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5-(3-Bromophenyl)-7-(6-morpholin-4-ylpyridin-3-yl)pyrido-[2,3-*d*]pyrimidin-4-ylamine: structure–activity relationships of 7-substituted heteroaryl analogs as non-nucleoside adenosine kinase inhibitors

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Abstract—4-Amino-5,7-disubstituted pyridopyrimidines are potent, non-nucleoside inhibitors of adenosine kinase (AK). We recently identified a potent, orally efficacious analog, **4** containing a 7-pyridylmorpholine substituted ring system as the key structural element of this template. In this report, we disclose the pharmacologic effects of five- and six-membered heterocyclic ring replacements for the pyridine ring in **4**. These replacements were found to have interesting effects on in vivo efficacy and genotoxicity as well as in vitro potency. We discovered that the nitrogen in the heterocyclic ring at C(7) is important for the modulation of mutagenic side effects (Ames assay).

1. Introduction

During cellular stress, local intracellular concentrations of adenosine (ADO) increase, which is followed by the active transport of ADO out of the cell and subsequent activation of ADO receptor (P1) subtypes.[1,2] Activation of ADO receptors produces a variety of homeostatic inhibitory cellular events that contribute to anti-nociceptive and anti-inflammatory actions in vivo.[3] Therapeutic exploitation of the beneficial effects of ADO receptor activation has focused on the development of receptor selective agonists.[4] Direct acting ADO A_1 receptor agonists for CNS indications, however, have suffered from cardiovascular side effects such as hypotension and bradycardia.[1]

An alternative approach to direct A_1 receptor activation is to modulate ADO concentration by inhibition of known ADO metabolic pathways including the inhibition of adenosine kinase (AK) and adenosine deaminase

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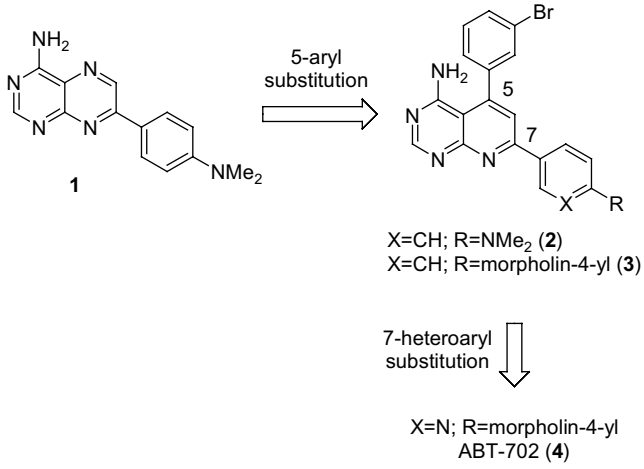


Chart 1. Development of HTS hit **1** to ABT-702 (**4**).

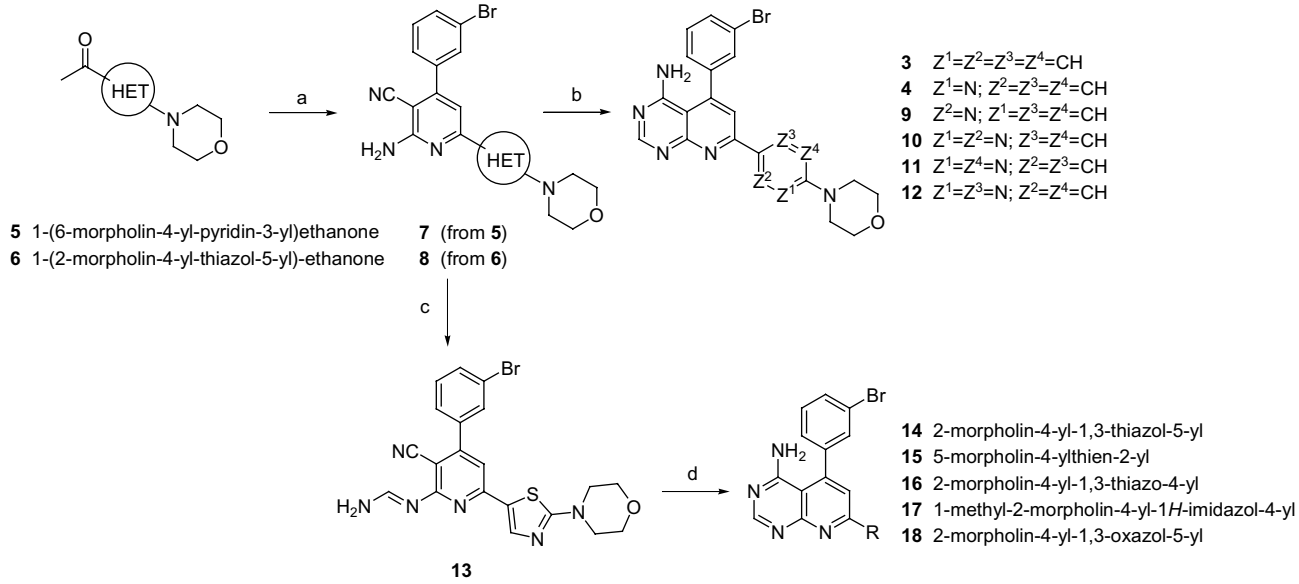
(ADA). AK is a ubiquitous enzyme that is primarily responsible for the intracellular phosphorylation of ADO to AMP.[5] Inhibition of AK, as compared to inhibition of ADA, was shown to produce higher levels of endogenous ADO release from nervous tissue and enhanced anti-convulsant and anti-nociceptive effects in animal models.[6]

Previous strategies from our laboratories[7] and others[8–22] have focused on the identification of nucleoside-like inhibitors. Recently, high-throughput screening of Abbott proprietary compounds identified a pteridine lead structure, **1** (Chart 1). Optimization[23,24] of this structure led to the conclusion that potent compounds of this series contained a substituted aryl ring at C(5) on the pyridopyrimidine ring core. The discovery of **2**[23] represented one of the early, potent, non-nucleoside inhibitors[25–27] of AK (IC_{50} = 5 nM (cytosolic); 167 nM (intact cell)), however, it displayed weak antihyperalgesic efficacy in vivo (ED_{50} = 30 μmol/kg, ip). Further optimization resulted in analog **3** with a morpholine residue located in the 4′-position. This analog potently inhibited AK (IC_{50} = 1.7 nM (cytosolic); 112 nM (intact cell)) and, in addition, showed improved thermal hyperalgesia efficacy in vivo (ED_{50} = 8 μmol/kg, ip), but lacked oral activity (data not shown). Incorporation of a morpholine substituted pyridine ring at C(7)[23,28,29] resulted in the discovery of ABT-702 (**4**) as the first, non-nucleoside, inhibitor of AK containing a 4-amino-5-bromophenyl-7-(4-aminosubstituted pyridyl)pyrido[2,3-*d*]pyrimidine scaffold.[24] ABT-702 is orally active to reduce pain and inflammation in animal models.[28,29] Expansion of the morpholine residue to include other amino groups revealed a significant effect on oral bioavailability[30] as well as locomotor side effects.[31] ABT-702, however, was found to be clastogenic in an in vitro Chinese Hamster micronucleus assay.[32] In the current report, we wish to report new data associated with changes in the heteroaromatic ring at the C(7) position. Our initial goal was to observe the influence of five- and six-membered heterocyclic ring replacements at C(7) on in vitro potency, in vivo efficacy and pharmacokinetic characteristics. Unexpectedly, we observed an influence on mutagenic side effects (Ames assay) depending on the placement of nitrogen atoms in the heteroaromatic ring.

2. Results

2.1. Chemistry

The synthesis of **4**[23] and other pyridopyrimidine AK inhibitors are shown in Scheme 1. Condensation of 2-(3-bromobenzylidene)malononitrile[33,34] with ketone, **5** provided pyridine ring, **7**.[35] Completion of the pyridopyrimidine ring core followed one of the two methods. In the first method, the pyridine ring was refluxed with

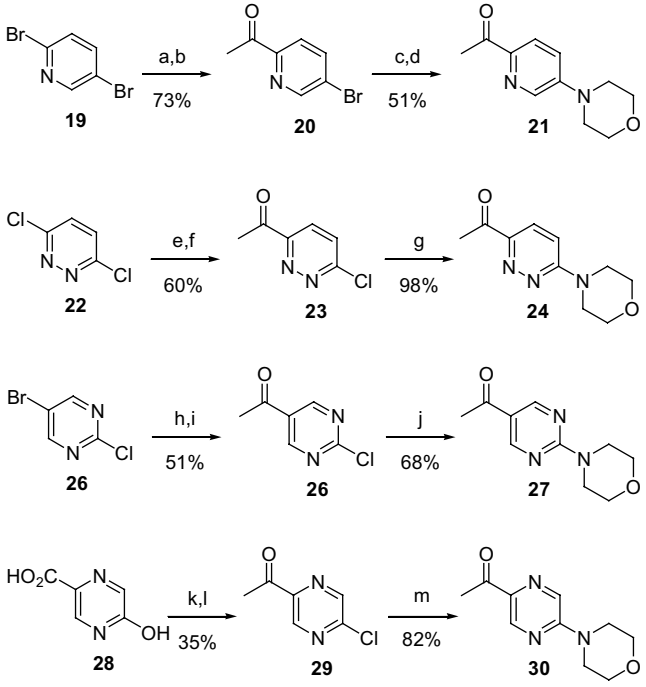


Scheme 1. Reagents and conditions: (a) 2-(3-bromobenzylidene)malononitrile, NH_4OAc, DCE, reflux; (b) formamide, 180 °C; (c) $CH(OEt)_3$, NH_4SO_4, reflux; NH_3, EtOH, rt; (d) *o*-DCB, reflux.

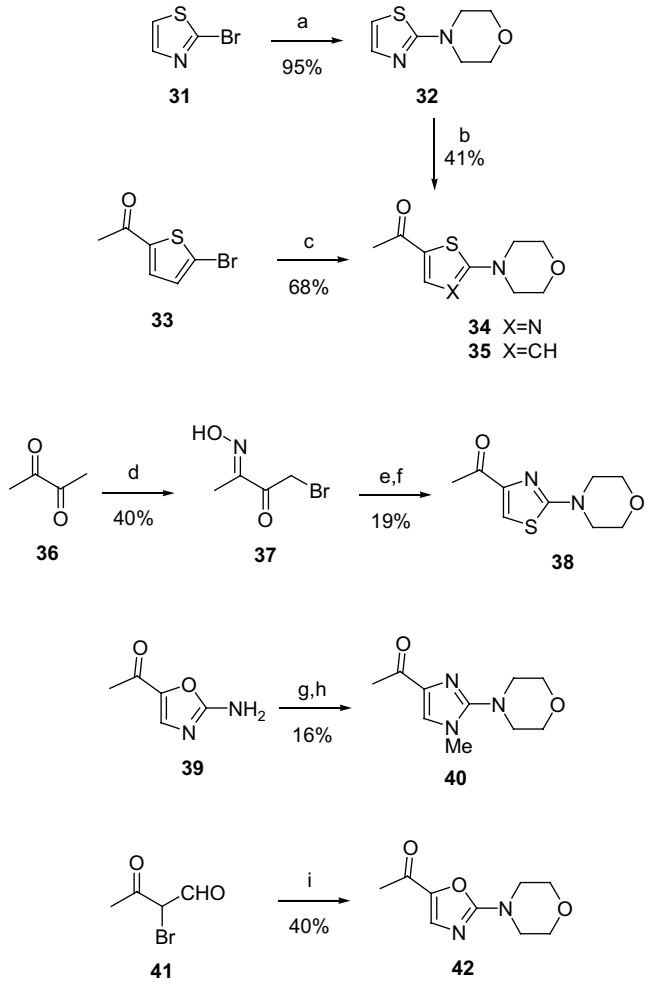
formamide to form the desired pyridopyrimidine ring analogs **3**, **4**, and **9**–**12**.[36] Some analogs, however, did not cyclize efficiently with formamide and were thus subjected to a slightly longer, two-step sequence. First, amidine, **13** was constructed by the treatment of **8** with triethylorthoformate followed by reaction with ammonia. The amidine was then heated in *o*-dichlorobenzene to effect the ring closure and provide the desired pyridopyrimidines, **14**–**18**.[37] Syntheses of all required ketones of structure type **5** and **6** are delineated in Schemes 2 and 3.

