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Imidazolyl benzimidazoles and imidazo[4,5-*b*]pyridines as potent p38α MAP kinase inhibitors with excellent in vivo antiinflammatory properties

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Abstract—Herein we report investigations into the p38α MAP kinase activity of trisubstituted imidazoles that led to the identification of compounds possessing highly potent in vivo activity. The SAR of a novel series of imidazopyridines is demonstrated as well, resulting in compounds possessing cellular potency and enhanced in vivo activity in the rat collagen-induced arthritis model of chronic inflammation.
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Over the last decade, the pursuit of p38α MAP kinase inhibitors has received an extraordinary level of attention in the pharmaceutical industry[1] and in the medicinal chemistry community.[2] A unique combination of well-established pharmacology, clinical efficacy,[3–6] and the opportunity to utilize structure-based drug design[7] has made this a highly attractive target for therapeutic intervention.[8,9]

There is overwhelming evidence indicating that p38α plays a dominant role in the pathogenesis of acute and chronic inflammatory responses.[1,2] Activation of p38α occurs in monocytes and macrophages under different stress-related stimuli. Subsequent phosphorylation of downstream effectors and transcriptional factors leads to the biosynthesis of potentially deleterious pro-inflammatory cytokines such as TNFα and IL-1β.[10] The clinical proof of concept in rheumatoid arthritis achieved with VX-745[3] and BIRB-796[5] validates the MAP kinase pathway as a useful mechanism for intervention in inflammatory disease.

We have previously reported the design and discovery of a 2-aminobenzimidazole-based series of potent and highly selective p38α inhibitors.[11] Awareness of potential CYP activity associated with the imidazole central core[12] led to an SAR around the imidazole C-2, and recognition that sterically bulky groups such as *tert*-butyl, 2,6-dichlorophenyl, or 2,6-difluorophenyl resulted in decreased inhibition of CyP3A4. The lead compound **1** had low nanomolar activity in both ATP competitive enzyme binding and inhibition of TNFα release in macrophages. Expansion of the SAR identified **2**

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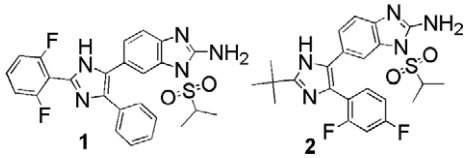
which showed excellent in vivo activity in the rat collagen-induced arthritis model (CIA) as compared to the p38 reference compounds BIRB-796, SB242235, and RWJ-67657. Herein we report investigations into the SAR of substitution on C-2, 4, and 5 of the imidazole to identify additional compounds possessing in vivo activity. The activity of a series of imidazopyridines is demonstrated as well, resulting in compounds with potency in the cellular and in vivo assays.



