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Itk kinase inhibitors: Initial efforts to improve the metabolical stability and the cell activity of the benzimidazole lead

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

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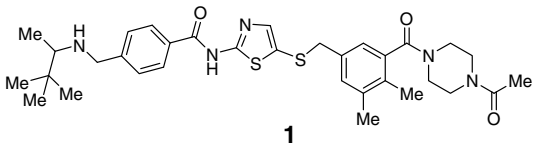
ABSTRACT

Previously, we reported a series of novel benzimidazole based Itk inhibitors that exhibited excellent enzymatic potency and selectivity but low microsomal stability. Employing a structure based approach a new series of inhibitors with comparable potency and selectivity to the original series and with a potential for improved microsome stability was identified.

The Tec-family of tyrosine kinases (Bmx, Btk, Itk, Rlk/Txk, and Tec) comprises five highly homologous proteins that are primarily expressed in T-cells and other hemopoietic cell types, three of which, Itk, Rlk/Txk, and Tec, are expressed in T-cells. Itk plays a critical function during the initial steps of T-cell-receptor signaling resulting in a cascade of downstream signaling pathways leading to T-cell activation, differentiation and the production of cytokines such as IL-2 and IFN-γ.[1–5] A selective inhibitor of Itk should prevent T-cell activation and thus have broad application for the treatment of T-cell-dependent processes such as autoimmune and inflammatory diseases as well as organ transplant rejection.[6–13]

Bristol Myers Squibb disclosed a series of 2-amino-5-[(thiomethyl)aryl]thiazoles, exemplified by compound **1**, which were potent and selective Itk inhibitors.[14,15] Compound **1** was shown to be effective at inhibiting IL-2 production in mice in a dose dependent manner (50 and 100 mpk, sc) following an intravenous injection of anti-CD3 antibody (Fig. 1).

We recently reported the discovery of a series of benzimidazoles (**2** and **3**) which are potent Itk kinase inhibitors (Fig. 2).[16]



1

Figure 1. Structure of ITK inhibitor reported by Bristol Myers Squibb.

Several of these compounds displayed excellent selectivity for inhibition of Itk over other kinases. The benzimidazole class Itk inhibitors were however rapidly metabolized in human liver microsomal condition. In vitro metabolite identification studies revealed the cyclohexyl C4 as the primary metabolic position, and the key pathway for metabolism was shown to be hydroxylation at the C4 site. Introduction of gem difluorides (**3**) at C4 dramatically improved the metabolic stability and eliminated the lability of a short in vitro half-life. However, the addition of the fluorines resulted in a loss of cellular potency presumably due to a decrease in cellular penetration since there was no observed loss in enzymatic activity. Encouraged by these preliminary results, we focused our efforts on identifying a replacement for the cyclohexyl group that maintained metabolic stability coupled with good

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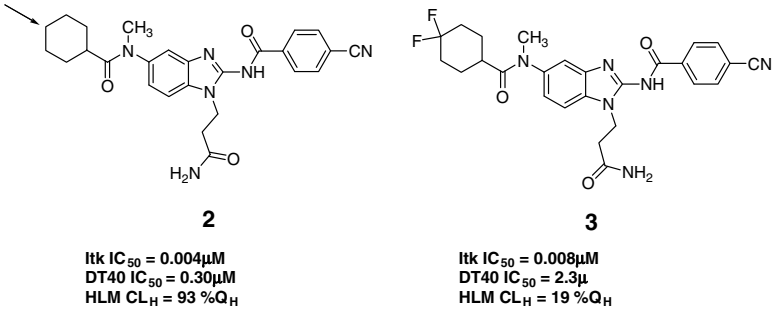


2

Itk IC_{50} = 0.004μM
DT40 IC_{50} = 0.30μM
HLM CL_H = 93 %Q_H

3

Itk IC_{50} = 0.008μM
DT40 IC_{50} = 2.3μ
HLM CL_H = 19 %Q_H

Figure 2. Structures of benzimidazole lead ITK inhibitors.

enzyme and cellular activity. In this paper, we present our initial efforts in our efforts to discovery novel, selective and improved metabolically stable inhibitors of Itk.

The crystal structure of the compound **4**/Itk complex revealed that the cyclohexyl ring is situated in a pocket located on the surface of the protein adjacent to the ATP binding site.[17] The cyclohexyl group binds in a hydrophobic subsite consisting of Phe437, Leu379, Ile369 and the methylene chains of Lys387 and Gln367. The C-5 anilide linkage adopts a cis-orientation with the carbonyl pointing toward the solvent and the *N*-methyl group is situated toward the exposed cleft region (Fig. 3).

