5,6,7-Trisubstituted 4-Aminopyrido[2,3-*d*]pyrimidines as Novel Inhibitors of Adenosine Kinase

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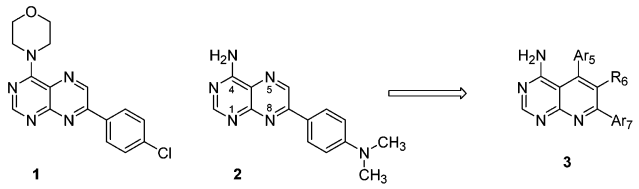
The synthesis and structure−activity relationship of a series of 5,6,7-trisubstituted 4-aminopyrido[2,3-*d*]pyrimidines as novel nonnucleoside adenosine kinase inhibitors is described. A variety of alkyl, aryl, and heteroaryl substituents were found to be tolerated at the C5, C6, and C7 positions of the pyridopyrimidine core. These studies have led to the identification of analogues that are potent inhibitors of adenosine kinase with in vivo analgesic activity.

Introduction

Adenosine kinase (AK) is a ubiquitous intracellular enzyme which catalyzes the phosphorylation of adenosine (ADO) to adenosine monophosphate and therefore is a key enzyme in the control of cellular concentrations of ADO. Adenosine has been characterized as a homeostatic modulator of cellular activity[1] and, as such, has several important physiological effects in the central nervous system, functioning as an endogenous anticonvulsant[2,3] and a neuroprotective agent.[4] Adenosine has also been implicated in modulating transmission in pain pathways in the spinal cord.[5] During periods of excessive cellular activity or tissue trauma extracellular ADO release is enhanced which activates specific P1 purinergic receptors (A_1, A_{2A}, A_{2B}, and A_3) to elicit a variety of responses which tend to decrease cellular activity and restore cellular function toward normal.[6,7] Because ADO has a half-life measured in seconds[8] in extracellular fluids, its endogenous actions are highly localized. Therefore, selective inhibition of AK represents an attractive approach to enhance the release of endogenous ADO to the extracellular space, thus benefiting from its neuroprotective, anticonvulsant, and antinociceptive effects.

High-throughput screening of the Abbott compound library aimed at identifying novel, nonnucleoside AK inhibitors produced two structurally related pteridine-like molecules, **1** and **2** (Figure 1), with low micromolar potency as AK inhibitors ($AK_{(enzyme)}$: IC_{50} = 0.4 μM). Both compounds were inactive at other receptor, enzyme and kinase screens, suggesting selectivity for AK.[9] The ability of **1** and **2** to inhibit adenosine phosphorylation by AK was confirmed and their selectivity further characterized within the AK program. Although compounds **1** and **2** were identified as AK inhibitors with modest potency, they were considerably weaker at inhibiting ADO phosphorylation in intact cells



	1	2
AK enzyme (IC_{50}, nM)	410 ± 78	440 ± 29
IMR-32 intact cell AK (IC_{50}, nM)	22,000 ± 1700	3600 ± 740

Figure 1. Adenosine kinase inhibitor high-throughput screening hits (**1** and **2**).

($AK_{(intact\ cell)}$: IC_{50} = 22 μM and 3.6 μM, respectively), indicating poor ability to penetrate the cell membrane. Screening of analogues of **1** and **2** as well as medicinal chemistry efforts directed at optimizing the potency and improving the membrane permeability of these AK inhibitors was initiated. While no other analogue was found to be more potent than the original hits, initial SAR studies of compound **2** yielded several important observations. Replacement of the dimethylamino group of **2** with a variety of other functionality (e.g., NH_2, NO_2, OCH_3) failed to improve AK inhibitory activity. However, installation of a phenyl substituent in the 6-position of the pteridine nucleus resulted in a greater than 15-fold increase in in vitro activity (IC_{50} = 25 nM vs 440 nM).[10] Additionally, it was found through synthetic efforts that the 5-nitrogen was not required, which not only resulted in increased compound output due to an improved synthetic scheme, but also yielded an alternative scaffold that allowed for substitution at that position as well. It was found that placement of a phenyl substituent at the 5-position gave a compound with very good AK inhibitory activity (IC_{50} = 7 nM, compound **22**, Table 1). These results opened the door to new chemistry strategies for optimizing the potency, as well as characterizing the physical chemical properties of these types of molecules, one of which was to substitute the 5-, 6-, and 7-positions around the pyridopyrimidine

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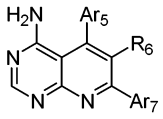
Table 1. In Vitro Biological Activity of Adenosine Kinase Inhibitors[a]



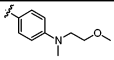
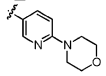
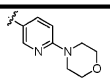
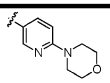
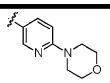
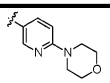
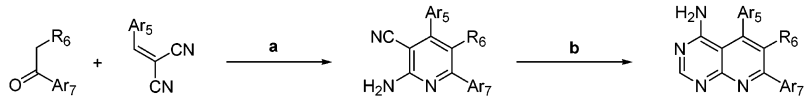
Compd	Ar_5	R_6	Ar_7	AK inhibition IC_{50}(nM) (enzyme)	AK inhibition IC_{50} (nM) (intact cells)
21	H	H		773 ± 58	> 10,000
22	Ph	H		7.0 ± 0.8	467 ± 33
23	H	Ph		350 ± 50	1500 ± 289
24				16 ± 7	250 ± 50
25				6.7 ± 4.2	> 1000
26				0.55 ± 0.15	49 ± 7
27				0.25 ± 0.05	288 ± 52
28				0.43 ± 0.13	25 ± 8
29				3.0 ± 1.0	163 ± 56
30				0.98 ± 0.37	367 ± 317
31				3.1 ± 1.7	350 ± 145
32				3.2 ± 1.2	53 ± 9
33				5.0 ± 1.7	130 ± 35
34				4.0 ± 1.2	155 ± 33

Table 1 (Continued)

Compd	Ar_5	R_6	Ar_7	AK inhibition IC_{50}(nM) (enzyme)	AK inhibition IC_{50} (nM) (intact cells)
35				5.0 ± 2.0	197 ± 61
36				37 ± 5	130 ±70
37				60 ± 20	625 ± 111
38				400 ± 115	> 1000
39				300 ± 100	> 1000
40				55 ± 16	625 ± 325
41				63 ± 23	450 ± 244

[a] All values are the mean ± SEM of at least three separate observations run in triplicate.

Scheme 1[a]



[a] Reagents and conditions: (a) NH_4OAc, *n*-butanol, reflux; (b) formamidine acetate, diglyme, 155 °C.

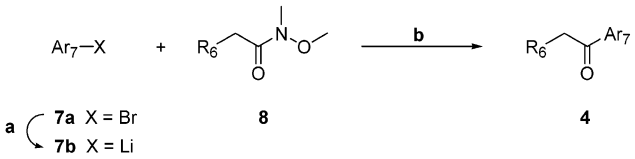
nucleus in a single molecule. It was this investigation which evolved into the trisubstituted pyridopyrimidine series **3**.

