Design and Synthesis of 4-Azaindoles as Inhibitors of p38 MAP Kinase

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Inhibition of the biosynthesis of proinflammatory cytokines such as tumor necrosis factor and interleukin-1 via p38 has been an approach toward the development of a disease modifying agent for the treatment of chronic inflammation and autoimmune diseases. The development of a new core structure of p38 inhibitors, 3-(4-fluorophenyl)-2-(pyridin-4-yl)-1*H*-pyrrolo[3,2-b] pyridine, is described. X-ray crystallographic data of the lead bound to the active site of p38 was used to guide the optimization of the series. Specific focus was placed on modulating the physical properties of the core while maintaining potent inhibition of p38. These efforts identified **42c** as a potent inhibitor of p38, which also possessed the required physical properties worthy of advanced studies.

Introduction

It is now known that the regulation of cytokine biosynthesis, including the proinflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα),[1] in various cell types is regulated through activation of p38 MAP kinase. Both TNFα and IL-1β are implicated in the onset of rheumatoid arthritis (RA) and in the progression of bone and joint destruction associated with RA.[2] Recent human clinical trials using IL-1ra (kineret), the natural receptor antagonist of IL-1β, and the biologic TNFα inhibitors (infliximab and etanercept) have clinically validated that modulation of these cytokines leads to dramatic improvement in inflammatory diseases such as RA and Crohn's disease.[3] Development of an orally active small molecule inhibitor of p38 is of extreme interest and is the focus of many pharmaceutical research programs.[4]

SmithKline Beecham's pioneering series of p38 inhibitors, pyridinylimidazoles, has been well documented in the literature and shown to be effective in inhibiting bone resorption, inflammation, delayed type hypersensitivity, as well as other immune and inflammatory based pathologies.[6,7] In this report, we describe one of the initial approaches toward developing novel proprietary leads in our p38 program based on structure guided design. An X-ray crystal structure of native unphosphorylated p38 MAP kinase was solved in-house as well as complexes with early diarylimidazole p38 inhibitors from SKB (SK&F86002) (Figure 1).

SK&F86002

Figure 1.

The mode of binding of this class has now been well documented in the literature.[7] These inhibitors make optimal interactions through their 4-pyridyl moiety (hydrogen bond with the main chain NH of Met 109), the 4-fluorophenyl group that optimally fills a hydrophobic pocket (partly created by specificity residue Thr 106 in p38) and the nitrogen of the central imidazole ring with the terminal nitrogen of Lys 53.[8,9] Published structures within the diaryl imidazole class of inhibitors in which the hydrogen bond to Lys 53 has been observed[10] suggest this interaction is required for the regioselective positioning of the pyridyl and the 4-fluorophenyl groups. As this Lys is a highly conserved ATP-binding residue,[11] targeting this interaction is unlikely to contribute to inhibitor selectivity; however, interactions with this Lys offer an opportunity to enhance the intrinsic potency of kinase inhibitors. We reasoned that an azaindole ring could incorporate a basic nitrogen within direct hydrogen binding distance to Lys 53 and serve as a suitable scaffold. 4-Azaindole **9** was synthesized, and the X-ray crystal structure of this compound bound to unphosphorylated p38 confirmed the direct hydrogen bonding distance of 2.8 Å (heteroatom to heteroatom) between the 4-indole nitrogen and the Lys 53 (Figure 3A). In this structure, Lys 53 maintains its hydrogen bond interaction with Glu 71. Compounds in the 4-azaindole class are extremely potent with IC_{50}'s in the low nanomolar range. Our lead compound **9** has an IC_{50} = 6 nM (compared with IC_{50} = 400 nM of SK&F86002). However, compound **9** was highly crystalline and poorly soluble.

To optimize the physical properties of the series, as well as the bioavailability of our lead, a number of

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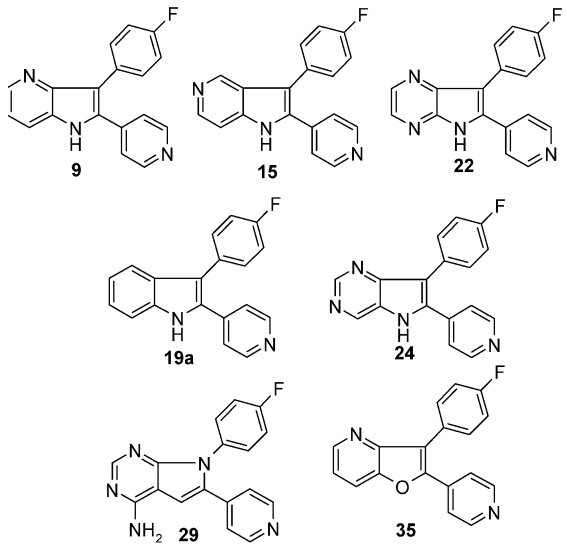


Figure 2. Core Indole Template Modifications.

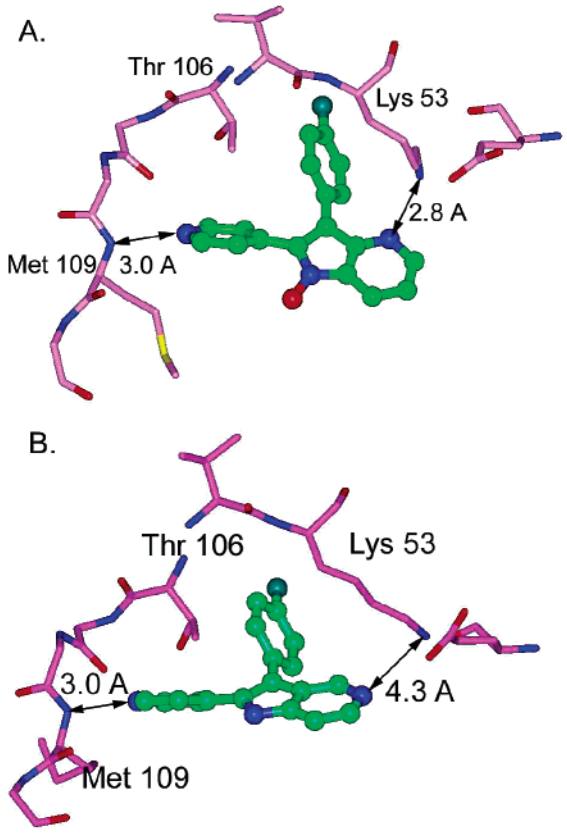


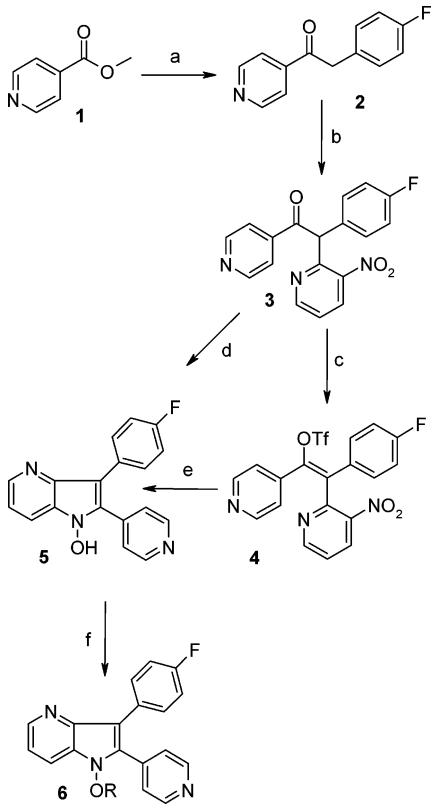
Figure 3. Crystal structure of (A) 4-azaindole **9** and (B) 5-azaindole **15** bound inside the ATP binding pocket of p38 MAP Kinase. Only the 4-azaindole is capable of direct hydrogen bonding with the terminal nitrogen of Lys 53.

azaindole template derivatives were synthesized for evaluation as listed in Figure 2.

Chemistry. Two synthetic routes to the 4-azaindole scaffold were developed. In the first (Scheme 1), the anion of ketone **2** was treated with 2-chloro-3-nitropyridine to afford the desired adduct **3** in reasonable yield. Reduction of the nitro group under various conditions including hydrogenation, tin chloride, titanium trichloride, and sodium dithionite resulted in cyclization of the intermediate *N*-hydroxylamine to the *N*-hydroxy 4-azaindole **5**. Attempts to directly convert **5** to **9** via reduction of the nitrogen−oxygen bond were unsuccessful. Conversion of the ketone **3** to the enol triflate **4** prior to nitro reduction still resulted in *N*-hydroxyazaindole **5** upon reduction of the nitro group.

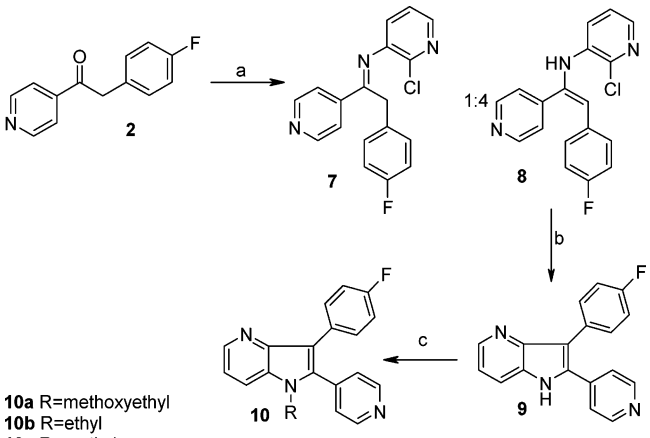
Compound **9** was readily obtained by the second route shown in Scheme 2. Ketone **2** was converted to a mixture of imine-enamine **7**−**8** via condensation with

Scheme 1. Synthesis of *N*-Hydroxy 4-Azaindoles[a]



[a] Reagents: (a) 4-Fluorophenylacetate, NaOEt, EtOH, reflux; (b) 2-Chloro-3-nitropyridine, NaH, DMF; (c) Tf_2O, pyridine, CH_2Cl_2, 0 °C; (d) Pd/C, H_2; (e) $SnCl_2 \cdot H_2O$, EtOAc, 50 °C; (f) RCl, NaH, DMF.

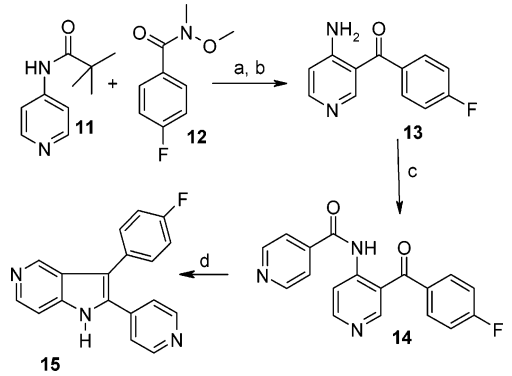
Scheme 2. Synthesis of 4-Azaindoles[a]



10a R=methoxyethyl
10b R=ethyl
10c R=methyl
10d R=hydroxyethyl
10e R=morpholinoethyl
10f R=pyrrolidinoethyl
10g R=piperidinoethyl
10h R=carboxymethyl

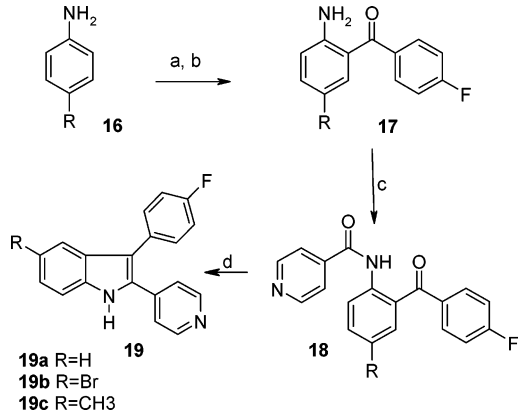
[a] Reagents: (a) 3-Amino-2-chloropyridine, *p*-TSA, toluene, reflux; (b) $PdCl_2(PPh_3)_2$, DABCO, DMF, 120 °C; (c) RI, NaH, DMF.

