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Discovery and Initial SAR of Imidazoquinoxalines as Inhibitors of the Src-Family Kinase p56^{Lck}

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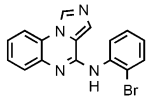
Abstract—We have identified a novel series of 1,5-imidazoquinoxalines as inhibitors of Lck with excellent potency (IC_{50}s < 5 nM) as well as good cellular activity against T-cell proliferation (IC_{50}s < 1 μM). Structure–activity studies demonstrate the requirement for the core heterocycle in addition to an optimal 2,6-disubstituted aniline group. © 2002 Published by Elsevier Science Ltd.

As a member of the Src-family of non-receptor tyrosine kinases, Lck is expressed predominantly in T cells and natural killer cells. Lck plays essential roles in both T cell development[1] and activation,[2] acting to initiate T cell antigen receptor (TCR) signaling that leads to cytokine production and T cell activation. Upon recognition of an antigen by the TCR, Lck phosphorylates the Immunoreceptor Tyrosine Activation Motif (ITAM) sequences in the invariant chains of T cell receptor, including those of the ζ-chain. Phosphorylation of the dual tyrosine residues of the ζ-chain ITAMs allows binding of the Syk family tyrosine kinase ZAP-70 via its tandem SH2 domains.[2c] Activation of ZAP-70 via its phosphorylation by Lck and the combined action of Lck, ZAP-70 and the related Src-family tyrosine kinase Fyn drive downstream signaling events that ultimately lead to T cell activation and proliferation. Inactivation of the Lck gene by genetic ablation or over expression of the dominant negative leads to early arrest of thymocyte development prior to expression of CD4, CD8, and the TCR.[1] In addition, loss of expression of Lck in Jurkat T cells results in the inability of these cells to respond to anti-TCR antibodies and the absence of Ca^{2+} mobilization required for T cell activation. Inhibition of Lck is considered potentially useful for the treatment of both chronic and acute T cell-mediated autoimmune and inflammatory disorders such as rheumatoid arthritis, multiple sclerosis, transplant rejection and delayed hypersensitivity (DTH) reactions. Several classes of Lck inhibitors, both of the catalytic and SH2 domains, have recently been reported.[3–11]



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IC_{50} (Lck) = 170 nM
IC_{50} (T-cell Prolif) = 17μM

Figure 1. Activity for imidazoquinoxaline **1**. Assay conditions described in ref 13.

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High throughput screening of our compound collection resulted in the identification of the 1,5-imidazoquinoxaline **1** (Fig. 1) as a relatively potent and selective Lck inhibitor. Although anilino-substituted quinoxalines are known inhibitors of tyrosine kinases,[12] fusion of an imidazo group to the quinoxaline ring represented a novel tyrosine kinase inhibitor motif. In addition, compound **1** displays modest potency against anti-CD3/anti-CD28 induced PBL proliferation[13b] and shows encouraging selectivity against non-Src family Tyr and Ser/Thr kinases.[13c] This report describes our preliminary efforts toward optimizing the enzymatic and cellular activity of this lead.

1,2-Imidazoquinoxaline **2**, the regioisomer of **1**, was first prepared, as a 1:1 mixture, by reaction of the precursor urea **3** with $POCl_3$ at reflux (Fig. 2). Compound **2** displays poor activity against Lck, indicating that the N-2 nitrogen of the imidazole ring plays an important role in enzyme binding, presumably as a hydrogen bond acceptor (vide infra).

