Aminopyridine-Based c-Jun N-Terminal Kinase Inhibitors with Cellular Activity and Minimal Cross-Kinase Activity†

Bruce G. Szczepankiewicz,* Christi Kosogof, Lissa T. J. Nelson, Gang Liu, Bo Liu, Hongyu Zhao, Michael D. Serby, Zhili Xin, Mei Liu, Rebecca J. Gum, Deanna L. Haasch, Sanyi Wang, Jill E. Clampit, Eric F. Johnson, Thomas H. Lubben, Michael A. Stashko, Edward T. Olejniczak, Chaohong Sun, Sarah A. Dorwin, Kristi Haskins, Cele Abad-Zapatero, Elizabeth H. Fry, Charles W. Hutchins, Hing L. Sham, Cristina M. Rondinone,‡ and James M. Trevillyan

Metabolic Disease Research, Global Pharmaceutical Research and Discovery Organization, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, Illinois 60064-6098

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The c-Jun N-terminal kinases (JNK-1, -2, and -3) are members of the mitogen activated protein (MAP) kinase family of enzymes. They are activated in response to certain cytokines, as well as by cellular stresses including chemotoxins, peroxides, and irradiation. They have been implicated in the pathology of a variety of different diseases with an inflammatory component including asthma, stroke, Alzheimer's disease, and type 2 diabetes mellitus. In this work, high-throughput screening identified a JNK inhibitor with an excellent kinase selectivity profile. Using X-ray crystallography and biochemical screening to guide our lead optimization, we prepared compounds with inhibitory potencies in the low-double-digit nanomolar range, activity in whole cells, and pharmacokinetics suitable for in vivo use. The new compounds were over 1000-fold selective for JNK-1 and -2 over other MAP kinases including ERK2, p38α, and p38δ and showed little inhibitory activity against a panel of 74 kinases.

Introduction

c-Jun N-terminal kinase-1 (JNK-1) is a member of the mitogen activated protein kinase (MAP kinase) family of enzymes responsible for the serine/threonine phosphorylation of intracellular targets. JNK-1 and the other JNK enzymes JNK-2 and JNK-3 are activated in response to cellular stresses such as heat shock, irradiation, hypoxia, chemotoxins, and peroxides. They are also activated in response to various cytokines and participate in the onset of apoptosis.[1,2] Up-regulation of JNK activity is associated with a number of different disease states. JNK-1 phosphorylation of IRS-1 at Ser^{307} has been shown to down-regulate insulin signaling in vitro.[3,4] Conversely, JNK-1 null mice maintain lower fasting plasma glucose and insulin levels compared to their wild-type litter mates when they are high-fat-fed, indicating that these animals are protected from the development of obesity-induced insulin resistance.[5] These results imply a role for JNK-1 in type 2 diabetes mellitus.[6]

JNK-2 null mice have been produced by two different laboratories, and there is a clear role for JNK-2 in immunological function.[7,8] JNK-2, often in concert with JNK-1, has been implicated in the pathology of autoimmune disorders such as rheumatoid arthritis[9] and asthma.[10−15] It also may play a role in cancer,[16] as well as ischemia-reperfusion injury following myocardial infarction[17] or stroke.[1] Inhibitors of JNK-1 and JNK-2 could be useful for treating these diseases, as well as a broad range of other diseases with an inflammatory component.

JNK-3 has been shown to mediate neuronal apoptosis and may therefore be involved in the pathology of neurodegenerative diseases. Inhibitors of JNK-3 could be useful for treating Parkinson's disease, Alzheimer's disease, epilepsy, stroke, and other diseases of the central nervous system.[1,18] JNK-3 knockout mouse studies lend further evidence to the role of JNK-3 in neurological disorders.[19] Thus, inhibitors of JNK activity have the potential to provide benefits for patients with many different chronic diseases. Small molecules with good potency, selectivity against a broad panel of kinases, and pharmacokinetic profiles sufficient to achieve good exposure in vivo would be ideal for probing the role of JNK function in these diseases.

The active sites of JNK-1, -2, and-3 are highly homologous, but each protein is the product of a different gene. At least 10 different JNK isoforms have been identified, and all of these are splice variants of JNK-1, -2, or -3. JNK-1 and -2 are ubiquitously expressed in human tissues, while JNK-3 is restricted to the brain, heart, and testis. Some cellular proteins phosphorylated by JNK enzymes include the gene transcription factors c-Jun, c-Fos, ATF-2, and Elk-1;[2] the insulin receptor substrates IRS-1[13] and IRS-2; Shc; and Gab-1. JNK enzymes themselves must be phosphorylated to carry out their functions. The enzymes MKK-4 and MKK-7 are known to phosphorylate and activate JNK-1 and -2.[1,2] Some of these biochemical events can serve as the basis for assaying JNK activity in vitro.

There has been considerable effort to identify JNK inhibitors over the past several years, and several detailed reports have appeared[20−26] (Figure 1). Still more examples have appeared in the patent literature.[27,28] In this work, we report a series outside the scope of the previous work. The new compounds are potent JNK inhibitors that bind to the ATP site in an unusual manner. They also display remarkable selectivity vs several dozen other kinases, perhaps because of their special binding mode with JNK-1. These JNK inhibitors may be suitable for use in animal studies, providing a critical tool to elucidate the role of JNK in vivo.

Chemistry

Most of compounds in this study were prepared by appropriate substitution of diaminopyridine **1**, available in one step from malononitrile and $HBr_{(g)}$[29] (Scheme 1). Addition−elimination with sodium ethoxide at the 2-bromo position gave ether **2**.[30]

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* To whom correspondence should be addressed. Phone: (847) 935-1559. Fax: (847) 938-1674. E-mail: bruce.szczepankiewicz@abbott.com.

‡ Current Address: Hoffman-La Roche Inc., Metabolic Diseases, 340 Kingsland Street, Nutley, NJ 07110.

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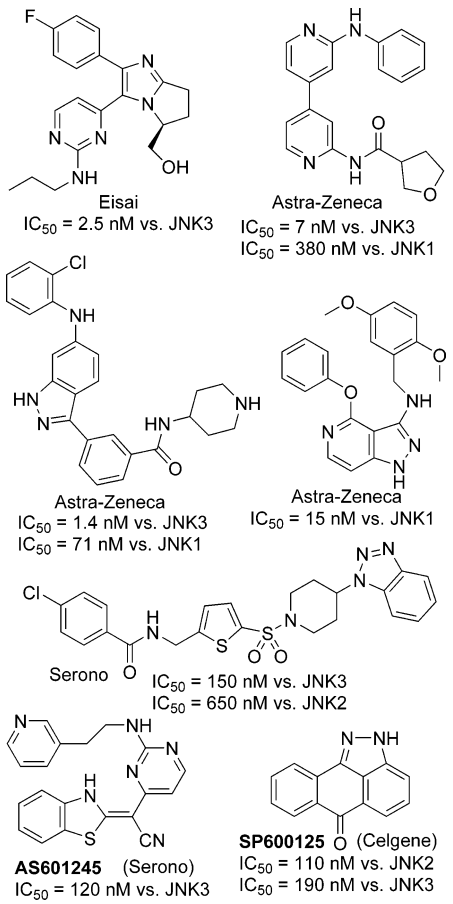
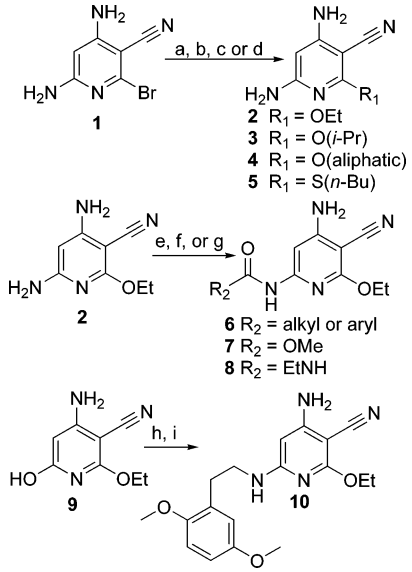


Figure 1. Previously disclosed c-Jun N-terminal kinase inhibitors.

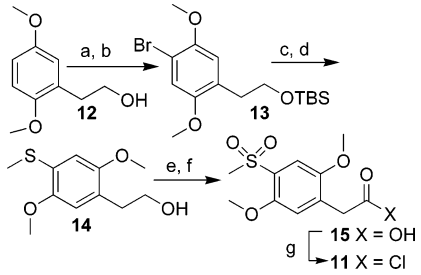
Scheme 1. Introduction of 2-Pyridyl Substituents and N-6 Elaboration[a]



[a] Reagents and conditions: (a) **1** → **2**, 1 M NaOEt in EtOH, 150 °C; (b) **1** → **3**, 1 M *i*-PrONa in *i*-PrOH, 150 °C; (c) **1** → **4**, ca. 1 M Na alkoxide in parent alcohol or dioxane, 150 °C; (d) **1** → **5**, *n*-BuSNa, DMF, 120 °C; (e) **2** → **6** acid chloride, pyridine/CH_2Cl_2, −78 or 25 °C; (f) **2** → **7**, methyl chloroformate, $(i\text{-Pr})_2$EtN, THF, 25 °C; (g) **2** → **8**, (i) triphosgene, $(i\text{-Pr})_2$EtN, THF, 25 °C; (ii) $EtNH_2$ THF, 25 °C; (h) $PhN(Tf)_2$, Et_3N, CH_2Cl_2, (i) (2,5-dimethoxyphenyl)ethylamine, DMSO, 110 °C.

Similarly, sodium isopropoxide gave ether **3**, while other alkoxides gave alkyl ethers **4**, and *n*-butylthiol sodium salt gave thioether **5**. Acylation of ether **2** with acid chlorides was regioselective, providing N-6 amides **6**. Methylchloroformate and triphosgene also reacted with diaminopyridine **2** at N-6, providing carbamate **7** and, upon treatment of the N-6 isocyanate with ethylamine, urea **8**. Since diaminopyridine **2** is rather electron-deficient, N-6 did not participate in alkylation or reductive amination reactions. To prepare an N-6 alkyl derivative, we started with hydroxypyridine **9**.[31] A triflate ester was prepared using $PhN(Tf)_2$, and then addition−elimination with (2,5-dimethoxyphenyl)ethylamine proceeded smoothly to provide phenethylaminopyridine **10**.

Scheme 2. Preparation of Acid Chloride **11**[a]



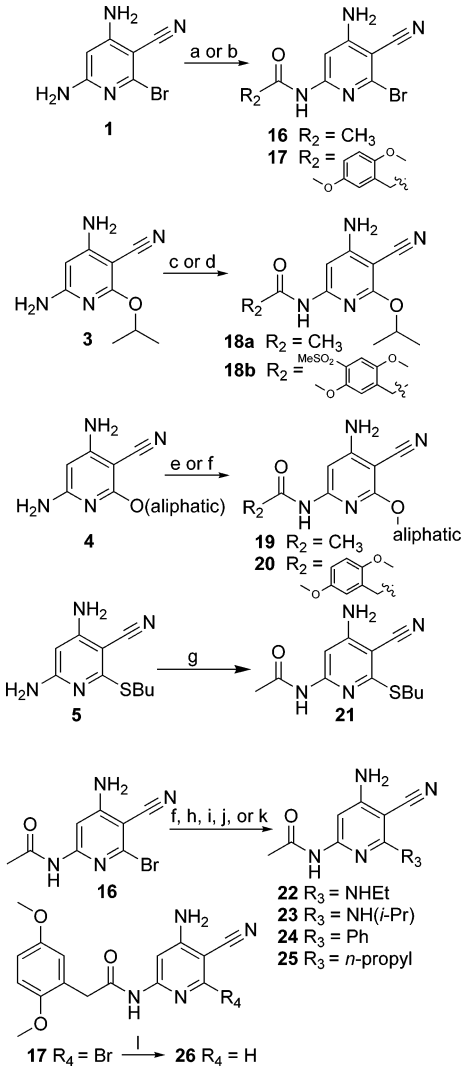
[a] Reagents and conditions: (a) Br_2, AcOH, 25 °C; (b) TBSCl, imidazole, DMF; (c) (i) *n*-BuLi/hexanes, THF, −78 °C; (ii) MeSSMe, −78 to 0 °C; (d) TBAF, THF, 25 °C; (e) H_2O_2, TFA; (f) 2M H_2CrO_4, acetone, 25 °C; (g) $SOCl_2$, reflux.

Most of the acid chlorides either were commercially available or prepared in one step from a readily available acid and either thionyl chloride or oxalyl chloride. Acid chloride **11** required a lengthier sequence (Scheme 2). Starting from 2-(2,5-dimethoxyphenyl)ethanol[32] (**12**), electrophilic bromination and then protection of the alcohol with TBSCl gave silyl ether **13**. Halogen−metal exchange with *n*-butyllithium was followed by addition of dimethyl disulfide and then removal of the silyl protecting group with fluoride ion to provide thioether **14**. Oxidation of the thioether to the sulfone was accomplished with hydrogen peroxide in trifluoroacetic acid, and then oxidation of the alcohol to the acid with Jones' reagent provided acid **15**. Treatment with thionyl chloride gave acid chloride **11**, which was used for subsequent acylations.

We prepared amides **16** and **17** from bromopyridine **1** and acetyl chloride or (2,5-dimethoxyphenyl)acetyl chloride, respectively (Scheme 3). Attempts to prepare C-2 ethers from bromide **16** or **17** via alkoxide addition−elimination resulted in hydrolysis of the amide, so we elected to install the C-2 alkoxy groups first and then acylate the resulting ethers. By use of conditions similar to those employed for the acylation of bromopyridine **1**, ethers **3** and **4** and thioether **5** were acylated with acid chlorides to provide acetamides **18** and **19**, (2,5-dimethoxyphenyl)acetamides **20**, and amidothioether **21**. Other C-2 substituents could be installed without hydrolysis of the N-6 amide, so bromides **16** and **17** were suitable starting materials for the preparation of analogues **22**−**26**. Addition−elimination of ethylamine or isopropylamine to bromide **16** gave triaminopyridine **22** or **23**, respectively. Suzuki coupling of bromide **16** with phenylboronic acid gave biarylamide **24**, while Negishi coupling with *n*-propylzinc iodide gave propylpyridine **25**. Reduction of bromide **17** with zinc/HCl provided C-2 unsubstituted pyridine **26**.

Acylation of aminopyridine **2** with bromoacetyl bromide cleanly gave bromoamide **27** (Scheme 4). This intermediate allowed us to further explore the SAR about the N-6 amide, using nucleophilic substitution of the bromide to rapidly introduce further diversity to the series. The best results were found with amines, so bromide **27** was treated with different primary or secondary alkylamines to provide analogues **28**. Cyclic amines also smoothly displaced bromide **27**, providing

Scheme 3. Synthesis of Pyridine C-2 Analogues[a]



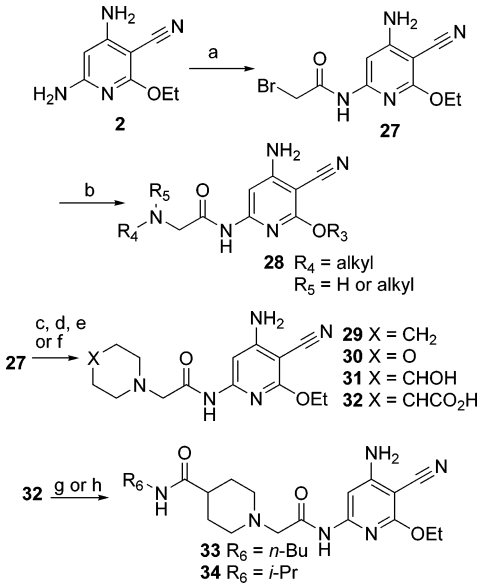
[a] Reagents and conditions: (a) **1** → **16**, acetyl chloride, pyridine, 25 °C; (b) **1** → **17**, 2,5-dimethoxyphenylacetyl chloride, pyridine/CH_2Cl_2, −78 °C; (c) **3** → **18a**, acetyl chloride, pyridine, 25 °C; (b) **3** → **18b**, acid chloride **11**, pyridine/CH_2Cl_2, −78 °C; (e) **4** → **19**, acetyl chloride, pyridine/CH_2Cl_2, −78 or 25 °C; (f) **4** → **20**, 2,5-dimethoxyphenylacetyl chloride, pyridine/CH_2Cl_2, −78 °C; (g) acetyl chloride, pyridine; (h) **7** → **22**, $EtNH_2$, NMP, 150 °C; (i) **7** → **23**, *i*-$PrNH_2$, NMP, 150 °C; (j) **7** → **24**, $PhB(OH)_2$, Na_2CO_3, $Pd(PPh_3)_4$, DMF/THF/H_2O; (k) **7** → **25**, *n*-propyl-ZnI, $Pd(OAc)_2$, (*o*-tol)$_3$P, DMF, 90 °C; (l) Zn, 1 M $HCl_{(aq)}$/DMF.

piperidine **29**, morpholine **30**, hydroxypiperidine **31**, and carboxypiperidine **32**. Carboxypiperidine **32** could be further elaborated by preparing amides from the carboxylic acid. Standard peptide coupling conditions were sufficient to produce amides **33** and **34**.

Exploration of the pyridine C-3 position required a different synthesis of the core (Scheme 5). To prepare 3-chloropyridine **35**, we began by heating 4-amino-2,6-dichloropyridine (**36**) with sodium ethoxide to displace the C-2 chloride. Then a carboxylate was installed at C-6 via Pd-mediated carbonylation in methanol, providing methyl ester **37**. Chlorination with *N*-chlorosuccinimide proceeded at C-3, and then the resulting intermediate was stirred with methanolic ammonia to give an amide. This was subjected to Hoffmann rearrangement conditions to provide diaminopyridine **38**. Acylation with acid chloride **11** proceeded as for other aminopyridines, giving amide **35**.

