Design of Adenosine Kinase Inhibitors from the NMR-Based Screening of Fragments

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A strategy is described for designing high-affinity ligands using information derived from the NMR-based screening of fragments. The method involves the fragmentation of an existing lead molecule, identification of suitable replacements for the fragments, and incorporation of the newly identified fragments into the original scaffold. Using this technique, novel substituents were rapidly identified and incorporated into lead inhibitors of adenosine kinase that exhibited potent in vitro and in vivo activities. This approach is a valuable strategy for modifying existing leads to improve their potency, bioavailability, or toxicity profile and thus represents a useful technique for lead optimization.

Introduction

The identification of lead molecules that bind with high affinity to a given molecular target is a critical step in drug discovery, and many new technologies are emerging to aid in overcoming this important hurdle in the discovery process.[1−4] However, even after the identification of such a lead molecule, the incorporation of sufficient levels of specificity, bioavailability, and safety is still an arduous process and is responsible for the failure of nearly 50% of all putative drug candidates.[5] Traditional medicinal chemistry approaches for improving the pharmacokinetic (PK) profile of lead compounds typically involve the incorporation of a wide variety of substituents. However, this demands extensive synthetic efforts, and unfortunately, the majority of these analogues are often less active than the original lead compound.

In principle, a fragment-based approach can be used to aid in the identification of fragments that could be incorporated into a lead inhibitor without negatively affecting its potency but that may impact the PK profile or other characteristics of the resulting compound (e.g., improved solubility, reduced toxicity, etc.). Such an approach could significantly decrease the synthetic efforts required to improve the PK of lead compounds as the chemistry can focus on incorporating only the optimized fragments, without expending the time and cost to explore suboptimal substituents. However, the identification of such weakly binding fragments is a difficult process. In general, conventional screening methods cannot reliably detect the binding of low-molecular-weight fragments due to the high concentrations of compound required. In addition, no information on the binding location is obtained from these assays to guide the synthetic efforts. An alternative approach for the identification of suitable fragments is through the use of molecular modeling. However, the problems associated with the computational prediction of ligand binding reduces the reliability of such approaches.

Here we describe an experimental fragment-based approach for the modification of high-affinity leads. The method involves the fragmentation of existing high-affinity ligands followed by the identification of suitable replacements through NMR-based screening.[6,7] The methodology is demonstrated by the design and synthesis of nonnucleoside inhibitors of adenosine kinase (AK), a 39-kDa protein primarily responsible for the intracellular metabolism of adenosine.[8] These compounds have potential use as anticonvulsant and antinociceptive agents.[8−10]

Results and Discussion

Description of the Approach. The general outline for a fragment-based approach to lead modification is shown in Figure 1. First, an existing high-affinity ligand is fragmented into its components, and the fragment to replace is identified (Figure 1a). The binding affinity and binding site location of the chosen fragment are then determined by analyzing changes in $^{15}N-^{1}H$ HSQC spectra of the ^{15}N-labeled protein upon addition of the test molecule.[6,7,11] Next, NMR-based screening is used to identify molecules that bind to the same site as the chosen fragment and which would be predicted to have favorable characteristics compared to the existing substituent (Figure 1b). Finally, the fragments identified from the screen are incorporated into the original lead molecule (Figure 1c).

Fragmentation of AK Inhibitors. The pyridopyrimidine **1** is a novel, nonnucleoside inhibitor of AK that has been demonstrated to have potent antinociceptive activity in both acute and chronic animal pain models.[12,13] However, it was desired to modify this compound to increase solubility and overcome side effects that limit its usefulness. One site for potential optimization is that occupied by the bromophenyl moiety of **1**. Replacements

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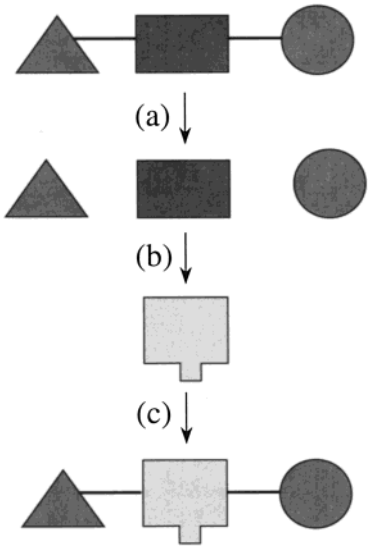


Figure 1. Summary of the fragment optimization approach in which (a) a lead molecule is fragmented into component molecules, (b) an alternative fragment is identified, and (c) the alternative fragment is incorporated back into the original lead.

