Piperazine Derivatives of [1,2,4]Triazolo[1,5-*a*][1,3,5]triazine as Potent and Selective Adenosine A_{2a} Receptor Antagonists

Chi B. Vu,* Bo Peng, Gnanasambandam Kumaravel, Glenn Smits, Xiaowei Jin, Deepali Phadke, Thomas Engber, Carol Huang, Jennifer Reilly, Stacy Tam, Donna Grant, Gregg Hetu, Liqing Chen, Jianbo Zhang, and Russell C. Petter

Department of Medicinal Chemistry, Biogen Idec, Inc., 14 Cambridge Center, Cambridge, Massachusetts 02142

Received February 24, 2004

The [1,2,4]triazolo[1,5-*a*]triazine derivative **3**, more commonly known in the field of adenosine research as ZM-241385, has previously been demonstrated to be a potent and selective adenosine A_{2a} receptor antagonist, although with limited oral bioavailability. This [1,2,4]triazolo[1,5-*a*]-triazine core structure has now been improved by incorporating various piperazine derivatives. With some preliminary optimization, the A_{2a} binding affinity of some of the best piperazine derivatives is almost as good as that of compound **3**. The selectivity level over the adenosine A_1 receptor subtype for some of the more active analogues is also fairly high, >400-fold in some cases. Many compounds within this piperazine series of [1,2,4]triazolo[1,5-*a*]triazine have now been shown to have good oral bioavailability in the rat, with some as high as 89% (compound **35**). More significantly, some piperazines derivatives of [1,2,4]triazolo[1,5-*a*]-triazine also possessed good oral efficacy in rodent models of Parkinson's disease. For instance, compound **34** was orally active in the rat catalepsy model at 3 mg/kg. In the 6-hydroxydopamine-lesioned rat model, this compound was also quite effective, with a minimum effective dose of 3 mg/kg po.

Introduction

In recent years, the adenosine receptors have emerged as potential drug targets.[1] These receptors have been extensively characterized and divided into four different subtypes (A_1, A_{2a}, A_{2b}, and A_3).[2] Selective agonists[3] and antagonists[4] of these four adenosine receptor subtypes have been shown to elicit a variety of biological responses that could be beneficial in a number of clinical settings. The A_{2a} receptor is abundant in the basal ganglia, a region of the brain that has been shown to be important in motor function.[5] There are currently a number of pharmacological studies that demonstrate that modulation of the A_{2a} receptor will produce significant changes in motor movement.[6,7] In rats, intracerebroventricular administration with a selective A_{2a} receptor agonist such as CGS-21680 induces catalepsy, a condition that shares some similarities with symptoms of human disorders such as Parkinson's disease.[7] Furthermore, this cataleptic condition could be reversed by the use of selective adenosine A_{2a} receptor antagonists.[7,8] Although clinical data are not yet available, selective antagonism of the A_{2a} receptor in a primate model of Parkinson's disease ameliorates motor depression. Specifically, in marmosets, where MPTP was used to induced motor disabilities similar to those exhibited in Parkinsonian patients, oral administration with the selective A_{2a} receptor antagonist **1** (KW-6002) at 10 mg/kg has been shown to improve disability without causing hyperactivity or dyskinesia.[9]

Compound **1** represents the successful optimization of the classical xanthine template.[10] It is currently being evaluated in clinical trials for the treatment of Parkinson's disease. Considerable efforts have also been devoted to identifying non-xanthine adenosine A_{2a} receptor antagonists.[11] Previous non-xanthine leads from the literature include compound **2** (SCH-58261) and compound **3** (ZM-241385) (Figure 1).[12,13] Since its disclosure in the early 1990s, significant improvement has been made with the tricyclic derivative **2**.[14] Most notable is the recent report regarding the use of piperazine derivatives to increase the oral bioavailability of this tricyclic template.[15,16] Compound **3** has previously been shown to be a highly potent and selective adenosine A_{2a} receptor antagonist.[17] However, because of the lack of oral bioavailability, compound **3** has been of limited utility in pharmacological assays.[18] Subsequent work centering around these [1,2,4]triazolo[1,5-*a*]triazines has not resulted in an improvement in oral bioavailability.[19] In connection with our recent interest in adenosine A_{2a} receptor antagonists, we reexamined this bicyclic template. The main objective was to identify new derivatives that are not only orally bioavailable but also capable of crossing the blood−brain barrier in order to achieve efficacy in various rodent models of Parkinson's disease. On the basis of the promising in vivo activity of piperazine derivatives in a related series of A_{2a} receptor antagonists,[15,16] we incorporated this diamine into the [1,2,4]triazolo[1,5-*a*]triazine template (general structure **4**).

Results and Discussion

Scheme 1 illustrates how compounds of the general structure **4** were prepared. Sulfone **5**[13] was first exposed to an excess of piperazine in order to obtain compound **6**. This could then be treated with a variety of aldehydes under reductive amination conditions to afford piperazine derivatives **9**−**45**. In cases where the corresponding

* To whom correspondence should be addressed. Telephone: 617-679-3861. Fax: 617-679-2616. E-mail: chi.vu@biogenidec.com.

10.1021/jm0498405 CCC: $27.50
Published on Web 07/13/2004

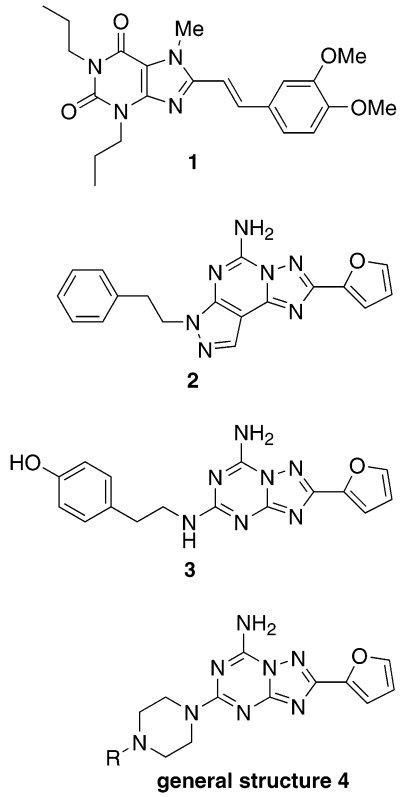
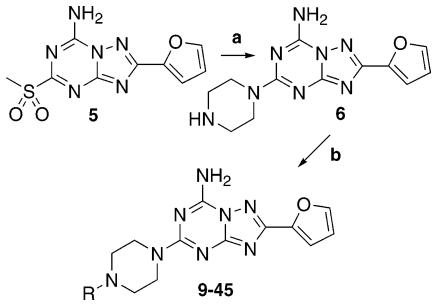


Figure 1.

Scheme 1[a]



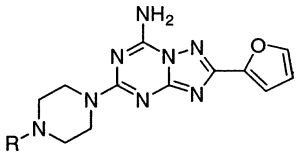
[a] Reagents and conditions: (a) 5 equiv of piperazine, CH_3CN, reflux; (b) RCHO, $Na(OAc)_3BH$, CH_2Cl_2, room temp; or RCH_2Cl, Et_3N, CH_3CN, room temp. For compounds **7** and **8**, phenylpiperazine and benzylpiperazine were condensed, respectively, with **3**.

