N-[6-Amino-2-(heteroaryl)pyrimidin-4-yl]acetamides as A_{2A} Receptor Antagonists with Improved Drug Like Properties and in Vivo Efficacy

Marion C. Lanier,[#] Manisha Moorjani,[#] Zhiyong Luo,[#] Yongsheng Chen,[#] Emily Lin,[#] John E. Tellew,[#] Xiaohu Zhang,[#] John P. Williams,[#] Raymond S. Gross,[†] Sandra M. Lechner,[‡] Stacy Markison,[‡] Tanya Joswig,[‡] William Kargo,[‡] Jaime Piercey,[‡] Mark Santos,[§] Siobhan Malany,[§] Marilyn Zhao,[†] Robert Petroski,[‡] María I. Crespo,[⊥] José-Luis Díaz,[⊥] John Saunders,[#] Jenny Wen,[||] Zhihong O'Brien,[||] Kayvon Jalali,[||] Ajay Madan,[||] and Deborah H. Slee*[,#]

Departments of Medicinal Chemistry, Pharmacology, Neuroscience, Chemical Development, and Preclinical Development, Neurocrine Biosciences, 12780 El Camino Real, San Diego, California 92130, and Almirall Research Center, Almirall, Ctra. Laureà Miró, 408-410, E-08980 St. Feliu de Llobregat, Barcelona, Spain

Received July 21, 2008

In the present article, we report on a strategy to improve the physical properties of a series of small molecule human adenosine 2A (hA_{2A}) antagonists. One of the aromatic rings typical of this series of antagonists is replaced with a series of aliphatic groups, with the aim of disrupting crystal packing of the molecule to lower the melting point and in turn to improve the solubility. Herein, we describe the SAR of a new series of water-soluble 2,4,6-trisubstituted pyrimidines where R^1 is an aromatic heterocycle, R^2 is a short-chain alkyl amide, and the typical R^3 aromatic heterocyclic substituent is replaced with an aliphatic amino substituent. This approach significantly enhanced aqueous solubility and lowered the log *P* of the system to provide molecules without significant hERG or CYP liabilities and robust in vivo efficacy.

Introduction

On the basis of its distribution in the brain and interaction with dopamine receptors, the human A_{2A} (hA_{2A}[a]) receptor has emerged as a novel nondopaminergic target to treat Parkinson's disease (PD).[1] There is accumulating evidence to suggest that antagonists of this receptor will improve the motor dysfunction associated with PD while reducing the severe side effects that result from long-term dopaminergic therapies. Many companies are interested in this therapeutic approach, and currently several A_{2A} antagonists are in clinical development.[2] The xanthine derivative Istradefylline[3] (**1**, also known as KW-6002, Figure 1) was discovered by Kyowa Hakko Kogyo Corporation. This molecule represents the adenosine A_{2A} receptor antagonist that has advanced the furthest in terms of clinical development. Schering-Plough has developed a series of purine analogues, and SCH-420814[4] (**2**, Figure 1) is in phase II clinical trials. Biogen Idec initiated a phase II clinical trial for PD with the non-xanthine A_{2A} antagonist BIIB014 (also known as V-2006) in May 2007 (structure not disclosed).[5] Similarly Synosia is also in phase II clinical trials with SYN115 (structure not disclosed).[6]

Our research efforts have focused on the development of trisubstituted pyrimidines as human A_{2A} (hA_{2A}) antagonists. Previously we reported on compound **3**[7] (Figure 2), where a pyrimidine core is substituted at the 2 and 4 positions by an aromatic or heteroaromatic ring and at the 6 position by a simple amide.

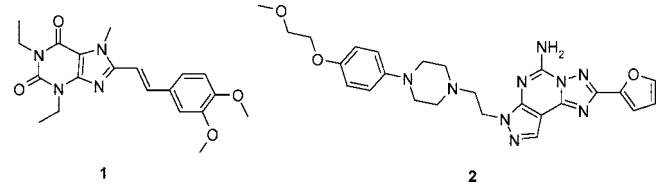


Figure 1. Examples of A_{2A} antagonists in clinical development.

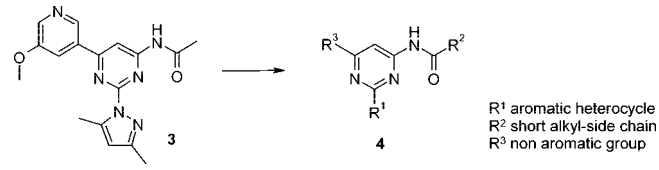


Figure 2. Novel approach proposed to improve the solubility of the pyrimidine series.

Compound **3** showed good in vitro and in vivo profiles (hA_{2A} K_i = 2.3 nM, hcAMP IC_{50} = 85 nM, 82-fold selectivity over the human A_1 (hA_1) receptor; efficacious in the rat haloperidol-induced catalepsy (HIC) model at 1 mg/kg), but its poor solubility (<0.01 mg/mL at pH ≥ 2) hindered further development. We attributed this poor solubility to the observed high melting point (283 °C) indicating a high crystal lattice energy. In our earlier work[8] we described adding polar or basic substituents to improve drug like properties and in particular to increase aqueous solubility. In parallel, we also pursued a strategy of replacing one of the aromatic rings with an aliphatic group, which we expected would disrupt crystal packing, lower the melting point, and in turn improve the solubility. Herein, we report a new series of water-soluble 2,4,6-trisubstituted pyrimidines where R^1 is an aromatic heterocycle, R^2 is a short-chain alkyl amide, and the typical R^3 aromatic heterocyclic substituent is replaced with an aliphatic amino substituent (compound **4**, Figure 2).

Chemistry

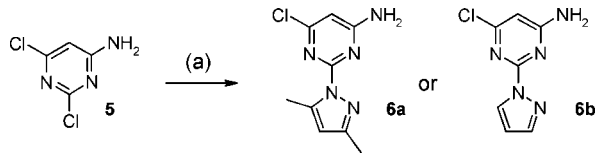
Preparation of intermediates **6a** and **6b** (Scheme 1) was carried out through the reaction of either 3,5-dimethylpyrazole

* To whom correspondence should be addressed. Phone: 858 617-7849. Fax: 858 617-7925. E-mail: dslee@neurocrine.com.
[#] Department of Medicinal Chemistry, Neurocrine Biosciences.
[†] Department of Chemical Development, Neurocrine Biosciences.
[‡] Department of Neuroscience, Neurocrine Biosciences.
[§] Department of Pharmacology, Neurocrine Biosciences.
[⊥] Almirall Research Center.
[||] Department of Preclinical Development, Neurocrine Biosciences.

[a] Abbreviations: hA_{2A}, human adenosine 2A; hA_1, human adenosine 1; HIC, haloperidol induced catalepsy; hERG, human ether-a-go-go related gene; CYP, cytochrome P450; PBS, phosphate buffered saline; SAR, structure activity relationship; po, oral dosing; 6-OHDA, 6-hydroxydopamine.

10.1021/jm800908d CCC: $40.75
Published on Web 01/13/2009

Scheme 1[a]



[a] Reagents and conditions: 3,5-dimethylpyrazole (for **6a**) or pyrazole (for **6b**), cesium carbonate, dioxane, reflux, 39% yield.

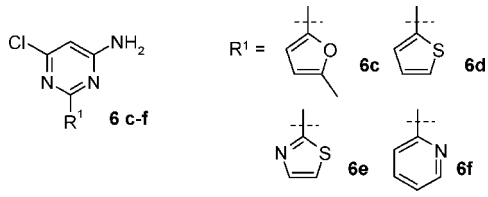
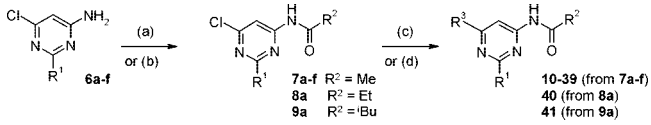


Figure 3. Common key intermediates used for final compound synthesis.

Scheme 2[a]



[a] Reagents and conditions. (a) R^2 = Me: Ac_2O, AcOH, 90 °C, 18 h, 83% yield. (b) R^2 = Et or ^{t}Bu: propionyl chloride or isovaleryl chloride, pyridine, DMF, room temp, 12 h. (c) R^3 = amine: amine, dioxane, 80 °C, 2 h. (d) R^3 = pyrrolidin-1-yl-2-one: 2-pyrrolidinone, palladium acetate, xanthpos, cesium carbonate, toluene, 100 °C, 18 h.

or pyrazole with 2,4-dichloro-6-aminopyrimidine **5** in the presence of cesium carbonate in dioxane at reflux. Preparation of intermediates **6c**−**f** (Figure 3) was carried according to the procedures described previously.[9]

Amines **6a**−**f** were then acetylated using acetic anhydride in acetic acid to give **7a**−**f**, respectively. Compound **6a** was also reacted with propionyl or isovaleryl chloride in the presence of pyridine to give **8a** and **9a**, respectively (Scheme 2). The displacement of the chlorine atom of the pyrimidine intermediates (**7a**−**f**, **8a**, and **9a**) was carried out either in the presence of the desired amine in refluxing dioxane or in the presence of a lactam using palladium-mediated coupling conditions, as outlined in Scheme 2, to give the final products **10**−**41** (Tables 1−5).