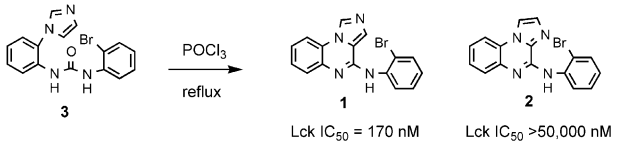
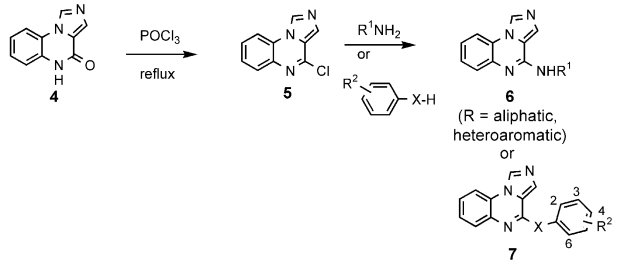
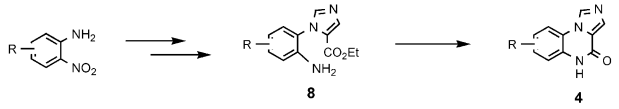


Figure 2. Preparation and activity of 1,2-imidazo regioisomer.

Having confirmed that the regioisomeric 1,5-imidazoquinoxaline is the more potent scaffold of the two, we next examined replacement of the bromoaniline moiety by other aromatic and non-aromatic amines. The requisite compounds were readily prepared by reaction of the 2-chloroimidazoquinoxaline intermediate **5** with the appropriate amines, anilines (where X = NH), phenols (where X = O) and thiophenols (where X = S) (Scheme 1). The intermediate **5** was obtained from reaction of imidazoquinoxalinone **4** with $POCl_3$. Precursor **4** in turn was prepared by methods recently reported from these labs.[14,15]



Scheme 1.



Scheme 2.

Table 1.

Compd	R^1 or R^2	X	Lck IC_{50} (nM)[13a]
1	2-Br	NH	170
6a	Cyclohexyl	na[a]	3170
6b	Cyclopropyl	na	3170
6c	*n*-Propyl	na	2680
6d	*n*-Hexyl	na	> 12,500
6e	Benzyl	na	> 12,500
6f	3-Cl-phenethyl	na	4390
6g	3-Pyridyl	na	820
6h	4-Pyridyl	na	> 12,500
7a	2-Br	O	3700
7b	2-Br	S	> 12,500
7c	2,6-di-Me	—	> 12,500
7d	2-Cl	N-Me	> 12,500
7e	2-Br	–NHCO—	60
7f	2-Me	–NHCO—	> 12,500
7g	2,6-di-Cl	–NHCO—	810

[a]Not applicable.

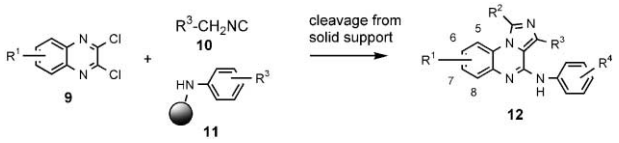
Alternatively, intermediate **4** could be obtained from 1-arylimidazole-5-carboxylates **8**[16] via a base-catalyzed intramolacular cyclization process (Scheme 2).

Approximately 160 compounds were prepared in this effort using parallel synthesis and led to the following SAR: (1) Replacement of the 2-bromoaniline of **1** with aliphatic and arylalkyl amines (**6a**–**f**) resulted in substantial losses in activity against Lck. (2) Both oxygen (**7a**) and sulfur (**7b**) linkers were significantly less potent than the amine linker. (3) Biaryl analogue (**7c**) and *N*-methyl analogue (**7d**) displayed poor activity suggesting that the aniline N-H is important for activity. Interestingly, while the amide **7e** displays similar potency to **1**, **7f** and **7g** are substantially less active. Finally, a quick survey of heterocyclic replacements for the aniline phenyl group reveals a significant difference in potency between nitrogen regioisomers (**6g** vs **6h**) (Table 1).

Modification of the substitution on the aniline phenyl ring led to a dramatic change in potency (Table 2). Removal of the 2-bromo-substituent of **1** causes a 5-fold loss in activity (**7h**). Exchange of 2-fluoro for 2-bromo (**7i**) results in a 2-fold loss of activity whereas replacing

Table 2.

