Bioorganic & Medicinal Chemistry Letters 21 (2011) 2759–2763

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Inhalation by design: Dual pharmacology β-2 agonists/M3 antagonists for the treatment of COPD

Lyn H. Jones [a,*], Helen Baldock [a], Mark E. Bunnage [a], Jane Burrows [b], Nick Clarke [c], Michele Coghlan [c], David Entwistle [b], David Fairman [d], Neil Feeder [b], Craig Fulton [b], Laura Hilton [a], Kim James [a], Rhys M. Jones [d], Amy S. Kenyon [a], Stuart Marshall [a,b,c], Sandra D. Newman [a], Rachel Osborne [a], Sheena Patel [c], Matthew D. Selby [a], Emilio F. Stuart [c], Michael A. Trevethick [c], Karen N. Wright [c], David A. Price [a]

[a] Sandwich Chemistry, World Wide Medicinal Chemistry, Pfizer, Ramsgate Road, Sandwich CT13 9NJ, UK
[b] Pharmaceutical Sciences, Pfizer, Ramsgate Road, Sandwich CT13 9NJ, UK
[c] Allergy and Respiratory Biology, Pfizer, Ramsgate Road, Sandwich CT13 9NJ, UK
[d] Pharmacokinetics, Dynamics and Metabolism, Pfizer, Ramsgate Road, Sandwich CT13 9NJ, UK

ARTICLE INFO

Article history:
Received 3 September 2010
Revised 25 October 2010
Accepted 26 October 2010
Available online 31 October 2010

Keywords:
COPD
Inhalation
Dual pharmacology
MABA
Crystallinity

ABSTRACT

This paper describes the successful design and development of dual pharmacology β-2 agonists-M3 antagonists, for the treatment of chronic obstructive pulmonary disorder using the principles of 'inhalation by design'. A key feature of this work is the combination of balanced potency and pharmacodynamic duration with desirable pharmacokinetic and material properties, whilst keeping synthetic complexity to a minimum.

Chronic bronchitis and emphysema are components of chronic obstructive pulmonary disease (COPD) that damages the airways of the lungs leading to reduced air flow. COPD is a major cause of disability and is predicted to be the third largest cause of death by 2030.[1] Treatments for COPD include the use of bronchodilators that increase lung air flow, the two major classes being inhaled β-2 adrenergic agonists and muscarinic M3 antagonists. Long acting β-2 agonists (LABAs) and long acting muscarinic antagonists (LAMAs) have been, and continue to be, developed to enable once-daily inhalation dosing. Significant efficacy improvements can be seen from the combination of LABAs and LAMAs.[2] Moreover, triple therapy, from the optimal synergistic combination of a LABA, LAMA and an inhaled corticosteroid, represents an exciting new treatment paradigm for this debilitating condition.[3] However, the complexity of combining three different drugs that operate via three distinct mechanisms into a single device for inhalation dosing, such that patient compliance is high, is considerable. To facilitate the triple therapy concept, we and others,[4] have pursued a strategy to incorporate muscarinic antagonism and β-2 agonism into a single molecule (MABA), such that combination with an ICS could be achieved in a dry powder inhaler. Indeed, a number of dual combination inhalation therapies are already available for the treatment of COPD.

A clear advantage of inhalation is the potential to target the active drug directly to the site of action and, importantly, minimize the potential for side effects associated with systemic drug exposure. Effective inhaled drugs require a number of essential attributes to be secured: very low dose and optimal material properties (to facilitate delivery), suitable solubility and pharmacokinetics, and the desired pharmacodynamic duration of action in the lung. Although the medicinal chemistry associated with inhaled drug discovery is distinct from classical oral drug design, many of the key insights and learnings may be transferable into more mainstream medicinal chemistry.

To design a dual pharmacology molecule[5] we chose a strategy to conjugate a β-2 agonist motif onto that of an M3 antagonist. This would create larger molecules, with high lipophilicity, that are likely to possess high metabolism and low oral absorption to minimize potential systemically-driven side effects from the swallowed component following inhalation. A caveat of this strategy is the potential to create a highly synthetically complex molecule, that would prohibit rapid development, and therefore enabling synthesis was a key component of our medicinal chemistry design.