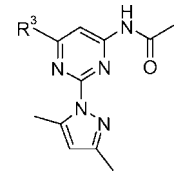
Synthesis of compound **43** where R^2 is an amine or **44** where R^2 is an alkoxy group was accomplished via first displacing the chlorine atom of the aminopyrimidine intermediate **6a** with (*R*)-2-methoxymethylpyrrolidine to give intermediate **42**, followed by reaction with triphosgene and addition of either isopropylamine (for **43**) or methanol (for **44**) (Scheme 3). All final compounds were purified by HPLC.

Results and Discussion

From previous experience with the pyrimidine system,[9] it was apparent that a wide range of substitution was tolerated at the R^3 position of the molecule (Figure 2, general structure **4**). While we were unable to get a crystal structure of the lead compound **3**, we were able to get a crystal structure of a closely related compound **45**[10] (hA_{2A} K_i = 1.9 nM) (Figure 4), which showed that the pyrimidine system was planar, helping to explain the high melting points and low solubility observed for the initial leads.

With the aim of reducing the planarity of the system, and the hope of improving physical properties, we set out to further explore the structure activity relationship (SAR) around the R^3 group with the goal of replacing the typical aromatic R^3 moiety with an aliphatic group (Table 1). By use of the versatile chloro intermediates **7a**−**f**, **8a**, and **9a** as starting points, a diverse set of amine-linked side chains which included cyclic and open chain amines and lactams was incorporated into the structure at the R^3 position. The initial results were encouraging, and it was found that compounds with a simple diethylamine **10**, unsubstituted pyrrolidine **11**, or piperidine **12** moiety at R^3 were quite potent lead like molecules with hA_{2A} receptor affinity of approximately 100 nM and selectivity on the order of 45-fold over the human A_1 receptor (hA_1) (Table 1). While the ethylamine analogue **10** had similar activity and selectivity to the cyclic systems, subsequent attempts to improve upon this with additional open chain analogues proved unfruitful (data not shown), and the ethylamine remained the most potent in this subseries. Interestingly the lactam analogue **13** had significantly better affinity for the hA_{2A} receptor (hA_{2A} K_i = 12 nM) and was found to be slightly more selective (74-fold) for the hA_{2A} receptor over the hA_1 receptor than the corresponding pyrrolidine **11**. We hypothesized that the carbonyl of the lactam might be picking up a key hydrogen bonding interaction with the receptor which accounted for this increase in potency. Unfortunately, it was observed that in general lactam analogues

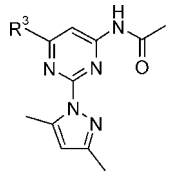
Table 1[c]

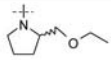
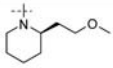
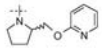


Compound	R^3	K_i (nM) ± SEM hA_{2A}[a]	K_i (nM) ± SEM hA_1[b]	Selectivity hA_1/hA_{2A}
10		92±3	4466±1105	49
11		110±9	4954±586	45
12		78±7	3364.3±497	43
13		12±1	913±71	76
14		313±29	8124±2166	26
15		1867±681	>10000	ND
16		4.2±0.8	323±10	77

[a] Displacement of specific [^{3}H]**46** ([^{3}H]-ZM 241385, Figure 7)[12] binding at human A_{2A} receptors expressed in HEK293 cells. [b] Displacement of specific [^{3}H]**47** ([^{3}H]-DPCPX, Figure 7)[13] binding at human A_1 receptors expressed in HEK293 cells. [c] Data are expressed as geometric mean values of at least two runs ± the standard error measurement (SEM). ND: not determined.

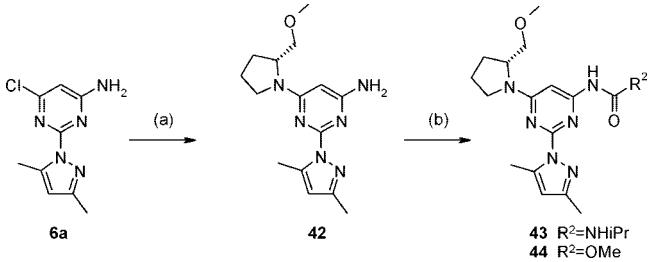
Table 2[c]



R³	Compound	Configuration	K_i (nM) ± SEM hA_{2A}[a]	hA_1[b]	Selectivity hA_1/hA_{2A}
	**11**	-	110±9	4954±586	45
	**17**	*RS*	28±1	1037±298	37
	**18**	*RS*	26±3	222±45	8
	**19**	*R*	205±1	2934±24	14
	20	*S*	>10000	ND	ND
	**21**	*RS*	9.0±1.3	320±42	35
	22	*R*	4.7±0.8	162±18	34
	23	*S*	324±34	4468±40	14
	**24**	*RS*	455±27	4186±918	9
	**25**	*R*	25.8±0.2	1533±408	59
	**26**	*R*	275±126	4695±538	17
	**27**	*R*	47±9	1640±151	35
	**28**	*R*	40±0.4	64±0.3	1.5
	29	*S*	439±9	12100±419	28

[a] Displacement of specific [^{3}H]**46** binding at human A_{2A} receptors expressed in HEK293 cells. [b] Displacement of specific [^{3}H]**47** binding at human A_1 receptors expressed in HEK293 cells. [c] Data are expressed as geometric mean values of at least two runs ± the standard error measurement (SEM). ND: not determined.

Scheme 3[a]



[a] Reagents and conditions: (a) (*R*)-2-methoxymethylpyrrolidine, dioxane, 80 °C, 2 h; (b) triphosgene, pyridine, DCM, 0 °C, 0.5 h, then isopropylamine (for **43**) or methanol (for **44**).

were prone to chemical instability over time, and lactam ring opening readily occurred under basic conditions. When tested further in secondary assays, it was found that the lactams also suffered from poor functional activity[11] (IC_{50} = 1000 nM for

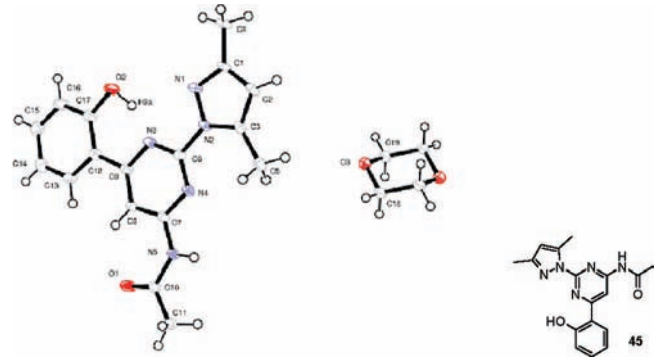
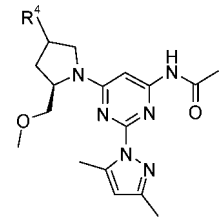


Figure 4. Crystal structure of a pyrimidine dimethylpyrazole containing lead molecule **45** showing the planarity of the system.

Table 3[c]



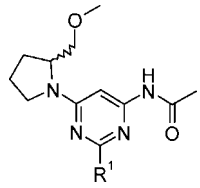
Compound	R⁴	K_i (nM) ± SEM hA_{2A}[a]	hA_1[b]	Selectivity hA_1/hA_{2A}
30	*trans*-OMe	7.5 ± 3.7	352 ± 117	47
31	*cis*-OMe	23 ± 3	620 ± 180	27

[a] Displacement of specific [^{3}H]**46** binding at human A_{2A} receptors expressed in HEK293 cells. [b] Displacement of specific [^{3}H]**47** binding at human A_1 receptors expressed in HEK293 cells. [c] Data are expressed as geometric mean values of at least two runs ± the standard error measurement (SEM).

inhibition of hcAMP release for **13**). The idea that there might be alternative stable analogues that could take advantage of this potential interaction was pursued further, and the focus shifted to incorporation of heteroatoms either into the aliphatic system or as substituents off the ring system. Incorporation of heteroatoms into the ring system as exemplified by the morpholine analogue **14** (hA_{2A} K_i = 313 nM) and the *N*-methylpiperazine analogue **15** (hA_{2A} K_i = 1867 nM) proved unsuccessful, as was the case with other polar substituents such as amino, amide (data not shown), and ester moieties (e.g., compounds **19** and **20**, Table 2). In contrast, adding lipophilic groups such as the fused phenyl ring in compound **16** boosted both binding for the human A_{2A} receptor (4.2 nM) and selectivity over the hA_1 receptor (77-fold). However, as expected, the strategy of adding lipophilicity to gain potency severely compromised physical properties, leading again to poorly soluble compounds (the solubility of compound **16** in phosphate buffer at pH 7.2 was 0.01 mg/mL).

