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

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Piperazine derivatives of 2-furanyl[1,2,4]triazolo[1,5-a][1,3,5]triazine have recently been demonstrated to be potent and selective adenosine A_{2a} receptor antagonists with oral activity in rodent models of Parkinson's disease. We have replaced the piperazinyl group with a variety of linear, monocyclic, and bicyclic diamines. Of these diamines, (*R*)-2-(aminomethyl)pyrrolidine is a particularly potent and selective replacement for the piperazinyl group. With this diamine component, we have been able to prepare numerous analogues with low nanomolar affinity toward the A_{2a} receptor and good selectivity with respect to the A_1 receptor (>200-fold in some cases). Selected analogues from this series of [1,2,4]triazolo[1,5-a][1,3,5]triazine have now been shown to be orally active in the mouse catalepsy model.

Introduction

Adenosine has long been recognized as an important mediator of numerous biological functions both in the nervous system and in the peripheral tissues. Adenosine can elicit very specific physiological responses when it interacts with a particular adenosine receptor. The adenosine receptors have been divided into four different subtypes (A_1, A_{2a}, A_{2b}, and A_3),[1] and selective interaction with each of these receptors through the use of agonists[2] or antagonists[3] has become an increasingly attractive paradigm for therapeutic intervention. The adenosine A_{2a} receptors are widely expressed in the basal ganglia.[4] Modulation of these receptors, via the action of selective A_{2a} receptor agonists or antagonists, will produce fairly significant changes in motor function.[5] Human Parkinson's disease is a motor disorder that arises from the progressive loss of dopaminergic nigrostriatal neurons of the basal ganglia.[6] There are currently a number of pharmacological models that can recapitulate many of the symptoms displayed in Parkinsonian patients such as bradykinesia, tremor, and rigidity, and A_{2a} receptor antagonists appeared to have a beneficial effect in many of these models.[5,7−9] For instance, in rats, catalepsy is a behavioral condition that shares some similarity to human Parkinson's disease.[10] Fairly sustained catalepsy can be induced in rats by the use of a selective A_{2a} receptor agonist or a dopamine antagonist such as haloperidol or reserpine.[8] Administration with selective A_{2a} receptor antagonists has been known to reverse this cataleptic condition in rats very effectively.[8] In marmosets, MPTP and a combination of L-dopa and benserazide can be used to induce motor disabilities that are comparable to patients with Parkinson's disease.[9] Oral administration with a selective A_{2a} receptor antagonist to these marmosets has recently been shown to reverse motor disability in a dose-dependent manner.[9]

Compound **1** (KW-6002) is a xanthine-based adenosine A_{2a} receptor antagonist.[8,11] Nonxanthine-based A_{2a} receptor antagonists are also being extensively studied by other investigators.[12] Among the standard reference compounds not derived from xanthine, compound **2** (SCH-58261) and compound **3** (ZM-241385) are two of the most widely used (Figure 1).[13,14] Originally disclosed in the early 1990s, both of these compounds are fairly selective toward A_{2a}, although both suffered other critical drawbacks such as a lack of oral bioavailability.[15] Much work has taken place since then to improve the pharmacological properties of these compounds, and it appears that orally active compounds can now be obtained using these core structures.[16] Our laboratory has studied extensively the [1,2,4]triazolo[1,5-a][1,3,5]-triazine core structure of compound **3**. We recently demonstrated that piperazine derivatives of type **4** were selective and potent adenosine A_{2a} receptor antagonists with oral efficacy in rodent models of Parkinson's disease.[17] As an extension of this series, we began to explore the possibility of replacing the piperazine with the hope of retaining the A_{2a} binding affinity and selectivity over other adenosine receptors while improving the pharmacokinetic properties.

Our synthetic approach to this problem was inspired by earlier work in the quinolone antibacterials. One of the major advances in the quinolone research occurred in the 1980s with the introduction of norfloxacin and ciprofloxacin, both of which contained a critical piperazine moiety.[18] In later generations of quinolone antibiotics, the piperazine is replaced by a variety of diamines. This type of structural modification allowed the in vitro potency to be retained, and in some cases, an improvement in pharmacokinetic properties was observed. With our current lead series **4**, we felt that a systematic scanning of different diamines could be just as fruitful. We selected compound **5** as a lead to improve upon since its modest binding affinity toward A_{2a} of 180 nM would allow us to definitively identify superior replacements for the piperazine. To isolate the influence of the diamines, we fixed the methyl isoxazole capping group and the 2-furanyl[1,2,4]triazolo[1,5-a][1,3,5]triazine moiety.

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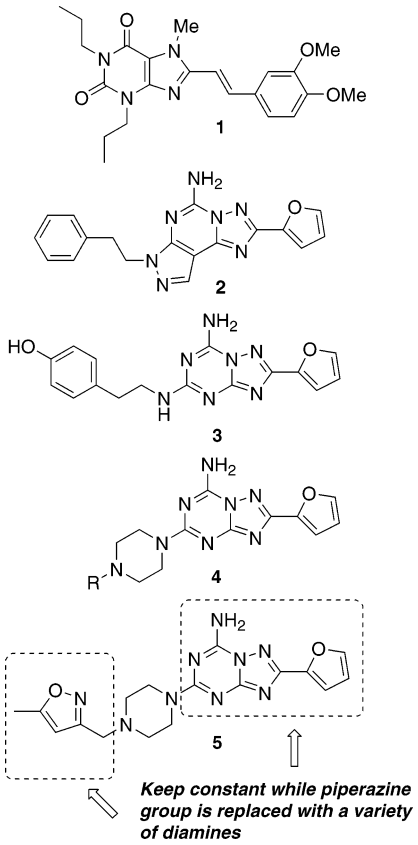


Figure 1.

