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Discovery and initial SAR of inhibitors of interleukin-1 receptor-associated kinase-4

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Abstract—High-throughput screening of a small-molecule compound library resulted in the identification of a novel series of *N*-acyl 2-aminobenzimidazoles that are potent inhibitors of interleukin-1 receptor-associated kinase-4.

Interleukin-1 (IL-1) receptor-associated kinase-4 (IRAK-4) is a serine/threonine kinase acting downstream of members of the Toll/IL-1 receptor (TIR) family which act as mediators in signal transduction. IRAK-4 shares the domain structure of the other IRAKs[1–4] and has been shown to activate similar signal transduction pathways, namely the NF-κB and MAPK pathways.[4] In contrast to the other IL-1 receptor-associated kinases, IRAK-1,[1] IRAK-2,[2] and IRAK-M,[3] IRAK-4 has been shown to require its kinase activity in order to activate NF-κB.[4] Endogenous IRAK-4 interacts with IRAK-1 and TRAF6 in an IL-1-dependent manner but is not redundant with IRAK-1, suggesting a central role for IRAK-4 in the early signal transduction of Toll/IL-1 receptors. IRAK-4 knockout mice have been characterized, demonstrating that IRAK-4 is indispensable for IL-1 signal transduction,[5] and reconstitution experiments with IRAK-4-deficient cell lines have confirmed the dependence of IRAK-4 on its kinase activity for signal transduction.[6]

Recently, several human patients who lack full-length IRAK-4 expression have been identified. These patients suffer from a compromised immune response, further supporting IRAK-4 inhibition as a means of addressing inflammatory diseases.[7,8] The clinically demonstrated utility of IL-1 antagonists as anti-inflammatory therapeutics,[9] combined with the properties of the IRAK-4 kinase described above, makes IRAK-4 an interesting target for intervention by an orally administered small-molecule drug.

We identified *N*-acyl-2-aminobenzimidazoles **1** (IRAK-4 IC_{50} = 4.0 μM) and **2** (IC_{50} = 2.0 μM) following high-throughput screening of IRAK-4 against a small-molecule library (Fig. 1).

Acyl-2-aminobenzimidazole analogs **3**–**48** were prepared as outlined in Scheme 1.[10] Treatment of 2-aminoanilines **49** with cyanogen bromide[11] gave the 2-aminobenzimidazoles **50**. Treatment of **50** (X=H) with 3-nitrobenzenesulfonyl chloride in $NaOH/H_2O$ gave the sulfonamide **5**, while coupling of **50** with the appropriate benzoic acid with HBTU/HOBT gave *N*-acyl-2-aminobenzimidazoles **51**. Reduction of **51** (X=H, Y=NO_2) with $NaBH_4$ in toluene at 100 °C gave the *N*-benzyl-2-aminobenzimidazole **3**. General conditions were developed for the alkylation of **51** with a variety of alkyl halides. Thus, treatment of **51** with the appropriate alkyl halide in the presence of potassium carbonate in acetone/DMF/H_2O (5:1:0.1) and at temperatures from room temperature to 40 °C gave the final products **6**–**46** in moderate to excellent yields.

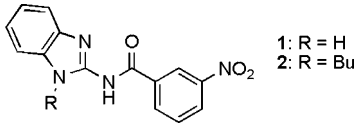

Figure 1. Initial IRAK-4 inhibitor hits.

Keywords: IRAK-4; Kinase; Inflammation; Kinase inhibitor.
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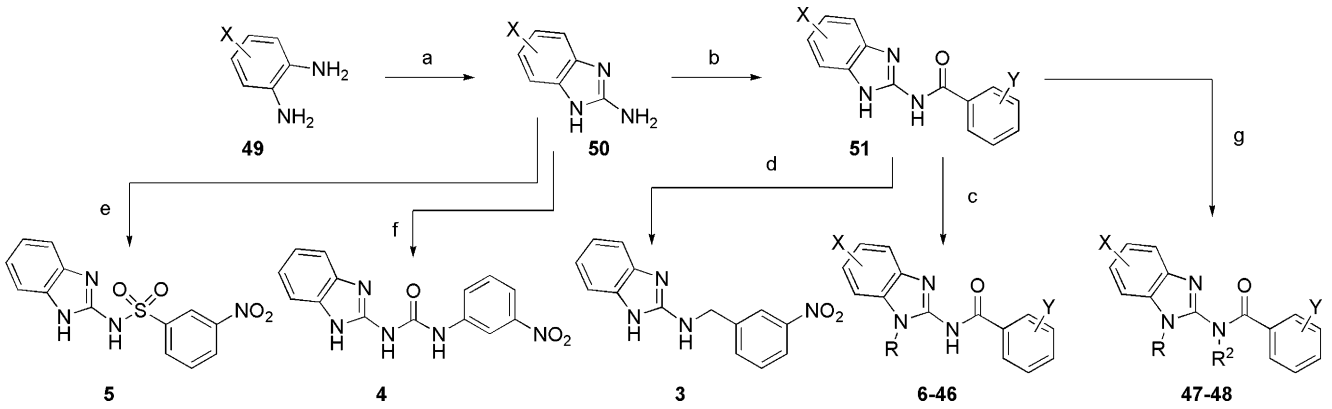