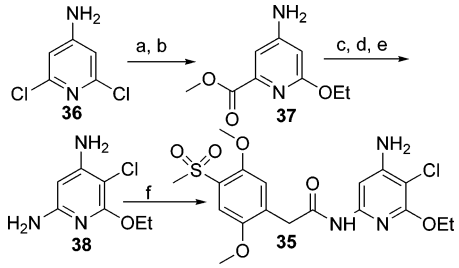
Changes at C-4 and C-5 were accomplished via other methods (Scheme 6). To prepare 4-methylpyridine **39**, we subjected chloropyridine **40**[33] to the same ethoxide addition−elimination

Scheme 4. Preparation of Aminoacetamides[a]



[a] Reagents and conditions: (a) bromoacetyl bromide, pyridine, CH_2Cl_2, −78 °C; (b) 1° or 2° amine, DMF, 25 °C; (c) **27** → **29**, piperidine, DMF, 25 °C; (d) **27** → **30**, morpholine, DMF, 25 °C; (e) **27** → **31**, 4-hydroxypiperidine, DMF, 25 °C; (f) **27** → **31**, (i) methyl isonipecotate, DMF, 25 °C; (ii) K_2CO_3, aqueous MeOH; (g) **32** → **33**, *n*-$BuNH_2$, TBTU, DMF, 25 °C; (h) **32** → **34**, *i*-$PrNH_2$, TBTU, DMF, 25 °C.

Scheme 5. Synthesis of Chloropyridine Analogue **35**[a]



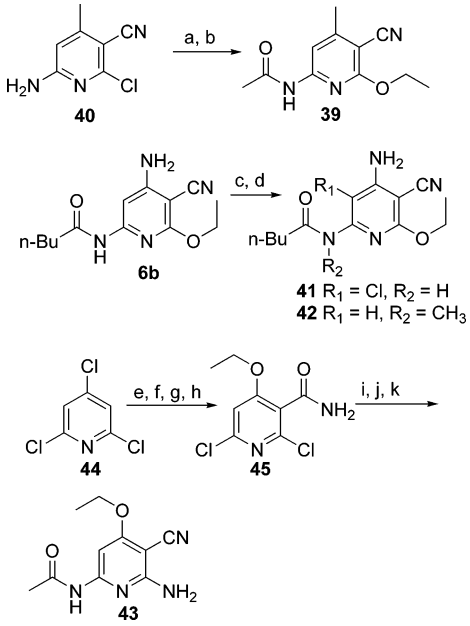
[a] Reagents and conditions: (a) NaOEt, EtOH, 150 °C; (b) $PdCl_2CH_2Cl_2$dppf, Et_3N, 100 atm of CO, CH_3OH, 100 °C; (c) NCS; (d) NH_3, CH_3OH, 25 °C; (e) NaOCl, NaOH, *i*-PrOH, 25 °C; (f) acid chloride **11**, pyr, CH_2Cl_2, −78 °C.

conditions as we did for bromopyridine **1** and then followed with N-6 acylation. Starting with pentanoylamide **6b**, treatment with sulfuryl chloride gave 5-chloropyridine **41**. The N-6 methylation was also straightforward starting from amide **6b**, giving tertiary amide **42**. To prepare isomeric pyridine **43**, we started with 2,4,6-trichloropyridine (**44**)[34] and sodium ethoxide. After isolation of the 4-ethoxypyridine, lithiation and carboxylation were followed by conversion to the corresponding nicotinamide **45** using standard conditions. Dehydration of the amide with $POCl_3$ provided the nicotinonitrile, and then the C-2 and C-6 amino groups were simultaneously installed under autoclave conditions. Acylation completed the synthesis of amidopyridine **43**.

Results and Discussion

In our work, the basis for the biochemical assay was JNK-1 mediated phosphorylation of ATF-2.[35] High-throughput screening identified acetamide **6a**, with IC_{50} = 750 nM (Table 1). Competition experiments confirmed that this inhibitor binds competitively and reversibly to the ATP site. This compound demonstrated a remarkable selectivity profile, showing little cross-reactivity with any of 74 other non-c-Jun-N-terminal kinases. Additionally, it showed some inhibition of c-Jun

Scheme 6. Modification of Pyridine C-4, C-5, and N-1 Positions and N-6 Alkylation[a]



[a] Reagents and conditions: (a) NaOEt, EtOH, 150 °C; (b) AcCl, pyr, 25 °C; (c) **6b** → **41**, SO_2Cl_2, CH_2Cl_2, 25 °C; (d) **6b** → **42**, NaH, CH_3I, DMF, 25 °C; (e) KOH, EtOH, reflux; (f) (i) *n*-BuLi, THF −78 °C; (ii) CO_2, −78 to 25 °C; (g) $SOCl_2$, reflux; (h) concentrated NH_4OH, THF, 25 °C; (i) $POCl_3$, imidazole, pyr, 0 °C; (j) concentrated NH_4OH, dioxane, 130 °C; (k) AcCl, pyr, 25 °C.

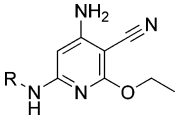
phosphorylation in hepG2 cells. The rapid synthesis of the core and the modular nature of analogue synthesis allowed us to quickly explore the SAR about the C-2 and C-6 positions.

Exploration of the amide position revealed that groups larger than the original acetamide **6a** could improve potency (**6b**). Branching at the α-position diminished activity (**6c**), but *β*-branching was better tolerated (**6d**). Replacement of the amide α-carbon with oxygen or nitrogen completely abolished activity, as did removal of the amide carbonyl group (**7**−**10**). Phenylacetamide **6e** was slightly more potent than acetamide **6a**, but further exploration of the amide region was facilitated by the ready availability of a variety of substituted phenylacetic acids. Hydrophobic substituents gave some improvement in activity (**6f**−**k**), but the best gains were found with methoxy substituents. Methoxy regioisomers **6l**, **6m**, and **6n** were all more active than unsubstituted phenylacetamide **6e**. Further gains in potency could be found upon dimethoxy substitution of the phenyl ring, with 2,5-dimethoxy analogue **6o** being the optimal arrangement. Disubstitution with other groups such as methyl (**6p**) or bromide (**6q**) did not match these gains in potency. Additional substituents in the **6o** 4-phenyl position were tolerated. Acetamide **6r**, sulfone **6s**, and bromide **6t** were roughly equipotent with **6o**, while nitro compound **6u** was slightly less potent than amide **6o**. The optimal N-6 amide was the (2,5-dimethoxy-4-methylsulfonylphenyl)acetamide **6s**.

A second set of N-6 amides was prepared from bromoamide **27** (Table 2). Since inhibitors could be prepared in one step from bromoamide **27**, we were able to rapidly assemble and evaluate a set of 2-aminoacetamides. N,N-Disubstitution was preferred because tertiary amines **28a** and **28b** were more active than secondary amine **28c**. Linear aliphatic amines were generally less potent than amide **6a**, but we made some gains by displacing bromoamide **27** with more hydrophilic amines. Hydroxyethylamine **28d**, piperidine **29**, morpholine **30**, and hydroxypiperidine **31** all were more potent than acetamide **6a**. Further modification of the piperidine scaffold revealed additional gains. Piperidine 4-carboxylic amides were somewhat more potent than hydroxypiperidine **31**, with examples **33** and **34** giving IC_{50} values below 100 nM. The most potent example from this set of aminoacetamides was *n*-butylamidopiperidine **33**.

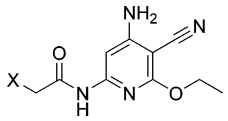
Three crystal structures of JNK have appeared previously, facilitating the use of X-ray crystallography to determine the

Table 1. SAR about Amide **6**



entry	R	JNK-1 IC_{50} (nM)	SD	JNK-2 IC_{50} (nM)	SD	pCjun EC_{50} (nM)	SD
6a	MeCO	750	43	1100	110	6800	3000
6b	*n*-BuCO	350	63	690	370		
6c	*i*-PrCO	4000	770	8400	800		
6d	*i*-BuCO	1400	110	3500	590		
6e	$PhCH_2CO$	570	140	770	130		
6f	2-(Cl)$PhCH_2CO$	1100	300	4900	1800		
6g	3-(Cl)$PhCH_2CO$	300	70	900	520		
6h	4-(Cl)$PhCH_2CO$	580	170	610	270		
6i	2-(Me)$PhCH_2CO$	400	120	740	420		
6j	3-(Me)$PhCH_2CO$	420	56	790	200		
6k	4-(Me)$PhCH_2CO$	190	4	360	89	3400	960
6l	2-(MeO)$PhCH_2CO$	120	47	260	110	1400	840
6m	3-(MeO)$PhCH_2CO$	210	95	310	56	2500	550
6n	4-(MeO)$PhCH_2CO$	180	59	274	120	1400	340
6o	2,5-(diMeO)$PhCH_2CO$	45	3	160	40	920	660
6p	2,5-(diMe)$PhCH_2CO$	4400	670	>10000			
6q	2,5-(diBr)$PhCH_2CO$	3000	1600	>10000			
6r	4-AcNH-2,5-(diMeO)$PhCH_2CO$	35	16	26	7	280	200
6s	4-($MeSO_2$)-2,5-(diMeO)$PhCH_2CO$	38	5	150	21	1300	170
6t	4-Br-2,5-(diMeO)$PhCH_2CO$	77	38	160	48	890	160
6u	4-NO_2-2,5-(diMeO)$PhCH_2CO$	69	14	100	1	910	110
7	MeOCO	>10000		>10000			
8	EtNHCO	>10000		>10000			
10	2,5-(diMeO)$PhCH_2CH_2$	>10000		>10000			

Table 2. SAR of Aminoacetamides



entry	X	JNK-1 IC_{50} (nM)	SD	JNK-2 IC_{50} (nM)	SD	pCjun EC_{50} (nM)	SD
28a	di(Et)N	2700	880	6300	2000		
28b	di(*n*-butyl)N	3500	350	>10000			
28c	*n*-butylNH	6300	2400	>10000			
28d	(*n*-propyl)(2-hydroxyethyl)N	160	46	740	120	5500	290
29	1-piperidinyl	510	43	3400	670		
30	4-morpholinyl	270	28	1200	250		
31	4-hydroxy-1-piperidinyl	300	150	640	340		
33	4-(*n*-butylaminocarbonyl)-1-piperidinyl	69	19	180	7	>10000	
34	4-(*i*-propylaminocarbonyl)-1-piperidinyl	87	21	130	16	3900	1100

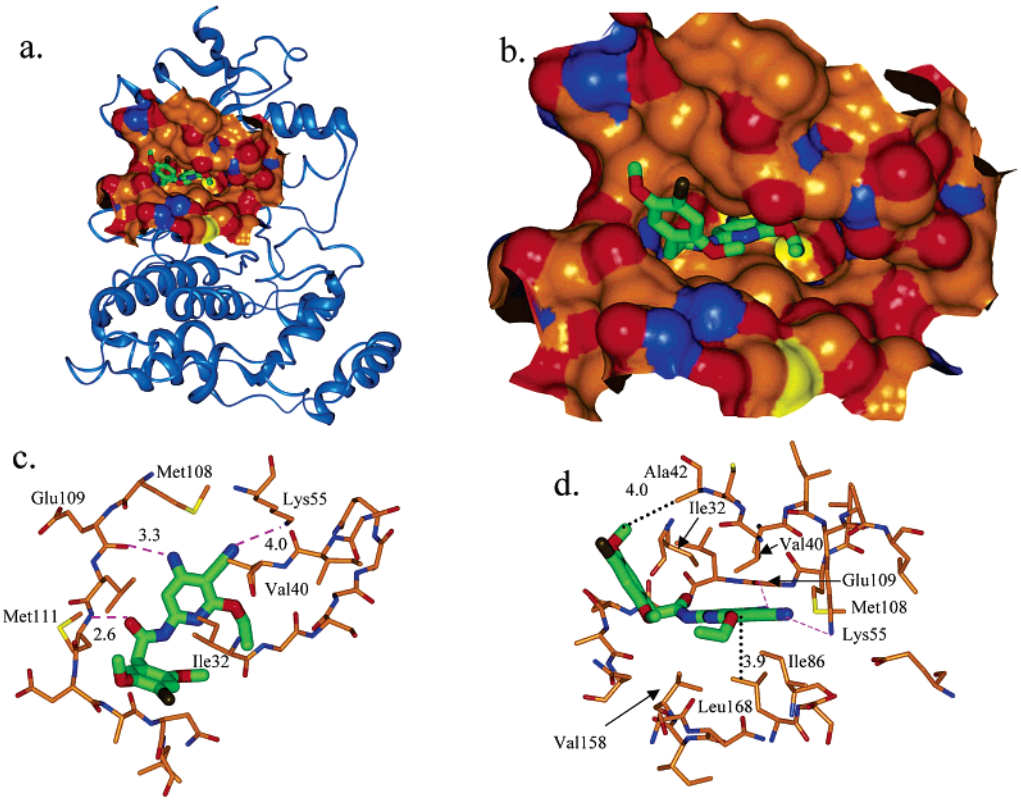
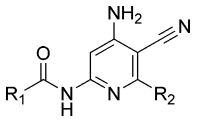


Figure 2. X-ray crystal structure of inhibitor **6t** with JNK-1 with protein carbon in orange, inhibitor carbon in green, nitrogen in blue, oxygen in red, sulfur in yellow, and bromine in black. Purple lines denote hydrogen bonds, and black lines denote hydrophobic interactions: (a) ribbon diagram of **6t** bound to JNK-1 with the ATP-binding site highlighted; (b) space filling diagram of **6t** bound to JNK-1; (c) stick diagram of **6t**−JNK-1 complex with some ATP-site amino acid residues labeled and interatomic distances in angstroms shown, with some residues removed for clarity; (d) alternative view of **6t**−JNK-1 complex with the pyridine ring shown edge-on. Additional amino acid residues and interatomic distances in angstroms are shown.

mode of binding for our inhibitors.[36−38] An X-ray crystal structure of bromide **6t** bound to JNK-1 reveals some of the critical binding interactions between the ligand and the enzyme (Figure 2). The inhibitor occupies the ATP binding site, with the pyridine ring placed deep within the adenosine binding region. The C-4 amine forms a weak hydrogen bond with Glu109, but the hydrogen-bonding interaction is not optimal because the C-4 amine and Glu109 do not lie in the same plane. The pyridine ring itself lies in a hydrophobic pocket defined by Ile86, Leu168, Val158, Val40, and Ile32. The nitrile protrudes further into this pocket and lies 4.0 Å from Lys55, forming a weak hydrogen bond with the side chain amine. The ethyl ether is oriented out of the adenosine binding region and toward the ribose binding region on the surface of the protein. There are no clear hydrogen-bonding interactions between the ether oxygen and the protein. The inhibitor amide carbonyl oxygen lies 2.6 Å from the backbone amide NH group of Met111 but not in the same plane as the backbone amide. Thus, the inhibitor makes two out-of-plane hydrogen bonds with the hinge region (residues 109−111). The phenyl ring makes a hydrophobic interaction with Ile32, while the 4-bromo and 5-methoxy substituents are pointed toward the solvent. Additional residues on the protein surface offer the potential for hydrogen-bonding interactions with substituents in the 4- or 5-phenyl position. It is possible that hydrogen bond acceptors in the 4-phenyl position (e.g., **6r** and **6s**) take advantage of these interactions. Unfortunately we could not confirm this hypothesis because high-quality X-ray data could not be obtained with inhibitors bearing such 4-phenyl substituents.

The X-ray data revealed that the ethyl ether was pointed toward the ribose binding region of JNK-1. The ribose binding site is rich in potential hydrogen-bonding interactions, and so

Table 3. Pyridine C-2 SAR



entry	R_1	R_2	JNK-1 IC_{50} (nM)	SD	JNK-2 IC_{50} (nM)	SD	pCjun EC_{50} (nM)	SD
6a	Me	EtO	750	43	1100	110	6800	3000
17	2,5-(diMeO)$PhCH_2$	Br	1700	53	>10000			
18a	Me	*i*-PrO	310	1	470	140		
18b	(2,5-(diMeO)-4-($MeSO_2$)-Ph)CH_2	*i*-PrO	14	2	64	2	650	24
19a	Me	(2-Me_2N)CH_2CH_2O	>10000		>10000			
19b	Me	(2-CO_2H)CH_2CH2O	4800	190	4900	2400		
19c	Me	(3-tetrahydrofuryl)O	890	88	2900	400		
19d	Me	BuO	2100	1000	4663	1000		
19e	Me	MeO	2200	120	4500	920		
20a	2,5-(diMeO)$PhCH_2$	$HOCH_2CH_2O$	74	11	240	15	1600	520
20b	2,5-(diMeO)$PhCH_2$	(MeO)CH_2CH_2O	110	13	270	38	1400	920
20c	2,5-(diMeO)$PhCH_2$	(2-$MeSO_2$)CH_2CH_2O	400	45	740	70		
21	Me	*n*-BuS	8900	690	>10000			
22	Me	NHEt	2400	300	>10000			
23	Me	NH(*i*-Pr)	910	200	3000	410		
24	Me	Ph	1900	37	7700	1400		
25	Me	*n*-Pr	>10000		>10000			
26	2,5-(diMeO)$PhCH_2$	H	2600	58	5100	1100		

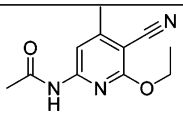
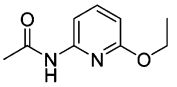
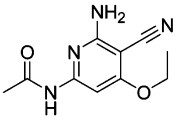
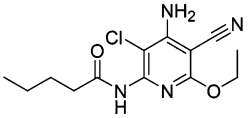
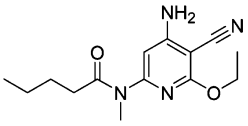
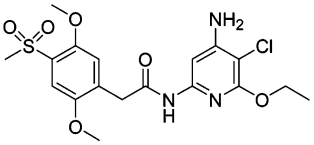
accessing this region was the focus of SAR efforts about the ether. Believing that more polar groups would provide stronger interactions with the hydrogen-bonding groups available in the ribose binding pocket, we added a variety of functional groups to the end of the ethyl chain (Table 3). 2-Hydroxyethyl and 2-methoxyethyl ethers **20a** and **20b** were both approximately as potent as ethyl ether **6o**, but there was no gain in potency with these modifications. Other changes we explored at the ethyl 2-position included methyl sulfone **20c**, amine **19a**, and carboxylic acid **19b**. In each case, installation of these groups diminished activity. Variation in the spacing between the ether oxygen atom and the distal functional group also failed to improve potency. In the hope of mimicking the ribose furan skeleton, we prepared tetrahydrofuryl analogue **19c**, but this too was less potent than ethyl ether **6a**. Despite these attempts, efforts to access the nearby ribose binding pocket did not result in significant gains in potency. An ether linkage was optimal at C-2 because bromide **17**, thioether **21**, amine (**22** and **23)**, aryl **24**, alkyl **25**, or hydrogen **26** substitution gave compounds with diminished activity. Variation of the alkyl ether chain length showed that neither a longer alkyl ether (**19d)** nor a methyl ether (**19e)** provided additional potency. However, a branched ether did provide a boost in potency. Isopropyl ether **18a** showed about a 2-fold improvement over the corresponding ethyl ether **6a**. The optimal group at the C-2 position was the isopropyl ether. When this was combined with the optimal C-6 amide, the resulting compound **18b** showed $IC_{50} = 14$ nM.

