Journal of Medicinal Chemistry

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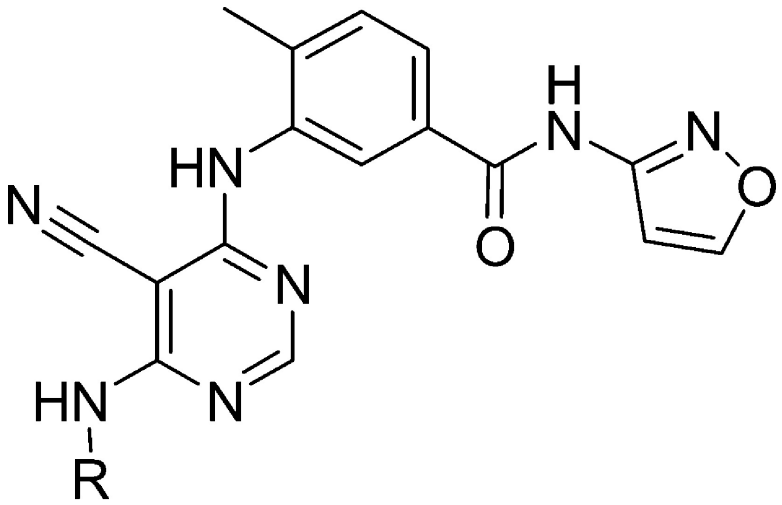
J. Med. Chem., **2005**, 48 (20), 6261-6270• DOI: 10.1021/jm0503594 • Publication Date (Web): 13 September 2005



R = isopropyl or cyclopentyl

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5-Cyanopyrimidine Derivatives as a Novel Class of Potent, Selective, and Orally Active Inhibitors of p38α MAP Kinase

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Received April 15, 2005

A novel class of 5-cyanopyrimidine-based inhibitors of p38α MAP kinase has been investigated. Analogues optimized through SAR iterations display low nanomolar enzymatic and cellular activity. The in vivo efficacy of this class of p38 inhibitors was demonstrated by **3a** and **3b** (>50% reduction in TNF levels when orally dosed at 5 mg/kg, 5 h prior to LPS administration in an acute murine model of inflammation). For **3a** and **3b**, the previously identified *N*-methoxybenzamide moiety (**1**) was replaced with *N*-(isoxazol-3-yl)benzamide, thereby providing increased metabolic stability. Cyanopyrimidine **3a** demonstrated 100% oral bioavailability in mouse. High p38 kinase selectivity versus over 20 kinases was observed for analogue **3b**. Direct hydrogen bonding of the cyano nitrogen of the 5-cyanopyrimidine core to the backbone NH of Met109 was confirmed by X-ray crystallographic analysis of **3a** bound to p38α.

Introduction

p38 mitogen-activated protein kinase (MAPK) is a member of a serine-threonine protein kinase family that also includes extracellular-regulated protein kinase (ERK) and c-Jun NH_2-terminal kinase (JNK). Members of this family have a homologous threonine-X-tyrosine amino acid sequence motif with X equal to glycine for p38, glutamic acid for ERK, and proline for JNK. A common feature of this family is that the activation of its members requires dual phosphorylation of threonine and tyrosine residues.[1] Four isoforms of p38 are known: p38α and p38β are widely expressed in eukaryotic cells including endothelial and inflammatory cells. Expression of p38γ is found in skeletal muscle, and p38δ is predominantly expressed in the small intestine, kidney, and lung tissue.[2] p38 MAP kinase plays a crucial role in regulating the biosynthesis of many inflammatory cytokines including tumor necrosis factor alpha (TNFα) and interleukin-1 (IL-1β).[3] Excessive production of TNFα and IL-1β is implicated in many inflammatory diseases.[4] The blockade of TNFα function by biological agents such as etanercept (Enbrel), a soluble TNFα receptor, and infliximab (Remicade), a TNFα antibody, is clinically proven to be effective in the treatment of rheumatoid arthritis, Crohn's disease, and psoriasis.[5] As a result, discovery and development of small molecule p38 MAP kinase inhibitors as orally active therapeutic agents for the treatment of inflammatory diseases has been a goal of numerous pharmaceutical research groups, and many classes of p38 MAP kinase inhibitors have been reported.[6]

We recently disclosed the discovery of a class of triaminotriazine aniline amides as novel p38 MAP kinase inhibitors[7] SAR modification led to the identification of **1** as a potent inhibitor of p38α (Figure 1). Triazine-based **1** was orally active in vivo and demonstrated unique H-bonding interactions relative to known p38 kinase inhibitors at the time. However, this compound is metabolically unstable, resulting in a poor pharmacokinetic (PK) profile. The pendant methoxyamide moiety proved to be the major cause of the in vivo metabolic instability.

X-ray crystallography revealed that the binding interactions of 1 and the p38α enzyme include three key hydrogen bonds and two hydrophobic interactions, as depicted in Figure 1. Two of the three hydrogen bonding interactions arise from the methoxyamide moiety of the inhibitor. There is a hydrogen bond between the methoxyamide carbonyl oxygen and the backbone NH of Asp168 and a hydrogen bond between the methoxyamide NH and the side chain carboxylate of Glu71. The third important hydrogen bond occurs between a triazine ring nitrogen and the backbone NH of Met109, through a bridged water molecule.

We were interested in modifying **1** so that the through-water hydrogen bond was replaced by a direct interaction between a cyano-substiututed pyrimidine group (e.g. **2**) and the Met109 backbone N−H. Successful displacement of active-site water molecules with a cyano functionality has been previously demonstrated with inhibitors of scytalone dehydratase[8] and epidermal growth factor receptor kinase.[9] In this paper, we present the synthesis and SAR of 5-cyanopyrimidine derivatives as inhibitors of p38α MAP kinase. Inhibitors such as **3a** and **3b** identified from this study not only showed efficacy in an acute murine model of inflammation, but also demonstrated appropriate metabolic and pharmacokinetic profiles for an orally active drug.

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10.1021/jm0503594 CCC: $30.25
Published on Web 09/13/2005

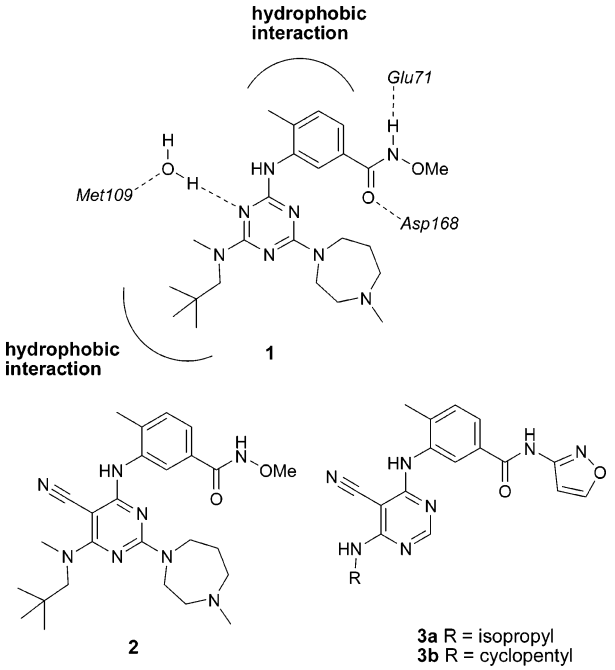
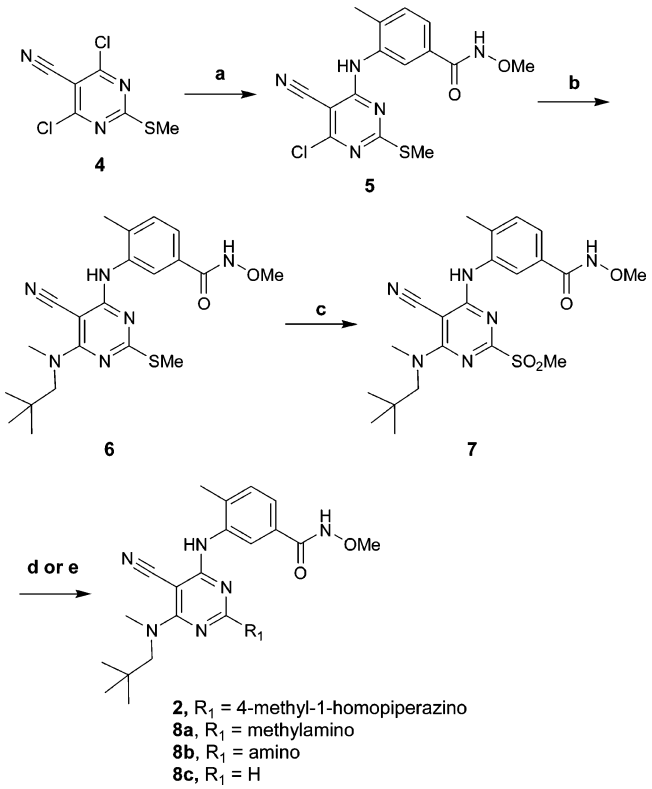


Figure 1.

Scheme 1[a]

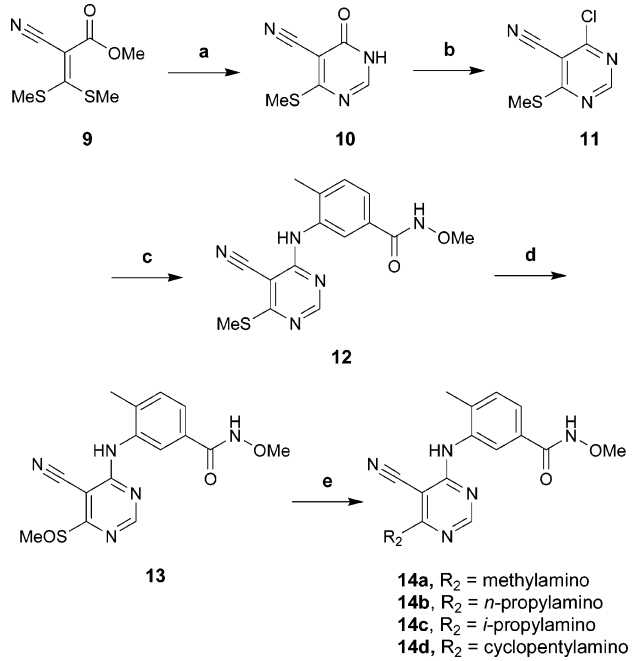


[a] Reagent and conditions: (a) *N*-methoxy-3-amino-4-methylbenzamide hydrochloride, (*i*-Pr)$_2$NEt, DMF, rt; (b) *N*-methylneopentylamine hydrochloride, (i-Pr)$_2$NEt, DMF, rt; (c) Oxone, H_2O/MeOH, rt; (d) appropriate amines, 50−70 °C; (e) LiBHEt$_3$, THF, −78 °C.