Ketone **21** was obtained from dibromide **19** (Scheme 2) by a palladium coupling with trimethylsilyl acetylene utilizing Sonogashira's protocol[38] to give the desired acetylenic intermediate. The acetylene was hydrolyzed under acidic conditions to give bromo ketone **20**, which was followed by protection as the dioxolane. The resulting bromide was reacted with morpholine in a palladium catalyzed coupling[39,40] and subsequently reacted under acidic conditions to give ketone, **21**. Ketone **24** was available from dichloropyridazine **22**. Palladium-mediated coupling with tributyl(1-ethoxyvinyl)tin followed by acidic hydrolysis gave intermediate chloro ketone (60% yield), which was displaced with morpholine to provide ketone, **24**. Synthesis of pyrimidine ketone, **27**, proceeded from 5-bromo-2-chloropyrimidine[41] through a similar synthetic sequence developed for ketone **24**. Synthesis of pyrazine ketone, **30**, was achieved from hydroxy acid **28** by treatment with thionyl chloride followed by the addition of methyl magnesium malonate and decarboxylation to afford the intermediate chloro ketone, **29**. Formation of **29** was followed by morpholine displacement and proceeded without incident to give **30** in 29% overall yield.

The synthesis of all required five-membered ring heterocyclic ketones are shown in Scheme 3. Thiazole **34** was obtained by the addition of morpholine to 2-bromothiazole followed by metallation with *n*-butyllithium and trapping with acetic anhydride in 39% overall yield. Thiophene ketone, **35**, was available from commercial bromide, **33** by reaction with morpholine at 145 °C in a sealed tube to provide the desired product in 68% yield.



Scheme 2. Reagents and conditions: (a) TMSCCH, $PdCl_2(PPh_3)_2$, CuI, CH_3CN, Et_3N, rt, 1 h, 91%; (b) $Hg(OAc)_2$, H_2SO_4, H_2O, acetone, reflux, 1 h, 80%; (c) $HOCH_2CH_2OH$, *p*-TsOH, PhH, reflux, 3 h, 86%; (d) morpholine, $Pd_2(dba)_3$, BINAP, NaO*t*-Bu, PhMe, reflux, 6 h; 4 M HCl, THF, 59%; (e) tributyl(1-ethoxyvinyl)tin, $Pd_2(dba)_3$, P(2-furyl)$_3$, DMF, 80 °C, 4 h; (f) 1 N HCl, acetone, rt, 2 h; (g) morpholine, EtOH, reflux, 4 h; (h) tributyl(1-ethoxyvinyl)tin, $PdCl_2(PPh_3)_2$, DMF, 70 °C, 2 h, 60%; (i) 1 N HCl, acetone, rt, 18 h; reflux, 1 h, 85%; (j) morpholine, sealed tube, 90 °C, 2 h, 68%; (k) $SOCl_2$, DMF, reflux, 16 h; (l) $MgCl_2$, $CH_2(CO_2Me)_2$, Et_3N, PhMe, rt to 160 °C; (m) morpholine, DMSO, rt, 3 h.



Scheme 3. Reagents and conditions: (a) morpholine, sealed tube, 100 °C, 3 d; (b) *n*-BuLi, THF, −78 °C; Ac_2O, −60 °C, 1 h; (c) morpholine, sealed tube 145 °C; (d) Br_2, hydroxylamine hydrochloride, Na_2CO_3, H_2O; (e) morpholine-4-carbothioic acid amide, EtOH, reflux, 2 h, 99%; (f) H_2SO_4, EtOH, $NaNO_2$, 50 °C, 2 h, 19%; (g) morpholine, H_2O, reflux, 33%; (h) NaH, MeI, THF, rt, 1.5 h, 49%; (i) morpholine-4-carboxylic acid amide, acetone, rt, 1 h; reflux, 1 h.

Formation of thiazole **38** (regioisomer of ketone **34**) was achieved from 2,3-butanedione by treatment with bromine followed by hydroxylamine hydrochloride to give unstable oxime, **37** in 40% yield. Cyclization with morpholine-4-carbothioic acid amide[42] in refluxing EtOH and deprotection provided **38** in 19% yield.[43] Compound **40** was synthesized from known oxazole **39**[44] by, first, displacement and rearrangement with morpholine in 33% yield followed by methylation to give the desired imidazole ketone in 49% yield. Oxazole **42** was available from a cyclization of morpholine-4-carboxylic acid amide[45] and known[46] bromo ketone **41** in refluxing acetone in 46% yield.

2.2. Biology

Compounds were evaluated in vitro for their ability to inhibit cytosolic AK (AK_{cyt}) as well as ADO phosphorylation in intact cells (AK_{cell}).[28] Analgesic activity was measured utilizing the carrageenan-induced thermal hyperalgesia model in rats (thermal hyperalgesia).[29,47] Compounds were also evaluated for mutagenicity in an Ames miniscreen assay using the procedure as described earlier[48] and clastogenicity in a V79 Chinese Hamster lung cell micronucleus assay.[32]

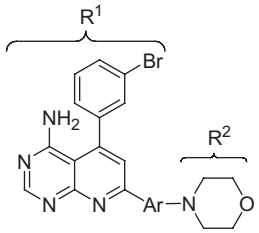
3. Discussion

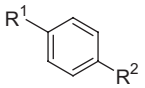
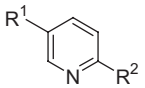
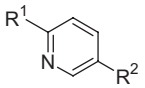
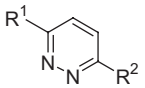
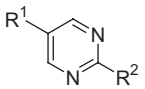
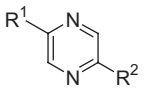
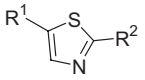
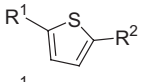
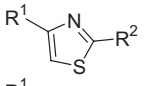
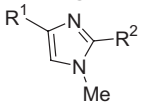
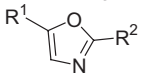
From earlier studies,[23,30] we observed that compounds similar to the phenyl morpholine analog, **3**, and pyridyl morpholine, **4**, display similar in vitro potency but pyridyl analogs, like **4**, show superior thermal hyperalgesia efficacy in vivo (**4**: ED_{50} = 0.6 μmol/kg, ip; ED_{50} = 5 μmol/kg, po). Two conclusions were drawn from these earlier studies. First, the pyridine ring in **4** is critical for bioavailability and oral activity. Second, in vitro potency was not greatly influenced by changes at the 4′-amino position on the pyridine ring; a fact that is contrary to the observation at C(5).[24,49]

Extension (Table 1) to other six-membered heteroaromatic rings (**9**–**12**) at C(7) showed that all were equipotent (AK_{cyt}) with the phenyl analog, **3**, and the pyridine compound, **4** in inhibiting AK. Inhibition of AK_{cell} showed the same trend as in the purified enzyme assay, however, the absolute activity values were about 10 times less potent. The factors contributing to the lack of cell penetration for these AK inhibitors are not well understood[23,24,30,31,49] and does not seem to be confined to the 5,7-substituted pyrido[2,3-*d*]pyrimidine scaffold.[25,50] To expand the scope of the 7-heteroaryl compounds, a series of five-membered ring analogs were completed and tested.

While changes in the spatial orientation of the morpholine ring relative to the 7-aryl substituent are tolerated to some degree (**18** vs **4**), clearly, some five-membered heterocyclic analogs (**15**–**17**) show reduced potency (AK_{cyt}). Additionally, the bioisosteres **14** (to **4**) and **15** (to **3**) exhibit an interesting trend depending on nitrogen position. Thiazole **14** is nearly equipotent (AK_{cyt} IC_{50} = 12 nM) with pyridine **4** but thiophene **15** is less active (AK_{cyt} IC_{50} = 86 nM) than the phenyl analog, **3**. Additionally, it appears that if the nitrogen atom is in a favorable position within the five-membered ring, other heteroatoms are tolerated. For example, if the sulfur atom in **14** is replaced with oxygen, the activity of the oxazole analog **18** (AK_{cyt} IC_{50} = 19 nM) was retained. Thus, contrary to the six-membered ring heterocycles, in vitro potency in five-membered ring heterocycles seems to be influenced by both the position of the morpholine ring and the placement of the heteroatoms within the ring.

Table 1. In vitro characterization of AK inhibitors **3**, **4**, **9**–**12**, **14**–**18** in cytosolic and intact cell assays



Compound	Ar	AK inhibition (IC_{50})[a]	
		Cytosolic	Intact cell
3		2 ± 0.8	112 ± 61
4		2 ± 0.5	50 ± 8
9		14 ± 30	75 ± 25
10		3 ± 2	30 ± 10
11		6 ± 2	79 ± 28
12		4 ± 2	37 ± 25
14		12 ± 4	127 ± 73
15		86 ± 18	105 ± 14
16		55 ± 19	81 ± 2
17		94 ± 30	785[b]
18		19 ± 3	159 ± 10

[a] Mean values for inhibitors (IC_{50} in nM) calculated from at least three determinations ± standard error of the mean (SEM). Inhibition of AK and ADO phosphorylation assays as described by Jarvis et al.[28]
[b] n = 1.

Table 2. In vivo characterization of AK inhibitors **3**, **4**, **9**–**12**, **14**–**16**, and **18**

Compound	Thermal hyperalgesia[a], ED_{50}[b]	Formalin[a], ED_{50}[b]
3	8	10
4	0.6	10
9	8	8
10	3	30
11	5	>30
12	20	>30
14	3	nd[c]
15	>10	nd
16	25	nd
18	>10	nd

[a] Values represent compounds administered by ip injection.
[b] ED_{50} values (in μmol/kg) reported for compounds for which a complete dose-response relationship was obtained with at least six animals (rats) at each dose, $p < 0.05$.
[c] nd = not determined.

The effect on AK inhibition in intact cells with these five-membered ring heterocyclic analogs was also more pronounced compared to the six-membered ring counterparts. The thiazole **14**, imidazole **17** and oxazole **18** were approximately 10 times less potent in AK_{cell} compared to their activity in the AK_{cyt} assay. The thiazole regioisomer, **16**, and thiophene **15** analogs, however, were nearly equipotent in both assays. These results suggest that the presence of the nitrogen atom is not enough to explain the increased cell penetration and that the position of the heteroatom may also be a requisite for potent AK_{cell} inhibition.

The effect of the five- and six-membered heterocycle modifications on in vivo efficacy is shown in Table 2. Earlier studies showed that the C(7) modification of phenyl compound, **3** to the pyridine analog, **4**, had a favorable impact on in vivo efficacy in the thermal hyperalgesia model when administered by intraperitoneal (ip) injection. This could be attributed to the 2-fold increase in AK_{cell} going from **3** to **4**. Further heterocyclic modifications do not seem to provide any observable increase in efficacy. In fact, several of these analogs display decreased activity (**15**, **16**, and **18**) after ip administration. We suspected that the pharmacokinetics associated with each analog might be responsible for the observed trends in the in vivo efficacy. Table 3 shows the intravenous PK profile for analogs **4**, **10**, and **12**. The decreased in vivo activity of compounds **10** and **12** (compared to **4**) could be attributed with their respective plasma clearance values (Table 3).

Evaluation of these non-nucleoside AK inhibitors for mutagenicity (Table 4) revealed a clear signal for the 7-phenyl derivatives, such as **3**, in the bacterial reverse mutation assay (Ames assay)[48] after an S-9 mix[51] activation. The introduction of a nitrogen atom and its position in the aromatic ring were found to play a key role in the Ames toxicity. The 3′-pyridine analog, **4**, displays no mutagenic activity after S-9 mix activation. If, however, the nitrogen is moved to the 2′-position (e.g., **9**), mutagenicity is observed. It appears that a nitrogen atom in the 3′ position is critical to avoid mutagenic activity. Diazines **10** and **12** contain a 3′ nitrogen and display no mutagenic activity even after S-9 mix activation. The only five-membered ring heterocycle that was evaluated in this Ames assay was the 3′-pyridyl bioisostere, thiazole **14**. It, also, displayed no mutagenic side effects. To further expand on this observation, other amino substituted 7-aryl and heteroaryl pyrido pyrimidines were tested for their mutagenicity in the Ames assay (Table 5). Again, the same trend was observed as in the morpholine case. Dimethylamino phenyl analog, **2** and 2′-pyridyl compound, **44** were both found to be mutagenic; while the heteroaryl, dimethylamino analogs, **43**, **45**, and **46** (all with a 3′-nitrogen) were non-mutagenic. Less electron rich nitrogen analogs, such as **47**–**49** and **51** also follow this same general trend.