Changes elsewhere about the diaminopyridine core generally were not tolerated (Table 4). As expected from the X-ray structure, replacement of the 4-amino group with a methyl substituent resulted in a loss of activity (**39**). Simultaneous deletion of the C-3 nitrile and the C-4 amino groups (**46**)[39] likewise abolished JNK inhibition. The pyridine nitrogen atom of **6u** did not appear to be interacting with the protein surface, so we prepared 2,6-diaminopyridine **43** in the hope of gaining another H-bond with the hinge region. Unfortunately, **43** was devoid of activity. Chlorination of the unsubstituted C-5 position also was not tolerated (**41**). N-Methylation of the N-6 amide abolished activity as well (**42**). One change that was successful involved replacement of the C-3 nitrile with a chloride. Chloropyridine **35** showed an IC_{50} value of 36 ± 14 nM, comparable to cyanopyridine **6s**. Lead amide **6a** therefore contains the optimal ring pattern substitution with a C-2 ether, a C-3 nitrile, a C-4 amine, and a C-6 amide.

Compounds with $IC_{50} < 200$ nM vs JNK-1 were screened for their ability to inhibit c-Jun phosphorylation in HepG2 cells (Tables 1−4). Because c-Jun is a known substrate for JNK-1, this assay provides a direct measurement of JNK-1 inhibition in whole cells. In general, compounds showed EC_{50} values about 15-fold less potent than their IC_{50} values. There was no correlation between inhibitor membrane permeability (data not shown) and the EC_{50} to IC_{50} ratio. This indicates that cell penetration alone does not explain the drop in cellular potency relative to the ATF-2 phosphorylation assay. The intracellular concentration of ATP (10 mM) is greater than that used in the in vitro assays (5 μM), and this may account for most of the discrepancy between the two assay formats. Most of the phenylacetamides tested showed cellular activity, with the relative potencies in the enzymatic assay translating to a similar rank order in whole cells.

Perhaps the most remarkable feature of this series is its exquisite kinase selectivity profile. Previously, investigators at Serono published JNK inhibitors with some kinase selectivity, and these represent the most selective JNK inhibitors in the literature.[22,25] We screened several compounds against a panel of 74 different kinases and found that cross-reactivity was minimal, with the exception of JNK-2 and JNK-3 (Table 5). K_i values for JNK-1 were in the single-digit nanomolar range for the most potent compounds, but these showed measurable activity against only a few other members of the panel. Where cross-reactivity could be found, the compounds were well over 100-fold selective. A possible explanation for this selectivity profile can be discerned from the crystal structure of the enzyme−inhibitor complex. Most kinase inhibitors form strong and clearly defined hydrogen bonds to the hinge region.[40−42] Our compounds form two suboptimal hydrogen bonds with the JNK-1 hinge region, deriving much of their potency from other interactions within the ATP binding site (Figure 2). The weak hydrogen-bonding interaction with the hinge region could limit the ability of our JNK inhibitors to bind to other kinases.[43] Although the kinases we screened represent only about 15% of the human kinome, this level of selectivity is uncommon among

Table 4. Pyridine N-1, C-3, C-4, and C-5 Modifications and N-6 Amide Alkylation

entry	Structure	JNK-1 IC_{50} (nM)	SD	JNK-2 IC_{50} (nM)	SD	pCjun EC_{50} (nM)	SD
39		>10000		>10000			
46		>10000		>10000			
43		>10000		>10000			
41		>10000		>10000			
42		>10000		>10000			
35		36	14	70	25	1750	15

kinase inhibitors. Such selectivity offers an important advantage for probing the role of JNK inhibition in vivo.

Pharmacokinetic profiles were determined for JNK inhibitors **6o**, **6s**, **18b**, and **35** in Sprague-Dawley rats (Table 6). Inhibitor **6o** showed a short half-life of roughly 1 h, with rapid clearance and barely measurable bioavailability. Microsomal incubation studies revealed that oxidative metabolism was very rapid with this compound, indicating that the pharmacokinetic profile could be improved if oxidative metabolism could be suppressed. Attempts to improve the microsomal stability of these phenylacetamides revealed that substitution of the phenylacetamide with a methylsulfonyl group greatly lowered the rate of oxidative metabolism. Inhibitor **6s**, which bears a 4-methylsulfonyl group on the 2,5-dimethoxyphenyl ring, showed a somewhat better half-life than **6o** and markedly better bioavailability. Although it is not clear whether this is due to improved first-pass metabolism or better absorption, the presence of the methylsulfone group clearly is beneficial to the pharmacokinetic profile of the JNK inhibitors. The most potent JNK-1 inhibitor, compound **18b**, was less orally bioavailable than **6s**, and it was also cleared more rapidly than ethyl ethers **6o** and **6s**. This is evidence that the isopropyl ether is more metabolically labile than the corresponding ethyl ether. 3-Chloropyridine analogue **35** showed the best oral bioavailability (F = 49%), half-life (2.1 h), and drug exposure level (AUC = 1.47 μg·h/mL) of the four compounds we evaluated. The oral bioavailability and reasonable drug exposures demonstrated with **6s** and **35** make them candidates for further in vivo testing in JNK-related disease models.

Conclusion

We have identified several potent JNK inhibitors with excellent kinase selectivity profiles. X-ray crystallographic data indicate that the hydrogen-bonding network of these kinase ATP-site ligands is different from many other kinase inhibitors, and the unusual binding mode may be responsible for their kinase selectivity. We have prepared compounds **6s** and **35** with cellular activity and oral bioavailability. These selective JNK inhibitors can serve as important probes to discern what role JNK plays in a host of diseases. Evaluation of the new compounds in a variety of disease models will give us a better understanding of what the physiological implications of JNK inhibition are, and we are eager to make these inhibitors available for such studies. Our own in vivo results with JNK inhibitors in inflammation and diabetes models will be disclosed at a later date.

Experimental Section

General. Thin-layer chromatography systems were the same as those used for column chromatography, with $R_f \approx 0.3$. Column chromatography was performed under 5 psi of N_2 using 230−400 mesh silica gel or with an Analogix Intelli Flash 280 MPLC system using prepacked, disposable silica gel columns. Analytical HPLC traces and compounds purified by HPLC were obtained by elution through a C_{18} reverse-phase column with a gradient of 5 mM aqueous ammonium acetate/acetonitrile or 0.1% aqueous TFA/acetonitrile as eluants. Concentration gradients started at 0% acetonitrile and 100% aqueous TFA or at 5% acetonitrile and 95% aqueous medium. Microwave heating was performed using a Personal Chemistry Emrys Optimizer microwave apparatus, and reaction pressures were not allowed to exceed 20 atm. ^{1}H NMR data are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; b, broad), number of protons, coupling constants in Hz.

4,6-Diamino-2-ethoxynicotinonitrile (2).[30] To a 5 mL sealable heavy-walled glass tube suitable for microwave heating containing 5 mL of absolute ethanol was added 150 mg (6.5 mmol) of sodium

Table 5. Kinase Selectivity Data for Diaminopyridine-Based JNK Inhibitors (K_i in nM)[a]

	K_i (nM)							K_i (nM)					
kinase	**6a**	**6o**	**6s**	**24**	**18b**	**35**	kinase	**6a**	**6o**	**6s**	**24**	**18b**	**35**
AKT1	>8200	>8200	>8200	>8200	>8200	>8200	JAK3	>1700	>1700	>1700	>1700	>1700	>1700
AKT2	>8400	>8400	>8400	>8400	>8400	>8400	*JNK-1*	*190*	*2*	*3*	*550*	*1*	*3*
AKT3	>9500	>9500	>9500	>9500	>9500	>9500	*JNK-2*	*440*	*4*	*9*	*1900*	*4*	*13*
AMPK	>9800	>9800	>9800	>9800	>9800	>9800	*JNK-3*	*520*	*52*	*72*	*3300*	*18*	*61*
Abl	>710	>710	>710	>710	>710	>710	Kdr	>8200	>8200	>8900	>8200	>8900	>8900
Aurora1	>6900	>6900	>6900	>6900	>6900	>6900	Lck	>2700	>2700	>2700	>2700	>2700	>2700
Aurora2	>6700	>6700	>6700	>6700	>6700	>6700	Limk1	>9600	>9600	>9600	>9600	>9600	>9600
Blk	>1500	>1500	>1500	>1500	>1500	>1500	Lyn	>1700	>1700	>1700	>1700	>1700	>1700
CDC2	>8900	>8900	>8900	>8900	>8900	>8900	MAPK(APK2)	>8400	>8400	>8400	>8400	>8400	>8400
CDC42	>9500	>9500	>9500	>9500	>9500	>9500	MK3	>9200	>9200	>9200	>9200	>9200	>9200
CDK2	>8600	*820*	>8600	*1300*	>8600	>8600	Mst2	>8800	>8800	>8800	>8800	>8800	>8800
CDK5	>9300	>9300	>9300	>9300	>9300	>9300	Nek2	>920	>920	>9200	>9200	>9200	>9200
CHK1	>8800	>8800	>8800	>8800	>8800	>8800	*P70S6K*	>6700	*3400*	>6700	>6700	>6700	>6700
CHK2	>9500	>9500	>9500	>9500	>9500	>9500	PAK1	>9700	>9700	>9700	>9700	>9700	>9700
CK2	>5800	>5800	>5800	>5800	>5800	>5800	PAK4	>5500	>5500	>5500	>5500	>5500	>5500
CSF1R	>2500	>2500	>2500	>2500	>2500	>2500	PDK1	>3800	>3800	>3800	>3800	>3800	>3800
CTAK1	>6700	>6700	>6700	>6700	>6700	>6700	PKA	>7500	>7500	>7500	>7500	>7500	>7500
CaMK4	>8600	>8600	>8600	>8600	>8600	>8600	PKCδ	>8900	>8900	>8900	>8900	>8900	>8900
CK1δ	>7800	>7800	>7800	>7800	>7800	>7800	PKCγ	>8800		>8800	>8800	>8800	>8800
Dyrk1A	>6900	>6900	>6900	>6900	>6900	>6900	PKCζ	>8300	>8300	>8300	>8300	>8300	>8300
EGFR	>1800	>1800	>1800	>1800	>1800	>1800	Pim1	>9200	>9200	>9200	>9200	>9200	>9200
EMK	>7500	>7500	>7500	>7500	>7500	>7500	Pim2	>1400	>1400	>1400	>1400	>1400	>1400
ERK2	>8800	*4700*	*5800*	>8800	*3900*	>8800	*Pkd2*	*2500*	*5300*	>9000	>9000	*950*	>9000
ErbB2	>1400	>1400	>1400	>1400	>1400	>1400	Plk1	>8900	>8900	>8900	>8900	>8900	>8900
FGFR	>4700	>4700	>4700	>4700	>4700	>4700	Plk3	>8000	>8000	>8000	>8000	>8000	>8000
FGFR3	>1900	>1900	>1900	>1900	>1900	>1900	Rock1	>9100	>9100	>9100	>9100	>9100	>9100
Flt1	>440	>440	>440	>440	>440	>440	Rock2	>7500	>7500	>7500	>7500	>7500	>7500
Flt3	>2500	>2500	>2500	>2500	>2500	>2500	*RSK2*	>7400	>7400	>7400	>7400	*3300*	>7400
Flt4	>7100	>7100	>7100	>7100	>7100	>7100	SGK	>9400	>9400	>9400	>9400	>9400	>9400
Fyn	>810	>810	>810	>810	>810	>810	Src	>1900	>1900	>1900	>1900	>1900	>1900
GSK3α	>8900	>8900	>8900	>8900	>8900	>8900	TrkA	>2500	>2500	>2500	>2500	>2500	>2500
GSK3β	>7100	>7100	>7100	>7100	>7100	>7100	TrkB	>4500	>4500	>4500	>4500	>4500	>4500
ITK	>1500	>1500	>1500	*970*	>1500	>1500	Tyk2	>1900	>1900	>1900	>1900	>1900	>1900
IKKα	>3800	>3800	>3800	>3800	>3800	>3800	ZipK	>1400	>1400	>1400	>1400	>1400	>1400
IKKβ	>3200	>3200	>3200	>3200	>3200	>3200	cKit	>1200	>1200	>1200	>1200	>1200	>1200
insulin R	>5200	>5200	>5200	>5200	>5200	>5200	cMet	>2900	>2900	>2900	>2900	>2900	>2900
IRAK4	>9900	>9900	>9900	>9900	>9900	>9900	p38δ	>6900	>6900	>6900	>6900	>6900	>6900
JAK2	>1500	>1500	>1500	>1500	>1500	>1500	p38γ	>7500	>7500	>7500	>7500	>7500	>7500

[a] Kinases showing measurable inhibition are in italics.

Table 6. Pharmacokinetic Parameters for JNK Inhibitors in Sprague-Dawley Rats (5 mg/kg Dose)

	iv					po				
compd	$t_{1/2}$[a]	V_{ss}[b]	V_b[c]	AUC[d]	CL_p[e]	$t_{1/2}$[f]	C_{max}[g]	T_{max}[h]	AUC[i]	F[j]
6o	1.4	1.2	2.6	4.1	1.2	1.1	0.31	0.033	0.11	2.7
6s	2	1.5	4.7	3.5	1.5	1.5	1.36	0.5	1.1	31
18b	2	1.6	8.6	2.2	3.0	2.9	0.08	0.75	0.12	5.7
35	0.8	1.6	2.0	3.0	1.7	2.1	1.42	0.67	1.5	49

[a] Intravenous half-life (hours). [b] Intravenous volume of distribution at steady state (L/kg). [c] Intravenous volume of distribution (L/kg). [d] Area under the curve or total drug exposure after intravenous dosing from $t = 0$ to $t = \infty$ (μg·h/mL). [e] Plasma clearance after intravenous dosing ((L/h)/kg). [f] Oral half-life (hours). [g] Maximum plasma concentration after oral dosing (μM). [h] Time from oral dosing to maximum plasma concentration (hours). [i] Area under the curve or total drug exposure after oral dosing from $t = 0$ to $t = \infty$ (μg·h/mL). [j] Oral bioavailability (%).

metal. The mixture was stirred until all of the sodium had reacted, and then 1.00 g (4.71 mmol) of 2-bromo-4,6-diaminonicotinonitrile (**1**)[29] was added. The tube was sealed, and the mixture was heated with a microwave apparatus at 150 °C for 10 min, then cooled and diluted with 15 mL of water. The orange precipitate that formed was washed with water until pH 7 was attained and then dried in vacuo at 105 °C to give 0.545 g (65%) of a light-orange solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.19 (s, 2H), 6.14 (s, 2H), 5.32 (s, 1H), 4.23 (q, 2H, $J = 7.1$ Hz), 1.25 (t, 3H, $J = 7.1$ Hz); MS (ESI) $m/e = 151$ (M − Et + H)$^+$, 179 (M + H)$^+$.

4,6-Diamino-2-isopropoxynicotinonitrile (3). To a 5 mL sealable heavy-walled glass tube suitable for microwave heating containing 5 mL of 2-propanol was added 150 mg (6.5 mmol) of sodium metal. The mixture was stirred and heated under N_2 until all of the sodium had reacted. Then 1.00 g (4.71 mmol) of **1** was added. The mixture was heated at 160 °C for 20 min and then concentrated in vacuo. The residue was taken up in 10 mL of water and then extracted with ethyl acetate (3 × 10 mL). The combined organic layers were extracted with saturated $NaHCO_{3(aq)}$ (1 × 10 mL) and then brine (1 × 10 mL), dried over $MgSO_4$, filtered, and concentrated to a solid. This was purified via MPLC, eluting with a 30−50% ethyl acetate/hexanes gradient to give 380 mg (42%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.16 (s, 2H), 6.11 (s, 2H), 5.30 (s, 1H), 5.11−5.28 (septet, $J = 6.1$ Hz, 1H), 1.23 (d, $J = 6.1$ Hz, 6H). MS (ESI) $m/e = 151$ (M − Pr + H)$^+$, 193 (M + H)$^+$.

