2-Amino-*N*-pyrimidin-4-ylacetamides as A_{2A} Receptor Antagonists: 1. Structure−Activity Relationships and Optimization of Heterocyclic Substituents

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Previously we have described a novel series of potent and selective A_{2A} receptor antagonists (e.g., **1**) with excellent aqueous solubility.[1] While these compounds are efficacious A_{2A} antagonists in vivo, the presence of an unsubstituted furyl moiety was a cause of some concern. In order to avoid the potential metabolic liabilities that could arise from an unsubstituted furyl moiety, an optimization effort was undertaken with the aim of replacing the unsubstituted furan with a more metabolically stable group while maintaining potency and selectivity. Herein, we describe the synthesis and SAR of a range of novel heterocyclic systems and the successful identification of a replacement for the unsubstituted furan moiety with a methylfuran or thiazole moiety while maintaining potency and selectivity.

Introduction

The adenosine receptors are G-protein-coupled receptors of which four subtypes have so far been identified: A_1, A_{2A}, A_{2B}, and A_3. Through these receptors, adenosine regulates a wide range of physiological functions such as motor function, sleep, anxiety, pain, and psychomotor activity.[2] While all adenosine receptor subtypes are present in the brain, A_{2A} receptor expression is the highest in a few brain regions, including the striatum and nucleus accumbens.[3] A_{2A} receptors have been shown to modulate the release of GABA in the striatum, which appears to regulate the activity of medium spiny neurons. By reduction of GABA output, A_{2A} antagonism helps restore normal function in the basal ganglia following dopamine depletion. Thus, A_{2A} receptor antagonists may be a useful treatment for neurodegenerative movement disorders such as Parkinson's and Huntington's diseases,[4] dyskinesias such as those caused by prolonged use of neuroleptic and dopaminergic drugs,[5] and dystonias such as restless leg syndrome.[6]

Interestingly, many potent A_{2A} receptor antagonists including compounds **1**, **26**, and **27**[1] (Table 1), and many of the non-xanthine A_{2A} receptor antagonists described in the literature, contain an unsubstituted furan moiety including the clinical candidate **2** (SCH420814, Figure 1).[7] While compound **1** is a potent and selective (hA_{2A}) receptor antagonist (human A_{2A} K_i = 2.7 nM, 100-fold selective for human A_{2A} over human A_1) with in vivo efficacy,[1] there was some concern that the presence of an unsubstituted furyl moiety could be a metabolic liability. It is well documented that unsubstituted furans can be metabolized to form reactive intermediates that have the potential to react and form protein adducts that can result in liver toxicity, among other adverse reactions.[8,9] To address this concern, other heterocycles and substituted furyl moieties were explored as alternatives to a simple furan, with the aim of finding a moiety with an in vitro profile similar to that of compound **1** but with less likelihood of producing reactive metabolites.[9]

Chemistry

The methods used to synthesize pyrimidine analogues **1** and **26**−**40** are described below. In most cases the appropriate commercially available nitrile **3** was converted to the carboxyamidine **4** via treatment with sodium methoxide in methanol at room temperature, followed by reaction with ammonium chloride. These carboxyamidines were then converted to the 6-chloropyrimidin-4-amines **8a**−**d** via one of two routes. The first route was via the 6-hydroxypyrimidin-4-amine intermediates **5b**−**d**, through reaction of the carboxyamidines **4b**−**d** with ethyl cyanoacetate in the presence of sodium methoxide. The resulting intermediates **5b**−**d** were then treated with phosphorus oxychloride to give the key intermediates **8b**−**d**. Alternatively, the carboxyamidine **4a** was reacted with diethyl malonate in methanol, in the presence of sodium ethoxide, to yield the corresponding pyrimidine-4,6-diol **6a**. Reaction with phosphorus oxychloride in the presence of *N,N*-diisopropylethylamine yielded the 4,6-dichloropyrimidine intermediate **7a**, which was then treated with ammonium hydroxide in methanol under reflux to yield intermediate **8a** as shown in Scheme 1. These intermediates were then further derivatized to install R^2 as described in Schemes 2 and 3.

The chlorine moiety of **8a**−**d** can be displaced with pyrazole (Scheme 2) to give analogues **9a**−**d**. This reaction was slower, and yields were reduced when substituted pyrazoles were used.

To overcome this, an alternative approach was used for the synthesis of the methyl-substituted pyrazole analogues, which consisted of first displacing the chloro moiety of **8a**−**d** with hydrazine to give intermediates **10a**−**d**, followed by cyclization with pentane-2,4-dione to give the dimethylpyrazole analogues **11a**−**d**. Intermediate **10d** was also reacted with 4-dimethylami-

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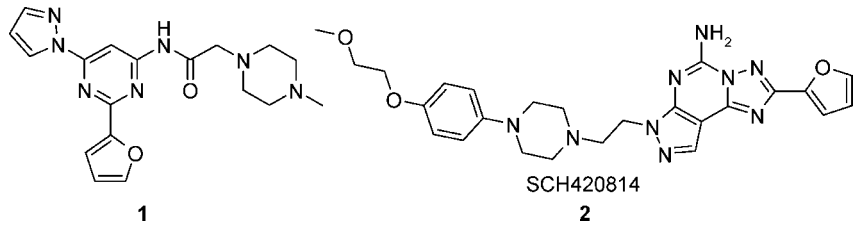
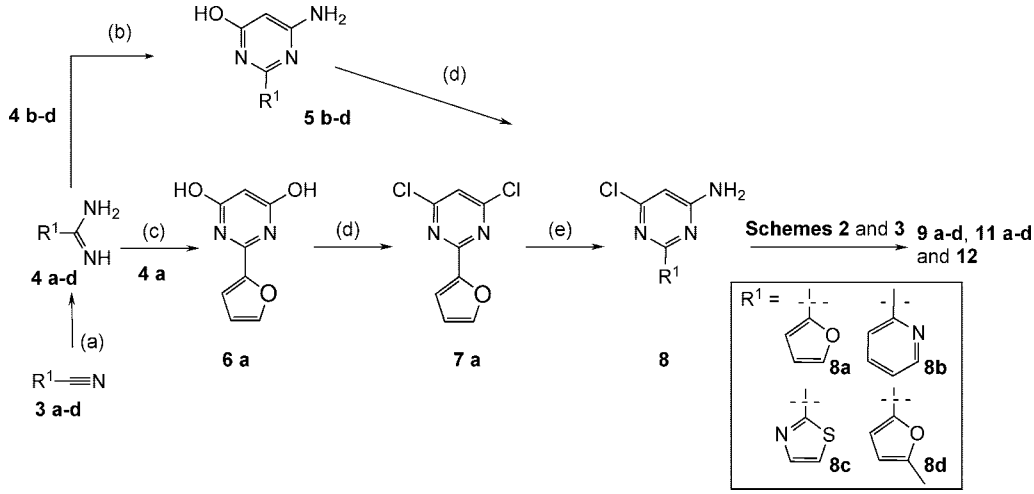


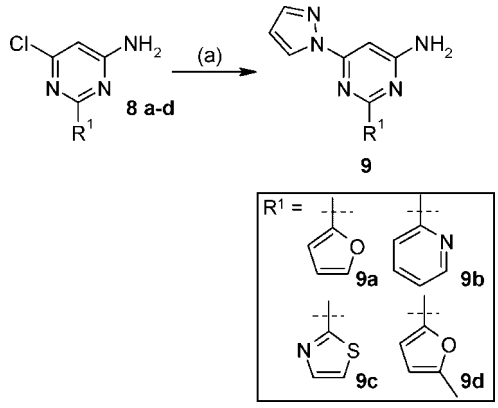
Figure 1. Representative analogue **1** of lead series and a clinical compound **2**.

Scheme 1[a]



[a] Reagents and conditions: (a) NaOMe (1.1 equiv), NH_4Cl, MeOH, room temp up to 68 h, 87–96%; (b) ethyl cyanoacetate, NaOMe, EtOH, 70 °C, 6–12 h, 60–80%; (c) NaOEt, diethylmalonate, room temp to reflux, 12–60 h, 78%; (d) $POCl_3$, 90 °C, 3–12 h, 33–87%; (e) NH_4OH, MeOH, 80 °C, 20 h, 76%.

Scheme 2[a]



[a] Reagents and conditions: (a) pyrazole; $CsCO_3$, DMF, 85 °C, 21 h, 55%.

nobut-3-en-2-one at room temperature to give the 5-methylpyrazole analogue **12**.

For the synthesis of carbon-linked heterocycles at R^2, the carboxyamidines **4a**, and **4d** were reacted with 3-oxo-3-thiazol-2-ylpropionic acid ethyl ester **13a**, and the commercially available thiophene carboxyamidine **4e** was reacted with 3-oxo-3-pyridin-2-ylpropionic acid ethyl ester **13b** to give intermediates **14a**−**c**. Ester **13b** was commercially available; however, compound **13a** was synthesized through reaction of the methyl ketone **17** with diethyl carbonate, using sodium hydride as the base. The resulting pyrimidin-4-ol intermediates of formula **14a**−**c** could then be converted to the amine in two steps via treatment with phosphorus oxychloride to give **15a**−**c**, followed by reaction with ammonium hydroxide, to yield the corresponding 6-heteroarylpyrimidin-4-amines **16a**−**c** as outlined in Scheme 4. While this second route was less versatile, it was more convenient for the analogues when R^2 is a carbon-linked thiazole or pyridine.

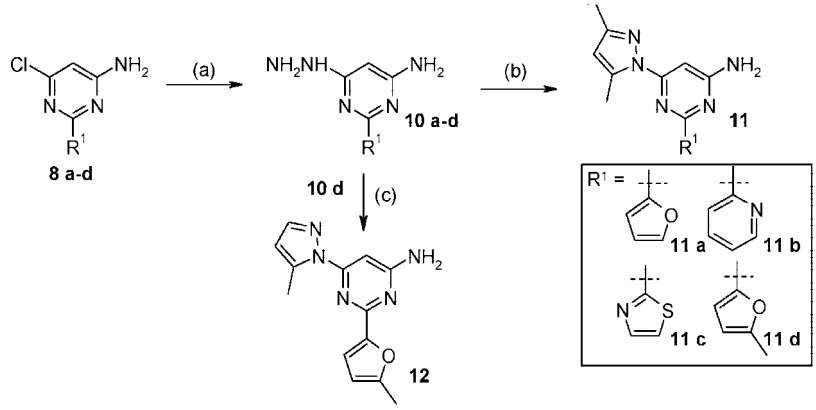
Additional analogues were synthesized from the commercially available 4-amino-2,6-dichloropyrimidine **18** (Scheme 5). Intermediate **18** was reacted with pyrazole in dimethylformamide in the presence of cesium carbonate to give the 2,6-disubstituted analogue **19**. The *N*-[6-chloro-2-(3,5-dimethylpyrazol-1-yl)pyrimidin-4-yl]acetamide **20** was made from compound **18** by reaction with hydrazine followed by cyclization with pentan-2,4-dione and subsequent acetylation using acetic anhydride. Suzuki coupling of **20** with phenylvinylboronic acid yielded the olefin intermediate **21**, which upon oxidative cleavage with ozone/dimethyl sulfide afforded aldehyde **22**,[10] which was used without purification and cyclized with TosMIC or methyl TosMIC in MeOH in the presence of potassium carbonate, to give intermediate **23** or **24** respectively, with in situ removal of the *N*-acetyl group.[11] Negishi coupling[12] of **20** with thiazole in THF in the presence of BuLi, $ZnCl_2$, and tetrakis(triphenylphosphine)palladium(0) afforded **25** with concurrent cleavage of the *N*-acetyl group.