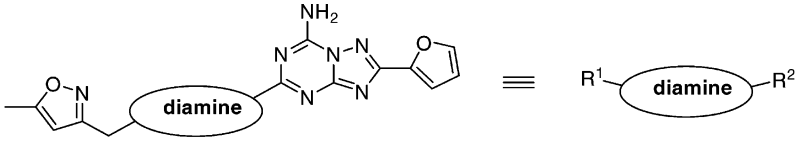
Results and Discussion

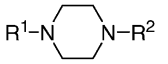
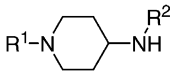
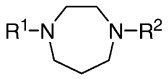
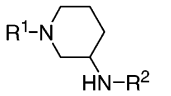
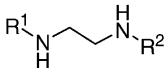
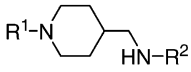
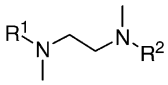
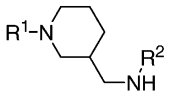
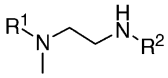
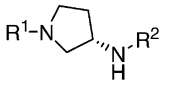
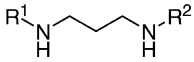
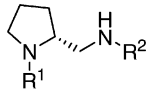
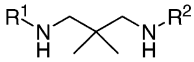
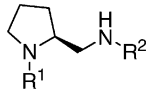
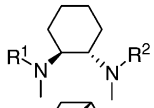
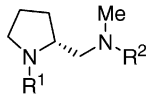
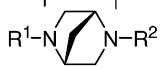
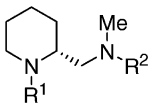
Table 1 lists the diamines used to replace the piperazine group. Schemes 1 and 2 describe how these derivatives were prepared. For symmetrical amines, such as the ones used to prepare compounds **9**−**11** and **13**−**16**, an excess of the unprotected diamine could be condensed directly onto sulfone **6** (Scheme 1).[14] Purification by column chromatography removes the unreacted diamine, and then, alkylation with the mesylate derivative of (5-methyl-isoxazol-3-yl)methanol afforded the desired derivatives. For unsymmetrical amines such as the ones used to prepare compounds **12** and **17**−**25**, the BOC-protected diamines were used. The BOC-protected diamines were commercially available with the exception of the two used to prepare compounds **24** and **25**. These two diamines were prepared by taking the appropriate BOC-protected amino acids and converting them to the corresponding amides through the reaction with methylamine in the presence of EDC/HOBT and N-methylmorpholine. These amides were then reduced to the desired N-methyl amines using BH_3·THF.

As shown in Table 1, homopiperazine (compound **9**) was fairly similar to piperazine and a slight gain of A_{2a} binding affinity was observed. The ethylenediamine linker represented another obvious starting point, and only a modest loss in A_{2a} binding affinity was observed (compound **10**). Methylating the amino group as in **11** and **12** did improve the A_{2a} binding. Replacing the ethylenediamine linker with a longer chain as in **13** and **14** resulted in a substantial loss of A_{2a} binding affinity. A substantial loss of A_{2a} binding affinity was also observed when the piperazino group was replaced with the more constrained amines such as those shown in **15** and **16**. When the piperazino group was replaced with a 4-aminopiperidine group as in **17**, a loss in A_{2a} binding affinity was observed. That loss in A_{2a} activity was even more pronounced when 3-aminopiperidine was used to replace the piperazine (compound **18**). A similar loss in A_{2a} activity was also observed when 4-(aminomethyl)piperidine was used (compound **19**). Interestingly, when a 3-(aminomethyl)piperidine group was used to replace the piperazine, as in **20**, no significant loss in A_{2a} activity was observed. We then turned our attention to the pyrrolidine ring. With 3-aminopyrrolidine, there was a fairly significant loss in A_{2a} activity (compound **21**). However, when (*R*)-2-(aminomethyl)-pyrrolidine was used, as in **22**, a fairly significant increase in A_{2a} binding affinity was observed. Interestingly, the (*S*)-isomer of 2-(aminomethy)pyrrolidine (compound **23**) was not as active as the (*R*)-isomer. Adding an extra methyl group as in **24** also increased the binding affinity toward A_{2a}, and when the pyrrolidine ring was changed to a piperidine ring as in **25**, a comparable A_{2a} activity was observed.

After having identified (*R*)-2-(aminomethyl)pyrrolidine as a good replacement for the piperazine, we then turned our attention to optimizing the capping group. Table 2 lists analogues with substituted benzyl capping groups, and Table 3 lists analogues with a variety of heterocycles as capping groups. In general, analogues prepared using the (*R*)-2-(aminomethy)pyrrolidine were more active against A_{2a} than those prepared using piperazine as the diamine component.[12] As shown in Tables 2 and 3, most of the analogues that we prepared had a K_i value of less than 50 nM against the adenosine A_{2a} receptor. The SAR, however, was slightly different with these (*R*)-2-(aminomethyl)pyrrolidine derivatives. As illustrated in compounds **26**−**28**, *meta* substitution appeared to be more favorable than either *ortho* or *para* substitution and compound **27** had a K_i of 14 nM against the A_{2a} receptor. With the piperazine series, *ortho* substitution was better than either *meta* or *para* substitution.[17] For comparison purposes, a small set of piperazine derivatives of the general structure **4** is shown in Table 4 (compounds **57**−**60**). Even though the *meta* substitution appeared to be favorable, having two chloro substituents, as in **29**, resulted in a decrease in A_{2a} binding affinity. Having two chloro substituents at the *ortho* and *para* positions, as in **30**, was, however, comparable to one chloro substituent at the *meta* position, and a K_i of 11 nM against A_{2a} was obtained. For compound **30**, the K_i for the adenosine A_1 receptor was determined to be 1700 nM (>150-fold selectivity between A_{2a} and A_1 receptors). As shown later on with different analogues, many compounds within this series of (*R*)-2-(aminomethyl)pyrrolidine displayed a very high level of selectivity over the adenosine A_1 receptor. The methyl and fluoro substituents were comparable to the chloro substituent in terms of A_{2a} activity (compare compounds **31** and **32** with compound **27**). With compound **33**, when two fluorine atoms were installed at the *meta* positions, a slight loss of A_{2a} activity was observed. This was similar to the pattern observed above with the chloro derivative **27**. Moving one of the fluorine atoms from the *meta* position to the *ortho* position, as in **34**, did not result in any significant changes in A_{2a} activity. The *ortho*, *para* substitution pattern shown in compound **35** was slightly more

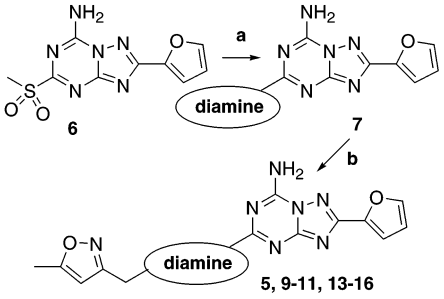
Table 1. Variation of the Diamino Portion of Substituted [1,2,4]Triazolo[1,5-a][1,3,5]triazines[a]



