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Molecular Design, Synthesis, and Structure–Activity Relationships Leading to the Potent and Selective P56^{lck} Inhibitor BMS-243117

Jagabandhu Das,* James Lin, Robert V. Moquin, Zhongqi Shen, Steven H. Spergel, John Wityak, Arthur M. Doweyko, Henry F. DeFex, Qiong Fang, Suhong Pang, Sidney Pitt, Ding Ren Shen, Gary L. Schieven and Joel C. Barrish

Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543-4000, USA

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Abstract—A series of structurally novel benzothiazole based small molecule inhibitors of p56^{lck} were prepared to elucidate their structure–activity relationships (SARs), selectivity and cell activity in the T-cell proliferation assay. BMS-243117 (compound **2**) is identified as a potent, and selective Lck inhibitor with good cellular activity ($IC_{50} = 1.1\ \mu M$) against T-cell proliferation.

p56^{lck}(Lck), a member of the Src family of non-receptor protein tyrosine kinases is expressed primarily in T-lymphocytes and natural killer cells. In T cells, Lck is absolutely required for T-cell antigen receptor (TCR) signaling, culminating in IL-2 gene expression and effector functions.[1] T-cells lacking Lck are shown to be severely impaired in TCR tyrosine phosphorylation and subsequent activation via the TCR.[2] Selective inhibitors of Lck may have potential therapeutic utility in the treatment of T cell mediated disorders such as autoimmune and inflammatory diseases and in the prevention of solid organ transplant rejection.[3]

Over the past decade, development of small molecule Lck inhibitors[4] has been an area of major pharmaceutical research. High-throughput screening of our in-house compound collection identified thiazole **1** as a modestly potent Lck inhibitor (Fig. 1). In this communication, we describe structure activity studies leading to a novel benzothiazole template, that have culminated in the discovery of BMS-243117 (**2**) as a potent, and selective Lck inhibitor having excellent activity in vitro and good activity in a T-cell proliferation assay. The synthesis, and SAR studies leading to BMS-243117 and analogues are described.

The synthesis of this class of compounds follows a general synthetic route that is illustrated in Scheme 1. Accordingly, 2-amino-6-carbethoxybenzothiazole[5] was treated with excess of di-*tert*-butyl dicarbonate and a catalytic amount of 4-dimethylaminopyridine in dichloromethane to form the corresponding *tert*-butyl carbamate. Saponification with aqueous sodium hydroxide formed acid **3** which was coupled to a wide variety of anilines **4** under standard coupling conditions to form anilides **6** outlined in Table 3. Alternatively carboxylic acid **3** was converted to its acid chloride (CH_2Cl_2, oxalyl chloride, catalytic DMF, rt) and then condensed with the anilines **4** (THF, *i*-Pr_2NEt, 0 °C) to form anilides **5**. Hydrolysis of the *tert*-BOC group with trifluoroacetic acid afforded the 2-amino-benzothiazole analogues **6**. This synthetic route is quite general and was used for the synthesis of related analogues (Table 2).

A solution-phase parallel synthesis approach was used to prepare a large number of carboxamides **7** (Table 4). 2-Aminobenzothiazole **6** was either treated with the corresponding carboxylic acid anhydride in dichloromethane and pyridine or coupled with a carboxylic acid under standard activating conditions to form carboxamides **7** (Scheme 2).

