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Design and Synthesis of Potent, Selective, and Orally Bioavailable Tetrasubstituted Imidazole Inhibitors of p38 Mitogen-Activated Protein Kinase

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Novel potent and selective diarylimidazole inhibitors of p38 MAP (mitogen-activated protein) kinase are described which have activity in both cell-based assays of tumor necrosis factor-α (TNF-α) release and an animal model of rheumatoid arthritis. The SAR leading to the development of selectivity against c-Raf and JNK2α1 kinases is presented, with key features being substitution of the 4-aryl ring with *m*-trifluoromethyl and substitution of the 5-heteroaryl ring with a 2-amino substituent. Cell-based activity was significantly enhanced by incorporation of a 4-piperidinyl moiety at the 2-position of the imidazole which also enhanced aqueous solubility. In general, oral bioavailability of this class of compounds was found to be poor unless the imidazole was methylated on nitrogen. This work led to identification of **48**, a potent (p38 MAP kinase inhibition IC_{50} 0.24 nM) and selective p38 MAP kinase inhibitor which inhibits lipopolysaccharide-stimulated release of TNF-α from human blood with an IC_{50} 2.2 nM, shows good oral bioavailability in rat and rhesus monkey, and demonstrates significant improvement in measures of disease progression in a rat adjuvant-induced arthritis model.

Introduction

Tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) are proinflammatory cytokines[1] produced in response to infection and other cellular stresses. Although TNF-α in appropriate amounts plays an important role in the host immune response, excess levels are thought to underlie a number of serious inflammatory diseases.[2,3] Recent clinical data, obtained with either chimeric TNF-α antibodies[4−7] or soluble TNF-α receptor[8] in the treatment of rheumatoid arthritis and Crohn's disease, has confirmed the key role of TNF-α in these inflammatory conditions. While the complete biochemical pathway for the production of TNF-α and IL-1 in response to inflammatory stimuli has yet to be elucidated, p38 MAP (mitogen-activated protein) kinase has been shown to play a role in this process.[9] p38 MAP kinase is one member of a group of serine−threonine kinases that are activated via dual phosphorylation of a TXY motif (TGY in the case of p38, TEY in ERK2, and TPY in JNK).[10] This phosphorylation is carried out by dual specificity kinases (MKK3 and MKK6[11] in the case of p38) in response to extracellular stimuli such as osmotic shock, endotoxin (lipopolysaccharide, LPS), UV light, or cytokines.[12] Previous work has demonstrated that small-molecule inhibitors of p38 MAP kinase such as SB203580 (**1**)[13] can reduce TNF-α levels.

Our goal was to develop selective small-molecule inhibitors of p38 MAP kinase that could be tested as a therapy for inflammatory conditions resulting from excess cytokine production. One obstacle to overcome is selectivity for p38 kinase inhibition over the vast number of other cellular kinases. The prototypical inhibitor of p38 kinase, **1**, which has shown activity in a number of animal models of rheumatoid arthritis[14] demonstrated limited selectivity in our kinase enzyme assays (Table 1), in contrast to reported data.[12] Specifically, significant inhibitions of c-Raf and JNK2α1 kinases were observed. In addition **1** was weak as an inhibitor of TNF-α production in human monocytes (IC_{50} 84 ± 11 nM, $n = 32$) and in human whole blood (IC_{50} 1000 ± 175 nM, $n = 2$). The key objectives for our effort were to increase selectivity, particularly with respect to c-Raf and JNK2α1, and to reduce the IC_{50} for inhibition of TNF-α in human whole blood, thereby minimizing required drug levels in a therapeutic setting. From a physical chemistry perspective, we sought to improve aqueous solubility of these inhibitors (**1**: 0.0019 mg/mL at pH 7.4, 0.043 mg/mL at pH 5).

In this paper we describe our investigations into the structure−activity relationship (SAR) of a series of imidazole inhibitors of p38 MAP kinase and the evolution of the aminopyridine derivative **48** as an extremely potent and selective inhibitor of p38 MAP kinase. **48** also demonstrates good oral bioavailability in two species, reduces LPS-stimulated production of TNF-α in human whole blood at low-nanomolar concentrations, and is active in an animal model of rheumatoid arthritis. The approach taken was to carry out separate optimiza-

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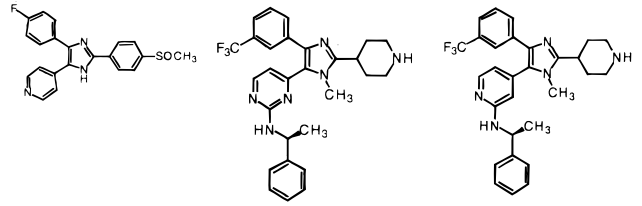
Table 1. Kinase Selectivity of Key Compounds[a]

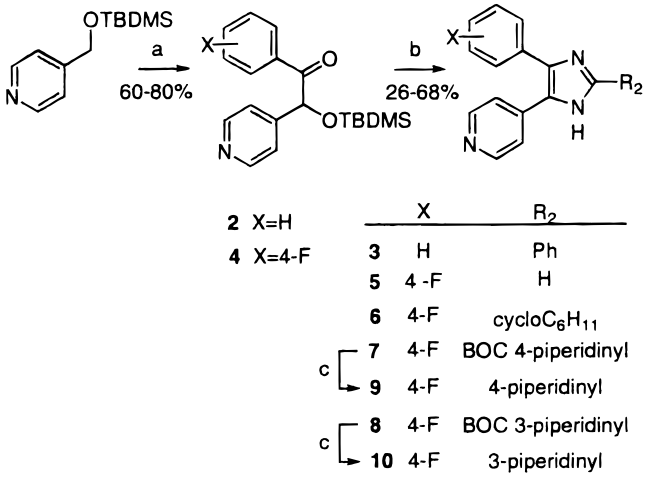


1 **39** **48**

	IC_{50} (*n*)		
kinase	**1**	**39**	**48**
p38[b]	39 ± 11 (70)	0.11 ± 0.046 (21)	0.19 ± 0.08 (29)
c-Raf[d]	330 ± 155 (7)	>1000 (2)	810 ± 100 (2)
JNK2α1[c]	290 ± 110 (4)	675 (1)	1170 ± 790 (2)
JNK2α2	1900 ± 28 (2)	1550 (1)	2200 ± 1030 (3)
Lck[d]	3450 ± 2000 (2)	>20000 (1)	>10000 (1)
EGF[d]	96000 ± 8000 (2)	>10000 (1)	>10000 (1)
MEK[d]	61000 ± 6000 (2)	>10000 (1)	>10000 (1)
PKA[d]	50000 (1)	>24000 (1)	>1500 (1)
PKC[d]	75000 ± 35000 (2)	>6000 (1)	>1500 (1)

[a] IC_{50} values for inhibition of kinases are in nM (±SD (*n*)). [b] Reference 32. [c] Determined as in ref 13, replacing p38 with JNK2α2 or JNK2α1 and using 2 μM ATP. [d] Carried out as described in the Experimental Section.

Scheme 1[a]



[a] Reagents: (a) LDA/THF then $XPhCON(CH_3)OCH_3$; (b) R_2CHO, $Cu(OAc)_2$, NH_4OAc, AcOH, reflux; (c) HCl, EtOAc, MeOH.

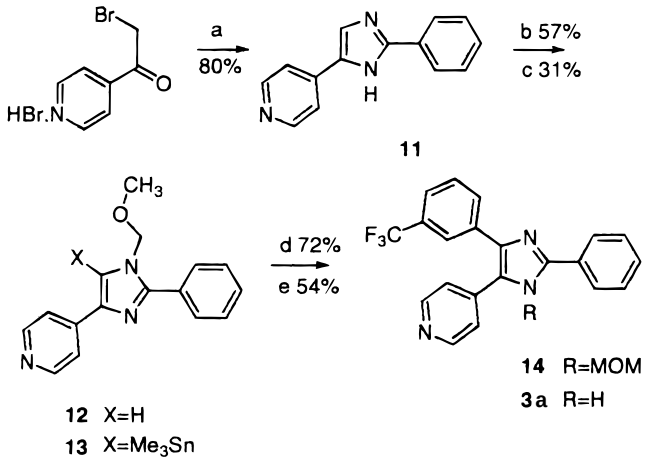
tion of each of the imidazole substituents independently and then establish their additivity.

Chemistry

2-Phenylimidazole derivatives were prepared by two different routes. In the first (Scheme 1), the anion of 4-[(*tert*-butyldimethysilyloxy)methyl]pyridine was condensed with an appropriate Weinreb amide,[15] and the resulting protected benzoins **2** and **4** were condensed with ammonium acetate and the desired aldehyde in acetic acid to give **3d**, **3g**, **3h**, **3k**, **3m**, **3p**, and **3q** (Table 2) and **5**−**8** (Table 4). HCl deprotection of **7** and **8** afforded piperidines **9** and **10**.

In a second approach which allows for ready variation of the 4-aryl substituent, a stannane coupling reaction was used as the key step (Scheme 2). 2-Phenyl-4-(4-pyridyl)imidazole (**11**) was prepared from 4-(bromoacetyl)-pyridine hydrobromide[16] and excess benzamidine in DMF. Protection of the imidazole as the methoxymethyl ether afforded a 3:1 mixture of regioisomers favoring

Scheme 2[a]



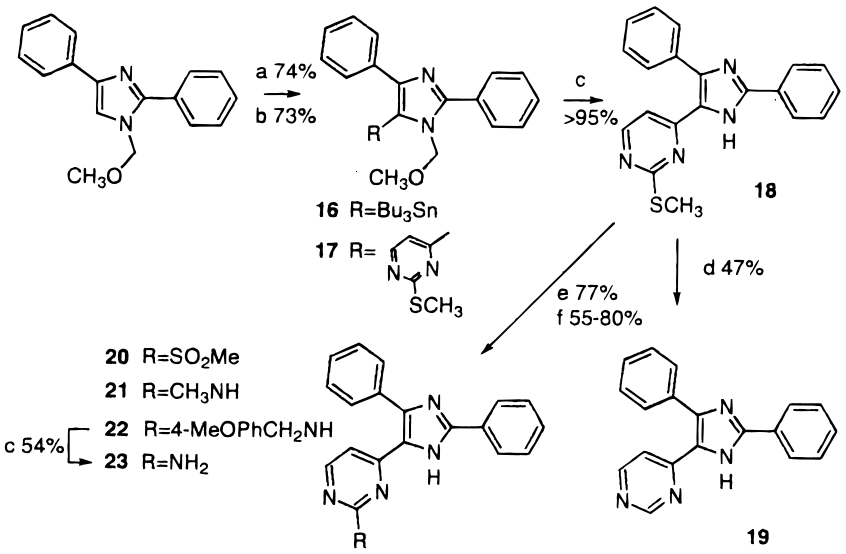
[a] Reagents: (a) $PhC(=NH)NH_2$, DMF, 40 °C; (b) NaH, THF, 0 °C then methoxymethyl chloride; (c) *n*-BuLi, THF, −78 °C then Me_3SnCl; (d) 3-CF_3PhI, DMF, $(Ph_3P)_4Pd$, 80 °C; (e) 6 M HCl, reflux.

the less hindered isomer **12**. Deprotonation at the 5-position with *n*-butyllithium and quenching with trimethyltin chloride afforded the stannane **13** which underwent smooth palladium-catalyzed coupling[17] with 3-iodo(trifluoromethyl)benzene to give **14** and deprotection with aqueous HCl to afford **3a**.

