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Disubstituted pyrimidines as Lck inhibitors

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ABSTRACT

We have developed a family of 4-benzimidazolyl-*N*-piperazinethyl-pyrimidin-2-amines that are subnanomolar inhibitors of Lck. A subset of these Lck inhibitors, with heterocyclic substituents at the benzimidazole C5, are also low-nanomolar inhibitors of cellular IL2 release.

P56^{Lck} (Lck) is a member of the Src family of non-receptor protein tyrosine kinases. Lck is expressed almost exclusively in T-cells and its catalytic activity is required for T-cell signal transduction.[1] A potent Lck kinase inhibitor would inhibit T-cell activation, and thus could be useful as an immunosuppressive agent for the prevention of graft rejection following organ transplantation, or for treating autoimmune diseases such as rheumatoid arthritis and psoriasis. Therefore, Lck is an attractive target for therapeutic intervention.[2]

High-throughput screening for a nascent medicinal chemistry program aimed at identifying an Lck inhibitor disclosed disubstituted pyrimidine **1** as a promising lead.[3] Optimization of the α-methylbenzylamine substructure of **1** led to the piperidine naphthyl urea **2**, which was highly potent against Lck activity in vitro, and moderately active in our cellular assay for IL2 release. Optimization of the benzimidazole substructure of compound **1** resulted in C5-substituted inhibitors such as pyridine **3**, which was a potent inhibitor of Lck activity in vitro and a moderate inhibitor of cellular IL2 release. Combination of the optimized substructures of **2** and **3** proved synergistic: Compound **4** is a potent inhibitor of both Lck activity and cellular IL2 release (Fig. 1).

Our initial structure–activity investigations were directed at the 2-benzylamino group of our screening lead, compound **1**. As shown in Table 1, the diminished activities of the enantiomeric compound (**5**), as well as of the des-methyl analog (**6**) underscore the importance of the benzylic methyl substitution, as well as the preferred (*S*) stereochemistry.

The increased potency of the *m*-nitrobenzylamine analog **7** relative to **1** prompted further exploration of this position. As inhibitory activity was not dependent on the presence of an aromatic

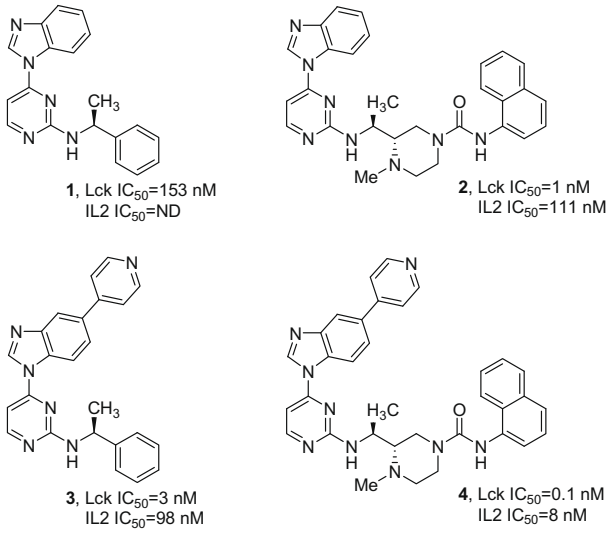

Figure 1. Disubstituted pyrimidines as inhibitors of Lck.

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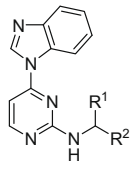
Table 1
2-Aminopyrimidine derivatives



Compd	R^1	R^2	Lck IC_{50}[4] (nM)
1	(*S*)-Me	Ph	153
5	(*R*)-Me	Ph	1770
6	H	Ph	827
7	(*S*)-Me	*m*-NO_2–Ph	47
8	(*S*)-Me	Cyclohexyl	243

ring (note cyclohexyl-substituted compound **8**), we chose to investigate heterocyclic replacements and, in particular, piperidines. All three of the regioisomeric methylamino-*N*-Cbz-piperidines were prepared in order to determine the optimum regioisomer. The Cbz-substituted piperidine **10**, with the nitrogen at the 3 position, exhibited activity comparable to that of **6**, the des-methyl analog of **1** (Table 2); this result dovetailed with the results for *m*-nitrobenzylamine **7** (Table 1).

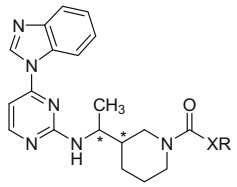
We turned next to the installation of an α-methyl group on piperidine **10**. As expected, addition of the α-methyl group resulted in a marked increase in activity. The more active diastereomer **12a** showed inhibitory activity comparable to our lead **1**.

The piperidine scaffold was examined further via derivatization of **12a**. Removal of the Cbz group followed by methylation or benzylation under standard conditions resulted in loss of activity.[5] Recognizing the significance of the carbonyl linkage to the piperidine, we next prepared a variety of urea, amide, and carbamate analogs using standard acylation chemistry.

