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N-(3-(Phenylcarbamoyl)arylpyrimidine)-5-carboxamides as potent and selective inhibitors of Lck: Structure, synthesis and SAR

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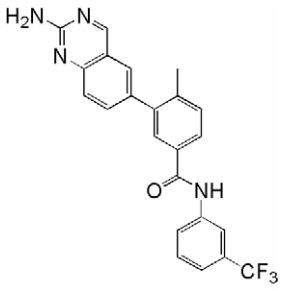
Abstract—*N*-3-(Phenylcarbamoyl)arylpyrimidine-5-carboxamides are a novel class of selective Lck inhibitors. This series of compounds derives its selectivity from a hydrogen bond with the gatekeeper Thr316 of the enzyme. X-ray co-crystal structural data, structure–activity relationships, and the synthesis of these inhibitors are reported herein.

T cell receptor (TCR) signal transduction pathways play a role in the adaptive immune response through gene regulation events, which lead to cytokine release, proliferation, and survival of antigen-specific T cells. A number of protein kinases have been shown to be potentiators of the TCR signal transduction pathways; among them are Src family members, Lck (lymphocyte-specific kinase) and Fyn.[1] Mice and humans with Lck mutations exhibit defective T cell maturation and signaling.[2] This observation suggests that Lck inhibition may be a means of treating T cell-mediated autoimmune and inflammatory diseases and transplant graft rejection.

While several groups[3] have reported the discovery and development of potent Lck inhibitors with efficacy in in vivo models of inflammation, attaining selectivity over structurally related kinases has been an ongoing challenge which has only recently been addressed.[3b]

We recently reported a series of potent, nonselective inhibitors of Lck, represented by 2-aminoquinazoline **1** (Fig. 1).[3f] The 2-aminoquinazolines exhibited excellent potency, desirable pharmacokinetic properties, and efficacy in a mouse model of inflammation. However, after extensive SAR studies, it was possible to attain only modest selectivity over Tie-2, Jak3, KDR, and other kinases. We believed further selectivity could be achieved through additional modifications to this scaffold.



1
IC_{50} (Lck) = 0.2 nM
IC_{50} (KDR) = 2 nM
IC_{50} (p38α) = 6 nM
IC_{50} (Jak3) = 20 nM
IC_{50} (Tie-2) = 2 nM

Figure 1. Representative example of 2-aminoquinazoline series (**1**).

The X-ray co-crystal structure of **1** bound to Lck was solved to 2.0 Å resolution (Fig. 2). Binding of the inhibitor in the ATP-binding site induced the protein to as-

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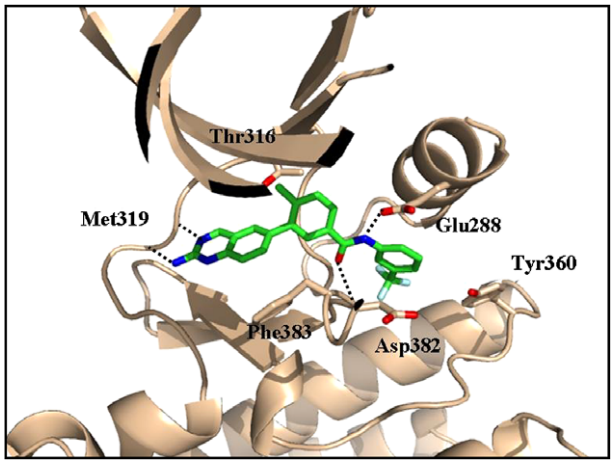


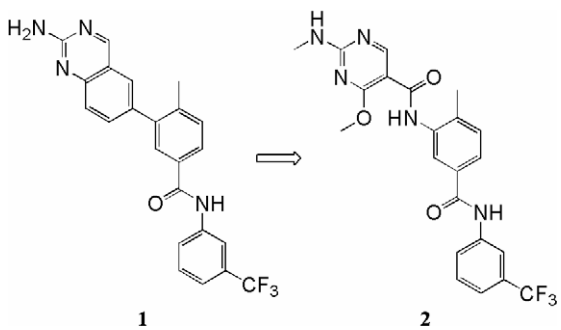
Figure 2. Co-crystal structure of Lck with **1** (green). Oxygen atoms are shown in red, and nitrogen atoms are blue.