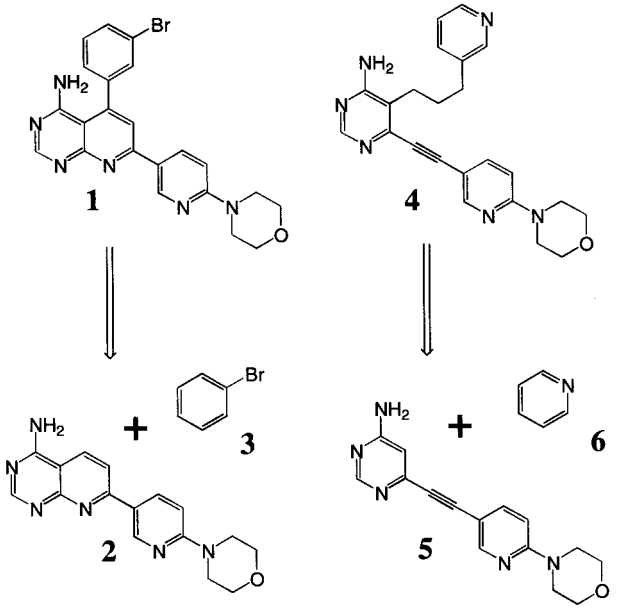


Figure 2. Fragmentation of AK inhibitors **1** and **4** into compounds that bind to a first site (**2** and **5**) and a second site (**3** and **6**).

for the bromophenyl moiety were sought which would maintain or improve potency both in vitro and in vivo as well as confer improved PK properties to the inhibitor.

To apply the NMR-based screening approach shown in Figure 1 in the search for bromophenyl replacements, a suitable "core" molecule was needed that would allow access to the binding site but reduce undesired binding to other areas in the active site. In addition, since $^{15}N-^{1}H$ HSQC spectra were utilized to detect ligand binding, those cross-peaks in the spectrum that could serve as markers for the binding of potential replacements needed to be identified. As shown in Figure 2, **1** can be fragmented into molecules that represent a "core" structure (**2**) and a substituent (**3**) for which modification is desired. Thus, molecules that bind to the same

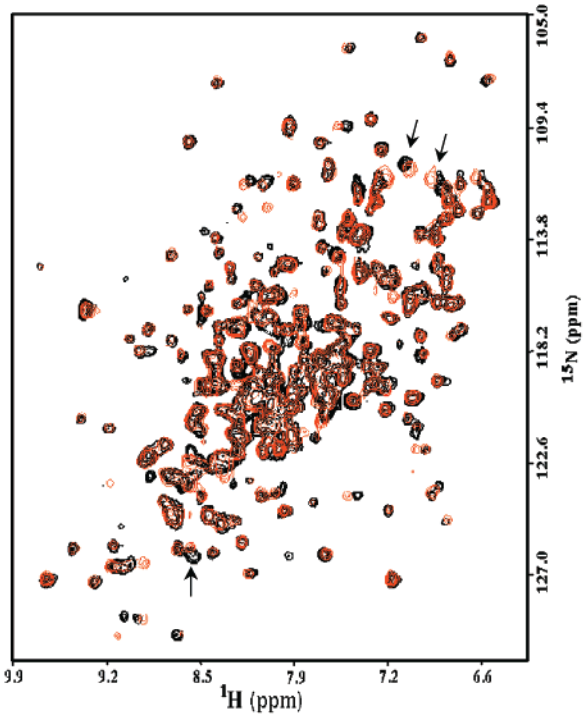
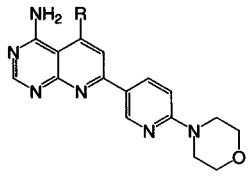