The aminopyrimidine intermediates **9a**−**d**, **11a**−**d**, **12**, **16a**−**c**, **19**, and **23**−**25** described above were then converted to the final compounds via the route outlined in Scheme 6. Each aminopyrimidine was reacted with chloroacetyl chloride to give the corresponding intermediate, which was then used crude and reacted with methylpiperazine to give compounds **1** and **26**−**40** (Table 1).

Results and Discussion

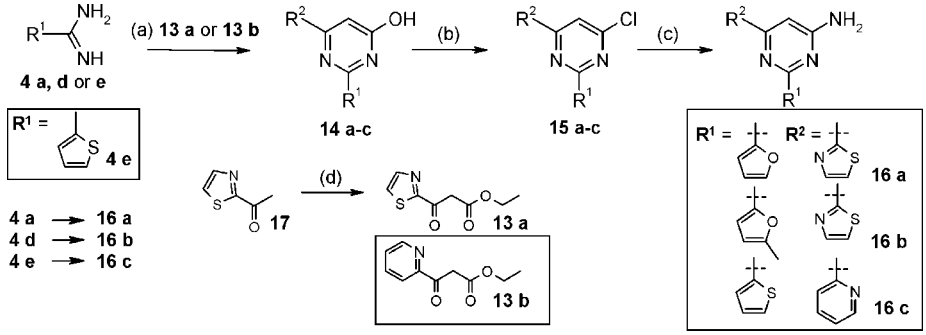
After the initial discovery of compound **1**, further investigation into the in vitro metabolism of this compound identified what were believed to be small amounts of the downstream

Scheme 3[a]



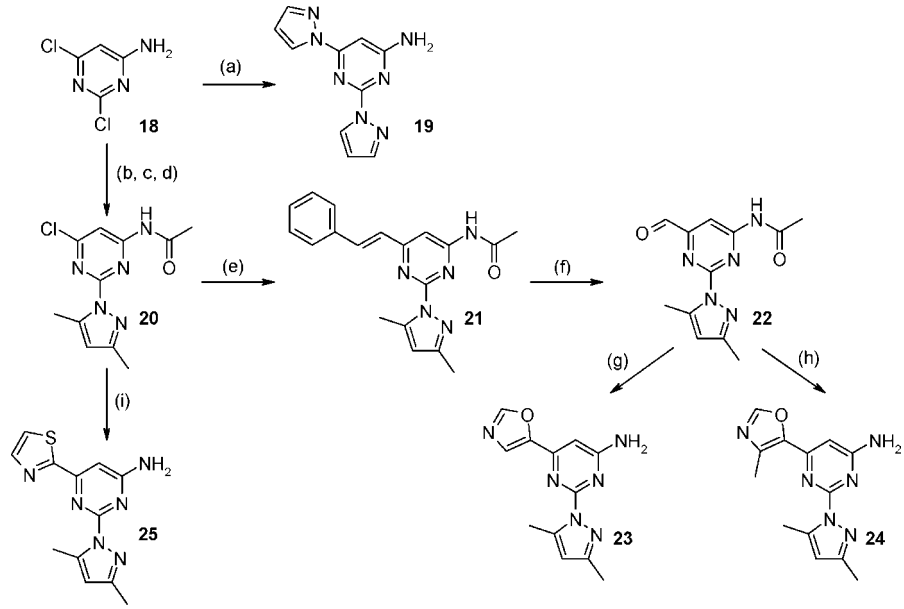
[a] Reagents and conditions: (a) N_2H_4, EtOH, 90 °C, 2 h, 80%; (b) pentane-2,4-dione, 0–90 °C, 2 h, 72%; (c) 4-dimethylaminobut-3-en-2-one, HCl, dioxane, room temp, 30%.

Scheme 4[a]



[a] Reagents and conditions: (a) KO*t*-Bu, (1.5 equiv), *t*-BuOH, 135 °C, 12 h, 50%; (b) $POCl_3$, 90 °C, 24 h, 66%; (c) NH_4OH, EtOH, 120 °C, 3 h, 53%; (d) diethyl carbonate (5 equiv), NaH, 90 °C, 2 h, 56% after distillation.

Scheme 5[a]

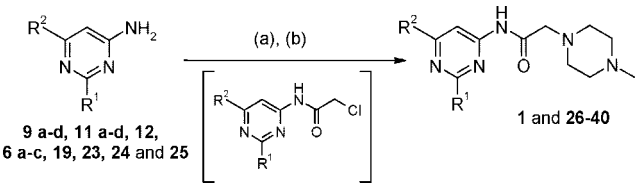


[a] Reagents and conditions: (a) pyrazole, Cs_2CO_3, DMF, 120 °C, 15 h, 60%; (b) N_2H_4, NMP, 60 °C, 2 h; (c) pentane-2,4-dione, 0–60 °C, 2 h, 66% (two steps); (d) Ac_2O, AcOH, 90 °C, 18 h, 83%; (e) *trans*-2-phenylvinylboronic acid, tetrakis(triphenylphosphine)palladium(0), Na_2CO_3, H_2O/dioxane, 90 °C, 16 h, 85%; (f) O_3, Me_2S, CH_2Cl_2/MeOH; (g) TosMIC, K_2CO_3, MeOH, 80 °C, 16 h, 50% (over two steps); (h) Me-TosMIC, K_2CO_3, MeOH, 80 °C, 16 h, 50% (over two steps); (i) thiazole, BuLi, $ZnCl_2$, THF, −78 °C, then tetrakis(triphenylphosphine)palladium(0), 80 °C, 2 h, 70%.

products that would result from the metabolism of the furan moiety (Figure 2). While this was not observed in human hepatocytes and does not always lead to toxicity, the literature is rich in reports of drugs containing furan moieties that cause toxicities that are attributed to reactive intermediates derived from metabolism of the furan moiety.[13]

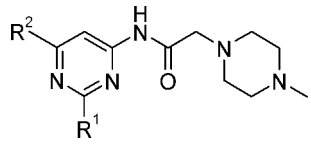
To address this concern, alternative heterocycles and substituted furyl moieties were explored with the goal of finding an

Scheme 6[a]



[a] Reagents and conditions: (a) chloroacetyl chloride, pyridine, DCM, room temp, 2–12 h; (b) methylpiperazine, DIPEA, room temp, 6–12 h, 14–82%.

Table 1



Compound	R^1	R^2	K_i (nM) hA_{2A}[a] ± SEM	hA_1[a] ± SEM	hA_1/hA_{2A}
1			2.7 ± 0.2	270 ± 60	100
26			0.9 ± 0.1	220 ± 30	244
27			2.0 ± 0.2	1700 ± 200	850
28			135 ± 35	850 ± 120	6
29			14 ± 3	390 ± 10	28
30			12 ± 2	850 ± 230	71
31			51 ± 2	720 ± 280	14
32			24 ± 5	389 ± 9	16
33			131 ± 9	4200 ± 1100	32
34			27 ± 4	1700 ± 590	63
35			1000 ± 40	>10000	-
36			190 ± 10	>10000	-
37			17 ± 3	850 ± 100	50
38			53.4 ± 0.3	930 ± 120	17
39			25.5 ± 0.5	4400 ± 330	173
40			9 ± 1	2000 ± 300	222

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

analogue with a similar in vitro and in vivo profile to the lead compound **1**, but with reduced risk of producing chemically reactive metabolites. As can be seen from Table 1, this proved to be somewhat challenging. Simply adding a methyl group to the furan of compound **1** to give compound **28** had a large negative effect on potency, reducing binding to the hA_{2A} receptor from 2.7 to 135 nM (45-fold). Interestingly, the addition of methyl groups to the pyrazole at R^2 of compound **28**, as in examples **29** and **30**, counteracts this effect and brings the potency back from 135 nM to 14 and 12 nM, respectively. In addition, the selectivity for the hA_{2A} receptor over the human A_1 (hA_1) receptor also improves, from 6-fold to 28-fold for compound **29** and to 71-fold for compound **30**. Selectivity over the hA_1 receptor was desired because the A_1 receptor is present in cardiac muscle and, as a result, may represent a cardiotoxicity risk.[14] Replacing the pyrazole at R^2 of compound **29** with thiazole (compound **31**) increased potency slightly, but selectivity remained poor. The combination of thiophene at R^1 with 2-pyridyl at R^2 gave a quite potent compound (**32**) (K_i = 24 nM), but again, selectivity over the hA_1 receptor was modest. The symmetrical bis-pyrazole analogue **33** was less active than compound **1** (hA_{2A} K_i = 131 vs 2.7 nM), indicating that the nitrogen of a pyrazole was less tolerated than the oxygen of the furan moiety. In some cases the heterocycles appear to be interchangeable as with compounds **34** and **40**, which have similar in vitro profiles (K_i values of 27 and 9 nM, respectively). For these compounds it is possible that the bulky dimethylpyrazole moiety dictates the binding mode, and forces the pyrimidine core to rotate to accommodate these substituents. In other words, the heterocycles and side chain are still able to bind to the same regions of the A_{2A} receptor.

The oxazole analogues **35** and **36** were poor hA_{2A} receptor antagonists, indicating that the presence of a nitrogen atom at the 3-position was not tolerated. A pyrazole at R^2 in combination with a 2-thiazole (compound **37**) or 2-pyridyl (compound **38**) at R^1 gave moderately potent analogues with activities of 17 and 53 nM, respectively. Consistent with previous observations, both activity and selectivity were significantly improved when these heterocycles (2-pyridyl and 2-thiazole) were combined with dimethylpyrazole at R^2. The analogue with 2-pyridyl at R^1 in combination with dimethylpyrazole (compound **39**) had a K_i of 26 nM against the hA_{2A} receptor and was 173-fold selective for hA_{2A} over the hA_1 receptor. The analogue with 2-thiazole at R^1 in combination with dimethylpyrazole at R^2 (compound **40**) had a K_i of 9 nM against the hA_{2A} receptor and was 222-fold selective over the hA_1 receptor. In general the compounds described were found to be functional antagonists in a cAMP assay[15] (compound **26**, IC_{50} of 103 nM); however, the binding assay was used routinely for lead optimization because of the variability and low throughput of the functional assay.

