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2,6-Diaryl-4-phenacylaminopyrimidines as potent and selective adenosine A_{2A} antagonists with reduced hERG liability

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Abstract—In this report, the design and synthesis of a series of pyrimidine based adenosine A_{2A} antagonists are described. The strategy and outcome of expanding SAR exploration to attenuate hERG and improve selectivity over A_1 are discussed. Compound **33** exhibited excellent potency, selectivity over A_1, and reduced hERG liability.

Adenosine receptors belong to the superfamily of G-protein coupled receptors and are divided into four subtypes: A_1, A_{2A}, A_{2B}, and A_3.[1] These four receptors are linked to the secondary messenger adenylyl cylase. Both subtypes A_{2A} and A_{2B} stimulate adenylyl cyclase, whereas A_1 and A_3 inhibit this enzyme.[2] 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.[3] Not surprisingly, antagonists of the A_{2A} receptor have demonstrated efficacy in models of Parkinson's disease (PD) in addition to exhibiting neuroprotective properties. Parkinson's disease is a debilitating motor disorder arising from the progressive degeneration of dopaminergic neurons in the nigrostriatal pathway.[4] Current dopamine replacement therapies for PD lack neuroprotective benefits and suffer from poor long-term control and undesirable side effects, namely dyskinesia (involuntary movements). In recent clinical trials, the most advanced A_{2A} antagonist, KW-6002 (istradefylline) from Kyowa Hakko Kogyo, showed efficacy in alleviating symptoms of the disease.[5] In addition, Schering–Plough has progressed SCH 420814 into Phase II clinical trials and Vernalis/Biogen Idec are in Phase II clinical trials with V2006 (structure not disclosed) (Fig. 1).[6]

Previously, we have reported on the discovery of a new class of A_{2A} antagonists, based on a pyrimidine core (Fig. 2).[7] Exploration around the pyrimidine core was carried out, optimizing the R^2 and R^3 positions with heterocycles and the R^1 position with amines. Although potent and selective compounds were discovered, improvements in several areas were desired. The incorporation of basic groups within the R^1 substituent gave compounds with desirable physical properties. However, on further characterization some of the most promising compounds exhibited inhibition of the hERG channel (Fig. 3).[7,8] It has been cited that blockade of the hERG K^+ channel can lead to prolongation of the heart rate-corrected QT interval, which in turn elevates the risk for cardiac arrhythmia and can lead to *torsades de points* (sudden death).[9] In addition to attenuating hERG

Keywords: A_{2A} antagonists; Adenosine receptor; Parkinson's disease; Phenacylaminopyrimidines; hERG.
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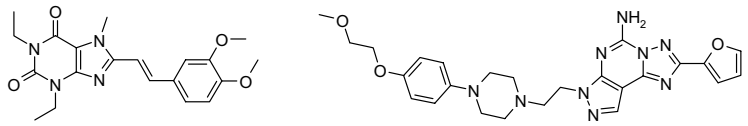
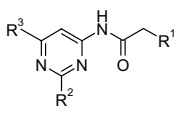
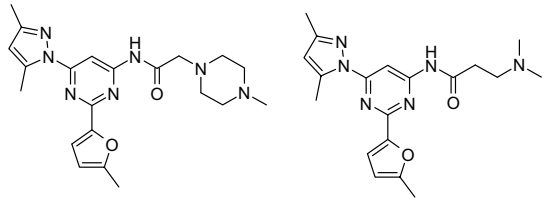


Figure 1. Examples of A_{2A} antagonists in clinical development.



1

Figure 2. Pyrimidine core.