A related stannane coupling-based route was used for the preparation of 5-(4-pyrimidinyl)-2-phenylimidazole derivatives **19**−**23** (Scheme 3). Palladium-catalyzed coupling of the stannyl derivative **16** with 4-iodo-2-(methylthio)pyrimidine[18] and subsequent acid hydrolysis of **17** afforded **18**. Reductive removal of the methylthio substituent was then accomplished with Raney nickel to give the unsubstituted derivative **19**. Introduction of amino substituents was carried out by first oxidizing the sulfide **18** to the corresponding sulfone **20**, for which Oxone was found to be the most convenient reagent, followed by displacement with the desired amine. The parent amino-substituted compound **23** was then prepared by acid hydrolysis of the 4-methoxybenzylamino derivative **22**.

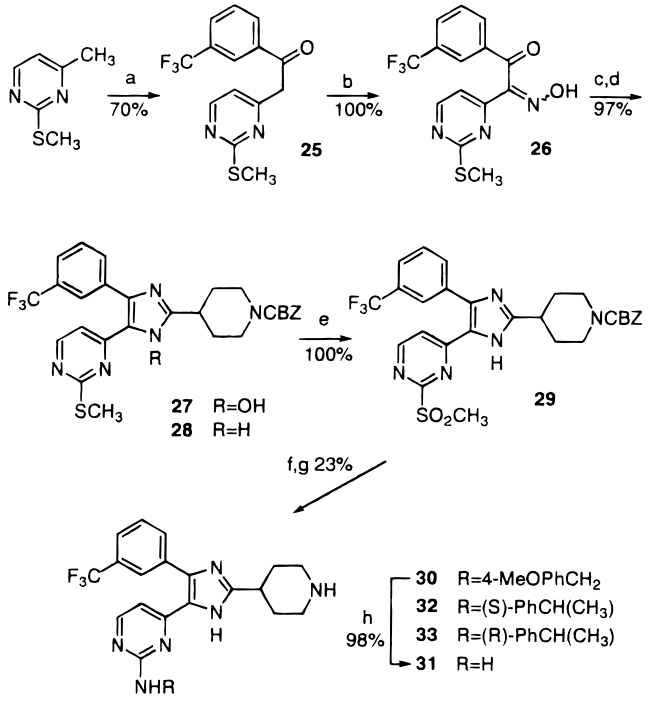
While a preparation of compounds combining the 2-piperidine and 5-pyrimidinyl substituents could be envisaged using chemistry related to that in Scheme 3, the necessary intermediate corresponding to **16** would require several synthetic steps to prepare. An alternative route which avoided tin chemistry and proved readily applicable to large-scale synthesis was developed (Scheme 4). Deprotonation of the methyl group of 4-methyl-2-(methylthio)pyrimidine[19] was readily carried out with LDA at −78 °C and the resulting anion quenched with the Weinreb amide **24** of 3-(trifluoromethyl)benzoic acid to provide the ketone **25**. α-Oximination was carried out in high yield by treatment with sodium nitrite[20] in an acetic acid, THF, water solvent system to afford a mixture of oxime isomers **26**. Both isomers participated in the key cyclization reaction when treated with CBZ piperidine-4-carboxaldehyde[21] and ammonium acetate in acetic acid at reflux, to afford the hydroxyimidazole **27**. Several methods are available for the reduction of hydroxyimidazoles, including $P(OMe)_3$,[22] Raney nickel-catalyzed hydrogenation,[23] and aqueous titanium trichloride.[24] Hydrogenation would be incompatible with the CBZ functionality and further

Scheme 3[a]



[a] Reagents: (a) *n*-BuLi, THF, −78 °C then Bu_3SnCl; (b) 4-iodo-2-(methylthio)pyrimidine, DMF, $(Ph_3P)_4Pd$, 80 °C; (c) 6 M HCl, reflux; (d) Raney Ni, EtOH, H_2O, reflux; (e) Oxone, MeOH, H_2O; (f) RNH_2, neat, 100 °C.

Scheme 4[a]



[a] Reagents: (a) LDA, THF, −78 °C then 3-$CF_3PhCON(CH_3)OCH_3$ (**24**); (b) $NaNO_2$, AcOH, THF, H_2O; (c) CBZ piperidine-4-carboxaldehyde, NH_4OAc, AcOH, reflux; (d) $TiCl_3$, MeOH, H_2O, reflux; (e) Oxone, MeOH, H_2O; (f) RNH_2, neat, 140 °C; (g) HBr, AcOH or H_2, Pd/C, *i*-PrOH; (h) 3 M HCl, reflux.

complicated by the presence of the (methylthio)pyrimidine. Treatment of the crude cyclization product **27** in methanol with an aqueous HCl solution of titanium(III) chloride proved rapid and high yielding. The 2-pyrimidinyl position of the product **28** was then activated toward nucleophilic displacement by oxidation to the sulfone **29** with Oxone in aqueous methanol. Displacement was carried out with different amines, if necessary in a sealed tube for volatile examples, and the CBZ group deprotected with HBr/AcOH or by hydrogenolysis to afford **30**, **32**, and **33**. The parent aminopyrimidine analogue **31** was conveniently prepared by treatment of the 4-methoxybenzyl derivative **30** with aqueous HCl at reflux.

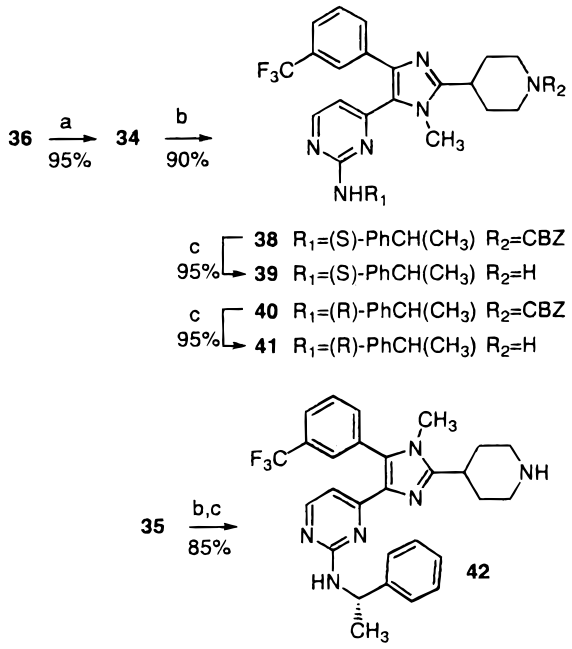
Initial attempts to prepare the corresponding *N*-methylimidazole derivatives focused on methylation of sulfone **29** using iodomethane and cesium carbonate in DMF. This provided the undesired regioisomer **35** with less than 5% of the desired product **34** (Table 3). The sulfide **28** under the same conditions yielded 25% of the desired isomer **36**, and this ratio was not altered by using methyl triflate as the electrophile. In contrast treatment of **28** with DMF dimethyl acetal in toluene at reflux formed predominantly the desired isomer **36**. Oxidation of **36** with Oxone, displacement of the sulfone **34** with amine, and deprotection afforded methylated analogues **39** and **41** (Scheme 5). In a similar manner, the regioisomer (**42**) of **39** was prepared from the other methylation product **37**.

The analogue **48**, in which the pyrimidine ring is replaced by pyridine, was prepared by an analogous route from 2-fluoro-4-methylpyridine (Scheme 6) with two significant differences. In the key cyclization step, only one of the oxime isomers was consumed when the reaction was carried out at 80 °C, and heating for sufficient time at higher temperatures in an effort to utilize the other isomer resulted in concomitant conversion of the desired product to the corresponding pyridone, presumably by nucleophilic displacement of the fluoropyridine by acetate and hydrolysis to the pyridone on workup. While sodium nitrite in aqueous acetic acid provided a 1:1 mixture of oxime regioisomers, use of *tert*-butyl nitrite in ethanol containing HCl[25] gave >90% of the more reactive isomer. In the second difference, attempted methylation of the imidazole ring of fluoropyridine gave <25% yield of the desired isomer, and the methylation was instead carried out on the amine displacement product **46**, which afforded 61% of the desired regioisomer **47**. Hydrogenolysis of the CBZ group then gave pyridine analogue **48**.

Biological Results and Discussion

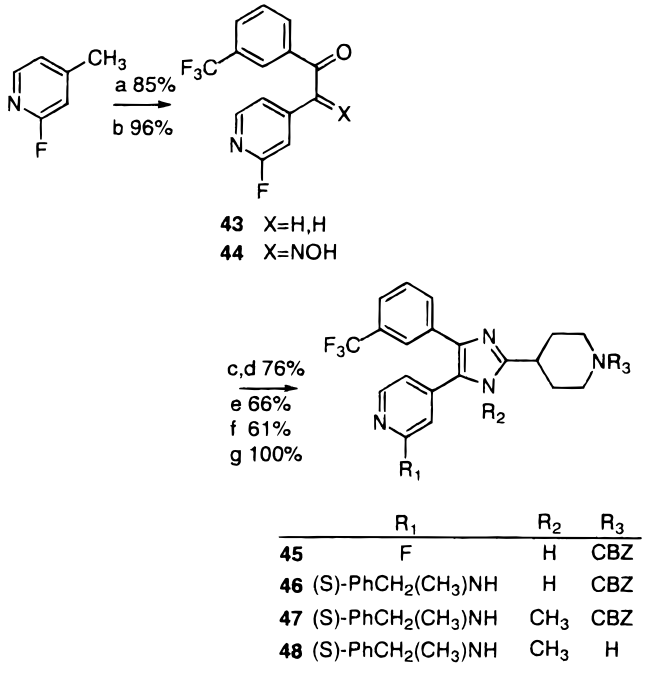
An aryl group at the 4-position was important for p38 kinase inhibition, with the 4-isopropyl analogue **3q**

Scheme 5[a]



[a] Reagents: (a) Oxone, MeOH, H_2O; (b) RNH_2, neat, 150 °C; (c) H_2, Pd/C, 2-propanol.

Scheme 6[a]

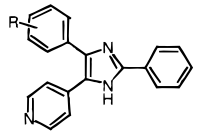


	R_1	R_2	R_3
45	F	H	CBZ
46	(S)-$PhCH_2(CH_3)NH$	H	CBZ
47	(S)-$PhCH_2(CH_3)NH$	CH_3	CBZ
48	(S)-$PhCH_2(CH_3)NH$	CH_3	H

[a] Reagents: (a) LDA, THF, −78 °C then 3-$CF_3PhCON(CH_3)OCH_3$ (**25**); (b) *t*-BuONO, EtOH, HCl; (c) CBZ piperidine-4-carboxaldehyde, NH_4OAc, AcOH, 80 °C; (d) $TiCl_3$, MeOH, H_2O; (e) RNH_2, neat, 150 °C; (f) Cs_2CO_3, MeI, DMF; (g) H_2, Pd/C, 2-propanol.

showing significantly reduced activity and the cyclohexyl compound **3p** showing no significant inhibitory activity (Table 2). While a number of small substituents were tolerated on the 4-phenyl, at the 2-, 3-, and 4-positions, our key goal at the time was to focus on pursuing selectivity. As a result we chose to focus on the 3-trifluoromethyl substituent present in **3a**, which provided a modest 2-fold increase in p38 potency but was accompanied by 30-fold selectivity over Raf rather than the somewhat more active compounds **3e** and **3n** which showed equal potency versus p38 and c-Raf.

