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The development of 2-benzimidazole substituted pyrimidine based inhibitors of lymphocyte specific kinase (Lck)

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Abstract—This communication details the synthesis, biological activity, and binding mode of a novel class of 2-benzimidazole substituted pyrimidines. The most potent analogs disclosed showed low nanomolar activity for the inhibition of Lck kinase and a representative analog was co-crystallized with Hck (a structurally related member of the Src family kinases).

Lck is a 56-kD Src family protein tyrosine kinase (PTK) that plays a critical role in the development and activation of T cells including T-cell antigen receptor (TCR) phosphorylation (an event necessary for signal transduction in the T cell signaling cascade of the T-cell receptor).[1a,1b] Activation of this cascade ultimately results in the production of cytokines such as interleukin-2 (IL-2) and IFNγ.[1b,1c,1d] The production of these cytokines results in further activation and proliferation of T lymphocytes to generate an immune response. Unlike the widespread expression of some other Src family PTKs, Lck expression is restricted to T-cells and natural killer (NK) cells.[1d] As such the inhibition of Lck has been proposed as a potential treatment for a number of autoimmune diseases where T-cells are thought to play an important role such as rheumatoid arthritis (RA), inflammatory bowel disease (IBD), psoriasis, systemic lupus erythematosus (SLE), and organ graft rejection.[1e]

Screening efforts in our laboratories identified 4-benzo[*d*]isoxazole compounds **1a**–**b** as moderate Lck inhibitors (Fig. 1). Initial work directed at improving

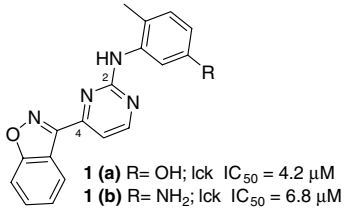

Figure 1. Initial benzo[*d*]isoxazole containing lead molecule.

the potency of these lead compounds led to the development of a facile SAR strategy incorporating benzimidazole substituted pyrimidines. This communication details the synthesis, biological activity, and binding mode of a novel class of 2,4,6-trisubstituted pyrimidine derivatives based on the initial lead benzo[*d*]isoxazole **1**. The binding mode of these trisubstituted pyrimidine inhibitors was also determined from X-ray co-crystallography experiments in the related hematopoietic cell kinase (Hck), a member of the Src family kinases.[1a,b]

The synthesis of the lead 2,4-disubstituted pyrimidines (**1a**–**b**) is outlined in Scheme 1. 4-Iodo-2-methylthiopyrimidine[2a] (**2**) was treated with isopropyl magnesium chloride followed by addition of 2-fluoro-benzaldehyde to give alcohol **3**.[2b,2c] Oxidation of this material with MnO_2 afforded the corresponding ketone which was condensed with hydroxyl amine resulting in oxime **4**.

Keywords: Kinase; Lck; Hck; Lymphocyte specific kinase; Hematopoietic cell kinase; Src family kinase; T cell.
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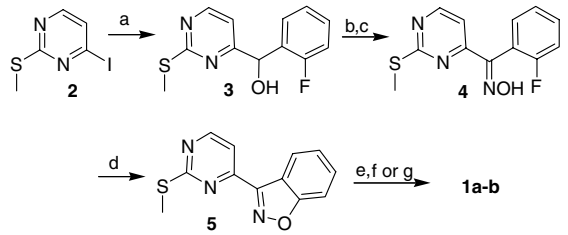
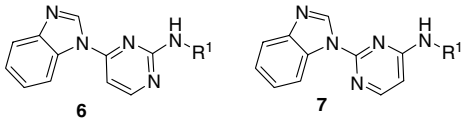


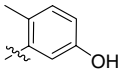
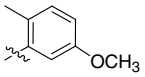
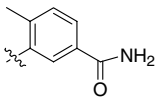
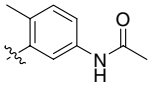
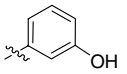
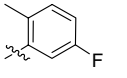
Scheme 1. Preparation of compounds **1a**–**b**. Reagents and conditions: (a) isopropyl magnesium chloride 2 M, THF −40 °C; then 2-fluorobenzaldehyde, 34%; (b) MnO_2, DCM, 24 h, 97%; (c) hydroxylamine·HCl, pyridine, 95 °C, 3 h; (d) NaH, DMF 165 °C, 0.5 h, 54% (2 steps); (e) *m*-CPBA, DCM, 0 °C, 0.25 h; (f) 3-amino-4-methylphenol, CH_3CN, 155 °C, microwave, 15% (2 steps **1a**); (g) 4-methylbenzene-1,3-diamine, CH_3CN, 150 °C, microwave, 0.5 h, HPLC separation of isomers, 1% (2 steps **1b**).