Chemistry

The synthesis of 5-cyanopyrimidine derivative **2** and its analogues **8a**−**c** are shown in Scheme 1. 5-Cyano-4,6-dichloro-2-methylthiopyrimidine (**4**), prepared according to a literature procedure,[10] was reacted with

Scheme 2[a]



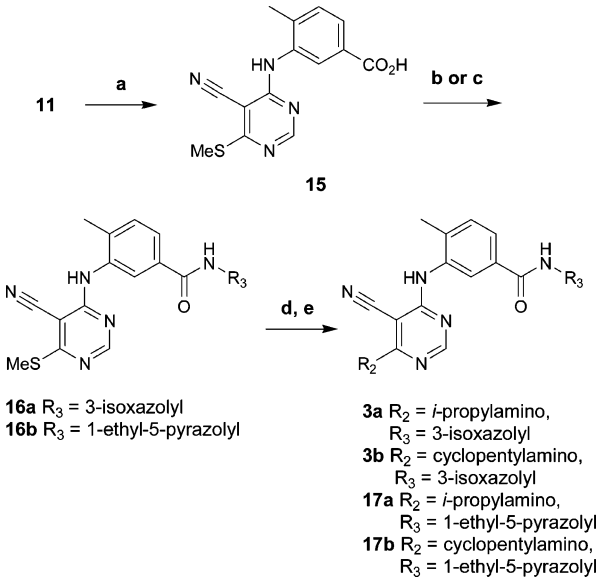
[a] Reagent and conditions: (a) formamidine hydrochloride, NEt$_3$, EtOH; (b) POCl$_3$, reflux; (c) *N*-methoxy-3-amino-4-methylbenzamide hydrochloride, (*i*-Pr)$_2$NEt, DMF, 65 °C; (d) Oxone, MeOH/H_2O, 0 °C; (e) appropriate amines, 1,4-dioxane, rt.

N-methoxy-3-amino-4-methylbenzamide hydrochloride[7] in the presence of Hünigs base at room temperature to afford **5**. Treatment of **5** with *N*-methylneopentylamine at room temperature provided **6**. Reaction of **6** with Oxone gave sulfone **7**, which served as a common precursor to compounds **2** and **8a**−**c** by either heating **7** with the corresponding amines (**2**, **8a**,**b**) or reduction with superhydride (LiBHEt$_3$) at −78 °C (**8c**).

Scheme 2 outlines the synthesis of **14a**−**d**. The cyanopyrimidine ring system **10** was constructed from commercially available methyl 3,3-bis(methylthio)-2-cyanoacrylate (**9**) and formamidine hydrochloride with triethylamine in refluxing ethanol.[11] Phosphorus oxychloride converted **10** into chlorocyanopyrimidine **11**. Heating **11** with *N*-methoxy-3-amino-4-methylbenzamide hydrochloride in the presence of Hünigs base in DMF supplied **12**. Oxidation of **12** using Oxone at 0 °C over 5 min resulted in the formation of sulfoxide **13**. However, if the oxidation was allowed to proceed for a longer period of time, **13** was further oxidized to the corresponding sulfone. This proved to be too reactive and unstable to be isolated. Compounds **14a**−**d** were readily prepared by reacting **13** with the corresponding amines at room temperature.

For the preparation of **3a**,**b** and **17a**,**b**, chlorocyanopyrimidine **11** was heated with 3-amino-4-methylbenzoic acid in DMF to give **15** (Scheme 3). The conversion of **15** to **16a** required generation of the acid chloride followed by treatment of 3-aminoisoxazole in the presence of pyridine. Amide **16b** could be obtained via a direct coupling reaction of the carboxylic acid **15** with 5-amino-1-ethylpyrazole in the presence of benzotriazole-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent). Conversion to the corresponding sulfones was carried out by treatment of **16a** and **16b** with *m*-chloroperbenzoic acid (mCPBA) in THF.

Scheme 3[a]



[a] Reagent and conditions: (a) 3-amino-4-methylbenzoic acid, DMF, 55 °C; (b) i. thionyl chloride, ii. 3-aminoisoxazole or 5-amino-1-phenylpyrazole, pyridine; (c), 5-amino-1-ethylpyrazole, BOP reagent, 1-methylmorpholine; (d) mCPBA, THF; (e) isopropylamine or cyclopentylamine, 1,4-dioxane, 150 °C, microwave.

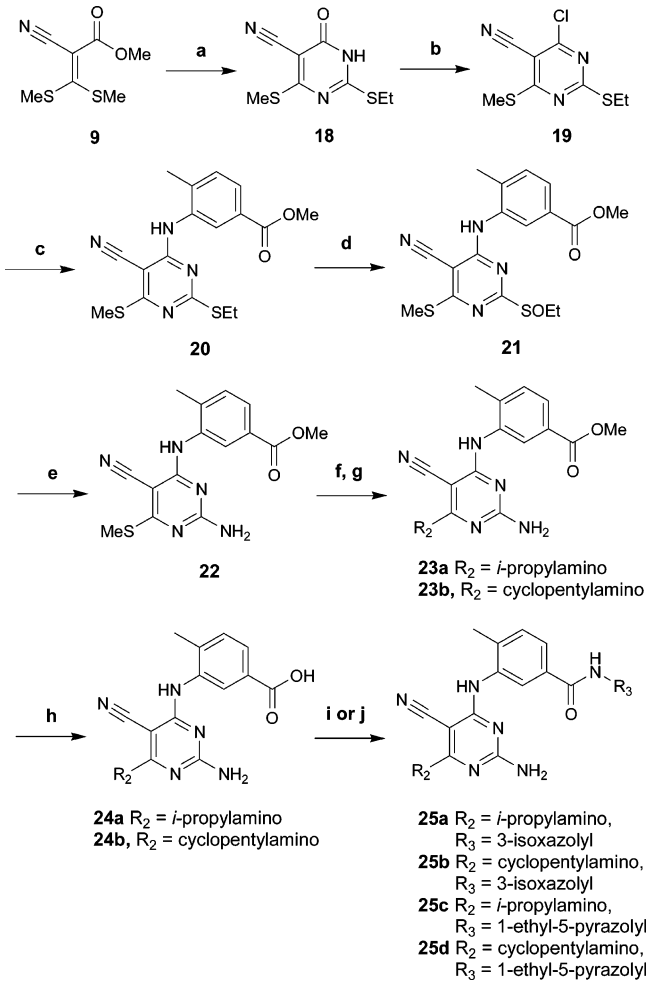
Subsequent reaction with isopropylamine or cyclopentylamine in 1,4-dioxane at 150 °C under microwave conditions completed the syntheses of **3a**,**b** and **17a**,**b**.

In the process of synthesizing **25a**−**d** (Scheme 4), the key intermediate **20** was prepared from commercially available **9** via an analogous sequence to that used for the synthesis of **12**, as previously described in Scheme 2. This intermediate contains both a 2-ethylthio and a 4-methylthio on the pyrimidine ring. The ethylthio group could be selectively oxidized to ethylthionyl with 1 equiv of mCPBA at 0 °C within 1 h.[12] The product **21** was heated with 2 N ammonia in isopropyl alcohol to provide **22**. The methyl sulfide **22** was then oxidized with excess mCPBA to the corresponding methyl sulfone and was subsequently converted, under microwave heated conditions, to **23a** and **23b** with isopropylamine and cyclopentylamine, respectively. Hydrolysis of esters **23a** and **23b** to the corresponding acids **24a** and **24b** was effected with LiOH at room temperature, with the cyano group remaining intact. The conversion of **24a** and **24b** to **25a**−**d** was accomplished either through the use of the BOP reagent or via an acid chloride intermediate in a manner similar to the transformation of **15** to **16a**,**b** in Scheme 3.

Results and Discussion

Compounds were evaluated for their ability to inhibit phosphorylation of substrate (myelin basic protein) by recombinant human p38α. A peripheral blood mononuclear cell assay (hPBMC) was used to measure the ability of compounds to inhibit LPS-induced TNFα production in human primary cells. Compound **2** was prepared to test the concept of replacing the water involved in the hydrogen bond to Met109 with the nitrogen of a nitrile. It was found that **2** displayed a K_i value of 0.057 nM versus 3.7 for **1** (60-fold difference) The cellular activity (TNFα IC_{50}) for **2** was 3.3 nM. However, **2** was cytotoxic to PHA blast cells (0% control

Scheme 4[a]

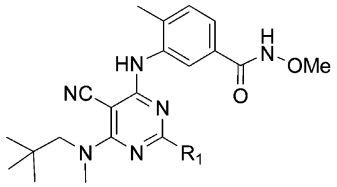


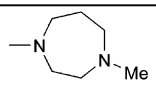
[a] Reagent and conditions: (a) 2-ethyl-2-thiopseudourea hydrochloride, $(i\text{-Pr})_2NEt$, EtOH, reflux; (b) $POCl_3$, reflux; (c) methyl 3-amino-4-methylbenzoate, DMF, 65 °C; (d) mCPBA (1.15 equiv), THF, 0 °C; (e) NH_3/*i*-PrOH, 65 °C; (f) mCPBA (excess), THF, rt; (g) isopropylamine or cyclopentylamine, 1,4-dioxane, microwave, 150 °C; (h) LiOH, THF/MeOH/H_2O, rt; (i) i. thionyl chloride, ii. 3-aminoisoxazole, pyridine; (j), 5-amino-1-ethylpyrazole, BOP reagent, 1-methylmorpholine, DMF.

after 24 h of treatment at 30 μM), and therefore the cellular activity of 3.3 nM was questionable. Also, similar to triaminotriazine **1**, cyanopyrimidine **2** was not stable to liver microsomes. To address these issues, an SAR study of **2**, focused on the replacement of the *N*-methyl-1-homopiperazino (R_1), *N*-methylneopentylamino (R_2), and methoxy (R_3), was systematically undertaken.