Overall, compounds **30** and **40** appeared to represent the most promising leads. While compound **30** still contained a furyl moiety, the methyl group would be expected to significantly attenuate the minor metabolism of the furan that was observed with compound **1**. Additional metabolite identification to support this will be discussed in the following paper. Compound **40** showed some weak inhibition of CYP3A4 (IC_{50} of 13 μM); however, compound **30** appeared to have no significant CYP inhibition potential in the same dose range. Therefore compound **30** was chosen for further lead optimization for in vivo efficacy, as will be discussed in the accompanying paper.

Conclusion

We have developed a novel series of potent and selective human adenosine A_{2A} receptor antagonists, which have excellent

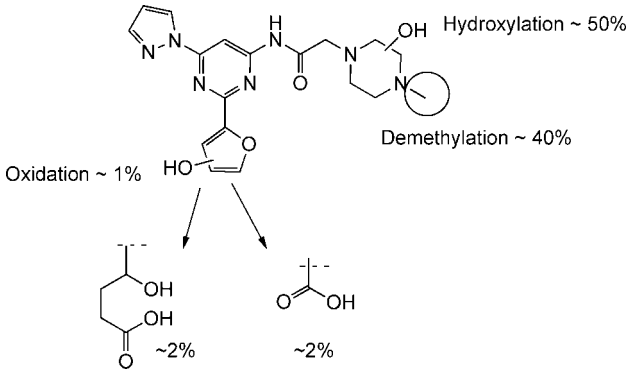


Figure 2. Metabolites of compound **1** after incubation with human liver microsomes. Tentative assignment of peaks observed by LC−MS.

physical properties for drug development. These compounds were optimized to remove an unsubstituted furan moiety with the objective of reducing the risk of forming chemically reactive metabolites. From this SAR study a number of potential lead compounds were identified. Compounds **30** and **40** were the most potent and had promising selectivity. While these compounds were not quite as potent as compound **1**, the potency and selectivity were in a suitable range for these compounds to be considered for further characterization and lead optimization.

Experimental Section

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. Analytical HPLC−MS method 1 was as follows; platform, Agilent 1100 series equipped with an autosampler, a UV detector (220 and 254 nm), a MS detector (APCI); HPLC column, YMC ODS AQ, S-5, 5 μm, 2.0 mm × 50 mm cartridge; HPLC gradient, 1.0 mL/min from 10% acetonitrile in water to 90% acetonitrile in water in 2.5 min, maintaining 90% for 1 min. Both acetonitrile and water have 0.025% TFA. Analytical HPLC−MS method 2 was as follows: platform, Agilent 1100 series equipped with an autosampler, an UV detector (220 and 254 nm), a MS detector (APCI); HPLC column, Phenomenex Synergi-Max RP, 2.0 mm × 50 mm column; HPLC gradient, 1.0 mL/min from 5% acetonitrile in water to 95% acetonitrile in water in 13.5 min, maintaining 95% for 2 min. Both acetonitrile and water have 0.025% TFA. Analytical HPLC−MS method 3 was as follows: platform, Agilent 1100 series equipped with an autosampler, an UV detector (220 and 254 nm), a MS detector (electrospray); HPLC column, XTerra MS, C_{18}, 5 μm, 3.0 mm × 250 mm column; HPLC gradient, 1.0 mL/min from 10% acetonitrile in water to 90% acetonitrile in water in 46 min, jump to 99% acetonitrile and maintain 99% acetonitrile for 8.0 min. Both acetonitrile and water have 0.025% TFA. Analytical HPLC−MS method 4: platform, Dionex equipped with an autosampler, an UV detector (220 and 254 nm), a MS detector (APCI); HPLC column, Phenomenex C18 4.6 mm × 150 mm; HPLC gradient, 2.5 mL/min from 5% acetonitrile in water to 90% acetonitrile in water in 9.86 min, 12.30 min run. Both acetonitrile and water have 0.04% NH_4OH. Analytical HPLC−MS method 5 was as follows: platform Agilent 1100 series equipped with an autosampler, an UV detector (220 and 254 nm), a MS detector (APCI); HPLC column, Phenomenex Fusion RP, 2.0 mm × 50 mm column; HPLC gradient, 1.0 mL/min. Solvent C is 6 mM ammonium formate in water, and solvent D is 25% acetonitrile in methanol. The gradient runs from 5% D (95% C) to 95% D (5% C) in 6.43 min with a 1.02 min hold at 95% D followed by a return and hold at 5% D for 1.52 min. Analytical HPLC−MS method 6 was as follows: platform, Agilent equipped with an autosampler, an UV detector (220 and 254 nm), a MS detector (APCI); HPLC column, Waters XTerraMS C18, 3.0 mm × 250 mm; HPLC gradient, 1.0 mL/min from 10% acetonitrile in water to 90% acetonitrile in water in 46 min, maintaining 90% for 7.0 min. Both acetonitrile and water have 0.025% TFA. Analytical HPLC-MS method 7 was as follows: platform, Agilent equipped with an autosampler, an UV detector (220 and 254 nm), a MS detector (APCI); HPLC column, Waters XTerraMS C18, 3.0 mm × 250 mm; HPLC gradient, 1.0 mL/min from 10% acetonitrile in water to 90% acetonitrile in water in 46 min, maintaining 90% for 7.0 min. Acetonitrile has 0.1% NH_4OH, and water has 0.05% NH_4OH. Preparative HPLC−MS was as follows: platform, Dionex equipped with a Gilson 215 autosampler/fraction collector, UV detector, and a Dionex MSQ mass detector; HPLC column, Phenomenex Synergy Max-RP, 21.2 mm × 50 mm; HPLC gradient, 35 mL/min, 5% acetonitrile in water to 95% acetonitrile in water in 17.7 min. Both acetonitrile and water have 0.05% TFA.

2-Furancarboxamidine (HCl) (4a). To a solution of sodium methoxide (55.5 mmol) in methanol (50 mL) was added 2-furonitrile **3a** (5.0 g, 53.2 mmol). The mixture was stirred at room temperature for 3 h. To the resulting solution was slowly added ammonium chloride (3.14 g, 58.7 mmol), and the mixture was stirred at room temperature until the reaction was complete (up to 68 h). The resulting suspension was filtered and the solvent removed under reduced pressure. The solid obtained was washed with ethyl ether (3 × 25 mL) to give compound **5** as an off-white solid (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).

Pyridine-2-carboxamidine (HCl) (4b). The title compound **4b** was obtained as a pale-yellow solid starting from 2-pyridinenitrile using the procedure described for **4a** above. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.81 (m, 1H), 8.40 (d, J = 7.8, 1H), 8.02 (dt, J = 8.0, 1.8, 1H), 7.78 (m, 1H).

Thiazole-2-carboxamidine (HCl) (4c). See ref 16.

5-Methyl-2-furancarboxamidine (HCl) (4d). The title compound **4d** (3.71 g, 87%) was obtained as a pale-yellow solid starting from 5-methyl-2-furonitrile (2.85 g) using the procedure described for **4a** above. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.49 (bs, 4 H), 7.64 (d, J = 3.6, 1H), 6.36 (d, J = 3.6, 1H), 2.27 (s, 3H).

Thiophene-2-carboxamidine (HCl) (4e). 4e is commercially available.

6-Hydroxy-2-(pyridin-2-yl)pyrimidin-4-amine (5b). To ethanol (100 mL) was added pyridine-2-carboxamidine (HCl) **4b** (20.8 g, 130 mmol, 1.1 equiv) and NaOMe/MeOH solution (100 mL, 30% W, 5 equiv). The mixture was stirred at room temperature for 30 min. Ethyl cyanoacetate (13 mL, 120 mmol, 1 equiv) was added, and the reaction mixture was heated at 90 °C for 16 h. The reaction mixture was concentrated to dryness and resuspended in water (70 mL), and 4 M HCl was added to adjust the pH to 5. The mixture was stirred at 0 °C for 30 min, filtered, and washed with cold water (2 × 20 mL) and the solid obtained dried under vacuum at 90 °C for 12 h. Intermediate **5b** was obtained as a golden solid (18.1 g, 73%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.70 (m, 1H), 8.20 (dd, J = 8.1, 0.9, 1H), 8.02 (dt, J = 7.6, 1.8, 1H), 7.47 (ddd, J = 7.4, 4.9, 1.3, 1H), 6.64 (s, 1H). LCMS-1: t_R = 1.55 min. MS: m/z 189.0 $[M + H]^+$, expected 189 $[M + H]^+$.

6-Hydroxy-2-(1,3-thiazol-2-yl)pyrimidin-4-amine (5c). Compound **5c** was prepared using the same procedure as for **5b** above except that the amidine **4c** was used instead of **4b**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.07 (d, J = 3.0, 1H), 8.05 (d, J = 3.0, 1H), 5.46 (s, 1H).

6-Hydroxy-2-(5-methyl-2-furyl)pyrimidin-4-amine (5d). Compound **5d** was prepared using the same procedure as for **5b** above except that the amidine **4d** was used instead of **4b**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.35 (d, J = 3.6, 1H), 6.29 (d, J = 3.3, 1H), 4.95 (s, 1H), 2.32 (s, 3H).

2-(2-Furyl)pyrimidine-4,6-diol (6a). To a solution of sodium ethoxide (19.1 mmol) in ethanol (90 mL) was slowly added

compound **4a** (5.6 g, 38.2 mmol). The mixture was stirred at room temperature for 30 min, and then diethyl malonate (4.87 g, 30.4 mmol) was added. 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 M HCl. 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 **6a** 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 (7a). To a suspension of compound **6a** (3.0 g, 16.8 mmol) was added *N,N*-diisopropylethylamine (3.85 g, 29.8 mmol) and phosphorus oxychloride (17 mL). 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), a 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 **7a** as a gray solid (3.15 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 (8a). A suspension of compound **7a** (2.0 g, 9.3 mmol) in methanol (14 mL) and 30% ammonium hydroxide (27 mL) was heated at 80 °C 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 **8a** was obtained as an off-white solid (1.48 g, 76%). ^{1}H NMR (300 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).

