Bioorganic & Medicinal Chemistry Letters 19 (2009) 4471–4475

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

SAR of a series of inhaled A_{2A} agonists and comparison of inhaled pharmacokinetics in a preclinical model with clinical pharmacokinetic data

Simon J. Mantell *, Peter T. Stephenson, Sandra M. Monaghan, Graham N. Maw, Michael A. Trevethick, Michael Yeadon, Don K. Walker, Matthew D. Selby, David V. Batchelor, Stuart Rozze, Helene Chavaroche, Arnaud Lemaitre, Karen N. Wright, Lynsey Whitlock, Emilio F. Stuart, Patricia A. Wright, Fiona Macintyre

Pfizer Global Research and Development, Sandwich Laboratories, Ramsgate Road, Kent, CT13 9NJ, United Kingdom

ARTICLE INFO

Article history:
Received 26 February 2009
Revised 7 May 2009
Accepted 8 May 2009
Available online 12 May 2009

Keywords:
A2A
COPD
Pharmacokinetics
Inhalation

ABSTRACT

COPD is a major cause of mortality in the western world. A_{2A} agonists are postulated to reduce the lung inflammation that causes COPD. The cardiovascular effects of A_{2A} agonists dictate that a compound needs to be delivered by inhalation to be therapeutically useful. The pharmacological and pharmacokinetic SAR of a series of inhaled A_{2A} agonists is described leading through to human pharmacokinetic data for a clinical candidate.

The administration by inhalation of therapeutic agents that have the lung as their target organ is a well-accepted clinical practice. For example, the majority of currently prescribed asthma treatments, for example, β-agonists and inhaled corticosteriods, are all delivered by the inhaled route.[1] Chronic Obstructive Pulmonary Disease (COPD) affects 10–24 million adults in the USA.[2] This disease is characterised by chronic cough, mucus hypersecretion, breathlessness and a gradual decline in lung function. Neutrophils are implicated in the initiation, maintenance and symptomatology of COPD,[3] and are believed to play an important pathophysiological role.[4] Bronchial neutrophilia is the most significant cellular change in the disease, which correlates with airflow obstruction. Furthermore the most important risk factor for the development of COPD is cigarette smoking. However, since systemically available A_{2A} agonists were known to induce cardiovas-

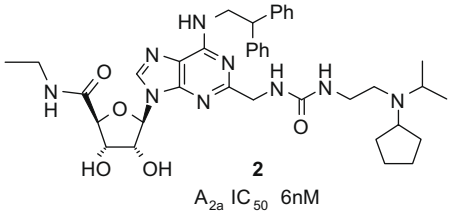


Figure 2. Structure of urea containing lead.

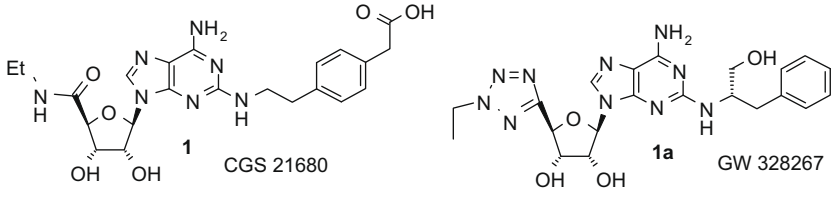


Figure 1. The structure of CGS 21680 and GW 328267.

* Corresponding author. Tel.: +44 01304 641375.
E-mail address: simon.mantell@pfizer.com (S.J. Mantell).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.05.027

