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Pyrimidinylimidazole Inhibitors of p38: Cyclic N-1 Imidazole Substituents Enhance p38 Kinase Inhibition and Oral Activity

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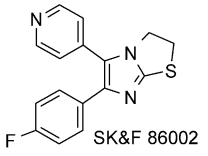
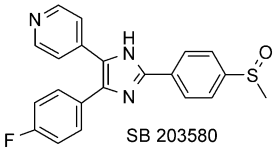
Abstract—Optimization of a series of N-1-cycloalkyl-4-aryl-5-(pyrimidin-4-yl)imidazole inhibitors of p38 kinase is reported. Oral administration of inhibitors possessing a cyclohexan-4-ol or piperidin-4-yl group at N-1 in combination with alkoxy, amino(alkyl), phenoxy and anilino substitution at the 2-position of the pyrimidine was found to potently inhibit LPS-induced TNF in mice and rats. The selectivity of these new inhibitors for p38 kinase versus eight other protein kinases is high and in all cases exceeds that of SB 203580.

The pyridinylimidazole class of antiinflammatory agents (e.g., SK&F 86002 and SB 203580) selectively inhibit the stress-activated p38α MAP kinase (CSBP2, RK).[1,2] Signaling through p38α is required for synthesis of several proinflammatory proteins (e.g., IL-1, TNF, COX-2, IL-6, and IL-8) and is also known to mediate numerous other aspects of cell physiology.[3] Selective inhibitors of p38 have shown beneficial effects in several animal models of disease, for example, rheumatoid arthritis and septic shock.[4,5] Recently, p38α has received widespread attention as an attractive target for the development of therapeutic agents, and several research teams have undertaken the challenge to find p38 inhibitors suitable for clinical development.



SK&F 86002



SB 203580

The importance of the 4-(pyridin-4-yl)-5-phenyl-imidazole substructure for p38α inhibition is well established.[6] X-ray crystallographic and mutagenesis studies of p38α with these inhibitors have revealed the molecular basis for much of the observed SAR.[7,8] These studies locate the pyridin-4-yl group in the adenine binding pocket of ATP with a hydrogen bond between the pyridinyl nitrogen and the amide NH of Met109. That the hydrogen bond to the pyridyl nitrogen is important for inhibition is illustrated by the >100-fold loss in affinity resulting from substitution with a 2- or 3-pyridine or a phenyl.[6] We have also reported that the 4-pyridinyl group is an important factor contributing to P450 inhibition and described a series of pyrimidinylimidazole p38α inhibitors with reduced inhibition of P450.[9] In this communication we report new pyrimidinylimidazoles which are more potent p38 MAP kinase inhibitors and which demonstrate improved in vivo activity. Recently, Liverton et al. at Merck have reported the optimization of a related series of C-2 alkyl substituted pyrimidinylimidazoles which also display improved efficacy.[9]

Boehm et al. reported that an alpha-branched N-1 substituent on the imidazole improves the oral activity of 1-alkyl-4-(aryl)-5-(pyridin-4-yl)imidazoles relative to the unbranched compounds.[10] In the present communication, this observation has been applied in combination

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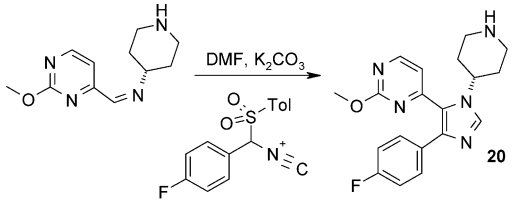
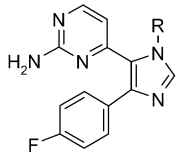
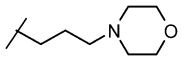
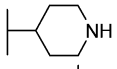
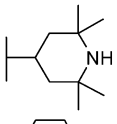
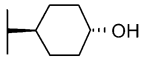
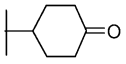
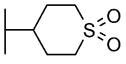
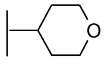
with a 2-substituted pyrimidin-4-yl replacement for the pyridin-4-yl group, towards the goal of achieving improved in vitro and in vivo potency. Desiring not to introduce chirality, compounds with symmetrical, non-chiral, cyclic groups at N-1 were preferentially targeted for synthesis.

All the compounds described herein were prepared using either the previously published imine-tosylisonitrile cycloaddition reaction conditions (TBD/CH_2Cl_2) to form the imidazole,[9] or more preferably, the preformed imine was treated with the isonitrile in DMF using solid K_2CO_3 as the base.[11]



Representative compounds are illustrated in Table 1, along with their IC_{50} for inhibition of p38α and the ED_{50} for inhibition of murine TNF synthesis induced by lipopolysaccharide (LPS). A substantial increase in p38α potency was seen for the piperidinyl (**3** and **4**) and cyclohexyl (**5**) compounds. These analogues also demonstrated good in vivo activity in the murine assay for inhibition of LPS-induced TNF.