6-Chloro-2-(pyridin-2-yl)pyrimidin-4-amine (8b). To **5b** (14.0 g, 74.5 mmol) in a sealed tube (350 mL) was added $POCl_3$ (75 mL). The mixture was heated at 90 °C for 16 h. The mixture was cooled to room temperature and poured onto ice (500 mL volume). Solid sodium carbonate was added to adjust the pH to 7. The mixture was extracted twice with 300 mL of ethyl acetate (300 mL). The organic layers were combined, washed with sodium bicarbonate and brine, dried (Na_2SO_4), and concentrated to a yellow solid (5.1 g, 33%). ^{1}H NMR (300 MHz, $CDCl_3$ + 10% CD_3OD): δ 8.70 (m, 1H), 8.41 (m, 1H), 7.90 (dt, J = 7.7, 1.6, 1H), 7.45 (ddd, J = 7.5, 4.8, 1.2, 1H), 6.44 (s, 1H). LCMS-1: t_R =1.71 min. MS: m/z 206.9 $[M + H]^+$, expected 207 $[M + H]^+$.

6-Chloro-2-(thiazol-2-yl)pyrimidin-4-amine (8c). Intermediate **8c** was prepared from **5c** according to the procedure described for **8b** above. ^{1}H NMR (300 MHz, $CDCl_3$ + 10% CD_3OD): δ 7.92 (d, J = 3.3, 1H), 7.52 (d, J = 3.0, 1H), 6.44 (s, 1H).

6-Chloro-2-(5-methylfuran-2-yl)pyrimidin-4-ylamine (8d). Intermediate **8d** was prepared from **5d** according to the procedure described for **8b** above. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.07 (d, J = 3.3, 1H), 6.40 (s, 1H), 6.21 (dd, J = 3.0, 1.2, 1H), 2.86 (bs, 2H), 2.36 (s, 3H). LCMS-1: t_R = 2.18 min. MS: m/z 210 $[M + H]^+$, expected 210 $[M + H]^+$.

2-(2-Furyl)-6-(1*H*-pyrazol-1-yl)pyrimidin-4-amine (9a). To a solution of compound **8a** (1.0 g, 5.1 mmol) in anhydrous DMF (20 mL) was added pyrazole (0.7 g, 10.2 mmol) and cesium carbonate (3.34 g, 10.2 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 **9a** as an off-white solid (0.64 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 = 4.24 min (98%). LCMS-3: t_R = 6.40 min. MS: m/z 228 $[M + H]^+$, expected 228 $[M + H]^+$.

6-Pyrazol-1-yl-2-pyridin-2-ylpyrimidin-4-ylamine (9b). Intermediate **9b** was prepared from **8b** according to the procedure described for **9a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.86–8.83 (m, 1H), 8.71 (dd, J = 2.4, 0.6, 1H), 8.57 (dt, J = 8.1, 1.2, 1H), 7.92–7.86 (m, 1H), 7.79–7.78 (m, 1H), 7.46–7.42 (m, 1H), 7.04 (s, 1H), 6.49 (dd, J = 2.7, 1.5, 1H), 6.36 (bs, 1H, NH_2). LCMS-1: t_R = 1.83 min. MS: m/z 239 $[M + H]^+$, expected 239 $[M + H]^+$.

***N*-(6-Pyrazol-1-yl-2-thiazol-2-ylpyrimidin-4-yl)amine (9c).** Intermediate **9c** was prepared from **8c** according to the procedure described for **9a**. ^{1}H NMR (300 MHz, $CDCl_3$ + 10% CD_3OD): δ 8.56 (d, J = 2.4, 1H), 7.86 (d, J = 3.0, 1H), 7.69 (m, 1H), 7.50 (d, J = 3.0, 1H), 6.54 (m, 1H), 6.98 (s, 1H), 6.41 (m, 1H). LCMS-5: t_R = 4.02 min. LCMS-2: t_R = 4.32 min. MS: m/z 245 $[M + H]^+$, expected 245 $[M + H]^+$.

2-(5-Methyl-2-furyl)-6-(1*H*-pyrazol-1-yl)pyrimidin-4-amine (9d). Intermediate **9d** was prepared from **8d** according to the procedure described for **9a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.63 (d, J = 2.7, 1H), 7.74 (d, J = 1.5, 1H), 7.23 (d, J = 3.6, 1H), 6.85 (s, 1H), 6.46 (dd, J = 2.7, 1.8, 1H), 6.18 (m, 1H), 5.36 (bs, 2H), 2.46 (s, 3H).

6-(3,5-Dimethylpyrazol-1-yl)-2-(furan-2-yl)pyrimidin-4-ylamine (11a). To a solution of intermediate **8a** (1.0 g, 5.1 mmol) in absolute EtOH (4.5 mL) was added anhydrous hydrazine (0.32 mL, 10.2 mmol). The mixture was heated at 90 °C for 22 h to give **10a**. The mixture was then cooled to 0 °C. 2,4-Pentanedione (1.05 mL, 10.2 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 to dryness. 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 **11a** as a white solid (0.94 g, 72%). LCMS-1: t_R = 2.44 min. MS: m/z 256 $[M + H]^+$, expected 256 $[M + H]^+$.

6-(3,5-Dimethylpyrazol-1-yl)-2-pyridin-2-ylpyrimidin-4-ylamine (11b). Intermediate **11b** was prepared from **8b** according to the procedure described for **11a**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.80 (d, J = 4.5, 1H), 8.41 (d, J = 7.8, 1H), 8.18 (ddd, J = 7.8, 7.5, 1.2, 1H), 7.71–7.75 (m, 1H), 7.04 (s, 1H), 6.22 (s, 1H), 2.77 (s, 3H), 2.22 (s, 3H). LCMS-4: t_R = 6.59 min. MS: m/z 267 $[M + H]^+$, expected 267 $[M + H]^+$.

6-(3,5-Dimethylpyrazol-1-yl)-2-thiazol-2-ylpyrimidin-4-ylamine (11c). Intermediate **11c** was prepared from **8c** according to the procedure described for **11a**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.10 (d, J = 3.3, 1H), 7.90 (d, J = 3.6, 1H), 6.86 (s, 1H), 6.15 (s, 1H), 2.74 (s, 3H), 2.20 (s, 3H). LCMS-1: t_R = 2.43 min. MS: m/z 273 $[M + H]^+$, expected 273 $[M + H]^+$.

6-(3,5-Dimethylpyrazol-1-yl)-2-(5-methylfuran-2-yl)pyrimidin-4-ylamine (11d). Intermediate **11d** was prepared using the procedure described for **11a** except that intermediate **8d** was used instead of intermediate **8a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.12 (d, J = 3.0, 1H), 6.80 (s, 1H), 6.15 (m, 1H), 6.00 (s, 1H), 5.10 (bs, 2H), 2.76 (s, 3H), 2.44 (s, 3H), 2.28 (s, 3H). LCMS-2: t_R = 5.75 min. MS: m/z 270 $[M + H]^+$, expected 270 $[M + H]^+$.

2-(5-Methylfuran-2-yl)-6-(5-methylpyrazol-1-yl)pyrimidin-4-ylamine (12). To 10 mL of ethanol was added intermediate **8d** (3.0 g, 14.3 mmol), DIEA (4.6 g, 35.8 mmol, 2.5 equiv), and anhydrous hydrazine (0.92 g, 28.6 mmol, 2.0 equiv). The reaction mixture was stirred at 90 °C for 48 h. The mixture was cooled to room temperature and concentrated under vacuum. The residue was dissolved in anhydrous dioxane (30 mL). A solution of 4-dimethylaminobut-3-en-2-one (8.22 g, 72.6 mmol, 5 equiv) in 10 mL of dioxane was added slowly at room temperature. Then 1 M aqueous HCl (2.4 mL) was added and the mixture stirred at room temperature overnight. Solvents were evaporated, the residue dissolved in ethyl acetate, quenched with saturated sodium bicarbonate (20 mL) and extracted with ethyl acetate (2 × 30 mL). The aqueous solution was washed with an additional aliquot of ethyl acetate (20 mL). The organic layers were combined and dried (Na_2SO_4), then concentrated under reduced pressure. The residue was purified by column chromatography, eluting with ethyl acetate/hexanes, 30–70%, to give **12** as a pale-yellow solid (1.2 g, 30%). ^{1}H NMR (300 MHz, $CDCl_3$ + 10% CD_3OD): δ 7.61 (d, J = 3.3,

1H), 7.58 (d, J = 1.2, 1H), 6.93 (s, 1H), 6.26 (d, J = 3.3, 1H), 6.20 (bs, 1H), 2.73 (s, 3H), 2.44 (s, 3H). LCMS-1: t_R = 2.09 min. MS: m/z 256 $[M + H]^+$, expected 256 $[M + H]^+$.

3-Oxo-3-thiazol-2-ylpropionic Acid Ethyl Ester (13a). To a solution of 60% sodium hydride (95.4 mmol) in diethyl carbonate (90 mL) was slowly added 2-acetylthiazole **17** (5.0 g, 39.4 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 **13a** 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 (14a). To a solution of potassium *tert*-butoxide (0.57 g, 6.03 mmol) in butanol (2 mL) was added compound **4a** (0.85 g, 4.26 mmol) and 3-oxo-3-thiazol-2-ylpropionic acid ethyl ester **13a** (0.75 g, 4.69 mmol). The mixture was heated at 135 °C for 3 h. The crude reaction was poured into water (20 mL) and acidified with 10% HCl (25 mL). The resulting solid was filtered, washed with water (2 × 25 mL), and dried. The title compound **14a** was obtained as an off-white solid (0.64 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).

2-(5-Methyl-2-furyl)-6-(1,3-thiazol-2-yl)pyrimidin-4-ol (14b). Intermediate **14b** was prepared according to the procedure described for **14a** except that 5-methyl-2-furancarboxamidine **4d** was used instead of **4a**. ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.03 (d, J = 2.8, 1H), 7.98 (d, J = 2.8, 1H), 7.44 (d, J = 2.8, 1H), 6.77 (s, 1H), 6.38 (d, J = 2.8, 1H), 2.45 (s, 3H).

2-(Thien-2-yl)-6-(pyridin-2-yl)pyrimidin-4-ol (14c). Intermediate **14c** was prepared according to the procedure described for **14a** except that thiophen-2-carboxamidine **4e** was used instead of **4a** and that 3-oxo-3-pyridin-2-ylpropionic acid ethyl ester **13b** was used instead of 3-oxo-3-thiazol-2-ylpropionic acid ethyl ester **13a**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.74 (d, J = 4.8, 1H), 8.38 (d, J = 7.8, 1H), 8.22 (d, J = 3.9, 1H), 8.11 (m, 1H), 7.91 (d, J = 4.8, 1H), 7.62 (m, 1H), 7.25 (dd, J = 4.8, 3.9, 1H), 7.17 (s, 1H).