* Corresponding author. Tel.: +44 1304 644 256; fax: +44 1304 651 821.
E-mail address: lyn.jones@pfizer.com (L.H. Jones).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.10.132

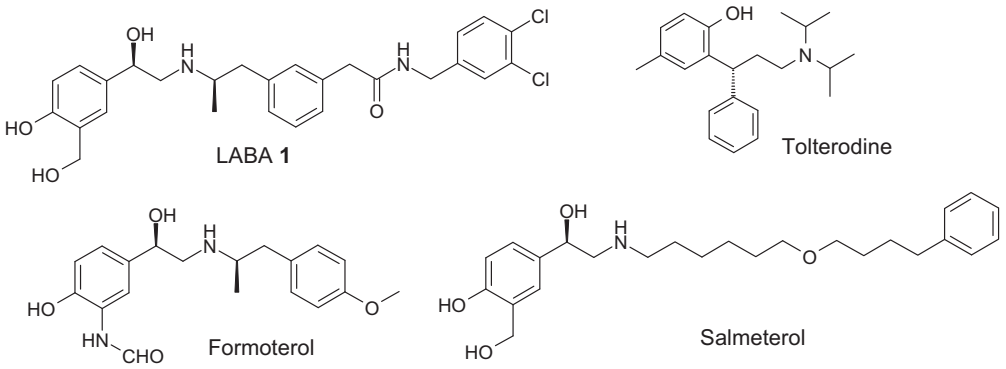
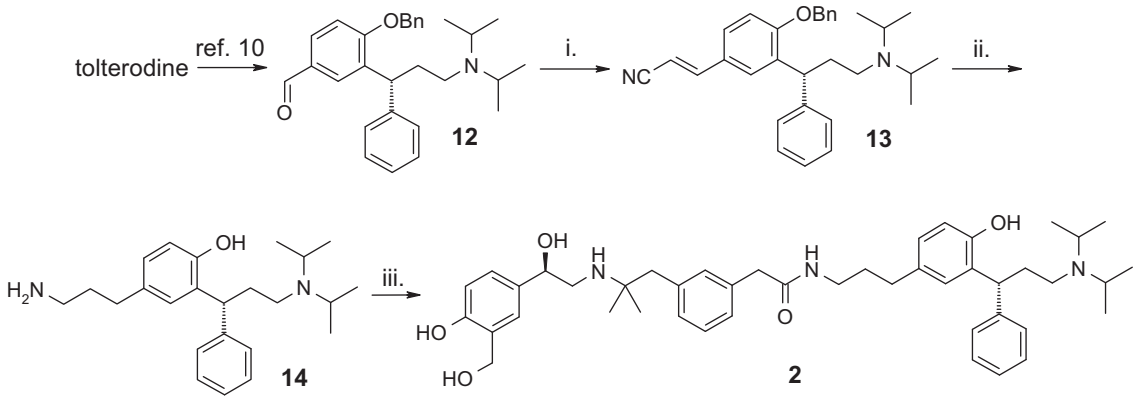


Figure 1. β-2 Agonists and the M3 antagonist tolterodine.



Scheme 1. Synthesis of the amide-tethered series. Reagents: (i) MeCN, KOH aq; (ii) Pd/C, H_2, AcOH; (iii) Acid, WSCDI, pyridine.

Novel saligenin LABAs, exemplified by compound **1** (Fig. 1), were described previously, that possess impressive pharmacological profiles.[6] An in vitro β-2 wash-off assay was developed to provide evidence of a long duration of action (a small shift in potency when the receptor is washed),[7] that was confirmed via prolonged functional antagonism of electrically field stimulated guinea-pig trachea. Low membrane permeability was achieved through the introduction of hydrogen-bonding groups,[8] particularly through the benzylamide, that also ensures high first-pass metabolism. Moreover, the availability of bulk material for this template and the ability to analog the benzylamide provided an attractive synthetic starting point to explore conjugation of the M3 motif.

We decided the potent M3 antagonist tolterodine (Fig. 1) was an ideal fragment to incorporate into our design of a dual pharmacology agent. We have previously described the ability to functionalise the toluene methyl group to create a fluorometric antimuscarinic for assay development.[9] Consequently, the structure–activity relationships also suggest this position of the tolterodine template may be resilient to conjugation to a β-2 motif.

