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Discovery and Initial SAR of 2-Amino-5-carboxamidothiazoles as Inhibitors of the Src-family Kinase p56^{Lck}

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Abstract—A novel series of 2-amino-5-carboxamidothiazoles were identified as inhibitors of Lck. Structure–activity studies demonstrate the structural requirements for potent Lck activity. Cyclopropylamide **11d** is a potent Lck inhibitor having sub-micromolar activity in a PBL proliferation assay.

The tyrosine kinase p56^{Lck} is a member of the Src family of kinases expressed predominantly in T cells and NK cells. It plays essential roles in T cell development,[1] activation,[2] and T cell antigen receptor (TCR) signaling. Lck phosphorylates the immunoreceptor tyrosine activation motif (ITAM) sequences of the invariant chains (including the ζ chain) within the TCR. Once phosphorylated, the ζ chain binds ZAP-70 via its SH2 domains.[2c] Phosphorylation of ZAP-70 by Lck, and the combined action of ZAP-70, Lck, and the related Src family kinase Fyn drives downstream signaling events and leads ultimately to calcium flux, and T cell activation and proliferation. Genetics experiments using Lck−/− mice have validated Lck as an immunosuppressive molecular target.[1a] These mice display a SCID-like syndrome and are unable to reject skin grafts despite the presence of peripheral T cells.[3] In addition, over expression of a dominant negative form of Lck leads to early arrest of thymocyte development prior to expression of CD4, CD8, and the TCR.[1b] A selective Lck inhibitor should inhibit T cell activation and have potential utility in the treatment of acute and chronic T cell mediated autoimmune and inflammatory disorders such as solid organ transplant graft rejection, multiple sclerosis, rheumatoid arthritis, psoriasis, and delayed type hypersensitivity reactions. Recent reviews describe inhibitors and highlight the therapeutic potential of Src family and Lck inhibition.[4]

We recently reported the discovery of a novel series of imidazoquinoxalines as Lck kinase domain inhibitors.[5]

In addition, high throughput screening of our compound collection led to the identification of 2-aminothiazole **1** as a weakly active Lck inhibitor (Fig. 1). In this report we describe our initial efforts toward optimizing the Lck activity of this lead.

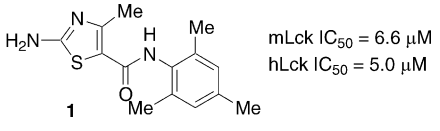

Figure 1. Activity for 2-aminothiazole **1**.

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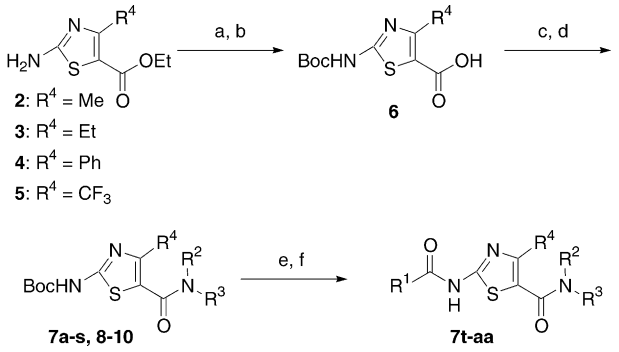
Screening was initiated using mouse Lck (mLck), and subsequently continued using human Lck (hLck). Since Lck from these two species has a sequence homology of 84.4%,[6] it was expected that this change would have little bearing on the resulting SAR. Indeed, the bridging assay data revealed a good correlation between the mouse and human assay results.[7]

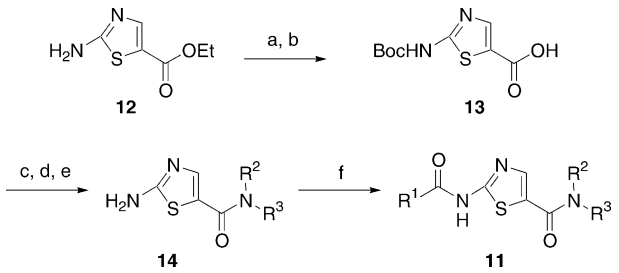
The thiazoles studied were synthesized as shown in Schemes 1 and 2. In Scheme 1, the commercially available 4-methyl- (**2**), 4-ethyl- (**3**), 4-phenyl- (**4**), and 4-trifluoromethyl- (**5**) ethyl 2-aminothiazole-5-carboxylates were converted to their corresponding Boc-derivatives followed by ester saponification. Activation of the resulting thiazole-5-carboxylates **6** through formation of the acyl chloride followed by reaction with various amines then afforded the desired thiazole derivatives **7**–**10**.

