Lead Optimization of 4-Acetylamino-2-(3,5-dimethylpyrazol-1-yl)-6-pyridylpyrimidines as A_{2A} Adenosine Receptor Antagonists for the Treatment of Parkinson's Disease

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4-Acetylamino-2-(3,5-dimethylpyrazol-1-yl)-pyrimidines bearing substituted pyridyl groups as C-6 substituents were prepared as selective adenosine hA_{2A} receptor antagonists for the treatment of Parkinson's disease. The 5-methoxy-3-pyridyl derivative **6g** (hA_{2A} K_i 2.3 nM, hA_1 K_i 190 nM) was orally active at 3 mg/kg in a rat HIC model but exposure was poor in nonrodent species, presumably due to poor aqueous solubility. Follow-on compound **16a** (hA_{2A} K_i 0.83 nM, hA_1 K_i 130 nM), bearing a 6-(morpholin-4-yl)-2-pyridyl substituent at C-6, had improved solubility and was orally efficacious (3 mg/kg, HIC) but showed time-dependent cytochrome P450 3A4 inhibition, possibly related to morpholine ring metabolism. Compound **16j** (hA_{2A} K_i 0.44 nM, hA_1 K_i 80 nM), bearing a 6-(4-methoxypiperidin-1-yl)-2-pyridyl substituent at C-6, was sparingly soluble but had good oral exposure in rodent and nonrodent species, had no cytochrome P450 or human ether-a-go-go related gene channel issues, and was orally efficacious at 1 mg/kg in HIC and at 3 mg/kg for potentiation of L-dopa-induced contralateral rotations in 6-hydroxydopamine-lesioned rats.

Introduction

Extracellular adenosine regulates a wide range of functions in higher organisms, in which the effects are mediated by a family of four class A (rhodopsin-like) GPCRs,[a,1] adenosine receptors known as A_1, A_{2A}, A_{2B}, and A_3.[2] Caffeine, a nonselective adenosine receptor antagonist, has been used for millennia for its effects on wakefulness and alertness, and in recent years, epidemiological studies have shown that consumption of caffeine is associated with a decreased risk of developing Parkinson's disease, although a true neuroprotective effect has not been demonstrated.[3] The neurological effects of caffeine are due in large part to its activity at human adenosine 2A (hA_{2A}) receptors, which are abundant in the nucleus accumbens, olfactory tubercle, and striatum, where they are colocalized with dopamine D_2 receptors.[4] It has been shown that antagonists of A_{2A} receptors in the striatum can potentiate the response of dopamine agonists acting at D_2 receptors in the same location. This has led to the hypotheses that A_{2A} antagonists, either alone or in combination with dopamine agonists, can have a role in the treatment of neurodegenerative movement disorders such as Parkinson's disease and Huntington's disease, and there is substantial preclinical evidence in support of this hypothesis.[5] Accordingly, the pharmaceutical industry has made a substantial investment in recent years to develop selective, orally available A_{2A} antagonists.[6] At least three A_{2A} antagonists are currently undergoing clinical trials for Parkinson's symptomology.[7]

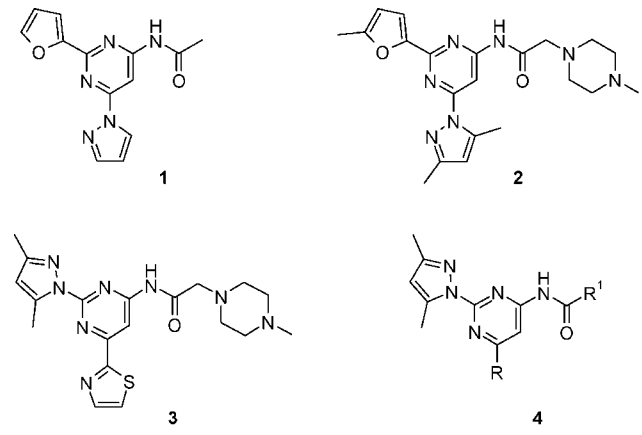

Figure 1. Lead acylaminopyrimidine hA_{2A} antagonists. R = phenyl, substituted phenyl, pyridyl or substituted pyridyl, R^1 = small alkyl or alkoxy.

Previously we reported work on a series of 4-acylaminopyrimidine A_{2A} antagonists based on **1** (Figure 1).[8] This compound has high affinity for the hA_{2A} and rat A_{2A} (rA_{2A}) receptors but has poor selectivity over the human A_1 (hA_1) receptor (hA_{2A} K_i 0.6 nM, hA_1 K_i 9.2 nM), blockade of which could lead to diuresis and various cardiovascular effects.[9] The simple furan substituent in this series of compounds also represents a toxicology risk.[10] Recently, we reported on a series of active

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[a] hA_{2A}, human adenosine 2A; hA_1, human adenosine 1; HIC, haloperidol induced catalepsy; GPCR, G-protein coupled receptor; MED, minimal effective dose; hERG, human ether-a-go-go related gene; CYP, cytochrome P450.

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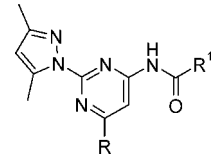
Table 1. Human A_{2A} and A_1 Binding Affinity and Other Data for Compounds **6a**−**6h**, **7**, and **9a**−**9d**



			$K_i \pm$ SEM (nM)[a]			Cl_{int} (mL/min kg)		
compd	R	R^1	hA_{2A}[b]	hA_1[c]	hA_1/hA_{2A}	HLM	RLM	CYP-3A4 IC_{50}(μM)
6a	Ph	CH_3	87 ± 9	290 ± 110	3.3	ND	ND	43
6b	3-pyridyl	CH_3	120 ± 4	2100 ± 20	18	ND	ND	>20
6c	4-pyridyl	CH_3	40 ± 0	820 ± 150	21	ND	ND	8.5
6d	3-MeO-Ph	CH_3	0.92 ± 0.13	65 ± 8	71	ND	ND	0.74
6e	2-F-3-MeO-Ph	CH_3	0.60 ± 0.04	110 ± 9	187	36	360	>20
6f	3,5-di-MeO-Ph	CH_3	0.19 ± 0.05	22 ± 2	116	9.3	110	0.38
6g	5-MeO-3-pyridyl	CH_3	2.3 ± 0.2	190 ± 20	82	35	120	18
6h	5-OH-3-pyridyl	CH_3	2.9 ± 0.5	195 ± 100	67	41	140	18
7	5-(2-methoxy-ethoxy)-3-pyridyl	CH_3	3.1 ± 0.6	110 ± 13	36	3.0	43	14
9a	5-MeO-3-pyridyl	(morpholin-4-yl)-methyl	4.0 ± 0.1	940 ± 170	235	3.0	150	>20
9b	5-MeO-3-pyridyl	Et	1.8 ± 0.4	70 ± 22	39	29	110	17
9c	5-MeO-3-pyridyl	OMe	7.4 ± 0.6	780 ± 60	106	16	93	16
9d	5-MeO-3-pyridyl	OEt	7.8 ± 1.8	620 ± 100	79	38	59	20

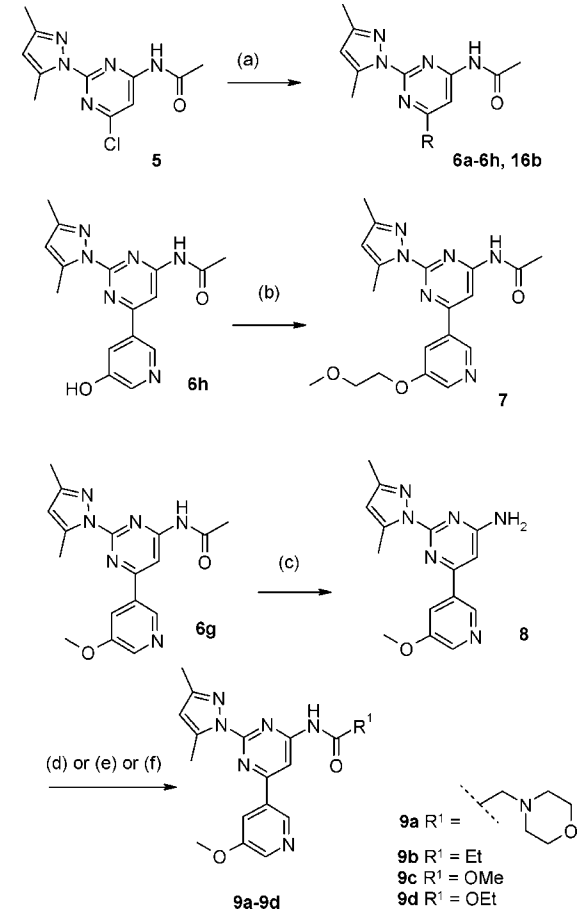
[a] Data are expressed as geometric means of at least two runs ± the standard error of measurement (SEM). [b] Displacement of specific [^{3}H]-**27** ([^{3}H]-ZM241385, Figure 3)[21] binding at hA_{2A} receptors expressed in HEK293 cells. [c] Displacement of specific [^{3}H]-**28** ([^{3}H]-DPCPX, Figure 3)[22] binding at hA_1 receptors expressed in HEK293 cells; HLM = human liver microsomes; RLM = rat liver microsomes; ND = not determined.

and selective compounds such as **2** (hA_{2A} K_i 12 nM, hA_1 K_i 850 nM), in which the furyl group has been substituted or replaced entirely. During the course of that work, it became clear that one of the preferred pyrimidine C-6 substituents, 3,5-dimethylpyrazol-1-yl, could also serve well as a C-2 furan replacement, as in **3** (hA_{2A} K_i 27 nM, hA_1 K_i 1700 nM).[11]

Compounds with amine-bearing substituents on the acyl group such as **2** tend to be blockers of the hERG channel,[12−14] blockade of which has been associated with cardiovascular safety risks.[15] Neutral lipophilic acyl groups yield active and selective compounds[16] with reduced hERG liability, but these compounds tend to be poorly soluble and are not generally orally active in rat haloperidol-induced catalepsy (HIC), an in vivo pharmacological model used to assess reversal of the effects of the dopamine D_2 antagonist haloperidol.[17] Elaborate acyl groups, whether neutral or basic, tend to improve selectivity over hA_1, but they also tend to decrease activity at the rA_{2A} receptor, complicating interpretation of in vivo results. We reasoned that combining the C-2 dimethylpyrazole substituent of **3** with a simple 4-acylamino group, as in **1**, might allow us to discover compounds that had a combination of good human and rat A_{2A} activity, good selectivity over hA_1, and low hERG inhibitory activity. We recently reported preliminary results from this approach, in which we explored substituted phenyl substituents at the C-6 position.[18] We now report the extension of this work to pyridyl compounds of general structure **4** (Figure 1), in which R^1 is a small alkyl or alkoxy group. This work resulted in the discovery of a highly active and selective series of compounds, the best of which (**6g** and **16j**) displayed good in vivo oral activity in rat models for Parkinson's disease and were advanced to preclinical development. The details of this investigation are disclosed herein.