Additional focus on pyrrolidines substituted with simple alkyl and alkoxy side chains in an attempt to balance activity, selectivity, and solubility (Table 2) led to some exciting results. A small alkyl chain such as a methyl (**17**) or an *n*-propyl (**18**) at the 2 position of the pyrrolidine gave a 4-fold boost in activity compared to the unsubstituted pyrrolidine **11**. Further, the methoxymethyl (MOM, **21**) was even better with a K_i value for the human A_{2A} receptor equal to 9.0 nM for the racemic mixture. After synthesis of the single enantiomers it was found that the *R* enantiomer **22** was significantly more active and selective for the human A_1 receptor than the *S* enantionmer **23** (hA_{2A} K_i of 4.7 and 324 nM, and 34- and 14-fold selective vs hA_1, respectively). These results again suggested that addition of an appropriately placed hydrogen bond accepting group was

Table 4[c]



Compound	Configuration	R^1	K_i (nM) ± SEM		Selectivity
			hA_{2A}[a]	hA_1[b]	hA_1/hA_{2A}
22	*R*		4.7±0.8	162±18	34
23	*S*		324±34	4468±40	14
32	*R*		2.7±0.7	10.5±0.4	4
33	*S*		119±7	215±27	2
34	*R*		47±17	203±62	4.3
35	*S*		27±4	325±87	12
36	*R*		20±5	150±20	7.5
37	*S*		15±3	354±60	23
38	*R*		46±1	69±6	1.5
39	*S*		50±4	125±28	2.5

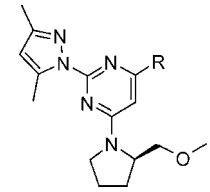
[a] Displacement of specific [^{3}H]**46** binding at human A_{2A} receptors expressed in HEK293 cells. [b] Displacement of specific [^{3}H]**47** binding at human A_1 receptors expressed in HEK293 cells. [c] Data are expressed as geometric mean values of at least two runs ± the standard error measurement (SEM).

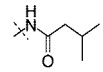
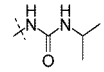
beneficial and that the chirality was important for both potency and selectivity. Extension of the methyl to ethyl (**24**) or addition of heterocyclic substituents (**28**) was not well tolerated. Extending the carbon chain between the piperidine ring and the oxygen by one carbon to give **26** (hA_{2A} K_i = 275 nM, 17-fold selectivity vs hA_1) resulted in a sharp loss of both activity and selectivity. Removal of the methyl group to reveal a hydroxyl moiety (**25**) resulted in a 4-fold loss in potency, and additional substitution on the pyrrolidine ring (**27**) did not improve the profile.

Additional SAR around the pyrrolidine ring concentrated on combining the MOM group at the 2 position of the pyrrolidine ring with a second simple substitution around the ring, with the aim of further refining the activity and selectivity of the molecule (examples shown in Table 3). Combining the *R*-2-MOM of **22** (hA_{2A} K_i = 4.7 nM) with a 4-OMe group maintained potency against the human A_{2A} receptor in the case of the trans diastereoisomer **30** (hA_{2A} K_i = 7.5 nM, 47-fold selective over hA_1). The cis diasteroisomer **31** was not as active (hA_{2A} K_i = 23 nM) and was also slightly less selective over the hA_1 receptor (27-fold selective). The same pattern was observed for the 4-Me analogues (data not shown). In summary, from a limited exploration of substitution at the 4-position of the pyrrolidine ring no improvement was seen, and compound **22** remained the most attractive lead molecule.

The R^1 region of the molecule was also revisited. The 3,5-dimethylpyrazole was replaced by various heterocycles such as 5-methylfuran, thiophene, thiazole, and pyridine in combination with the 2-MOM pyrrolidine (Table 4). The 3,5-dimethylpyrazole **22**, **23**, and the 5-methylfuran **32**, **33** were the most

Table 5[c]



Compound	Configuration	R	K_i (nM) ± SEM		Selectivity
			hA_{2A}[a]	hA_1[b]	hA_1/hA_{2A}
42	*R*		261±10	2473±383	9.5
22	*R*		4.7±0.8	162±18	34
40	*R*		5.9±1.1	64±9	11
41	*R*		50±2	92±9	2
43	*R*		43±29	1238±140	29
44	*R*		19±1	960±185	50

[a] Displacement of specific [^{3}H]**46** binding at human A_{2A} receptors expressed in HEK293 cells. [b] Displacement of specific [^{3}H]**47** binding at human A_1 receptors expressed in HEK293 cells. [c] Data are expressed as geometric mean values of at least two runs ± the standard error measurement (SEM).

sensitive to the configuration of the MOM group, and in general the 3,5-dimethylpyrazole analogues displayed an increase in selectivity for the human A_{2A} receptor over the human A_1 receptor. In the case of the 2-thiophene, 2-thiazole, and 2-pyridine analogues (compounds **34**−**39**), the stereochemistry of the MOM group had little or no influence on the binding at the human A_{2A} receptor and the selectivity over the human A_1 receptor was greatly decreased.

Finally, the R^2 region was explored (Table 5). Compound **42** (the deacetylated amine analogue of **22**) showed a K_i value for the human A_{2A} receptor of 261 nM and was 9.5-fold selective over the human A_1 receptor, indicating that the carbonyl of the acetate group of compound **22** was essential for activity. Extension of the alkyl group to give compounds **40** and **41** reduced selectivity and also potency in the case of **41**. A urea (**43**) or a carbamate (**44**) was tolerated but again had an inferior profile compared to compound **22**. After extensive synthetic efforts to further optimize the series, it was concluded that the simple 2-methylmethoxypyrrolidine analogue **22** had the most attractive profile in terms of activity and selectivity. In terms of physical properties compound **22** had a low molecular weight (344 g/mol), a measured log *D* of 2.2, and good solubility (1 mg/mL at pH 7.4 as the free base and >6.7 mg/mL as the HCl salt). The melting point for compound **22** was significantly lower than that of the lead molecule **3** (186 °C vs 283 °C), which we believe is largely responsible for the improvement in physical properties. Overall, compound **22** has the profile of an efficient molecule[14] with excellent physical properties for development and hence was further investigated in preclinical and efficacy assays.

Additional characterization of compound **22** for human A_{2A} functional activity, rat A_{2A} receptor binding affinity, metabolic stability, membrane permeability, cytochrome P450 (CYP450)

Table 6. In Vitro Data for Compound **22**

parameter	value
hA_{2A} K_i (nM) ± SEM[a,d]	4.7 ± 0
hcAMP IC_{50} (nM) ± SEM[b,d]	85 ± 16
rA_{2A} K_i (nM) ± SEM[c,d]	48 ± 10
CYP 3A4/2D6/2C9/2C19 inhibition (μM)	>50
CYP 1A2 inhibition (μM)	11
scaled intrinsic clearance (human liver microsomes) ((mL/min)/kg)	17
scaled intrinsic clearance (rat liver microsomes) ((mL/min)/kg)	74
Caco-2 rate × 10^{-6} cm/s [B > A/A > B ratio]	70 [0.7]
hERG patchclamp block at 10 μM (%)	19
solubility in PBS (mg/mL)	>6.7 (HCl salt)

[a] Displacement of specific [^{3}H]**46** binding at human A_{2A} receptors expressed in HEK293 cells. [b] hA_{2A} receptor antagonism of 3-[4-[2-[[6-amino-9-[(2*R*,3*R*,4*S*,5*S*)-5-(ethylcarbamoyl)-3,4-dihydroxyoxolan-2-yl]purin-2-yl]amino]ethyl]phenyl]propanoic acid (CGS-21680)[15] stimulated cAMP production. [c] Displacement of specific [^{3}H]**46** binding at rat A_{2A} receptors expressed in CHO cells. [d] Data are expressed as geometric mean values of at least two runs ± the standard error measurement (SEM).

Table 7. Plasma and Brain Exposure of **22** after Oral Administration at a Dose of 10 mg/kg in Rats[a]

time point (h)	brain levels (ng/g)	plasma levels (ng/mL)	B/P ratio
1	863 ± 530	1604 ± 716	0.51 ± 0.13
4	116 ± 71	333 ± 148	0.33 ± 0.08
24	BQL	BQL	NA

[a] BQL: below the limits of quantitation. NA: not available.

inhibition, human ether-a-go-go related gene (hERG) inhibition, and solubility (as the HCl salt) is summarized in Table 6. Compound **22** showed no significant inhibition of the major CYP450 enzymes, CYP3A4, 2D6, 2C9, 2C19, but was found to be a weak inhibitor of CYP1A2 (11 μM). Metabolic stability was good when incubated with human and rat liver microsomes, and no significant hERG channel block was observed at the concentrations tested. Importantly, compound **22** had very good solubility in phosphate buffered saline (PBS) as the HCl salt. Although binding at the rat A_{2A} receptor was 10 times weaker than at the human receptor, the potency of the compound was still sufficient to show efficacy in rat in vivo models.

Compound **22** was then characterized in a discrete rat pharmacokinetic study with 10 mg/kg oral dosing (po) and showed good exposure in both plasma and brain (Table 7). The T_{max} was found to be around 1 h (0.9 h) and the compound was rapidly eliminated. After 4 h, the brain levels were significantly decreased (863 ng/g at 1 h and 116 ng/g at 4 h) and were below the limits of quantitation at 24 h.

