Discovery of 4-Amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3-*d*]pyrimidine, an Orally Active, Non-Nucleoside Adenosine Kinase Inhibitor

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Adenosine (ADO) is an endogenous homeostatic inhibitory neuromodulator that reduces cellular excitability at sites of tissue injury and inflammation. Inhibition of adenosine kinase (AK), the primary metabolic enzyme for ADO, selectively increases ADO concentrations at sites of tissue trauma and enhances the analgesic and antiinflammatory actions of ADO. Optimization of the high-throughput screening lead, 4-amino-7-aryl-substituted pteridine (**5**) (AK IC_{50} = 440 nM), led to the identification of compound **21** (4-amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido [2,3-*d*]pyrimidine, ABT-702), a novel, potent (AK IC_{50} = 1.7 nM) non-nucleoside AK inhibitor with oral activity in animal models of pain and inflammation.

Introduction

Extracellular concentrations of the endogenous neuromodulator, adenosine (ADO), are increased under conditions of metabolic stress and trauma (e.g., pain, inflammation, tissue damage, ischemia, seizure activity, etc.) and act to limit tissue damage and restore normal function[1] by activating members of the P1 receptor family, A_1, A_{2A}, A_{2B}, and A_3 receptors.[2] Increased extracellular ADO concentrations can result in the inhibition of excitatory amino acid (glutamate) release, suppression of free radical formation, and neutrophil adhesion, depending on the phenotype of the tissue involved.[3] Since ADO has a half-life on the order of seconds in physiological fluids,[4] its extracellular actions are restricted to the tissue and cellular sites where it is released.[5] The effects of extracellular ADO are terminated by its reuptake and phosphorylation by ADO kinase (AK; ATP: adenosine 5′-phosphotranferase, EC 2.7.1.20) and via deamination by adenosine deaminase (ADA).[6] By preventing ADO phosphorylation, AK inhibition increases intracellular ADO concentrations, altering the equilibrium of the bidirectional transport systems responsible for ADO reuptake with the net effect of increasing the local concentration of ADO in the extracellular compartment.[7] AK inhibition is a more effective mechanism for increasing extracellular ADO levels than inhibition of the catabolic enzyme ADA, as shown by the comparative efficacy of AK and ADA inhibitors in reducing seizure and nociceptive activity in vivo.[8,9]

All published AK inhibitors are purine nucleoside analogues and include 5′-deoxy-5′-amino-ADO **1**,[10] 5′-deoxy-5-iodotubercidin **2**,[11] clitocine **3**,[12] and GP-3269 **4**[13] (Figure 1). The present article describes the optimization of a novel non-nucleoside AK inhibitor, compound **5**, identified as a high-throughput screening lead, to yield 4-amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3-*d*]pyrimidine **21**, a potent (IC_{50} = 1.7 nM) and selective AK inhibitor with oral activity in animal models of pain and inflammation.

Chemistry

Compounds **5**−**10** were prepared from the condensation of 4,5,6-triamonopyrimidine and phenylglyoxals in refluxing 1,4-dioxane as previously described[14] and which is shown in Scheme 1. The synthesis of 6-substituted pteridine **11** was accomplished using similar synthetic methods as illustrated in Scheme 1. The diketone was prepared by oxidation of the corresponding substituted acetophenone by selenium oxide in refluxing 1,4-dioxane.

Synthesis of the 6-substituted pyridopyrimidine analogues (**12**−**16**) was prepared through iodination of 4,6-diaminopyrimidine in DMF to yield 4,6-diamino-5-iodopyrimidne and followed by Suzuki coupling reaction with substituted vinylboronic acid to yield 4,6-diamino-5-vinylpyrimidine. The final cyclization process was furnished by aza-Cope rearrangement of 4,6-diamino-5-vinylpyrimidine and benzaldehyde in diphenyl ether to yield 6-substituted pyridopyrimidine analogues, which is illustrated by Scheme 2.

