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Discovery of 4-amino-5,6-biaryl-furo[2,3-*d*]pyrimidines as inhibitors of Lck: Development of an expedient and divergent synthetic route and preliminary SAR

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Abstract—4-Amino-5,6-biaryl-furo[2,3-*d*]pyrimidines were identified as potent non-selective inhibitors of Lck. A novel, divergent, and practical synthetic route was developed to access derivatives from bifunctional intermediates. Lead optimization was guided by X-ray crystallographic data, and preliminary SAR led to the identification of compounds with improved cellular potency and selectivity.

The lymphocyte-specific kinase (Lck) is a member of the Src family of cytoplasmic tyrosine kinases and is expressed specifically in T cells and natural killer (NK) cells. Genetic evidence from gene knockout mice and human mutations demonstrates that Lck activity is critical for T cell receptor (TCR)-mediated signaling, leading to normal T cell development and activation.[1] Selective inhibition of Lck is expected to offer a new therapy for the treatment of graft rejection and/or T cell-mediated autoimmune and inflammatory disease such as rheumatoid arthritis, psoriasis, multiple sclerosis, atherosclerosis, and delayed-type hypersensitivity reactions. In accordance with our ongoing efforts to identify small molecule inhibitors of Lck for the development of new therapeutic agents, we recently disclosed two promising chemical series.[2] Likewise, others have shown that potent and bioavailable Lck antagonists have in vivo inhibitory activities in models of T cell-dependent immune responses.[3]

4-Amino-5,6-biaryl-furo[2,3-*d*]pyrimidines **1**–**3** were identified as promising leads for the Lck program during a high throughput screen of our kinase-preferred compound collection (Table 1). This scaffold originated in our Ack1 oncology program[4] and has previously been shown to possess inhibitory activity against other kinases, including DDR2,[5] EGFR,[6] Tie-2,[7] KDR,[7] adenosine kinase,[8] and Chk1.[9] Furthermore, lead compound **3** exhibited respectable pharmacokinetic properties in rats (Table 2). Herein we present an extension of this chemical series toward the identification of several Lck inhibitors with promising cellular potency and selectivity. Moreover, we disclose a novel, expedient, and divergent synthetic route that allows for the introduction of diversity at C4 and C6 from bifunctional intermediates.

A co-crystal structure of **3** and Lck revealed a rather unusual binding mode (Fig. 1).[10] Compound **3** occupies the ATP binding site, making two H-bond contacts to

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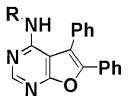
Table 1. Lead compounds **1**–**3** identified from high throughput screening of Ack1 inhibitors (IC_{50}, μM)[a]



	R	Ack1	Lck	MLR[b]	IL-2[c]
1		0.523	1.21	2.13	—
2		0.024	0.122	8.95	2.41
3		0.181	0.081	1.71	0.69

[a] Values are means of two or more separate determinations run in duplicate.
[b] huMLR, human mixed lymphocyte reaction.
[c] IL-2: Anti-CD3/CD28-induced T cell IL-2 secretion assay.

Table 2. Rat pharmacokinetic properties of **3**[a]

iv[b]		po[c]	
CL	887 mL/h/kg	%*F*	15
V_{ss}	2767 mL/kg	C_{max}	180 ng/mL
$t_{1/2}$	2.37 h	AUC_{0-inf}	1037 ng h/mL

[a] $n = 3$ animals per study.
[b] Dosed intravenously at 1 mg/kg in DMSO to male Sprague–Dawley rats.
[c] Dosed orally at 5 mg/kg as a suspension in 2% HPMC/1% Tween80/97% water to male Sprague–Dawley rats.

