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Synthesis and SAR of Novel Imidazoquinoxaline-Based Lck Inhibitors: Improvement of Cell Potency

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Abstract—A series of anilino(imidazoquinoxaline) analogues bearing solubilizing side chains at the 6- and 7-positions of the fused phenyl ring has been prepared and evaluated for inhibition against Lck enzyme and of T-cell proliferation. Significant improvement of the cellular activity was achieved over the initial lead, compound **2**.

The cytoplasmic, non-receptor Src-family tyrosine kinase Lck plays a crucial role in T-cell antigen receptor (TCR) phosphorylation, a process required for the activation of protein tyrosine kinase signal transduction cascades and its down-stream events.[1] Studies have shown that T-cells that lack Lck are severely impaired in TCR tyrosine phosphorylation and unable to be activated via the TCR.[2] Consequently, there has been a continuous effort to develop small molecule inhibitors against Lck for the treatment of T-cell mediated autoimmune and inflammatory diseases.

High-throughput screening of the BMS compound collection led to the discovery of **1**, a structurally novel inhibitor of Lck. Subsequent structure–activity relationship studies on the 1,5-imidazoquinoxaline core have led to the identification of compound **2**, a lead analogue in this series with excellent inhibitory activity against the isolated enzyme as well as in a T-cell proliferation assay[3] (Fig. 1).

In this paper, we describe our efforts in exploring modifications of the fused phenyl ring of the imidazoquinoxaline core. Our primary objectives were to further enhance the cellular activity and to improve the physical properties of this series of inhibitors.

Chemistry

Our preliminary investigation focused on evaluating various substituents on the phenyl ring of the imidazoquinoxaline core. As outlined in Scheme 1, two general approaches were taken to prepare the key intermediate, the imidazo[1,5-*a*]quinoxalin-4(5*H*)-one **4**, using the chemistry developed in this lab.[4,5] Reaction of symmetrical or unsymmetrical *ortho*-diaminobenzenes with glyoxylate followed by selective alkylation with PMB-Cl yielded the *N*-protected quinoxalin-2(1*H*)-one **3** as a major product. In the cases where the unsymmetrical diaminobenzenes were used, the desired regio-isomers were usually obtained by silica gel chromatography separation/purification. Subsequent reaction with Tos-MIC/NaH followed by deprotection of PMB group furnished desired imidazo-quinoxalinone **4** (**Route A**). Alternatively, treatment of the substituted *ortho*-fluoroanilines with carbonyl-imidazole dimer[5] in the presence of a base gave intermediate **5**. The intramolecular cyclization of **5** was accomplished under the basic conditions to generate **4** (**Route B**). Treatment of **4** with $POCl_3$

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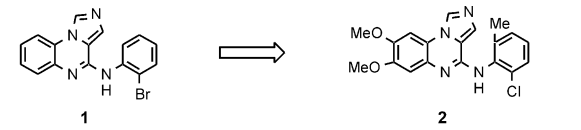


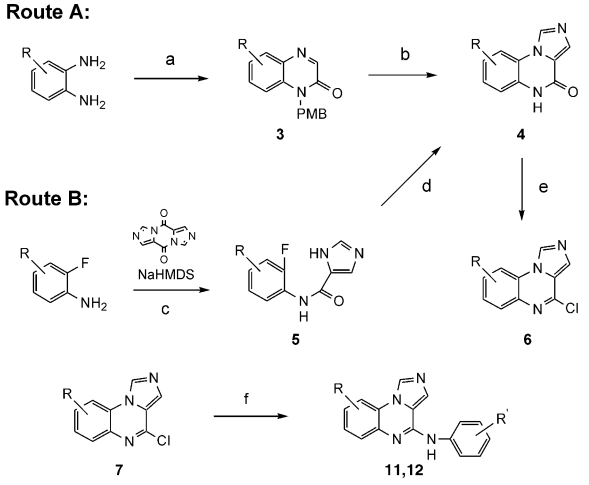
Figure 1. Activity for imidazoquinoxoline **1** and **2**.

generated the desired chloride **6**. Analogues **7a**–**v** were prepared from **6** by displacement of the chloride with the corresponding aniline in the presence of a base (Scheme 1).