Cpds	diamine	A_{2a} Ki(nM)	Cpds	diamine	A_{2a} Ki (nM)
5		180	**17**		270
9		52	**18**		680
10		200	**19**		560
11		130	**20**		150
12		79	**21**		>500
13		>1000	**22**		25
14		>1000	**23**		64
15		>1000	**24**		12
16		>1000	**25**		14

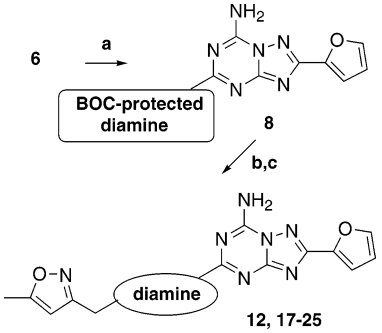
[a] For the A_{2a} receptor, the membranes were prepared from rat brain tissues and the radioligand binding assay was performed using the radioligand [^{3}H]ZM-241385. All K_i values were calculated from binding curves generated from the mean of three determinations per concentration, with the variation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

Scheme 1[a]



[a] Reagents and conditions: (a) Symmetrical diamine, CH_3CN, reflux (for example, piperazine and homopiperazine were used, respectively, in the preparation of **5** and **9**). (b) Mesylate of (5-methyl-isoxazol-3-yl)methanol, Et_3N, CH_2Cl_2, room temperature.

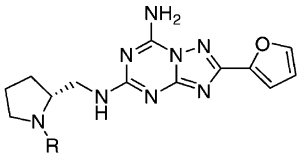
Scheme 2[a]

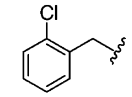
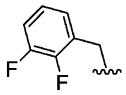
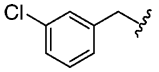
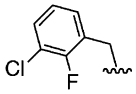
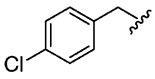
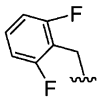
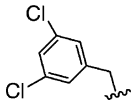
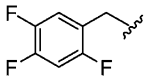
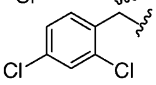
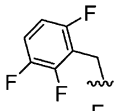
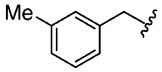
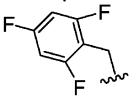
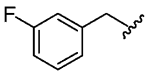
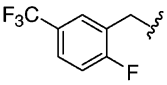
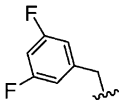
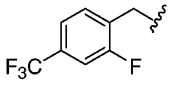
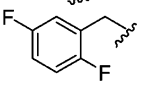
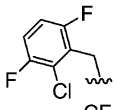
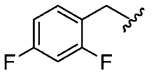
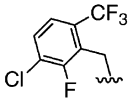


[a] Reagents and conditions: (a) BOC-protected diamine, CH_3CN, Et_3N, reflux (for example, in the preparation of **17**, 4-amino-1-BOC-piperidine was used). (b) 25% TFA, 75% CH_2Cl_2, room temperature. (c) Mesylate of (5-methyl-isoxazol-3-yl)methanol, Et_3N, CH_2Cl_2, room temperature.

effective, and a K_i of 14 nM against A_{2a} was obtained. The more effective difluoro substitution patterns were either *ortho*, *meta* (compound **36**) or *ortho*, *ortho* (compound **38**) where low nanomolar binding affinity toward A_{2a} was observed. Because the chloro substituent was comparable to the fluoro substituent, compound **37** was essentially equivalent to **36**. Adding one more fluoro substituent as in **39**−**41** was still acceptable, and fairly potent compounds could be obtained. Compound **41**, in particular, was not only very potent against A_{2a} ($K_i = 2$ nM)but also very selective over the A_1 receptor (400-fold selective). When one of the fluoro groups was

Table 2. Benzyl Capping Groups[a]



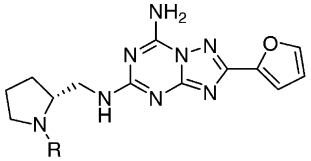
Cpds	R	A_{2a} Ki (nM)	A_1 Ki (nM)	Cpds	R	A_{2a} Ki (nM)	A_1 Ki (nM)
26		39	>250	**36**		5	>250
27		14	>250	**37**		7	>250
28		39	>250	**38**		4	430
29		57	>250	**39**		8	>250
30		11	1700	**40**		4	>250
31		30	>250	**41**		2	800
32		25	>250	**42**		4	>250
33		36	>250	**43**		5	>250
34		30	>250	**44**		4	>250
35		14	1000	**45**		8	>250

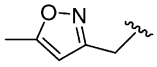
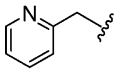
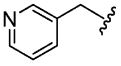
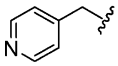
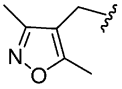
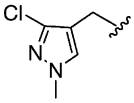
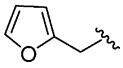
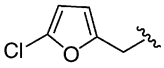
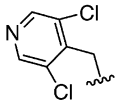
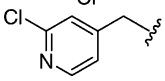
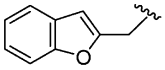
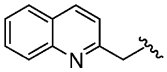
[a] For the A_{2a} receptor, the membranes were prepared from rat brain tissues and the radioligand binding assay was performed using the radioligand [^{3}H]ZM-241385. For the A_1 receptor, the membranes were prepared from rat cerebral cortex and the radioligand binding assay was performed using the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

replaced with a trifluoromethyl group, as in compounds **42**, **43**, and **45**, no significant loss of A_{2a} binding affinity was observed and fairly potent compounds could still be obtained. For comparison, we have also determined the A_{2a} and A_1 binding affinities for a number of reference compounds. In our radioligand binding assay, we used compound **2** as the control compound. The A_{2a} K_i for compound **2** was 37 nM, and the A_1 K_i was 390 nM. As additional points of reference, the binding affinities for compounds **1** and **3** were determined. For compound **1**, the A_{2a} K_i was 42 nM and the A_1 K_i was 930 nM. For compound **3**, the A_{2a} K_i was 0.9 nM and the A_1 K_i was 680 nM. This clearly indicated that in our preliminary round of optimization, we had identified compounds with better A_{2a} binding affinity than those displayed with compounds **1** and **3**. The potency and selectivity level of compound **41** were comparable to those of compound **3**, one of the most potent and selective adenosine A_{2a} antagonists disclosed to date.