Table 3. Pharmacokinetic data[a] for AK inhibitors **4**, **10**, and **12**

Compound	$T_{1/2}$	V_β	CL_p
4	0.9	1.0	0.8
10	1.2	3.0	1.7
12	1.2	3.0	1.7

[a] IV dose in rat at 5 μmol/kg. Units: $T_{1/2}$ (h); V_β (L/kg); CL_p (L/h kg).

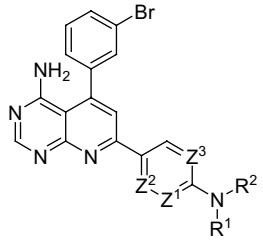
Table 4. Genetic toxicology data for AK inhibitors **3**, **4**, **9**–**12**, and **14**

Compound	Ames bacterial reversion assay[a]		In vitro micronucleus (MN) assay[b]	
	NA[c]	A[d]	NA	A
3	–	+	nd[e]	nd
4	–	–	+	nd
9	–	+	–	–
10	–	–	–	–
11	nd	nd	–	–
12	–	–	–	–
14	–	–	+	nd

[a] Ames miniscreen was performed at 1–10,000 μg compound/plate as described.[48,60] Positive responses are defined as at least 2-fold increases over background colony counts for TA-98 and TA-100 and at least 3-fold increases over background for TA-1535 and TA-1538.
[b] V79 Chinese hamster lung cell micronucleus assay was performed as described earlier.[32] Positive micronucleus responses are defined as at least 3-fold increase in percent micronucleated cells relative to solvent controls.
[c] NA = with no S-9 mix activation.
[d] A = with S-9 mix activation.
[e] nd = not determined.

In view of the need for S-9 activation in order to establish mutagenic side effects of some of these analogs, metabolism studies were carried out on 3′-pyridyl analog, **4** as well as phenyl derivative, **2** in an attempt to identify metabolites that could account for the observed toxicity. Results from these studies revealed metabolism primarily at the amino portion (morpholine or dimethylamino) of the molecule, which was shown to be non-mutagenic (e.g., **48**, Table 5). No additional metabolites were observed with **2** that could account for the mutagenicity. At this time, it is not clear how the 3′-nitrogen influences the parent molecule to eliminate the mutagenicity. Some explanations could involve an effect on the conformation of the 7-heteroaryl ring relative to the pyridopyrimidine core through some chelation

Table 5. Genetic toxicology data for AK inhibitors **2** and **43–52**



Compound	R^1	R^2	Z^1	Z^2	Z^3	Ames[a]	MN[b]	AK inhibition (IC_{50})[c]	
								Cytosolic	Intact cell
2	Me	Me	CH	CH	CH	+	–	6	47
43	Me	Me	N	CH	CH	–	+	1	47
44	Me	Me	CH	N	CH	+	–	3	73
45	Me	Me	N	N	CH	–	–	1	48
46	Me	Me	N	CH	N	–	+	6	80
47	Me	CHO	CH	CH	CH	+	+	2	77
48	Me	CHO	N	CH	CH	–	+	2	68
49	Me	CHO	CMe	CH	CH	+	+	10	103
50	Me	H	CH	CH	CH	+	+	3	74
51	CHO	H	CH	CH	CH	+	+	4	175
52	H	H	CH	CH	CH	+	–	8	275

[a] Assay with S-9 mix activation.
[b] Assay without S-9 mix activation.
[c] Mean values for inhibitors (IC_{50} in nM) calculated from at least three determinations (standard error of the mean (SEM) $\leqslant$10%).

involving a nitrogen of the 7-heteroaryl substituent and the 8-amino nitrogen of the pyridopyrimidine core with some other ion present. The lack of genotoxicity could also be the result of specific electronic factors attributed to the electron deficient nature of the 3′-substituted heterocycle. Further expansion of this series will be necessary in order to more fully understand these observations.

As part of further toxicological evaluation, compounds were also screened in an in vitro micronucleus (MN) assay.[32] For the array of compounds in Tables 3 and 4, clastogenicity was observed for both aryl and six-membered heteroaryl analogs, but not in all. The five-membered ring heterocycles, like **14**, also do not seem to offer an advantage in eliminating clastogenicity. The conclusion from these studies is that there does not seem to be a clear trend (as in the Ames assay) to predict clastogenic activity in this series. An important observation, however, is that these clastogenic side effects do not appear related to the mechanism of AK inhibition.

4. Conclusion

During the course of these studies, we were able to identify several 7-heteroaryl analogs that potently inhibited AK. These new compounds had similar in vitro profiles, however, some derivatives displayed lowered in vivo efficacy (ip), compared to **4**, to reduce nociception in an animal model of inflammatory hyperalgesia. Initial pharmacokinetic analysis seems to suggest that these differences (of **10** and **12**, for example) may be explained by the increased clearance values in rat. Substitution of the heterocyclic 7-substituent was found to influence genotoxicity. Placement of nitrogen in the 3′ position seems to eliminate mutagenic side effects although the role of this substitution pattern and its effect on toxicity is unclear. Clastogenetic effects did not seem to follow a trend as observed in the Ames assay. The genotoxicity, however, does not appear to be inherent to this series or mechanism based.

5. Experimental

5.1. General procedures

General experimental procedures and techniques are reported elsewhere.[52]

5.2. Chemical procedures

5.2.1. 1-[6-(4-Morpholinyl)-3-pyridinyl]ethanone (5). A solution of $MgCl_2$ (20.4 g, 0.214 mol) in toluene (220 mL) at rt was treated with Et_3N (101 mL, 0.722 mol) followed by dimethyl malonate (41.2 mL, 0.361 mol). After 1 h, 6-chloronicotinoyl chloride (52.8 g, 0.300 mol) was added as a solution in toluene (40 mL) and stirring continued for 20 min. The reaction was quenched with concentrated HCl and H_2O. The aqueous layer was extracted with Et_2O, the organic phases combined, dried, and concentrated. The resulting white solid was taken up in DMSO (350 mL) and H_2O (20 mL) and the reaction heated to 150 °C for 2 h. The mixture was cooled to rt, H_2O added, and the solid filtered and dried to give 40.2 g (78%) of 1-(6-chloro-3-pyridinyl)ethone as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ 8.94 (dd, 1H, $J = 2.5$, 0.7), 8.20 (dd, 1H, $J = 8.4$, 2.6), 7.45 (dd, 1H, $J = 8.1$, 0.7), 2.62 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 195.3, 155.6, 150.1, 138.0, 131.1, 124.5, 26.6. MS (DCI/NH_3) *m/z* 173 $(M+NH_4)^+$, 156 $(M+H)^+$.

A suspension of 1-(6-chloro-3-pyridinyl)ethone (40.0 g, 0.257 mol) in EtOH (300 mL) was treated with morpholine (47.1 mL, 0.540 mol) and the mixture heated to reflux for 4 h. Excess EtOH was removed under reduced pressure and the residue taken up in H_2O and extracted with CH_2Cl_2. The organic layers were combined, washed with 1 N HCl, dried, and concentrated to provide 36.0 g (68%) of **5** as a light tan solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.75 (d, 1H, J = 2.6), 8.03 (dd, 1H, J = 9.2, 2.6), 6.61 (d, 1H, J = 9.2), 3.80 (m, 4H), 3.67 (m, 4H), 2.50 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.1, 160.4, 150.5, 137.1, 123.0, 105.3, 66.4, 44.8, 25.8. MS (DCI/NH_3) *m/z* 207 $(M+H)^+$.

5.2.2. 2-Amino-4-(3-bromophenyl)-6-[2-(4-morpholinyl)-5-pyridyl]nicotinonitrile (7). A suspension of **5** (15.0 g, 72.7 mmol), 2-(3-bromobenzylidene)malononitrile[33,34] (20.3 g, 87.1 mmol) and ammonium acetate (29.7 g, 385 mmol) in 1,2-dichloroethane (150 mL) was heated to reflux for 10 h. The mixture was cooled, filtered, washed with H_2O, and dried to provide 20.3 g (64%) of **7** as a yellow solid. This material was used without further purification in the next step. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.94 (d, 1H, J = 2.0), 8.29 (dd, 1H, J = 9.1, 2.7), 7.86 (dd, 1H, J = 1.7, 1.7), 7.71 (m, 2H), 7.51 (dd, 1H, J = 7.8, 7.8), 7.27 (s, 1H), 6.94 (m, 3H), 3.71 (m, 4H), 3.59 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.7, 159.4, 157.2, 152.7, 147.6, 139.3, 136.2, 132.2, 130.9, 130.7, 127.4, 122.2, 121.8, 117.0, 107.7, 106.1, 85.2, 65.9, 44.7. MS (DCI/NH_3) *m/z* 436/438 $(M+H)^+$.

5.2.3. 5-(3-Bromophenyl)-7-[6-(4-morpholinyl)-3-pyridinyl]pyrido[2,3-*d*]pyrimidin-4-ylamine (4). A suspension of **7** (15.0 g, 34.4 mmol) in formamide (200 mL) was heated to 180 °C for 10 h. The reaction mixture was cooled, H_2O added, and filtered. The solid was purified by silica gel chromatography (first column elution with 10% MeOH–CH_2Cl_2; second column elution with 5% H_2O–CH_3CN) to provide 6 g (38%) of **4** as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.08 (d, 1H, J = 2.4), 8.53 (s, 1H), 8.47 (dd, 1H, J = 9.2, 2.4), 7.85 (m, 2H), 7.78 (m, 1H), 7.56 (m, 2H), 6.98 (d, 1H, J = 9.1), 3.72 (m, 4H), 3.62 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.9, 159.7, 159.6, 158.8, 158.1, 148.0, 147.6, 140.4, 136.5, 132.0, 131.3, 130.9, 127.9, 122.2, 122.1, 119.1, 106.3, 104.5, 65.9, 44.7. HRMS calcd for $C_{22}H_{20}{}^{79}BrN_6O$: 463.0882; observed: 463.0888. Anal. Calcd for $C_{22}H_{19}BrN_6O{\cdot}0.5\ H_2NC(O)H$: C, 55.62; H, 4.25; N, 18.74. Found: C, 55.43; H, 4.27; N, 18.76.

5.2.4. 5-(3-Bromophenyl)-7-[4-(4-morpholinyl)phenyl]pyrido[2,3-*d*]pyrimidin-4-ylamine (3). Following the procedure for **7** substituting 1-[4-(4-morpholinyl)phenyl]-ethanone (Aldrich) for **5** and purification by silica gel chromatography (elution with 15% MeOH–CH_2Cl_2) to give 2-amino-4-(3-bromophenyl)-6-(4-morpholin-4-ylphenyl)nicotinonitrile (14%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (d, 2H, J = 8.8), 7.73 (dd, 1H, J = 1.7, 1.7), 7.61 (m, 2H), 7.39 (dd, 1H, J = 7.8, 7.8), 7.10 (s, 1H), 6.96 (d, 2H, J = 9.2), 5.30 (br s, 2H), 3.88 (m, 4H), 3.28 (m, 4H).

Following the procedure for **4** substituting 2-amino-4-(3-bromophenyl)-6-(4-morpholin-4-ylphenyl)nicotinonitrile for **7** and purification by silica gel chromatography (elution with 15% MeOH–CH_2Cl_2) to provide **3** (36%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.73 (s, 1H), 8.26 (d, 2H, J = 9.2), 7.72 (ddd, 1H, J = 7.1, 2.1, 2.1), 7.68 (m, 1H), 7.61 (s, 1H), 7.44 (m, 2H), 6.98 (d, 2H, J = 8.8), 3.88 (m, 4H), 3.31 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.9, 160.1, 159.8, 158.1, 152.6, 147.4, 140.6, 132.0, 131.3, 130.9, 128.8, 127.9, 127.1, 122.2, 119.4, 114.1, 104.4, 65.9, 47.2. HRMS calcd for $C_{23}H_{20}{}^{79}BrN_5O$: 462.0929; observed: 462.0935. Anal. Calcd for $C_{23}H_{20}BrN_5O$: C, 59.75; H, 4.36; N, 15.14. Found: C, 59.44; H, 4.21; N, 14.98.

