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2,6-Diaryl-4-acylaminopyrimidines as potent and selective adenosine A_{2A} antagonists with improved solubility and metabolic stability

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ABSTRACT

In this report, the strategy and outcome of expanding SAR exploration to improve solubility and metabolic stability are discussed. Compound **35** exhibited excellent potency, selectivity over A_1 and improved solubility of >4 mg/mL at pH 8.0. In addition, compound **35** had good metabolic stability with a scaled intrinsic clearance of 3 mL/min/kg (HLM) and demonstrated efficacy in the haloperidol induced catalepsy model.

Adenosine is considered to be one of the human body's most important neuromodulators, in both the central and peripheral nervous systems.[1] The effects of this purine nucleoside are modulated via four receptor subtypes: A_1, A_{2A}, A_{2B} and A_3.[2] These four subtypes belong to the family of seven trans-membrane G-protein coupled receptors (GPCRs).[3] Adenosine A_{2A} receptors are highly distributed in the central nervous system and are found in abundance in the basal ganglia, a region of the brain associated with motor function.[4] A number of A_{2A} receptor antagonists have been shown to improve motor disabilities in animal models of Parkinson's disease.[5] Parkinson's disease is a debilitating motor disorder arising from the progressive degeneration of dopaminergic neurons in the nigrostriatal pathway.[6] Unfortunately, current dopamine replacement therapies for Parkinson's disease suffer from poor long term control and undesirable side effects, namely dyskinesia (involuntary movements). A number of companies have progressed A_{2A} antagonists into clinical trials including KW-6002 (istradefylline) from Kyowa Hakko Kogyo, which showed efficacy in alleviating symptoms of the disease in Phase II clinical trials.[7] In addition, Schering-Plough has progressed SCH 420814 into Phase II clinical trials (Fig. 1).[8]

Previously, we reported on a series of non-xanthine based A_{2A} antagonists which incorporated a pyrimidine core. A number of compounds from our initial exploration, including compound **1**, showed good in vivo efficacy in rat models of Parkinson's disease.[8] However, as this series of compounds contained an unsubstituted furan ring, we sought to replace this heterocycle (Fig. 2).[9] It has been well documented that unsubstituted furan rings can be metabolized to form reactive intermediates, which in turn can form protein adducts leading to liver toxicity.[10] A number of heterocycles were surveyed in the context of the right hand side methyl piperazine. Although compound **2** was less active than the starting lead **1**, replacement of the furan with a dimethyl pyrazole was tolerated and significantly decreased binding against the A_1 receptor. In addition, we found that by removing the bulky right hand side methyl piperazine (**3**), the potency against the A_{2A} receptor was increased. We hoped to further benefit from these findings by utilizing the dimethyl pyrazole as a furan replacement and eliminating the need for a bulky right hand side substituent. In an effort to further increase potency, an SAR exploration was undertaken to replace the left hand side heterocycle with previously unexplored substituted phenyl groups.

Compounds **8**–**35** were prepared from intermediate **4**[9] according to the general synthesis outlined in Scheme 1. Compounds **8**–**22** were prepared in one step by Suzuki coupling with suitable

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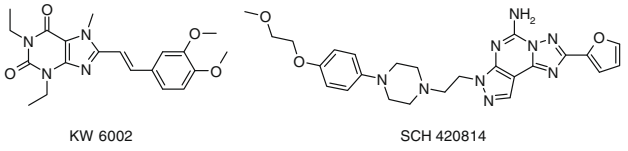
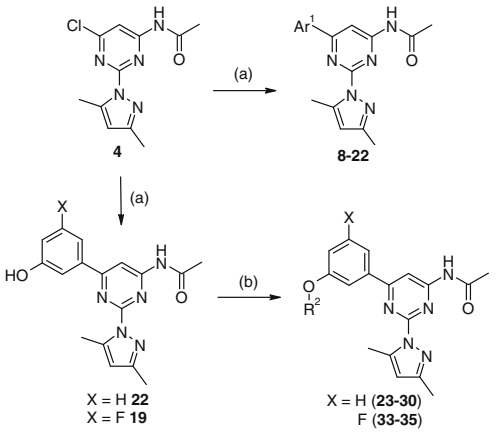


Figure 1. Examples of A_{2A} antagonists in clinical development.

commercially available boronic acids. For compounds **23**–**30** and **33**–**35**, intermediate **4** was first reacted with either 3-hydroxyphenylboronic acid (**23**–**30**) or 3-fluoro-5-hydroxyphenylboronic acid (**33**–**35**) by Suzuki coupling to yield intermediates **22** and **19**, respectively. The resulting intermediates were reacted with alcohols via a Mitsunobu reaction to yield final compounds. Likewise, compounds **31**–**32** were prepared by coupling intermediate **4** with 3-(hydroxymethyl)phenylboronic acid, followed by Mitsunobu reaction with the appropriate alcohol in a similar fashion to compounds 23–30 and 33–35.