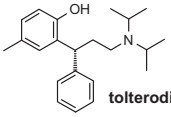
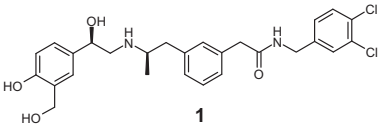
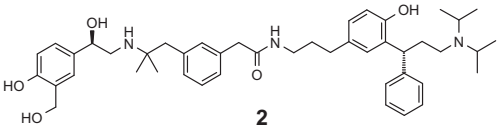
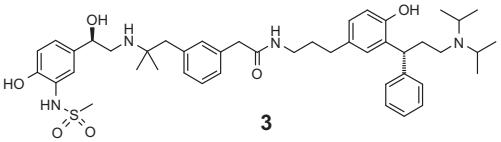
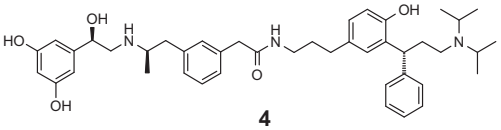
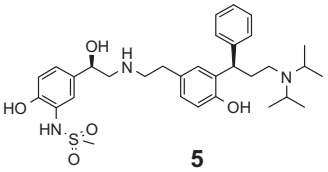
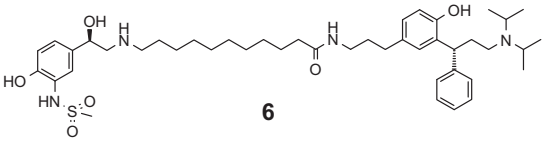
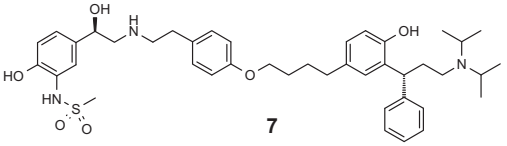
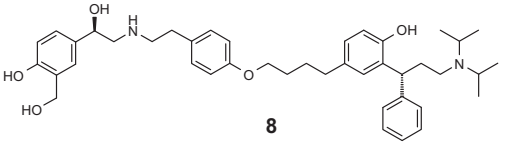
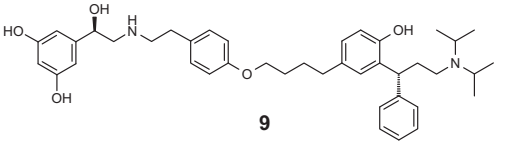
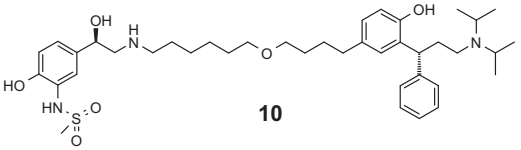
The synthesis commenced with benzyl protection of the tolterodine phenol and subsequent oxidation of the toluene group to the benzaldehyde **12** (Scheme 1).[10] Homologation with deprotonated acetonitrile provided the unsaturated nitrile **13** that was reduced and benzyl-deprotected to the amine **14** in one step. Amidation yielded the saligenin **2** in a very efficient total synthesis, in just four steps from an antimuscarinic drug. Other amide-linked MABAs were prepared in an analogs manner.

Amide **2** possessed remarkable β-2 and M3 potency and the β-2 duration, as assessed in the wash-off assay, was comparable to the lead saligenin LABA **1** (Table 1). We then assessed M3 duration by measuring offset kinetics using a dilution-offset methodology whereby the offset is inferred from the on rate of co-administered ^{3}H-NMS (*N*-methyl scopolamine) and is expressed as the time taken to reach 50% of total ^{3}H-NMS for solvent treated membranes.[11] Interestingly, although the offset kinetics were considerably improved over tolterodine itself (Table 1), we believed that further improvements could be made to the M3 off rate of saligenin **2**.

We explored the SAR of this amide series by investigating the effect of the β-2 agonist trigger head group, and prepared the sulfonamide **3** and resorcinol **4** derivatives.[12] These compounds possessed similar profiles to the saligenin **2** and disappointingly retained the mediocre M3 kinetics (Table 1). Similarly, the truncated derivative **5** achieved potent antimuscarinic and β-2 effects, but lacked pharmacological duration against both receptors in this case. When a long aliphatic chain was used to link the β-2 and M3 motifs, there was a significant loss of β-2 potency (compound **6**). All derivatives possessed rapid metabolic turnover, as assessed in human liver microsomes predicting for high first pass metabolism and low systemic exposure.