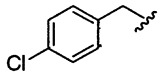
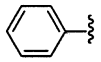
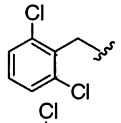
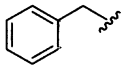
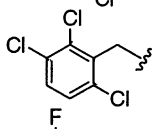
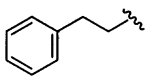
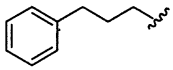
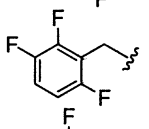
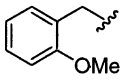
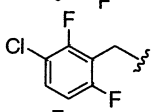
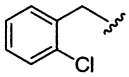
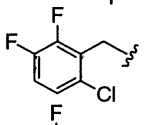
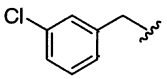
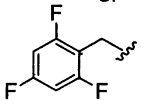
aldehyde was not available, the intermediate **6** could be alkylated instead with the desired halide or mesylate. Table 1 summarizes the examples having substituted or unsubstituted phenyl or benzyl derivatives as capping group R. For practical purposes, we chose the 2-furanyl as the constant aryl substituent of the [1,2,4]-triazolo[1,5-*a*]triazine core. This was based on some previously available SAR studies that indicated that 2-furanyl was optimal at that particular position.[20] The A_{2a} and A_1 binding affinities for these compounds are summarized in Table 1. For the A_{2a} receptor, membranes were prepared from rat brain tissues and the radioligand binding assay was performed using the radioligand [^{3}H]ZM-241385. For the A_1 receptor, membranes were prepared from rat cerebral cortex and the radioligand binding assay was performed using the radioligand [^{3}H]DPCPX. As a positive control for these radioligand binding assays, we routinely employed compound **2**, which had a K_i value at A_{2a} receptors of 37 nM and a K_i value at A_1 receptors of 390 nM. As additional points of reference, the binding affinities for compounds **1** and **3** were also determined. For compound **1**, the K_i value at A_{2a} receptors was 42 nM and the K_i value at A_1 receptors was 930 nM. Similarly, for compound **3**, the K_i value at A_{2a} receptors was 0.9 nM and the K_i value at A_1 receptors was 680 nM. Compound **6** was essentially inactive, indicating that some form of capping group was needed for A_{2a} activity. When a phenyl group was installed as a capping group, the optimal chain length was explored with compounds **7**−**10**. Substituted benzyl derivatives appeared to be optimal, which was convenient because a large number of substituted benzaldehydes and benzyl halides were commercially available. Looking closely at the type of substitution pattern on the phenyl ring, we observed that electron-withdrawing groups such as chloro and fluoro were more favorable than electron-donating groups such as OMe (compare compounds **11** and **12**). With chlorine as the aromatic substituent, ortho substitution as in **12** was clearly superior to either meta or para substitution (**13** and **14**, respectively). With two chlorine atoms, as in **15**, the binding affinity toward A_{2a} improved somewhat. When three chlorine atoms are used (compound **16**), a more dramatic improvement in A_{2a} binding affinity was observed. Substituting fluoro for chloro was better for A_{2a} activity, as evident by the 7-fold improvement in affinity between compounds **15** and **17**. Again, trisubstitution on the phenyl ring increased the binding affinity even more (compound **18**). When a chlorine atom was substituted in the appropriate position, as in **20**, there was a marked improvement in binding affinity toward A_{2a}. With the *o*,*o*,*p*-substitution pattern, as in **21**, a very potent and selective adenosine A_{2a} receptor antagonist was obtained.

Table **2** lists the compounds where the phenyl group has been replaced with a heterocyclic group. Unsubstituted heterocycles, in general, worked reasonably well, and fairly potent and selective A_{2a} antagonists were obtained (compounds **22**−**27**). As observed previously in the substituted phenyl derivatives, substitution at the 2-position of a heterocycle will improve the A_{2a} activity (compare compounds **27** and **28**; also compare compound **22** with compounds **29** and **30**). Compounds **31** and **32** illustrated a more dramatic improvement in A_{2a} binding affinity when two methyl groups were placed in the correct position of the heterocycle. A similar trend was observed with the pyrazine derivatives **33** and **34**. With the pyridine ring as the heterocyclic component, a similar trend was observed favoring analogues having substitution at the 2-position as in **38** and **39**. Sustituting two chlorine atoms at the 2-position (compound **40**) resulted in a very potent A_{2a} receptor antagonist. When the pyridine ring was replaced with the quinoline ring as in **41**−**45**, again, a similar trend was observed. The 2-chloro-substituted quinoline derivative **44** showed a 10-fold increase in A_{2a} activity, and that activity was diminished slightly with an additional Me group on the quinoline ring (compound **45**).

There are a number of rodent models for Parkinson's disease, and we employed three of them to evaluate the in vivo activity of these piperazine derivatives. As a preliminary evaluation for both oral bioavailability and

Table 1. Phenyl Derivatives as Capping Groups[a]



cpds	R	A_{2a} Ki (nM)	A_1 Ki (nM)	cpds	R	A_{2a} Ki (nM)	A_1 Ki (nM)
6		>5000	NT	14		1100	>500
7		190	>500	15		114	4500
8		140	>500	16		18	840
9		1500	>500	17		16	>500
10		1300	>500	18		10	1500
11		380	>500	19		30	1300
12		180	>500	20		5	500
13		1200	>500	21		3	1300

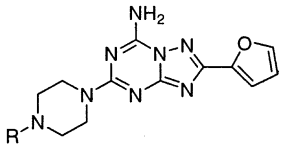
[a] For the A_{2a} receptor, membranes were prepared from rat brain tissues and the radioligand binding assay was performed using the radioligand [^{3}H]ZM-241385. For the A_1 receptor, membranes were prepared from rat cerebral cortex and the radioligand binding assay was performed using the radioligand [^{3}H]DPCPX. K_i values were calculated from binding curves generated from the mean of three determinations per concentration, with variation in individual values of <15%.

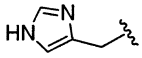
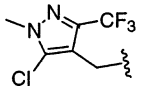
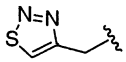
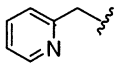
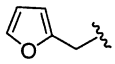
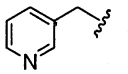
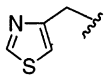
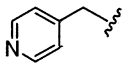
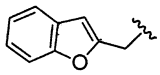
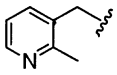
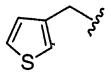
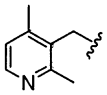
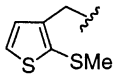
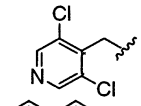
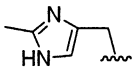
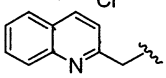
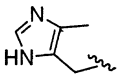
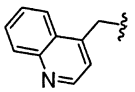
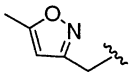
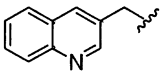
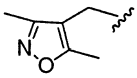
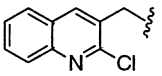
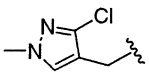
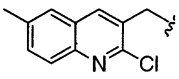
central nervous system (CNS) activity, we used the mouse catalepsy model. In this model, mice (CD-1, 25−30 g) were injected subcutaneously with 3 mg/kg of haloperidol in order to induce catalepsy. This is a behavioral condition in which the animal is unable to correct an externally imposed posture.[21] With this type of study, a test compound was determined to be efficacious when it allowed the animals to correct the externally imposed posture within a certain time period (refer to the Experimental Section for a more detailed description of the mouse catalepsy model). Since catalepsy shares some similarity to symptoms of Parkinson's disease, this type of behavioral study represents a relatively quick way to assess efficacy. The sample requirement for the mouse catalepsy study was low (3 mg), making this assay a convenient, moderately high-throughput screen for both oral and CNS activity. As a point of reference, the full dose response for the xanthine derivative **1** was determined in this mouse catalepsy model and its ED_{50} was 1 mg/kg po. Since compound **1** has been selected for clinical testings, we arbitrarily set this dose as a highly desirable dose of efficacy for our piperazine analogues.