4-Chloro-2-(2-furyl)-6-(1,3-thiazol-2-yl)pyrimidine (15a). A suspension of compound **14a** (0.63 g) in phosphorus oxychloride (20 mL) was refluxed for 24 h. The solvent was removed under reduced pressure, and ice and water were slowly added. The resulting solid was filtered, washed with 2 M sodium hydroxide, and dried. Purification by column chromatography with silica gel and methylene chloride as eluent gave compound **15a** as an off-white solid (0.44 g, 66%). LCMS-1: t_R = 2.73 min. MS: m/z 263.9 $[M + H]^+$, expected 264 $[M + H]^+$.

4-Chloro-2-(5-methyl-2-furyl)-6-(1,3-thiazol-2-yl)pyrimidine (15b). Intermediate **15b** was prepared from **14b** according to the procedure described for **15a**. ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.03 (d, J = 4.8, 1H), 7.81 (s, 1H), 7.53 (d, J = 3.2, 1H), 7.31 (d, J = 3.2, 1H), 6.15 (d, J = 4.8, 1H), 2.41 (s, 3H).

4-Chloro-2-(thien-2-yl)-6-(pyridin-2-yl)-pyrimidine (15c). Intermediate **15c** was prepared from **14c** according to the procedure described for **15a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.74 (td, J = 4.8, 0.9, 1H), 8.59 (d, J = 8.1, 1H), 8.23 (s, 1H), 8.13 (dd, J = 3.9, 1.2, 1H), 7.93 (dt, J = 7.6, 1.7, 1H), 7.54 (dd, J = 5.2, 1.4, 1H), 7.47 (ddd, J = 7.6, 4.7, 1.0, 1H), 7.17 (dd, J = 5.0, 3.8, 1H).

2-(2-Furyl)-6-(1,3-thiazol-2-yl)pyrimidin-4-amine (16a). A suspension of compound **15a** (0.25 g) in ethanol (22 mL) and 30% ammonium hydroxide (22 mL) was heated at 120 °C in a pressure reactor for 3 h. 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 **16a** as an off-white solid (0.12 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-(5-Methyl-2-furyl)-6-(1,3-thiazol-2-yl)pyrimidin-4-amine (16b). Intermediate **16b** was prepared from **15b** according to the procedure described for **16a**. ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.03 (dd, J = 3.0, 1.0, 1H), 7.93 (dd, J = 3.0, 1.0, 1H), 7.28 (bs, 2H), 7.08 (d, J = 3.4, 1H), 6.99 (m, 1H), 6.29 (dd, J = 3.0, 1.0, 1H), 2.38 (s, 3H).

6-Pyridin-2-yl-2-thiophen-2-ylpyrimidin-4-ylamine (16c). Intermediate **16c** was prepared from **15c** according to the procedure described for **16a**. ^{1}H NMR (300 MHz, $CDCl_3$ + 10% CD_3OD): δ 8.54 (d, J = 4.2, 1H), 8.44 (d, J = 7.8, 1H), 7.94 (s, 1H), 7.84 (t, J = 7.6, 1H), 7.40 (d, J = 5.4, 1H), 7.36 (m, 1H), 7.18 (m, 1H), 7.06 (m, 1H). LCMS-1: t_R = 1.94 min. MS: m/z 255 $[M + H]^+$, expected 255 $[M + H]^+$.

2,6-Bis(1*H*-pyrazol-1-yl)pyrimidin-4-amine (19). To a solution of 2,6-dichloro-pyrimidin-4-amine **18** (81 mg, 0.5 mmol) in anhydrous DMF (1 mL) was added pyrazole (68 mg, 1.0 mmol) and cesium carbonate (0.32 g, 1.0 mmol). The mixture was heated at 120 °C for 15 h. The solution was poured into water (10 mL) and extracted with ethyl acetate (2 × 5 mL). The organic layer was washed with water (2 × 5 mL) and brine (5 mL) and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was purified by preparative TLC plate, eluting with 3% methanol in methylene chloride, to give the title compound **19** as an off-white solid (66 mg, 60%). ^{1}H NMR (300 MHz, $CDCl_3$ + 10% CD_3OD): δ 8.61 (m, 1H), 8.50 (m, 1H), 7.71 (m, 1H), 6.68 (m, 1H), 6.72 (s, 1H), 6.42 (m, 2H). LCMS-5: t_R = 3.82 min. LCMS-2: t_R = 4.10 min. MS m/z 228 $[M + H]^+$, expected 228 $[M + H]^+$.

***N*-[6-Chloro-2-(3,5-dimethylpyrazol-1-yl)pyrimidin-4-yl]acetamide (20).** 4-Amino-2,6-dichloropyrimidine **18** (40 g, 0.24 mol, 1 equiv) was dissolved in *N*-methylpyrrolidinone (200 mL). The slurry was heated to 60 °C, and anhydrous hydrazine (19 mL, 0.61 mol, 2.5 equiv) was added slowly over 1.5 h. The mixture was cooled to room temperature, and 2,4-pentanedione (63 mL, 0.61 mol, 2.5 equiv) was added slowly, with cooling to keep the reaction temperature below 50 °C. After 1 h, the mixture was heated to 50 °C and then ethanol (200 mL) was added, followed by water (400 mL). Once the water addition was complete, the reaction mixture was cooled to room temperature, and the solid was collected by filtration. The solid was washed with ethanol/water (3 × 200 mL) and dried under vacuum at 60 °C overnight. The tan solid obtained was a mixture of the desired regioisomer (43.2 g, 85% purity by HPLC area at 254 nm) and the 4-dimethylpyrazole regioisomer. The product was recrystallized from hot THF/*i*-PrOAc to give 6-chloro-2-(3,5-dimethyl-pyrazol-1-yl)pyrimidin-4-ylamine as a white solid (66%). LCMS-3: t_R = 1.97 min. MS: m/z 223.9 $[M + H]^+$, expected 224 $[M + H]^+$.

The 6-chloro-2-(3,5-dimethylpyrazol-1-yl)pyrimidin-4-ylamine (40 g, 0.18 mol, 1 equiv) was dissolved in acetic acid (200 mL, 0.9 mol, 0.5 equiv) and stirred. Acetic anhydride (80 mL, 0.8 mol, 4.7 equiv) was added and the mixture heated to 90 °C overnight. Once the reaction was complete, the mixture was cooled to room temperature and water (16 mL) was added over 30 min. The product was collected via filtration, washed with water (4 × 75 mL), and dried under vacuum at 50 °C overnight. Compound **20** was obtained as an off-white crystalline solid (AcOH solvate) (48.2 g, 0.15 mol, 83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.07 (s, 1H), 8.03 (s, 1H), 6.03 (s, 1H), 2.65 (s, 3H), 2.30 (s, 3H), 2.22 (s, 3H), 2.12 (s, 3H). LCMS-1: t_R = 2.11 min. MS: m/z 265.9 $[M + H]^+$, expected 265.9 $[M + H]^+$.

***N*-[6-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-2-((*E*)-styryl)pyrimidin-4-yl]acetamide (21).** A mixture of intermediate **20** (3.26 g, 10 mmol), phenylvinylboronic acid (2.3 g, 15 mmol), and sodium carbonate (4.3 g, 40 mmol) in dioxane/water (9/1, 100 mL) was degassed with bubbling N_2 for 15 min. Tetrakis(triphenylphosphine)palladium(0) (0.58 g, 0.5 mmol) was added and the mixture heated at 90 °C for 16 h. The solution was poured into water (20 mL) and extracted with ethyl acetate (2 × 50 mL). The organic layer was washed with water (2 × 15 mL) and brine (15 mL) and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography

with 20−35% acetone in methylene chloride to give the title compound **21** as a yellow solid (2.83 g, 85%). LCMS-1: $t_R = 2.41$ min. MS: *m/z* 334 $[M + H]^+$, expected 334 $[M + H]^+$.

***N*-[2-Formyl-6-(3,5-dimethyl-1*H*-pyrazol-1-yl)pyrimidin-4-yl]acetamide (22).** Intermediate **21** (0.2 g, 0.8 mmol) was dissolved in MeOH/DCM (4/1, 10 mL) and cooled to −78 °C, and O_3 was bubbled in for 5 min. After the solution was flushed with N_2 for 10 min, dimethyl sulfide (0.2 mL) was added, and the mixture was allowed to warm to room temperature. The solution was evaporated with N_2 purging to give crude aldehyde **22**, which was used directly without purification in the following reactions.

2-(1,3-Oxazol-5-yl)-6-(3,5-dimethyl-1*H*-pyrazol-1-yl)pyrimidin-4-amine (23). A mixture of intermediate **22** (104 mg, 0.4 mmol), TOSMIC (160 mg, 0.8 mmol), and potassium carbonate (170 mg, 1.2 mmol) in MeOH (10 mL) was heated at 80 °C for 16 h. MeOH was evaporated, and the residue was partitioned between ethyl acetate and water. The organic layer was washed with brine and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was purified by preparative TLC with 5% methanol in methylene chloride to give **23** as a yellow solid (51 mg, 50% over two steps). ^{1}H NMR (300 MHz, $CDCl_3$ + 10% CD_3OD): δ 7.99 (s, 1H), 7.65 (s, 1H), 6.58 (s, 1H), 6.00 (s, 1H), 2.62 (s, 3H), 2.25 (s, 3H). LCMS-5: $t_R = 4.04$ min. LCMS-2: $t_R = 3.87$ min. MS: *m/z* 257 $[M + H]^+$, expected 257 $[M + H]^+$.

2-(4-Methyl-1,3-oxazol-5-yl)-6-(3,5-dimethyl-1*H*-pyrazol-1yl)pyrimidin-4-amine (24). Intermediate **22** (208 mg, 0.8 mmol) was reacted with Me-TOSMIC (336 mg, 1.6 mmol) according to the procedure described for intermediate **23** to give intermediate **24** as a yellow solid (108 mg, 50% over two steps). LCMS-1: $t_R = 1.88$ min. MS: *m/z* 271 $[M + H]^+$, expected 271 $[M + H]^+$.