Scheme 3. Synthesis of 5-Azaindole[a]



[a] Reagents: (a) *n*-BuLi, THF; (b) aq. HCl, reflux; (c) isonicotinoyl chloride, pyridine, CH_2Cl_2, 0 °C; (d) $TiCl_3$, Mg, pyridine, DME, relux.

Scheme 4. Synthesis of Indoles[a]



[a] Reagents: (a) 4-fluorobenzonitrile, BCl_3, $AlCl_3$, toluene, reflux; (b) aq. HCl, reflux; (c) Isonicotinoyl chloride, pyridine, CH_2Cl_2, 0 °C; (d) $TiCl_3$, Zn, pyridine, DME, reflux.

3-amino-2-chloropyridine with azeotropic removal of water. The desired indole was then formed via an intramolecular Heck reaction in extremely high yield.[12] Direct alkylation of indoles **5** and **9** with the corresponding alkyl halides or diazomethane gave the desired derivatives **6** and **10.**

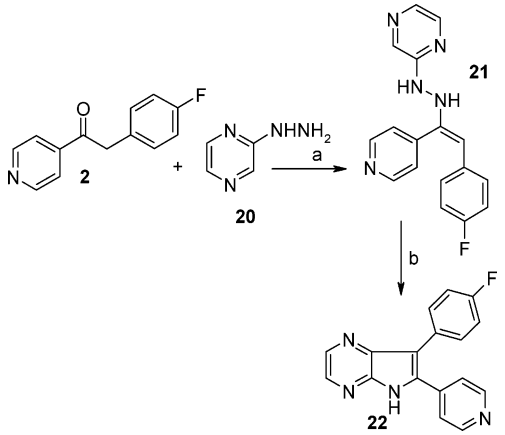
The general approach for 5-azaindole **15** and indole **19** is outlined in Schemes 3 and 4. 4-(Pivaloylamino) pyridine **11** was ortho lithiated using reported conditions[14] and was subsequently trapped with commercially available 4-fluoro-*N*-methoxy-*N*-methyl benzamide to give adduct **13**. Treatment with isonicotinoyl chloride in dichloromethane in the presence of pyridine afforded the oxoamide **14**, which was then subjected to reductive coupling with $TiCl_3$/Mg under the "instant method conditions" described by Fustner et al.[15] to yield 5-azaindole **15**. Indoles **19a**−**c** were prepared in similar fashion.

Both 4,7-diazaindole and 4,6-diazaindole derivatives were prepared by Fisher indole reactions.[15] The 4,7-diazaindoles **22 (**Scheme 5) will be described in greater detail in a separate paper.[16]

The preparation of 4,6-diazaindole **24** is outlined in Scheme 6. Ketone **2** was converted to its hydrazone **23** using standard conditions. The hydrazone **23** was then coupled with 5-bromopyrimidine via palladium catalysis using conditions initially described by Hartwig.[17]

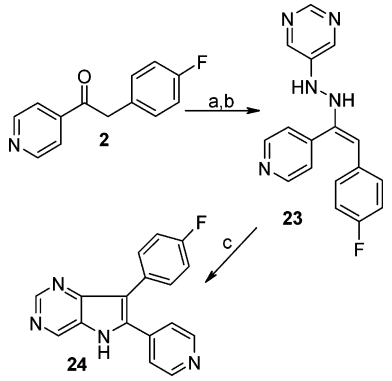
The construction of 7-amino-4,6-diazaindole **29** involved the bromination of 4-acetyl pyridine **25** with

Scheme 5. Synthesis of 4,7 Diazaindoles[a]



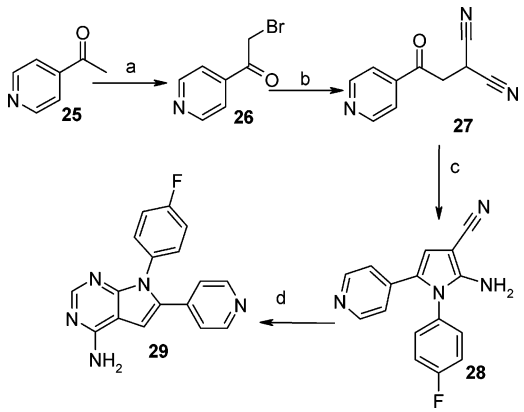
[a] Reagents: (a) *p*-TSA, benzene, reflux; (b) diethylene glycol, 250 °C.

Scheme 6. Synthesis of 4,6-Diazaindoles[a]



[a] Reagents: (a) NH_2NH_2, EtOH; (b) 5-bromopyrimidine, NaOtBu, $PdCl_2$(DPPF), DPPF, toluene, 100 °C; (c) diethylene glycol, reflux.

Scheme 7. Synthesis of 7-Amino-4,6-Diazaindole[a]

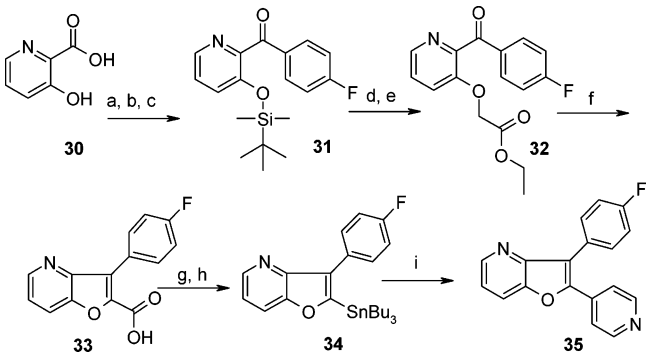


[a] Reagents: (a) bromine, HBr, AcOH, 0-5 °C; (b) malononitrile, NaOHaq, EtOH; (c) 4-fluoroaniline, AcOH, 100 °C; (d) formamide, formic acid, DMF, reflux.

bromine and HBr[18] followed by nucleophilic substitution with malononitrile. This reaction proved to be difficult and after experimenting with various conditions, and sodium hydroxide as base in ethanol gave the best results. Condensation of **27** with 4-fluoroaniline under acidic conditions afforded **28**. Subsequent treatment of **28** with formamide using literature conditions[19] gave the desired product **29** (Scheme 7).

The 4-azabenzofuran template was synthesized starting from esterification of commercial 5-hydroxypicolinic

Scheme 8. Synthesis of 4-Azabenzofuran[a]



[a] Reagents: (a) EtOH, H_2SO_4, benzene; (b) TBSCl, imidazole, CH_2Cl_2; (c) 4-fluorophenyl magnesium bromide; (d) TBAF, THF, 0 °C; (e) ethyl bromoacetate, K_2CO_3, acetone, reflux; (f) NaOEt, toluene, reflux; (g) Cu, quinoline, reflux; (h) TMEDA, *n*-BuLi, Bu_3SnCl, THF; (i) 4-bromopyridine.

acid followed by protection of the phenol as the *tert*-butyldimethylsilyl ether.[20] Treatment of the ester with 4-fluoromagnesium bromide afforded ketone **31** in good yield. Removal of the silyl protecting group, [20] subsequent reaction with ethyl bromoacetate, and cyclization to the benzofuran **33** were accomplished using literature conditions.[21] Decarboxylation[22] followed by formation of the tributylstannane using known procedures[23] gave adduct **34**. The installation of the 4-pyridyl ring was completed using Stille conditions[24] and gave the desired 4-azabenzofuran **35** (Scheme 8). Although the preparation of this compound could be envisaged using reductive coupling with $TiCl_3$/Zn, we thought the stannane coupling reaction used in the last step would allow for ready variation of the pyridyl group.

Our initial route to 4-azaindole (Scheme 2) did not allow for derivation of the 2-pyridyl ring; therefore, the synthesis was modified to incorporate a masked chloropyridine via *N*-oxide **36**. Ketone **2** was oxidized with methyltrioxorhenium[25] to afford *N*-oxide **36** in good yield. However, subsequent condensation with 3-amino-2-chloropyridine under a variety of conditions gave adduct **39** in poor yields due to thermal decomposition of the product. Dramatic increases in yield was accomplished by prior conversion of the ketone to the dimethyl acetal **38**[26] which could then undergo condensation with 3-amino-2-chloropyridine at lower temperature to give **39**. Intramolecular Heck reaction using DABCO as base in DMF gave **40** in moderate yield. Further improvement in yield was achieved using DIPEA in propionitrile. The requisite chloride in compound **41** was formed via treatment of *N*-oxide **40** with phosphorus oxychloride. Nucleophilic displacement of the chloride with the desired alkylamine afforded the amino substituted 4-azaindoles **42** (Scheme 9).

Results and Discussion

The 4-azaindole class of inhibitors bind to both p38 α and p38 β isoforms as well as to both the activated and unactivated form of p38 α as reported with similar diaryl heterocyclic classes of p38 inhibitors.[6]

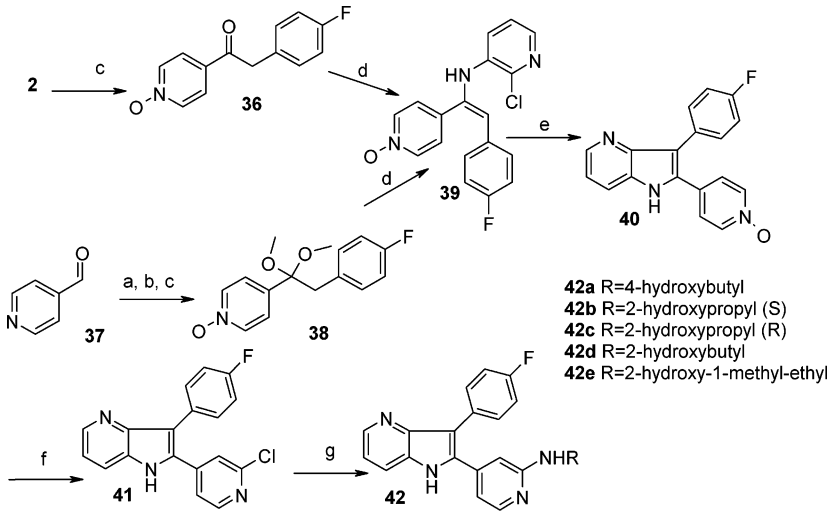
These compounds do not inhibit either p38 γ or p38 δ isoforms. [28] The γ and δ isoforms have a larger Met residue at position 106, whereas the α and β isoforms have a smaller Thr at this position. It is thought that Met side-chain blocks access to the hydrophobic pocket occupied by the 4-fluorophenyl group of the azaindole inhibitors, and hence the inhibitors are not effective against the γ and δ isoforms.

To optimize our lead, we investigated a number of heterocyclic derivatives of the 4-azaindole template (Table 1). Compounds **15** and **19a**, both lacking the 4-indole nitrogen, illustrate the importance of the direct hydrogen bond interaction between the nitrogen of the 4-azaindole ring and Lys 53. In the crystal structure of 5-azaindole **15** bound to unphosphorylated p38 enzyme, the distance between the nitrogen of Lys 53 and the nitrogen of the 5-azaindole ring is 4.3 Å, as compared with 2.8 Å in the 4-azaindole **9** structure (Figure 3B). Presumably, the loss of this hydrogen bond interaction explains the dramatic loss in potency (400-fold). Similarly, absence of nitrogen altogether decreases inhibition as is shown with indole **19a**. Compounds **22** and **35** maintain the same regioselective positioning of the pyridyl, 4-fluorophenyl rings and the 4-nitrogen of the indole ring. The loss in potency, however, may be explained by the reduced electron density of the 4-nitrogen in these heterocyclic systems relative to compound **9**. Finally, compounds **24** and **29** show a dramatic loss in potency perhaps due to a combination of reduced electron density on the 4-nitrogen as well as potential repulsive interactions between the enzyme and the 6-azaindole nitrogen. On the basis of these results, we primarily focused on the optimization of 4-azaindole series.

