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Bioorganic & Medicinal Chemistry Letters 16 (2006) 2411–2415
Bioorganic & Medicinal Chemistry Letters

Discovery and SAR of 2-amino-5-[(thiomethyl)aryl]thiazoles as potent and selective Itk inhibitors

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Received 3 January 2006; revised 24 January 2006; accepted 26 January 2006
Available online 14 February 2006

Abstract—A series of structurally novel aminothiazole based small molecule inhibitors of Itk were prepared to elucidate their structure–activity relationships (SARs), selectivity and cell activity in inhibiting IL-2 secretion in a Jurkat T-cell assay. Compound **2** is identified as a potent and selective Itk inhibitor which inhibits anti-TCR antibody induced IL-2 production in mice in vivo.

Mammalian immunity relies on the activation of T cells upon antigen presentation. Following the engagement of the T-cell antigen receptor (TCR), T-cell activation is initiated by sequential activation of three distinct classes of non-receptor protein tyrosine kinases, namely the Src family kinases (Lck and Lyn), the Syk family kinases (ZAP-70 and Syk), and the Tec family kinases (Itk, Txk, and Tec).[1] Inhibition of any of these kinases will impede the initiation signals and block the T-cell activation following antigen presentation.

Itk (interleukin-2-inducible T-cell kinases), also known as Emt or Tsk, is expressed mainly in T-, natural killer, and mast cells. Itk is tyrosine phosphorylated and activated in response to cross linking of TCR, CD28 or CD2, and has been implicated in thymocyte development and activation of T cells through TCR and CD28 engagement. Within the cell, Itk is regulated by membrane recruitment, followed by Lck phosphorylation, and then autophosphorylation.[2] Gene knockout studies have revealed that mice lacking Itk have fewer number of T cells, especially CD4+ T cells, and matured T cells isolated from these mice exhibit defective TCR mediated responses such as calcium mobilization, IL-2 secretion, and proliferation.[3] In addition, Itk-deficient mice are unable to establish functional Th2 cells resulting in their inability to clear parasitic infections dependent upon a Th2 response.[4] These studies suggest that a selective Itk inhibitor should be useful as an immunosuppressive and/or anti-inflammatory agent and may be an attractive modulator of dysregulated allergic pathways mediated by Th2 cells.[5]

Directed screening of our internal compound libraries[6,8] using an HTRF assay identified the aminothiazole **1** as a selective ATP-competitive Itk inhibitor.[7] In this report, we outline our initial efforts in optimizing this series leading to compound **2** as a potent and selective Itk inhibitor in vitro with excellent activity in reducing IL-2 production, and T cell proliferation in both human and mouse cells (Figure 1). In addition, **2** is shown to suppress anti-TCR antibody induced IL-2 production in mice in vivo.

The synthesis of these compounds follows a general synthetic route that is illustrated with the preparation of **2** in Scheme 1. Accordingly, 2-amino-4-thiocyanatothiazole[8] was treated with 2 equiv of sodium borohydride in ethanol at 0–5 °C for 2 h and then reacted with methyl 5-chloromethyl-2,3-dimethylbenzoate[9] at rt for 16 h to form a methyl ester, which was saponified with aq. sodium hydroxide solution in methanol under reflux to form acid **3** in 72% overall yield.

Keywords: Selective Itk inhibitors; Thiazoles.
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doi:10.1016/j.bmcl.2006.01.115

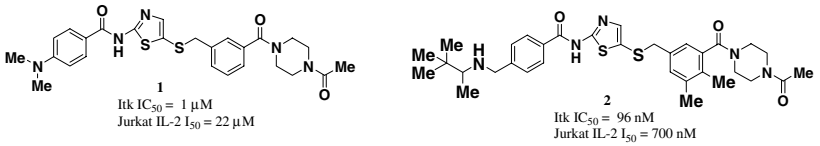
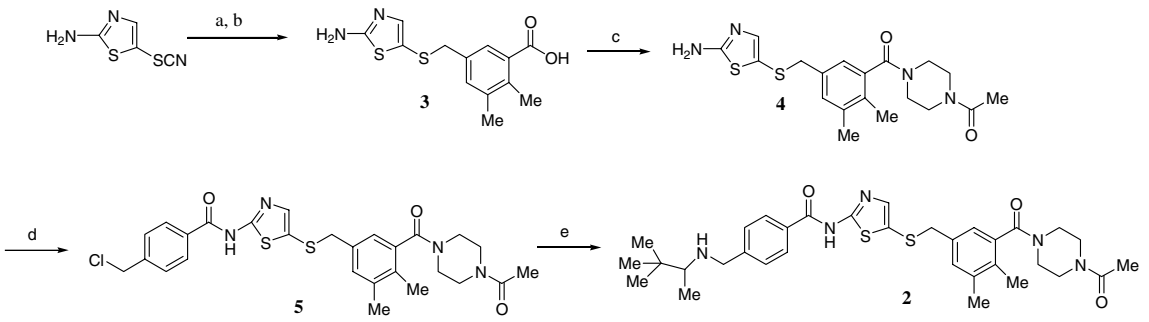