Chemistry

The synthesis of 5,6,7-trisubstituted 4-aminopyrido-[2,3-*d*]pyrimidines was accomplished using two distinctly different synthetic routes. In the first route shown in Scheme 1, ketone **4** and dicyanoethylene **5** were combined with ammonium acetate in *n*-butanol at reflux to give the intermediate aminocyanopyridine **6** in 20−50% yield, presumably via Michael addition of the in situ formed enamine of **4** to **5** followed by cyclization on one of the nitrile groups and air oxidation leading to the aromatic pyridine ring. Intermediate **6** was then subsequently reacted with formamidine acetate in diglyme at 155 °C to form the aminopyrimidine ring of the desired trisubstituted pyridopyrimidine **3** in 15−50% yield.

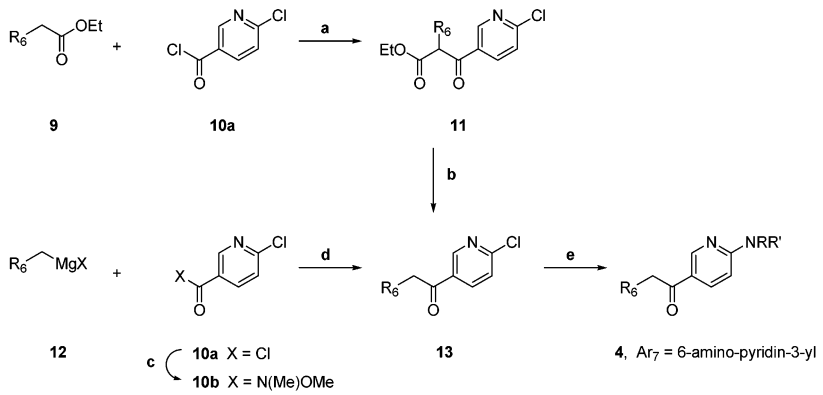
The requisite ketones **4** and dicyanoethylenes **5** were prepared by standard methods in separate steps. Several different synthetic routes were utilized for the preparation of **4** as shown in Schemes 2 and 3. The first method (Scheme 2), which was useful for analogues where the 7-position substituent was phenyl or thienyl, involved addition of an aryllithium reagent **7b**, prepared via direct lithiation or metal−halogen exchange, to an appropriately substituted Weinreb amide[11] **8** to give after workup the desired ketone **4**. For cases where the Ar_7 substituent was 2-amino-substituted pyridyl, two procedures were employed, both starting with 6-chloronicotinyl chloride as shown in Scheme 3. The first procedure, when R_6 was frequently aryl, utilized a condensation reaction between an appropriately sub-

Scheme 2[a]



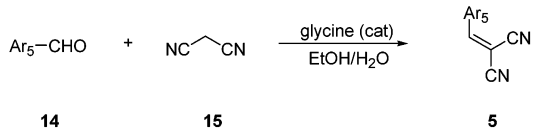
[a] Reagents and conditions: (a) *n*-BuLi; (b) −78 °C, THF.

Scheme 3[a]

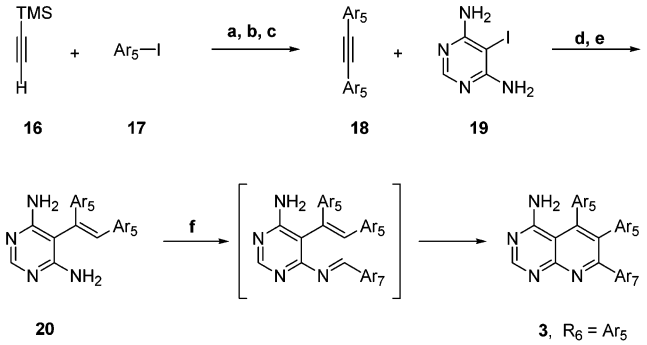


[a] Reagents and conditions: (a) $LiN(TMS)_2$, THF; (b) $DMSO/H_2O$, 155 °C; (c) NH(Me)OMe·HCl, Et_3N, CH_2Cl_2, 0 °C; (d) −78 °C to room temp., THF; (e) NRR′, ethanol/reflux or H_2O/100 °C sealed tube.

Scheme 4



Scheme 5[a]



[a] Reagents and conditions: (a) $(PPh_3)_2PdCl_2$, CuI, Et_3N; (b) (aq)K_2CO_3/MeOH; (c) Ar_5I, $(PPh_3)_2PdCl_2$, CuI, Et_3N; (d) catecholborane, THF, reflux; (e) $(PPh_3)_4Pd$, (aq)Na_2CO_3, (aq)NaOH, reflux; (f) Ar_7CHO, 1,2,4-trichlorobenzene, △, air.

stituted ethyl acetate **9** and acyl chloride **10a** to yield *β*-ketoester **11** which was decarboxylated at elevated temperature to give the desired ketone **13**. Alternatively, when R_6 was frequently alkyl, an appropriate Grignard reagent **12** was reacted with **10** after Weinreb amide formation to give intermediate **13**. Ketone **13** was then converted to the desired ketone **4** by reaction with the requisite amine in ethanol at reflux or in water at 100 °C in a sealed tube. The dicyanoethylenes **5** were prepared by reacting an aryl aldehyde with malononitrile using glycine as the catalyst (Scheme 4).[12]

Scheme 5 illustrates an alternate route for preparing pyridopyrimidines **3** wherein the substituents at C5 and C6 were the same moiety. A bis-substituted acetylene derivative **18**, prepared via tandem palladium coupling reactions of trimethylsilylacetylene and the appropriately substituted iodobenzene **17**, was treated with catecholborane followed by Suzuki coupling of the resulting boronic acid with 4,6-diamino-5-iodopyrimidine (**19**) to give the trisubstituted alkene **20**. The desired products **3** were then obtained via cyclization by aza-Cope rearrangement of the intermediate formed by reaction of **20** with an aryl aldehyde at high temperature in 1,2,4-trichlorobenzene or diphenyl ether as described previously.[10]

Results and Discussion

Table 1 summarizes the effect of placing three substituents simultaneously around the pyridopyrimidine core, and in general these data indicate good tolerability. A variety of aryl or heteroaryl groups at the 5-position could be incorporated into the molecule, leading to an increase in AK potency as compared to the unsubstituted derivatives **21** and **23**. Increasing the lipophilicity of these groups by adding substituents such as the isopropyl moiety in compound **25** tended to yield more potent analogues, indicating the importance of this interaction. Additionally, although it was found that incorporation of a phenyl substituent at the 6-position of the pteridine ring system of **2** boosted the in vitro activity more than 15-fold (IC_{50} = 25 nM vs 440 nM),[10] a comparison of compounds **21**, **22**, and **23** suggested that in the pyridopyrimidine series the 5-substituent played a more important role than the 6-substituent with regard to enzymatic AK inhibitory activity. Perhaps this was due to better alignment of the 5-substituent in the lipophilic pocket of the binding site in the AK enzyme. Thus, the tolerability of a group at the 6-position provided the potential for utilizing this substituent not only for improving AK activity but also to alter the physical chemical characteristics of the molecule to ultimately improve such properties as cell penetration, solubility, and pharmacokinetics.