The initial 4-azaindole lead **9**, while being a potent inhibitor of p38 kinase with an IC_{50} of 6 nM, is a crystalline solid with a high melting point (>285 °C as the free base) and poor aqueous solubility (only 80 μg/mL as the HCl salt). We reasoned that substitution of the indole nitrogen would reduce the crystallinity of the template, and thus a number of derivatives were synthesized to optimize the physical properties of the series while attempting to maintain intrinsic potency. (Table 2).

The X-ray structure revealed that substitution at the indole nitrogen would be well tolerated as this position is solvent accessible and does not interact directly with the protein. However, a clear preference for small alkyl substituents is evident from the data (Table 2). Small neutral alkyl groups such as ethyl **10b** and methoxyethyl **10a** are best tolerated relative to the unsubstituted indole **9**. Attempts to further increase solubility with attachment of basic amines via an alkyl linker resulted in even greater loss of potency **10e**−**g**. Subsequent X-ray structures revealed that substituted alkyl derivatives such as morpholino ethyl **10e** preferred to displace the methionine 109 side chain and increase hydrophobic contacts with the hinge portion of p38 (perhaps explaining the preference for strictly hydrophobic moieties at this position). While the crystal structure reveals hydrophobic interactions between the morpholine ring of **10e** and the enzyme, significant desolvation penalty likely explains the lower intrinsic potency of this and similar analogues. Additionally, installation of acidic functionality at different distances from the indole templates was not at all tolerated, i.e., **10h**. While some intrinsic potency was lost with N-alkylation, the dramatic reduction in crystallinity more than compensated for this loss and in vivo efficacy was

Scheme 9. Synthesis of Amino Substituted 4-Azaindoles[a]



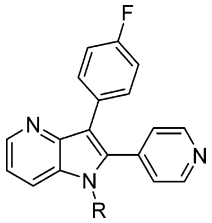
[a] Reagents: (a) $CH(OMe)_3$, H_2SO_4, MeOH; (b) 4-fluorobenzyl chloride, *n*-BuLi, THF; (c) CH_3ReO_3 (VII), H_2O_2, CH_2Cl_2; (d) 3-amino-2-chloropyridine, *p*-TSA, 60 °C; (e) $PdCl_2(PPh_3)_2$, DIPEA, CH_3CH_2CN, 98 °C; (f) $POCl_3$, reflux; (g) H_2NR, 120−140 °C.

Table 1. Effect of Indole Templates

compound	P_{38} inhibition assay IC_{50}[a] (nM)	rat LPS % inhibition @30 mg/kg
9	6.5	46
15	3100	
19a	1800	
22	53	98
24	895	26[b]
29	3300	
35	86	44

[a] IC_{50}'s were calculated from the dose−response curves. Positive control: SB202190 IC_{50} of 310 nM. [b] %inhibition @10 mg/kg.

Table 2. Effect of Substitution at the Indole Nitrogen[a]



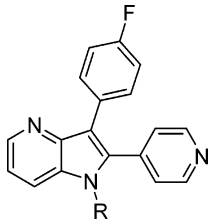
	R	P_{38} inhibition assay IC_{50} (nM)
9	H	6
6	methoxy	68
10a	methoxyethyl	21
10b	ethyl	24
10c	methyl	98
10d	hydroxyethyl	172
10e	morpholinoethyl	185
10f	pyrrolidinoethyl	217
10g	piperidinoethyl	1970
10h	carboxymethyl	23600

[a] IC_{50}'s were calculated from the dose−response curves. Positive control: SB202190 IC_{50} of 310 nM.

established in this series (Table 3) in a rat model of LPS-stimulated TNFα synthesis.

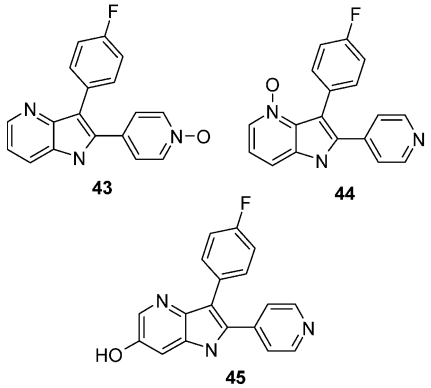
While in vivo efficacy was initially established with this template, the compounds in this series were prone to rather high metabolic clearance rates. In vitro studies with hepatic microsomal protein identified three sites of oxidative metabolism of azaindole **9**. The 4-pyridyl nitrogen **43** and the 4-azaindole nitrogen **44** were the major sites of metabolic transformation (confirmed by

Table 3. In Vivo Effect of Substitution at the Indole Nitrogen



compound	rat LPS % inhibition @ 30 mg/kg
9	46
10b	41
10c	53
10e	79
10f	86
10g	75
10h	50

Scheme 10. Azaindole **9** Metabolites



independent synthesis) and hydroxylation of the 6-position of the azaindole ring **45** was observed as a minor in vitro metabolite (Scheme 10). Metabolites **43** and **44** were significantly less active (IC_{50} = 2.5 and 6.7 μM respectively), consistent with the binding mode idente, and synthetic focus was concentrated on substitution of the pyridyl ring at the ortho position to the nitrogen. We postulated that steric hindrance may reduce the rate of *N*-oxide formation. The X-ray crystal structures suggested that monosubstituted amines could be toler-

Table 4. In Vitro and In Vivo Effects of Aminoalkyl Substitutions

compound	P38 inhibition assay[a] (IC_{50} nM)	rat LPS % inhibition @10 mg/kg
42a	5	69
42b	1	88
42c	1	76
42d	3	85
42e	3	88

[a] IC_{50}'s were calculated from the dose−response curves. Positive control: SB202190 IC_{50} of 310 nM.

Table 5. Abbreviated Pharmacokinetic Parameters after Single Dose 10 mg/kg PO and IV Doses of **42b** and **42c**[a]

	42b		**42c**	
	PO	IV	PO	IV
T_{max} (h)	1.00	<1.00	1.00	<1.00
C_{max}	6.9	10.3	2.8	8.8
$T_{1/2}$ (h)	1.5	1.1	1.5	2.9
AUC 0−24h	20.0	23.4	8.4	27.5
CL (L kg^{-1} h^{-1})		1.2		1.0
%*F*	85.6		30.5	

[a]Administered to female Hanover−Wistar rats. Units: C_{max} (μM), AUC (μM h).

Table 6. RO3206145 **42b** Biological and Physical Data

P38 IC_{50}	THP-1 IC_{50}	HWB IC_{50}	rat LPS ED_{50}
1.29 nM	1.70 nM	210 nM	2 ± 0.3 mg/kg
aqueous solubility: >25 μg/mL	log *P* (o/w): 3.5	Caco-2 permeability: 5.8×10^{-5} cm/s (A → B)	molecular weight: 362 g/mol

Table 7. Selectivity Profile of Compound **42b**

kinase	kinase assay IC_{50} (μM)
MAPKAP-2	>30
IKK β	>30
IRAK-1	>30
IRAK-4	>30
CDK1,2,3,4	>25
KDR	>10
PKC	>10
JNK-1	>10
PKA	>10
$P55^{Fyn}$	>10
ERK	>10

ated next to the pyridyl nitrogen atom and perhaps form a bidentate hydrogen bonding motif with Met 109.[8,9]

Mono hydroxyalkylamine derivatives were potent inhibitors of p38 both in vitro and in vivo. Optimal potency was achieved when the hydroxyl group was located two carbons from the exocyclic amine. The chirality of the hydroxyl group did not alter the intrinsic potency of the inhibitor (**42b** vs **42c**, Table 4). Differences in plasma exposure in rodents, however, between the enantiomers were observed. Compound **42b** had greater plasma exposure (higher C_{max} and AUC) than compound **42c** (Table 5). Compound **42b** was evaluated for kinase selectivity against a panel of related enzymes including ERK and JNK-1 and was quite selective for p38 MAP kinase (Table 7). On the basis of these results, and the desirable drug-like physical properties (Table 6) of **42b**, this inhibitor was selected for advanced preclinical evaluation.

In conclusion, a novel scaffold for inhibition of p38 MAP kinase was identified by exploiting the hydrogen bond to Lys 53 using structure guided design. A number of variants of the 4-azaindole were synthesized; however, none were identified to be as potent as this core scaffold. Attempts to improve the potency and physical properties of the lead **9** via alkylation of the indole nitrogen successfully identified derivatives with both in vitro and in vivo efficacy. Unfortunately, these analogues suffered from high rates of oxidative metabolic clearance directed principally at the pyridine ring. The X-ray crystal structure provided insight into the design of well-tolerated substituted pyridine derivatives which provided steric bulk around the pyridine nitrogen. Compound **42b** was identified as a potent, selective orally bioavailable inhibitor of p38 which possessed desirable physical properties.

Experimental Section

Reagents and solvents were obtained from commercial suppliers and were used without further purification. Flash chromatography was performed with Merck silica gel 60 (230−400 mesh), and reaction progress was determined by thin-layer chromatography (TLC) using Analtec 250-μm silica gel plates. Visualization was done with UV light (254 nm) or iodine. Yields are of purified compounds and were not optimized. ^{1}H NMR measurements were recorded at 75.40 MHz using a Bruker AMX 300 instrument in DMSO with tetramethylsilane as the internal standard. Melting points recorded were uncorrected. Elemental analyses were within ±0.4% except for compound **19a** for which carbon and nitrogen analysis exhibits ±0.46 and ±0.45% errors and **24** for which hydrogen analysis exhibits ±0.47% error. Purity of compounds **43** and **44** was determined using a Micromass Platform LC, single quadruple mass spectrometer with a electrospray (ES) probe using a mobile phase of acetonitrile/water with 0.1% TFA.

Synthesis of 3-(4-Fluorophenyl)-1-hydroxy-2-(pyridin-4-yl)-1H-pyrrolo[3,2-b]pyridine (5). Sodium metal (5.06 g, 210 mmol) was dissolved in absolute ethanol (150 mL), and then a solution of methyl isonicotinate (20.55 g, 150 mmol) and 4-fluorophenylacetonitrile (20.25 g, 150 mmol) in absolute ethanol (50 mL) was added in one portion. The reaction mixture was heated to reflux for 3 h and then cooled to room temperature. The reaction mixture was poured into ice water (300 mL), and the pH was adjusted to 3 with 10% hydrochloric acid. The yellow precipitate was filtered and dried in vacuo to give 24 g of cyano ketone. This material was suspended in 48% hydrobromic acid (90 mL) and heated to reflux. After 8 h, the reaction mixture was cooled to room temperature and carefully poured into ice water (300 mL). The pH was adjusted to 7−8 with ammonium hydroxide. The product was extracted into ethyl acetate (3 × 100 mL), and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentration in vacuo to give 1-(pyridin-4-yl)-2-(4-fluorophenyl)ethanone (11.8 g) as a tan solid.^{1}H NMR (DMSO-d_6) d: 4.26 (s, 2 H) 7.04 (t, J = 8.67 Hz, 2 H), 7.21 (dd, J = 8.72 Hz, J = 5.31 Hz, 2 H), 7.17 (dd, J = 4.42 Hz, J = 1.68 Hz, 2 H), 8.81 (dd, J = 4.43 Hz, J = 1.67 Hz, 2 H).