With heterocyclic capping groups, 3-substitution, again, appears to be slightly more favorable than 2-substitution. This was first illustrated with the three pyridinyl analogues (compounds **46**−**48**). Having two methyl groups as in **49** certainly was not as favorable as having one methyl group at the 3-position, as in compound **22**. When a halogen was substituted onto a heterocyclic ring as in **50**, an increase in A_{2a} activity was observed. A similar increase in A_{2a} activity was also observed with the two furans **51** and **52** (again, note the increase in A_{2a} activity when the chlorine atom was installed at the appropriate position on the capping furan ring). Perhaps a more dramatic illustration of the use of the 3-substitution pattern is illustrated with compounds **53** and **54**. Clearly, having two chlorine

Table 3. Heterocyclic Capping Groups[a]



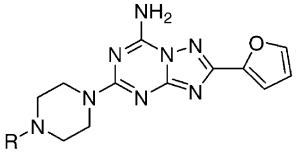
Cpds	R	A_{2a} Ki (nM)	A_1 Ki (nM)
22		25	>250
46		15	>250
47		7	>250
48		37	>250
49		516	>250
50		7	>250
51		18	>250
52		3	>250
53		22	>250
54		4	>250
55		12	>250
56		17	>250

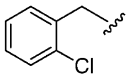
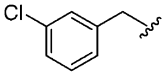
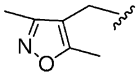
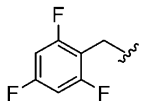
[a] Refer to Table 2 for membrane preparation and details regarding the radioligand binding assay. All K_i values were calculated from binding curves generated from the mean of three determinations per concentration, with the variation in individual values of <15%.

atoms at the 2- and 6-positions was not as potent as having just one chlorine atom at the 3-position. Finally, slightly larger capping groups, such as benzofuran and quinoline, were still acceptable and fairly potent analogues could still be obtained (compounds **55** and **56**).

For an initial evaluation of both oral bioavailability and CNS activity, we used a mouse catalepsy model.[17] This is a well-established efficacy model for Parkinson's disease in which catalepsy is induced by haloperidol. A detailed description of this model has been disclosed

Table 4. Piperazine Derivatives of [1,2,4]Triazolo[1,5-a][1,3,5]triazine[a]



cpds	R	A_{2a} Ki (nM)	A_1 Ki (nM)
57		180	>500
58		1200	>500
59		18	2900
60		3	1300

[a] Taken in part from ref 17. Refer to Table 2 for membrane preparation and details regarding the radioligand binding assay.

previously (additional details could also be found in the Experimental Section below). The ease of observation and the speed of the response make this assay a convenient and relatively high-throughput screen of in vivo activity.

All of the compounds listed in Tables 2 and 3 were tested in this mouse catalepsy model. The xanthine derivative **1** was employed as the positive control. A full dose−response curve for compound **1** was determined, and its ED_{50} was 1 mg/kg p.o. All 34 new analogues shown in Tables 2 and 3 were tested in the mouse catalepsy model, and eight of these showed good oral activity at 10 mg/kg: **30**, **33**, **35**, **38**, **41**, **43**, **52**, and **54**. Oral activity at 3 mg/kg was not observed with any of these (*R*)-2-(aminomethyl)pyrrolidine analogues. This is in sharp contrast to the piperazine series **4** where oral activity at 3 mg/kg was observed in the mouse catalepsy model for a number of analogues. Analogues derived from (*R*)-2-(aminomethyl)pyrrolidine in general appeared to be highly potent and selective in vitro but were not as potent in vivo as those derived from piperazine. Figures 2 and 3 clearly show that the piperazine analogue **60** and compound **1** were superior to analogues derived from (*R*)-2-(aminomethyl)pyrrolidine. In these studies, both **60** and **1** were dosed orally at a lower dose of 3 mg/kg.

On the basis of the sustained oral activity at the 120 min time point, compound **38** was selected for additional profiling in the rat. In the rat catalepsy model, the full dose−response for **38** was determined and its ED_{50} was 10 mg/kg when administered orally. The pharmacokinetic properties of **38** were also determined using standard protocols. Here, rats were dosed both IV and orally at 1 mg/kg and plasma samples were taken at nine time points over 24 h. Compound **38** did not appear to be orally bioavailable since no parent compound was detected in the plasma ($\%F = 0\%$). Compound **38**,

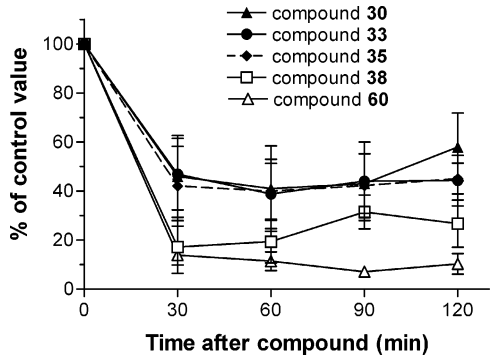


Figure 2. Mouse catalepsy data. For the mouse catalepsy study, CD-1 mice (25−30 g) were injected subcutaneously with 3 mg/kg of haloperidol in order to induce catalepsy. Compounds **30**, **33**, **35**, and **38** were dosed orally at 10 mg/kg. Compound **60** was dosed orally at 3 mg/kg. All test compounds were formulated as the hydrochloride salt and dissolved in saline before administration to the animals. Catalepsy was measured at four time points over a period of 2 h. The data represent the mean of six animals per group. Catalepsy was measured as the time in seconds until the animal 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. These values are shown on the *y*-axis.