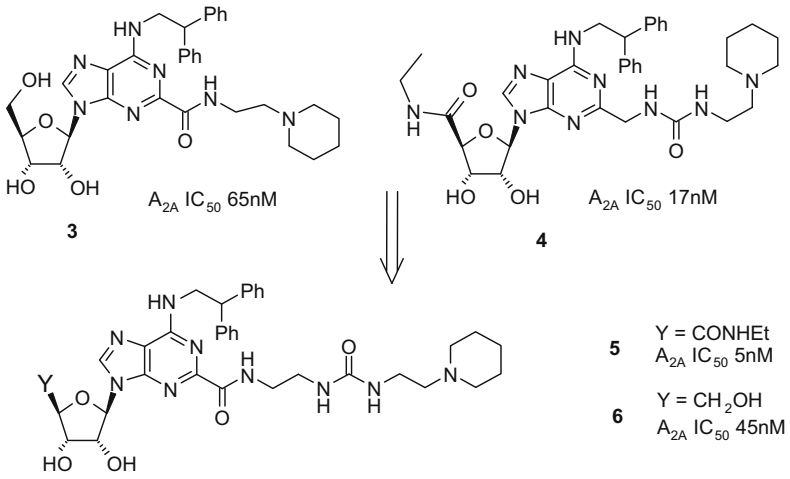


Figure 3. Combining purine linked amide and purine linked methylene urea A_{2A} agonist series.

cular effects, in particular hypotension, in laboratory animals,[5] inhaled administration of the agent was targeted from the outset. Intratracheal administration[6] of the adenosine A_{2A} agonist, CGS 21680 **1** inhibited lung inflammation in the rat but was associated with falls in blood pressure.[7] This has been supported by recent clinical trial data with the inhaled adenosine A_{2A} agonist GW 328267 **1a** which caused hypotension and tachycardia at doses that limited clinical utility.[8]

By optimisation of the pharmacokinetic and physiochemical properties of the compounds in order to minimise systemic

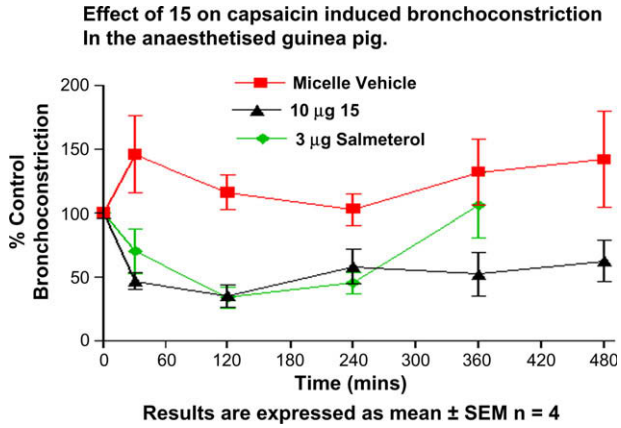


Figure 4. Duration of action of **15**.

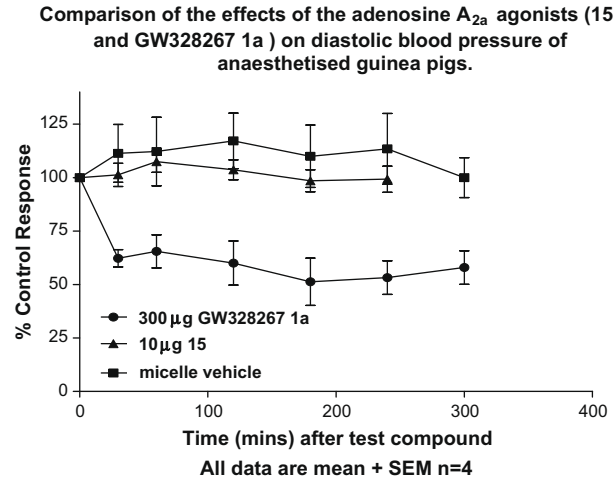
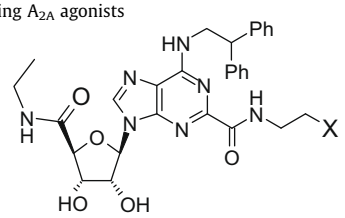


Figure 5. Side effect liability of **15**.