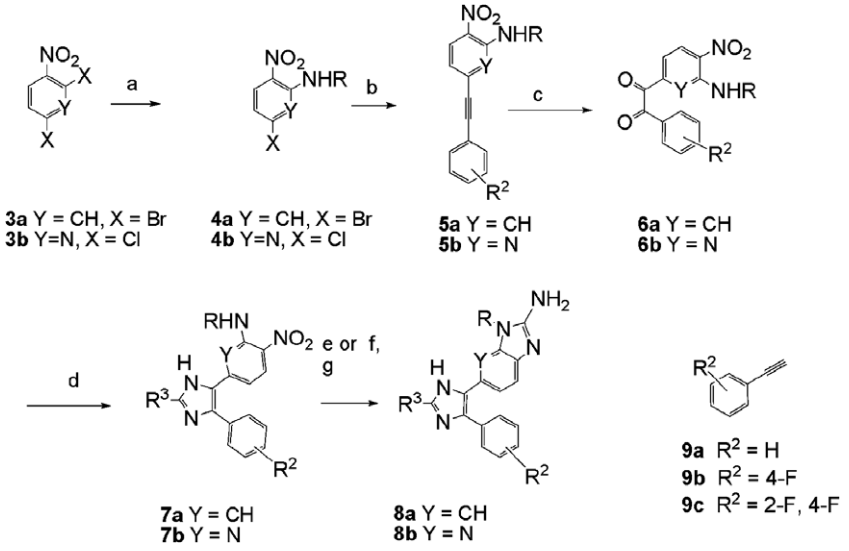
1 **2**

The route for preparation of *N*-sulfonyl analogs of **1** (**20**, **24**–**34**) has been reported previously.[11] The *N*-alkyl derivatives in Tables 1–3 were prepared as shown in Scheme 1. Beginning with nucleophilic aromatic substitution by an alkyl amine on the dihalides **3a**[13] or **3b**, palladium catalyzed coupling of the halides **4a** or **4b** with aryl alkynes (**9a**–**c**) was followed by oxidation using $KMnO_4$, leading to diketones **6a** and **6b**. Treatment of the diketones with an aldehyde in the presence of excess ammonium acetate afforded the trisubstituted imidazoles **7a** and **7b**. Finally, reduction of the nitro moiety by either sodium dithionite or tin dichloride provided an amine, which was cyclized by treatment with cyanogen bromide to form the C-2 amino benzimidazoles **8a** or imidazopyridines **8b**. Alkylamino benzimidazole products **10**, **11**, and **14** were prepared via a modified sequence (not shown) in which amino benzimidazoles **8a** were converted into chlorides via a Sandmeyer reaction utilizing *tert*-butyl nitrite and copper chloride. The chlorides were converted to the alkyl amine in reaction with the corresponding alkyl amine in THF. Frequently, these basic compounds were converted to their mesylate salts for assay.

Inhibition of p38α was determined using recombinant human p38α in a standard filter binding protocol using ATP[γ-^{33}P] and EGFR 21-mer peptide as substrate. Functional inhibition of TNFα in murine peritoneal macrophages was determined using LPS stimulation in the presence of test compounds. To assess p38α activity in cells more directly, RAW 264.7 cells were treated with test compounds and then stimulated with anisomycin. The level of p38α activity was detected using a phospho-MAPKAPK-2 (pMK2) (Thr 334) antibody which reacted with a residue specifically phosphorylated by p38α. The compounds appeared to have greater potency in the TNFα macrophage assay due to JNK2α2 activity,[11] and therefore we focused on the more direct read-out of p38 cellular activity, the pMK2 cELISA, to prioritize compounds for in vivo study.[14]

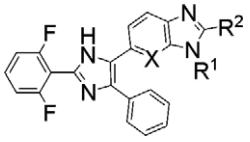
In comparison to the initial lead **1**, mono- and dialkylation of the benzimidazole C-2′ amino group resulted in decreased potency in both the enzyme and cellular assays for **10** and **11**. Removal of the sulfonyl group at N-1′ of the benzimidazole also diminishes activity (**13**), but in this instance, C-2′ amino alkylation of **14** recovers 2- to 3-fold activity in the enzyme and cell.

Placing an alkyl group at N-1′ rather than on the C-2′ amine is also well tolerated (**15**–**18**), but a phenyl group is not (**19**). Interestingly, the C-2′ amino moiety is not essential for enzyme activity (**12** vs **13** and **14**; **15** and **17** vs **16**), and its impact on cellular activity appears dependent on substitution at N-1′. Introduction of nitrogen into the 6-membered ring of the benzimidazole



Scheme 1. General synthetic scheme for imidazolyl benzimidazoles and imidazopyridines. Reagents and conditions: (a) RNH_2 (1.5 equiv), Na_2CO_3 (1.75 equiv), EtOH, rt, 18 h (84–94%); (b) **9a**–**c** (1.5 equiv), Ph_3P, $Pd(OAc)_2$, Et_3N, 70–80 °C, 1–3 h (71–85%); (c) $KMnO_4$, $MgSO_4$/$NaHCO_3$, acetone, 0 °C, 1–2 h (50–82%); (d) R_3–CHO (2 equiv), NH_4OAc (15 equiv), AcOH, 80 °C (62–97%); (e) $Na_2S_2O_4$, concd NH_4OH, rt, 2 h; (f) $SnCl_2{\cdot}2H_2O$, EtOH, EtOAc, 70 °C, 2 h; (g) CNBr (1.1 equiv), LiOMe (1.5 equiv), 1,2-dichloroethane (43–59% for the two-step reduction/cyclization process). Some compounds were isolated as mesylate salts: MsOH, MeOH.