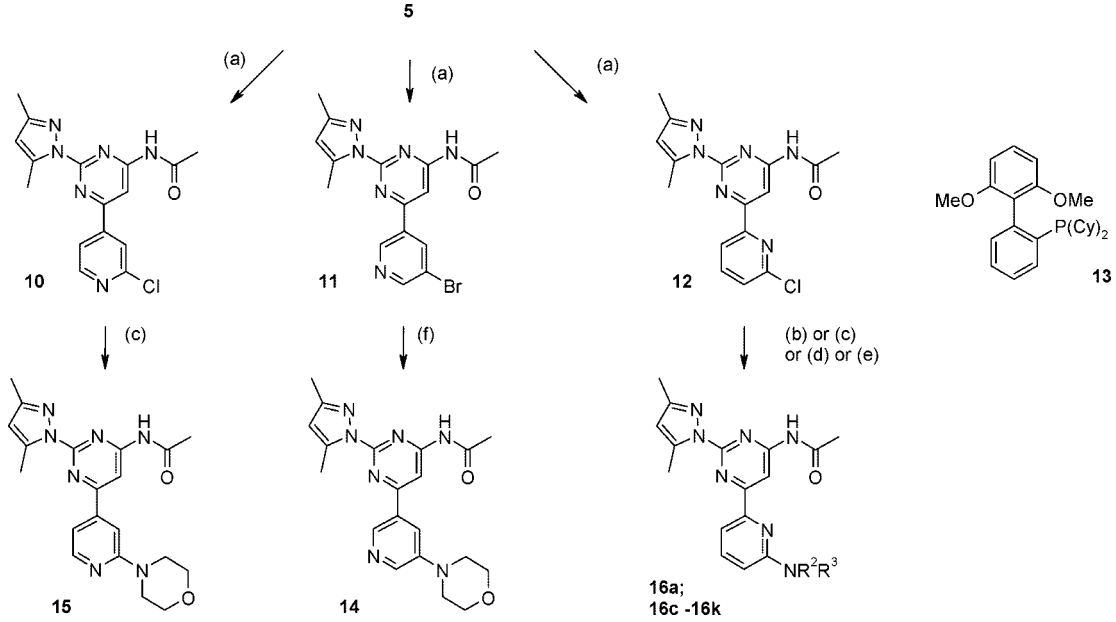
Chemistry. The compounds shown in Table 1 were prepared from **5**[11] according to the methods described in Scheme 1. Compounds **6a**−**6h** bear simple 6-aryl substituents and were prepared in one step by Suzuki coupling with suitable commercially available boronic acids or esters (Scheme 1). Compound **7** was prepared by Mitsunobu alkylation of **6h**. Compound **9a** was prepared by acylation of **8** with chloroacetyl chloride, followed by displacement with morpholine. Compound **9b** was prepared by acylation of **8** with propionyl chloride. Because direct reaction of **8** with chloroformates was unsuc-

Scheme 1[a]



[a] Reagents and conditions: (a) $RB(OR')_2$, $Pd(Ph_3P)_4$, K_2CO_3, dioxane/water, 90 °C; (b) 2-methoxyethanol, DEAD, Ph_3P; (c) K_2CO_3, MeOH, r.t.; (d) $ClCH_2COCl$, pyridine; morpholine; (e) EtCOCl, pyridine, DCM; (f) triphosgene, DCM; methanol or ethanol.

Scheme 2[a]



[a] Reagents and conditions: (a) $RB(OR')_2$, $Pd(Ph_3P)_4$, K_2CO_3, dioxane/water, 90 °C; (b) amine, DMA, 190 °C, microwave; (c) amine, DMSO, 100 °C; (d) amine hydrochloride, NMP, TEA, 160 °C, 80 min, microwave; (e) amine hydrochloride, dioxane, Pd_2dba_3, K_3PO_4, ligand **13**, 100 °C; (f) morpholine, DMSO, CuI, L-proline, K_2CO_3, 100 °C.

cessful, we prepared **9c** and **9d** by reaction of **8** with triphosgene, followed by treatment with methanol or ethanol, respectively.

The aminopyridine-substituted compounds of Table 4 were generally prepared from halopyridyl intermediates **10**, **11**, and **12** by either thermal or metal-catalyzed amine displacement reactions (Scheme 2). In the thermal reaction, under a variety of conditions, the desired acetylamino products were typically accompanied by the corresponding deacetylated primary amine products. The halopyridyl intermediates were obtained by Suzuki reaction of **5** with commercially available halopyridyl boronate esters. Compound **16b** was obtained by direct Suzuki reaction of **5** with 6-aminopyridin-2-ylboronic acid pinacol ester (Scheme 1).

In the case of compound **17**, the displacement routes of Scheme 2 failed, so an alternate route involving de novo synthesis of the pyrimidine nucleus was used (Scheme 3). Potassium *tert*-butoxide promoted reaction of acetonitrile with cyanopyridine **20b** provided enaminonitrile **21b**. Subsequent condensation[19] with thiourea, methylation, and oxidation with oxone provided sulfone **24b**. Displacement of the methanesulfonyl group with 3,5-dimethylpyrazole was problematic; however, displacement with hydrazine, followed by condensation with 2,4-pentanedione, provided the pyrazole **26b** in acceptable yield. Acetylation furnished final compound **17**. Variations of the route in Scheme 3 were used to perform scale-up syntheses of **16a** (Table 4) and other compounds in this general series. Depending on the substrate, the oxidation of sulfide **23** to sulfone **24** could be accompanied by oxidation of the amine side chain to generate an *N*-oxide. The parent amine was regenerated via reduction with hydrazine (or another reaction component) during the subsequent sulfone displacement step.

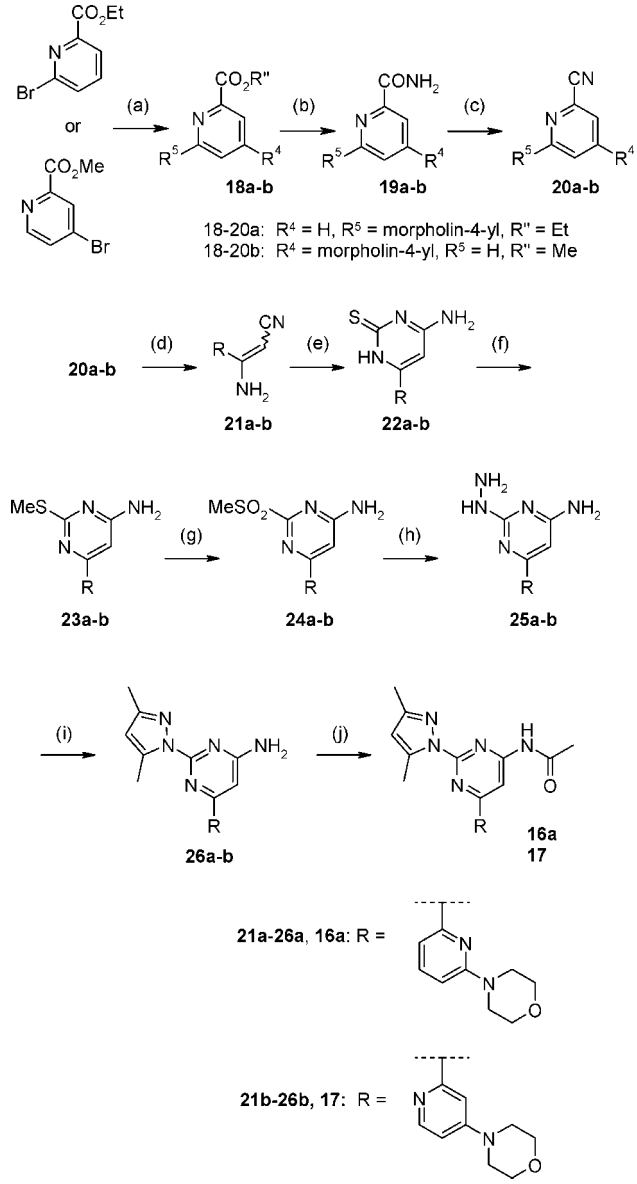
Results and Discussion

The prototypical phenyl and pyridyl compounds **6a**, **6b**, and **6c** were found to be moderately potent binders at the hA_{2A} receptor but had poor selectivity versus hA_1 (Table 1). We performed an extensive survey of substituted phenyl groups at the pyrimidine C-6 position with the goal of improving potency and selectivity against the hA_1 receptor. This survey revealed that a variety of substitution patterns gave compounds with hA_{2A} activity in the 10−50 nM range but typically with low to fair selectivity over hA_1 (5−30×). The key finding from this survey was that a hydrogen bond accepting group in the phenyl 3-position, such as fluoro or methoxy, improved both hA_{2A} binding activity and selectivity over hA_1. Thus compounds such as **6d**, **6e**, and in particular **6f** were among the most active and selective compounds in the series (Table 1). As expected for these very weakly basic compounds, little or no hERG inhibition was seen in this series.[20]

Further profiling of **6e** and **6f** (Tables 1 and 2) indicated that both compounds had moderate to good stability in human (HLM) and rat (RLM) liver microsomal assays and had good human functional antagonist activity in a cAMP assay,[23] validating the design premise for the series. Compound **6f** was poorly soluble and had poor permeability in an in vitro Caco-2 cell permeability assay ($p = 24$ nm/s), while **6e** had reasonable solubility in a high-throughput solubility assay in pH 7.4 phosphate-buffered saline (PBS). Compound **6e** also was found to have good Caco-2 cell permeability ($p = 260$ nm/s). In the rat HIC model, however, orally dosed (PO) **6e** was inactive, presumably due to lack of exposure (Table 2). Compound **6f**, in spite of its in vitro profile, had good oral exposure and was quite efficacious in the HIC model, having a minimal effective dose (MED) of 1 mg/kg (Table 2). Unfortunately, these and other 3-methoxyphenyl compounds interacted with CYP3A4, either through direct inhibition or in the case of **6f**, through time-dependent increase of CYP3A4 inhibition, indicating potent inhibition by a metabolite.

Our discovery efforts thus turned to finding a compound with the efficacy and selectivity profile of **6f** but with a more favorable metabolism profile. Because the simple 3-pyridyl compound **6b** was similar in activity to phenyl compound **6a** and appeared to have somewhat less CYP3A4 inhibitory activity, we made methoxypyridine **6g**. This compound was a potent antagonist of hA_{2A} and was reasonably selective versus hA_1

Scheme 3[a]



[a] Reagents and conditions: (a) morpholine, DMA, Et_3N, 100 °C; (b) NH_3, MeOH, sealed tube, 75 °C; (c) $(CF_3CO)_2O$, DCM, Et_3N, 0 °C to r.t.; (d) t-BuOK, MeCN, toluene, r.t.; (e) thiourea, NaOEt, EtOH, sealed tube, 100 °C; (f) NaOH, H_2O, MeI, r.t.; (g) oxone, MeOH, H_2O, $NaHCO_3$; (h) H_2NNH_2, EtOH, reflux; (i) 2,4-pentanedione, EtOH, r.t.; (j) AcCl, pyridine, DCM, r.t.

yet was only a weak inhibitor of CYP3A4 and a weak blocker of the hERG channel (IC_{50} 24 μM, electrophysiology assay). It had good functional activity and was orally efficacious at 1 mg/kg in HIC. We prepared a series of analogues of **6g** in which we varied the methoxy group (**6h**, **7**) or the acyl group (**9a**−**9d**); however, none of these compounds was superior overall to **6g**, having either lower selectivity (**7**, **9b**) or poor in vivo efficacy in the HIC model (**6h**, **9a**, **9c**, **9d** Table 2). Compound **6g** had poor aqueous solubility (<0.01 mg/mL at pH ≥ 2) but had reasonable oral exposure in the rat (AUC_{0-24} 1430 ng h/mL at 10 mg/kg, free base, 25% PEG-400 in D5W).[25] Exposure at elevated doses (30−300 mg/kg) in rat was sufficient for 14 day toxicological studies in that species (Table 3). However, exposure in nonrodent species (dog, cynomolgus monkey, marmoset) was extremely low using a variety of formulations, precluding further development. The extremely high melting point (283 °C) of **6g** free base no doubt contributed to the solubility and exposure difficulties.[26] Compound **6g** (pK_a 3.5) could be formulated as a hydrochloride salt, but this resulted in no overall improvement in pharmacokinetics.

