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# Discovery of pyrimidine benzimidazoles as Lck inhibitors: Part I

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ABSTRACT

A series of 4-amino-6-benzimidazole-pyrimidines was designed to target lymphocyte-specific tyrosine kinase (Lck), a member of the Src kinase family. Highly efficient parallel syntheses were devised to prepare analogues for SAR studies. A number of these 4-amino-6-benzimidazole-pyrimidines exhibited single-digit nanomolar $IC_{50}$s against Lck in biochemical and cellular assays. These 4-amino-6-benzimidazole-pyrimidines represent a new class of tyrosine kinase inhibitors.



Lymphocyte-specific kinase (Lck) is a non-receptor protein tyrosine kinase in the Src-family with restricted expression in T cells and natural killer (NK) cells.[1] Lck plays a critical role in the initial steps of T-cell receptor (TCR) signaling.[2] Activation of TCR signaling by Lck triggers a cascade of downstream signaling pathways, leading to the production of cytokines such as interleukin-2 (IL-2) and interferon-γ.[3,4] Because of the restricted expression pattern of Lck, selective inhibitors of Lck would be expected to exhibit improved safety profiles over currently available immunosuppressive agents, which possess non-lymphocyte-related toxicities. Therefore, there have been extensive efforts to develop selective Lck inhibitors for the treatment of autoimmune disease and organ transplant rejection.[5,6]

A number of potent Lck inhibitors have been reported.[7] In this paper, we discuss the design, synthesis and preliminary structure–activity relationships (SAR) of a novel class of potent Lck inhibitors based on 4-amino-6-benzimidazole-pyrimidines.

The design of this new class of Lck inhibitors was based on a dihydropyrimido [4,5-*d*] pyrimidinone series of kinase inhibitors originally reported by Hoffmann-La Roche[8] (Fig. 1). Molecular modeling studies on the binding mode of these dihydropyrimido [4,5-*d*] pyrimidinones to Lck guided the selection of some important features for our design (Fig. 2). Compound **A** binds to the ATP binding site of Lck. The N3 and adjacent NH of compound **A** form bidentate hydrogen bonds to the backbone of Met319 in the Lck hinge region. Phenyl ring II is positioned in the kinase specificity pocket.[9] Also, based on our research result of this dihydropyrimido [4,5-*d*] pyrimidinone scaffold,[10] the pyrimidine N1 of compound **A** does not form any hydrogen bonds and can be replaced with a carbon without loss of protein kinase inhibitory activity (data not shown). Based on this structural information, we designed compound **B**, where the central ring is replaced with a pseudo-ring constrained by an intramolecular hydrogen bond between the pyrimidine N and the benzimidazole NH group.[11] The 4,6-pyrimidine substitution pattern allows retention of the bidentate hinge hydrogen bonds. This intramolecular hydrogen bond is expected to position the phenyl ring II of compound **B** into the kinase specificity pocket, as in compound **A**. This design strategy was expected to allow scaffold B to mimic the binding conformation of

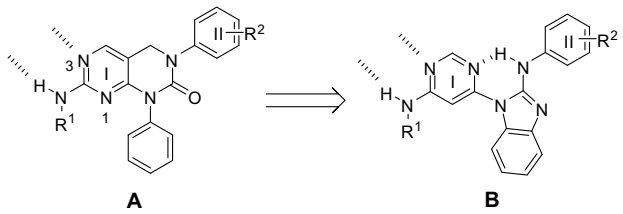


**Figure 1.** Rational design of pyrimidine benzimidazoles.

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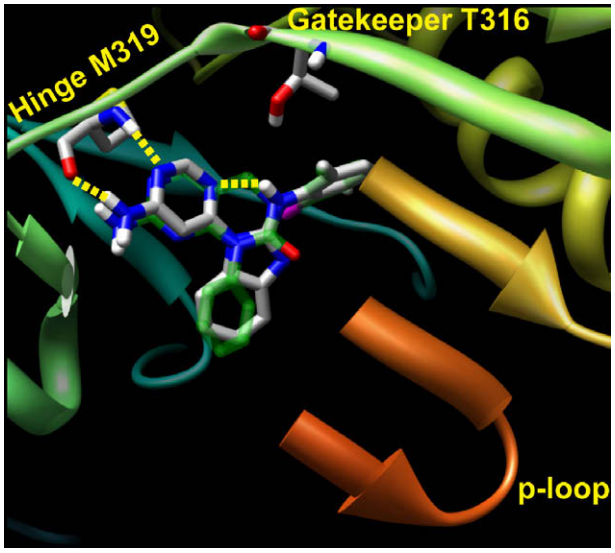
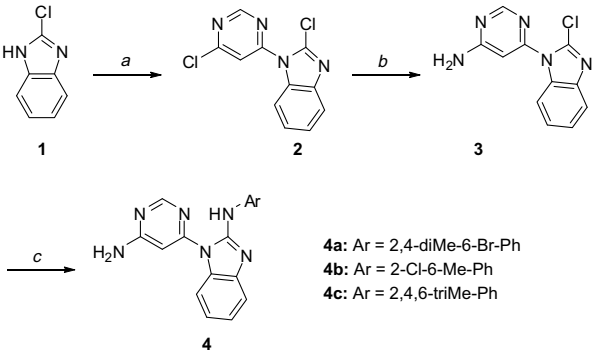