Table 1
SAR of urea containing A_{2A} agonists



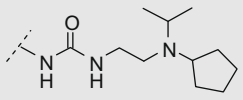
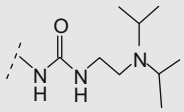
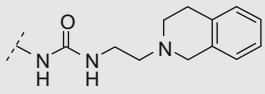
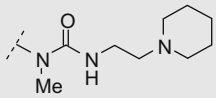
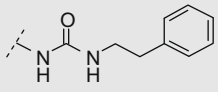
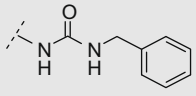
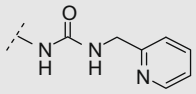
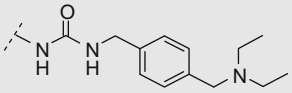
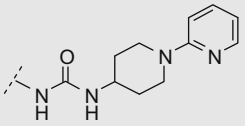
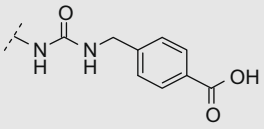
Compound	X=	A_{2a} IC_{50}[12]
7		7
8		5
9		10
10		305
11		10
12		6
13		4

Table 1 (*continued*)

Compound	X=	A_{2a} IC_{50}[12]
14		5
15		4
16		0.4

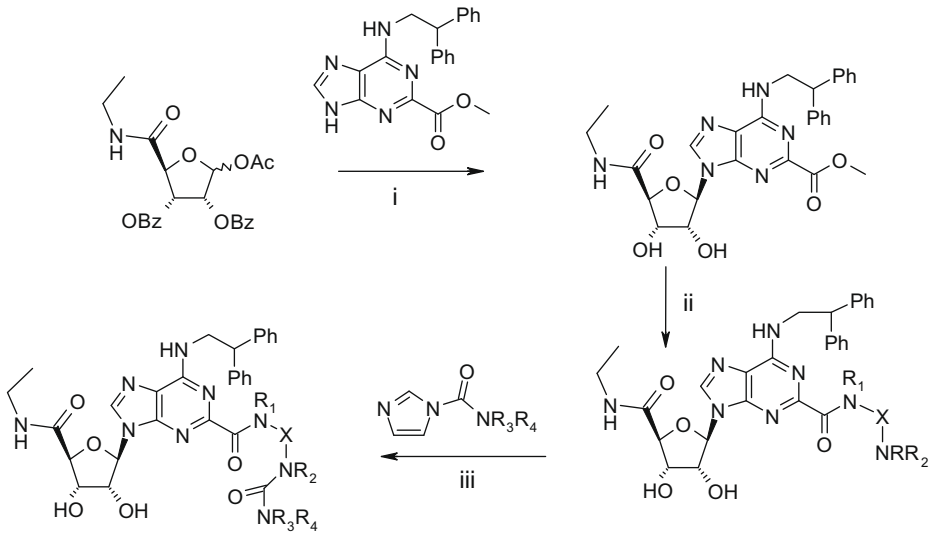
exposure after delivery by inhalation, we hoped to identify an efficacious compound with an acceptable therapeutic index. In a previous publication in this journal we described our strategy for achieving lung selectivity and the SAR of a series of A_{2A} agonists, leading up to the discovery of **2**.[9]