Figure 1. Itk inhibitory activity of aminothiazoles **1** and **2**.

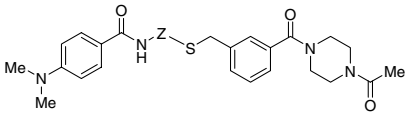


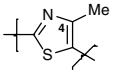
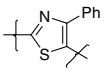
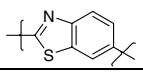
Scheme 1. Reagents and conditions: (a) $NaBH_4$, EtOH, methyl 5-chloromethyl-2,3-dimethylbenzoate, 87%; (b) MeOH, 1 N aq. NaOH, Δ, 86%; (c) *N*-acetylpiperazine, BOP reagent, 4-methylmorpholine, DMF, 60 °C, 88%; (d) 4-chloromethylbenzoyl chloride, Py, CH_2Cl_2; (e) 3,3-dimethyl-2-butanamine, DMF, 55 °C, 30% overall yield in two steps.

Reaction of acid **3** with *N*-acetylpiperazine in DMF in the presence of BOP reagent and 4-methylmorpholine at 50 °C for 5 h afforded compound **4** (88% yield), which was treated with 4-chloromethylbenzoyl chloride in dichloromethane in the presence of pyridine at rt for 16 h to form benzamide **5**. Benzamide **5** was heated with 6 equiv. of 3,3-dimethyl-2-butanamine in DMF at 55 °C for 16 h to form **2** (BMS-488516) in 30% overall yield in two steps.

Aminothiazole **1** and its analogs were tested for their ability to inhibit the phosphorylation of an exogenous

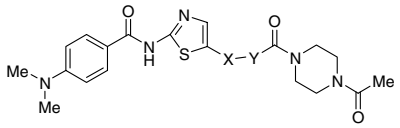
Table 1. SAR for thiazole ring modification



Compound	Z	Itk inhibition IC_{50}[6,a] (μM)
1		1.0
1a		>50
1b		39
1c		>50
1d		>50
1e		>50
1f		>50
1g		>50

[a] Values throughout are means of three independent experiments, standard deviation ±10%.

Table 2. SAR for linker modification



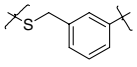
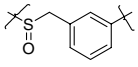
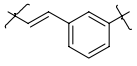
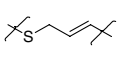
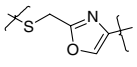
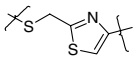
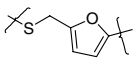
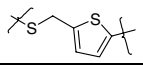
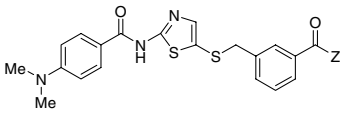
Compound	X–Y	Itk inhibition IC_{50}[6] (μM)
1		1.0
1h		>50
1i		5.0
1j		4.6
1k		1.7
1l		1.6
1m		1.6
1n		0.6

Table 3. SAR for amide modification



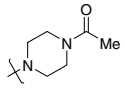
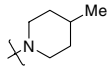
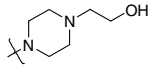
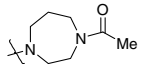
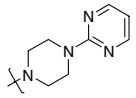
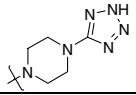
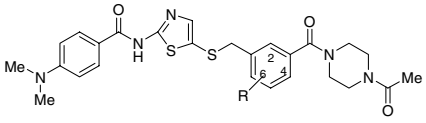
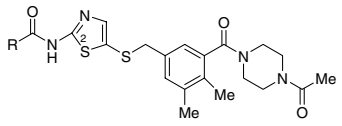
Compound	Z	Itk inhibition IC_{50}[6] (μM)
1		1.0
1o	NHPh	>50
1p		11.2
1q	$NH(CH_2)_2NHAc$	>50
1r		2.2
1s		2.1
1t		2.6
1u		0.9
1v	NMe_2	14.8
1w		1.44

