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Discovery of 2-amino-6-carboxamidobenzothiazoles as potent Lck inhibitors

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Abstract—A novel series of 2-amino-6-carboxamidobenzothiazole was discovered to have potent Lck inhibitory properties. A highly efficient chemistry was developed. Also described are the detailed SAR study and the BaF3 cell line profiling for this series.

Lymphocyte specific protein tyrosine kinase (Lck), a member of the Src family of non-receptor protein tyrosine kinases, is predominantly expressed in T-lymphocytes and natural killer cells.[1,2] Lck plays an essential role in the T cell receptor (TCR) signal transduction pathway.[3] It phosphorylates the ζ chain of the TCR complex on specific tyrosine residue located within a motif termed the immunoreceptor tyrosine activation motif (ITAM), resulting in coupling of TCR with protein tyrosine kinase ZAP-70 via its Src homology-2 (SH2) domains.[1] Subsequent phosphorylation of ZAP-70 by Lck[4] triggers a series of downstream cascade events that ultimately leads to cytokine release, T cell activation and proliferation.[5] Genetically modified mice with Lck mutations exhibit defects in T cell maturation and signalling.[6] These findings indicate that Lck inhibitors should inhibit T cell activation and therefore be useful therapies for T cell-mediated autoimmune diseases and graft rejection. Additionally, a selective Lck inhibitor will offer a more desirable safety profile compared to other immuno-suppressive agents (e.g., cyclosporine or steroids currently used for treating T cell-mediated autoimmune and inflammatory diseases) since Lck is exclusively expressed in lymphoid cells.[2]

Developing small molecule Lck inhibitors has been the focus of major pharmaceutical research in recent years.[7] Potent and bioavailable Lck inhibitors have been identified and demonstrated in vivo efficacy in several models of T cell dependent immune responses.[8] Our research efforts towards modifying the 2-amino-6-carboxamidobenzothiazole series previously reported by Bristol-Myers Squibb[9] resulted in a novel scaffold with potent Lck inhibitory properties. Described here are the chemistry for analogue synthesis, structure–activity relationship (SAR) study and kinase profiling for this series.

As shown in Scheme 1, the chemistry started by coupling of 2-*tert*-butoxycarbonylaminobenzothiazole-6-carboxylic acid (**1a**) with *N*-(4-amino-3-methylphenyl)-3-trifluoromethylbenzamide (**1b**) in the presence of HATU to provide compound **2**. After removing the Boc group with TFA, the amino intermediate **3** was treated with different isocyanates to generate the urea analogues **4a**–**e**. Numerous carbamates **4f**–**j** were also generated by reacting compound **3** with different chloroformates. Several aminoalkyl and heteroaryl groups were attached to the –NH at the C-2 position via chemistry described in Scheme 2. First, mixing 2-aminobenzothiazole-6-carboxylic acid methyl ester (**5**) with *tert*-butyl nitrite and $CuBr_2$ provided bromo ester **6**, which was saponified to carboxylic acid **7**. Treating this intermediate with oxalyl chloride and compound **1b** in sequence resulted in bisamide **8**. Then, the 2-bromide was displaced with various amines to generate analogues **9a**–**h**. For the synthesis of compounds **9d**–**h**, the addition of NaH was necessary in order to generate a more active N anion.