We sought a followup to **6g** that would have improved nonrodent exposure, which we hoped to achieve by increasing aqueous solubility and/or decreasing the melting point.[27] We reasoned that introducing a weakly basic or polar substituent onto the pyridine ring could increase solubility by disrupting crystal packing and/or increasing polarity. To this end, we prepared a series of morpholine-substituted pyridine isomers **14**, **15**, **16a**, and **17** (Table 4). Compound **17** had only moderate binding affinity, while **14** had poor functional activity (cAMP IC_{50} 213 nM). Compound **15** was reasonably active and selective; however, the 3-morphinyl-2-pyridyl isomer **16a** stood out, having very potent hA_{2A} binding affinity, high selectivity over hA_1, and very good functional activity (Table 2). Solubility of **16a** (0.14 mg/mL in PBS) was also improved over that of **6g**.

Fixing on the 3-substituted-2-pyridyl isomeric relationship, we then surveyed other amines in place of the morpholine of **16a**, with illustrative examples shown in Table 4. A simple amino group (**16b**) gave weaker binding, while some acyclic amines such as dimethylamine (**16c**) gave compounds with high binding affinity and selectivity although with relatively poor human functional activity (cAMP IC_{50} 156 nM, Table 2). Cyclic aliphatic amines such as pyrrolidine (**16d**) or piperidine (**16e**) gave potent compounds but with reduced selectivity. Homomorpholine **16f** was less active and had poor RLM stability. *N*-Methylpiperazine derivative **16g** was the most potent compound in the series, both in terms of binding affinity and also in terms of functional activity (Table 2). In addition, it was highly selective over hA_1 (175×) and its weakly basic amino group (measured pK_a 7.6) provided options for formulation, although actual solubility (0.24 mg/mL, PBS) was similar to that of **16a**. Compound **16g** was studied in the rat HIC model, where, disappointingly, it showed no inhibition of catalepsy. Exposure was somewhat low (Table 2) in spite of good stability in the RLM assay and reasonable in vitro permeability (MDCK-MDR assay, p = 154 nm/s). However, comparison with **6g** indicates that the poor efficacy for **16g** is clearly not due to modest exposure alone and is consistent with a trend we have observed in which basic compounds, in spite of good plasma and gross brain exposure, have generally weak efficacy in the rat HIC model.[17] Compound **16g** was also found to be a potent blocker of the hERG channel (IC_{50} 540 nM, electrophysiology assay).

Returning to the relatively nonbasic morpholine **16a**, we found that it had a good overall in vitro profile (Tables 2 and 4) and showed good efficacy in the HIC model with a MED of 3 mg/kg. This compound was inactive at the hERG channel and was not a potent inhibitor of CYP3A4 under normal assay conditions. However, **16a** was found to have time-dependent inhibition of CYP3A4 and was further found to form glutathione adducts when incubated with HLM in the presence of glutathione, implying metabolic generation of a reactive species. Metabolite identification studies (HLM) indicated that a substantial portion of the metabolism took place on the morpholine ring.[28]

We then set out to replace the morpholine ring with a group that would not be susceptible to the metabolic issues seen with **16a**. Since we had observed that an oxygen atom (presumably a hydrogen bond acceptor) distal to the amine nitrogen improved selectivity, we incorporated a number of oxygen-bearing cyclic and acyclic amines in place of the morpholine. In particular,

Table 2. Secondary Data for Select Compounds

compd	Rat HIC MED (mg/kg)	HIC % reversal[a]	C_{plasma}[a] (ng/mL)	C_{brain}[a] (ng/g)	rA_{2A}[b] K_i (nM)	hcAMP[c] IC_{50} (nM)	solubility[d] (mg/mL)	measured $logD_{7.4}$	measured pK_a
6e[e]			ND	ND	12	32	0.17	2.0	ND
6f	1[f]	49	1330	1150	3	17	<0.02	3.2	ND
6h[e]			ND	ND	62	57	ND	ND	ND
6g	1	64	264	154	32	64	ND	2.4	3.5
9a[e]			ND	ND	30	49	ND	2.4	5.3, 3.6
9b	1	69	860	1380	21	31	ND	2.8	3.6
9c	10	46	ND	ND	68	167	ND	ND	ND
9d[e]			ND	ND	82	196	ND	ND	ND
16a	3[f]	55	150	170	10	13	0.14	ND	2.9
16g[e]			53	91	7	7	0.24	ND	7.6
16c	30		290	180	20	156	ND	ND	ND
16h	10	70	220	870	14	70	ND	ND	ND
16j	1[f]	88	150	560	1.5	18	0.03	3.6	3.4
16k	30		16	9	10	22	0.20	ND	ND

[a] At time = 2 h after 10 mg/kg oral dose. [b] Displacement of specific [^{3}H]-**27** binding at rA_{2A} receptors expressed in CHO cells. [c] hA_{2A} receptor antagonism of 3-[4-[2-[[6-amino-9-[(2*R*,3*R*,4*S*,5*S*)-5-(ethylcarbamoyl)-3,4-dihydroxy-oxolan-2-yl]purin-2-yl]amino]ethyl]phenyl]propanoic acid (CGS-21680)[24] stimulated cAMP. [d] Solubility in PBS at pH 7.4. [e] Inactive at a dose of 30 mg/kg PO. [f] Lowest dose tested; ND = not determined.

Table 3. Plasma Exposure of **6g** in Rats Following Oral Dosing (Vehicle: 25% PEG-400 in D5W)

dose (mg/kg)	C_{max} (ng/mL)	AUC_{0-24} (ng h/mL)
10	410	1430
30	2600	18700
100	3400	28900
300	4200	36800

we chose methoxyalkyl-substituted amines, reasoning that the metabolically "soft" methoxy group might divert metabolism from the aminopyridine center. Selected compounds are listed in Table 4. Acyclic derivative **16h** was a potent and reasonably selective hA_{2A} antagonist and was efficacious in the HIC model at 10 mg/kg. The methoxyazetidine **16i** was similarly selective and had even more potent binding affinity. However, the 4-methoxypiperidine compound **16j** had the most interesting in vitro profile and was selected for detailed characterization.

Compound **16j** was a highly potent A_{2A} antagonist at both the human and rat receptors with greater than 100× selectivity over hA_1. Compound **16j** did not show time-dependent inhibition of CYP3A4 and did not generate glutathione adducts upon incubation with HLM in the presence of glutathione. Metabolic stability in HLM and RLM assays was decreased relative to **16a** but remained reasonable. As anticipated, the major metabolite observed from incubation with HLM was the *O*-demethylated compound **16k**, which turned out to be a potent and selective hA_{2A} antagonist as well (Table 4). Solubility for **16j** was still poor (0.03 mg/mL, PBS); however, its melting point was 140 °C, substantially lower than for **6g**, indicating weaker crystal packing and the potential for more rapid absorption.

In spite of its relatively poor solubility, **16j** demonstrated efficacy in two Parkinson's related behavioral models. **16j** was efficacious down to a dose of 1 mg/kg PO in HIC and demonstrated 88% inhibition of catalepsy at 10 mg/kg, a dose at which plasma exposure (150 ng/mL) was moderate but brain exposure (560 ng/g) was relatively high (Table 2). We also examined the ability of **16j** to potentiate L-dopa induced rotational behavioral in unilaterally 6-hydroxydopamine-lesioned rats (Figure 2). **16j** dose-dependently potentiated L-dopa induced rotations with an MED of 3 mg/kg when dosed orally.

In escalating dose pharmacokinetic studies in the rat, exposure of **16j** reached a plateau at moderate doses using a variety of formulations, limiting the scope of safety studies. However, adequate exposure of **16j**, for safety screening, was achieved in the mouse with 5% TPGS[29] in 0.5% methylcellulose at pH 3.7 as vehicle (AUC_{0-24} 25,700 ng h/mL at 300 mg/kg PO). Solubility in this formulation was 1 mg/mL. In dogs, acceptable exposure was also observed (AUC_{0-24} 3,400 ng h/mL at 30 mg/kg PO) using 10% PEG400 in 0.25% methylcellulose as vehicle. On the basis of the above data, **16j** was selected for further preclinical evaluation.

Hydroxypiperidine metabolite **16k** had very low exposure when dosed orally at 10 mg/kg (Table 2) and thus had low efficacy in the rat HIC model at this dose. It is interesting to note, however, that **16k** strongly inhibited catalepsy at a dose of 30 mg/kg PO (90% inhibition, Table 2). Exposure at this dose was moderate (plasma and brain levels 180 ng/mL and 91 ng/g, respectively, Table 2), suggesting that circulating **16k** could contribute to the efficacy of **16j** in vivo.

Conclusion

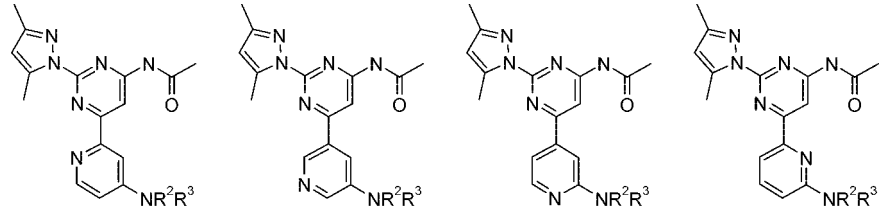
In summary, we have prepared a series of potent and selective 4-acetylamino-2-(3,5-dimethylpyrazol-1-yl)pyrimidine hA_{2A} antagonists with substituted pyridines as the C-6 substituent. Selected compounds had good in vivo activity in the rat HIC model of Parkinson's symptomology. The advanced compound **6g** was precluded from further development due to solubility-related low exposure in nonrodent species. Successor compound **16a**, with a more elaborate morpholine substituent on the pyridine ring, was more soluble and showed promising activity but also showed time-dependent CYP3A4 inhibition and generated glutathione adducts when incubated with HLM in the presence of glutathione. Methoxypiperidine **16j**, with a readily metabolized methoxy group, was free of the reactive metabolite issues seen with **16a** and had a superior in vitro and in vivo profile. Although **16j** still suffered from poor thermodynamic aqueous solubility, its melting point was significantly lower than that of **6g**, allowing for more rapid absorption with proper formulation. Exposure in mouse and dog was sufficient for **16j** to be chosen for further preclinical development, which included 14-day safety screening. The results of these studies will be reported in due course.

Experimental Section

Biology. Human and rat A_{2A} and human A_1 binding assays, metabolism studies, hERG assays, membrane permeability assays, pharmacokinetic assays, and HIC efficacy studies were performed as described previously.[14]

L-dopa Induced Rotations in 6-Hydroxydopamine Lesioned Rats. Male Wistar rats (Charles River; 250 g) were pretreated 30 min before surgery with desipramine and pargyline to ensure a

Table 4. Data for Compounds **17**, **14**, **15**, and **16a−16k**



17 **14** **15** **16a-16k**

compd	NR^2R^3	$K_i \pm$ SEM (nM)[a] hA$_{2A}$[b]	hA$_1$[c]	hA$_1$/hA$_{2A}$	Cl_{int} (mL/min kg) HLM	RLM	CYP-3A4 IC_{50}(μM)
17	morpholin-4-yl	87 ± 7	4600 ± 350	53	ND	ND	ND
14	morpholin-4-yl	3.0 ± 0.8	220 ± 90	74	12	150	>20
15	morpholin-4-yl	12 ± 1	580 ± 170	48	ND	ND	ND
16a	morpholin-4-yl	0.83 ± 0.1	130 ± 13	155	3.0	25	>20
16b	amino	7.9 ± 0.5	440 ± 15	57	ND	ND	>20
16c	*N*,*N*-dimethyl-amino	0.61 ± 0.07	81 ± 12	133	54	1600	25
16d	pyrrolidin-1-yl	0.26 ± 0.01	7.2 ± 4	28	ND	ND	>20
16e	piperidin-1-yl	0.56 ± 0.05	31 ± 5	54	ND	ND	ND
16f	homomorpholin-4-yl	2.3 ± 0.4	280 ± 20	121	94	1000	>20
16g	4-methylpiperazin-1-yl	0.71 ± 0.11	120 ± 10	175	3.0	25	>20
16h	(2-methoxy-ethyl)-methyl-amino	0.72 ± 0.13	55 ± 17	76	52	490	2.5
16i	3-methoxy-azetidin-1-yl	0.47 ± 0.06	34 ± 15	72	25	160	11
16j	4-methoxy-piperidin-1-yl	0.44 ± 0.1	85 ± 24	193	41	540	>20
16k	4-hydroxy-piperidin-1-yl	1.9 ± 0.2	470 ± 110	247	43	230	>20

[a] Data are expressed as geometric means of at least two runs ± the standard error of measurement (SEM); [b] Displacement of specific [^{3}H]-**27** binding at human A_{2A} receptors expressed in HEK293 cells; [c] Displacement of specific [^{3}H]-**28** binding at human A_1 receptors expressed in HEK293 cells; HLM = Human liver Microsomes; RLM = Rat liver Microsomes; ND = not determined.