A similar approach was utilized to prepare the urea derivatives **8** (Scheme 3). 2-Aminobenzothiazole **6** was treated with an isocyanate in dichloromethane and pyridine to form urea **8**. Alternatively **6** was treated with phenyl chloroformate in aqueous THF in the presence

*Corresponding author. Tel.: +1-609-252-5068; fax: +1-609-252-6804; e-mail: jagabandhu.das@bms.com

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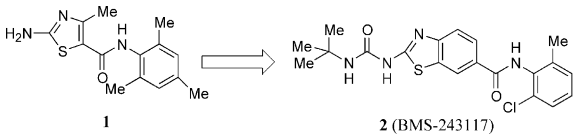
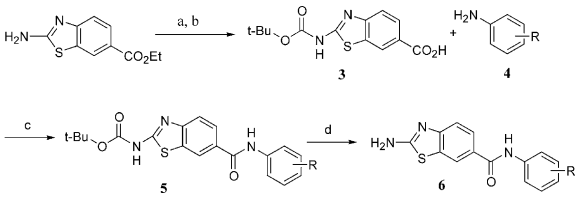


1
Lck IC_{50} = 3.2 μM

2 (BMS-243117)
Lck IC_{50} = 4 nM
T-cell Prolif. IC_{50} = 1.1 μM

Figure 1. Lck inhibitory activity of thiazole **1** and benzothiazole **2**.



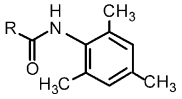
Scheme 1. (a) CH_2Cl_2, DMAP, $(BOC)_2O$, 72%; (b) MeOH, 1 N aq NaOH, 91%; (c) DMF, HATU, *i*-Pr_2NEt, 55–85%; (d) CF_3CO_2H, 72–95%.

of potassium bicarbonate to form a phenyl carbamate which was treated with an amine in THF to form the urea **8**.

Compound **2** (BMS-243117) and its analogues were tested for their ability to inhibit the phosphorylation of an exogenous substrate[6] by human Lck enzyme (IC_{50}, Tables 1–5).

With compound **1** as the initial lead, the effect of the modification of the core thiazole ring was studied and these results are summarized in Table 1. Thiazole **1** and its *tert*-butylcarbamate derivative **9** were found to be equipotent in inhibiting human Lck. Replacement of the thiazole nucleus in **1** with a benzothiazole ring system (**10**) resulted in 5-fold loss in potency but the corresponding *tert*-butylcarbamate analogue **5a** was an order of magnitude more potent than **1**. Similar replacement of the

Table 1. SAR for core nucleus modification



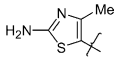
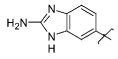
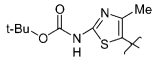
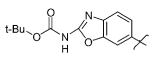
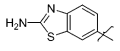
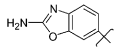
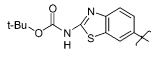
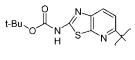
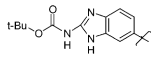
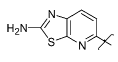
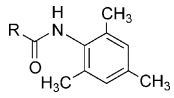
Compd	R	Lck inhibition IC_{50}, μM[6]	Compd	R	Lck inhibition IC_{50}, μM[6]
1		3.2	**12**		> 12.5
9		3.0	**13**		7.25
10		15	**14**		23
5a		0.29	**15**		> 12.5
11		3.23	**16**		> 12.5

Table 2. SAR for benzothiazole core modification



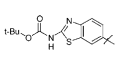
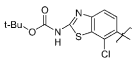
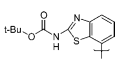
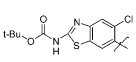
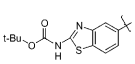
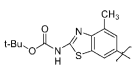
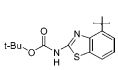
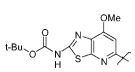
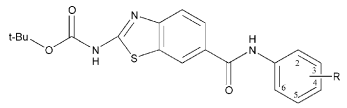
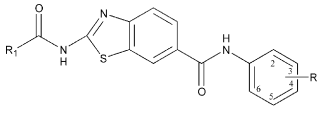
Compd	R	Lck inhibition IC_{50}, μM[6]	Compd	R	Lck inhibition IC_{50}, μM[6]
5a		0.29	**20**		4.4
17		> 12.5	**21**		> 12.5
18		> 12.5	**22**		5.6
19		> 12.5	**23**		> 12.5