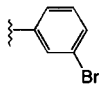
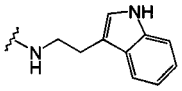
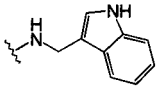
Figure 3. $^{1}H-^{15}N$ HSQC spectra acquired on AK in the presence of **4** (black contours) and **5** (red contours). Arrows indicate cross-peaks that can serve as markers for compounds that bind to the same subsite as the propylpyridyl moiety of **4**.

subsite as bromobenzene (**3**) can be incorporated into the core molecule. While **2** is a suitable core structure for the incorporation of mimetics, it is not ideal for identifying replacements using NMR-based screening since the proton at the 5-position may sterically occlude binding to the bromophenyl subsite. Thus, an alternative core structure was used for the screening experiments based on the acetylene-containing compound **4** that also binds tightly to AK (unpublished results). This compound can be fragmented to yield a core structure (**5**) that should not interfere with binding to the desired subsite (Figure 2). Next, the cross-peaks in the $^{15}N-^{1}H$ HSQC spectrum of AK that can serve as markers for the binding of potential mimetics were identified. This was done from an analysis of the differential chemical shift changes observed for AK complexed to **1** vs **2** and **4** vs **5**. A select subset of peaks in the HSQC spectrum was observed to exhibit differential chemical shift changes (Figure 3) which identify the binding site of the bromophenyl and pyridyl moieties of **1** and **4**, respectively. In addition, these cross-peaks were confirmed to be markers for the binding of the bromphenyl or pyridyl group by titrating pyridine against AK in the presence of **5**. It was observed that these same cross-peaks exhibited chemical shift changes upon the addition of pyridine ($K_D = 37$ mM). No chemical shift changes were observed upon the addition of pyridine to a sample of AK in the presence of **1** or **4**, confirming that pyridine binds competitively with the bromobenzene moiety of **1** and the pyridyl moiety of **4**.

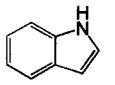
Identification of Replacements. Using NMR-based screening, more than 2000 compounds were tested for binding to AK in the presence of saturating amounts (1 mM) of **5**. A number of compounds were identified which bound to the protein and caused chemical shift changes

Table 1. Pyridopyrimidine Inhibitors of AK

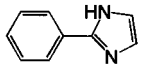


No.	R	AK IC_{50} (nM)	Intact cell AK inhibition IC_{50} (nM)	Hyperalgesia ED_{50} (μmol/kg, i.p.)
1		1.7 ± 0.5	51 ± 8	0.6
15		23 ± 2	920 ± 1	-
16		10 ± 2	371 ± 95	3

in the marker peaks for the bromobenzene/pyridine binding site, including indole (**7**) and 2-phenylimidazole (**8**).



7
K_D = 3 mM



8
K_D = 3 mM

In fact, these compounds bind to AK with 10-fold better affinities than pyridine itself.[14] In addition, these compounds bind to AK in the presence of the pyridopyrimidine core structure **2**. However, these compounds do not bind to AK in the presence of **1** or **4**, providing additional evidence that indole and 2-phenylimidazole bind to the same subsite as the bromophenyl and pyridine moieties of **1** and **4**, respectively. Both of these compounds can serve as interesting replacements for the bromophenyl moiety of **1** since they can potentially impart improved PK properties while maintaining or improving the affinity for AK.

Incorporation of Replacements. In the absence of detailed structural information on the binding of **7** and **8** to AK in the presence of **2** or **5**, information from a series of competitive binding experiments was used to assess the approximate distance between the core structures and the new substituents. Based on simple modeling studies, the simultaneous binding of indole in the presence of **2** indicated a minimum distance of 3−4 Å between the C5 carbon on the pyridopyrimidine core and the indole. The competitive binding observed between indole and **1** required at least one steric contact between the bromophenyl substituent and the indole, which would place a maximum distance of 7−8 Å between potential attachment sites. Interestingly, even an S-Me substituent at the C5 position blocked indole binding, reducing the maximum distance to 5−6 Å. With this information, simple linkers containing 3−5 heteroatoms were designed which would attach the indole substituent to the C5 position of the pyridopyrimidine core. Such linkers can span distances consistent with the competitive binding data (approximately 4−6 Å).