Compound **2** showed high potency and projected selectivity for lung over cardiovascular effects. However, when a solution of the compound in an organic solvent was washed with a saturated aqueous solution of sodium hydrogen carbonate **2** appeared to form a carbonate adduct that was stable to the buffer used in our human neutrophil assay and to dosing to our in-vivo model.[10] This complex had a lower affinity for the A_{2A} receptor than the parent. Since the compound will be exposed to carbon dioxide in the lung the observation of adduct formation meant that **2** could not be progressed as a clinical candidate as it would be very difficult to estimate doses required for efficacy. Thus, our A_{2A} SAR exploration continued with the objective of finding a compound with a similar pharmacological and pharmacokinetic profile to **2** but without the problem of carbon dioxide complexation. The first step in achieving these objectives was to determine which of the structural features was responsible for carbon dioxide absorption. Therefore the tendency of a number of structures related to **2** to absorb carbon dioxide was evaluated. This study consisted of trying to form the complex by washing a solution of the uncomplexed compound in an organic solvent with saturated aqueous sodium hydrogen carbonate solution, drying the organic phase with anhydrous magnesium sulphate, filtering and solvent removal. The ^{1}H NMR was inspected to determine whether it was different to a spectrum taken immediately prior to washing with the sodium hydrogen carbonate solution. These fairly crude experiments indicated that the presence of the urea linked through to a basic nitrogen by a two carbon chain led to carbon dioxide complexation. As described previously,[9] the SAR of the series from which **2** is derived is fairly tight, altering the urea portion or removing the basicity of the nitrogen loses activity. Therefore, it became necessary to look at changing **2** more substantially in order to move away from the carbon dioxide absorption properties. Previous SAR[9] had also shown that potent compounds could be obtained if a basic substituent was linked through to the purine via an amide **2**. Therefore, this modification was incorporated into the series containing a urea in the side chain (Figs. 1–5).

Adding together the purine sidechains in **4** and **3** gave two potent compounds **5**, where Y = CONHEt, and **6**, where Y = OH. Compound **5** was significantly more active than **6** so the series Y = CONHEt was pursued. **5** and all the others in Table 1 were prepared using the synthetic route in Scheme 1.[11]

A series of compounds where the urea containing sidechain of **5** was modified were prepared.

Incorporation of the urea containing sidechain that gave good activity in **2** to give **7** gave similar potency. The comparable activity of **7** to **9** demonstrates that there is a substantial amount of flexibility in what substituents on the basic nitrogen will give activity. However, methylation of the urea portion of **5** to give **10** causes a drop off in activity. With the knowledge that there is flexibility in the SAR of the basic nitrogen substituents but that this may be more limited in the urea portion, a series of compounds that did not contain the basic centre in **5** were synthesised. The benzyl **12** and phenethyl **13** derivatives were synthesized. The benzylic derivative **12**, was found to be slightly more potent. The close in SAR around the benzylic substituent of **12** was then examined. Replacement of the phenyl in **12** with 2-pyridyl to give **13**



Scheme 1. Reagents and conditions: (i) *N*,*O*-Bis(trimethylsilylacetamide, then purine and TMSOTf, toluene, reflux, 2.5 h; (ii) sodium carbonate, methanol, rt, 4 h; (iii) HNR_1–X–NRR_2, 105 °C; (iv) toluene/isopropyl alcohol 4:1, reflux.

Table 2
Potency, molecular properties and side affect liability for analogues

Compound	A_{2A} IC_{50} (nM)	HLM Cl_{int}[a] (μl/min/mg)	Rat Clu[b] (ml/min/kg)	TPSA[c]	Log *D* pH 7.4	MW	Free C_{max}[d] (nM)
2	6	135	3500	179	2.0	713	1.8
8	5	81	750	208	0.9	755	12.3
15	4	>255	5500	221	3.4	778	0.2

[a] Human Liver Microsome (HLM) stability given as intrinsic clearance (Cl_{int}).
[b] Rat unbound clearance from IV bolus PK at 1 mg/kg.
[c] Topological polar surface area.
[d] Free C_{max} was dose normalised to 1 mg/kg IT, all compounds were delivered as solutions.

Table 3
Pharmacokinetics properties of compound **15** in healthy male humans

Dose (μg)	*n*	C_{max}[a] (nM)
100	7	0.45
200	3	0.30
200	6	0.30
400	9	0.19
800	10	0.19
1600	7	0.12

[a] Free C_{max} was dose normalised to 1 mg/kg.