4,6-Diamino-2-butylsulfanylnicotinonitrile (5). To a suspension of 60% NaH (16 mg, 0.4 mmol) in DMF (1 mL) was added *n*-butylthiol (22 μL, 0.2 mmol) followed by **1** (43 mg, 0.2 mmol). The mixture was heated with an oil bath at 120 °C for 30 min. Then it was cooled and partitioned between ethyl acetate and water. The organic phase was washed with brine (×3), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified via silica gel chromatography, eluting with ethyl acetate/hexane (1:1) to provide the thioether as a pale-yellow solid (23 mg, 52%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.28 (s, 2H), 6.19 (s, 2H), 5.42 (s, 1H), 3.09 (t, $J = 7.1$ Hz, 2H), 1.63−1.52 (m, 2H), 1.44−1.32 (m, 2H), 0.89 (t, $J = 7.1$ Hz, 3H); MS (ESI) $m/e = 223$ (M + H)$^+$; 221 (M − H)$^-$.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)acetamide (6a).** To 50 mg (0.28 mmol) of **2** dissolved in 1 mL of pyridine was added 60 μL (0.84 mmol) of acetyl chloride. The mixture was stirred at ambient temperature for 2 h and then diluted with 7 mL of water. The precipitate was filtered and washed with 5 mL of water. The

supernatant was extracted with ethyl acetate (3 × 5 mL). Then the combined ethyl acetate layers were back-extracted with brine (1 × 5 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to a solid. This was combined with the precipitated product, dissolved in methanol/ethyl acetate, and concentrated. Most of the remaining pyridine was removed by coevaporation with toluene in vacuo. The product was purified via silica gel chromatography, eluting with 50% ethyl acetate/hexanes to give 31 mg (50%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.73 (s, 1H), 7.17 (s, 1H), 6.88 (s, 2H), 4.32 (q, 2H, J = 7.1 Hz), 2.07 (s, 3H), 1.29 (t, 3H, J = 7.1 Hz); MS (ESI) m/e = 221 $(M + H)^+$, m/e = 219 $(M - H)^-$. Anal. ($C_{10}H_{12}N_4O_2$) C, H, N.

Pentanoic Acid (4-Amino-5-cyano-6-ethoxypyridin-2-yl)amide (6b). To 50 mg (0.28 mmol) of **2** in 1 mL of pyridine was added 7 drops of valeryl chloride via pipet. After being stirred at ambient temperature for 18 h, the mixture was diluted with 7 mL of water and stirred for 1 h. The suspension was subjected to centrifugation (800*g* for 1 min), and then the solvent was decanted. After resuspension and centrifugation once more, the product was purified via reversed-phase HPLC (aqueous TFA gradient). The purified product was dissolved in 5 mL of ethyl acetate, extracted with $NaHCO_{3(aq)}$ (1 × 1 mL), then brine (1 × 1 mL), dried over $MgSO_4$, filtered, and concentrated to 21 mg (29%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.03 (s, 1H), 7.20 (s, 1H), 6.87 (s, 2H), 4.33 (q, J = 7.1 Hz, 2H), 2.36 (t, J = 7.5 Hz, 2H), 1.39−1.73 (m, 2H), 1.16−1.42 (m, 5H), 0.88 (t, J = 7.3 Hz, 3H); MS (ESI) m/e = 263 $(M + H)^+$. Anal. ($C_{13}H_{18}N_4O_2$) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)isobutyramide (6c).** This was prepared according to the same procedure described for **6a**, substituting isobutyryl chloride for acetyl chloride. The crude product was recrystallized from methanol to give 40 mg (69%) of a white solid. TLC (30% ethyl acetate/hexanes); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.20 (s, 1H), 6.87 (s, 2H), 4.33 (q, 2H, J = 7.1 Hz), 2.75 (m, 1H, J = 6.8 Hz), 1.29 (t, 3H, J = 7.1 Hz), 1.05 (d, 6H, J = 7.1 Hz); MS (ESI) m/e = 221 $(M - Et + H)^+$, 249 $(M + H)^+$, 247 $(M - H)^-$. Anal. ($C_{12}H_{16}N_4O_2$) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-3-methylbutyramide (6d).** This was prepared according to the same procedure described for **6a**, substituting 3-methylbutyryl chloride for acetyl chloride. The crude product was purified by reverse-phase HPLC, eluting with a gradient of 5−100% CH_3CN in 0.1% aqueous TFA to give 12 mg (16%) of a white solid. TLC (30% ethyl acetate/hexanes); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.01 (s, 1H), 7.22 (s, 1H0, 6.87 (s, 2H), 4.33 (q, 2H, J = 7.1 Hz), 2.25 (d, 2H, J = 7.1 Hz), 2.03 (m, 1H), 1.29 (t, 3H, J = 7.1 Hz), 0.90 (d, 6H, J = 6.4 Hz); MS (ESI) m/e = 263 $(M + H)^+$, 261$(M - H)^-$. Anal. ($C_{13}H_{18}N_4O_2$) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-phenylacetamide (6e).** This was prepared according to the same procedure described for **6a**, substituting phenylacetyl chloride for acetyl chloride. The crude product was recrystallized from methanol to give 8 mg (11%) of a white solid. TLC (30% ethyl acetate/hexanes); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.31 (s, 1H), 7.26 (m, 5H), 7.16 (s, 1H), 6.89 (s, 2H), 4.34 (q, 2H, J = 7.1 Hz), 3.70 (s, 2H), 1.30 (t, 1H, J = 7.1 Hz,); MS (ESI) m/e = 269 $(M - Et + H)^+$, 297 $(M + H)^+$, 295 $(M - H)^-$. Anal. ($C_{16}H_{16}N_4O_2$) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-(2-chlorophenyl)acetamide (6f).** To a solution of 29 mg (0.17 mmol) of (2-chlorophenyl)acetic acid in 0.6 mL of CH_2Cl_2 was added 20 μL (0.23 mmol) of oxalyl chloride. The mixture was allowed to stand at ambient temperature for 20 min, and then the acid chloride solution was added to 30 mg (0.17 mmol) of **1** in 0.6 mL of pyridine at ambient temperature. The mixture was swirled and then allowed to stand at ambient temperature for 1 h. Next, 1 mL of water was added. Then the mixture was concentrated to dryness in vacuo. The residue was partitioned between EtOAc (10 mL) and water (5 mL), and then the water layer was removed. The organic phase was extracted with water (1 × 5 mL), saturated $NaHCO_{3(aq)}$ (1 × 5 mL), and brine (1 × 5 mL), dried over $MgSO_4$, filtered, and concentrated to a solid. This was recrystallized from methanol to give 10 mg (18%) of white crystals. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.38 (s, 1H), 7.41 (m, 2H), 7.31 (m, 2H), 7.13 (s, 1H), 6.89 (s, 2H), 4.35 (q, 2H, J = 7.1 Hz), 3.90 (s, 2H), 1.31 (t, 3H, J = 7.1 Hz); MS (ESI) m/e = 303 $(M - Et + H)^+$, 331 $(M + H)^+$, 329 $(M - H)^-$. Anal. ($C_{16}H_{15}ClN_4O_2$) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-(3-chlorophenyl)acetamide (6g).** This was prepared according to the same procedure as **6f**, substituting (3-chlorophenyl)acetic acid for (2-chlorophenyl)acetic acid. The product was purified via reverse-phase HPLC, eluting with a 0−70% CH_3CN gradient in 0.1% aqueous TFA. Yield, 8 mg (14%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.35 (s, 1H), 7.38 (m, 1H, J = 1.7 Hz), 7.31 (m, 3H), 7.15 (s, 1H), 6.90 (s, 2H), 4.34 (q, 2H, J = 7.0 Hz), 3.73 (s, 2H), 1.31 (t, 3H, J = 7.1 Hz); MS (ESI) m/e = 303 $(M - Et + H)^+$, 331 $(M + H)^+$, 329 $(M - H)^-$. Anal. ($C_{16}H_{15}ClN_4O_2$·0.07TFA) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-(4-chlorophenyl)acetamide (6h).** This was prepared according to the same procedure as **6f**, substituting (4-chlorophenyl)acetic acid for (2-chlorophenyl)acetic acid. Yield, 8 mg (14%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.33 (s, 1H), 7.35 (m, 4H), 7.15 (s, 1H), 6.90 (s, 2H), 4.34 (q, 2H, J = 7.1 Hz), 3.71 (s, 2H), 1.30 (t, 3H, J = 7.1 Hz); MS (ESI) m/e = 303 $(M - Et + H)^+$, 331 $(M + H)^+$, 329 $(M - H)^-$. Anal. ($C_{16}H_{15}ClN_4O_2$·0.15CH_3OH) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-*o*-tolylacetamide (6i).** This was prepared according to the same procedure as **6f**, substituting (2-methylphenyl)acetic acid for (2-chlorophenyl)-acetic acid. Yield, 10 mg (10%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.28 (s, 1H), 7.19 (t, 1H, J = 7.5 Hz), 7.16 (s, 1H), 7.07 (m, 3H), 6.89 (s, 2H), 4.34 (q, 2H, J = 7.1 Hz), 3.65 (s, 2H), 2.28 (s, 3H), 1.30 (t, 3H, J = 7.1 Hz); MS (ESI) m/e = 283 $(M - Et + H)^+$, 311 $(M + H)^+$, 309 $(M - H)^-$. Anal. ($C_{17}H_{18}N_4O_2$) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-*m*-tolylacetamide (6j).** This was prepared according to the same procedure as **6f**, substituting (3-methylphenyl)acetic acid for (2-chlorophenyl)-acetic acid. The product was purified via MPLC, eluting with 30% ethyl acetate in hexanes followed by trituration with ethyl acetate. Yield, 10 mg (10%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.31−10.28 (s, 1H), 7.20 (t, 1H, J = 7.6 Hz), 7.16 (s, 1 H), 7.13−7.08 (m, 2H), 7.05 (d, 1H, J = 7.3 Hz), 6.89 (s, 2 H), 4.34 (q, 2H, J = 7.0 Hz), 3.65 (s, 2H), 2.28 (s, 3H), 1.30 (t, 3H, J = 7.0 Hz); MS (ESI) m/e = 283 $(M - Et + H)^+$, 311 $(M + H)^+$, 309 $(M - H)^-$. Anal. ($C_{17}H_{18}N_4O_2$·0.11EtOAc) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-*p*-tolylacetamide (6k).** This was prepared according to the same procedure as **6f**, substituting (4-methylphenyl)acetic acid for (2-chlorophenyl)-acetic acid. The product was purified by trituration with hot 2-propanol. Yield, 20 mg (19%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.26 (s, 1H), 7.15 (m, 5H), 6.88 (s, 2H), 4.34 (q, 2H, J = 7.1 Hz), 3.64 (s, 2H), 2.27 (s, 3H), 1.30 (t, 3H, J = 7.1 Hz); MS (ESI) m/e = 283 $(M - Et + H)^+$, 311 $(M + H)^+$, 309 $(M - H)^-$. Anal. ($C_{17}H_{18}N_4O_2$·0.09*i*-PrOH) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-(2-methoxyphenyl)acetamide (6l).** This was prepared according to the same procedure as **6f**, substituting (2-methoxyphenyl)acetic acid for (2-chlorophenyl)acetic acid. The product was purified by trituration with hot 2-propanol. Yield, 10 mg (9%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.10 (s, 1H), 7.24 (m, 1H), 7.17 (dd, 1H, J = 7.5, 1.7 Hz), 7.13 (s, 1H), 6.97 (d, 1H, J = 7.1 Hz), 6.89 (m, 3H), 4.34 (q, 2H, J = 7.1 Hz), 3.76 (s, 3H), 3.68 (s, 2H), 1.31 (t, 3H, J = 7.1 Hz); MS (ESI) m/e = 299 $(M - Et + H)^+$, 327 $(M + H)^+$, 325 $(M - H)^-$. Anal. ($C_{17}H_{18}N_4O_3$·0.08EtOAc) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-(3-methoxyphenyl)acetamide (6m).** This was prepared according to the same procedure as **6f**, substituting (3-methoxyphenyl)acetic acid for (2-chlorophenyl)acetic acid. The product was recrystallized from 1 mL of methanol to give 9 mg (16%) of a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.28 (s, 1H), 7.22 (m, 1H), 7.16 (m, 1H), 6.89 (m, 4H), 6.81 (m, 1H), 4.34 (q, 2H, J = 7.1 Hz), 3.74 (s, 3H), 3.67 (s, 2H), 1.31 (t, 3H, J = 7.1 Hz); MS (ESI) m/e = 299 $(M - Et + H)^+$, 327 $(M + H)^+$, 349 $(M + Na)^+$. Anal. ($C_{17}H_{18}N_4O_3$) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-(4-methoxyphenyl)acetamide (6n).** This was prepared according to the same procedure as **6f**, substituting (4-methoxyphenyl)acetic acid for (2-chlorophenyl)acetic acid. Yield, 9 mg (16%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.24 (s, 1H), 7.23 (d, 2H, J = 8.8 Hz), 7.15 (s, 1H), 6.87 (d, 4H, J = 8.8 Hz), 4.34 (q, 2H, J = 7.1 Hz), 3.72 (s, 3H), 3.62 (s, 2H), 1.30 (t, 3H, J = 7.1 Hz); MS (ESI) m/e = 299 (M − Et + H)$^+$, 327 (M + H)$^+$, 349 (M + Na)$^+$. Anal. ($C_{17}H_{18}N_4O_3$·0.17CH_3OH) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-(2,5-dimethoxyphenyl)acetamide (6o).** This was prepared according to the same procedure as **6f**, substituting (2,5-dimethoxyphenyl)acetic acid for (2-chlorophenyl)acetic acid. The product was purified by recrystallization from ethanol. Yield, 11 mg (18%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.10 (s, 1H), 7.12 (s, 1H), 6.90 (m, 3H), 6.79 (m, 2H), 4.34 (q, 2H, J = 7.1 Hz), 3.70 (s, 3H), 3.69 (s, 3H), 3.66 (s, 2H), 1.31 (t, 3H, J = 7.1 Hz); MS (ESI) m/e = 329 (M − Et + H)$^+$, 357 (M + H)$^+$, 355 (M − H)$^-$. Anal. ($C_{18}H_{20}N_4O_4$·0.10EtOH) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-(2,5-dimethylphenyl)acetamide (6p).** This was prepared according to the same procedure as **6f**, substituting 2,5-dimethylphenylacetic acid for (2-chlorophenyl)acetic acid. The product was purified by trituration with hot methanol. Yield, 9 mg (16%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.26 (s, 1H), 7.15 (s, 1H), 7.04 (m, 2H), 6.93 (m, 3H), 4.35 (q, 2H, J = 7.1 Hz), 3.70 (s, 2H), 2.23 (s, 3H), 2.21 (s, 3H), 1.31 (t, 3H, J = 7.1 Hz); MS (ESI) m/e = 297 (M − Et + H)$^+$, 325 (M + H)$^+$, 323 (M − H)$^-$. Anal. ($C_{18}H_{20}N_4O_2$·0.09EtOAc) C, H, N.

2,5-Dibromobenzyl Alcohol. To an ice-cooled solution of 2.80 g (10.0 mmol) of 2,5-dibromobenzoic acid in 5 mL of THF was added 11 mL of 1.0 M BH_3·THF. The mixture was warmed to ambient temperature and stirred for 24 h. The mixture was cooled with an ice bath. Then 4 mL of water was added, and the solution was stirred until bubbling ceased. The solvents were removed in vacuo, and then the residue was taken up in 30 mL of diethyl ether. This was extracted with 20 mL of 1 M NaOH and then with 2 M NaOH (2 × 10 mL) and brine (1 × 10 mL), dried, and concentrated to 2.49 g (94%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.65 (d, J = 2.4 Hz, 1H), 7.53 (d, J = 8.5 Hz, 1H), 7.40 (dd, J = 8.5, 2.4 Hz, 1H), 5.58 (t, J = 5.4 Hz, 1H), 4.49 (d, J = 5.4 Hz, 2H).

2,5-Dibromobenzyl Chloride. To a solution of 2.49 g (9.36 mmol) of 2,5-dibromobenzyl alcohol in 15 mL of DMF was added 794 mg (18.7 mmol) of lithium chloride. After the lithium chloride had dissolved, 1.2 mL (16.4 mmol) of thionyl chloride was added. After spontaneously warming, the solution was stirred at ambient temperature for 1.5 h and then poured into 75 mL of water. The precipitate was collected, washed with water (2 × 20 mL), and then taken up in 50 mL of hexanes. The residual water was drained, and the organic layer was extracted with brine (1 × 10 mL), dried, and concentrated to 2.42 g (91%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.88 (d, J = 2.4 Hz, 1H), 7.64 (d, J = 8.5 Hz, 1H), 7.51 (dd, J = 8.5, 2.4 Hz, 1H), 4.79 (s, 2H); MS (ESI) m/e = 305 (M + Na)$^+$.

(2,5-Dibromophenyl)acetonitrile.[44] To 2.42 g (8.51 mmol) of 2,5-dibromobenzyl chloride in 10 mL of DMSO was added 600 mg (9.2 mmol) of powdered potassium cyanide. The mixture was stirred at ambient temperature for 24 h and then poured into 75 mL of water. The aqueous mixture was stirred for 30 min, and then the precipitate was collected. The crude product was purified via silica gel chromatography (90:10 hexane/EtOAc) to give 1.16 g (50%) of a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.77 (d, J = 2.4 Hz, 1H), 7.66 (d, J = 8.5 Hz, 1H), 7.53 (dd, J = 8.5, 2.4 Hz, 1H), 4.08 (s, 2H); MS (ESI) m/e = 273 (M − H)$^-$.

2,5-Dibromophenylacetic Acid. To 274 mg (1.0 mmol) of (2,5-dibromophenyl)acetonitrile was added 4 mL of 60% (w/w) $H_2SO_{4(aq)}$. The mixture was stirred at reflux for 3 h and then was cooled, diluted with 10 mL of water, and extracted with diethyl ether (2 × 15 mL). The combined ether layers were back-extracted with 1 M NaOH (2 × 5 mL), and then the combined NaOH layers were back-extracted with ether (1 × 5 mL). The ether layers were set aside, and the NaOH layer was made acidic by addition of 12 M $HCl_{(aq)}$ (ca. 1.5 mL). The acidic suspension was then extracted with diethyl ether (3 × 5 mL). Then a final set of ether layers was back-extracted with brine (1 × 5 mL), dried, and concentrated to 173 mg of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.57 (s, 1H), 7.66 (d, J = 2.7 Hz, 1H), 7.56 (d, J = 8.8 Hz, 1H), 7.42 (dd, J = 8.8, 2.7 Hz, 1H), 3.74 (s, 1 H); MS (ESI) m/e = 249 (M − CO_2H)$^-$.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-(2,5-dibromophenyl)acetamide (6q).** This was prepared according to the same procedure as **6f** from 100 mg of **1** and 170 mg of 2,5-dibromophenylacetic acid. The product was purified via reversed-phase HPLC followed by trituration with hot methanol. Yield, 20 mg (8%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.42 (s, 1H), 7.64 (d, J = 2.7 Hz, 1H), 7.56 (d, J = 8.5 Hz, 1H), 7.42 (dd, J = 8.5, 2.4 Hz, 1H), 7.11 (s, 1H), 6.90 (s, 2H), 4.35 (q, J = 7.1 Hz, 2H), 3.93 (s, 2H), 1.32 (t, J = 7.1 Hz, 3H); MS (ESI) m/e = 450 (M + H)$^+$. Anal. ($C_{16}H_{14}Br_2N_4O_2$·0.39H_2O) C, H, N.