In addition to the mouse catalepsy model, we also made use of the rat catalepsy model. This type of rat study also allowed us to correlate the in vivo activity with the in vitro binding data, which were obtained using rat membranes. As with the rat model, haloperidol was used to induce catalepsy. Male Sprague-Dawley rats (225−275 g) responded to haloperidol at a much lower dose, and 1 mg/kg sc was sufficient to induce the same cataleptic state observed in mice. The xanthine derivative **1** was tested in this rat catalepsy model, and its ED_{50} was determined to be 1 mg/kg po. Since our piperazine analogues are derivatives of the triazolotriazine lead **3**, this compound was also tested in both the mouse and rat catalepsy model. Compound **3** showed no oral activity at 10 mg/kg in both of these catalepsy models.

As an additional measure of efficacy, we employed the 6-hydroxydopamine-lesioned rat model.[7] This is a frequently used rodent model for Parkinson's disease in which efficacy is defined as the ability to potentiate

Table 2. Heterocyclic Capping Groups[a]



cpds	R	A_{2a} Ki (nM)	A_1 Ki (nM)	cpds	R	A_{2a} Ki (nM)	A_1 Ki (nM)
22		320	>500	**34**		14	1100
23		96	>500	**35**		110	2700
24		47	>500	**36**		280	>500
25		54	720	**37**		140	>500
26		74	650	**38**		48	>500
27		75	NT	**39**		36	>500
28		35	NT	**40**		2	670
29		220	>500	**41**		73	>500
30		130	>500	**42**		85	7400
31		180	>500	**43**		57	670
32		18	2900	**44**		6	250
33		58	420	**45**		12	360

[a] Refer to Table 1 for rat membrane preparation and details regarding the radioligand binding assay.

L-Dopa-induced turning behavior. In this model, rats were anesthetized and injected in the left medial forebrain with 6-hydroxydopamine. Following a recovery period, these animals were screened for contralateral rotation in response to apomorphine (0.1 mg/kg, sc). Animals that showed robust contralateral rotations during the test period were then selected for the behavioral study. For this part of the study, the animals were administered first with the test compounds. Thirty minutes later, the animals were given L-Dopa (3.7 mg/kg, ip) and the peripheral decarboxylase inhibitor benserazide (25 mg/kg, ip) and the contralateral rotations were counted for the next 2 h. Efficacious compounds were defined as those that increased the contralateral turning behavior of L-Dopa-administered rats. In this model, the minimum effective dose (MED) for the xanthine derivative **1** was determined to be 3 mg/kg po.

In general, compounds with a K_i value at A_{2a} receptors of less than 100 nM and a reasonable level of selectivity over the adenosine A_1 receptor were first tested in this mouse catalepsy model. Thus, compounds **15**−**21**, **24**−**28**, **33**−**35**, **38**−**45** were evaluated for anticataleptic activity in this mouse model. The results were summarized in Table 3. Among the halogenated benzyl derivatives **15**−**21**, oral activity at either 10 or 3 mg/kg was observed initially in the mouse catalepsy model. Compound **15**, for instance, showed oral activity at 10 mg/kg in the mouse catalepsy model. It was then tested in the rat catalepsy model where the ED_{50} was deter-

Table 3. Summary of Efficacy Data in Rodent Models of Parkinson's Disease[a]

compd	mouse catalepsy po	rat catalepsy po (ED_{50}, mg/kg)	rat 6-hydroxydopamine po (MED, mg/kg)
15	active @ 10 mg/kg	10	10
16	active @ 3 mg/kg	NT	NT
17	active @ 3 mg/kg	NT	NT
18	active @ 3 mg/kg	NT	NT
19	active @ 10 mg/kg	NT	NT
20	active @ 10 mg/kg	10	>10
21	active @ 3 mg/kg	3	10
24	inactive @ 10 mg/kg	NT	NT
25	active @ 3 mg/kg	NT	NT
26	active @ 3 mg/kg	>3	NT
27	inactive @ 10 mg/kg	NT	NT
28	inactive @ 10 mg/kg	NT	NT
32	NT	10	>10
33	active @ 3 mg/kg	NT	NT
34	active @ 3 mg/kg	3	3
35	active @ 10 mg/kg	10	>10
38	active @ 3 mg/kg	>3	NT
39	active @ 3 mg/kg	NT	NT
40	active @ 3 mg/kg	NT	NT
41	inactive @ 10 mg/kg	NT	NT
42	NT	10	NT
43	active @ 10 mg/kg	NT	NT
44	active @ 3 mg/kg	>3	NT
45	active @ 10 mg/kg	>10	NT
3	inactive @ 10 mg/kg	>10	NT
1	active @ 1 mg/kg	1	3

[a] For the mouse catalepsy study, CD-1 mice (25−30 g) were injected subcutaneously with 3 mg/kg of haloperidol in order to induce catalepsy. For the rat catalepsy study, male Sprague-Dawley rats (225−275 g) were treated with haloperidol (1 mg/kg sc) in order to induce catalepsy. Additional details regarding the 6-hydroxydopamine model can be found in the Experimental Section as well as in Figure 3. In all of these studies, test compounds, formulated as the hydrochloride salt, were dissolved in saline and administered by oral gavage.

mined to be 10 mg/kg po. When compound **15** was tested in this 6-hydroxydopamine model, the MED was determined to be 10 mg/kg po. Compound **20** was substantially more active than compound **15** in the in vitro binding assay. However, in both the mouse and rat catalepsy model, compound **20** appeared to be equipotent to **15** and the ED_{50} was also 10 mg/kg po. Interestingly, when **20** was tested in the 6-hydroxydopamine model, it was essentially ineffective when it was administered orally at 10 mg/kg. Among the halogenated benzyl derivatives shown in Table 3 (**15**−**21**), a number of these showed oral activity at 3 mg/kg in the mouse catalepsy model. We selected compound **21** for additional profiling. In the rat catalepsy model, compound **21** was still fairly potent and an ED_{50} of 3 mg/kg po was observed. However, when **21** was tested in the 6-hydroxydopamine model, the MED was determined to be slightly higher, at 10 mg/kg po. The trend of lower oral activity in the 6-hydroxydopamine model would be observed later with different compounds (see discussion below), and it appeared that this model was a more stringent test for efficacy than the rat catalepsy model.