Table 1. Enzymatic and cellular activity for *N*-1′-substituted aminobenzimidazoles (IC_{50}, nM)



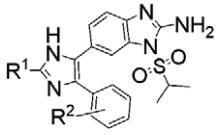
Compound	R^1	R^2	X	p38α	Macrophage TNFα	cELISA pMK2
1	*i*-$PrSO_2$	NH_2	CH	4.9	10.3	45.7
10	*i*-$PrSO_2$	NHEt	CH	156	527	—[c]
11	*i*-$PrSO_2$	N(Me)Bn	CH	2489	234	—[c]
12[a]	H	H	CH	88.4	419	766
13	H	NH_2	CH	131	1326	—[c]
14	H	NHEt	CH	56.4	493	—[c]
15	*i*-Bu	H	CH	16.2	51.9	—[c]
16[a]	*i*-Bu	NH_2	CH	9.9	47.5	78.3
17[b]	*i*-Bu	Me	CH	11.2	338	—[c]
18[a]	CH_2-*t*-Bu	NH_2	CH	6.0	3.5	126
19	Ph	NH_2	CH	158	—[c]	—[c]
20[b]	*i*-$PrSO_2$	NH_2	N	3.5	3.7	298
21[b]	*i*-Bu	NH_2	N	2.5	1.3	35.0
22[b]	*i*-Bu	H	N	4.5	26.7	69.7
23[b]	CH_2-*t*-Bu	NH_2	N	6.4	<9	—[c]

[a] Bismesylate salt.
[b] Monomesylate salt.
[c] Not tested.

provided a series of imidazopyridines with potent TNFα macrophage activity (**20**–**23**), and was independent of substitution at N-1′ and C-2′.

Table 2 demonstrates the impact of variation of substitution at the imidazole C-2 by aromatics or methyl, while maintaining the amino benzimidazole substitution constant. No substitution (R^1) is required for enzymatic activity (**24**), but it does improve cellular potency (**2**). An embedded electronegative atom (pyridyl) is well tolerated in **25**, but a decrease in enzymatic and cellular potency is observed when the same position is substituted by an electronegative chloride (**26**). An electron withdrawing group at the ortho site is comparable to that at para (**26** vs **27**), hence we focused on the ortho positions because of earlier findings that demonstrated ortho substitution decreased CYP activity. Best within this series is the 2,6-dichloro analog **30**, which shows enhanced cellular potency relative to the other disubstituted analogs, **28** and **29**. An aromatic group at C-4 of the imidazole has been shown to occupy the lipophilic specificity pocket of the ATP binding site,[10,15] and addi-

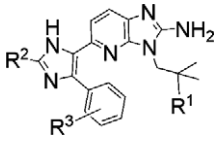
Table 2. Enzymatic and cellular activity for 1-(isopropylsulfonyl)benzimidazole-2-amines (IC_{50}, nM)



Compound	R^1	R^2	p38α	Macrophage TNFα	cELISA pMK2
2[c]	*t*-Bu	2,4-F_2	4.4	6.2	26.2
24	H	H	4.2	31.8	195
25	4-Pyr	H	6.1	14.1	62.3
26	4-Cl–C_6H_5	H	34.7	71.0	376
27	2-CF_3–C_6H_4	H	26.1	33.1	284
28[a]	2-Cl–6-F–C_6H_4	H	14.5	11.2	74.5
29[b]	2-Cl–6-CF_3–C_6H_4	H	8.5	1.9	398
30[b]	2,6-Cl_2–C_6H_4	H	15.3	2.6	38.9
31[b]	2,6-Cl_2–C_6H_4	4-F	6.7	5.3	27.5
32[a]	Me	H	20.4	186.4	—[c]
33[b]	Me	4-F	7.1	103.5	225
34[b]	Me	2,4-F_2	8.8	46.5	59.7

[a] Bismesylate salt.
[b] Monomesylate salt.
[c] Not tested.