The 4-amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3-*d*]pyrimidine (**21**) was prepared from the condensation of 3-bromobenzylidene-malononitrile with acetopyridine and ammonium acetate to produce the amino cyano pyridine intermediate **22**. The final cyclization of **22** to the product **21** was furnished by heating in formamide (Scheme 3). [15]

Biology

AK inhibition was measured at 23 °C in a 100 μL reaction mixture in triplicate containing 64 mM Tris

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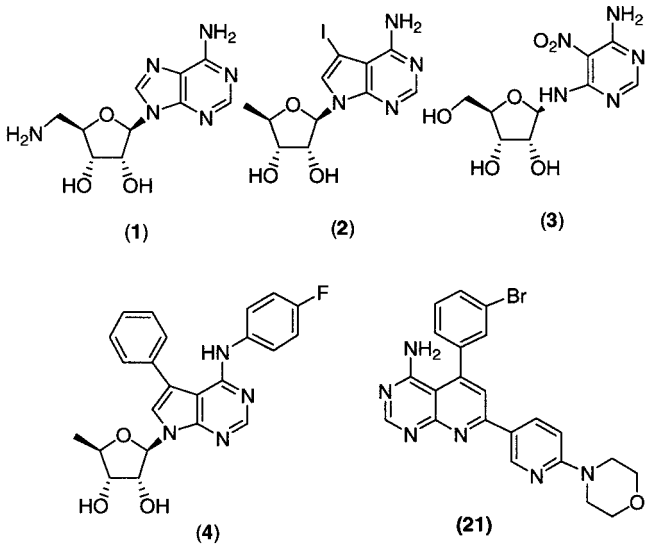
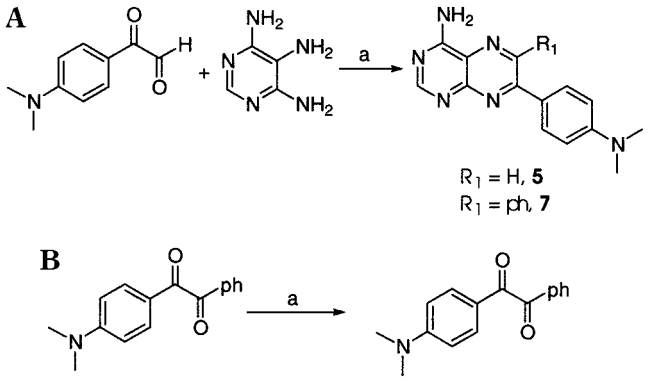


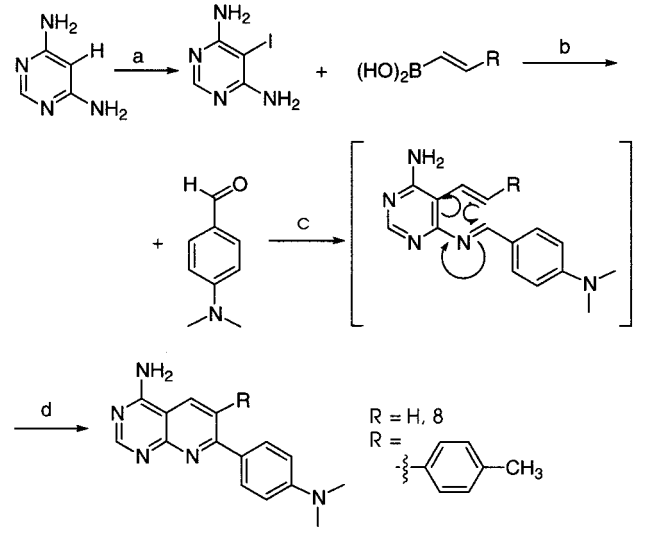
Figure 1. Structures of nucleoside AK inhibitors and **21** (ABT-702).

Scheme 1[a]



[a] Reagents and conditions for A: (a) dioxane/H_2O/Δ. For B: (a) SeO_2/dioxane/Δ.