The 4-des(alkyl)thiazoles **11** were prepared according to Scheme 2. Starting from ethyl 2-aminothiazole-5-carboxylate (**12**),[8] protection of the amine as the Boc-derivative followed by saponification afforded acid **13**. This material was activated through formation of the acyl chloride, coupled with an appropriate aniline derivative, and deprotected to give amine **14**. Amine **14** was then acylated to afford the corresponding urea, carbamate, or amide derivatives **11**.

Simply protecting the 2-amino group of **1** as the Boc-derivative **7a** resulted in a 2-fold improvement in Lck



Scheme 1. Synthesis of 4-substituted thiazole derivatives: (a) Boc_2O, DMAP, THF; (b) NaOH, MeOH (aq); (c) $(COCl)_2$, DMF, THF; (d) iPr_2Net, THF; (e) TFA, CH_2Cl_2; (f) R′OCOCl, DMAP, THF or PhOCOCl, THF followed by $NHR'_1R'_2$ or RCOCl, DMAP, THF.



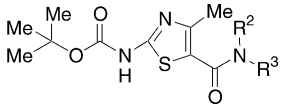
Scheme 2. Synthesis of 4-hydridothiazole derivatives: (a) Boc_2O, DMAP, THF; (b) NaOH, MeOH (aq); (c) $(COCl)_2$, DMF, THF; (d) R^2R^3NH, iPr_2NEt, THF; (e) TFA, CH_2Cl_2; (f) R^1OCOCl, DMAP, THF or PhOCOCl, THF followed by $NHR^1R^{1'}$ or R^1COCl, DMAP, THF.

activity. Our SAR studies continued with a round of parallel synthesis in which the 2,4,6-trimethylaniline moiety of **7a** was replaced with a diverse set of amines. As summarized in Table 1, this survey revealed a narrow SAR that favored certain secondary arylamides (**7a**–**e**) over analogues having a secondary or tertiary alkylamide (**7f**–**j**). Furthermore, the importance of small substituents on the 2- and 6-positions of the arylamide is clearly evident as **7a**–**e** have superior Lck activity versus those analogues lacking one or both substituents (**7k**–**o**) or having sterically demanding groups at these positions (**7p**–**s**). In this respect, the SAR parallels that observed with other series of inhibitors.[5a,9] These substituents likely serve to orient the aniline ring in a conformation orthogonal to the thiazole (vide infra).

We next focussed on developing the SAR with respect to the thiazole 2-amino group (Table 2). A large number of amides, carbamates, and ureas were prepared using a parallel synthesis strategy. This effort resulted in Lck inhibitors having greatly improved activity. A reduction in steric bulk appeared to be beneficial (**7a** vs **7t**). Certain aryl and heteroaryl amides also showed good activity (**7u**, **7v**), and alkylureas **7w** and **7x** were particularly active. In a direct comparison of 2,4,6-trimethylanilines (**7u**, **7v**, **7x**) with their 2-chloro-6-methyl aniline counterparts (**7y**, **7z**, **7aa**), the former were 2–20-fold more active than the latter against Lck.