2

Ki (nM) hA_{2A} 12
hA_1/hA_{2A} 71
hERG IC_{50} (nM) 950

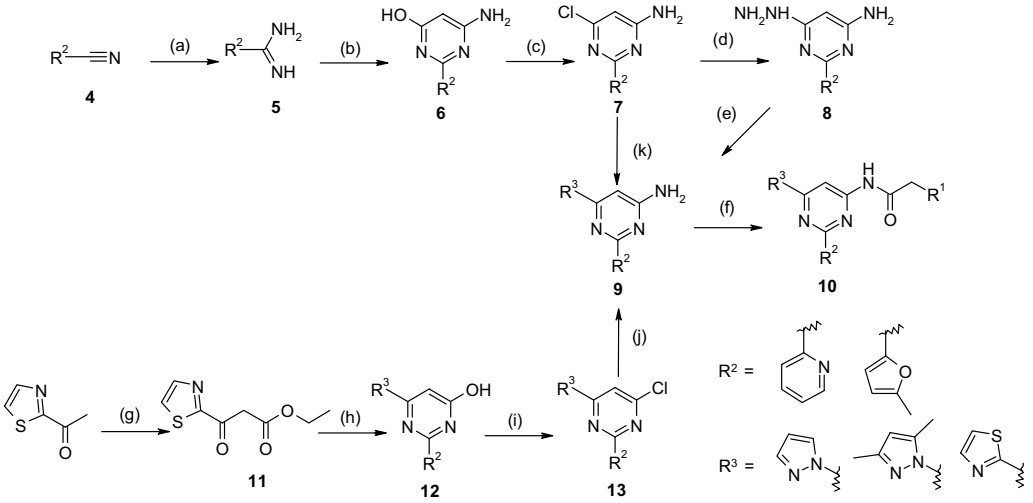
3

Ki (nM) hA_{2A} 2
hA_1/hA_{2A} 99
hERG IC_{50} (nM) 653

Figure 3. Potent A_{2A} antagonists that suffer from hERG inhibition.

inhibition, we wanted to improve selectivity over the A_1 receptor. The A_1 receptor is present in cardiac muscle and as a result, may represent a cardio-toxicity risk.[1] To address these issues, we broadened the scope of our right-hand side exploration to incorporate previously unexplored substituted phenyl groups.

Compounds **14**–**33** were prepared according to the general synthesis outlined in Scheme 1. Starting with either 5-methyl-2-furonitrile (compounds **14**–**25**) or 2-cyanopyridine (compounds **27** and **29**–**33**), the corresponding carboxyamidine **5** was synthesized by treatment with sodium methoxide at room temperature followed by reaction with ammonium chloride. The resulting carboxyamidine was then treated with ethyl cyanoacetate in the presence of sodium methoxide to yield 6-hydroxypyrimidin-4-amine intermediate **6**, which was then treated with phosphorus oxychloride at reflux in the presence of *N*,*N*-diisopropyl ethylamine to give intermediate **7**. The chloro-moiety of intermediate **7** was then displaced with hydrazine and cyclized with pentane-2-4-dione to give **9** with dimethylpyrazole at R^3. Alternatively, displacement of the chloro on **7** with pyrazole in the presence of cesium carbonate yields **9** with pyrazole at R^3. In the case of compounds **26** and **28** which have a thiazole at position R^2, a slightly modified synthesis was followed. Starting with 2-acetylthiazole, 3-oxo-3-thiazol-2-yl-propionic acid ethyl ester **11** was generated by treatment with diethyl carbonate and sodium hydride. Intermediate **11** was then reacted with carboxyamidines of formula **5**, in the presence of potassium *tert*-butoxide to yield pyrimidin-4-ol intermediate **12**. The pyrimidin-4-ol could then be treated with phosphorus oxychloride to give **13**, followed by reaction with ammonium hydroxide to give **9**. Final compounds were made by reacting the appropriate phenyl acetic acid with