5.2.5. 1-(5-Bromo-2-pyridinyl)ethanone (20). A solution of 2,5-dibromopyridine (20.0 g, 84.4 mmol) and trimethylsilyl acetylene (8.84 g, 90.0 mmol) in Et_3N/CH_3CN (1:1, 150 mL) at rt was treated with dichlorobis(triphenylphosphine)palladium(II) ($PdCl_2(PPh_3)_2$; 1.19 g, 1.70 mmol) and CuI (320 mg, 1.70 mmol). After 1 h, the mixture was concentrated and the residue purified by silica gel chromatography (elution with 50% EtOAc–hexanes) to provide 19.5 g (91%) of 5-bromo-2-[(trimethylsilyl)ethynyl]pyridine as a light orange solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (dd, 1H, J = 2.4, 0.6), 8.03 (dd, 1H, J = 8.3, 2.4), 7.48 (dd, 1H, J = 8.2, 0.7), 0.23 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.7, 140.4, 139.2, 128.6, 120.2, 103.2, 95.4, −0.6. MS (DCI/NH_3) *m/z* 254/256 $(M+H)^+$.

A solution of 5-bromo-2-[(trimethylsilyl)ethynyl]pyridine (10.2 g, 40.1 mmol) in acetone/H_2O (8:1, 150 mL) at rt was treated with mercuric acetate (17.9 g, 42.0 mmol). After 15 min, the reaction was treated with 3 M H_2SO_4 (40 mL) and heated to reflux for 1 h. The mixture was cooled, neutralized with 2 M NaOH, and extracted with EtOAc. The organic layers were combined, dried (Na_2SO_4), concentrated, and the residue purified by silica gel chromatography (elution with 50% EtOAc–hexanes) to provide 5.90 g (80%) of **20** as a light green solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86 (d, 1H, J = 1.9), 8.26 (dd, 1H, J = 8.6, 2.5), 7.88 (d, 1H, J = 8.6), 2.63 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 198.7, 151.8, 150.2, 140.4, 125.2, 123.2, 25.8. MS (DCI/NH_3) *m/z* 200/202 $(M+H)^+$.

5.2.6. 1-[5-(4-Morpholinyl)-2-pyridinyl]ethanone (21). A solution of **20** (2.50 g, 13.6 mmol) and ethylene glycol (3.72 g, 60.0 mmol) in benzene (100 mL) was treated with a catalytic amount of *p*-toluenesulfonic acid, fitted with a Dean–Stark condenser and the reaction heated to reflux for 3 h. The mixture was cooled, washed with aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated. The residue was purified by silica gel chromatography (elution with 50% EtOAc–hexanes) to give 2.47 g of 5-bromo-2-(2-methyl-1,3-dioxolan-2-yl)pyridine (86%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69

(dd, 1H, $J = 2.2$, 0.7), 8.06 (dd, 1H, $J = 8.3$, 2.5), 7.51 (dd, 1H, $J = 8.3$, 0.6), 4.03 (m, 2H), 3.85 (m, 2H), 1.63 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.3, 149.6, 139.3, 121.3, 119.6, 107.7, 64.6, 24.4. MS (DCI/NH_3) m/z 244/246 $(M+H)^+$.

A solution of 5-bromo-2-(2-methyl-1,3-dioxolan-2-yl)-pyridine (3.80 g, 17.9 mmol), morpholine (2.80 g, 32.0 mmol), sodium *tert*-butoxide (3.46 g, 36.0 mmol), $Pd_2(dba)_3$ (275 mg, 0.300 mmol), and BINAP (373 mg, 0.599 mmol) in toluene (20 mL) was heated to reflux for 6 h. The reaction mixture was cooled, poured into aqueous $NaHCO_3$ and extracted with CH_2Cl_2, and concentrated. The residue was taken up in THF (100 mL) and treated with 4 M HCl (10 mL) and stirred for 2 h at rt. The mixture was neutralized with 2 M NaOH and extracted with CH_2Cl_2. The organic layers were combined, concentrated, and the residue purified by silica gel chromatography to provide **21** in 59% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, 1H, $J = 2.7$), 7.94 (d, 1H, $J = 8.9$), 7.17 (dd, 1H, $J = 8.9$, 2.7), 3.87 (m, 4H), 3.34 (m, 4H), 2.64 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.3, 148.4, 144.4, 134.8, 122.5, 119.6, 66.0, 46.7, 25.1. MS (DCI/NH_3) m/z 207 $(M+H)^+$.

5.2.7. 5-(3-Bromophenyl)-7-[5-(4-morpholinyl)-2-pyridinyl]pyrido[2,3-*d*]pyrimidin-4-ylamine (9). Following the procedure of compound **7** substituting **21** for **5** provided 2-amino-4-(3-bromophenyl)-6-[5-(4-morpholinyl)-2-pyridyl]nicotinonitrile in 71% yield. Material was used directly in the next reaction.

Following the procedure for compound **4** substituting 2-amino-4-(3-bromophenyl)-6-[5-(4-morpholinyl)-2-pyridyl]nicotinonitrile for **5** and purification by silica gel chromatography (gradient elution with CH_2Cl_2 to 10% MeOH–CH_2Cl_2) to provide 1.22 g (52%) of **9**. This material was converted into the HCl salt. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.09 (br d, 1H), 8.82 (s, 1H), 8.35 (d, 1H, $J = 2.5$), 8.30 (d, 1H, $J = 8.9$), 8.24 (s, 1H), 7.82 (s, 1H), 7.77 (d, 1H, $J = 8.0$), 7.56 (m, 3H), 7.34 (s, 1H), 3.73 (m, 4H), 3.38 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.5, 158.5, 152.1, 150.0, 149.1, 148.1, 139.0, 137.8, 134.1, 133.1, 131.5, 131.5, 128.1, 124.1, 122.6, 122.2, 122.1, 104.1, 65.7, 46.3. MS (DCI/NH_3) m/z 207 $(M+H)^+$. Anal. Calcd for $C_{22}H_{19}BrN_6O{\cdot}3$ HCl: C, 46.14; H, 3.87; N, 14.67. Found: C, 46.34; H, 4.19; N, 14.30.

5.2.8. 1-(6-Chloro-3-pyridazinyl)ethanone (23). A solution of ethoxyvinyltin (21.5 g, 59.6 mmol), tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$, 670 mg, 0.73 mmol), tri-2-furylphosphine (670 mg, 2.85 mmol), and 3,6-dichloropyridazine (7.5 g, 50.3 mmol) was stirred in DMF (60 mL) at 80 °C for 4 h. Reaction mixture was poured into 100 mL of aqueous KF and Et_2O (200 mL) and stirred for 30 min. The solids were filtered through a pad of Celite® and the filtrate concentrated. The residue was taken up in acetone (270 mL) and treated with 1 N HCl (50 mL) at rt. After 2 h, the mixture was transferred to a separatory funnel and extracted with Et_2O (×3). The organic phases were combined, washed with aqueous $NaHCO_3$, H_2O, brine; dried (Na_2SO_4) and concentrated. The residue was purified by silica gel chromatography (elution with 20% EtOAc–hexanes) to provide 4.71 g (60%) of **23** as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (d, 1H, $J = 8.8$), 7.67 (d, 1H, $J = 8.8$), 2.88 (s, 3H). MS (DCI/NH_3) m/z 174 $(M+NH_4)^+$, 157 $(M+H)^+$.

5.2.9. 1-[6-(4-Morpholinyl)-3-pyridazinyl]ethanone (24). Following the procedure for compound **5** substituting **23** for 1-(6-chloro-3-pyridinyl)ethone to give **24** in 98% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, 1H, $J = 9.6$), 6.90 (d, 1H, $J = 9.5$), 3.86 (m, 4H), 3.80 (m, 4H), 2.78 (s, 3H). MS (ESI) m/z 208 $(M+H)^+$.

5.2.10. 5-(3-Bromophenyl)-7-[6-(4-morpholinyl)-3-pyridazinyl]pyrido[2,3-*d*]pyrimidin-4-ylamine (10). Following the procedure for compound **7** substituting **24** for **5** to afford 2-amino-4-(3-bromophenyl)-6-[6-(4-morpholinyl)-3-pyridazinyl]nicotinonitrile (45%) as a brown powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (d, 1H, $J = 9.5$), 8.10 (s, 1H), 7.82 (dd, 1H, $J = 1.9$, 1.9), 7.64 (m, 2H), 7.39 (dd, 1H, $J = 7.7$, 7.7), 6.98 (d, 1H, $J = 9.6$), 5.33 (br s, 2H), 3.88 (m, 4H), 3.78 (m, 4H). MS (DCI/NH_3) m/z 437/439 $(M+H)^+$.

Following the procedure for **4** substituting 2-amino-4-(3-bromophenyl)-6-[6-(4-morpholinyl)-3-pyridazinyl]-nicotinonitrile for **7** to provide **10** (79%) as a brown powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.59 (s, 1H), 8.41 (d, 1H, $J = 9.9$), 8.26 (s, 1H), 7.85 (s, 1H), 7.82 (m, 1H), 7.53 (m, 3H), 3.75 (m, 4H), 3.73 (m, 4H). HRMS calcd for $C_{21}H_{18}BrN_7O$: 464.0829; observed: 464.0829. Portion of this sample converted to the HCl salt for biological testing. Anal. Calcd for $C_{21}H_{18}BrN_7O{\cdot}2.0$ HCl: C, 47.92; H, 3.77; N, 18.63. Found: C, 47.77; H, 3.76; N, 18.97.

5.2.11. 1-(2-Chloro-5-pyrimidinyl)ethanone (26). A solution of 2-chloro-5-bromopyrimidine[41] (1.93 g, 10.0 mmol) and tributyl(1-ethoxyvinyl)tin (3.71 mL, 11.0 mmol) in DMF (20 mL) at rt was added $PdCl_2(PPh_3)_2$ (140 mg) and the mixture heated to 70 °C for 2 h. The reaction was cooled and aqueous KF added and stirring continued overnight. The mixture was poured into H_2O and extracted with Et_2O. The organic phases were combined, concentrated, and purified by silica gel chromatography (elution with 50% hexane–CH_2Cl_2) to provide 2-chloro-5-(1-ethoxyvinyl)pyrimidine in 60% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.81 (s, 2H), 4.73 (d, 1H, $J = 3.3$), 4.40 (d, 1H, $J = 3.3$), 3.95 (q, 2H, $J = 6.9$), 1.43 (t, 3H, $J = 7.0$).

A solution of 2-chloro-5-(1-ethoxyvinyl)pyrimidine (1.10 g, 5.96 mmol) in acetone (20 mL) was added 1 N HCl (5 mL) and the mixture stirred at rt for 18 h then heated to reflux for 1 h. The reaction was cooled, extracted with Et_2O, dried, and concentrated to give **26** (85%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.11 (s, 2H), 2.66 (s, 3H).

5.2.12. 1-[2-(4-Morpholinyl)-5-pyrimidinyl]ethanone (27). A mixture of ketone **26** and morpholine was combined into a sealed tube and heated to 90 °C for 2 h. The

mixture was cooled, concentrated, and the residue purified by silica gel chromatography (elution with CH_2Cl_2) to provide **27** in 68% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.84 (s, 2H), 3.95 (m, 4H), 3.77 (m, 4H), 2.48 (s, 3H). MS (DCI/NH_3) *m*/*z* 208 $(M+H)^+$.

5.2.13. 5-(3-Bromophenyl)-7-[2-(4-morpholinyl)-5-pyrimidinyl]pyrido[2,3-*d*]pyrimidin-4-ylamine (11). Following the procedure for compound **7** substituting **27** for **5** to provide 2-amino-4-(3-bromophenyl)-6-[2-(4-morpholinyl)-5-pyrimidinyl]nicotinonitrile in 38% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.11 (s, 2H), 7.86 (m, 1H), 7.70 (m, 2H), 7.52 (dd, 1H, J = 7.8), 7.30 (s, 1H), 7.02 (br s, 2H), 3.82 (m, 4H), 3.70 (m, 4H). MS (DCI/NH_3) *m*/*z* 437/439 $(M+H)^+$.

