Bioorganic & Medicinal Chemistry Letters 18 (2008) 5545–5549

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery, SAR and X-ray structure of 1*H*-benzimidazole-5-carboxylic acid cyclohexyl-methyl-amides as inhibitors of inducible T-cell kinase (Itk)

Kevin J. Moriarty [c], Hidenori Takahashi [a,*], Steven S. Pullen [b], Hnin Hnin Khine [b], Rosemarie H. Sallati [b], Ernest L. Raymond [b], Joseph R. Woska Jr. [b], Deborah D. Jeanfavre [b], Gregory P. Roth [d], Michael P. Winters [c], Lei Qiao [c], Declan Ryan [c], Renee DesJarlais [c], Darius Robinson [c], Matthew Wilson [c], Mark Bobko [c], Brian N. Cook [a], Ho Yin Lo [a], Peter A. Nemoto [a], Mohammed A. Kashem [a], John P. Wolak [a], André White [a], Ronald L. Magolda [a], Bruce Tomczuk [c]

[a] *Department of Medicinal Chemistry, Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, PO Box 368, Ridgefield, CT 06877, USA*
[b] *Department of Immunology and Inflammation, Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, PO Box 368, Ridgefield, CT 06877, USA*
[c] *Johnson & Johnson, PRDUS, LLC, Springhouse, PA 19477, USA*
[d] *Burnham Institute for Medical Research at Lake Nona, Orlando, FL 32819, USA*

ARTICLE INFO

Article history:
Received 15 July 2008
Revised 29 August 2008
Accepted 3 September 2008
Available online 7 September 2008

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

Keywords:
SAR
Itk
Inhibitor
Benzimidazole

ABSTRACT

A series of novel potent benzimidazole based inhibitors of interleukin-2 T-cell kinase (Itk) were prepared. In this report, we discuss the structure–activity relationship (SAR), selectivity, and cell-based activity for the series. We also discuss the SAR associated with an X-ray structure of one of the small-molecule inhibitors bound to ITK.

Antigen recognition by T-cells is the initiating stimulus for T-cell activation. Activation of T-cells 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]

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 reduced secretion of the cytokines IL-2, IL-4, IL-5, IL-10 and IFN-γ.[5–7] Also, the immunological symptoms of allergic asthma are attenuated in $Itk^{-/-}$ mice and lung inflammation, eosinophil infiltration and mucous production are drastically reduced in response to challenge with the allergen OVA.[8] Together, these studies have defined an important role for Itk in TCR signaling leading to thymic development and cytokine gene expression.

In addition to the discovery of the role this kinase plays in this important signaling pathway, Bristol–Myers Squibb (BMS) recently disclosed a series of 2-amino-5-[(thiomethyl)aryl]thiazoles, exemplified by compound **1**, which are potent and selective Itk inhibitors.[4,9] 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).

Our interest in kinase-regulated approaches to inflammatory diseases prompted us to evaluate the potential of Itk as a therapeutic target. A screening program was initiated that identified compound **2** as a hit structure. This compound was shown to be an adenosine 5′-triphosphate (ATP) competitive inhibitor of Itk, with an IC_{50} of 0.04 μM in the Itk kinase assay and an IC_{50} = 1.4 μM in the DT40 cell-based assay.[10] In this report, we discuss our initial efforts in optimizing this series leading to potent and selective

* Corresponding author. Tel.: +1 203 798 5049; fax: +1 203 798 5297.
E-mail address: hidenori.takahashi@boehringer-ingelheim.com (H. Takahashi).

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.09.015

inhibitors of Itk in vitro with activity in reducing IL-2 production in mouse and human cells.

The general synthetic route for the compounds is shown in Scheme 1 and is illustrated with the preparation of **2**. The commercially available 4-fluoro-3-nitro-phenylamine (**3**) was acylated with cyclohexanecarbonyl chloride in the presence of pyridine to give **4**. The secondary amide was then converted to the *N*-methyl amide by alkylation with methyl iodide in the presence of NaH at room temperature to give **5**. The fluoride was displaced with 3-aminopropanamide at 50 °C overnight and the nitro group was then reduced with $NiCl_2/NaBH_4$ in methanol to give the dianilino compound **6** in quantitative yield.[11] Compound **6** was treated with cyanogen bromide, and the resulting 2-amino-1*H*-benzimidazole **7** was acylated with thiophene-2-carboxylic acid using PyBOP/DIEA in DMF produced compound **2** in overall yield of 50%.