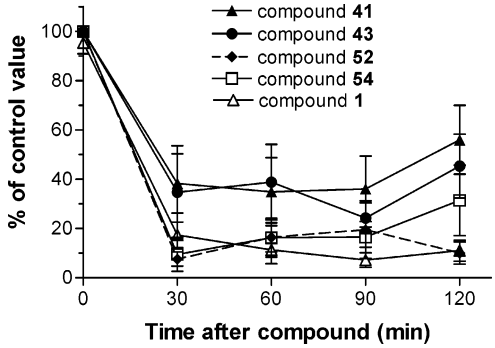


Figure 3. Mouse catalepsy data. Refer to Figure 2 for additional details regarding the mouse catalepsy study. Compounds **41**, **43**, **52**, and **54** were dosed orally at 10 mg/kg. Compound **1** was dosed orally at 3 mg/kg.

however, still had a reasonably long IV half-life of 1.7 h and a fairly large volume of distribution of 7 L/kg. Future work in this series will focus on the determination of active metabolites that may be responsible for the observed efficacy in the catalepsy models. In addition, modifications to the basic [1,2,4]triazolo[1,5-a]-triazine core structure will be carried out in order to improve the overall pharmacological properties of these (*R*)-2-(aminomethyl)pyrrolidines.

In summary, we have shown how the piperazine of **4** could be replaced effectively with a number of different diamines without experiencing any loss in in vitro potency. Among the diamines that were examined, (*R*)-2-(aminomethyl)pyrrolidine emerged as a particularly effective replacement. The basic [1,2,4]triazolo[1,5-a]-triazine core structure could now be modified effectively by using the appropriately substituted (*R*)-2-(aminomethyl)pyrrolidines. An initial round of optimization has resulted in the discovery of a number of potent and selective adenosine A_{2a} receptor antagonists. In in vitro binding assays, compound **41**, for example, was about as active as compound **3**, one of the most potent and selective adenosine A_{2a} receptor antagonists disclosed to date. Even though some of these new [1,2,4]triazolo-[1,5-a]triazines have now been shown to be orally active in rodent models for Parkinson's disease, further optimization is needed to improve their oral bioavailability. These results will be disclosed in due course.

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 a HP 1100 series, 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 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.

2-Furan-2-yl-5-[4-(5-methyl-isoxazol-3-ylmethyl)[1,4]-diazepan-1-yl][1,2,4]triazolo[1,5-a][1,3,5]triazin-7-ylamine (9). In a typical procedure, 0.7 mmol of 2-furan-2-yl-5-methanesulfonyl[1,2,4]triazolo[1,5-a][1,3,5]triazin-7-ylamine (**6**)[14] was suspended in 10 mL of CH_3CN along with 5 equiv of homopiperazine. 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 intermediate homopiperazine.

In a separate flask, (5-methyl-isoxazol-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 containing 0.14 mmol of the homopiperazine derivative prepared above 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 **9**: ^{1}H NMR (DMSO-d_6): δ 8.20 (br s, 2 H), 7.80 (d, J = 1.0 Hz, 1 H), 7.00 (d, J = 3.6 Hz, 1 H), 6.68 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 6.30 (s, 1 H), 4.20 (br s, 2 H), 3.60−2.70 (m, 10 H), 2.35 (s, 3H) ppm. MS m/z = 396 amu ($M^+ + H$). Anal. ($C_{18}H_{21}N_9O_2$) C, H, N.

General Procedure for the Preparation of Compounds 10, 11, and 13−16. The same procedure outlined in the preparation of compound **9** was used to prepare compounds **10**, **11**, and **13**−**16**. The appropriate symmetrical diamine was used in each case (see examples below).

N^5-(2-(N-Methyl-N-((5-methylisoxazol-3-yl)methyl)amino)ethyl)-2-(furan-2-yl)-N^5-methyl[1,2,4]triazolo[1,5-a]-[1,3,5]triazine-5,7-diamine (11). The same procedure outlined in the preparation of compound **9** was used to prepare compound **11**. *N*,*N*′-Dimethylethylenediamine was used instead of homopiperazine. Spectroscopic data for **11**: ^{1}H NMR (DMSO-d_6): δ 8.30 (br s, 2 H), 7.80 (d, J = 1.0 Hz, 1 H), 6.90

(d, J = 3.6 Hz, 1 H), 6.50 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 6.30 (s, 1 H), 4.60 (br s, 2 H), 3.60−3.10 (m, 4 H), 2.75 (s, 3 H), 2.35 (s, 3H), 2.25 (s, 3H) ppm. MS m/z = 384 amu (M^+ + H). High-resolution MS (M^+ + H) calcd for $C_{17}H_{22}N_9O_2$, 384.1896; found, 384.1885. HPLC retention time = 3.58 min (HP1100 analytical HPLC, gradient elution at a rate of 2 mL/min, 5% aqueous CH_3CN to 95% aqueous CH_3CN in 10 min).

2-(Furan-2-yl)-5-(5-((5-methylisoxazol-3-yl)methyl)-2,5-diaza-bicyclo[2.2.1]heptan-2-yl)[1,2,4]triazolo[1,5-a][1,3,5]-triazin-7-amine (16). The same procedure outlined in the preparation of compound **9** was used to prepare compound **16**. Commercially available (*1S*,*4S*)-(+)-2,5-diazabicyclo[2.2.1]-heptane was used instead of homopiperazine. Spectroscopic data for **16**: ^{1}H NMR (DMSO-d_6): δ 8.20 (br s, 2 H), 7.80 (d, J = 1.0 Hz, 1 H), 7.00 (d, J = 3.6 Hz, 1 H), 6.60 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 6.30 (s, 1 H), 4.80 (br s, 2 H), 4.20−3.20 (m, 8 H), 2.35 (s, 3H) ppm. MS m/z = 394 amu (M^+ + H). High-resolution MS (M^+ + H) calcd for $C_{18}H_{20}N_9O_2$, 394.1740; found, 394.1720. HPLC retention time = 3.23 min (HP1100 analytical HPLC, gradient elution at a rate of 2 mL/min, 5% aqueous CH_3CN to 95% aqueous CH_3CN in 10 min).