**Figure 2.** Binding mode of compound **4b** to Lck based on modeling studies. This model was constructed by docking[12] compound **4b** into crystal structure of Lck [PDB ID code: 1QPE] [13] based on the binding mode of structurally similar compounds co-crystallized with c-Abl[14] and c-Kit.[15] Compound **B** overlaps with **4b** very well and is shown as green and blue.

scaffold A but perhaps might allow for subtle conformational changes that could influence kinase specificity and potency.

To simplify our exploration, we chose $R^1$ = H, and compounds **4a–c** were synthesized according to Scheme 1. First, coupling of 4,6-dichloropyrimidine and 2-chlorobenzimidazole (**1**) in the presence of NaH in DMF gave intermediate **2**. Selective displacement of the 4-Cl on the pyrimidine ring of **2** was readily achieved by reaction with excess ammonia in isopropanol at 50 °C to give aminopyrimidine **3** in excellent yield. The 2-Cl on the benzimidazole ring of **3** was rather inert to displacement by aniline under basic conditions, even under Pd-catalyzed conditions. However, the reaction was readily accomplished under acid–catalyzed conditions, giving rise to analogues **4a–4c**.

All analogues were tested for their ability to inhibit Lck enzyme activity using an Lck Lance assay.[16] Compounds that potently inhibited Lck in the enzymatic assay were then tested to determine their cellular activity using a cell proliferation assay in a TEL-Lck-transformed Ba/F3 cell line.[17] The TEL-Lck fusion construct includes the catalytic domain of Lck and the TEL oligomerization domain. TEL-induced oligomerization of TEL-Lck resulted in the




**Scheme 1.** Synthesis of compounds **4a–c**. Reagents and conditions: (a). 4,6-dichloropyrimidine (2.5 equiv), NaH (1.5 equiv), DMF, 0–25 °C, 12 h, 56%; (b). $NH_3$ (excess), isopropanol, 50 °C, 12 h, 92%; (c). aniline (1.5 equiv), $CH_3SO_3H$ (2.0 equiv), *N*,*N*-dimethylimidazolinone, 90 °C, 2 h, 65–85%.

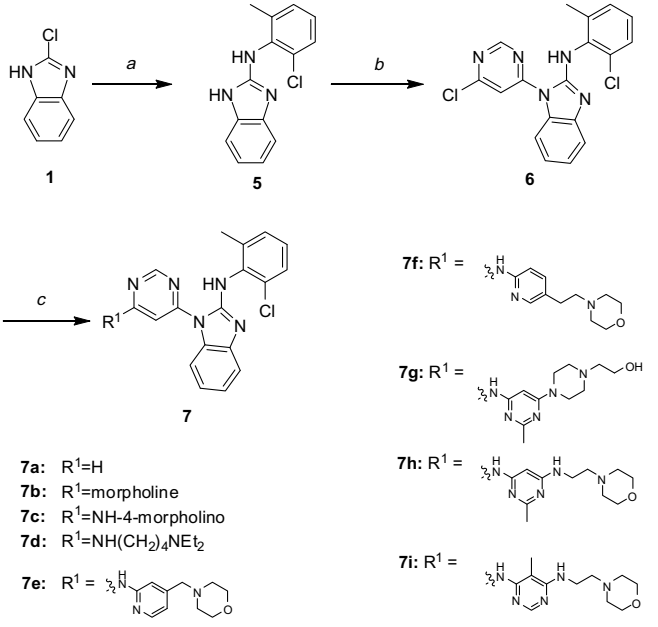
**Table 1**
$IC_{50}$ (μM) for inhibition of Lck kinase activity for **4a–c**

| Compound | Lck Lance | Ba/F3/Tel-Lck |
|---|---|---|
| **4a** | 1.355 | 1.167 |
| **4b** | 0.514 | 0.438 |
| **4c** | 1.785 | 0.583 |

constitutive activation of its tyrosine kinase activity and conferred cytokine-independent proliferation to the interleukin-3-dependent Ba/F3 hematopoietic cell line.

Sixteen anilines with different substitution patterns were chosen to react with compound **3**. A selection of the compounds that inhibited Lck kinase activity as measured using the Lck enzyme assay and Lck-dependent proliferation assay are shown in Table 1. Most of the other compounds exhibited very weak or no Lck inhibitory activity and are not listed here. Analysis of the SAR revealed that a 2,6-substitution pattern on aniline ring is important for Lck inhibition. Although its Lck inhibitory activity is moderate, compound **4b** was the most promising compound to emerge from this initial set of compounds and was chosen for further optimization.

