Articles

Identification of Novel, Water-Soluble, 2-Amino-*N*-pyrimidin-4-yl Acetamides as A_{2A} Receptor Antagonists with In Vivo Efficacy

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Potent adenosine hA_{2A} receptor antagonists are often accompanied by poor aqueous solubility, which presents issues for drug development. Herein we describe the early exploration of the structure–activity relationships of a lead pyrimidin-4-yl acetamide series to provide potent and selective 2-amino-*N*-pyrimidin-4-yl acetamides as hA_{2A} receptor antagonists with excellent aqueous solubility. In addition, this series of compounds has demonstrated good bioavailability and in vivo efficacy in a rodent model of Parkinson's disease, despite having reduced potency for the rat A_{2A} receptor versus the human A_{2A} receptor.

Introduction

The effects of adenosine are mediated through at least four specific cell membrane receptors classified as A_1, A_{2A}, A_{2B}, and A_3. These receptors belong to the G protein-coupled receptor family.[1] The A_1 and A_3 receptors decrease cellular cAMP levels through their coupling to G_i proteins, which inhibit adenylate cyclase. In contrast, A_{2A} and A_{2B} receptors couple to $G_{s/olf}$ proteins that activate adenylate cyclase and increase intracellular levels of cAMP. While A_1 receptors are widely distributed in most areas of the brain and peripheral organs,[1] A_{2A} receptors are highly concentrated in the dorsal striatum, nucleus accumbens, and olfactory tubercle.[2] This unique cellular location of the A_{2A} receptor provides the anatomical basis for the adenosine−dopamine interaction that underlies the motor symptomatic benefits of A_{2A} receptor antagonists in Parkinson's disease.

Adenosine A_{2A} receptors modulate the release of GABA in the striatum, which appears to regulate the activity of medium spiny neurons. By reducing GABA output, A_{2A} antagonism helps restore normal function in the basal ganglia following dopamine depletion. Thus, hA_{2A} receptor antagonists may be a useful treatment for neurodegenerative movement disorders such as Parkinson's and Huntington's disease,[3] dystonias such as restless leg syndrome,[4] and dyskinesias such as those caused by prolonged use of neuroleptic and dopaminergic drugs.[5]

The development of potent and selective antagonists of the hA_{2A} receptor has challenged the medicinal chemistry research community for a number of years,[6] however, several potent and selective structures are now emerging. Kyowa Hakko Kogyo, Schering-Plough, and Vernalis/Biogen have taken hA_{2A} receptor antagonist drugs into the clinic for the treatment of Parkinson's disease. Kyowa has recently filed an NDA for compound **1**,[7] (KW6002, Figure 1), Schering-Plough has taken compound **2** (SCH420814, Figure 1)[8] into phase II clinical trials, and Vernalis/Biogen have entered phase II trials with V2006 (structure not yet disclosed).[9]

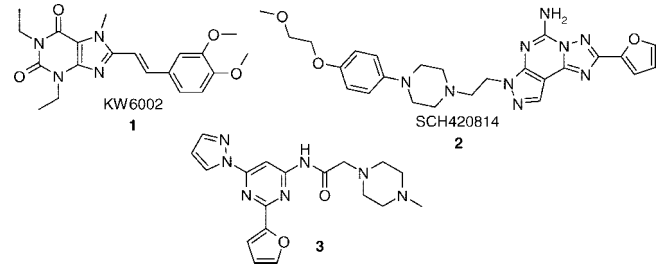

Figure 1. hA_{2A} antagonists in clinical development (**1** and **2**) and lead compound **3**.

Poor solubility is a common issue with many adenosine antagonists, for example, **2** is reported to have solubility of less than 10 ng/mL at pH 7.4.[8] The work herein describes a novel series of potent, selective, and highly soluble hA_{2A} antagonists for the treatment of Parkinson's disease, as represented by compound **3** in Figure 1.

Chemistry

The pyrimidine analogues, **3**, **17**, **18**, and **20–23** were synthesized according to a number of methods, as described below in Schemes 1–4. The method used to make key intermediates involved conversion of the commercially available nitrile **4** to the corresponding carboxyamidine **5** via treatment with sodium methoxide in methanol at room temperature,

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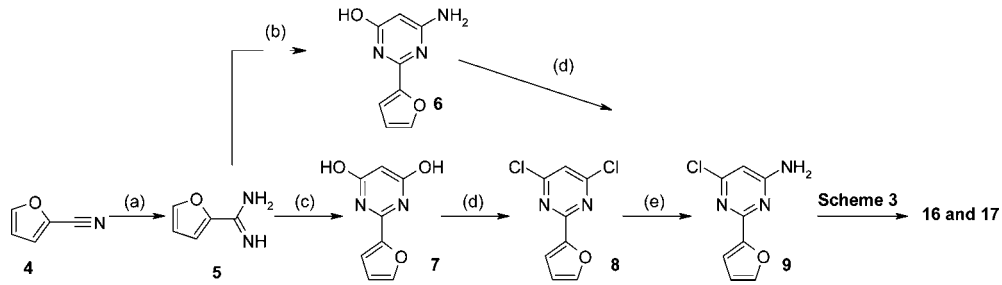
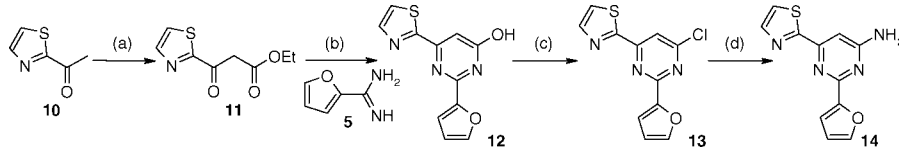
Scheme 1[a]