To a solution of 1-(pyridin-4-yl)-2-(4-fluorophenyl)ethanone (4.0 g, 18.6 mmol) and 2-chloro-3-nitropyridine (6.50 g, 41.13 mmol) in dimethylformamide (50 mL) at 0 °C was added sodium hydride (1.65 g, 41 mmol, 60% in oil) under argon atmosphere. The reaction mixture was warmed to room temperature and stirred for an hour. The reaction mixture was quenched with water (100 mL) and the product extracted into ethyl acetate (2 × 100 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give a dark brown oil. Purification by flash column chromatography (10 to 50% ethyl acetate/ hexanes) gave 1-(pyridin-4-yl)-2-(4-fluorophenyl)-2-(3-nitro-2-pyridyl) ethanone as a brown oil (4.08 g).

A solution of 1-(pyridin-4-yl)-2-(4-fluorophenyl)-2-(3-nitro-2-pyridyl)ethanone (2.0 g, 5.93 mmol) and pyridine (0.52 g, 6.53 mmol) in dichloromethane (30 mL) was added to a cold solution of trifluoromethanesulfonic anhydride (1.1 mL, 6.53

mmol) in dichloromethane (7 mL) at 0 °C. After 1 h, the reaction mixture was poured into water (50 mL) and the product extracted into dichloromethane. The combined extracts were washed with sodium bicarbonate solution and brine and dried over $MgSO_4$. Concentration in vacuo gave a brown oil which was purified by flash column chromatography (50−60% ethyl acetate/ hexanes) to give trifluoromethanesulfonic acid 2-(4-fluorophenyl)-2-(3-nitropyridin-4-yl) vinyl ester as a light tan oil (1.56 g). ^{1}H NMR (DMSO-d_6) d: 7.17−7.10 (m, 4 H), 7.49 (dd, J = 8.32 Hz, J = 4.73 Hz, 1 H), 7.69 (dd, J = 8.96 Hz, J = 5.28 Hz, 2 H), 8.19 (dd, J = 8.32 Hz, J = 1.56 Hz, 1 H), 8.51 (d, J = 6.09 Hz, 2 H), 8.93 (dd, J = 4.72 Hz, J = 1.56 Hz, 1 H).

To a solution of trifluoromethanesulfonic acid 2-(4-fluorophenyl)-2-(3-nitropyridin-4-yl)vinyl ester (1.5 g, 3.20 mmol) in ethyl acetate (50 mL) was added stannous chloride dihydrate (2.98 g, 12.8 mmol), and the reaction mixture was warmed to 50 °C. After 1 h, the warm solution was treated with saturated sodium bicarbonate solution (10 mL) and filtered through Celite. The filtrate was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. Purification by flash chromatography (dichloromethane−95% dichloromethane/methanol gradient) gave 3-(4-fluorophenyl)-1-hydroxy-2-(pyridin-4-yl)-1H-pyrrolo[3,2-b]pyridine as a tan solid (700 mg).^{1}H NMR (DMSO-d_6) δ: 7.18 (t, J = 8.48 Hz, 2 H), 7.32 (dd, J = 8.27 Hz, J = 4.54, 1 H) 7.51−7.46 (m, 4 H), 7.95 (dd, J = 8.27 Hz, J = 1.44 Hz, 1 H), 8.46 (dd, J = 4.53 Hz, J = 1.44 Hz, 1 H), 8.56 (dd, J = 4.47 Hz, J = 1.62 Hz, 2 H), 11.57 (s, 1 H). Ms (EI/CI) *m/z*: (M+H) 305. Anal. ($C_{18}H_{12}OFN_3$·0.2 H_2O) C,H,N.

3-(4-Fluoro-phenyl)-1-methoxy-2-pyridin-4-yl-1*H*-pyrrolo[3,2-*b*]pyridine (6). ^{1}H NMR (DMSO-d_6) δ: 3.72 (s, 3 H), 7.07 (t, J = 8.53 Hz, 2 H) 7.30−7.26 (m, 1 H), 7.50−7.45 (m, 4 H), 7.84 (d, J = 8.37 Hz, 1 H), 8.58 (d, J = 4.61 Hz, 1 H), 8.67 (d, J = 6.01 Hz, 2 H). Ms (EI/CI) *m/z*: (M+H) 319. Anal. ($C_{19}H_{14}OFN_3$) C,H,N.

3-(4-Fluorophenyl)-2-(pyridin-4-yl)-1H-pyrrolo[3,2-b]-pyridine (9). To a solution of 1-(pyridin-4-yl)-2-(4-fluorophenyl)ethanone (5.20 g, 24 mmol) and the corresponding 3-amino-2-chloropyridine (4.04 g, 31.4 mmol) in toluene (150 mL) was added *p*-toluenesulfonic acid monohydrate (457 mg, 24 mmol), and the reaction mixture was brought to reflux with Dean−Stark removal of toluene/water azeotrope. After 24 h, toluene was removed in vacuo and the residue was suspended in ethyl acetate. The precipitate was collected by vacuum filtration to give the corresponding (2-chloropyridin-3-yl)-[2-(4-fluorophenyl)-1-(pyridin-4-yl)-vinyl]amine (5.0 g) as a tan solid. The filtrate was concentrated in vacuo and purified by flash column chromatography (50−80% ethyl acetate:hexanes gradient) to give additional 1.50 g of product.

To a solution of (2-chloropyridin-3-yl)-[2-(4-fluorophenyl)-1-(pyridin-4-yl)-vinyl]amine (6.0 g, 18.5 mmol) and DABCO (6.2 g, 55 mmol) in dimethylformamide (75 mL) was added bis triphenylphosphine palladium (II) chloride (650 mg, 0.926 mmol), and the reaction mixture was heated at 120 °C under argon atmosphere. After 4 h, dimethylformamide was removed in vacuo and the residue was heated in ethyl acetate/methanol mixture. The product was filtered off to give a green solid which was redissolved in boiling methanol/chloroform mixture and treated with charcoal. The solution was filtered through a pad of Celite and the filtrate was concentrated to give 3-(4-fluorophenyl)-2-(pyridin-4-yl)-1H-pyrrolo[3,2-b]pyridine (5.27 g) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ: 7.29−7.21 (m, 3 H), 7.47 (dd, J = 4.52 Hz, J = 1.66 Hz, 2 H), 7.53 (dd, J = 9.00 Hz, J = 5.64, 2 H), 7.88 (dd, J = 8.23 Hz, J = 1.42 Hz, 1 H), 8.41 (dd, J = 4.50 Hz, J = 1.39 Hz, 1 H), 8.61 (d, J = 6.03 Hz, 2 H) 12.04 (s, 1 H). Ms (EI/CI) *m/z*: (M+H) 289. Anal. ($C_{18}H_{12}FN_3$·1/4H_2O) C,H,N.

General Procedure for the alkylation of 3-(4-fluorophenyl)-2-(pyridin-4-yl)-1H-pyrrolo[3,2-b]pyridine. Compounds (10a−h). Sodium hydride (60% w/v dispersion in mineral oil, 14 mmol) was added to a cold solution of 3-(4-fluorophenyl)-2-(pyridin-4-yl)-1H-pyrrolo[3,2-b]pyridine (1.4 mmol) in DMF (10 mL). The mixture was stirred at room temperature for 30 min and then cooled to 0−5 °C. A solution of the appropriate alkyl bromide (3.5 mmol) was then added, and the resulting mixture allowed to stir at room temperature for 18 h. The mixture was quenched with water (100 mL) and extracted into ethyl acetate (2 × 20 mL). The combined organic layers were washed with water and dried over sodium sulfate, filtered, and concentrated under vacuum, and the product was redissolved in ethyl acetate (5 mL) and HCl (1.0 M in ether) was added. The precipitate was collected by filtration and dried in vacuo to give products **10a**−**h**.

3-(4-Fluorophenyl)-1-methoxy-ethyl-2-pyridin-4-yl-1H-pyrrolo[3,2-b]pyridine (10a). ^{1}H NMR (DMSO-d_6) δ: 3.19 (s, 3 H), 3.69 (br, 2 H), 4.66 (br, 2 H), 7.07 (t, J = 8.50 Hz, 2 H), 7.34−7.30 (m, 2 H), 7.82 (dd, J = 8.28 Hz, J = 5.61 Hz, 1 H) 8.06 (d, J = 5.72 Hz, 2 H), 8.78 (d, J = 5.53 Hz, 1 H) 8.90 (d, J = 8.40 Hz, 1 H), 8.98(d, J = 5.55 Hz, 2 H). Ms (EI/CI) *m/z*: (M+H) 347. Anal. ($C_{21}H_{18}FN_3O$·2HCl ·11/2H_2O) C,H,N.

3-(4-Fluorophenyl)-1-ethyl-2-pyridin-4-yl-1H-pyrrolo-[3,2-b]pyridine (10b). ^{1}H NMR (DMSO-d_6) δ: 1.13(t, J = 7.12 Hz, 3 H), 4.17 (q, J = 7.11 Hz, 2 H), 7.12 (t, J = 8.97 Hz,, 2 H), 7.31 (dd, J = 8.34 Hz, J = 4.55 Hz, 1 H), 7.50 (m, 4 H), 8.11 (dd, J = 8.35 Hz, J = 1.39 Hz, 1 H) 8.48 (dd, J = 4.53 Hz, J = 1.29 Hz, 1 H), 8.73 (dd, J = 4.40 Hz, J = 1.59 Hz, 2 H). Ms (EI/CI) *m/z*: (M+H) 316. Anal. ($C_{20}H_{16}FN_3$·2HCl) C,H,N.

3-(4-Fluorophenyl)-1-methyl-2-pyridin-4-yl-1H-pyrrolo-[3,2-b]pyridine (10c). ^{1}H NMR (DMSO-d_6) δ: 3.87(s, 3 H), 7.23 (t, J = 8.92 Hz, 2 H), 7.41 (dd, J = 8.82 Hz, J = 5.53 Hz, 2 H), 7.71−7.67 (m, 3 H), 8.64 (dd, J = 5.24 Hz, J = 1.06 Hz, 1 H), 8.68 (d, J = 8.40 Hz, 1 H) 8.84 (d, J = 5.54 Hz, 2 H). Ms (EI/CI) *m/z*: (M+H) 302. Anal. ($C_{19}H_{14}FN_3$·1.5HCl) C,H,N.

3-(4-Fluorophenyl)-1-hydroxyethyl-2-pyridin-4-yl-1H-pyrrolo[3,2-b]pyridine (10d). ^{1}H NMR (DMSO-d_6) δ: 3.60 (br, 2 H), 4.43 (br, 2 H), 7.26 (t, J = 8.87 Hz, 2 H), 7.40 (dd, J = 8.73 Hz, J = 5.53 Hz, 2 H), 7.80−7.75 (m, 1 H), 7.84 (d, J = 6.09 Hz, 2 H) 8.69 (d, J = 5.30 Hz, 1 H), 8.90−8.86 (m, 3 H).Ms (EI/CI) *m/z*: (M+H) 333. Anal. ($C_{20}H_{16}FN_3O$·3HCl) C,H,N.

3-(4-Fluorophenyl)-1-morpholinoethyl-2-pyridin-4-yl-1H-pyrrolo[3,2-b]pyridine (10e). ^{1}H NMR (DMSO-d_6) δ: 2.20 (t, J = 4.54 Hz, 4 H), 2.52−2.48 (m, 2 H), 3.46 (t, J = 4.58 Hz, 4 H), 4.03 (t, J = 4.97 Hz, 2 H), 7.20 (t, J = 8.92 Hz, 2 H), 7.40 (dd, J = 8.31 Hz, J = 4.57 Hz, 1 H) 7.53−7.47 (m, 4 H), 8.13(dd, J = 8.30 Hz, J = 1.39 Hz, 1 H), 8.51 (dd, J = 4.58 Hz, J = 1.37 Hz, 1 H), 8.70 (dd, J = 4.46, J = 1.6, 2 H). Ms (EI/CI) *m/z*: (M+H) 418. Anal. ($C_{24}H_{23}FN_4$ O·2HCl·1H_2O) C,H,N.