The possibility of replacing the *N*-methyl-1-homopiperazino group in **2** was first explored while maintaining the *N*-methylneopentylamino and *N*-methoxybenzamide functional groups. An important finding with the cyanopyrimidine scaffold was that the 1-homopiperazino group could be replaced with small amino groups such as methylamino and amino without significantly altering the p38α enzyme activity. Unlike in the triaminotriazine series, where an analogous modification resulted in a 4-fold reduction in enzyme activity, **8a** and **8b** displayed essentially the same p38 enzyme activity as **2**, as seen in Table 1. It was further discovered that the 1-homopiperazino group could be completely removed without a substantial loss of p38α

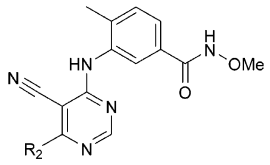
Table 1. In Vitro Activity of **2** and **8a**−**c**

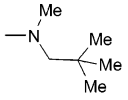
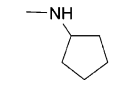


Compd	-R_1	P38α K_i (nM)[a]	hPBMC TNFα IC_{50} (nM)[b]
2		0.057	3.3[c]
8a	—NHMe	0.047	64
8b	—NH_2	0.050	17
8c	—H	0.15	42

[a] $n = 4$, variation in individual values, <20%. [b] $n = 3$, variation in individual values, <25%. [c] Cytotoxic @ 30 micromolar.

Table 2. In Vitro Activity of **14a**−**d** versus that of **8c**



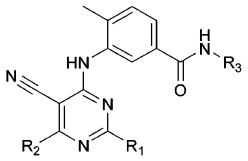
Compd	-R_2	P38α K_i(nM)[a]	hPBMC TNFα IC_{50} (nM)
8c		0.15	42
14a	—NHMe	16	> 250
14b	—$NHPr^n$	0.97	158
14c	—$NHPr^i$	0.61	4.6
14d		0.42	4.1

[a] $n = 4$, variation in individual values, <20%. [b] $n = 3$, variation in individual values, <25%.

activity to afford **8c** (p38α K_i = 0.15 nM). The IC_{50} values of **8a**, **8b**, and **8c** in inhibiting LPS induced TNFα production in cells were 64, 17, and 42 nM, respectively. Furthermore, **8a**, **8b**, and **8c** were found not to be cytotoxic (≥73% control after 24 h of treatment at 30 μM). It appeared that the observed cytotoxicity seen with **2** was due to the presence of the basic 1-homopiperazino group.

Table 2 contains SAR results for R_2 while maintaining R_1 as hydrogen and R_3 as methoxy. The *N*-methylneopentylamino group on **2** is expected to occupy a hydrophobic pocket. Replacement of this group with a much smaller, less hydrophobic methylamino group led to a 100 fold less potent p38α inhibitor **14a**. The cellular IC_{50} of **14a** was greater than 250 nM. The use of *n*-propylamino in place of methylamino improved the p38α K_i to 0.97 nM (**14b**). The cellular activity for this compound was also improved (IC_{50} = 158 nM) compared to **14a**. α-Branched alkyl or cycloalkylamines such as *i*-propylamino (**14c**) and cyclopentylamino (**14d**) resulted in good enzymatic (K_i = 0.61 nM and 0.40 nM, respectively) and cellular (IC_{50} = 4.6 nM and 4.1 nM, respectively) potencies. These analogues not only reduced the overall hydrophobicity and molecular weight but also improved the cellular potency by 8−9-fold. This

Table 3. In Vitro Activity of **3a**,**b**, **17c**,**d**, and **25a**−**d** versus that of **14c**,**d**



Compd	-R_1	-R_2	-R_3	P38α Ki (nM)[a]	hPBMC TNFα IC_{50} (nM)
14c	—H	—$NHPr^i$	—OMe	0.61	4.6
14d	—H		—OMe	0.41	4.1
3a	—H	—$NHPr^i$		0.41	8.7
3b	—H			1.6	3.5
17c	—H	—$NHPr^i$		15	130
17d	—H			14	72
25a	—NH_2	—$NHPr^i$		0.057	18
25b	—NH_2			0.16	1.5
25c	—NH_2	—$NHPr^i$		1.5	67
25d	—NH_2			1.9	21

[a] n = 4, variation in individual values, <20%. [b] n = 3, variation in individual values, <25%.

was not the case with our previous triaminotriazine series, where a tertiary amino of R_2 was essential for cellular activity.

After identifying the appropriate replacements for R_1 and R_2, a study to replace the *N*-methoxybenzamide moiety with more stable functionalities was undertaken. For this SAR study, R_1 was fixed as hydrogen or amino, and R_2 was selected from either isopropylamino or cyclopentylamino. As with our previous triaminotriazine series,[7] the replacement of *N*-methoxybenzamide with *N*-alkylbenzamide in the cyanopyrimidine series caused significant losses in both p38 enzyme and cell activity (data not shown). However, *N*-(isoxazol-3-yl)benzamide analogues were found to be highly potent p38α inhibitors and demonstrated very good cell activity. As seen in Table 3, **3a** was comparable to **14c** in both the p38α enzyme and cellular assays, with the p38α K_i = 0.41 nM and the TNFα IC_{50} = 8.7 nM. Similarly, **3b** had enzymatic and cellular activities (p38α K_i = 1.6 nM; TNFα IC_{50} = 3.5 nM) comparable to that of **14d**. Compounds **25a** and **25b** differ from **3a** and **3b** due to the introduction of an amino group at R_1. As expected, **25a** and **25b** were very potent p38 inhibitors (p38α K_i = 0.057 nM and 0.16 nM, respectively) and were effective in cells (TNFα IC_{50} = 18 nM and 1.5 nM, respectively). 1-Ethyl-5-pyrazolyl was found not to be

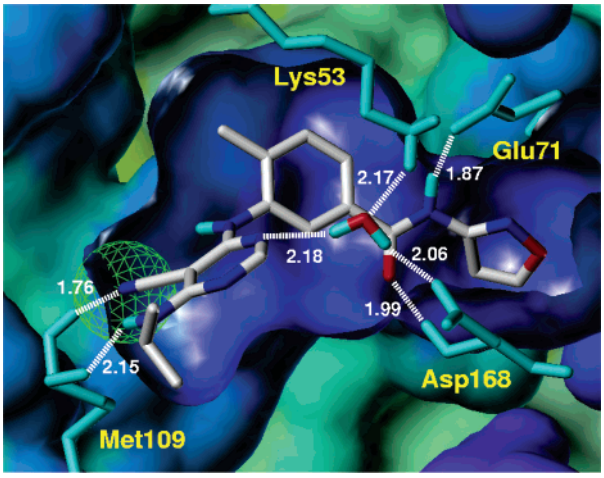


Figure 2. Binding interactions between **3a** and unphosphorylated p38α based on X-ray crystallographic analysis. Hydrogen bond distances are given in angstroms. The green sphere denotes the location of the water molecule found in the p38-bound triaminotriazine **1**.

as good as 3-isoxazolyl for the replacement of methoxy, as judged by the activities of **17c** (p38α K_i = 15 nM; TNFα IC_{50} = 130 nM) and **17d** (p38α K_i = 14 nM; TNFα IC_{50} = 72 nM), which were 24- and 34-fold less active in the enzyme assay, and 28- and 18-fold less active in the cellular assay, respectively, compared to **14c** and **14d**. However, the p38α enzyme activity of **17c** and **17d** could be improved by changing R_1 from hydrogen to amino, as shown by **25c** (p38α IC_{50} = 1.5 nM; TNFα IC_{50} = 67 nM) and **25d** (p38α IC_{50} = 1.9 nM; TNFα IC_{50} = 21 nM). It is noticed that generally, when R1 is amino, analogues containing cyclopentylamino as the R_2 group demonstrate improved cell activity compared to analogues where R_2 is *i*-propylamine, even though the enzyme activity for both compounds is similar. This is true for **25d** versus **25c**, and **25b** vs **25a**. This is not as pronounced when R1 is hydrogen (**17c** vs **17d** and **14c** vs **14d**).

Thus far we have described the identification and SAR of a novel series of in vitro potent, 5-cyanopyrimidine-based p38 inhibitors. To confirm our originally proposed binding mode for this series, an X-ray crystallographic study of **3a** bound to purified, unphosphorylated p38α was completed.[13] The binding interactions between **3a** and the p38α enzyme are illustrated in Figure 2. The proposed hydrogen bond between the cyano nitrogen of **3a** and the Met109 backbone NH of p38 is evident (1.76 Å). A secondary hydrogen bond between the *i*-propylamino NH and the Met109 carbonyl oxygen is observed (2.15 Å). The remaining interactions of **3a** with p38α remain similar to that of **1** as follows: The pendant amido group provides two more H-bonds with Asp168 (1.99 Å) and Glu71 (1.87 Å). Hydrophobic interactions include the angular methylaniline group of **3a** seated in a deep hydrophobic pocket, while the pendant *i*-propyl participates in a weaker hydrophobic interaction at the mouth of the binding site.

The in vivo activity of 5-cyanopyrimidine-based p38 inhibitors was demonstrated by assessment of **3a** and **3b** in an acute murine model of TNFα inhibition. Mice were dosed orally with **3a** and **3b** at 5 mg/kg, 5 h prior to LPS administration. The TNFα levels were measured 90 min later. As shown in Figure 3, **3a** and **3b** are both orally active, inhibiting the production of TNFα by 60% and 83%, respectively.