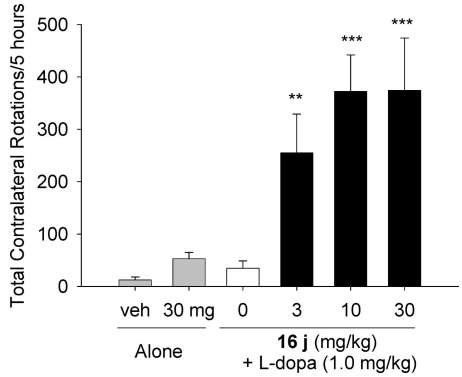
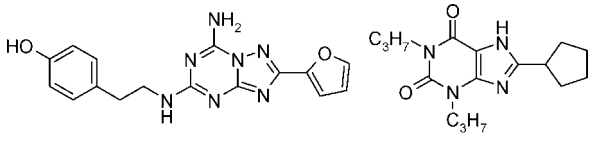


Figure 2. Potentiation of L-dopa induced rotations in unilaterally 6-hydroxydopamine-lesioned rats by orally dosed **16j**.



27 **28**

Figure 3. Structures of antagonists used in binding assays.

maximal effect of the 6-hydroxydopamine (6-OHDA). Then 8 μg of 6-OHDA was injected over 4 min into the medial forebrain bundle (+2.4 mm lateral to the midline, −2.2 mm anterior to bregma (AP), −8.1 mm ventral to the dura surface). After a 3 week recovery, the lesion was verified by measuring rotational behavior in response to apomorphine hydrochloride (0.5 mg/kg sc). Rats that failed to make more than 300 rotations in a 1 h test period were excluded from the study. Rats that passed the apomorphine screen underwent L-dopa sensitization; they were treated with L-dopa (10 mg/kg ip) three times separated by 3−5 days. If rats did not respond to L-dopa by increasing rotations on two of the three sensitization days, they were excluded from the experiment. Each rat underwent each treatment in a randomized design. Test days were separated by 2−3 days. On each test day, rats were habituated to the rotational chambers for 30 min, injected with carbidopa (12 mg/kg ip), 30 min later they were given the treatment, then 30 min later treated with L-dopa (10 mg/kg ip). Rotational behavioral was then measured over the next 5 h.

CYP2D6 and CYP3A4 Inhibition Screen. Recombinant CYP2D6 or CYP3A4 were incubated with a selective marker substrate (AMMC (3-[2-(*N*,*N*-diethyl-*N*-methylammonium)-ethyl]-7-methoxy-4-methylcoumarin) for CPY2D6 and BFC (7-benzyloxy-4-trifluoromethylcoumarin) for CYP3A4) in a NADPH generating system in 96-well assay plates with a total incubation volume of 200 μL/well. Incubations were carried out using NCE concentrations of 0.16, 0.8, 4.0, and 200 μM. The NADPH generating system for the CYP2D6 assay consisted of 75 mM potassium phosphate buffer (pH 7.4), 0.5 mM magnesium chloride, 0.01 mM nicotinamide adenine dinucleotide phosphate (NADP), 0.5 mM glucose-6-phosphate (G-6-P), 1 unit/mL glucose-6-phosphate dehydrogenase (G-6-PD). The NADPH generating system for the CYP3A4 assay consisted of 75 mM potassium phosphate buffer (pH 7.4), 3.0 mM magnesium chloride, 1.0 mM NADP, 5.0 mM gluc G-6-P, and 1 unit/mL G-6-PD.

Reactions were initiated with the addition of the enzyme solution and the samples are incubated at 37 °C for 30 min. Reactions were stopped by adding 75 μL of ice-cold 80% acetonitrile/20% 0.5 M Tris Base. The amount of AHMC formed from CYP2D6 catalyzed *O*-demethylation of AMMC and the amount of 7-HFC formed from CYP3A4 catalyzed dealkylation of BFC were detected using a 96-well microplate fluorescence reader.

Metabolic Stability Screen. Pooled human liver microsomes (0.5 mg/mL; n > 10; mixed gender) were incubated at 37 °C with the NCE in the presence of an NADPH-generating system containing 50 mM, potassium phosphate buffer (pH 7.4), 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 in six 1.2 mL titer tube plates using a 1 μM concentration of each NCE (containing up to 0.01% DMSO) with a total volume of 250 μL per incubation. Each plate representing a single time point contains 96 Titertube Micro Tubes allowing for duplicates of 48 compounds 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 an internal standard for LC-MS).

Precipitated proteins were removed by centrifugation for 35 min at 3500 rpm at 5 °C, and the supernatant fluid (~0.1 mL) was analyzed by LC-MS-MS to determine the amount of parent compound remaining. The in vitro initial rates of metabolism are scaled using constants such as 45 mg microsomal protein/gm liver, 20 g liver/kg body weight, and 20 mL/min/kg liver blood flow to predict systemic clearance and % bioavailability. These calculations from nonlinear regression assume that liver metabolism and not absorption is the determinant of Bioavailability.

Time-Dependent Inhibition of CYP450 Enzymes (1A2, 2C9, 2C19, 2D6, 3A4). Pooled human liver microsomes ($N = 10$) were preincubated with the NCE at 37 °C for 0 and 30 min in mixtures containing potassium phosphate buffer (50 mM, pH 7.4), $MgCl_2$ (3 mM), and EDTA (1 mM) with an NADPH-generating system (1 mM NADP, 5 mM G-6-P, and 1 unit/mL G-6-PD) at the final concentrations indicated. After the preincubation period, the marker substrates (1A2: phenacetin at 50 μM; 2C9: diclofenac at 4 μM; 2C19: S-mephenytoin at 35 μM; 2D6: dextromethorphan at 5 μM; 3A4: midazolam at 3 μM) were added, and the incubation was continued for 10 min (30 min for 1A2). Several concentrations (e.g., 5−7 concentrations) of the NCE were studied, which normally cover a range that spans at lease 2 orders of magnitude (e.g., 0.5−100 μM). Incubations were stopped by the addition of a stop reagent (e.g., acetonitrile with internal standard). Precipitated proteins were removed by centrifugation (3500 rpm for 35 min at 5 °C). Aliquots of supernatant fractions were analyzed by LC-MS-MS to determine the amounts of the metabolites of the marker substrates (acetaminophen, 4′-hydroxydiclofenac, 4′-hydroxymephenytoin, dextrorphan, 1′-hydroxymidazolam, respectively).

In Vitro Absorption Studies in Caco-2 Cells. Caco-2 cells (approximately 1.35×10^6 cells/mL) were thawed and transferred to a Corning Costar T-75 flask (Cambridge, MA) with 25 mL of DMEM supplemented with 10 mM HEPES buffer, 1 mM sodium pyruvate, 2 mM L-glutamine, 0.1 mM MEM nonessential amino acids solution, and 10% FBS for subculture. After subculture to a minimum of 69 passages, Caco-2 cells were seeded (~67000 cells) onto the apical side of microporous polycarbonate membranes in 24-well Costar Transwell plates and cultured for at least 21 days to allow for formation of a confluent monolayer (diffusional barrier) and optimal expression of P-glycoprotein at 37 °C, 95% humidity and 5% CO_2.

Incubation of Caco-2 cell monolayer with 20 μM of a NCE (containing up to 0.1% DMSO) in a solution containing 99% HBSS, 1% HEPES, 0.1 μM propranolol, and 25 μM PEG 400 (1.0 mL for basolateral chamber and 0.3 mL for apical chamber) was conducted at 37 °C, 95% humidity with gentle rotary shaking for 60 min. After the incubation the contents of the apical and basolateral chambers were collected in a 96-deep well plate and analyzed by LC-MS-MS to determine the amount of parent compound in each chamber. Digoxin was used as a positive control for P-glycoprotein-mediated transport, and propranolol and PEG 400 were used as high permeability internal standard and low permeability internal standard for quantitation, respectively.

Chemistry. Reagents, starting materials, and solvents were purchased from commercial suppliers and used as received. Concentration refers to evaporation under vacuum using a Büchi rotatory evaporator. Reaction products were purified, when necessary, by flash chromatography on silica gel (40−63 μm) with the solvent system indicated. Spectroscopic data were recorded on a Varian Mercury 300 MHz spectrometer or a Bruker Avance 500 MHz spectrometer. Elemental analyses were done by Robertson Microlit Laboratory, Madison, NJ, or Numega Resonance Laboratories, San Diego, CA. High-resolution mass spectrometry was performed by the Scripps Research Institute Mass Spectrometry Facility, La Jolla, CA. Melting points were recorded on a Büchi 535 apparatus. Preparative HPLC-MS: Platform, Dionex equipped with a Gilson 215 autosampler/fraction collector, UV detector, and a Dionex MSQ mass detector; HPLC column, Phenomenex Synergy Max-RP, 21.2 mm × 50 mm. HPLC gradient: 35 mL/min, acetonitrile/water, 8−30 min run times. Both acetonitrile and water have 0.05% TFA.

General Suzuki Coupling Method: *N*-[6-(6-Chloropyridin-2-yl)-2-(3,5-dimethyl-pyrazol-1-yl)-pyrimidin-4-yl]-acetamide (12). A mixture of **5** (1.8 g, 6.8 mmol), 6-chloropyridin-2-boronic acid pinacol ester (2.27 g, 9.5 mmol), potassium carbonate (1.88 g, 13.6 mmol), dioxane (35 mL), and water (3.5 mL) was sparged with nitrogen gas for 5 min. Tetrakis(triphenylphosphine)palladium(0) (785 mg, 0.68 mmol) was added, the mixture was sparged with nitrogen gas for an additional 10 min, then the vessel was sealed and heated with stirring at 90 °C for 16 h. Water (50 mL) was added, and the resulting solid was collected, rinsed with water and ethyl acetate, and then dried under vacuum to provide the title compound (1.53 g, 4.5 mmol, 66%) as a pink solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.94 (s, 1H), 8.87 (s, 1H), 8.26 (d, $J = 8$, 1H), 7.81 (t, $J = 8$, 1H), 7.44 (d, $J = 8$, 1H), 6.14 (s, 1H), 2.83 (s, 3H), 2.43 (s, 3H), 2.28 (s, 3H). LCMS-2: $t_R = 6.39$ min. MS: m/z 343, 345 $[M + H]^+$, expected 343, 345 $[M + H]^+$.

***N*-[6-(2-Chloropyridin-4-yl)-2-(3,5-dimethyl-pyrazol-1-yl)-pyrimidin-4-yl]-acetamide (10).** Prepared according to the procedure for **12** from 6-chloropyridin-2-boronic acid pinacol ester (40% crude yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.58 (d, $J = 5$, 1H), 8.55 (s, 1H), 7.99 (s, 1H), 7.87 (dd, $J = 1,5$, 1H), 6.19 (s, 1H), 6.03 (s, 1H), 2.84 (s, 3H), 2.47 (s, 3H), 2.30 (s, 3H). LCMS-2: $t_R = 5.99$ min. MS: m/z 343, 345 $[M + H]^+$, expected 343, 345 $[M + H]^+$.