Table 3. SAR for aniline modification

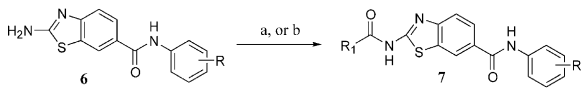


Compd	R	Lck inhibition IC_{50}, μM[6]	Compd	R	Lck inhibition IC_{50}, μM[6]
5a	2,4,6- Me_3	0.29	**5i**	2-Br,6-Me	4.27
5b	2-Me,4-Br	>12.5	**5j**	5-Br, 2,4,6- Me_3	>12.5
5c	2-Cl,6-Me	0.07	**5k**	2,6- Me_2,3-*i*Pr	>12.5
5d	2,6- Me_2	0.09	**5l**	2,6- $(OMe)_2$	>12.5
5e	2-Me,6-$(CH_2)_2OH$	>12.5	**5m**	2,6-Me_2,4-CO_2Me	3.12
5f	2-OH,6-Ac	>12.5	**5n**	2-Br,4,6- Me_2	0.20
5g	2-Br,3,4,6- Me_3	>12.5	**5o**	2,6-Me_2,4-CH_2OH	0.31
5h	2,6-Me_2,4-Br	0.11	**5p**	2,6-Me_2,4-Ph	9.65

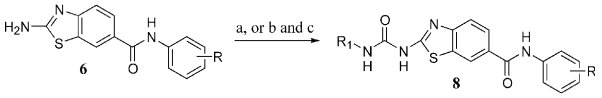
Table 4. SAR for amide modification



Compd	R	R_1	Lck inhibition IC_{50}, μM[6]
7a	2,4,6-Me_3	Me	0.12
7b	2,4,6-Me_3	Ph	>12.5
7c	2,4,6-Me_3	*i*-Propyl	0.70
7d	2,4,6-Me_3	Cyclopropyl	0.02
7e	2,6-Me_2	Cyclopropyl	0.015
7f	2,6-Me_2	*i*-Propyl	1.80
7g	2,6-Me_2	2-Me-cyclopropyl	0.011
7h	2-Cl,6-Me	Cyclopropyl	0.009
7i	2-Cl,6-Me	Cyclobutyl	0.666
7j	2-Cl,6-Me	2-Tetrahydrofuryl	0.272
7k	2-Cl,6-Me	*o*-MePh–CH_2	0.565
7l	2-Cl,6-Me	2-Me-cyclopropyl	0.007
7m	2-Cl,6-Me	*trans*-2-Ph-cyclopropyl	0.027
7n	2-Cl,6-Me	1-Me-cyclopropyl	0.337
7o	2-Cl,6-Me	Cyclopropyl-CH_2	0.085
7p	2-Cl,6-Me	*trans*-2-Me_2NCH_2-cyclopropyl	0.007
7q	2-Cl,6-Me	*trans*-2-CH_2OH-cyclopropyl	0.003
7r	2-Cl,6-Me	2-Pyrrolidinyl-CH_2-cyclopropyl	0.011



Scheme 2. (a) $(R_1CO)_2O$, DMAP, THF, Py; (b) R_1CO_2H, HOAt, EDAC, THF, *i*-Pr_2NEt, 45 °C.

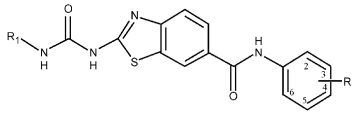


Scheme 3. (a) R_1NCO, CH_2Cl_2, Py; (b) PhOCOCl, THF, aq $KHCO_3$; (c) R_1NH_2, THF.

thiazole nucleus with a benzimidazole, benzoxazole, or 7-azabenzothiazole ring system led to significant attenuation in activity in vitro. Compound **5a** served as the starting point for further optimization.

Table 2 outlines the SAR observed with the carboxamide regio-isomers of compound **5a** or effect of substitution in the phenyl ring of compound **5a**. None of these modifications led to any improvement in potency.