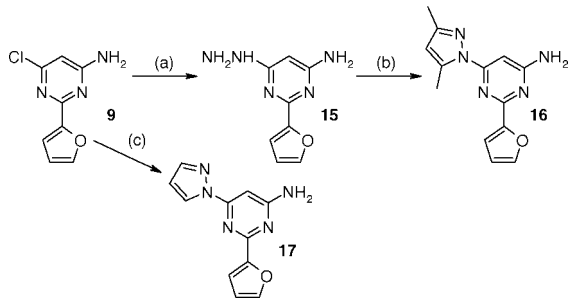
[a] Reagents and conditions: (a) NaOMe, (1.1 equiv), NH_4Cl, MeOH, rt, 6–12 h, 96%; (b) ethyl cyanoacetate, NaOMe, EtOH, 67%; (c) NaOEt, diethylmalonate, rt−reflux, 12–60 h, 78%; (d) $POCl_3$, DIPEA, 90 °C, 3–12 h, 87%; (e) NH_4OH, MeOH, 80 °C, 12 h, 76%.

Scheme 2[a]



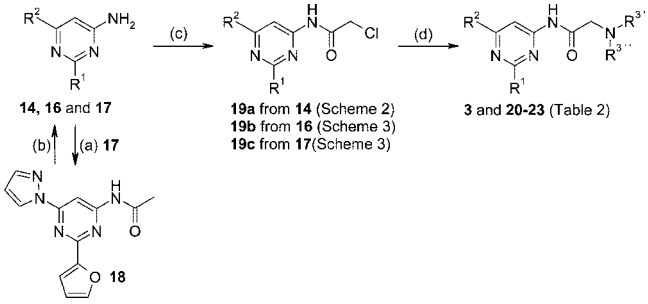
[a] Reagents and conditions: (a) diethyl carbonate (5 equiv), NaH, 80 °C, 12 h, 56%; (b) KO^{t}Bu, (1.5 equiv), ^{t}BuOH, 90 °C, 12 h, 50%; (c) $POCl_3$, DIPEA, 90 °C, 3–12 h, 66%; (d) NH_4OH, MeOH, 80 °C, 12 h, 53%.

Scheme 3[a]



[a] Reagents and conditions: (a) N_2H_4, EtOH, 90 °C; (b) pentane-2,4-dione, 0−90 °C, 2 h, 72%; (c) pyrazole, Cs_2CO_3, DMF, 85 °C, 21 h, 55%.

Scheme 4[a]



[a] Reagents and conditions: (a) acetylchloride, pyridine, DCM, rt, 12 h, 80–95%; (b) KOH $_{(aq)}$, dioxane, 90 °C, 30 min−2 h, 90–95%; (c) chloroacetyl chloride, pyridine, DCM, rt, 2–12 h, 60–85%; (d) primary or secondary amine, Et_3N, rt, 6–12 h.

followed by reaction with ammonium chloride. The resulting carboxyamidine was then reacted with diethyl malonate in methanol in the presence of sodium ethoxide to yield the corresponding pyrimidine-4,6-diol **7**. Intermediate **7** was then reacted with phosphorus oxychloride, in the presence of *N*,*N*-diisopropyl-ethylamine to yield the 4,6-dichloropyrimidine **8**, which was then treated with refluxing ammonium hydroxide in methanol to yield the 6-chloropyrimidin-4-amine intermediate **9**. Alternatively, **9** can be formed in two steps (from **5**) via reaction with ethyl cyanoacetate to give intermediate **6**, followed by treatment with phosphorus oxychloride. This latter route

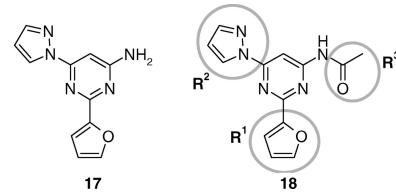


Figure 2. Lead structures **17** and **18**, with three groups R^1, R^2, and R^3 for optimization.

worked well, but on larger scale, the phosphorus oxychloride reaction step was cleaner with intermediate **7** than with **6**, therefore, this slightly longer route was preferred. Further reaction of **9** to install R^2 (as defined in Figure 2) is illustrated in Scheme 3.

There are a number of commercially available ethyl 3-oxo-propanoates, however, **11** was synthesized via the reaction of the desired methyl ketone, in this case, **10**, with diethyl carbonate, using sodium hydride as the base to give 3-oxo-3-thiazol-2-yl-propionic acid ethyl ester **11** in good yield (Scheme 2). Compound **11** was then reacted with the carboxyamidine **5** to install R^2 directly. After treatment with phosphorus oxychloride to give **13**, reaction with ammonium hydroxide yielded the corresponding 6-heteroaryl-pyrimidin-4-amine intermediate **14** (Scheme 2). While this second route was less versatile, it was more convenient for the thiazole analogue. Intermediate **14** was further manipulated, as described in Scheme 4.

Derivatization of the key 6-chloropyrimidin-4-amine intermediate **9** was carried out to give a number of analogues, as shown in Scheme 3. The dimethyl-substituted pyrazole analogue was synthesized by first displacing the chloride with hydrazine to give **15**, followed by cyclization with pentane-2,4-dione to give intermediate **16**. Alternatively, the chloride can be displaced with pyrazole directly to give intermediate **17**.

The aminopyrimidine intermediates **14**, **16**, and **17** described above were then converted to the final compounds via the routes outlined in Scheme 4. Compound **17** was acylated using acetylchloride and pyridine to give compound **18**. Compounds

Table 1.

cmpd	K_i (nM) ± SEM hA_{2A}[a]	hA_1[b]	hA_1/hA_{2A}
17	2.9 ± 0.1	56 ± 1	20
18	0.6 ± 0.4	9.2 ± 0.6	15

[a] Displacement of specific [^{3}H]-ZM241385 binding at human A_{2A} receptors expressed in HEK293. [b] Displacement of specific [^{3}H]-DPCPX binding at human A_1 receptors expressed in HEK293 cells. Data are expressed as mean values of at least three runs ± SEM.