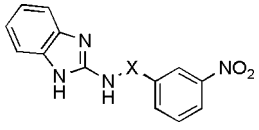
Scheme 1. Reagents and conditions: (a) BrCN, MeOH/H_2O, 42–84%; (b) benzoic acid, HBTU, HOBT, NMM, DMF, 56–97%; (c) alkyl halide, K_2CO_3, acetone/DMF/H_2O 5:1:0.1, 39–100%; (d) $NaBH_4$, toluene, 100 °C, 16%; (e) 3-nitrobenzenesulfonyl chloride, 1 M NaOH/THF, 87%; (f) 3-NO_2PhNCO, DMF, rt, 1 h, 34%; (g) excess alkyl halide, K_2CO_3, acetone/DMF/H_2O 5:1:0.1, 53% (**48**), 88% (**47**).

Initial efforts sought to establish the minimum pharmacophore required for IRAK-4 inhibition. Investigation of the amide functionality was carried out by carbonyl replacement (Table 1). Compared to **1**, removal of the amide carbonyl (**3**) resulted in a substantial reduction of IRAK-4 inhibition, as did replacement of the amide moiety with urea and sulfonamide groups (**4** and **5**), indicating that the amide connection between the two aromatic groups is beneficial for activity.

We next turned to substituent modification on the benzamide ring (Table 2). Removal or replacement of the 3-nitro group was found to be detrimental for potency in a limited set of analogs of **1** (**6**–**9**) or the *N*-alkyl substituted lead **2** (**10**–**13**).

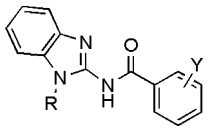
Our investigation into the pharmacophore required for IRAK-4 inhibition then moved to the benzimidazole group (Tables 3 and 4). Replacement of the fused benzene ring with the corresponding 2 and 3 pyridyl analogs resulted in a substantial reduction in activity (**14**–**15**). Similarly, replacement of the benzimidazole nitrogen with sulfur such as in benzothiazole **16** also reduced potency. The effect of substituents on the benzimidazole ring indicated a sharp SAR, and in a limited set of analogs substitution in the 5-position led to improvements in potency (Table 4, **19**–**20**), while substitution at the 4-position (**24**, **27**) or disubstitution at the 5- and 6-positions (**25**–**26**, **28**) led to less active compounds.

Table 1. Inhibition of IRAK-4 by selected analogs: linker replacements



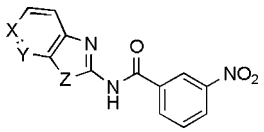
Compound	X	IRAK-4% inhibition at 30 μM	IRAK-4 IC_{50} (μM)[12]
1	–CO–		4.0
3	$–CH_2–$	8%	—
4	–CONH–	15%	—
5	$–SO_2–$	45%	—

Table 2. Inhibition of IRAK-4 by selected analogs: amide aryl substituent replacements



Compound	R	Y	IRAK-4% inhibition at 30 μM	IRAK-4 IC_{50} (μM)[12]
1	H	3-NO_2		4.0
6	H	3-CN		13.6
7	H	H	11%	—
8	H	3-NO_2-4-F		6.5
9	H	3-NO_2-4-Me		12
2	Bu	3-NO_2		2
10	Bu	3-Cl		30
11	Bu	3,4-di-Cl		3
12	Bu	4-NO_2	8%	—
13	Bu	4-OMe		6.5

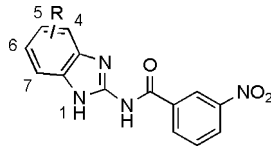
Table 3. Inhibition of IRAK-4 by selected analogs: benzimidazole modifications



Compound	X	Y	Z	IRAK-4% inhibition at 30 μM	IRAK-4 IC_{50} (μM)[12]
1	CH	CH	NH		4.0
14	CH	N	NH	39	—
15	N	CH	NH		30
16	CH	CH	S	11	—