Substituents at the 6-position could be aryl or alkyl. Substitution on the 6-phenyl ring which yielded potent analogues ranged from lipophilic groups such as isopropyl (compound **25**) and fluoro (compound **35**) to hydrophilic groups such as 3,4-dimethoxy and dimethylamino (e.g., **31** and **36**). Alkyl groups at the 6-position gave potent analogues provided they were not too sterically demanding. For example, smaller groups such as *n*-pentyl (compound **40**) and cyclopropyl (compound **37**) had good AK inhibitory activity (IC_{50} = 55 nM and 60 nM, respectively), albeit approximately 12-fold lower than the case where R_6 is aryl (e.g., **33**). However, branched alkyl groups, such as isopropyl, or larger cycloalkyl groups such as cyclohexyl, led to a 60 to 100-fold decrease in AK potency as compared to aryl (Table 1, compounds **38** and **39**). In addition, the 6-alkyl analogues tended to have weaker ability to inhibit ADO

Table 2. In Vivo Analgesic Activity of Selected 5,6,7-Trisubstituted Pyridopyrimidines[a]

compd	AK IC_{50} (nM)	intact cell IC_{50} (nM)	hotplate analgesia, % MPE
24	16	250	77*
32	3.2	53	50*
33	5.0	130	56*

[a] % MPE = maximal protective effect ([postdrug latency] − [vehicle latency])/([maximum latency] − [vehicle latency]) × 100%, where maximum (cutoff) latency was 180 s. Values represent mean within ± 8%. *Indicates $p < 0.05$ compared to vehicle-treated mice.[15]

phosphorylation in intact cells, indicating poor cell penetration. Other groups that could be placed at the 6-position included benzyl (compound **41**, $IC_{50} = 63$ nM) as well as the ethyl acetate derivative **29** which gave a single-digit nanomolar analogue.

A variety of aromatic and heteroaromatic groups at the 7-position were equally tolerated, with the 2-thiophene moiety generally giving the most potent analogues. Compound **26**, for example, was 29-fold more potent than the corresponding dimethylaminophenyl derivative **24** and about 6-fold more potent than the dimethylaminopyridyl analogue **32**. In fact, in many cases such compounds as **26**, **27**, and **28** had subnanomolar affinity to inhibit enzymatic AK activity, while essentially all others were in the single-digit nanomolar range. Despite the high potency of the 7-thienyl compounds, the amino-substituted pyridines, for example compounds **30**, **33**, and **34**, were the most significant 7-position substituents, as these groups not only yielded potent analogues but also provided the potential for improving the aqueous solubility of these compounds, a problem that plagued the earlier analogues. From the developing structure−activity relationship studies of this and other series, it was found that a wide variety of substituents could be placed on the 7-aryl ring para to the pyridopyrimidine core without any loss of AK activity.[14] As a result groups such as dimethylamino, morpholino, and *N*-methyl-*N*-methoxyethyl were incorporated into the 7-pyridyl substituent which yielded very potent AK inhibitors, while at the same time more readily allowing for hydrochloride salt formation and somewhat improved aqueous solubility.

Selected compounds were screened for analgesic activity in animal pain models such as the mouse hotplate test and rat formalin test, and some showed significant effects. For example, compounds **24**, **32**, and **33** all showed statistically significant acute analgesic effects in the mouse hotplate test at 30 μmol/kg ip (Table 2). In addition, **32** and **33** showed a statistically significant ($p < 0.05$) 38% and 39% reduction in formalin-induced nociception at the 10 μmol/kg ip dose, respectively.

Only very modest improvements in aqueous solubility were able to be achieved through various substitutions at the 6-position or by incorporation of the aminopyridyl substituents at Ar_7. Therefore, despite the excellent in vitro and encouraging in vivo data associated with this series of compounds, their poor solubility and the presumed poor pharmacokinetic properties precluded further development of the trisubstituted pyridopyrimidines.

In conclusion, we have developed a series of 5,6,7-trisubstituted 4-aminopyrido[2,3-*d*]pyrimidines as novel nonnucleoside AK inhibitors. These compounds showed very potent inhibitory activity at the AK enzyme, some with sub-nanomolar potency. A wide variety of substituted aryl groups at the 5-position, and substituted aryl and alkyl groups at the 6-position were evaluated and yielded potent analogues. At the 7-position, the 2-thiophene moiety generally gave the most potent analogues, often in the sub-nanomolar range. In an effort to improve the solubility of these compounds a variety of amino-substituted pyridyl groups were installed at the 7-position which resulted in potent AK inhibitors, but had only a modest effect on the aqueous solubility. In addition, some analogues showed statistically significant analgesic activity in the mouse hotplate and rat formalin animal models of pain.

Experimental Section

^{1}H NMR spectra were obtained at 300 MHz using tetramethylsilane as internal standard. The mass spectra (electron spray ionization (ESI) and dissolvable chemical ionization (DCI)) and high-resolution mass spectra were recorded on Finnigin-4000 instruments. Elemental combustion analyses were within ±0.4% of theoretical values and were performed by Robertson Microlit Laboratories. Flash column chromatography was carried out on EM Science silica gel 60 (230−400 mesh). Reactions were routinely conducted under inert atmosphere (N_2) using commercial high purity solvents as received.

General procedures for the preparation of compounds **21**−**23** were followed as described in ref 10.

General Procedures for the Preparation of Compounds 24−27: 1,2-Bis(4-isopropylphenyl)acetylene (18; Ar_5 = 4-isopropylphenyl). To a solution of 4-iodoisopropylbenzene (12.3 g, 50 mmol) in triethylamine (150 mL) were added trimethylsilylacetylene (5.89 g, 60 mmol), dichlorobis(triphenylphosphine)palladium(II) (0.70 g, 1 mmol), and copper(I) iodide (1.5 g, 7.9 mmol). The reaction was stirred at room temperature for 18 h, diluted with hexanes, and filtered. The filtrate was evaporated under reduced pressure to give crude 1-(4-isopropylphenyl)-2-trimethylsilylacetylene. This crude product was dissolved in methanol (100 mL), aqueous 1 M potassium carbonate solution (25 mL) was added, and the reaction was stirred at room temperature for 2 h. The reaction mixture was then diluted with water and extracted with pentane. The organic layers were combined, dried with magnesium sulfate, and evaporated under reduced pressure without heating to give crude 4-isopropylphenyl acetylene. This crude acetylene was then dissolved in triethylamine (100 mL). 4-Iodoisopropylbenzene (12.3 g, 50 mmol), dichlorobis(triphenylphosphine)-palladium(II) (0.70 g, 1 mmol), and copper(I) iodide (1.5 g, 7.9 mmol) were added. The reaction was stirred at room temperature for 2 days, heated to reflux for 1 h, cooled, diluted with hexanes, and filtered. The filtrate was evaporated under reduced pressure. The residue was filtered through a pad of silica gel with hexanes, and the solvent was evaporated to give 11.40 g (87%) of the title compound. ^{1}H NMR ($CDCl_3$): δ 7.45 (m, 4H), 7.20 (m, 4H), 2.91 (m, 2H), 1.25 (d, 12H, $J = 7.0$ Hz). MS (DCI/NH_3): m/z 263 $(M + H)^+$.