Table 5. SAR for urea modification



Compd	R	R_1	Lck inhibition IC_{50}, μM[6]
8a	2,4,6-Me_3	Me	0.13
8b	2,4,6-Me_3	Ph	0.38
8c	2,4,6-Me_3	Bn	1.73
8d	2,4,6-Me_3	Cyclopropyl	0.30
8e	2,4,6-Me_3	*n*-Bu	0.33
8f	2,4,6-Me_3	*t*-Bu	0.07
8g	2,6-Me_2	Cyclopropyl	0.09
8h	2,6-Me_2	*t*-Bu	0.10
8i	2,6-Me_2	1,1-Me_2propyl	0.08
8j	2-Cl,6-Me	Cyclopropyl	0.09
8k	2-Cl,6-Me	Cyclopentyl	0.045
8l	2-Cl,6-Me	2-Furyl-CH_2	0.039
8m	2-Cl,6-Me	α-Et-Bn	0.019
8n	2-Cl,6-Me	2-Pyridinyl	2.01
8o	2-Cl,6-Me	2-Pyrimidinyl	>3.125
8p	2-Cl,6-Me	*p*-F-Ph	0.123
8q	2-Cl,6-Me	*p*-F-Bn	0.087
2	2-Cl,6-Me	*t*-Bu	0.004
8s	2-Cl,6-Me	1,1-Me_2propyl	0.055
8t	2-Cl,6-Me	$Me_2N(CH_2)_2$	0.03
8u	2-Cl,6-Me	*N*-Me-2-pyrrolidinyl-$(CH_2)_2$	0.007
8v	2-Cl,6-Me	2,2,6,6-Me_4-4-piperidinyl	0.004

In order to further optimize the potency, we investigated the effect of substitution on the aniline ring of **5a** (Table 3). The methyl substituent at C4 can be replaced with hydrogen (**5d**), bromine (**5h**) or hydroxymethyl group (**5o**) without any loss of activity. In contrast, a carbomethoxy group (**5m**) or a bulky phenyl ring at this position is not tolerated. Addition of a substituent at either C3 or C5 is quite detrimental for activity (**5g**, **5j**, **5k**). A methyl group at C2 of **5d** can be substituted with chlorine (**5c**) but replacement with bromine (**5i**) or hydroxyethyl (**5e**) resulted in significant loss of potency. The importance of 2,6-substitution in the aniline ring is demonstrated by the fact that compound **5b** is more than 150-fold less potent than **5h**. This finding is consistent with findings reported earlier with other Lck inhibitors.[4a,c] It appears that only a small alkyl or halogen substituent is tolerated at C2 and C6 since replacement of a chlorine by bromine at C2 (**5i**) or

introduction of polar functions (**5f**, **5l**) has led to significant losses of activity. Because of the optimal potency of the 2,4,6-trimethylaniline (**5a**), 2,6-dimethylaniline (**5d**), and 2-chloro-6-methylaniline (**5c**) analogues, further optimization was carried out on these series.

Table 4 outlines the SAR observed with the carboxamide modification at the C2 amine of the benzothiazole nucleus. In the 2,4,6-trimethylaniline series, acetamide **7a** was roughly equipotent as the carbamate **5a**. Corresponding *iso*-propyl amide **7c** was about 6-fold less potent while the benzamide **7b** was significantly less potent than **7a**. Cyclopropyl amide **7d** was the most potent analogue in this series. A similar trend was observed with the 2,6-dimethylanilines. Cyclopropyl amides (**7h**, **7l**, **7p–q**) in the 2-chloro-6-methylaniline series turned out to be the most potent lck inhibitors. Replacement of the cyclopropyl group by cyclobutyl (**7I**), tetrahydrofuryl (**7j**) or *o*-MeBn (**7k**) was detrimental for activity as was the introduction of a methyl substituent at C1 of the cyclopropyl ring (**7n**) or insertion of a methylene linker (**7o**). Substitution at C2 of the cyclopropyl ring was well tolerated as was the introduction of a polar functionality at this position. Compounds **7l**, and **7p–q** were identified to be the most potent analogues in the carboxamide series.