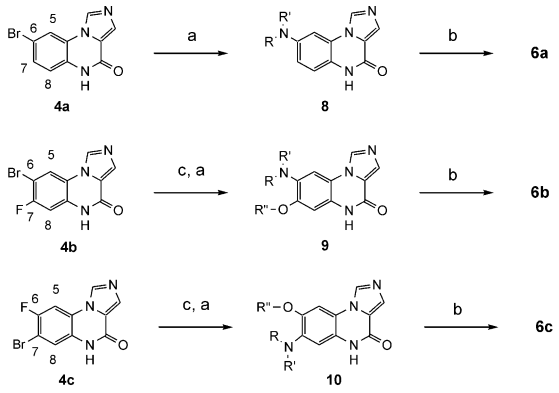
Utilizing the appropriately substituted intermediate **4a**–**c** allowed for introduction of various polar groups on the fused phenyl ring. For example, reaction of 6-bromo-imidazoquinoxalin-4(5*H*)-one **4a** with amines, in the presence of palladium (0) catalyst, afforded substituted anilines **8**. Further reaction of **8** with $POCl_3$ yielded the desired imidazoquinolinechloride **6a**. Similarly, the 6-bromo-7-fluoroimidazoquinoxalin-4(5*H*)-one **4b** and its regio-isomer **4c** were sequentially treated with an alkoxide, followed by a palladium (0)-catalyzed amination reaction affording **9** and **10**. Subsequent chlorination provided the desired intermediate **6b** and **6c** (Scheme 2). Finally, analogue **11a** was obtained from **2** via removal of methyl groups with BBr_3. Both **11b** and **11c** were obtained by bis-alkylation of **11a** with dibromomethane and/or dibromoethane in the presence of a base.

Results and Discussion

Introduction of side chains bearing polar and/or weakly basic amine-based functional groups into a bicyclo-heteroaryl scaffold is well documented for inhibitors[6] of protein tyrosine kinases. Such appendages often lead to a minimal change in intrinsic potency, provided that the side chains are properly positioned such that the polar



Scheme 1. PMB = *p*-MeO-benzyl: (a) ethyl glyocylate, toluene, reflux, then PMB–Cl, NaH, THF; (b) Tos-MIC, NaH, then TfOH, TFA, anisol; (c) see the above Scheme; (d) K_2CO_3, DMA or DBU, DMF; (e) $POCl_3$, reflux; (f) aniline, base, THF.



Scheme 2. (a) RR'NH, $Pd(dba)_2$, BINAP, NaO*t*-Bu, Tol; (b) $POCl_3$, reflux; (c) R″-OH, NaH, DMSO.

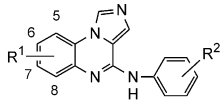
functionality is pointed toward solvent. Beneficial effects are often noted, such as enhancement of cellular activity as a result of improved cell permeability, as well as improvement of other physical chemical properties, for example aqueous solubility. Our objective was to examine a number of analogues, with a variety of functional residues appended to the fused phenyl moiety, to establish the effects on inhibition of Lck and T-cell proliferation in human PBLs.

Using a parallel synthesis approach, a large number of differentially substituted phenyl analogues of **2** were prepared and evaluated with the initial goal of identifying the most appropriate site(s) for further elaboration. Table 1 lists the structures and in vitro biological activities of selected anilino(imidazoquinoxaline) analogues from this study.

Replacement of the 2-chloro-6-methyl aniline of **2** with 2,6-di-methyl aniline resulted in no change in enzyme activity, but led to a modest loss in cellular activity (**2** vs **2a**). The di-phenolic analogue **11a** was significantly less potent (loss of >13-fold in Lck inhibition). Both the 6,7-methylenedioxy (**11b**) and the 6,7-ethylenedioxy analogues (**11c**) displayed decreased potency (2- to 5-fold) against Lck as well as reduced cellular activity (3-fold) in comparison to **2**. Systematic exploration of substitution with a *mono*-methoxy moiety on the fused phenyl revealed that substitution at 5-, 6-, and 7- positions was allowed (**11d**–**f** and **11h**), although increasing the steric bulk at the 5-position appears detrimental to the potency (**11h** vs **11i**). However, substitution at the 8-position was not tolerated (**11g**). Introduction of an electron-withdrawing group at the 5-, 6-, or 7-positions (**11j**–**p** and **11s**–**v**) also resulted in a significant loss of Lck inhibitory activity. As a general trend, substitution at the 5-, 6- and 7-positions with an electron-donating group is favored for the binding affinity (**2**, **2a**, **11a**–**f**, **11q**–**r**) whereas no substitution is favored at the 8-position. While the initial study suggested that the 6- and 7-positions might be the suitable sites for further substitution, none of the modifications led to any improvement in cellular activity.