Keywords: Lck inhibitor; 2-Amino-6-carboxamidobenzothiazole.
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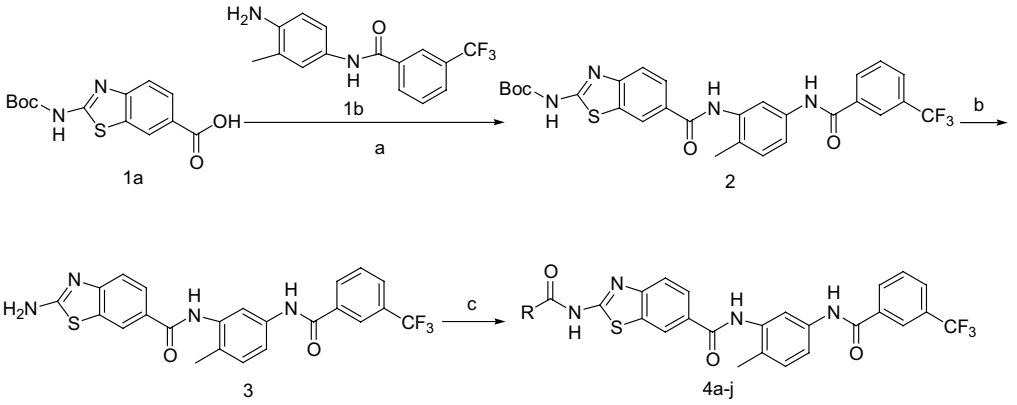
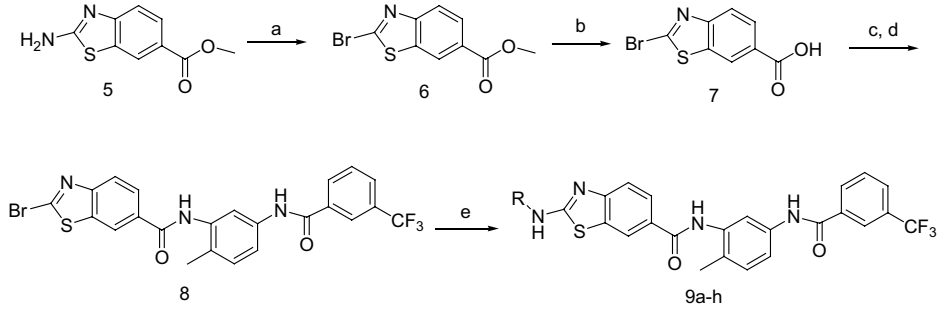


Scheme 1. Synthesis of analogues **4a**–**j**. Reagents: (a) *N*,*N*-diisopropylethylamine, HATU, DMF, 100%; (b) TFA, 85%; (c) RNCO for **4a**–**e**, 80–90% or RO(CO)Cl for **4f**–**j**, 40–60%.

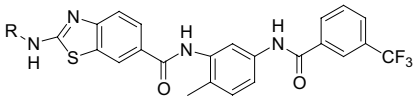


Scheme 2. Synthesis of analogues **9a**–**h**. Reagents: (a) *tert*-BuONO, $CuBr_2$, CH_3CN, 88%; (b) LiOH, THF/H_2O (1:1), 85%; (c) $(COCl)_2$, CH_2Cl_2; (d) **1b**, *iso*-Pr_2NEt, CH_2Cl_2, 97%; (e) RNH_2, DMF, 60–90%.

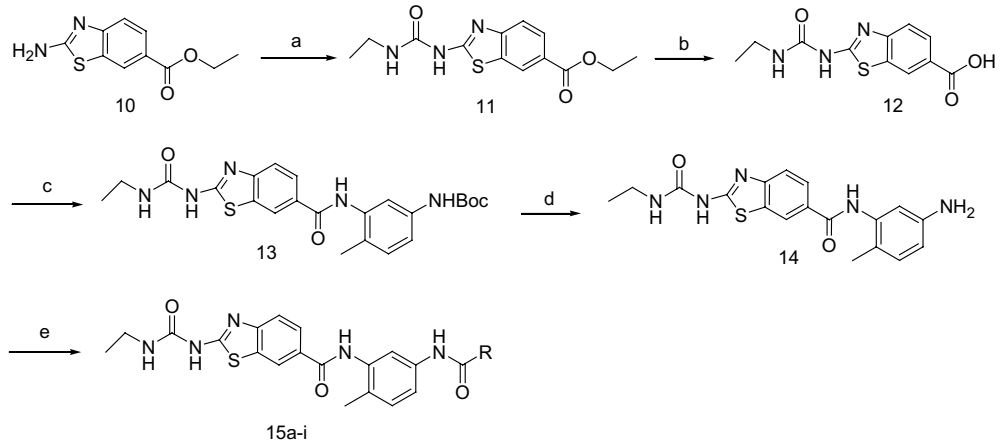
All analogues were tested for their cellular activity using a TEL-Lck transformed BaF3 cell line that was stably transfected with a Cytomegalovirus immediate early promoter upstream of a luciferase gene. Briefly, the 3′ portion of Lck was fused to the 5′ region of TEL, a gene encoding a member of the ETS transcription factor family. The TEL-Lck fusion protein includes the catalytic domain of Lck and the TEL-specific oligomerization domain. TEL-induced oligomerization of TEL-Lck resulted in the constitutive activation of its tyrosine kinase activity and conferred cytokine-independent proliferation to the interleukin-3-dependent BaF3 hematopoietic cell line. Transformed BaF3 cells were plated in 384-well plates (25,000 cells per well) and incubated with serial dilutions of inhibitor or DMSO for 48 h. Luciferase expression was used as a measure of cell proliferation/survival and was evaluated with the Bright-Glo Luciferase Assay System (Promega, Madison, WI).