Two compounds (**15** and **16**) were prepared which incorporated linkers between the indole and the C5 position of **2** as shown in Scheme 1. As only the approximate distance and not the site of attachment to the indole could be predicted with the available data, the placement was chosen based on accessible synthetic routes. Both **15** and **16** exhibited nanomolar inhibitory activity against AK in an in vitro cell-free enzyme assay (IC_{50} values of 23 and 10 nM for **15** and **16**, respectively, as compared to 1.7 nM for **1**). Compound **16** also exhibits good activity in an intact cell ADO phosphorylation assay (IC_{50} value of 370 nM, as compared to 51 nM for **1**). Significantly, **16** exhibited promising in vivo activity, with an ED_{50} value of 3 μmol/kg in a carrageenan-induced thermal hyperalgesia assay.[13] Thus, the indole moiety is a novel substituent for pyridopyrimidine inhibitors of AK that maintains potency both in vitro and in vivo.

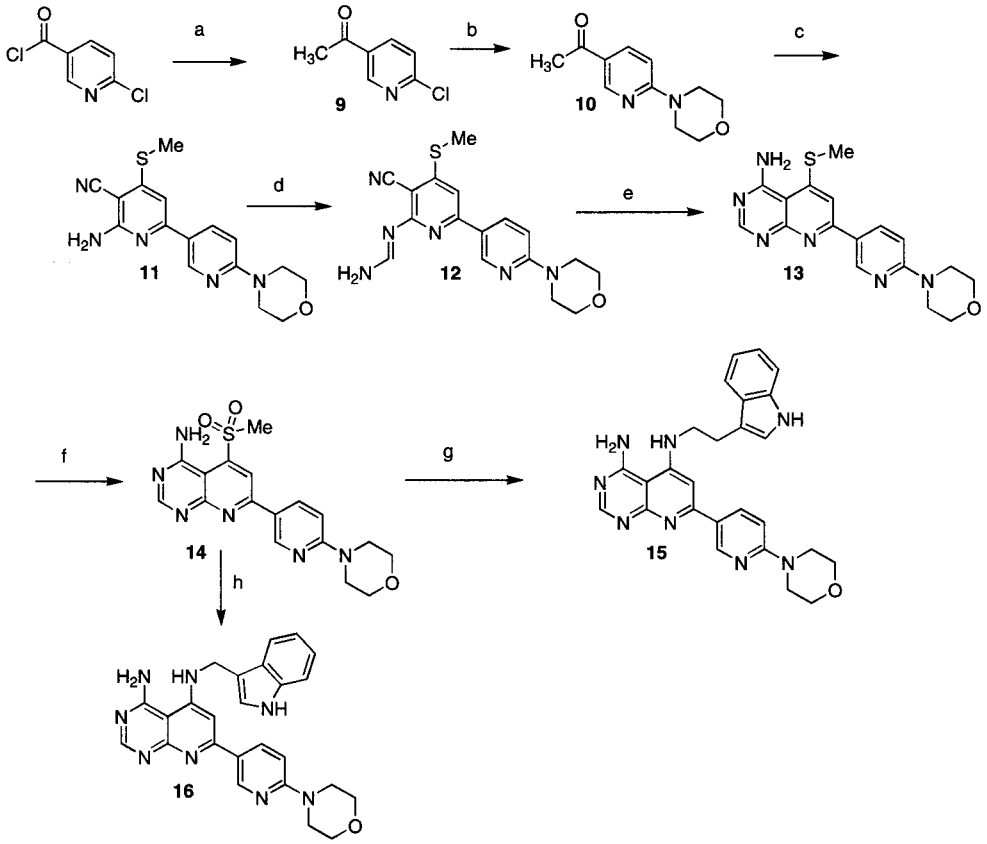
Conclusions

The use of fragment-based approaches to lead modification can significantly decrease the time and effort involved in modifying high-affinity ligands. Using NMR, thousands of potential mimetics with widely diverse functionalities can be rapidly analyzed for binding to the protein. This is difficult to achieve using synthetic approaches, as multiple synthetic routes would have to be developed to incorporate substituents with a variety of functional groups. Instead, using a fragment-based strategy, the chemistry effort can be focused only on those fragments that bind to the site of interest and have properties that may improve the PK profile of the lead compound. In the example presented here, more than 2000 potential replacements were tested for binding to AK in less than 3 weeks of NMR time. Only two compounds were synthesized based on the NMR binding data and modeling studies, both of which maintained high affinity for the target while containing substantially different substituents compared to the initial lead. This demonstrates the utility of using NMR-based screening to explore diversity space that may not be sampled using conventional synthetic approaches. This technique should be applicable to a wide variety of systems. All that is required is a fragment of the lead molecule that is potent and soluble enough to fully saturate the first site (e.g., when the K_D value of the ligand is 10-fold lower than its solubility). In addition, this strategy can be used iteratively to explore multiple substituents and cores for a single high-affinity lead. Therefore, fragment-based modification strategies that employ NMR-based screening are expected to greatly facilitate the drug discovery process.