Following the procedure for compound **4** substituting 2-amino-4-(3-bromophenyl)-6-[2-(4-morpholinyl)-5-pyrimidinyl]nicotinonitrile for **7** and purification by silica gel chromatography (elution with 2% MeOH–CH_2Cl_2) to provide **11** in 10% yield. This material was converted to the HCl salt. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.32 (s, 1H), 8.88 (s, 1H), 8.19 (s, 1H), 7.94 (m, 1H), 7.82 (m, 1H), 7.60 (m, 3H), 3.88 (m, 4H), 3.70 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6/DCl) δ 162.8, 160.6, 158.1, 157.6, 151.5, 149.5, 148.6, 137.0, 132.5, 131.3, 130.8, 127.8, 122.0, 121.0, 117.2, 102.7, 65.5, 43.6. MS (DCI/NH_3) *m*/*z* 464/466 $(M+H)^+$. Anal. Calcd for $C_{21}H_{18}BrN_7O\cdot 1.2$ HCl: C, 49.64; H, 3.81; N, 19.30. Found: C, 49.69; H, 3.76; N, 18.90.

5.2.14. 1-[5-(4-Morpholinyl)-2-pyrazinyl]ethanone (30). A solution of 5-hydroxypyrazine-2-carboxylic acid (Lonza; 6.70 g, 47.8 mmol) in thionyl chloride (40 mL) was treated with DMF (0.4 mL) and the reaction warmed to reflux for 16 h. The volatiles were removed under reduced pressure and the residue was used directly in the next reaction.

A suspension of magnesium chloride (2.67 g, 28.0 mmol), dimethyl malonate (7.70 g, 58.3 mmol), and Et_3N (11.72 g, 115.8 mmol) in toluene (70 mL) was stirred for 1 h at rt. To this suspension was slowly added the acid chloride in 50 mL of toluene and stirring continued for 2 h. The reaction was quenched with H_2O, the acidity adjusted to pH 5 and the mixture extracted with CH_2Cl_2. The extracts were combined, concentrated, dissolved in DMSO (125 mL), H_2O (3 mL), and the mixture heated to 160 °C for 90 min. The reaction was cooled and portioned between H_2O and CH_2Cl_2. The organic phase was dried (Na_2SO_4), concentrated, and purified by silica gel chromatography to provide **29** (35%), which was used directly in the next reaction.

A solution of **29** (2.65 g, 16.9 mmol) in DMSO (30 mL) was treated with morpholine (3.48 g, 39.9 mmol) and stirred at rt for 3 h. The mixture was poured into H_2O (100 mL) and extracted with CH_2Cl_2. The organic layers were combined, dried, concentrated, and the residue purified by silica gel chromatography (elution with 50% hexanes–EtOAc) to give 2.87 g (82%) of **30**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.79 (d, 1H, J = 1.5), 8.05 (d, 1H, J = 1.5), 3.83 (m, 4H), 3.77 (m, 4H), 2.60 (s, 3H). MS (DCI/NH_3) *m*/*z* 208 $(M+H)^+$.

5.2.15. 5-(3-Bromophenyl)-7-[5-(4-morpholinyl)-2-pyrazinyl]pyrido[2,3-*d*]pyrimidin-4-ylamine (12). Following the procedure for compound **7** substituting **30** for **5** provided 2-amino-4-(3-bromophenyl)-6-[5-(4-morpholinyl)-2-pyrazinyl]nicotinonitrile in 61% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.97 (d, 1H, J = 1.1), 8.39 (d, 1H, J = 1.4), 7.82 (dd, 1H, J = 2.0, 2.0), 7.75 (m, 1H), 7.63 (m, 1H), 7.52 (dd, 1H, J = 8.1, 8.1), 7.41 (s, 1H), 7.07 (br s, 2H), 3.72 (m, 4H), 3.68 (m, 4H). MS (DCI/NH_3) *m*/*z* 437/439 $(M+H)^+$.

Following the procedure for compound **4** substituting 2-amino-4-(3-bromophenyl)-6-[5-(4-morpholinyl)-2-pyrazinyl]nicotinonitrile for **7** and purification by silica gel chromatography (elution with 10% MeOH–CH_2Cl_2) provided **12**. This material was converted to the HCl salt. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.19 (d, 1H, J = 1.1), 8.56 (s, 1H), 8.42 (d, 1H, J = 1.1), 7.98 (s, 1H), 7.81 (m, 2H), 7.57 (m, 2H), 3.73 (m, 8H). MS (DCI/NH_3) *m*/*z* 464/466 $(M+H)^+$. Anal. Calcd for $C_{21}H_{18}BrN_7O\cdot 1.47$ HCl: C, 48.70; H, 3.79; N, 18.93. Found: C, 49.07; H, 3.82; N, 18.55.

5.2.16. 1-[2-(4-Morpholinyl)-1,3-thiazol-5-yl]ethanone (34). 2-Bromothiazole (12.63 g, 77.00 mmol) in morpholine (30.0 mL) was sealed into a tube and heated to 100 °C for 3 days. The mixture was cooled, partitioned between H_2O and CH_2Cl_2, the layers separated, and the organic phase dried (Na_2SO_4) and concentrated to afford 12.5 g (95%) of 2-morpholinothiazole[53] as a brown oil. Material was used directly in the next reaction. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.19 (d, 1H, J = 3.7), 6.86 (d, 1H, J = 3.7), 3.71 (m, 4H), 3.37 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.0, 139.7, 108.5, 65.7, 48.7. MS (DCI/NH_3) *m*/*z* 171 $(M+H)^+$.

A solution of 2-morpholinothiazole (7.20 g, 42.3 mmol) in THF (80 mL) at −78 °C was treated with *n*-BuLi (2 M in hexanes, 23.5 mL). After 30 min, the reaction mixture was transferred via cannula to a solution of acetic anhydride (10 mL) in THF (50 mL) at −60 °C and stirred for 1 h. The slurry was then warmed to room temperature for an additional 30 min, quenched with saturated sodium bicarbonate, and extracted with Et_2O. The organic phase was dried (Na_2SO_4), concentrated, and purified by silica gel chromatography (elution with 50% CH_2Cl_2–EtOAc) to provide 3.70 g (41%) of **34**.[54] ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (s, 1H), 3.71 (m, 4H), 3.53 (m, 4H), 2.39 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 188.7, 174.8, 149.2, 128.5, 65.3, 48.0, 25.7. MS (DCI/NH_3) *m*/*z* 213 $(M+H)^+$.

5.2.17. 2-Amino-4-(3-bromophenyl)-6-[2-(4-morpholinyl)-1,3-thiazol-5-yl]nicotinonitrile (8). A slurry of **34** (4.91 g, 23.1 mmol) and ammonium acetate (9.75 g, 127 mmol) in 1,2-dichloroethane (50 mL) was treated with 2-(3-bromobenzylidene)malononitrile[33,34] (10.78 g, 46.3 mmol) and the mixture heated to reflux overnight. The solution was cooled to room temperature, hexanes (50 mL) added, and stirring continued for 3 h. The solid was

collected by filtration, washed with methanol, and dried to provide 4.57 g (45%) of **8** as an orange solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.81 (m, 1H), 7.72 (m, 1H), 7.63 (m, 1H), 7.50 (dd, 1H, J = 7.8, 7.8), 7.21 (s, 1H), 6.92 (br s, 2H), 3.73 (m, 4H), 3.49 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6/DCl) δ 170.3, 160.5, 153.1, 151.4, 138.8, 133.3, 132.8, 131.1, 131.0, 127.7, 125.8, 122.1, 116.7, 107.6, 86.1, 65.1, 49.2. MS (DCI/NH_3) m/z 442/444 $(M+H)^+$.

5.2.18. *N*-[4-(3-Bromophenyl)-3-cyano-6-(2-morpholin-4-yl-thiazol-5-yl)-pyridin-2-yl]-formamidine (13). A solution of **8** (1.50 g, 3.39 mmol) and triethylorthoformate (34 mL) with a catalytic amount of ammonium sulfate was heated to reflux for 6 h. The dark mixture was cooled, ammonia in EtOH (2 M, 70 mL) added, and the mixture stirred overnight. The solid product was collected by filtration and dried to provide 1.17 g (73%) of **13** as a yellow solid. MS (DCI/NH_3) m/z 469/471 $(M+H)^+$.

5.2.19. 5-(3-Bromophenyl)-7-[2-(4-morpholinyl)-1,3-thiazol-5-yl]pyrido[2,3-*d*]pyrimidin-4-ylamine (14). A solution of **13** (2.49 g, 5.30 mmol) in *o*-dichlorobenzene (15 mL) was heated to reflux overnight. The reaction mixture was cooled to room temperature, the solid collected by filtration, and purified by silica gel chromatography (elution with 3% MeOH–CH_2Cl_2) to provide 1.06 g (43%) of **14** as a product as a yellow solid. mp: >280 °C. ^{1}H NMR (400 MHz, DMSO-d_6/DCl) δ 9.86 (br s, 1H), 8.77 (s, 1H), 8.49 (s, 1H), 8.03 (s, 1H), 7.86 (s, 1H), 7.83 (m, 1H), 7.58 (m, 2H), 6.96 (br s, 1H), 3.77 (m, 4H), 3.60 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6/DCl) δ 173.6, 162.8, 156.5, 151.5, 150.2, 147.5, 145.9, 137.7, 132.8, 131.4, 131.2, 128.0, 124.8, 122.4, 120.9, 102.0, 65.1, 48.0. MS (DCI/NH_3) m/z 469/471 $(M+H)^+$. Anal. Calcd for $C_{20}H_{17}BrN_6OS$: C, 51.18; H, 3.65; N, 17.91. Found: C, 51.15; H, 3.76; N, 17.83.

5.2.20. 1-[5-(4-Morpholinyl)-2-thienyl]ethanone (35). A solution of 5-acetyl-2-bromothiophene (8.01 g, 39.1 mmol) and morpholine (16 mL) was heated to 145 °C overnight. The reaction mixture was cooled and partitioned between H_2O and CH_2Cl_2. The organic phase was concentrated and purified by silica gel chromatography (elution with 30% hexane–EtOAc) to give 5.61 g (68%) of **35**.[54] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, 1H, J = 4.4), 6.06 (d, 1H, J = 4.4), 3.84 (m, 4H), 3.28 (m, 4H), 2.46 (s, 3H). MS (DCI/NH_3) m/z 212 $(M+H)^+$.

5.2.21. 5-(3-Bromophenyl)-7-[5-(4-morpholinyl)-2-thienyl]-pyrido[2,3-*d*]pyrimidin-4-ylamine (15). Following the procedure for compound **8** substituting **35** for **34** provided 2-amino-4-(3-bromophenyl)-6-(5-morpholin-4-ylthiophen-2-yl)nicotinonitrile in 38% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.79 (m, 1H), 7.73 (m, 2H), 7.62 (m, 1H), 7.49 (dd, 1H, J = 7.8, 7.8), 7.12 (s, 1H), 6.80 (br s, 2H), 6.27 (d, 1H, J = 4.0), 3.75 (m, 4H), 3.19 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.8, 160.6, 154.9, 151.8, 139.4, 132.1, 130.7 (overlapped), 129.5, 127.4, 127.2, 121.8, 117.3, 106.2, 105.5, 83.2, 65.3, 49.6. MS (DCI/NH_3) m/z 441/443 $(M+H)^+$.

Following the procedure for compound **13** substituting 2-amino-4-(3-bromophenyl)-6-(5-morpholin-4-ylthiophen-2-yl)nicotinonitrile for **8** provided *N*-[4-(3-bromophenyl)-3-cyano-6-(5-morpholin-4-ylthiophen-2-yl)pyridin-2-yl]formamidine in 93% yield as an orange solid. MS (DCI/NH_3) m/z 468/470 $(M+H)^+$.

Following the procedure for compound **14** substituting *N*-[4-(3-bromophenyl)-3-cyano-6-(5-morpholin-4-ylthiophen-2-yl)pyridin-2-yl]formamidine for **13** provided **15** in 12% yield as an orange solid. mp: 265 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45 (s, 1H), 7.90 (d, 1H, J = 4.4), 7.77 (m, 2H), 7.69 (s, 1H), 7.52 (m, 2H), 6.29 (d, 1H, J = 4.4), 3.76 (m, 4H), 3.26 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6/DCl) δ 167.0, 163.2, 158.2, 152.0, 151.1, 147.4, 138.4, 135.7, 133.7, 132.3, 132.0, 128.6, 124.5, 123.3, 121.0, 107.4, 101.7, 66.0, 50.0. MS (ESI) m/z 468/470 $(M+H)^+$. Anal. Calcd for $C_{21}H_{18}BrN_5OS$: C, 53.85; H, 3.87; N, 14.95. Found: C, 53.59; H, 3.97; N, 14.62.