Compound **22** was subsequently tested for efficacy in two in vivo rat models. First, the ability of **22** to reverse haloperidol induced catalepsy (HIC) in rodent was evaluated. In this assay, compound **22** (3, 10, 30 mg/kg, po) was administered 2 h prior to the assay and significantly reduced descent latency at the 10 and 30 mg/kg doses (Figure 5). Exposure at all doses was acceptable (Table 8).

Compound **22** was also evaluated for its ability to potentiate L-dopa induced rotational behavior in the 6-hydroxydopamine (6-OHDA) unilateral lesioned rat. Lesioned, L-dopa sensitized rats (three treatments with 10 mg/kg L-dopa) were tested in a repeated measure, randomized design for responsiveness to vehicle, L-dopa (1 mg/kg), and **22** (3, 10, 30 mg/kg, po) in combination with L-dopa. As shown in Figure 6, compound **22** dose-dependently increased rotational behavior compared to L-dopa alone, with significant effects at both the 10 and 30 mg/kg doses.

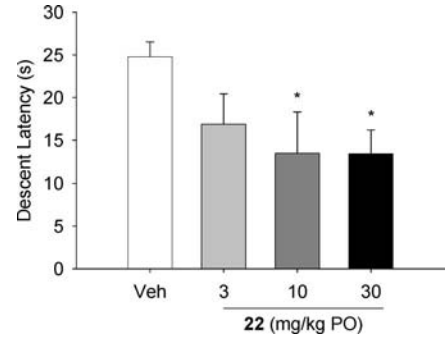


Figure 5. Effect of compound **22** (2 h after dose) on the mean decent-latency (±standard error) in seconds on haloperidol-induced catalepsy in the bar test at 3, 10, and 30 mg/kg, po: (*) vehicle versus treated rats, $p < 0.05$.

Table 8. Plasma and Brain Exposure for Compound **22**, at 2 h After Oral Dosing, in Rats used in the HIC Model

dose (mg/kg)	plasma (ng/mL)	brain (ng/g)	B/P ratio
3	98	34	0.37
10	1062	488	0.46
30	4638	2024	0.45

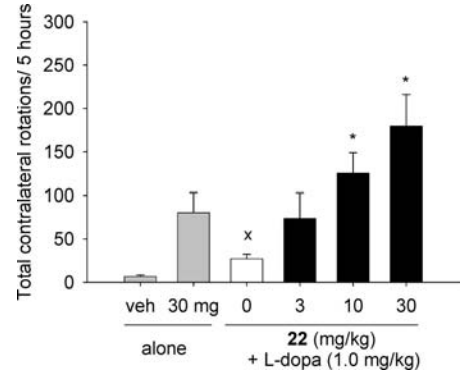
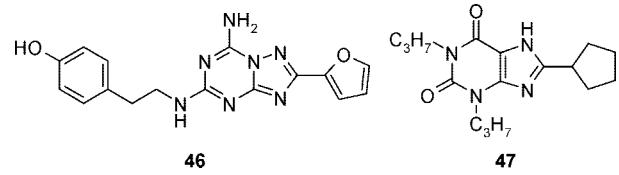


Figure 6. Potentiation of L-dopa induced rotations in unilaterally 6-OHDA-lesioned rats by **22** dosed orally at 30 mg/kg alone and 0, 3, 10, and 30 mg/kg plus L-dopa. The "×" notes that a 1 mg/kg dose of L-dopa causes a significant increase in rotations when compared to vehicle alone.



46 **47**

Figure 7. Structures of antagonists **46** and **47** used in binding assays.

The behavioral study in the HIC model was carried out 2 h after oral dosing of **22**, and the total contralateral rotation count with 6-OHDA was taken 5 h after oral dosing of **22**. Although the peak concentration was reached well before the time of measurement in both animal models, the in vivo concentration was still sufficient to account for the observed efficacy. Given that binding affinity of **22** to the human A_{2A} receptor is at least 10 times better than in the rat, it is expected that efficacy in humans should be observed at lower doses. Compound **22** was further assayed for off-target activity at Cerep[16] in 25 binding assays and 17 enzyme assays

and was found only to have activity on the closely related A_{2B} and A_3 receptors (hA_{2B} K_i = 145 nM; hA_3 K_i = 19 nM).

Conclusion

We have developed an atypical novel series of potent A_{2A} receptor antagonists with a reduced number of aromatic heterocycles. This decreases the planarity of the system and results in compounds with good pharmaceutical properties for drug development. Compound **22** demonstrates that it is possible to make hA_{2A} receptor antagonists with very good physical properties, good functional activity at the human A_{2A} receptor, and with no CYP or hERG liabilities. Despite a rat A_{2A} binding K_i of approximately 50 nM, compound **22** showed oral efficacy in two animal models at a dose of 10 mg/kg, and exhibited good exposure without the need of formulation. This work illustrates how less hydrophobic compounds can be developed as small molecule antagonists for the human A_{2A} receptor. Compound **22** was selected to advance into safety screening studies and for further detailed preclinical evaluation. These results will be reported in due course.

Experimental Section

Human and rat A_{2A} and A_1 binding assays,[7] metabolism studies,[8] hERG assays,[17] pharmacokinetic assays,[6] cytochrome P450 inhibition,[6] Caco-2 permeability assays,[6] HIC and 6-OHDA efficacy studies,[7] and solubility studies[8] were performed as described previously.

Chemistry. Reagents, starting materials, and solvents were purchased from commercial suppliers and used as received. Concentration refers to evaporation under vacuum using a Büchi rotatory evaporator. Reaction products were purified, when necessary, by flash chromatography on silica gel (40−63 μm) with the solvent system indicated. All NMR spectroscopic data were recorded on a Varian Mercury 300 MHz spectrometer. Coupling constants J are reported in Hz. The elemental analysis was done by Robertson Microlit Laboratory, Madison, NJ. Descriptions of analytical HPLC-MS methods 1−5 are given in Supporting Information. Preparative HPLC-MS platform: Dionex, equipped with a Gilson 215 autosampler/fraction collector, UV detector, and a Dionex MSQ mass detector. HPLC column: Phenomenex Synergy Max-RP, 21.2 mm × 50 mm. HPLC gradient: 35 mL/min, 5% acetonitrile in water to 95% acetonitrile in water in 17.7 min. Both acetonitrile and water have 0.05% TFA.

6-Chloro-2-(pyrazol-1-yl)pyrimidin-4-amine (6b). 6-Amino-2,4-dichloropyrimidine (25.0 g, 164 mmol, 1 equiv), pyrazole (15.5 g, 228 mmol, 1.5 equiv), and cesium carbonate (16.4 g, 228 mmol, 1.5 equiv) were heated at reflux in dioxane (150 mL) for 3 days. The reaction was allowed to cool to room temperature and filtered over Celite. The Celite was washed with dioxane (300 mL), and the filtrate was concentrated under vacuum. The residue was slurried with dichloromethane for 16 h and then filtered to give the desired-product as an off-white solid (8.1 g). The operation was repeated with the mother liquor to get a second crop (3.6 g) (39% overall yield). ^{1}H NMR ($CDCl_3$): δ 8.52 (d, J = 2.1, 1H), 7.78 (d, J = 0.6, 1H), 7.65 (d, J = 1.8, 1H), 6.45 (dd, J = 2.1 and 1.8, 1 H), 5.8 (bs, NH_2). LCMS-1: t_R = 3.03 (98%). MS: m/z 196.0 $[M + H]^+$, expected 196.0 $[M + H]^+$.

Intermediate **6a** was prepared according to the same procedure using 3,5-dimethylpyrazole in place of pyrazole.

***N*-[6-Chloro-2-(3,5-dimethylpyrazol-1-yl)pyrimidin-4-yl]acetamide (7a).**[9] 6-Chloro-2-(3,5-dimethylpyrazol-1-yl)pyrimidin-4-ylamine **6a** (40.0 g, 0.18 mol, 1 equiv) was dissolved in acetic acid (200 mL, 0.9 mol, 5 equiv) and stirred at ambient temperature. Acetic anhydride (80 mL, 0.8 mol, 4.7 equiv) was added, and the mixture was heated at 90 °C overnight. Once the reaction was complete, the mixture was cooled to room temperature and water (16 mL) was added over 30 min. The mixture was then filtered through filter paper, and the filter cake was washed with water (4 × 75 mL). The solid was dried in a vacuum oven at 50 °C overnight. The acetic acid solvate of **7a** was obtained as an off-white crystalline solid (48.2 g, 0.15 mol, 83%). ^{1}H NMR ($CDCl_3$): δ 9.07 (s, 1H), 8.03 (s, 1H), 6.03 (s, 1H), 2.65 (s, 3H), 2.30 (s, 3H), 2.22 (s, 3H), 2.12 (s, 3H).

Intermediates **7b**−**f** were prepared according to the same procedure using the corresponding intermediates **6b**−**f** as starting materials.

***N*-(6-Chloro-2-pyrazol-1-ylpyrimidin-4-yl)acetamide (7b).** ^{1}H NMR ($CDCl_3$): δ 8.54 (d, J = 2.4, 1H), 8.41 (s, 1H), 8.09 (s, 1H), 7.79 (bs, 1H), 6.49 (dd, J = 2.7, 0.9, 1H), 2.24 (s, 3H).