***N*-[6-(5-Bromopyridin-3-yl)-2-(3,5-dimethyl-pyrazol-1-yl)-pyrimidin-4-yl]-acetamide (11).** Prepared according to the procedure for **12**, from 3-bromopyridine-5-boronic acid. Purification by chromatography (9:1 DCM/methanol eluant) yielded the title compound as a white solid (11% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.26 (d, $J = 2$, 1H), 8.80 (d, $J = 2$, 1H), 8.65 (s, 1H), 8.50 (t, $J = 2$, 1H), 8.47 (s, 1H), 6.09 (s, 1H), 2.78 (s, 3H), 2.35 (s, 3H), 2.26 (s, 3H). LCMS-2: $t_R = 6.04$ min. MS: m/z 387, 389 $[M + H]^+$, expected 387, 389 $[M + H]^+$.

***N*-[2-(3,5-Dimethyl-pyrazol-1-yl)-6-phenyl-pyrimidin-4-yl]-acetamide (6a).** Prepared according to the procedure for **12** from phenylboronic acid. Purification by HPLC yielded the title compound (10 mg, 30% yield). A sample was further purified by preparative TLC (1:1 hexanes/acetone eluant). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.26 (br s, 1H), 8.45 (s, 1H), 8.06−8.09 (m, 2H), 7.56−7.59 (m, 3H), 6.16 (s, 1H), 2.68 (s, 3H), 2.19 (s, 3H), 2.18 (s, 3H). LCMS-2: $t_R = 6.68$ min. (97%); MS: m/z 308 $[M + H]^+$, expected 308 $[M + H]^+$.

***N*-[2-(3,5-Dimethyl-pyrazol-1-yl)-6-pyridin-3-yl-pyrimidin-4-yl]-acetamide (6b).** Prepared according to the procedure for **12** from 3-pyridylboronic acid. Purification by HPLC yielded the title compound (5 mg, 6% yield) as the TFA salt. LCMS-2: $t_R = 3.69$ min. (95%); MS: m/z 309 $[M + H]^+$, expected 309 $[M + H]^+$. LCMS-4: $t_R = 6.15$ min (100%). MS: m/z 309 $[M + H]^+$, expected 309 $[M + H]^+$.

***N*-[2-(3,5-Dimethyl-pyrazol-1-yl)-6-pyridin-4-yl-pyrimidin-4-yl]-acetamide (6c).** Prepared according to the procedure for **12** from 4-pyridylboronic acid. Purification by HPLC yielded the title compound (17 mg, 21%) as the TFA salt. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.85−8.87 (m, 2H), 8.55 (s, 1H), 8.10−8.12 (m, 2H), 6.20 (s, 1H), 2.70 (s, 3H), 2.21 (s, 6H). LCMS-4: $t_R = 6.13$ min (99%). MS: m/z 309 $[M + H]^+$, expected 309 $[M + H]^+$.

***N*-[2-(3,5-Dimethyl-pyrazol-1-yl)-6-(3-methoxyphenyl)-pyrimidin-4-yl]-acetamide (6d).** Prepared according to the procedure for **12** from 3-methoxyphenylboronic acid. Purification by HPLC yielded the title compound (34 mg, 39%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.51 (br s, 1H), 8.46 (s, 1H), 7.69−7.72 (m, 2H), 7.42 (t, $J = 8$, 1H), 7.05−7.08 (m, 1H), 6.07 (s, 1H), 3.89 (s, 3H), 2.81 (s, 3H), 2.35 (s, 3H), 2.24 (s,3H). LCMS-2: $t_R = 6.78$ min (99%). MS: m/z 338 $[M + H]^+$, expected 338 $[M + H]^+$.

***N*-[2-(3,5-Dimethyl-pyrazol-1-yl)-6-(2-fluoro-3-methoxy-phenyl)-pyrimidin-4-yl]-acetamide (6e).** Prepared according to the procedure for **12** from 2-fluoro-3-methoxyphenylboronic acid. The crude product was taken up in 9:1 DCM/methanol, filtered, and then the filtrate was concentrated to yield a solid. This solid was triturated with ether to yield the title compound as a pale-yellow solid (230 mg, 43%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.28 (s, 1H), 8.45 (s, 1H), 7.50 (m, 1H), 7.35 (m, 2H), 6.17 (s, 1H), 3.90

(s, 3H), 2.62 (s, 3H), 2.20 (s, 3H), 2.19 (s, 3H). LCMS-3: t_R = 21.16 min (100%). MS: *m/z* 356 $[M + H]^+$, expected 356 $[M + H]^+$. Anal. ($C_{18}H_{18}FN_5O_2$) C, H, N.

***N*-[6-(3,5-Dimethoxy-phenyl)-2-(3,5-dimethyl-pyrazol-1-yl)-pyrimidin-4-yl]-acetamide (6f).** Prepared according to the procedure for **12** from 3,5-dimethoxyphenylboronic acid pinacol ester. Purification as for **6e** yielded the title compound as a white solid (296 mg, 54%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.42 (s, 1H), 7.18 (m, 2H), 6.75 (m, 1H), 6.17 (s, 1H), 3.86 (s, 6H), 3.35 (s, 3H), 2.68 (s, 3H), 2.19 (s, 3H). LCMS-3: t_R = 22.43 min (100%). MS: *m/z* 368 $[M + H]^+$, expected 368 $[M + H]^+$. Anal. ($C_{19}H_{21}N_5O_3$) C, H, N.

***N*-[2-(3,5-Dimethyl-pyrazol-1-yl)-6-(5-methoxy-pyridin-3-yl)-pyrimidin-4-yl]-acetamide (6g).** Prepared according to the procedure for **12** from 4.8 g (18 mmol) **5** and 5-methoxypyridyl-3-boronic acid pinacol ester. The crude reaction mixture was concentrated and the residue suspended in a mixture of ethyl acetate and water. The mixture was filtered, and then the resulting solid was slurried in ether. Filtration yielded the title compound (2.93 g, 48%). A portion of this material (1.5 g, 4.4 mmol) was suspended in hexafluoroisopropanol (20 mL) and treated with 2 M hydrogen chloride in ether (2.7 mL, 5.4 mmol) at rt. The sample was concentrated, then twice taken up in 100 mL water and lyophilized to dryness to provide the title compound as the hydrochloride salt dihydrate. ^{1}H NMR (300 MHz, CD_3OD): δ 9.27 (d, J = 1, 1H), 8.85 (m, 1H), 8.70 (d, J = 3, 1H), 8.65 (s, 1H), 6.23 (s, 1H), 4.15 (s, 3H), 2.76 (s, 3H), 2.35 (s, 3H), 2.29 (s, 3H). LCMS-3: t_R = 13.50 min (100%). MS: *m/z* 339 $[M + H]^+$, expected 339 $[M + H]^+$. Anal. ($C_{17}H_{18}N_6O_2 \cdot HCl \cdot 2H_2O$) C, H, N.

***N*-[6-(5-Hydroxypyridin-3-yl)-2-(3,5-dimethyl-pyrazol-1-yl)-pyrimidin-4-yl]-acetamide (6h).** A mixture of 3-bromo-5-hydroxypyridine (1.0 g, 5.7 mmol), bis(pinacolato)diboron (1.75 g, 6.9 mmol), potassium acetate (1.7 g, 17 mmol), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) ($PdCl_2 \cdot$dppf, 380 mg, 0.47 mmol), and anhydrous dioxane (15 mL) was sparged with nitrogen and then heated in a sealed tube for 5 h. Additional bis(pinacolato)diboron (1.75 g, 6.9 mmol) and $PdCl_2 \cdot$dppf (380 mg, 0.47 mmol) were added and the reaction heated for an additional 24 h. Ethyl acetate was added, and the reaction mixture was poured onto celite and washed with ethyl acetate. The filtrate was discarded. The solid cake was then washed with 1:1 DCM/methanol, and then methanol. The filtrate was concentrated to provide a crude mixture of 5-hydroxypyridyl-3-boronic acid and its pinacol ester (400 mg).

A mixture of the crude boronic acid/ester from above (400 mg), **5** (765 mg, 2.9 mmol), tris(dibenzylideneacetone)dipalladium(0) (132 mg, 0.14 mmol), cesium carbonate (1.9 g, 5.8 mmol), tri-*tert*-butylphosphonium tetrafluoroborate salt (84 mg, 0.30 mmol), THF (10 mL), and water (2 mL) was sparged with nitrogen for 10 min and then heated at reflux under nitrogen for 5 h. The cooled reaction mixture was filtered and the solid rinsed with THF and water. The collected solid (200 mg) was taken up in 1:1 DCM/methanol, then filtered and the filtrate concentrated. The residue was taken up in THF (10 mL) and water (2 mL) and stirred with Smopex-110 resin (Alfa-Aesar no. 44724) for 16 h. The mixture was filtered to remove the resin, rinsed with THF/water, methanol, and DCM. The filtrate was concentrated, then azeotroped with ethanol, and then dried under vacuum to provide the product as a yellow solid (158 mg, 27% yield). A sample was purified by preparative HPLC to provide the TFA salt. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.41 (s, 1H), 8.87 (br s, 1H), 8.53 (m, 1H), 8.48 (s, 1H), 8.34 (br s, 1H), 6.20 (s, 1H), 2.69 (s, 3H), 2.21 (s, 6H). LCMS-3: t_R = 10.96 min (100%). MS: *m/z* 325 $[M + H]^+$, expected 325 $[M + H]^+$.

***N*-[6-(6-Amino-pyridin-2-yl)-2-(3,5-dimethyl-pyrazol-1-yl)-pyrimidin-4-yl]-acetamide (16b).** Prepared according to the procedure for **12** from 6-aminopyridin-2-ylboronic acid pinacol ester. HPLC purification provided the TFA salt (68 mg, 28%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.38 (dd, J = 1,5, 1H), 8.19 (s, 1H), 8.09 (s, 1H), 7.70−7.61 (m, 2H), 7.48 (d, J = 8, 1H), 6.97−7.01 (m, 1H), 6.02 (s, 1H), 2.63 (s, 3H), 2.33 (s, 3H), 2.21 (s, 3H). LCMS-2: t_R = 3.65 min (100%). MS: *m/z* 324 $[M + H]^+$, expected 324 $[M + H]^+$.

6-(5-Methoxypyridin-3-yl)-2-(3,5-dimethyl-pyrazol-1-yl)-pyrimidin-4-ylanine (8). A mixture of **6g** (1.05 g, 3.1 mmol), potassium carbonate (325 mesh, 1.29 g, 9.3 mmol), and methanol (45 mL) was stirred at rt for 16 h and then was filtered and concentrated. The residue was chromatographed (9:1 DCM/methanol eluant) to provide the title compound (845 mg, 92%) as a solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.79 (d, J = 1, 1H), 8.40 (d, J = 3, 1H), 7.87 (dd, J = 1,3, 1H), 6.72 (s, 1H), 6.05 (s, 1H), 5.40 (br s, 2H), 3.92 (s, 3H), 2.76 (s, 3H), 2.35 (s, 3H). LCMS-4: t_R = 3.08. MS: *m/z* 297 $[M + H]^+$, expected 297 $[M + H]^+$.