We next focused our attention on introducing polar moieties to 6- and 7-positions, with the objective to

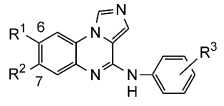
Table 1. Inhibition of Lck (enzyme) and T-cell proliferation (human PBLs)



11a-v

No.	Syn. route	R^1	R^2	Lck IC_{50} (nM)[3]	T-cell prolif. IC_{50} (nM)[3]
2	**A**	6,7-di-OMe	2-Cl, 6-Me	2	670
2a	**A**	6,7-di-OMe	2,6-di-Me	2.4	1100
11a	**A**	6,7-di-OH	2-Cl, 6-Me	4	>9000
11b	**A**	6,7-OCH_2O	2-Cl, 6-Me	4	2000
11c	**A**	6,7-$O(CH_2)_2O$	2-Cl, 6-Me	10	2200
11d	**A**	6-OMe	2-Cl, 6-Me	3	1400
11f	**A**	7-OMe	2-Cl, 6-Me	8.7	2600
11g	**A**	8-OMe	2-Cl, 6-Me	280	—
11h	**A**	5-OMe	2-Cl, 6-Me	9.4	2300
11i	**A**	5-BnO	2-Cl, 6-Me	240	—
11j	**A**	5-NO_2	2,6-di-Me	100	—
11k	**A**	5-NH_2	2,6-di-Me	70	—
11l	**B**	6-F	2-Cl, 6-Me	26	—
11m	**B**	6-Br	2-Cl, 6-Me	15	—
11n	**A**	6-CO_2Me	2-Cl, 6-Me	26	—
11o	**A**	6-NO_2	2, 6-di-Cl	24	—
11p	**A**	6-CN	2-Cl, 6-Me	100	—
11q	**A**	6-NH_2	2-Cl, 6-Me	7	1600
11r	**A**	6-NHAc	2-Cl, 6-Me	3	1400
11s	**B**	7-Br	2-Cl, 6-Me	14	—
11t	**A**	7-NH_2	2-Cl, 6-Me	21	—
11u	**A**	7-NHAc	2-Cl, 6-Me	11	—
11v	**A**	7-$CONH_2$	2-Cl, 6-Me	30	—

Table 2. Inhibition of Lck (enzyme) and T-cell proliferation (human PBLs)



12a-z

No.	Syn. Route	R^1	R^2	R^3	Lck enzyme[b] IC_{50} (nM)[3]	T-cell prolif.[c] IC_{50} (nM)[3]
12a	**B**[a]	NMe_2	H	2-Cl, 6-Me	5	2000
12b	**B**[a]	NEt_2	H	2-Cl, 6-Me	2	2600
12c	**B**[a]	NHEt	H	2-Cl, 6-Me	10	2200
12d	**B**[a]	$NHCH_2CH_2NMe_2$	H	2-Cl, 6-Me	6	880
12e	**B**[a]	$NHCH_2CH_2$–morpholine	H	2-Cl, 6-Me	7	1300
12f	**B**[a]	$NHCH_2CH_2CH_2$–morpholine	H	2-Cl, 6-Me	3	760
12g	**B**[a]	Morpholine	H	2-Cl, 6-Me	4	780
12h	**B**[a]	Piperazine	H	2-Cl, 6-Me	3	380
12i	**B**[a]	*N*-Me-piperazine	H	2-Cl, 6-Me	1	240
12j	**B**[a]	*N*-Et piperazine	H	2-Cl, 6-Me	2	270
12k	**B**[a]	*N*-Formyl piperazine	H	2-Cl, 6-Me	9	530
12l	**B**[a]	3,5-di-Me-piperazine	H	2-Cl, 6-Me	3	340
12m	**B**[a]	*N*-Me-homopiperazine	H	2-Cl, 6-Me	9	540
12n	**B**[a]	H	NEt_2	2-Cl, 6-Me	9	1700
12o	**B**[a]	H	$NHCH_2CH_2NMe_2$	2-Cl, 6-Me	10	520
12p	**B**[a]	H	$NHCH_2CH_2CH_2NMe_2$	2-Cl, 6-Me	9	480
12q	**B**[a]	H	Morpholine	2-Cl, 6-Me	6	1000
12r	**B**[a]	H	Piperazine	2-Cl, 6-Me	11	1000
12s	**B**[a]	H	*N*-Me-piperazine	2-Cl, 6-Me	8	720
12t	**B**[a]	H	3,5-di-Me-piperazine	2-Cl, 6-Me	13	570
12u	**B**[a]	OCH_2CH_2–morpholine	OMe	2-Cl, 6-Me	36	—
12v	**B**[a]	3,5-di-Me-piperazine	OMe	2-Cl, 6-Me	18	760
12w	**B**[a]	OMe	OCH_2CH_2–morpholine	2-Cl, 6-Me	2.4	470
12x	**B**[a]	OMe	$NHCH_2CH_2$–morpholine	2-Cl, 6-Me	5.4	280
12y	**B**[a]	OMe	$NHCH_2CH_2NMe_2$	2-Cl, 6-Me	1.7	190
12z	**B**[a]	OMe	$NHCH_2CH_2NMe_2$	2-Cl, 6-F	5	240