4,6-Diamino-5-(1,2-bis(4-isopropylphenyl)ethenyl)pyrimidine (20; Ar_5 = 4-isopropylphenyl). 1,2-Bis(4-isopropylphenyl)acetylene (11.40 g, 43 mmol) was dissolved in 50 mL of THF, catecholborane (1 M, 50 mL) in THF was added, and the mixture was heated at reflux for 30 h. The mixture was cooled, then 4,6-diamino-5-iodopyrimidine,[10] 30 mL of saturated aqueous sodium bicarbonate, 20 mL of 3 N aqueous sodium hydroxide, and 1.00 g (0.87 mmol) tetrakis(triphenylphosphine)palladium(0) were added. The mixture was heated to reflux for 18 h, cooled, diluted with water, then extracted with ethyl acetate. The organic layers were combined, dried with magnesium sulfate, and the solvent evapo-

rated. The residue was chromatographed on silica gel with 2.5% to 5% (19:1 ethanol:ammonium hydroxide) in ethyl acetate to give the desired product (4.53 g, 28% yield). ^{1}H NMR (DMSO-d_6): δ 7.81 (s, 1H), 7.26 (d, 2H, J = 8.1 Hz), 7.12 (m, 4H), 7.06 (d, 2H, J = 8.1 Hz), 6.57 (s, 1H), 5.71 (bs, 4H), 2.84 (m, 2H), 1.18 (m, 12H). MS (DCI/NH_3): m/z 273 $(M + H)^+$.

4-Amino-5,6-bis(4-isopropylphenyl)-7-(4-dimethylaminophenyl)pyrido[2,3-*d*]pyrimidine (25). A sample of 4,6-diamino-5-(1,2-bis(4-isopropylphenyl)ethenyl)pyrimidine (745 mg, 2 mmol) was dissolved in 20 mL of 1,2,4-trichlorobenzene containing 4-dimethylaminobenzaldehyde (0.89 g, 6 mmol), and approximately 1 g of 4 Å molecular sieves was added to the reaction mixture. The mixture was heated to reflux for 20 h, cooled, and filtered through a pad of Celite. The filtrate was applied directly to a silica gel chromatography column, which was eluted with 2.5% (19:1 ethanol:ammonium hydroxide) in ethyl acetate to give the desired product (186 mg, 18.5% yield). ^{1}H NMR (DMSO-d_6): δ 8.51 (s, 1H), 7.70 (bs, 1H), 7.26 (d, 2H, J = 9 Hz), 7.17 (d, 2H, J = 8 Hz), 7.12 (d, 2H, J = 8 Hz), 6.90 (d, 2H, J = 8 Hz), 6.83 (d, 2H, J = 8 Hz), 6.49 (d, 2H, J = 9 Hz), 4.70 (bs, 1H), 2.87 (s, 6H), 2.84 (m, 1H), 2.72 (m, 1H), 1.12 (d, 6H, J = 6.8 Hz), 1.06 (d, 6H, J = 6.8 Hz). MS (DCI/NH_3): m/z 502 $(M + H)^+$. Anal. Calcd ($C_{33}H_{35}N_5$·0.25EtOAc): C, H, N.

4-Amino-5,6-diphenyl-7-(4-dimethylaminophenyl)pyrido[2,3-*d*]pyrimidine (24). Compound **24** was synthesized using iodobenzene and the process described for the synthesis of **25**. ^{1}H NMR (DMSO-d_6): δ 8.53 (s, 1H), 7.75 (bs, 1H), 7.37−7.25 (m, 5H), 7.24 (d, 2H, J = 9 Hz), 7.15 (m, 3H), 6.98 (m, 2H), 6.50 (d, 2H, J = 9 Hz), 4.50 (bs, 1H), 2.88 (s, 6H). MS (DCI/NH_3): m/z 418 $(M + H)^+$. Anal. Calcd ($C_{27}H_{23}N_5$·$0.5H_2O$): C, H, N.

4-Amino-5,6-diphenyl-7-(thien-2-yl)pyrido[2,3-*d*]pyrimidine (26). Compound **26** was synthesized using iodobenzene, 2-thiophenecarboxaldehyde, and the process described for the synthesis of **25**. ^{1}H NMR (DMSO-d_6): δ 8.55 (s, 1H), 7.85 (bs, 1H), 7.65 (m, 1H), 7.40−7.20 (m, 8H), 7.16 (m, 2H), 6.84 (m, 1H), 6.14 (m, 1H), 4.50 (bs, 1H). MS (DCI/NH_3): m/z 381 $(M + H)^+$. Anal. Calcd ($C_{23}H_{16}N_4S$·$0.3H_2O$): C, H, N.

4-Amino-5,6-bis(3-fluoro-4-methylphenyl)-7-(thien-2-yl)pyrido[2,3-*d*]pyrimidine Hydrochloride (27). Compound **27** was synthesized using 3-fluoro-4-methyliodobenzene, 2-thiophenecarboxaldehyde, and the process described for the synthesis of **25**. ^{1}H NMR (DMSO-d_6): δ 9.77 (bs, 1H), 8.90 (s, 1H), 7.84 (m, 1H), 7.43−6.85 (m, 7H), 6.52 (m, 1H), 5.83 (bs, 1H), 2.21 (s, 6H). MS (DCI/NH_3): m/z 445 $(M + H)^+$. Anal. Calcd ($C_{25}H_{18}F_2N_4S$·1.9HCl): C, H, N.

General Procedures for the Preparation of Compounds 28−31: 4-Bromo-2-(2,2-dicyanoethenyl)thiophene (5; Ar_5 = 4-bromothien-2-yl). Compound **5** was synthesized following the method of Bastus.[12] 4-Bromo-2-thiophenecarboxaldehyde (6.92 g, 36.2 mmol) and malononitrile (2.39 g, 36.2 mmol) were dissolved in 100 mL of 1:1 EtOH:H_2O. A small spatula of glycine was added, and the reaction was stirred at ambient temperature for 30 min. The precipitated product was collected by suction filtration, washed with water, and dried under vacuum overnight. The result was 8.38 g (97%) of the desired product. ^{1}H NMR ($CDCl_3$): δ 7.77 (m, 1H), 7.74 (m, 2H). MS (DCI/NH_3): m/z 238/240 $(M + H)^+$.

2-(3,4-Dimethoxyphenyl)-1-(thien-2-yl)ethanone (4; R_6 = 3,4-dimethoxyphenyl, Ar_7 = thien-2-yl). (3,4-Dimethoxyphenyl)acetic acid (13.0 g, 66.4 mmol) was suspended in 200 mL of anhydrous CH_2Cl_2 followed by addition of EDCI (15.3 g, 79.7 mmol), HOBt (20.6 g, 152 mmol), triethylamine (8.06 g, 79.7 mmol), and *N*,*O*-dimethylhydroxylamine hydrochloride (6.48 g, 66.4 mmol). The reaction was stirred 3 days at ambient temperature after which the solvent was evaporated at reduced pressure. The residue was partitioned between EtOAc and water. The organic layer was washed with aq HCl, sat. $NaHCO_3$, brine, dried (Na_2SO_4), and concentrated in vacuo to give 10.5 g (66%) of *N*-methyl-*N*-methoxy-(3,4-dimethoxyphenyl)acetamide as a pale brown oil.