***N*-[2-(3,5-Dimethyl-pyrazol-1-yl)-6-(5-methoxy-pyridin-3-yl)-pyrimidin-4-yl]-2-morpholin-4-yl-acetamide (9a).** A solution of **8** (1.0 g, 3.4 mmol) and pyridine (0.32 mL, 4.1 mmol) in dichloromethane (70 mL) was treated at rt with chloroacetyl chloride (0.30 mL, 3.7 mmol). The mixture was stirred at rt for 2 h then partitioned against 10% aqueous sodium hydrogensulfate solution. The aqueous layer was extracted with dichloromethane, and then the combined dichloromethane layers were washed with brine, dried over sodium sulfate, filtered, and concentrated. The crude product was triturated with ethyl acetate to provide a solid, which was dissolved in dichloromethane (30 mL). Morpholine (0.57 g, 6.6 mmol) was added, and the resulting solution was stirred at rt for 16 h. The mixture was concentrated and the residue purified by silica gel chromatography (95/3/0.1 DCM/methanol/aqueous ammonia eluant) to provide the title compound as a beige solid (1.1 g, 76% yield). The free base was dissolved in dichloromethane and treated with an excess of hydrogen chloride in ether. Concentration provided the HCl salt as a beige solid (1.09 g) ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.85 (s, 1H), 8.55 (s, 1H), 8.38 (s, 1H), 7.98 (s, 1H), 6.20 (s, 1H), 4.37 (s, 2H), 3.94 (s, 3H), 3.80−4.00 (m, 4H), 3.20−3.60 (m, 4H), 2.70 (s, 3H), 2.20 (s, 3H). LCMS-4: t_R = 7.00 min (98.4%). MS: *m/z* 424 $[M + H]^+$, expected 424 $[M + H]^+$; Anal. ($C_{21}H_{25}N_7O_3 \cdot HCl \cdot H_2O$) C, H, N.

***N*-[2-(3,5-Dimethyl-pyrazol-1-yl)-6-(5-methoxy-pyridin-3-yl)-pyrimidin-4-yl]-propionamide (9b).** A mixture of **8** (300 mg, 1.0 mmol), pyridine (0.15 mL, 1.8 mmol), and DCM (3 mL) was treated with propionyl chloride (188 mg, 2.0 mmol) at rt and stirred at rt for 68 h. Aqueous sodium bicarbonate was added, and the mixture was extracted with four portions of DCM. The combined organic extracts were washed with aqueous sodium hydrogensulfate and brine, dried over magnesium sulfate, and then a few drops of methanol were added and the mixture was filtered and concentrated. The residue was purified by chromatography (97:3 DCM/MeOH eluant) to provide the title compound as a solid (200 mg, 52%). The free base was dissolved in DCM (30 mL) and then was treated with 2 M hydrogen chloride in ether (0.285 mL, 0.57 mmol). The mixture was concentrated and dried under vacuum to provide the title compound (200 mg) as the HCl salt. ^{1}H NMR (300 MHz, CD_3OD): δ 9.35 (d, J = 2, 1H), 8.96 (m, 1H), 8.78 (d, J = 3, 1H), 8.73 (s, 1H), 6.32 (s, 1H), 4.19 (s, 3H), 2.81 (s, 3H), 2.60 (q, J = 8, 2H), 2.41 (s, 3H), 1.24 (t, J = 8, 3H). LCMS-3: t_R = 15.08 min (98.6%). MS: *m/z* 353 $[M + H]^+$, expected 353 $[M + H]^+$.

[2-(3,5-Dimethyl-pyrazol-1-yl)-6-(5-methoxy-pyridin-3-yl)-pyrimidin-4-yl]-carbamic acid methyl ester (9c). A solution of triphosgene (300 mg, 1.0 mmol) in dichloromethane (3 mL) was added to an ice-cold suspension of **8** (300 mg, 1.0 mmol) and pyridine (0.60 mL, 7.4 mmol) in DCM (3 mL). The mixture was stirred at 0 °C for 90 min, then methanol (0.30 mL, 7.4 mmol) was added and the mixture was stirred at rt for 68 h. The reaction mixture was concentrated and the residue partially purified by preparative HPLC. The product was converted to the free base by extraction with DCM from aqueous sodium bicarbonate, then the residue was purified by preparative TLC (95:5 DCM/MeOH eluant) to provide the title compound as a white solid (52 mg, 15%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.21 (s, 1H), 8.84 (d, J = 1, 1H), 8.50 (d, J = 2, 1H), 8.25 (s, 1H), 7.95 (br s, 1H), 6.17 (s, 1H), 3.95 (s, 3H), 3.76 (s, 3H), 2.67 (s, 3H), 2.21 (s, 3H). LCMS-2: t_R = 4.49 min (100%). MS: *m/z* 355 $[M + H]^+$, expected 355 $[M + H]^+$.

[2-(3,5-Dimethyl-pyrazol-1-yl)-6-(5-methoxy-pyridin-3-yl)-pyrimidin-4-yl]-carbamic acid ethyl ester (9d). Prepared according to the procedure for **9c** from **8** (80 mg, 0.27 mmol) and ethanol. Following HPLC purification, the product was dissolved in DCM and treated with 2 M HCl in ether (0.30 mL, 0.6 mmol). The mixture was concentrated to provide the title compound as the HCl salt (57 mg, 52% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.14 (s, 1H), 8.86 (br s, 1H), 8.54 (d, $J = 2$, 1H), 8.25 (s, 1H), 8.02 (br s, 1H), 6.16 (s, 1H), 4.21 (q, $J = 7$, 2H), 3.95 (s, 3H), 2.65 (s, 3H), 2.19 (s, 3H), 1.26 (t, $J = 7$, 3H). LCMS-4: $t_R = 7.62$ min (100%). MS: m/z 369 $[M + H]^+$, expected 369 $[M + H]^+$.

***N*-{2-(3,5-Dimethyl-pyrazol-1-yl)-6-[5-(2-methoxy-ethoxy)-pyridin-3-yl]-pyrimidin-4-yl}-acetamide (7).** Diethyl azodicarboxylate (DEAD, 61 mg, 0.35 mmol) was added to a solution of **6h** (75 mg, 0.23 mmol), 2-methoxyethanol (26 mg, 0.74 mmol), and triphenylphosphine (91 mg, 0.34 mmol) in THF (1 mL) at rt. The mixture was stirred at rt for 16 h. Additional DEAD (22 mg), triphenylphosphine (30 mg), and 2-methoxyethanol (10 mg) were added, and the mixture was stirred at rt for an additional 4 h. The mixture was concentrated and purified by preparative HPLC to yield the product as the TFA salt (22 mg, 19%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.41 (s, 1H), 8.82 (d, $J = 1$, 1H), 8.50 (d, $J = 1$, 1H), 8.46 (s, 1H), 7.95 (s, 1H), 6.19 (s, 1H), 4.29−4.32 (m, 2H), 3.70−3.73 (m, 2H), 3.33 (s, 3H), 2.69 (s, 3H), 2.21 (s, 3H), 2.20 (s, 3H). LCMS-2: $t_R = 4.35$ min (95.9%). MS: m/z 383 $[M + H]^+$, expected 383 $[M + H]^+$.

***N*-[2-(3,5-Dimethyl-pyrazol-1-yl)-6-(2-morpholin-4-yl-pyridin-4-yl)-pyrimidin-4-yl]-acetamide (15).** A mixture of **10** (50 mg, 0.15 mmol), morpholine (26 mg, 0.30 mmol), and DMA (1 mL) was heated in a microwave reactor at 190 °C for 20 min. Direct purification of the reaction mixture by preparative HPLC furnished the title compound as the TFA salt. This material was converted to the free base by extraction with DCM from aqueous sodium bicarbonate to yield the free base as a yellow solid (10 mg, 17% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.62 (s, 1H), 8.49 (s, 1H), 8.38 (d, $J = 6$, 1H), 7.39−7.31 (m, 2H), 6.11 (s, 1H), 3.87 (t, $J = 5$, 4H), 3.63 (t, $J = 5$, 4H), 2.79 (s, 3H), 2.38 (s, 3H), 2.26 (s, 3H). LCMS-2: $t_R = 3.91$ min (100%). MS: m/z 394 $[M + H]^+$, expected 394 $[M + H]^+$.

***N*-(2-(3,5-Dimethyl-pyrazol-1-yl)-6-{6-[(2-methoxyethyl)-methylamino]-pyridin-2-yl}-pyrimidin-4-yl)-acetamide (16h).** Prepared according to the procedure for **15** starting from **12** (500 mg, 1.5 mmol) and *N*-(2-methoxyethyl)methylamine (1.0 g, 11 mmol). Aqueous sodium bicarbonate was added to the crude reaction mixture, and the mixture was extracted with DCM. The combined organic extracts were dried over sodium sulfate, filtered, and concentrated. The residue was purified by preparative TLC (9:1 DCM/MeOH with 1% NH_4OH as eluant) to provide the title compound (70 mg 12%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.93 (s, 1H), 8.31 (s, 1H), 7.65−7.57 (m, 2H), 6.68 (d, $J = 8$, 1H), 6.06 (s, 1H), 3.87 (t, $J = 6$, 3H), 3.72 (t, $J = 5$, 3H), 3.39 (s, 3H), 3.19 (s, 3H), 2.81 (s, 3H), 2.35 (s, 3H), 2.22 (s, 3H). LCMS-2: $t_R = 4.96$ min (100%). MS: m/z 396 $[M + H]^+$, expected 396 $[M + H]^+$.

***N*-[2-(3,5-Dimethyl-pyrazol-1-yl)-6-(4-methoxy-3,4,5,6-tetrahydro-2*H*-[1,2′]bipyridinyl-6′-yl)-pyrimidin-4-yl]-acetamide (16j).** A mixture of **12** (300 mg, 0.85 mmol), powdered potassium phosphate (650 mg, 3.1 mmol), tris(dibenzylideneacetone)dipalladium(0) (39 mg, 0.038 mmol), 4-methoxypiperidine hydrochloride (264 mg, 1.74 mmol), 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl (72 mg, 0.18 mmol), and dioxane (10 mL) was sparged with nitrogen gas for 5 min, and then the reaction vessel was sealed and the mixture heated with stirring at 100 °C for 16 h. Water and ethyl acetate were added to the cooled reaction mixture, and then the mixture was filtered and the aqueous phase extracted twice with ethyl acetate. The combined organic extracts were dried over sodium sulfate, filtered, and concentrated to provide a yellow oil. Preparative HPLC, followed by neutralization of product fractions with solid sodium bicarbonate and extraction with ethyl acetate, provided a brown solid. Trituration with ethyl acetate provide the title compound as a yellow-brown solid (80 mg, 22%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.20 (s, 1H), 8.87 (s, 1H), 7.76−7.62 (m, 2H), 7.06 (d, $J = 9$, 1H), 6.16 (s, 1H), 4.04−4.08 (m, 2H), 3.44−3.46 (m, 1H), 3.34 (s, 3H), 3.24−3.34 (m, 2H), 2.69 (s, 3H), 2.20 (s, 3H), 2.18 (s, 3H), 1.92−1.96 (m, 2H), 1.47−1.49 (m, 2H). LCMS-3: $t_R = 22.07$ min (100%). MS: m/z 422 $[M + H]^+$, expected 422 $[M + H]^+$. Anal. ($C_{22}H_{27}N_7O_2 \cdot {}^1/_4$HOAc) C, H, N.

***N*-[2-(3,5-Dimethyl-pyrazol-1-yl)-6-(5-morpholin-4-yl-pyridin-3-yl)-pyrimidin-4-yl]-acetamide (14).** A mixture of **11** (150 mg, 0.39 mmol), morpholine (150 mg, 1.7 mmol), potassium carbonate (150 mg, 1.1 mmol), L-proline (50 mg, 0.43 mmol), copper(I) iodide (50 mg, 0.26 mmol), and DMSO (3 mL) was heated in a sealed tube with stirring at 100 °C for 16 h. The cooled reaction mixture was diluted with methanol, filtered, and purified by prep HPLC. Extraction of product fractions (DCM/aqueous sodium bicarbonate), followed by two preparative TLC purifications (9:1 DCM/methanol eluant, then ethyl acetate eluant) provided the free base (yield 3.4 mg, 2%). LCMS-2: $t_R = 3.88$ min (95%). MS: m/z 394 $[M + H]^+$, expected 394 $[M + H]^+$.