Experimental Section

Protein Preparation. The short form of the human AK gene[15] was cloned into the pET28 vector (Novagen). The resulting plasmid, pETAK-WT, permitted expression of a His-tagged (thrombin cleavable) form of AK from the T7 lac promoter and was used to transform *E. coli* strain BL21(DE3) [F^- *ompT* $hsdS_B(r_B^-m_B^-)$ *gal dcm* (DE3)] (Novagen). A 1-mL glycerol stock of *E. coli* BL21(DE3)/pETAK-WT was used to inoculate a flask containing 150 mL of M9 minimal media supplemented with kanamycin (50 mg/L). Cells were grown

Scheme 1[a]



[a] Reagents and conditions: (a) 1. dimethyl malonate, toluene, Et_3N, $MgCl_2$, rt, 4 h, 2. DMSO, 150−160 °C, 2 h, 85% for 2 steps; (b) morpholine, EtOH, reflux, 12 h, 84%; (c) 3,3-bis(methylthio)-2-cyanoacrylonitrile, NaO*t*-Bu, DMSO−THF, 0 °C, 2 h, 29%; (d) $HC(OEt)_3$, $(NH_4)_2SO_4$, reflux, 2 h, 73%; (e) *o*-dichlorobenzene, reflux, 5 h, 82%; (f) aq solution of oxone, CH_2Cl_2−MeOH, 72%; (g) tryptamine, DMSO, 80 °C, 2 h, 16%; (h) 3-(methylamino)indole, DMSO, 80 °C, 2 h, 7%.

(30 °C, 225 rpm) until the culture OD reached 0.22. At this point, the entire preculture was transferred to a New Brunswick Scientific (Edison, NJ) Micros fermenter containing 18 L of media. The fermenter media consisted of (per L): 11.32 g $Na_2HPO_4{\cdot}7H_2O$, 3 g KH_2PO_4, 0.5 g NaCl, 1 mL 1% DF-60 antifoam, 1.5 g $^{15}NH_4Cl$, 4 g glucose, 2 mL 1 M $MgSO_4$, 0.1 mL 1 M $CaCl_2$, 0.5 mL thiamine-HCl (10 mg/mL), 0.02 mL $FeSO_4$ (40 mg/mL), 2 mL kanamycin (25 mg/mL), and 0.633 mL trace element solution (per L in 5 N HCl: 10 g $MnSO_4{\cdot}H_2O$, 10 g $AlCl_3{\cdot}H_2O$, 4 g $CoCl_2$, 2 g $ZnSO_4{\cdot}7H_2O$, 2 g $Na_2MoO_4{\cdot}2H_2O$, 1 g $CuCl_2{\cdot}2H_2O$, 0.5 g H_3BO_4). The temperature was controlled at 30.0 °C. The dissolved oxygen concentration (DO_2) was maintained at 45% air saturation through a cascaded control loop which increased the agitation speed when the DO_2 concentration dropped below 45%. The pH was allowed to drop until 50 min post-induction, at which point it was controlled at the existing pH (6.65) for the remaining portion of the run through automatic addition of 4 N H_2SO_4 and 4 N KOH. At an OD of 1.5, expression of AK was induced via addition of 1 mM IPTG. The glucose concentration was maintained between 0.5 and 2.0 g/L during the entire expression phase through feeding of a 30% glucose solution. Cells were harvested 5.75 h post-induction, pelleted, and stored at −80 °C. This procedure routinely generated 12 g wet cell/L.