After having examined the in vivo efficacy of compounds **15** and **21**, we then turned our attention to their pharmacokinetic (PK) properties. The data are summarized in Table 4. For the rat PK study, we employed standard protocols, and compounds were dosed both iv and po at 1 mg/kg. Plasma samples were then taken 9 times over a period of 24 h and analyzed by extraction and LC/MS. Compound **15** had a low oral bioavailability of 2%; its oral half-life was 1.8 h, and its volume of distribution (V_{dss}) was 2.8 L/kg. Likewise, compound **21** also had a low level of oral bioavailability of 3%. Even though its oral half-life was shorter at 0.9 h, its volume of distribution was still fairly high, at 3.4 L/kg, indicating a good measure of tissue distribution.

Table 4. Summary of Rat PK Data[a]

compd	F (%)	$T_{1/2}$ (h)	V_{dss} (L/kg)
15	2	1.8	2.8
21	3	0.9	3.4
34	7	0.5	1.8
35	89	1.9	1.1
42	87	2.5	0.8
44	59	1.4	0.5
45	46	1.0	0.5
1	60	4.1	3.7

[a] Rat PK experiments were carried out using standard protocols. Compounds were dosed both iv and orally at 1 mg/kg. Test compounds were formulated as the hydrochloride salt and dissolved in saline. Plasma samples were taken at 9 time points over a period of 24 h. F refers to the % oral bioavailability. V_{dss} refers to the volume of distribution. Both $T_{1/2}$ and V_{dss} were determined from the oral administration.

For compounds with higher oral bioavailability, we turned to analogues with an additional basic site at the capping groups, as in **35** and **38**−**45**. The dibasic derivatives in general showed very good oral efficacy in the mouse catalepsy model. The activity in the rat model, however, was more variable. The pyridine analogue **35**, for example, had a very high level of oral bioavailability of 89%. Its oral half-life was still reasonably long, at 1.9 h; and its volume of distribution was 1.1 L/kg. Even though **35** was orally active at 10 mg/kg in both the mouse and rat catalepsy model, it was essentially ineffective at 10 mg/kg when it was given orally to rats in the more challenging 6-hydroxydopamine model. Similarly, the quinoline analogues **42**, **44**, and **45** all showed a fairly high level of oral bioavailability (Table 4, F ranging from 46% to 87%). However, these dibasic derivatives all failed to show compelling CNS activity in the various rat models of efficacy.

Of the remaining compounds that were tested, compound **34** was particularly interesting. Even though its oral bioavailability in the rat was still rather low, at 7%, and its oral half-life was still rather short, at 0.5 h, compound **34** showed very robust oral activity at 3 mg/kg in both the mouse and rat catalepsy models. More significantly, when tested in the rat 6-hydroxydopamine model, compound **34** had a MED of 3 mg/kg po. This level of efficacy was comparable to that of the xanthine derivative **1**, the clinical candidate from Kyowa Hakko. Figures 2 and 3 summarize the in vivo data for compound **34** along with the benchmark compound **1**. In Figure 2, the rat catalepsy data for compounds **34** and **1** are shown along with those for compound **15**. Note that compounds **34** and **1** were both substantially more potent than compound **15**. For this study, compounds **34** and **1** were dosed orally at 3 and 1 mg/kg, respectively, whereas compound **15** was dosed orally at a higher dose of 10 mg/kg. Figure 3 summarizes the efficacy data for **34** and **1** in the 6-hydroxydopamine-lesioned rat model. In this study, both compounds showed an MED of 3 mg/kg po. We have since evaluated compound **34** against the four cloned human adenosine receptor subtypes and were pleased with the level of selectivity (A_{2a} K_i = 100 nM; A_1 K_i = 10 μM; A_{2b} K_i =

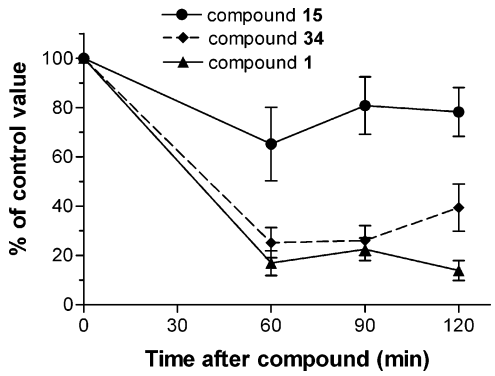


Figure 2. Rat catalepsy data. Male Sprague-Dawley rats (225−275 g) were injected with haloperidol (1 mg/kg sc) in order to induce catalepsy. Test compounds, formulated as the hydrochloride salts, were dosed orally. Compounds **34** and **1** were dosed at 3 and 1 mg/kg po, respectively. Compound **15** was dosed at 10 mg/kg po. The data represent the mean of six animals per group. Catalepsy was measured as the time in seconds until the animals removed at least one forepaw from the bar, with a maximum value of 120 s per test. Values for each animal in the drug-treated groups were expressed as the percentage of the mean value for the vehicle-treated control group at that time point.

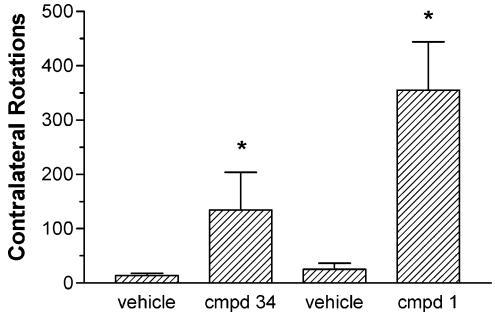


Figure 3. Efficacy data for the 6-hydroxydopamine rotation model. Male Sprague-Dawley rats (225−250 g) were injected in the left medial forebrain with 6-hydroxydopamine. Following a recovery period, rats were screened for rotational response to apomorphine (0.1 mg/kg sc). Only rats showing robust contralateral rotation were used for the behavioral study. Six animals were used per group. Compounds **34** and **1** were dissolved in 2% DMSO in H_2O and 0.5% methyl cellulose, respectively, and dosed orally. Thirty minutes after dosing of the test compounds, all rats were given L-Dopa (3.7 mg/kg, ip) and benserazide (25 mg/kg, ip). Contralateral rotations were then counted for the next 2 h. The minimum effective dose is shown above for **34** and **1** (MED = 3 mg/kg po).

28 μM; A_3 K_i > 10 μM). The selectivity level of the A_{2b} receptor was quite significant because the lead compound **3** displayed little selectivity toward this receptor (A_{2b} K_i = 30 nM).[19] Future work in this series will focus on understanding how low oral bioavailability in the rat and high oral efficacy in the mouse are consistently observed among the various derivatives. Specifically, the drug level in the brain and the presence of active metabolites will have to be examined in order to account for the observed oral efficacy in rodent models of Parkinson's disease.