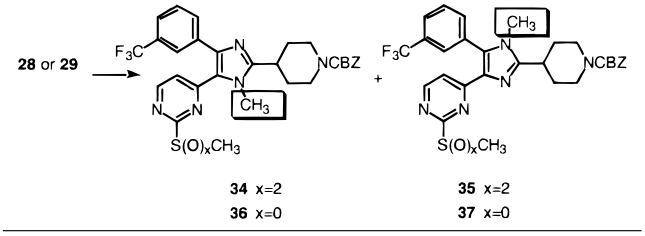
Table 2. 4-Position Substitution



compd	R	IC_{50} (nM) p38	IC_{50} (nM) c-Raf	prep method[a]	anal.
3a	3-CF_3	86	2600	B	C,H,N
3b	4-CF_3	200	1400	B	C,H,N
3c	2-Cl	150	610	B	C,H,N
3d	3-Cl	130	90	A	C,H,N
3e	4-Cl	48	56	B	C,H,N
3f	4-CN	970	86	B	C,H,N
3g	4-OCH_3	300	950	A	C,H,N
3h	4-HO	1400	390	A	C,H,N
3i	4-CO_2CH_3	>10000	320	B	C,H,N
3j	4-Ph	1000	2500	B	C,H,N
3k	3-HO	140	23	A	C,H,N
3l	3-F	495	3000	B	C,H,N
3m	3-OCH_3	550	210	A	C,H,N
3n	3,4-DiCl	28	20	B	C,H,N
3o	3,5-DiCl	137	1600	B	C,H,N
3p	cycloC_6H_{11}	>10000	570	A	C,H,N
3q	isopropyl	2100	>10000	A	C,H,N

[a] Method A: prepared according to Scheme 1. Method B: prepared according to Scheme 2.

Table 3. Regiochemistry of Methylation



starting material	conditions	product	ratios[a]
29	Cs_2CO_3, MeI, DMF	**34**, <5%	**35**, >95%
28	Cs_2CO_3, MeI, DMF	**36**, 25%	**37**, 75%
28	Cs_2CO_3, MeOTf, DMF	**36**, 25%	**37**, 75%
28	DMF dimethyl acetal	**36**, 71%[b]	**37**, 29%

[a] Ratios determined by HPLC. [b] Isolated yield after chromatography 65%.

Examining SAR at the 2-position (Table 4), the 2-unsubstituted compound **5** demonstrated that the 2-aryl moiety of **1** does not contribute significantly to potency or selectivity. Preparation of the cyclohexyl analogue **6** reinforced this and demonstrated tolerance for nonplanar groups at this position. Selectivity was not, however, improved by this modification. Based on this observation and the desire to generate compounds with improved aqueous solubility, the 4-piperidinyl analogue **9** was prepared and found to provide improved inhibition of p38 MAP kinase combined with enhanced selectivity. Preparation of the 3-piperidinyl derivative **10** demonstrated that the nitrogen position could be altered with only minor effects on activity.

In the third independent study of substituents, data from the 5-(4-pyrimidinyl) series of compounds (**19**, **21**−**23**) clearly demonstrated that c-Raf activity could be significantly reduced by utilizing the pyrimidine ring and that the inhibition of p38 MAP kinase was tolerant of a relatively large substituent on the 2-amino group,

Table 4. Kinase Inhibition Data

compd	IC_{50} (nM)			
	p38	c-Raf	JNK2α1	TNF-α whole blood
1	39	330	290	1000
3	180	380 ± 115 (2)	130 ± 0 (2)	ND
3a	86	2600 (1)	910 ± 50 (2)	>100000 (2)
5	170	1300 ± 280 (2)	195 ± 7 (2)	>10000 (2)
6	70	135 ± 7 (2)	29 ± 1 (2)	34 ± 1.4 (2)
9	9	80 ± 15 (2)	210 (1)	175 ± 7 (2)
10	19	110 ± 60 (2)	140 ± 14 (2)	585 ± 275 (2)
19	700	5000 ± 2900 (2)	1420 ± 340 (2)	>100000 (2)
21	120	2100 ± 1260 (4)	175 ± 7 (2)	>100000 (2)
23	57	750 ± 360 (2)	180 ± 0 (2)	>100000 (2)
22	38	1000 ± 280 (2)	98 ± 17 (2)	>100000 (2)
31	7.0	36% @ 1000 (2)	3300 ± 3600 (2)	190 (1)
30	7.6	66% @ 1000 (1)	735 ± 21 (2)	290 ± 0 (2)
32	0.75	3500 (1)	1750 (1)	6 ± 0.6 (2)
33	0.97	4500 (1)	295 (1)	0.4 (1)
41	1.5	48% @ 5000 (1)	300 (1)	50 ± 23 (4)
39	0.11 ± 0.046 (21)	>1000 (2)	675 (1)	4.3 ± 2.9 (12)
48	0.19 ± 0.08 (29)	810 ± 100 (2)	1170 ± 790 (2)	2.8 ± 1.5 (4)
42	1220	26% @ 5000 (1)	37% @ 40000	24000 ± 6000 (2)

an observation that would prove useful in modifying the physical properties of later compounds in order to gain oral bioavailability.

On the basis of the data from these three separate studies, a series of compounds (**30**−**33**) were prepared with the optimal features at the 2-, 4-, and 5-positions of the imidazole. The parent aminopyrimidine derivative **31** clearly demonstrated that not only were the incremental increases in p38 inhibition additive, but the improvements in selectivity were also combined to give greater than 100-fold selectivity versus both c-Raf and JNK2α1. Once again the 4-methoxybenzylamino substituent in **30** was tolerated, and (*R*)- and (*S*)-α-methylbenzylamino analogues **41** and **39**, respectively, were prepared based on the hypothesis that removing the electron-donating methoxy substituent and increasing steric congestion at the benzylic position would lead to reduced oxidative metabolic debenzylation in later in vivo studies. The (*S*)-enantiomer **32** inhibited p38 MAP kinase with an IC_{50} of 0.75 nM, without any concomitant increase in c-Raf or JNK2α1, resulting in a selectivity ratio of >1000. As demonstrated by the enantiomer **33**, the chirality of the methyl substituent has no significant effect on activity. Methylation of the imidazole, however, caused a significant difference in p38 MAPK kinase inhibition between enantiomers, with the (*S*)-enantiomer **39** being the more active p38 MAP kinase inhibitor (IC_{50} 0.11 nM) and the (*R*)-enantiomer **41** being 30-fold less active. The critical importance of the position of the methyl group on the imidazole was exemplified by the preparation of the regioisomer of **39** (**42**) which showed >20 000-fold reduced inhibition of p38 MAP kinase. The pyridine analogue **48** of pyrimidine **39** proved to have similar activity in the enzyme inhibition assay.

Cell-Based Assays of TNF-α Release. To select a compound for testing in animal models of rheumatoid arthritis, activity of the compounds in inhibiting the LPS-induced release of TNF-α from human whole blood was evaluated (Table 4). The shift of potency in this assay relative to the p38 kinase inhibition assay provides information on the varying cell permeability and protein binding of the compounds. For this reason there is not a simple relationship between IC_{50} in the p38 kinase inhibition assay and the whole cell data. The triaryl systems prepared (**3a**, **5**, **19**, **21**−**23**) all showed minimal inhibition of TNF-α production, which may be a consequence of the anticipated high protein binding of these largely planar compounds. Breaking up the planarity without large effects on lipophilicity can have a dramatic effect as shown by the cyclohexyl analogue **6** which has whole blood activity comparable to the kinase assay. The piperidine derivatives **9** and **10** show an approximately 20-fold shift in whole cells. The pyrimidine analogues **30**−**33**, **39**, and **41** showed a 1−80-fold range of attenuation in the whole cell assay.

Table 5. Pharmacokinetic Parameters of Selected p38 Kinase Inhibitors

compd	iv $t_{1/2}$ (min)	Vd_{sss} (L/kg)	Cl_{pl} (mL/min/kg)	po C_{max} (μM)	F (%)
31	7.9 ± 1.3	1.23 ± 0.07	167 ± 3.3	ND	0
32	1 ± 0.1	6.5 ± 0.7	146 ± 46	ND	0
39	294	12.7	38.3	0.78	64
48	171	12.3	63	0.39	85

Pharmacokinetics and Drug Metabolism. Initial screening of several compounds for both intravenous and oral pharmacokinetics was carried out in the rat (Table 5). The initial product of combining independent optimization studies, **31**, was evaluated and found to show a disappointingly short iv half-life and no oral bioavailability. The drug is cleared extremely rapidly, and HPLC analysis showed complete excretion of intact compound in both urine (80%) and bile (20%), possibly resulting from the low log *P* value of 0.39. A simple relationship between log *P* and oral bioavailability was ruled out in this series of compounds when it was found that the much more lipophilic **32** (log *P* > 3.9) showed the same profile of short intravenous half-life coupled with no oral bioavailability. In complete contrast, the methylated analogue **39** showed a highly desirable profile with a greatly increased iv $t_{1/2}$, coupled with good oral bioavailability. Compound **39** has an intermediate lipophilicity (log *P* 2.37), in the range commonly associated with improved prospects for oral bioavailability. The pyridine analogue **48** showed a similar pharmacokinetic profile (Figure 1) and lower clearance combined with a lower volume of distribution.

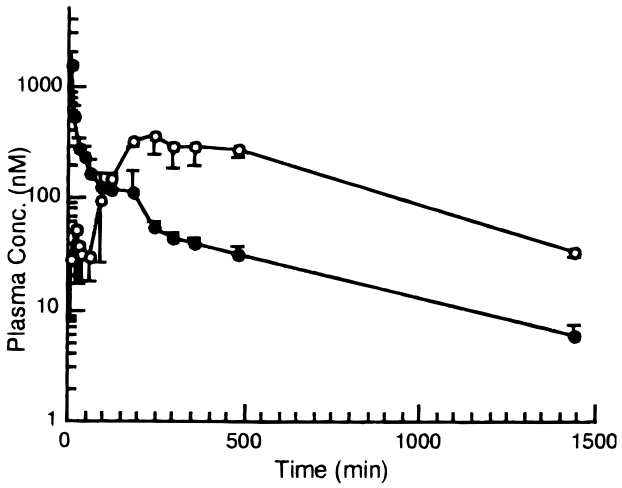


Figure 1. Mean plasma concentrations of **48** after iv (2 mg/kg, closed circles) or oral (10 mg/kg, open circles) administration; $n = 3$, ±SD dosed to male Sprague−Dawley rats.

