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5-Aminomethyl-1*H*-benzimidazoles as orally active inhibitors of inducible T-cell kinase (Itk)

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This paper is dedicated to the memory of Dr. Ronald L. Magolda.

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ABSTRACT

A series of novel 5-aminomethyl-1*H*-benzimidazole based inhibitors of Itk were prepared. Structure–activity relationships, selectivity and cell activity are reported for this series. Compound **2**, a potent and selective antagonist of Itk, inhibited anti-CD3 antibody induced IL-2 production in vivo in mice.

Antigen recognition by T-cells is the initiating stimulus for T-cell activation. T-cell activation can lead to proliferation, secretion of cytokines, and initiation of regulatory and cytolytic effector functions. Engagement of the T-cell antigen receptor (TCR) results in the rapid recruitment and activation of three classes of non-receptor tyrosine kinases, the Src family (Lck and Lyn), the Syk family (Zap-70 and Syk) and the Tec family (Itk, Txk, and Tec).[1–3] Inhibiting any of these tyrosine kinases would potentially impede T-cell activation following antigen presentation by blocking the signaling cascade.[4]

Interleukin-2 (IL-2)-inducible T-cell kinase (Itk) is a key member of the Tec kinase family and a number of factors point to the importance of this kinase in immune disease. Deletion of Itk in mice results in reduced TCR-induced proliferation and secretion of the cytokines IL-2, IL-4, IL-5, IL-10, and IFN-γ.[5–7] In a mouse model of allergic asthma, lung inflammation, eosinophil infiltration, and mucous production are drastically reduced in response to challenge with the allergen OVA in the absence of Itk.[8] Furthermore, the Itk gene is reported to be more highly expressed in peripheral blood T-cells from patients with moderate or severe atopic dermatitis than in controls or patients with mild atopic dermatitis.[9,10] These studies suggest that a selective Itk inhibitor may be useful as an anti-inflammatory or immunosuppressive agent for the treatment of dysregulated pathways mediated by Th2 cells.

Thiazole inhibitors of Itk were disclosed recently,[11,12] as were the hit to lead studies that eventually led to the benzimidazole series of compound **1** which we previously identified.[13] Compound **1** had an IC_{50} value of 12 nM in the Itk DELFIA-based enzyme assay, IC_{50} of 0. 3343 μM in the DT40 Ca^{2+} flux cellular assay and exhibited good selectivity against a panel of protein kinases.[14] Compound **1** was not toxic (TC_{50} > 10 μM; Jurkat) and showed good stability against both human and rat liver microsomes. In the course of exploring the functionality necessary for Itk activity, the 5-amide was converted to a methylamino moiety **2** (Fig. 1). Although less potent than **1** in the DELFIA assay (46 nM), it had similar potency (0.41 μM) in the DT40 cellular assay. Compound **2** became the starting point for a new lead series, and this report details the SAR of this novel series.[15]

The general synthetic route to this novel benzimidazole series is illustrated by the preparation of **2** (Scheme 1). Accordingly, 4-fluoro-3-nitrobenzylalcohol **3** was converted to **4** by treatment with

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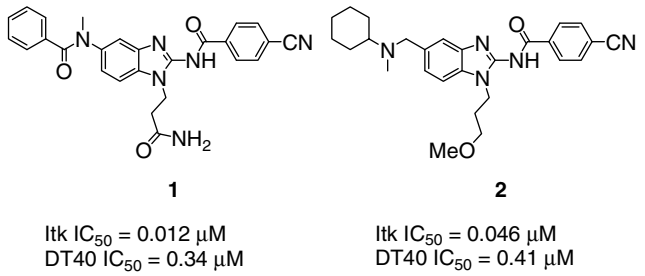
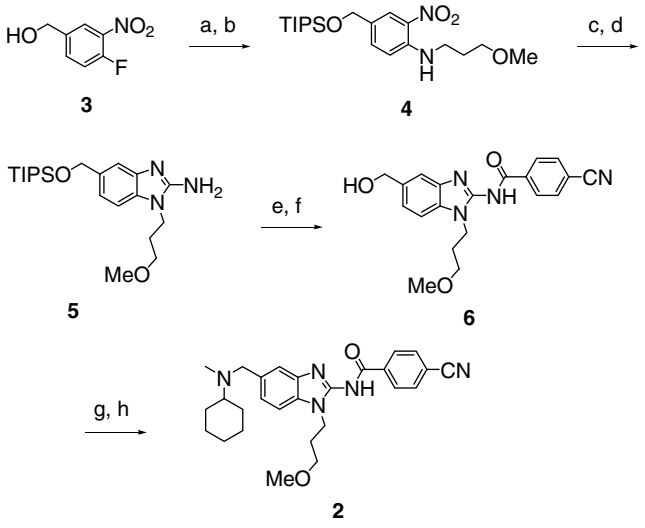


Figure 1. Itk inhibitory activity for compounds **1** and **2**.