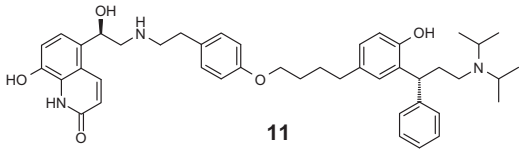
We next decided to explore structural and physicochemical-kinetics relationships of the linker on M3 offset and focused on replacing the polar amide moiety with an ether linkage. In particular, we concentrated on the aryl–alkyl and alkyl–alkyl ether connectivities present in the potent β-2 agonists formoterol and salmeterol (Fig. 1).[13] The functional tether from the tolterodine motif was created using 'bromotolterodine' **15**,[14] that was homologated using Heck reaction conditions to provide **16** (Scheme 2). Hydrogenation of the olefin and benzyl deprotection, followed by acid-mediated cleavage of the Boc group, revealed amine **17**, that was subsequently coupled to bromide **18**.[14] Debenzylation and

Table 1
Pharmacological and metabolic profiles of tolterodine, LABA **1** and MABAs 2-**11**[a]

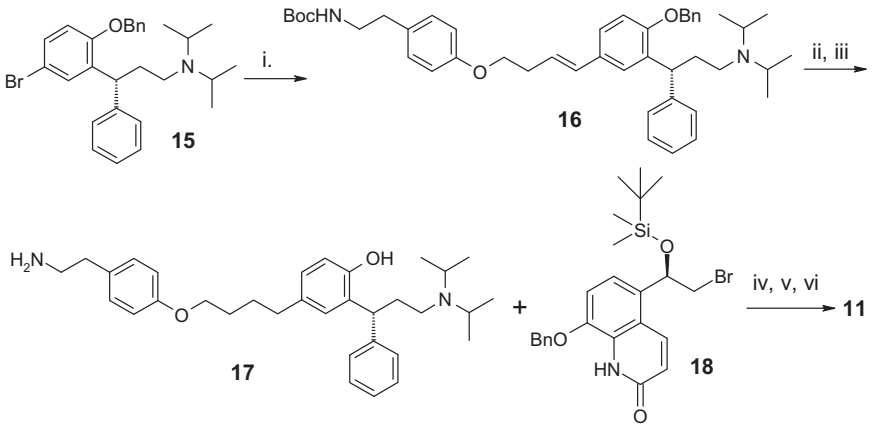
Compound	β-2 EC_{50}[b] (nM)	β-2-fold shift[b]	M3 K_i (nM)	M3 offset[c] (min)	MW	*c* Log *P*	HLM[d] (μl/min/mg)
 tolterodine	n/a	n/a	3.6	<15	325	5.2	163
 **1**	1.1	2.9	n/a	n/a	517	3.1	>440
 **2**	17	2.8	0.091	355	723	5.3	263
 **3**	8.1	1.1	0.276	166	787	5.2	109
 **4**	15	0..7	0.397	135	695	5.3	>440
 **5**	10	19	6.10	<15	583	3.4	nd
 **6**	116	7.3	1.45	nd	781	6.3	87
 **7**	13	1.9	0.634	>1200	732	6.3	67
 **8**	129	2.6	0.765	>1200	669	6.4	54
 **9**	173	nd	4.7	nd	654	6.9	nd
 **10**	5.7	0.7	0.99	>1200	712	5.6	57

(*continued on next page*)

Table 1 (*continued*)

Compound	β-2 EC_{50}[b] (nM)	β-2-fold shift[b]	M3 K_i (nM)	M3 offset[c] (min)	MW	*c* Log *P*	HLM[d] (μl/min/mg)
 **11**	2.4	1.1	0.305	>1200	706	6.6	74

[a] nd = not determined; n/a = not applicable.
[b] Washed and unwashed agonist potency of compounds at the β-2 adrenoceptor, by measuring their ability to stimulate intracellular cAMP production in CHO-hβ-2 cells.
[c] Offset kinetics from human cloned M3 receptor using [^{3}H] *N*-methyl scopolamine (dilution method).
[d] Clearance values in human liver microsomes.



Scheme 2. Synthesis of the ether-tethered series. Reagents: (i) Olefin[14], P(*o*-toluene)$_3$, Pd(OAc)$_2$, Hünigs base, MeCN; (ii) H_2, Pd/C, EtOH; (iii) 2 N HCl/Et_2O, EtOH; (iv) $NaHCO_3$, MeCN; (v) ammonium formate, Pd(OH)$_2$, MeOH; (vi) $Et_3N{\cdot}3HF$, MeOH, THF.