5.2.22. 1-[2-(4-Morpholinyl)-1,3-thiazol-4-yl]ethanone (38). 2,3-Butanedione (30 mL, 355 mmol) at 0 °C was treated with 10 drops of bromine (*caution*: delayed exothermic reaction). After 20 min, additional bromine (12.2 mL, 237 mmol) was added dropwise at such a rate as to maintain reaction temperature between 20 and 30 °C. Vacuum distillation (10 mmHg; fractions between 37 and 48 °C) provided 16.26 g (41%) of 1-bromo-2,3-butanedione compound as a yellow oil. A solution of 1-bromo-2,3-butanedione (16.26 g, 98.55 mmol) in H_2O at 0 °C was treated dropwise with a solution of hydroxylamine hydrochloride (6.90 g, 99.29 mmol) and sodium carbonate (4.20 g, 39.63 mmol) in H_2O (38 mL). After 1 h, the reaction was extracted with CH_2Cl_2, concentrated to one fourth the original volume, cooled, and filtered to provide 7.08 g (40%) of **37** as an unstable (freezer) white solid.

A solution of known morpholine-4-carbothioic acid amide[42] (2.66 g, 18.2 mmol) and 1-bromobutane-2,3-dione-3-oxime (3.29 g, 18.3 mmol) in EtOH (5.5 mL) was heated to reflux for 2 h. The reaction mixture was cooled to room temperature and the solid filtered, washed with EtOH, and dried to provide 4.16 g (99%) of the desired intermediate morpholine hydroxylamine compound as pink solid. MS (DCI/NH_3) m/z 228 $(M+H)^+$.

A solution of 1-(2-morpholinothiazol-5-yl)ethanone-oxime (6.00 g, 26.4 mmol) in H_2O (250 mL), sulfuric acid (27 mL), and EtOH (26 mL) at 50 °C was treated dropwise with sodium nitrite (1.97 g, 28.5 mmol) as a solution in H_2O (50 mL). After 2 h, the mixture was cooled to 0 °C, neutralized with ammonium hydroxide and extracted with CH_2Cl_2. The organic phases were concentrated and purified by silica gel chromatography (elution with 10% EtOAc–CH_2Cl_2) to give 1.06 g (19%) of **38** as a pink solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.77 (s, 1H), 3.72 (m, 4H), 3.41 (m, 4H), 2.44 (s, 3H). MS (DCI/NH_3) m/z 213 $(M+H)^+$.

5.2.23. 5-(3-Bromophenyl)-7-[2-(4-morpholinyl)-1,3-thiazol-4-yl]pyrido[2,3-*d*]pyrimidin-4-ylamine (16). Following the procedure for compound **8** substituting **38** for **34** provided 2-amino-4-(3-bromophenyl)-6-(2-morpholin-4-ylthiazol-4-yl)nicotinonitrile in 74% yield as yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.80 (m, 1H), 7.75 (m, 1H), 7.61 (m, 1H), 7.55 (s, 1H), 7.52 (dd, 1H, J = 7.8, 7.8), 7.24 (s, 1H), 7.01 (br s, 2H), 3.72 (m, 4H), 3.43 (m, 4H). MS (DCI/NH_3) m/z 442/444 $(M+H)^+$.

Following the procedure for compound **13** substituting 2-amino-4-(3-bromophenyl)-6-(2-morpholin-4-ylthiazol-4-yl)nicotinonitrile for **8** provided *N*-[4-(3-bromophenyl)-3-cyano-6-(2-morpholin-4-ylthiazol-4-yl)pyridin-2-yl]-formamidine in 83% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.77 (m, 1H), 8.22 (m, 1H), 7.76 (m, 4H), 7.60 (m, 1H), 7.52 (m, 2H), 3.72 (m, 4H), 3.46 (m, 4H). MS (DCI/NH_3) m/z 469/471 $(M+H)^+$.

Following the procedure for compound **14** substituting *N*-[4-(3-bromophenyl)-3-cyano-6-(2-morpholin-4-ylthiazol-4-yl)pyridin-2-yl]formamidine for **13** provided **16** in 40% yield as a tan solid. mp: 278–282 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.55 (s, 1H), 7.86 (s, 1H), 7.81 (m, 3H), 7.56 (m, 2H), 3.72 (m, 4H), 3.47 (m, 4H). MS (DCI/NH_3) m/z 469/471 $(M+H)^+$. Anal. Calcd for $C_{20}H_{17}BrN_6OS{\cdot}0.6\ H_2O$: C, 50.03; H, 3.82; N, 17.50. Found: C, 49.77; H, 3.92; N, 17.28.

5.2.24. 1-[1-Methyl-2-(4-morpholinyl)-1*H*-imidazol-4-yl]-ethanone (40). A solution of 5-acetyl-2-aminooxazole[44] (7.00 g, 55.5 mmol) and morpholine (20 mL) in H_2O (14 mL) was heated to reflux overnight. The reaction was cooled to room temperature, concentrated and triturated with EtOAc, filtered, and dried to provide 3.52 g (33%) of the desired compound. A slurry of sodium hydride (60% in oil, 590 mg, 14.7 mmol) and methyl iodide (0.86 mL, 13.7 mmol) in THF (20 mL) at room temperature was added a solution of 1-[2-(4-morpholinyl)-1*H*-imidazol-4-yl]ethanone (2.44 g, 12.5 mmol) in DMF (13 mL) and stirring continued for 1.5 h. Reaction quenched with EtOH, H_2O added and extracted with CH_2Cl_2. The organic phases were combined, dried (Na_2SO_4), concentrated, and the residue purified by silica gel chromatography (elution with 25% CH_2Cl_2–EtOAc) to provide 1.27 g (49%) of **40**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 (s, 1H), 3.72 (m, 4H), 3.53 (s, 3H), 3.01 (m, 4H), 2.30 (s, 3H). MS (DCI/NH_3) m/z 210 $(M+H)^+$.

5.2.25. 5-(3-Bromophenyl)-7-[1-methyl-2-(4-morpholinyl)-1*H*-imidazol-4-yl]pyrido[2,3-*d*]pyrimidin-4-ylamine (17). Following the procedure for compound **8** substituting **40** for **34** provided 2-amino-4-(3-bromophenyl)-6-(1-methyl-2-morpholin-4-yl-1*H*-imidazol-4-yl)nicotinonitrile in 75% yield as an orange solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.76 (m, 1H), 7.73 (m, 1H), 7.59 (m, 1H), 7.56 (s, 1H), 7.53 (m, 1H), 7.06 (s, 1H), 6.79 (br s, 2H), 3.72 (m, 4H), 3.55 (s, 3H), 3.02 (m, 4H). MS (DCI/NH_3) m/z 439/441 $(M+H)^+$.

Following the procedure for compound **13** substituting 2-amino-4-(3-bromophenyl)-6-(1-methyl-2-morpholin-4-yl-1H-imidazol-4-yl)nicotinonitrile for **8** provided *N*-[4-(3-bromo-phenyl)-3-cyano-6-(1-methyl-2-morpholin-4-yl-1*H*-imidazol-4-yl)-pyridin-2-yl]-formamidine. This material was used directly in the next reaction.

A solution of *N*-[4-(3-bromophenyl)-3-cyano-6-(1-methyl-2-morpholin-4-yl-1*H*-imidazol-4-yl)pyridin-2-yl]-formamidine in 30% sodium methoxide in MeOH (5 equiv) was heated to reflux. After 2 h, the reaction was cooled filtered and the solid purified by silica gel chromatography (gradient CH_2Cl_2; 2% MeOH–CH_2Cl_2; 4% MeOH–CH_2Cl_2) to provide **17** (48%) as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.50 (s, 1H), 7.87 (s, 1H), 7.79 (m, 2H), 7.63 (s, 1H), 7.54 (m, 2H), 3.73 (m, 4H), 3.60 (s, 3H), 3.04 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6/DCl) δ 164.0, 152.6, 152.5, 150.4, 150.1, 150.0, 137.5, 133.9, 132.1 (overlapped), 128.8, 125.9, 125.6, 123.1, 122.8, 104.5, 66.2, 49.5, 36.1. MS (DCI/NH_3) m/z 466/468 $(M+H)^+$. Anal. Calcd for $C_{21}H_{20}BrN_7O{\cdot}0.7\ H_2O$: C, 52.66; H, 4.50; N, 20.47. Found: C, 52.38; H, 4.46; N, 20.30.

5.2.26. 1-[2-(4-Morpholinyl)-1,3-oxazol-5-yl]ethanone (42). A solution of 2-bromo-3-oxobutyraldehyde[46] (6.00 g, 36.4 mmol) and morpholine-4-carboxylic acid amide[45] (9.01 g, 69.2 mmol) in acetone (40 mL) was stirred at rt for 1 h then heated to reflux for 1 h. The solvent removed under reduced pressure and the residue purified by silica gel chromatography (gradient elution with 10% EtOAc–CH_2Cl_2 to EtOAc) to provide 2.85 g (40%) of the desired compound as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.99 (s, 1H), 3.68 (m, 4H), 3.53 (m, 4H), 2.26 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.1, 163.0, 144.7, 139.8, 65.5, 45.2, 25.2. MS (DCI/NH_3) m/z 197 $(M+H)^+$.

5.2.27. 5-(3-Bromophenyl)-7-[2-(4-morpholinyl)-1,3-oxazol-5-yl]pyrido[2,3-*d*]pyrimidin-4-ylamine (18). Following the procedure for compound **8** substituting **42** for **34** provided 2-amino-4-(3-bromophenyl)-6-(2-morpholin-4-yloxazol-5-yl)nicotinonitrile in 59% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.79 (dd, 1H, J = 2.1, 2.1), 7.74 (m, 1H), 7.62 (s, 1H), 7.61 (m, 1H), 7.50 (dd, 1H, J = 7.8, 7.8), 6.97 (br s, 2H), 6.93 (s, 1H), 3.70 (m, 4H), 3.53 (m, 4H). MS (DCI/NH_3) m/z 426/428 $(M+H)^+$.

Following the procedure for compound **13** substituting 2-amino-4-(3-bromophenyl)-6-(2-morpholin-4-yloxazol-5-yl)nicotinonitrile for **8** provided *N*-[4-(3-bromophenyl)-3-cyano-6-(2-morpholin-4-yloxazol-5-yl)pyridin-2-yl]-formamidine in 86% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.65 (m, 1H), 8.20 (m, 1H), 7.81 (m, 1H), 7.74 (m, 3H), 7.64 (m, 1H), 7.51 (dd, 1H, J = 8.1, 8.1), 7.23 (s, 1H), 3.70 (m, 4H), 3.54 (m, 4H). MS (DCI/NH_3) m/z 453/455 $(M+H)^+$.

Following the procedure for compound **14** substituting *N*-[4-(3-bromophenyl)-3-cyano-6-(2-morpholin-4-yl-oxazol-5-yl)pyridin-2-yl]formamidine for **13** provided **18** in 31% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.50 (s, 1H), 7.95 (s, 1H), 7.80 (m, 3H), 7.54 (m, 4H), 3.72 (m, 4H), 3.57 (m, 4H). ^{13}C NMR

(100 MHz, DMSO-d_6/DCl) δ 163.7, 161.4, 152.5, 151.4, 150.8, 149.4, 142.8, 137.8, 133.7, 132.5, 132.1, 131.9, 128.5, 123.1, 121.2, 103.4, 65.5, 45.9. MS (DCI/NH_3) m/z 453/455 $(M+H)^+$. Anal. Calcd for $C_{20}H_{17}BrN_6O_2 \cdot 0.3\ H_2O$: C, 52.37; H, 3.87; N, 18.37. Found: C, 52.01; H, 3.69; N, 18.26.

5.2.28. 5-(3-Bromophenyl)-7-(4-dimethylaminophenyl)-pyrido[2,3-*d*]pyrimidin-4-ylamine (2). Following the procedure for **7** substituting 1-[4-(dimethylamino)-phenyl]ethanone for **5** and purification by silica gel chromatography (elution with 15% MeOH–CH_2Cl_2) gave 2-amino-4-(3-bromophenyl)-6-(4-dimethylamino-phenyl)nicotinonitrile (54%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.03 (d, 2H, J = 8.9), 7.84 (dd, 1H, J = 1.1, 1.1), 7.73 (m, 1H), 7.65 (m, 1H), 7.51 (dd, 1H, J = 8.1, 8.1), 7.18 (s, 1H), 6.85 (br s, 2H), 6.77 (d, 2H, J = 9.2), 2.99 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.6, 159.0, 152.3, 151.6, 139.6, 132.0, 130.9, 130.7, 128.4, 127.4, 124.3, 121.8, 117.2, 111.4, 107.6, 84.2, 39.7. MS (DCI/NH_3) m/z 393/395 $(M+H)^+$.