Frozen cell pellets were thawed in the presence of five volumes of lysis buffer containing 50 mM Tris, 50 mM imidazole, 500 mM KCl, 10% glycerol, 1% 2-mercaptoethanol, 1 mM $MgCl_2$, 0.25 mg lysozyme/mL, 3000 IU *S. marcesens* endonuclease/mL (American International Chemical), 0.2 mM 4-(2-aminoethyl)benzenesulfonyl fluoride (Boehringer Mannheim), 1 μg/mL aprotinin, 10 μg/mL leupeptin, 10 μg/mL pepstatin-A, pH 7.5 (chemicals from Sigma Chemical Co., unless otherwise indicated). Following a 30-min incubation with lysozyme, the *E. coli* cells were broken by sonication (500 W) in 30-s pulses for 10 min. All steps were at 4 °C, unless otherwise indicated. Cell fragments and inclusion bodies were sedimented by centrifugation at 27000*g* for 30 min, and the clear supernatants were frozen until use.

AK was purified using Ni^{2+} affinity and anion-exchange chromatography. Cell lysates were loaded onto nickel-nitrilotriacetic acid chelate resin (Invitrogen) columns equilibrated with buffer containing 50 mM Tris, 50 mM imidazole, 500 mM KCl, 10% glycerol, 1% 2-mercaptoethanol, pH 7.8. Protein bound to the column was eluted using a linear gradient of imidazole from 50 to 1000 mM in 10 column volumes with elution of AK beginning at 100 mM imidazole at 1 mL/min. Fractions containing AK activity were pooled and dialyzed against buffer containing 10 mM KCl, 50 μM adenosine, 1 mM DTT, 10 mM Tris, pH 7.8, in preparation for anion-exchange chromatography and frozen at −80 °C until use. After dialysis, the eluent was applied to the Source Q resin (Pharmacia Biotech) column (2.6 × 15 cm) and eluted by linear gradient formed with 200 mM KCl in 20 column volumes at 1.5 mL/min. Active fractions were pooled and concentrated to 3−5 mg/mL in a stirred cell using YM10 membrane (Amicon). The concentrated protein was dialyzed 18 h at 4 °C against buffer containing 10% glycerol, 50 mM KCl, 1 mM DTT, 1 mM NaN_3, 20 mM Tris, pH 7.5, and stored at −80 °C.

NMR Spectroscopy. NMR samples were composed of uniformly ^{15}N-labeled AK at 0.4 mM in a H_2O/D_2O (9:1) solution containing 20 mM BisTris, 10 mM DTT, pH 7.0. Ligand binding was detected at 30 °C by acquiring sensitivity-enhanced ^{15}N HSQC spectra[16] on 500 μL of 0.4 mM AK in the presence and absence of added compound. Compounds were added as solutions in perdeuterated DMSO. A Bruker sample changer was used on a Bruker AMX500 spectrometer. Compounds were initially tested at 5.0 mM each, and binding was determined by monitoring changes in the ^{15}N HSQC spectra. Dissociation constants were obtained for selected compounds by monitoring the chemical shift changes as a function of

ligand concentration. Data were fit using a single-binding-site model. A least-squares grid search was performed by varying the values of K_D and the chemical shift of the fully saturated protein.

Biological Assays. Assays for AK inhibition and ADO phosphorylation in intact cells (IMR-32) cells were conducted using radiochemical methods as described by Jarvis et al.[12] 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 hindpaw.[13] 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.[17] All animal handling and experimental protocols were approved by an institutional animal care and use committee (IACUC).