3-(4-Fluorophenyl)-1-pyrrolidinoethyl-2-pyridin-4-yl-1H-pyrrolo[3,2-b]pyridine (10f).^{1}H NMR (DMSO-d_6) δ: 1.74−1.70 (m, 4 H), 2.38 (br, 4 H), 2.69 (t, J = 7.50 Hz, 2 H), 4.26 (t, J = 7.51 Hz, 2 H), 6.99 (t, J = 8.84 Hz, 2 H), 7.24 (dd, J = 8.34 Hz, J = 4.59 Hz, 1 H) 7.33 (dd, J = 4.41 Hz, J = 1.65 Hz, 2 H), 7.44−7.37 (m, 2H), 7.79 (dd, J = 8.31 Hz, J = 1.27 Hz, 1 H), 8.58 (dd, J = 4.60, J = 1.30 Hz, 1 H), 8.70 (dd, J = 4.40 Hz, J = 1.64 Hz, 2 H). Ms (EI/CI) *m/z*: (M+H) 387. Anal. ($C_{24}H_{23}FN_4O$) C,H,N.

3-(4-Fluorophenyl)-1-piperidinoethyl-2-pyridin-4-yl-1H-pyrrolo[3,2-b]pyridine (10 g). ^{1}H NMR (DMSO-d_6) δ: 1.40−1.38 (m, 2 H), 1.53−1.46 (m, 4 H), 2.27 (t, J = 5.10 Hz, 4 H), 2.52 (t, J = 7.25 Hz, 2 H), 4.22 (t, J = 7.25 Hz, 2 H), 6.98 (t, J = 8.81 Hz, 2 H) 7.23 (dd, J = 8.24 Hz, J = 4.60 Hz, 1 H), 7.35 (dd, J = 4.40 Hz, J = 1.66 Hz, 2 H), 7.41 (dd, J = 8.81 Hz, J = 5.50 Hz, 2 H), 7.78 (dd, J = 8.32 Hz, J = 1.31 Hz, 1 H), 8.58 (dd, J = 4.60 Hz, J = 1.39 Hz, 1 H), 8.70 (dd, J = 4.40 Hz, J = 1.65 Hz, 2 H). Ms (EI/CI) *m/z*: (M+H) 400. Anal. ($C_{25}H_{28}FN_4$) C,H,N.

3-(4-Fluorophenyl)-2-pyridin-4-yl)-1H-pyrrolo[3,2-c]-pyridine (15). To a solution of 4-pivaloylaminopyridene (7.0 g, 39 mmol) in tetrahydrofuran (100 mL) was added *n*-butyllithium (39.3 mL, 98 mmol, 2.5 M solution in tetrahydrofuran) at −78 °C and quenched with a solution of *N*-methoxy-*N*-methyl-4-fluorobenzamide (7.9 g, 43 mmol) in 100 mL of tetrahydrofuran. The reaction mixture was warmed to room temperature and poured into water. The product was extracted with ethyl acetate (3 × 100 mL). The organic layers were washed with brine, dried over sodium sulfate, and evaporated

to dryness. The residue was purified by flash column chromatography (10% ethyl acetate/hexanes) to afford *N*-[3-(4-fluorobenzoyl)-4-pyridinyl]-2,2-dimethylpropanamide (10 g).

A solution of *N*-[3-(4-fluorobenzoyl)-4-pyridinyl]-2, 2-dimethylpropanamide (2.85 g, 9.5 mmol) in 3 N aqueous HCl (15 mL) was heated to reflux overnight. After cooling the sample to room temperature, the reaction mixture was washed with ether, and the aqueous layer was separated and neutralized with potassium carbonate. The product was extracted into ethyl acetate, dried over potassium carbonate/sodium carbonate, and concentrated. The residue was purified by flash chromatography (5% methanol/ methylene chloride) to afford 4-amino-3-(4-fluorobenzoyl) pyridine (1.58 g).

4-Amino-3-(4-fluorobenzoyl) pyridine (1.5 g, 7.0 mmol) was suspended in methylene chloride (90 mL) and pyridine (2.24 g, 28 mmol). The reaction mixture was cooled to 0 °C, and nicotinoyl chloride (1.4 g, 7.6 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirring continued for 4 h. Methylene chloride was added and the white precipitate was filtered and dried in vacuo to yield 4-isonicotinoylamide-3-(4-fluorobenzoyl)pyridine (1.7 g).

A suspension of 4-isonicotinoylamide-3-(4-fluorobenzoyl)-pyridine (300 mg, 0.75 mmol), titanium trichloride (6.3 mL, 6.3 mmol, 1.0 M solution in dichloromethane/tetrahydrofuran 2:1), magnesium (309 mg, 12.7 mmol), and pyridine (0.62 mL, 8.0 mmol) in ethylene glycol dimethyl ether (50 mL) was refluxed for 1 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate, and a solution of 5% sodium bicarbonate was added. The reaction mixture was vigorously stirred overnight and then filtered through a pad of Celite. The organic layer was separated and concentrated in vacuo. Purification by flash chromatography (5% methanol/ methylene chloride) gave 3-(4-fluorophenyl)-2-pyridin-4-yl)-1H-pyrrolo[3,2-c]pyridine as a tan solid (30 mg).^{1}H NMR (DMSO-d_6) δ: 7.32 (t, J = 8.92 Hz, 2 H), 7.49−7.41(m, 4 H), 7.56 (dd, J = 5.87 Hz, J = 0.88 Hz, 1 H), 8.32 (d, J = 5.87 Hz, 1 H), 8.60 (dd, J = 4.56 Hz, J = 1.59 Hz, 2 H), 8.80 (d, J = 0.86 Hz, 1 H) 12.49 (br, 1 H). Ms (EI/CI) *m/z*: (M+H) 289. Anal. ($C_{18}H_{12}FN_3$·0.4H_2O) C,H,N.

General Procedure for the Preparation of Compounds (19a−c). To boron trichloride (18 mmol, 1.0 M in xylenes) at 0 °C under nitrogen atmosphere, a solution of the appropriate aniline (16.1 mmol) and 4-fluorobenzonitrile (24.1 mmol) in toluene (18 mL) was added slowly. After addition was complete, aluminum trichloride (18 mmol) was added and the reaction was heated to reflux for 12 h. The reaction mixture was cooled to 0 °C and water (1 mL) followed by 10% HCl (18 mL) was added and the reaction was brought to reflux for 2 h, cooled to room temperature, and the precipitate was collected by vaccum filtration; the solid was suspended in water/5% sodium hydroxide (20 mL/20 mL). After stirring of the sample for 1 h, the solid was isolated by filtration and dried in vacuo to give products **17a−c**.

To (2-amino-phenyl)-(4-fluoro-phenyl)methanone (16.13 mmol) and pyridine (2 mL) in dichloromethane (10 mL) at 0 °C was added isonicotinoyl chloride (6.75 mmol) in one portion, the suspension was stirred at room temperature for 3 h, then diluted with dichloromethane, washed with 5% sodium bicarbonate, dried over sodium sulfate, and concentrated under vacuo. The residue was purified by flash chromatography using 5% methanol in dichloromethane as eluent to afford compounds **18a−c**.

A suspension of **18a−c** (3.12 mmol), titanium trichloride (20.3 mmol, 1.0M solution in dichloromethane/tetrahydrofuran 2:1), and zinc (13 mmol) in tetrahydrofuran (50 mL) was refluxed for 2.5 h. The reaction mixture was cooled to room temperature, filtered through a short plug of silica gel, and the silica gel was washed with ethyl acetate and dichloromethane. The filtrate was concentrated in vacuo and purified by flash chromatography using 5% methanol in dichloromethane as eluent to afford compounds **19a−c**.

3-(4-Fluorophenyl)-2-pyridin-4-yl-1H-indole (19a). ^{1}H NMR (DMSO-d_6) δ: 7.21−7.10 (m, 3 H), 7.33−7.29 (m, 3 H), 7.39 (dd, J = 8.77 Hz, J = 5.53 Hz, 2 H), 7.47 (d, J = 8.14 Hz, 1 H), 7.58 (d, J = 8.22 Hz, 1 H), 8.54 (d, J = 5.76 Hz, 2 H). Ms (EI/CI) *m/z*: (M+H) 288. Anal. ($C_{20}H_{13}FN_2$·0.8 HCl) C,H,N.

5-Bromo-3-(4-fluorophenyl)-2-pyridin-4-yl-1H-indole (19b). ^{1}H NMR (DMSO-d_6) δ: 7.42−7.10 (m, 7 H), 7.48 (d, J = 8.64 Hz, 1 H), 7.52 (s, 1 H), 8.56 (d, J = 5.56 Hz, 2 H), 12.06 (s, 1 H). Ms (EI/CI) *m/z*: (M+H) 366. Anal. ($C_{19}H_{12}BrFN_2$·0.15H_2O) C,H,N.

3-(4-Fluorophenyl)-5-methyl-2-pyridin-4-yl-1H-indole (19c). ^{1}H NMR (DMSO-d_6) δ: 2.40(s, 3 H), 7.22−7.15 (m, 4 H), 7.42−7.33 (m, 3 H), 7.77 (br, 2 H), 8.55 (br, 2 H), 11.21 (s, 1 H). Ms (EI/CI) *m/z*: (M+H) 302. Anal. ($C_{20}H_{15}FN$) C,H,N.

7-(4-Fluorophenyl)-6-pyridin-4-yl-5H-pyrrolo[3,2-d]pyrimidine (24). A mixture of 2-(4-fluorophenyl)-1-pyridin-4-yl-ethyldiazene (1.68 g, 7 mmol), 5-bromopyridine (1.8 g, 11.24 mmol), sodium *tert*-butoxide (1.1 g, 11.24 mmol), [1,1′-bis (diphenylphosphino)ferrocene] dichloropalladium (II) complex with dichloromethane (0.30 g, 0.40 mmol),), 1,1′-bis (diphenylphosphino)ferrocene (0.62 g, 1.12 mmol) in toluene (10 mL) was stirred at 100 °C under argon atmosphere. After 12 h, the reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated in vacuo and the residue was purified by flash chromatography using hexanes−acetone (3:2) as eluent to afford *N*-[2-(4-fluorophenyl)-1-pyridin-4-yl-ethylidene]-*N*′-pyrimidin-5-yl-hydrazine (0.42 g). ^{1}H NMR (DMSO-d_6) δ: 4.12 (s, 2 H), 7.04 (t, J = 8.55 Hz, 3 H), 7.17 (dd, J = 8.61 Hz, J = 5.22 Hz, 3 H), 7.65 (dd, J = 4.71 Hz, J = 1.51 Hz, 2 H), 8.03 (s, 1 H), 8.63−8.51 (m, 4 H), 8.80(s, 1 H). Ms (EI/CI) *m/z*: (M+H) 307.