Similarly, functionalization of the C2 amino group as a urea also led to some very potent Lck inhibitors in this series (Table 5). Some of the interesting SAR findings are: (1) in the 2,4,6-trimethylaniline series methyl urea **8a** was more potent than the corresponding phenyl (**8b**), *n*-butyl (**8e**), cyclopropyl (**8d**) and the benzyl (**8c**) analogues. More importantly, replacement of the methyl by *tert*-butyl group (**8f**) was well tolerated. The corresponding analogue (**8h**) in the 2,6-dimethylaniline series was equipotent to **8f** as were the homologated analogue **8i** and the cyclopropyl urea **8g**. As in the carboxamide series the ureas in the 2-chloro-6-methylaniline series were the most potent Lck inhibitors. The cyclobutyl (**8k**) and tetrahydrofurylmethyl (**8l**) ureas were roughly equipotent, while the α-ethylbenzyl analogue (**8m**) was 5-fold more potent than the cyclopropyl analogue (**8j**). Heteroaryl ureas (**8n**, and **8o**) were significantly less potent. In contrast, the phenyl (**8p**) and benzyl (**8q**) urea modifications were tolerated. The *tert*-butyl urea **2** was one of the most potent inhibitors in this series. SAR pattern observed with the urea analogues was in some cases different from the one observed with the carboxamides. In general the cyclopropyl amide analogues (**7d**, **7e**, and **7h**) were significantly more potent than the corresponding urea analogues (**8d**, **8g**, and **8j**). However, as in the carboxamide series, introduction of polar functionality also resulted in some of the most potent urea analogues (**8u**, **8v**).

The most potent carboxamide and urea analogues (**2**, **7l**, **7p**, **7q**, **8u**, and **8v**) were tested for their selectivity against several kinases and for cell activity in a T-cell proliferation assay (Table 6). All these compounds showed some degree of selectivity against other members of the Src family kinases. Urea **2** (BMS-243117) is the most selective Lck inhibitor in this series. In addition, **2** was highly selective against several additional receptor and non-receptor tyrosine kinases, and serine/threonine kinases (>6000-fold selectivity over cdk2, IGF1R, Kdr, and p38 kinases). Compound **2** inhibited anti-CD3/anti-CD28 induced PBL proliferation with an IC_{50} of 1.1 μM.

Table 6. Enzyme selectivity of selected lck inhibitors

Compd	Lck IC_{50}, nM[6]	Src[a]	Fyn[a]	Hck[a]	Blk[a]	Lyn[a]	Fgr[a]	T-Cell prolif. IC_{50}, μM[6]
2	4	158	32	960	84	330	60	1.1
7l	7	57	1.5	92	46	5	3	1.58
7p	7	57	23	650	47	100	10	3.53
7q	3	12	5	NT	NT	22	7	1.45
8u	7	200	3	>600	300	150	17	0.96
8v	4	84	0.5	325	16	185	6	2.80

[a]Selectivity ratio: IC_{50}/IC_{50} (Lck); NT, not tested.

A binding model for these benzothiazole Lck inhibitors was developed based on the published coordinates of the activated Lck kinase domain bound to ANP (phosphoaminophosphonic acid-adenylate ester, a non-hydrolyzable ATP mimic).[7] As illustrated in Figure 2, BMS-243117 (**2**) binds in an extended conformation to the ATP-binding site of Lck. The 2,6-disubstituted aniline of **2** occupies a clearly defined hydrophobic pocket (common to all Src family kinases) which is not occupied by ATP. The 2,6-disubstitution skews the aniline ring out of plane with respect to the benzothiazole core and this in turn allows the phenyl ring to fit into the narrow and angular hydrophobic pocket. The SAR observed regarding the aniline substitution pattern in this series is consistent with this proposed model. In addition, compound **2** makes several productive hydrogen bond interactions within the active site: the NH of the aniline is hydrogen bonded to Thr316 side-chain hydroxyl, the benzothiazole nitrogen is in H-bond contact with Tyr318, and the two NHs of the urea moiety are in contact with Met319, and Glu320, respectively. The high degree of selectivity exhibited by the urea analogue **2** is not clearly understood but it may be due to differences in the amino acid side chains found at the edge of the ATP binding pocket in other kinases. For example, Lck residues Glu320 and Asn321 are replaced