3 and **20**−**23** (Table 2) were made via reaction of the corresponding intermediates **19a**, **b**, or **c** with the appropriate amine.

Results and Discussion

Compound **17** was identified as an antagonist of the hA_{2A} receptor with a K_i of 2.9 nM (Figure 2).

The main deficiencies of this molecule appeared to be the lack of selectivity for hA_{2A} versus hA_1 (20-fold), the relatively poor metabolic stability in human liver microsomes (scaled intrinsic clearance 73 mL/min/kg), and the limited aqueous solubility (0.08 mg/mL in PBS). Interestingly, acylation of compound **17** to give compound **18** led to a significant increase in potency (K_i 0.6 nM, Table 1). Although the selectivity for hA_{2A} over hA_1 was not significantly enhanced, this finding opened up an area for further exploration, and a series of modifications were made via acylation of the aromatic amine. Incorporation of polar substituents in the R^3 region, in an attempt to immediately address the issues of solubility, gave encouraging results.

The addition of a single amino moiety to give compound **20** (Table 2) improved physical properties and potency against hA_{2A} was maintained. Further analogues were made and it was found that the incorporation of a morpholine ring (compound **21**) both maintained potency and enhanced the selectivity for hA_{2A} over hA_1. Replacement of the morpholine with a piperazine ring to give compound **3** further increased selectivity and gave a highly soluble compound when converted to the HCl salt (>25 mg/mL in water). In addition, compound **3** had a measured Log D of 2.2 and pK_a of 7.9[10] (distal piperazine nitrogen), with a molecular weight of 367, which are good physical properties for achieving oral bioavailability and cell permeability. Metabolic stability, measured in human liver microsomes, was also much improved (scaled intrinsic clearance 6 mL/min/kg) over that of **17**, presumably due to the increased hydrophilic nature of this compound. Indeed, compound **3** had good bioavailability in rat (*%F* 79, AUC 2870 ng·h/mL, from a 10 mg/kg oral dose, unpublished data). As can be seen from the brain exposure at 2 h (Table 3), compound **3** and the closely related and physically similar analogue compound **22** also have good exposure in the brain. In addition, these compounds showed no significant inhibition of the major cytochrome P_{450} enzymes (>10 μM, CYP2D6 and CYP3A4). Compound **3** was also assayed against the human A_{2B} and human A_3 receptors at CEREP[11] and was found to bind with K_i values of 35 and 660 nM, respectively.

The effect of subtle variations to the R^2 region was also explored (see Figure 2 and Scheme 4). Both the thiazole (compound **22**) and the dimethyl pyrazole (compound **23**) ring system variants were well tolerated at the R^2 position, with compound **23** having 864-fold selectivity for hA_{2A} over hA_1. This is presumably due to the ability of hA_{2A} to tolerate the increased steric bulk of the methyl groups, whereas for hA_1, this increase in bulk cannot be accommodated. Compounds **3**, **22**, and **23** were selected to be further studied for in vivo efficacy. The haloperidol-induced catalepsy (HIC) model in rat was used as the primary assay to screen compounds for efficacy. Compound **1** is reported to work in this model down to 0.3 mg/kg.[12] As shown in Figure 3, all three analogues **3**, **22**, and **23** were efficacious at the screening dose of 10 mg/kg p.o. It is worth noting that the hA_{2A} receptor antagonist activity for these compounds was on the order of 10-fold less for the rat A_{2A} (rA_{2A}) receptor than for the hA_{2A} receptor (Table 3). In contrast, compound **1** was found to be slightly less potent against hA_{2A} than rA_{2A} (K_i 6 and 2 nM, respectively).

Conclusions

We have developed a novel series of potent and selective adenosine hA_{2A} antagonists that contain basic amines that confer excellent physical properties for drug development. Although in most cases the compounds were less potent against the rA_{2A} receptor, we were able to demonstrate in vivo efficacy for this series in a rat model of haloperidol-induced catalepsy. Further optimization and evaluation of this series in other models of Parkinson's disease will be reported in due course.

Experimental Section

Solubility Assay Protocol. To determine solubility of compounds, approximately 1 mg of sample was weighed into a Falcon centrifuge tube (15 mL), and the weight was recorded to 0.001 mg. Assay medium (200 μL, phosphate buffered saline (PBS), pH 7.4, or water, etc.) was added, and the sample was sonicated for 10 min and then shaken overnight. The sample was then centrifuged, and the supernatant was analyzed by HPLC to determine the concentration of sample in solution. The concentration in solution was then calculated based on a standard curve generated from known dilutions of authentic sample.

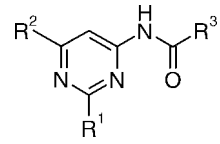
Biology Experimental

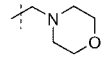
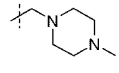
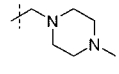
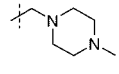
Pharmacology. Adenosine A_1 and A_{2A} Receptor Binding Assays. Receptor Cloning. The coding sequence of the human A_1 and A_{2A} receptor was amplified from a human brain cDNA library by the polymerase chain reaction. Each amplicon was cloned into the pcDNA5/FRT/V5-His-TOPO expression vector (Invitrogen), and the sequence was confirmed using an ABI 3100 automated sequencer (Applied Biosystems). Each expression construct was transfected into Flp-In HEK cells (Invitrogen) using Lipofectamine 2000 (Invitrogen). Cells stably expressing either the human A_1 or A_{2A} receptor were selected using 1 mg/mL hygromycin in complete DMEM.