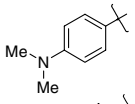
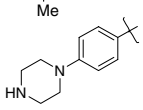
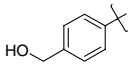
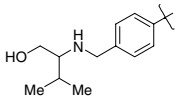
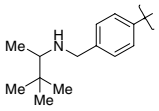
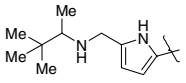
Table 4. SAR for thioaryl modification



Compound	R	Itk inhibition IC_{50}[6] (μM)
1	H	1.0
1x	2-Me	37
1y	4-Me	0.38
1z	5-Me	0.26
1aa	6-Me	15
1ab	5-NO_2	13
1ac	5-NH_2	0.54
1ad	5-NHAc	14
1ae	4-NH_2	0.73
1af	4-NHAc	2.42
1ag	4-NO_2	1.09
1ah	4-Cl	1.03
1ai	4-OMe	0.35
1aj	5-OMe	0.35
1ak	5-Cl	0.72
1al	4,5-Di-Me	0.30

Table 5. SAR for amide modification



Compound	R	Itk inhibition C_{50}, μM,[6]	Jurkat IL-2 inhibition C_{50} [6,a] (μM)
1al		0.3	2.0
1am		0.04	13.5
1an		0.32	5.7
1ao		0.096	2.2
2		0.09	0.7
1ap		0.14	2.4
1aq		0.035	0.7

[a] Values are means of three independent experiments, standard deviation ±20%.

substrate (GST-fused SLP-76)[6] using a recombinant Itk kinase domain as source of the enzyme (IC_{50}, Tables 1–5).

Table 1 outlines the SAR observed with the thiazole ring modification. Introduction of a substituent in the 4-position of the thiazole (**1a** and **1b**) resulted in a significant loss of potency. Replacement of the thiazole ring with isosteric five-membered ring heterocycles (**1c** and **1d**) or six-membered ring systems (**1e** and **1f**) or a benzothiazole ring (**1g**) was equally unsuccessful thereby indicating that the thiazole ring system was optimal for Itk inhibitory activity.

Table 2 summarizes relevant SAR for the thioalkylaryl linker. Oxidation of the thioether linkage to its sulfoxide (**1h**) resulted in complete loss of potency. Replacement of the thioether moiety with an olefin (**1i**) or substitution of the aryl ring with an olefin (**1j**) led to an approximately 5-fold loss in activity. The phenyl ring can be replaced with several 5-membered heterocycles (**1k**–**1n**) without any loss of Itk inhibitory potency.

To optimize the potency, we next turned to the modification of the *N*-acetyl-piperazine amide moiety (Table 3). Replacement with benzamide (**1o**) or *N*,*N*-dimethyl amide (**1v**) groups significantly attenuated the potency. Substitution of the piperazine ring with an 2-acetami-

Table 6. Enzyme selectivity of selected Itk inhibitors

Compound	Itk IC_{50}[6] (nM)	B_{max}[a]	Btk[a]	Tec[a]	Txk[a]	Lck[a]	Syk[a]	Cdk2[a]
2	96	>500	>500	>500	>500	270	>500	33
1aq	35	1000	>1400	325	90	90	>1400	300

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

doethylenediamine side chain (**1q**) was also not tolerated. Replacement of the *N*-acetylpiperazine with a piperidine group (**1p**) also significantly reduced the potency. However, the piperazine ring can be replaced with a homopiperazine (**1s**) or a morpholine moiety (**1t**) without any loss of potency. Finally substitution of the acetyl group attached to the piperazine ring with either a tetrazole (**1w**), a pyrimidine ring (**1u**) or hydroxyethyl group (**1r**) was tolerated.

Introduction of substituents on the central phenyl ring had a significant effect on Itk inhibitory activity of this series of analogs (Table 4). A methyl group scan demonstrated that such substitution at either C4- or C5-position (**1y** and **1z**) led to a 3- and 4-fold increase in potency, respectively. However, such substitution at C2 (**1x**) or C6 (**1aa**) was detrimental to activity. A general SAR trend was observed regarding substituents at the C4- and C5-positions. The nature and size of the substituents at C4 had a minor effect on potency. In contrast, Itk inhibitory activity was more sensitive with regard to substituents at C5. An electron-deficient function (**1ab**) or a large group (**1ad**) was not tolerated. A small alkyl substituent (**1y** and **1z**), or electron-rich groups (**1ac**, **1ae**, **1ai**, and **1aj**) at C4 and C5 led to the more potent analogs in this series. 4,5-Dimethyl-substituted analog (**1a**l) was also identified as a potent Itk inhibitor.