***N*-[6-(6-Dimethylamino-pyridin-2-yl)-2-(3,5-dimethyl-pyrazol-1-yl)-pyrimidin-4-yl]-acetamide (16c).** A mixture of **12** (400 mg), dimethylamine hydrochloride (300 mg), and DMF (6 mL) was heated in a microwave reactor at 200 °C for 20 min. The cooled reaction mixture was diluted with methanol, filtered, and partially purified by preparative HPLC. Extraction of product fractions (DCM/aqueous sodium bicarbonate), followed by preparative TLC purification (9:1 $CHCl_3$/MeOH eluant) provided the free base as a yellow solid (70 mg, 17% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.99 (s, 1H), 8.32 (s, 1H), 7.57−7.65 (m, 2H), 6.66 (dd, $J = 9,1$, 1H), 6.06 (s, 1H), 3.19 (s, 6H), 2.81 (s, 3H), 2.36 (s, 3H), 2.23 (s, 3H). LCMS-2: $t_R = 4.57$ min (100%). MS: m/z 352 $[M + H]^+$, expected 352 $[M + H]^+$.

***N*-[2-(3,5-Dimethyl-pyrazol-1-yl)-6-(6-pyrrolidin-1-yl-pyridin-2-yl)-pyrimidin-4-yl]-acetamide (16d).** A mixture of **12** (100 mg, 0.38 mmol), pyrrolidine (0.8 mL), and DMSO (1 mL) was heated in a sealed tube at 90 °C for 64 h. Preparative HPLC purification yielded the deacetylated product (90 mg, 69%) as the TFA salt. The salt was converted to the free base by extraction with DCM from aqueous sodium bicarbonate (yield 65 mg). This material was reacetylated by treatment with pyridine (32 mg) and acetyl chloride (17 mg) in DCM (5 mL) at rt for 1 h. The reaction mix was concentrated and subjected to preparative HPLC purification. Extraction (DCM/aqueous sodium bicarbonate) provided the free base (17 mg, 12% yield) as a yellow solid. LCMS-2: $t_R = 4.43$ min (100%). MS: m/z 378 $[M + H]^+$, expected 378 $[M + H]^+$. ESHRMS m/z 378.2040, $[M + H]^+$ requires 378.2037.

***N*-[2-(3,5-Dimethyl-pyrazol-1-yl)-6-(3,4,5,6-tetrahydro-2*H*-[1,2′]bipyridinyl-6′-yl)-pyrimidin-4-yl]-acetamide (16e).** Prepared according to the procedure for **15** from **12** (50 mg, 0.15 mmol) and piperidine. Direct purification of the reaction mixture by preparative HPLC furnished the title compound (3 mg, 5% yield) as the TFA salt. LCMS-2: $t_R = 6.22$ min (98%). MS: m/z 392 $[M + H]^+$, expected 392 $[M + H]^+$. ESHRMS m/z 392.2188, $[M + H]^+$ requires 392.2193.

***N*-[2-(3,5-Dimethyl-pyrazol-1-yl)-6-(6-[1,4]oxazepan-4-yl-pyridin-2-yl)-pyrimidin-4-yl]-acetamide (16f).** Prepared according to the procedure for **15** from **12** (50 mg, 0.15 mmol), homomorpholine hydrochloride (42 mg, 0.3 mmol), and triethylamine (0.042 mL, 0.3 mmol). Direct purification of the reaction mixture by preparative HPLC furnished the title compound (17 mg, 28% yield) as the TFA salt. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.92 (s, 1H), 8.44 (s, 1H), 7.59−7.63 (m, 2H), 6.68 (d, $J = 8$, 1H), 6.06 (s, 1H), 3.84−3.92 (m, 4H), 3.71 (t, $J = 6$, 2H), 2.80 (s, 3H), 2.35 (s, 3H), 2.23 (s, 3H), 2.10 (t, $J = 5$, 2H), 0.8−1.2 (m, 2H). LCMS-1: $t_R = 2.33$ min (100%). MS: m/z 408 $[M + H]^+$, expected 408 $[M + H]^+$.

***N*-{2-(3,5-Dimethyl-pyrazol-1-yl)-6-[6-(4-methyl-piperazin-1-yl)-pyridin-2-yl]-pyrimidin-4-yl}-acetamide (16g).** Prepared according to the procedure for **15** from **12** (45 mg, 0.13 mmol) and *N*-methylpiperazine. Direct purification of the reaction mixture by preparative HPLC furnished the title compound as the TFA salt. This material was converted to the free base by extraction with

DCM from aqueous sodium bicarbonate to yield the free base as a yellow solid (12 mg, 23%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.92 (s, 1H), 8.33 (s, 1H), 7.72 (d, $J = 8$, 1H), 7.64 (d, $J = 8$, 1H), 6.80 (d, $J = 8$, 1H), 6.07 (s, 1H), 3.71 (t, $J = 5$, 4H), 2.80 (s, 3H), 2.55 (t, $J = 5$, 4H), 2.36 (s, 3H), 2.35 (s, 3H), 2.23 (s, 3H). LCMS-3: $t_R = 15.11$ min (100%). MS: m/z 407 $[M + H]^+$, expected 407 $[M + H]^+$.

***N*-{2-(3,5-Dimethyl-pyrazol-1-yl)-6-[6-(3-methoxy-azetidin-1-yl)-pyridin-2-yl]-pyrimidin-4-yl}-acetamide (16i).** Prepared according to the procedure for **15** from **12** (50 mg, 0.15 mmol), 3-methoxyazetidine hydrochloride (37 mg, 0.30 mmol), and DIPEA (0.053 mL, 0.30 mmol). Direct purification of the reaction mixture by preparative HPLC furnished the title compound (3 mg, 5% yield) as the TFA salt. LCMS-2: $t_R = 4.67$ min (90%). MS: m/z 394 $[M + H]^+$, expected 394 $[M + H]^+$. ESHRMS m/z 394.1995, $[M + H]^+$ requires 394.1986.

***N*-[2-(3,5-Dimethyl-pyrazol-1-yl)-6-(4-hydroxy-3,4,5,6-tetrahydro-2*H*-[1,2′]bipyridinyl-6′-yl)-pyrimidin-4-yl]-acetamide (16k).** Prepared according to the procedure for **15** from **12** (500 mg, 1.5 mmol) and 4-hydroxypiperidine (300 mg, 2.9 mmol). Direct purification of the reaction mixture by preparative HPLC furnished the title compound as the TFA salt. This material was converted to the free base by extraction with DCM from aqueous sodium bicarbonate to yield the free base as a yellow solid (45 mg, 8% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.19 (s, 1H), 8.85 (s, 1H), 7.60−7.73 (m, 2H), 7.04 (d, $J = 9$, 1H), 6.16 (s, 1H), 4.72 (d, $J = 5$, 1H), 4.09−4.14 (m, 2H), 3.70−3.75 (m, 1H), 3.15−3.22 (m, 2H), 2.67 (s, 3H), 2.19 (s, 3H), 2.17 (s, 3H), 1.79−1.85 (m, 2H), 1.37−1.44 (m, 2H). LCMS-3: $t_R = 16.99$ min (100%). MS: m/z 408 $[M + H]^+$, expected 408 $[M + H]^+$. Anal. ($C_{21}H_{25}N_7O_2$) C, H, N.

Ethyl 6-(Morpholin-4-yl)pyridine-2-carboxylate (18a). Morpholine (4.17 mL, 47.9 mmol, 1.05 equiv) was added to a solution of ethyl 6-bromopyridine-2-carboxylate (10.5 g, 45.6 mmol, 1.0 equiv) and triethylamine (9.5 mL, 68.4 mmol, 1.5 equiv) in *N*,*N*-dimethylacetamide (20 mL). The mixture was heated at 100 °C in a sealed tube for 14.5 h. Aqueous sodium bicarbonate was added, and the mixture was extracted with ethyl acetate. The organic layer was dried over sodium sulfate, filtered, and concentrated to a brown oil. Chromatography (3:1 hexanes/EtOAc eluant) gave the product as a yellow solid (6.5 g, 60% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 (t, $J = 7$, 1H), 7.47 (d, $J = 7$, 1H), 6.85 (d, $J = 7$, 1H), 4.39 (q, $J = 7$, 2H) 3.85 (t, $J = 5$, 4H), 3.59 (t, $J = 5$, 4H), 1.42 (t, $J = 7$, 3H). LCMS-1: $t_R = 2.35$ min. MS: m/z 237 $[M + H]^+$, expected 237 $[M + H]^+$.

6-(Morpholin-4-yl)pyridine-2-carboxamide (19a). 18a (6.3 g, 26.7 mmol) was dissolved in 7N ammonia in methanol (150 mL). The solution was heated with stirring in a sealed tube at 75 °C for 16 h. The mixture was concentrated, and the solid residue was coevaporated with methanol, then ethyl acetate. The solid product was dried under vacuum to provide the title compound (5.46 g) as an off-white solid, which was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$ + 1 drop CD_3OD): δ 7.60 (t, $J = 8$, 1H), 7.59 (d, $J = 8$, 1H), 6.89 (d, $J = 8$, 1H), 3.85 (t, $J = 5$, 4H), 3.54 (t, $J = 5$, 4H). LCMS-1: $t_R = 1.97$ min. MS: m/z 208 $[M + H]^+$, expected 208 $[M + H]^+$.

6-(Morpholin-4-yl)pyridine-2-carbonitrile (20a). A suspension of **19a** (5.4 g, 26 mmol) and triethylamine (7.3 mL, 52 mmol) in dichloromethane (100 mL) was cooled in an ice bath and treated dropwise with trifluoroacetic anhydride (4.0 mL, 29 mol). The mixture was allowed to stir at 0 °C for 15 min, then was allowed to warm to rt. After 30 min, additional trifluoroacetic anhydride (0.5 mL, 3.6 mmol) was added, and the mixture was stirred for another 30 min. Aqueous sodium bicarbonate solution was added, and the mixture was extracted with dichloromethane. The organic layer was dried over sodium sulfate, filtered, and concentrated to a solid, which was triturated with 3:1 hexanes/ethyl acetate to provide the title compound (3.69 g) as a white solid. A second crop (0.66 g, slightly yellow solid) was obtained from the mother liquor. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56 (dd, $J = 7,9$, 1H), 7.02 (d, $J = 7$, 1H), 6.83 (d, $J = 9$, 1H), 3.82 (t, $J = 5$, 4H), 3.56 (t, $J = 5$, 4H). LCMS-1: $t_R = 2.38$ min. MS: m/z 190 $[M + H]^+$, expected 190 $[M + H]^+$.

3-Amino-3-(6-morpholin-4-yl-pyridin-2-yl)-acrylonitrile (21a). A mixture of **20a** (7.43 g, 39 mmol, 1.0 equiv), anhydrous toluene (100 mL), and potassium *t*-butoxide (13.2 g, 118 mmol, 3.0 equiv) was stirred without external heating or cooling while anhydrous acetonitrile (4.1 mL, 79 mmol, 2.0 equiv) was added dropwise over 10 min. The thick yellow mixture was stirred for an additional 2 h. Aqueous sodium bicarbonate solution was added (exothermic), and then the mixture was extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered, and concentrated to provide the title compound as a brown oil (9.6 g), which was used without purification. LCMS-1: $t_R = 2.38$ min. MS: m/z 231 $[M + H]^+$, expected 231 $[M + H]^+$.