A solution of *N*-[2-(4-fluorophenyl)-1-pyridin-4-yl-ethylidene]-*N*′-pyrimidin-5-yl-hydrazine (0.79 g, 2.57 mmol) in diethylene glycol (6 mL) was heated to reflux for 6 h. Then cooled to room temperature and stirred for 12 h in a mixture of ether and brine. The organic layer was separated, dried over sodium sulfate, and purified by flash chromatography eluting with 5% methanol in dichloromethane to 15% methanol in dichloromethane to afford 7-(4-fluorophenyl)-6-pyridin-4-yl-5H-pyrrolo[3,2-d]pyrimidine (0.1 g) ^{1}H NMR (DMSO-d_6) δ: 7.10 (t, J = 8.66 Hz, 2 H), 7.36−7.29 (m, 4 H), 8.15 (s, 1 H), 8.47 (dd, J = 5.31 Hz, J = 1.50 Hz, 2 H), 9.11 (s, 1 H). Ms (EI/CI) *m/z*: (M+H) 289. Anal. ($C_{17}H_{11}FN_4$·0.3 EtOAc·0.6 H_2O) C,H,N.

7-(4-Fluoro-phenyl)-6-pyridin-4-yl-7H-pyrrolo[2,3-d]-pyrimidin-4-ylamine (29). Malononitrile 0.061 g (0.92 mmol) was dissolved in 2 mL of ethanol. (0.260 g, 0.93 mmol) 2-bromo-1-pyridin-4-yl-ethanone hydrobromide was added. (0.074 g 1.9 mmol) of sodium hydroxide in 1 mL of water was added to the stirring mixture. 3 mL more of water was added. The resulting precipitate was filtered and dried in vacuo to yield 0.090 g (53% yield) of 2-(2-Oxo-2-pyridin-4-yl-ethyl)-malononitrile (**27**). mp 207.0−215.0 °C. ^{1}H NMR (DMSO-d_6): δ 8.85 (d, J = 6.0 Hz, 2 H), 7.86 (d, J = 6.0 Hz, 2 H), 5.13 (t, J = 6.0 Hz, 1 H), 4.11 (d, J = 6.0 Hz, 2 H); ^{13}C NMR (DMSO-d_6): d 194.6, 150.9, 140.6, 121.1, 114.0, 38.5, 17.7. MS *m/z* 185 (M^+).

A solution of 0.350 g (1.89 mmol) of 2-(2-Oxo-2-pyridin-4-yl-ethyl)-malononitrile and 1.05 g (9.45 mmol) of 4-fluoroaniline in 10 mL of acetic acid was heated to 100 °C for 18 h. This was cooled and poured into 100 g of ice. This was made basic by the careful addition of sat. aqueous sodium bicarbonate. This was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. Purification by flash chromatography (3% methanol/dichloromethane) followed by repurification by flash chromatography (gradient elution with 30% acetone/hexane brought to 100% acetone) gave 0.100 g (19% yield) of 2-amino-1-(4-fluoro-phenyl)-5-pyridin-4-yl-1H-pyrrole-3-carbonitrile (28). ^{1}H NMR ($CDCl_3$): δ 8.34 (d, J = 4.9 Hz), 7.26−7.19 (m, 4 H), 6.79 (d, J = 4.9 Hz, 2 H), 6.62 (s, 1 H), 4.22 (s, 2 H). MS *m/z* 278 (M^+).

2-Amino-1-(4-fluoro-phenyl)-5-pyridin-4-yl-1H-pyrrole-3-carbonitrile 0.048 g (0.17 mmol) was dissolved in 1.05 mL of formamide, 0.35 mL of dimethylformamide, and 0.14 mL of formic acid. This was heated to reflux for 3 h. This was cooled and partitioned between ethyl acetate and saturated aqueous

sodium bicarbonate. The organic layer was washed with water, washed with brine, dried over anhydrous sodium sulfate, and concentated in vacuo. Purification by flash chromatography (5% methanol/dichloromethane) gave 0.026 g (49% yield) of 7-(4-fluoro-phenyl)-6-pyridin-4-yl-7H-pyrrolo[2,3-d]pyrimidin-4-ylamine (**29**). ^{1}H NMR (DMSO-d_6): δ 8.48 (d, J = 6.2 Hz, 2 H), 8.08 (s, 1 H), 7.34−7.29 (m, 4 H), 7.15 (s, 1 H), 7.13 (d, J = 6.2 Hz, 2 H). MS m/z 305 (M^+).

3-(4-Fluorophenyl-2-(pyridin-4yl)furo[3,2-b]pyridine (35). 3-Hydroxypicolinic acid (12.5 g, 900 mmol) was suspended in a mixture of ethanol (300 mL) and benzene (100 mL). Sulfuric acid (5 mL) was added and the reaction mixture was heated at reflux with azeotropic removal of water via Dean−Stark trap. After the reaction was complete, the organics were removed in vacuo. The residue was dissolved in water, basified with sodium carbonate, and extracted into ethyl acetate. The ethyl acetate layer was dried over $MgSO_4$, concentrated in vacuo to give ethyl 3-hydroxypicolinate (15 g).

A mixture of ethyl 3-hydroxypicolinate (15 g, 900 mmol), tributylsilyl chloride (16.23 g, 110 mmol), and imidazole (8.0 g, 120 mmol) in methylene chloride was stirred overnight under a nitrogen atmosphere. Water was added and the methylene chloride layer was separated and concentrated in vacuo. Purification on a silica gel column using ethyl acetate−hexane (1:4) as the eluant gave ethyl 3-(tributylsililoxy)-picolinate as a solid (20 g).

A solution of ethyl 3-(tributylsililoxy)picolinate (19 g, 35 mmol) in tetrahydrofuran (100 mL) was cooled to 0 °C and 4-fluorophenylmagnesium chloride (52 mL, 1.0 M in tetrahydrofuran) was added dropwise. After 30 min, the reaction mixture was quenched with water and the product was extracted with ethyl acetate (3 × 50 mL). The organic layer was dried over sodium sulfate and concentrated in vacuo. Purification on a silica gel column using ethyl acetate−hexane (2:98) as the eluant gave 2-(4-fluorobenzoyl)-3-(tributylsilyloxy)pyridine (3.75 g).

A solution of 2-(4-fluorobenzoyl)-3-(tributylsilyloxy)pyridine (3.75 g, 11.3 mmol) in tetrahydrofuran (17 mL) was cooled to 0 °C and tetrabutylammonium fluoride (17 mL, 1.0 M in tetrahydrofuran) was added. After 2 h, the reaction mixture was diluted with ethyl acetate. The organic layer was washed with sodium bicarbonate and brine, and dried over sodium sulfate. The organics were removed in vacuo, and the residue was purified on a silica gel column using ethyl acetate−hexane (1:4) as the eluant to give 2-(4-fluorobenzoyl)-3-hydroxypyridine (2.2 g).

A mixture of give 2-(4-fluorobenzoyl)-3-hydroxypyridine (2.2 g, 11.3 mmol), ethyl bromoacetate (1.6 mL, 14.15 mmol), and potassium carbonate (4.34 g, 31.4 mmol) in acetone (40 mL) was heated at reflux. After 3 h, the reaction was cooled to room temperature, filtered, and concentrated in vacuo to give ethyl-2-[2-(4-fluorobenzoyl)pyridin-3-yloxy]acetate (3.5 g, 11.5 mmol). ^{1}H NMR (DMSO-d_6) δ: 1.25 (s, 3 H), 4.21 (q, J = 7.15 Hz, 2 H), 4.67 (s, 2 H), 7.13 (t, J = 8.70 Hz, 2 H), 7.28 (d, J = 6.74 Hz, 1 H), 7.39 (dd, J = 8.55 Hz, J = 4.61 Hz, 2 H), 7.94 (dd, J = 8.93 Hz, J = 5.47 Hz, 2 H), 8.34 (dd, J = 5.82 Hz, J = 1.2 Hz, 1 H).

This was added to a suspension of sodium ethoxide (1.51 g, 22.6 mmol) in toluene (25 mL). The reaction was heated at reflux under an argon atmosphere. After 12 h, the reaction was cooled to room temperature and the product was extracted into water. The aqueous layer was acidified with hydrochloric acid to give 2-carboxy-3-(4-fluorophenyl)-furo[3,2-b]pyridine as a solid (1.8 g).

A mixture of 2-carboxy-3-(4-fluorophenyl)-furo[3,2-b]pyridine as a solid (1.8 g, 7 mmol) and copper metal (0.56 g, 8.81 mmol) in quinoline (10 mL) was heated at reflux. After 45 min, the reaction was cooled to room temperature and the product was extracted into water. The aqueous solution was acidified with hydrochloric acid and acetic acid. The product was filtered and dissolved in ether. The ethereal solution was dried over sodium sulfate and concentrated in vacuo. Purification by flash chromatography using ethyl acetate−hexanes (1:9) as the eluent gave 3-(4-fluorophenyl)-furo[3,2-b]pyridine as a solid (0.85 g).^{1}H NMR (DMSO-d_6) δ: 7.21−7.13 (m, 2H), 7.29 (dd, J = 8.36 Hz, J = 4.72 Hz, 1 H), 7.80 (dd, J = 8.36 Hz, J = 1.33 Hz, 1 H), 8.70−8.02 (m, 2 H), 8.08 (s, 1 H), 8.65 (dd, J = 4.75 Hz, J = 1.32 Hz, 1 H). Ms (EI/CI) m/z: (M+H) 213.

A solution of 3-(4-fluorophenyl)-furo[3,2-b]pyridine (0.40 g, 1.84 mmol) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (0.33 g, 7.2 mmol) in tetrahydrofuran (15 mL) was cooled to −78 °C, *n*-butyllithium (1.1 mL, 2.5 M in hexanes, 2.7 mmol) was added, and the reaction mixture was allowed to warm to room temperature. After 1.5 h, the reaction was recooled to −78 °C and *n*-tributyltin chloride (0.5 mL, 1.84 mmol) was added. The reaction was warmed to 25 °C and then quenched with aqueous ammonium chloride. The product was extracted into ether and the ethereal layer dried over sodium sulfate and concentrated in vacuo. Purification by flash chromatography using hexanes, followed by ethyl acetate−hexanes (5:95) as the eluant gave 2-(*n*-tributyltin-3-(4-fluorophenyl)-furo[3,2-b]-pyridine (0.72 g).

A mixture of 2-(*n*-tributyltin)-3-(4-fluorophenyl)-furo[3,2-b]-pyridine (0.72 g, 1.4 mmol), tetrakis(triphenylphosphine)-palladium (II) (0.165 g, 0.14 mmol), and bromopyridine (1.4 g, 7.15 mmol) in xylenes (20 mL) was heated at reflux under argon atmosphere. After 12 h, the reaction mixture was cooled to room temperature and purified by flash chromatography eluting with 5% ethyl acetate in hexanes to give 3-(4-fluorophenyl)-2-(pyridin-4-yl)-furo[3,2-b]pyridine. Recrystallization from ethyl acetate−hexanes mixture gave 0.15 g of pure material. ^{1}H NMR (DMSO-d_6) δ: 7.39 (t, J = 8.90 Hz, 2 H), 7.51 (dd, J = 8.41 Hz, J = 4.69 Hz, 1 H), 7.57 (dd, J = 6.70 Hz, J = 1.66 Hz, 2 H), 7.68−6.64 (m, 2 H), 8.19 (dd, J = 8.41 Hz, J = 1.24 Hz, 1 H), 8.60 (dd, J = 4.68 Hz, J = 1.48 Hz, 1 H), 8.66 (dd, J = 4.56 Hz, J = 1.68 Hz, 2 H). Ms (EI/CI) m/z: (M+H) 290. Anal. ($C_{18}H_{11}N_2FO$) C,H,N.