2-(1,3-Thiazol-2-yl)-6-(3,5-dimethyl-1*H*-pyrazol-1-yl)pyrimidin-4-amine (25). To a solution of thiazole (70 μL, 1.0 mmol) in anhydrous THF (2 mL) at −78 °C was added *n*-BuLi (1.6 M in hexane, 1.0 mL, 1.6 mmol) followed by stirring for 15 min. $ZnCl_2$ (0.5 M in THF, 6.6 mL, 3.3 mmol) was added, and the mixture was stirred for 1 h and then allowed to warm to −20 °C. Intermediate **20** (65 mg, 0.2 mmol) and tetrakis(triphenylphosphine)palladium(0) (46 mg, 0.04 mmol) were added, and the mixture was heated at 80 °C for 2 h. The solution was then poured into 1 M HCl (10 mL) and extracted with ethyl acetate (2 × 10 mL). The organic layer was washed with water (2 × 5 mL) and brine (5 mL) and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was purified by preparative TLC plate, eluting with 5% methanol in methylene chloride to give **25** as yellow solid (0.19 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$ + 10% CD_3OD): δ 7.90 (d, $J = 3.3$, 1H), 7.53 (d, $J = 3.0$, 1H), 7.06 (s, 1H), 6.09 (s, 1H), 2.72 (s, 3H), 2.32 (s, 3H). LCMS-5: $t_R = 4.90$ min. LCMS-2: $t_R = 4.16$ min. MS: *m/z* 273 $[M + H]^+$, expected 273 $[M + H]^+$.

General Method for Final Compounds 1 and 26–40. To dichloromethane (5 mL) were added compound **9a** (0.3 g 1.3 mmol), chloroacetyl chloride (0.22 g, 0.20 mmol, 1.5 equiv), and pyridine (0.16 g). The reaction mixture was stirred at room temperature for 2 h. The reaction was quenched with saturated sodium bicarbonate (5 mL) and extracted with dichloromethane (3 × 15 mL). The organic layers were combined and dried (Na_2SO_4), then concentrated to give 2-chloro-*N*-(2-furan-2-yl-6-pyrazol-1-ylpyrimidin-4-yl)acetamide as a yellow solid (0.4 g, 100% crude yield), which was used crude in the next reaction.

To dichloromethane (10 mL) were added 2-chloro-*N*-(2-furan-2-yl-6-pyrazol-1-ylpyrimidin-4-yl)acetamide (1.6 g, 5.3 mmol), DIPEA (1.85 mL, 10.6 mmol, 2.0 equiv), and methylpiperazine (2.0 equiv). The reaction mixture was stirred at room temperature overnight. The reaction was quenched with water (5 mL) and extracted with dichloromethane (3 × 15 mL). The organic layers were combined and dried (Na_2SO_4), then concentrated. The residue was purified by column chromatography on silica gel (DCM with methanol gradient from 0 to 5%) to give *N*-(2-Furan-2-yl-6-pyrazol-1-ylpyrimidin-4-yl)-2-(4-methylpiperazin-1-yl)acetamide (**1**) as a pale yellow solid (70%). ^{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). LCMS-7: $t_R = 13.25$ min (99%). 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.

The following compounds were prepared using the same general method as described for compound **1**:

***N*-(2-Furan-2-yl-6-thiazol-2-ylpyrimidin-4-yl)-2-(4-methylpiperazin-1-yl)acetamide (26).** Intermediate **16a** was reacted with chloroacetyl chloride followed by methylpiperazine according to the procedure described for compound **1** to give compound **26** as a slightly yellow solid (59%). ^{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-4: $t_R = 22.58$ min (100%). MS: *m/z* 385 $[M + H]^+$. Anal. ($C_{18}H_{20}N_6O_2S \cdot 2HCl$) C, H, N.

***N*-[6-(3,5-Dimethylpyrazol-1-yl)-2-furan-2-ylpyrimidin-4-yl]-2-(4-methylpiperazin-1-yl)acetamide (27).** Intermediate **11a** was reacted with chloroacetyl chloride followed by methylpiperazine according to the procedure described for compound **1** to give compound **27** as an off-white solid (76%). ^{1}H NMR (free base) (300 MHz, CD_3OD): δ 8.45 (s, 1H), 7.76 (dd, $J = 1.5$, 0.6, 1H), 7.31 (dd, $J = 3.6$, 0.6, 1H), 6.65 (dd, $J = 3.6$, 1.5, 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-4: $t_R = 17.00$ min (100%). LCMS-6: $t_R = 26.5$. MS: *m/z* 396 $[M + H]^+$, expected 396 $[M + H]^+$.

***N*-[2-(5-Methylfuran-2-yl)-6-pyrazol-1-ylpyrimidin-4-yl]-2-(4-methylpiperazin-1-yl)acetamide (28).** Intermediate **9d** was reacted with chloroacetyl chloride followed by methylpiperazine according to the procedure described for compound **1** to give compound **28** as a yellow solid (64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.63 (s, 1H), 8.65 (s, 1H), 8.55 (s, 1H), 7.80 (s, 1H), 7.30 (s, 1H), 6.49 (s, 1H), 6.21 (s, 1H), 3.23 (s, 2H), 2.48 (s, 4H), 2.33 (s, 4H), 2.17 (s, 3H). LCMS-2: $t_R = 4.52$ min. LCMS-5: $t_R = 5.43$ min. MS: *m/z* 382 $[M + H]^+$.

***N*-[2-(5-Methylfuran-2-yl)-6-(5-methylpyrazol-1-yl)pyrimidin-4-yl]2-(4-methylpiperazin-1-yl)acetamide (29).** Intermediate **12** was reacted with chloroacetyl chloride followed by methylpiperazine according to the procedure described for compound **1** to give compound **29** as a yellow solid (55%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.45 (s, 1H), 7.72 (s, 1H), 7.21 (d, $J = 3.6$, 1H), 6.38 (s, 2H), 6.35 (d, $J = 3.6$, 1H), 2.79 (bs, 4H), 2.76 (s, 3H), 2.45–2.50 (m, 4H), 2.38 (s, 3H). LCMS-2: $t_R = 4.79$ min. LCMS-5: $t_R = 5.74$ min. MS: *m/z* 396 $[M + H]^+$, expected 396 $[M + H]^+$.

***N*-[6-(3,5-Dimethylpyrazol-1-yl)-2-(5-methylfuran-2-yl)pyrimidin-4-yl]-2-(4-methylpiperazin-1-yl)acetamide (30).** Intermediate **11d** was reacted with chloroacetyl chloride followed by methylpiperazine according to the procedure described for compound **1** to give compound **30** as a white solid (82%). ^{1}H NMR (300 MHz, CD_3OD): δ 8.42 (s, 1H), 7.20 (d, $J = 3.3$, 1H), 6.26 (dd, $J = 3.0$, 0.9, 1H), 6.13 (bs, 1H), 3.28 (s, 2H), 2.76 (s, 3H), 2.58–2.76 (m, 8H), 2.43 (s, 3H), 2.35 (s, 3H), 2.27 (s, 3H). LCMS-2: $t_R = 5.27$ min. LCMS-6: $t_R = 19.17$ min. MS: *m/z* 410 $[M + H]^+$, expected 410 $[M + H]^+$.

***N*-[2-(5-Methylfuran-2-yl)-6-thiazol-2-ylpyrimidin-4-yl]-2-(4-methylpiperazin-1-yl)acetamide (31).** Intermediate **16b** was reacted with chloroacetyl chloride followed by methylpiperazine according to the procedure described for compound **1**. The product was purified by HPLC to give compound **31** as the TFA salt (15%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.62 (s, 1H), 8.74 (s, 1H), 8.02 (d, $J = 3.0$, 1H), 7.54 (d, $J = 3.0$, 1H), 7.34 (d, $J = 3.6$, 1H), 6.22 (dd, $J = 3.6$, 0.9, 1H), 3.24 (s, 2H), 2.62–2.80 (m, 8H), 2.48 (s, 3H), 2.43 (s, 3H). LCMS-5: $t_R = 5.36$ min (100%). LCMS-2: $t_R = 4.57$ min. MS: *m/z* 399 $[M + H]^+$, expected 399 $[M + H]^+$.

***N*-[2-(Thiophen-2-yl)-6-pyridin-2-ylpyrimidin-4-yl]-2-(4-methylpiperazin-1-yl)acetamide (32).** Intermediate **16c** was reacted with chloroacetyl chloride followed by methylpiperazine according to the procedure described for compound **1**. The product was purified by HPLC to give compound **32** as a TFA salt (48%). ^{1}H NMR (300 MHz, $CDCl_3$ + 10% CD_3OD): δ 8.86 (s, 1H), 8.69 (td, $J = 4.8$, 0.9, 1H), 8.49 (d, $J = 8.1$, 1H), 8.02 (dd, $J = 3.4$, 1.4,

1H), 7.87 (dt, $J = 7.6, 1.8$, 1H), 7.47 (dd, $J = 5.1, 0.9$, 1H), 7.41 (ddd, $J = 7.4, 4.7, 1.4$, 1H), 7.13 (dd, $J = 5.1, 3.9$, 1H), 3.39 (s, 2H), 3.18 (m, 4H), 3.02 (m, 4H), 2.84 (s, 3H). LCMS-2: $t_R = 4.5$ min. LCMS-5: $t_R = 5.7$ min. MS: *m/z* 395 $[M + H]^+$, expected 395 $[M + H]^+$.

***N*-(2,6-Dipyrazol-1-ylpyrimidin-4-yl)-2-(4-methylpiperazin-1-yl)acetamide (33).** Intermediate **19** was reacted with chloroacetyl chloride followed by methylpiperazine according to the procedure described for compound **1**. The product was purified by HPLC to give compound **33** as the TFA salt (35%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.56 (t, $J = 3.0$, 2H), 8.51 (s, 1H), 7.75 (m, 2H), 6.48 (m, 2H), 3.36 (s, 2H), 3.32 (m, 4H), 3.00 (m, 4H), 2.79 (s, 3H). LCMS-2: $t_R = 3.65$ min. LCMS-5: $t_R = 4.71$ min. MS: *m/z* 368 $[M + H]^+$, expected 368 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-thiazol-2-ylpyrimidin-4-yl]-2-(4-methylpiperazin-1-yl)acetamide (34).** Intermediate **25** was reacted with chloroacetyl chloride followed by methylpiperazine according to the procedure described for compound **1**. The product was purified by HPLC to give compound **34** as the TFA salt (38%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.64 (bs, 1H), 8.82 (s, 1H), 8.04 (d, $J = 3.3$, 1H), 7.56 (d, $J = 3.0$, 1H), 6.10 (s, 1H), 3.29 (s, 2H), 2.99–2.89 (m, 8H), 2.80 (s, 3H), 2.62 (s, 3H), 2.36 (s, 3H). LCMS-2: $t_R = 4.24$ min. LCMS-5: $t_R = 5.07$ min. MS: *m/z* 413 $[M + H]^+$, expected 413 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-oxazol-5-ylpyrimidin-4-yl]-2-(4-methylpiperazin-1-yl)acetamide (35).** Intermediate **23** was reacted with chloroacetyl chloride followed by methylpiperazine according to the procedure described for compound **1**. The product was purified by HPLC to give compound **35** as the TFA salt (14%). ^{1}H NMR (300 MHz, $CDCl_3$ + 10% CD_3OD): δ 8.41 (s, 1H), 8.11 (s, 1H), 8.08 (s, 1H), 6.31 (s, 1H), 3.36 (s, 2H), 2.87 (m, 8H), 2.70 (s, 3H), 2.56 (s, 3H), 2.25 (s, 3H). LCMS-2: $t_R = 3.74$ min. LCMS-5: $t_R = 4.85$ min. MS: *m/z* 397 $[M + H]^+$, expected 397 $[M + H]^+$.