Synthesis of 4-Amino-5-sulfonylmethyl-7-(6-morpholinopyridin-3-yl)pyrido[2,3-*d*]pyridine (14). To a slurry of $MgCl_2$ (38.7 g, 0.4 mol) in toluene (460 mL) were added triethylamine (138.6 g, 1.4 mol) and dimethyl malonate (90 g, 0.7 mol) and the resulting slurry stirred at ambient temperature for 1.5 h. Then a solution of 6-chloronicotinoyl chloride (100 g, 0.57 mol) in toluene (75 mL) was added within 30 min; the mixture stirred for another 2 h and was neutralized with 6 N HCl (100 mL). Aqueous phase was separated and extracted with diethyl ether. Combined organic phases were concentrated, dissolved in DMSO (520 mL)- H_2O (21 mL) and heated to 150−160 °C for 2 h. The reaction mixture was cooled and diluted with water. The resulting solid was filtered, washed twice with water and dried in vacuo to give 72 g (85%) of the ketone **9** as a tan solid: ^{1}H NMR ($CDCl_3$) δ 8.94 (d, J = 3.0 Hz, 1H), 8.20 (dd, J = 3.0 and 9.0 Hz, 1H), 7.25 (d, J = 9.0 Hz, 1H), 2.52 (s, 3H); MS *m*/*e* 156 (M + 1).

A solution of **9** (45.6 g, 0.3 mol) and morpholine (80 mL) in EtOH (800 mL) was refluxed for 12 h. The solution was cooled to ambient temperature, volatiles were removed in vacuo and the remaining solid was partitioned between ethyl acetate and water. The organic phase was additionally washed twice with water, separated, dried over $MgSO_4$ and concentrated to give 50 g (84%) of **10**: ^{1}H NMR (DMSO-d_6) δ 8.73 (d, J = 3.0 Hz, 1H), 8.0 (dd, J = 3.0 and 9.0 Hz, 1H), 6.90 (d, J = 9.0 Hz, 1H), 3.70 (m, 4H), 3.64 (m, 4H), 2.46 (s, 3H); MS *m*/*e* 206 (M + 1).

To a 0 °C slurry of NaO*t*-Bu (7.50 g, 78 mmol) in DMSO (50 mL) and THF (50 mL) was added a solution of **10** (12.1 g, 59 mmol) in THF (25 mL) followed by commercially available 3,3-bis(methylthio)-2-cyanoacrylonitrile (10.0 g, 59 mmol). The mixture was allowed to stir at 0 °C for 2 h, quenched with a solution of AcOH (6 mL) in H_2O (50 mL) and stirred 15 min. Then water (300 mL) was added, concentrated and extracted with dichloromethane. Organic phase was dried over $MgSO_4$, concentrated, and dissolved in dichloroethane (60 mL) and AcOH (60 mL) and ammonium acetate (45.7 g, 593 mmol) was added. After the resulting mixture was refluxed for 12 h, it was allowed to cool to ambient temperature and was poured into 1 N NaOH (750 mL). The mixture was extracted twice with dichloromethane, dried over $MgSO_4$, and concentrated in vacuo and the residue was chromatographed (ethyl acetate−hexane, 50:50) to yield 5.63 g (29%) of **11** as a tan solid: ^{1}H NMR (DMSO-d_6) δ 8.90 (d, J = 3.0 Hz, 1H), 8.23 (dd, J = 3.0 and 9.0 Hz, 1H), 6.99 (s, 1H), 6.91 (d, J = 9.0 Hz, 1H), 5.80 (s, 2H), 3.70 (m, 4H), 3.56 (m, 4H), 2.65 (s, 3H); MS *m*/*e* 328 (M + 1).

A slurry of **11** (5.6 g, 17.1 mmol) and ammonium sulfate (0.073 g) in triethyl orthoacetate (20 mL) was refluxed for 2 h. After cooling to ambient temperature, a 2 M solution of NH_3 in EtOH (30 mL) was added and stirred at ambient temperature for 14 h. The mixture was concentrated, diluted with hexane and filtered. The precipitate was washed with hexanes, then diethyl ether and air-dried to yield 4.51 g (72.5%) of the desired amidine **12** as a light brown solid: ^{1}H NMR ($CDCl_3$) δ 8.90 (m, 1H), 8.82 (broad s, 1H), 8.18 (m, 1H), 7.40 (s, 1H), 6.77 (d, J = 9.0 Hz, 1H), 3.85 (m, 4H), 3.64 (m, 4H), 2.63 (s, 3H); MS *m*/*e* 355 (M + 1).