The inhibition of cell proliferation results is shown in Table 1. Compound **3** with an unsubstituted amino group only showed moderate cellular potency with an IC_{50} of 170 nM. Converting the amine to urea resulted in more potent compounds. A 10-fold increase in potency was observed with simple alkyl and carbocyclic ureas (**4a**, **4b**, **4c**), though the potency improvement for aryl and heteroaryl ureas (**4d**, **4e**) was marginal. Transforming the amine to carbamate was also beneficial to cellular potency. As seen with urea analogues, alkyl carbamates (**4f**, **4g**, **4h**) were more anti-proliferative than aryl carbamates (**4i**, **4j**). In particular, compound **4h** had a single-digit nM IC_{50}, possibly due to its high cellular permeability. Adding amino groups (**9a**–**c**) not only improved solubility, but also in-

Table 1. Cellular activity of compounds **3**, **4a**–**j**, **9a**–**h**



Compound	R	Cellular activity IC_{50} (μM)
3	H	0.170
4a	$CH_3NHC(O)$	0.021
4b	$CH_3CH_2NHC(O)$	0.016
4c	CyclopentylNHC(O)	0.016
4d	PhNHC(O)	0.094
4e	Pyrid-3-ylNHC(O)	0.125
4f	$CH_3OC(O)$	0.030
4g	IsopropylOC(O)	0.030
4h	$CH_3OCH_2CH_2OC(O)$	0.008
4i	PhOC(O)	0.112
4j	4-MethoxyphenylOC(O)	0.131
9a	*N*,*N*-$(CH_3)_2NCH_2CH_2CH_2$	0.081
9b	1-Methylpiperazin-4-ylCH_2CH_2	0.058
9c	Morpholin-4-yl$CH_2CH_2CH_2$	0.078
9d	4-Methylpyridin-2-yl	0.016
9e	4,6-Dimehylpyrimidin-2-yl	0.053
9f	2,4-Dimethylpyrimidin-6-yl	0.0006
9g	Pyrazin-2-yl	0.004
9h	5-Methylisoxazol-3-yl	0.008

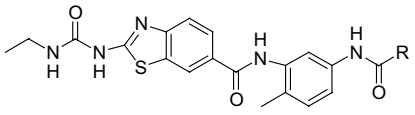


Scheme 3. Synthesis of analogues **15a**–**i**. Reagents: (a) EtNCO, THF, 86%; (b) LiOH, 1,4-dioxane/H_2O (1:1), 92%; (c) 5-*tert*-butoxycarbonylamino-2-methylaniline, HATU, Et_3N, DMF, 82%; (d) TFA, 92%; (e) RC(O)OH, HATU, Et_3N, DMF, 82%.

creased cellular potency. Attaching heteroaryls (**9d**–**h**) generated the most potent analogues, for example, compounds **9d** and **9e** had single-digit nM IC_{50} and the IC_{50} for compound **9f** was <1 nM, indicating there might be additional interactions between the Lck ATP binding pocket and these heteroaryls.

The second stage of our SAR study was focused on the right side benzoyl functionality while fixing the left side as an ethyl urea group. Using the chemistry shown in Scheme 3, 2-aminobenzothiazole-6-carboxylic acid ethyl ester (**10**) was reacted with ethyl isocyanate to generate compound **11**, which was saponified to carboxylic acid **12**. It was then coupled with compound 5-*tert*-butoxycarbonylamino-2-methyl-aniline to provide amide **13**. After removing the Boc group with TFA, the amino intermediate **14** was coupled with various carboxylic acids to provide analogues **15a**–**i**. The cellular data are displayed in Table 2. Adding F to the C-3 position of the 5-CF_3-phenyl ring (**15a**) increased potency slightly while the effect of 4-methylimidizol-1-yl (**15b**) at the same position was minimal. Attaching solubilizing group 4-methylpiperizin-1-yl (**15c**) to the C-3 position barely changed the potency. On the other hand, 4-Cl on the 5-CF_3-phenyl ring (**15d**) seemed to be harmful, resulting in a threefold loss in potency. The negative impact by substituting the CF_3 with CH_3O (**15e**), Me (**15f**) or Cl (**15g**) was even more severe. Replacing 3-CF_3-phenyl group with 2-*tert*-butylpyridin-4-yl group (**15h**) or 2-*tert*-butylthien-5-yl (**15i**) had very little effect in terms of cellular inhibition.