Membrane Preparation. Crude membranes were prepared from Flp-In HEK cells transfected with either the human A_1 or A_{2A} receptor by resuspending cells in lysis buffer (50 mM Tris-HCl pH 7.4, 5 mM EDTA, 10 mM $MgCl_2$) and disrupting under N_2 at a pressure of 900 psi (Parr Cell disruption bomb, cat. #4639) for 30 min on ice, followed by differential centrifugation. The resulting crude membrane pellet was resuspended in assay buffer (50 mM Tris-HCl pH 7.4, 1 mM EDTA, 10 mM $MgCl_2$). Membrane protein concentration was determined by Bradford assay. Membranes of cloned rat A_{2A} receptor produced in CHO cells were obtained from Perkin-Elmer (SignalScreen, Lot no. 6110536-09). Membrane aliquots were stored at −80 °C.

Binding Assay. An aliquot of membranes (1–2 μg of protein) was preincubated for 30 min at RT in the presence of 10 μg/mL adenosine deaminase (type IV calf spleen, Sigma). Membranes were then incubated for 90 min with either 1.0 nM [^{3}H]DPCPX (120.00 Ci/mmol Perkin-Elmer NET 974) for the A_1 membranes or 2.0 nM [^{3}H]-ZM 241385 (27.40 Ci/mmol Tocris R1036) for the A_{2A} human and rat membranes in the

Table 2.



Cmpd	R^1	R^2	R^3	Ki (nM) ± SEM hA_{2A}[a]	Ki (nM) ± SEM hA_1[b]	hA_1/hA_{2A}
20				5.2 ± 2.4	28 ± 2	5
21				1.6 ± 0.2	63 ± 8	39
3				2.7 ± 0.2	266 ± 60	99
22				0.9 ± 0.1	218 ± 30	238
23				2.0 ± 0.2	1730 ± 200	864

[a] See Table 1. Data are expressed as mean values of at least three runs ± SEM. [b] See Table 1. Data are expressed as mean values of at least three runs ± SEM.

Table 3.

cmpd	hA_{2A}[a] K_i (nM) ± SEM	rA_{2A}[b] K_i (nM) ± SEM	concn in rat brain[c] (ng/g)
3	2.7 ± 0.2	26 ± 4	2700
22	0.9 ± 0.1	11 ± 2	3200
23	2.0 ± 0.2	14 ± 2	600

[a] See Table 1. [b] Displacement of specific [^{3}H]-ZM241385 binding at rat A_{2A} receptors expressed in CHO cells. Data are expressed as mean values of at least three runs ± SEM. [c] At 2 h after dosing with a 10 mg/kg oral (p.o.) dose.

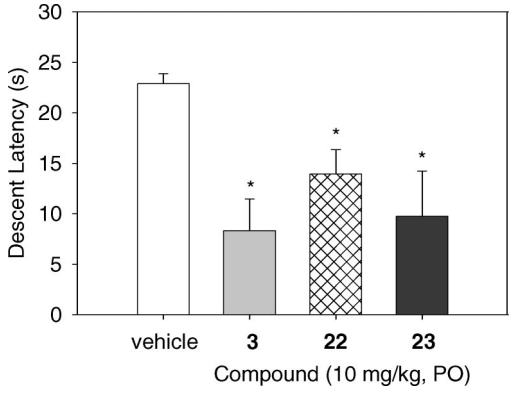


Figure 3. Effect of compounds **3**, **22**, and **23** in the haloperidol-induced catalepsy bar test. Mean descent latency (±standard error) in seconds. The vehicle bar represents the mean and standard error from 3 experiments. *Vehicle vs treated rats, $p < 0.05$.

presence of varying concentrations of competing ligand. Nonspecific binding was determined in the presence of excess (1 μM) DPCPX or CGS15943 for the A_1 and A_{2A} membranes, respectively. Bound and free ligands were separated by rapid vacuum filtration using a Packard 96-well cell harvester onto UniFilter GF/C filter plates (Perkin Elmer) that had been pretreated with 0.5% polyethyleneimine. The filter plates were than washed 3 × 200 μL with 50 mM Tris-HCl and 50 mM NaCl, pH 7.4. Bound radioligand was determined by scintillation counting using a TopCount-NXT (Packard). Binding data were analyzed by nonlinear, least-squares curve fitting algorithms using GraphPad Prism (GraphPad Software, Inc., San Diego, CA) or ActivityBase (IDBS, Guildford, Surrey, U.K.). K_i values were calculated from IC_{50} values using the Cheng−Prusoff equation (Cheng and Prusoff, 1973).[13]

For A_1 membrane assay, ^{3}H-DPCPX measured $K_d = 1.0 \pm 0.5$ nM and $B_{max} = 8 \pm 4$ pmol/mg by Scatchard Analysis. Control DPCPX (Tocris, 0439) $K_i = 1.6 \pm 0.7$ nM. For human A_{2A} membrane assay, ^{3}H-ZM241385 measured $K_d = 0.22 \pm 0.20$ nM and $B_{max} = 33 \pm 8$ pmol/mg by Scatchard Analysis. Control NBI 80634 $K_i = 0.25 \pm 0.04$ nM. For rat A_{2A} membrane assay, ^{3}H-ZM241385 measured $K_d = 0.33 \pm 0.20$ nM and $B_{max} = 2.4$ pmol/mg by Scatchard Analysis. Control NBI 80634 $K_i = 1.2 \pm 0.3$ nM.