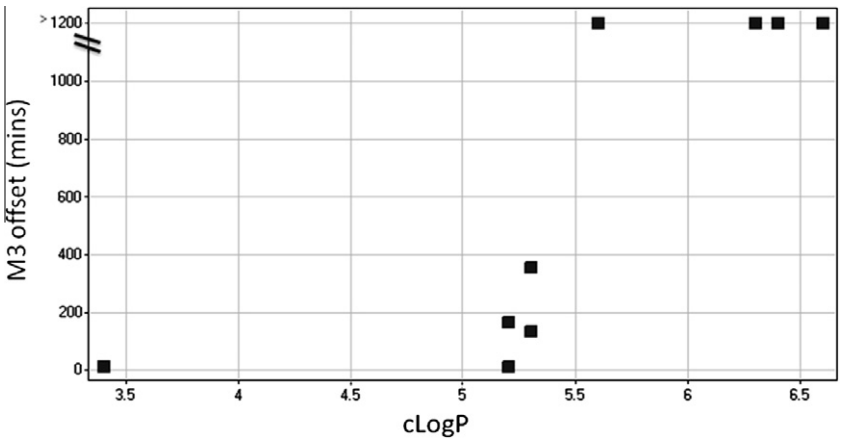


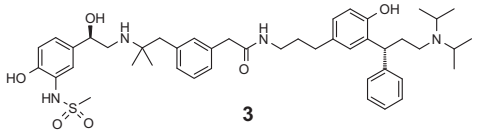
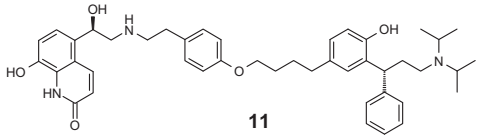
Figure 2. Relationship between M3 offset kinetics and lipophilicity (compounds **2**, **3**, **4**, **5**, **7**, **8**, **10**, **11** and tolterodine).

silyl ether deprotection furnished MABA **11** in an efficient manner. Other ether-linked MABAs were prepared using an analogs methodology.

All derivatives in the ether series were potent dual pharmacology MABAs, although β-2 potency for the saligenin **8** and resorcinol **9** was lower than expected (Table 1). Other β-2 head groups investigated were sulfonamide (**6** and **10**) and quinolinone (**11**).[12] Pleasingly, the M3 offset kinetics for this series were very impressive and a link with lipophilicity appears possible, (Figure 2). Again, the desired metabolic instability has been retained across the series (HLM >50 μl/min/mg).

We then assessed the potency of our key compounds in the guinea-pig trachea (GPT) model using electric field stimulation to release endogenous acetylcholine.[6] The drive for bronchoconstriction in this model operates through both β-2 and M3 mechanisms and therefore values here reflect combined bronchodilatory effects. A 'duration of action' was defined as the time taken for the muscle tone at a submaximal concentration of the compound to recover by 50% of the inhibition induced. As expected the amide **3** and ether **11** are potent bronchodilators in this model with respectable duration of action (Table 2).[15] To delineate the M3 drive, the β-2 antagonist propranolol was added to the EFS model to block the

Table 2
Ex vivo pharmacological profiles of MABAs[a]

Compound	GPT EFS EC_{50}[b] (nM)	GPT EFS duration $T_{50\%}$[b] (h)	GPT EFS + propranolol EC_{50}[c] (nM)	GPT EFS + propranolol duration[c] (h)	GPT histamine EC_{50}[d] (nM)
 **3**	1.3	>6.5	5.3	4.8	2.1
 **11**	4.1	>7.7	4.0	>10	4.0

[a] All data are geometric means with 95% CI, $n = 4$.
[b] GPT = Guinea pig trachea. EFS = Electric field stimulation. Potency and duration for β-2 and M3 components of bronchodilation. Submaximal concentration used for the duration is that which gives 70% inhibition of the EFS response.
[c] Potency and duration for M3 component of bronchodilation.
[d] Potency for β-2 component of bronchodilation.

β-2 effect. Amide **3** was found to retain the potency, but lost pharmacological duration, in line with the fast offset M3 kinetics. However, ether **11** retains both potency and duration of action, resulting from the long M3 offset. To confirm the β-2 pharmacological component, the GPT assay was modified to use histamine as the bronchoconstricting agent.[16] Both **3** and **11** are potent bronchodilators in this model as expected.

Ether **11** effected a dose-related inhibition of methacholine-induced bronchoconstriction in dogs[17] when dosed intratracheally, with an ED_{50} of 30 μg, duration of action >16 h and a therapeutic index over cardiovascular side effects (heart rate and contractility) threefold superior to salmeterol in this model, further supporting the exciting potential of this novel MABA.

