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Inhaled adenosine A_{2A} receptor agonists for the treatment of chronic obstructive pulmonary disease

Simon J. Mantell,* Peter T. Stephenson, Sandra M. Monaghan, Graham N. Maw, Michael A. Trevethick, Michael Yeadon, Ruth F. Keir, Don K. Walker, Rhys M. Jones, Matthew D. Selby, David V. Batchelor, Stuart Rozze, Helene Chavaroche, Tim J. Hobson, Peter G. Dodd, Arnaud Lemaitre, Karen N. Wright and Emilio F. Stuart

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

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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. A strategy of minimizing side-effect liability by maximizing systemic clearance was followed and pharmacological and pharmacokinetic SAR of a series of inhaled A_{2A} agonists described. A sevenfold improvement in potency and 150-fold reduction in side-effect liability over the lead compound CGS-21680, were obtained.

The administration via 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 via the inhaled route. However, since the development of inhaled agents is both more lengthy and costly compared to oral approaches, the choice of this mode of delivery is usually only made when an oral approach is not viable. In both of the above examples, this reason is one of safety, with inhalation providing a greater therapeutic index over unwanted systemic side effects than would be obtained by dosing through the oral route. Thus, inhalation has allowed the exploitation of clinically efficacious mechanistic approaches that would otherwise not be viable.[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. Currently available therapies for COPD are mainly inhaled bronchodilators (β-2 agonists, muscarinic antagonists) and corticosteroids either alone or in combination and when dosed chronically they improve lung function. Whilst more research with combination therapies of bronchodilator plus corticosteroid may show increased benefit compared to monotherapy[3] at the moment corticosteroids are known to work well to control asthma but their effects on COPD are less pronounced. Thus, there is a clear need to develop more effective anti-inflammatory approaches to treat COPD.

COPD is a disease that is characterised by chronic inflammation. Neutrophils are implicated in the initiation, maintenance and symptomatology of COPD, 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. Exposure of experimental animals to cigarette smoke and other irritants, such as ozone or sulphur dioxide, leads to rapid accumulation of neutrophils in pulmonary tissue. [5] Activated neutrophils can damage lung tissue by release of reactive oxygen species (superoxide) and granule products such as Human Neutrophil Elastase (HNE), amongst other inflammatory mediators.[6] In addition, activated neutrophils synthesise and release arachidonate products such as leukotriene B_4 (LTB_4). The latter is a potent chemo-attractant that recruits additional neutrophils to the

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* Corresponding author. E-mail: simon.mantell@pfizer.com

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inflammatory focus,[7] whereas superoxide and HNE can adversely affect pulmonary extracellular matrix.[7] HNE has also been shown to act as a potent mucus secretagogue.[7] Several lines of evidence suggest that agonists of the A_{2A} receptor might represent an attractive target to treat lung inflammation in COPD. For instance, stimulation of A_{2A} receptors on the surface of human neutrophils inhibits many pro-inflammatory processes,[8] and mice deficient in the A_{2A} receptor have enhanced lung inflammation in response to an inflammatory stimulus[9] suggesting that adenosine A_{2A} agonists may represent a novel therapy to treat lung inflammation. However, since systemically available A_{2A} agonists were known to induce cardiovascular effects, in particular hypotension, in laboratory animals,[10] inhaled administration of the agent was targeted from the outset. Intratracheal administration of the adenosine A_{2A} agonist, CGS-21680 **1**, inhibited lung inflammation in the rat but was associated with falls in blood pressure.[11] By optimisation of the pharmacokinetic and physiochemical properties of the compounds in order to minimise systemic exposure, we hoped to identify an efficacious compound with an acceptable therapeutic index.

The pharmacokinetic objectives for a compound delivered by inhalation to act in the lung are very different from those for an orally administered agent. The relevant pharmacokinetic processes are represented schematically in Figure 1.

Following inhalation, the drug may deposit in the lung or be swallowed. Drug in the lung is able to exert the desired pharmacological action. The material that has been swallowed may be absorbed into systemic circulation. It has been documented that after the inhalation of drugs a significant contribution to the systemic effects can come from absorption of the swallowed portion.[12] Therefore, to prevent this swallowed portion from contributing to side-effect liability, it is desirable to reduce drug oral bioavailability. This can be done by limiting absorption across the gut wall and ensuring high hepatic turnover of the absorbed fraction.[13] Once systemic effects from orally absorbed drug have been minimised the drug concentrations in the lung will greatly exceed those systemically and this situation will be maintained provided that the rate of clearance of the compound is greater than absorption. In order to avoid systemic side effects, it is desirable for compounds to be cleared by mechanisms that do not result in the formation of pharmacologically active metabolites (see Figs. 2 and 3).