2-(4-Fluoro-phenyl)-1-(1-hydroxy-pyridin-4-yl)-ethanone (36). To a solution of 2-(4-fluoro-phenyl)-1-pyridin-4-yl-ethanone (30 g, 0.14 mol) in methylene chloride (60 mL) was added methyltrioxorhenium (180 mg, 0.7 mmol). The mixture was stirred for 5 min, then hydrogen peroxide (30 mL, 30% aqueous solution) was added, and the mixture was stirred at room temperature for 18 h. The reaction was partitioned between 10% solution of sodium sulfate and ethyl acetate, the organic layer was separated, dried over sodium sulfate, and concentrated, and the residue was purified by flash chromatography eluting with methylene chloride:methanol 95:5 to afford 24 g of the desired product. ^{1}H NMR (DMSO-d_6) δ: 7.05 (t, J = 8.68 Hz, 2 H), 7.18−7.22 (m, 2 H), 7.82 (d, J = 7.36 Hz, 2 H), 8.22 (d, J = 6.36 Hz, 2 H).

4-[2-(4-Fluoro-phenyl)-1,1-dimethoxy-ethyl]-pyridin-1-ol (38). To a solution of the ketal (74 g, 283.2 mmol) and methyltrioxorhenium (400 mg, 1.6 mmol, from Strem Chemicals) in CH_2Cl_2 (150 mL), cooled below 10 °C, was added dropwise a 30% solution of hydrogen peroxide (100 mL, 1.16 mole) maintaining the temperature below 15 °C. After the addition was completed, the mixture was stirred at room temperature for 3−4 h and diluted with water. The organic phase was separated and added to a sodium sulfite solution (10%) maintaining the temperature between 20 and 30 °C (**caution:** low-temperature quenching does not destroy all the peroxide). The organic phase was washed with water, dried over sodium sulfate, filtered, and evaporated. The solid was triturated with hexane and filtered to afford pure *N*-oxide (76.8 g, 97.8% yield).

4-[1-(2-Chloro-pyridin-3-ylamino)-2-(4-fluoro-phenyl)-vinyl]-pyridin-1-ol (39). Procedure A. A mixture of 2-(4-fluoro-phenyl)-1-(1-hydroxy-pyridin-4-yl)-ethanone (24 g, 0.104 mol), 3-amino-2-chloropyridine (26.7 g, 0.208 mol), *p*-toluensulfonic acid (500 mg) in dicloroethane (210 mL) under argon was refluxed passing through a bed of sieves. After 12 h, the reaction mixture was washed with 5% sodium bicarbonate solution (75 mL) dried over sodium sulfate, and concentrated under vacuo, and the residue was purified by flash chromatography eluting with methylene chloride:methanol 97:3, to afford 11 g of 4-[1-(3-chloro-pyridin-4-ylimino)-2-(4-fluoro-phenyl)-ethyl]-pyridin-1-ol.

Procedure B. A solution of the *N*-oxide (32.95 g, 118.83 mmol) and 3-amino-2-chloropyridine (30 g, 233.4 mmol) in acetonitrile (300 mL) and chlorotrimethylsilane (50 mL, 393.96 mmol), under nitrogen, was heated at 65 °C for 4 h. After the reaction was completed, the mixture was concentrated under vacuum, the residue diluted with dichloromethane, and the suspension added to a mixture of dichloromethane and a solution of sodium carbonate (10% in water); the pH was maintained above 7 by addition of solid sodium carbonate. The organic phase was separated, washed with water, dried over sodium sulfate, filtered, and evaporated. The residue was suspended in *tert*-butyl methyl ether and filtered to give the enamine (28.8 g, 71% yield). ^{1}H NMR (DMSO-d_6) δ: 3.34 (s, 2H), 6.74 (d, J = 8.01 Hz, 1H), 7.03−7.07 (m, 1H), 7.16 (d, J = 8.77 Hz, 2H), 7.54 (d, J = 7.25 Hz, 2 H), 7.58−7.63 (m, 2 H), 7.71 (dd, J = 1.53 Hz, J = 3.05 Hz, 1 H), 8.19 (d, J = 6.87 Hz, 2 H).

2-(2-Chloro-pyridin-4-yl)-3-(4-fluoro-phenyl)-1H-pyrrolo-[3,2-b]pyridine (40). Procedure A. A mixture of 4-[1-(3-chloro-pyridin-4-ylimino)-2-(4-fluoro-phenyl)-ethyl]-pyridin-1-ol (11 g, 32.2 mmol), DABCO (10.83 g, 96.56 mmol), and dichlorobis (triphenylphosphine) palladium (1.13 g, 1.6 mmol) in DMF (160 mL) was warmed to 120 °C under argon for 3 h. The reaction was cooled to room temperature concentrated under vacuum and the residue was suspended in ethyl acetate, the solid was filtered and washed with ethyl acetate and methylene chloride:methanol 95:5, and then dried under vacuum to afford 10.1 g of 4-[3-(4-fluoro-phenyl)-1H-pyrrolo-[3,2-b]pyridin-2-yl]-pyridin-1-ol.

Procedure B. To a stirred suspension of enamine **39** (50.0 g, 126 mmol) and $(PPh_3)_2PdCl_2$ (3.6 g, 5.1 mmol) in 1 L of degassed EtCN was added $EtNiPr_2$ (30 mL, 172 mmol) and the resultant mixture was heated to 98−100 °C. The reaction mixture briefly becomes a homogeneous golden brown solution, and then gradually clouds as product **40** precipitates. After 5 h at 98 °C, the mixture was allowed to cool slowly to ambient temperature. The precipitated cream-colored product (a highly fluorescent compound) was collected by filtration, rinsed with MeCN (50 mL), and dried (50 °C, 27″ Hg with N_2 bleed, 72 h). ^{1}H NMR (DMSO-d_6) δ: 7.21−7.29 (m, 3 H), 7.46 (d, J = 7.36 Hz, 2 H), 7.54−7.58 (m, 2 H), 7.85 (dd, J = 1.43 Hz, J = 6.75 Hz, 1 H), 8.26 (d, J = 7.36 Hz, 2 H), 8.38 (dd, J = 1.45 Hz, J = 3.07 Hz, 1 H).

2-(2-Chloro-pyridin-4-yl)-3-(4-fluoro-phenyl)-1H-pyrrolo-[3,2-b]pyridine (41). The indole *N*-oxide (20.85 g, 68.29 mmol) in phosphorus oxychloride (160 mL, 1.72 mol) was heated, under nitrogen at reflux for 20 min, cooled, and the excess of reagent distilled off under vacuum.. The residue was loaded on silica gel (75 g) by dissolution in methanol and evaporation then chromathographed on silica gel (500 g) with increasing amounts of methanol (1, 2, 5, and 10%) in dichloromethane. Fractions containing the desired product were evaporated to give the final product (20 g, 90% yield). ^{1}H NMR (DMSO-d_6) δ: 7.14 (t, J = 8.83 Hz, 2 H), 7.20 (dd, J = 4.56 Hz, J = 8.24 Hz, 1 H) 7.35 (dd, J = 1.54 Hz, J = 3.73 Hz, 1 H) 7.52 (dd, J = 5.50 Hz, J = 8.85 Hz, 2 H) 7.60 (s, 1 H),) 7.83 (dd, J = 6.78 Hz, J = 1.45 Hz, 1 H) 8.29 (d, J = 5.28 Hz, 1 H) 8.47 (dd, J = 1.42 Hz, J = 3.12 Hz, 1 H) 11.73 (s, 1 H).

1-{4-[3-(4-Fluoro-phenyl)-1H-pyrrolo[3,2-b]pyridin-2-yl]-pyridin-2-ylamino}-propan-2-ol (42b). 2-(2-Chloro-pyridin-4-yl)-3-(4-fluoro-phenyl)-1H-pyrrolo[3,2-b]pyridine (5.55 g, 0.017 mol) was suspended in *S*-(+)-1-amino-2-propanol and the mixture was heated at 140 °C After 48 h, the reaction mixture was cooled to room temperature, concentrated under vacuum, and the residue was purified by column chromatography eluting with dichloromethane methanol 97:3, triturated with ethyl acetate, and filtered to afford 3.64 g of product ^{1}H NMR (DMSO-d_6) δ: 1.14 (d, J = 5.97 Hz, 3 H), 3.17−3.29 (m, 1 H), 3.29−3.35 (m, 1 H), 3.82−3.87 (m, 1 H,) 6.74 (dd, J = 1.48 Hz, J = 5.04 Hz, 1 H), 7.23−7.32 (m, 3 H), 7.37 (q, J = 4.67 Hz, 2 H), 7.91 (d, J = 6.62 Hz, 1 H), 8.45 (d, J = 3.34 Hz, 1 H), 12.67 (s,1 H). Ms (EI/CI) m/z: (M+H) 363. Anal. ($C_{22}H_{21}FN_4$ O·2 HCl·0.55 H_2O) C,H,N.

4-{4-[3-(4-Fluoro-phenyl)-1H-pyrrolo[3,2-b]pyridin-2-yl]-pyridin-2-ylamino}-butan-1-ol. (42a). ^{1}H NMR (DMSO-d_6) δ: 1.48−1.61 (m, 4 H), 3.38−3.45 (m, 4 H), 6.73 (d, J = 5.32 Hz, 1 H), 7.31−7.40 (m, 2 H) 7.55−7.60 (m, 2 H), 7.71−7.76 (m, 1 H), 7.93 (br, 1 H), 8.60−8.65 (m, 2 H), 14.14 (br, 1 H). Ms (EI/CI) m/z: (M+H) 377. Anal. ($C_{22}H_{21}FN_4O$·1HCl·3/2 H_2O) C,H,N.

1-{4-[3-(4-Fluoro-phenyl)-1H-pyrrolo[3,2-b]pyridin-2-yl]-pyridin-2-ylamino}-propan-2-ol (42c). ^{1}H NMR (DMSO-d_6) δ: 1.14 (d, 5.97 Hz, 3 H), 3.17−3.29 (m, 1 H), 3.29−3.35 (m, 1 H), 3.82−3.87 (m, 1 H,) 6.74 (dd, J = 1.48 Hz, J = 5.04 Hz, 1 H), 7.23−7.32 (m, 3 H), 7.37 (q, J = 4.67 Hz, 2 H), 7.91 (d, J = 6.62 Hz, 1 H), 8.45 (d, J = 3.34 Hz, 1 H), 12.67 (s,1 H). Ms (EI/CI) m/z: (M+H) 363. Anal. ($C_2H_{21}FN_4O$·2HCl) C,H,N.

1-{4-[3-(4-Fluoro-phenyl)-1H-pyrrolo[3,2-b]pyridin-2-yl]-pyridin-2-ylamino}-butan-2-ol. (42d). ^{1}H NMR (DMSO-d_6) δ: 3.14 (t, J = 7.38 Hz, 3 H) 1.26−1.46 (m, 2 H) 3.10−3.26 (m, 2 H) 3.50 (br, 1 H) 6.51 (d, J = 5.32 Hz, 1 H) 6.58 (t, J = 5.48 Hz, 1 H) 6.64 (s, 1 H) 7.19−7.25 (m, 2 H) 7.54−7.58 (m, 2 H) 7.82 (d, J = 6.92 Hz, 1 H) 7.96 (d, J = 5.32 Hz, 1 H) 8.38 (d, J = 3.17 Hz, 1 H) 11.84 (s, 1 H). Ms (EI/CI) m/z: (M+H) 377. Anal. ($C_{22}H_{21}FN_4O$·1 HCl·3/2 H_2O) C,H,N.

2-{4-[3-(4-Fluoro-phenyl)-1H-pyrrolo[3,2-b]pyridin-2-yl]-pyridin-2-ylamino}-propan-1-ol. (42e). ^{1}H NMR (DMSO-d_6) δ: 1.17 (d, J = 6.44 Hz, 3 H), 3.39−3.57 (m, 2 H), 4.01−4.04 (m, 1 H), 6.70 (d, J = 6.73 Hz, 1 H) 7.31−7.37 (m, 3 H), 7.55−7.59 (m, 3 H), 7.91 (d, J = 6.70 Hz, 1 H), 8.37 (d, J = 8.09 Hz, 1 H), 8.57 (d, J = 4.86 Hz, 1 H) 13.45 (br, 1 H). Ms (EI/CI) m/z: (M+H) 363. Anal. Calc. for $C_{21}H_{19}FN_4O$·2HCl·1/4 H_2O: C,H,N.