Compounds **7bb** and **7cc** (Fig. 2) serve to demonstrate the importance of the amide NH moieties to Lck activity. Each compound was inactive when tested at a concentration of 50 μM. As a comparison, **7a** had an IC_{50} of 1.5–3 μM. From analysis of the docking of **11e** into

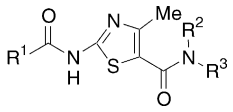
Table 1. 2-*t*-Butyloxycarbonylamino-4-methylthiazole 5-carboxamide SAR



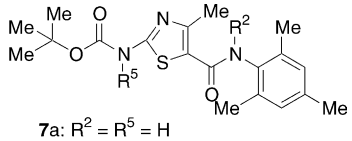
Compd	R^2	R^3	mLck	hLck
			IC_{50}, μM[a]	IC_{50}, μM[a]
7a	H	2,4,6-Me_3 aniline	3	1.5
7b	H	2-Cl, 6-Me aniline	9.6	28
7c	H	2,6-Me_2 aniline	7.5	4.4
7d	H	4-Br, 2,6-Me_2 aniline	2.4	1.4
7e	H	2,6-Cl_2 aniline	—	12.5
7f	H	2,2-Me_2 propylamine	>50	—
7g	H	1,2-Me_2 propylamine	>50	>50
7h	H	2,5-Me_2 3-pyrroline	>50	—
7i	—	4-Formylpiperazine	>50	—
7j	—	Cyclohexylmethylamine	>50	—
7k	H	Aniline	>50	—
7l	H	2,4-Cl_2 aniline	>50	—
7m	H	2-NO_2 aniline	>50	—
7n	H	3-OMe, 5-CF_3 aniline	>50	—
7o	H	2-Me aniline	45.7	—
7p	H	2-Me, 6-*i*Pr aniline	40.3	—
7q	H	2,6-iPr_2 aniline	>50	—
7r	H	2-OMe, 6-Me aniline	24.3	—
7s	H	2-*t*Bu, 6-Me aniline	>50	—

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

Table 2. 4-Methylthiazole 2-amino substituent SAR



Compd	R^1	R^2	R^3	mLck	hLck
				IC_{50}, μM	IC_{50}, μM
7t	MeO–	H	(2,4,6-Me_3)Ph	0.72	0.7
7u	2-Furyl–	H	(2,4,6-Me_3)Ph	—	0.36
7v	Phenyl–	H	(2,4,6-Me_3 Ph	0.32	0.80
7w	MeNH–	H	(2,4,6-Me_3)Ph	0.17	0.24
7x	*n*-BuNH–	H	(2,4,6-Me_3)Ph	0.070	0.030
7y	2-Furyl–	H	(2-Cl, 6-Me)Ph	—	0.71
7z	Phenyl–	H	(2-Cl, 6-Me)Ph	—	4.5
7aa	*n*-BuNH–	H	(2-Cl, 6-Me)Ph	—	0.62



7a: $R^2 = R^5 = H$
7bb: $R^2 = Me$, $R^5 = H$
7cc: $R^2 = H$, $R^5 = Me$

Figure 2. *N*-Methyl analogues **7bb** and **7cc**.

the ATP binding site of the published X-ray structure,[10] we surmise that these amide NH groups may be engaged in significant hydrogen bonding interactions (Fig. 3); the aniline NH appears to be favorably positioned for a hydrogen bonding interaction with the Thr316 side-chain hydroxyl, while the 2-aminothiazole moiety appears in close contact for hydrogen bonding with the protein backbone carbonyl and NH of Met319. This binding mode is also consistent with the observed preference for 2,6-disubstitution of the aniline. Such substitution ensures that the aniline adopts a conformation permitting it to occupy an angular hydrophobic pocket common to tyrosine kinases.

Turning our attention to the 4-position of the thiazole, it was found in a series of compounds in which the 2-amino substituent was Boc that groups larger than methyl were not well tolerated. Analogues **8**–**11a** in Table 3 were inactive when tested at a concentration of 30 μM. In an apparent SAR paradox, while pursuing the 4-des(alkyl) series further it was found that when Boc was replaced by certain amides (**11b**,**c**), these compounds had roughly similar activities to their 4-methyl counterparts **7y** and **7z**. Furthermore, the combination of a cyclopropylamide with the 4-des(alkyl) core as in **11d** and **11e** resulted in the discovery of the most highly active Lck inhibitors from this series. In a direct comparison of **11d** with the corresponding 4-methylthiazole **7dd**, **11d** was found to have a 40-fold potency advantage. In a PBL proliferation assay using costimulation with anti-CD3 and anti-CD28, **11d** had an IC_{50} of 0.88 μM, while **11e** had an IC_{50} of 1.8 μM.