sume the 'DFG-out' conformation.[4] This orientation allows the trifluoromethylphenyl ring to access an extended hydrophobic pocket. van der Waals interactions between hydrophobic residues in this pocket and the aryl ring of the inhibitor are responsible for the excellent potency observed in this series. Several other interactions are visible in the co-crystal structure. The 2-amino functional group and N3 of the quinazoline are involved in hydrogen bonds with Met319 in the hinge region of the protein. The amide functionality of **1** donates a hydrogen bond to Glu288 of the C-helix and accepts an NH hydrogen bond from Asp382 of the DFG sequence.

Based on this structural information, we hypothesized that selectivity could be gained by exploiting a sequence difference between Lck and related kinases, particularly KDR, Jak3, and Tie-2 (Table 1). We designed an inhibitor (**2**, Fig. 3) to be isosteric with the quinazolines but containing an appropriately placed carbonyl to engage in a hydrogen bond with the hydroxyl group of the Thr316 gatekeeper residue in Lck. The gatekeeper residues of KDR, Jak3, and various other kinases are precluded from participating in this hydrogen bonding interaction (Table 1). On the other hand, p38α also

Table 1. Gatekeeper residues in Lck and other kinases

Lck	KDR	p38α	Jak3	Tie-2
Thr	Val	Thr	Met	Ile



1 2

Figure 3. Progression from quinazoline **1** to pyrimidine amide **2**.

has threonine as its gatekeeper residue, which could potentially limit selectivity. Herein, we describe the structure–activity relationships and synthesis of *N*-(3-(phenylcarbamoyl)aryl)pyrimidine-5-carboxamides, a novel class of selective inhibitors of Lck.

Compound **2** (Table 2) and subsequent compounds were tested for Lck inhibition in a homogeneous time-resolved fluorescent (HTRF) kinase assay. For the purpose of evaluating kinase selectivity, the compounds were also tested against KDR, p38α, Jak3, and Tie-2. Cellular activity was measured by testing the compounds for the inhibition of T cell activation in a human mixed lymphocyte reaction (MLR).[5]

While compound **2** exhibits modest potency in the Lck enzyme inhibition assay (IC_{50} = 53 nM) and promising selectivity (>50-fold) over KDR, p38α, Jak3, and Tie-2 (Table 2), it showed >10 μM cellular potency in our MLR assay. We sought to improve the potency by synthesizing variations of the trifluoromethylphenyl ring that showed promise in our 2-aminoquinazoline SAR studies.[3f] However, we found that none of these compounds (exemplified by **3** and **4**, Table 2) exhibited sub-micromolar cellular activity.

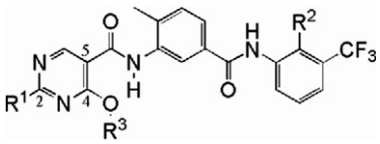
We further modified the scaffold to create additional contacts with the enzyme in order to achieve greater enzyme and cellular potency. To engage the ribose pocket of the enzyme, the methoxy group at the 4-position of the pyrimidine was replaced by a phenoxy group (**5**, Table 2). While compound **5** offered comparable enzyme potency and maintained excellent selectivity, no increase in cellular potency was realized.

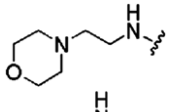
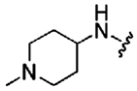
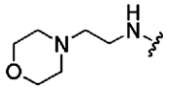
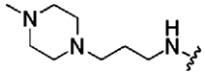
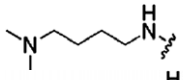
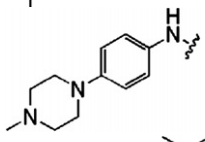
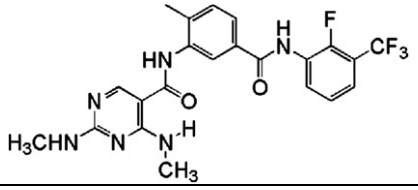
We concurrently examined the effect of substitution on the hinge-binding region of the pyrimidine. Functionality at the 2-position of the pyrimidine should be easily accommodated by the protein as evidenced by the co-crystal structures of related compounds with Lck[3f] and other kinases.[6]