Table 3. Enzymatic and cellular activity for imidazopyridine derivatives (IC_{50}, nM)



Compound	R^1	R^2	R^3	p38α	Macrophage TNFα	cELISA pMK2
21[b]	H	2,6-F_2–C_6H_4	H	2.5	1.3	35.0
35[b]	H	2,6-Cl_2–C_6H_4	H	3.7	1.7	64.7
36[a]	H	*t*-Bu	H	5.1	54.5	66.4
37[b]	H	2,6-Cl_2–C_6H_4	2,4-F_2	3.9	<9.0	31.2
38[b]	H	*t*-Bu	2,4-F_2	3.9	49.2	41.8
39[b]	CH_3	2-Cl, 6-F–C_6H_4	H	3.2	<9.0	39
40[a]	CH_3	*t*-Bu	4-F	7.0	5.2	34.3
41[b]	CH_3	2,6-F_2–C_6H_4	4-F	7.2	<9.0	—[c]
42[b]	CH_3	2,6-Cl_2–C_6H_4	4-F	3.6	<9.0	23.0
43[b]	CH_3	*t*-Bu	2,4-F_2	2.3	24.1	20.7

[a] Bismesylate salt.
[b] Monomesylate salt.
[c] Not tested.

tion of halogens to the C-4 aryl improves both enzymatic and cellular potency. A stepwise analysis of the impact of halogenation on the C-4 aromatic was conducted in the series **32**–**34**, with a 2-fold improvement in enzymatic and macrophage TNFα activity as fluorines are added to the phenyl ring. Fluorination increases activity in the pMK2 cELISA approximately 4-fold (**33** and **34**).

The imidazopyridines **20**–**23** possessed significant enzymatic and cellular potency, and the series was expanded. Gains in cellular potency were achieved as we focused on the elements that had been found to be preferred on the imidazole: at C-2, a di-ortho substituted aromatic ring or *tert*-butyl, and at C-4, a fluorinated phenyl (Table 3). Varying R^1 as isobutyl (**21**, **35**–**38**) or neopentyl (**39**–**43**) provided compounds that were similarly potent in the enzyme, as all were single-digit nanomolar inhibitors. Greater differences in potency were manifested in the cellular assays. Aromatic substitution at R^2 of the imidazole was preferred in the isobutyl series for cellular potency (**35** and **37**), as the *tert*-butyl analogs **36** and **38** were 50-fold less potent in the macrophage assay. However, the neopentyl analogs **40**–**42** tolerated *tert*-butyl and dihalo aromatics equally well in the cellular assays. An exception is **43**, for which the 2,4-difluorophenyl at C-4 did not provide an increase in cellular potency as it had in the methyl imidazole series **32**–**34**.

To evaluate how the cell activity translated to in vivo acute TNFα inhibition, compounds were orally dosed in Balb/c mice followed by iv LPS administration after 2 h. From these dose response studies, a threshold minimum 50% effective dose ($TMED_{50}$) was determined. The compounds were also evaluated in a 14-day chronic model of inflammation, the rat CIA,[16] and activity was assessed by effect on paw swelling and by histopathology scores (bone erosion and cartilage destruction). In the mouse model, the imidazopyridines **35** and **40** showed improved potency relative to the benzimidazoles. In the rat CIA model, both exposure and half-life in rat appeared to influence activity. Compounds **1** and **35** were significantly less potent than the *tert*-butyl analogs **2** and **40** in both paw swelling and histology $TMED_{50}$ (Table 4).

In summary, we have shown that substitution at C-2 of the imidazole by di-ortho substituted aromatics or *tert*-butyl provides analogs with good activity in the enzyme and cellular assays, and that cellular potency can be increased with addition of fluorines to the imidazole C-4 aromatic ring. Conversion of the benzimidazole warhead into an imidazopyridine results in increased in vivo potency in an acute inflammation model, but activity in the chronic model may be more dependent on the pharmacokinetic properties of the compounds.

Table 4. In vivo activity of selected compounds[a]

Compound	Mouse TNFα inh. (po, iv LPS), $TMED_{50}$ (mg/kg)	CIA, paw swelling $TMED_{50}$[b] (mg/kg)	CIA, histology, $TMED_{50}$[b] (mg/kg)	Rat oral AUC_{0-inf} (10 mg/kg) (μM h)	Rat oral $t_{1/2}$ (h)
1	5.2	>30	>30	5.5	5
2	2.2	1.5	8.1	14.6	8
35	<2.5	15	>30	29.9	4
40	<1.0	1.5	1.5	87.5	10

[a] Vehicle: 1%CMC/0/25% Tween 80 in water.
[b] Oral bid dosing for 14 days, therapeutic, $p < 0.05$ for all cases.

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