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Pyrrolo[2,3-*d*]pyrimidines Containing an Extended 5-Substituent as Potent and Selective Inhibitors of lck II

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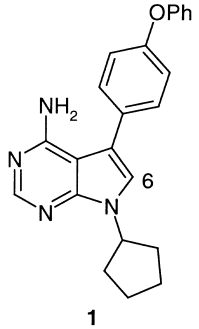
Abstract—Pyrrolo[2,3-*d*]pyrimidines containing a 5-(4-phenoxyphenyl) substituent are novel, potent and selective inhibitors of lck in vitro. Exploration of C-6 position of the pyrrolo[2,3-*d*]pyrimidine and the terminal phenyl group structure–activity relationship (SAR) is detailed. Compound **1** is orally active in animal models.

lck, an src-family tyrosine kinase expressed primarily in T lymphocytes, plays an essential role in the immune response.[1] Productive T-cell activation is characterized by the appearance of a hyperphosphorylated ζ-chain and by phosphorylation and catalytic activation of the syk family ZAP-70 kinase by lck.[2,3] Selective inhibition of lck function therefore represents an attractive target for therapeutic intervention in the treatment of autoimmune and inflammatory diseases and also in organ transplantation.

The previous paper in this journal[4] detailed our discovery of a novel series of pyrrolo[2,3-*d*]pyrimidines containing a 5-(4-phenoxyphenyl) substituent, typified by compound **1**, as potent and selective inhibitors of lck in vitro.



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This paper documents our initial explorations centered around the terminal phenyl ring and the 6-position of the pyrrolo[2,3-*d*]pyrimidine in an attempt to identify more potent and selective inhibitors of lck.

Two crystal structures of the catalytic domain of lck in its active state (Y394 phosphorylated) have been published.[5,6] The structure of two src-family kinases, in their inactive states (Y394 unphosphorylated) has also been determined; namely src[7] and hck.[8,9] Comparison of these structures suggests that there may be more structural difference between inactive forms of different src-family kinases thus affording an increased opportunity for obtaining selectivity.[9]

We screened inhibitors using two forms of the lck kinase, lck (64–509) and lckcd. As detailed in the preceding paper, we determined that lck (64–509) is in the inactive state while lckcd is in the active state. Compounds inhibited the inactive form of lck more potently than the active form. The phosphorylation state of Y394 is responsible for these differences.

The proposed binding mode of **1** to lck (64–509) in our homology model delineated in the preceding paper suggests that the hydrophobic pocket in lck (64–509) is occupied by the phenoxyphenyl moiety of **1** and results in increased potency against lck (64–509) and also selectivity against lckcd. This is one of the regions we hoped to exploit.

Results and Discussion

The structural requirement at the 6-position of the pyrrolo[2,3-*d*]pyrimidine was probed with a diverse set of

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functional groups (Table 1).[10] Compounds **1**–**11** in Table 1 are potent inhibitors of lck (64–509) exhibiting eroded potency against lckcd. With the exception of compounds **7** and **9**, they are selective for lck (64–509) over kdr and tie-2. All compounds demonstrate selectivity for lck (64–509) and lckcd over src and are competitive with ATP (data not shown).

Although full kinase inhibition data were not generated for all compounds, some general trends are worthy of note. Within electronically similar functional groups, there is a steric component at C-6 as evidenced by comparison of the primary alcohol **6** to the sterically more demanding secondary alcohol **7**; the latter being 25-fold less potent against lck (64–509) than the former. This trend is also seen for the 6-H analogue **1** compared to the less active 6-methyl inhibitor **4**. This stringency does not manifest itself for the receptor tyrosine kinases kdr or tie-2.