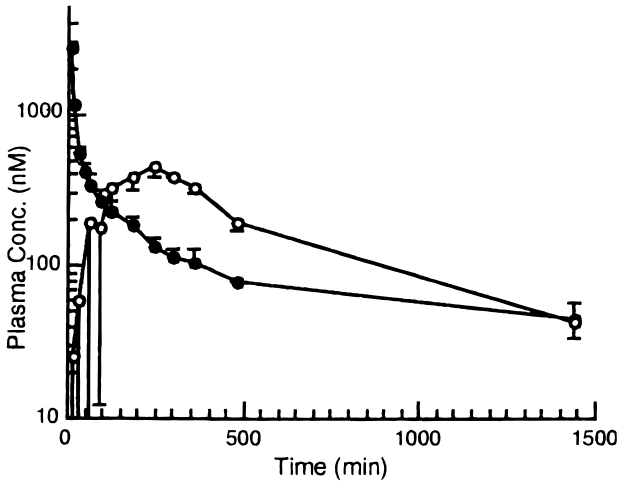


Figure 2. Mean plasma concentrations of **48** after iv (1 mg/kg, closed circles) or oral (2 mg/kg, open circles) adminstration; $n = 3$, ±SD dosed to fasted male rhesus monkeys.

One significant and unexpected difference noted between **39** and **48** was that while neither compound inhibited rat p450, the pyrimidine analogue **39**, but not pyridine **48**, proved to be a modest time-dependent inhibitor of p450 (CYP3A4) in human liver microsomes. As a result, **48** emerged as the leading candidate for further evaluation, and oral bioavailability in rat (Figure 1) and in fasted male rhesus monkey (Figure 2) was determined as 85% and 86%, respectively.

In Vivo Efficacy Model. On the basis of combination of p38 kinase inhibition, selectivity, and whole blood, pharmacokinetic, and p450 inhibition data above, aminopyridine analogue **48** was selected for evaluation in two in vivo models. In a murine LPS challenge model, **48** had an ED_{50} for reduction of TNF-α production of 0.06 mg/kg ($n = 2$) when given intravenously at the same time as LPS dosing and an ED_{50} of 0.6 mg/kg ($n = 2$) when administered po 6 h prior to dosing with LPS.

The efficacy of **48** was also determined in rat adjuvant-induced arthritis[26] at total daily doses of 2, 6, 20, and 60 mg/kg per os b.i.d. from day 1 (prophylactic regimen). **48** produced a dose-dependent inhibition of secondary paw swelling at day 21 with an ED_{50} on day 21 of 8.2 mg/kg/day. At 60 mg/kg/day, **48** inhibited secondary paw swelling by 76%. Joint destruction was assessed using radiographic total scores (RTS). The RTS on day 21 of primary (PP) and secondary (SP) paws of rats at both 20 mg/kg/day (RTS-PP 7.2 ± 2.2, RTS-SP 3.3 ± 1.8) and 60 mg/kg/day (RTS-PP 3.2 ± 1.4, RTS-SP 2.1 ± 0.6) or indomethacin at 1 mg/kg/day (RTS-PP 5.5 ± 2.0, RTS-SP 4.9 ± 2.0) were significantly less than those from rats administered vehicle (RTS-PP 13.0 ± 3.0, RTS-SP 10.2 ± 2.2).

Compound **48** was also effective in the adjuvant-induced arthritis model when given therapeutically beginning on day 14 through day 21. **48** inhibited the swelling of the secondary paw dose dependently with an ID_{50} value of 17.5 mg/kg/day. The primary and secondary paw RTS of all arthritic rats were significantly greater than those of nonadjuvant control rats on day 21. The RTS of primary and secondary paws of rats administered **48** at 20 and 60 mg/kg/day (RTS-PP 9.4 ± 1.8 and 8.1 ± 3.0, RTS-SP 6.2 ± 2.5 and 3.4 ± 0.5, respectively) were significantly less than those from rats administered vehicle (RTS-PP 13.0 ± 3.0, RTS-SP 10.2 ± 2.2). These results indicate that when **48** is administered at a total daily dose of 60 mg/kg/day b.i.d. progression of tissue destruction is almost completely prevented.

Conclusion

A highly potent and selective inhibitor (**39**) of p38 MAP kinase has been identified by combining the SAR data from several independent studies of the imidazole substituents. Incorporating a methyl group at N-1 of the imidazole improved selectivity and oral bioavailability. Comparison with the corresponding aminopyridine analogue (**48**) demonstrated that the two compounds were similar in their biological profiles, with the exception of moderate p450 inhibition shown by **39** in human liver microsomes. **48** was evaluated and found to show significant reductions in measures of disease progression in a functional model of arthritis and represents a potential candidate to permit the evaluation of a p38 MAP kinase inhibitor as a new therapy for the treatment of inflammatory diseases mediated by TNF-α.

Experimental Section

General. All reagents and solvents were of commercial quality and used without further purification unless indicated otherwise. All reactions were carried out under an inert atmosphere of nitrogen or argon. ^{1}H NMR spectra were obtained on a Varian Unity 300 spectrometer or a Varian Unity-Plus 500 spectrometer. Chemical shifts are reported in parts per million relative to TMS as internal standard. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter using a 1-dm cell. Melting points were determined in open glass capillaries using a Thomas-Hoover UniMelt melting point apparatus and are uncorrected. Microanalyses were carried out on a Perkin-Elmer 2400-II instrument. Log *P* values were determined by the traditional shake flask method partitioning between octanol and pH 7.4 buffer. Relative concentrations were then determined by HPLC analysis.

2-(*tert*-Butyldimethylsilanyloxy)-1-phenyl-2-pyridin-4-ylethanone (2). To a stirred solution of lithium diisopropylamide (8.4 mmol) in THF (25 mL) at −78 °C was added a solution of 4-[(*tert*-butyldimethylsilyloxy)methyl]pyridine[27] (1.34 g, 6.0 mmol) in THF (10 mL). After 5 min, a solution of *N*-methoxy-*N*-methylbenzamide (0.89 g, 5.4 mmol) in THF (10 mL) was added; the reaction mixture was allowed to warm to

room temperature, poured into saturated aqueous sodium bicarbonate solution, and extracted with EtOAc. The organic phase was washed with brine and dried over $MgSO_4$ and the solvent evaporated. Purification by flash column chromatography (25−100% EtOAc−hexane) afforded ketone **2** as an oil: ^{1}H NMR ($CDCl_3$) δ 0.10 (s, 6H), 0.90 (s, 9H), 5.64 (s, 1H), 7.3−7.5 (m, 5H), 7.95 (d, J = 8 Hz, 2H), 8.58 (d, J = 6.5 Hz, 2H).

2,4-Diphenyl-5-(4-pyridyl)imidazole (3). A solution of **2** (655 mg, 2.0 mmol), ammonium acetate (1.54 g, 20 mmol), benzaldehyde (212 mg, 2.0 mmol), and cupric acetate (726 mg, 4.0 mmol) in acetic acid (15 mL) was heated to reflux for 1.5 h. The cooled reaction mixture was poured into ice/ammonium hydroxide, stirred for 10 min, and partitioned with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$ and solvent evaporated. Purification by flash column chromatography eluting with EtOAc followed by crystallization from EtOAc/hexane afforded 160 mg (27%) of **3**: ^{1}H NMR ($CDCl_3$) δ 7.4−7.7 (m, 10H), 8.01 (d, J = 7.4 Hz, 2H), 8.41 (d, J = 7.4 Hz, 2H). Anal. ($C_{20}H_{15}N_3$) C, H, N.

2-(*tert*-Butyldimethylsilanyloxy)-1-(4-fluorophenyl)-2-pyridin-4-ylethanone (4). This compound was prepared as described in the synthesis of **2** (75%): ^{1}H NMR ($CDCl_3$) δ 0.11 (s, 6H), 0.90 (s, 9H), 5.60 (s, 1H), 7.03 (t, J = 8.55 Hz, 2H), 7.45 (d, J = 5.1 Hz, 2H), 8.0 (m, 2H), 8.59 (m, 2H).

5-(4-Fluorophenyl)-4-(4-pyridyl)imidazole (5): paraformaldehyde, yield 0.25 g, 68%; mp 243−245 °C; ^{1}H NMR (CD_3OD) δ 7.19 (brt, 2H), 7.45 (m, 3H), 7.88 (s, 1H), 8.42 (brs, 2H). Anal. ($C_{14}H_{13}FN_3 \cdot 0.5H_2O$) C, H, N.

2-Cyclohexyl-5-(4-fluorophenyl)-4-(4-pyridyl)imidazole (6): cyclohexanecarboxaldehyde, yield 0.125 g, 26%; mp 279−280 °C; ^{1}H NMR (CD_3OD) δ 1.3−2.1 (m, 10H), 2.81 (tt, J = 8.8, 3.4 Hz, 1H), 7.16 (t, J = 8.0 Hz, 2H), 7.3−7.6 (m, 4H), 8.39 (brs, 2H). Anal. ($C_{20}H_{20}FN_3$) C, H, N.

2-[4-(*N*-(*tert*-Butyloxycarbonyl)piperidinyl)]-4-(4-pyridyl)-5-(4-fluorophenyl)imidazole (7): *N*-(*tert*-butyloxycarbonyl)piperidine-4-carboxaldehyde,[28] 0.28 g, 46%; mp 157−160 °C; ^{1}H NMR (CD_3OD) δ 1.48 (s, 9H), 1.7−1.9 (m, 2H), 1.9−2.05 (m, 2H), 2.82−3.08 (m, 2H), 4.21 (d, J = 13.2 Hz, 2H), 7.10−7.25 (m, 2H), 7.45−7.50 (m, 4H), 8.4 (s, 2H). Anal. ($C_{24}H_{27}FN_4O_2 \cdot 0.70H_2O$) C, H, N.

2-[3-(*N*-(*tert*-Butyloxycarbonyl)piperidinyl)]-4-(4-pyridyl)-5-(4-fluorophenyl)imidazole (8): *N*-(*tert*-butyloxycarbonyl)piperidine-3-carboxaldehyde,[26] mp 193−194 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H), 1.45−1.70 (m, 1H), 1.75−2.00 (m, 2H), 2.08−2.20 (m, 1H), 2.70−3.00 (m, 1H), 3.12−3.22 (m, 1H), (d, J = 13.4 Hz, 1H), 4.26 (d, J = 10.7 Hz, 1H), 7.10−7.25 (m, 2H), 7.40−7.55 (m, 4 H), 8.40 (s, 2H). Anal. ($C_{24}H_{27}FN_4O_2 \cdot 0.20H_2O$) C, H, N.