In Vivo Assays. Selected compounds were tested for their effects on haloperidol-induced catalepsy, measured with the bar test. Separate groups of rats were used to test each compound ($n = 24$/experiment). Male Wistar rats (Charles River) were habituated to the vivarium for 1 week prior to testing. Rats were weighed (~220 g at time of testing) and administered test compound orally (p.o.) 2 h prior to the bar test. The vehicle for all test compounds was 5% cremophor in sterile water given at a volume of 5 mL/kg. One hour prior to the bar test, rats were given an intraperitoneal (i.p.) injection of haloperidol (1.5 mg/kg in a 10 mL/kg of sterile water). The bar test consisted of 3 and 30 s trials spaced 3 min apart. During a test, the rat's forepaws were placed on an 8.0 cm high metal bar. The descent latency of one paw from the bar was measured and recorded (in seconds) as the score for a given trial. The descent latency from each of the three trials was averaged to obtain the mean descent latency for each rat. Plasma and brain were collected following the haloperidol-induced catalepsy test (samples collected 2 h post dose), and samples were analyzed to determine exposure (the method is described below in Preclinical Assays).

Preclinical Assays. Metabolic Stability Assay in Rat and Human Liver Microsomes. Pooled male human and rat

liver microsomes (0.5 mg/mL for human and 0.1 mg/mL for rat; $n > 10$; mixed gender) were incubated at 37 °C with the NCE in the presence of an NADPH-generating system containing 50 mM, pH 7.4, potassium phosphate buffer, 3 mM magnesium chloride, 1 mM EDTA, 1 mM NADP, 5 mM G-6-P, and 1 unit/mL G-6-PD. Incubations were conducted with 1 μM of each NCE (0.01% DMSO), with a total volume of 250 μL in duplicate at each time point (0, 5, 10, 20, 40, and 60 min). Reactions were stopped by the addition of 0.3 mL of acetonitrile containing a proprietary internal standard. Precipitated proteins were removed by centrifugation for 15 min at 3000 rpm, and the supernatant fluid (~0.1 mL) was analyzed by LC/MS for the % of parent compound remaining. The in vitro initial rates of metabolism were scaled using constants, such as microsomal protein/gm liver, gm liver/kg body weight, and liver blood flow, to predict systemic clearance and % bioavailability. These calculations from nonlinear regression assume that liver metabolism, not absorption, is the determinant of bioavailability.

Screening PK Protocol for Sample Extraction and Analysis To Determine Brain Exposure in Rat at the 2 h Time Point. Sample extraction and analysis to determine brain exposure in rat at the 2 h time point was carried out as follows. Brain tissue sample analysis was conducted with a liquid chromatography in tandem with a mass spectrometric detector (LC/MS/MS) method. Half of the brain (left or right) was weighed and homogenized in 2.0 mL of ACN/H_2O/FA (formic acid; v/v/v 60/40/0.1) containing 50 μL internal standard (10 μg/mL NBI 67377). The homogenates were centrifuged at 3000 rpm for 20 min, and the supernatant was collected for LC/MS/MS analysis. Brain standards were prepared by spiking 1.0 mL of a series of premade calibration standard aqueous solutions (prepared in 60/40 ACN/H_2O), 1.0 mL of ACN/H_2O/FA (v/v/v 60/40/0.2), and 50 μL internal standard (10 μg/ml) into half-untreated rat brains. Standards for brain were processed and analyzed at the same time and in exactly the same way as the analytical samples.

Bioanalytical Analysis. The LC/MS/MS system was equipped with an Agilent 1100 HPLC system coupled to a CTC PAL autoinjector (Leap Technologies, Carrboro, NC) and a Sciex API 4000 triple quadruple mass spectrometer (Toronto, Canada). Samples were eluted at 0.45 mL/min using a mobile phase consisting of solution A (0.1% formic acid in water) and solution B (0.1% formic acid in acetonitrile) with a fast gradient (0–0.1 min, 5% B; 0.1–1.0 min, 5 to 95% B; hold to 2.5 min; 2.5–2.7 min, 95–5% B; hold to 3.5 min) and a column temperature of 20 °C. The HPLC column used for this assay was Agilent Zorbax SB-C18 column, 2.1 × 50 mm, with a particle size of 5 μm. Electrospray ionization source was used for detection at a positive MRM (multiple reaction monitoring) mode with the ion pair of 368.18→113.05. Quantification was performed by fitting peak area ratios (peak of parent analyte vs peak of the internal standard) to a weighted ($1/x$) linear calibration curve.

Experimental for Intermediate Synthesis and Analytical Methods. Reagents, starting materials, and solvents were purchased from commercial suppliers and used as received. Concentration refers to evaporation under vacuum using a Büchi rotatory evaporator. Reaction products were purified, when necessary, by flash chromatography on silica gel (40–63 μm) with the solvent system indicated. Spectroscopic data were recorded on a Varian Mercury 300 MHz spectrometer, Bruker DPX-250 spectrometer, or Bruker Avance 500 MHz spectrometer. The elemental analysis was done by Robertson Microlit Laboratory, Madison, NJ. Melting points were recorded on a Büchi 535 apparatus. Descriptions of analytical HPLC-MS methods 1–6 are given in Supporting Information; preparative HPLC-MS; platform, Dionex, equipped with a Gilson 215 autosampler/fraction collector, UV detector, and a Dionex MSQ mass detector; HPLC column, Phenomenex Synergy Max-RP, 21.2 × 50 mm. HPLC gradient, 35 mL/min, 5% acetonitrile in water to 95% acetonitrile in water in 17.7 min. Both acetonitrile and water have 0.05% TFA.