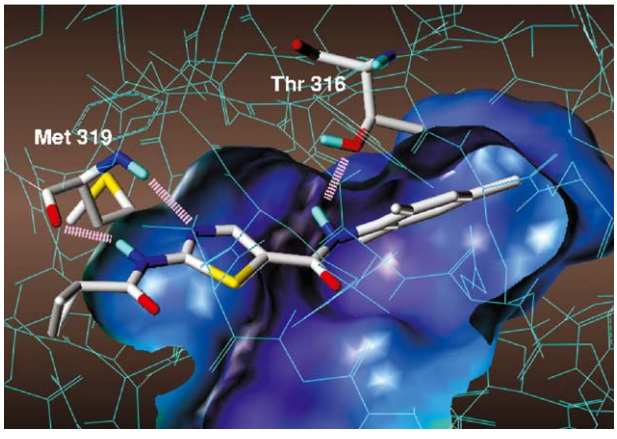
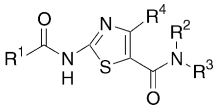


Figure 3. Proposed binding interactions of **11e** with Lck kinase domain.

Table 4 shows representative selectivity data for **11d** from a panel of potential kinase targets. Excellent selectivity was observed for the receptor tyrosine and serine/threonine kinases from this panel. However, significant selectivity was not observed for the other seven Src family members (Src, Fyn, and Hck data shown) nor for Jak3.

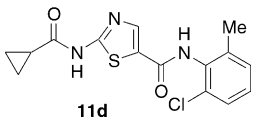
In summary, we identified a novel thiazole lead as a weak Lck inhibitor through high-throughput screening of our in-house compound collection. Through parallel synthesis and SAR studies, the Lck activity of this lead

Table 3. Thiazole 4-position SAR



Compd	R^1	R^2	R^3	R^4	hLck
					IC_{50} (μM)
7a	$(CH_3)_3CO$–	H	(2,4,6-Me_3)Ph	Me	1.5
8	$(CH_3)_3CO$–	H	(2,4,6-Me_3)Ph	Et	>30
9	$(CH_3)_3CO$–	H	(2,4,6-Me_3)Ph	Ph	>30
10	$(CH_3)_3CO$–	H	(2,4,6-Me_3)Ph	CF_3	>30
11a	$(CH_3)_3CO$–	H	(2,4,6-Me_3)Ph	H	>30
11b	2-Furyl–	H	(2-Cl, 6-Me)Ph	H	3.1
11c	Phenyl–	H	(2-Cl, 6-Me)Ph	H	0.89
11d	c-Pr–	H	(2-Cl, 6-Me)Ph	H	0.035
11e	c-Pr–	H	(2,4,6-Me_3)Ph	H	0.018
7dd	c-Pr–	H	(2-Cl, 6-Me)Ph	Me	1.4

Table 4. Kinase activity of **11d**



11d

Kinase	IC_{50} (μM)	Fold selectivity	Kinase	IC_{50} (μM)	Fold selectivity
Lck	0.035	—	Cdk2	>25	>700
Fyn	0.071	—	p38	25	700
Src	0.010	—	FGF	>25	>700
Hck	0.31	10	KDR	>25	>700
Jak3	0.044	—	HER1	10	300
FAK	>25	>7000	HER2	>25	>700

was optimized and a set of structural features associated with potent activity established. We identified **11d** as a potent Lck inhibitor having sub-micromolar activity in a PBL proliferation assay. When tested against a panel of potential kinase targets, **11d** was determined to be a potent pan-Src family/Jak3 kinase inhibitor. However, excellent selectivity was observed against kinases from several other kinase families. Further development of the SAR, and optimization of the cell and in vivo activity of this series will be reported in due course.

Acknowledgements

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