Several amine-containing functional groups were incorporated into our scaffold at the 2-position of the pyrimidine. Varying alkyl chain lengths and amine structure led to inhibitors **6**–**10** with Lck enzyme potency in the range of 19–101 nM (Table 2). We also observed an increase in cellular potency (MLR IC_{50} = 2.7 μM for **9**) through the addition of these groups. While attachment of a 2-morpholinoethyl amine increased cellular potency in **8** (MLR IC_{50} = 4.9 μM), no improvement was observed in the corresponding 4-OPh analog **6** (MLR IC_{50} = 10 μM). We were encouraged to find that incorporation of 4-(4-methylpiperazin-1-yl)aniline led to **11** which has greater potency in the Lck enzyme assay (IC_{50} = 0.6 nM). This represents an 80-fold increase over **2**, while maintaining >400-fold selectivity over the other enzymes in our kinase panel. Importantly, compound **11** also exhibited vastly improved potency in the MLR cellular assay (IC_{50} = 107 nM).

We then designed compound **12**, which presents an N–H hydrogen bond donor to the gatekeeper, Thr316, in con-

Table 2. SAR of pyrimidine amide series[a]



Compound	R^1	R^2	R^3	IC_{50} (nM)					
				Lck	KDR	p38α	Jak3	Tie-2	MLR
2	$NHCH_3$	H	CH_3	53	8330	2720	>25,000	>25,000	>10,000
3	$NHCH_3$	F	CH_3	7	>25,000	254	>25,000	>25,000	>10,000
4	$NHCH_3$	CH_3	CH_3	44	>25,000	>10,000	>25,000	>25,000	>10,000
5	$NHCH_3$	H	Ph	25	>25,000	ND	>25,000	>25,000	>10,000
6		H	Ph	41	>25,000	ND	>25,000	>25,000	>10,000
7		H	CH_3	101	>25,000	ND	>25,000	>25,000	ND
8		H	CH_3	22	2030	ND	>25,000	>25,000	4920
9		CH_3	CH_3	19	>25,000	ND	>25,000	>25,000	2710
10		CH_3	CH_3	45	>25,000	ND	>25,000	>25,000	ND
11		F	CH_3	0.6	255	856	334	3440	107
12				84	6679	2.4	>25,000	>25,000	ND

[a] IC_{50} values are means of two or more separate determinations, in duplicate.

trast to our other inhibitors (**2**–**11**) that present a hydrogen bond acceptor. Our objective was to determine whether the enzyme would tolerate this alternative hydrogen bonding partner. Compound **12** adopts its conformation through rotation around the C5–C7 bond, and this new conformation is enforced by an internal hydrogen bond between the carbonyl oxygen and an aminomethyl group at the 4-position of the pyrimidine. The effect of this modification on enzyme potency is notable; while retaining potency on Lck, **12** proved to be a potent inhibitor of p38α (IC_{50} = 2 nM). This observation can be explained by the structural differences between the threonine gatekeeper residues of p38α and Lck (vide infra).

X-ray crystallographic studies contributed to our understanding of the selectivity observed in this new series compared to the 2-aminoquinazoline series. A co-crystal structure of Lck with **11** was solved to 2.3 Å resolution (Fig. 4a).[7] As predicted, the pyrimidine amide oxygen is involved in a hydrogen bond with the gatekeeper residue, Thr316 (OH–O distance = 2.5 Å), providing an explanation for the selectivity observed over KDR, Tie-2, and Jak3. The three-dimensional structures of quinazoline **1** and pyrimidine **11** are similar, in which the pyrimidine and pseudo-ring of **11** (generated through intramolecular hydrogen bonding) occupy the same space as the quinazoline of **1** (Fig. 4b). Another important similarity between the structures of **1** and **11** in Lck is that the dihedral angle between the hinge-binding ring and the tolyl ring is 90°, which is the preferred geometry based on the enzyme structure. Compounds **1** and **11** participate in the same hydrogen bonding interactions with the hinge residue Met319 as well as Asp382 and Glu288. However, unlike quinazoline **1**, pyrimidine **11** does not induce the kinase to adopt the 'DFG out' conformation. Instead, in this unusual binding mode, the DFG motif may be prevented from adopting the 'DFG out' conformation because of interference with the 4-methoxy group on the pyrimidine. In the 'DFG out' conformation, the phenyl sidechain of Phe383 would be less than 3.0 Å from the 4-methoxy group of **11**. This interference may result in the displacement of the DFG motif, thereby causing the sidechain of Tyr360 to shift toward solvent. In this conformation, Phe383 of the DFG motif is involved in a hydrophobic