Following the procedure for **4** substituting 2-amino-4-(3-bromophenyl)-6-(4-dimethylaminophenyl)nicotino-nitrile for **7** and purification by silica gel chromatography (elution with 15% MeOH–CH_2Cl_2) to provide **2** (52%) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.51 (s, 1H), 8.20 (d, 2H, J = 9.0), 7.82 (m, 1H), 7.78 (m, 1H), 7.75 (s, 1H), 7.55 (m, 2H), 6.81 (d, 2H, J = 9.1), 3.02 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.9, 160.5, 159.9, 158.1, 152.0, 147.2, 140.7, 132.1, 131.3, 131.0, 128.9, 127.9, 124.2, 122.2, 119.2, 111.7, 104.1, 39.3. MS (DCI/NH_3) m/z 420/422 $(M+H)^+$. Anal. Calcd for $C_{21}H_{18}BrN_5 \cdot 1.5\ H_2O$: C, 56.39; H, 4.73; N, 15.66. Found: C, 56.24; H, 4.33; N, 15.47.

5.2.29. 5-(3-Bromophenyl)-7-(6-dimethylaminopyridin-3-yl)pyrido[2,3-*d*]pyrimidin-4-ylamine (43). Following the procedure for compound **5** substituting dimethylamine for morpholine gave 1-[6-(dimethylamino)-3-pyridinyl]-ethone as a yellow solid in 81% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.72 (d, 1H, J = 2.2), 7.96 (dd, 1 H J = 9.2, 2.6), 6.68 (dd, 1H, J = 9.1, 0.7), 3.12 (s, 6H), 2.44 (s, 3H). MS (DCI/NH_3) m/z 165 $(M+H)^+$.

Following the procedure for compound **7** substituting 1-[6-(dimethylamino)-3-pyridinyl]ethone for **5** with the following modified work up. After the reaction was judged complete by TLC, the solvent was removed under reduced pressure, the residue taken up in MeOH–CH_2Cl_2, filtered, and dried to provide 2-amino-4-(3-bromophenyl)-6-[2-dimethylamino-5-pyridyl]nicot-inonitrile (28%). MS (DCI/NH_3) m/z 394/396 $(M+H)^+$.

Following the procedure for compound **4** substituting 2-amino-4-(3-bromophenyl)-6-[2-dimethylamino-5-pyridyl]-nicotinonitrile for **7** to provide compound **43** in 73% yield. This material was converted to the HCl salt. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.04 (dd, 1H, J = 2.4, 0.7), 8.52 (s, 1H), 8.40 (dd, 1H, J = 9.0, 2.4), 7.84 (m, 1H), 7.80 (s, 1H), 7.78 (ddd, 1H, J = 7.5, 2.1, 2.1), 7.57 (ddd, 1H, J = 7.8, 1.6, 1.6), 7.54 (m, 1H), 6.75 (dd, 1H, J = 8.9, 0.7), 3.13 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.9, 159.7, 159.6, 159.1, 158.1, 148.2, 147.4, 140.5, 136.1, 132.0, 131.3, 130.9, 127.9, 122.2, 120.5, 118.9, 105.4, 104.3, 37.6. MS (DCI/NH_3) m/z 421/423 $(M+H)^+$. Anal. Calcd for $C_{20}H_{17}BrN_6 \cdot 3.0\ HCl \cdot 2.0\ H_2O$: C, 42.39; H, 4.27; N, 14.83. Found: C, 42.06; H, 4.44; N, 15.15.

5.2.30. 5-(3-Bromophenyl)-7-(5-dimethylaminopyridin-2-yl)pyrido[2,3-*d*]pyrimidin-4-ylamine (44). To a solution of 3-dimethylaminopyridine[55] (3.53 g, 28.9 mmol) in CH_2Cl_2 (100 mL) at 0 °C was added NBS (5.34 g, 30.0 mmol) and the reaction stirred for 3 h. The mixture was washed with saturated $NaHCO_3$, dried ($MgSO_4$), and concentrated. The residue was purified by silica gel chromatography (elution with 20% EtOAc–hexanes) to provide 2.67 g (46%) of (5-bromopyridin-2-yl)di-methylamine. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (d, 1H, J = 3.3), 7.25 (d, 1H, J = 8.8), 6.86 (dd, 1H, J = 8.8, 3.3), 2.96 (s, 6H). MS (DCI/NH_3) m/z 201/203 $(M+H)^+$.

To a solution of (5-bromopyridin-2-yl)dimethylamine (2.67 g, 13.4 mmol) in CH_3CN (20 mL) and Et_3N (20 mL) was added $PdCl_2(PPh_3)_2$ (175 mg, 0.25 mmol), CuI (48 mg, 0.25 mmol) and trimethylsilyl acetylene (2.46 g, 25.0 mmol), and the mixture stirred at rt for 48 h. The mixture was diluted with a mixture of hexanes and EtOAc and filtered through Celite®. The filtrate was concentrated and crude dimethyl(6-trimethylsilanylethyn-ylpyridin-3-yl)amine used directly in the next reaction.

A solution of dimethyl(6-trimethylsilanylethynylpyridin-3-yl)amine in acetone (25 mL) and H_2O (1 mL) was added 8 M H_2SO_4 (4 mL) followed by $Hg(OTf)_2$ (6.40 g, 15.0 mmol) and the reaction heated to reflux for 4 h cooled to rt and stirring continued overnight. The mixture was diluted with EtOAc and H_2O, filtered through Celite® and the filtrated partitioned, and extracted with EtOAc then CH_2Cl_2. The organic phases were combined, dried ($MgSO_4$), and concentrated. The residue was purified by silica gel chromatography (elution with 25% EtOAc–hexanes) to give 960 mg of 1-(5-dimethylaminopyridin-2-yl)ethone. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (d, 1H, J = 3.1), 7.96 (d, 1H, J = 8.8), 6.95 (dd, 1H, J = 8.8, 3.3), 3.10 (s, 6H), 2.63 (s, 3H). MS (DCI/NH_3) m/z 165 $(M+H)^+$.

Following the procedure for compound **7** substituting 1-(5-dimethylaminopyridin-2-yl)ethone for **5** to provide 6-amino-4-(3-bromophenyl)-5′-dimethylamino-[2,2′]bipyr-idyl-5-carbonitrile (88%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.19 (s, 1H), 8.17 (d, 1H, J = 5.8), 7.81 (dd, 1H, J = 1.7, 1.7), 7.74 (m, 1H), 7.63 (m, 1H), 7.54 (s, 1H), 7.51 (dd, 1H, J = 8.2, 8.2), 7.20 (dd, 1H, J = 9.2, 3.1), 6.97 (br s, 2H), 3.03 (s, 6H). MS (DCI/NH_3) m/z 394/396 $(M+H)^+$.

Following the procedure for compound **4** substituting 6-amino-4-(3-bromophenyl)-5′-dimethylamino-[2,2′]bipyr-idyl-5-carbonitrile for **7** to provide compound **44** in 25% yield. This material was converted to the HCl salt. ^{1}H

NMR (300 MHz, DMSO-d_6) δ 8.54 (s, 1H), 8.40 (d, 1H, J = 8.8), 8.21 (d, 1H, J = 2.9), 8.12 (s, 1H), 7.79 (m, 2H), 7.57 (m, 2H), 7.28 (dd, 1H, J = 9.2, 3.3), 3.07 (s, 6H). MS (DCI/NH_3) m/z 421/423 $(M+H)^+$.

5.2.31. 5-(3-Bromophenyl)-7-(6-dimethylaminopyridazin-3-yl)pyrido[2,3-*d*]pyrimidin-4-ylamine (45). Following the procedure for compound **5** substituting **23** for 1-(6-chloro-3-pyridinyl)ethone and dimethylamine hydrochloride for morpholine to give to provide 1-[6-(dimethylamino)-3-pyridazinyl]ethanone in 77% yield as yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, 1H, J = 9.5), 6.80 (d, 1H, J = 9.5), 3.28 (s, 6H), 2.77 (s, 3H). MS (DCI/NH_3) m/z 166 $(M+H)^+$.

Following the procedure for compound **7** substituting 1-[6-(dimethylamino)-3-pyridazinyl]ethanone for **5** to provide 2-amino-4-(3-bromophenyl)-6-[6-(dimethylamino)-3-pyridazinyl]nicotinonitrile in 44% yield as an orange solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (d, 1H, J = 9.8), 8.12 (s, 1H), 7.83 (dd, 1H, J = 2.0), 7.64 (m, 2H), 7.37 (dd, 1H, J = 7.8, 7.8), 6.88 (d, 1H, J = 9.5), 3.27 (s, 6H). MS (DCI/NH_3) m/z 395/397 $(M+H)^+$.

Following the procedure for **4** substituting 2-amino-4-(3-bromophenyl)-6-[6-(dimethylamino)-3-pyridazinyl]-nicotinonitrile for **7** to give **45** in 74% yield as a tan powder. This material was converted to the HCl salt. mp: >230 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.76 (s, 1H), 8.33 (d, 1H, J = 4.4), 8.18 (m, 1H), 7.77 (m, 1H), 7.70 (m, 1H), 7.53 (m, 1H), 7.45 (dd, 1H, J = 7.7, 7.7), 7.28 (m, 1H), 3.12 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.7, 158.1, 156.9, 152.4, 150.3, 149.5, 146.0, 137.7, 133.3, 131.6, 131.5, 128.1, 127.6, 122.6, 121.9, 116.4, 105.0, 38.7. HRMS calcd for $C_{19}H_{16}{}^{79}BrN_7$: 422.0729; observed: 422.0726. Anal. Calcd for $C_{19}H_{16}BrN_7 \cdot 1.6$ HCl: C, 47.48; H, 3.69; N, 20.40. Found: C, 47.41; H, 3.72; N, 20.57.

5.2.32. 5-(3-Bromophenyl)-7-(2-dimethylaminopyrimidin-5-yl)pyrido[2,3-*d*]pyrimidin-4-ylamine (46). To a solution of 5-bromo-2-(dimethylamino)pyrimidine[56] (8.11 g, 40.3 mmol; from 2-dimethylaminopyrimidine[57]) in DMF (80 mL) at rt was added tributyl(1-ethoxyvinyl)stannane (15.0 mL, 44.4 mmol) followed by Pd(PPh_3)Cl_2 (560 mg, 0.80 mmol) and the mixture heated to 90 °C for 2 h. The reaction was quenched with aqueous KF and extracted with Et_2O. The organic layers were combined, dried, and concentrated to provide [5-(1-ethoxyvinyl)pyrimidin-2-yl]dimethylamine. The resulting vinyl ether was used without further purification.

To a solution of [5-(1-ethoxyvinyl)pyrimidin-2-yl]dimethylamine in acetone (80 mL) was added 1 N HCl (20 mL) and the reaction stirred at rt overnight. The reaction was concentrated and the residue partitioned between H_2O and Et_2O. The organic layers were combined, concentrated, and purified by column chromatography (100% CH_2Cl_2) to provide 3.86 g (58% yield) of 1-(2-dimethylaminopyrimidin-5-yl)ethanone. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.84 (s, 2H), 3.28 (s, 6H), 2.47 (s, 3H).

Following the procedure for compound **7** substituting 1-(2-dimethylaminopyrimidin-5-yl)ethanone for **5** to provide 2-amino-4-(3-bromophenyl)-6-(2-dimethylaminopyrimidin-5-yl)nicotinonitrile in 27% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.07 (s, 2H), 7.86 (dd, 1H, J = 1.7, 1.7), 7.74 (br d, 1H, J = 8.5), 7.66 (br d, 1H, J = 7.8), 7.51 (dd, 1H, J = 7.8, 7.8), 7.28 (s, 1H), 7.03 (s, 2H), 3.20 (s, 6H).