2-(4-Acetylamino-2,5-dimethoxyphenyl)-*N*-(4-amino-5-cyano-6-ethoxypyridin-2-yl)acetamide (6r). To a suspension of 250 mg (0.623 mmol) of **6u** in 10 mL of glacial acetic acid and 2 mL of water at 90 °C was added 500 mg (7.65 mmol) of Zn dust. The mixture was heated at 90 °C for 30 min. Then the amber solution was decanted from the excess Zn, and the solvents were removed in vacuo. The residue was suspended in water amd filtered, and the precipitate was washed with additional water. The product was purified by dissolution in 10 mL of MeOH and 0.5 mL of 1 M $HCl_{(aq)}$, and then addition of 0.3 mL of 2 M NaOH gave a yellow precipitate. This was filtered, washed with methanol, and dried in vacuo to give 100 mg (43%) of the aminophenylacetamide. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.78 (s, 1H), 7.12 (s, 1H), 6.88 (s, 2H), 6.67 (s, 1H), 6.35 (s, 1H), 4.69 (s, 2H), 4.32 (q, J = 6.9 Hz, 2H), 3.68 (s, 3H), 3.64 (s, 3H), 3.51 (s, 2H), 1.30 (t, J = 7.1 Hz, 3H); MS (ESI) m/e = 372 (M + H)$^+$.

To a solution of 20 mg (0.054 mmol) of *N*-(4-amino-5-cyano-6-ethoxypyridin-2-yl)-2-(4-amino-2,5-dimethoxyphenyl)acetamide in 1 mL of pyridine and 1 mL of CH_2Cl_2 at −78 °C was added a solution of 100 μL (1.40 mmol) of acetyl chloride in 1 mL of CH_2Cl_2. The mixture was stirred at −78 °C for 5 min. Then 2 mL of water was added, and the mixture was warmed until the ice melted. The solvents were removed in vacuo, and the residue was suspended in 4 mL of water. This was placed in a centrifuge for 1 min at 2000 rpm. Then the water was decanted. This was repeated with 4 mL portions of water until the aqueous suspension was at neutral pH. The resulting wet solid was taken up in methanol and concentrated to dryness. The solid was recrystallized from methanol to give 5 mg (22%) of the acetamide as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.04 (s, 1H), 9.11 (s, 1H), 7.72 (s, 1H), 7.12 (s, 1H), 6.92 (s, 1H), 6.87 (s, 2H), 4.34 (q, J = 7.1 Hz, 2H), 3.76 (s, 3H), 3.66 (s, 3H), 3.65 (s, 2H), 2.08 (s, 3H), 1.31 (t, J = 7.1 Hz, 3H); MS (ESI) m/e = 414 (M + H)$^+$. Anal. ($C_{20}H_{23}N_5O_5$·0.53H_2O) C, H, N.

***N*-(4-Amino-5-cyano-6-isopropoxypyridin-2-yl)-2-(4-methanesulfonyl-2,5-dimethoxyphenyl)acetamide (6s).** This was prepared from **2** and acid chloride **11** according to the procedure for **6f**. The mixture was run at −78 °C. Yield, 34 mg (25%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.27 (s, 1H), 7.30 (s, 1H), 7.26 (s, 1H), 7.10 (s, 1H), 6.88 (s, 2H), 4.35 (q, J = 7.1 Hz, 2H), 3.89 (s, 3H), 3.82 (s, 2H), 3.76 (s, 3H), 3.24 (s, 3H), 1.32 (t, J = 7.1 Hz, 3H); MS (ESI) m/e = 435 (M + H)$^+$. Anal. ($C_{19}H_{22}N_4O_6S$·0.25CH_3OH) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-(4-bromo-2,5-dimethoxyphenyl)acetamide (6t).** This was prepared according to the same procedure as **6f** from 100 mg (0.562 mmol) of **1** and 202 mg (0.734 mmol) of 4-bromo-2,5-dimethoxyphenylacetic acid.[45] Trituration with methanol gave 46 mg (19%) of a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.16 (s, 1H), 7.18 (s, 1H), 7.11 (s, 1H), 7.04 (s, 1H), 6.87 (s, 2H), 4.34 (q, J = 7.0 Hz, 2H), 3.77

(s, 3H), 3.71 (s, 3H), 3.69 (s, 2H), 1.31 (t, $J = 7.0$ Hz, 3H); MS (ESI) $m/e = 436$ $(M + H)^+$. Anal. ($C_{18}H_{19}BrN_4O_4 \cdot 0.12EtOAc$) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-(2,5-dimethoxy-4-nitrophenyl)acetamide (6u).** To 241 mg (1.00 mmol) of (2,5-dimethoxy-4-nitrophenyl)acetic acid[46] was added 2 mL of thionyl chloride. The mixture was stirred at reflux for 1 h, during which time the starting acid dissolved completely. Then the excess thionyl chloride was concentrated in vacuo to give a yellow solid. This was taken up in 3 mL of CH_2Cl_2 and concentrated in vacuo to give the acid chloride. To a solution of 356 mg (2.00 mmol) of **1** in 6 mL of pyridine and 6 mL of CH_2Cl_2 at −78 °C was added a solution of 1.0 g (3.9 mmol) of (2,5-dimethoxy-4-nitrophenyl)acetyl chloride in 6 mL of CH_2Cl_2. The mixture was stirred at −78 °C for 20 min, and then 5 mL of water was added. The mixture was warmed until the ice had melted, and then the solvents were removed in vacuo. The residue was taken up in 75 mL of ethyl acetate and extracted with 1 M HCl (3 × 10 mL). Next, 30 mL of hexanes was added, and the organic phase was extracted with saturated $NaHCO_{3(aq)}$ (3 × 10 mL) and then brine (1 × 10 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to a yellow solid. This was triturated with 10 mL of hot methanol to give 479 mg (60%) of the phenylacetamide as fine yellow crystals. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.30 (s, 1H), 7.49 (s, 1H), 7.32 (s, 1H), 7.10 (s, 1H), 6.89 (s, 2H), 4.35 (q, $J = 7.1$ Hz, 2H), 3.86 (s, 3H), 3.82 (s, 2H), 3.77 (s, 3H), 1.32 (t, $J = 7.1$ Hz, 3H); MS (ESI) $m/e = 401$ $(M + H)^+$. Anal. ($C_{18}H_{19}N_5O_5 \cdot 0.58H_2O$) C, H, N.

(4-Amino-5-cyano-6-ethoxypyridin-2-yl)carbamic Acid Methyl Ester (7). To a solution of 50 mg (0.28 mmol) of **1** in 1 mL of tetrahydrofuran was added 100 μL (0.57 mmol) of *N*,*N*-diisopropylethylamine and then three drops of methylchloroformate. The mixture was stirred at ambient temperature for 5 min and then diluted with 7 mL of water. The precipitate was collected, washed with water, and recrystallized from methanol to give 17 mg (24%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.87 (s, 1H), 6.89 (s, 1H), 6.86 (s, 2H), 4.30 (q, $J = 7.1$ Hz, 2H), 3.65 (s, 3H), 1.28 (t, $J = 7.1$ Hz, 3H); MS (ESI) $m/e = 237$ $(M + H)^+$. Anal. ($C_{10}H_{12}N_4O_3 \cdot 0.4CH_3OH$) C, H, N.

1-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-3-ethylurea (8). To a solution of 50 mg (0.28 mmol) of **1** in 1 mL of tetrahydrofuran was added 100 μL (0.57 mmol) of *N*,*N*-diisopropylethylamine and then 28 mg (0.094 mmol) of triphosgene. The white suspension was stirred at ambient temperature for 15 min, and then 0.5 mL (0.5 mmol) of 1.0 M ethylamine in THF was added. The suspension cleared, and the solution was stirred at ambient temperature for 5 min. The mixture was concentrated in vacuo, and the white solid residue was suspended in 5 mL of water. This was extracted with warm ethyl acetate (15 mL). Then the ethyl acetate layer was back-extracted with water (1 × 5 mL) and brine (1 × 5 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to a solid. This was recrystallized from ethyl acetate to give an impure solid containing some symmetrical urea. Pure product was obtained by reversed-phase HPLC to give 7 mg (10%) of the urea as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.88 (s, 1H), 7.32 (t, $J = 5.1$ Hz, 1H), 6.75 (s, 2H), 6.55 (s, 1H), 4.27 (q, $J = 7.1$ Hz, 2H), 3.02−3.22 (m, 2H), 1.30 (t, $J = 7.1$ Hz, 3H), 1.06 (t, $J = 7.1$ Hz, 3H); MS (ESI) $m/e = 250$ $(M + H)^+$. Anal. ($C_{11}H_{15}N_5O_2S \cdot 0.18H_2O$) C, H, N.

4-Amino-6-[2-(2,5-dimethoxyphenyl)ethylamino]-2-ethoxynicotinonitrile (10). To a solution of 1.1 g (6.1 mmol) of 4-amino-2-ethoxy-6-hydroxynicotinonitrile (**9**)[31] in 10 mL of CH_2Cl_2 was added 1.4 g (14 mmol) of Et_3N and then 2.8 g (7.0 mmol) of *N*-phenyl-bis(trifluoromethanesulfonimide). The mixture was stirred at ambient temperature for 4 h, and then it was extracted with water, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified via MPLC (0−60% ethyl acetate gradient in hexanes) to give 900 mg (60%) of trifluoromethanesulfonic acid 4-amino-5-cyano-6-ethoxypyridin-2-yl ester. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.66 (broad s, 2H), 6.30 (s, 1H), 4.31 (q, $J = 7.2$ Hz, 2H), 1.30 (t, $J = 7.2$ Hz, 3H).

To 31 mg (0.10 mmol) of trifluoromethanesulfonic acid 4-amino-5-cyano-6-ethoxypyridin-2-yl ester and 91 mg (0.50 mmol) of 2-(2,5-dimethoxyphenyl)ethylamine was added DMSO (250 μL). The mixture was heated in a sealed vessel in a microwave reactor at 110 °C for 30 min. The mixture was taken up in EtOAc (20 mL) and washed with H_2O (2 × 15 mL). The organic layer was concentrated in vacuo and purified by MPLC (20−60% ethyl acetate gradient in hexanes) to provide 4-amino-6-[2-(2,5-dimethoxyphenyl)ethylamino]-2-ethoxynicotinonitrile (27 mg, 79%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.84−6.90 (m, 2H), 6.75 (t, $J = 3.39$ Hz, 1H), 6.73 (s, 1H), 6.13 (s, 2H), 5.32 (s, 1H), 4.30 (q, $J = 6.89$ Hz, 2H), 3.73 (s, 3H), 3.67 (s, 3H), 3.28−3.30 (m, 2H), 2.75 (dd, $J = 8.48$, 6.10 Hz, 2H), 1.28 (t, $J = 7.12$ Hz, 3H); MS (ESI) $m/e = 365$ $(M + Na)^+$, 343 $(M + H)^+$. Anal. ($C_{18}H_{22}N_4O_3$) C, H, N.

(4-Methanesulfonyl-2,5-dimethoxyphenyl)acetyl Chloride (11). To 300 mg (1.09 mmol) of acid **15** was added 4 mL of thionyl chloride. The mixture was heated at reflux for 20 min and then concentrated in vacuo. The residue was taken up in 5 mL of CH_2Cl_2 and concentrated 2 times to give the acid chloride.

[2-(4-Bromo-2,5-dimethoxyphenyl)ethoxy]-*tert*-butyldimethylsilane (13). To a solution of 3.16 g (12.1 mmol) of 2-(4-bromo-2,5-dimethoxy)phenylethanol (**12**)[47] in 15 mL of *N*,*N*-dimethylformamide was added 1.65 g (24.2 mmol) of imidazole and then 2.19 g (14.5 mmol) of *tert*-butyldimethylsilyl chloride. The mixture was stirred at ambient temperature for 1.5 h, and then it was poured into 100 mL of water. The aqueous suspension was extracted with hexanes (3 × 20 mL). Then the combined hexanes layers were back-extracted with water (1 × 20 mL), 5% (w/w) NH_4OH (2 × 20 mL), 1 M $HCl_{(aq)}$ (2 × 20 mL), and brine (1 × 20 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to an oil. This was heated at 110 °C at ca. 1 mmHg for 1 h to remove any volatile impurities, giving 4.38 g (96%) of the silyl ether as a colorless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.14 (s, 1H), 6.96 (s, 1H), 3.76 (s, 3H), 3.74 (s, 3H), 3.72 (t, $J = 6.8$ Hz, 2H), 2.72 (t, $J = 6.8$ Hz, 2H), 0.82 (s, 9H), −0.04 (s, 6H).

2-(2,5-Dimethoxy-4-methylsulfanylphenyl)ethanol (14). To a solution of 4.37 g (11.6 mmol) of **13** in 25 mL of tetrahydrofuran at −78 °C and under N_2 was added 6 mL (15 mmol) of 2.5 M *n*-butyllithium in hexanes. The mixture was stirred at −78 °C for 10 min, and then 2.3 mL (25.5 mmol) of dimethyl disulfide was added. The mixture was warmed to ambient temperature over 30 min. Then 3 mL of water was added, and the solution was concentrated in vacuo. The residue was taken up in 50 mL of hexanes, extracted with water (3 × 20 mL) and then brine (1 × 20 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to a white solid. This was taken up in 30 mL of tetrahydrofuran, and to the solution was added 4.5 g (ca. 15.7 mmol) of tetrabutylammonium fluoride hydrate. After 2.5 h, the solution was concentrated in vacuo to a solid. This was washed with water (3 × 20 mL) and then hexanes (2 × 20 mL). The solid was dissolved in ethyl acetate, dried over $MgSO_4$, and filtered. The ethyl acetate was removed in vacuo, and the residue was purified via silica gel chromatography, eluting with a 20−40% ethyl acetate/hexanes gradient to give 1.70 g (64%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.79 (s, 1H), 6.72 (s, 1H), 4.58 (t, $J = 5.3$ Hz, 1H), 3.76 (s, 3H), 3.74 (s, 3H), 3.46−3.57 (m, 2H), 2.68 (t, $J = 7.3$ Hz, 2H), 2.39 (s, 3H); MS (ESI) $m/e = 211$ $(M - OH)^+$.

(4-Methanesulfonyl-2,5-dimethoxyphenyl)acetic Acid (15). To a solution of 1.70 g (7.44 mmol) of 2-(2,5-dimethoxy-4-methylsulfanylphenyl)ethanol in 12 mL of trifluoroacetic acid was added 1.82 mL (17.8 mmol) of 9.8 M $H_2O_{2(aq)}$. The mixture was stirred at ambient temperature for 4 h, and then $NaHSO_{3(aq)}$ was added to destroy any excess H_2O_2, until starch-KI paper showed no excess oxidant. The mixture was concentrated in vacuo, and the residue was taken up in 50 mL of methanol. To the solution was added 6 g (43.4 mmol) of K_2CO_3 and 10 mL of water. Then the mixture was stirred at ambient temperature for 10 min. The solvents were removed in vacuo, and the residue was taken up in 50 mL of ethyl acetate. This was filtered to remove the inorganic salts. Then the salts were washed with ethyl acetate (2 × 5 mL). The combined filtrate and washings were extracted with brine (2 × 20 mL), water

(2 × 20 mL), saturated $NaHCO_{3(aq)}$ (1 × 20 mL), and again with brine (1 × 20 mL), dried over $MgSO_4$, filtered, and concentrated to 1.33 g (69%) of a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.27 (s, 1H), 7.16 (s, 1H), 4.67 (t, J = 5.4 Hz, 1H), 3.89 (s, 3H), 3.79 (s, 3H), 3.51−3.66 (m, 2H), 3.21 (s, 3H), 2.79 (t, J = 7.0 Hz, 2H); MS (ESI) m/e = 261 $(M + H)^+$.

To a solution of 1.33 g (5.11 mmol) of 2-(4-methanesulfonyl-2,5-dimethoxyphenyl)ethanol in 25 mL of acetone was added 6.5 mL (13.0 mmol) of 2.0 M $H_2CrO_{4(aq)}$. After 1 h, an additional 2 mL (4.0 mmol) of 2.0 M $H_2CrO_{4(aq)}$ was added. Then the mixture was stirred for another 2 h. The excess chromic acid was quenched by addition of 10 mL of water, followed by $NaHSO_{3(s)}$ to discharge the yellow-orange color. The solvents were removed in vacuo, and the residue was taken up in 25 mL of water and then extracted with ethyl acetate (3 × 35 mL). The combined ethyl acetate layers were back-extracted with water (2 × 10 mL) and brine (1 × 10 mL), dried over $MgSO_4$, filtered, and concentrated to a solid. This was recrystallized from 30 mL of ethyl acetate to give 453 mg (32%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.36 (s, 1H), 7.30 (s, 1H), 7.26 (s, 1H), 3.89 (s, 3H), 3.78 (s, 3H), 3.62 (s, 2H), 3.24 (s, 3H); MS (ESI) m/e = 275 $(M + H)^+$.