***N*-[2-(3,5-Dimethylpyrazol-1-yl)-6-(4-methyloxazol-5-yl)pyrimidin-4-yl]-2-(4-methylpiperazin-1-yl)acetamide (36).** Intermediate **24** was reacted with chloroacetyl chloride followed by methylpiperazine according to the procedure described for compound **1**. The product was purified by HPLC to give compound **36** as the TFA salt (28%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.50 (bs, 1H), 8.39 (s, 1H), 7.96 (s, 1H), 6.09 (s, 1H), 3.34 (s, 2H), 3.17 (m, 4H), 2.80 (s, 3H), 2.71 (s, 3H), 2.66 (s, 3H), 2.35 (s, 3H), 1.74 (m, 4H). LCMS-2: $t_R = 4.02$ min. LCMS-5: $t_R = 5.13$ min. MS: *m/z* 411 $[M + H]^+$, expected 411 $[M + H]^+$.

2-(4-Methylpiperazin-1-yl)-*N*-(6-pyrazol-1-yl-2-thiazol-2-ylpyrimidin-4-yl)acetamide (37). Intermediate **9c** was reacted with chloroacetyl chloride followed by methylpiperazine according to the procedure described for compound **1**. The product was purified by HPLC to give compound **37** as the TFA salt (40%). ^{1}H NMR (300 MHz, $CDCl_3$ + 10% CD_3OD): δ 8.65 (d, $J = 3.0$, 1H), 8.64 (s, 1H), 7.99 (d, $J = 3.6$, 1H), 7.78 (d, $J = 1.5$, 1H), 7.59 (d, $J = 3.0$, 1H), 6.49 (dd, $J = 2.7, 1.8$, 1H), 3.42 (s, 2H), 3.36 (m, 4H), 3.07 (m, 4H), 2.82 (s, 3H). LCMS-2: $t_R = 3.78$ min. LCMS-5: $t_R = 4.75$ min. MS: *m/z* 385 $[M + H]^+$, expected 385 $[M + H]^+$.

2-(4-Methylpiperazin-1-yl)-*N*-(6-pyrazol-1-yl-2-pyridin-2-ylpyrimidin-4-yl)acetamide (38). Intermediate **9b** was reacted with chloroacetyl chloride followed by methylpiperazine according to the procedure described for compound **1**. The product was purified by HPLC to give compound **38** as the TFA salt (32%). ^{1}H NMR (DMSO): δ 8.88 (d, $J = 2.7$, 1H), 8.76 (d, $J = 4.2$, 1H), 8.60 (s, 1H), 8.55 (d, $J = 7.8$, 1H), 8.03 (dt, $J = 8.1, 1.8$, 1H), 7.93 (d, $J = 0.9$, 1H), 7.59 (dd, $J = 4.8, 0.9$, 1H), 6.66 (s, 1H), 3.50 (s, 2H), 3.35–3.45 (m, 4H), 3.0–3.2 (m, 4H), 2.47 (s, 3H). LCMS-2: $t_R = 2.81$ min. LCMS-5: $t_R = 6.34$ min. MS: *m/z* 379 $[M + H]^+$, expected 379 $[M + H]^+$.

***N*-[6-(3,5-Dimethylpyrazol-1-yl)-2-pyridin-2-ylpyrimidin-4-yl]-2-(4-methylpiperazin-1-yl)acetamide (39).** Intermediate **11b** was reacted with chloroacetyl chloride according to the procedure described for compound **1** to give 2-chloro-*N*-[6-(3,5-dimethylpyrazol-1-yl)-2-pyridin-2-ylpyrimidin-4-yl]acetamide. ^{1}H NMR (TFA salt) (300 MHz, DMSO): δ 8.77 (dd, $J = 0.9, 4.2$, 1H), 8.52 (s, 1H), 8.39 (dd, $J = 0.9, 8.1$, 1H), 8.05 (ddd, $J = 7.8, 7.5, 1.8$, 1H), 7.60 (m, 1H), 6.26 (s, 1H), 4.47 (s, 2H), 2.82 (s, 3H), 2.25 (s, 3H). LCMS-2: $t_R = 4.31$ min. LCMS-5: $t_R = 5.53$ min. MS: *m/z* 343 $[M + H]^+$, expected 343 $[M + H]^+$.

2-Chloro-*N*-[6-(3,5-dimethylpyrazol-1-yl)-2-pyridin-2-ylpyrimidin-4-yl]acetamide was then reacted with methylpiperazine according to the procedure described for compound **1**. The product was purified by HPLC to give compound **39** as the TFA salt (50%). LCMS-2: $t_R = 3.81$ min. LCMS-5: $t_R = 5.53$ min. MS: *m/z* 407 $[M + H]^+$, expected 407 $[M + H]^+$.

***N*-[6-(3,5-Dimethylpyrazol-1-yl)-2-thiazol-2-ylpyrimidin-4-yl]-2-(4-methylpiperazin-1-yl)acetamide (40).** Intermediate **11c** was reacted with chloroacetyl chloride followed by methylpiperazine according to the procedure described for compound **1**. The product was purified by HPLC to give compound **40** the TFA salt (31%). ^{1}H NMR (300 MHz, CD_3OD): δ 8.63 (s, 1H), 8.05 (d, $J = 3.0$, 1H), 7.85 (d, $J = 3.6$, 1H), 6.14 (s, 1H), 3.31 (s, 2H), 2.80 (s, 3H), 2.60–2.80 (m, 8H), 2.35 (s, 3H), 2.26 (s, 3H). LCMS-6: $t_R = 15.96$ min. LCMS-7: $t_R = 22.66$ min. MS: *m/z* 413 $[M + H]^+$, expected 413 $[M + H]^+$.

Biology Experimental Section. 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 sequence 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, catolog no. 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 room temperature 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 presence of varying concentrations of competing ligand. Nonspecific binding was determined in the presence of excess (1 μM) of 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 (PerkinElmer) that had been pretreated with 0.5% polyethyleneimine. The filter plates were then washed with 3 × 200 μL of 50 mM Tris-HCl, 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).[17] (1) For the A_1 membrane assay, the results are as follows: ^{3}H-DPCPX measured $K_d = 1.0 \pm 0.5$ nM; $B_{max} = 8 \pm 4$ pmol/mg by Scatchard analysis; control DPCPX (Tocris, 0439) $K_i = 1.6 \pm 0.7$ nM. (2) For the human A_{2A} membrane assay, the results are as follows: ^{3}H-ZM241385 measured $K_d = 0.22 \pm 0.20$ nM; $B_{max} = 33 \pm 8$ pmol/mg by Scatchard analysis; control NBI 80634 $K_i = 0.25 \pm 0.04$ nM. (3) For the rat A_{2A} membrane assay, the results are as

follows: ^{3}H-ZM241385 measured $K_d = 0.33 \pm 0.20$ nM; $B_{max} = 2.4$ pmol/mg by Scatchard analysis, control NBI 80634 $K_i = 1.2 \pm 0.3$ nM.

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 percentage of parent compound remaining. The in vitro initial rates of metabolism were scaled using constants, such as microsomal protein/g of liver, g of liver/kg of body weight, and liver blood flow, to predict systemic clearance and maximum predicted percent bioavailability. These calculations from nonlinear regression assume that liver metabolism alone is the determinant of bioavailability.

Metabolite Identification Assay in Human Liver Microsomes. Human liver microsomes (HLM) were purchased from a commercial source (XenoTech LLC, Kansas City, KS) as a mixed gender pool from 10 donors. Liver microsomal incubations for metabolite identification were conducted using a 50 μM concentration of the NCE at a microsomal protein concentration of 0.5 mg/mL in a 50 mM potassium phosphate buffer in the presence of an NADPH-generating system composed of 1.0 mM NADP+, 3.0 mM $MgCl_2$, 5.0 mM G6P, and 3.0 units/mL G6PDH. A 50 mM stock solution of the NCE in DMSO was used to achieve a final concentration 50 μM, with the final concentration of DMSO being less than 0.1% v/v. All concentrations are relative to a final incubation volume of 1 mL.

Incubations were conducted for 0, 30, 60, and 120 min at 37 °C in a shaking water bath and were terminated by adding 1 mL of ice-cold ACN. After the incubation suspensions are thoroughly vortexed for 1 min and centrifuged at 3000 rpm for 20 min. The resultant supernatant fractions were kept at −80 °C before LC/MS analysis.

LC/MS analysis was carried out on an Agilent 1100 LC system coupled to a Finnigan LCQ ion trap mass spectrometer. The Agilent 1100 LC systems consisted of a binary pump, a diode array detector, a column heater, and a vacuum degasser/mobile phase tray. The LC columns were typically a YMC ODS-AQ column, 150 mm × 2 mm i.d., 5 μm particle size, and were operated at 45 °C. A typical mobile phase used consisted of mobile phase A (0.1% formic acid in deionized water) and mobile phase B (0.1% formic acid in acetonitrile). Gradients were generally about 25 min long and were optimized for separation of each NCE and its metabolites. Flow rates were typically 0.40 mL/min. The mass spectrometer was operated in (+)-ESI mode, and data dependent MS−MS spectra were obtained for structural characterization.

In Human Liver Hepatocytes. Human hepatocytes were purchased from a commercial source (XenoTech LLC, Kansas City, KS). Cryopreservation vials containing hepatocytes are removed from the liquid nitrogen storage unit and quickly placed in a water bath at 37 °C for 2.0 min. Hepatocytes are prepared with Hepatocyte Isolation Kit (XenoTech LLC) according to the protocol provided by the manufacturer (see manufacturer's appendix). Contents of the cryopreservation vials are gently poured into tube A containing Percoll solution and rinsed with 1.67 mL of tube B. The cell suspension is mixed by gentle inversion and centrifuged at 500–700 rpm for 5 ± 0.5 min at room temperature. The cell pellets are gently resuspended with 5 mL of tube B by inversion. The viability of hepatocytes is recorded. The volume of cell suspension is adjusted with tube B to obtain a cell concentration of approximately 1.0×10^6 to 4.0×10^6 cells/mL. The cell suspension is centrifuged again at 400–600 rpm for 3 ± 0.5 min at room temperature. The cell pellets are gently resuspended with 2 mL Krebs Henseleit buffer (Sigma) by inversion. The cells suspension is adjusted with Krebs Henseleit buffer to get a cell concentration of approximately 2.5×10^6 cells/mL. If rat hepatocytes are used or the incubation time is over 2 h, it is suggested that Waymouth's medium be used instead of Krebs Henseleit buffer.