2-Furancarboxamidine (5). To a solution of sodium methoxide (3.0 g, 56 mmol) in methanol (50 mL) in a round-bottomed flask equipped with a stirrer bar was added 2-furonitrile (5.0 g, 53 mmol). The mixture was stirred at room temperature for 3 h. To the resulting solution was slowly added ammonium chloride (3.0 g, 60 mmol), and the mixture was stirred at room temperature for 68 h. The resulting suspension was filtered, and the solvent was removed under reduced pressure. The solid obtained was washed with diethyl ether (3 × 25 mL) to give compound **5** as an off-white solid (HCl salt; 7.5 g, 96%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.22 (s, 3H), 8.19 (s, 1H), 7.89 (d, J = 3.8, 1H), 6.88–6.86 (m, 1H).

2-(2-Furyl)pyrimidine-4,6-diol (7). To a solution of sodium ethoxide (10.8 g, 200 mmol) in ethanol (90 mL) was slowly added compound **5** (5.6 g, 38 mmol). The mixture was stirred at room temperature for 30 min, and then diethyl malonate (5.0 g, 30 mmol) was added. The flask was fitted with a condenser and the suspension was refluxed for 32 h. The solvent was removed under reduced pressure, and the residue was suspended in water (100 mL) and acidified to pH = 6 with 5 N hydrochloric acid. The resulting solid was filtered and washed with water (50 mL), ethanol/ethyl ether (4:1, 25 mL), and ethyl ether (2 × 25 mL). Compound **7** was obtained as a pale yellow solid (4.2 g, 78%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.80 (s, 1H), 7.40 (d, J = 3.4, 1H), 6.60–6.70 (m, 1H), 5.00 (s, 1H).

4,6-Dichloro-2-(2-furyl)pyrimidine (8). To a suspension of compound **7** (3.0 g, 17 mmol) was added *N*,*N*-diisopropylethylamine (3.9 g, 30 mmol) and phosphorus oxychloride (17 mL). The flask was fitted with a condenser and the mixture was refluxed for 3 h. The solvent was removed under reduced pressure and methylene chloride (50 mL) and ice were slowly added. The organic layer was washed with water (2 × 25 mL), saturated solution of sodium bicarbonate (2 × 25 mL), and brine and dried (Na_2SO_4). The solvent was removed under reduced pressure to give compound **8** as a gray solid (3.2 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68 (s, 1H), 7.46 (d, J = 3.4, 1H), 7.22 (s, 1H), 6.63–6.61 (m, 1H).

6-Chloro-2-(2-furyl)pyrimidin-4-amine (9). A suspension of compound **8** (2.0 g, 9.3 mmol) in methanol (14 mL) and 30% ammonium hydroxide (27 mL) was heated in a pressure reactor for 20 h. The solvent was partially removed under reduced pressure. The resulting solid was filtered, washed with water (25 mL) and ethyl ether (25 mL), and dried. Compound **9** was obtained as an off-white solid (1.5 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 (s, 1H), 7.28 (d, J = 3.7, 1H), 6.54 (m, 1H), 6.31 (s, 1H), 5.21 (bs, 2H).

2-Chloro-*N*-(2-furan-2-yl-6-pyrazol-1-yl-pyrimidin-4-yl)-acetamide (19c). To 5 mL of dichloromethane were added compound **17** (0.3 g 1.3 mmol), chloroacetyl chloride (0.2 g, 0.2 mmol), and pyridine (0.2 g, 0.2 mmol). The reaction mixture was stirred at room temperature for 2 h. The reaction was quenched with 5 mL of saturated sodium bicarbonate and extracted with dichloromethane (3 × 15 mL). The organic layers were combined and dried over sodium sulfate, then concentrated to give compound **19c** as a yellow solid (0.4 g, 100% crude yield), which was used crude in subsequent reactions.

3-Oxo-3-thiazol-2-yl-propionic Acid Ethyl Ester (11). To a solution of 60% sodium hydride (3.8 g, 95 mmol) in diethyl carbonate (90 mL) was slowly added 2-acetylthiazole **10** (5.0 g, 40 mmol). The resulting solution was stirred at room temperature for 1 h and at 90 °C for 2 h. The reaction mixture was poured into ice/water and acetic acid (5 mL) was added. The mixture was extracted with ethyl acetate (2 × 75 mL). The organic layer was washed with water (2 × 50 mL) and brine (50 mL) and dried (Na_2SO_4), and the solvent was removed under reduced pressure. Distillation under reduced pressure gave the title compound **11** as a pale orange oil (4.4 g, 56%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.99 (d, J = 5.3, 1H), 7.71 (d, J = 5.3, 1H), 4.15 (m, 4H), 1.23 (t, 3H).

2-(2-Furyl)-6-(1,3-thiazol-2-yl)pyrimidin-4-ol (12). To a solution of potassium *tert*-butoxide (0.6 g, 6.0 mmol) in *tert*-butanol (2 mL) were added compound **5** (0.9 g, 4.3 mmol) and compound **11** (0.8 g, 4.7 mmol). The mixture was heated at 135 °C for 3 h. The crude reaction was poured into water (20 mL) and acidified with 10% hydrochloric acid (25 mL). The resulting solid was filtered, washed with water (2 × 25 mL), and dried. The title compound **12** was obtained as an off-white solid (0.6 g, 50%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.05–8.15 (m, 3H), 7.64 (d, J = 2.8, 1H), 6.90 (s, 1H), 6.78 (d, J = 2.8, 1H).

4-Chloro-2-(2-furyl)-6-(1,3-thiazol-2-yl)pyrimidine (13). A suspension of compound **12** (0.6 g) in phosphorus oxychloride (20 mL) was refluxed for 24 h. The solvent was removed under pressure and ice and water were slowly added. The resulting solid was filtered, washed with 2 N sodium hydroxide, and dried. Purification by column chromatography with silica gel and methylene chloride as eluent gave compound **13** as an off-white solid (0.4 g, 66%).