Table 1. N-1 Cyclic-4-(4-F-phenyl)-5-(pyrimidin-4-yl)imidazoles

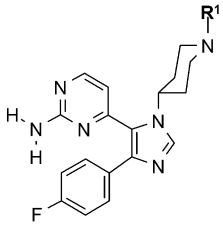
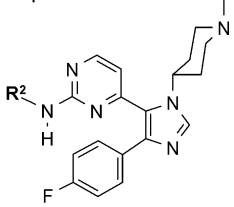
Compd		p38 α IC_{50} (nM)	Murine ED_{50} (mg/kg)[a]
1		480	5.2
2		170	−26%*
3		20	8.0
4		58	9.0
5		83	7.3
6		160	13
7		390	−13% NS
8		230	−50% ***

[a]Compounds were dosed orally using the published protocol.[12] Data given either as ED_{50} or % change in the LPS-induced TNF signal from controls at the screen dose of 50 mg/kg; significant difference from controls NS not significant, $*p<0.05$, $**p<0.01$, $***p<0.001$.

Piperidine **3** (SB 220025) was initially chosen for more detailed investigation (Table 2). Alkyl groups (Me, *i*Pr) and electron withdrawing substituents (CH_2CF_3 and CO_2Et) on the piperidine nitrogen (R^1) had a minor, but negative effect on p38α kinase activity. This trend was reversed for benzyl substitution, which afforded the most potent compound in this series (**13**). The in vivo activity of these compounds in the mouse following oral administration also decreased with R^1 substitution, the only exception being the *N*-methylpiperidine (**9**). Since maximal in vivo activity was achieved with the methyl substituted piperidine (**9**), the effect of substitution on the 2-aminopyrimidine nitrogen (R^2) was explored holding R^1 as methyl. In general, substitution of the amine improved p38 inhibition (**9** compared to **14**–**19**), but not in vivo activity. Exceptions were the significantly reduced p38α activity of the *N*-piperidinyl compound (**19**) and the high in vitro and in vivo potency of the anilinopyrimidine (**17**). In summary, although *N*-alkylation (R^1 and R^2) did not in most instances significantly improve p38α potency, it did in some cases improve oral activity. For example, the bis-*N*-methyl compound (**14**), a 100 nM p38α inhibitor, was one of the most potent inhibitors of LPS-induced TNF production in the mouse. Improved oral activity was also observed for the *N*-methyl analogues **9** and **14** ($ED_{50}=4$ and 5.5 mg/kg) relative to the parent **3** ($ED_{50}=20$ mg/kg) for the inhibition of LPS-induced TNF in the rat.[13]

The examples in Table 3 incorporate at N-1 of the imidazole the preferred unsubstituted piperidine group determined in Table 2 along with the *trans*-cyclohexan-4-ol (Table 1) in combination with the preferred 2-aminopyrimidine groups and several of the corresponding 2-oxy-pyrimidine analogues. We have previously reported that the 2-methoxypyrimidine analogue of compound **1**

Table 2. Optimization of piperidinyl nitrogen substitution

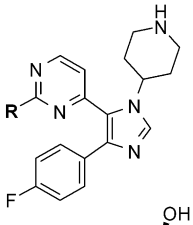
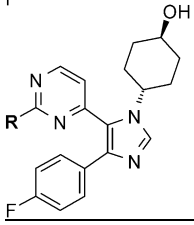
	Compd	R^1	p38 α IC_{50} (nM)	Murine ED_{50} (mg/kg[a])
	**3**	H	19	8.0
	9	Me	86	4.4
	10	*i*-Pr	170	−65%***
	11	CH_2CF_3	42	−82%***
	12	CO_2Et	33	−54%***
	13	Bn	8.5	17
		R^2		
	**14**	Me	100	2.4
	15	Et	9.6	11
	16	$HOCH_2CH_2$–	53	16
	17	Ph	3.0	0.42
	18	Bn	45	10
	19	Piperidin-4-yl	610	−62%***

[a]Same as Table 1.

possesses similar in vitro and in vivo activity and in the present case this trend holds for the 2-alkoxypyrimidines in Table 3.[9] The equivalent potency of the 2-alkoxy and 2-aminopyrimidines is evident for compounds from both the N-1-piperidin-4-yl (compare **20** and **24**) and N-1-*trans*-cyclohexan-4-ol (compare **26** and **28**) series. In comparison to the prototypical p38 inhibitor, SB 203580, the compounds in Table 3 are significantly more potent inhibitors of murine LPS-induced TNF ($ED_{50} = 15$ mg/kg for SB 203580).[4] Unlike the 2-alkoxy and 2-aminopyrimidines, the anilinopyrimidines (**25** and **29**) are approximately 10-fold more potent as inhibitors of p38α than the corresponding oxygen substituted phenoxypyrimidines (**23** and **27**).[14]