In summary, we have shown how the basic [1,2,4]-triazolo[1,5-*a*]triazine core structure could be improved by incorporating the appropriately substituted piperazines. After some preliminary optimization, we have been able to prepare a number of highly potent and selective adenosine A_{2a} receptor antagonists. Selected compounds from this series have now been shown to be orally active in three different rodent models of Parkinson's disease. Efforts are still underway to identify compounds from this series with the right combination of high oral bioavailability and potent anticataleptic activity. The results will be disclosed in future publications.

Experimental Section

General Information. All proton nuclear magnetic resonance spectra were determined in the indicated solvent using a 400 MHz Bruker with the appropriate internal standard. Low-resolution MS were performed on a Micromass/single quadrupole/electrospray platform. High-resolution MS were performed on a MALDI-TOF MS (Voyager-DE STR, Perseptive Biosystems) in the reflector mode with delayed extraction and an accelerating voltage of 20 kV. Each spectrum was an average of 100 laser shots, and the experimental monoisotopic $M^+ + H$ value was calculated by averaging five spectra. Elemental analyses were carried out at Quantitative Technologies Inc. (QTI, Whitehouse, NJ). Unless indicated otherwise, reagent-grade chemicals and solvents were purchased from Aldrich, Lancaster, Fisher, or Maybridge. Analytical HPLC analysis was carried out using an HP 1100 series instrument, with a 100 mm × 4.6 mm ID YMC column with S-5 μm packing (catalog no. AM-301). Preparative HPLC was carried out using a Gilson platform equipped with a UV/visible detector and an automatic fraction collector. Preparative HPLC columns were 50 mm × 20 mm IC YMC column with S-5 μm packing. HPLC solvents (H_2O and CH_3CN) were buffered with 0.1% TFA.

5-(4-Benzylpiperazin-1-yl)-2-furan-2-yl[1,2,4]triazolo-[1,5-*a*][1,3,5]triazin-7-ylamine (8). In a typical procedure, 5 mmol of 2-furan-2-yl-5-methanesulfonyl[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-ylamine (sulfone **5**)[13] was suspended in 10 mL of CH_3CN along with 1.5 equiv of benzylpiperazine and 2 equiv of Et_3N. The reaction mixture was stirred under reflux for 2 h. It was then cooled to room temperature and concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 and washed with H_2O and brine, dried (Na_2SO_4), and concentrated under reduced pressure. The resulting crude product was purified by column chromatography (SiO_2, 95% CH_2Cl_2, 4% MeOH, 1% Et_3N) to afford the titled compound **8**. ^1H NMR (DMSO-d_6) δ 7.60 (d, J = 1.0 Hz, 1 H), 7.3 (m, 5H), 7.28 (br s, 2 H), 7.22 (d, J = 3.6 Hz, 1 H), 6.68 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 3.6 (br s, 2 H), 2.5 (br s, 4 H), 1.6 (br s, 4 H) ppm. MS m/z = 377 amu ($M^+ + H$). Anal. ($C_{19}H_{18}N_8O$) C, H, N.

7-Furan-2-yl-2-piperazin-1-ylimidazo[1,2-*a*][1,3,5]triazin-4-ylamine (Intermediate 6). In a typical procedure, 18 mmol of 2-furan-2-yl-5-methanesulfonyl[1,2,4]triazolo[1,5-*a*][1,3,5]-triazin-7-ylamine (sulfone **5**)[13] was suspended in 50 mL of CH_3CN along with 5 equiv of piperazine. The reaction mixture was stirred under reflux for 2 h. It was then cooled to room temperature and concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 and washed with H_2O and brine, dried (Na_2SO_4), and concentrated under reduced pressure. The resulting crude product was purified by column chromatography (SiO_2, 95% CH_2Cl_2, 4% MeOH, 1% Et_3N) to afford the title compound, 7-furan-2-yl-2-piperazin-1-ylimidazo[1,2-*a*][1,3,5]triazin-4-ylamine (intermediate **6**). ^1H NMR (DMSO-d_6) δ 8.2 (br s, 2 H), 7.85 (d, J = 1.0 Hz, 1 H), 7.07 (d, J = 3.6 Hz, 1 H), 6.68 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 3.20−2.75 (m, 8 H) ppm. MS m/z = 287 amu ($M^+ + H$).

General Method for Reductive Amination. In a typical reductive amination procedure, 7-furan-2-yl-2-piperazin-1-ylimidazo[1,2-*a*][1,3,5]triazin-4-ylamine (0.15 mmol) was dissolved in 4 mL of CH_2Cl_2 along with 2 equiv of the desired aldehyde and 25 μL of glacial acetic acid. The reaction mixture was stirred at room temperature for 30 min, and sodium triacetoxyborohydride (4 equiv) was added in a single portion. The resulting reaction mixture was then stirred at room temperature for 18 h. It was then concentrated under a stream

of N_2 and purified by preparative HPLC using a mixture of aqueous CH_3CN that has been buffered with 0.1% TFA. Most of the derivatives shown in Tables 1 and 2 (with the exception of compounds **7**, **8**, **31**, and **32**) were prepared using this reductive amination condition and the appropriate aldehyde. As an example, to prepare compound **35**, pyridine-2-carboxaldehyde was used. Spectroscopic data for **35**: ^{1}H NMR (DMSO-d_6) δ 8.52 (d, J = 6.0 Hz, 1 H), 7.64−7.60 (m, 1 H), 7.50−7.19 (m, 4 H), 6.84 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 4.09−4.06 (m, 4 H), 3.89 (br s, 2 H), 2.51−2.41 (m, 4 H) ppm. MS m/z = 378 amu (M^+ + H). Anal. ($C_{18}H_{19}N_9O$) C, H, N.

General Method for Alkylation of Intermediate 6. To prepare compound **31**, (5-methylisoxazol-3-yl)methanol (32 mg, 0.28 mmol) was dissolved in 4 mL of CH_2Cl_2 along with 1.3 equiv of Et_3N. The solution was cooled in an ice bath, and methanesulfonyl chloride (1.2 equiv) was added. The reaction mixture was warmed to room temperature and stirred for 45 min. It was then quenched with brine, and the two layers were separated. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to afford the mesylate derivative. This mesylate was then added to a solution of 7-furan-2-yl-2-piperazin-1-ylimidazo[1,2-*a*][1,3,5]triazin-4-ylamine (0.14 mmol) and Et_3N (0.3 mmol) in 3 mL of CH_3CN. The resulting reaction mixture was stirred at room temperature for 18 h. It was then concentrated and purified by preparative HPLC using a mixture of aqueous CH_3CN that has been buffered with 0.1% TFA. Spectroscopic data for **31**: ^{1}H NMR (DMSO-d_6) δ 7.90 (br s, 2 H), 7.80 (d, J = 1.0 Hz, 1 H), 7.05 (d, J = 3.6 Hz, 1 H), 6.68 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 6.30 (s, 1 H), 3.65 (m, 2 H), 3.20−2.75 (m, 8 H), 2.35 (s, 3H) ppm. MS m/z = 382 amu (M^+ + H). Anal. ($C_{17}H_{19}N_9O_2$) C, H, N.