2-(2-Furyl)-6-(1,3-thiazol-2-yl)pyrimidin-4-amine (14). A suspension of compound **13** (0.3 g) in ethanol (22 mL) and 30% ammonium hydroxide (22 mL) was heated at 120 °C in a pressure reactor for 2 h 30 min. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate (50 mL). The resulting solution was washed with water (2 × 25 mL) and brine (25 mL) and dried (Na_2SO_4), and the solvent was removed under reduced pressure. Purification by trituration with ethyl ether gave compound **14** as an off-white solid (0.1 g, 53%). ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.05 (d, J = 3.0, 1H), 7.95 (d, J = 3.0, 1H), 7.85 (d, J = 1.5, 1H), 7.28 (bs, 2H), 7.18 (d, J = 3.4, 1H), 7.05 (s, 1H), 6.64 (dd, J = 3.0, 1.0, 1H).

2-Chloro-*N*-(2-furan-2-yl-6-thiazol-2-yl-pyrimidin-4-yl)-acetamide (19a). Compound **19a** was prepared according to the procedures for **19c** above, except that intermediate **14** was used instead of intermediate **17** to give the title compound **19c**, a yellow solid (quantitative yield) that was used crude in subsequent reactions. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.53 (s, 1H), 8.11 (d, J = 3.0, 1H), 8.04 (d, J = 3.0, 1H), 7.25 (d, J = 3.6, 1H), 6.36 (d, J = 3.3, 1H), 4.44 (s, 2H), 2.39 (s, 3H). LCMS-1: t_R = 2.34 (100%). MS: m/z 335 $[M + H]^+$, expected 335 $[M + H]^+$.

6-(3,5-Dimethyl-pyrazol-1-yl)-2-furan-2-yl-pyrimidin-4-ylamine (16). To a solution of intermediate **9** (1.0 g, 5.0 mmol) in absolute EtOH (4.5 mL) was added anhydrous hydrazine (0.3 mL, 10 mmol). The mixture was heated at 90 °C for 22 h in a sealed flask. The reaction was then cooled to room temperature and placed in an ice bath at 0 °C, while 2,4-pentanedione (1.0 mL, 10 mmol) was added slowly. The reaction mixture was heated at 90 °C for 2 h. Upon consumption of the hydrazine intermediate, the reaction was evaporated completely. The crude mixture was dissolved in CH_2Cl_2 (50 mL) and water (25 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (4 × 25 mL). The combined organic layers were washed with brine (25 mL), dried over magnesium sulfate, filtered, and concentrated. The crude product was purified by flash chromatography using 1:1 EtOAc/hexanes to give the title compound **16** as a white solid (0.9 g, 72%). LCMS-1: t_R = 2.44 (100%). MS: m/z 256 $[M + H]^+$, expected 256 $[M + H]^+$.

2-Chloro-*N*-[6-(3,5-dimethyl-pyrazol-1-yl)-2-furan-2-yl-pyrimidin-4-yl]-acetamide (19b). Compound **19b** was prepared according to the procedures for **19c** above, except that intermediate **16** was used instead of intermediate **17** to give the title compound **19b** as a pale yellow solid (64% yield), which was used crude in subsequent reactions. LCMS-1: t_R = 2.89 (100%). MS: m/z 332 $[M + H]^+$, expected 332 $[M + H]^+$.

2-(2-Furyl)-6-(1*H*-pyrazol-1-yl)pyrimidin-4-amine (17). To a solution of compound **9** (1.0 g, 5.1 mmol) in anhydrous DMF (20 mL) was added pyrazole (0.7 g, 10 mmol) and cesium carbonate (3.3 g, 10 mmol). The mixture was heated at 85 °C for 21 h. The solution was poured into water (50 mL) and extracted with ethyl acetate (2 × 25 mL). The organic layer was washed with water (2 × 25 mL) and brine (25 mL) and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The resulting solid was purified by column chromatography with silica gel, eluting with methylene chloride/methanol (3%), to give compound **17** as an off-white solid (0.6 g, 55%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.63 (d, J = 3.0, 1H), 7.75 (d, J = 1.2, 1H), 7.61 (s, 1H), 7.31 (d, J = 3.6, 1H), 6.90 (s, 1H), 6.57–6.55 (m, 1H), 6.48–6.46 (m, 1H), 5.12 (bs, 2H). LCMS-2: t_R = 4.2 (98%). LCMS-3: t_R = 6.4 (98%). MS: m/z 228 $[M + H]^+$, expected 228 $[M + H]^+$.

***N*-(2-Furan-2-yl-6-pyrazol-1-yl-pyrimidin-4-yl)-acetamide (18).** To a solution of compound **17** (0.3 g, 1.3 mmol) in methylene chloride (7 mL) was added pyridine (0.2 g, 2.6 mmol) and acetyl chloride (0.2 g, 2.6 mmol). The mixture was stirred at room temperature for 5 h, and more pyridine (52 mg, 0.7 mmol) and acetyl chloride (52 mg, 0.7 mmol) was added. The reaction was allowed to stand for 1.5 h at room temperature. The solution was diluted with methylene chloride (20 mL), washed with 10% sodium hydroxide (2 × 10 mL) and brine (10 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure. Purification by column chromatography with silica gel, eluting with ethyl acetate/*n*-hexane (1:3), gave compound **18** as an off-white solid (0.33 g, 92%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.64 (d, J = 2.1, 1H), 8.53 (s, 1H), 8.21 (bs, 1H), 7.81 (bs, 1H), 7.62 (bs, 1H), 7.35 (d, J = 2.1, 1H), 6.61–6.58 (m, 1H), 6.51–6.49 (m, 1H), 2.25 (s, 3H). LCMS-2: t_R = 5.6 (99%). LCMS-4: t_R = 4.1 (97%). MS: m/z 270 $[M + H]^+$, expected 270 $[M + H]^+$.