Because of its excellent potency, further optimization was focused on the 4,5-it di-Me analog (**1al**). We concentrated on SAR studies at the thiazole 2-carboxamide functionality (Table 5). Replacement of the *N*,*N*-di-Me group at the *para* position of the benzamide with a piperazine ring (**1am**) resulted in a 7-fold increase in enzyme activity in vitro. Despite its excellent in vitro potency compound **1am** was 7-fold less potent than compound **1al** in a Jurkat cell-based IL-2 production inhibition assay. Similarly the corresponding benzyl alcohol analog (**1an**) was 3-fold less potent in the Jurkat cell assay, despite being equipotent to compound **1an** against Itk. A pronounced increase in biochemical and cellular potency was observed on introduction of a large hydrophobic aminomethyl moiety at the *para* position of the benzamide group. Compound **2** was identified as a potent Itk inhibitor (IC_{50} = 90 nM) with a Jurkat T-cell IL-2 IC_{50} value of 700 nM. Furthermore, replacement of the benzamide group in **1al** with a pyrrolo-2-carboxamide group (**1ap**) retained both biochemical and cell potencies of the parent analog. Introduction of a large hydrophobic aminomethyl group at the C5-position of the pyrrole ring led to analog **1aq**, which was equipotent with **2** in both biochemical and cell-based assays. Compounds **1aq** and **2** were thus selected for further characterization.

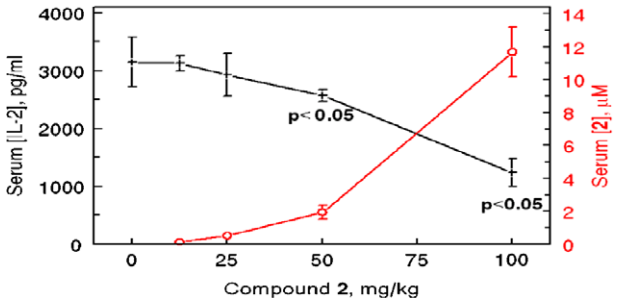


Figure 2. Dose-dependent inhibition of anti-CD3 antibody induced serum IL-2 production in mice (data in black). Serum concentrations of **2** (data in red).

Compounds **1aq** and **2** were tested for their selectivity over a panel of kinases (Table 6). Compound **2** showed a better selectivity profile than **1aq** with respect to other Tec family kinases. It was >500-fold selective against all Tec family kinases and showed at least 30-fold selectivity against all other protein kinases tested. Furthermore, **2** also exhibited competitive kinetics with respect to ATP in its inhibition of Itk.[6]

Based on its potent enzyme activity and selectivity, **2** was selected for further evaluation. Compound **2** inhibited anti-CD3-antibody induced IL-2 secretion both in human Jurkat T-cells (IC_{50} = 0.70 ± 0.18 µM), PBMC (IC_{50} = 0.65 ± 0.14 µM), and in murine EL4 cells (IC_{50} = 0.25 ± 0.07 µM) and splenocytes (IC_{50} = 1.36 ± 0.40 µM). Compound **2** was also tested for its ability to inhibit IL-2 production in vivo in mice following intravenous injection of anti-CD3 antibody. Analog **2** dose dependently suppressed IL-2 production in serum (50 and 100 mpk, sc) in line with its serum drug concentrations (Figure 2).

In summary, starting from a screening lead **1** with modest biochemical and cell potency we identified a series of potent and selective Itk inhibitors as exemplified by **2** through a systematic SAR approach. We also demonstrated the potency of this analog in an in vivo mouse model. To our knowledge, **2** is one of the first examples of a potent and selective small molecule Itk inhibitor reported to date and should serve as a useful tool in investigating the role of Itk in T cell signaling.

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1. (a) Kane, L. P.; Lin, J.; Weiss, A. *Curr. Opin. Immunol.* **2000**, *12*, 242; (b) Samelson, L. E. *Ann. Rev. Immunol.* **2002**, *20*, 371; (c) Ohashi, P. S. *Nat. Rev. Immunol.* **2002**, *2*, 427.
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