[a]Compounds **6a–c** (Scheme 2) were used as the intermediates.
[b]Concentration to inhibit by 50% the phosphorylation of an exogenous substrate by human Lck enzyme.
[c]Concentration to inhibit by 50% T-cell proliferation induced by anti-CD3/anti-CD28 co-stimulation in PBLs. CsA gave an IC_{50} of 115 nM in this assay.

improve in vitro potency against T-cell proliferation. Table 2 illustrates the results from some of the representative analogues prepared in this series.

Substitution with simple amines at either the 6- or 7-position initially proved to be ineffective in achieving this goal (**11q**, **12a**–**c**, **11t**, and **12n**). Almost all analogues displayed significantly reduced T-cell activity, even though they all showed comparable inhibitory activity against Lck as **2**. Replacement of the simple amine of **11q** with a side chain containing a basic amine moiety resulted in a modest improvement of cell activity (**12d**). Compounds **12d**, **12f**, and **12g** are essentially equally potent to **2** when examined for inhibition of T-cell proliferation. *A significant improvement in cellular activity resulted from substitution at the 6-position with the bulky secondary amines*. All the piperazine substituted analogues displayed excellent cellular potency (**12h**–**l**), with a nearly 3-fold improvement for **12i** over **2**.

Substitution with amines at the 7-position displayed a similar trend as that observed for the 6-position series, although the magnitude of the improvement was less significant. Overall, these analogues appear to be less potent in the T-cell proliferation assay than their 6-substituted counterparts (**12g** vs **12q**, **12h** vs **12r**, **12i** vs **12s**, and **12l** vs **12t**).

We also explored the effect of incorporation of a second small, electron-donating group into the fused phenyl ring of the imidazoquinoxaline core. Interestingly, the 6-amino-7-methoxy di-substituted analogues were significantly less potent (6-fold) than their corresponding mono-amino-substituted analogues (**12u** vs **12d** and **12v** vs **12l**), whereas their regio-isomers, the 6-methoxy-7-amino analogues (**12w**–**z**) showed similar or better enzyme potency than the mono-amino substituted counterparts. Most significantly, compound **12y** displayed potent cellular activity (IC_{50} <200 nM!) in the T-cell proliferation assay. Compound **13**, prepared according to a similar procedures outlined in Scheme 2, represents the most cell potent analogue from this series, as illustrated in Figure 2.

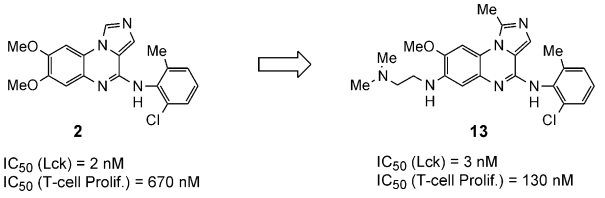


Figure 2. Activity for imidazoquinoxaline **2** and **12**.

In summary, we have described a promising series of anilinoimidazoquinoxalines, represented by **12y** and **13**, with excellent enzyme activity (IC_{50} <5 nM). In particular, significant improvement of activity against T-cell proliferation (IC_{50} <200 nM) was achieved from the initial lead, compound **2**, by incorporation of polar and weakly basic amine-bearing side chains into the 7-position of the fused phenyl ring of the imidazo-quinoxaline core. Full characterization of these compounds in vivo in pharmacokinetic and pharmacodynamic models will be disclosed in due course.

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