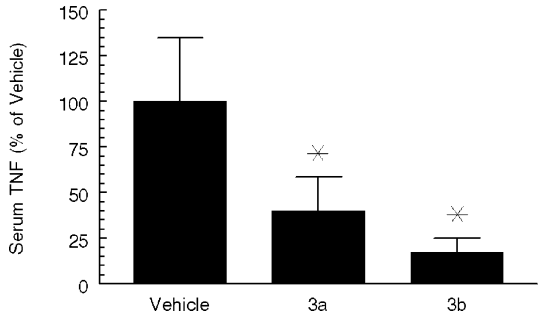


Figure 3. LPS-induced TNF inhibition by **3a** and **3b** in mouse. BALB/c female mice (Harlan), 6−8 weeks of age, were used. Compound was dissolved in poly(ethylene glycol) (MW = 300; PEG300) and administered to mice (n = 8/treatment) by oral gavage in a volume of 0.1 mL. Control mice received PEG300 alone ("Vehicle"). Five hours later, mice were injected intraperitoneally with 50 μg/kg lipopolysacchride (LPS; *E. coli* O111:B4; Sigma). Blood samples were collected 90 min after LPS injection. Serum was separated and analyzed for the level of TNF-α by commercial ELISA assay (BioSource) according to the manufacturer's instructions. Data shown are mean ± SD.

Table 4. Kinase Selectivity of **3b**

kinase	IC_{50} (μM)	kinase	IC_{50} (μM)
p38α	0.0041	IKK	>10
p38β	<0.1	JAK3	>50
p38γ	>30	Lck	>50
p38δ	>30	MEK	>25
Akt	>50	Met	>50
CaMKII	>50	MK2	>50
cdk2	>100	PKC	>50
Itk	>50	Raf	>30
ERK	>5	Syk	>50
FGF	>50	KDR	>30
GSK3	>50	HER1	>50
HER2	>50	PKA	>50
IGF-IR	>50		

To determine kinase selectivity, **3b** was tested for inhibitory activity versus over 20 varied kinases. The results are reported in Table 4. Compound **3b** did not show significant activity against any of the tested kinases except p38α and p38β. In most cases, the IC_{50} values were greater than 30−50 μM, indicating a selectivity of over 7000-fold. ERK is closely related to p38, and it was inhibited by **3b** with an IC_{50} of greater than 5 μM. This is over 1200-fold compared to its activity against p38α.

Additional profiling of **3a** indicates that it is not cytotoxic to PHA blast cells (82% of control after 24 h of treatment at 30 μM) and has excellent Caco-2 permeability (239 nm/s). Compound **3a** is stable in liver microsomes with a metabolic rate of 0.022 nmol/min·mg-protein in rats and 0.012 nmol/min·mg-protein in humans. Pharmacokinetic evaluation in mice revealed that **3a** has a $t_{1/2}$ of 1.7 h and a clearance of 1.2 L/h·kg after intravenous dosing. The oral bioavailability of **3a** was approximately 100% after administration of a solution dose in PEG400.

Conclusions

In conclusion, we have developed a series of 5-cyano-4,6-diaminopyrimidine derivatives as a novel class of p38α MAP kinase inhibitors. Optimized analogues such

as **3a** and **3b** possess potent in vitro and in vivo activities and display appropriate metabolic and pharmacokinetic profiles for an orally active drug. A representative example (**3b**) displays high kinase selectivity versus over 20 kinases. Finally, X-ray crystallographic analysis of **3a** bound to unphosphorylated p38α has confirmed the proposed binding interactions of these novel inhibitors.

Experimental Section

Chemistry. Proton and carbon magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded either on a Bruker Avance 400 or a JEOL Eclipse 500 spectrometer and are reported in ppm relative to the reference solvent of the sample in which they were run. HPLC and LCMS analyses were conducted using a Shimadzu LC-10AS liquid chromatograph and a SPD UV-vis detector at 220 nm with the MS detection performed with a Micromass Platform LC spectrometer. HPLC analyses were performed using the following conditions: Ballistic YMC S5 ODS 4.6 × 50 mm column with a binary solvent system where solvent A = 10% methanol, 90% water, 0.2% phosphoric acid and solvent B = 90% methanol, 10% water, and 0.2% phosphoric acid, flow rate = 4 mL/min, linear gradient time = 4 min, start %B = 0, final %B = 100. LCMS analyses were performed using the following conditions: Phenomenex 5 μm C18 4.6 × 50 mm column with a binary solvent system where solvent A = 10% methanol, 90% water, 0.1% trifluoroacetic acid and solvent B = 90% methanol, 10% water, and 0.1% trifluoroacetic acid, flow rate = 4 mL/min, linear gradient time = 2 min, start %B = 0, final %B = 100. Preparative reverse-phase HPLC purifications were performed using the following conditions: Ballistic YMC S5 ODS 20 × 100 mm column with a binary solvent system where solvent A = 10% methanol, 90% water, 0.1% trifluoroacetic acid and solvent B = 90% methanol, 10% water, and 0.1% trifluoroacetic acid, flow rate = 20 mL/min, linear gradient time = 10 min, start %B = 20, final %B = 100. Fractions containing the product were concentrated in vacuo to remove the methanol and neutralized with aqueous sodium bicarbonate. The products were collected by suction filtration or extracted with ethyl acetate and subsequent concentration.

All reagents were purchased from commercial sources and used without further purification unless otherwise noted. All reactions were performed under an inert atmosphere. Reactions run in aqueous media were run under an ambient atmosphere unless otherwise noted.

3-(6-Chloro-5-cyano-2-(methylthio)pyrimidin-4-ylamino)-*N*-methoxy-4-methylbenzamide (5). To a solution of *N*-methoxy-3-amino-4-methylbenzamide hydrochloride (0.27 g, 1.4 mmol) in DMF (2.4 mL) at room temperature was added diisopropylethylamine (0.19 mL, 1.1 mmol), and the mixture was stirred until homogeneous, then 4,6-dichloro-2-(methylthio)pyrimidine-5-carbonitrile (**4**) (0.30 g, 1.4 mmol), prepared according to a literature procedure,[10] was added. After 18 h at room temperature, the mixture was diluted with water (10 mL) and the solid was collected by vacuum filtration. The solid was washed with additional water and was dried in vacuo to afford **5** (0.37 g, 72% yield) as an off-white solid; 88% purity by HPLC; LCMS (EI) *m/z* Calcd for $C_{15}H_{15}ClN_5O_2S$ $[M + H]^+$ 364.06. Found: 364.32. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.75 (s, 1H), 10.11 (s, 1H), 7.71 (s, 1H), 7.63 (d, J = 1.4 Hz, 1H), 7.61 (d, J = 1.4 Hz, 1H), 3.70 (s, 3H), 2.24 (s, 3H), 2.22 (s, 3H).

3-(5-Cyano-6-(methyl(neopentyl)amino)-2-(methylthio)-pyrimidin-4-ylamino)-*N*-methoxy-4-methylbenzamide (6). To a mixture of **5** (0.31 g, 0.85 mmol) and *N*-methylneopentylamine hydrochloride (0.18 g, 1.3 mmol) in DMF (3 mL) at room temperature was added diisopropylethylamine (0.45 mL, 2.6 mmol). After stirring for 4 h at room temperature, the mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 × 30 mL). The combined extracts were diluted with hexanes (60 mL) and washed with water (3 × 10 mL) and brine (20 mL). After drying over anhydrous sodium sulfate, the solution was filtered and concentrated in vacuo to afford **6** (0.34 g, 93% yield) as a pale yellow solid; 96% purity by HPLC; LCMS (EI) *m/z* Calcd for $C_{21}H_{29}N_6O_2S$ $[M + H]^+$ 429.20. Found: 429.56; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.69 (br s, 1H), 8.43 (s, 1H), 7.46 (d, J = 7.9 Hz, 1H), 7.28 (s, 1H), 7.12 (s, 1H), 3.89 (s, 3H), 3.72 (s, 2H), 3.47 (s, 3H), 2.46 (s, 3H), 2.36 (s, 3H), 0.99 (s, 9H).

3-(5-Cyano-6-(methyl(neopentyl)amino)-2-(methylsulfonyl)pyrimidin-4-ylamino)-*N*-methoxy-4-methylbenzamide (7). To a solution of **6** (70 mg, 0.16 mmol) in methanol (1.75 mL) at room temperature was added a slurry of Oxone (0.40 g, 0.65 mmol) in water (0.75 mL), and the resulting mixture was stirred at room temperature for 16h, then concentrated in vacuo. The resulting residue was suspended in water (5 mL), and the solid was collected by vacuum filtration. After washing with additional water (3 × 3 mL), the solid was dried in vacuo to afford **7** (62 mg, 83% yield) of the title compound as a white solid; 88% purity by HPLC; LCMS (EI) *m/z* Calcd for $C_{21}H_{29}N_6O_4S$ $[M + H]^+$ 461.90. Found: 461.33. ^{1}H NMR (400 MHz, $MeOD_4$): δ 7.85 (d, J = 1.6 Hz, 1H), 7.62 (dd, J = 7.9, 1.6 Hz, 1H), 7.42 (d, J = 7.9 Hz, 1H), 3.83 (s, 2H), 3.82 (s, 3H), 3.56 (s, 3H), 3.08 (s, 3H), 2.34 (s, 3H), 1.04 (s, 9H).

3-(5-Cyano-6-(methyl(neopentyl)amino)-2-(4-methyl-1,4-diazepan-1-yl)pyrimidin-4-ylamino)-*N*-methoxy-4-methylbenzamide Trifluoroacetic Acid Salt (2). A solution of **7** (51 mg, 0.11 mmol) and *N*-methylhomopiperazine (0.04 mL, 0.33 mmol) in *N*-methylpyrrolidinone (0.25 mL) was heated at 70 °C for 3 h. After cooling to room temperature, the mixture was purified by reverse-phase preparative HPLC to afford the title compound (16 mg, 29%) as a pale yellow solid; 97% purity by HPLC; HRMS (ESI) *m/z* Calcd for $C_{26}H_{37}N_8O_2$ $[M - H]^+$ 493.3039. Found: 493.3052. ^{1}H NMR (400 MHz, $MeOD_4$): δ 8.26 (s, 1H), 7.32 (dd, J = 7.9, 1.7 Hz, 1H), 7.21 (d, J = 7.9 Hz, 1H), 3.76−3.58 (m, 4H), 3.70 (overlapping s, 3H), 3.60 (overlapping s, 2H), 3.31 (s, 3H), 2.64−2.49 (m, 4H), 2.28 (s, 3H), 2.24 (s, 3H), 1.91−1.79 (m, 2H), 0.89 (s, 9H).