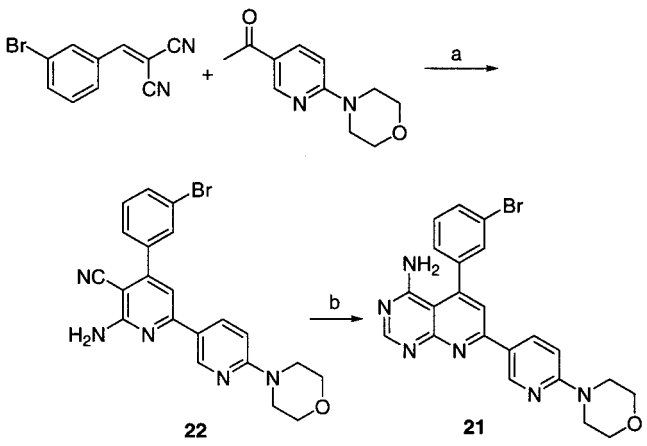
Scheme 2[a]



[a] Reagents and conditions: (a) I_2/DMF; (b) $Pd(PPh_3)_4$/DMF; (c) PhOPh/Δ; (d) air.

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, U.K.), and appropriate volumes of rat brain cytosol as a source of AK as previously

Scheme 3

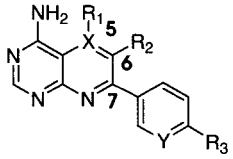


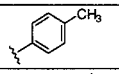
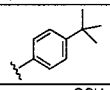
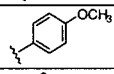
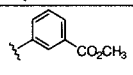
described.[16] After incubation for 15 min, the reaction was terminated by aliquoting 40 μL of the reaction mixture onto DE-81 anion exchange filter disks. The filter disks were air-dried, washed in 2 mM ammonium formate, and dried again. Bound radioactivity was determined by standard scintillation spectrometry. A 96-well plate high-throughput screening assay was derived from this methodology using [^{3}H]-6-methylmercaptopurineribofuranoside (Amersham, Arlington Heights, IL) as an AK substrate that is not susceptible to catabolism by ADO deaminase. 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 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. Nociceptive paw flinching in rats (Formalin test) was assessed 30 min following an intraplantar injection of 5% formalin (50 μL) into the right hind paw.[17]

Results and Discussion

The non-nucleoside AK inhibitor **5** was identified as the result of screening the Abbott compound library for inhibition of AK. Compound **5** inhibited AK activity (IC_{50} = 440 nM, Table 1) but was considerably weaker in inhibiting ADO phosphorylation in intact cells (IC_{50} = 3600 nM), indicating poor ability to penetrate the cell membrane. Initial SAR studies on **5** replacing the dimethylamino group with different functional groups failed to improve AK inhibitory activity. For example, 4-H (**6**), 4-NH_2 (**7**), 4-NO_2 (**8**), 4-OCH_3 (**9**), and 4-morpholino (**10**) showed either weaker or similar in vitro potency compared to **5** (Table 1). However, phenyl substitution in the 6-position of **5** resulted in a greater than 15-fold increase in in vitro activity (Table 1, compound **11**, IC_{50} = 25 nM). Given that compound **11** showed improved AK inhibitory activity relative to **5**, comparison of **5** and **11** with the known nucleoside AK inhibitor, 5′deoxy-5-iodotubercidin **2**, suggested that the 6-phenyl ring of **11** might overlap with the lipophilic

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



Compd	R_1	R_2	R_3	X	Y	AK inhibition IC_{50} (nM)	Inhibition of ADO Phosphorylation in intact cells IC_{50} (nM)
1						15± 7	6330 ± 880
2						1.6 ± 1.1	22.8 ± 33.3
3						2000 ± 316	26,000 ± 10,400
4						2.8 ± 0.7	134 ± 27
5		H	$-N(CH_3)_2$	N	C	440 ± 64	3600 ± 1592
6		H	H	N	C	5500 ± 707	>10,000
7		H	$-NH_2$	N	C	1750 ± 1060	>10,000
8		H	$-NO_2$	N	C	4750 ± 3180	>10,000
9		H	$-OCH_3$	N	C	2000 ± 1000	>10,000
10		H		N	C	400 ± 283	1000 ± 1000
11		-Ph	$-N(CH_3)_2$	N	C	25 ± 21	333 ± 58
12		H	$-N(CH_3)_2$	C	C	773 ± 58	> 10,000
13			$-N(CH_3)_2$	C	C	73.3 ± 30.6	500 ± 200
14			$-N(CH_3)_2$	C	C	62.5 ± 24.7	>1,000
15			$-N(CH_3)_2$	C	C	91.7 ± 67.9	567 ± 404
16			$-N(CH_3)_2$	C	C	66.7 ± 35.1	700 ± 28
17	-Ph	H	$-N(CH_3)_2$	C	C	7.0 ± 0.8	467 ± 577
18	-cyclohexyl	H	$-N(CH_3)_2$	C	C	8.3 ± 1.2	467 ± 231
19		H	$-N(CH_3)_2$	C	C	5 ± 2	167 ± 58
20		H	$-N(CH_3)_2$	C	N	1.8 ± 0.9	32 ± 6
21		H		C	N	1.7 ± 0.5	50 ± 8