2-(4-Piperidinyl)-4-(4-pyridyl)-5-(4-fluorophenyl)imidazole (9). Hydrogen chloride was passed into a cooled (0 °C) solution of **7** (140 mg, 0.33 mmol) in EtOAc (5 mL) and MeOH (1 mL) for 30 min, and the volatiles were evaporated. The residue was partitioned between saturated aqueous $NaHCO_3$ and DCM (×2). The combined extracts were dried over $MgSO_4$, and the solvent was evaporated to give a solid which was triturated with EtOAc and filtered to afford 30 mg (28%) of **9**: mp 267−270 °C; ^{1}H NMR (CD_3OD) δ 1.83 (qd, J = 12.6, 3.3 Hz, 2H), 1.95−2.05 (m, 2H), 2.75 (brd, J = 10.6 Hz, 2H), 2.97 (tt, J = 12.3, 3.7 Hz, 1H), 3.1−3.3 (m, 2H), 7.18 (t, J = 9.0 Hz, 2H), 7.4−7.5 (m, 4H), 8.40 (d, J = 6.2 Hz, 2H). Anal. ($C_{19}H_{19}N_4F \cdot 0.40H_2O$) C, H, N.

2-(3-Piperidinyl)-4-(4-pyridyl)-5-(4-fluorophenyl)imidazole (10). This was prepared from **8** as described in the synthesis of **9**: mp 267−270 °C; ^{1}H NMR (CD_3OD) δ 1.75−1.90 (m, 2H), 1.95−2.05 (m, 2H), 2.68−2.80 (m, 2H), 2.92−3.04 (m, 2H), 3.12−3.22 (m, 2H), 7.18 (t, J = 8.8 Hz, 2H), 7.41−7.50 (m, 4H), 8.40 (d, J = 6.2, 2H). Anal. ($C_{19}H_{19}N_4F \cdot 0.40H_2O$) C, H, N.

2-Phenyl-4-(4-pyridyl)imidazole (11). 4-(Bromoacetyl)-pyridine hydrobromide (10 g, 3.55 mmol) and benzamidine (25 g, 20.8 mmol) were dissolved in DMF (150 mL) at room temperature in DMF and heated to 40 °C for 45 min. The cooled mixture was poured into saturated $NaHCO_3$ and extracted with EtOAc (×3), the extracts were dried over $MgSO_4$, and the solvent was evaporated. Purification by flash column chromatography eluting with 5−10% MeOH/DCM afforded **11** as a solid: 6.2 g (80%); mp 212−214 °C; ^{1}H NMR (DMSO-d_6) δ 7.38 (t, J = 7.3 Hz, 1H), 7.47 (t, J = 7.8 Hz, 2H), 7.77 (d, J = 5.9 Hz, 2H), 7.99 (d, J = 7.6 Hz, 2H), 8.03 (s, 1H), 8.50 (d, J = 5.6 Hz, 2H). Anal. ($C_{14}H_{11}N_3 \cdot 0.1H_2O$) C, H, N.

1-(Methoxymethyl)-2-phenyl-4-(4-pyridyl)imidazole (12). Sodium hydride (690 mg, 60% dispersion, 28.8 mmol) was added to a stirred solution of **11** (5.3 g, 24 mmol) in THF (200 mL) at 0 °C, and after 10 min, methoxymethyl chloride (1.95 g, 24 mmol) was added. The reaction mixture was allowed to warm to room temperature, stirred for 1 h, poured into saturated aqueous $NaHCO_3$, and extracted with EtOAc (×3). The combined extracts were dried over $MgSO_4$, the solvent was evaporated, and the residue was purified by flash column chromatography eluting with 5−10% MeOH/DCM to afford **12** (3.3 g, 57%): ^{1}H NMR ($CDCl_3$) δ 3.42 (s, 3H), 5.29 (s, 2H), 7.2−7.85 (m, 8H), 8.60 (d, J = 4.6 Hz, 2H).

1-(Methoxymethyl)-2-phenyl-4-(4-pyridyl)-5-(trimethylstannyl)imidazole (13). *n*-Butyllithium (4.4 mL, 1.6 M in hexane, 11 mmol) was added to a cooled (−78 °C) solution of **12** (2.65 g, 10 mmol) in THF (100 mL), and after 5 min, chlorotrimethyltin (10 mL, 1 M in THF, 10 mmol) was added. After 5 min, the reaction mixture was poured into saturated aqueous $NaHCO_3$ and extracted with EtOAc (×2). The organic phases were dried over $MgSO_4$, the solvent was evaporated, and the residue was purified by flash column chromatography eluting with EtOAc, to afford 1.2 g (31%) of **13**: ^{1}H NMR ($CDCl_3$) δ 0.33 (s, 9H), 3.16 (s, 3H), 5.28 (s, 2H), 7.4−7.7 (m, 7H), 8.60 (d, J = 5.8 Hz, 2H).

1-(Methoxymethyl)-2-phenyl-4-(4-pyridyl)-5-[3-(trifluoromethyl)phenyl]imidazole (14). A mixture of **13** (200 mg, 0.59 mmol), 3-iodobenzotrifluoride (192 mg, 0.71 mmol), and tetrakis(triphenylphosphine)palladium(0) (68 mg, 0.059 mmol) in dry DMF was heated to 80 °C for 1 h, cooled to room temperature, and partitioned between saturated aqueous $NaHCO_3$ (150 mL) and EtOAc (3 × 75 mL). The combined organic phases were dried over $MgSO_4$, the solvent was evaporated, and the residue was purified by flash column chromatography eluting with EtOAc to afford 180 mg of solid. Crystallization from ether gave 40 mg (16%) of **14**: ^{1}H NMR ($CDCl_3$) δ 3.26 (s, 3H), 4.91 (s, 2H), 7.42 (d, J = 6.1 Hz, 2H), 7.46−7.56 (m, 3H), 7.60−7.94 (m, 6H), 8.46 (d, J = 6.1 Hz, 2H). Anal. ($C_{23}H_{18}F_3N_3O \cdot 0.15H_2O$) C, H, N.

2-Phenyl-4-(4-pyridyl)-5-[3-(trifluoromethyl)phenyl]-imidazole (3a). A mixture of **14** (140 mg, 0.327 mmol) and 6 M HCl (10 mL) was heated to reflux for 2 h. The cooled mixture was poured into saturated aqueous $NaHCO_3$ (150 mL) and 1 M NaOH (50 mL) and partitioned with EtOAc (3 × 100 mL), The combined extracts were dried over $MgSO_4$, and solvent was evaporated. Crystallization of the crude product from EtOAc afforded 67 mg (54%) of **3a**: mp 228−230 °C; ^{1}H NMR (CD_3OD) δ 7.4−7.58 (m, 5H), 7.64 (t, J = 7.6 Hz, 1H), 7.72 (d, J = 7.2 Hz, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.86 (s, 1H), 8.03 (d, J = 6.8 Hz, 2H), 8.48 (d, J = 5.4 Hz, 2H). Anal. ($C_{21}H_{14}F_3N_3$) C, H, N.

2,5-Diphenyl-1-(methoxymethyl)-5-(tributylstannyl)-imidazole (16). *n*-Butyllithium (870 μL, 2.18 mmol) was added to a cooled (−78 °C) solution of 2,5-diphenyl-1-(methoxymethyl)imidazole[29] (523 mg, 1.98 mmol) in THF (10 mL). After 15 min, tributyltin chloride (590 μL, 2.18 mmol) was added; the reaction mixture was stirred for 20 min, poured into saturated aqueous $NaHCO_3$, and extracted with EtOAc. The organic phase was dried over Na_2SO_4, the solvent evaporated, and the residue purified by flash column chromatography eluting with 2:1 hexane:EtOAc to give 813 mg (74%) of **16**: ^{1}H NMR ($CDCl_3$) δ 0.8−1.6 (m, 27H), 3.14 (s, 3H), 5.23 (s, 2H), 7.20−7.70 (m, 10H).

2,5-Diphenyl-1-(methoxymethyl)-5-[4-(2-(methylthio)-pyrimidinyl)]imidazole (17). A mixture of **16** (5.0 g, 9.04 mmol), 4-iodo-2-(methylthio)pyrimidine (3.417 g, 9.04 mmol), and tetrakis(triphenylphosphine)palladium(0) (2.088 g, 1.807 mmol) in dry DMF (100 mL) was heated to 80 °C for 20 h. The reaction mixture was cooled to room temperature, poured

into water, and extracted with EtOAc (3 × 75 mL), the extracts were dried over Na_2SO_4, the solvent was evaporated, and the residue was purified by flash column chromatography eluting with 2:1 hexane:EtOAc to give 2.549 g (73%) of **17**: ^{1}H NMR ($CDCl_3$) δ 2.59 (s, 3H), 3.20 (s, 3H), 5.65 (s, 2H), 6.93 (d, J = 5.1 Hz, 1H), 7.25−7.40 (m, 3H), 7.45−7.60 (m, 5H), 7.70−7.90 (m, 2H), 8.36 (d, J = 5.1 Hz, 1H).

2,5-Diphenyl-5-[4-(2-(methylthio)pyrimidinyl)]imidazole (18). A mixture of **17** (1.918 g, 4.937 mmol) and 3 M HCl (30 mL) was heated to reflux for 1.5 h, cooled, and concentrated to a solid which was partitioned between EtOAc and saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4 and the solvent evaporated to give 1.811 g of a solid. A small portion of this was recrystallized from EtOAc/hexane to give a pure sample of **18** as a yellow solid: mp 156−158 °C; ^{1}H NMR ($CDCl_3$) δ 2.63 (s, 3H), 7.04 (d, J = 5.4 Hz, 1H), 7.30−7.60 (m, 6H), 7.67 (dd, J = 7.8,1.6 Hz, 2H), 7.98 (dd, J = 7.8,1.6 Hz, 2H), 8.48 (d, J = 5.1 Hz, 1H), 10.44 (br s, 1H). Anal. ($C_{20}H_{16}N_4S\cdot 0.15H_2O$) C, H, N.

2,5-Diphenyl-5-(4-pyrimidinyl)imidazole (19). A mixture of **18** (200 mg, 0.581 mmol), Raney nickel (0.46 g), EtOH (20 mL), and water (10 mL) was heated to reflux for 20 h. The reaction mixture was filtered, the solvent evaporated, and the residue purified by flash column chromatography eluting with 1:1 $CHCl_3$:EtOAc to provide 82 mg (47%) of **19** as a white solid: mp 232−234 °C; ^{1}H NMR (DMSO-d_6) δ 7.72−7.85 (m, 6H), 8.03 (d, J = 7.1 Hz, 2H), 8.32 (d, J = 5.1 Hz, 1H), 8.44 (d, J = 7.6 Hz, 2H), 9.05 (d, J = 5.4 Hz, 2H), 9.22 (s, 1H). Anal. ($C_{19}H_{14}N_4\cdot 0.20H_2O$) C, H, N.

2,5-Diphenyl-5-[4-(2-(methylsulfonyl)pyrimidinyl)]imidazole (20). A cooled (0 °C) solution of **18** (1.36 g, 3.95 mmol) in MeOH (20 mL) was treated with a solution of Oxone (7.28 g, 11.84 mmol) in H_2O (20 mL) and stirred at room temperature for 4 h. The reaction mixture was partitioned between EtOAc and saturated aqueous $NaHCO_3$ (×3), the organic phases were dried over Na_2SO_4 and evaporated, and the residue was recrystallized from EtOAc to give 1.134 g (77%) of **20** as a yellow solid: mp 228−230 °C; ^{1}H NMR (CD_3OD) δ 2.93 (br s, 3H), 7.40−7.60 (m, 7H), 7.66 (dd, J = 7.6, 1.8 Hz, 2H), 8.05 (d, J = 6.63 Hz, 2H), 8.80 (br s, 1H). Anal. ($C_{20}H_{16}N_4O_2S\cdot 0.20H_2O$) C, H, N.