3-(5-Cyano-6-(methyl(neopentyl)amino)-2-(methylamino)pyrimidin-4-ylamino)-*N*-methoxy-4-methylbenzamide (8a). A solution of **7** (50.0 mg, 0.109 mmol) in 2 M $MeNH_2$/THF (3.0 mL, 6.0 mmol) was heated at 50 °C in a sealed tube for 30 min and then concentrated under vacuum. The residue was subjected to preparative HPLC to afford **8a** (30.9 mg, 69% yield) as a white solid. 100% purity by HPLC; HRMS (ESI) *m/z* Calcd for $C_{21}H_{29}N_7O_2$ $[M + H]^+$ 412.2583. Found: 412.2455. ^{1}H NMR (500 MHz, DMSO-d_6, 80 °C): δ 11.3 (s, 1H), 7.87 (s, 1H), 7.46 (d, J = 7.7 Hz, 1H), 7.28 (d, J = 7.7 Hz, 1H), 6.65 (s, br., 1H), 3.70 (s, 3H), 3.67 (s, 2H), 3.33 (s, 3H), 2.70 (d, J = 4.4 Hz, 3H), 2.24 (s, 3H), 0.95 (s, 9H).

3-(2-Amino-5-cyano-6-(methyl(neopentyl)amino)pyrimidin-4-ylamino)-*N*-methoxy-4-methylbenzamide (8b). A solution of **7** (50.0 mg, 0.109 mmol) in 2 M NH_3/*i*-PrOH (3.0 mL, 6.0 mmol) was heated at 65 °C in a sealed tube for 30 min and then concentrated under vacuum. The residue was subjected to preparative HPLC to afford **8b** (29.5 mg, 68% yield) as a white solid. 100% purity by HPLC; HRMS (ESI) *m/z* Calcd for $C_{20}H_{27}N_7O_2$ $[M + H]^+$ 398.2526. Found: 398.2303. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.6 (s, 1H), 8.29 (s, 1H), 7.68 (s, 1H), 7.48 (d, J = 8.2 Hz, 1H), 7.28 (d, J = 8.2 Hz, 1H), 6.46 (s, 2H), 3.68 (s, 3H), 3.62 (s, 2H), 3.28 (s, 3H), 2.19 (s, 3H), 0.91 (s, 9H); ^{13}C NMR (500 MHz, DMSO-*d*6): δ 164.5, 164.4, 163.4, 161.3, 137.8, 137.6, 129.9, 129.8, 125.2, 123.7, 119.2, 62.8, 61.2, 59.9, 42.0, 34.0, 27.7, 17.7.

3-(5-Cyano-6-(methyl(neopentyl)amino)pyrimidin-4-ylamino)-*N*-methoxy-4-methylbenzamide (8c). To a solution of **7** (60.0 mg, 0.130 mmol) in THF (4 mL) at −78 °C was added super hydride ($LiBHEt_3$) (1.0 M in THF, 0.65 mL, 0.65 mmol, −78 °C) over 2 min. The mixture was stirred at −78 °C for 30 min before it was poured into water (40 mL). The resulting mixture was stirred at room temperature for 30 min and then extracted with AcOEt (3 × 30 mL). The combined extract was washed with brine and concentrated under vacuum. The residue was subjected to preparative HPLC to afford **8c** (31.7 mg, 64% yield) as a white solid. 100% purity

by HPLC; HRMS (ESI) *m/z* Calcd for $C_{20}H_{26}N_6O_2$ $[M + H]^+$ 383.2372. Found: 383.2194. ^{1}H NMR (500 MHz, DMSO-*d*6): δ 11.7 (s, 1H), 9.00 (s, 1H), 8.01 (s, 1H), 7.64 (s, 1H), 7.54 (d, J = 8.2 Hz, 1H), 7.33 (d, J = 8.2 Hz, 1H), 3.72 (s, 2H), 3.68 (s, 3H), 3.38 (s, 3H), 2.18 (s, 3H), 0.92 (s, 9H); ^{13}C NMR (500 MHz, DMSO-*d*6): δ 163.8, 163.2, 163.0, 157.6, 138.5, 137.1, 130.0, 129.9, 125.9, 124.4, 117.0, 69.1, 63.0, 60.4, 42.4, 34.0, 27.7, 17.6.

4-(Methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (10). A mixture of 3,3-bis(methylthio)-2-cyanoacrylate (**9**) (10.1 g, 49.8 mmol), formamidine hydrochloride (4.40 g, 54.6 mmol), and triethylamine (11.4 mL, 81.8 mmol) in ethanol (300 mL) was heated at reflux for 5 h and then concentrated under vacuum to dryness. To the residue was added water (30 mL), and the mixture was acidified with 6 N HCl to pH 1. The precipitating material was collected by suction filtration, washed with water, and dried over drierite under vacuum to afford **10** (7.64 g) as a white solid. This product was 85% pure by HPLC, but it was used in the next step without further purification.

4-Chloro-6-(methylthio)pyrimidine-5-carbonitrile (11). An initially heterogeneous mixture of **10** (7.64 g, 85%, 35.0 mmol) and $POCl_3$ (50 mL) was heated at reflux for 7 h, and then the excess $POCl_3$ was removed under vacuum. The residue was diluted with AcOEt and washed twice with cold water, saturated $NaHCO_3$ solution, and brine. The solution was dried over anhydrous $MgSO_4$ and concentrated under vacuum to dryness to afford **11** (7.74 g) as a yellow solid. This product was 85% pure by HPLC, but it was used in the next step without further purification.

3-(5-Cyano-6-(methylthio)pyrimidin-4-ylamino)-*N*-methoxy-4-methylbenzamide (12). A mixture of **11** (1.90 g, 90%, 9.21 mmol), *N*-methoxy-3-amino-4-methylbenzamide hydrochloride (2.87 g, 13.2 mmol), and *N*,*N*-diisopropylethylamine (2.3 mL, 13.2 mmol) in DMF (15 mL) was heated at 65 °C for 5 h. After cooling to room temperature the solution was poured into water (150 mL). The resulting mixture was basified with saturated $NaHCO_3$ solution to pH 9 and was extracted with AcOEt (4 × 80 mL). The combined extract was washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum. The reside was subjected to silica gel chromatography (85% AcOEt/hexane) to afford **12** (2.15 g, 53% yield from **9**) as a pale solid. 100% purity by HPLC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.7 (s, 1H), 9.75 (s, 1H), 8.44 (s, 1H), 7.62 (s, 1H), 7.61 (d, J = 8.4, 1H), 7.38 (d, J = 8.4 Hz, 1H), 3.70 (s, 3H), 2.59 (s, 3H), 2.18 (s, 3H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 173.8, 163.6, 160.6, 158.8, 139.7, 136.6, 130.9, 130.8, 127.0, 125.9, 114.1, 86.2, 63.6, 18.2, 12.7.

3-(5-Cyano-6-(methylsulfinyl)pyrimidin-4-ylamino)-*N*-methoxy-4-methylbenzamide (13). To a slurry of **12** (0.500 g, 1.52 mmol) in MeOH (15 mL) was added a solution of Oxone (3.74 g, 6.08 mmol) in water (15 mL) at 0 °C over 3 min. The mixture was stirred at 0 °C for 5 min and then neutralized with saturated $NaHCO_3$ to pH 8. The mixture was diluted with water (30 mL) and extracted with AcOEt (4 × 30 mL). The combined extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum to give crude **13** (0.310 g) as a pale yellow solid. This crude product was 60% pure by HPLC, but it was used in the next step without further purification. LCMS (EI) *m/z* Calcd. for $C_{15}H_{15}N_5O_3S$ $[M + H]^+$ 346.09. Found: 346.38.

3-(5-Cyano-6-(methylamino)pyrimidin-4-ylamino)-*N*-methoxy-4-methylbenzamide (14a). A solution of **13** (40 mg, 60%, 0.070 mmol) and 2 N methylamine/THF (0.17 mL, 0.34 mmol) in 1,4-dioxane (1.5 mL) was stirred at room temperature for 10 min and then concentrated under vacuum. The residue was subjected to preparative HPLC to provide **14a** (16 mg, 26% yield from **12**) as a white solid. 100% pure by HPLC. HRMS (ESI) *m/z* Calcd for $C_{15}H_{16}N_6O_2$ $[M + H]^+$ 313.1535. Found: 313.1411. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.7 (s, 1H), 9.12 (s, 1H), 8.07 (s, 1H), 7.60 (s, 1H), 7.57 (d, J = 7.8, 1H), 7.50 (d, J = 4.2 Hz, 1H), 7.34 (d, J = 7.8 Hz, 1H), 3.69 (s, 3H), 2.87 (d, J = 4.2 Hz, 3H), 2.18 (s, 3H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 163.8, 163.5, 162.3, 160.0, 139.6, 137.5, 130.7, 130.5, 126.9, 125.2, 115.6, 69.2, 63.6, 28.3, 18.3.

3-(5-Cyano-6-(propylamino)pyrimidin-4-ylamino)-*N*-methoxy-4-methylbenzamide (14b). A solution of **13** (50 mg, 60%, 0.087 mmol) and *n*-propylamine (36 μL, 0.44 mmol) in 1,4-dioxane (1.5 mL) was stirred at room temperature for 10 min and then concentrated under vacuum. The residue was subjected to preparative HPLC to provide **14b** (19 mg, 23% yield from **12**) as a white solid. 100% pure by HPLC. HRMS (ESI) *m/z* Calcd for $C_{17}H_{20}N_6O_2$ $[M + H]^+$ 341.1848. Found: 341.1716. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.7 (s, 1H), 9.10 (s, 1H), 8.04 (s, 1H), 7.60 (s, 1H), 7.59−7.55 (m, 2H), 7.34 (d, J = 7.9 Hz, 1H), 3.69 (s, 3H), 3.34 (m, 3H), 2.18 (s, 3H), 1.54 (m, 2H), 0.86 (t, J = 7.4 Hz, 3H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 163.8, 163.1, 162.5, 159.9, 139.5, 137.5, 130.7, 130.5, 126.9, 125.2, 115.6, 69.0, 63.6, 22.7, 18.3, 11.6.