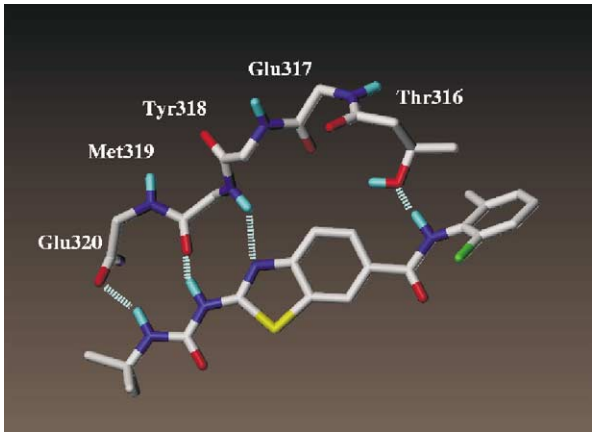


Figure 2. Proposed conformation of **2** bound to the ATP binding site of Lck.

in Src by Ser 320 and Lys321, respectively, and in Fyn by Asn320 and Lys321, respectively.

In summary, we have developed a novel series of benzothiazole Lck inhibitors based on our initial thiazole lead **1**. SAR studies identified several carboxamide and urea analogues as potent and selective inhibitors. BMS-243117 (**2**) was shown to be a potent, and selective lck inhibitor with good potency in a T-cell proliferation assay.

References and Notes

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6. (a) *Lck enzyme assay*: Recombinant Lck expressed as a His-tagged protein in insect cells using a baculovirus expression system and purified by nickel affinity chromatography was incubated in kinase buffer (20 mM MOPS, pH 7, 10 mM $MgCl_2$) in presence of the test compound. The reaction was initiated by addition of substrates to the final concentration of 1 μM ATP, 3.3 μCi/mL [^{33}P}g-APT, and 0.1 mg/mL acid denatured enolase, and stopped after 10 min by addition of 10% trichloroacetic acid, 100 mM sodium pyrophosphate followed by 2 mg/mL bovine serum albumin. The labeled enolase protein substrate was precipitated at 4 °C, harvested onto Packard Unifilter plates and counted in a Topcount scintillation counter. (b) Enzyme assays for Src, Fyn, Hck, Blk, Lyn, and Fgr were run under the same conditions as for Lck. (c) *PBL proliferation assay*: A 96-well plate was coated with monoclonal antibody to CD3 (G19-4), the antibody was allowed to bind, and the plate was washed. Normal human peripheral blood T-cells were added to the wells along with the test compound and anti-CD28 (E.3) antibody. After 3 days, [^{3}H]-thymidine was added to the cells, after further incubation, the cells were harvested and counted in a scintillation counter.
7. (a) Two crystal structures of the catalytic domain of Lck (Tyr394 phosphorylated) were published. See: Yamaguchi, H.; Hendrickson, W. A. *Nature* **1996**, *384*, 484. (b) Zhu, X.; Kim, J. L.; Newcomb, J. R.; Rose, P. E.; Stover, D. R.; Toledo, L. M.; Zhao, H.; Morgenstern, K. A. *Structure* **1999**, *7*, 651. Protein Data Bank (1QPC pdb, Research Collaboratory for Structural Bioinformatics.