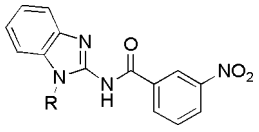
Having established the requirement for the amide linkage and substituent effects on the benzamide and benzimidazole rings, the SAR around benzimidazole *N*-alkyl substitution was explored (Table 5). As was expected from the 2-fold improvement in potency

Table 4. Inhibition of IRAK-4 by selected analogs: benzimidazole substituent modification



Compound	R	IRAK-4% inhibition at 30 μM	IRAK-4 IC_{50} (μM)[12]
1	H		4.0
17	5-Cl		6.0
18	5-F		4.5
19	5-CH_3		0.80
20	5-OCH_3		1.5
21	5-CO_2CH_3		25
22	5-$SO_2(CH_2)_2Me$		4.0
23	5-NO_2	29	—
24	4-NO_2	25	—
25	5,6-di-F		30
26	5,6-di-Cl	10	—
27	4,5-di-F	24	—
28	5,6-di-CH_3		8.0

Table 5. Inhibition of IRAK-4 by selected analogs: *N*-alkyl substitution



Compound	X	IRAK-4% Inhibition at 30 μM	IRAK-4 IC_{50} (μM)[12]
1	H		4.0
2	Bu		2.0
29	Et		2.0
30	^{i}Pr		1.0
31	Allyl		1.0
32	Pentyl		20
33	$(CH_2)_2$Cyclobutyl		2.0
34	$(CH_2)_2OMe$		1.0
35	$(CH_2)_2OEt$		2.0
36	CH_2CO_2Et		0.40
37	$(CH_2)_2CO_2Et$		1.0
38	α-(γ-Butyrolactone)		0.55
39	(2-Tetrahydrofuranyl)methyl		0.50
40	$(CH_2)_2OH$		0.40
41	$(CH_2)_3OH$		0.15
42	CH_2CO_2H	38	—
43	$(CH_2)_2CO_2H$		4.0
44	$(CH_2)_3NMe_2$		4.0
45	$(CH_2)_2NEt_2$		3.0
46	$(CH_2)_2$morpholinyl		0.20

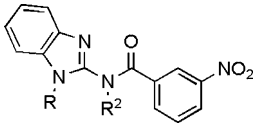
between the *N*-butyl lead **2** over the unsubstituted lead **1**, *N*-substitution with linear alkyl groups led to a modest 2- to 4-fold improvement in potency for ethyl (**29**) and allyl (**31**) over the unsubstituted lead **1**, but extension of the chain to pentyl (**32**, IC_{50} = 20 μM) led to a 10-fold decrease in potency relative to the *N*-butyl lead compound **2**. This sharp decrease in potency may be attributed to decreased solubility, given that the ethoxyethyl analog **35** (IC_{50} = 2 μM) has similar potency to **2**. The *N*-isopropyl compound **30** (IC_{50} = 1 μM) indicated that α-substitution was allowed on the *N*-alkyl substituent, while the ethylenecyclobutyl substituted compound **33** indicated room for increased steric bulk removed from the nitrogen of the benzimidazole.

Replacement of the *N*-alkyl group with more polar substituents led to additional improvements in potency. The acetoester **36** (IC_{50} = 0.4 μM) had 5-fold greater potency than the *N*-butyl lead **2**, as did the *N*-α-(γ-butyrolactone) analog **38** (IC_{50} = 0.55 μM) and *N*-(2-tetrahydrofuranyl)methyl analog **39** (IC_{50} = 0.5 μM). Increasing polarity and solubility further, the N-linked straight chain alcohols (e.g., **40**) resulted in improved potency, leading to the potent *N*-propanol analog **41** (IC_{50} = 0.15 μM), a 13-fold increase in potency over the *N*-butyl lead **2**. Carboxylic acid substitution was allowed with the proper spacer length (**43**) although no gain in potency was obtained over the lead, and similar results were observed for substitution with strongly basic amines (**44**–**45**). Finally, reducing the basicity of the amine functionality by replacement with the less basic *N*-ethylenemorpholine moiety greatly improved potency (**46**, IC_{50} = 0.20 μM), resulting in a 10-fold improvement over *N*-butyl lead **2**.

The importance of the free amide N–H was also investigated (Table 6). Replacement of the amide N–H was found to eliminate activity at the highest concentration tested (30 μM) in the case of the *N*-allyl substituted compound (cf. **31**, **47**), and replacement of the amide N–H with methyl was equally deleterious in the case of the potent analog **38** (cf. **38**, R_2=H, IC_{50} = 0.55 μM; **48**, R_2=Me, IC_{50} > 30 μM).