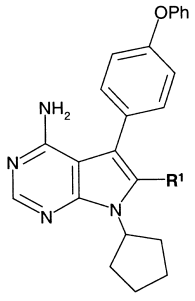
The installation of electron withdrawing (e.g., **2** and **5**), electron donating (**4**), acidic (**10**) or basic functionalities (**9**) does not improve potency for lck (64–509) over the reference compound **1**. The morpholinomethyl analogue **12** shows a significant drop in potency and is the least potent inhibitor of lck of this set of compounds. Compound **12** exhibits no selectivity over the two receptor tyrosine kinases kdr and tie-2. In summary, irrespective of the nature, modification at the 6-position results in a reduction in lck in vitro potency.

Modifications to the terminal phenyl ring were explored in an effort to access beneficial protein contacts in the hydrophobic pocket. For historical reasons, analogues were made in the N-7 cyclopentyl, *tert*-butyl and also the isopropyl series; data are shown in Table 2. As can be seen from compounds **19**–**23**, **25**, and **27**, installation of functionality at the 2-position negatively impacts potency for lck (64–509). A similar trend is seen for the parent src-family member although the effect against tie-2 and kdr is insignificant. Substitution at the 4-position has a variable effect on lck (64–509) potency with the 4-CN (**24**) and 4-COOH (**28**) exhibiting significant erosion; the latter example being greater than 100-fold less potent than its parent **13**. Analysis of this set of compounds indicates that modifications in the terminal ring do not impact tie-2 potency more than 11-fold. In contrast, significant perturbation is seen for kdr inhibition.

Within this set of compounds there were no examples with an improved profile over compound **1**, hence a more detailed in vitro profiling of compound **1** was warranted. Table 3 depicts inhibition data against three further src-family members, blk, fyn, and lyn. Additionally, the datum for the negative-regulatory tyrosine kinase, csk[11] is shown. All data are at 1 mM ATP. Aside from src itself, the highest selectivity (greater than 25-fold) within the src members was observed against lyn; less selectivity was seen against the other representatives blk and fyn. We did not generate counterscreen data against the remainder of the src family. Elucidation of a structural explanation for the diversity of selectivity within the src family awaits a co-crystal structure determination. Greater than 300-fold selectivity was seen for lck (64–509) against csk.

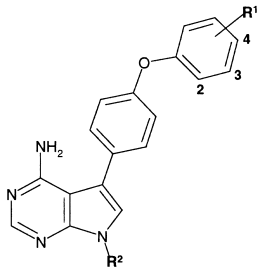
Compound **1** is a potent inhibitor of IL-2 production in cells[4] and has progressed to in vivo settings. Compound **1** inhibits T-cell receptor stimulated (α-CD3 mAb) IL-2 production in mice at low doses ($ED_{50} = 4$ mg/kg) after ip administration. However, efficacy is greatly reduced after oral administration ($ED_{50} = 25$ mg/kg) which is

Table 1. Inhibition of lck (64–509), lckCD, src, kdr and tie-2 for compounds **1**–**12** (IC_{50} values, μM)



		lck (64–509)		lckcd		src	kdr	tie-2
Compound	R^1	5 μM ATP	1 mM ATP	5 μM ATP	1 mM ATP	1 mM ATP	5 μM ATP	5 μM ATP
1	H	< 0.001	0.016	0.002	1.07	>50	1.57	1.98
2	Cl	0.011	0.38	0.06	8.1	>50	16.9	23.4
3	Br	0.03	0.075	2.29	11.7	NT	1.60	11.1
4	Me	0.26	NT	NT	NT	NT	3.35	1.81
5	CN	0.004	0.069	0.91	15.2	>50	3.2	0.50
6	CH_2OH	0.017	0.29	NT	18.3	>50	1.08	0.36
7	$CH(OH)CH_3$	0.47	NT	NT	NT	NT	1.86	0.61
8	CH_2OMe	0.31	NT	NT	NT	NT	4.24	2.26
9	CH_2NH_2	0.144	3.09	0.93	25.1	>50	0.677	0.13
10	COOH	0.020	1.06	NT	33.5	>50	9.82	0.41
11	$CONH_2$	0.030	26.2	NT	43.2	>50	9.11	0.58
12	CH_2 (morpholino)	1.49	NT	NT	NT	NT	3.68	1.02