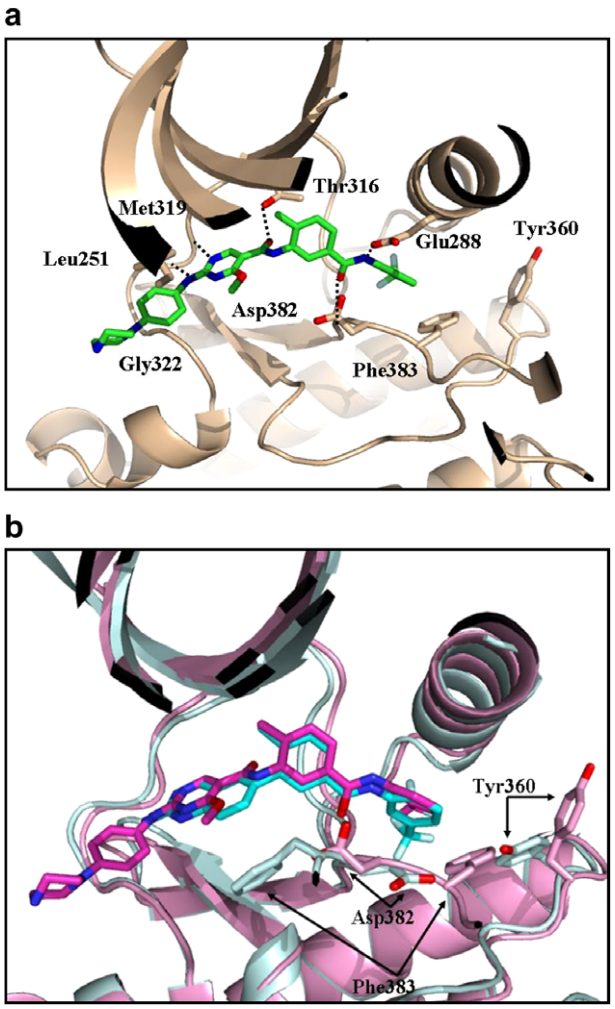


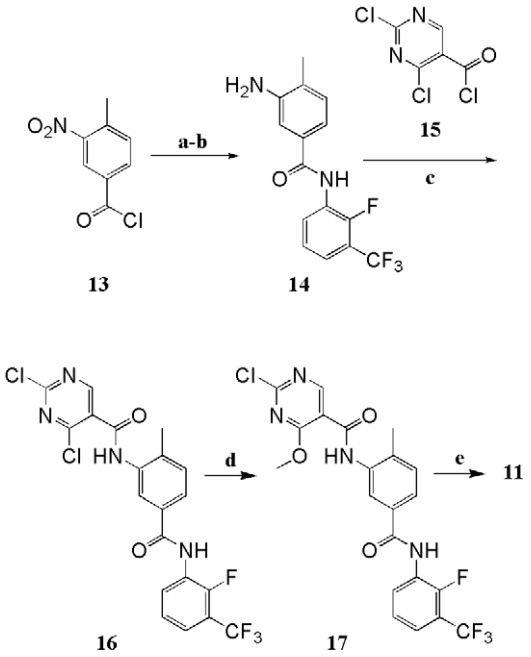
Figure 4. (a) Co-crystal structure of Lck with **11** (green). (b) Overlay of **1** and Lck (cyan) and **11** and Lck (magenta). Oxygen atoms are shown in red and nitrogen atoms are blue.

edge-to-face interaction with the 2-fluoro-3-trifluoromethyl phenyl ring of **11** (3.4 Å).