3-(5-Cyano-6-(isopropylamino)pyrimidin-4-ylamino)-*N*-methoxy-4-methylbenzamide (14c). 14c was prepared in exactly the same manner as **14b**. White solid; 21% yield from **12**; 100% pure by HPLC. HRMS (ESI) *m/z* Calcd for $C_{17}H_{20}N_6O_2$ $[M + H]^+$ 341.1845. Found: 341.1713. ^{1}H NMR (500 MHz, DMSO-d_6, 80 °C) δ 11.4 (s, 1H), 8.05 (s, 1H), 7.64 (s, 1H), 7.55 (d, J = 7.7 Hz, 1H), 7.31 (d, J = 7.7 Hz, 1H), 6.83, (s, 1H), 4.36 (m, 1H), 3.70 (s, 3H), 2.20 (s, 3H), 1.20 (d, J = 6.6 Hz, 6H). ^{13}C NMR (500 MHz, DMSO-d_6): δ 163.3, 162.1, 161.7, 159.3, 138.9, 137.0, 130.1, 130.0, 126.3, 124.6, 115.0, 68.6, 63.0, 42.6, 21.9, 17.7.

3-(5-Cyano-6-(cyclopentylamino)pyrimidin-4-ylamino)-*N*-methoxy-4-methylbenzamide (14d). 14d was prepared in exactly the same manner as **14b**. White solid; 22% yield from **12**; 100% pure by HPLC. HRMS (ESI) *m/z* Calcd for $C_{19}H_{22}N_6O_2$ $[M + H]^+$ 367.2004. Found: 367.1874. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.7 (s, 1H), 9.05 (s, 1H), 8.03 (s, 1H), 7.58 (s, 1H), 7.54 (d, J = 7.7, 1H), 7.32 (s, J = 7.7 Hz, 1H), 4.41 (m, 1H), 3.67 (s, 3H), 2.16 (s, 3H), 1.90−1.86 (m, 2H), 1.68−1.62 (m, 2H), 1.58−1.49 (m, 4H).

3-(5-Cyano-6-(methylthio)pyrimidin-4-ylamino)-4-methylbenzoic Acid (15). A solution of **11** (5.00 g, 90%, 24.2 mmol) and 3-amino-4-methylbenzoic acid (4.57 g, 30.2 mmol) in DMF (35 mL) was heated at 65 °C for 20 h. After cooling to room temperature, the solution was poured in water (300 mL), and the resulting mixture was neutralized with saturated $NaHCO_3$ solution to pH 5. The precipitating material was collected by suction filtration, washed with water, and dried over drierite under vacuum to provide **15** (5.89 g, 60% yield from **9**) as a beige solid. 98% purity by HPLC; LCMS (EI) *m/z* Calcd for $C_{14}H_{12}N_4O_2S$ $[M + H]^+$ 301.07. Found: 301.05; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.9 (s, br., 1H), 9.71 (s, 1H), 8.43 (s, 1H), 7.77 (d, J = 7.7, 1H), 7.76 (s, 1H), 7.40 (d, J = 7.7 Hz, 1H), 2.58 (s, 3H), 2.19 (s, 3H).

3-(5-Cyano-6-(methylthio)pyrimidin-4-ylamino)-*N*-(isoxazol-3-yl)-4-methylbenzamide (16a). An initially heterogeneous mixture of **15** (2.50 g, 8.32 mmol) and thionyl chloride (50 mL) was stirred at room temperature for 1 h and then the excess thionyl chloride was evaporated under vacuum. The trace amount of thionyl chloride was removed by adding toluene (5 mL) to the residue and repeating the evaporation process. The residue thus obtained was dissolved in CH_2Cl_2 (100 mL), and to this solution was added 3-aminoisoxazole (1.23 mL, 16.6 mmol) and pyridine (1.68 mL, 20.8 mmol). The mixture was heated at reflux for 1 h, during which period a large amount of precipitate formed. The solvent was removed under vacuum, and to the residue was added water (60 mL). The insoluble material was collected by suction filtration, washed with water, and dried over drierite under vacuum to provide **16a** (2.34 g, 77% yield) as a pale yellow solid; 94% purity by HPLC; LCMS (EI) *m/z* Calcd for $C_{17}H_{14}N_6O_2S$ $[M + H]^+$ 367.09. Found: 367.11.

3-(5-Cyano-6-(methylthio)pyrimidin-4-ylamino)-*N*-(1-ethyl-1*H*-pyrazol-5-yl)-4-methylbenzamide (16b). A mixture of **15** (300 mg, 1.00 mmol), 5-amino-1-ethylpyrazole (333 mg, 3.00 mmol), BOP reagent (663 mg, 1.50 mmol), and *N*-methylmorpholine (0.49 mL, 4.46 mmol) in DMF (3 mL) was heated at 60 °C for 16 h. After cooling to room temperature, the mixture was poured into water (30 mL), and the resulting mixture was extracted with AcOEt (3 × 30 mL). The combined

extract was washed with water, 0.5 N AcOH aqueous solution, saturated $NaHCO_3$ solution, and brine. The solution was dried over $MgSO_4$, and concentrated to dryness under vacuum to provide **16b** (420 mg) as a yellow powder; 80% pure by HPLC; LCMS (EI) *m/z* Calcd for $C_{19}H_{19}N_7OS$ $[M + H]^+$ 394.14. Found: 394.04.

3-(5-Cyano-6-(isopropylamino)pyrimidin-4-ylamino)-*N*-(isoxazol-3-yl)-4-methylbenzamide (3a). To a solution of **16a** (0.300 g, 0.819 mmol) in THF (20 mL) was added mCPBA (57−86%, 0.650 g, 2.15−3.24 mmol) at room temperature in one portion. The mixture was stirred at room temperature for 6 h and concentrated under vacuum. The residue was stirred with 5% $Na_2S_2O_3$ (30 mL) at room temperature for 10 min, and the aqueous solution was removed by decantation. This process was repeated once. To the residue thus obtained was added saturated $NaHCO_3$ solution, and the insoluble product (sulfone intermediate) (0.389 g) was collected by suction filtration, washed with water, and dried over drierite under vacuum.

A mixture of the above obtained sulfone intermediate (0.120 g) and isopropylamine (0.11 mL) in 1,4-dioxane (1.8 mL) was heated at 150 °C under microwave for 15 min, and then concentrated under vacuum. The residue was subjected to preparative HPLC to afford **3a** (33.2 mg, 35% yield from **16a**) as a pale solid; 100% purity by HPLC; HRMS (ESI) *m/z* Calcd for $C_{19}H_{19}N_7O_2$ $[M + H]^+$ 368.1800. Found: 367.1661. ^1H NMR (400 MHz, DMSO-d_6) δ 11.4 (s, 1H), 9.11 (s, 1H), 8.85 (s, 1H), 8.06 (s, 1H), 7.93 (s, 1H), 7.87 (d, J = 8.1 Hz, 1H), 7.41 (d, J = 8.1 Hz, 1H), 7.22 (d, J = 8.1 Hz, 1H), 7.05 (s, 1H), 4.38 (m, 1H), 2.23 (s, 3H), 1.18 (d, J = 6.6 Hz, 6H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 165.0, 162.7, 162.2, 160.4, 158.4, 140.4, 137.6, 131.3, 130.7, 128.0, 126.3, 115.6, 100.2, 69.2, 42.7, 22.4, 18.4.

3-(5-Cyano-6-(cyclopentylamino)pyrimidin-4-ylamino)-*N*-(isoxazol-3-yl)-4-methylbenzamide (3b). 3b was prepared in the same manner as **3a**; Beige solid; 32% yield from **16a** 99% purity by HPLC; HRMS (ESI) *m/z* Calcd for $C_{21}H_{21}N_7O_2$ $[M + H]^+$ 404.1957. Found: 404.1815. ^1H NMR (500 MHz, DMSO-d_6) δ 11.3 (s, 1H), 9.08 (s, 1H), 8.83 (s, 1H), 8.04 (s, 1H), 7.92 (s, 1H), 7.85 (d, J = 8.2 Hz, 1H), 7.39 (d, J = 8.2 Hz, 1H), 7.31 (d, J = 7.1 Hz, 1H), 4.41 (m, 1H), 2.21 (s, 3H), 1.88 (m, 2H), 1.68 (m, 2H), 1.58−1.49 (m, 4H).

3-(5-Cyano-6-(isopropylamino)pyrimidin-4-ylamino)-*N*-(1-ethyl-1*H*-pyrazol-5-yl)-4-methylbenzamide (17a). 17a was prepared in the same manner as **3a**; Pale yellow solid; 6% yield from **16b** 100% purity by HPLC; HRMS (ESI) *m/z* Calcd for $C_{21}H_{24}N_8O_2$ $[M + H]^+$ 405.2273. Found: 367.2137.

3-(5-Cyano-6-(cyclopentylamino)pyrimidin-4-ylamino)-*N*-(1-ethyl-1*H*-pyrazol-5-yl)-4-methylbenzamide (17b). 17b was prepared in the same manner as **3a**; Pale solid; 8% yield from **16b** 100% purity by HPLC; HRMS (ESI) *m/z* Calcd for $C_{23}H_{26}N_8O$ $[M + H]^+$ 431.2430. Found: 431.2312.

2-(Ethylthio)-4-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (18). A mixture of **9** (10.0 g, 49.2 mmol), 2-ethyl-2-thiopseudourea hydrobromide (10.0 g, 54.0 mmol), and *N*,*N*-diisopropylethylamine (14.0 mL, 80.4 mmol) in ethanol (300 mL) was heated at reflux for 24 h and then concentrated under vacuum. To the residue was added water (60 mL), and the resulting mixture was acidified with 1 N HCl to pH 1. The insoluble material was collected by suction filtration, washed with water, and dried over drierite under vacuum to provide **18** (10.4 g) as a beige solid. This product was 65% purity by HPLC, but it was used in the next step without further purification.