2-Lithiothiophene (1.0 M in THF, 33.0 mL, 33.0 mmol) was added dropwise to a solution of the oil obtained above (5.26 g, 22.0 mmol) in 75 mL of anhydrous THF at −78 °C. The reaction was allowed to proceed 90 min., then diluted with 100 mL of Et_2O and poured into 1 N aq HCl. The aqueous phase was extracted with Et_2O, and the combined organic fraction was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (25% EtOAc/hexanes) yielded 2.91 g (50%) of the title compound. ^{1}H NMR ($CDCl_3$): δ 7.77 (dd, 1H, J = 3.6, 1.2 Hz), 7.64 (dd, 1H, J = 5.1, 1.2 Hz), 7.13 (dd, 1H, J = 5.1, 3.6 Hz), 6.84 (m, 3H), 4.14 (s, 2H), 3.88 (s, 3H), 3.86 (s, 3H). MS (DCI/NH_3): m/z 263 $(M + H)^+$, 280 $(M + NH_4)^+$.

3-Cyano-4-(4-bromothien-2-yl)-5-(3,4-dimethoxyphenyl)-6-(thien-2-yl)-2-pyridineamine (6; Ar_5 = 4-bromothien-2-yl, R_6 = 3,4-dimethoxyphenyl, Ar_7 = thien-2-yl). 2-(3,4-Dimethoxyphenyl)-1-(thien-2-yl)ethanone (1.56 g, 5.95 mmol), 4-bromo-2-(2,2-dicyanoethenyl)thiophene (1.71 g, 7.13 mmol), and NH_4OAc (1.15 g, 14.9 mmol) were combined in *n*-BuOH (10 mL) and heated to reflux. After 24 h the reaction mixture was cooled, diluted with EtOAc, and washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography (40% EtOAc/hexanes) gave the desired product (0.76 g) which was shown to contain minor unidentifiable side products which could not be separated. ^{1}H NMR ($CDCl_3$): δ 7.40−6.60 (m, 8H), 5.36 (bs, 2H), 3.91 (s, 3H), 3.72 (s, 3H). MS (DCI/NH_3): m/z 498/500 $(M + H)^+$.

4-Amino-5-(4-bromothien-2-yl)-6-(3,4-dimethoxyphenyl)-7-(thien-2-yl)pyrido[2,3-*d*]pyrimidine Hydrochloride (28). 3-Cyano-4-(4-bromothien-2-yl)-5-(3,4-dimethoxyphenyl)-6-(thien-2-yl)-2-pyridineamine (750 mg, 1.50 mmol) and formamidine acetate (312 mg, 3.00 mmol) were taken up in 10 mL of diglyme and heated to 155 °C. Additional formamidine acetate (1 equiv) was added at 90 min intervals over a total of 6 h, then heating was continued overnight. The cooled reaction mixture was then partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography (3.5% MeOH/CH_2Cl_2) gave a brown residue which was dissolved in a small amount of CH_2Cl_2 followed by addition of Et_2O to precipitate the desired product (209 mg, 26%). This material was converted to the hydrochloride salt using ethanolic HCl followed by precipitation with Et_2O and filtration of the product. ^{1}H NMR (DMSO-d_6): δ 9.91 (bs, 1H), 8.89, (s, 1H), 7.88 (m, 1H), 7.83 (d, 1H, J = 5.1 Hz), 7.27 (m, 1H), 7.02 (m, 1H), 6.96 (d, 1H, J = 9.0 Hz), 6.85 (d, 1H, J = 2.1 Hz), 6.70 (m, 2H), 6.47 (bs, 1H), 3.78 (s, 3H), 3.64 (s, 3H). MS (DCI/NH_3): m/z 525/527 $(M + H)^+$. Anal. Calcd ($C_{23}H_{17}BrN_4O_2S_2$·1.1HCl): C, H, N.

4-Amino-5-(3-bromophenyl)-6-ethoxycarbonylmethyl-7-(thien-2-yl)pyrido[2,3-*d*]pyrimidine Hydrochloride (29). Compound **29** was synthesized using ethylsuccinyl chloride, 3-bromo-(2,2-dicyanoethenyl)benzene,[12] and the process described for the synthesis of **28**. ^{1}H NMR (DMSO-d_6): δ 9.88 (bs, 1H), 8.87 (s, 1H), 8.0 (m, 1H), 7.9 (m, 1H), 7.75−7.6 (m, 3H), 7.43 (m, 1H), 7.32 (m, 1H), 6.13 (bs, 1H), 4.01 (q, 2H, J = 7 Hz), 3.75 (m, 2H), 1.09 (t, 3H, J = 7 Hz). MS (DCI/NH_3): m/z 469/471 $(M + H)^+$. Anal. Calcd ($C_{21}H_{17}BrN_4O_2S$·HCl): C, H, N.

4-Amino-5-(4-bromothien-2-yl)-6-(3,4-dimethoxyphenyl)-7-(4-(*N*-methyl-*N*-(2-methoxyethyl)amino)phenyl)pyrido-[2,3-*d*]pyrimidine Hydrochloride (30). Compound **30** was synthesized using 1-bromo-4-(*N*-methyl-*N*-(2-methoxyethyl)-amino)benzene and the process described for the synthesis of **28**. ^{1}H NMR (DMSO-d_6): δ 9.71 (bs, 1H), 8.85 (s, 1H), 7.86 (m, 1H), 7.40 (m, 1H), 7.34 (d, 2H, J = 9 Hz), 6.79 (m, 2H), 6.59 (d, 2H, J = 9 Hz), 6.48 (dd, 1H, J = 8.1, 1.7 Hz), 6.31 (bs, 1H), 3.69 (s, 3H), 3.60 (s, 3H), 3.55−3.40 (m, 4H), 3.22 (s, 3H), 2.92 (s, 3H). MS (DCI/NH_3): m/z 606/608 $(M + H)^+$. Anal. Calcd ($C_{29}H_{28}BrN_5O_3S$·1.4HCl): C, H, N.

4-Amino-5-(3-bromophenyl)-6-(3,4-dimethoxyphenyl)-7-(4-(dimethylamino)phenyl)pyrido[2,3-*d*]pyrimidine Dihydrochloride (31). Compound **31** was synthesized using 3-bromo-(2,2-dicyanoethenyl)benzene,[12] 1-bromo-4-(dimethylamino)benzene, and the process described for the synthesis of **28**. ^{1}H NMR (DMSO-d_6): δ 9.90 (bs, 1H), 8.92 (s, 1H), 7.61

(m, 1H), 7.48 (d, 2H, J = 9 Hz), 7.45−7.3 (m, 2H), 6.80−6.67 (m, 2H), 6.60 (d, 2H, J = 9 Hz), 6.45−6.35 (m, 2H), 5.96 (bs, 1H), 3.65 (s, 3H), 3.55 (bd, 3H), 2.93 (s, 6H). MS (DCI/NH_3): m/z 556/558 $(M + H)^+$. Anal. Calcd ($C_{29}H_{26}BrN_5O_2$·2HCl): C, H, N.