As shown in Table 3, the activity of phenyl urea **15** was significantly improved over that of our lead compound **1**. Phenyl carbamate **17** was fourfold less potent than **15**, while the phenyl acetamide **16** lost much of its potency. These results indicated a preference for a pendant urea group. Therefore, we assessed a number of substituted aryl urea compounds. Among the analogs prepared, the 1-naphthyl urea compound **19** proved the most active. The individual enantiomers of **19** were separated by chiral HPLC (Chiralcel OJ) and the more active enantiomer, **20**, exhibited subnanomolar potency. Unfortunately, although compound **20** was an extremely potent inhibitor of Lck activity, its activity in our cellular assay for IL2 release was poor (IC_{50} = 1758 nM).[6]

In an attempt to improve the activity of our Lck inhibitors in our cellular assay, we turned to heterocyclic replacements of the piperidine ring. For our initial screen, we prepared the synthetically

Table 3
Ureas, carbamates, and amides



Compd	X	R	Lck IC_{50}[4] (nM)
13	NH	Me	242
14	NH	Cyclohexyl	1260
15	NH	Ph	11
16	CH_2	Ph	800
17	O	Ph	46
18	NH	2-Naphthyl	40
19 (***rac***)	NH	1-Naphthyl	1
20 (***S,S***)[a]	NH	1-Naphthyl	0.4

[a] Proposed stereochemistry. The (*S*)-methyl enantiomer is more active in the α-methylbenzylamine series, and the (*S,S*)-methylpiperazine is the more potent enantiomer (see below).

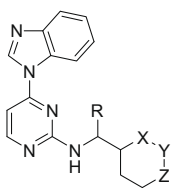
more accessible des-methyl compounds **21**–**25**.[7] As shown in Table 4, piperazine **25** was tenfold more potent than the corresponding des-methyl piperidine naphthyl urea, **22**.

Introduction of the potency-enhancing methyl group into compound **25** provided compounds 2**6a** and **26b** (Table 5). Comparison of diastereomers **26a** and **26b** shows that the relative configuration of **26a**, with the two methine protons *syn*, significantly enhances the potency of the compound as an inhibitor of both Lck activity and cellular IL2 release. Thus, the remaining analogs shown in Table 5 (**27–35**) were synthesized in the same relative configuration as **26a**. None of these analogs improved on **26a** in either biochemical or cellular potency. Taken together, the data in Tables 3 and 5 pointed to the 1-naphthyl urea as the optimal substituent at this position.

Separation of the enantiomers of **26a** via chiral HPLC (Chiralpak OD) showed that the (*S,S*) enantiomer—compound **36** (Table 6)—was approximately sixfold more potent than the racemate **26a**. Table 6 summarizes the biochemical and cellular activity of several (*S,S*)-piperazines. Replacement of the R^1 methyl in compound **6** with an ethyl (**37**) did not significantly change the potency, but larger alkyl groups, such as isobutyl (**38**) or hexyl (**39**) led to significantly weaker inhibitors of IL2 release. Other changes at R^1, such as pyridyl, ethyl ester, and acetyl substituents, were also detrimental to inhibitor potency and cellular activity.

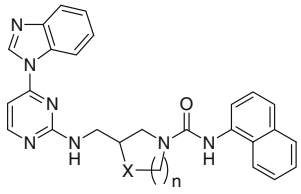
Although piperazine **36** is the most potent inhibitor of Lck activity in this series (IC_{50} = 0.06 nM), it is only a 111-nM inhibitor of cellular IL2 release, corresponding to a nearly 2000-fold shift in po-

Table 2
Piperidine regioisomers



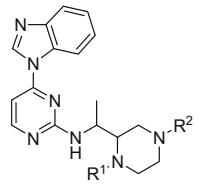
Compd	R	X	Y	Z	Lck IC_{50}[4] (nM)
9	H	NCbz	CH_2	CH_2	>20,000
10	H	CH_2	NCbz	CH_2	650
11	H	CH_2	CH_2	NCbz	>20,000
12a	Me	CH_2	NCbz	CH_2	144
12b	Me	CH_2	NCbz	CH_2	2723

Table 4
Des-methyl heterocycles



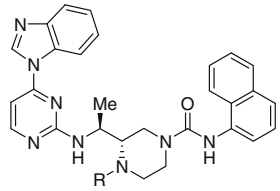
Compd	X	N	Lck IC_{50}[4] (nM)
21	CH_2	1	>10,000
22	CH_2	2	106
23	O	2	203
24	NH	2	110
25	NCH_3	2	10