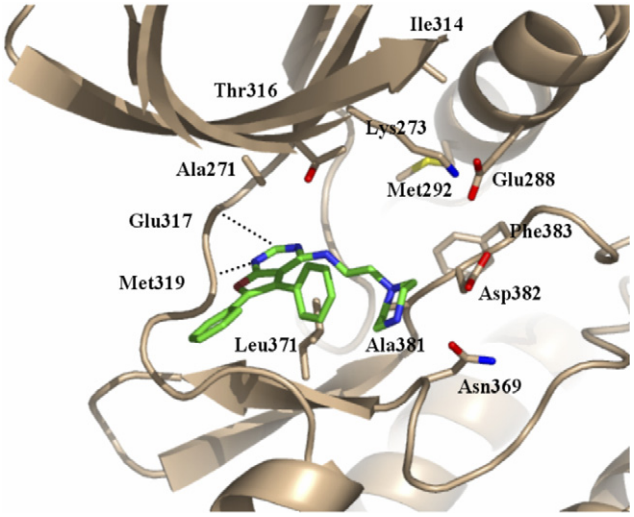


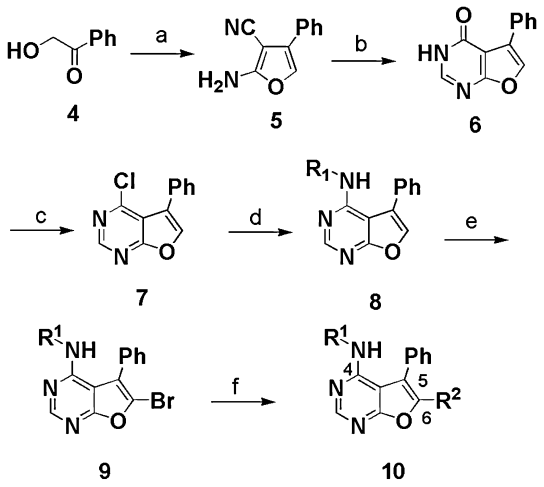
Figure 1. X-Ray co-crystal structure of **3** (green) and Lck. Oxygen atoms, red; nitrogen atoms, blue.

the hinge region: the pyrimidine N1 binds to the NH of Met319 (3.3 Å), and the carbonyl of Glu317 (3.6 Å) accepts the CH in the 2-position. The two phenyl rings are aligned edge-to-face, while the ethylpiperazine makes conformation-stabilizing van der Waals interactions with several residues, approaching but not entering the hydrophobic pocket. Since the phenyl at C6 is projected toward solvent, we reasoned that the introduction of an ionizable group at the *para*-position of this ring might improve cell permeability and potency, and oral bioavailability.

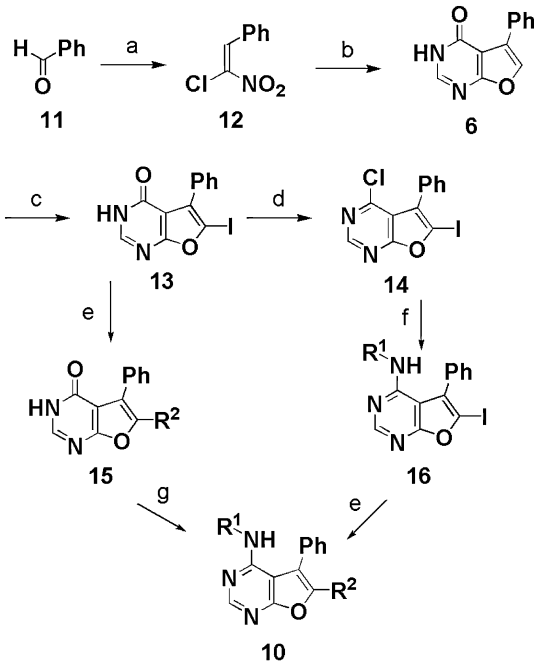
Our original synthetic route, depicted in Scheme 1, makes use of minor modifications to established procedures.[4,11] Although this route was sufficient, we sought an alternative strategy that would allow for later-stage variation of both the amine head group (R^1) at C4 and the aryl group (R^2) at C6.

The modified route (Scheme 2) utilizes a more reproducible and scalable method for the construction of the 5-phenylfuropyrimidinone **6**.[9,12] Regioselective iodination of **6**, followed by reaction with $POCl_3$, affords the key 4-chloro-6-iodo intermediate **14** in 57% yield over two steps. Compound **14** can be reacted with amines in the presence of base to generate penultimate 4-amino-6-iodo compounds **16** for scanning R^2 at C6 via Suzuki chemistry. Alternatively, iodide **13** can be reacted under Suzuki conditions with boronates to afford the C6-substituted pyrimidinones **15**. Intermediates **15** are used for scanning R^1 at C4 via a two-step chlorination-S_NAr sequence, which avoids purification of the intermediate C4-chloride and affords final compounds **10** in 50–60% overall yield from **15**.