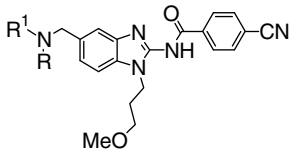
Scheme 1. General synthetic scheme for the synthesis of 5-aminomethylbenzimidazoles. Reagents and conditions: (a) TIPSOTf, 2,6-lutidine, CH_2Cl_2, rt, 24 h; (b) 3-methoxypropan-1-amine, DIEA, CH_3CN, rt to 50 °C, 24 h, 61% over two steps; (c) NH_4HCO_2, 10% Pd/C, ethanol, rt, 3 h, 91%; (d) BrCN, ethanol, rt, 24 h, 88%; (e) 4-cyanobenzoyl chloride, DIEA, DMAP, CH_2Cl_2, rt, 24 h, 77%; (f) 0.01 N HCl, dioxane, 95 °C, 3 h, 92%; (g) MnO_2, acetone, rt, 24 h, 84%; (h) *N*-methylcyclohexanamine, $NaBH(OAc)_3$, $(EtO)_3CH$, CH_2Cl_2, rt, 24 h, 42%.

TIPSOTf and 2,6-lutidine followed by displacement of the fluorine with 3-methoxypropylamine. The nitro group was reduced with ammonium formate and 10% Pd/C, and the resulting diamine cyclized to the 2-aminobenzimidazole **5** with cyanogen bromide. Acylation of the 2-amino with 4-cyanobenzoylchloride followed by TIPS deprotection with dilute HCl at 95 °C gave **6**. The resulting benzyl alcohol **6** was oxidized to the aldehyde with MnO_2, and lastly reductive amination with cyclohexylmethylamine in the presence of sodium triacetoxyborohydride provided the desired compound **2**.

All compounds were screened for inhibitory activity against Itk in a DELFIA assay. For the purposes of determining selectivity, testing of selected compounds was conducted against related kinases Tec, Bmx, Txk, and Btk as well as IRK (insulin receptor kinase), Syk and Lyn. Selected compounds were tested for cellular activity by measuring inhibition of Ca^{2+} flux in DT40 cells[13] and the inhibition of T-cell receptor induced IL-2 production in human T-cells.

In an effort to identify pharmacophores that would give improved potency against Itk we focused our initial explorations on the *N*-methylcyclohexyl group (Table 1). Removal of the cyclohexyl group (**7**) resulted in a 4-fold loss in potency while removal of the methyl (**8**) showed no effect, as did replacement of the cyclohexyl with phenyl (**9**). Confirming that large hydrophobic groups are preferred at this position, the only replacement that afforded a slight increase in potency was the introduction of cyclopentylmethyl (**10**)

Table 1
Modifications of the *N*-methylcyclohexyl group

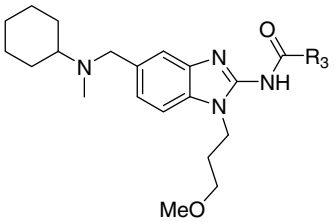


Compound	R^1	R	Itk IC_{50} (μM)
2	Cyclohexyl	CH_3	0.046
7	H	CH_3	0.17
8	Cyclohexyl	H	0.045
9	Phenyl	H	0.048
10	Cyclopentylmethyl	CH_3	0.034
11	2-Methoxyethyl	H	0.081
12	4-(*N*-Methylpiperidine)methyl	H	0.11
13	R^1 and R = morpholine		0.069

in place of cyclohexyl. Changing R^1 to less hydrophobic groups, 2-methoxyethyl (**11**) and 4-(*N*-methylpiperidine)methyl (**12**), resulted in a 2- and 3-fold loss in potency, respectively. Replacement of the *N*-methylcyclohexyl group with a morpholine (**13**) resulted in a slight loss in potency compared to **2**. This SAR data is consistent with the binding mode of compounds similar to **1** that were crystallized with Itk.[16] In the crystal structure, the equivalent hydrophobic group of **1** (the left side phenyl) interacts with mostly hydrophobic residues on the surface of the protein.