4-Amino-6-(6-morpholin-4-yl-pyridin-2-yl)-1H-pyrimidine-2-thione (22a). A suspension of **21a** (9.5 g, est 35 mmol) and thiourea (5.6 g, 74 mmol) in absolute ethanol (100 mL) was treated with sodium ethoxide solution (21 wt %, Aldrich, 27.5 mL, 74 mmol). The brown mixture was heated in a sealed tube at 100 °C for 49.5 h. The cooled reaction mixture was poured onto 10% aqueous sodium dihydrogen phosphate solution (200 mL) chilled in an ice bath. The mixture was filtered. The filter cake was washed with water and then air-dried briefly to provide the title compound (34.6 g) as a tan gummy solid. This material was used without further purification. A sample was dried further for analysis. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.9 (br s, 1H), 7.7−7.8 (m, 3H), 7.37 (d, $J = 8$, 1H), 7.05 (d, $J = 8$, 1H), 6.62 (s, 1H), 3.72 (m, 4H), 3.52 (m, 4H). LCMS-1: $t_R = 1.97$ min. MS: m/z 290 $[M + H]^+$, expected 290 $[M + H]^+$.

2-Methylsulfanyl-6-(6-morpholin-4-yl-pyridin-2-yl)-pyrimidin-4-ylamine (23a). A suspension of **22a** (34.5 g, est 30 mmol) in 5% aqueous sodium hydroxide (100 mL, 125 mmol) was stirred at rt while iodomethane (1.86 mL, 30 mmol) was added in one portion. A light-tan precipitate formed. After 45 min, the mixture was treated with 10% aqueous sodium dihydrogen phosphate solution (final pH 9), and then the solid was collected, washed with water, and air-dried to provide the title compound (9.95 g) as a tan solid, still wet with water. A sample was dried further for analysis. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.59−7.72 (m, 2H), 7.08 (s, 1H), 6.99 (brs, 2H), 6.92 (d, $J = 8$, 1H), 3.75 (m, 4H), 3.52 (m, 4H), 2.48 (s, 3H). LCMS-1: $t_R = 2.06$ min. MS: m/z 304 $[M + H]^+$, expected 304 $[M + H]^+$.

2-Methanesulfonyl-6-[6-(4-oxy-morpholin-4-yl)-pyridin-2-yl]-pyrimidin-4-ylamine (24a). A suspension of **23a** (9.9 g, est 25 mmol) in methanol (200 mL) and water (50 mL) was cooled in an ice bath and treated dropwise with a solution of oxone (20.7 g) in water (100 mL) over 10 min. The temperature was kept below 20 °C during the addition. Saturated sodium bicarbonate solution (75 mL) was then added dropwise over 5 min (effervescence), and the ice bath was removed. After 1 h, an additional portion of solid oxone (5.5 g) was added. After an additional 2 h, dichloromethane (200 mL) was added and the mixture was filtered. The filter cake was washed with water and air-dried to provide the first product fraction as a tan solid (7.0 g). The two phases of the filtrate were separated. The aqueous phase was adjusted to pH > 8 and was extracted with dichloromethane and ethyl acetate. The combined organic extracts were dried over sodium sulfate, filtered, and concentrated to provide the second product fraction (1.3 g) as a yellow solid. A slurry of product fractions one (6.8 g) and two (1.3 g) in ethanol was concentrated to dryness to provide the title compound (7.3 g) as a tan solid. ^{1}H NMR (300 MHz, DMSO-d_6) major species: δ 8.58 (d, $J = 8$, 1H), 8.41 (d, $J = 8$, 1H), 8.27 (t, $J = 8$, 1H), 7.98 (br s, 2H), 7.60 (s, 1H), 4.41 (t, $J = 11$, 2H), 4.19 (t, $J = 11$, 2H), 3.82 (d, $J = 11$, 2H), 3.38 (s, 3H), 2.95 (d, $J = 11$, 2H). LCMS-1: $t_R = 1.60$ min. MS: m/z 352 $[M + H]^+$, expected 352 $[M + H]^+$.

2-(3,5-Dimethyl-pyrazol-1-yl)-6-(6-morpholin-4-yl-pyridin-2-yl)-pyrimidin-4-ylamine (26a). A suspension of **24a** (7.3 g, 19.4 mmol) in ethanol (200 mL) was treated with anhydrous hydrazine (6 mL, 189 mmol). The mixture was refluxed for 16 h. The mixture (containing crude arylhydrazine **25a**) was allowed to cool to rt,

and then pentane-2,4-dione (20.5 mL, 200 mmol) was added dropwise over 30 min without external cooling. The mixture was heated at 70 °C for 1 h and then was allowed to cool and the solvent was removed. The resulting tan solid (30.5 g) was slurried with water (150 mL) at 40 °C for 15 min and then was allowed to cool. The cooled mixture was filtered, and the filter cake was rinsed with water. The resulting filter cake was slurried with ethyl acetate and then concentrated to dryness to provide the product **26a** (5.9 g) as a tan solid, which contained the desired product in addition to 3,5-dimethylpyrazole (LCMS analysis). A sample was further purified for analysis as follows: The crude product was dissolved in 1 M hydrochloric acid and then the solution was washed three times with EtOAc. 1N Sodium hydroxide solution was added until a white precipitate formed and then the mixture was extracted with DCM. The DCM extract was dried over sodium sulfate, filtered, and then concentrated to provide **26a** as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) major species: δ 7.72 (t, J = 8, 1H), 7.60 (d, J = 8, 1H), 7.28 (br s, 2H), 7.25 (s, 1H), 6.97 (d, J = 8, 2H), 6.05 (s, 1H), 3.74 (m, 4H), 3.54 (m, 4H), 2.60 (s, 3H), 2.16 (s, 3H). LCMS-1: t_R = 2.27 min. MS: m/z 352 $[M + H]^+$, expected 352 $[M + H]^+$.

***N*-[2-(3,5-Dimethyl-pyrazol-1-yl)-6-(6-morpholin-4-yl-pyridin-2-yl)-pyrimidin-4-yl]-acetamide (16a).** Acetyl chloride (0.035 mL, 0.45 mmol) was added at rt to a stirred mixture of the amine (115 mg, 0.33 mmol) and pyridine (0.053 mL, 0.66 mmol) in DCM (3 mL). The mixture was stirred at rt for 2.5 h, and then brine was added and the mixture was extracted with dichloromethane. The combined extracts were dried over sodium sulfate, filtered, and concentrated. The residue was purified by chromatography (1:1 hexanes/THF eluant) to provide the title compound (110 mg, 85% yield) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.21 (s, 1H), 8.85 (s, 1H), 7.79−7.68 (m, 2H), 7.05 (d, J = 8.3, 1H), 6.15 (s, 1H), 3.75 (t, J = 45.7, 4H), 3.55 (t, J = 4.9, 4H), 3.31 (s, 3H), 2.22 (s, 3H), 2.16 (s, 3H). LCMS-2: t_R = 6.07 min (100%). MS: m/z 394 $[M + H]^+$, expected 394 $[M + H]^+$.

Methyl 2-(morpholin-4-yl)pyridine-4-carboxylate (18b). Prepared from methyl 4-chloropyridin-2-carboxylate (4.5 g, 26 mmol) and morpholine according to the procedure of **18a**, except that the reaction product was not purified by chromatography. Brown solid (yield 3.3 g, 54%), LCMS-1: t_R = 0.43 min. MS: m/z 223 $[M + H]^+$, expected 223 $[M + H]^+$.

2-(Morpholin-4-yl)pyridine-4-carboxamide (19b). Prepared from **18b** (3.3 g, 15 mmol) according to the procedure of **19a**. The crude solid product was washed with methanol and then ether to provide the title compound as an off-white solid (yield 2.0 g, 65%). LCMS-1: t_R = 0.33 min. MS: m/z 208 $[M + H]^+$, expected 208 $[M + H]^+$.

2-(Morpholin-4-yl)pyridine-4-carbonitrile (20b). Prepared from **19b** (2.0 g, 9.6 mmol) according to the procedure of **20a**, except that the crude solid was triturated with EtOAc/ether. White solid (1.45 g, 79%). LCMS-1: t_R = 0.98 min. MS: m/z 190 $[M + H]^+$, expected 190 $[M + H]^+$.

3-Amino-3-(4-morpholin-4-yl-pyridin-2-yl)-acrylonitrile (21b). Prepared from **20b** (1.45 g, 7.6 mmol) according to the procedure of **21a**. The crude product was triturated with ether to provide the title compound as a yellow solid (1.77 g, 100%). LCMS-1: t_R = 0.91 min. MS: m/z 231 $[M + H]^+$, expected 231 $[M + H]^+$.

4-Amino-6-(4-morpholin-4-yl-pyridin-2-yl)-1*H*-pyrimidine-2-thione (22b). Prepared from **21b** (1.5 g, 6.5 mmol) according to the procedure of **22a**, except that the reaction time was 24 h. White solid (1.1 g, 58%). LCMS-1: t_R = 0.64 min. MS: m/z 290 $[M + H]^+$, expected 290 $[M + H]^+$.

2-Methylsulfanyl-6-(4-morpholin-4-yl-pyridin-2-yl)-pyrimidin-4-ylamine (23b). Prepared from **22b** (1.0 g, 3.5 mmol) according to the procedure for **23a**. Off-white solid (690 mg, 66%). LCMS-1: t_R = 1.91 min. MS: m/z 304 $[M + H]^+$, expected 304 $[M + H]^+$.

2-Methanesulfonyl-6-[4-(4-morpholin-4-yl)-pyridin-2-yl]-pyrimidin-4-ylamine (24b). Prepared from **23b** (330 mg, 1.1 mmol) according to the procedure for **24a**. The reaction mixture was filtered and washed with methanol, and then the filtrate was concentrated to provide a yellow foam (370 mg, 100%). The major species present was tertiary amine title compound; the *N*-oxide was minor. LCMS-1: t_R = 1.71 min. MS: m/z 336 $[M + H]^+$, expected 336 $[M + H]^+$.

2-(3,5-Dimethyl-pyrazol-1-yl)-6-(4-morpholin-4-yl-pyridin-2-yl)-pyrimidin-4-ylamine (26b). Prepared from **24b** (370 mg, 1.1 mmol) according to the procedure for **26a**, with modifications. Following treatment with hydrazine, the reaction mixture was filtered and the solid washed with methanol. The combined filtrate was concentrated to provide the intermediate hydrazinopyrimidine **25b** as a yellow foam. This was then treated with pentane-2,4-dione in ethanol according to the procedure of **26a**. Final purification by chromatography (9:1 DCM/methanol eluant) provided the title compound **26b** (110 mg, 29% from **21b**) as a yellow solid. LCMS-1: t_R = 2.05 min. MS: m/z 352 $[M + H]^+$, expected 352 $[M + H]^+$.

***N*-[2-(3,5-Dimethyl-pyrazol-1-yl)-6-(4-morpholin-4-yl-pyridin-2-yl)-pyrimidin-4-yl]-acetamide (17).** Prepared according to the procedure for **16a** from **26b** (110 mg, 0.31 mmol). Purification of the crude material by preparative HPLC, followed by conversion to the free base (DCM/aqueous sodium bicarbonate extraction) yielded the title compound (15 mg, 12% yield) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.94 (s, 1H), 8.44 (d, J = 6.0, 1H), 8.31 (s, 1H), 7.85 (d, J = 2.4, 1H), 6.77 (dd, J = 6.0, 2.4, 1H), 6.08 (s, 1H), 3.87 (t, J = 4.8, 4H), 3.37 (t, J = 4.8, 4H), 2.79 (s, 3H), 2.36 (s, 3H), 2.23 (s, 3H). LCMS-2: t_R = 3.99 min (100%). MS: m/z 394 $[M + H]^+$, expected 394 $[M + H]^+$.

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

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