Selected SAR from our C6 scan is collected in Tables 3 and 4. As hypothesized, the introduction of ionizable, solubilizing groups at the *para*-position of the C6 phenyl led to an improvement in cellular potency over the parent compounds **2** and **3**. These derivatives also exhibited a 2 to 20-fold increase in potency at the enzyme level. Modeling suggests that this potency improvement is due to charge-charge interactions between the tertiary amines and the carboxylic acid side chain of Asp326 (Fig. 2).[13] In general, compounds containing the (*S*)-*N*-methyl-THF head group at C4 were equipotent on Ack1 and Lck (**17**, **18**, **20**, and **21**). In this series, selectivity over Ack1 was marginal at best (e.g., **19**; five-fold

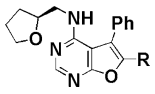


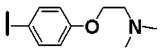
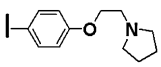
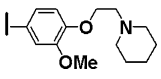
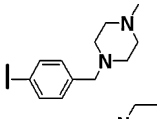
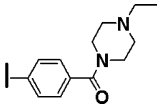
Scheme 1. Reagents and conditions: (a) malononitrile, NEt_3, DMF, 0 °C → rt, 70%; (b) HCO_2H, Ac_2O, reflux, 61%; (c) $POCl_3$, benzene, reflux, 90%; (d) R^1NH_2, DIPEA, *i*-PrOH, reflux, 70–92%; (e) NBS, KOAc, 80%; (f) $R^2B(Pin)$, 5% $Pd(PPh_3)_4$, K_2CO_3, DME/H_2O, 75 °C, 30–60%.



Scheme 2. Reagents and conditions: (a) bromo(nitro)methane, Me_2N-H·HCl, KF, xylenes, reflux, 80%; (b) pyrimidine-4,6-diol, DBU, EtOH, reflux, 70%; (c) NIS, CH_3CN/DME, reflux, 85%; (d) $POCl_3$, NEt_3, DCE, 80 °C, 67%; (e) $R^2B(Pin)$, 10% $Pd(dppf)Cl_2$, K_2CO_3, DMF/H_2O, 80 °C, 60–73%. (f) R^1NH_2, DIPEA, *i*-PrOH, reflux, 70–90%; (g) 1—$POCl_3$, reflux; 2—R^1NH_2, CH_3CN/CH_2Cl_2, reflux, 50–60% over two steps.

Table 3. Select SAR: variations at C6 with (*S*)-*N*-methyl-THF head group at C4 (IC_{50}, μM)[a]

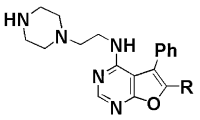


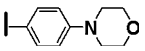
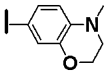
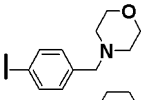
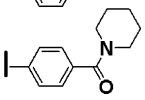
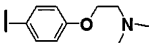
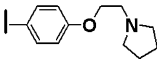
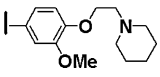
	R	Ack1	Lck	MLR[b]	IL-2[c]
17		0.011	0.006	0.464	0.119
18		0.016	0.011	0.670	0.435
19		0.038	0.008	0.825	0.193
20		0.007	0.006	0.337	0.293
21		0.013	0.011	0.437	2.420

[a] Values are means of two or more separate determinations run in duplicate.
[b] huMLR, human mixed lymphocyte reaction.
[c] IL-2: Anti-CD3/CD28-induced T cell IL-2 secretion assay.

selective). With the *N*-ethyl-piperazine head group, however, a variety of R groups imparted some degree of selectivity over Ack1 (**23**, **24**, and **25**; up to four-fold). Phenoxyethylamines (e.g., **26**, **27**, and **28**) proved most

Table 4. Select SAR: variations at C6 with *N*-ethyl-piperazine head group at C4 (IC_{50}, μM)[a]



	R	Ack1	Lck	MLR[b]	IL-2[c]
22		0.017	0.011	0.227	—
23		0.097	0.042	3.084	—
24		0.091	0.027	0.138	—
25		0.085	0.022	0.121	—
26		0.148	0.014	0.360	0.468
27		0.098	0.009	0.227	0.430
28		0.586	0.022	3.223	2.634

[a] Values are means of two or more separate determinations run in duplicate.
[b] huMLR, human mixed lymphocyte reaction.
[c] IL-2: Anti-CD3/CD28-induced T cell IL-2 secretion assay.