In order to compare the lung activity of a compound with its activity in the systemic circulation a model of

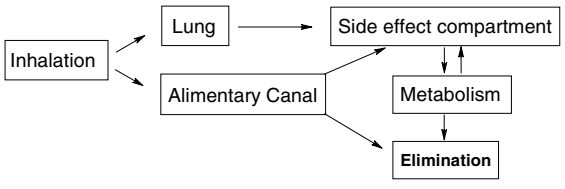


Figure 1. Processes affecting the distribution of inhaled drug.

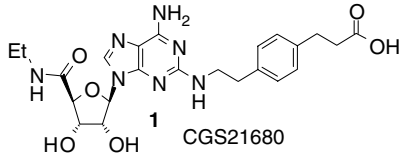


Figure 2. The structure of CGS-21680.

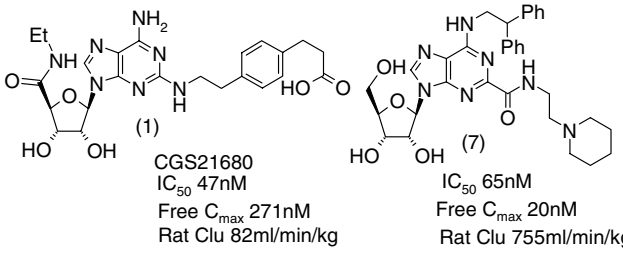


Figure 3. Structures and systemic levels of CGS21680 and compound **7**.

activity within the lung 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 A_{2A} agonists to inhibit capsaicin-induced bronchoconstriction in the anaesthetised guinea pig.[14] In this model intratracheal dosing of the test compound was followed by i.v. administration of capsaicin at hourly intervals to induce bronchoconstriction. The inhibition of this bronchoconstriction by the A_{2A} agonist was used to establish a time course for activity of the compound within the lung. Simultaneous measurement of cardiovascular parameters during the experiment allowed for any potential side effects arising from systemic exposure of the A_{2A} agonist to be observed. Benchmarks in this assay were provided by the β-agonists salbutamol and salmeterol which had durations of action of ~2 and 5 h, respectively, correlating well with the clinically used dosing regimes of q.i.d. and b.i.d., respectively. With our target dosing frequency being no more than twice daily, ideally once-daily dosing, duration of action in this model in excess of 5 h was set as our target criteria. 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 at this dose and hence the effect of potency on side effect liability cancels out.

The standard A_{2A} agonist **1** was used as an initial benchmark for both in vitro and in vivo studies.

The anti-neutrophil activity of this compound was assessed by its ability to inhibit fLMP-stimulated superoxide production in isolated human neutrophils, an

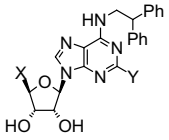
adenosine A_{2A} receptor mediated process. In this assay **1** showed good potency with an IC_{50} of 47 nM. In the guinea pig capsaicin-induced bronchoconstriction model, a 300 μg dose of **1** produced near maximal inhibition of bronchoconstriction at 15 min post dose. However the effect was short lived, with the level of inhibition returning to baseline values by 60 min, an inferior profile even compared to the clinically short acting β-agonist salbutamol. At the same time as effects on bronchoconstriction were observed there were cardiovascular effects, with falls in diastolic blood pressure of up to 30%. This profile indicated the compound that had high plasma levels and thus systemic side effects. This was confirmed in rat inhaled pharmacokinetic studies where, following a normalised 1 mg/kg intratracheal dose, a high free systemic C_{max} of 271 nM was observed. In order to reduce systemic levels and thus side effects a decision was taken to increase systemic clearance of free drug. Our strategy to achieve this was to increase the molecular weight and lipophilicity of the molecules, properties considered to be consistent with increased enzyme and transporter-mediated clearance. However, modulating these properties may affect other factors that will influence systemic levels and how rapidly the compounds permeate from the lung into systemic circulation. This strategy was put into place by replacement of the primary amine on the purine ring of **1** with diphenylethylamine[15] and the carboxylic acid functionality in the phenethylamine side-chain with alkyl and alkylamino groups.

Initially our attempts to increase the lipophilicity and molecular weight of **1** led to a reduction in potency, compounds **2** and **3** were 20-fold weaker. Methoxy compound **3** was found to be similar in potency to the N-ethylcarboxamide derivative **2**. Compounds **4**–**7** demonstrate that reasonable potency was obtained with a basic centre in various positions of the sidechain. Although **7** did not have our desired level of potency its pharmacokinetic profile was determined to test our hypothesis that increasing unbound clearance by raising molecular weight and lipophilicity would reduce free systemic levels after administration by inhalation.

The free C_{max} of **7** is 10-fold lower than compound **1** and therefore it should exhibit an improved safety profile. The compound showed no effects on diastolic blood pressure after administration of the same dose by inhalation in the guinea pig model. Our next objective was to increase the potency of compound **7**. The SAR in Table 1 had already demonstrated that good activity could be obtained with basic amines in various positions of the purine sidechain. Thus a series of ureas that contained a basic centre was prepared (see Table 2).