General Procedure for Preparation of Compounds 32−37: 1-(6-Chloropyridin-3-yl)-2-phenylethanone (13; R_6 = phenyl). A solution of ethyl phenylacetate (10.3 g, 62.8 mmol, neat) was added dropwise to a solution of lithium bis-(trimethylsilyl)amide (75 mL, 75 mmol) in 150 mL of THF at −78 °C. The reaction was stirred for 60 min followed by addition of 6-chloronicotinyl chloride (13.3 g, 75 mmol, solid) in one portion. The reaction was stirred an additional 60 min, then quenched with saturated ammonium chloride solution. The mixture was diluted with Et_2O, poured into water, and the aqueous phase was extracted with Et_2O. The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated in vacuo to 22 g crude product as a light yellow solid. This material was dissolved in DMSO (200 mL) and H_2O (10 mL), and the solution was heated to 155 °C for 3 h. The reaction was then cooled and poured into water, and the product was extracted with Et_2O. The combined Et_2O layers were washed with water, brine, dried ($MgSO_4$), and concentrated under vacuum. The product was purified by trituration with 30% EtOAc/hexanes which gave 7.79 g (54%) of the title compound. ^{1}H NMR ($CDCl_3$): δ 8.99 (d, 1H, J = 2.1 Hz), 8.21 (dd, 1H, J = 8.4, 2.1 Hz), 7.43 (d, 1H, J = 8.4 Hz), 7.39−7.22 (m, 5H), 4.27 (s, 2H). MS (DCI/NH_3): m/z 232 $(M + H)^+$.

1-(6-Morpholinopyridin-3-yl)-2-phenylethanone (4; R_6 = phenyl, Ar_7 = 6-morpholinopyridin-3-yl). The ketone **13** from above (2.18 g, 9.41 mmol) and morpholine (2.46 mL, 28.3 mmol) were dissolved in 20 mL of absolute ethanol, and the mixture was heated to reflux for 18 h. The volatiles were then removed under vacuum, and the residue was partitioned between Et_2O and saturated $NaHCO_3$. The Et_2O layer was washed with brine, dried (Na_2SO_4), and concentrated under vacuum to give the title compound (2.62 g, 99%). ^{1}H NMR ($CDCl_3$): δ 8.85 (d, 1H, J = 2.7 Hz), 8.08 (dd, 1H, J = 9.6, 2.7 Hz), 7.36−7.19 (m, 5H), 6.60 (d, 1H, J = 9.6 Hz), 4.17 (s, 2H), 3.80 (m, 4H), 3.66 (m, 4H). MS (DCI/NH_3): m/z 283 $(M + H)^+$.

3-Cyano-4-(3-bromophenyl)-5-phenyl-6-(6-morpholinopyridin-3-yl)-2-pyridineamine (6; Ar_5 = 3-bromophenyl, R_6 = phenyl, Ar_7 = 6-morpholinopyridin-3-yl). A solution of 1-(6-morpholinopyridin-3-yl)-2-phenylethanone (1.30 g, 4.60 mmol), 3-bromo-(2,2-dicyanoethenyl)benzene (1.61 g, 6.91 mmol), and NH_4OAc (0.89 g, 11.5 mmol) in 15 mL of *n*-butanol was heated to reflux. After 24 h the reaction mixture was cooled to ambient temperature followed by addition of Et_2O. The resulting precipitate was collected by filtration, washed with Et_2O, and dried under vacuum. The result was 0.57 g of the desired product which was found to also contain some of the starting ketone. ^{1}H NMR ($CDCl_3$): δ 8.20 (d, 1H, J = 2.1 Hz), 7.40−7.20 (m, 3H), 7.10 (m, 4H), 7.02 (m, 1H), 6.83 (m, 2H), 6.36 (d, 1H, J = 9 Hz), 5.30 (bs, 2H), 3.77 (m, 4H), 3.47 (m, 4H). MS (DCI/NH_3): m/z 512/514 $(M + H)^+$.

4-Amino-5-(3-bromophenyl)-6-phenyl-7-(6-morpholinopyridin-3-yl)pyrido[2,3-*d*]pyrimidine Dihydrochloride (34). 3-Cyano-4-(3-bromophenyl)-5-phenyl-6-(6-morpholinopyridin-3-yl)-2-pyridineamine (566 mg, 1.10 mmol) and formamidine acetate (573 mg, 5.50 mmol) were taken up in 15 mL of diglyme and heated to 150 °C. Additional formamidine acetate (1 equiv) was added at 90 min intervals over a total of 6 h, then heating was continued overnight. The cooled reaction mixture was then partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography (5% MeOH/CH_2Cl_2) gave a brown residue which was dissolved in a small amount of CH_2Cl_2 followed by addition of Et_2O to precipitate the product. This material was converted to the hydrochloride salt using ethanolic HCl followed by precipitation with Et_2O and filtration of the product (102 mg, 15%). ^{1}H NMR (DMSO-d_6): δ 10.05 (bs, 1H), 8.97 (s, 1H), 8.15 (d, 1H, J = 2.4 Hz), 7.58 (dt, 1H, J = 7.5, 1.7 Hz), 7.53 (d, 1H, J = 2.4 Hz), 7.49 (m, 1H), 7.34 (m, 2H), 7.20 (s, 3H), 7.05 (m, 2H), 6.76 (d, 1H, J = 9.5 Hz), 6.12 (bs, 1H), 3.64 (m, 4H), 3.50 (m, 4H). MS (DCI/NH_3): m/z 539/541 $(M + H)^+$. Anal. Calcd ($C_{28}H_{23}BrN_6O$·2HCl): C, H, N.

4-Amino-5,6-diphenyl-7-(6-(dimethylamino)pyridin-3-yl)pyrido[2,3-*d*]pyrimidine Hydrochloride (32). Compound **32** was synthesized using (2,2-dicyanoethenyl)benzene,[12] ethyl phenylacetate, dimethylamine, and the process described for the synthesis of **34**. ^{1}H NMR (DMSO-d_6): δ 10.1 (bs, 1H), 8.98 (s, 1H), 8.13 (d, 1H, J = 2.0 Hz), 7.55 (dd, 1H, J = 9.2, 2.0 Hz), 7.43 (m, 3H), 7.29 (m, 2H), 7.18 (d, 3H), 7.08 (m, 2H), 6.83 (m, 1H), 5.80 (bs, 1H), 3.14 (s, 6H). MS (DCI/NH_3): m/z 419 $(M + H)^+$. Anal. Calcd ($C_{26}H_{22}N_6$·1.8HCl): C, H, N.

4-Amino-5-(3-chlorophenyl)-6-phenyl-7-(6-(dimethylamino)-pyridin-3-yl)-pyrido[2,3-*d*]pyrimidine Dihydrochloride (33). Compound **33** was synthesized using 3-chloro-(2,2-dicyanoethenyl)benzene,[12] ethyl phenylacetate, dimethylamine, and the process described for the synthesis of **34**. ^{1}H NMR (DMSO-d_6): δ 10.06 (bs, 1H), 8.98 (s, 1H), 8.10 (d, 1H, J = 2.4 Hz), 7.55 (dd, 1H, J = 9.6, 2.4 Hz), 7.43 (m, 2H), 7.36 (m, 1H), 7.30−7.15 (m, 4H), 7.09 (m, 1H), 7.03 (m, 1H), 6.76 (bd, 1H), 6.15 (bs, 1H), 3.10 (s, 6H). MS (DCI/NH_3): m/z 453 $(M + H)^+$. Anal. Calcd ($C_{26}H_{21}ClN_6$·2HCl): C, H, N.