In the absence of structural information, the SAR results presented above may be combined to provide some insight into the binding of this novel class of inhibitors with IRAK-4. The preference for the amide carbonyl linker indicates its possible involvement in hydrogen bonding to the hinge backbone of IRAK-4, while the requirement for the amide N–H may indicate a possible hydrogen bond to the kinase, or potentially, the requirement that the inhibitors be able to populate a tautomeric form via abstraction of this hydrogen. Given the fairly wide tolerance for size and the preference for polar

Table 6. Inhibition of IRAK-4 by selected analogs: amide NH replacement



Compound	R	R^2	IRAK-4 IC_{50} (μM)[12]
31	Allyl	H	1.0
47	Allyl	Allyl	>30
38	α-(γ-Butyrolactone)	H	0.55
48	α-(γ-Butyrolactone)	Me	>30

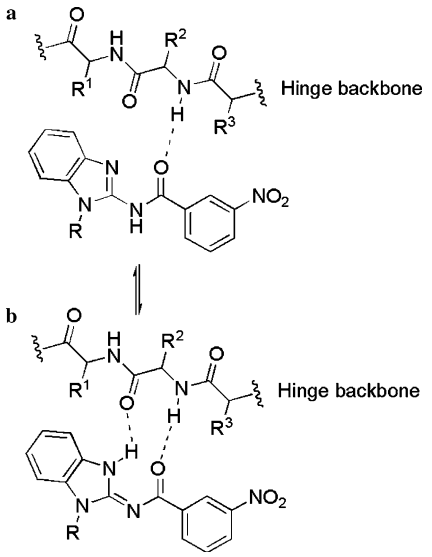


Figure 2. Proposed binding of acylbenzimidazole tautomeric forms to IRAK-4.

groups (i.e., **41**, **46**) in the *N*-alkyl substituent, it is likely that this group is pointing toward solvent or the phosphate binding loop in the kinase domain, although the equipotence of both acidic (i.e., **43**) and basic (i.e., **44**) functionalities when placed at the same distance from the benzimidazole ring would appear to indicate the former. When these results are combined together, they may be used to form a rough model of the binding of these inhibitors to IRAK-4 (Fig. 2).

Having identified a series of potent inhibitors of IRAK-4, several of the compounds were profiled for selectivity against other kinases. Inhibitors **36** (IRAK-4 $IC_{50} = 0.4\ \mu M$) and **46** (IRAK-4 $IC_{50} = 0.2\ \mu M$) were found to have IC_{50}'s greater than the highest concentration tested (10 μM) against a panel of 27 other kinases, including the most closely homologous (outside of the IRAK family) Lck and $pp60^{SRC}$. Additionally, compounds **36** and **46** did not show any signs of cytotoxicity in a 72 h proliferation assay in HeLa cells ($ED_{50} > 30\ \mu M$). Significant inhibition of IRAK-1 was observed with both compounds (IRAK-1 $IC_{50} = 0.75$ and 0.3 μM, respectively), not unexpectedly, given the high homology between these two kinases.

In summary, screening of a small-molecule library resulted in the discovery of a series of novel acyl-2-aminobenzimidazole inhibitors of IRAK-4. SAR studies resulted in significant improvement of potency, and established the key pharmacophore of the series. The amide functionality was found to be important for IRAK-4 inhibition, as was the benzimidazole ring, indicating the probable kinase binding motif as described in Figure 2. Modification of the benzamide aryl ring via substituent replacement demonstrated that some changes were allowed in this region, although the 3-nitro substituent remained the best substitution in terms of potency. Modification of the benzimidazole ring by substituent studies showed that improvements in potency resulted from modification of the 5-position, which may be important for future studies to optimize ADME and potency parameters. The most effective modifications resulted from optimization of the *N*-alkyl substituent, which gave >10-fold improvements in potency, resulting in the potent IRAK-4 inhibitors **41** ($IC_{50} = 150$ nM) and **46** ($IC_{50} = 200$ nM). The acyl-2-aminobenzimidazole scaffold reported herein represents a unique kinase binding motif, previously unreported in the literature, and further studies with this scaffold and its utility in the inhibition of IRAK-4 will be reported in subsequent publications.

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12. IRAK-4 enzyme activity was determined by its ability to phosphorylate a biotinylated synthetic peptide substrate (RRRVTSPARRS, sequence derived from GFAP) in a chemiluminescent ELISA. Assays were performed in streptavidin-coated 96-well plate format utilizing recombinant full length IRAK-4 and 10 μM ATP. IC_{50} values for inhibition of IRAK-4 were calculated from a minimum of two separate determinations each performed in duplicate.