Treatment of this crude material with NaH followed by heat afforded intermediate **5**.[3] Oxidation of the thiogroup on compound **5** with Oxone® and displacement of the resultant sulfone/sulfoxide mixture generated the final products **1a**–**b**.[4]

To more quickly expand the SAR of pyrimidines **1** and to overcome synthetic difficulties with this scaffold, a benzimidazole group was substituted for the 4-benzo[*d*]isoxazole moiety. The resulting phenol **6a** (Table 1) proved to be a significantly more potent inhibitor (Lck IC_{50} = 193 nM)[5a] compared to **1a**. Interestingly, the corresponding methyl ether **6b** and amides **6c**–**d** displayed greatly attenuated activity. Pyrimidine **6e** which did not contain the 4-methyl group on the C2 anilino substituent (compare **6a** vs **6e**) was devoid of activity.

Table 1. IC_{50} values for derivatives **6a**–**m**, **7a**–**c**



Compound	R^1	Lck IC_{50}[a] (nM)
6a		193
7a		24
6b		>10,000
7b		7700
6c		5664
6d		8640
6e		>10,000
7c		>10,000

[a] IC_{50}'s were determined with a commercial Proflour assay (Promega corp., Cat. #1271).

Our attention subsequently focused on understanding the role of the pyrimidine ring nitrogen atoms (N1 and N3) in the potency observed. Regioisomeric analogs **7a**–**c** were synthesized using modified literature conditions and the results are presented in Table 1.[6]

Derivative **7a** which transposed the substituents at C2 and C4 relative to original compound **6a** displayed greater potency (Lck IC_{50} = 24); however, the C4 C6 isomerically substituted compound (**8**, Fig. 2) was devoid of any Lck activity.[7]

With such a potent lead molecule (**7a**) we again attempted to introduce alternatively functionalized anilines at C4. However both the 5-methoxy (**7b**) and 5-fluoro aniline (**7c**) derivatives showed greatly attenuated activity (Table 1). The presence of both a 4-methyl and phenolic hydroxyl on the C2 anilino substituent appeared crucial for good activity.

Our efforts were next directed at adding functionality to improve the poor aqueous solubility (4 μg/ml) of scaffold **7a**.[8] A series of 2,4,6-trisubstituted pyrimidines were synthesized which maintained both the C2 and C4 groups found on **7a** while introducing various basic amine substituents to the C6 position of the pyrimidine core (Tables 2 and 3). We reasoned that basic amine substituents could be tolerated at position C6 after examining the active site of Lck in complex with inhibitors disclosed in the literature.[9] These groups may impart greater aqueous solubility and added potency through interactions with proximal acidic residues.

The synthesis of these 2,4,6-trisubstituted pyrimidines is outlined in Scheme 2. 4,6-Dichloro-2-methylsulfanyl-pyrimidine and 5-methoxy-2-methyl-phenylamine were heated at 140 °C to afford intermediate **10**. Oxidation of the thiol group in **10** with Oxone® followed by displacement of the resultant sulfone/sulfoxide mixture with sodium benzimidazolate and the subsequent phenol deprotection gave **11**. The 6-chloro group on this pyrimidine was then displaced with various amines and sodium alkoxides to give **12a**–**p**. Analogs **12q**–**r** resulted from Suzuki–Miyaura coupling of the corresponding vinyl heterocycle and **11**.[10]

The initially synthesized compounds **12a**–**e** containing simple substituents at the C6 position were somewhat

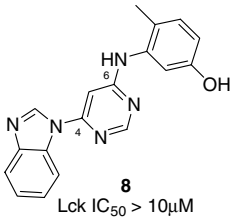


Figure 2. Compound **8**.