General Method for Compounds 3, 20, and 21. To 10 mL of dichloromethane were added compound **19c** (1.6 g, 5.3 mmol), DIPEA (1.8 mL, 10 mmol), and either dimethylamine, morpholine, or 4-methyl piperazine (2.0 equiv). The reaction mixture was stirred at room temperature overnight. The reaction was quenched with 5 mL of water and extracted with dichloromethane (3 × 15 mL). The organic layers were combined and dried over sodium sulfate and then concentrated. The residue was purified by column chromatography on silica gel (DCM with methanol gradient from 0 to 5%) to give the following:

***N*-(2-Furan-2-yl-6-pyrazol-1-yl-pyrimidin-4-yl)-2-(4-methyl-piperazin-1-yl)-acetamide (3).** Compound **3** as a pale yellow solid (70% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.56 (d, J = 1.2, 1H), 7.39 (s, 1H), 7.24 (s, 1H), 7.02 (s, 1H), 6.45 (d, J = 2.3, 1H), 6.21 (s, 1H), 6.09 (s, 1H), 4.01 (s, 2H), 3.82–3.74 (m,

4H), 3.60–3.48 (m, 4H), 3.02 (s, 3H). MS: *m/z* 368 $[M + H]^+$, expected 368 $[M + H]^+$. Anal. ($C_{18}H_{21}N_7O_2 \cdot 2HCl \cdot 0.9H_2O$) C, H, N.

2-Dimethylamino-*N*-(2-furan-2-yl-6-pyrazol-1-yl-pyrimidin-4-yl)-acetamide (20). Compound **20** as a pale yellow solid (50% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.87 (s, 1H), 8.65 (d, $J = 3$, 1H), 8.62 (s, 1H), 7.81 (d, $J = 1$, 1H), 7.65 (d, $J = 1$, 1H), 7.37 (d, $J = 3.6$, 1H), 6.59 (dd, $J = 3.6$, 1.8, 1H), 6.50 (dd, $J = 3.0$, 1.5, 1H), 3.16 (s, 2H), 2.39 (s, 6H). LCMS-2: $t_R = 3.8$ (98%). LCMS-4: $t_R = 5.2$ (100%). MS: *m/z* 313 $[M + H]^+$, expected 313 $[M + H]^+$.

***N*-(2-Furan-2-yl-6-pyrazol-1-yl-pyrimidin-4-yl)-2-morpholin-4-yl-acetamide (21).** Compound **21** as a white foam (0.5 g, 27% yield). ^{1}H NMR (300 MHz, CD_3OD): δ 8.65 (s, 1H), 8.32 (s, 1H), 7.98 (s, 1H), 7.69 (s, 1H), 7.31 (s, 1H), 6.59 (s, 1H), 6.54 (s, 1H), 3.80–4.15 (m, 4H), 3.30–3.75 (m, 4H), 3.30 (s, 2H). LCMS-5: $t_R = 13.0$ (99%). LCMS-6: $t_R = 22.7$, MS: *m/z* 355 $[M + H]^+$, expected 355 $[M + H]^+$. Anal. ($C_{17}H_{18}N_6O_3 \cdot HCl \cdot {}^1/_4H_2O$) C, H, N.

***N*-(2-Furan-2-yl-6-thiazol-2-yl-pyrimidin-4-yl)-2-(4-methyl-piperazin-1-yl)-acetamide (22).** Compound **19a** was reacted with methyl piperazine, according to the procedure described for compounds **3**, **20**, and **21** above, to give compound **22** as a slightly yellow solid (59% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.70 (s, 1H), 8.79 (s, 1H), 8.02 (d, $J = 3$, 1H), 7.67 (d, $J = 1.8$, 1H), 7.55 (d, $J = 3$, 1H), 7.43 (d, $J = 3.9$, 1H), 6.61 (dd, $J = 3.6$, 1.8, 1H), 3.22 (s, 2H), 2.66 (m, 4H), 2.56 (m, 4H), 2.34 (s, 3H). LCMS-5: $t_R = 22.6$ (100%). MS: *m/z* 385 $[M + H]^+$, expected 385 $[M + H]^+$. Anal. ($C_{18}H_{20}N_6O_2S \cdot 2HCl$) C, H, N.

***N*-[6-(3,5-Dimethyl-pyrazol-1-yl)-2-furan-2-yl-pyrimidin-4-yl]-2-(4-methyl-piperazin-1-yl)-acetamide (23).** Intermediate **19b** was reacted with methyl piperazine according to the procedure described for compounds **3**, **20** and **21**, to give compound **23** as an off-white flaky solid (76% yield). ^{1}H NMR (free base; 300 MHz, CD_3OD): δ 7.76 (dd, $J = 1.5$, 0.6, 1H), 7.31 (dd, $J = 3.6$, 0.9, 1H), 6.65 (dd, $J = 3.6$, 1.8, 1H), 6.13 (s, 1H), 3.29 (s, 2H), 2.77 (s, 3H), 2.54–2.75 (m, 8H), 2.35 (s, 3H), 2.27 (s, 3H). LCMS-5: $t_R = 17.0$ (100%).

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Supporting Information Available: Copies of NMR and LCMS data are available on final compounds, along with detailed descriptions of HPLC conditions for compound analysis. This material is available free of charge via the Internet at http://www.pubs.acs.org.

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