4-Amino-5-(3-bromophenyl)-6-(4-fluorophenyl)-7-(6-morpholinopyridin-3-yl)pyrido[2,3-*d*]pyrimidine Dihydrochloride (35). Compound **35** was synthesized using 3-bromo-(2,2-dicyanoethenyl)benzene,[11] ethyl 4-fluorophenylacetate, and the process described for the synthesis of **34**. ^{1}H NMR (DMSO-d_6): δ 10.05 (bs, 1H), 8.97, (s, 1H), 8.17 (d, 1H, J = 2.7 Hz), 7.61 (m, 1H), 7.50 (m, 2H), 7.37 (t, 1H, J = 7.8 Hz), 7.31 (m, 1H), 7.15−7.0 (m, 4H), 6.79 (d, 1H, J = 9.5 Hz), 6.16 (bs, 1H), 3.65 (m, 4H), 3.52 (m, 4H). MS (DCI/NH_3): m/z 557/559 $(M + H)^+$. Anal. Calcd ($C_{28}H_{22}BrFN_6O$·2.1HCl): C, H, N.

4-Amino-5-(3-chlorophenyl)-6-(4-dimethylaminophenyl)-7-(6-morpholinopyridin-3-yl)pyrido[2,3-*d*]pyrimidine (36). Compound **36** was synthesized using 3-chloro-(2,2-dicyanoethenyl)benzene,[12] ethyl 4-(dimethylamino)phenylacetate, and the process described for the synthesis of **34**. ^{1}H NMR (DMSO-d_6): δ 8.72 (bs, 1H), 8.28 (d, 1H, J = 2.6 Hz), 7.80 (dd, 1H, J = 8.8, 2.6 Hz), 7.30 (m, 2H), 7.20 (bs, 1H), 7.06 (m, 1H), 6.70 (d, 2H, J = 8.5 Hz), 6.45 (m, 3H), 5.15 (bs, 2H), 3.78 (m, 4H), 3.51 (m, 4H), 2.88 (s, 6H). MS (DCI/NH_3): m/z 538 $(M + H)^+$. Anal. Calcd ($C_{30}H_{28}ClN_7O$·0.1H_2O): C, H, N.

4-Amino-5-(4-bromothien-2-yl)-6-cyclopropyl-7-(6-(dimethylamino)pyridin-3-yl)pyrido[2,3-*d*]pyrimidine Dihydrochloride (37). Compound **37** was synthesized using 4-bromo-2-(2,2-dicyanoethenyl)thiophene,[12] ethyl 2-cyclopropyl acetate, dimethylamine, and the process described for the synthesis of **34**. ^{1}H NMR ($CDCl_3$): δ 8.71 (d, 1H, J = 2 Hz), 8.67 (s, 1H), 8.23 (dd, 1H, J = 9, 2 Hz), 7.58 (d, 1H, J = 1.5 Hz), 7.14 (d, 1H, J = 1.5 Hz), 6.63 (d, 1H, J = 9 Hz), 5.45 (b, 2H), 3.20 (s, 6H), 1.98 (m, 1H), 0.73 (m, 2H), 0.13 (m, 2H). MS (DCI/NH_3): m/z 467/469 $(M + H)^+$. Anal. Calcd ($C_{21}H_{19}BrN_6S$·2.3HCl): C, H, N.

General Procedure for Preparation of Compounds 38−41: 1-(6-Chloropyridin-3-yl)-3-phenylpropanone (13; R_6 = benzyl). A sample of 6-chloronicotinyl chloride (15.4 g, 87.4 mmol) was added to a mixture of *N*,*O*-dimethylhydroxylamine hydrochloride (9.38 g, 96.2 mmol) and triethylamine (36.6 mL, 262 mmol) in 200 mL of CH_2Cl_2 cooled to 0 °C. The reaction was stirred for 2 h, then poured into water. The separated organic layer was washed with brine, dried (Na_2SO_4), and concentrated under vacuum to give 14.6 g of the intermediate Weinreb amide as a light brown oil. A sample of the intermediate amide (4.09 g, 20.4 mmol) in 100 mL of THF was cooled to −78 °C followed by dropwise addition of phenethylmagnesium chloride (30.6 mL, 30.6 mmol, 1 M in THF). The reaction was allowed to warm to ambient temperature and stir 3 h after which it was quenched by 1 N aq HCl. The mixture was partitioned between Et_2O and saturated $NaHCO_3$. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by flash chromatography eluting with 30% EtOAc/hexanes which gave

3.77 g (75%) of the desired product. ^{1}H NMR ($CDCl_3$): δ 8.92 (d, 1H, J = 2.7 Hz), 8.18 (dd, 1H, J = 8.4, 2.7 Hz), 7.44 (d, 1H, J = 8.4 Hz), 7.37−7.18 (m, 5H), 3.30 (m, 2H), 3.08 (m, 2H). MS (DCI/NH_3): m/z 246 $(M + H)^+$.

1-(6-Morpholinopyridin-3-yl)-3-phenylpropanone (4; R_6 = benzyl, Ar_7 = 6-morpholinopyridin-3-yl). A solution of ketone **13** from above (3.77 g, 15.3 mmol) and morpholine (5.35 mL, 61.4 mmol) in 30 mL of absolute ethanol was heated to reflux for 18 h. The volatiles were then removed under vacuum, and the residue was partitioned between Et_2O and saturated $NaHCO_3$. The Et_2O layer was washed with brine, dried (Na_2SO_4), and concentrated under vacuum to give 4.50 g (100%) of the title compound. ^{1}H NMR ($CDCl_3$): δ 8.78 (m, 1H), 8.05 (dd, 1H, J = 9.3, 2.1 Hz), 7.34−7.16 (m, 5H), 6.60 (d, 1H, J = 9.3 Hz), 3.81 (m, 4H), 3.67 (m, 4H), 3.19 (m, 2H), 3.05 (m, 2H). MS (DCI/NH_3): m/z 297 $(M + H)^+$.

3-Cyano-4-(3-bromophenyl)-5-benzyl-6-(6-morpholinopyridin-3-yl)-2-pyridineamine (6; Ar_5 = 3-bromophenyl, R_6 = benzyl, Ar_7 = 6-morpholinopyridin-3-yl). A solution of 1-(6-morpholinopyridin-3-yl)-3-phenylpropanone (1.68 g, 5.67 mmol), 3-bromo-(2,2-dicyanoethenyl)benzene[12] (1.98 g, 8.50 mmol), and NH_4OAc (1.09 g, 14.2 mmol) in 15 mL of *n*-butanol was heated to reflux. After 16 h the reaction mixture was cooled and concentrated in vacuo. Flash chromatography (50% EtOAc/hexanes) gave 0.84 g (28%) of the desired product. ^{1}H NMR ($CDCl_3$): δ 8.36 (d, 1H, J = 2.1 Hz), 7.61 (dd, 1H, J = 9.3, 2.1 Hz), 7.48 (m, 1H), 7.20−7.07 (m, 5H), 6.97(m, 1H), 6.67−6.56 (m, 3H), 5.25 (bs, 2H), 3.83 (m, 6H), 3.55 (t, 4H, J = 5.1 Hz). MS (DCI/NH_3): m/z 526/528 $(M + H)^+$.