Following the procedure for **4** substituting 2-amino-4-(3-bromophenyl)-6-(2-dimethylaminopyrimidin-5-yl)-nicotinonitrile for **7** to give **46** in 50% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.93 (br s, 1H), 9.29 (s, 2H), 8.87 (s, 1H), 8.16 (s, 1H), 7.94 (dd, 1H, J = 1.7, 1.7), 7.82 (ddd, 1H, J = 7.8, 1.7, 1.7), 7.62 (ddd, 1H, J = 7.8, 1.7, 1.7), 7.57 (dd, 1H, J = 7.8, 7.8), 7.32 (br s, 1H), 3.26 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.2, 161.6, 158.9, 157.8, 151.8, 150.0, 148.9, 137.5, 132.9, 131.7, 131.1, 128.2, 122.4, 121.2, 116.7, 103.0, 36.9.

5.2.33. *N*-{4-[4-Amino-5-(3-bromophenyl)pyrido[2,3-*d*]-pyrimidin-7-yl]phenyl}-*N*-methylformamide (47). To a solution of 4′-fluoroacetophenone (10.0 g, 72.4 mmol) in DMSO (20 mL) was added methylamine (40% in H_2O, 65 mL), the reaction flask fitted with a reflux condenser and the mixture heated to 90 °C for 18 h. The reaction was cooled, H_2O added, the solid filtered, dried, and purified by chromatography (gradient elution: 0–60% EtOAc–hexanes) to provide 6.12 g (57%) of 1-(4-methylaminophenyl)ethanone[58] as a light yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.72 (AA′BB′, 2H, J = 8.8), 6.59 (m, 1H), 6.54 (AA′BB′, 2H, J = 8.8), 2.73 (d, 3H, J = 5.1), 2.39 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.1, 153.8, 130.5, 124.8, 110.5, 29.2, 25.8. MS (DCI/NH_3) m/z 150 $(M+H)^+$.

Following the procedure for compound **7** substituting 1-(4-methylaminophenyl)ethanone for **5** to provide 2-amino-4-(3-bromophenyl)-6-(4-methylaminophenyl)nicotinonitrile (39%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.96 (AA′BB′, 2H, J = 8.8), 7.83 (dd, 1H, J = 1.7, 1.7), 7.72 (ddd, 1H, J = 7.8, 1.7, 1.0), 7.64 (ddd, 1H, J = 8.1, 1.7, 1.7), 7.50 (dd, 1H, J = 8.1, 8.1), 7.13 (s, 1H), 6.81 (br s, 2H), 6.59 (AA′BB′, 2H, J = 8.8), 6.24 (q, 1H, J = 5.1), 2.74 (d, 3H, J = 5.1). ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.6, 159.2, 152.3, 151.8, 139.6, 132.0, 130.74, 130.66, 128.6, 127.4, 124.0, 121.8, 117.3, 111.1, 107.4, 83.9, 29.3. MS (ESI) m/z 378/380 $(M+H)^+$.

Following the procedure for **4** substituting 2-amino-4-(3-bromophenyl)-6-(4-methylaminophenyl)nicotinonitrile for **7** to give **47** in 51% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.03 (br s, 1H), 8.92 (s, 1H), 8.83 (s, 1H), 8.46 (AA′BB′, 2H, J = 8.8), 8.27 (s, 1H), 7.98 (m, 1H), 7.83 (m, 1H), 7.59 (m, 4H), 7.46 (br s, 1H), 3.30 (s, 3H). MS (DCI/NH_3) m/z 435/437 $(M+H)^+$.

5.2.34. *N*-{5-[4-Amino-5-(3-bromophenyl)pyrido[2,3-*d*]-pyrimidin-7-yl]pyridin-2-yl}-*N*-methylformamide (48). To a solution of 6-chloro-3-acetylpyridine[23] in DMSO (5 M) was added methylamine (40% in H_2O; 5 eq) the

reaction flask sealed and the mixture heated to 90 °C for 18 h. The reaction was cooled, H_2O added, the solid filtered, and dried to provide 1-(6-methylaminopyridin-3-yl)ethanone in 67% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.71 (d, 1H, J = 2.4), 8.02 (dd, 1H, J = 9.2, 2.4), 6.40 (d, 1H, J = 9.2), 3.30 (m, 1H), 3.00 (d, 3H, J = 5.1), 2.50 (s, 3H).

To formic-pivalic anhydride[59] (2 eq) at 0 °C was added 1-(6-methylaminopyridin-3-yl)ethanone (1 eq) as a solution in CH_2Cl_2 (1 M), the reaction warmed to rt and stirred for 5 h. The reaction was washed with aqueous sodium hydroxide, dried (Na_2SO_4), and concentrated to provide *N*-(5-acetylpyridin-2-yl)-*N*-methylformamide (70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.63 (s, 1H), 8.94 (d, 1H, J = 2.2), 8.30 (dd, 1H, J = 8.8, 2.2), 7.08 (d, 1H, J = 8.5), 3.38 (s, 3H), 2.63 (s, 3H).

Following the procedure for compound **7** substituting *N*-(5-acetylpyridin-2-yl)-*N*-methylformamide for **5** to provide *N*-[6-amino-4-(3-bromophenyl)-5-cyano[2,3′]bipyridinyl-6′-yl]-*N*-methylformamide. Material was used directly in the next reaction.

Following the procedure for **4** substituting *N*-[6-amino-4-(3-bromophenyl)-5-cyano[2,3′]bipyridinyl-6′-yl]-*N*-methylformamide for **7** to give **48** in 31% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.47 (s, 1H), 9.31 (d, 1H, J = 2.2), 8.76 (dd, 1H, J = 8.8, 2.2), 8.59 (s, 1H), 8.03 (s, 1H), 7.90 (dd, 1H, J = 1.8, 1.8), 7.80 (ddd, 1H, J = 7.4, 1.8, 1.8), 7.57 (m, 3H), 3.32 (s, 3H).

5.2.35. *N*-4-[4-Amino-5-(3-bromophenyl)pyrido[2,3-*d*]pyrimidin-7-yl]-2-methylphenyl-*N*-methylformamide (49). A solution of 2-fluorotoluene (10.0 g, 90.8 mmol) and acetyl chloride (6.64 mL, 93.4 mmol) in dry CH_2Cl_2 (50 mL) was treated with $AlCl_3$ (15.3 g, 115 mmol), portionwise, and the reaction stirred at rt for 24 h. To complete reaction, the mixture was heated to reflux in the last 2 h. The reaction mixture was cooled, quenched with H_2O and extracted with CH_2Cl_2. Organic layers combined, concentrated, and the residue purified by silica gel chromatography (elution with 100% hexanes then 100% CH_2Cl_2) to provide 1-(4-fluoro-3-methylphenyl)ethanone (82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, 1H, J = 7.5), 8.00 (m, 1H), 7.27 (dd, 1H, J = 8.7), 2.78 (s, 3H), 2.53 (s, 3H).

To a solution of 1-(4-fluoro-3-methylphenyl)ethanone (4.59 g, 30.2 mmol) in DMSO (7 mL) was added methylamine (40% in H_2O, 7 mL), the reaction sealed in a tube and heated to 96 °C for 18 h. The mixture was cooled, poured into H_2O, extracted with CH_2Cl_2, the organic phases combined, and concentrated. The residue was purified by silica gel chromatography (elution with 80% CH_2Cl_2–hexanes) to provide 1-(3-methyl-4-methylaminophenyl)ethanone (31%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (dd, 1H, J = 8.1, 1.7), 7.71 (m, 1H), 6.56 (d, 1H, J = 8.5), 2.97 (s, 3H), 2.51 (s, 3H), 2.16 (s, 3H).

Following the procedure for compound **7** substituting 1-(3-methyl-4-methylaminophenyl)ethanone for **5** to provide 2-amino-4-(3-bromophenyl)-6-(3-methyl-4-methylaminophenyl)nicotinonitrile (36%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, 1H, J = 8.5, 2.0), 7.84 (m, 1H), 7.74 (dd, 1H, J = 1.7, 1.7), 7.64 (m, 1H), 7.59 (m, 1H), 7.40 (dd, 1H, J = 8.1, 8.1), 7.07 (s, 1H), 6.67 (d, 1H, J = 8.5), 2.98 (s, 3H), 2.21 (s, 3H).

Following the procedure for **4** substituting 2-amino-4-(3-bromophenyl)-6-(3-methyl-4-methylaminophenyl)-nicotinonitrile for **7** to give **48** (54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.32 (m, 1H), 8.21 (s, 1H), 8.11 (dd, 1H, J = 8.5, 2.2), 7.75 (ddd, 1H, J = 7.4, 1.8, 1.8), 7.68 (m, 2H), 7.47 (m, 2H), 7.26 (d, 1H, J = 8.5), 5.31 (br s, 2H), 3.25 (s, 3H), 2.38 (s, 3H).

5.2.36. 5-(3-Bromophenyl)-7-(4-methylaminophenyl)pyrido[2,3-*d*]pyrimidin-4-ylamine (50). A solution of **47** in concentrated HCl was stirred overnight at rt. The reaction mixture was lyophilized to provide **50** in quantitative yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.75 (br s, 1H), 8.82 (s, 1H), 8.21 (AA′BB′, 2H, J = 8.8), 8.00 (s, 1H), 7.91 (s, 1H), 7.80 (m, 1H), 7.57 (m, 2H), 7.06 (br s, 1H), 6.69 (AA′BB′, 2H, J = 8.8), 2.80 (s, 3H).

5.2.37. *N*-{4-[4-Amino-5-(3-bromophenyl)pyrido[2,3-*d*]-pyrimidin-7-yl]phenyl}formamide (51) and 7-(4-aminophenyl)-5-(3-bromophenyl)pyrido[2,3-*d*]pyrimidin-4-ylamine (52). Following the procedure for compound **7** substituting 1-(4-aminophenyl)ethanone (Aldrich) for **5** to provide 2-amino-6-(4-aminophenyl)-4-(3-bromophenyl)nicotinonitrile (44%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.89 (AA′BB′, 2H, J = 8.5), 7.83 (dd, 1H, J = 1.7, 1.7), 7.72 (br d, 1H, J = 8.1), 7.64 (br d, 1H, J = 7.8), 7.50 (dd, 1H, J = 7.8, 7.8), 7.11 (s, 1H), 6.79 (br s, 2H), 6.61 (AA′BB′, 2H, J = 8.8), 5.65 (br s, 2H).

Following the procedure for **4** substituting 2-amino-6-(4-aminophenyl)-4-(3-bromophenyl)nicotinonitrile for **7** to give **51** and **52** as a separable mixture. **51**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.49 (s, 1H), 8.56 (s, 1H), 8.34 (m, 1H), 8.32 (AA′BB′, 2H, J = 8.8), 7.88 (m, 2H), 7.80 (m, 1H), 7.76 (AA′BB′, 2H, J = 8.8), 7.56 (m, 2H). **52** HCl salt: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.82 (s, 1H), 8.16 (AA′BB′, 2H, J = 8.8), 7.99 (s, 1H), 7.91 (dd, 1H, J = 1.5, 1.5), 7.80 (ddd, 1H, J = 7.7, 1.5, 1.5), 7.60 (ddd, 1H, J = 8.1, 1.1, 1.1), 7.56 (m, 1H), 6.74 (AA′BB′, 2H, J = 8.8).

5.3. Biological procedures

Assays for AK inhibition and ADO phosphorylation in intact cells (IMR-32) cells were conducted using radiochemical methods as described by Jarvis et al.[28] Inflammatory hyperalgesia was assessed in male Sprague–Dawley rats (200-400 g, Charles River, Wilmington, MA) 2 h after an injection of 100 μL of a 1% solution of λ-carrageenan (Sigma Chemical Co., St. Louis, MO) into the plantar surface of the right hind paw.[29] A hyperalgesic response to thermal stimulation was determined using a commercially available paw thermal stimulator (UARDG, Department of Anesthesiology, University of California, San Diego, La Jolla, CA) modeled after that described by Hargreaves et al.[47] All animal handling and experimental protocols were

approved by an institutional animal care and use committee (IACUC).

5.4. Genetic toxicology

Assays for bacterial reverse mutation (Ames assay) using a Salmonella strain (TA-98) with and without S-9 mix metabolic activation were conducted as previously described.[48] A positive response was defined as at least a two-fold increase the number of mutant colonies in the presence of test compound (2000 μg/well) as compared to vehicle controls. Additional assays were conducted to assess clastogenic and aneugenic potential using a Chinese hamster lung V79 cell micronucleus assay.[32] In this assay, a positive response was defined as at least a three-fold increase in the percent of micronucleated cells in the presence of test compound (5000 μg/well) rela- tive to vehicle controls and/or a clear concentration dependent response.

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