Alternatively, if the halide is available, as in the synthesis of compound **32**, 1.5 equiv of 4-chloromethyl-3,5-dimethylisoxazole could be added to a solution of 7-furan-2-yl-2-piperazin-1-ylimidazo[1,2-*a*][1,3,5]triazin-4-ylamine (0.14 mmol) and Et_3N (0.3 mmol) in 3 mL of CH_3CN directly. The resulting reaction mixture was stirred at room temperature for 18 h. It was then concentrated and purified by preparative HPLC using a mixture of aqueous CH_3CN that has been buffered with 0.1% TFA. Spectroscopic data for **32**: ^{1}H NMR (DMSO-d_6) δ 7.60 (d, J = 1.0 Hz, 1 H), 7.28 (br s, 2 H), 7.22 (d, J = 3.6 Hz, 1 H), 6.68 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 3.8 (br s, 2 H), 2.2−3.2 (m, 8H), 1.6 (br s, 6H). MS m/z = 396 amu (M^+ + H). Anal. ($C_{18}H_{21}N_9O_2$) C, H, N.

Biological Assays. A_{2a} Receptor. Membranes were prepared from rat brain tissues purchased from Pel-Freez. Tissues were homogenized in buffer A (10 mM EDTA, 10 mM Na−HEPES, pH 7.4) supplemented with protease inhibitors (10 μg/mL benzamidine, 100 μM PMSF, and 2 μg/mL each of aprotinin, pepstatin, and leupeptin) and centrifuged at 20000*g* for 20 min. Pellets were resuspended and washed twice with buffer HE (10 mM Na HEPES, 1 mM EDTA, pH 7.4, plus protease inhibitors). Final pellets were resuspended in buffer HE, supplemented with 10% (w/v) sucrose and protease inhibitors, and frozen in aliquots at −80 °C. Protein concentrations were measured using a BCA protein assay kit (Pierce).

Rat A_1 Receptor. Membranes were prepared from rat cerebral cortex isolated from freshly euthanized rats. Tissues were homogenized in buffer A (10 mM EDTA, 10 mM Na−HEPES, pH 7.4) supplemented with protease inhibitors (10 μg/mL benzamidine, 100 μM PMSF, and 2 μg/mL each of aprotinin, pepstatin, and leupeptin) and centrifuged at 20000*g* for 20 min. Pellets were resuspended and washed twice with buffer HE (10 mM Na HEPES, 1 mM EDTA, pH 7.4, plus protease inhibitors). Final pellets were resuspended in buffer HE, supplemented with 10% (w/v) sucrose and protease inhibitors, and frozen in aliquots at −80 °C. Protein concentrations were measured using a BCA protein assay kit (Pierce).

Radioligand Binding Assays. Membranes (40−70 μg membrane protein), radioligands, and varying concentrations of competing ligands were incubated in triplicates in 0.1 mL of buffer HE plus 2 units/mL adenosine deaminase for 2.5 h at 21 °C. Radioligand [^{3}H]DPCPX was used for competition binding assays on A_1 receptors, and [^{3}H]ZM241385 was used for A_{2a} adenosine receptors. Nonspecific binding was measured in the presence of 10 μM NECA for A_1 receptors or 10 μM XAC for A_{2A} receptors. Binding assays were terminated by filtration over Whatman GF/C glass fiber filters using a BRANDEL cell harvester. Filters were rinsed three times with 3−4 mL of ice-cold 10 mM Tris-HCl, pH 7.4, and 5 mM $MgCl_2$ at 4 °C and were counted in a Wallac β-counter.

Analysis of Binding Data. For K_i determinations, competition binding data were fit to a single-site binding model and plotted using Prizm GraphPad. The Cheng−Prusoff equation $K_I = IC_{50}/(1 + [I]/K_D)$ was used to calculate K_I values from IC_{50} values, where K_I is the affinity constant for the competing ligand, [I] is the concentration of the free radioligand, and K_D is the dissociation constant for the radioligand.

Catalepsy Experiments. Haloperidol-induced catalepsy was used to mimic the effects of Parkinson's disease in rats and mice. Animals were injected with haloperidol, which causes immobility. A test compound was then administered orally, and the compound's ability to reverse these Parkinson-like symptoms was analyzed.

Mouse Catalepsy Study. In this model, mice (CD-1, 25−30 g) were injected subcutaneously with 3 mg/kg of haloperidol in order to induce catalepsy. This is a behavioral condition in which the animal is unable to correct an externally imposed posture.[21] With this type of study, a test compound was determined to be efficacious when it allowed the animals to correct the externally imposed posture within a certain time period. For the mouse catalepsy study, the animals' forelimbs were placed on an aluminum bar suspended horizontally 4.5 cm above the surface of the bench in order to create an unnatural posture. Catalepsy-free mice, i.e., those without haloperidol, should be able to come down from the horizontal bar almost immediately. On the other hand, cataleptic mice, when placed in this awkward position, were unable to come down from the bar over a period of 120 s or more. With haloperidol, the induced catalepsy was sufficiently severe that the animals usually would remain in this state for 3 h or more. A test compound was then administered orally by gavage. Orally efficacious compounds were defined as those that allowed the mice to come down from the bar within 60 s. The animals must then remain in this catalepsy-free state for at least 60 min. In other words, when placed back on the horizontal bar 60 min after drug administration, the animals should still be able to place one of its forelimbs back on the bench within 60 s. Six animals were used per group, and data were analyzed by one factor analysis of variance with Dunnett's "*t*" test used to make post hoc comparisons.

Rat Catalepsy Study. Male Sprague-Dawley rats (225−275 g) were injected with haloperidol (1 mg/kg sc). Every 30 min for the next 3 h, catalepsy was measured using the bar test.[21] In this test, the rats' forelimbs were placed on an aluminum bar (1 cm in diameter) suspended horizontally 10 cm above the surface of the bench. The elapsed time until the rat places one forepaw back on the bench is measured, with a maximum time of 120 s allowed. Once rats show a stable baseline cataleptic response (about 3 h after haloperidol injection), test compound or vehicle alone is administered by oral gavage, and catalepsy was measured by the bar test every 30 min for the next 2 h. Efficacious compounds were defined as those that allowed the rats to come down from the horizontal bar within 60 s and then remain in the same catalepsy-free state over the next 60 min. Six animals were used per group, and data were analyzed by one factor analysis of variance with Dunnett's "*t*" test used to make post hoc comparisons.

6-Hydroxydopamine Rotation Model. Male Sprague-Dawley rats (225−250 g) were given a stereotaxic injection of 6-hydroxydopamine hydrochloride into the left medial forebrain bundle under brevital (45 mg/kg)/isoflurane anesthesia. Injections were placed 4.0 mm anterior to λ, 1.3 mm lateral to the midline, and 8.4 mm ventral to the surface of skull at the site of injection. 6-Hydroxydopamine·HCl was injected at dose

of 8 μg (weight of salt) in 4 μL of saline with 0.02% ascorbate over 8 min using a syringe pump. The cannula was left in place for an additional 1 min and withdrawn slowly to minimize reflux up the tract. Rats were kept warm on a heating pad until they woke from anesthesia.