Docking studies using the above crystal structure indicated that a phenyl ring would be readily accommodated in the hydrophobic pocket and offered the possibility of replacing the cyclohexyl ring with a potentially less susceptible pharmacophore. Our efforts in the modification of the benzimidazole series, to improve its metabolic stability focused on replacing the cyclohexyl ring with aryl rings.

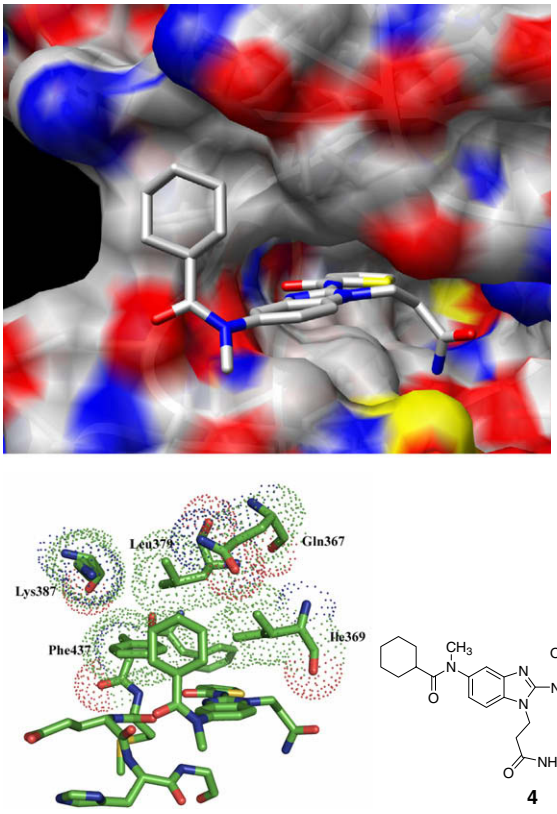
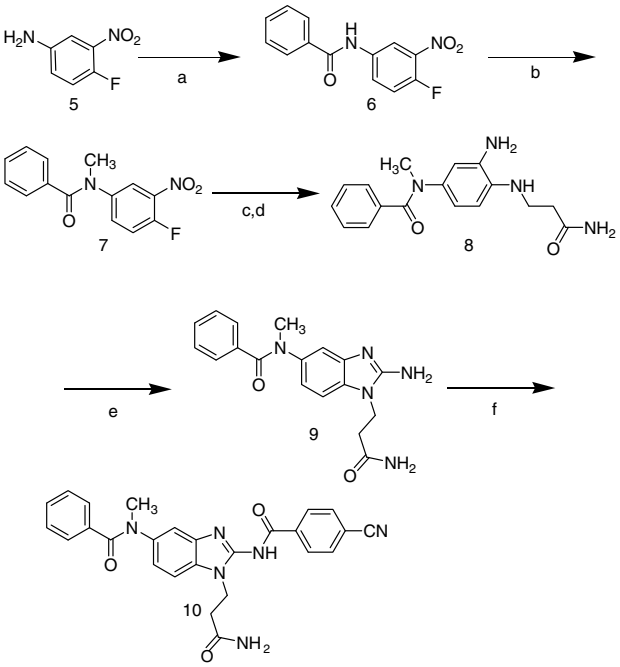


Figure 3. X-ray structure of compound **4** bound in Itk.



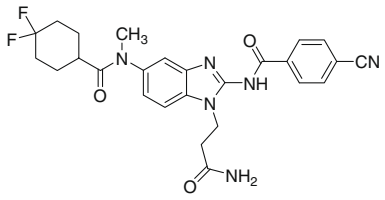
Scheme 1. Representative synthetic scheme of 2-amino-1*H*-benzimidazole class Itk inhibitors. Reagents and conditions: (a) C_6H_5COCl, pyridine, CH_2Cl_2, 0 °C to rt, 99%; (b) excess CH3I, NaH, THF, rt, 24 h, 96%; (c) $H_2NCH_2CH_2CONH_2$, DIEA, CH_3CN, 50 °C, 24 h, 92%; (d) $NiCl_2{\cdot}6H_2O$, $NaBH_4$, CH_3OH, rt, 24 h, 86%; (e) BrCN, EtOH; rt, 24 h, 83%; (e) (*p*-CN)$C_6H_4CO_2H$, PyBOP, DIEA, DMF, rt, 24 h, 93%.