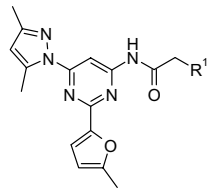
Scheme 1. Regents and conditions: (a) NaOMe, NH_4Cl, MeOH, rt, 6–12 h, 87–96%; (b) ethyl cyanoacetate, NaOMe, EtOH, 70 °C, 6–12 h, 60–80%; (c) $POCl_3$, DIPEA, 90 °C, 3–12 h, 50–80%; (d) N_2H_4, EtOH, 90 °C, 4 h, 80%; (e) pentane-2,4-dione, 0–90 °C, 2 h, 72%; (f) substituted phenylacetic acid, oxalyl chloride, DMF, pyridine, rt, 4–12 h, 20–80%; (g) diethyl carbonate, NaH, 80 °C, 12 h, 50–70%; (h) carboxyamidine, KO^{t}Bu, ^{t}BuOH, 135 °C, 12 h, 50%; (i) $POCl_3$, DIPEA, 90 °C, 3–12 h, 50–80%; (j) NH_4OH, MeOH, 80 °C, 12 h, 80–95%; (k) pyrazole, cesium carbonate, 150 °C, 6 h, 80%.

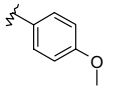
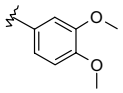
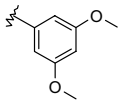
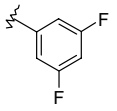
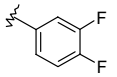
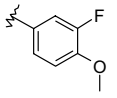
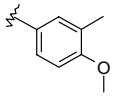
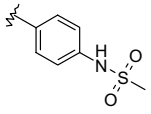
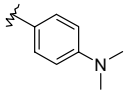
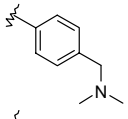
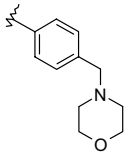
oxalyl chloride in the presence of DMF to generate the acid chloride. The resulting acid chlorides were then reacted with intermediate **9** in the presence of pyridine to yield compounds of formula **10**.

Replacement of the right-hand side amine with a substituted phenyl group increased the lipophilicity. To counter this increase, substitution was limited to more polar groups. The 4-methoxy-substituted phenyl (**14**) was potent (K_i of 4 nM) although selectivity of ~60-fold with respect to A_1 was less than desired (Table 1). Incorporation of a second methoxy substituent as in the 3,4-dimethoxyphenyl (**15**), and 3,5-dimethoxyphenyl (**16**) derivatives improved selectivity while maintaining potency (A_1/A_{2A} of 180 and 487, respectively). When the 3,5-dimethoxyphenyl was replaced by small electron-withdrawing groups such as 3,5-difluorophenyl (**17**), a loss in selectivity was observed. A similar trend was noted upon incorporation of 3,4-difluorophenyl (**18**). Further attempts to include more polar substituents on the phenyl ring led to the incorporation of a *para* sulfonamide group (**21**). Although once again potency was maintained with a K_i of 5 nM, selectivity was lower, being only 90-fold over A_1. In general, electronics and polarity of the phenyl substituent(s) did not greatly affect A_{2A} binding, however, A_1 selectivity was more sensitive to these changes. Compounds **22**–**24** showed promising potency and selectivity; however, all three exhibited inhibition of *CYP*3A4 or 2D6 (IC_{50} < 5 μM).[11] The most promising compound from this set, compound **16**, showed very good binding activity with a K_i of 2 nM and selectivity of 487-fold. Profiling of compound **16** in secondary assays showed that it was not a potent inhibitor of the major *CYP* enzymes (*CYP*3A4 and 2D6 IC_{50} > 5 μM). In addition, in a patch clamp assay, compound **16** did not show significant inhibition of the hERG channel (IC_{50} > 3 μM). By replacing the basic amine side chain with a substituted phenyl group, we successfully attenuated the hERG liability. However, this compound exhibited poor solubility of <0.1 mg/mL at pH 2.[12] In an effort to increase solubility, an SAR exploration was undertaken to vary the heterocycles at the 2 and 6 positions on the pyrimidine core.