Three weeks after surgery, rats were screened for rotational response to apomorphine (0.1 mg/kg sc) in an automated rotometer; only rats showing robust contralateral rotation were included in the study. Six animals were used per group. One week after apomorphine testing, rats were given compound **31**, KW-6002, or the corresponding vehicle solutions (2% DMSO in H_2O for compound **31**; 0.5% methyl cellulose for KW-6002) in the case of controls and placed in the rotometers. Thirty minutes later, all rats were given L-Dopa (L-dihydroxyphenylalanine, 3.7 mg/kg, ip) and the peripheral decarboxylase inhibitor benserazide (25 mg/kg, ip), and contralateral rotations were counted for the next 2 h. The total number of contralateral rotations in the drug-treated groups was compared to that in the corresponding vehicle-treated control group using the Mann−Whitney rank sum test.

Note: All experiments using animals were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee. The IACUC protocol number for all the catalepsy studies is 105-00, and the corresponding number for the 6-hydroxydopamine studies is 77-98.

Acknowledgment. We thank Azita Kaffashan for carrying out the high-resolution MS. Ronald Savage and Zhaoyang Li provided additional information regarding rat pharmacokinetic data.

Supporting Information Available: Spectroscopic data for additional compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Müller, C. E.; Scior, T. Adenosine Receptors and their Modulators. *Pharm. Acta Helv.* **1993**, *68*, 77−111. (b) DeNinno, M. P. Adenosine. *Annu. Rep. Med. Chem.* **1998**, *33*, 111−121.

(2) Fredholm, B. B.; Abbraccio, M. P.; Burnstock, G.; Daly, J. W.; Harden, T. K.; Jacobson, K. A.; Leff, P.; Williams, M. Nomenclature and Classification of Purinoceptors. *Pharmacol. Rev.* **1994**, *46*, 143−156.

(3) (a) Müller, C. E. Adenosine Receptor Ligands−Recent Developments Part I. Agonists. *Curr. Med. Chem.* **2000**, *7*, 1269−1288. (b) Keeling, S. E.; Albinson, F. D.; Ayres, B. E.; Butchers, P. R.; Chambers, C. L.; Cherry, P. C.; Ellis, F.; Ewan, G. B.; Gregson, M.; Knight, J.; Mills, K.; Ravenscroft, P.; Reynolds, L. H.; Sanjar, S.; Sheehan, M. J. The Discovery and Synthesis of Highly Potent, A_{2a} Receptor Agonists. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 403−406. (c) Zablocki, J. A.; Palle, V. P.; Varkhedkar, V.; Ibrahim, P.; Ahmed, H.; Gao, Z.; Ozeck, M.; Wu, Y.; Zeng, D.; Wu, L.; Belardinelli, L.; Leung, K.; Chu, N.; Blackburn, B. SAR of Partial Adenosine A_1 Agonists and Their Potential as Supraventricular Anti-Arrhythmic Agents. *Abstracts of Papers*, Division of Medicinal Chemistry, 224th National Meeting of the American Chemical Society, Boston, MA, August 18−22, 2002; American Chemical Society: Washington, DC, 2002; Abstract 420. (d) DeNinno, M. P.; Masamune, H.; Chenard, L. K.; DiRico, K. J.; Eller, C.; Etienne, J. B.; Tickner, J. E.; Kennedy, S. P.; Knight, D. R.; Kong, J.; Oleynek, J.; Tracey, W. R., Hill, R. J. 3′-Aminoadenosine-5′-uronamides: Discovery of the First Highly Selective Agonist at the Human Adenosine A_3 Receptor. *J. Med. Chem.* **2003**, *46*, 353−355.

(4) (a) Müller, C. E. A_{2a} Adenosine Receptor Antagonists. Future Drugs for Parkinson's Disease? *Drugs Future* **2000**, *25*, 1043−1052. (b) Gellai, M.; Schreiner, G. F.; Ruffolo, R. R., Jr.; Fetcher, T.; Dewolf, R.; Brooks, D. P. CVT-124, a Novel Adenosine A1 Receptor Antagonist with Unique Diuretic Activity. *J. Pharmacol. Exp. Ther.* **1998**, *286*, 1191−1196. (c) Gottlieb, S. S.; Skettino, S. L.; Wolff, A.; Beckman, E.; Fisher, M. L.; Freudenberger, R.; Gladwell, T.; Marshall, J.; Cines, M.; Bennett, D.; Littschwager, E. B. Effects of BG-9719 (CVT-124), an A_1-Adenosine Receptor Antagonist, and Furosemide on Glomerular Filtration Rate and Natriuresis in Patients with Congestive Heart Failure. *J. Am. Coll. Cardiol.* **2000**, *35*, 56−69. (d) Müller, C. A.; Thorand, M.; Qurishi, R.; Diekmann, M.; Jacobsen, K. A.; Padgett, W. L.; Daly, J. W. Imidazo[2,1-*i*]purin-5-ones and Related Tricyclic Water-Soluble Purine Derivatives: Potent A_{2a}- and A_3-Adenosine Receptor Antagonists. *J. Med. Chem.* **2002**, *45*, 3440−3450. (e) Linden, J.; Figler, R. A.; Wang, G.; Jones, D. R. A_{2b} Adenosine Receptor Antagonists for Asthma and Diabetes *Abstract of Papers*, Division of Medicinal Chemistry, 224th National Meeting of the American Chemical Society, Boston, MA, August 18−22, 2002; American Chemical Society: Washington, DC, 2002; Abstract 418. (f) Press, N. J.; Fozard, J. R.; Beer, D.; Heng, R.; di Padova, F.; Tranter, P.; Trifilieff, A.; Walker, C.; Keller, T. New Highly Potent and Selective Adenosine A_3 Receptor Antagonist. *Abstract of Papers*, Division of Medicinal Chemistry, 224th National Meeting of the American Chemical Society, Boston, MA, August 18−22, 2002; American Chemical Society: Washington, DC, 2002; Abstract 419.

(5) (a) Jarvis, M. F.; Williams, M. Direct Autoradiographic Localization of Adenosine A_2 Receptors in the Rat Brain using the A_2-Selective Agonist, [^{3}H]CGS 21680. *Eur. J. Pharmacol.* **1989**, *168*, 243−246. (b) Martinez-Mir, M. I.; Probst, A.; Palacios, J. M. Adenosine A_{2a} Receptors: Selective Localization in the Human Basal Ganglia and Alterations with Disease. *Neuroscience* **1991**, *42*, 697−706. (c) Svenningsson, P.; Le Moine, C.; Fisone, G.; Fredholm, B. B. Distribution, Biochemistry and Function of Striatal Adenosine A_{2a} Receptors. *Prog. Neurobiol.* **1999**, *59*, 355−396. (d) Hurley, M. J.; Mash, D. C.; Jenner, P. Adenosine A_{2a} Receptor mRNA Expression in Parkinson's Disease. *Neurosci. Lett.* **2000**, *291*, 54−58.

(6) Kanda, T.; Shiozaki, S.; Shimada, J.; Suzuki, F. Nakamura, J. KF 17837: A Novel Selective Adenosine A_{2a} Antagonist with Anticataleptic Activity. *Eur. J. Pharmacol.* **1994**, *256*, 263−268.