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Supporting Information Available: NMR and LCMS data on key final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Slee, D. H.; Zhang, X.; Moorjani, M.; Lin, E.; Lanier, M. C.; Chen, Y.; Rueter, J. K.; Lechner, S. M.; Markison, S.; Malany, S.; Joswig, T.; Santos, M.; Gross, R. S.; Williams, J. P.; Castro-Palomino, J. C.; Crespo, M. I.; Prat, M.; Gual, S.; Wen, J.; O'Brien Z.; Diaz, J. L.; Saunders, J. Identification of novel, water soluble, 2-amino-*N*-pyrimidin-4-ylacetamides as A_{2A} receptor antagonists with in vivo efficacy. *J. Med. Chem.*, **2008**, *51*, 400–406.

(2) (a) Fredholm, B. B.; Abbracchio, M. P.; Burnstock, G.; Daly, J. W.; Harden, T. K.; Jacobson, K. A.; Leff, P.; Williams, M. Nomenclature and classification of purinoceptors. *Pharmacol. Rev.* **1994**, *46*, 143–156. (b) Fredholm, B. B.; IJzerman, A. P.; Jacobson, K. A.; Klotz, K. N.; Linden, J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev* **2001**, *53*, 527–552. (c) Klotz, K.-N. Adenosine receptors and their ligands. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2000**, *362*, 382–391. (d) Jacobson, K. A.; Gao, A.-G. Adenosine receptors as therapeutic targets. *Nat. Rev.* **2006**, *5*, 247–264.

(3) (a) Svenningsson, P.; Le Moine, C.; Fisone, G.; Fredholm, B. B. Distribution, biochemistry and function of striatal adenosine A_{2A} receptors. *Prog. Neurobiol.* **1999**, *59*, 355–396. (b) Moreau, J.-L.; Huber, G. Central adenosine A2A receptors: an overview. *Brain Res. Rev.* **1999**, *31*, 65–82.

(4) (a) Tuite, P.; Riss, J. Recent developments in the pharmacological treatment of Parkinson's disease. *Expert Opin. Invest. Drugs* **2003**, *12*, 1335–1352. (b) Popoli, P.; Pintor, A.; Domenici, M. R.; Frank, C.; Tebano, M. T.; Pezzola, A.; Scarchilli, L.; Quarta, D.; Reggio, R.; Malchiodi-Albedi, F.; Falchi, M.; Massotti, M. Blockade of striatal adenosine A2A receptor reduces, through a presynaptic mechanism, quinolinic acid-induced excitotoxicity: possible relevance to neuroprotective interventions in neurodegenerative diseases of the striatum. *J. Neurosci.* **2002**, *22*, 1967–1975. (c) Mangiarini, L.; Sathasivam, K.; Seller, M.; Cozens, B.; Harper, A.; Hetherington, C.; Lawton, M.; Trottier, Y.; Lehrach, H.; Davies, S. W.; Bates, G. P. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* **1996**, *87*, 493–506. (d) Hickey, M. A.; Gallant, K.; Gross, G. G.; Levine, M. S.; Chesselet, M. F. Early behavioral deficits in R6/2 mice suitable for use in preclinical drug testing. *Neurobiol. Dis.* **2005**, *20*, 1–11. (e) Popoli, P.; Blum, D.; Martire, A.; Ledent, C.; Ceruti, S.; Abbracchio, M. P. Functions, dysfunctions and possible therapeutic relevance of adenosine A(2A) receptors in Huntington's disease. *Prog. Neurobiol.* **2007**, *81*, 331–348. (f) Scattoni, M. L.; Valanzano, A.; Pezzola, A.; March, Z. D.; Fusco, F. R.; Popoli, P.; Calamandrei, G. Adenosine A2A receptor blockade before striatal excitotoxic lesions prevents long term behavioural disturbances in the quinolinic rat model of Huntington's disease. *Behav. Brain. Res.* **2007**, *176*, 216–221. (g) Blum, D.; Hourez, R.; Galas, M. C.; Popoli, P.; Schiffmann, S. N. Adenosine receptors and Huntington's disease: implications for pathogenesis and therapeutics. *Lancet Neurol.* **2003**, *2*, 366–374.

(5) (a) Jenner, P. Pathophysiology and biochemistry of dyskinesia: clues for the development of non-dopaminergic treatments. *J. Neurol.* **2000**, *247*, 43–50. (b) Bibbiani, F.; Oh, J. D.; Petzer, J. P.; Castagnoli, N., Jr.; Chen, J. F.; Schwarzschild, M. A.; Chase, T. N. A2A antagonist prevents dopamine agonist-induced motor complications in animal models of Parkinson's disease. *Exp. Neurol.* **2003**, *184*, 285–294. (c) Kanda, T; Jackson, M. J.; Smith, L. A.; Pearce, R. K.; Nakamura, J.; Kase, H.; Kuwana, Y.; Jenner, P. Combined use of the adenosine A(2A) antagonist KW-6002 with L-DOPA or with selective D1 or D2 dopamine agonists increases antiparkinsonian activity but not dyskinesia in MPTP-treated monkeys. *Exp. Neurol.* **2000**, *162*, 321–327.

(6) Happe, S.; Sauter, C.; Klosch, G.; Saletu, B.; Zeitlhofer, J. Gabapentin versus ropinirole in the treatment of idiopathic restless legs syndrome. *Neuropsychobiology.* **2003**, *48*, 82–86.

(7) (a) Vu, C. B. Recent advances in the design and optimization of adenosine A2A receptor antagonists. *Curr. Opin. Drug Discovery Dev.* **2005**, *8*, 458–468. (b) Yuzlenko, O.; Kiec-Kononowicz, K. Potent adenosine A1 and A2A receptors antagonists: recent developments. *Curr. Med. Chem.* **2006**, *13*, 3609–3625. (c) Cristalli, G.; Cacciari, B.; Dal, B. D.; Lambertucci, C.; Moro, S.; Spalluto, G.; Volpini, R. Highlights on the development of A2A adenosine receptor agonists and antagonists. *ChemMedChem.* **2007**, *2*, 260–281. (d) Baraldi, P. G.; Tabrizi, M. A.; Romagnoli, R.; El-Kashef, H.; Preti, D.; Bovero, A.; Fruttarolo, F.; Gordaliza, M.; Borea, P. A. Pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine template: organic and medicinal chemistry approach. *Curr. Org. Chem.* **2006**, *10*, 259–275. (e) Neustadt, B. R.; Hao, J.; Lindo, N.; Greenlee, W. J.; Stamford, A. W.; Tulshian, D.; Ongini, E.; Hunter, J.; Monopoli, A.; Bertorelli, R.; Foster, C.; Arik, L.; Lachowicz, J.; Ng, K.; Feng, K.-I. Potent, selective, and orally active adenosine A2A receptor antagonists: arylpiperazine derivatives of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1376–1380. (f) Dowling, J. E.; Vessels, J. T.; Haque, S.; Chang, H. X.; van Vloten, K.; Kumaravel, G.; Engber, T.; Jin, X.; Phadke, D.; Wang, J.; Ayyub, E.; Petter, R. C. Synthesis of [1,2,4]triazolo[1,5-*a*]pyrazines as adenosine A2A receptor antagonists. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4809–4813.

(8) (a) Evans, D. C.; Watt, A. P.; Nicoll-Griffith, D. A.; Baillie, T. A. Drug−protein adducts: an industry perspective on minimizing the potential for drug bioactivation in drug discovery and development. *Chem. Res. Toxicol.* **2004**, *17*, 3–16. (b) Kalgutkar, A. S.; Soglia, J. R. Minimising the potential for metabolic activation in drug discovery. *Expert Opin. Drug Metab. Toxicol.* **2005**, *1*, 91–142.

(9) Dalvie, D. K.; Kalgutkar, A. S.; Khojasteh-Bakht, S. C.; Obach, R. S.; O'Donnell, J. P. Biotransformation reactions of five-membered aromatic heterocyclic rings. *Chem. Res. Toxicol.* **2002**, *15*, 269–299.

(10) (a) Evans, D. A.; Fitch, D. M. Asymmetric synthesis of phorboxazole B. Part II: synthesis of the C1−C19 subunit and fragment assembly. *Angew. Chem., Int. Ed.* **2000**, *39*, 2536–2540. (b) Mylari, B. L.; Oates, P. J.; Beebe, D. A.; Brackett, N. S.; Coutcher, J. B.; Dina, M. S.; Zembrowski., W. J. Sorbitol dehydrogenase inhibitors (SDIs): a new potent, enantiomeric SDI, 4-[2-(1*R*-hydroxy-ethyl)-pytimidin-4-yl]-piperazine-1-sulfonic acid dimethylamide. *J. Med. Chem.* **2001**, *44*, 2695–2700.

(11) Van Leusen, A. M.; Wildemean, J. Chemistry of sulfonylmethyl isocyanides. 14. Synthesis of 1,3-thiazoles from carbon disulfide and tosylmethyl isocyanide under phase-transfer conditions. *Synthesis* **1977**, 501.

(12) Bell, A. S.; Roberts, D. A.; Ruddock, K. S. A synthesis of 2- and 4 (5)-(2-pyridinyl)imidazoles by palladium-catalyzed cross-coupling reactions. *Tetrahedron Lett.* **1988**, *29*, 5013.

(13) (a) Dalvie, D. K.; Kalgutkar, A. S.; Khojasteh-Bakht, S. C.; Obach, R. S.; O'Donnell, J. P. Biotransformation reactions of five-membered aromatic heterocyclic rings. *Chem. Res. Toxicol.* **2002**, *15*, 269–299. (b) Evans, D. C.; Watt, A. P.; Nicoll-Griffith, D. A.; Baillie, T. A. Drug−protein adducts: an industry perspective on minimizing the potential for drug bioactivation in drug discovery and development. *Chem. Res. Toxicol.* **2004**, *17*, 3–16. (c) Kalgutkar, A. S.; Soglia, J. R. Minimising the potential for metabolic activation in drug discovery. *Expert Opin. Drug Metab. Toxicol.* **2005**, *1*, 91–142.

(14) Jacobson, K. A.; Zhan-Guo, G. Adenosine receptors as therapeutic targets. *Nat. Rev. Drug Discovery* **2006**, *5*, 247.

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

(16) Tommasi, R. A.; Macchia, W. M.; Parker, D. T. Novel synthesis of heterocyclic aryl amidines. *Tetrahedron Lett.* **1998**, *39*, 5947–5950.

(17) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50% inhibition (I50) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

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