4-Amino-5-(3-bromophenyl)-6-benzyl-7-(6-morpholinopyridin-3-yl)pyrido[2,3-*d*]pyrimidine Dihydrochloride (41). A solution of 3-cyano-4-(3-bromophenyl)-5-benzyl-6-(6-morpholinopyridin-3-yl)-2-pyridineamine (830 mg, 1.58 mmol) and formamidine acetate (822 mg, 7.90 mmol) in 15 mL of diglyme was heated to 150 °C. Additional formamidine acetate (1 equiv) was added at 90 min intervals over a total of 6 h and heating was continued overnight. The cooled reaction mixture was then partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography (5% MeOH/CH_2Cl_2) gave a brown residue which was dissolved in a small amount of CH_2Cl_2 followed by addition of Et_2O to precipitate the desired product. This material was converted to the hydrochloride salt using ethanolic HCl followed by precipitation with Et_2O and filtration of the product. (620 mg, 63%). ^{1}H NMR (DMSO-d_6): δ 9.94 (bs, 1H), 8.92, (s, 1H), 8.40 (d, 1H, J = 2.4 Hz), 7.83 (dd, 1H, J = 8.8, 2.4 Hz), 7.74 (m, 1H), 7.46 (m, 2H), 7.34 (m, 1H), 7.18−7.05 (m, 3H), 6.93 (d, 1H, J = 9.2 Hz), 6.67 (m, 2H), 6.10 (bs, 1H), 4.00 (m, 2H), 3.68 (m, 4H), 3.55 (m, 4H). MS (DCI/NH_3): m/z 553/555 $(M + H)^+$. Anal. Calcd ($C_{29}H_{25}BrN_6O$·2HCl): C, H, N.

4-Amino-5-(4-bromothien-2-yl)-6-isopropyl-7-(6-morpholinopyridin-3-yl)pyrido[2,3-*d*]pyrimidine Dihydrochloride (38). Compound **38** was synthesized using 3-bromo-(2,2-dicyanoethenyl)benzene,[12] isobutylmagnesium chloride, and the process described for the synthesis of **41**. ^{1}H NMR (DMSO-d_6): δ 10.0 (bs, 1H), 8.90 (s, 1H), 8.32 (d, 1H, J = 2 Hz), 7.92 (m, 1H), 7.80 (m, 2H), 7.66 (t, 1H, J = 8 Hz), 7.58 (d, 1H, J = 8 Hz), 7.04 (d, 1H, J = 9 Hz), 5.84 (bs, 1H), 3.74 (m, 4H), 3.59 (m, 4H), 3.35−3.05 (m, 1H), 0.9 (m, 6H). MS (DCI/NH_3): m/z 505/507 $(M + H)^+$. Anal. Calcd ($C_{25}H_{25}BrN_6O$·2.2HCl): C, H, N.

4-Amino-5-(3-bromophenyl)-6-cyclohexyl-7-(6-(dimethylamino)-pyridin-3-yl)pyrido[2,3-*d*]pyrimidine Dihydrochloride (39). Compound **39** was synthesized using 3-bromo-(2,2-dicyanoethenyl)benzene,[12] cyclohexylmethylmagnesium chloride, and the process described for the synthesis of **41**. ^{1}H NMR (DMSO-d_6): δ 8.48 (s, 1H), 8.19 (d, 1H, J = 2.6 Hz), 7.86 (dt, 1H, J = 7.4, 1.8 Hz), 7.81 (bs, 1H), 7.66−7.52 (m, 3H), 6.76 (d, 1H, J = 8.8 Hz), 4.4 (bs, 1H), 3.11 (s, 6H), 2.65 (m, 1H), 1.65−1.35 (m, 5H), 1.15−0.95 (m, 2H), 0.85−0.55 (m, 3H). MS (DCI/NH_3): m/z 503/505 $(M + H)^+$. Anal. Calcd ($C_{26}H_{27}BrN_6$·2HCl): C, H, N.

4-Amino-5-(3-bromophenyl)-6-pentyl-7-(6-(dimethylamino)-pyridin-3-yl)-pyrido[2,3-*d*]pyrimidine Dihydrochloride (40). Compound **40** was synthesized using 3-bromo-(2,2-dicyanoethenyl)benzene,[12] *n*-hexylmagnesium chloride, and the process described for the synthesis of **41**. ^{1}H NMR (DMSO-d_6): δ 8.23 (d, 1H, J = 2.4 Hz), 7.69 (m, 2H), 7.63 (bs, 1H), 7.49 (t, 1H, J = 8 Hz), 7.40 (d, 1H, J = 8 Hz), 6.78 (bs, 2H), 6.70 (d, 1H, J = 9 Hz), 3.07 (s, 6H), 2.31 (m, 2H), 1.02 (m, 2H), 0.85 (m, 4H), 0.59 (t, 3H, J = 7 Hz). MS (DCI/NH_3): m/z 491/493 $(M + H)^+$. Anal. Calcd ($C_{25}H_{27}BrN_6$·2HCl): C, H, N.

AK Inhibition Assay. AK inhibition was measured at 23 °C in a 100 μL reaction mixture in triplicate containing 64 mM Tris HCl (pH 7.5), 0.2 mM $MgCl_2$, 1 mM ATP, 0.2 μM [U-^{14}C]-adenosine or [2-^{3}H]-adenosine (Amersham International, Bucks, United Kingdom) and appropriate volumes of rat brain cytosol as a source of AK as previously described.[9] After incubation for 15 min, the reaction was terminated by aliquoting 40 μL of the reaction mixture onto DE−81 anion exchange filter disks. The filter disks were air-dried, washed in 2 mM ammonium formate, and dried again. Bound radioactivity was determined by standard scintillation spectrometry. A 96-well plate high-throughput screening assay was derived from this methodology using [^{3}H]-6-methylmercaptopurine (Amersham) as an AK substrate that is not susceptible to catabolism by ADO deaminase.

Intact Cell ADO Phosphorylation Assay. Assays for ADO phosphorylation in intact cells were conducted using confluent IMR-32 human neuroblastoma cells (ATCC, Gaithersburg, MD). Appropriate concentrations of test compounds (10^{-11} to 10^{-4} M) were added to each cell culture well and incubated in 400 μL of warm Gey's Balanced Salt Solution for 10 min. The reaction run in triplicate was initiated by the addition of 50 μL of 2 μM [U-^{14}C]-adenosine. After a 20 min incubation, the assay buffer was rapidly aspirated and the cells were quickly frozen by the addition of excess liquid nitrogen. A 50 μL aliquot of the thawed supernatant was placed onto DE−81 filter disks and processed as described above.

In Vivo Evaluation of AK Inhibitors. Acute thermal nociception was assessed in mice (n = 6−8 per group) using the 55 °C hotplate test as previously described.[9] Nociceptive paw flinching in rats (Formalin test, n = 6 per group) was assessed 30 min following an intraplantar injection of 5% formalin (50 μL) into the right hindpaw.[13] Compounds were administered intraperitoneally 30 min before nociceptive testing.

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