As the product profile of a MABA requires formulation into a dry powder inhaler, either as a standalone agent, or as a combination therapy with an ICS, it is imperative to have the desired material properties. Following an extensive sitting-drop salt screen,[18] the fumarate salt of **11** was identified as a crystalline hit and converted on scale-up to a 1:1 fumarate hydrate (melting point 151.8–153.3 °C), thus enabling further formulation development of this compound. Additionally, ether **11** was clean in in vitro genetic toxicity testing (AMES and micronucleus) and wide-ligand profiling against a broad protein panel at CEREP, highlighting the exquisite selectivity that can be achieved in this dual pharmacological approach. Rat pharmacokinetics experiments on **11**, when combined with the poor membrane permeability and high metabolic instability,[19] predict negligible human oral bioavailability (<5%) thus minimizing potential risks from systemic exposure.

In conclusion, we have created novel MABAs using the principles of 'inhalation by design'. Balanced potency and pharmacological duration was combined with high metabolic clearance, low oral bioavailability, desirable material properties and minimized synthetic complexity, culminating in compound **11**. Further elaboration and development of the ether-tethered series will appear elsewhere.

Acknowledgements

We thank Farhat Hussain for investigating the scale-up of the quinolinone β-2 head group, and Paul Glossop, Graham Lunn and John Harvey for useful discussions.

References and notes

1. http://www.who.int/respiratory/copd/en/.
2. Cazzola, M.; Molimard, M. *Pul. Pharm. Ther.* **2010**, *23*, 257.
3. Welte, T. *Int. J. Clin. Pract.* **2009**, *63*, 1136.
4. (a) Ray, N. C.; Alcaraz, L. *Exp. Opin. Ther. Patents* **2009**, *19*, 1; (b) Cazzola, M.; Matera, M. G. *Br. J. Pharmacol.* **2008**, *155*, 291.
5. (a) Morphy, R.; Rankovic, Z. *J. Med. Chem.* **2005**, *48*, 6523; (b) Morphy, R.; Rankovic, Z. *J. Med. Chem.* **2006**, *49*, 4961; (c) Morphy, R.; Rankovic, Z. *Curr. Pharm. Des.* **2009**, *15*, 587.
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. Summerhill, S.; Stroud, T.; Nagendra, R.; Perros-Huguet, C.; Trevethick, M. *J. Pharm. Tox. Methods* **2008**, *58*, 189.
8. Kerns, E. H.; Di, L. *Drug-like Properties—Concepts, Structure Design and Methods—From ADME to Toxicity Optimization*; Elsevier: Burlington, 2008.
9. Jones, L. H.; Randall, A.; Napier, C.; Trevethick, M.; Sreckovic, S.; Watson, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 825.
10. Kumar, Y.; Prasad, M.; Nayyar, K.; Sharma, N. WO2005012227.
11. Watson, J.; Strawbridge, M.; Brown, R.; Campany, K.; Coghlan, M.; Trevethick, M. *Fund. Clin. Pharm.* **2008**, *22*(Suppl. 2), 69.
12. Glossop, P. A.; Price, D. A. *Ann. Rep. Med. Chem.* **2006**, *41*, 237.
13. Alikhani, V.; Beer, D.; Bentley, D.; Bruce, I.; Cuenoud, B. M.; Fairhurst, R. A.; Gedeck, P.; Baberthuer, S.; Hayden, C.; Janus, D.; Jordan, L.; Lewis, C.; Smithies, K.; Wissler, E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4705.
14. Jones, L. H.; James, K.; Price, D. A. WO2007107828.
15. Patel, S., Marshall, S., Summerhill, S.; Strawbridge, M.; Stanley, M.; Stuart, E.; Clarke, N.; Trevethick, M.; Yeadon, M.; Perros-Huguet, C. Presented at the European Respiratory Society Congress, Barcelona, September 2010; Poster 1232.
16. Roux, F. J.; Grandordy, B.; Douglas, J. S. *Am. J. Respir. Crit. Care Med.* **1996**, *153*, 1489.
17. Wright, K.; Davies, N.; Clarke, N.; Yeadon, M. Perros-Huguet, C. Presented at the European Respiratory Society Congress, Vienna, September 2009; Poster 2060.
18. Clark, D. *Am. Pharm. Rev.* **2004**, *7*, 76.
19. Rat intravenous pharmacokinetics (dose 2 mg/kg): AUC 2670 ng h/mL; clearance 12.6 mL/min/kg.