To optimize lead compound **4b**, we further explored the SAR on $R^1$. A series of analogues **7a–i** were synthesized following Scheme 2. Briefly, reacting 2-chlorobenzimidazole with 2-chloro-6-methylaniline under acid-catalyzed conditions gave compound **5** in excellent yield. Compound **5** was then coupled to 4,6-dichloropyrimidine in the presence of NaH to provide compound **6**. It is interesting to note that use of DIEA as a base in this step led exclusively to the other regioisomer as a result of the *exo* aniline NH of **5** attacking the chlorine on the pyrimidine. Compound **7a** was obtained by hydrogenation on Pd/C, and compounds **7b–i** were obtained by direct amination (**7b–7d**) with various amines or Pd-catalyzed coupling (**7e–7i**) with various heteroaromatic amines.




**Scheme 2.** Synthesis of compounds **6**–**7i**. Reagents and conditions: (a) 2-chloro-6-methylaniline (1.5 equiv), $CH_3SO_3H$ (2.0 equiv), *N*,*N*-dimethylimidazolinone, 90 °C, 2 h, 75%; (b) 4,6-dichloropyrimidine (2.5 equiv), NaH (1.5 equiv), DMF, 0–80 °C, 1 h, 80%; (c) i—For compound **7a**, $H_2$/Pd/C, 65%; ii—For compounds **7b–d**, amine (3.0 equiv), isopropanol, 50 °C, 1 h, 80–95%; iii—For compounds **7e–i**, aniline (1.2 equiv), $Pd_2dba_3$ (0.02 equiv), Xantphos (0.04 equiv), $K_3PO_4$ (2.0 equiv), dioxane, 90 °C, 12 h, 60–85%.

**Table 2**
$IC_{50}$ (μM) for inhibition of Lck kinase activity for **6-7i**

| Compound | Lck Lance | Ba/F3/Tel-Lck |
|---|---|---|
| **6** | >2.5 | 5.704 |
| **7a** | 2.09 | N/A |
| **7b** | >2.5 | >10 |
| **7c** | >2.5 | >10 |
| **7d** | 0.789 | 5.036 |
| **7e** | 0.014 | 0.017 |
| **7f** | 0.031 | 0.034 |
| **7g** | 0.025 | 0.007 |
| **7h** | 0.005 | 0.013 |
| **7i** | 0.119 | 0.359 |

**Table 3**
Kinase selectivity profiling of compound **7f** and **7g** ($IC_{50}$ μM)

| Compound | **7f** | **7g** |
|---|---|---|
| Lck Lance | 0.031 | 0.025 |
| Ba/F3/Tel-Lck | 0.034 | 0.007 |
| Ba/F3/Tel-Lyn | 0.215 | 0.03 |
| Ba/F3/Tel-Src | 0.147 | 0.005 |
| Ba/F3/Tel-KDR | 1.012 | 0.356 |
| Ba/F3/Tel-InsR | 5.165 | 2.345 |

The inhibition data for compounds **6–7i** are shown in Table 2. It is not surprising to see that compounds **6, 7a** and **7b** all lost potency compared with compound **4b**, because they all lack the hydrogen-bond-donating NH group required for strong Lck hinge binding according to our proposed binding mode. It is also interesting to note that compound **6** is less potent than compound **7a**. It can be rationalized that the electron withdrawing effect of –Cl makes its adjacent pyrimidine nitrogen less basic and therefore weakens the hydrogen bonding of that nitrogen with the Lck hinge region.

From the perspective of electronic effects, the amino group in **7c** and **7d** should make N1 more basic than that in **4b**. Both **7c** and **7d** are expected to form stronger hydrogen bonds and therefore have better inhibitory activity on Lck. However, compounds **7c** and **7d** both have much weaker potency compared to compound **4b**. From the binding mode, the substitution on the amino group of the pyrimidine is exposed to solvent and should be well-tolerated. To rationalize these data, we performed a conformational analysis of these compounds (Fig. 3). In order for compound **7c** and **7d** to form bidentate hydrogen bonds to the Lck hinge region, they must assume conformation **I** as shown in Figure 3. However, a relatively larger R group would introduce a steric clash with $H_a$, which would make conformation **II** more favorable. In conformation **II,** $H_b$ cannot form hydrogen bond to the hinge, and the R-group would interfere with the hydrogen bond between N1 and the hinge. This also explains why compounds **7b** and **7c** have even weaker inhibition compared to compound **7a**.