A solution of **12** (12.6 g, 35.5 mmol) in *o*-dichlorobenzene (100 mL) was refluxed for 5 h. The solution was cooled to ambient temperature and diluted with diethyl ether (100 mL). The resulting solid was filtered and washed with diethyl ether and air-dried. The solid was then added to dichloromethane−methanol (100 mL, 4:1), stirred vigorously, and filtered and the filtrate concentrated in vacuo to give 10.3 g (82%) of the desired pyridopyrimidine **13** as a yellow solid: ^{1}H NMR (DMSO-d_6) δ 9.05 (d, J = 3.0 Hz, 1H), 8.43 (s, 1H), 8.40 (d, J = 3.0 Hz, 1H), 7.65 (s, 2H), 6.90 (d, J = 6.0 Hz, 1H), 3.73 (m, 4H), 3.61 (m, 4H), 2.84 (s, 3H); MS *m*/*e* 355 (M + 1).

To a solution of **13** (3.5 g, 10 mmol) in dichloromethane−methanol (400 mL, 1:1) was added a solution of oxone (18.5 g, 30 mmol) in water (125 mL) over 10 min with vigorous stirring. Then a solution of saturated $NaHCO_3$ (75 mL) was added, stirred for 45 min and the resulting mixture diluted with dichloromethane and water. The organic layer was separated, washed with water and brine, dried over $MgSO_4$ and concentrated to yield 2.8 g (72%) of desired sulfone **14** as a light brown solid: ^{1}H NMR (DMSO-d_6) δ 9.04 (d, J = 3.0 Hz, 1H), 8.60 (s, 1H), 8.42 (dd, J = 3.0 and 6.0 Hz, 1H), 8.38 (s, 1H), 7.05 (d, J = 6.0 Hz, 1H), 3.93 (m, 4H), 3.65 (m, 7H); MS *m*/*e* 387 (M + 1).

Synthesis of 4-Amino-5-(*N*-aminoethyl-3-indolyl)-7-(6-morpholinopyridin-3-yl)pyrido[2,3-*d*]pyridine (15). A solution of **14** (1.6 g, 3 mmol) and tryptamine (2.4 g, 15 mmol) in DMSO (30 mL) was heated to 80 °C for 2 h. The mixture was allowed to retain ambient temperature, diluted with brine and extracted twice with dichloromethane. The organic layer was dried over $MgSO_4$, concentrated in vacuo and the residue chromatographed (methanol−dichloromethane, 2:98) to obtain 0.22 g (16%) of **15** as a tan solid: ^{1}H NMR (acetone-d_6) δ 8.84 (s, 1H), 8.13 (d, J = 9.0 Hz, 1H), 7.95 (broad s, 1H), 7.54 (d, J = 9.0 Hz, 1H), 7.35 (d, J = 9.0 Hz, 1H), 7.28 (s, 1H), 7.08 (m, 1H), 7.00 (m, 1H), 6.85 (s, 1H), 6.79 (d, J = 9.0 Hz, 1H), 3.55 (m, 6H), 3.20 (t, J = 6.0 Hz, 2H); MS *m*/*e* 467 (M + 1). Anal. ($C_{26}H_{26}N_8O\cdot0.5H_2O$) C, H, N.

Synthesis of 4-Amino-5-(*N*-aminomethyl-3-indolyl)-7-(6-morpholinopyridin-3-yl)pyrido[2,3-*d*]pyridine (16). The title compound was prepared in 7% yield from the sulfone and corresponding amine[18] as described in the last synthesis: ^{1}H NMR (acetone−DMSO-d_6) δ 8.88 (d, J = 3.0 Hz, 1H), 8.24 (dd, J = 3.0 and 9.0 Hz, 1H), 7.80 (s, 1H), 7.60 (d, J = 6.0 Hz, 1H), 7.35 (s, 1H), 7.30 (d, J = 6.0 Hz, 1H), 7.01 (m, 2H), 6.91 (t, J = 6.0 Hz, 1H), 6.75 (d, J = 6.0 Hz, 1H), 4.68 (s, 2H), 3.63 (4H), 3.48 (m, 4H); MS *m*/*e* 453 (M + 1); IR (KBr) 3346, 2857, 1596, 1118 cm^{-1}. Anal. ($C_{25}H_{24}NO\cdot1.0H_2O$) C, H; N: calcd, 23.81; found, 23.21.

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