Compd	R^2	X	Lck IC_{50} (nM)[13a]
1	2-Br	NH	170
7h	H	NH	890
7i	2-F	NH	390
7j	3-F	NH	1330
7k	2-Cl	NH	60
7l	2-OMe	NH	5520
7m	2-NO_2	NH	> 12,500
7n	4-Br	NH	> 12,500
7o	2-Cl, 4-Me	NH	240
7p	2-Cl, 4,6-di-Me	NH	30
7q	2,4,6-tri-Me	NH	40
7r	2,6-di-Me	NH	16
7s	2,6-di-Br	NH	50
7t	2,6-di-Cl	NH	9
7u	2,6-di-F	NH	360
7v	2-Cl, 6-Me	NH	9
7w	2,6-di-Et	NH	1690



Scheme 3.

2-bromo with 2-chloro (**7k**) led to a 3-fold increase in potency. However, replacement of the bromine with electron-donating (**7l**) or electron-withdrawing (**7m**) groups yielded compounds with >10-fold reduction in activity. As a general trend, *ortho* substitution is much more favored over *meta* (**7i** vs **7j**) or *para* (**1** vs **7n**). Most significantly, when both *ortho* positions of the aniline are substituted by small groups, the potency increased by more than an order of magnitude, as demonstrated by three 2,6-disubstituted analogues **7r**, **7t** and **7v**. The 2,6-dibromo substituted analogue (**7s**) displays a 3-fold reduction of potency compared to its dichloro counterpart (**7t**). Surprisingly, the difluoro analogue (**7u**) showed a 40-fold loss of activity, indicating the importance of steric bulk at both *ortho* positions. On the other hand, there also appears to be a size limit for such *ortho*-substitution. The 2,6-diethyl analogue (**7w**) is more than 100-fold less active than its dimethyl counterpart **7r**. When the substituent is small in size, 2,4,6-trisubstitution is tolerable, as evidenced by the activity of compounds **7p** and **7q**.

Both **7r** and **7v** display improved potency against anti-CD3/anti-CD28 induced PBL proliferation[13b] with IC_{50}'s of 3000 and 7000 nM, respectively. Since **7r** and **7s** represent the most potent aniline analogues prepared in this series, the 2-chloro-6-methyl and 2,6-dimethyl anilines were used in our subsequent SAR studies.

Next, we turned our attention to the substitution on the imidazole ring. Compounds **12a–k** were prepared by methods analogous to those described earlier[14,15] and by an automated synthetic approach using a solid-phase support[17] as outlined in Scheme 3.

Substitution at the 4-position (R^3) of the imidazole ring with a small alkyl group is tolerable (**7k** vs **12b**) whereas substitution with a larger phenyl residue (**12a**) led to a complete loss in potency. Similarly, potency remains unchanged when a small alkyl group was placed at the 2-position (R^2) of the imidazole (**1** vs **12c**). In addition, small polar groups are also tolerable at this position (**12e–g** vs **12h–k**). Finally, incorporation of small, electron-rich functional groups at the open positions of the fused phenyl ring[18] further enhances potency against the enzyme (**12l**, **12m** vs **7r**, **7s**), suggesting the potential of improvement in activity via substitution at this portion of the molecule. Follow-up SAR at the fused phenyl ring is the subject of a subsequent report (Table 3).

Our SAR study is consistent with the binding model which was developed based on the published coordinates of activated Lck kinase domain bound to ANP (phosphoaminophosponic acid-adenylate ester, a non-hydrolyzable ATP mimic).[19] As shown in Figure 3, we believe that the 2,6-disubstitution of the aniline of **1** enables the phenyl ring to adopt a preferred orientation wherein the ring is skewed out of plane with respect to the tricyclic system. This conformation permits the ring to occupy an angular hydrophobic pocket common to many tyrosine kinases. QSAR analyses of phenyl ring substitution patterns correlated small size, di-*ortho* substitution and electron-withdrawing properties to higher observed Lck affinities, underscoring the choice of 2-chloro-6-methyl substitution in the present series.[20] In addition, two critical hydrogen bonding interactions are thought to be involved: the nitrogen of the imidazole ring acting as an acceptor to backbone Met 319 N-H and the N-H of the aniline acting as a donor to the Thr 316 hydroxyl oxygen.