***N*-(4-Amino-6-bromo-5-cyanopyridin-2-yl)acetamide (16).** Bromopyridine **1** (100 mg, 0.47 mmol) was dissolved in 1.0 mL of anhydrous pyridine. AcCl (225 μL, 2.35 mmol) was added dropwise slowly to control heat evolution. The reaction mixture was shaken overnight at ambient temperature. Water (5 mL) was added to the reaction mixture. The resulting brown solid was filtered, washed with cold water, and dried in a vacuum oven to give 100 mg (83%) of the acetamide. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.65 (s, 1H), 7.51 (s, 1H), 7.30 (s, 2H), 2.06 (s, 3H); MS (ESI) m/e = 255 $(M + H)^+$, 257 $(M + H)^+$.

***N*-(4-Amino-6-bromo-5-cyanopyridin-2-yl)-2-(2,5-dimethoxyphenyl)acetamide (17).** This was prepared according to the procedure described for **6f**, substituting 456 mg of (2,5-dimethoxyphenyl)acetic acid for (2-chlorophenyl)acetic acid and 212 mg (1.00 mmol) of bromopyridine **1** for ethoxypyridine **2**. Recrystallization from EtOAc gave 73 mg (19%) of a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.69 (s, 1H), 7.47 (s, 1H), 7.28 (s, 2H), 6.88 (m, 1H), 6.78 (m, 2H), 3.69 (s, 3H), 3.68 (s, 3H), 3.65 (s, 2H); MS (ESI) m/e = 391, 393 $(M + H)^+$. Anal. ($C_{16}H_{15}BrN_4O_3$·0.12EtOAc) C, H, N.

***N*-(4-Amino-5-cyano-6-isopropoxypyridin-2-yl)acetamide (18a). 18a** was prepared according to the procedure for **6a**, substituting isopropyl ether **3** for ethyl ether **2**. The product was recrystallized from methanol to give 12 mg (11%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.05 (s, 1H), 7.15 (s, 1H), 6.84 (s, 2H), 5.27 (m, 1H), 2.06 (s, 3H), 1.28 (d, 6H, J = 6.4 Hz); MS (ESI) m/e = 193 $(M - Pr + H)^+$, 235 $(M + H)^+$, 233 $(M - H)^-$. Anal. ($C_{11}H_{14}N_4O_2$) C, H, N.

***N*-(4-Amino-5-cyano-6-isopropoxypyridin-2-yl)-2-(4-methanesulfonyl-2,5-dimethoxyphenyl)acetamide (18b). 18b** was prepared according to the procedure for **6f** from 160 mg (0.547 mmol) of acid chloride **11** and 55 mg (0.29 mmol) of **3**. The acylation was performed at −78 °C. Recrystallization from methanol gave 49 mg (38%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.25 (s, 1H), 7.30 (s, 1H), 7.26 (s, 1H), 7.08 (s, 1H), 6.86 (s, 2H), 5.30 (m, 1H), 3.89 (s, 3H), 3.82 (s, 2H), 3.76 (s, 3H), 3.24 (s, 3H), 1.30 (d, J = 6.4 Hz, 6H); MS (ESI) m/e = 449 $(M + H)^+$. Anal. ($C_{20}H_{24}N_4O_6S$·0.33CH_3OH) C, H, N.

***N*-[4-Amino-5-cyano-6-(2-(dimethylamino)ethoxy)pyridin-2-yl]acetamide (19a).** To 2 mL of dimethylaminoethanol in a sealable tube suitable for microwave heating was added 40 mg (1.7 mmol) of sodium. The mixture was heated gently until all of the sodium had reacted. Then it was cooled to ambient temperature. Next, 212 mg (1.0 mmol) of **1** was added, and the mixture was heated at 150 °C for 15 min in a microwave reactor. The solvent was removed in vacuo, and the residue was taken up in 2 M NaOH and then extracted with ethyl acetate (3×). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated to an oily residue. This was purified via silica gel chromatography, eluting with CH_2Cl_2/MeOH/concentrated NH_4OH (10:1:0.1) to give 50 mg (23%) of a yellow foam. The foam was taken up in 0.6 mL of pyridine and 0.6 mL of CH_2Cl_2 and then cooled to −78 °C. Next, 16 mL (0.23 mmol) of acetyl chloride was added, and the mixture was stirred at −78 °C for 10 min. Water (1 mL) was added, and the mixture was allowed to warm until the ice had melted. Then the solvents were removed in vacuo. The residue was taken up in 5 mL of 2 M $NaOH_{(aq)}$ and then extracted with ethyl acetate (6 × 5 mL). The combined organic layers were back-extracted with brine (1 × 5 mL), dried over $MgSO_4$, filtered, and concentrated to a solid. Recrystallization from methanol gave 12 mg (20%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.12 (s, 1H), 7.23 (s, 1H), 6.94 (s, 2H), 4.40−4.43 (t, J = 5.83 Hz, 2H), 2.64−2.67 (t, J = 5.83 Hz, 2H), 2.27 (s, 6H), 2.13 (s, 3H); MS (ESI) m/e = 264 $(M + H)^+$, 262 $(M - H)^-$. Anal. ($C_{12}H_{17}N_5O_2$) C, H, N.

3-(6-Acetylamino-4-amino-3-cyanopyridin-2-yloxy)propionic Acid (19b). 4,6-Diamino-2-(3-hydroxypropoxy)nicotinonitrile was prepared according to the procedure described for **2**, substituting propylene glycol for ethanol. The yield was 617 mg (63%). From 200 mg (0.96 mmol) of this glycol ether and 400 μL (5.63 mmol) of acetyl chloride, both N-6 acylation and O acylation were accomplished according to the procedure described for **6a**. To the intermediate acetoxypropyl ester in 10 mL of warm 95:5 MeOH/H_2O was added 400 mg (2.89 mmol) of K_2CO_3. The hydrolysis was complete upon dissolution of the starting ester with gentle heating. The solvents were removed in vacuo, and then the residue was taken up in 30 mL of ethyl acetate and 5 mL of water. Gentle heating was necessary to dissolve the product. The layers were separated, and the organic layer was extracted with 1 M $HCl_{(aq)}$ (1 × 5 mL), saturated $NaHCO_{3(aq)}$ (2 × 5 mL), and brine (1 × 5 mL), dried, and concentrated to 135 mg (56%) of a white solid. To a suspension of 50 mg (0.20 mmol) of this alcohol in 5 mL of 2-butanone was added 1 mL (2.0 mmol) of Jones' reagent. The mixture was stirred at ambient temperature for 2.5 h, and then the solvent was removed in vacuo. The residue was suspended in 5 mL of water, and then $NaHSO_{3(s)}$ was added until the solution was blue. The precipitate was collected, washed with water, suspended in ethyl acetate, and concentrated in vacuo to 25 mg (47%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.34 (s, 1H), 10.12 (s, 1H), 7.19 (s, 1H), 6.90 (s, 2H), 4.45 (t, J = 6.4 Hz, 2H), 2.70 (t, J = 6.4 Hz, 2H), 2.07 (s, 3H); MS (ESI) m/e = 265 $(M + H)^+$, 263 $(M - H)^-$. Anal. ($C_{11}H_{12}N_4O_4$·0.08EtOAc) C, H, N.

***N*-[4-Amino-5-cyano-6-(tetrahydrofuran-3-yloxy)pyridin-2-yl]-acetamide (19c).** A sealable tube suitable for microwave heating was charged with 176 mg (2.00 mmol) of 3-hydroxytetrahydrofuran, 3 mL of dioxane, and 60 mg (1.5 mmol) of 60% NaH in mineral oil. The mixture was stirred for 1 h, and then 213 mg (1.00 mmol) of **1** was added. The mixture was heated in a microwave reactor at 190 °C for 10 min and then concentrated in vacuo. The residue was taken up in 10 mL of ethyl acetate and extracted with water (2 × 5 mL) and brine (1 × 5 mL), dried, and concentrated. The product was purified via MPLC, eluting with a 50−100% ethyl acetate in hexanes gradient to give 44 mg (20%) of the ether as a solid. From 44 mg (0.20 mmol) of this ether and 5 drops of acetyl chloride, the product was prepared according to the procedure for **6a**. Recrystallization from MeOH gave 10 mg (19%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.06 (s, 1H), 7.18 (s, 1H), 6.92 (s, 2H), 5.46 (m, 1H), 3.82 (m, 4H), 2.22 (m, 1H), 2.07 (s, 3H), 1.99 (m, 1H); MS (ESI) m/e = 263 $(M + H)^+$. Anal. ($C_{12}H_{14}N_4O_3$) C, H, N.

***N*-(4-Amino-6-butoxy-5-cyanopyridin-2-yl)acetamide (19d).** 4,6-Diamino-2-butoxynicotinonitrile was prepared according to the procedure for **3**, substituting *n*-butanol for 2-propanol. The butyl ether was purified via silica gel chromatography, eluting with 50% ethyl acetate/hexanes to give a white solid. This was acylated according to the procedure for **6a**. The product was recrystallized from 1 mL of methanol to give 16 mg (27%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.07 (s, 1H), 7.17 (s, 1H), 6.89 (s, 2H), 4.27 (t, 2H, J = 6.6 Hz), 2.06 (s, 3H), 1.67 (m, 2H), 1.40 (m, 2H), 0.93 (t, 3H, J = 7.5 Hz); MS (ESI) m/e = 193 $(M - Bu + H)^+$, 249 $(M + H)^+$, 247 $(M - H)^-$. Anal. ($C_{12}H_{16}N_4O_2$) C, H, N.

***N*-(4-Amino-5-cyano-6-methoxypyridin-2-yl)acetamide (19e).** 4,6-Diamino-2-methoxynicotinonitrile was prepared according to the procedure for **2**, substituting methanol for ethanol. The methyl ether was purified via silica gel chromatography, eluting with 60:40 hexanes/ethyl acetate to give 114 mg (70%) of a white solid. To a solution of 50 mg (0.30 mmol) of the methyl ether in 1 mL of pyridine was added 3 drops of acetyl chloride. The mixture was stirred for 2 h at ambient temperature, and then 10 mL of water was added. After the mixture was stirred for 10 min, the precipitate was collected and washed with water. Recrystallization from 1 mL of methanol gave 17 mg (27%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.13 (s, 1H), 7.19 (s, 1H), 6.92 (s, 2H), 3.85 (s, 3H), 2.07 (s, 3H); MS (ESI) m/e = 207 $(M + H)^+$, 205 $(M - H)^-$. Anal. ($C_9H_{10}N_4O_2 \cdot 0.13H_2O$) C, H, N.

***N*-[4-Amino-5-cyano-6-(2-hydroxyethoxy)pyridin-2-yl]-2-(2,5-dimethoxyphenyl)acetamide (20a).** 4,6-Diamino-2-(2-hydroxyethoxy)nicotinonitrile was prepared according to the procedure for **3**, substituting ethylene glycol for 2-propanol. Purification of the pyridyl ether was not necessary. Acylation of 100 mg (0.51 mmol) of the pyridyl ether was accomplished according to the same procedure as for **6f**, substituting (2,5-dimethoxyphenyl)acetic acid for (2-chlorophenyl)acetic acid and running the reaction at −78 °C. The product was purified by reversed-phase HPLC, eluting with a 5−100% CH_3CN gradient in 0.1% $TFA_{(aq)}$ to give 13 mg (7%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.10 (s, 1H), 7.13 (s, 1H), 6.90 (m, 3H), 6.79 (m, 2H), 4.81 (t, 1H, J = 5.4 Hz), 4.31 (m, 2H), 3.69 (m, 8H), 3.66 (s, 2H); MS (ESI) m/e = 329 $(M - EtOH + H)^+$, 373 $(M + H)^+$, 371 $(M - H)^-$. Anal. ($C_{18}H_{20}N_4O_5 \cdot 0.49$TFA) C, H, N.

***N*-[4-Amino-5-cyano-6-(2-methoxyethoxy)pyridin-2-yl]-2-(2,5-dimethoxyphenyl)acetamide (20b).** 4,6-Diamino-2-(2-methoxyethoxy)nicotinonitrile was prepared according to the procedure for **3**, substituting 2-methoxyethanol for 2-propanol. Purification of the pyridyl ether was not necessary. Acylation was accomplished according to the same procedure as for **6f**, substituting (2,5-dimethoxyphenyl)acetic acid for (2-chlorophenyl)acetic acid. The product was recrystallized from 3 mL of ethyl acetate to give 30 mg (38%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.11 (s, 1H), 7.14 (s, 1H), 6.90 (m, 3H), 6.79 (m, 2H), 4.42 (m, 2H), 3.70 (s, 3H), 3.69 (s, 3H), 3.64 (m, 4H), 3.31 (s, 3H); MS (ESI) m/e = 329 $(M - CH_2CH_2OCH_3 + H)^+$, 387 $(M + H)^+$, 385 $(M - H)^-$. Anal. ($C_{19}H_{22}N_4O_5$) C, H, N.

***N*-[4-Amino-5-cyano-6-(2-methanesulfonylethoxy)-2-pyridinyl]-2-(2,5-dimethoxyphenyl)acetamide (20c).** 4,6-Diamino-2-(2-methylsulfanylethoxy)nicotinonitrile was prepared according to the procedure for **3**, substituting 2-methylthioethanol for 2-propanol. Purification via silica gel chromatography (1:1 EtOAc/hexanes) gave 186 mg (83%). Acylation was accomplished according to the procedure for **6f**, substituting (2,5-dimethoxyphenyl)acetic acid for (2-chlorophenyl)acetic acid. The reaction was run at −78 °C. Purification via silica gel chromatography (40% EtOAc/hexanes) gave 67 mg (76%). To the amide (19 mg, 0.049 mmol) dissolved in 2 mL of glacial acetic acid was added 6 equiv of 30% hydrogen peroxide. After being heated at 50 °C for 2 h, the mixture was stirred at ambient temperature for 8 h. Water was added. Then the precipitate was collected, washed with additional water, and dried in vacuo to give 7.2 mg (34%) of a white powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.10 (s, 3H), 3.31 (s, 2H), 3.66 (s, 2H), 3.69 (d, J = 5.49 Hz, 6H), 4.63 (t, J = 5.80 Hz, 2H), 6.78−6.83 (m, 2H), 6.90 (d, J = 8.54 Hz, 1H), 7.01 (s, 2H), 7.18 (s, 1H), 10.21 (s, 1H). MS (ESI) m/e = 435 (M + H), 433 (M − H).

***N*-(4-Amino-6-butylsulfanyl-5-cyanopyridin-2-yl)acetamide (21).** This was prepared according to the procedure for **6a**, substituting **5** for **2** and using a volume of CH_2Cl_2 equal to the amount of pyridine. The reaction was run at −78 °C for 1 h. The product was isolated as a pale-yellow solid (yield 100%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.13 (s, 1H), 7.27 (s, 1H), 6.91 (s, 2H), 3.19 (t, J = 7.1 Hz, 2H), 2.10 (s, 3H), 1.64−1.52 (m, 2H), 1.45−1.35 (m, 2H), 0.89 (t, J = 7.1 Hz, 3H); MS (ESI) 265 $(M + H)^+$, 263 $(M - H)^-$. Anal. ($C_{12}H_{16}N_4OS$) C, H, N.

***N*-(4-Amino-5-cyano-6-(ethylamino)pyridin-2-yl)acetamide (22).** To a solution of 1 g (22 mmol) of ethylamine in 5 mL of *N*-methylpyrrolidinone in a sealable tube suitable for microwave heating was added 250 mg (0.98 mmol) of **16**. The mixture was heated in a microwave apparatus at 150 °C for 10 min and then poured into 50 mL of water. The suspension was extracted with ethyl acetate (3 × 10 mL). Then 25 mL of hexanes was added, and the organic layer was back-extracted with water (2 × 10 mL) and brine (1 × 10 mL), dried, and concentrated to a waxy solid. This was triturated with diethyl ether and then recrystallized from methanol to give 15 mg (7%) of white crystals. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.73 (s, 1H), 6.82 (s, 1H), 6.46 (s, 2H), 6.32 (t, J = 5.8 Hz, 1H), 3.34 (m, 2H), 2.04 (s, 3H), 1.09 (t, J = 7.0 Hz, 3H); MS (ESI) m/e = 220 $(M + H)^+$. Anal. ($C_{10}H_{13}N_5O \cdot 0.08CH_3OH$) C, H, N.

***N*-(4-Amino-5-cyano-6-isopropylaminopyridin-2-yl)acetamide (23).** This was prepared according to the procedure for **22** from 167 mg of 2-propylamine and 50 mg of **16**. The mixture was heated at 200 °C for 30 min. The product was purified via silica gel chromatography, eluting with 35% EtOAc/hexanes to give 15 mg (33%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.74 (s, 1H); 6.83 (s, 1H), 6.47 (s, 2H), 5.83 (d, J = 8.24 Hz, 1H), 4.25−4.30 (m, 1H), 2.04 (s, 3H), 1.14 (d, J = 6.40 Hz, 6H); MS (ESI) m/e = 234 $(M + H)^+$, 232 $(M - H)^-$. Anal. ($C_{11}H_{15}N_5O \cdot 0.1H_2O$) C, H, N.

***N*-(4-Amino-5-cyano-6-phenylpyridin-2-yl)acetamide (24).** A mixture of bromide **16** (45 mg, 0.18 mmol), Na_2CO_3 (38 mg, 0.35 mmol), and $Pd(PPh_3)_4$ (10 mg, 0.009 mmol) was stirred in DMF/THF/H_2O (1:1:0.5) under nitrogen in a microwave reactor vial. Phenylboric acid (26 mg, 0.21 mmol) was added. The resulting mixture was capped and heated at 130 °C for 20 min in a microwave reactor. The crude mixture was partitioned between EtOAc and water. The organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated in vacuo. The crude residue was purified by reversed-phase HPLC. The purified product was transferred to a vial in methanol and then concentrated to give 33 mg (72%) of the phenylpyridine. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.44 (s, 1H), 7.74−7.68 (m, 2H), 7.55 (s, 1H), 7.52−7.46 (m, 3H), 6.98 (s, 2H), 2.08 (s, 3H); MS (ESI) m/e 253 $(M + H)^+$, 251 $(M - H)^-$. Anal. ($C_{14}H_{12}N_4O \cdot 0.11CH_3OH$) C, H, N.