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

iodo substituent of **2** (Figures 2 and 3). Replacement of the 5-position nitrogen of **5** with carbon was considered an alternative scaffold to the pteridine pharmacophore and had the potential to offer better access to the iodo lipophilic pocket defined by compound **2** (Figure 3). Further SAR investigation between the 6-substituted pridopyrimidines **12**−**16** and the 5-substituted pyridopyrimidines **18**−**20** suggested that these compounds interacted with AK in similar relative orientations. However, 5-substituted pyridopyrimidines showed a greater than 80-fold improvement in in vitro potency as compared to **5** and were at least 10-fold more active as AK inhibitors as compared to the 6-substitutents analogues. These data indicate that the 5-position substituted pyridopyrimidines offered improved alignment relative to **2** as compared to the 6-position substitutions (Figure 4). That the 5-substituent provided an additional lipophilic interaction was confirmed by the increased AK inhibitor potency of the 5-cyclohexyl derivative **18** as compared to **13**. Extensive SAR studies using the 4-amino-7-(4-dimethylaminophenyl)-pyridopyrimidine core led to the identification of the 5-(3-bromophenyl) analogue **19** as an optimal substituent to improve in vitro potency with high affinity to inhibit

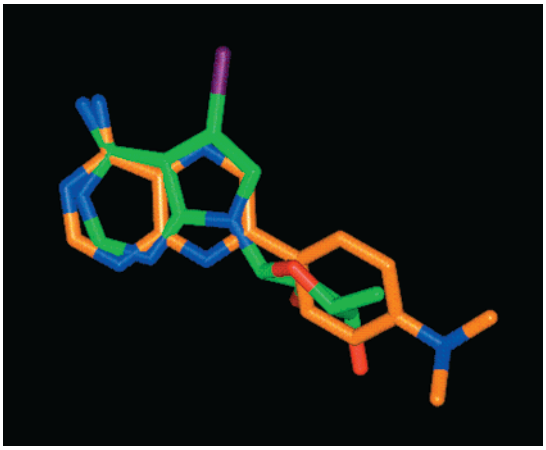


Figure 2. Superposition of compounds **2** (green) and **5** (yellow).

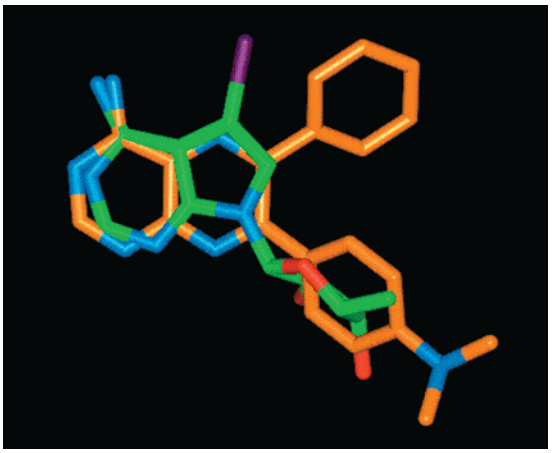


Figure 3. Superposition of compounds **2** and **11** (yellow).