Table 3.

No.	R1	R2	R3	R4	IC_{50} (nM)[13a]
1	H	H	H	2-Br	170
7k	H	H	H	2-Cl	60
7r	H	H	H	2,6-di-Me	16
7s	H	H	H	2-Cl, 6-Me	9
12a	H	H	Ph	2-Br	>12,500
12b	H	H	Me	2-Cl	30
12c	H	Me	H	2-Br	180
12d	7,8-di-MeO	Me	H	2-Cl, 6-Me	7.4
12e	7,8-di-MeO	CH_2OH	H	2-Cl, 6-Me	6.2
12f	7,8-di-MeO	CHO	H	2-Cl, 6-Me	10
12g	7,8-di-MeO	CHN=OH	H	2-Cl, 6-Me	4.3
12h	7,8-di-MeO	CHOHMe	H	2-Cl, 6-Me	53
12i	7,8-di-MeO	CHOH*i*-Pr	H	2-Cl, 6-Me	238
12j	7,8-di-MeO	$(CH_2)_3OH$	H	2-Cl, 6-Me	69
12k	7,8-di-MeO	C(=O)NHEt	H	2-Cl, 6-Me	665
12l	7,8-di-MeO	H	H	2, 6-di-Me	2.4
12m	7,8-di-MeO	H	H	2-Cl, 6-Me	2

In summary, we have identified novel Lck inhibitors with a 1,5-imidazoquinoxaline template, as represented by **1**, using high throughput screening of our in-house compound collection. Optimization of **1** led to compound **12m** with excellent enzymatic activity against Lck (IC_{50} = 2 nM) and good potency blocking T cell proliferation[13b] (IC_{50} = 0.67 μM). Structure–activity studies demonstrate the requirement for the core heterocyclic scaffold in addition to an optimal 2,6-disubstituted aniline moiety. Further characterization of **12m** and related SAR will be reported in due course.

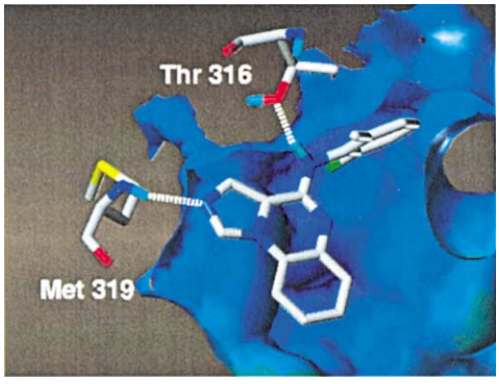


Figure 3. Inhibitor **1** modeled into the ATP-binding site of Lck illustrating hydrogen bond interactions between Thr 316 and Met 319 and the ligand. The ortho-disubstituted aniline occupies a deep hydrophobic pocket.

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13. (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 is incubated in kinase buffer (20 mM MOPS, pH7, 10 mM $MgCl_2$) in the presence of the test compound. The reaction was initiated by the 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 was stopped after 10 min by the addition of 10% trichloro-acetic 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 scintilla-tion counter. (b) *PBL Proliferation Assay*: A 96-well plate was coated with a 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 test compound plus anti-CD28 (E.3) antibody. After 3 days, the [^{3}H]-thymidine was added to the cells, and after further incubation, the cells were harvested and counted in a scintillation counter. (c) Preliminary study showed that compound **1** displays selectivity against other non-Src-family Tyr and Ser/Thr kinases (unpublished data).
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