***N*-(6-Chloro-2-(5-methyl-2-furan-2-ylpyrimidin-4-yl))acetamide (7c).** ^{1}H NMR ($CDCl_3$): δ 8.21 (bs, 1H), 7.97 (s, 1H), 7.24 (d, J = 3.6, 1H), 6.18 (dd, J = 3.6, 0.9, 1H), 2.43 (d, J = 0.9, 3H), 2.22 (s, 3H).

***N*-[(6-Chloro-2-thiophen-2-yl)pyrimidin-4-yl]acetamide (7d).** ^{1}H NMR ($CDCl_3$): δ 8.00 (s, 1H), 7.98 (dd, J = 3.6, 1.2, 1H), 7.50 (dd, J = 5.1, 1.2, 1H), 7.13 (dd, J = 4.8, 0.9, 1H), 2.27 (s, 3H).

***N*-(6-Chloro-2-thiazol-2-ylpyrimidin-4-yl)acetamide (7e).** ^{1}H NMR ($CDCl_3$): δ 8.42 (bs, 1H), 8.20 (s, 1H), 8.02 (d, J = 3.0, 1H), 7.58 (d, J = 3.0, 1H), 2.25 (s, 3H).

***N*-(6-Chloro-2-pyridin-2-ylpyrimidin-4-yl)acetamide (7f).** ^{1}H NMR ($CDCl_3$): δ 8.80 (d, J = 4.5, 1H), 8.51 (d, J = 7.3, 1H), 8.42 (bs, 1H), 8.23 (s, 1H), 7.87 (dt, J = 7.3, 1.8, 1H), 7.43 (dd, J = 4.5, 1.2, 1H), 2.24 (s, 3H).

***N*-[6-Chloro-2-(3,5-dimethylpyrazol-1-yl)pyrimidin-4-yl]propionamide (8a).** Propionyl chloride (1.4 mL, 16.3 mmol, 1.2 equiv) was added slowly to cold dimethylformamide (30 mL, ice bath) followed by addition of anhydrous pyridine (1.3 mL, 16.3 mmol, 1.2 equiv) and intermediate **6a** (3.0 g, 13.6 mmol, 1 equiv). The mixture was allowed to warm to room temperature and stirred overnight. After completion of the reaction, the reaction mixture was neutralized with a saturated aqueous solution of sodium bicarbonate and the product extracted with dichloromethane (3 × 50 mL). The organic layers were combined, dried over magnesium sulfate, and concentrated. Purification by column chromatography eluting with dichloromethane with 2% methanol yielded the desired product **8a** as a light-brown solid (3.4 g, 75%). ^{1}H NMR ($CDCl_3$): δ 8.26 (bs, 1H), 8.06 (s, 1H), 6.04 (s, 1H), 2.66 (s, 3H), 2.43 (q, J = 7.5, 2H), 2.33 (s, 3H), 1.64 (bs, 1H), 1.24 (t, J = 7.5, 3H).

***N*-[6-Chloro-2-(3,5-dimethylpyrazol-1-yl)pyrimidin-4-yl]-3-methylbutyramide (9a).** Intermediate **9a** was prepared by reacting intermediate **6a** with isovaleryl chloride in a similar way as for intermediate **8a**. The residue was purified by liquid chromatography using a mixture of 1:1 ethyl acetate/hexanes to afford **9a** as a white solid in similar yield. ^{1}H NMR ($CDCl_3$): δ 8.09 (s, 1H), 6.05 (s, 1H), 2.66 (s, 3H), 2.31 (s, 3H), 2.30−2.16 (m, 3H), 1.01 (d, J = 6, 6H). LCMS-1: t_R = 5.51 (97%). MS: m/z 308.3 $[M + H]^+$, expected 308.3 $[M + H]^+$.

General Method for Compounds 10−31. Intermediate **7a** (50 mg, 0.19 mmol, 1 equiv) was dissolved in dry dioxane (2 mL) followed by addition of the appropriate amine (1.2 equiv, 0.23 mmol). The mixture was heated at 80 °C for 2 h, cooled to room temperature, filtered, and purified by HPLC.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-diethylamino-1-yl-pyrimidin-4-yl]acetamide (10).** ^{1}H NMR (DMSO-d_6): δ 8.09 (bs, 1H), 7.17 (s, 1H), 5.97 (s, 1H), 3.55−3.40 (m, 4H), 2.64 (s, 3H), 2.30 (s, 3H), 2.14 (s, 3H), 1.20 (t, J = 6.9, 6H). LCMS-2: t_R = 5.08 (100%). MS: m/z 303.1 $[M + H]^+$, expected 303.1 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-pyrrolidin-1-ylpyrimidin-4-yl]acetamide (11).** ^{1}H NMR (DMSO-d_6): δ 10.65 (s, 1H), 7.01 (s, 1H), 6.06 (s, 1H), 3.50 (brm, 2H), 3.35 (brm, 2H), 2.57 (s, 3H), 2.15 (s, 3H), 2.09 (s, 3H), 1.96 (brm, 4H). LCMS-3: t_R = 5.21 (100%). MS: m/z 301.0 $[M + H]^+$, expected 301.0 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-piperidin-1-ylpyrimidin-4-yl]acetamide (12).** ^{1}H NMR ($CDCl_3$): δ 7.11 (s, 1H), 6.13 (s, 1H), 3.80−3.70 (m, 4H), 2.67 (s, 3H), 2.34 (s, 6H), 1.82−1.64 (m, 6H). LCMS-2: t_R = 5.38 (100%). MS: m/z 314.9 $[M + H]^+$, expected 315.4 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-(2-oxopyrrolidin-1-yl)pyrimidin-4-yl]acetamide (13).** A mixture of intermediate **6a** (50 mg, 0.19 mmol, 1 equiv), pyrrolidinone (81 mg, 0.95 mmol, 5 equiv), palladium acetate (5 mg, 0.02 mmol, 0.1 equiv), 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (17 mg, 0.03 mmol, 0.15 equiv), and cesium carbonate (68 mg, 0.21 mmol, 1.1 equiv) was heated in dry toluene (2 mL) at 100 °C overnight. After return to room temperature and filtration, the mixture was purified by HPLC. ^{1}H NMR ($CDCl_3$): δ 8.92 (s, NH), 8.60 (s, 1H), 7.26 (s, 1H), 4.06 (t, J = 6.9, 2H), 2.68 (t, J = 6.9, 2H), 2.64 (s, 3H), 2.31 (s, 3H), 2.19 (s, 3H), 2.15 (t, J = 6.9, 2H). LCMS-2: t_R = 4.60 (96%). MS: m/z 315.2 $[M + H]^+$, expected 315.3 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-morpholin-4-ylpyrimidin-4-yl]acetamide (14).** ^{1}H NMR ($CDCl_3$): δ 7.20 (s, 1H), 6.16 (s, 1H), 3.85−3.70 (brm, 8H), 2.69 (s, 3H), 2.28 (s, 3H), 2.33 (s, 3H). LCMS-1: t_R = 6.19 (100%). MS: m/z 317.3 $[M + H]^+$, expected 317.4 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-(4-methylpiperazin-1-yl)pyrimidin-4-yl]acetamide (15).** ^{1}H NMR ($CDCl_3$): δ 8.10 (bs, 1H), 7.26 (s, 1H), 5.98 (s, 1H), 3.70 (t, J = 5.1, 4H), 2.62 (s, 2H), 2.49 (t, J = 5.1, 4H), 2.35 (s, 3H), 2.31 (s, 3H), 2.15 (s, 3H). LCMS-2: t_R = 2.53 (100%). MS: m/z 330.1 $[M + H]^+$, expected 330.4 $[M + H]^+$.

***N*-[6-(3,4-Dihydro-1*H*-isoquinolin-2-yl)-2-(3,5-dimethylpyrazol-1-yl)pyrimidin-4-yl]acetamide (16).** ^{1}H NMR (DMSO-d_6): δ 10.68 (s, 1H), 7.35 (s, 1H), 7.30−7.22 (m, 1H), 7.22−7.15 (m, 4H), 4.71 (brs, 2H), 3.79 (brm, 2H), 2.92 (t, J = 5.7, 2H), 2.59 (s, 3H), 2.15 (s, 3H), 2.10 (s, 3H). LCMS-2: t_R = 6.57 (100%). MS: m/z 363.0 $[M + H]^+$, expected 363.4 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-(2-methylpyrrolidin-1-yl)pyrimidin-4-yl]acetamide (17).** ^{1}H NMR ($CDCl_3$): δ 6.68 and 6.61 (2s, 1H), 6.14 (s, 1H), 4.47 and 4.16 (2m, 1H), 3.83−3.90 (m, 2H), 2.69 (brs, 3H), 2.34 (s, 3H), 2.32 (s, 3H), 2.20−1.78 (m, 4H), 1.28 and 1.26 (2s, 3H). LCMS-1: t_R = 7.91 (100%). MS: m/z 315.6 $[M + H]^+$, expected 315.4 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-(2-propylpyrrolidin-1-yl)pyrimidin-4-yl]acetamide (18).** ^{1}H NMR ($CDCl_3$): δ 6.89 (s, 1H), 6.12 (s, 1H), 4.27 (brs, 1H), 3.85 (brd, J = 36, 2H), 3.56 (brd, J = 49.2, 2H), 2.69 (s, 3H), 2.34 (s, 6H), 2.20−1.60 (m, 4H), 1.45−1.20 (m, 2H), 1.06−0.90 (m, 3H). LCMS-1: t_R = 9.27 (97%). MS: m/z 343.6 $[M + H]^+$, expected 343.5 $[M + H]^+$.