Replacement of the left hand side heterocycle with a simple phenyl group (**8**) showed modest A_{2A} activity but poor selectivity over the A_1 receptor (K_i of 87 nM and A_1/A_{2A} of 7). However, encouraged that some potency remained, we further explored various substituents. By the addition of a methoxy group (**9–11**), potency and selectivity were greatly increased. In particular, substitution in the *ortho* (**9**) and *meta* (**10**) positions gave very potent compounds with K_is of 2 and 1 nM, respectively, and selectivity of ~70-fold over A_1. A further boost in potency came upon the addition of another methoxy substituent, as in the case of 3,5-dimethoxy phenyl (**18**). Compound **18** not only showed an increase in potency (K_i of 0.2 nM) but the A_1 selectivity was 111-fold. In addition to having very good potency and selectivity, compound **18** was potent in an A_{2A} functional assay (cAMP IC_{50} of 29 nM).[11] Due to the promising in vitro profile, compound **18** was selected for in vivo efficacy evaluation. The haloperidol induced catalepsy (HIC) model in rat was used as the primary assay to screen compounds for efficacy.[12] Compound **18** showed good efficacy in the HIC model with a minimum efficacious dose of 1 mg/kg p.o., however, it was determined to have poor aqueous solubility (<0.1 mg/mL at pH 8.0).[13] In addition, upon further profiling, compound **18** showed time dependant inhibition of *CYP*3A4. Compound **21** also showed promising potency and selectivity with a K_i of 3 nM and selectivity of 164-fold over A_1. Unfortunately, compound **21** also exhibited poor aqueous solubility of <0.1 mg/mL at pH 8.0. From this initial survey, we determined that *meta* substitution off of the phenyl ring was preferred. Also, the incorporation of a hydrogen bond acceptor, as in the case of the 3-methoxyphenyl and 3,5-dimethoxyphenyl, increased A_{2A} potency. However, poor solubility of the most promising compounds, and the inhibition of a major *CYP* enzyme for compound **18**, prevented further development. As such, an SAR exploration was initiated to improve solubility while maintaining potency at A_{2A}. The idea was to introduce a basic center off of the phenyl group, while maintaining the preferred *meta* substitution pattern and a hydrogen bond acceptor at that site, in the form of an oxygen atom (see Table 2).



Scheme 1. Reagents and conditions: (a) $Ar^1B(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane, 100 °C, 12 h, 60–90%; (b) R^2OH, DEAD, PPh_3, THF, rt, 12 h, 70–85%.

By replacing the methoxy group with an *N*-dimethylamino ethoxy (**23**), we obtained a potent and selective compound. Solubility testing at pH 8.0 revealed that incorporation of an amine did in fact increase the solubility significantly to >4 mg/mL. To reduce the number of rotatable bonds in an attempt to improve drug like properties,[14] cyclized analogues of compound **23** were made, exploring ring size and substitution off of the basic nitrogen. The methyl pyrrolidine compound **24**, showed an increase in A_{2A} potency and selectivity over A_1 as compared to the straight chain analogue **23**. Extending the substitution off of the pyrrolidine to ethyl (**25**) and isopropyl (**26**) resulted in similar potency as the methyl version (**24**) but a slight loss of selectivity from 191-fold over A_1 to 167-fold was observed. As the six membered piperidine compounds did not significantly increase potency or selectivity (**27**), the single enantiomers of compound **24** were made. The S enantiomer (**30**) showed slightly better potency than the R enantiomer (**29**) with a K_i of 2 nM versus 4 nM. A more significant improvement was seen in the selectivity over A_1 as the S isomer was 320-fold selective, double the selectivity of the R isomer. Further in vitro profiling of compound **24** revealed similar human functional activity to compound **18** (cAMP IC_{50} of 42 nM). Unfortunately, this compound showed poor metabolic stability in human liver microsomes with a scaled intrinsic clearance value of 54 mL/min/kg.[15] As this series proved to have good potency and selectivity while greatly improving the solubility (compound **24** >4 mg/mL at pH 8.0), we turned to improving the poor metabolic stability. From our initial survey, we determined that fluorine atoms were well tolerated off of the phenyl ring (**19–21**). The addition of an electron withdrawing group such as a fluorine atom may help to stabilize the electronic rich phenyl group, thereby improving the metabolic stability (see Table 3).