Having established the requirement for the *N*-methylcyclohexyl position of the benzimidazole, the SAR around the R^2 substituent was explored (Table 2). In another series (**1**), this position was found to have a profound influence on the selectivity against insulin receptor kinase (IRK). Substitution of *p*-cyano with *p*-CF_3 (**14**) resulted in a complete loss of kinase activity while replacement with *p*-chloro (**15**) had no effect demonstrating that there is a size limitation at this position. In addition, both **2** and **15** had excellent selectivity against IRK. Replacement of the *p*-cyano with the *m*-cyano (**16**) resulted in a 2-fold increase in potency while the *m*-bromo (**17**) gave a 26-fold loss in potency compared to **2**. It is interesting to note that the IRK selectivity decreased substantially with *meta*-substitution. Heterocycles 5-isoxazole (**18**) and 4-pyridyl (**19**) were both well tolerated and had good selectivity versus IRK, and 3-pyridyl substitution (**20**) resulted in a 6 nM compound with excellent selectivity against IRK.

Table 2
SAR of C-2 amide linkage



Compound	R^2	Itk IC_{50} (μM)	Selectivity versus IRK[a]
2	*p*-CN-Phenyl	0.046	>217
14	*p*-CF_3-Phenyl	>10	1
15	*p*-Cl-Phenyl	0.046	>217
16	*m*-CN-Phenyl	0.020	17
17	*m*-Br-Phenyl	1.2	6.6
18	5-Isoxazole	0.047	115
19	4-Pyridyl	0.036	64
20	3-Pyridyl	0.006	163

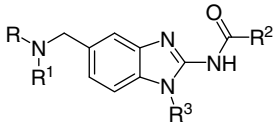
[a] Selectivity ratio IC_{50} IRK/IC_{50} Itk.

Having identified a set of optimum pharmacophores for the C-2 amide linkage (R^2) of the benzimidazole as well as the most favorable changes at the C-5 position (R and R^1), we next undertook a rapid evaluation of the N-1 (R^3) site in-conjunction with changes to R, R^1, and R^2 (Table 3). First, keeping R, R^1, and R^2 constant as CH_3, cyclohexyl and *p*-CN-phenyl, respectively, changing R^3 from 3-methoxypropyl (**2**) to β-alanine amide (**21**) gave an almost equipotent compound, while changing to 3-(*N*1-imidazo)propyl (**22**) or 3-hydroxypropyl (**23**) gave a slight increase in potency. Another example, 3-(*N*1-imidazo)propyl (**24**) for 3-methoxypropyl (**18**) when R^2 is 5-isoxazole, also gave a negligible change in potency. These results confirm that for the conservative changes to R^3 shown, there is an almost flat SAR. This is consistent with the crystal structure of compounds such as **1** wherein this group does not interact with the protein and extends toward solvent.[15] Interestingly, two changes that by themselves did not give an increase in potency alone, R^1 to phenyl and R^2 to 5-isoxazole (**26**), gave a 15-fold potency increase when combined, 3 nM versus Itk.

Five compounds were selected for further evaluation in the DT40 cell-based Ca^{2+} flux inhibition assay (Table 4). Compounds **2**, **8**, **15**, and **24** showed good activity in the cell-based assay, all with IC_{50}s of approximately 0.5 μM. Of these, **2** and **8** were selected for further study. Compound **26** showed outstanding potency in the DT40 cell assay, 75 nM, mimicking its performance in the enzyme assay, and was also selected for further study.

Compounds **2, 8**, and **26** were tested for selectivity against a panel of kinases (Table 5). All showed >50-fold selectivity against the kinases screened with the exception of Txk. Compounds **2** and **8** showed selectivity of about 20-fold against Txk while **26** showed much improved selectivity against Txk.