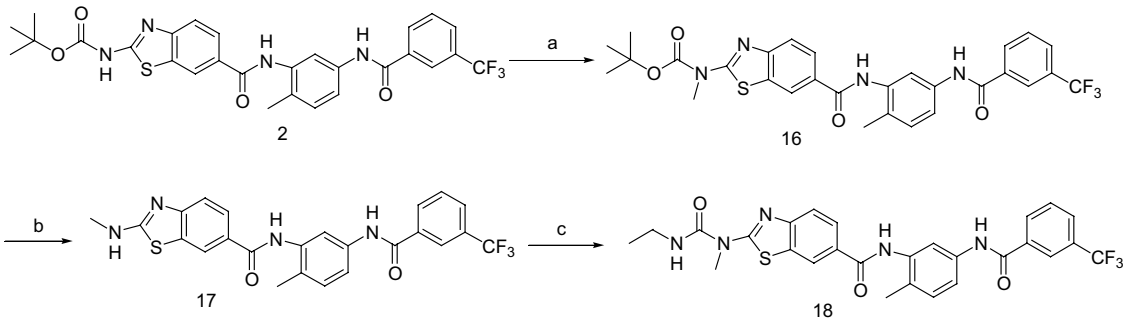
Table 2. Cellular activity for compounds **15a**–**i**



Compound	R	Cellular activity IC_{50} (μM)
15a	3-F-5-CF_3-phenyl	0.007
15b	3-(4-Methylimidazol-1-yl)-5-CF_3-phenyl	0.015
15c	3-(4-Methylpiperizin-1-yl)-5-CF_3-phenyl	0.019
15d	4-Cl-3-CF_3-phenyl	0.050
15e	3-Methoxyphenyl	0.158
15f	3-Methylphenyl	0.112
15g	3-Chlorophenyl	0.104
15h	2-*tert*-Butylpyridin-4-yl	0.009
15i	2-*tert*-Butylthien-5-yl	0.012

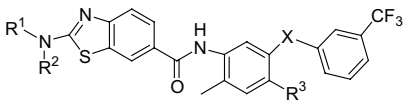
Using chemistry in Scheme 4, compounds **16** and **18** were synthesized to explore whether the 2-NH is essential for cellular activity. Methylation of the 2-NH of compound **2** under Mitsunobu conditions afforded analogue **16**. Next, the Boc was removed with TFA to generate intermediate **17**, which was reacted with ethyl isocyanate to afford analogue **18**. Methylation of the 2-NH resulted in a dramatic loss in cellular potency for both carbamate **16** and urea **18** (data shown in Table 3), demonstrating this NH is crucial for Lck potency. A synthesis was also developed to examine the effect of bromination of the middle ring. As shown in Scheme 5, intermediate **3** was reacted with *tert*-butyl nitrite and $CuBr_2$ to generate dibromo compound **19**. Next, displacement of the 2-Br with 2-(morpholin-4-yl)ethylamine provided analogue **20**, which was nearly inactive in BaF3 cells (see Table 3), showing that adding Br to the middle ring was detrimental to Lck potency. Finally, we examined the effect of reversing the amide group at the right side. By similar chemistry in Scheme 1, compounds **21** and **22** were synthesized, both of which exhibited several fold lower cellular potency relative to their corresponding analogues **4b** and **4c**.

Compounds **4b**, **4c**, **4f** and **4h** were also tested in two different Jurkat cell lines that have the interleukin 2 (IL-2) promoter driving luciferase. In the Jurkat IL-2 anti-CD3+28 assay, the T cell receptor is stimulated by incubating cells with plate bound anti-CD3+CD28 antibodies, leading to an increase in IL-2 promoter activity. Therefore, Lck inhibitors would block this activation. On the other hand, a Jurkat IL-2 PMA/Iono assay is used to measure general off target effects, as the IL-2 promoter is stimulated by PMA and ionomycin in a T cell