The effects of changing the substitution at the R^3 and R^2 positions were investigated, with particular focus on reducing the log *P* contribution of these heterocycles (Table 2). Replacing dimethyl pyrazole at R^3 (**14**) with pyrazole (**25**) decreased the calculated log *P* by one unit (from *c*log *P* 2.7 to 1.7). Unfortunately, a 5-fold decrease in selectivity and 2-fold decrease in binding was observed. When R^2 was pyridine or methyl furan, incorporation of a thiazole group at R^3 again led to decreased selectivity as compared to dimethyl pyrazole at R^3 (**27** vs **28**, **14** vs **26**). Maintaining the dimethyl pyrazole at R^3 and replacing the methyl furan by a 2-pyridyl group **27** increased potency (K_i of 1 nM) and kept selectivity (A_1/A_{2A} 66-fold) compared to compound **14** (K_i of 4 nM, A_1/A_{2A} 61-fold). In addition to maintaining the binding profile and lowering the *c*log *P*, the 2-pyridyl compound **27** exhibited lower *CYP*3A4 inhibition with an IC_{50} of 1.8 μM (as compared to the methyl furan analog **14**, IC_{50} of 300 nM). Furthermore, incorporation of the pyridine ring enabled us to make hydrochloride salts of the final compounds which in turn could im-

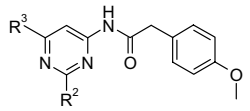
Table 1. Binding affinities of **14**–**24** toward the human A_{2A} receptor and selectivity over the human A_1 receptor



Compound	R^1	K_i (nM) hA_{2A}[a]	hA_1/hA_{2A}
14		4	61
15		2	180
16		2	487
17		5	126
18		5	152
19		3	129
20		3	411
21		5	90
22		2	162
23		0.2	80
24		0.8	179

[a] Displacement of specific [^{3}H]-ZM241385 binding at human A_{2A} expressed in HEK293 cells. Displacement of specific [^{3}H]-DPCPX binding at human A_1 receptors expressed in HEK293 cells. Data are expressed as geometric means of at least three runs with a standard deviation less than or equal to 20%.[10]

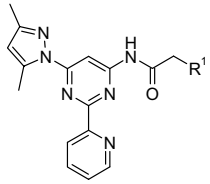
Table 2. Binding affinities of **25**–**28** towards the human A_{2A} receptor, selectivity over the human A_1 receptor and calculated log *P* values

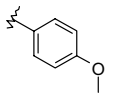
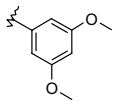
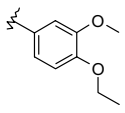
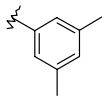
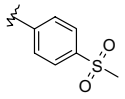


Compound	R^3	R^2	K_i (nM) hA_{2A}[a]	hA_1/hA_{2A}	*c*log *P*[b]
25			10	12	1.7
26			6	12	3.2
27			1	66	2.3
28			1	18	2.9

[a] See footnotes of Table 1.
[b] Calculated log *P* values using ACD/Labs log *P* database.[13]

Table 3. Binding affinities of **29**–**33** towards the human A_{2A} receptor, selectivity over the human A_1 receptor and *CYP*3A4 inhibition



Compound	R^1	K_i (nM) hA_{2A}[a]	hA_1/hA_{2A}	*CYP*3A4 inh. IC_{50}[b] (μM)
29		1	66	1.8
30		1	477	1.3
31		1	257	7
32		3	186	>10
33		1	148	>10

[a] See footnotes of Table 1.
[b] See note 11.

prove solubility. As 2-pyridyl seemed like a promising alternative to methyl furan at the R^2 position, this series was further explored.