Table 2. Inhibition of lck (64–509), lckcd, src, kdr and tie-2 for compounds **13**–**28** (IC_{50} values, μM)

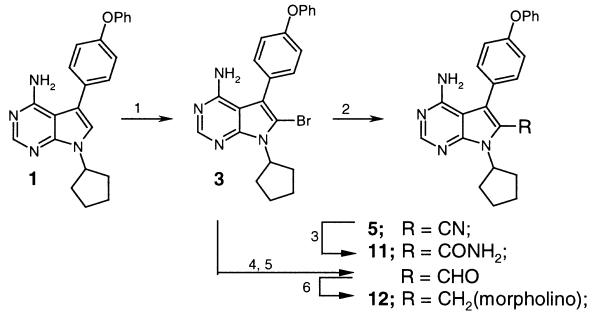


			lck (64–509)		lckcd		src	kdr	tie-2
Compound	R^2	R^1	5 μM ATP	1 mM ATP	5 μM ATP	1mM ATP	5 μM ATP	5 μM ATP	5 μM ATP
13	*i*-Propyl	H	< 0.001	0.011	0.01	NT	0.048	0.43	0.43
14	*t*-Butyl	H	0.002	0.075	0.014	NT	0.61	2.29	2.68
15	*t*-Butyl	4-OMe	< 0.001	NT	0.07	NT	NT	1.31	1.22
16	*t*-Butyl	4-OH	< 0.001	0.03	0.017	NT	0.25	2.0	1.8
17	*t*-Butyl	4-NH_2	< 0.003	0.003	0.019	NT	0.075	0.90	0.90
18	*t*-Butyl	3-NH_2	< 0.003	NT	0.015	NT	0.085	0.40	1.5
19	*i*-Propyl	2-NH_2	0.005	1.01	0.15	9.12	0.554	3.3	0.40
20	Cyclopent	2-OMe	0.006	2.11	0.02	7.1	0.068	0.50	0.60
21	Cyclopent	2-OH	0.008	0.633	0.33	3.13	4.0	0.09	0.56
22	*i*-Propyl	2-NO_2	< 0.003	0.79	0.038	4.13	0.208	0.90	1.90
23	*i*-Propyl	2-CN	< 0.003	0.459	NT	2.53	0.77	0.161	0.239
24	*i*-Propyl	4-CN	0.040	5.55	NT	24.8	3.10	0.129	0.97
25	*i*-Propyl	2-CH_2OH	< 0.003	0.529	NT	10.01	0.224	0.144	0.162
26	*i*-Propyl	4-CH_2OH	< 0.003	0.751	NT	16.04	0.45	0.029	0.246
27	*i*-Propyl	2-COOH	0.942	15.8	NT	NT	26.1	5.06	0.677
28	*i*-Propyl	4-COOH	0.131	1.31	NT	NT	6.9	6.89	0.43

Table 3. Inhibition of lyn, blk, fyn and csk (IC_{50} values, μM)

Compound	lck (64–509)	blk	fyn	lyn	csk
1	0.016	0.066	0.126	0.42	5.18

presumed to reflect poor intestinal absorption in the latter regimen. Inhibition of antigen specific T-cell immune responses was also seen for compound **1**. After administration of compound **1** twice daily (100 mg/kg po) for 3 days during the in vivo priming phase, a 70% inhibition of IFNγ production was seen upon subsequent antigen-specific (KLH) challenge of lymphocytes from the draining lymph nodes in vitro. Further in vivo characterization of compound **1** and analogues will be published subsequently.