All compounds were screened for inhibitory activity against Itk in a DELFIA assay.[10] For determining selectivity, selected compounds were also tested against the related kinases Tec, Bmx, Txk, Btk as well as insulin receptor kinase (IRK), Syk and Lyn. Selected compounds were tested for cellular activity by measuring inhibition of Ca^{2+} flux in DT40 cells, and the inhibition of IL-2 production in human and murine T-cells following TCR stimulation with anti-CD3 antibody.

Compound **2** had an IC_{50} = 0.04 μM in the Itk kinase assay with a DT40 cell-based assay IC_{50} of 0.9 μM). Compound **2** showed no activity against Btk, Lyn and Syk, however, **2** showed activity against IRK (IC_{50} = 0.14 μM). In an effort to identify pharmacophores that would provide both improved selectivity and potency against Itk, we explored replacements for the 2-thiophene ring. As shown in Table 1, replacement of the 2-thiopene with 2-furyl, 5-isoxazole, 3-pyridyl, or 4-pyridyl resulted in an improvement in activity against Itk. Additionally, selectivity over IRK was also improved. Similarly, a replacement of the 2-thiophene with a phenyl ring (**12**) was also tolerated, albeit with only a small improvement in IRK selectivity versus **2**. We turned to substituent modifications on the benzamide (Table 1). *ortho*-Substitution was found to be detrimental for activity (**18,** IC_{50} = 3.3 μM**)** while substitution at the *meta* or *para*-positions proved to be the most beneficial. Small electron-donating and withdrawing groups were well tolerated showing little difference in Itk inhibitory activity. Increasing a size at the *para*-position was not tolerated and lead to a loss in activity with increasing size of the substituent. Carboxylic acid substitution (**25**) at the *para*-position of the benzamide was also not tolerated leading to a >100-fold loss in activity relative to compound **2**. Selectivity against IRK was also influenced by the substitution pattern around the benzamide ring. The *para*-position (**14, 15, 17** and **22**) was found to be the preferred substitution yielding analogs with >1000-fold selectivity. This same trend was also seen with the pyridine analogs (**8** vs **11**). These results were consistent with our previously proposed rationale for the improved selectivity of the *para*-substituents. 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.[10]

Having established the requirement for the benzamide substituent at the C-2 position of the benzimidazole, the SAR around the benzimidazole N-1 position was explored. Table 2 summarizes

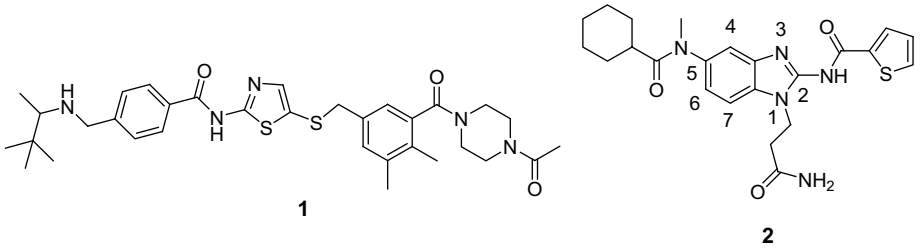
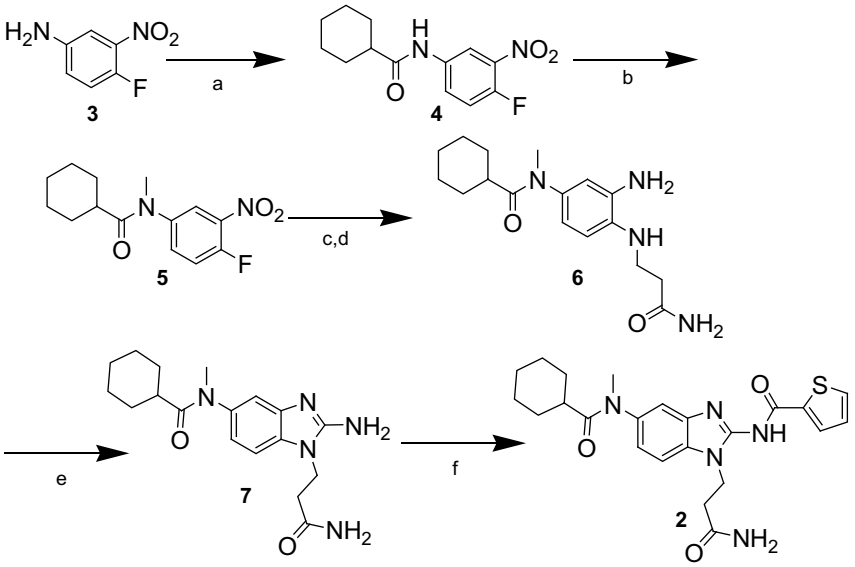
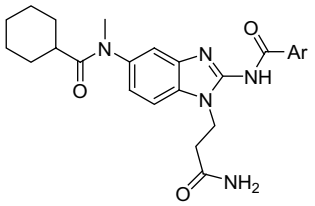