The observation that compounds **2**–**11** are selective over p38α, despite its threonine gatekeeper residue, can be explained by examining co-crystal structures of inhibitors bound to p38α. In Lck, the Thr316 sidechain is positioned to donate a hydrogen bond to the inhibitor through the γ-OH. However, published structures of p38α show that the gatekeeper threonine hydroxyl group is involved in a bridging hydrogen bond network with main chain atoms from strands β5 and β6.[8] In this conformation, the gatekeeper threonine is available to engage a hydrogen bond donor, but not a hydrogen bond acceptor, such as compound **11**. This argument also applies to the potency on p38α observed for compound **12**, since the threonine oxygen lone pairs can form a hydrogen bond with the NH of the pyrimidine amide.

X-ray crystallographic studies also assisted us in accounting for the increase in potency we observe for compound **11** compared to other compounds in this series (Fig. 4a). The phenyl ring appended to the aminopyrimidine of **11** is involved in van der Waals interactions with the residues succeeding the hinge region of Lck: with the α carbon of Gly322 (3.1 Å) and with Tyr318 (3.2 Å) and Met319 (3.0 Å). Furthermore, the phenyl ring of **11** also participates in a favorable hydrophobic interaction with the Leu251 residue of the glycine-rich loop. Due to similar interactions with the residues succeeding the hinge region, a degree of potency is regained on tyrosine kinases such as Tie-2 and KDR. Conversely, the analogous sequence in p38α (Met109-Gly110-Ala111-Asp112) adopts a conformation that does not interact favorably with the inhibitor due to the absence of one amino acid (Gly). Therefore, compound **11** exhibits increased selectivity over p38α.

The synthesis of the 3-(phenylcarbamoyl)arylpyrimidine-5-carboxamides is illustrated in Scheme 1.[9] Commercially available 4-methyl-3-nitrobenzoyl chloride **13** is treated with 2-fluoro-3-trifluoromethyl aniline in the presence of base, followed by reduction of the nitro group, to provide the substituted 3-amino-4-methyl-*N*-phenylbenzamide (**14**). The aminobenzamide is then reacted with the known 2,4-dichloropyrimidine-5-carbonyl chloride (**15**)[10] in the presence of Hunig's base to furnish dichloropyrimidine **16**. Addition of one equivalent of sodium methoxide to the more reactive 4-position of the pyrimidine provides the penultimate intermediate **17**. Finally, 4-(4-methylpiperazin-1-yl)aniline is added to incorporate the amino hinge binding element at the 2-position of the pyrimidine to yield compound **11**.



Scheme 1. Preparation of 3-(phenylcarbamoyl)arylpyrimidine-5-carboxamide **11**. Reagents and conditions: (a) 2-fluoro-3-trifluoromethyl aniline (1.05 equiv), DIPEA (2.0 equiv), THF (0.30 M), rt; (b) H_2 (1 atm), Pd/C (30% by wt), MeOH (0.10 M), rt, 64% yield (over two steps); (c) **15** (1.1 equiv), DIPEA (2.0 equiv), EtOAc (0.50 M), rt, 82% yield; (d) NaOMe (0.95 equiv), CH_3CN (0.10 M), 0 °C; (e) 4-(4-methylpiperazin-1-yl)aniline (1.1 equiv), TFA (2.0 equiv), THF/DMF (5:1, 0.17 M), 70 °C, 13% yield (over two steps).

In conclusion, we have discovered a new series of potent inhibitors of Lck with enhanced selectivity in comparison to their quinazoline predecessors, based on a rationally designed hydrogen bonding interaction with the gatekeeper threonine residue of the enzyme. We also found that aryl functionality at the 2-position of the pyrimidine can provide much improved enzyme and cellular potency. This study has provided us with a promising lead molecule **11** on which further studies can be based. In addition, the discovery that **11** induces a unique conformation of the DFG sequence of Lck may lead to new opportunities for inhibitor design.

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