increased potency slightly, as did addition of a basic substituent onto the phenyl ring to give **14**. Addition of a carboxylic acid to the phenyl ring in **12** to give **16** increased potency 15 fold. Incorporation of a piperidylpyridine sidechain gave a potent compound, **15.** The 2-aminopyridine in **15** is basic enough to allow salt formation and hence aid solid form selection but because of conformational restriction of the sidechain it was hypothesised that it might not form a complex with carbon dioxide. **15** was found not to complex carbon dioxide and was progressed to in vivo studies. In order to compare the duration of action of a compound in the lung with its activity in the systemic circulation a model where A_{2A} activity in the lung and the systemic circulation could be determined simultaneously was required. Unfortunately, due to differences in A_{2A} receptor pharmacology between man and common laboratory animals, the development of such an assay with a neutrophil-dependant endpoint was not possible. An alternative model was thus adopted based on the ability of intrathecally dosed A_{2A} agonists to inhibit intravenously dosed capsaicin induced bronchoconstriction in the anaesthetised guinea pig.[13]

Compound **14** showed a >5 h duration in the capsaicin induced bronchoconstriction model at the same dose that showed no effects on diastolic blood pressure after intrathecal administration in the guinea pig model.

This is a significantly extended duration of action compared to salmeterol and indicates that the compound could be expected to be dosed twice daily in the clinic at worst and most likely once a day. It is unclear why the duration of action is extended. Possible factors include compound onset/offset kinetics,[14] an exosite binding receptor as postulated for salmeterol,[15] partitioning of lipophilic amines into the lipid bilayers of smooth muscle,[16] poor permeability from the lung into systemic circulation or slow dissolution of a compound that has crystallised after inhalation. The overall systemic side-effect liability for an A_{2A} inhaled agent is thought to be dependent upon the free plasma C_{max} achieved following inhalation. Side effect liability was assessed by comparing the compounds normalised free C_{max} (i.e., free C_{max}/dose) subsequently referred to as free C_{max} in this text. This assumes that the required dose will be proportional to the potency of the compound, the free plasma concentrations will also scale proportionally to this dose and hence the effect of potency on side effect liability cancels out. As can be seen in Table 3 the first compound in the series profiled **8**, was found to have a higher C_{max} than **2**. This was concerning but consistent with our previous observations[9] that C_{max} is at least partly driven by unbound clearance (clearance/free fraction). The unbound clearance for **8** is 750 ml/min/kg, approximately fivefold lower than for **2** and the C_{max} of **8** is approximately fivefold higher. Comparison of **2** with compound **15** also shows that increasing unbound clearance reduces C_{max} but there appears a much greater reduction in C_{max} than would be expected from the increase in unbound clearance, there is only a 1.6-fold increase in unbound clearance but a ninefold drop in the C_{max}. This shows that it is unlikely that increasing clearance is the sole contributor to reducing C_{max} for compound **15** and that increasing the lipophilicity and molecular weight of the molecules is impacting on other factors that affect C_{max}, most likely slower transfer from lung to blood. Contribution to systemic levels of the compounds in Table 2 by absorption from the gut is highly unlikely given the very high Topological Polar Surface Area (TPSA) of the compounds.[17] The passive transcellular diffusion rate of compound **15** was found to be negligible in a Parallel Artificial Membrane Permeation assay, further supporting our hypothesis that none of the compounds in Table 2 would be significantly absorbed from the gut. **15** was taken forward into phase I studies in man.

Healthy male subjects (age 18–45 years) in 2 cohorts received single escalating doses of **15** administered by an aerosol device over the dose range 100–1600 μg. Plasma drug concentrations were determined at doses above 100 μg with a validated analytical method using HPLC with mass spectrometric detection. Pharmacodynamic measurements of haemodynamic parameters were recorded up to 4 h post dose along with standard safety evaluation at times during the study. Mean half-life values for **15** were in the range 3–6 h across the dose range studied. Exposure increased with increasing dose although in a subproportional manner (Table 3). C_{max} values were similar to those observed in the preclinical model.

There were no clinically significant effects on sitting blood pressure measurements or peak expiratory flow rate. A dose dependent increase in sitting pulse rate was observed between 200 and 1600 μg, these effects were generally not considered to be clinically significant.