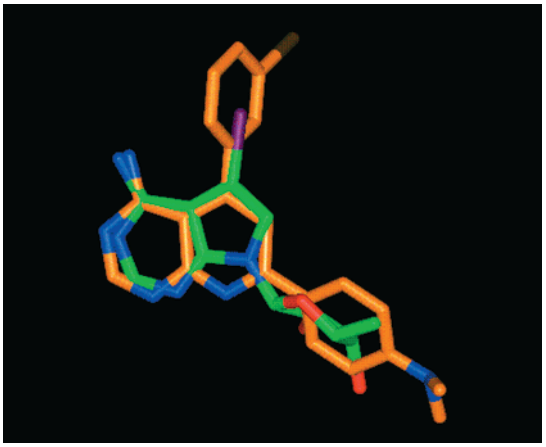


Figure 4. Superposition of compounds **2** and **19** (yellow).

enzymatic AK activity and to inhibit ADO phosphorylation in intact cells (Table 1). Compound **19** also dose dependently reduced nociception in the persistent phase of the formalin test (ED_{50} = 1 μmol/kg; ip).[17] However, compound **19** was not active following oral administration. In an attempt to improve oral activity, heteroatoms were introduced into the 7-phenyl ring. The 7-pyridyl analogue **20**, like **19**, potently inhibited AK and ADO phosphorylation in intact cells (Table 1); however, oral analgesic activity was not improved (21% reduction of formalin-induced nociception, 100 μmol/kg, po). Dealkylation of the dimethylamino substituent on the 7-aryl ring was considered to be the possible cause of the metabolic liability of compound **20** ($t_{1/2}$ = 0.4 h after iv dosing). Additional SAR was focused on the amino substituent of the pyridyl ring with the finding that the morpholine analogue **21** had significantly enhanced in vivo activity (formalin induced nociception ED_{50} = 60 μmol/kg, po)[17] and the plasma half-life was extended to 1 h after iv dosing (data not shown).

4-Amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3-*d*]pyrimidine **21** was active both in inhibiting AK (IC_{50} = 1.7 nM) and ADO phosphorylation in the intact cells (IC_{50} = 50 nM) (Table 1). Compound **21** was also highly selective for AK inhibition as compared to other sites of ADO action including ADA, ADO receptors, and ADO transport sites.[16] Compound **21** had dose-dependent antinociceptive and antiinflammatory actions in a variety of animal models of nociceptive, inflammatory, and neuropathic pain.[16, 17]

In conclusion, compound **21** is the first of a novel class of potent, selective, non-nucleoside, orally active AK inhibitors that have potent antinociceptive effects in animal models.

Experimental Section

Melting points (uncorrected) were determined in open capillary tubes with a Buchi 510 apparatus. ^{1}H NMR was measured on X-300 Hz spectrometers, using tetramethylsilane as internal standard. Positive ion fast atom bombardment (FAB), electron spray ionization (ESI), or dissolvable chemical ionization (DCI) were obtained on Finnigin-4000 instruments. High-resolution mass spectra were measured on a Finnigin-4000 spectrometer. Flash column chromatography was carried out on EM science silica gel 66 (230−400 mesh). Elemental combustion analyses were within ±0.4% of theoretical values and obtained from Roberson Microlit Laboratories, Inc. The following abbreviations are used in the Experimental Section: THF for tetrahydrofuan, $CDCl_3$ for deuteriochloroform, DMSO-d_6 for deuteriodimethyl sulfoxide, CH_2Cl_2 for methylene chloride, DMF for *N*,*N*-dimethylformamide, and TFA for trifluoroacetic acid. Reactions were routinely conducted under inert gas (N_2) unless otherwise indicated.

General procedures for compounds **5**−**11** were followed as described in ref 14.

General Procedures for Compounds 13−16: 5-Iodo-4,6-diaminopyrimidine. 4,6-Diaminopyrimidine hemisulfate monohydrate (26.13 g, 147.5 mmol) and K_2CO_3 (30.58 g, 221.3 mmol) were suspended in water (400 mL), and iodine (41.19g, 162.3 mmol) and DMF (100 mL) were added. The solution was heated at 45 °C for 23 h. After cooling, a 2 M solution of $Na_2S_2O_3$ (15 mL) was added, and the solution turned clear. The white solid was then collected by filtration and washed with water (3 × 20 mL), and the solid was dried under high vacuum to yield 33.1 g of product (90%): ^{1}H NMR (300 MHz, DMSO-d_6) d; MS (DCI/NH_3) *m*/*z* 237 $(M + H)^+$.