***N*-(4-Amino-5-cyano-6-propylpyridin-2-yl)acetamide (25).** To 2.1 g (32 mmol) of freshly activated zinc dust was added 5.4 mL of DMF and then 930 mg of *n*-propyl iodide. The mixture was stirred under a nitrogen atmosphere at 85 °C for 20 min and then cooled, and the excess zinc was allowed to settle.

To 128 mg of **16**, 6 mg (0.027 mmol) of palladium(II) acetate, and 30 mg (0.098 mmol) of tri(*o*-tolyl)phosphine was added 0.5 mL of DMF. The mixture was put under N_2 and stirred, and then 2.0 mL of the *n*-propylzinc iodide solution was added via syringe. The mixture was heated at 90 °C for 10 min and then poured into a stirred mixture of 10 mL of water and 10 mL of ethyl acetate. The metal salts were filtered away through diatomaceous earth, and then the layers were separated. The organic layer was extracted with water (2 × 10 mL) and brine (1 × 10 mL), dried over $MgSO_4$, filtered, and concentrated to a solid. This was purified via silica gel chromatography, eluting with 50:50 hexanes/ethyl acetate and loading as a solution in hot ethyl acetate to give 34 mg (31%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.29 (s, 1H), 7.39 (s, 1H), 6.82 (s, 2H), 2.63 (m, 2H), 2.07 (m, 3H), 1.67 (m, 2H), 0.92 (t, 3H, J = 7.5 Hz); MS (ESI) m/e 219 $(M + H)^+$, 217 $(M - H)^-$. Anal. ($C_{11}H_{14}N_4O$) C, H, N.

***N*-(4-Amino-5-cyanopyridin-2-yl)-2-(2,5-dimethoxyphenyl)acetamide (26).** To a solution of 38 mg (0.097 mmol) of **17** in 3 mL of DMF was added 1 mL of 1 M HCl. To this was added 50 mg (0.81 mmol) of Zn dust. The mixture was stirred for 30 min and then 1 mL of 1 M HCl was added. After an additional 30 min, the mixture was decanted from excess Zn and was concentrated in vacuo. The residue was taken up in 20 mL of warm ethyl acetate and extracted with 1 M HCl (3 × 5 mL) and then brine (1 × 5 mL), dried, and concentrated to 5 mg (17%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.33 (s, 1H), 8.21 (s, 1H), 7.46 (s,

1H), 6.95 (s, 2H), 6.89 (d, $J = 8.8$ Hz, 1H), 6.80 (m, 2H), 3.69 (m, 3H), 3.69 (s, 3H), 3.66 (s, 2H); MS (ESI) $m/e = 313$ $(M + H)^+$. Anal. ($C_{16}H_{16}N_4O_3 \cdot 0.35EtOAc \cdot 0.47H_2O$) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-bromoacetamide (27).** To a solution of 1.03 g (5.77 mmol) of **1** in 12 mL of pyridine and 12 mL of CH_2Cl_2 at −78 °C was added a solution of 1.41 g (6.99 mmol) of bromoacetyl bromide in 10 mL of CH_2Cl_2. The mixture was stirred at −78 °C for 10 min. Then 5 mL of water was added, and the mixture was allowed to warm until the ice had melted. The mixture was diluted with 200 mL of water and then extracted with ethyl acetate (3 × 30 mL). The combined ethyl acetate layers were back-extracted with 1 M $HCl_{(aq)}$ (2 × 30 mL), water (1 × 30 mL), and brine (1 × 30 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to 1.45 g (84%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.50 (s, 1H), 7.16 (s, 1H), 6.99 (s, 2H), 4.33 (q, $J = 7.1$ Hz, 2H), 4.11 (s, 2H), 1.30 (t, $J = 7.1$ Hz, 3H); MS (ESI) $m/e = 299$ $(M + H)^+$, 301 $(M + H)^+$.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-diethylaminoacetamide (28a).** To a solution of 30 mg (0.10 mmol) of **27** in 0.5 mL of DMF was added 16 μL (0.15 mmol) of *N*,*N*-diethylamine and then 52 μL (0.3 mmol) of *N*,*N*-diisopropylethylamine. The mixture was stirred for 1 h and then concentrated in vacuo. The residue was recrystallized from methanol to give 15 mg (51%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.50 (s, 1H), 7.18 (s, 1H), 6.99 (s, 2H), 4.25−4.32 (q, $J = 7.1$ Hz, 2H), 3.17 (s, 2H), 2.56−2.62 (q, $J = 7.1$ Hz, 4H), 1.27−1.31 (t, $J = 7.1$ Hz, 3H), 0.97−1.02 (t, $J = 7.1$ Hz, 6H); MS (ESI) $m/e = 292$ $(M + H)^+$, 290 $(M - H)^-$. Anal. ($C_{14}H_{21}N_5O_2$) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-dibutylaminoacetamide (28b).** To a solution of 30 mg (0.10 mmol) of *N*-(4-amino-5-cyano-6-ethoxypyridin-2-yl)-2-bromoacetamide in 0.5 mL of DMF was added 25 μL (0.15 mmol) of *N*,*N*-dibutylamine and then 52 μL (0.3 mmol) of *N*,*N*-diisopropylethylamine. The mixture was stirred for 1 h and then diluted with H_2O and filtered. The residue was recrystallized from methanol to give 16 mg (60%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.53 (s, 1H), 7.16 (s, 1H), 7.00 (s, 2H), 4.25−4.32 (q, $J = 6.8$ Hz, 2H), 3.16 (s, 2H), 2.48−2.52 (m, 4H), 1.32−1.41 (m, 8H), 1.27−1.31 (t, $J = 6.8$ Hz, 3H), 0.84−0.89 (t, $J = 7.1$ Hz, 6H); MS (ESI) $m/e = 348(M + H)^+$, 346 $(M - H)^-$. Anal. ($C_{18}H_{29}N_5O_2$) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-butylaminoacetamide (28c). 28c** was prepared according to the procecdure for **28a**, substituting *n*-butylamine for *N*,*N*-diethylamine. The crude product was recrystallized from methanol to give 15 mg (51%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.19 (s, 1H), 6.96 (s, 2H), 4.26−4.33 (q, $J = 6.78z$, $J = 7.12$ Hz, 2H), 3.32 (s, 2H), 3.25 (s, 2H), 1.35−1.41 (m, 4H), 1.27−1.31 (t, $J = 7.12$ Hz, 3H), 0.85−0.90 (t, $J = 7.12$ Hz, 3H); MS (ESI) $m/e = 292$ $(M + H)^+$, 290 $(M - H)^-$. Anal. ($C_{14}H_{21}N_5O_2 \cdot 0.22CH_3OH$) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-[(2-hydroxyethyl)propylamino]acetamide (28d). 28d** was prepared according to the procedure for **28b**, substituting *N*-(2-hydroxyethyl)-*N*-propylamine for *N*,*N*-diethylamine. The crude product was recrystallized from methanol to give 10 mg (38%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.76 (s, 1H), 7.19 (s, 1H), 6.96 (s, 1H), 4.62 (s, 1H), 4.26−4.30 (q, $J = 7.01$ Hz, 2H), 3.48 (s, 2H), 3.25 (s, 2H), 2.60−2.62 (t, $J = 5.80$ Hz, 2H), 1.40−1.44 (q, $J = 7.01$ Hz, 2H), 1.27−1.29 (t, $J = 7.02$ Hz, 3H), 0.87−0.9 (t, $J = 7.32$ Hz, 3H); MS (ESI) $m/e = 322$ $(M + H)^+$, 320 $(M - H)^-$. Anal. ($C_{15}H_{23}N_5O_3$) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-piperidin-1-ylacetamide (29). 29** was prepared according to the procedure for **28a**, substituting piperidine for *N*,*N*-diethylamine. Recrystallization from methanol gave 15 mg (49%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.51 (s, 1H), 7.18 (s, 1H), 6.98 (s, 2H), 4.30 (q, $J = 7.1$ Hz, 2H), 3.09 (s, 2H), 2.46 (m, 4H), 1.53 (m, 4H), 1.41 (m, 2H), 1.30 (t, $J = 7.0$ Hz, 3H); MS (ESI) $m/e = 304$ $(M + H)^+$. Anal. ($C_{15}H_{21}N_5O_2 \cdot 0.26CH_3OH$) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-morpholin-4-ylacetamide (30). 30** was prepared according to the procedure for **28a**, substituting morpholine for *N*,*N*-diethylamine. Recrystallization from methanol gave 13 mg (43%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.63 (s, 1H), 7.18 (s, 1H), 6.97 (s, 2H), 4.31 (q, $J = 7.1$ Hz, 2H), 3.60 (m, 4H), 3.17 (s, 2H), 2.51 (m, 4H), 1.30 (t, $J = 7.0$ Hz, 3H); MS (ESI) $m/e = 306$ $(M + H)^+$. Anal. ($C_{14}H_{19}N_5O_3 \cdot 0.17CH_3OH$) C, H, N.

***N*-(4-Amino-5-cyano-6-ethoxypyridin-2-yl)-2-(4-hydroxypiperidin-1-yl)acetamide (31). 31** was prepared according to the procedure for **28a**, substituting 4-hydroxypiperidine for *N*,*N*-diethylamine. Recrystallization from methanol gave 12 mg (38%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.52 (s, 1H), 7.18 (s, 1H), 6.98 (s, 2H), 4.60−4.61 (d, $J = 4.07$ Hz, 1H), 4.27−4.34 (q, $J = 7.12$ Hz, $J = 7.12$ Hz, 2H), 3.44−3.51 (m, 1H), 3.11 (s, 2H), 3.11 (s, 2H), 2.70−2.76 (m, 2H), 2.21−2.29 (m, 2H), 1.71−1.75 (m, 2H), 1.36−1.48 (m, 2H), 1.27−1.32 (t, $J = 7.12$ Hz, 3H); MS (ESI) $m/e = 320$ $(M + H)^+$, 318 $(M - H)^-$. Anal. ($C_{15}H_{21}N_5O_3$) C, H, N.

1-[(4-Amino-5-cyano-6-ethoxypyridi-2-ylcarbamoyl)methyl]-piperidine-4-caroxylic Acid (32). To a solution of 2.0 g (6.7 mmol) of **27** in 20 mL of DMF was added 1.8 mL (13.3 mmol) of methyl isonipecotate and 3.5 mL (20.0 mmol) of *N*,*N*-diisopropylethylamine. The mixture was stirred at ambient temperature for 2 h. It was concentrated under high vacuum with mild heating, and the resulting solid was recrystallized from MeOH to give 1.9 g (77%) of a pale-yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.53 (s, 1H), 7.18 (s, 1H), 6.97 (s, 2H), 4.27−4.34 (q, $J = 7.12$ Hz, 2H), 3.61 (s, 3H), 3.14 (s, 2H), 2.78−2.82 (m, 2H), 2.20−2.31 (m, 3H), 1.81−1.86 (m, 2H), 1.58−1.66 (m, 2H), 1.27−1.32 (t, $J = 7.12$ Hz, 3H); MS (ESI) $m/e = 362$ $(M + H)^+$, 360 $(M - H)^-$.

The solid was taken up in MeOH (40 mL). Then a solution of 1.4 g (10.5 mmol) of K_2CO_3 in 10 mL of H_2O was added. The hydrolysis mixture was heated at 50 °C for 2 h. The solvents were removed in vacuo. Then the residue was taken up in H_2O and acidified with 1 M HCl to pH 5. The precipitate was filtered, rinsed with H_2O, and dried on the filter to give 1.1 g (51%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.17 (s, 1H), 9.51 (s, 1H), 7.19 (s, 1H), 6.96 (s, 2H), 4.27−4.34 (q, $J = 7.12$ Hz, 2H), 3.13 (s, 2H), 2.78−2.82 (m, 2H), 2.19−2.26 (m, 3H), 1.79−1.84 (m, 2H), 1.51−1.64 (m, 2H), 1.27−1.32 (t, $J = 7.12$ Hz, 3H); MS (ESI) $m/e = 348$ $(M + H)^+$, 346 $(M - H)^-$.

1-[(4-Amino-5-cyano-6-ethoxypyridin-2-ylcarbamoyl)methyl]-piperidine-4-carboxylic Acid Butylamide (33). To a solution of **32** (30 mg, 0.086 mmol) in DMF (0.4 mL) was added butylamine (9 μL, 0.086 mmol), triethylamine (24 μL, 0.17 mmol), and *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) (41 mg, 0.13 mmol). After being stirred for 1 h at ambient temperature, the mixture was diluted with H_2O. Then the precipitate was filtered. The crude solid was recrystallized from MeOH to give 20 mg (57%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.47 (s, 1H), 7.68−7.72 (t, $J = 5.42$ Hz, 1H), 7.19 (s, 1H), 6.97 (s, 2H), 4.27−4.34 (q, $J = 7.12$ Hz, 2H), 3.12 (s, 2H), 2.99−3.05 (m, 2H), 2.83−2.89 (m, 2H), 2.05−2.19 (m, 3H), 1.60−1.64 (m, 4H), 1.22−1.39 (m, 7H), 0.83−0.88 (t, $J = 7.12$ Hz, 3H); MS (ESI) $m/e = 403$ $(M + H)^+$, 401 $(M - H)^-$. Anal. ($C_{20}H_{30}N_6O_3 \cdot 0.24CH_3OH$) C, H, N.

1-[(4-Amino-5-cyano-6-ethoxypyridin-2ylcarbamoyl)methyl]-piperidine-4-carboxylic Acid Isopropyl Amide (34). 34 was prepared according to the procedure for **33**, substituting isopropylamine for *n*-butylamine. The crude solid was recrystallized from MeOH to give 17 mg (51%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.47 (s, 1H), 7.58 (d, $J = 7.80$ Hz, 1H), 7.19 (s, 1H), 6.97 (s, 2H), 4.27−4.34 (q, $J = 7.12$ Hz, 2H), 3.77−3.84 (m, 1H), 3.12 (s, 2H), 2.84−2.89 (m, 2H), 2.11−2.18 (m, 2H), 2.02−2.08 (m, 1H), 1.56−1.64 (m, 4H), 1.28−1.32 (t, $J = 7.12$ Hz, 3H), 1.02 (d, $J = 6.78$ Hz, 6H); MS (ESI) $m/e = 389$ $(M + H)^+$, 387 $(M - H)^-$. Anal. ($C_{19}H_{28}N_6O_3$) C, H, N.

***N*-(4-Amino-5-chloro-6-ethoxypyridin-2-yl)-2-(4-methanesulfonyl-2,5-dimethoxyphenyl)acetamide (35).** To a solution of 50 mg of crude **38** in 1 mL of CH_2Cl_2 and 0.3 mL of pyridine at −78 °C was added a solution of 53 mg (0.18 mmol) of acid chloride **11** in 1 mL of CH_2Cl_2. The mixture was stirred at −78 °C for 20 min. Then 2 mL of water was added, and the mixture was allowed

to warm until the ice melted. The solvents were removed in vacuo, and then the residue was taken up in 20 mL of diethyl ether. The solution was extracted with water (1 × 5 mL), 1 M $HCl_{(aq)}$ (2 × 5 mL), saturated $NaHCO_{3(aq)}$ (2 × 5 mL), and brine (1 × 5 mL), dried over $MgSO_4$, filtered, and concentrated to a solid. This was recrystallized from methanol to give 30 mg (38%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.98 (s, 1H), 7.30 (s, 1H), 7.26 (s, 1H), 7.14 (s, 1H), 6.20 (s, 2H), 4.31 (q, 2H, J = 7.1 Hz), 3.89 (s, 3H), 3.79 (s, 2H), 3.77 (s, 3H), 3.24 (s, 3H), 2.50 (t, 3H, J = 7.1 Hz); MS (ESI) m/e = 444 $(M + H)^+$, 442 $(M - H)^-$. Anal. ($C_{18}H_{22}ClN_3O_6S$) C, H, N.

4-Amino-6-ethoxypyridine-2-carboxylic Acid Methyl Ester (37). To 1.08 g (6.6 mmol) of 4-amino-2,6-dichloropyridine (**36**) in 1.5 mL of EtOH was added 2.7 mL (6.6 mmol) of 2.5 M EtONa in EtOH. The mixture was heated in a sealed tube at 150 °C with an oil bath for 6 h. Water was added, and the mixture was extracted with EtOAc. The extracts were concentrated and purified via MPLC, eluting with a gradient of 10−30% ethyl acetate in hexanes to give 1.0 g (88%) of the ethyl ether. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.24 (broad s, 2H), 6.19 (s, 1H), 5.76 (s, 1H), 4.13 (q, J = 7.2 Hz, 2H), 1.23 (t, J = 7.2 Hz, 3H). To a solution of 3.0 g (17 mmol) of the ethyl ether in 20 mL of MeOH was added 360 mg (0.44 mmol) of $PdCl_2(CH_2Cl_2)$dppf and then 4.9 mL (35 mmol) of Et_3N. The mixture was heated at 100 °C under 100 psi of CO for 6 h. The solvent was removed in vacuo. Then the crude product was purified via MPLC, eluting with a gradient of 10−40% ethyl acetate in hexanes to give 2.4 g (72%) of methyl ester **37**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.96 (s, 1H), 6.22 (broad s, 2H), 5.95 (s, 1H), 4.21 (q, J = 7.2 Hz, 2H), 3.78 (s, 3H), 1.26 (t, J = 7.2 Hz, 3H).

To a solution of 400 mg (2.0 mmol) of **37** in 4 mL of DMF was added *N*-chlorosuccinimide (267 mg, 2.0 mmol). The mixture was stirred at ambient temperature for 3 days and then diluted with water. The precipitate was collected and purified via MPLC, eluting with a gradient of 10−30% ethyl acetate in hexanes to give 220 mg (48%) of 4-amino-5-chloro-6-ethoxypyridine-2-carboxylic acid methyl ester. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.17 (s, 1H), 6.55 (broad s, 2H), 4.33 (q, J = 7.2 Hz, 2H), 3.80 (s, 3H), 1.31 (t, J = 7.2 Hz, 3H).