***(R)*-1-[6-Acetylamino-2-(3,5-dimethylpyrazol-1-yl)pyrimidin-4-yl]pyrrolidine-2-carboxylic Acid Methyl Ester (19).** ^{1}H NMR ($CDCl_3$): δ 8.15 (bs, 1H), 7.13 (s, 1H), 5.95 (s, 1H), 4.73 (d, J = 9, 1H), 3.81−3.65 (m, 1H), 3.66 (s, 3H), 3.58−3.44 (m, 1H), 2.52 (s, 3H), 2.29 (s, 3H), 2.14 (s, 3H), 2.18−2.05 (m, 2H), 1.88−1.78 (m, 2H). LCMS-2: t_R = 4.87 (100%). MS: m/z 359.2 $[M + H]^+$, expected 359.4 $[M + H]^+$.

***(S)*-1-[6-Acetylamino-2-(3,5-dimethylpyrazol-1-yl)pyrimidin-4-yl]pyrrolidine-2-carboxylic Acid Methyl Ester (20).** ^{1}H NMR ($CDCl_3$): δ 8.18 (bs, 1H), 7.14 (s, 1H), 5.95 (s, 1H), 4.73 (d, J = 9, 1H), 3.81−3.65 (m, 1H), 3.66 (s, 3H), 3.58−3.44 (m, 1H), 2.52 (s, 3H), 2.29 (s, 3H), 2.15 (s, 3H), 2.20−2.00 (m, 2H), 2.00−1.80 (m, 2H). LCMS-3: t_R = 4.20 (98%). MS: m/z 359.1 $[M + H]^+$, expected 359.4 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-(2-methoxymethylpyrrolidin-1-yl)pyrimidin-4-yl]acetamide (21).** LCMS-2: t_R = 5.04 (99%). MS: m/z 344.9 $[M + H]^+$, expected 345.4 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-(2-(*R*)-methoxymethylpyrrolidin-1-yl)pyrimidin-4-yl]acetamide (22).** ^{1}H NMR (DMSO-d_6): δ 6.82 (s, 1H), 6.14 (s, 1H), 4.38 (brs, 1H), 3.60−3.20 (brm, 4H), 3.25 (s, 3H), 2.57 (s, 3H), 2.17 (s, 3H), 2.12 (s, 3H), 2.05−1.90 (m, 4H). LCMS-4: t_R = 18.17 (100%). MS: m/z 345.2 $[M + H]^+$, expected 345.4 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-(2-(*S*)-methoxymethyl)pyrrolidin-1-ylpyrimidin-4-yl]acetamide (23).** ^{1}H NMR ($CDCl_3$): δ 7.04 and 6.92 (s, 1H), 6.11 and 6.05 (s, 1H), 3.74−3.37 (m, 2H), 3.37 (s, 3H), 2.68 (s, 3H), 2.34 (s, 3H), 2.32 (s, 3H), 2.24−1.94 (m, 4H). LCMS-1: t_R = 6.33 (98%). MS: m/z 345.2 $[M + H]^+$, expected 345.4 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-(2-ethoxymethylpyrrolidin-1-yl)pyrimidin-4-yl]acetamide (24).** ^{1}H NMR ($CDCl_3$): δ 7.02 and 6.89 (2s, 1H), 6.12 (s, 1H), 4.46 and 4.22 (2brs, 1H), 3.66−3.32 (brm, 4H), 3.46 (q, J = 6.9, 2H), 2.69 (s, 3H), 2.34 (s, 3H), 2.33 (s, 3H), 2.22−1.96 (m, 4H), 1.18 (t, J = 6.9, 3H). LCMS-1: t_R = 8.02 (100%). MS: m/z 359.7 $[M + H]^+$, expected 359.4 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-((*R*)-2-hydroxymethylpyrrolidin-1-yl)pyrimidin-4-yl]acetamide (25).** ^{1}H NMR ($CDCl_3$): δ 7.52 (s, 1H), 6.14 (s, 1H), 4.14 (brs, 1H), 3.78−3.45 (brm, 4H), 2.68 (s, 3H), 2.37 (s, 3H), 2.33 (s, 3H), 2.27−2.05 (m, 4H). LCMS-3: t_R = 4.57 (100%). MS: m/z 331.2 $[M + H]^+$, expected 331.4 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-[(*R*)-2-(2-methoxyethyl)piperidin-1-yl]pyrimidin-4-yl]acetamide (26).** LCMS-3: t_R = 5.83 (96%). MS: m/z 373.0 $[M + H]^+$, expected 373.5 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-((*R*)-2-methoxymethyl-5-methylpyrrolidin-1-yl)pyrimidin-4-yl]acetamide (27).** ^{1}H NMR ($CDCl_3$): δ 6.81 (brs, 1H), 6.14 (s, 1H), 4.46 (brs, 1H), 4.13 (brd, J = 36.9, 1H), 3.65−3.39 (m, 2H), 3.36 (s, 3H), 2.69 (s, 3H), 2.35 (s, 3H), 2.34 (s, 3H), 2.19−1.78 (m, 4H), 1.37 and 1.35 (2s, 3H). LCMS-3: t_R = 5.50 (95%). MS: m/z 359.2 $[M + H]^+$, expected 359.4 $[M + H]^+$. LCMS-1: t_R = 3.61 (100%). MS: m/z 359.3 $[M + H]^+$, expected 359.4 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-[(*R*)-2-(pyridin-2-yloxymethyl)pyrrolidin-1-yl]pyrimidin-4-yl]acetamide (28).** ^{1}H NMR ($CDCl_3$): δ 8.09 (d, J = 5.1, 1H), 7.70−7.57 (m, 1H), 6.99−6.80 (m, 2H), 6.74 (s, 1H), 6.11 (s, 1H), 4.72 (brs, 1H), 4.55−4.47 (m, 1H), 4.42−4.34 (m, 1H), 3.82−3.48 (m, 1H), 2.63 (s, 3H), 2.36 (s, 3H), 2.34 (s, 3H), 2.34−2.08 (m, 4H). LCMS-2: t_R = 5.63 (100%). MS: m/z 407.9 $[M + H]^+$, expected 408.5 $[M + H]^+$. LCMS-1: t_R = 3.80 (100%). MS: m/z 408.3 $[M + H]^+$, expected 408.5 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-[(*S*)-2-(pyridin-2-yloxymethyl)pyrrolidin-1-yl]pyrimidin-4-yl]acetamide (29).** LCMS-2: t_R = 5.66 (100%). MS: m/z 408.0 $[M + H]^+$, expected 408.5 $[M + H]^+$. LCMS-5: t_R = 2.23 (100%). MS: m/z 408.8 $[M + H]^+$, expected 408.5 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-((*S*)-4-methoxy-(*R*)-2-methoxymethylpyrrolidin-1-yl)pyrimidin-4-yl]acetamide (30).** ^{1}H NMR ($CDCl_3$): δ 6.81 (d, J = 30.6, 1H), 6.13 (s, 1H), 4.44 (d, J = 30.6, 1H), 4.20 (brs, 1H), 3.72 (dd, J = 12.3 and 5.1, 2H), 3.66−3.35 (brm, 2H), 3.33 (s, 3H), 3.30 (s, 3H), 2.67 (d, J = 10.2, 3H), 2.34 (s, 6H), 2.30−2.10 (m, 2H). LCMS-2: t_R = 4.85 (100%). MS: m/z 375.4 $[M + H]^+$, expected 375.4 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-((*R*)-4-methoxy-(*R*)-2-methoxymethylpyrrolidin-1-yl)pyrimidin-4-yl]acetamide (31).** ^{1}H NMR ($CDCl_3$): δ 6.75 (s, 1H), 6.15 (s, 1H), 4.54−3.42 (m, 4H), 3.36 (s, 6H), 2.70 (s, 3H), 2.36 (s, 3H), 2.34 (s, 3H), 2.45−2.05 (m, 2H). LCMS-2: t_R = 4.82 (99%). MS: m/z 375.3 $[M + H]^+$, expected 375.4 $[M + H]^+$. LCMS-1: t_R = 3.15 (100%). MS: m/z 375.2 $[M + H]^+$, expected 375.4 $[M + H]^+$.

Compounds **32**−**39** were prepared according to the same procedure used to prepare compound **10**, using **7c**−**f** to the yield the desired product in similar yields.