***p*-Methyl-styrylboronic Acid.** 4-Ethynyl-4-methyl-benzene (5 mmol) was dissolved in 5 mL of dry THF, and catecholborane (5 mL, 1 M in THF) was added dropwise at 0 °C. The solution was heated to reflux for 1.5 h, and the solvent was removed under vacuum. The solution was quenched with 1 M HCl (10 mL), and this solution was used directly for the next step.

5-(*p*-Methyl-styryl)-4,6-diaminopyrimidine. To a solution of 5-iodo-4,6-diaminopyrimidine (1 mmol) in 50 mL of dioxane (or DME) were added substituted boronic acid (5 mmol), 5% of $Pd(PPh_3)_4$, and 1 M Na_2CO_3 (10 mL). The reaction mixture was heated at 80 °C for 12 h. After workup, the crude mixture was purified by column chromatography (using 5% MeOH/CH_2Cl_2 as eluent) to give the product (yield 45−86%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75 (s, 1H), 7.6 (d, 2 H, J = 7.5 Hz), 7.4 (d, 2 H, J = 7.5 Hz), 6.9 (q, 2 H, J = 16.5, 15 Hz), 2.45 (s, 3 H); MS (DCI/NH_3) *m*/*z* 215 $(M + H)^+$.

4-Amino-6-(4-methylphenyl)-7-(4-(dimethylamino)-phenyl)pyrido[2,3-*d*]pyrimidine (13). 4,6-Diamino-5-(2-phenylethenyl)pyrimidine (150 mg) was suspended in 10 mL of phenylether with 1.2 equiv of 4-(dimethylamino)benzaldehyde and 1.5 g of 4 Å molecular sieves. The solution was heated to 170 °C for 4 h and then cooled to room temperature. The reaction mixture was loaded on the top of a silica gel column and eluted with hexane to remove phenylether followed by 5% MeOH/CH_2Cl_2 as eluent to give 130 mg of the product. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.75 (s, 1 H), 7.98 (s, 1 H), 7.5 (d, 2 H, J = 9 Hz), 7.18 (m, 4 H), 6.55 (d, 2H, J = 9 Hz), 5.9 (bs, 2H), 2.8 (s, 6H), 2.49 (s, 3H); MS (DCI/NH_3) m/z $(M + NH_4)^+$, 156 $(M + H)^+$. Anal. Calcd for $C_{22}H_{21}N_5$: C, 74.34; H, 5.96; N, 19.7. Found: 74.52; H, 5.84; N, 19.7.

6-Chloro-3-acetylpyridine. As per the procedure described in *Tetrahedron* **1992**, *48*, 9233, we have prepared 100 g of this material. Triethylamine (EM Chemical Company, 183.2 g, 1.8 mol) followed by dimethyl malonate (Aldrich Chemical Company, 119 g, 0.9 mol) were added to a 2 L three-neck round-bottom flask with mechanical stirring containing magnesium chloride (Aldrich Chemical Company, 51 g, 0.53 mol) in dry tolene (700 mL). The resulting gray and heterogeneous mixture was stirred at 25 °C for 1.5 h, and then 6-chloronicotinic acid chloride (Lancaster Ltd., 134 g, 0.75 mol) was added in the solid form slowly in small portions over 45 min. Stirring was continued for 40 min before concentrated HCl (261.0 g, 2.32 mol) was carefully added to quench the reaction. The toluene layer was separated and removed in vacuum to give a white needle-like solid. The solid was directly treated with DMSO (650 mL) and water (29 mL). The mixture was heated at 155 °C for approximately 3 h and cooled. It was then quenched with water, and the solid was collected by filtration. The solid was redissolved in CH_2Cl_2 and dried over $MgSO_4$, and the solvent was removed under vacuum to give the product as a white solid 94 g (80% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.94 (dd, 1 H, J = 2.5, 0.7 Hz), 8.20 (dd, 1 H, J = 8.4, 2.6 Hz), 7.45 (dd, 1 H, J = 8.1, 0.7 Hz), 2.62 (s, 3 H); ^{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 $(M + H)^+$.