5-Chloro-6-ethoxypyridine-2,4-diamine (38). To 231 mg (1.00 mmol) of the methyl ester was added 5 mL of 7 M NH_3 in CH_3OH. The solution was stirred at ambient temperature for 5 h and then concentrated in vacuo to give the amide as a solid (215 mg, 100%). To a suspension of 186 mg (0.866 mmol) of the amide in 3 mL of 2-propanol was added 2.6 mL (5.2 mmol) of 2 M $NaOH_{(aq)}$ and then 2 mL (1.64 mmol) of 0.82 M $NaOCl_{(aq)}$. The suspension cleared upon addition of NaOCl, and stirring was continued for 2 h at ambient temperature. The solvents were removed in vacuo. Then the residue was taken up in 5 mL of water and filtered through a short plug of diatomaceous earth. The filter pad was washed with water (3 × 1 mL). Then 1 M HCl was added to the combined filtrate and washings to adjust to pH 1. The pH was brought to >13 with 2 M $NaOH_{(aq)}$, and then the aqueous suspension was extracted with ethyl acetate (3 × 5 mL). The combined organic layers were back-extracted with brine (1 × 5 mL), dried over $MgSO_4$, filtered, and concentrated to 93 mg of an impure solid. This was used without further purification for subsequent acylations. ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.38 (s, 2H), 5.23 (d, 1H, J = 1.7 Hz), 5.18 (s, 2H), 5.13 (d, 1H, J = 1.7 Hz), 4.05 (q, 2H, J = 7.1 Hz), 1.19 (t, 3H, J = 7.1 Hz); MS (ESI) m/e = 154 $(M + H)^+$.

***N*-(5-Cyano-6-ethoxy-4-methylpyridin-2-yl)acetamide (39).** 6-Amino-2-chloro-4-methylnicotinonitrile (**40**)[33] (84 mg, 0.50 mmol) was subjected to the procedure described for the preparation of **2** to provide 58 mg (65%) of 6-amino-2-ethoxy-4-methylnicotinonitrile. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.88 (s, 2H), 5.95 (s, 1H), 4.30 (q, J = 7.1 Hz, 2H), 2.20 (s, 3H), 1.28 (t, J = 7.1 Hz, 3H); MS (ESI) m/e = 178 $(M + H)^+$. To a solution of 18 mg (0.10 mmol) of 6-amino-2-ethoxy-4-methylnicotinonitrile in 0.5 mL of CH_2Cl_2 was added 0.2 mL of pyridine and then 21 μL (0.3 mmol) of acetyl chloride. The mixture was stirred at ambient temperature for 15 min. Then brine (20 mL) was added, and the mixture was extracted with ethyl acetate (1 × 50 mL). The ethyl acetate layer was washed with 1 M $HCl_{(aq)}$ (1 × 30 mL), saturated $NaHCO_{3(aq)}$ (1 × 30 mL), and brine (2 × 30 mL), dried over $MgSO_4$, filtered, and concentrated to give 22 mg (100%) of the amide as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.62 (s, 1H), 7.69 (s, 1H), 4.42 (q, J = 7.1 Hz, 2H), 2.41 (s, 3H), 2.12 (s, 3H), 1.34 (t, J = 7.1 Hz, 3H); MS (ESI) m/e = 220 $(M + H)^+$. Anal. ($C_{11}H_{13}N_3O_2$·0.15EtOAc) C, H, N.

Pentanoic Acid (4-Amino-3-chloro-5-cyano-6-ethoxypyridin-2-yl)amide (41). To a solution of 20 mg (0.076 mmol) of **6b** in 1 mL of CH_2Cl_2 was added 100 μL (0.10 mmol) of SO_2Cl_2 in CH_2Cl_2. The mixture was stirred at ambient temperature for 10 min. Then 1 mL of 5% $NaHSO_{3(aq)}$ was added, and the CH_2Cl_2 was removed in vacuo. The residue was taken up in ethyl acetate (10 mL), extracted with water (1 × 5 mL) and then brine (1 × 5 mL), dried over $MgSO_4$, filtered, and concentrated. The product was purified via reversed-phase HPLC (5−95% CH_3CN in NH_4OAc gradient) to give 12 mg (53%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.85 (s, 1H), 7.17 (s, 2H), 4.30 (q, J = 7.0 Hz, 2H), 2.37 (t, J = 7.5 Hz, 2H), 1.45−1.68 (m, 2H), 1.21−1.44 (m, 5H), 0.88 (t, J = 7.3 Hz, 3H); MS (ESI) m/e = 297 $(M + H)^+$.Anal. ($C_{13}H_{17}ClN_4O_2$·0.2H_2O) C, H, N.

Pentanoic Acid (4-Amino-5-cyano-6-ethoxypyridin-2-yl)methylamide (42). To a solution of 25 mg (0.095 mmol) of **6b** in 1 mL of THF was added 4 mg (0.10 mmol) of 60% NaH in mineral oil. The mixture was stirred at ambient temperature for 5 min, and then 12 μL (0.19 mmol) of methyl iodide was added. The mixture was stirred at ambient temperature for 18 h and then concentrated in vacuo. The residue was taken up in 10 mL of diethyl ether and then extracted with water (2 × 3 mL) and brine (1 × 3 mL), dried over $MgSO_4$, filtered, and concentrated to a solid. The product was purified via silica gel chromatography, eluting with 40% ethyl acetate in hexanes to give 6.7 mg (26%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.06 (s, 2H), 6.39 (s, 1H), 4.30 (q, J = 7.1 Hz, 2H), 3.17 (s, 3H), 2.39 (t, J = 7.6 Hz, 2H), 1.42−1.56 (m, 2H), 1.16−1.36 (m, 5H), 0.83 (t, J = 7.3 Hz, 3H); MS (ESI) m/e = 277 $(M + H)^+$. Anal. ($C_{14}H_{20}N_4O_2$) C, H, N.

***N*-(6-Amino-5-cyano-4-ethoxypyridin-2-yl)acetamide (43).** To a solution of 210 mg (0.893 mmol) of nicotinamide **45** in 2.5 mL of pyridine was added 61 mg (0.89 mmol) of imidazole. The mixture was cooled with an ice bath. Then 170 μL (1.8 mmol) of $POCl_3$ was added, and stirring was continued for 1 h. The solvent was removed in vacuo, and then the residue was taken up in 10 mL of 1 M $HCl_{(aq)}$ and extracted with diethyl ether (3 × 5 mL). The combined ether layers were back-extracted with H_2O (1 × 5 mL) and then brine (1 × 5 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to a solid. This was purified via MPLC, eluting with 30% ethyl acetate in hexanes to give 99 mg (51%) of 2,6-dichloro-4-ethoxynicotinonitrile as a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.61 (s, 1H), 4.39 (q, J = 7.1 Hz, 2H), 1.38 (t, J = 7.1 Hz, 3H). To 150 mg (0.69 mmol) of the nitrile in 1 mL of dioxane was added 15 mL of 28% NH_4OH. The mixture was heated in an autoclave at 130 °C for 24 h. The solvents were removed in vacuo. Then the residue was taken up in 10 mL of ethyl acetate and extracted with H_2O (1 × 3 mL) and then brine (1 × 3 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The product was purified via MPLC, eluting with a 60−100% ethyl acetate/hexanes gradient to give 37 mg (29%) of a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.35 (s, 2H), 6.07 (s, 2H), 5.39 (s, 1H), 4.01 (q, J = 7.1 Hz, 2H), 1.31 (t, J = 7.1 Hz, 3H); MS (ESI) m/e = 235 $(M + H)^+$. To 10 mg (0.056 mmol) of 2,6-diamino-4-ethoxynicotinonitrile in 0.2 mL of pyridine was added 3 drops of acetyl chloride via pipet, while cooling with an ice bath. The mixture was stirred for 10 min and then diluted with 1 mL of H_2O to give a white precipitate. The solvents were decanted, and the solid was washed with 2 × 1 mL of H_2O, dried, and then recrystallized from methanol to give 3 mg (24%) of a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.25 (s, 1H), 7.20 (s, 1H), 6.47 (s, 2H), 4.11 (q, 2 H, J = 7.1 Hz), 2.07 (s, 3H), 1.33 (t, 3H, J = 7.1 Hz); MS (ESI) m/e = 221 $(M + H)^+$. Anal. ($C_{10}H_{12}N_4O_2$·0.15CH_3OH) C,H,N.

2,6-Dichloro-4-ethoxynicotinamide (45). To 1.82 g (10 0.0 mmol) of 2,4,6-trichloropyridine (**44**)[34] was added 6 mL of ethanol

and then 4 mL (10.2 mmol) of 2.54 M KOH in ethanol. The mixture was stirred at reflux for 30 min and then concentrated in vacuo. The residue was taken up in 10 mL of water and extracted with diethyl ether (2 × 10 mL). The combined ether layers were back-extracted with brine (1 × 10 mL), dried over $MgSO_4$, filtered, and concentrated to an oil. This was purified via silica gel chromatography, eluting with 2.5% ethyl acetate/hexanes to recover the less polar 2-ethoxy isomer and then with 5% ethyl acetate/hexanes to give 750 mg (39%) of 2,6-dichloro-4-ethoxypyridine. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.17 (s, 2H), 4.20 (q, J = 7.0 Hz, 2H), 1.32 (t, J = 7.0 Hz, 3H); MS (ESI) m/e = 191 $(M + H)^+$. To a solution of 450 mg (2.34 mmol) of 2,6-dichloro-4-ethoxypyridine in 8 mL of THF at −78 °C under N_2 was added 2 mL of 1.6 M *n*-butyllithium in hexanes. The mixture was stirred at −78 °C for 10 min, and then CO_2 gas was introduced into the mixture while warming to ambient temperature. The solvent was removed in vacuo, and then the residue was taken up in 10 mL of water and extracted with diethyl ether (2 × 10 mL). The aqueous layer was made acidic by addition of 5 mL of 1 M $HCl_{(aq)}$. Then it was extracted with ethyl acetate (3 × 10 mL). The combined ethyl acetate layers were back-extracted with brine (1 × 10 mL), dried over $MgSO_4$, filtered, and concentrated to an oil. (The oil contained a mixture of 2,6-dichloro-4-ethoxynicotinic acid and 2,6-dichloro-4-ethoxypyridine-3,5-dicarboxylic acid, which was used without purification.) The oil was taken up in 2 mL of thionyl chloride, and the mixture was heated at reflux for 30 min. Then the excess thionyl chloride was removed in vacuo. The residue was taken up in 2 mL of THF, and then 5 mL of 28% $NH_4OH_{(aq)}$ was added. The mixture was stirred at ambient temperature for 30 min, and then 10 mL of water was added. The suspension was extracted with ethyl acetate (3 × 10 mL). Then the combined ethyl acetate layers were back-extracted with brine (1 × 10 mL), dried over $MgSO_4$, filtered, and concentrated to an oily solid. This was suspended in ethyl acetate and filtered to remove the insoluble diamide. Concentration of the filtrate gave 210 mg (38%) of nicotinamide **45** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.91 (s, 1H), 7.69 (s, 1H), 7.34 (s, 1H), 4.22 (q, J = 7.1 Hz, 2H), 1.30 (t, J = 7.1 Hz, 3H); MS (ESI) m/e = 235 $(M + H)^+$.

ATF-2 Phosphorylation Assay. JNK-1, JNK-2, and JNK-3 were purchased from Upstate Cell Signaling Solutions (formerly Upstate Biotechnology).[47] Kinase reactions were performed in a 50 μL volume containing 10 ng/well JNK-1 (4.4 nM), 10 ng/well JNK-2 (4.1 nM), or 10 ng/well (3.8 nM) JNK-3, 1 μM BT-GST-ATF-2 substrate, and γ-[^{33}P]-ATP (5 μM, 400 μCi/μmol) in a buffer containing 20 mM MOPS, pH 7.2, 2 mM EGTA, 10 mM $MgCl_2$, 0.1% Triton X-100, and 1 mM dTT in a 96-well polypropylene plate. Reactions were carried out at 15−20 °C and stopped after 60 min with the addition of EDTA, giving a final concentration of 50 mM. The 30 μL aliquots of the quenched reactions were transferred to a Streptavidin FlashPlate containing 170 μL of PBS. The plate was incubated at ambient temperature for 30 min. The plate was then washed 4 times with PBS. The plate was then counted in a Perkin-Elmer Micro-Beta plate counter, 1 min per well.

Kinase Selectivity Assays. Recombinant protein kinases were either commercially obtained or expressed using the FastBac bacculovirus expression system (GIBCO BRL, Gaithersburg, MD) and purified using either nickel (his-tag) or glutathione (GST) affinity chromatography. Kinase assays were conducted in 24 μL volumes on 384-well microplates using TECAN liquid-handling automation. Ser/Thr-kinase assays were performed using a radioactive FlashPlate-based assay platform.[48] In this format, biotinylated substrate peptide (2 μM), γ-[^{33}P]-ATP (5 μM, 2 mCi/μmol), inhibitors (3−10000 nM in 2% DMSO), and enzyme were incubated for 1 h in buffer containing 25 mM Hepes, pH 7.5, 1 mM DTT, 10 mM $MgCl_2$, 100 μM Na_3VO_4, and 0.075 mg/mL Triton X-100, stopped with 80 μL of stop buffer containing 100 mM EDTA and 4 M NaCl, transferred to streptavidin-coated 384-well FlashPlates (Perkin-Elmer, Boston, MA), which were then washed 3 times and read using a TopCount microplate reader (Perkin-Elmer). Tyr kinase reactions were performed using a time-resolved fluorescence (HTRF) platform. In this format, biotinylated substrate peptide (0.5 μM), ATP (10 μM to 1 mM), inhibitors (3−10000 nM in 2% DMSO), and enzyme were incubated for 1 h in buffer containing 50 mM Hepes, pH 7.4, 1 mM DTT, 10 mM $MgCl_2$, 2 mM $MnCl_2$, 100 μM Na_3VO_4, and 0.01% BSA. Reactions were stopped with 50 μL of revelation buffer containing (final concentrations of) Eu-conjugated anti-pY-PT66 antibody (0.05 μg/mL) (CisBio), PycolLink Streptaviding-APC (0.001 μg/mL) (Prozyme), and 60 mM EDTA in a buffer containing 25 mM Hepes, pH 7.4, 250 mM KF, 0.005% Tween-20, and 0.05% BSA. Following 60 min of incubation with revelation buffer, the reactions were read using an Envision fluorescence microplate reader (Perkin-Elmer) with 615 nm excitation and 665 nm emission. IC_{50} values were determined from six compound titration curves, and corresponding K_i values were calculated using the Cheng−Prusoff equation $K_i = IC_{50}/(1 + ([ATP]/K_m))$.[49]

Cellular Assay (c-Jun Phosphorylation). HepG2 human hepatoma cells (ATCC) were cultured in low glucose MEM supplemented with 1× NEAA, 1× sodium pyruvate, and 10% FBS (all from Invitrogen, "complete media"). For P-c-Jun assays, cells were plated at 5 × 10^4 cells/well in 500 μL of complete media on 24-well collagen-coated plates and incubated overnight. Serial compound dilutions were made in DMSO at 100×, and then 5 μL was added directly to the media on the cells to provide the final inhibitor concentrations (final DMSO concentration in media was 1%). After 1 h, cells were stimulated with vehicle control or TNFα for 30 min and harvested in 70 μL of lysis buffer (TBS (54 mM Tris-HCl, pH 7.6, 150 mM NaCl, Sigma), 1% TritonX-100 (Sigma), 0.5% Nonidet P-40 (Sigma), 0.25% sodium deoxycholate (Sigma), 1 mM EDTA, 1 mM EGTA (Sigma), 0.5 mM sodium fluoride (Sigma), 1 mM pervanadate (Sigma), 1 μM microcystin (Calbiochem), 1 mM AEBSF (Roche), 1 tablet of complete EDTA Free-Mini inhibitor cocktail (Roche)) and frozen at −80 °C prior to use in the P-c-Jun assay. P-c-Jun ELISAs were performed as described by the manufacturer (Cell Signaling Technology) using 50 μL of cell extract.

X-ray Crystallography. Purified JNK-1-α1 isoform[50] with a C-His-tag extension was incubated with a 5× molar excess of the JIP-1 peptide.[36] The crystallization experiments were in the hanging drop vapor diffusion format at 4 °C. Hanging drops consisted of 2 μL of the protein/peptide solution plus 2 μL of well solution over 1 mL of reservoir. The reservoir contained 3 M $(NH_4)SO_4$ (2.8−3.1 M range) and 10−14% glycerol at a pH between 5.8 and 7.0. Cocrystallization with compound **6t** was achieved by dissolving DMSO stock solutions of **6t** (100 or 50 mM) into the protein/peptide solution in a 3-fold molar excess. The protein/JIP-1/compound **6t** complex was allowed to incubate for at least an hour on ice and was later set up to crystallize. Crystals grew in 4−7 days. Large crystals of the complex had the morphology of hexagonal bipyramids.

The structure was solved by molecular replacement using a model of residues 63−373 of the PDB entry 1jnk, corresponding to the structure of JNK-3. Data were collected at the IMCA-CAT facility using a Quantum210 detector and consisted of 100 1° frames with an exposure per frame of 5 s. Data were collected with the standard station software, processed with HKL2000, and the structure solution and refinement were done using CNX and QUANTA. The coordinates for the structure of the refined JNK-1/JIP-1/compound **6t** complex have been deposited with the RCSB collaborative (PDB) with entry code 2GMX.

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Supporting Information Available: Substrates, assay formats, and constructs for kinases in Table 5; elemental analysis data for compounds **6a**−**u**, **7**, **8**, **10**, **17**, **18a**,**b**, **19a**−**e**, **20a**,**b**, **21**−**26**, **28a**−**d**, **29**−**31**, **33**−**35**, **39**, and **41**−**43**. This material is available free of charge via the Internet at http://pubs.acs.org.

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