The 3-fluoro-5-(N-methyl-3-pyrrolidol) compound **33**, showed good potency with a K_i of 1 nM and selectivity of 135-fold over A_1. The addition of a 3-fluorine atom did not adversely affect the potency or the selectivity when compared to compound **24**. The

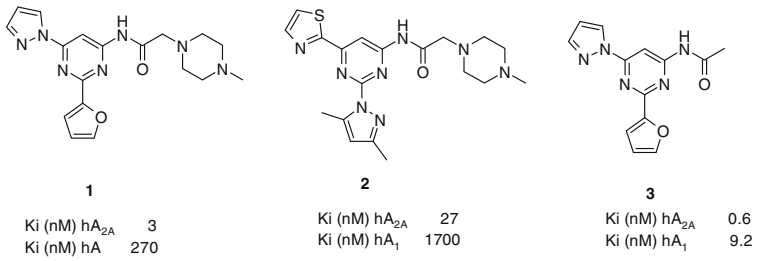
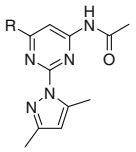
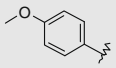
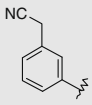
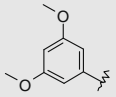
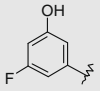
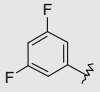
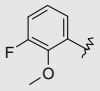


Figure 2. Potent A_{2A} antagonists

Table 1
Binding affinities of **8–21** towards the human A_{2A} receptor and selectivity over the human A_1 receptor

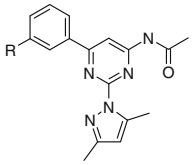


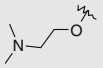
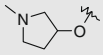
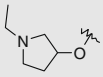
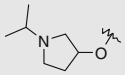
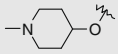
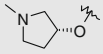
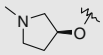
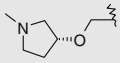
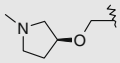
Compound	R	K_i (nM) hA_{2A}[a]	hA_1/hA_{2A}
8		87	7
9		2	71
10		1	72
11		17	44
12		7	59
13		2	10
14		2	50
15		2	40
16		8	43
17		19	46
18		0.2	111
19		2	54
20		4	64
21		3	164

[a] Displacement of specific [^{3}H]-DPCPX binding at human A_1 receptors expressed in HEK293 cells. Displacement of specific [^{3}H]-ZM241385 binding at human A_{2A} expressed in HEK293. Data are expressed as geometric means of at least three runs with a standard deviation less than or equal to 20%.[17]

racemic compound was followed up with the single isomers. As seen with the des-fluoro analogues, both the R (**34**) and S (**35**) isomers had similar A_{2A} potency to the racemic compound. However, a significant difference in selectivity over A_1 was noted with the R isomer being 125-fold selective and the S over 200-fold selective.

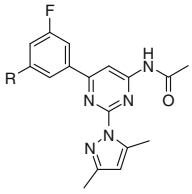
Table 2
Binding affinities of **22–32** towards the human A_{2A} receptor and selectivity over the human A_1 receptor

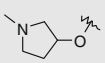
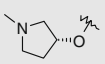
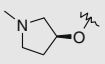


Compound	R	K_i (nM) hA_{2A}[a]	hA_1/hA_{2A}
22	OH	2	74
23		5	134
24		2	191
25		3	168
26		3	167
27		6	146
28		1	203
29		4	145
30		2	320
31		67	99
32		73	102

[a] See footnotes of Table 1.

Table 3
Binding affinities of **33–35** towards the human A_{2A} receptor, selectivity over the human A_1 receptor and intrinsic clearance



Compound	R	K_i (nM) hA_{2A}[a]	hA_1/hA_{2A}	CL_{int} (HLM) [b] (mL/min/kg)
33		1	135	3
34		2	125	3
35		1	218	3

[a] See footnotes of Table 1.
[b] See note 15.