Figure 1. Structures of reported Itk inhibitors.



Scheme 1. Representative synthetic scheme for 2-amino-1H-benzimidazole class Itk inhibitors. Reagents and conditions: (a) cyclohexanecarbonyl chloride, pyridine, CH_2Cl_2, 0 °C–rt, 98%; (b) excess CH_3I, NaH, THF, rt, 24 h, 94%; (c) 3-aminopropanamide, DIEA, CH_3CN, 50 °C, 24 h, 85%; (d) $NiCl_2$, $NaBH_4$, CH_3OH, rt, 24 h, 83%; (e) BrCN, EtOH, rt, 24 h, 88% (e) thiophene-2-carboxylic acid, PyBOP, DIEA, DMF, rt, 24 h, 86%.

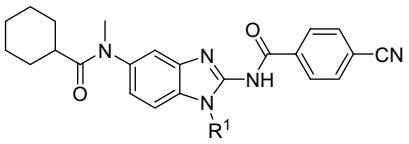
Table 1
Effects of thiophene replacements on ITK inhibition



Compound	Ar	Itk IC_{50} (μM)	IRK IC_{50} (μM)
2	2-Thiophene	0.040	0.140
8	3-Pyridyl	0.005	0.850
9	5-Isoxazole	0.010	1.8
10	2-Furyl	0.010	0.55
11	4-Pyridyl	0.025	4.9
12	C_6H_5	0.016	0.235
13	C_6H_4(*m*-Br)	0.009	0.095
14	C_6H_4(*p*-Br)	0.003	>10 μM
15	C_6H_4(*p*-Cl)	0.004	3.9
16	C_6H_4 (*m*-OCH_3)	0.010	0.550
17	C_6H_4 (*p*-OCH_3)	0.012	>10 μM
18	C_6H_4(*o*-CH_3)	3.3	NT
19	C_6H_4(*m*-CH_3)	0.056	0.550
20	C_6H_4(*p*-CH_3)	0.035	>10 μM
21	C_6H_4 (*m*-CN)	0.004	0.125
22	C_6H_4 (*p*-CN)	0.004	>10 μM
23	C_6H_4(*p*-$COCH_3$)	0.068	>10 μM
24	C_6H_4(*p*-CO_2CH_3)	0.25	NT
25	C_6H_4(*p*-CO_2H)	4.5	NT
26	C_6H_4(*p*-$CONH_2$)	0.52	NT
27	C_6H_4(*p*-CONH(CH_3))	>10	NT