Initial results showed a similar SAR trend with the dimethyl pyrazole/pyridine core (Table 3) as compared to the dimethyl pyrazole/methyl furan core (Table 1). The 3,5-dimethoxy phenyl analog **30** was potent (K_i of 1 nM) and exhibited increased selectivity over the monomethoxy phenyl **29** (Table 3). However, compound **30** exhibited inhibition of *CYP*3A4 (IC_{50} of 1.3 μM). As encouraging potency and selectivity was seen with electron-rich phenyl rings, variations of methoxy and methyl substituents were made. Both 3-methoxy-4-ethoxy phenyl **31** and 3,5-dimethylphenyl **32** demonstrated good potency (K_i of 1 and 3 nM, respectively) and selectivity over 100-fold against A_1. The *para*-sulfone substituent (**33**), showed good potency (K_i of 1 nM) and selectivity of 148-fold. Further in vitro profiling showed that compound **33** had good functional activity (hcAMP IC_{50} of 100 nM), good metabolic stability with an intrinsic clearance of 7 mL/min/kg (Human Liver Microsomes) (Table 4) and was not a potent *CYP*3A4 or 2D6 inhibitor ($IC_{50} > 5$ μM).[14] Head-to-head comparison with compound **16** illustrates that compound **33** exhibits increased metabolically stability and has a significantly reduced *c*log *P* (from 2.5 to 0.7). In addition, the HCl salt of compound **33** demonstrated good solubility of 0.5 mg/mL at pH 2; whereas, the free base of compound **16** had a solubility of <0.1 mg/mL at pH 2. Finally, compound **33** was tested in a hERG patch clamp assay and showed significantly less activity ($IC_{50} > 4$ μM) than our initial starting points, compounds **2** and **3** ($IC_{50} < 1$ μM), not to mention increased A_1 selectivity to almost 150-fold.

Table 4. Binding affinities towards the human A_{2A} receptor, selectivity over the human A_1 receptor, intrinsic clearance, and calculated log *P* values

Compound	hA_{2A}[a] K_i (nM)	hA_1/hA_{2A}	CL_{int} (HLM)[c] (mL/min/kg)	*c*log *P*[b]
16	2	487	78	2.5
31	1	257	47	3.4
32	3	185	113	2.7
33[16]	1	148	7	0.7

[a] See footnotes of Table 1.
[b] See footnotes of Table 2.
[c] See note 15.

In summary, we have expanded our scope of SAR around the pyrimidine core to incorporate non-basic amine side chains, namely substituted phenyls, in an attempt to attenuate hERG liability and improve selectivity over A_1. By balancing lipophilicity with potency and selectivity, we developed several promising adenosine A_{2A} antagonists. A number of compounds exhibited excellent potency and selectivity over A_1 to >100-fold. Furthermore, while maintaining excellent in vitro profiles we produced A_{2A} antagonists with good physiochemical properties and attenuated the hERG liability. Further optimization and evaluation of this series will be reported in due course.

Acknowledgments

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8. The hERG potassium current was recorded from a hERG/HEK cell line using established patch-clamp methods. The effects of test compounds on the hERG current were determined at the end of a 5-min application. Test compounds were tested at six concentrations (0.1 nM, 1 nM, 10 nM, 100 nM, 1 μM and 10 μM). Cisapride (30 nM) was used as a positive control.
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10. 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.
11. 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 two or three experiments.
12. 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, Buffer Solution pH 2.00) 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.
13. The calculated log *P* values stated were obtained using ACD/Labs Log *P* database, version 9.02 (2005), Advanced Chemistry Development Inc., Toronto, Ontario, Canada (http://www.acdlabs.com).
14. General experimental details for the human cAMP functional 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.
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16. Compound **33** was prepared in one step from 6-(3,5-Dimethyl-pyrazole-1-yl)-2-pyridin-2-yl-pyrimidin-4-ylamine (intermediate **9**), using the procedure outlined in Scheme 1 to yield the final compound as an HCL salt (65%): ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.77–8.78 (m, 1H), 8.52 (s, 1H), 8.37–8.39 (m, 1H), 8.04–8.07 (m, 1H), 7.89–7.92 (m, 2H), 7.57–7.65 (m, 3H), 6.23 (s, 1H), 3.99 (s, 2H), 3.20 (s, 3H), 2.80 (s, 3H), 2.22 (s, 3H). LCMS: t_R = 24.075 (100%); MS: *m*/*z* 463 $[M+H]^+$, expected 463 $[M+H]^+$.