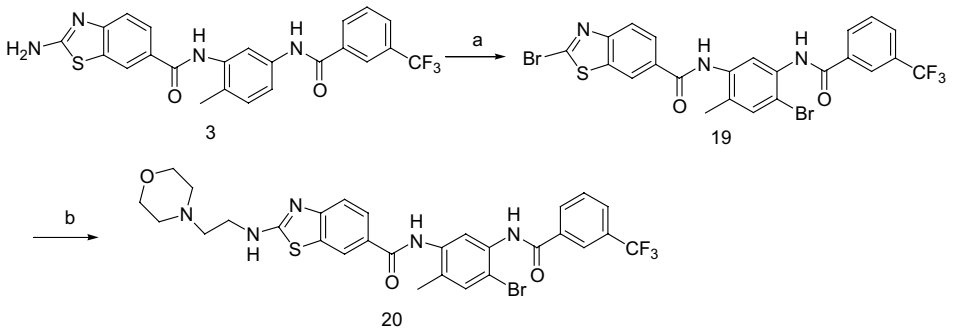


Scheme 4. Synthesis of analogues **16** and **18**. Reagents: (a) Ph_3P, DIAD, MeOH, THF, 53%; (b) TFA, 99%; (c) EtNCO, Et_3N, THF, 71%.

Table 3. Cellular activity for compounds **16**, **18**, **20**–**22**



Compound	R^1	R^2	R^3	–X–	Cellular activity IC_{50} (μM)
2	*tert*-ButylOC(O)	H	H	–NH(CO)–	0.036
16	*tert*-ButylOC(O)	CH_3	H	–NH(CO)–	0.955
18	$CH_3CH_2NHC(O)$	CH_3	H	–NH(CO)–	>10
20	2-(Morpholin-4-yl)CH_3CH_2	H	Br	–NH(CO)–	2.099
21	$CH_3CH_2NHC(O)$	H	H	–(CO)NH–	0.118
22	CyclopentylNHC(O)	H	H	–(CO)NH–	0.139



Scheme 5. Synthesis of analogue **20**. Reagents: (a) *tert*-BuONO, $CuBr_2$, CH_3CN, 72%; (b) 2-(morpholin-4-yl)ethylamine, DMF, 70%.

receptor independent manner. For the anti-CD3+28 assay, anti-mouse IgG was bound to the plate using a sodium bicarbonate buffer followed by incubation with OKT3 (mouse anti-human CD3) and mouse anti-human CD28 antibodies. Plates were then washed and 100,000 Jurkat IL-2-luc cells were plated out in 0.05 mL for 5 h. For the PMA/Iono assay, 50,000 cells were plated out in 0.025 mL followed by the addition of 0.025 mL of 40 ng/mL PMA and 1.5 μg/mL ionomycin. Luciferase activity was evaluated with the Bright-Glo Luciferase Assay System. As expected, these potent Lck inhibitors exhibited high potency in the Jurkat IL-2 anti-CD3+CD28 assay and low off target activity as determined in the Jurkat IL-2 PMA/Iono assay (see Table 4).

Compounds **4a** and **4g** were selected for screening against a panel of BaF3 cell lines transformed with different kinases. The results are shown in Table 5. Excellent selectivity was achieved against a number of structurally diverse kinases including JAK3, KDR, InsR, Alk, FGFR-3 and Flt3. Both compounds also showed good selectivity against Sky though their potency for two other Src family members Lyn and Src remains relatively high.

Table 4. Cellular activity of compounds **4b**, **4c**, **4f** and **4h**

Compound	Jurkat cell/anti-CD3+ CD28 IC_{50} (μM)	Jurkat cell/PMA/Iono IC_{50} (μM)
4b	0.044	0.373
4c	0.062	0.812
4f	0.036	0.851
4h	0.012	0.438

In summary, a novel series of 2-amino-6-carboxamidobenzothiazole was discovered to have potent

Table 5. BaF3 cell line profiling of compounds **4a** and **4g**

Compound	IC_{50} (μM)									
	BaF3/Lck	BaF3/Lyn	BaF3/Src	BaF3/Sky	BaF3/JAK3	BaF3/KDR	BaF3/InsR	BaF3/Alk	BaF3/FGFR3	BaF3/FLT3
4a	0.021	0.094	0.301	1.105	5.01	1.608	1.276	1.633	1.984	3.141
4g	0.030	0.268	0.517	2.436	>10	3.283	13.5	6.109	12.603	>10

Lck inhibitory properties. A highly efficient chemistry was developed. Many potent analogues were synthesized with urea, carbamate, heteroarylamine or alkylamine at the C-2 position. The SAR for the middle ring, the right side aryl group was also explored. The selectivity profile was herein included. Future studies will be directed towards determination of PK properties and in vivo efficacy.

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