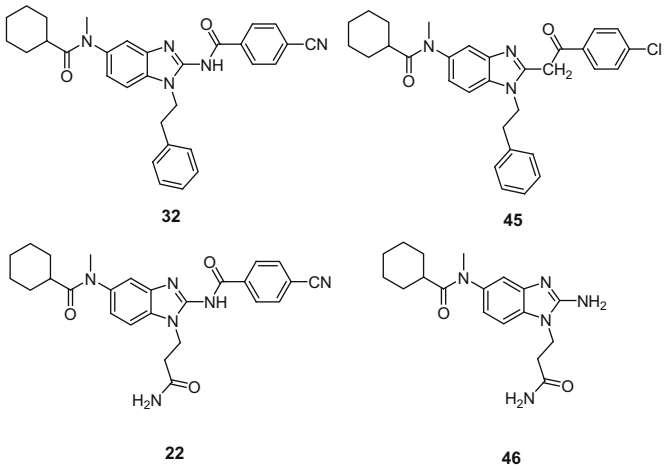
the SAR observed for substitution on the N-1 position. Substitution of the N-1 with linear alkyl groups lead to a 17-fold improvement in potency going from a methyl (**28**, IC_{50} = 0.27 μM) to a butyl (**29**, IC_{50} = 0.016 μM) but extension of the chain to heptyl (**30**, IC_{50} = 4.5 μM) resulted in a 17-fold loss in activity relative to compound **28**. The *N-iso*-propyl analog (**31**) indicated that the α-substitution was tolerated at the N-1 position. Replacement of the *N*-alkyl group with a phenethyl group (**32**, IC_{50} = 0.006 μM) led to an additional improvement in potency, however, substitution of the phenyl ring (**33**–**35**) was not tolerated and lead to a loss in

Table 2
SAR of N-1 substituents on ITK inhibition



Compound	R^1	Itk IC_{50} (μM)
28	CH_3	0.27
29	$(CH_2)_3CH_3$	0.016
30	$(CH2)_6CH_3$	4.5
31	CH $(CH_3)_2$	0.035
32	$CH_2CH_2C_6H_5$	0.006
33	$CH_2CH_2C_6H_4$(*p*-OCH_3)	0.089
34	$CH_2CH_2C_6H_4$(*o*-OCH_3)	0.69
35	$CH_2CH_2C_6H_3$(2,4-di-Cl)	2.0
36	$CH_2CH_2CH_2OCH_3$	0.016
37	$-CH_2CONH_2$	0.073
22	$-CH_2CH_2CONH_2$	0.004
38	$-CH_2CH_2CONH(CH_3)$	0.009
39	$-CH_2CH_2CON(CH_3)_2$	0.014
40	3-(1*H*-Imidazol-1-yl)propyl	0.004
41	2-(4-Methylpiperazin-1-yl)ethyl	0.061
42	3-(4-Methylpiperazin-1-yl)propyl	0.005
43	(Piperazin-1-yl)propan-1-one	0.005
44	(4-Methylpiperazin-1-yl)propan-1-one	0.018

Table 3
Importance of N–H group on C-2 for ITK inhibitory activity



Compound	Itk IC_{50} (μM)
32	0.006
45	>20
22	0.004
46	>20

activity. Shortening the spacer length between the N-1 of the benzimidazole and the side chain primary amide (**22** vs **37**) resulted in an 18-fold loss in potency while mono and di-substitution of the amide NH_2 of **22** resulted in a small loss in potency (2- to 4-fold). Replacement of the propyl amide group in compound **22** with polar groups was allowed with the proper spacer length (**41** vs **42**). The 1-(3-methoxypropyl) (**36**, IC_{50} = 0.016 μM) and (4-methylpiperazin-1-yl)propan-1-one (**44**, IC_{50} = 0.018 μM) groups showed good activity albeit with a small loss in potency in comparison to compound **22**. The 3-(1*H*-imidazol-1-yl)propyl (**40**, IC_{50} = 0.004 μM), 3-(4-methylpiperazin-1-yl)propyl (**42**, IC_{50} = 0.005 μM) and the (piperazin-1-yl)propan-1-one (**43**, IC_{50} = 0.005 μM) analogs on the other hand all had potencies similar to compound **22**.

The importance of the C-2 amide linkage was also explored (Table 3). Removal of the N–H group at C-2 (**45**, IC_{50} = >20 μM) resulted in a complete loss in activity. Although right hand side substituents of the molecules (**32** and **45**) are not identical, we can make an activity comparison since *p*-Cl and *p*-CN phenyl showed comparable ITK inhibitory activity (Table 2, compound **15** vs **22**). Similarly, complete removal of the carboxy aryl portion (**46**, IC_{50} = >20 μM) was found to be equally detrimental to the activity against Itk. The results indicated that the amide connection between the benzimidazole and the aromatic groups is critical for Itk inhibition.