The synthesis of these compounds follows a general synthetic route that is illustrated with the preparation of **10** in Scheme 1. The commercially available 4-fluoro-3-nitro-phenylamine (**5**) was acylated with benzoyl chloride in the presence of pyridine to give compound **6**. The resulting anilide was then reacted with excess methyl iodide in the presence of NaH at room temperature to give compound **7**. The fluoride was displaced with β-alanine amide at 50 °C for 12 h, and the nitro group was then reduced quantitatively with $NiCl_2$ $6H_2O/NaBH_4$[18] in methanol to give the phenylenediamine compound **8**. Compound **8** was then treated with cyanogen bromide in ethanol at room temperature overnight to form compound **9**. The 2-amino group was acylated with 4-cyanobenzoic acid using PyBOP/DIEA in DMF afforded **10** in 60% overall yield.

The benzimidazole **10** and its analogs were tested for their ability to inhibit the phosphorylation of substrate using a recombinant Itk kinase domain as the source of enzyme.[19]

Replacement of the cyclohexyl with a phenyl ring (**2** vs **10**) resulted in no loss in potency against Itk in the enzyme assay but lead to an approximately 2-fold improvement in the DT40 cellular assay[19] (Table 1). Substitution in the *para* position (**11**–**15**) was well tolerated in the Itk enzyme assay yielding analogs with com-

Table 1
SAR of substitutions on C-5 anilide position



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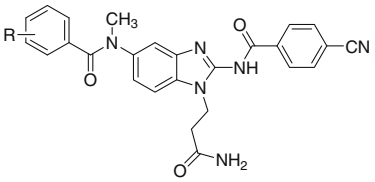


10-17

Compound	R	Itk IC_{50} (μM)	DT40 IC_{50} (μM)
3	—	0.008	2.3
10	H	0.003	0.34
11	*p*-F	0.002	0.33
12	*p*-Cl	0.012	0.26
13	*p*-CH_3	0.010	0.37
14	*p*-OCH_3	0.007	1.1
15	*p*-OCF_3	0.035	0.50
16	3-Pyridyl	0.004	0.72
17	4-Pyridyl	0.027	7.2

parable activity to **10**. Replacement of the phenyl ring with either a 3- or 4-pyridyl (**16** and **17**) was also well tolerated in the enzyme assay. Introduction of substitutions on the phenyl ring, however, had a significant effect on the in vitro cellular activity for this series. The *p*-fluoro (**11**), *p*-chloro (**12**), *p*-methyl (**13**), *p*-OCF_3 (**15**), and 3-pyridyl (**16**) groups gave compounds that had improved cellular activity compared to **10** while the, *p*-OCH_3 (**14**) and 4-pyridyl analogs (**17**) resulted in a loss of potency compared to **10**.

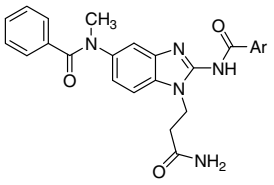
Generally, analogs **10–13** and **15** displayed the best balance of enzyme and cellular potencies. In the series, compounds with low *A*Log*P* (<2.8) and high TPSA > 100 Å^2 coupled with molecular weights >500 displayed a more pronounced biochemical to cellular assay shift in IC_{50} values (Table 2).[20] These results are suggestive of poor cellular permeability consistent with compounds in the literature with similar characteristics.[20] Interestingly, the region-isomers of 3- and 4-pyridyl analogs (compound **16** and **17**, respectively) showed a 10-fold difference in cell potency even though both have similar physicochemical properties.

Table 3 summarizes the relevant SAR observed for the Ar substitutions at C-2 position. From our previous SAR studies of the cyclohexyl on the left-hand side of the molecule derivatives, we

Table 2
Predicted physicochemical properties of C-5 anilide analogs

Compound	R	*A*Log*P*	TPSA (Å^2)	M_W
3	—	2.6	134	508.5
10	H	2.9	134	466.5
11	*p*-F	3.1	134	484.5
12	*p*-Cl	3.5	134	501.0
13	*p*-CH_3	3.3	134	480.5
14	*p*-OCH_3	2.8	143	496.5
15	*p*-OCF_3	5.0	143	550.5
16	3-Pyridyl	1.7	147	467.5
17	4-Pyridyl	1.7	147	467.5