Table 5
Racemic piperazines



Compd	R^1	R^2	Lck IC_{50}[4] (nM)	IL2 IC_{50}[6] (nM)
26a	Me	1-Np urea	0.25	361
26b	Me	1-Np urea	17.4	>10^3
27	Bn	1-Np urea	2.6	>10^3
28	SO_2Me	1-Np urea	11.8	>10^3
29	SO_2Ph	1-Np urea	26.5	>10^4
30	Cbz	1-Np urea	>10^3	>10^4
31	Me	2-Np urea	75.2	>10^3
32	Me	SO_2Me	22.5	>10^3
33	Me	Bn	501	>10^4
34	Me	Me	>10^3	>10^4
35	Me	H	>10^3	>10^4

Table 6
(*S*,*S*)-Piperazines

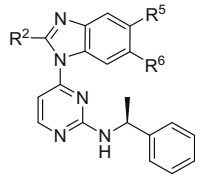


Compd	R	Lck IC_{50}[4] (nM)	IL2 IC_{50}[6] (nM)
36	Me	0.06	111
37	Et	0.07	154
38	*i*-Bu	0.2	538
39	Hexyl	1.8	>10^3
40	4-Pyridyl	0.2	546
41	CH_2CO_2Et	0.45	847
42	Ac	115	>10^4

tency from the enzyme to the cell. We turned our attention to the benzimidazole portion of the inhibitor in hopes of addressing this potency shift.

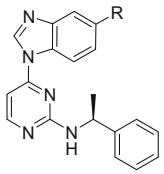
Concurrent with our studies on substitutions for the 2-benzylamino group of lead compound **1**, we also examined the effect of modifications of the benzimidazole moiety, as shown in Table 7. Substitution at C2 of the benzimidazole severely diminished activity against Lck, as exemplified by the 2-methyl analog **43**. Substitution of the 5- and 6-positions of the benzimidazole proved more promising. The regioisomeric methyl- (**44** and **45**) and amino- (**47** and **48**) analogs maintained activity, with the 6-methyl and 5-amino-regioisomers each being more potent than the parent compound.

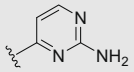
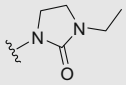
Table 7
Substituted benzimidazoles



Compd	R^2	R^5	R^6	Lck IC_{50}[4] (nM)
1	H	H	H	153
43	CH_3	H	H	>20,000
44	H	CH_3	H	128
45	H	H	CH_3	58
46	H	CH_3	CH_3	2073
47	H	NH_2	H	86
48	H	H	NH_2	19.5

Table 8
C5-Substituted benzimidazoles

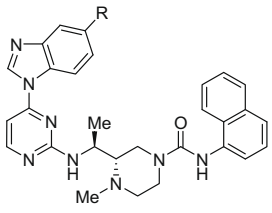


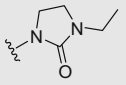
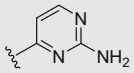
Compd	R	Lck IC_{50}[4] (nM)	IL2 IC_{50}[6] (nM)
3		3	98
49		1.5	111
50		1	167

Encouraged by the activity of compound **48**, and cognizant of the synthetic utility of the amino group, we prepared a variety of alkylamine, amide, sulfonamide, and urea derivatives, but none of these compounds showed cellular activity.[7] However, when we explored heterocyclic substituents at the benzimidazole C5, we eventually discovered a handful of compounds, shown in Table 8, with promising activity in our IL2 assay.

When these benzimidazole C5 substituents were incorporated into the piperazine naphthyl urea series, the resulting compounds were highly potent inhibitors of both Lck activity and cellular IL2 release. Compounds **51**, **52**, and **4** showed much less dramatic shifts in potency from the enzyme to the cell, as compared to the parent piperazine **36** (Table 9). In particular, pyridine-substituted compound **4** was a 0.12-nM inhibitor of Lck activity and an 8-nM inhibitor of IL2 release (corresponding to a 70-fold shift). Compound **4** is the most potent inhibitor of cellular IL2 release prepared to date in our laboratories.

Table 9
C5-Substituted benzimidazole (*S*,*S*)-piperazines



Compd	R	Lck IC_{50}[4] (nM)	IL2 IC_{50}[6] (nM)
51		0.4	42
52		0.07	16
4		0.12	8

In summary, we have developed a family of 4-benzimidazolyl-1-piperazinethyl-substituted pyrimidin-2-amines as Lck kinase inhibitors. We have shown that the preferred piperazine substituents are *N*1-naphthyl urea and *N*4-methyl, and we have determined the optimal relative and absolute stereochemistry of these chiral compounds. Further, we have shown that compound **4**, which combines the optimized piperazine-ethyl moiety at the pyrimidine C2 with the optimized benzimidazolyl substituent at the pyrimidine C4, is a potent inhibitor of both Lck kinase activity and cellular IL2 release.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.102.

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7. See Supplementary data for synthetic schemes and additional data.