The SAR around the amide linkage on C-2 provided some insight into the possible binding mode of these inhibitors to Itk. The loss of activity associated with a removal of either the amide N–H or carbonyl suggested an involvement in hydrogen bonding to the hinge region of the kinase. Two binding modes were envisioned; through the amide linkage **A** or through its tautomer **B** (Fig. 2). In binding mode **A** the N-1 substituent is oriented toward the solvent exposed region while in **B** the N-1 group is pointing toward the ribose pocket. The SAR of the phenethyl analogs appeared to indicate a potential size restriction consistent with the later binding mode. This hypothesis was confirmed by X-ray crystallography. A crystal structure of compound **2** bound to Itk was solved and showed the inhibitor adopting binding mode **B** (Fig. 3). The crystal structure of the **2**/Itk complex reveals that **2** binds to the protein in the same manner as binding mode **B** with the N-1 group situated

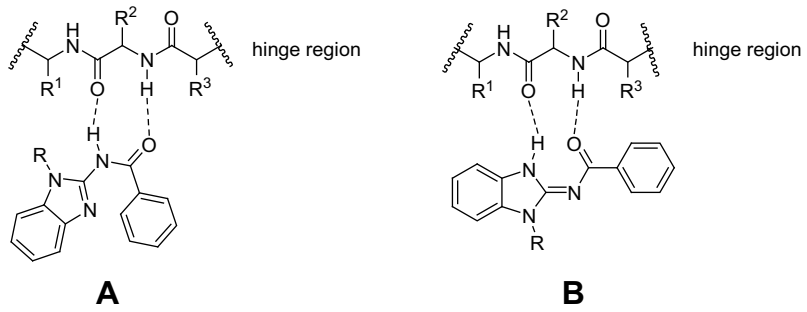


Figure 2. Proposed binding modes to Itk.

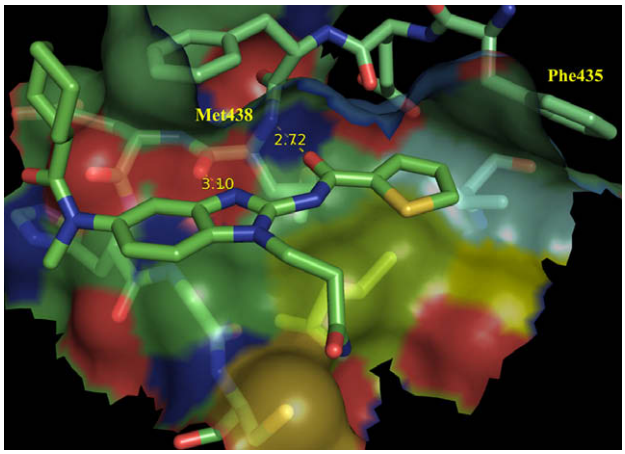


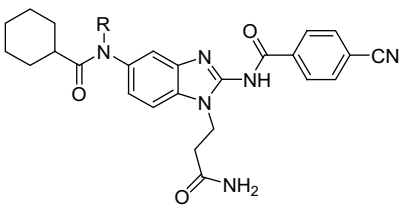
Figure 3. X-ray structure of compound **2** bound to Itk.

in the ribose site of the ATP pocket (Fig. 3).[12,13] The carbonyl of the C-2 amide makes a hydrogen bond with the N–H of Met438 while the N-3 makes a hydrogen bond interaction through the tautomeric form to the carbonyl of the Met438.

Our investigation moved next to the C-5 position of the benzimidazole ring. Introduction of an ethyl group at the R position (**47,** IC_{50} = 0.007 μM) did not negatively impact the enzymatic activity, however when the methyl group (**22,** IC_{50} = 0.004 μM) was replaced by an *iso*-propyl group (**48,** IC_{50} = 0.153 μM) there was a 38-fold loss in activity (Table 4).