2-(Furan-2-yl)-N^5-(((*R*)-1-((5-methylisoxazol-3-yl)methyl)pyrrolidin-2-yl)methyl)[1,2,4]triazolo[1,5-a][1,3,5]-triazine-5,7-diamine (22). In a typical procedure, 2.5 mmol of 2-furan-2-yl-5-methanesulfonyl[1,2,4]triazolo[1,5-a]triazin-7-ylamine (**6**)[14] was suspended in 20 mL of CH_3CN along with 5 mmol of (*R*)-2-aminomethyl-1-BOC-pyrrolidine (commercially available from Astatech). The reaction mixture was stirred under reflux for 2 h. It was then cooled to room temperature and concentrated under reduced pressure. The resulting residue was diluted with CH_2Cl_2 and washed with dilute 1% aqueous citric acid and brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification by chromatography (98% CH_2Cl_2, 2% MeOH) afforded 880 mg of the BOC-protected material. This material was dissolved in 6 mL of 25% TFA in CH_2Cl_2 and allowed to stand at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure to afford the TFA salt of 2-furan-2-yl-N^5-pyrrolidin-2-ylmethyl-[1,2,4]triazolo[1,5-a][1,3,5]triazine-5,7-diamine.

In a separate flask, (5-methyl-isoxazol-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 containing 0.15 mmol of the pyrrolidine salt prepared above and Et_3N (0.5 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 had been buffered with 0.1% TFA. Spectroscopic data for **22**: ^{1}H NMR (DMSO-d_6): δ 8.20 (br s, 2 H), 7.85 (d, J = 1.0 Hz, 1 H), 7.00 (d, J = 3.6 Hz, 1 H), 6.60 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 6.30 (s, 1 H), 4.20 (br s, 2 H), 3.60−3.10 (m, 9 H), 2.35 (s, 3H) ppm. MS m/z = 396 amu (M^+ + H). High-resolution MS (M^+ + H) calcd for $C_{18}H_{22}N_9O_2$, 396.1896; found, 396.1900. HPLC retention time = 3.63 min (HP1100 analytical HPLC, gradient elution at a rate of 2 mL/min, 5% aqueous CH_3CN to 95% aqueous CH_3CN in 10 min).

General Procedure for the Preparation of Compounds 12, 17−21, and 23. The same procedure outlined above in the preparation of compound **22** was used to prepare compounds **12**, **17**−**21**, and **23**. The appropriate BOC-protected diamine was used in each case (see examples below).

2-(Furan-2-yl)-N^5-(1-((5-methylisoxazol-3-yl)methyl)piperidin-4-yl)[1,2,4]triazolo[1,5-a][1,3,5]triazine-5,7-diamine (17). The same procedure outlined above in the preparation of compound **22** was used. However, 4-amino-1-BOC-piperidine was used instead of (*R*)-2-aminomethyl-1-BOC-pyrrolidine. Spectroscopic data for **17**: ^{1}H NMR (DMSO-d_6): δ 8.30 (br s, 2 H), 7.80 (d, J = 1.0 Hz, 1 H), 6.90 (d, J = 3.6 Hz, 1 H), 6.60 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 6.30 (s, 1 H), 4.50 (br s, 2 H), 3.60−1.80 (m, 9 H), 2.35 (s, 3H) ppm. MS m/z = 396 amu (M^+ + H). High-resolution MS (M^+ + H) calcd for $C_{18}H_{20}N_9O_2$, 396.1896; found, 396.1900. HPLC retention time = 3.37 min (HP1100 analytical HPLC, gradient elution at a rate of 2 mL/min, 5% aqueous CH_3CN to 95% aqueous CH_3CN in 10 min).

2-(Furan-2-yl)-N^5-(1-((5-methylisoxazol-3-yl)methyl)piperidin-3-yl)[1,2,4]triazolo[1,5-a][1,3,5]triazine-5,7-diamine (18). The same procedure outlined above in the preparation of compound **22** was used. However, racemic 3-amino-1-BOC-piperidine was used instead of (*R*)-2-aminomethyl-1-BOC-pyrrolidine. Spectroscopic data for **18**: ^{1}H NMR (DMSO-d_6): δ 8.30 (br s, 2 H), 7.80 (d, J = 1.0 Hz, 1 H), 6.90 (d, J = 3.6 Hz, 1 H), 6.60 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 6.30 (s, 1 H), 4.50 (br s, 2 H), 3.60−1.80 (m, 9 H), 2.35 (s, 3H) ppm. MS m/z = 396 amu (M^+ + H). High-resolution MS (M^+ + H) calcd for $C_{18}H_{20}N_9O_2$, 396.1896; found, 396.1885. HPLC retention time = 3.46 min (HP1100 analytical HPLC, gradient elution at a rate of 2 mL/min, 5% aqueous CH_3CN to 95% aqueous CH_3CN in 10 min).

2-(Furan-2-yl)-N^5-(1-((5-methylisoxazol-3-yl)methyl)-pyrrolidin-3-yl)[1,2,4]triazolo[1,5-a][1,3,5]triazine-5,7-diamine (21). The same procedure outlined above in the preparation of compound **22** was used. However, (*R*)-3-amino-1-BOC-pyrrolidine was used instead of (*R*)-2-aminomethyl-1-BOC-pyrrolidine. Spectroscopic data for **21**: ^{1}H NMR (DMSO-d_6): δ 8.20 (br s, 2 H), 7.85 (d, J = 1.0 Hz, 1 H), 7.00 (d, J = 3.6 Hz, 1 H), 6.60 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 6.30 (s, 1 H), 4.40 (br s, 2 H), 3.60−3.10 (m, 7 H), 2.35 (s, 3H) ppm. MS m/z = 382 amu (M^+ + H). High-resolution MS (M^+ + H) calcd for $C_{17}H_{20}N_9O_2$, 382.1740; found, 382.1726. HPLC retention time = 3.49 min (HP1100 analytical HPLC, gradient elution at a rate of 2 mL/min, 5% aqueous CH_3CN to 95% aqueous CH_3CN in 10 min).