In conclusion, a series of potent A_{2A} agonists have been identified. Compound **15** showed >8 h activity in a guinea pig lung based duration of action model and low side effect liability in a rat cardiovascular side effect liability model has been identified. Compound **15** has been taken through to the clinic and at doses predicted to have antineutrophil effects in the lung no clinically significant side effects were observed.

Acknowledgements

The authors of this manuscript would like to thank Petra Chaffe, Adrian Barnard, Gary Salmon, Rob Webster and Gill Allen for their invaluable contributions to generating the data in this manuscript. The senior author would like to highlight the identification of the chemical series disclosed herein by Peter Stephenson and the

identification of compound **15** by Peter Stephenson and David Batchelor as being critical contributions to this Letter.

References and notes

1. Dalby, R.; Suman, J. *Adv. Drug Delivery Rev.* **2003**, *55*, 779.
2. Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2006. Global Strategy for Diagnosis, Management and Prevention of COPD. www.goldcopd.com.
3. Wise, R. A.; Tashkin, D. P. *Am. J. Med.* **2007**, *120*, S4.
4. Stockley, R. A. *Quart. J. Med.* **1995**, *88*, 141.
5. Hutchison, A. J.; Webb, R. L.; Oei, H. H.; Ghai, G. R.; Zimmerman, M. B.; Williams, M. *J. Pharm. Exp. Ther.* **1989**, *251*, 47.
6. The micelle vehicle used for dosing the compounds in the in-vivo model is prepared by dissolving 1 mg of compound in 0.66 g of a 0.9% w/v aqueous sodium chloride solution and 0.39 g of a solution that was prepared by mixing 11.55 g of glycerol formol, 0.56 g of benzyl alcohol and 8.97 g of 12-hydroxystearate (BASF Aktiengesellschaft solutol HS15).
7. Fozard, J. R.; Ellis, K. M.; Villela Dantas, M. F.; Tigani, B.; Mazzoni, L. *Eur. J. Pharmacol.* **2002**, *438*, 183.
8. Trevethick, M. A.; Mantell, S. J.; Stuart, E. F.; Barnard, A.; Wright, K. N.; Yeadon, M. *Br. J. Pharmacol.* **2008**, *155*, 463.
9. Mantell, S. J.; Stephenson, P. T.; Monaghan, S. M.; Maw, G. N.; Trevethick, M. A.; Yeadon, M.; Keir, R. F.; Walker, D. K.; Jones, R. M.; Selby, M. D.; Batchelor, D. V.; Rozze, S.; Chavaroche, H.; Hobson, T. J.; Dodd, P. G.; Lemaitre, A.; Wright, K. N.; Stuart, E. F. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1284.
10. Ureas are known to form strong complexes with carboxylic acids in the solid phase: Schlenk, H.; Holman, R. T. *J. Am. Chem. Soc.* **1950**, *72*, 5001; Smith, G.; Baldry, K. E.; Byriel, K. A.; Kennard, C. H. L. *Aust. J. Chem.* **1997**, *50*, 727.
11. Synthetic procedures for all compounds are given in Mantell, S. J.; Stephenson, P. T. WO2001094368.
12. A_{2A} Screening details in WO2002000676.
13. Morimoto, H.; Yamashita, M.; Imazumi, K.; Matsida, A.; Ochi, T.; Seki, N.; Mizuhara, H.; Fujii, T.; Senoh, H. *Eur. J. Pharmacol.* **1993**, *240*, 121. All experiments using animals were carried out in compliance with UK Legislation and subject to local ethical review.
14. Disse, B.; Speck, G. A.; Rominger, K. L.; Witek, T. J.; Hammer, R. *Life Sci.* **1999**, *64*, 457.
15. Nials, A. T.; Sumner, M. J.; Johnson, M.; Coleman, R. A. *Br. J. Pharmacol.* **1993**, *108*, 507.
16. Anderson, G. P.; Linden, A.; Rabe, K. F. *Eur. Respir. J.* **1994**, *7*, 569.
17. Hou, T.; Wang, J.; Zhang, W.; Xu, X. *J. Chem. Inf. Model.* **2007**, *47*, 208.