2,5-Diphenyl-5-[4-(2-(methylamino)pyrimidinyl)]imidazole (21). A mixture of **20** (150 mg, 0.398 mmol) in EtOH (5 mL) and methylamine (5 mL) was heated to 140 °C in a sealed tube for 3 h. The cooled reaction mixture was partitioned between EtOAc and saturated $NaHCO_3$ (×3), the combined extracts were washed with H_2O and brine and dried over Na_2SO_4, and the solvent was evaporated. The residue was purified by flash column chromatography eluting with 95:5:0.5 DCM:MeOH:NH_4OH and the product thus obtained triturated with ether overnight and filtered to give 73 mg (56%) of **21** as a solid: mp 182−184 °C; ^{1}H NMR (CD_3OD) δ 2.9 (br m, 3H), 6.50 and 7.00 (br s, 1H total), 7.30−7.55 (m, 6H), 7.62 (d, J = 7.1 Hz, 2H), 8.02 (d, J = 7.1 Hz, 2H), 8.10 (br s, 1H). Anal. ($C_{20}H_{17}N_5\cdot 0.10H_2O$) C, H, N.

2,5-Diphenyl-5-[4-(4-(methoxybenzylamino)pyrimidinyl)]imidazole (22). A mixture of **20** (100 mg, 0.266 mmol) and 4-methoxybenzylamine (0.50 mL) was heated to 170 °C for 1 h. The cooled reaction mixture was partitioned between saturated aqueous $NaHCO_3$ and EtOAc (×3), the combined organic layers were washed with H_2O and brine and dried over Na_2SO_4, and the solvent was evaporated. The crude product was purified by flash column chromatography eluting with 95:5:0.5 DCM:MeOH:NH_4OH to afford **22** as a white solid: mp 158−160 °C; ^{1}H NMR (CD_3OD) δ 3.75 (s, 3H), 4.20 (br s, 1H), 4.50 (br s, 1H), 6.5−7.55 (m, 11 H), 7.60 (dd, J = 7.4, 1.8 Hz, 2H), 8.00 (d, J = 6.6 Hz, 2H), 8.10 (br s, 1H). Anal. ($C_{27}H_{23}N_5O\cdot 0.25H_2O$) C, H, N.

2,5-Diphenyl-5-[4-(2-aminopyrimidinyl)]imidazole (23). A mixture of **22** (70 mg, 0.161 mmol) and 6 M HCl (8 mL) was heated to 100 °C for 1 h. The cooled reaction mixture was evaporated to dryness and azeotroped with EtOAc (×3) and the residue purified by flash column chromatography eluting with 95:5:0.5 DCM:MeOH:NH_4OH to afford 27 mg (54%) of **23** as a solid: mp 119−121 °C; ^{1}H NMR (CD_3OD) δ 6.6 (br s, 1H), 7.40−7.55 (m, 7H), 7.59 (dd, J = 7.7,1.7 Hz, 2H), 8.00−8.10 (m, 3H). Anal. ($C_{19}H_{15}N_5\cdot 0.40Et_2O$) C, H, N.

***N*-Methyl-*N*-methoxy[3-(trifluoromethyl)phenyl]carboxamide (24).** To a suspension of *N*,*O*-dimethylhydroxylamine hydrochloride (58.2 g, 0.60 mol) in DCM (1 L) at 0 °C was added 3-(trifluoromethyl)benzoyl chloride (104.0 g, 0.50 mol), followed by a slow addition (≤5 °C) of triethylamine (152.3 mL, 1.09 mol). The reaction was stirred for 30 min at 5 °C and then allowed to warm to room temperature. The reaction was then washed with 5% aqueous citric acid (500 mL) and 5% aqueous $NaHCO_3$, the aqueous extracts were back-extracted with DCM (100 mL), the combined organic extracts were dried over Na_2SO_4, and the solvent was evaporated. The oil was redissolved in toluene (2 × 100 mL) and evaporated in vacuo to afford **24** (114.7 g, 98%) which was used in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 3.39 (s, 3H), 3.55 (s, 3H), 7.55 (t, J = 7.8 Hz, 1H), 7.72 (d, J = 7.8 Hz, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.98 (s, 1H).

2-[2-(Methylthio)pyrimidin-4-yl]-1-[3-(trifluoromethyl)-phenyl]ethanone (25). *n*-Butyllithium (209.7 mL, 2.5 M in hexane, 0.524 mol) was added to a solution of diisopropylamine (73.5 mL, 0.524 mol) in THF (980 mL) at −78 °C, followed after 5 min by a solution of 2-(methylthio)-4-methylpyrimidine (49 g, 0.35 mol) in THF (500 mL). After stirring for 15 min at −78 °C, a solution of **24** (89.67 g, 0.385 mol) in THF (400 mL) was added. After stirring for 5 min, the reaction was allowed to warm to 0 °C and quenched by pouring into water (2000 mL) and EtOAc (2000 mL). The layers were separated, and the aqueous layer was washed with EtOAc (1000 mL). The combined organic extracts were dried over Na_2SO_4, and the solvent evaporated to an oil/solid (133 g). Trituration with 10% ether/hexane (1000 mL) gave 76 g (70%) of **25**: ^{1}H NMR ($CDCl_3$) (mixture of keto and enol tautomers) δ 2.4−2.7 (m, 3H), 6.0−8.5 (m, 6H, rotamers).

1-[2-(Methylthio)pyrimidin-4-yl]-2-[3-(trifluoromethyl)-phenyl]ethane-1,2-dione 1-Oxime (26). To **25** (67 g, 0.215 mol) in acetic acid (1000 mL) were added THF (800 mL) and water (100 mL). The mixture was cooled to 5 °C and an aqueous solution (130 mL) of sodium nitrite (20.0 g, 0.290 mol) added dropwise while maintaining the temperature below 10 °C. Upon completion of addition, the reaction mixture was allowed to warm to room temperature for 1 h, concentrated to remove THF and acetic acid, and diluted with water (2000 mL) and EtOAc (2000 mL). The pH was adjusted to 8 with 3 N NaOH, the layers were separated, and the aqueous layer was washed with EtOAc (500 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to yield 73.2 g (100%) of **26** as an oil: ^{1}H NMR ($CDCl_3$) δ 1.60 (brs, 1H), 2.18 (s, 3H), 7.52 (d, J = 5.4 Hz, 1H), 7.66 (t, J = 8.6 Hz, 1H), 7.89 (d, J = 8.6 Hz, 1H), 8.06 (d, J = 8.6 Hz, 1H), 8.18 (s, 1H), 8.58 (d, J = 5.4 Hz, 1H).

5-[2-(Methylthio)pyrimidin-4-yl]-4-[3-(trifluoromethyl)-phenyl]-2-[4-(*N*-(benzyloxycarbonyl)piperidinyl)]imidazole (28). A mixture of ammonium acetate (333 g, 4.26 mol), **26** (72.5 g, 0.212 mol), and *N*-(benzyloxycarbonyl)piperidine-4-carboxaldehyde (63.0 g, 0.255 mol) in acetic acid (1400 mL) was heated to reflux for 2 h, cooled, and concentrated to remove most of the acetic acid. The residue was quenched into concentrated NH_4OH (400 mL), ice (1 kg), and EtOAc (1500 mL) and the pH adjusted to 9 with NH_4OH. The organic layer was separated and the aqueous layer washed with EtOAc (500 mL). The combined organic phases were dried over Na_2SO_4 and concentrated to 143.8 g of **27** as an oil. To a solution of the crude **27** in methanol (1700 mL) at 20 °C was slowly added titanium(III) chloride (223 mL, 0.258 mol, 15 wt % in 20−30% HCl) dropwise over 20 min. The reaction mixture was stirred for 3 h at 20 °C, concentrated to remove methanol, and quenched into ice (1 kg) and EtOAc (3000 mL). The pH was adjusted to 8 with concentrated NH_4OH. After stirring for 30 min the organic layer was removed and aqueous extract washed with EtOAc (2 × 1000 mL). The combined extracts were dried over Na_2SO_4 and concentrated to an oil (143 g). The oil was dissolved in DCM (50 mL) and chromatographed

on silica gel (4 kg) using 1:1 EtOAc/hexane to give 116.0 g (97% from oxime) of **28** as a yellow foam: ^{1}H NMR ($CDCl_3$) δ 1.7−2.2 (m, 4H), 2.58 (s, 3H), 3.0 (br m, 3H), 4.30 (brs, 2H), 5.12 (s, 2H), 6.95 (d, J = 5.5 Hz, 1H), 7.2−7.8 (m, 9H), 8.28 (d, J = 5.5 Hz, 1H), 10.18 (br s, 1H).

5-[2-(Methylsulfonyl)pyrimidin-4-yl]-4-[3-(trifluoromethyl)phenyl]-2-[4-(*N*-(benzyloxycarbonyl)piperidinyl)]-imidazole (29). An aqueous solution (75 mL) of Oxone (8.32 g, 0.0135 mol) was slowly added to a stirred solution of **28** (2.5 g, 0.0045 mol) in methanol (75 mL) at 20 °C. After stirring for 4 h, the reaction mixture was concentrated in vacuo to remove methanol, diluted with 10% aqueous $NaHCO_3$ (100 mL), and extracted with EtOAc (2 × 150 mL). The organic extracts were combined, dried over Na_2SO_4, and concentrated to give 2.75 g of **29**: ^{1}H NMR ($CDCl_3$) δ 1.8−2.2 (m, 4H), 2.7−3.1 (m, 3H), 3.35 (s, 3H), 4.30 (br s, 2H), 5.12 (s, 2H), 7.35 (m, 5H), 7.5−8.6 (m, 6H).

5-[2-(4-(Methoxybenzylamino)pyrimidinyl)]-4-[3-(trifluoromethyl)phenyl]-2-(4-piperidinyl)imidazole (30). A mixture of **29** (1.5 g, 2.56 mmol) and 4-methoxybenzylamine (3.51 g, 26 mmol) was heated in a pressure tube at 140 °C for 10 min. The mixture was cooled and residue purified by flash column chromatography eluting with 5:95 MeOH/DCM to give 1.52 g (92%) of the CBZ derivative of **30**; 30% HBr/AcOH (16 mL) was slowly added to a stirred solution of the intermediate (1.0 g, 1.56 mmol) in DCM (16 mL) and the reaction mixture stirred for 30 min and diluted with Et_2O (160 mL). The mixture was stirred for 1 h and filtered and the solid washed with ether (10 mL) and partitioned between saturated aqueous $NaHCO_3$ (40 mL) and DCM (50 mL). The aqueous layer was washed with methylene chloride (25 mL); the combined organic extracts were dried over Na_2SO_4 and concentrated to a foam (0.80 g) which was purified by flash column chromatography eluting with 95:10:2 DCM:MeOH:NH_4OH to give 180 mg (23%) of **30**: ^{1}H NMR ($CDCl_3$) δ 1.6−2.2 (m, 4H), 2.77 (m, 2H), 2.95 (m, 1H), 3.22 (m, 2H), 3.80 (s, 3H), 4.60 (br s, 2H), 5.44 (br s, 1H), 6.78 (br s, 1H), 6.90 (d, J = 8.1 Hz, 2H), 7.32 (d, J = 8.1 Hz, 1H), 7.42 (t, J = 8.1 Hz, 1H), 7.62 (d, J = 8.1 Hz, 1H), 7.80 (d, J = 8.1 Hz, 1H), 7.90 (s, 1H),), 8.12 (d, J = 5.8 Hz, 1H), 9.8 (br s, 1H). Anal. ($C_{35}H_{33}N_6O_2F_3$·0.20H_2O) C, H, N.