Table 3
SAR of N-1 position



Compound	R	R^1	R^2	R^3	Itk IC_{50} (μM)
2	CH_3	C_6H_{11}	*p*-CN-Phenyl	3-Methoxypropyl	0.046
21	CH_3	C_6H_{11}	*p*-CN-Phenyl	$-CH_2CH_2CONH_2$	0.041
22	CH_3	C_6H_{11}	*p*-CN-Phenyl	3-(*N*1-Imidazo) propyl	0.030
23	CH_3	C_6H_{11}	*p*-CN-Phenyl	$-CH_2CH_2CH_2OH$	0.027
18	CH_3	C_6H_{11}	5-Isoxazole	3-Methoxypropyl	0.047
24	CH_3	C_6H_{11}	5-Isoxazole	3-(*N*1-Imidazo)propyl	0.046
25	H	C_6H_{11}	5-Isoxazole	3-Methoxypropyl	0.053
26	H	C_6H_5	5-Isoxazole	3-Methoxypropyl	0.003

Table 4
DT40 cell assay

Compound	Itk IC_{50} (μM)	DT40 IC_{50} (μM)
2	0.046	0.41
8	0.045	0.65
15	0.046	0.60
24	0.046	0.54
26	0.003	0.075

Table 5
Enzyme selectivity of selected Itk inhibitors

Compound	Itk IC_{50} (μM)	IRK[a]	Btk[a]	Syk[a]	Bmx[a]	Txk[a]	Tec[a]
2	0.046	>217	61	>217	>217	24	>217
8	0.045	>222	70	>222	>222	22	>222
26	0.003	>3333	1658	>3333	>3333	149	68

[a] Selectivity ratio IC_{50}/IC_{50} Itk.

Table 6
Microsome stability study

Compound	RLM CL_H (%Q_H) $t_{1/2}$ (min)	HLM CL_H (%Q_H) $t_{1/2}$ (min)
2	3435	5433
8	1789	27,107
26	669	6422

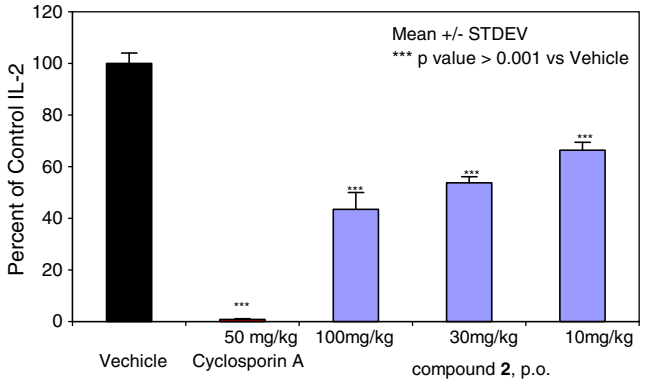


Figure 2. Effects of Itk inhibition on in vivo IL-2 production in anti-CD3 stimulated Balb/c mice.

All three compounds were incubated with human liver microsomes; **8** had excellent microsomal stability while **2** was good and **26** fair (Table 6). The stability in rat liver microsomes mimicked the human, although the $t_{1/2}$ for **26** was very short, only 9 min. For this reason, inhibitors **2** and **8** were chosen for evaluation in the functional assays.

Compounds **2** and **8** were evaluated in a human $CD4^+$ T-cell co-stimulation assay. Human $CD4^+$ T-cells were co-stimulated with immobilized anti-CD3 and anti-CD28 antibodies in the presence of compound or DMSO for 20 h. IL-2 and IFN-γ levels were then measured by ELISA. Compound **2** inhibited the anti-CD3 and CD28 antibody induced production of IL-2 (IC_{50} 0.6 μM) and IFN-γ (IC_{50} 1.1 μM) while **8** inhibited IL-2 and IFN-γ at IC_{50}s of 1.0 and 2.3 μM, respectively. Inhibitor **2** showed excellent selectivity against five isoforms of cytochrome P450, 1A2, 2C9, 2C19, 2D6, and 3A4 with IC_{50}s > 14 μM. A pharmacokinetic study of **2** in mice revealed an iv $t_{1/2}$ of 5 h, clearance of 43 mL/min/kg, and oral bioavailability of 17%. Compound **2** was also tested for its ability to inhibit IL-2 production in vivo in mice following iv injection of anti-CD3 antibody (Fig. 2). Inhibitor **2** dose-dependently inhibited IL-2 production in anti-CD3 stimulated Balb/c mice at oral doses of 10, 30, and 100 mg/kg.

In conclusion we identified a series of potent and selective inhibitors of Itk as exemplified by compound **2**. We have also shown that **2** demonstrated good activity in vivo in mice following oral administration. Continuing efforts focused on further optimizing the benzimidazole series are currently underway and will be reported at a later date.

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