(7) Fenu, S.; Pinna, A.; Ongini, E.; Morelli, M. Adenosine A_{2a} Receptor Antagonism Potentiates L-Dopa-Induced Turning Behavior and c-fos Expression in 6-Hydroxydopamine-Lesioned Rats. *Eur. J. Pharmacol.* **1997**, *26*, 143−147.

(8) Shiozaki, S.; Ichikawa, S.; Nakamura, J.; Kitamura, S.; Yamada, K.; Kuwana, Y. Actions of Adenosine A_{2a} Receptor Antagonist KW-6002 on Drug-Induced Catalepsy and Hypokinesia Caused by Reserpine or MPTP. *Psychopharmacology* **1999**, *147*, 90−95.

(9) Kanda, T.; Jackson, M. J.; Smith, L. A.; Pearce, R. K. B.; Nakamura, J.; Kase, H.; Kuwana, Y.; Jenner, P. Adenosine A_{2a} Antagonist: A Novel Anti-Parkinsonian Agent That Does not Provoke Dyskinesia in Parkinsonian Monkeys. *Ann. Neurol.* **1998**, *43*, 507−513. (b) Grondin, R.; Bedard, P. J.; Hadj, T. A.; Gregoire, L.; Mori, A.; Kase, H. Antiparkinsonian Effect of a New Selective Adenosine A_{2a} Receptor Antagonist in MPTP-Treated Monkeys. *Neurology* **1999**, *52*, 1673−1677.

(10) (a) Jacobson, K. A.; Gallo-Rodriguez, C.; Melman, N.; Fischer, B.; Maillard, M.; van Bergen, A.; van Galen, P. J. M.; Karton, Y. Structure−Activity Relationships of 8-Styrylxanthines as A_2-Selective Adenosine Antagonists. *J. Med. Chem.* **1993**, *36*, 1333−1342. (b) Shimada, J.; Koike, N.; Nonaka, H.; Shiozaki, S.; Yanagawa, K.; Kanda, T.; Kobayashi, H.; Ichimura, M.; Nakamura, J.; Kase, H.; Suzuki, F. Adenosine A_{2a} Antagonists with Potent Anti-Cataleptic Activity. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2349−2352.

(11) For a review on adenosine A_{2a} receptor antagonists, see the following. Baraldi, P. G.; Cacciari, B.; Spalluto, G.; Borioni, A.; Viziano, M.; Dionisotti, S.; Ongini, E. Current Developments of A_{2a} Adenosine Receptor Antagonists. *Curr. Med. Chem.* **1995**, *2*, 707−722.

(12) Zocchi, C.; Ongini, E.; Conti, A.; Monopoli, A.; Negretti, A.; Baraldi, P. G.; Dionisotti, S. The Non-Xanthine Heterocyclic Compound SCH 58261 Is a New Potent and Selective A_{2a} Adenosine Receptor Antagonist. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 398−404.

(13) (a) Jones, G.; James, R.; Hargreaves, R. B. 2-Furyl-triazolo[1,5-*a*]-[1,3,5]triazines and Pyrazolo[2,3-a][1,3,5]triazines. U.S. Patent 5,380,714, 1995. (b) Caulkett, P. W. R.; Jones, G.; McPartlin, M.; Renshaw, N. D.; Stewart, S. K.; Wright, B. Adenine Isosteres with Bridgehead Nitrogen. Part 1. Two Independent Syntheses of the [1,2,4]Triazolo[1,5-a][1,3,5]Triazine Ring System Leading to a Range of Substituents in the 2, 5 and 7 Positions. *J. Chem. Soc., Perkin Trans. 1* **1995**, 801−808.

(14) Baraldi, P. G.; Cacciari, B.; Romagnoli, R.; Spalluto, G.; Monopoli, A.; Ongini, E.; Varani, K.; Borea, P. A. 7-Substituted 5-Amino-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines as A_{2a} Adenosine Receptor Antagonists: A Study on the Importance of Modifications at the Side Chain on the Activity and Solubility. *J. Med. Chem.* **2002**, *45*, 115−126.

(15) (a) Neustadt, B. R.; Lindo, N. A.; Greenlee, W. J.; Tulshian, D.; Silverman, L. S.; Xia, J.; Boyle, C. D.; Chackalamannil, S. Adenosine A_{2a} Receptor Antagonists. WO 01/92264 A1, 2001. (b) Neustadt, B. R.; Liu, H. Triazolopyrimidine Derivatives Which Are Useful as Adenosine A_{2a} Antagonists for the Treatment of Parkinson's Disease and Other CNS Disorders. WO 03048163, 2003.

(16) Piperazine derivatives of triazolopyrimidines have also been reported recently as A_{2a} receptor antagonists. (a) Tsumuki, H.;

Junichi, S.; Imma, H.; Nakamura, N.; Nonaka, H.; Shiozaki, S.; Ichikawa, S.; Kanda, T.; Kuwana, Y.; Ichimura, M.; Suzuki, F. [1,2,4]Triazolo[1,5-*c*]pyrimidine Derivatives. U.S. patent 6,222,-035 B1, 2001. (b) Tsumuki, H.; Nakamura, A.; Shiozaki, S.; Ichimura, M.; Kuwana, Y.; Shimada, J. Remedies/Preventives for Parkinson's Disease. WO 99/43678 A1, 1999. (c) Shimada, J.; Imma, H.; Osakada, N.; Shiozaki, S.; Kanda, T.; Kuwana, Y. [1,2,4]Triazolo[1,5-*c*]pyrimidine Derivatives. WO 00/17201 A1, 2000.

(17) Ongini, E.; Dionisotti, S.; Gessi, S.; Irenius, E.; Fredholm, B. B. Comparison of CGS15943, ZM241385 and SCH58261 as Antagonists at Human Adenosine Receptors. *Arch. Pharmacol.* **1999**, *359*, 7−10.

(18) Poucher, S. M.; Keddie, J. R.; Brooks, R.; Shaw, G. R.; McKillop, D. Pharmacodynamics of ZM 241385, a Potent A_{2a} Adenosine Receptor Antagonist, after Enteric Administration in Rat, Cat and Dog. *J. Pharm. Pharmacol.* **1996**, *48*, 601−606.

(19) de Zwart, M.; Vollinga, R. C.; Beukers, M. W.; Sleegers, D. F.; Kunzel, J. K.; de Groote, M.; Ijzerman, A. P. Potent Antagonists for the Human Adenosine A_{2b} Receptor. Derivatives of the Triazolotriazine Adenosine Receptor Antagonist ZM241385 with High Affinity. *Drug Dev. Res.* **1999**, *48*, 95−103.

(20) Francis, J. E.; Cash, W. D.; Psychoyos, S.; Ghai, G.; Wenk, P.; Friedmann, R. C.; Atkins, C.; Warren, V.; Furness, P.; Hyun, J. L.; Stone, G. A.; Desai, M.; Williams, M. Structure−Activity Profile of a Series of Novel Triazoloquinazoline Adenosine Antagonists. *J. Med. Chem.* **1988**, *31*, 1014−1020.

(21) Sanberg, P. R.; Giordano, M.; Bunsey, M. D.; Norman, A. B. The Catalepsy Test: Its Ups and Downs. *Behav. Neurosci.* **1988**, *102*, 748−759.

JM0498405