5-(2-Aminopyrimidinyl)-4-[3-(trifluoromethyl)phenyl]-2-(4-piperidinyl)imidazole (31). A mixture of **30** (0.125 mg, 0.26 mmol) and 3 M HCl (35 mL) was heated to 100 °C for 12 h. The reaction was cooled, washed with diethyl ether (10 mL), and concentrated in vacuo to a solid. Trituration from 80% ether/ethanol (10 mL) gave the hydrochloride salt of **31** (0.102 g, 98%) as a yellow solid: mp 225−230 °C; ^{1}H NMR (CD_3OD) δ 2.1−2.4 (m, 4H), 3.2−3.6 (m, 5H), 7.02 (d, J = 6.6 Hz, 1H), 7.77 (t, J = 8.4 Hz, 1H), 7.89 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 8.4 Hz, 1H), 8.0 (s, 1H), 8.19 (d, J = 6.6 Hz, 1H).

(*S*)-5-[2-(1-Phenylethylamino)pyrimidin-4-yl]-4-[3-(trifluoromethyl)phenyl]-2-(4-piperidinyl)imidazole (32). This compound was prepared as described in the synthesis of **30** and **31**: mp 157−161 °C; $[\alpha]_D$ = −165.1° (MeOH). Anal. ($C_{27}H_{26}N_6F_3$·HCl·1.50H_2O) C, H, N.

(*R*)-5-[2-(1-Phenylethylamino)pyrimidin-4-yl]-4-[3-(trifluoromethyl)phenyl]-2-(4-piperidinyl)imidazole (33). This compound was prepared as described in the synthesis of **30** and **31**: mp 155−160 °C; $[\alpha]_D$ = +165.1° (MeOH). Anal. ($C_{27}H_{26}N_6F_3$·HCl·1.50H_2O) C, H, N.

5-[2-(Methylthio)pyrimidin-4-yl]-1-methyl-4-[3-(trifluoromethyl)phenyl]-2-[4-(*N*-(benzyloxycarbonyl)piperidinyl)]imidazole (36). To a stirred solution of **28** (24.0 g, 43.4 mmol) in toluene (300 mL) was added DMF dimethyl acetal (25 mL), and the mixture was heated to reflux for 48 h. The reaction was cooled, concentrated in vacuo to a foam, and chromatographed on silica gel (1 kg) using 1:33 acetone/DCM to give 16 g (65%) of **36**: mp 148−152 °C; ^{1}H NMR ($CDCl_3$) δ 2.0 (m, 4H), 2.60 (s, 3H), 2.98 (m, 3H), 3.80 (s, 3H), 4.35 (br s, 2H), 5.13 (s, 2H), 6.76 (d, J = 6.0 Hz, 1H), 7.3−7.45 (m, 6H), 7.5−7.6 (m, 2H), 7.78 (s, 1H), 8.33 (d, J = 6.0 Hz, 1H).

Additional elution of the above column chromatography with 1:9 methanol/DCM gave **37**: ^{1}H NMR ($CDCl_3$) δ 1.7 (s, 3H), 1.9−2.1 (m, 4H), 2.7−3.1 (m, 3H), 3.38 (s, 3H), 4.35 (br s, 2H), 5.18 (s, 2H), 7.3−7.44 (m, 5H), 7.5−7.74 (m, 5H), 8.39 (d, J = 6.0 Hz, 1H).

5-[2-(Methylsulfonyl)pyrimidin-4-yl]-1-methyl-4-[3-(trifluoromethyl)phenyl]-2-[4-(*N*-benzyloxycarbonyl)piperidinyl)]imidazole (34). This compound was prepared as described in the synthesis of **29**: ^{1}H NMR ($CDCl_3$) δ 2.0 (m, 4H), 3.01 (m, 3H), 3.40 (s, 3H), 3.95 (s, 3H), 4.34 (m, 2H), 5.14 (s, 2H), 7.24 (d, 1H), 7.3−7.4 (m, 5H), 7.48 (t, 1H), 7.60 (m, 2H), 7.76 (s, 1H), 8.58 (d, 1H).

(*S*)-5-[2-(1-Phenylethylamino)pyrimidin-4-yl]-1-methyl-4-[3-(trifluoromethyl)phenyl]-2-[4-(*N*-(benzyloxycarbonyl)piperidinyl)]imidazole (38). This compound was prepared as described in the synthesis of **30**: ^{1}H NMR ($CDCl_3$) δ 1.59 (d, 3H, J = 8.1 Hz), 1.8−2.0 (m, 4H), 2.8−3.1 (m, 3H), 3.32 (br s, 3H), 4.3 (br s, 2H), 5.1−5.2 (m, 3H), 5.55 (d, J = 6.6 Hz, 1H), 6.35 (d, J = 5.1 Hz, 1H), 7.2−7.4 (m, 11H), 7.48 (d, J = 7.6 Hz, 1H), 7.58 (d, J = 7.6 Hz, 1H), 7.79 (s, 1H), 8.12 (d, J = 5.1 Hz, 1H).

(*S*)-5-[2-(1-Phenylethylamino)pyrimidin-4-yl]-1-methyl-4-[3-(trifluoromethyl)phenyl]-2-(4-piperidinyl)imidazole (39). A solution of **38** (10.9 g, 0.017 mol) in 2-propanol (500 mL) containing 10% Pd/C (4.0 g) was hydrogenated at 1 atm of hydrogen for 24 h. The mixture was filtered, the catalyst was washed with 2-propanol (200 mL), the combined filtrates were concentrated, and the resulting foam (8.0 g, 0.0158 mol, 93% yield) was recrystallized from 400 mL of 50% methanol/water to give upon filtration and drying at 40 °C in vacuo 7.04 g of **39** (88%): mp 97−99 °C; ^{1}H NMR (CD_3OD) δ 1.58 (d, J = 7.6 Hz, 3H), 1.8−2.0 (m, 5H), 2.6−3.4 (m, 7H), 5.17 (m, 1H), 5.52 (brd, J = 6.9 Hz, 1H), 6.35 (d, J = 4.9 Hz, 1H), 7.2−7.3 (m, 6H), 7.48 (d, J = 7.8 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.81 (s, 1H). 8.11 (d, J = 5.2 Hz, 1H). Anal. ($C_{28}H_{29}N_6F_3$·0.75H_2O) C, H, N.

(*R*)-5-[2-(1-Phenylethylamino)pyrimidin-4-yl]-1-methyl-4-[3-(trifluoromethyl)phenyl]-2-(4-piperidinyl)imidazole (41). This was prepared as described in the synthesis of **39**. The product was isolated as the hydrochloride salt by dissolving 0.20 g of the free base in 1.6 mL of 1 N HCl (4.0 equiv) and 5 mL of water. The resulting solution was filtered and lyophilized to give 230 mg of solid: ^{1}H NMR (CD_3OD) δ 1.58 (br s, 3H), 2.3 (br m, 5H), 3.2−4.0 (m, 8H), 5.17 (m, 1H), 6.60 (s, 1H), 7.2−7.5 (m, 5H), 7.6−8.0 (m, 4H), 8.24 (s, 1H). Anal. ($C_{28}H_{29}N_6F_3$·3.0HCl·3.0H_2O) C, H, N.

(*S*)-4-[2-(1-Phenylethylamino)pyrimidin-4-yl]-1-methyl-5-[3-(trifluoromethyl)phenyl]-2-(4-piperidinyl)imidazole (42). This was prepared from **37** as described for the synthesis of **39**. Crystallization of the product as the sulfate salt was accomplished by dissolving 4.0 g of the free base in ethanol (40 mL) and adding 98% sulfuric acid (0.834 g, 1.05 equiv) dissolved in ethanol (10 mL). The resulting solid was filtered cold at 0 °C and dried in vacuo at 60 °C to give 4.0 g of the sulfate salt: mp 193−196 °C; ^{1}H NMR (CD_3OD) δ 1.25 (br d, J = 7.6 Hz, 3H), 2.20 (m, 4H), 3.1−3.4 (m, 3H), 3.46 (s, 3H), 3.60 (m, 2H), 4.28 (m, 1H), 6.9−7.3 (m, 6H), 7.48 (d, J = 7.8 Hz 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.81 (s, 1H). 8.11 (d, J = 5.2 Hz, 1H). Anal. ($C_{28}H_{29}N_6F_3$·H_2SO_4·0.80H_2O) C, H, N.

2-(2-Fluoropyridin-4-yl)-1-[3-(trifluoromethyl)phenyl]-ethanone (43). This was prepared as described in the synthesis of **25**: ^{1}H NMR ($CDCl_3$) δ 4.37 (s, 2H), 6.86 (s, 1H), 7.09 (d, J = 5.1 Hz, 1H), 7.67 (t, J = 7.8 Hz, 1H), 7.88 (d, J = 7.8 Hz, 1H), 8.18 (d, J = 9.3 Hz, 1H), 8.20 (d, J = 5.1 Hz, 1H), 8.25 (s, 1H).

1-(2-Fluoropyridin-4-yl)-2-[3-(trifluoromethyl)phenyl]-ethane-1,2-dione 1-Oxime (44). To a mixture of **43** (10.80 g, 0.038 mol) in ethanol (200 mL), at −10 °C, under argon, were added *tert*-butyl nitrite (5.0 mL, 0.042 mol) and hydrogen chloride (12.2 mL, 2.5 M in ethanol, 0.031 mol) dropwise while maintaining the temperature below −5 °C. The reaction was allowed to warm to room temperature, stirred for 2 h, concentrated, diluted with water (100 mL) and saturated aqueous $NaHCO_3$ (200 mL), and extracted with EtOAc (3 × 400 mL). The organic layers were washed with water (300 mL) and brine (300 mL), dried over Na_2SO_4, and concentrated to afford 11.4 g of **44** (96%): ^{1}H NMR (CD_3OD) δ 7.23 (s, 1H),

7.40 (d, $J = 5.1$ Hz, 1H), 7.71 (t, $J = 7.8$ Hz, 1H), 7.92 (d, $J = 8.1$ Hz, 1H), 8.24 (d, $J = 7.8$ Hz, 1H), 8.29 (d, $J = 5.3$ Hz, 1H), 8.31 (s, 1H).