Optimizing oral activity, as discussed above, and reducing hepatic P450 inhibition and dual 5-LO/COX inhibition, as reported in previous communications,[9,10] are key accomplishments toward our goal of discovering pyridinyl/pyrimidinylimidazoles suitable for clinical evaluation. In order to minimize potential undesirable side effects, an additional feature anticipated to be important for a clinical development candidate is high

Table 3. Optimization of the 2-pyrimidinyl substituent for preferred N-1 side chains

	Compd	R	p38α IC_{50} (nM)	Murine ED_{50} (mg/kg)[a]
	**20**	MeO	19	2.6
	21	EtO	79	0.25
	22	*i*-PrO	53	1.2
	23	PhO	20	0.84
	24	MeNH	27	3.0
	25	PhNH	1.4	0.60
	**26**	MeO	34	5.8
	27	PhO	12	4.2
	28	MeNH	18	6.5
	29	PhNH	1.5	−28%[b]

[a]Same as Table 1.
[b]The 28% inhibition at a dose of 5 mg/kg was not statistically significant from control.

Table 4. Kinase selectivity of selected pyrimidinylimidazoles

			IC_{50} (nM)	
Kinase	AA @ 106	SB 203580	**20**	**26**
P38α	Thr	48	19	34
ERK2	Gln	>50,000	>50,000	>50,000
JNK1	Met	5,000	>50,000	>50,000
JNK2β2	Met	2,000	>20,000	5,000
cdc2	Phe	>50,000	>100,000	>50,000
PKCα	Met	>100,000	83,000	83,000
PKCβ2	Met	>50,000	17,000	ND
cRaf	Thr	460	>50,000	>50,000
EGFR	Thr	10,000	>10,000	83,000
p56 Lck	Thr	20,000	>10,000	18,000

ND, not determined.

selectivity for the target kinase. The kinase selectivity of the pyridinylimidazole class was originally demonstrated with SB 203580.[2]

The structural basis for the p38α selectivity has been studied by several investigators all of whom have noted the importance of a single residue at position 106 in the ATP binding site of p38.[7,8] In p38α and β, both of which are sensitive to inhibition by SB 203580 and related pyridinylimidazoles, this residue is threonine. The small threonine residue at position 106 allows for the formation of an aryl binding pocket, which is not utilized by ATP, but which accommodates the 4-F-phenyl group of the pyridinylimidazoles. Hence, much of the specificity of the pyridinylimidalozes results from the inability of kinases with larger amino acid side-chains at position 106 to readily accommodate the 4-F-phenyl group. Other members of the MAP kinase family to which p38 belongs and the corresponding position 106 residue for these kinases are: (1) p38γ and δ (methonine), (2) Erk1 and 2 (glutamine), and (3) JNK1, 2 and 3 (methonine). These larger side-chain residues at position 106 are sufficient to make the ERK kinases and both the γ and δ forms of p38 insensitive to inhibition by SB 203580. In contrast, a more modest ~100-fold selectivity is seen for JNK1 and 2β2. Outside of the MAP kinase family selectivity is again >1000-fold for kinases having residues larger than threonine at position 106 (cdc2, PKCα, and PKCβ2), but is reduced for those kinases which, like p38α, have a threonine at position 106 (cRaf, EGFR, and p56 lck).[3] For the presently reported compounds, the best overall kinase selectivity was obtained for the 2-methoxypyrimidines, **20** and **26** (Table 4). The selectivity of these compounds is improved relative to SB 203580, with the reduced inhibition of cRaf and JNK1 being the most advantageous changes.

Compounds **20** (SB 242235) and **26** (SB 239063) have been used as tools to provide evidence supporting the potential therapeutic utility of p38 inhibitors in the treatment of rheumatoid arthritis,[15] stroke,[16,17] pulmonary inflammatory disease,[18,19] and virally-induced inflammation.[20] Based upon its overall profile, SB 242235 was chosen as a clinical development candidate for evaluation in the treatment of rheumatoid arthritis.[21]

References and Notes

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13. Further studies revealed improved bioavailability for the *N*-methyl compounds **9** and **14,** relative to **3**. When combined with the rapid *N*-dealkylation of the piperidines (**9** and **14**) to yield more potent inhibitors of p38 (**3** and **24**), this may explain the better oral activity of the weaker p38 inhibitors.
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