***N*-[6-((*R*)-2-Methoxymethylpyrrolidin-1-yl)-2-(5-methylfuran-2-yl)pyrimidin-4-yl]acetamide (32).** ^{1}H NMR (DMSO-d_6): δ 10.55 (s, 1H), 7.01 (d, J = 3, 1H), 6.26 (dd, J = 0.9, 2.1, 1H), 3.37−3.34 (m, 5H), 3.31 (s, 3H), 2.34 (s, 3H), 2.09 (s, 3H), 1.95 (brm, 4H). LCMS-3: t_R = 5.29 (100%). MS: m/z 331.1 $[M + H]^+$, expected 331.4 $[M + H]^+$.

***N*-[6-((*S*)-2-Methoxymethylpyrrolidin-1-yl)-2-(5-methylfuran-2-yl)pyrimidin-4-yl]acetamide (33).** ^{1}H NMR (DMSO-d_6): δ 10.52 (s, 1H), 6.99 (d, J = 3, 1H), 6.26 (d, J = 3, 1H), 3.37−3.34 (m, 5H), 3.31 (s, 3H), 2.34 (s, 3H), 2.08 (s, 3H), 1.95 (brm, 4H). LCMS-2: t_R = 4.16 (98%). MS: m/z 331.1 $[M + H]^+$, expected 331.4 $[M + H]^+$.

***N*-[6-((*R*)-2-Methoxymethylpyrrolidin-1-yl)-2-thiophen-2-ylpyrimidin-4-yl]acetamide (34).** ^{1}H NMR ($CDCl_3$): δ 8.39 (d, J = 3.6, 1H), 7.60 (d, J = 5.1, 1H), 7.27 (d, J = 5.1, 1H), 7.21 (t, J = 5.1, 1H), 3.71−3.40 (m, 5H), 3.86 (s, 3H), 2.31 (s, 1H), 2.30−1.95 (m, 4H). LCMS-1: t_R = 5.36 (97%). MS: m/z 332.9 $[M + H]^+$, expected 333.4 $[M + H]^+$.

***N*-[6-((*S*)-2-Methoxymethylpyrrolidin-1-yl)-2-thiophen-2-yl-pyrimidin-4-yl]acetamide (35).** ^{1}H NMR ($CDCl_3$): δ 8.43 (d, $J = 3.9$, 1H), 7.65 (d, $J = 3.9$, 1H), 7.27−7.19 (m, 2H), 4.64 (brs, 1H), 3.89 (brs, 1H), 3.69−3.40 (brm, 3H), 3.39 (s, 3H), 2.34 (s, 3H), 2.28−2.05 (m, 4H). LCMS-1: $t_R = 8.79$ (97%). MS: *m*/*z* 333.3 [M + H]$^+$, expected 333.4 [M + H]$^+$.

***N*-[6-((*R*)-2-Methoxymethylpyrrolidin-1-yl)-2-thiazol-2-ylpyrimidin-4-yl]acetamide (36).** ^{1}H NMR ($CDCl_3$): δ 8.05 (d, $J = 2.7$, 1H), 7.63 (d, $J = 2.7$, 1H), 7.14 (s, 1H), 4.60 (m, 1H), 4.20 (m, NH), 3.81 and 3.63 (AB syst, 2H), 3.62 and 3.50 (AB syst, 2H), 3.39 (s, 3H), 2.31 (s, 3H), 2.26−2.00 (m, 4H). LCMS-2: $t_R = 4.60$ (97%). MS: *m*/*z* 334.0 [M + H]$^+$, expected 334.4 [M + H]$^+$.

***N*-[6-((*S*)-2-Methoxymethylpyrrolidin-1-yl)-2-thiazol-2-ylpyrimidin-4-yl]acetamide (37).** ^{1}H NMR ($CDCl_3$): δ 8.12 (d, $J = 3.0$, 1H), 7.73 (d, $J = 3.0$, 1H), 6.97 (s, 1H), 4.65 (brs, 1H), 3.87 (brs, 1H), 3.69−3.35 (m, 3H), 3.39 (s, 3H), 2.37 (s, 3H), 2.29−1.95 (m, 4H). LCMS-2: $t_R = 4.60$ (99%). MS: *m*/*z* 334.2 [M + H]$^+$, expected 334.4 [M + H]$^+$. LCMS-1: $t_R = 3.15$ (100%).

***N*-[6-((*R*)-2-Methoxymethylpyrrolidin-1-yl)-2-pyridin-2-ylpyrimidin-4-yl]acetamide (38).** ^{1}H NMR ($CDCl_3$): δ 8.82 (d, $J = 4.2$, 1H), 8.40 (m, 1H), 7.99 (t, $J = 6.3$, 1H), 7.60 (t, $J = 6.3$, 1H), 7.10 (s, 1H), 4.70 (m, 1H), 4.25 (m, NH), 3.90 and 3.68 (AB syst, 2H), 3.62 and 3.50 (AB syst, 2H), 3.67 (s, 3H), 2.39 (s, 3H), 2.30−2.00 (m, 4H). LCMS-3: $t_R = 4.84$ (100%). MS: *m*/*z* 328.2 [M + H]$^+$, expected 328.4 [M + H]$^+$.

***N*-[6-((*S*)-2-Methoxymethylpyrrolidin-1-yl)-2-pyridin-2-ylpyrimidin-4-yl]acetamide (39).** ^{1}H NMR ($CDCl_3$): δ 8.78−8.84 (m, 1H), 8.41 (dd, $J = 16.8$ and 8.1, 1H), 8.05−7.95 (m, 1H), 7.68−7.57 (m, 1H), 6.78 (s, 1H), 4.71 (brs, 1H), 3.90 (brs, 1H), 3.70−3.42 (brm, 3H), 3.37 (s, 3H), 2.37 (s, 3H), 2.26−1.96 (m, 4H). LCMS-3: $t_R = 4.83$ (100%). MS: *m*/*z* 328.5 [M + H]$^+$, expected 328.4 [M + H]$^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-((*R*)-2-methoxymethylpyrrolidin-1-yl)pyrimidin-4-yl]propionamide (40).** Compound **40** was prepared by reacting intermediate **8a** with (*R*)-2-methoxymethylpyrrolidine using the method described for compound **10**. ^{1}H NMR (DMSO-d_6): δ 10.62 (s, 1H), 7.09 (s, 1H), 6.06 (s, 1H), 4.36 (brs, 1H), 3.80−3.40 (brm, 4H), 3.27 (brs, 3H), 2.56 (s, 3H), 2.41 (q, $J = 7.5$, 2H), 2.15 (s, 3H), 2.10−1.80 (m, 4H), 1.04 (t, $J = 7.5$, 3H). LCMS-3: $t_R = 5.56$ (100%). MS: *m*/*z* 359.1 [M + H]$^+$, expected 359.4 [M + H]$^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-((*R*)-2-methoxymethylpyrrolidin-1-yl)pyrimidin-4-yl]-3-methylbutyramide (41).** Compound **41** was prepared by reacting intermediate **9a** with (*R*)-2-methoxymethylpyrrolidine using the method described for compound **10**. ^{1}H NMR (DMSO-d_6): δ 10.62 (s, 1H), 6.07 (s, 1H), 3.37−3.34 (m, 5H), 3.27 (s, 3H), 2.57 (s, 3H), 2.30−2.28 (m, 3H), 2.16 (s, 3H), 1.96 (brm, 4H), 0.91 (d, $J = 6.6$, 6H). LCMS-3: $t_R = 6.11$ (100%). MS: *m*/*z* 387.1 [M + H]$^+$, expected 387.5 [M + H]$^+$.

2-(3,5-Dimethylpyrazol-1-yl)-6-((*R*)-2-methoxymethylpyrrolidin-1-yl)pyrimidin-4-ylamine (42). To ethanol (2 mL) was added 6-chloro-2-(3,5-dimethylpyrazol-1-yl)pyrimidin-4-ylamine **6a** (250 mg, 1.1 mmol), diisopropylamine (3 equiv), and (*R*)-2-methoxymethylpyrrolidine (3 equiv). The mixture was heated overnight at 80 °C to give compound **42**. The product was purified by HPLC. ^{1}H NMR ($CDCl_3$): δ 8.18 (m, 2H), 6.04 (s, 1H), 5.50 (bs, 1H), 4.25−4.15 (m, 1H), 4.10−3.80 (m, 2H), 3.60−3.40 and 3.40−3.20 (AB syst, 2H), 3.33 (s, 3H), 2.60 (s, 3H), 2.27 (s, 3H), 2.20−1.80 (m, 4H). LCMS-3: $t_R = 4.93$ (100%). MS: *m*/*z* 303.1 [M + H]$^+$, expected 303.4 [M + H]$^+$.

1-[2-(3,5-Dimethylpyrazol-1-yl)-6-((*R*)-2-methoxymethylpyrrolidin-1-yl)pyrimidin-4-yl]-3-isopropylurea (43). To **42** (75 mg) in dichloromethane (1 mL) at 0 °C was added dry pyridine (20 μL, 1 equiv) followed by triphosgene (75 mg, 1 equiv). After 30 min, isopropylamine (1 equiv) was added. The mixture was allowed to warm to room temperature and stirred overnight. Dichloromethane was removed by evaporation, the residue dissolved in methanol (1 mL), and the product compound **43** purified by HPLC. ^{1}H NMR (DMSO-d_6): δ 9.38 (s, 1H), 8.90 (s, 1H), 6.10 (s, 1H), 3.75−3.45 (m, 5H), 3.26 (s, 3H), 2.59 (s, 3H), 2.17 (s, 3H), 1.95 (brm, 4H), 1.16 (d, $J = 6.3$, 6H). LCMS-3: $t_R = 6.09$ (100%). MS: *m*/*z* 388.1 [M + H]$^+$, expected 388.5 [M + H]$^+$. LCMS-5: $t_R = 2.29$ (100%). MS: *m*/*z* 387.8 [M + H]$^+$, expected 388.5 [M + H]$^+$.