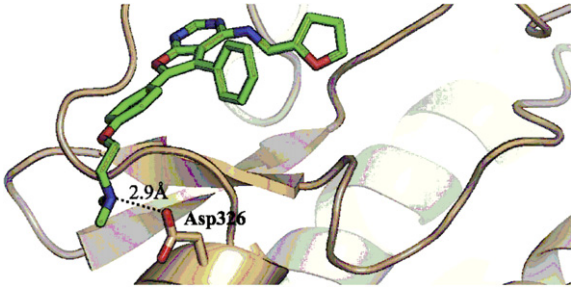


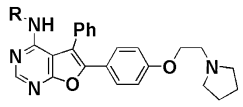
Figure 2. Model of **17** and Lck based on the co-crystal structure of **3** and Lck (Fig. 1).

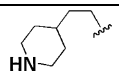
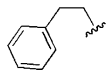
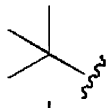
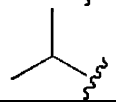
selective; up to 30-fold selectivity over Ack1 was observed with some compounds (e.g., **28**).

Based on the X-ray co-crystal structure of **3** and Lck (Fig. 1), we speculated that a hydrophobic substituent at C4 may be better tolerated than an ionizable head group. We therefore sought to reduce polarity and size by fixing the phenoxyethylpyrrolidine at C6 and examining lipophilic residues at C4 (Table 5). Initial results are promising as **29** exhibits 24-fold selectivity over Ack1 and **32** demonstrates significant size-reduction without a substantial loss in cellular potency.

Compounds **27** and **32**, which demonstrated good cellular potency and moderate selectivity over Ack1, were

Table 5. Select SAR: variations at the C4 amine with phenoxyethyl-pyrrolidine at C6 (IC_{50}, μM)[a]



	R	Ack1	Lck	MLR[b]	IL-2[c]
29		0.332	0.014	2.452	—
30		0.001	0.005	1.672	5.719
31		0.650	0.083	2.376	3.257
32		0.078	0.036	0.533	0.199

[a] Values are means of two or more separate determinations run in duplicate.
[b] huMLR, human mixed lymphocyte reaction.
[c] IL-2: Anti-CD3/CD28-induced T cell IL-2 secretion assay.

Table 6. Kinase Selectivity Summary for **27** and **32** (IC_{50}, μM)[a]

Kinase	**27**	**32**
Lck	0.009	0.036
Src	0.045	0.914
Ack1	0.098	0.078
EGFR	0.397	1.029
Jak3	0.960	1.426
ZAP70	0.992	3.272
KDR	3.096	0.537
IGFR	3.146	1.493
Jak2	>8.333	4.891
Tie-2	>25.000	>8.333

[a] Values are means of two or more separate determinations run in duplicate.

further profiled for inhibitory activity against a panel of receptor and non-receptor tyrosine kinases. Selectivity was generally good (>100-fold) to excellent (>1000-fold) over a range of enzymes (Table 6). As anticipated based on the close sequence homology among members of the Src family, compounds **27** and **32** exhibit modest selectivity over Src.

In summary, 4-amino-5,6-biaryl-furo[2,3-*d*]pyrimidines **1**–**3** were identified as potent, non-selective inhibitors of Lck. A novel and expeditious synthetic route allowed for rapid diversification of the core scaffold with focus on exploring SAR at the C4-amine head group and the C6-aryl group. This focused exploration was guided by X-ray crystallographic data and has led to the identification of compounds (**27** and **32**) with acceptable cellular potency and selectivity profiles. Lessons learned from the 4-amino-5,6-biaryl-furo[2,3-*d*]pyrimidines were invaluable in advancing a subsequent, closely related 2,3-diarylfuro[2,3-*b*]pyridine-4-amine series, which we introduce in this issue.[14]

Acknowledgments

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