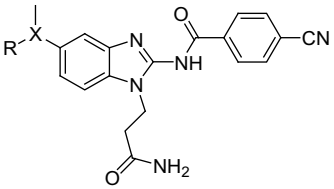
Replacement of the cyclohexyl ring with a methyl (**49**, IC_{50} = 0.059 μM), ethyl (**50**, IC_{50} = 0.32 μM) or *iso*-propyl (**51**, IC_{50} = 0.22 μM) resulted in a loss in potency. Replacement of the cyclohexyl ring with a cyclopropyl (**52**, IC_{50} = 0.046 μM) also resulted in a loss in potency while the cyclobutyl ring (**53**, IC_{50} = 0.008 μM) gave potency comparable to **22**. Substituting the cyclohexyl ring with a phenyl (**54**, IC_{50} = 0.020 μM) gave a compound with 5-fold less activity than compound **22**, while removal of the carboxy cyclohexyl group (**55**, IC_{50} = 0.076 μM) led to a 19-fold decrease in potency relative to compound **22**. Interestingly, replacement of the amide N with C–H (**56**, IC_{50} = 0.003 μM) did not attenuate the activity (Table 5).

Table 4
Modification of *N*-substitution on C-5 position



Compound	R	Itk IC_{50} (μM)
22	Me	0.004
47	Et	0.007
48	*iso*-Propyl	0.15

Table 5
SAR of C-5 substituents on Itk inhibition



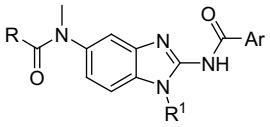
Compound	R	X	Itk IC_{50} (μM)
22	Cyclohexyl-CO–	N	0.004
49	CH_3CO–	N	0.059
50	Ethyl-CO–	N	0.32
51	*iso*-Propyl-CO–	N	0.22
52	Cyclopropyl-CO–	N	0.046
53	Cyclobutyl-CO–	N	0.008
54	Phenyl-CO–	N	0.020
55	–H	N	0.076
56	Cyclohexyl-CO–	CH	0.003

Table 6
Enzyme selectivity of selected Itk inhibitors

Compound	Lyn	IR[a]	Btk[a]	Syk[a]	Bmx[a]	Txk[a]	Tec[a]
15	>1000	>1000	>500	>1000	>1000	550	>1000
40	>1000	>1000	>1000	>1000	>1000	85	>1000
47	>1000	>1000	520	>1000	>1000	77	>1000

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

Table 7
Human liver microsomal stability results of selected compounds



Compound	R	R^1	Ar	HLM CL_H (% Q_H)
15	Cyclohexyl	$-CH_2CH_2CONH_2$	*p*-Cl phenyl	91
40	Cyclohexyl	3-(1*H*-imidazol-1-yl) propyl	*p*-CN phenyl	93
57	4-diF cyclohexyl	$-CH_2CH_2CONH_2$	*p*-CN phenyl	19

Having identified a series of potent inhibitors of Itk, a set of analogs were profiled against a panel of other kinases (Table 6). Compounds **15**, **40** and **47** (Itk, IC_{50} = 0.004, 0.004 and 0.007 μM, respectively) showed 77- to 1000-fold selectivity against all the kinases screened.

Based on the enzyme activity and selectivity, compounds **15** and **40** were selected for further evaluation. Inhibitors **15** and **40** both showed good activity in the DT40 cell-based Ca^{2+} flux inhibition assay with IC_{50}s of 0.32 and 0.51 μM, respectively. Compound **15** inhibited anti-CD3 antibody induced IL-2 secretion both in murine T_H1 cells (IC_{50} = 0.44 μM) and splenocytes (IC_{50} = 0.52 μM). Compound **15,** however, only weakly inhibited anti-CD3/anti-CD28 antibody induced IL-2 secretion in human $CD4^+$ T-cells (IC_{50} = 4.1 μM). Compound **40** inhibited IL-2 secretion in human $CD4^+$ T-cells (IC_{50} = 0.65 μM) and murine T_H1 cells (IC_{50} = 0.35 μM).

Compounds **15** and **40** were tested using human microsomes and both were found to be rapidly metabolized. Metabolite identification studies showed that the major metabolite was hydroxylation at the 4-position of the cyclohexyl ring. Introduction of gem-difluorides (**57**) at the 4-position resulted in a dramatic improvement in microsome stability and identified the cyclohexyl ring as the major liability of the series (Table 7).