6-Morpholinyl-3-acetylpyridine. 6-Chloro-3-acetylpyridine (28 g), 30 mL of morpholine, and 150 mL of EtOH were combined in a flask, and the reaction mixture was heated to reflux for 4 h with stirring. The reaction mixture was cooled, and the solvent was removed under vaccum and then poured into H_2O. The mixture was extracted with CH_2Cl_2 and washed with 0.5 M HCl two times and dried over $MgSO_4$. The solvent was removed to give 6-morpholinyl-3-acetylpyridine in 81% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.75 (d, 1 H, J = 2.6 Hz), 8.03 (dd, 1 H, J = 9.2, 2.6 Hz), 6.61 (d, 1 H, J = 9.2 Hz), 3.80 (m, 4 H), 3.67 (m, 4 H), 2.50 (s, 3 H); ^{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 $(M + H)^+$.

General Procedures for Compounds 17−21: 2-Amino-3-cyano-4-(3-bromophenyl)-6-(4-(2-morphlinoopyrido)-pyridine. In a Dean-Stark apparatus, 6-morpholinyl-3-acetylpyridine (6.95 g, 33.7 mmol), *m*-bromo-benzaldehyde (6.24g, 33.7 mmol), malononitrile (2.22 g, 33.7 mmol), and ammonium acetate (4.2 g) were combined in 40 mL of benzene and heated to reflux for 3 h. After cooling, the solvent was removed under vacuum to give a dark solid. The solid was triturated with methanol, filtered, and washed with methanol twice (20 mL) to give a light yellow solid 4.85 g (33% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.94 (d, 1 H, J = 2.0 Hz), 8.29 (dd, 1 H, J = 9.1, 2.7 Hz), 7.86 (dd, 1 H, J = 1.7, 1.7 Hz), 7.71 (m, 2 H), 7.51 (dd, 1 H, J = 7.8, 7.8 Hz), 7.27 (s, 1 H), 6.94 (m, 3 H), 3.71 (m, 4 H), 3.59 (m, 4 H); ^{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 $(M + H)^+$. TLC using 5% MeOH/CH_2Cl_2 as eluent: $R_f \sim 0.65$). The product TLC produced a light blue, highly fluorescent spot.

4-Amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3-*d*]pyrimidine (21). A total of 3.3 g of 2-amino-3-cyano-4-(3-bromophenyl)-6-(4-(2-morphlinoopyrido)pyridine was suspended in formamide (25 mL). The reaction was heated to reflux (using a heating mantel). After 1−2 h of refluxing (usually TLC monitoring is necessary: product R_f at 0.4−0.5 and SM at 0.7 with 10% MeOH/CH_2Cl_2), the reaction mixture was cooled to room temperature, and the reaction mixture was quenched with water to give a brown solid. The solid was then filtered and collected. There was product still remaining in the mother liquor. The solid was then purified by column chromatography using 10% MeOH/CH_2Cl_2 as eluent to give the desired product (1.2 g as a yellow solid, 31% yield). Futher purification of this compound by flash column chromatography using 5% H_2O/CH_3CN as eluent (R_f 0.3) gave 98% (HPLC: 35% 0.1 M NH_4OAc/65% CH_3CN) pure product in 89% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.08 (d, 1 H, J = 2.4 Hz), 8.53 (s, 1 H), 8.47 (dd, 1 H, J = 9.2, 2.4 Hz), 7.85 (m, 2 H), 7.78 (m, 1 H), 7.56 (m, 2 H), 6.98 (d, 1 H, J = 9.1 Hz), 3.72 (m, 4 H), 3.62 (m, 4 H); ^{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 $C_{22}H_{19}BrN_6O \cdot 0.5H_2NC(O)H$: C, 55.62; H, 4.25; N, 18.74. Found: C, 55.43; H, 4.27; N, 18.76.

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