4-(3-(4-Fluorophenyl)-1H-pyrrolo-[3,2-b]-pyridin-2-yl)-pyridin-1-oxide (43) and 4-(3-(4-Fluorophenyl)-2-pyridin-4-yl-1H-pyrrolo-[3,2-b]-pyridin-4-oxide (44). To a room temperature slurry of azaindole starting material (1 g, 3.46 mmol) in 10 mL of dichloromethane was added 72% *m*-peroxybenzoic acid (3 g, 10.4 mmol). The reaction mixture immediately dissolved. On chilling the sample with an ice bath, the exothermic solution precipitated into a yellow slurry which was stirred at room temperature. After 2 h, the reaction was quenched with sodium bisulfite and potassium carbonate, filtered, and evaporated in vacuo. The residue was triturated several times with minimum dichloromethane, acetone, or methanol into a large volume of hexanes, then purified by flash chromatography (5−50% methanol/dichloromethane) to yield the three products shown: 108 mg of 4-pyridyl N1-oxide (**43**). ^{1}H NMR (DMSO-d_6) δ: 7.15 (t, J = 8.59 Hz, 2 H), 7.48 (dd, J = 8.69 Hz, J = 6.50 Hz, 1 H), 7.72 (d, J = 7.37 Hz, 2 H), 7.84−7.99 (m, 2 H), 8.06−8.09 (dd, J = 6.48 Hz, J = 0.76 Hz, 1 H), 8.26 (d, J = 7.34 Hz, 2 H), 8.44 (d, J = 7.34 Hz, 2 H), 9.50 (s, 1 H). Ms (EI/CI) m/z: (M+H) 305. 15 mg of azaindole N4-oxide (**44**), ^{1}H NMR (DMSO-d_6) δ: 7.21−7.29 (m, 3 H), 7.46 (d, J = 7.35 Hz, 2 H), 7.53−7.58 (m, 2 H), 7.87 (dd, J = 8.13 Hz, J = 1.39 Hz, 2 H), 8.26 (d, J = 7.31 Hz, 2 H), 8.39 (dd, J = 4.51 Hz, J = 1.31 Hz, 1 H), 12.09 (s, 1 H). Ms (EI/CI) m/z: (M+H) 305.

Crystallization and Structure Determination. The full-length amino acid coding sequence of p38 (SWISS−PROT entry sw_hum:mk14_human), as a BamHI−XhoI DNA fragment, was inserted in the BamHI−SalI sites of the pQE9 vector (Qiagen), adding a 6× HIS tag at the N-terminus. Bacterial strain M15[pREP4] (Qiagen) was transformed with the cloned vector and grown in LB media at 37 °C until the cell density reached an A_{600} of 0.7. The culture was induced with IPTG and incubated at 23 °C for 16 h. The harvested bacterial cells were lysed by sonication in 50 mM $NaPO_4$ pH 8.0, 0.3 M NaCl, 1 mM EDTA, 0.5 μg/mL aprotonin, 1 μg/mL leupeptin, and 0.5 μg/mL Pefabloc. The supernatant was applied onto a nickel affinity column (Chelating Sepharose Fast Flow, Pharmacia). Unbound protein was washed off the column with lysis buffer minus EDTA followed by a second wash with 20 mM imidazole in the same buffer. Bound proteins were eluted with a 6 column-volume gradient from 20 to 275 mM imidazole with p38 eluting halfway through the gradient. The pooled p38 peak was dialyzed into 20 mM Tris

HCI pH 8.0, 10 mM $MgCI_2$, and 5 mM DTT and subsequently purified by chromatography over Mono Q HR 10/10 (Pharmacia). Column elution was accomplished with a 30 column-volume gradient from 0 to 400 mM NaCl in the same buffer. p38 eluted in two peaks with only the first peak showing monodisperse behavior by light scattering. The first peak was concentrated in an Amicon Stirrer cell and YM30 membrane to 2 mg/mL. The protein was then diluted 50-fold with 20 mM Tris pH 8.0, 0.2 M NaCl, 10 mM $MgCl_2$, and 5 mM DTT and concentrated again to final concentration of 50 mg/mL. The concentrated protein was frozen in 50 μL aliquots using liquid nitrogen and stored at −80 °C. Reproducible protein crystallization was achieved at 17 °C with crystal micro seeding; hanging drops consisted of a 1 to 1 mixture of 32 mg/mL protein and mother liquor, 50 mM Hepes pH 7.6, 50 mM $CaCl_2$, and 17% PEG 3350. Single crystals were transferred into mother liquor baths containing 10−100 μM inhibitor for 8−24 h. The soaked crystals were briefly exposed to cryosolvent (24% glycerol, 17% Peg 3350) and mounted into a liquid nitrogen stream for data collection at −180 °C.

p38 crystallized in space group $P2_12_12_1$ as reported previously.[10] Structure solution via molecular replacement was straightforward. For the complex between p38 and **5**, refinement against 2.1 Å data was performed using XPLOR and REFMAC5.[28] The final working R-factor was 20.1%, and the free R-factor was 25.1%. Details of the data collection methods and statistics are reported along with the deposited structure coordinates as entry 1OZ1 in the Protein Data Bank.[29]

Biological methods. p38 MAP kinase Inhibition. In Vitro Assay. Inhibition of human recombinant active p38α [5 nM] was tested by measuring the incorporation of ^{33}P from γ-[^{33}P]ATP [50 μM, 1 μCi] into myelin basic protein (MBP) [35 μM]. The assay was performed in a 96-well microtiter plate.

In 40 μL reaction volume, 26 μL diluted p38α [5 nM] in assay dilution buffer (ADB) (20 mM MOPS pH 7.4, 40 mM $MgCl_2$, 1 mM DTT, 25 mM β-glycerol phosphate, 5 mM EGTA, 1 mM sodium orthovanadate) were preincubated at room temperature for 10 min with 4 μL of test compounds [0−10 μM], dissolved in dimethyl sulfoxide (DMSO) [10%].

The kinase reaction was initiated by the addition of 10 μL of assay mix, containing ADB and γ-[^{33}P]ATP [50 μM, 1 μCi] and MBP [35 μM].

After 30 min at 30 °C, the reactions were stopped by the removal of 25 μL of reaction sample onto 200 μL of 0.75% phosphoric acid (H_3PO_4) containing, acid prewetted Multiscreen 96-well phosphocellulose filter plate (Millipore).

The plate membranes were washed three times with 200 μL of 0.75% H_3PO_4 for the removal of the free radionucleotide.

Fifty microliters of Microscint-20 scintillant (Packard) was added to each well, the plate sealed with plastic film, and counted in a Packard Topcount microplate scintillation counter.

50% inhibitory value was calculated by fitting the data to the equation:

$$\text{fractional activity} = 1/[I]/IC50 + 1$$

Induction of TNF Biosynthesis. THP-1 cells were suspended in culture medium [RPMI (Gibco-BRL, Gailthersburg, MD) containing 15% fetal bovine serum, 0.02 mM 2-mercaptoethanol], at a concentration of 2.5×10^6 cells/mL and then plated in 96-well plates (0.2 mL aliquots in each well). Test compounds were dissolved in DMSO and then diluted with the culture medium such that the final DMSO concentration was 5%. Twenty-five microliter aliquots of test solution or only medium with DMSO (control) were added to each well. The cells were incubated for 30 min at 37 °C. LPS (Sigma, St. Louis, MO) was added to the wells at a final concentration of 0.5 μg/mL, and cells were incubated for an additional 2 h. At the end of the incubation period, culture supernatants were collected and the amount of TNFα present was determined by a specific trapping ELISA assay using two anti-TNFα antibodies (2TNF−H22 and 2TNF−H34) as described.[30]

Human blood from volunteer donors who are medication-free for at least 14 days was collected into siliconized vacutainers containing heparin (19 units/mL). Test compounds were prepared as stock solutions in DMSO (6 mM). Six subsequent 3-fold serial dilutions are performed in DMSO to create a compound concentration curve. Dilute samples 1/20 in RPMI to reduce DMSO concentration. Twenty-five microliter aliquots of each concentration were dispensed in triplicate to a 96-well plate. A total of 200 μL of human whole blood was added to each well and incubated at 37 °C for 30 min. Twenty-five microliters of lipopolysaccharide (LPS, E. coli 0127; B8, Cat. No. L3129, Sigma Chem Co) dissolved in tissue culture media to yield a final concentration of 0.5 μg/mL was added to all wells except media control wells. The plates were incubated overnight at 37 °C, 5% CO_2. The plates were centrifuged and plasma was collected and stored in polypropylene plates. IL-1β specific ELISA (Antibody Solutions, Palo Alto, CA) was performed according to manufacturer's specifications immediately or samples were stored at 4 °C until analyzed.

Rat LPS Assay. Female Wistar/Han (CRL:WI) Rats (Charles River, Hollister, CA) were acclimated for at least one week. At −30 min, groups containing 8 rats each were dosed orally either with the test compounds in an aqueous vehicle containing 0.9% sodium chloride, 0.5% sodium carboxymethyl-cellulose, 0.4% polysorbate 80, 0.9% benzyl alcohol (CMC vehicle) or with vehicle only (control group). At 0 h, the rats were injected intraperitoneally with 50 μg/kg of LPS (Sigma, St. Louis, MO). After 1.5 h, the rats were euthanized by CO_2 inhalation, and blood was drawn by cardiocentesis and subsequently separated by centrifugation at 15600*g* for 5 min. Serum TNFα was quantified using a ELISA kit (Biosource International, Camarillo, CA.)

Rat Phamacokinetic Study. Female Wistar/Han (CRL: WI) Rats (Charles River, Hollister, CA) weighing between 180 and 220 g were used. Animals were allowed free access to a standard laboratory chow and tap water, and were housed in a constant temperature-humidity environment. Three rats per dose regime were administered either single 10 mg/kg IV bolus doses (50% cyclodextran/water) or single 10 mg/kg oral suspension doses prepared in aqueous vehicle containing 0.9% NaCl, 0.5% sodium carboxymethyl cellulose, 0.4% polysorbate 80 and 0.9% benzyl alcohol. Blood was collected from each rat anesthetized with CO_2:O_2 (60:40) via the orbital sinus or cardiac puncture at 1, 3, 6, 8, and 24 h after dosing. Plasma levels of test compounds were assayed by a LC/MS method. In this method, an aliquot of plasma was treated by mixing with actonitrile to precipitate protein, centrifuged to clarify the supernatant, then further diluted with formate buffer (50 mM), and injected onto an HPLC. Test compounds were separated from endogenous interfering substances and subsequently eluted from the HPLC column for mass spectrometric quantification.

Microsomal Incubations. Nicotine adenine dinucleotide phosphate (NADPH)-dependent transformation was studied with concentrations of 100 μM of test compound. Incubations were conducted at 37 °C, and contained 2 mg/mL of liver microsomal protein in pH 7.4, 40 mM phosphate buffer containing 3 mM magnesium chloride and 1.0 mM NADPH. Aliquots of the incubations were taken at specific time points over a 30 min incubation period. To stop the reaction the timed aliquots were added to an equal volume of acetonitrile containing an internal standard (IS). The IS−acetonitrile solution denatured and precipitated the microsomal protein. The protein was separated by centrifugation and an aliquot of the resultant supernatants were assayed by a HPLC/UV method. Incubations prepared without the addition of NADPH acted as controls.

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