[2-(3,5-Dimethylpyrazol-1-yl)-6-((*R*)-2-methoxymethylpyrrolidin-1-yl)pyrimidin-4-yl]carbamic Acid Methyl Ester (44). To **42** (75 mg) in dichloromethane (1 mL) at 0 °C was added dry pyridine (20 μL, 1 equiv) followed by triphosgene (75 mg, 1 equiv). After 30 min, methanol (1 equiv) was added. The mixture was allowed to warm up to room temperature and stirred overnight. Dichloromethane was removed by evaporation, the residue dissolved in methanol (1 mL), and the product compound **44** purified by HPLC. ^{1}H NMR ($CDCl_3$): δ 6.72 (s, 1H), 6.14 (s, 1H), 4.45 (m, 1H), 4.40−3.90 (m, 2H), 3.87 (s, 3H), 3.70−3.40 (m, 2H), 3.33 (s, 3H), 2.68 (s, 3H), 235 (s, 3H), 2.25−1.95 (m, 4H). LCMS-3: $t_R = 5.62$ (100%). MS: *m*/*z* 361.1 [M + H]$^+$, expected 361.4 [M + H]$^+$.

Acknowledgment. We thank Shawn Ayube, Chris Devore, Paddi Ekhlassi, and John Harman for analytical support.

Supporting Information Available: Compound purities, detailed descriptions of HPLC conditions used for purity assessment, and NMR and LCMS data for final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Kase, H.; Mori, A.; Jenner, P. Adenosine A_{2A}-receptor antagonists: beyond dopaminergic therapies for Parkinson's disease. *Drug Discovery Today: Ther. Strategies* **2004**, *1*, 51–57. (b) Xu, K.; Bastia, E.; Schwarzschild, M. Therapeutic potential of adenosine A_{2A} receptor antagonists in Parkinson's disease. *Pharmacol. Ther.* **2005**, *105*, 267–310.

(2) (a) Vu, C. B. Recent advances in the design and optimization of adenosine A_{2A} receptor antagonists. *Curr. Opin. Drug Discovery Dev.* **2005**, *8*, 458–468. (b) Cacciari, B.; Pastori, G.; Spalluto, G. Medicinal chemistry of A_{2A} adenosine receptor antagonists. *Curr. Top. Med. Chem.* **2003**, *3*, 403–411. (c) Yuzlenko, O.; Kiec-Kononowicz, K. Potent adenosine A_1 and A_{2A} receptors antagonists: recent developments. *Curr. Med. Chem.* **2006**, *13*, 3609–3625. (d) Jacobson, K. A.; Gao, A.-G. Adenosine receptors as therapeutic targets. *Nat. Rev.* **2006**, *5*, 247–264.

(3) Jenner, P. Istradefylline, a novel adenosine A_{2A} receptor antagonist, for the treatment of Parkinson's disease. *Expert Opin. Invest. Drugs* **2005**, *14*, 729–738.

(4) Neustadt, B. R.; Hao, J.; Lindo, N.; Greenlee, W. J.; Stamford, A. W.; Tulshian, D.; Ongini, E.; Hunter, J.; Monopoli, A.; Bertorelli, R.; Foster, C.; Arik, L.; Lachowicz, J.; Ng, K.; Feng, K.-I. Potent, selective, and orally active adenosine A_{2A} receptor antagonists: arylpiperazine derivatives of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1376–1380.

(5) Lightowler, S. Presented at the International Research Conference, Targeting Adenosine A_{2A} Receptors in Parkinson's Disease and Other CNS Disorders, Boston, MA, May 2006.

(6) Study To Evaluate SYN115 in Parkinson's Disease, Synosia Therapeutics. www.clinicaltrials.gov. March 14, 2008.

(7) Zhang, X.; Tellew, J. E.; Luo, Z.; Moorjani, M.; Lin, E.; Lanier, M. C.; Chen, Y.; Williams, J. P.; Saunders, J.; Lechner, S. M.; Markison, S.; Joswig, T.; Petroski, R.; Piercey, J.; Kargo, W.; Malany, S.; Santos, M.; Gross, R. S.; Wen, J.; Jalali, K.; O'Brien, Z.; Stotz, C. E.; Crespo, M. I.; Di´az, J. L.; Slee, D. H. Lead Optimization of 4-acetylamino-2-(3,5-dimethylpyrazol-1-yl)-6-pyridylpyrimidines as A2A adenosine antagonists for the treatment of Parkinson's disease. *J. Med. Chem.* **2008**, *51*, 7099–7110.

(8) 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.; Diaz, J.-L.; Wen, J.; O'Brien, Z.; Saunders, J. Identification of novel, water soluble 2-amino-*N*-pyrimidin-4-yl acetamides as A_{2A} antagonists with in vivo efficacy. *J. Med. Chem.* **2008**, *51*, 400–406.

(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.; Yang, C.; Castro-Palomino, J. C.; Crespo, M. I.; Prat, M.; Gual, S.; Diaz, J.-L.; Saunders, J. 2-Amino-*N*-pyrimidin-4-ylacetamides as A2A receptor antagonists: 1. Structure−activity relationships and optimization of heterocyclic substituents. *J. Med. Chem.* **2008**, *51*, 1719–1729.

(10) Crystal structures were generated at the UCSD Small-Molecule Crystallography Facility. The crystal system and space group assignments were unambiguous. All non-hydrogen atoms were

refined anisotropically by full-matrix least-squares (SHELXL-97). All hydrogen atoms, with the exception of the hydroxyl hydrogen H2a, were placed using a riding model. Their positions were constrained relative to their parent atom using the appropriate HFIX command in SHELXL-97. The hydroxyl hydrogen H2a was located from the difference map, and its location was refined isotropically. The target molecule cocrystallized with a half of molecule of 1,4-dioxane, which lies on the inversion center, to form the asymmetric unit. All software was contained in the APEX, SAINT, and SHELX software distributed by Bruker-AXS, Madison, WI. Crystal data for **45**: CCDC 710881, $C_{19}H_{21}N_5O_3$, triclinic, space group $P\bar{1}$, a = 8.0030 (9) Å, b = 9.9780 (12) Å, c = 11.6910 (14) Å, β = 77.992(2)°, V = 893.07 (18) Å^3, Z = 2, T = 100 K, R_{int} = 0.0409. R1 is 6.3% based on 3142 independent reflections.

(11) Selkirk, J. V.; Nottebaum, L. M.; Ford, I. C.; Santos, M.; Malany, S.; Foster, A. C.; Lechner, S. M. A novel cell-based assay for G-protein-coupled receptor-mediated cyclic adenosine monophosphate response element binding protein phosphorylation. *J. Biomol. Screening* **2006**, *11*, 351–358.

(12) 4-[2-(7-Amino-2-furan-2-yl[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-ylamino)ethyl]phenol (ZM241385): Cacciari, B.; Pastorin, G.; Spalluto, G. Medicinal chemistry of A2A adenosine receptor antagonists. *Curr. Top. Med. Chem.* **2003**, *3*, 403–411.

(13) 8-Cyclopentyl-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (DPCPX): Coates, J.; Sheehan, M. J.; Strong, P. 1,3-Dipropyl-8-cyclopentyl xanthine (DPCPX): a useful tool for pharmacologists and physiologists. *Gen. Pharmacol.* **1994**, *25*, 387–394.

(14) Hopkins, A. L.; Groom, C. R.; Alex, A. Ligand efficiency: a useful metric for lead selection. *Drug Discovery Today* **2004**, *9*, 430–431.

(15) 3-[4-[2-[[6-Amino-9-[(2*R*,3*R*,4*S*,5*S*)-5-(ethylcarbamoyl)-3,4-dihydroxy-oxolan-2-yl]purin-2-yl]amino]ethyl]phenyl]propanoic acid (CGS-21680): Cristalli, G.; Lambertucci, C.; Taffi, S.; Vittori, S.; Volpini, R. Medicinal chemistry of adenosine A2A receptor agonists. *Curr. Top. Med. Chem.* **2003**, *3*, 387–401.

(16) http://www.cerep.fr.

(17) Slee, D. H.; Moorjani, M.; Zhang, X.; 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.; Diaz, J.-L.; Jalali, K.; Sai, Y.; Zuo, Z.; Yang, C.; Wen, J.; O'Brien, Z.; Petroski, R.; Saunders, J. 2-Amino-*N*-pyrimidin-4-ylacetamides as A_{2A} receptor antagonists: 2. Reduction of hERG activity, observed species selectivity, and structure−activity relationships. *J. Med. Chem.* **2008**, *51*, 1730–1739.

JM800908D