Table 3
SAR of aromatic amide on C-2 position



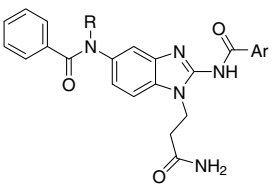
Compound	Ar	Itk IC_{50} (μM)	IRK IC_{50} (μM)
18	C_6H_5	0.003	0.12
10	C_6H_4(*p*-CN)	0.003	>10
19	C_6H_4(*p*-Cl)	0.006	>10
20	C_6H_3(3-CH_3,4-CN)	1.9	>10
21	5-Picolinonitrile	0.023	>10
22	5-Cyanothiophene	0.008	>10
23	2-Thiophene	0.014	0.11
24	5-Isoxazole	0.010	>10
25	3-Furyl	0.010	0.32

observed that modifications of the Ar position had a significant influence on the selectivity profile versus the insulin receptor kinase (IRK). Conscious of this, we profiled the 5-aryl analogs against both Itk and IRK. A similar selectivity profile was found for these derivatives. *para*-Substitution (**10**, **19**, and **21**) yielded inhibitors with both excellent Itk inhibitory potency and improved selectivity against IRK while the 2-thiophene (**23**) and the 3-furyl (**25**) showed diminished selectivity. The 5-cyanothiophene (**22**) and the 5-isoxazole (**24**) were also tolerated providing analogs with improved selectivity compared to compound **18** while di-substitution of the aryl ring (**20**) was found to be detrimental to activity. These results were consistent with our previously proposed rationale for the improved selectivity of the *para*-substitutents. The improved selectivity results from a steric interaction with the gatekeeper residue (Met) at the opening of the Selectivity Pocket of the insulin receptor kinase.[16]

We next focused our explorations on the N–CH_3 position of the 5-anilinde (Table 4). As a possible site of metabolism we focused on identifying potential replacements for the methyl group. The ethyl and propyl groups were all tolerated at this position. There was however a slight preference for the ethyl over the propyl indicating the previous size requirement at this position.[16]

Based on the enzyme potency, three compounds (**10**, **19**, and **27**) were selected for further evaluation. The compounds were screened for cellular activity, selectivity, microsome stability, and cytotoxicity activity (Tables 5 and 6). The inhibitors all exhibited

Table 4
SAR of N-substitutions at C-5 position



Compound	R	Ar	Itk IC_{50} (μM)
10	CH_3	C_6H_4(*p*-CN)	0.003
19	CH_3	C_6H_4(*p*-Cl)	0.006
26	CH_3	5-Isoxazole	0.010
27	Et	C_6H_4(*p*-CN)	0.007
28	Et	5-Isoxazole	0.027
29	Pr	C_6H_4(*p*-CN)	0.018
30	Pr	C_6H_4(*p*-Cl)	0.054

Table 5
Cellular activity, cytotoxicity and microsome stability of selected compounds

Compound	Itk IC_{50} (μM)	DT40 IC_{50} (μM)	Jurkat TC_{50} (μM)	HLM CL_H (%Q_H)	RLM CL_H (%Q_H)
10	0.003	0.34	>10	32	35
19	0.006	0.34	5.8	48	46
27	0.007	0.16	>10	57	nd

nd, not determined.

Table 6
Selectivity results of selected compounds

Compound	IRK IC_{50} (μM)	Lyn IC_{50} (μM)	Tec IC_{50} (μM)	Txk IC_{50} (μM)	Cyp 2C9 (μM)	Cyp 3A4 (μM)
10	>10	>10	>10	0.56	>30	>30
19	>10	>10	>10	0.50	>30	>30
27	>10	>10	>10	0.31	>30	>30

good cellular potency in the DT40 cellular assay. All the compounds exhibited improved stability compared to **2** against human microsomes, and greater than 500-fold separations between the DT40 IC_{50} and TC_{50} values. The compounds also showed excellent selectivity against IRK, Lyn, Tec and a 44- to 188-fold selectivity against Txk. Unfortunately although the compounds did show an improvement there still was a high degree of metabolism indicating additional susceptible sites on the compounds. There was however an observed 2-fold improvement when the N–CH_3 was replaced with a N–Et which did point to one of the possible sites of metabolism for the series. Additional profiling is currently underway to identify the other sites so additional corrective modifications can be carried out to improve the stability of the series.

In summary, starting from a series that showed excellent enzymatic potency and selectivity but poor microsomal stability, we identified a new series that retained excellent potency, selectivity and cellular activity and with the potential for improved microsome stability. Further improvements and modifications of the series focusing on improving the stability and leading to an orally active Itk inhibitor will be presented in forthcoming publications.

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