2-(Furan-2-yl)-N^5-methyl-N^5-(((*R*)-1-((5-methylisoxazol-3-yl)methyl)pyrrolidin-2-yl)methyl)[1,2,4]triazolo[1,5-a]-[1,3,5]triazine-5,7-diamine (24). The same procedure outlined above in the preparation of compound **22** was used. However, (*R*)-2-methylaminomethyl-1-BOC-pyrrolidine was used instead of (*R*)-2-aminomethyl-1-BOC-pyrrolidine. (*R*)-2-Methylaminomethyl-1-BOC-pyrrolidine was prepared as follows: (*R*)-BOC-proline (4.8 g, 22.3 mmol) was suspended in 100 mL of THF. EDC (5.13 g, 1.2 equiv) was added, followed by HOBT (3.62 g, 1.2 equiv) and N-methylmorpholine (3.7 mL, 1.5 equiv). The reaction mixture was stirred at room temperature for 30 min, and 35 mL of methylamine in THF (2.0 M solution in THF, 3 equiv) was added. The resulting reaction mixture was stirred at room temperature for 18 h. It was then concentrated under reduced pressure, and the residue was taken up in CH_2Cl_2. This solution was washed with diluted $NaHCO_3$, water, dilute 5% aqueous citric acid, and brine, dried (Na_2SO_4), and concentrated to afford 4.8 g of the crude carboxamide. This material was dissolved in 100 mL of anhydrous THF and cooled to 0 °C. A solution of borane−THF (53 mL of the 1.0 M solution in THF, 2.5 equiv) was added, and the reaction mixture was allowed to warm to room temperature on its own and stirred at room temperature for 18 h. It was then cooled to 0 °C and carefully quenched with 50 mL of methanol. The reaction mixture was concentrated under reduced pressure. The resulting residue was redissolved in 50 mL of methanol and 100 mL of EtOAc and concentrated under reduced pressure. This trituration with MeOH/EtOAc and concentration were repeated three more times to afford essentially a quantitative yield of (*R*)-2-methylaminomethyl-1-BOC-pyrrolidine. Spectroscopic data for **24**: ^{1}H NMR (DMSO-d_6): δ 8.20 (br s, 2 H), 7.85 (d, J = 1.0 Hz, 1 H), 7.00 (d, J = 3.6 Hz, 1 H), 6.60 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 6.30 (s, 1 H), 4.40 (br s, 2 H), 3.60−3.10 (m, 9 H), 2.90 (s, 3 H), 2.35 (s, 3H) ppm. MS m/z = 410 amu (M^+ + H). High-resolution MS (M^+ + H) calcd for $C_{19}H_{24}N_9O_2$, 410.2053; found, 410.2022. HPLC retention time = 3.86 min (HP1100 analytical HPLC, gradient elution at a rate of 2 mL/min, 5% aqueous CH_3CN to 95% aqueous CH_3CN in 10 min).

N^5-(((*R*)-1-(2-chlorobenzyl)pyrrolidin-2-yl)methyl)-2-(furan-2-yl)[1,2,4]triazolo[1,5-a][1,3,5]triazine-5,7-diamine (26). The TFA salt of 2-furan-2-yl-N^5-pyrrolidin-2-ylmethyl[1,2,4]triazolo[1,5-a][1,3,5]triazine-5,7-diamine, prepared as outlined above in the preparation of compound **22**, was used as the starting material. This TFA salt (2 mmol) was converted to the corresponding free amine first by dissolving in 5 mL of water and neutralized with 1 molar equivalent of aqueous NaOH. The resulting solution was concentrated to dryness to afford the desired pyrrolidine as a mixture of the free amine and sodium salt. This pyrrolidine mixture (0.15 mmol) was suspended in 4 mL of CH_2Cl_2 along with 2 equiv of 2-chlorobenzaldehyde and 25 μL of glacial acetic acid. The resulting 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 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 had been buffered with 0.1% TFA. Analytically pure samples could also be obtained by column chromatography (SiO_2) using a mixture of 95% CH_2Cl_2 and 5% MeOH. Spectroscopic data for **26**: ^{1}H NMR (DMSO-d_6): δ 8.20 (br s, 2 H), 7.80 (d, J = 1.0 Hz, 1 H), 7.30−7.10 (m, 4 H), 7.00 (d, J = 3.6 Hz, 1 H), 6.68 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 4.20 (br s, 2 H), 3.60−2.50 (m, 9 H) ppm. MS m/z = 426 amu (M^+ + H). Anal. ($C_{20}H_{21}ClN_8O$) C, H, N.

General Procedure for the Preparation of Compounds 27−45. The rest of the compounds shown in Table 2 (compounds **27**−**45**) were prepared using the same procedure outlined above in the preparation of compound **26** using the appropriate aldehyde (see examples below).

N^5-(((*R*)-1-(3-Fluorobenzyl)pyrrolidin-2-yl)methyl)-2-(furan-2-yl)[1,2,4]triazolo[1,5-a][1,3,5]triazine-5,7-diamine (32). The same procedure outlined above in the preparation of compound **26** was used. However, 3-fluorobenzaldehyde was used instead of 2-chlorobenzaldehyde. Spectroscopic data for **32**: ^{1}H NMR (DMSO-d_6): δ 8.20 (br s, 2 H), 7.80 (d, J = 1.0 Hz, 1 H), 7.30−7.10 (m, 4 H), 7.00 (d, J = 3.6 Hz, 1 H), 6.68 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 4.80 (br s, 2 H), 3.60−2.50 (m, 9 H) ppm. MS m/z = 409 amu (M^+ + H). Anal. ($C_{20}H_{21}FN_8O$) C, H, N.

N^5-(((*R*)-1-(2,6-Difluorobenzyl)pyrrolidin-2-yl)methyl)-2-(furan-2-yl)[1,2,4]triazolo[1,5-a][1,3,5]triazine-5,7-diamine (38). The same procedure outlined above in the preparation of compound **26** was used. However, 2,6-difluorobenzaldehyde was used instead of 2-chlorobenzaldehyde. Spectroscopic data for **38**: ^{1}H NMR (DMSO-d_6): δ 8.20 (br s, 2 H), 7.80 (d, J = 1.0 Hz, 1 H), 7.60 (br s, 1 H), 7.28−7.22 (m, 1 H), 6.91−6.86 (m, 2 H), 7.00 (d, J = 3.6 Hz, 1 H), 6.68 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 5.20 (br s, 2 H), 3.60−2.50 (m, 9 H) ppm. MS m/z = 427 amu (M^+ + H). Anal. ($C_{20}H_{20}F_2N_8O$) C, H, N

N^5-(((*R*)-1-(2,4,6-Trifluorobenzyl)pyrrolidin-2-yl)methyl)-2-(furan-2-yl)[1,2,4]triazolo[1,5-a][1,3,5]triazine-5,7-diamine (41). The same procedure outlined above in the preparation of compound **26** was used. However, 2,4,6-trifluorobenzaldehyde was used instead of 2-chlorobenzaldehyde. Spectroscopic data for **41**: ^{1}H NMR (DMSO-d_6): δ 8.20 (br s, 2 H), 7.80 (d, J = 1.0 Hz, 1 H), 6.91−6.86 (m, 2 H), 7.00 (d, J = 3.6 Hz, 1 H), 6.68 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 5.20 (br s, 2 H), 3.60−2.50 (m, 9 H) ppm. MS m/z = 427 amu (M^+ + H). Anal. ($C_{20}H_{19}F_3N_8O\cdot 1H_2O$) C, H, N.