In summary starting from a screening hit with moderate enzyme activity and poor cellular potency, we optimized the series using a systematic SAR approach leading to the identification of novel potent and selective Itk inhibitors. The obtained X-ray structure suggested that the inhibitors bind to the kinase through an extended tautomeric intermediate. Microsome studies showed that the cyclohexyl ring was the major site of metabolism and modification of the ring lead to an increased stability. Further improvements and modifications of the series focusing on improving the microsome stability and leading to an orally active Itk inhibitor will be presented in forthcoming publications.

References and notes

1. Kane, L. P.; Lin, J.; Weiss, A. *Curr. Opin. Immunol.* **2000**, *12*, 242.
2. Samelson, L. E. *Annu. Rev. Immunol.* **2002**, *20*, 371.
3. Ohashi, P. S. *Nat. Rev. Immunol.* **2002**, *2*, 427.
4. Lin, T. A.; McIntyre, K. W.; Das, J.; Liu, C.; O'Day, K. D.; Penhallow, B.; Hung, C. Y.; Whitney, G. S.; Shuster, D. J.; Yang, X.; Townsend, R.; Postelnek, J.; Spergel, S. H.; Lin, J.; Moquin, R. V.; Furch, J. A.; Kamath, A. V.; Zhang, H.; Marathe, P. H.; Perez-Villar, J. J.; Doweyko, A.; Killar, L.; Dodd, J. H.; Barrish, J. C.; Wityak, J.; Kanner, S. B. *Biochemistry* **2004**, 11056.
5. Liao, X. C.; Littman, D. R. *Immunity* **1995**, 757.
6. Schaeffer, E. M.; Debnath, J.; Yap, G.; McVicar, D.; Liao, X. C.; Littman, D. R.; Sher, A.; Varmus, H. E.; Lenardo, M. J.; Schwartzberg, P. L. *Science* **1999**, *284*, 638.
7. Miller, A. T.; Wilcox, H. M.; Lai, Z.; Berg, L. J. *Immunity* **2004**, *21*, 67.
8. Forssell, J.; Sideras, P.; Eriksson, C.; Malm-Erjefalt, M.; Rydell-Tormanen, K.; Ericsson, P. O.; Erjefalt, J. S. *Am. J. Respir. Cell Mol. Biol.* **2005**, *32*, 511.
9. Das, J.; Liu, C.; Moquin, R. V.; Lin, J.; Furch, J. A.; Spergel, S. H.; Doweyko, A. M.; McIntyre, K. W.; Shuster, D. J.; O'Day, K. D.; Penhallow, B.; Hung, C. Y.; Kanner, S. B.; Lin, T. A.; Dodd, J. H.; Barrish, J. C.; Wityak, J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4148.
10. (a) Kashem, M. A.; Nelson, R. M.; Yingling, J. D.; Pullen, S. S.; Prokopowicz, A. S., III; Jones, J. W.; Wolak, J. P.; Rogers, G. R.; Morelock, M. M.; Snow, R. J.; Homon, C. A.; Jakes, S. *J. Biomol. Screen.* **2007**, *12*, 70; (b) Snow, R. J.; Abeywardane, A.; Campbell, S.; Lord, J.; Kashem, M. A.; Khine, H. H.; King, J.; Kowalski, J. A.; Pullen, S. S.; Roma, T.; Roth, G. P.; Sarko, C. R.; Wilson, N. S.; Winters, M. P.; Wolak, J. P.; Cywin, C. L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3660.
11. Nose, A.; Kudo, T. *Chem. Pharm. Bull.* **1981**, *29*, 1159.
12. Bentzien, J. M.; Farmer, B., II; Pullen, S. S.; Studts, J. M.; White, A. WO 2005066335 A1 2005: 356 or X-ray crystallographic information.
13. White, A.; Abeywardane, A.; Cook, B. N.; Fuschetto, N.; Gautschi, E.; John, A.; Kroe, R. R.; Kronkaitis, A.; Li, Xiang; Pullen, S. S.; Roma, T.; Moriarty, K. J.; Roth, G. P.; Snow, R. J.; Studts, J. M.; Takahashi, H.; Farmer, B. T., II, *J. Biol. Chem.*, submitted for publication.