4-Chloro-2-(ethylthio)-6-(methylthio)pyrimidine-5-carbonitrile (19). A mixture of **18** (3.50 g, 65%, 10.0 mmol) and $POCl_3$ (35 mL) was heated at reflux for 1.5 h and then concentrated under vacuum. The residue was dissolved in AcOEt (200 mL) and washed with cold water, saturated $NaHCO_3$ solution, water, and brine. The solution was dried over $MgSO_4$ and concentrated under vacuum. The residue was subjected to column chromatography (silica gel, 3% AcOEt/hexane) to afford **19** (1.72 g, 42% yield from **9**) as a yellow solid. 98% purity by HPLC; ^1H NMR (400 MHz, $CDCl_3$) δ 3.12 (q, J = 7.4 Hz, 2H), 2.58 (s, 3H), 1.35 (t, J = 7.4 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 175.5, 175.3, 161.1, 113.0, 100.6, 26.4, 25.6, 14.4, 13.7.

Methyl 3-(5-Cyano-2-(ethylthio)-6-(methylthio)pyrimidin-4-ylamino)-4-methylbenzoate (20). A mixture of **19** (1.78 g, 7.24 mmol) and methyl 3-amino-4-methylbenzoate (1.50 g, 8.90 mmol) in DMF (15 mL) was heated at 65 °C for 2 days and then diluted with AcOEt (300 mL). The resulting solution was washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum. Column chromatography (silica gel, 15% AcOEt/hexane) of the residue provided **20** (1.90 g, 70% yield) as a white solid; 98% purity by HPLC; ^1H NMR (400 MHz, DMSO-d_6) δ 9.68 (s, 1H), 7.85 (s, 1H), 7.80(d, J = 8.0 Hz, 1H), 7.44 (d, J = 8.0 Hz, 1H), 3.84 (s, 3H), 2.84 (q, J = 7.3 Hz, 2H), 2.56 (s, 3H), 2.23 (s, 3H), 1.04 (t, J = 7.3 Hz, 3H).

Methyl 3-(5-Cyano-2-(methylsulfinyl)-6-(methylthio)-pyrimidin-4-ylamino)-4-methylbenzoate (21). To a solution of **20** (1.65 g, 4.41 mmol) in THF was added mCPBA (1.14 g, ≤77%, ≤5.09 mmol) at 0 °C in portions over 3 min. The resulting mixture was stirred at 0 °C for 1 h and then concentrated under vacuum to the half of its original volume. The residue was diluted with AcOEt (200 mL) and washed with saturated $NaHCO_3$ solution (twice) and brine. The solution was dried over anhydrous $MgSO_4$ and concentrated under vacuum to dryness to give crude **21** (1.78 g) as a white solid; This product was 60% pure by HPLC, but it was used in the next step without further purification.

Methyl 3-(2-Amino-5-cyano-6-(methylthio)pyrimidin-4-ylamino)-4-methylbenzoate (22). A mixture of the above obtained crude **21** (1.78 g, 60%) and 2 N NH_3/*i*-PrOH (20 mL) was heated at 65 °C in a sealed tube for 1 h. After cooling to room temperature, product precipitated from the solution, and collected by suction filtration to afford the first crop of **22** (0.500 g, 34% yield) as a beige solid. The filtrate was concentrated and the residue was subjected to preparative HPLC to provide the second crop of **22** (0.156 g, 11% yield); 97% purity by HPLC; LCMS (EI) *m/z* Calcd for $C_{15}H_{15}N_5O_2S$ $[M + H]^+$ 330.09. Found: 330.05; ^1H NMR (400 MHz, DMSO-d_6) δ 8.99 (s, 1H), 7.77 (s, 1H), 7.75 (d, J = 7.8 Hz, 1H), 7.40 (d, J = 7.8 Hz, 1H), 7.13 (s, br., 2H), 3.84 (s, 3H), 2.50 (s, 3H), 2.21 (s, 3H).

Methyl 3-(2-Amino-5-cyano-6-(isopropylamino)pyrimidin-4-ylamino)-4-methylbenzoate (23a). To a solution of **22** (0.500 g, 1.52 mmol) in THF (15 mL) was added mCPBA (≤77%, 0.740 g, ≤3.30 mmol) at room temperature in one portion. The resulting mixture was stirred at room temperature for 5 h before it was diluted with AcOEt (200 mL) and washed with saturated $NaHCO_3$ solution (twice) and brine. The solution was dried over anhydrous $MgSO_4$ and concentrated under vacuum to dryness. To the residue (sulfone intermediate) were added 1,4-dioxane (5 mL) and isopropylamine (0.53 mL, 6.22 mmol). The mixture was heated at 150 °C under microwave for 15 min. It was then diluted with AcOEt, washed with water (three times) and brine, and dried over anhydrous $MgSO_4$. The evaporation of solvent under vacuum provided **23a** (0.520 g, 100% yield) as a pale solid; 93% purity by HPLC; LCMS (EI) *m/z* Calcd for $C_{17}H_{20}N_6O_2$ $[M + H]^+$ 341.16. Found: 341.10; ^1H NMR (400 MHz, DMSO-d_6) δ 8.46 (s, 1H), 7.79 (s, 1H), 7.69 (d, J = 8.0 Hz, 1H), 7.36 (d, J = 8.0 Hz, 1H), 6.48 (s, 2H), 6.44 (d, J = 8.3 Hz, 1H), 4.33 (m, 1H), 3.84 (s, 3H), 2.22 (s, 3H), 1.15 (d, J = 6.6 Hz, 6H).

Methyl 3-(2-Amino-5-cyano-6-(cyclopentylamino)pyrimidin-4-ylamino)-4-methylbenzoate (23b). 23b was prepared in exactly the same manner as **23a**; white solid; 65% yield; 99% purity by HPLC; LCMS (EI) *m/z* Calcd for $C_{19}H_{22}N_6O_2$ $[M + H]^+$ 367.18. Found: 367.31; ^1H NMR (500 MHz, DMSO-d_6) δ 8.43 (s, 1H), 7.77 (s, 1H), 7.68 (d, J = 8.2 Hz, 1H), 7.34 (d, J = 8.2 Hz, 1H), 6.52 (d, J = 7.7 Hz, 1H), 6.46 (s, 2H), 4.38 (m, 1H), 3.82 (s, 3H), 2.20 (s, 3H), 1.86 (m, 2H), 1.67 (m, 2H), 1.53−1.47 (m, 4H).

3-(2-Amino-5-cyano-6-(isopropylamino)pyrimidin-4-ylamino)-4-methylbenzoic Acid (24a). To a solution of **23a** (100 mg, 0.294 mmol) in THF (5 mL) and MeOH (2 mL) was added a solution of LiOH (25.0 mg, 1.05 mmol) in water (2 mL) at room temperature. The mixture was stirred at room

temperature for 4 h and then concentrated under vacuum. The residue was diluted with water (2 mL) and neutralized to pH 6 with 1 N HCl. The resulting precipitate was collected by suction filtration, washed with water, and dried over drierite under vacuum to provide **24a** (80.0 mg, 83% yield) as a white solid; 94% purity by HPLC; LCMS (EI) *m/z* Calcd for $C_{16}H_{18}N_6O_2$ $[M + H]^+$ 327.15. Found: 327.31; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.8 (s, 1H), 8.41 (s, 1H), 7.72 (s, 1H), 7.66 (d, J = 7.7 Hz, 1H), 7.31 (d, J = 7.7 Hz, 1H), 6.45 (s, 2H), 6.39 (d, J = 8.3 Hz, 1H), 4.31 (m, 1H), 2.19 (s, 3H), 1.14 (d, J = 6.6 Hz, 6H).

3-(2-Amino-5-cyano-6-(cyclopentylamino)pyrimidin-4-ylamino)-4-methylbenzoic acid (24b). 24b was prepared in exactly the same way as **24a**; White solid; 100% purity by HPLC; LCMS (EI) *m/z* Calcd for $C_{18}H_{20}N_6O_2$ $[M + H]^+$ 353.16. Found: 353.31; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.9 (s, br., 1H), 8.58 (s, br, 1H), 7.74 (s, 1H), 7.70 (d, J = 7.8 Hz, 1H), 7.35 (d, J = 7.8 Hz, 1H), 6.65 (s, br., 2H), 4.39 (m, 1H), 2.21 (s, 3H), 1.89 (m, 2H), 1.69 (m, 2H), 1.57−1.48 (m, 4H).

3-(2-Amino-5-cyano-6-(isopropylamino)pyrimidin-4-ylamino)-*N*-(isoxazol-3-yl)-4-methybenzamide (25a). Thionyl chloride (3 mL) was cooled to 0 °C and then added to **24a** (60 mg, 0.18 mmol). The resulting mixture was stirred at room temperature for 1 h. The excess amount of thionyl chloride was evaporated under vacuum. The trace amount of thionyl chloride was removed by adding toluene (1 mL) to the residue and repeating the evaporation process. The residue thus obtained was dissolved in CH_2Cl_2 (5 mL), and to this solution was added at 3-aminoisoxazole (0.040 mL, 0.59 mmol) and pyridine (0.037 mL, 0.46 mmol). The whole was heated at reflux for 1 h and then concentrated under vacuum. The residue was subjected to preparative HPLC to afford **25a** (51 mg, 71%yield) as a white solid; 100% purity by HPLC; HRMS (ESI) *m/z* Calcd for $C_{19}H_{20}N_8O_2$ $[M + H]^+$ 393.1909. Found: 393.1772.