5-(2-Fluoropyridin-4-yl)-1-methyl-4-[3-(trifluoromethyl)phenyl]-2-[4-(*N*-(benzyloxycarbonyl)piperidinyl)]imidazole (45). This was prepared as described in the synthesis of **28** except that the first step was carried out at 80 °C instead of reflux: ^{1}H NMR (CD_3OD) δ 1.87 (m, 2H), 2.04 (d, $J = 12.9$ Hz, 2H), 3.06 (m, 3H), 4.29 (d, $J = 13.4$ Hz, 2H), 5.14 (s, 2H), 7.09 (s, 1H), 7.26 (d, $J = 4.5$ Hz, 1H), 7.38 (m, 5H), 7.63 (d, $J = 7.3$ Hz, 1H), 7.68 (d, $J = 7.6$ Hz, 1H), 7.70 (t, $J = 7.3$ Hz, 1H), 7.76 (s, 1H), 8.07 (d, $J = 5.1$ Hz, 1H).

(*S*)-5-[2-(1-Phenylethylamino)pyridin-4-yl]-4-[3-(trifluoromethyl)phenyl]-2-[4-(*N*-(benzyloxycarbonyl)piperidinyl)]imidazole (46). A mixture of **45** (40.0 g, 0.0763 mol) and (*S*)-(−)-(α)-methylbenzylamine (167 mL, 1.525 mol) was heated at 150 °C for 100 h, cooled to room temperature, poured in to 1.5 L of pH 4.5 solution of 10 N NaOH in 10% citric acid, and extracted with EtOAc (3 × 1 L). The combined extracts were washed with saturated aqueous $NaHCO_3$ and brine (750 mL), dried over Na_2SO_4, and concentrated to an oil which was purified by chromatography on silica gel (2 kg) eluting with 75% EtOAc/hexane to give **46** (31.5 g, 66%): ^{1}H NMR (CD_3OD) δ 1.48 (d, $J = 6.8$ Hz, 3H), 1.75−2.10 (m, 4H), 3.03 (m, 4H), 4.29 (d, $J = 13.4$ Hz, 2H), 4.63 (q, $J = 6.8$ Hz, 1H), 5.14 (s, 2H), 6.40 (s, 1H), 6.58 (d, $J = 5.4$ Hz, 1H), 7.14−7.37 (m, 10H), 7.61 (d, $J = 5.9$ Hz, 1H), 7.63 (d, $J = 7.3$ Hz, 1H), 7.72 (s, 1H), 7.86 (d, $J = 5.1$ Hz, 1H).

(*S*)-5-[2-(1-Phenylethylamino)pyridin-4-yl]-1-methyl-4-[3-(trifluoromethyl)phenyl]-2-[4-(*N*-(benzyloxycarbonyl)-piperidinyl)]imidazole (47). Iodomethane (1.88 mL, 0.030 mol) was added dropwise to a mixture of **46** (18.32 g, 0.029 mol) and cesium carbonate (18.32 g, 0.056 mol) in DMF (150 mL) at −30 °C. This reaction mixture was slowly warmed to room temperature over 3 h, diluted with EtOAc (1.5 L), washed with water (5 × 100 mL) and brine (300 mL), dried over Na_2SO_4, and concentrated to an oil. The oil was purified by column chromatography on 1.5 kg of silica gel, eluting with 5% acetone in DCM to give 11.50 g (61%) of **47**: ^{1}H NMR (CD_3OD) δ 1.53 (d, $J = 6.8$ Hz, 3H), 1.95 (m, 4H), 3.05 (br s, 2H), 3.17 (m, 1H), 3.40 (s, 3H), 4.29 (d, $J = 13.4$ Hz, 2H), 4.80 (q, $J = 6.8$ Hz, 1H), 5.14 (s, 2H), 6.32 (s, 1H), 6.44 (d, $J = 6.6$ Hz, 1H), 7.22−7.38 (m, 101H), 7.44 (t, $J = 7.8$ Hz, 1H), 7.48 (d, $J = 9.3$ Hz, 1H), 7.49 (d, $J = 7.8$ Hz, 1H), 7.66 (s, 1H), 7.99 (d, $J = 5.9$ Hz, 1H).

(*S*)-5-[2-(1-Phenylethylamino)pyridin-4-yl]-1-methyl-4-[3-(trifluoromethyl)phenyl]-2-(4-piperidinyl)imidazole (48). This was prepared as described for **39** and characterized as the sulfate salt: mp softens at 165 °C; $[\alpha]^D$ −45.60° ($c = 0.0109$ g/mL, MeOH); ^{1}H NMR (CD_3OD) δ 1.53 (d, $J = 6.8$ Hz, 3H), 2.16 (m, 4H), 3.17 (m, 3H), 3.40 (s, 3H), 3.56 (d, $J = 12.7$ Hz, 2H), 4.76 (q, $J = 6.8$ Hz, 1H), 6.47 (s, 1H), 6.54 (d, $J = 5.9$ Hz, 1H), 7.26 (m, 5H), 7.40 (t, $J = 7.7$ Hz, 1H) 7.49 (d, $J = 8.1$ Hz, 1H), 7.53 (d, $J = 7.6$ Hz, 1H), 7.71 (s, 1H), 7.97 (d, $J = 5.9$ Hz, 1H). Anal. ($C_{29}H_{30}N_5F_3 \cdot H_2SO_4 \cdot 2.45H_2O$) C, H, N.

Biological Methods. p38 purification and assays were carried out as described previously.[28] For compounds with $IC_{50} < 5$ nM, the published conditions were altered by lowering the enzyme concentration and increasing the incubation period by the same factor to give the same turnover.

Purification of Raf and Mek Kinases. Recombinant full-length human c-Raf with a carboxyl-terminal middle T-epitope tag, Glu-Tyr-Met-Pro-Met-Glu, was produced from baculovirus-infected Sf9 cells. For that purpose c-Raf was cloned into the pVL1393 vector and expressed using the BaculoGold system (PharMingen). Activated Raf was produced by coinfection of Sf9 cells with virus expressing c-Raf, Lck, and activated H-Ras (Val12) at a multiplicity of infection of 10:2:2. The cells were lysed by sonication in 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM Na_3VO_4, 50 mM NaF, 1 mM EDTA, 1 mM $MgCl_2$, 1% Triton X-100, protease inhibitor cocktail (10 μg/mL benzamidine, 5 μg/mL each of leupeptin, aprotinin, pepstatin), 1 mM AEBSF, 1 mM DTT, 5 mM sodium pyrophosphate, and 25 mM sodium glycerolphosphate (buffer A). The soluble fraction was loaded onto a GammaBind Plus column (Pharmacia) preloaded with 17 mg of anti-middle T-antibody/mL of resin. The column was washed with three column volumes of each of the following: buffer A; then 25 mM Tris (pH 8), 0.1 mM EGTA, 0.1 mM EDTA, protease inhibitor cocktail, 1 mM DTT, 1 mM Na_3VO_4, 5 mM sodium pyrophosphate, and 25 mM sodium glycerolphosphate (buffer B) containing 0.01% Triton X-100; and buffer B without Triton. Protein was eluted with 50 μg/mL Glu-Tyr-Met-Pro-Met-Glu peptide in buffer B. Peak fractions were pooled and aliquots stored at −70 °C in the presence of 0.1 mg/mL BSA and 20% glycerol.

Wild-type Mek1 and a kinase-inactive form of Mek1 (Lys97 to Ala substitution) with amino-terminal middle T-epitopes were cloned, expressed, and purified as described for c-Raf. Activated Mek1 was obtained by coinfection with viruses expressing c-Raf, Lck, and activated H-Ras at a multiplicity of infection of 10:2:2:2. Wild-type and a kinase-inactive form of Erk2 (Lys52 to Arg substitution) were expressed in *Escherichia coli* as GST-fusion proteins in pGEX vectors (Promega) and purified over glutathione resins. Wild-type GST-Erk2 was activated in vitro with activated Mek1 using reaction conditions as described above.

Raf, Mek, and Erk2 Kinase Assays. All three kinases were assayed in the same basic assay buffer containing 25 mM HEPES, pH 8.0, 5 mM $MgCl_2$, 0.5 mM EDTA, 0.2 mM Na_3VO_4, 0.1 mg/mL bovine serum albumin, 2 mM β-mercaptoethanol, and 10 μM [γ-^{33}P]ATP (20 μCi/mL). Raf, Mek, and Erk2 kinase assays used the following concentrations of substrates: 25 μg/mL kinase-inactive Mek1, 30 μg/mL kinase-inactive GST-Erk2, and 50 μg/mL myelin basic protein, respectively. Inhibitor dilutions were made in DMSO. Final DMSO concentrations were 2.5% for Mek assays and 5% for all others. Reactions were run at room temperature and terminated with an equal volume of 100 mM EDTA, 100 mM sodium pyrophosphate. Phosphorylated product was captured in 96-well Immobilon-P filter plates (Millipore). Filters were washed five times with H_2O and counted on a TopCount microplate scintillation counter (Packard).

PKA and PKC Kinase Assays. PKA and PKC were assayed at room temperature in the presence of 10 μM [^{33}P]-ATP. PKA and PKC (mix of isozymes) were assayed as recommended by the supplier (Upstate Biotechnology) using 10 μM kemptide (Sigma) and 70 μg/mL myelin basic protein (Upstate Biotechnology) as substrates, respectively. Reactions were terminated and quantitated as described for c-Raf, except that PKA reactions were stopped with 75 mM phosphoric acid, captured on 96-well P81 phosphocellulose filter plates (Millipore), and washed with phosphoric acid. EGF receptor tyrosine kinase (Promega) was assayed with poly(Glu,Ala,Tyr) (6:3:1) (Sigma) as substrate at a final concentration of 40 μM in a buffer containing 25 mM HEPES, pH 7.4, 5 mM $MgCl_2$, 2 mM $MnCl_2$. Phosphorylated peptide was captured and counted as described for Raf.

Lck Assays. These were carried out in a buffer containing 50 mM MOPS (pH 7.0), 1 mM DTT, 10 mM $MgCl_2$, 1 mg/mL BSA, 0.2 μCi of [^{33}P]ATP, 2 μM ATP, 2 μM Biotinyl-cdc2 peptide, and 1 nM Lck kinase domain. The reactions were incubated at 23 °C for 40 min, stopped with 100 mM EDTA, mixed with streptavidin-coated SPA beads, and counted on a Packard Top Count.

LPS Challenge Assay. Female CD1 mice (8/group, 8−10 weeks of age) were injected intraperitoneally with 10 μg of LPS and 800 mg/kg D-galactosamine in saline (0.5 mL). Compound was administered orally in 0.5 mL of 0.05 M aqueous citric acid 6 h before injection of LPS or intravenously in 0.2 mL of 0.5% methocel in saline immediately before LPS injection. Mice were sacrificed by CO_2 asphyxiation 90 min after LPS, and heparinized blood was collected by cardiac puncture. Blood was centrifuged at 1500*g* and 4 °C for 15 min, and plasma TNF-α levels were determined by ELISA (Genzyme).

Rat and Rhesus Pharmacokinetic Studies. These were carried out as described previously.[30]

In Vitro p450 Inhibition Studies. These were performed as described previously.[31]

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