Scheme 1. Reagents and conditions: (1) NBS, DMF, rt, 16 h, 94%; (2) $Zn(CN)_2$, P(2-furyl)$_3$, $Pd(dba)_3$, NMP, 90 °C, 18 h, 47%; (3) 1,4-dioxane, 2 N aq NaOH, 2 N aq KOH, 100 °C, 5 days, 52%; (4) *n*-$Bu_3SnCHCH_2$, $Pd(PPh_3)_4$, toluene, 100 °C, 18 h, 95%; (5) $NaIO_4$, OsO_4, 1,4-dioxane, H_2O, rt, 1 h, 63%; (6) morpholine, $NaHB(OAc)_3$, DCE, rt, 18 h, 51%.

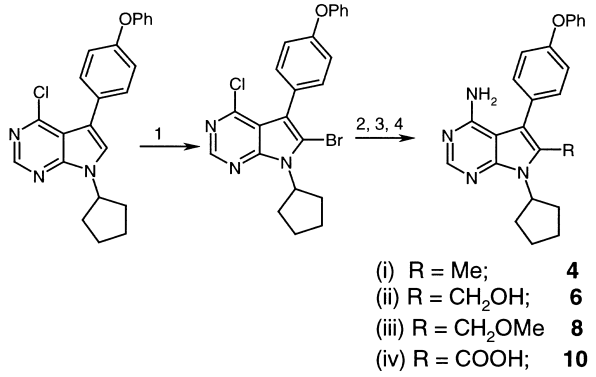
Synthesis

C-6 analogues

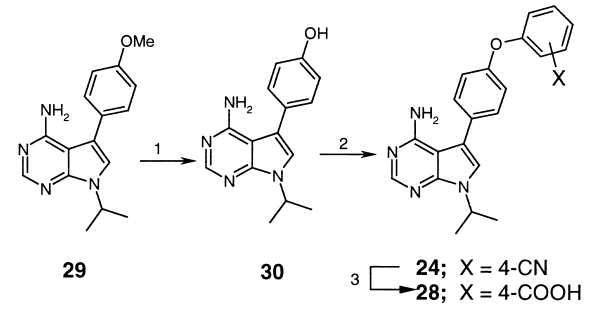
Two routes were used to prepare the pyrrolopyrimidine compounds described in this paper. Scheme 1 starts from compound **1** and exemplifies compounds **3**, **5**, **11**, and **12**. Initial installation of bromine at C-6 allowed manipulation to the cyanide **5** and primary amide **11** utilizing palladium catalysis. Stille coupling and Lemieux–Johnson oxidation presented the aldehyde allowing access to the aminomethyl derivative **12**. Other analogues are easily accessible by known routes. An additional route by lithium halogen exchange is shown in Scheme 2 for compounds **4**, **6**, **8**, and **10**.

Terminal phenyl analogues 13–28

A general route to representative compounds from Table 2 is shown in Scheme 3. Compound **29** was prepared in a manner analogous to that detailed for compound **1**. Demethylation unmasked the phenol **30** and subsequent reaction with an appropriate aryl fluoride generated many of the compounds in Table 2 after suitable further functional group interconversion. This is exemplified for carboxylic acid **28**.



Scheme 2. Reagents and conditions: (1) NBS, DMF, rt, 24 h, 95%; (2) *n*-BuLi, THF, −65 °C, 30 min; (3) (i) CH_3I; (ii) $(CH_2O)n$; (iii) $ClCH_2OMe$, (iv) CO_2; (4) 30% NH_4OH, 1,4-dioxane, 120 °C, 18 h.



Scheme 3. Reagents and conditions: (1) BBr_3, CH_2Cl_2; (2) 4-FC_6H_4CN, K_2CO_3, DMF, 120 °C, 4 h; (3) KOH, 1,4-dioxane, 120 °C, 18 h.

In summary, a novel series of pyrrolo[2,3-*d*]pyrimidines is described, many of which are selective for lck (64–509) over src. Compound **1** exhibits modest selectivity across a panel of src-family kinases and portrays oral activity in models of T-cell activation. Efforts are underway to maximize selectivity for lck against src-family members and also to enhance oral activity.

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