General Procedure for the Preparation of Compounds 46−48, 50−52, and 55−56. These compounds were prepared using the same reductive amination conditions that were outlined above in the preparation of compound **26**. The appropriate heterocyclic aldehyde was used (see example below).

2-(Furan-2-yl)-N^5-(((*R*)-1-((pyridin-2-yl)methyl)pyrrolidin-2-yl)methyl)[1,2,4]triazolo[1,5-a][1,3,5]triazine-5,7-diamine (46). The same procedure outlined above in the preparation of compound **26** was used. However, 2-pyridinecarboxaldehyde was used instead of 2-chlorobenzaldehyde. Spectroscopic data for compound **46**: ^{1}H NMR (DMSO-d_6): δ 8.5 (br s, 1 H), 8.20 (br s, 2 H), 7.80 (d, J = 1.0 Hz, 1 H), 7.60 (br s, 1 H), 7.50−7.22 (m, 3 H), 7.00 (d, J = 3.6 Hz, 1 H), 6.68 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 4.20 (br s, 2 H), 3.60−2.50 (m, 9 H) ppm. MS m/z = 392 amu (M^+ + H). Anal. ($C_{19}H_{21}N_9O$) C, H, N.

General Procedure for the Preparation of Compounds 49, 53, and 54. An alkylation procedure was used to prepare these compounds. The preparation of **49** is shown below. Compounds **53** and **54** were prepared using the same general procedure outlined in the preparation of **49** using the appropriate halide.

2-(Furan-2-yl)-N^5-(((*R*)-1-((3,5-dimethylisoxazol-4-yl)-methyl)pyrrolidin-2-yl)methyl)[1,2,4]triazolo[1,5-a][1,3,5]-triazine-5,7-diamine (49). The TFA salt of 2-furan-2-yl-N^5-pyrrolidin-2-ylmethyl[1,2,4]triazolo[1,5-a][1,3,5]triazine-5,7-diamine, prepared as outlined above in the preparation of compound **19**, was used as the starting material. This TFA salt (2 mmol) was converted to the corresponding free amine first by dissolving in 5 mL of water and neutralized with 1 molar equivalent of aqueous NaOH. The resulting solution was concentrated to dryness to afford the desired pyrrolidine as a mixture of the free amine and $NaCF_3CO_2H$. This pyrrolidine mixture (0.10 mmol) was suspended in 2 mL of CH_3CN along with 2 equiv of 4-chloromethyl-3,5-dimethyl-isoxazole and 3 equiv of Et_3N. The resulting reaction mixture was stirred at room temperature for 18 h and then concentrated under a stream of N_2. The resulting crude product was purified by preparative HPLC. Spectroscopic data for **49**: ^{1}H NMR (DMSO-d_6): δ 8.20 (br s, 2 H), 7.85 (d, J = 1.0 Hz, 1 H), 7.00 (d, J = 3.6 Hz, 1 H), 6.60 (dd, J = 3.6 Hz, 1.0 Hz, 1 H), 4.20 (br s, 2 H), 3.60−3.10 (m, 9 H), 2.35 (s, 3H), 2.10 (s, 3H) ppm MS m/z = 410 amu (M^+ + H). HPLC retention time = 3.10 min (HP1100 analytical HPLC, gradient elution at a rate of 2 mL/min, 5% aqueous CH_3CN to 95% aqueous CH_3CN in 10 min).

Formation of the Hydrochloride Salt for 30, 33, 35, 38, 41, 43, 52, 54, and 60. For each of these compounds, the corresponding hydrochloride salt was prepared as follows. Each compound (0.2 mmol) was dissolved in 10 mL of 1:1 CH_3CN/H_2O. Aqueous HCl (0.2 mL of a 1 N solution) was then added, and the resulting solution was lyophilized to afford the desired hydrochloride salt.

Biological Assays. 1. A_{2a} Receptor. The membranes were prepared from rat brain tissues purchased from Pel-Freez. The 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. The pellets were resuspended and washed twice with buffer HE (10 mM Na−HEPES, 1 mM EDTA, pH 7.4, plus protease inhibitors). The 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 the BCA protein assay kit (Pierce).

2. Rat A_1 Receptor. The membranes were prepared from rat cerebral cortex isolated from freshly euthanized rats. The 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. The pellets were resuspended and washed twice with buffer HE (10 mM Na−HEPES, 1 mM EDTA, pH 7.4, plus protease inhibitors). The final pellets were resuspended in buffer HE, supplemented with 10% (w/v) sucrose and protease inhibitors, and frozen in aliquots at −80 °C. The protein concentrations were measured using the BCA protein assay kit (Pierce).

3. Radioligand Binding Assays. Membranes (40−70 μg of membrane protein), radioligands, and varying concentrations of competing ligands were incubated in triplicate 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 competi-

tion 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. The binding assays were terminated by filtration over Whatman GF/C glass fiber filters using a BRANDEL cell harvester. The 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.

4. 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.

5. Catalepsy Experiments. Haloperidol-induced catalepsy was used to mimic the effects of Parkinson's disease in rats and mice. The animals were injected with haloperidol, which causes immobility. A test compound was then administered orally, and the compound's ability to reverse these Parkinson's-like symptoms was analyzed. 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 of the catalepsy studies is 105-00.

6. Rats. Male Sprague−Dawley rats (225−275 g) were injected with haloperidol (1 mg/kg s.c.). 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), the 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. Data were analyzed by one factor analysis of variance with Dunnett's *t*-test used to make post-hoc comparisons.

7. Mice. The method was identical for mice (CD-1; 25−30 g) except that the dose of haloperidol was 3 mg/kg s.c., and the bar was suspended 4.5 cm above the surface of the bench.

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

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