect on the β_2 adrenoreceptor and highly selective over the β_1 receptor. However, we were confident that within this expression we could deliver a more potent, selective



Scheme 1. Reagents and conditions: (a) aq LiOH, THF, 95%; (b) 1-adamantylamine, DIP, THF, 82%; (c) Pd/C, NH_4HCO_2, EtOH, 90%; (d) melt reaction with saligenin head group, 72%; (e) Pd/C, NH_4HCO_2, EtOH, 100%; (f) L-tartaric acid, EtOH, 55%.

compound to ensure we delivered a high quality alternative to **1**. It was clear that if we were to combine the required lipophilicity to drive duration of action with the exquisite potency we could obtain with cyclohexyl derived substituents we would need to be more creative than ring homologation as in **4**.

A review of the structure of marketed drugs containing cycloalkyl substituents was undertaken to design alternative expressions to the cycloalkyl substituent in **2**. From the anti-infectives literature amantidine **8** (Fig. 2) caused interest as an antiviral agent which specifically inhibits the replication of influenza A viruses at low concentrations. It was also encouraging that as a marketed agent amantidine would have been extensively reviewed for safety issues and this gave us confidence in the incorporation of an adamantyl group into our series. Adamantyl is a chemically robust expression which would be amenable to a variety of reaction conditions and so opened up the possibilities of being able to extensively reorder the synthetic route to give alternative crystalline intermediates.

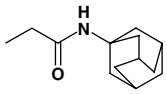
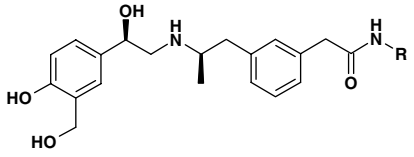


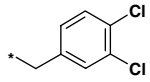
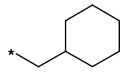
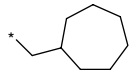
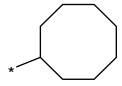
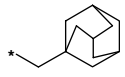
Figure 2. Amantidine **8**.

Incorporation of an adamantyl group into the homologated series generated **5** which displayed a reduction in potency, however, in the direct linked series **7** possessed an exciting profile with salmeterol-like potency and excellent β_1 selectivity. It is interesting to note that the alternative 2-adamantyl regioisomer **6** showed 3-fold lower potency in the primary assay suggesting how specific the binding is in this region of the receptor (Table 1).

Progression of **7** into the guinea pig tissue assay confirmed an extended duration of action comparable to **1**. Guinea pig in vivo studies showed that **7** had an identical pharmacological profile to that of the lead compound **1** following *intra-tracheal* dosing in terms of its potency and duration of action. With this data **7** was prioritised for full pharmacokinetic profiling and the initial work was to assess cell permeability as measured by

Table 1. Log *D*, cell potency and tissue duration of action for analogues



Compounds		log *D*	Potency[a] (pM)	β_1/β_2	HLM (min)	Duration of action[b] (h)
	**1**	2.6	30	6339	4	L
	**2**	1.5	20	6787	3	M
	**3**	2.0	118	1886	Not done	L
	**4**	1.9	200	192	2	L
	**5**	2.5	250	547	2	L
	**6**	2.1	190	451	2	L
	**7**	2.1	60	2211	2	L
Salmeterol	—	2.1	70	7885	—	L

[a] Potency and efficacy at human recombinant β adrenoreceptors expressed in CHO cells assessed as elevations in cyclic AMP ($n = 3$). In this assay all compounds appeared to be full agonists.

[b] A 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 ($n = 2$). The compounds were described as long (L) if they possessed a lead compound **1**-like duration and medium (M) if they possessed a duration of action shorter than **1**.

apical to basal flux rate through a monolayer of CaCo-2 cells. This assay suggested that not only did **7** possess intrinsically poor cell permeability but it was also highly effluxed by transporter proteins. This in vitro data combined with the low microsomal stability demonstrated that **7** should have low oral bioavailability due to poor absorption through the gut wall and high first pass metabolism. Analogue **7** was progressed into rat in vivo studies and following *intra venous* administration the volume of distribution was high (V_d = 14 L/kg), however, with a clearance of greater than liver blood flow (103 ml/min/kg) the measured half life was 1.6 h. An oral cross over was conducted and confirmed that in rat the oral bioavailability was <5%. This pharmacokinetic profile added to the confidence that any swallowed fraction of the inhaled dose in man would not contribute to the systemic exposure of **7**. Compound **7** was also screened for off target pharmacology and showed no significant affinity (<100 nM) for other receptors, enzymes or ion channels.

Attention now turned to reordering the synthetic route to maximise the number of crystalline intermediates and minimise synthetic complexity. Within the early discovery phase of the project the amide coupling was performed as the final step to allow rapid variation of the amine expression. However, there were no crystalline intermediates in the route which presented difficulties in purification when scaling up the final optimised compound. It was decided that with such a chemically robust adamantyl expression the amide coupling could be performed earlier in the route. This had the advantage that the new intermediate **10** required was highly crystalline and precipitated from the reaction solvents as the hydrochloride salt and could be isolated by filtration. Amide coupling of 1-adamantylamine and cleavage of the benzylamine chiral auxiliary generated **12** which could again be purified by recrystallisation. The synthesis was completed with installation of the saligenin expression, hydrogenation and cleavage of the benzyl protecting group and finally removal of the silicon protecting group. This could be achieved using L-tartaric acid which performed the protodesilylation and salt formation to give crystalline, high purity final material. This focus on route optimisation, collaboration with large scale chemistry experts and selection of a clinical candidate using synthetic expedience as a key criterion was a highly successful approach to ensure a rapid delivery of high quality material for subsequent clinical studies.

In conclusion, we have described our efforts to deliver a β_2-adrenoreceptor agonist that has a longer duration of action in pre-clinical models than salmeterol. 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 by the liver. Furthermore, through collaboration with experts in large scale chemistry **10** was designed with the goal of delivering an optimised synthetic route at time of nomination to expedite delivery of bulk material for clinical trials.

Acknowledgments

The authors recognise Michele Coghlan, Susan Summerhill and Karen Wright, for their expertise in generating and analysing the biological data.

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**, *153*, 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. Brown, A. D.; Bunnage, M. E.; Glossop, P. A.; James, K.; Jones, R.; Lane, C. A. L.; Lewthwaite, R. A.; Mantell, S.; Perros-Huguet, C.; Price, D. A.; Trevethick, M.; Webster, R. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4012.
7. Bennett, J. A.; Harrison, T. W.; Tattersfield, A. E. *Eur. Respir. J.* **1999**, *13*, 445.
8. Artursson, P.; Palm, K.; Luthman, K. *Adv. Drug Delivery Rev.* **1996**, *12*, 67.
9. Nials, A. T.; Sumner, M. J.; Johnson, M.; Coleman, R. A. *Br. J. Pharmacol.* **1993**, *108*, 507.
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.