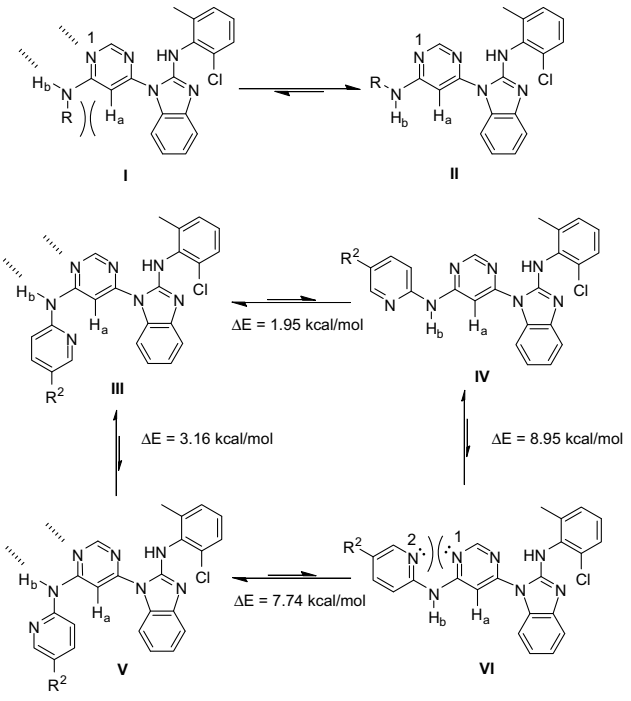



**Figure 3.** Conformation analysis of compounds **8a–i**.

Based on this conformational analysis, we designed compounds **7e** and **7f.** Computational analysis shows conformer **III** has lower energy than the other three conformers, which are also energy minima. Conformer **V** is significantly lower in energy ($\Delta E$ = 7.74 kcal/mol) than conformer **VI**. These energy favorable conformers **III** and **V** are ideally suited for hydrogen bonding to the hinge region.

The Lck inhibitory activity of compounds **7e** and **7f** confirmed our conformational analysis. Both compounds are very potent in both enzyme and cellular assays, and the enzyme data correlate very well with the cellular data for both compounds. Compound **7e** is about twice as potent as compound **7f**, suggesting that the solubility-enhancing morpholine group is better positioned *meta* relative to the NH group.

With this observation in mind, we further synthesized compounds **7g–i**, all of which have the water solubility-enhancing group bearing a tertiary nitrogen *meta* to the NH. Also, the pyridine ring of these compounds was changed to a pyrimidine ring, a similar modification to what can be found in the Src-family kinase inhibitor dasatinib. Both compounds **7g** and **7h** exhibit biochemical and cellular potency comparable to the most potent Lck inhibitors reported in the literature. Compound **7i** is 25-fold less potent than compound **7h**. This is probably due to the fact that the methyl group could have a steric clash with the hinge region of Lck.

The kinase selectivity profiles of compounds **7f** and **7g** are shown in Table 3. Although both compounds are quite potent inhibitors of other Src-family kinases such as Lyn and Src, they exhibited good selectivity over non-Src-family kinases KDR and InsR. Compound **7f** also showed moderate selectivity over the Src-family kinases Lyn and Src.

In summary, a promising new structural class of potent Lck inhibitors was discovered through rational design. Synthesis of these structures proceeds through an efficient scheme that is amenable to rapid analogue synthesis using widely available diversity elements. These Lck inhibitors represent a new class of tyrosine kinase inhibitors that are promising candidates for further optimization.

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16. (a) Hemmila, I. *J. Biomol. Screen.* **1999**, *4*, 303; b In a typical assay, 15 μL of assay buffer (50 mM Tris/HCl, pH 7.5, 0.05% Tween-20, 10 μM ATP, 20 mM $MnCl_2$, 20 μM $NaVO_3$, 2 mM DTT) containing 1 μM biotin labeled Src-family peptide substrate (biotin-lys-val-glu-lys-ile-gly-glu-gly-thr-tyr) were added to each well of a black 384-well plate. Fifty nanoliters of compound were added, followed by 5 μL of 40 ng/mL Lck enzyme (Upstate Biotech) in 50 mM Tris/HCl, pH 7.5, 0.05% Tween-20, 1% BSA, and the mixture was incubated at room temperature for 90 min. Ten microliters of 22.5 mM EDTA were added to stop the reaction followed by 45 μL of detection buffer [20 mM Tris/HCl, pH 7.5, 150 mM NaCl, 0.5% Tween-20, 0.1% BSA, 0.6 nM Lance Europium-labeled anti-phosphotyrosine antibody, 220 nM Allophycocyanin-Streptavidin conjugate (Perkin-Elmer, Waltham MA)]. The plates were incubated for 1 h in the dark then read on an Analyst (Molecular Devices, Sunnyvale CA) or ViewLux (Perkin-Elmer, Waltham MA) plate reader using the HTRF method.
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