3-(2-Amino-5-cyano-6-(cyclopentylamino)pyrimidin-4-ylamino)-*N*-(isoxazol-3-yl)-4-methylbenzamide (25b). 25b was prepared in the same manner as **25a**; White solid; 79% yield; 100% purity by HPLC; HRMS (ESI) *m/z* Calcd for $C_{21}H_{22}N_8O_2$ $[M + H]^+$ 419.2066. Found: 419.1955. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.3 (s, 1H), 8.79 (s, 1H), 8.36 (s, 1H), 7.87 (s, 1H), 7.74 (d, J = 7.7 Hz, 1H), 7.30 (d, J = 7.7 Hz, 1H), 6.99 (s, 1H), 6.47 (d, J = 7.7 Hz, 1H), 6.43 (s, 2H), 4.35 (m, 1H), 2.17 (s, 3H), 1.83 (m, 2H), 1.63 (m, 2H), 1.50−1.43 (m, 4H); ^{13}C NMR (500 MHz, DMSO-d_6) δ 164.6, 163.1, 163.0, 162.6, 159.7, 157.8, 139.4, 137.7, 130.6, 129.9, 127.0, 124.9, 117.2, 99.5, 60.7, 51.3, 31.8, 23.1, 17.9.

3-(2-Amino-5-cyano-6-(isopropylamino)pyrimidin-4-ylamino)-*N*-(1-ethyl-1*H*-pyrazol-5-yl)-4-methylbenzamide (25c). A mixture of **24a** (30 mg, 0.092 mmol), 5-amino-1-ethylpyrazole (35 mg, 0.32 mmol), BOP reagent (61 mg, 0.14 mmol), and *N*-methylmorpholine (0.045 mL, 0.41 mmol) in DMF (0.5 mL) was heated at 65 °C for 16 h. The mixture was diluted with AcOEt (40 mL), washed with water (twice) and brine, and dried over anhydrous $MgSO_4$. Purification by flash chromatography (silica gel, 80% AcOEt/hexane) provided **25c** (17 mg, 45% yield) as a white solid; 95% purity by HPLC; HRMS (ESI) *m/z* Calcd for $C_{21}H_{25}N_9O_2$ $[M + H]^+$ 420.2382. Found: 420.2262. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.1 (s, 1H), 8.42 (s, 1H), 7.84 (s, 1H), 7.71 (d, J = 7.7 Hz, 1H), 7.41 (d, J = 1.6 Hz, 1H), 7.36 (d, J = 7.7 Hz, 1H), 6.47 (s, 2H), 6.40 (d, J = 8.2 Hz, 1H), 6.18 (d, J = 1.6 Hz, 1H), 4.31 (m, 1H), 3.99 (q, J = 7.1 Hz, 2H), 2.21 (s, 3H), 1.29 (t, J = 7.1 Hz, 3H), 1.14 (d, J = 6.7 Hz, 6H); ^{13}C NMR (500 MHz, DMSO-d_6) δ 165.0, 162.9, 162.6, 162.5, 139.0, 137.6, 137.2, 135.0, 130.8, 129.9, 126.5, 124.6, 117.1, 100.7, 60.5, 42.4, 41.0, 22.0, 17.8, 14.5.

3-(2-Amino-5-cyano-6-(cyclopentylamino)pyrimidin-4-ylamino)-*N*-(1-ethyl-1*H*-pyrazol-5-yl)-4-methylbenzamide (25d). 25d was prepared in the same manner as **25c**; White solid; 45% yield; 96% purity by HPLC; HRMS (ESI) *m/z* Calcd for $C_{23}H_{27}N_9O$ $[M + H]^+$ 446.2539. Found: 446.2422. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.1 (s, 1H), 8.42 (s, 1H), 7.84 (s, 1H), 7.72 (d, J = 8.2 Hz, 1H), 7.41 (d, J = 1.6 Hz, 1H), 7.35 (d, J = 8.2 Hz, 1H), 6.52 (d, J = 7.7 Hz, 1H), 6.47 (s, 2H), 6.18 (d, J = 1.6 Hz, 1H), 4.39 (m, 1H), 3.98 (q, J = 7.2 Hz, 2H), 2.22 (s, 3H), 1.86 (m, 2H), 1.67 (m, 2H), 1.53−1.46 (m, 4H).

Generation of p38 Kinases. cDNAs of human p38 α, β isozymes were cloned using PCR technology. These cDNAs were subcloned in the pGEX expression vector (Pharmacia). GST-p38 fusion protein was expressed in *E. coli* and purified from bacterial pellets by affinity chromatography using gluthathione agarose. p38 fusion protein was activated by incubating with constitutively active MKK6. Active p38 was separated from MKK6 by affinity chromatography. Phosphorylated MKK6 was generated according to the literature.[13]

p38 Kinase Assay. The assay was performed in V-bottomed 96-well plates. The final assay volume was 60 μL which was from three 20-μL additions of enzyme, substrates (myelin basic protein (MBP) and ATP), and test compounds in assay buffer (50 mM Tris pH 7.5, 10 mM $MgCl_2$, 50 mM NaCl and 1 mM DTT). Bacterially expressed, activated p38 was preincubated with test compounds for 10 min prior to the initiation of reaction by adding substrates. The plates were incubated at room temperature for 45 min. The reaction was terminated by adding 5 μL of 0.5 M EDTA to each well. The reaction mixture was aspirated onto a prewet filtermat using a Skatron Micro96 Cell Harvester (Skatron), then washed with PBS. The filtermat was dried in a microwave oven for 1 min, coated with a layer of MeltilLex A scintillation wax (PerkinElmer), and counted on a Microbeta scintillation counter (Model 1450, PerkinElmer). The data were analyzed using the Prizm nonlinear least-squares regression (GraphPad Software). The final concentrations of reagents in the assays were [ATP], 1 μM; [[γ-^{33}P]ATP]], 3 nM, [MBP] (Sigma, M1891), 2 μg/well; [p38], 15 ng/well; [DMSO], 0.3%. This assay was used for initial determination of IC50 values for the compounds.

Alternate Enzyme Assay for Tight Binding Compounds. For K_i determinations, the p38 enzyme assay was modified to permit analysis of more potent compounds. Each reaction mixture consisted of a total volume of 240 μL and contained 1.07 ng/mL of the bacterially expressed recombinant p38-GST fusion protein, 66.7 μg/mL of myelin basic protein, 1 μM of ATP, and 2.4 μCi of [γ-33P]ATP (NEN). The mixtures also contained 20 mM HEPES, pH 7.4, 15 mM $MgCl_2$, 25 mM β-glycerophosphate, 0.1 mg/mL bovine serum albumin, and 1 mM DTT. The reaction mixtures were incubated at room temperature for 21 h, and kinase activity was determined by quantitation of the amount of radioactive phosphate transferred to myelin basic protein. The reactions were terminated by the addition of 60 μL of a solution of 50% trichloroacetic acid/250 mM sodium pyrophosphate. The samples were incubated on ice for 60 min to allow precipitation of the labeled substrate. The precipitated substrate was harvested using a Packard Harvester with a Unifilter-96, GF/C Filter Plate (Perkin-Elmer). Bound radiolabeled phosphate was quantitated using a TopCount 96-well liquid scintillation counter (Packard Instrument Co.). The concentration of the p38-GST fusion protein, 1.07 ng/mL, corresponds to a concentration of 15.9 pM of p38 enzyme. This concentration fell within the linear range of the enzyme assay. The enzyme assay run under these conditions maintained linearity over a range of 3.97 pM to 509 pM of p38. The p38 enzyme preparation assayed under these conditions retained full activity through the 21 h incubation time.

LPS-induced TNF Production in Human PBMC. Human PBMCs were isolated from whole blood collected from healthy donors. Blood was diluted into RPMI 1640 (Life Technologies) containing 2.5 mM EDTA (Life Technologies), 10 μg/mL polymyxin (Sigma), and then underlaid with ficoll (Accurate Scientific Co.) and centrifuged at 600*g* for 25 min. The interface was collected, and cells were washed twice and resuspended in RPMI, 10% FBS. Cells are then distributed (200 mL/well) into 96-well tissue culture treated plates (Falcon) at 1×10^6 cells/mL in RPMI, 10% FBS. Test compounds were added to appropriate wells and incubated with cells for 30 min. Cells were then stimulated by the addition of lipopolysaccharide (LPS, BioWhittaker), with a final concentration of 25 ng/mL, and incubated for 6 h at 37

°C, 5% CO_2. The cell supernatants were removed and assayed for TNF-α by ELISA (R&D Systems).

Inhibition of TNFα Release in Mice. BALB\c female mice, 6−8 weeks of age, were obtained from Harlan Laboratories and maintained ad libitum on water and standard rodent chow (Harlan Teklad). Mice were acclimated to ambient conditions for at least one week prior to use. For oral dosing, the compounds were prepared in a solution of 100% poly(ethylene glycol) (mw 300), and a dosing volume of 0.2 mL per mouse was administered by average 30 min prior to LPS injection (0.1 mL of LPS suspended at 10 μg/mL in PBS, administered ip). Blood samples were obtained 90 min after LPS injection. Serum was separated from clotted blood samples by centrifugation (5 min, 5000*g*, room temperature) and analyzed for levels of TNFα by ELISA assay (R&D Systems) according to the manufacturer's directions. Results are shown as mean ± SD of $n = 8$ mice per treatment group. All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee.

Cytotoxicity Assay. T cells in 1.25×10^6/mL human peripheral blood mononuclear cells (PBMC) isolated from normal volunteers were stimulated with 5 μg/mL phytohemaglutinin (PHA) for 4 d to generate dividing T cell blasts. The PHA blast cells at 1.5×10^6 per mL were incubated with 30 μM compound in RPMI media (Gibco) with 10% fetal bovine serum and a final dimethyl sulfoxide concentration of 0.003% for 24 h at 37 °C, 5% CO_2. Cytotoxicity was determined by the detection of metabolic activity using the AlamarBlue assay (Biosource).[14]

Metabolic Stability in Liver Microsomes. Incubations with rat and human liver microsomes (BD Gentest, Woburn, MA) were conducted at 1 mg/mL protein concentration, 3 μM compound in 56 mM phosphate buffer (pH 7.4), and 1 mM β-nicotinamide adenine dinucleotide phosphase (β-NADPH) at 37 °C for 1 h. Aliquots of incubation mixtures (0.2 mL) were taken at 0 and 15 min, and the reaction was quenched with one volume of acetonitrile. The rate of metabolism was calculated based on the fraction of parent disappearance by comparing 15 min to 0 min time points.

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JM0503594