Replacement of the terminal hydroxyl in compound **7** with N-ethylcarboxamide gave **10** which had led to a sevenfold increase in activity. Replacement of the amide portion of the sidechain in **10** with the methylene-linked urea in **8** led to a slight drop in potency. Increasing substitution around the basic nitrogen in **8** to give **9** and **11** improved potency slightly. The drop in activity on going from **11** to compound **12** indicated that there was a limit to how much increasing the size and lipophilicity of the amine substituents increased potency. Rat inhaled pharmacokinetic studies revealed that the free C_{max} of compound **11** was 11-fold lower than the free C_{max} for **7** and 150-fold lower than **1** (see Table 3). The systemic exposure from the swallowed portion of the dose for **1**, **7** and **11** is likely to be low as all 3 compounds exhibit low membrane permeability in the

Table 1. SAR of A_{2A} agonists



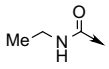
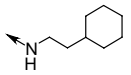
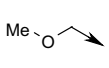
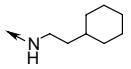
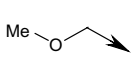
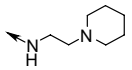
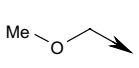
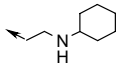
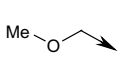
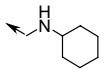
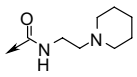
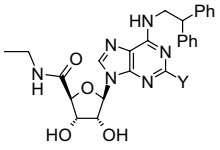
Compound	X	Y	A_{2A} IC_{50} nM[16]
2			1000
3			1010
4			60
5			28
6			100
7			65

Table 2. SAR of amine containing A_{2A} agonists



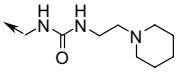
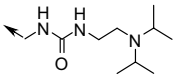
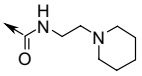
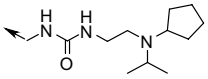
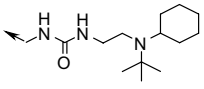
Compound	Y	A_{2A} IC_{50} nM
8		17
9		11
10		9
11		6
12		33

Table 3. Potency, molecular properties and side-effect liability for analogues

Compound	A_{2A} IC_{50} nM	HLM Cl_{int} (μl/min/mg)[a]	Rat Cl (ml/min/kg)[b]	Rat Clu (ml/min/kg)[b]	Pampa (10^{-6} cm/s)[c]	log *D* pH 7.4	MW	Free C_{max} (nM)[d]
1	47	<8.0	14	82	<0.2	−0.15	499	271
7	65	62.6	48	755	0.2	2.1	602	20
11	6	135	45	3500	<0.7	2.0	713	1.8

[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] Parallel Artificial Membrane Permeation Assay (PAMPA) for passive transcellular diffusion rate.
[d] Free C_{max} was dose-normalised to 1 mg/kg IT, all compounds were delivered as solutions.

in vitro PAMPA assay and moderate systemic clearance in the rat in vivo. The reason for the reduction in free C_{max} between compounds maybe the increase in unbound clearance. A 9-fold increase in unbound clearance correlates with a similar decrease, 14-fold, in free C_{max} going from **1** to compound **7**. However, an increase in unbound clearance of 43-fold results in a super proportional decrease in free C_{max} of 151-fold going from **1** to compound **11**. This shows that it is unlikely that increasing clearance is the sole contributor to reducing free C_{max} for compound **11** and that increasing the lipophilicity and molecular weight of the molecules is impacting on other factors that affect free C_{max}. Compound **11** showed a >5 h duration in the capsaicin induced bronchoconstriction model at the same dose that showed no effects on diastolic blood pressure after administration by inhalation in the guinea pig model. It is unclear why the duration of action is extended. Possible factors include compound onset/offset kinetics,[17] an exosite binding receptor as postulated for salmeterol,[18] partitioning of lipophilic amines into the lipid bilayers of smooth muscle,[19] poor permeability from the lung into systemic circulation or slow dissolution of a compound that has crystallised after inhalation. Pharmacokinetic studies in the rat showed that compound **7** was cleared by excretion of parent molecule via the bile and that the compound was not absorbed from the gastrointestinal tract. This clearance mechanism was also observed for a number of similar derivatives (and CGS-21680) and it is thus likely that compound **11** was similarly eliminated. However, since this was not determined experimentally, it is possible that active metabolites may have contributed to side-effect liability for compound **11**.

In conclusion, the pharmacokinetic objectives for a compound delivered by inhalation to act selectively in the lung are very different from those for an orally administered agent. Systemic drug concentrations are often irrelevant to the targeted pharmacological effect, but it is usually desirable to minimise systemic concentrations to reduce potential side effects. In this paper we have shown that a strategy of targeting high systemic clearance by increasing molecular weight and lipophilicity can lead to an optimal profile for an inhaled therapeutic agent. Compound **11** combines long pharmacological duration of action in the lung with low systemic plasma levels. Applying our strategy to a series of adenosine A_{2A} agonists has allowed the identification of compounds which may have utility in the treatment of COPD.

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