The more promising compound **35** was further profiled in vitro and found to have better functional activity than compound **18** with a cAMP IC_{50} of 15 nM. In addition, compound **35** showed

excellent solubility of >4 mg/mL at pH 8.0. Furthermore, by the introduction of a fluorine atom on the electron rich phenyl ring, the metabolic stability in human liver microsomes was significantly increased from 54 mL/min/kg (scaled intrinsic clearance) for compound **24** to 3 mL/min/kg for compound **35**. In addition, compound **35** was not a potent inhibitor of the major *CYP* enzymes (*CYP*3A4 and 2D6).[16] Due to the promising in vitro profile, good solubility and improved metabolic stability, compound **35** was assayed in the rat efficacy model (HIC), and demonstrated a significant effect at 30 mg/kg p.o.

In summary, we have found potent and selective A_{2A} antagonists which show efficacy in the haloperidol induced catalepsy model. By introducing small basic phenyl substituents, we greatly improved solubility from <0.1 mg/mL at pH 8.0 to >4 mg/mL. In addition, a significant increase in metabolic stability was achieved while maintaining a promising in vitro profile. We have identified a potent, soluble, and metabolically stable series with the best compound showing significant efficacy in the HIC model at 30 mg/kg.

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9. Slee, D. H.; Chen, Y.; Zhang, X.; Moorjani, M.; Lanier, M. C.; Lin, E.; Rueter, J. K.; Williams, J. P.; Lechner, S. M.; Markison, S.; Malany, S.; Santos, M.; Gross, R. S.; Jalali, K.; Sai, Y.; Zuo, Z.; Castro-Palomino, J. C.; Crespo, M. I.; Prat, M.; Gual, S.; Díaz, J. L.; Saunders, J. *J. Med. Chem.* **2008**, *51*, 1719.
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11. General experimental details for this assay may be found in the following reference Selkirk, J. V.; Nottebaum, L. M.; Ford, I. C.; Santos, M.; Malany, S.; Foster, A. C.; Lechner, S. M. *J. Biomol. Screen.* **2006**, *11*, 351.
12. General experimental details for this assay may be found in the following reference Slee, D. H.; Zhang, X.; Moorjani, M.; Lin, E.; Lanier, M. C.; Chen, Y.; Rueter, J. K.; Lechner, S. M.; Markison, S.; Malany, S.; Joswig, T.; Santos, M.; Gross, R. S.; Williams, J. P.; Castro-Palomino, J. C.; Crespo, M. I.; Prat, M.; Gual, S.; Díaz, J. L.; Jalali, K.; Sai, Y.; Zuo, Z.; Yang, C.; Wen, J.; O'Brien, Z.; Saunders, J. *J. Med. Chem.* **2008**, *51*, 400.
13. To determine solubility of compounds, approximately 1 mg of sample was weighed into a 15 mL Falcon Centrifuge tube, and the weight recorded to 0.001 mg. Assay medium, (200 μL, Phosphate Buffer Solution pH 8.0) was added and the sample sonicated for 10 min, then shaken overnight. The sample was then centrifuged and the supernatant was analyzed by HPLC to determine the concentration of sample in solution. The concentration in solution was then calculated based on a standard curve generated from known dilutions of authentic sample.
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15. General experimental details for this assay may be found in the following reference Guo, Z.; Zhu, Y. F.; Gross, T. D.; Tucci, F. C.; Gao, Y.; Moorjani, M.; Connors, P. J.; Rowbottom, M. W.; Chen, Y.; Struthers, R. S.; Xie, Q.; Saunders, J.; Reinhart, G.; Chen, T. K.; Bonneville, A. L. K.; Chen, C. *J. Med. Chem.* **2004**, *47*, 1259.
16. Inhibition assays were carried out using microsomes isolated from transfected cells expressing only *CYP*3A4, and in the presence of the fluorescent substrate BFC. Ketoconazole was used as a positive control. The *CYP*2D6 assay was carried out in the presence of the fluorescent substrate, AMMC. Quinidine was used as a positive control. All compounds described with an $IC_{50} < 30$ μM were assayed in 2 or 3 experiments.
17. On each assay plate, a standard antagonist of comparable affinity to those being tested was included as a control for plate-to-plate variability. Overall K_i values were highly reproducible, the standard deviations were less than or equal to 20%. All compounds reported were assayed in 3–6 independent experiments.