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Pyrrolo[2,3-*d*]pyrimidines Containing Diverse N-7 Substituents as Potent Inhibitors of Lck

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Abstract—A series of pyrrolo[2,3-*d*]pyrimidines was synthesized and evaluated as inhibitors of Lck. Lck accommodates a diverse set of substituents at N-7. Altering the substituent at N-7 provided compound **13**, an orally available lck inhibitor which inhibited TCR mediated IL-2 production after oral dosing.

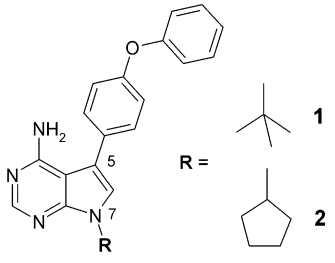
Lck, a src family tyrosine kinase expressed primarily in T lymphocytes, provides a critical function during the initial steps of T-cell receptor (TCR) signaling.[1] A cascade of downstream signaling pathways ultimately leads to T-cell activation and the production of cytokines such as interleukin-2 (IL-2) and IFNγ.[2,3] A selective inhibitor of lck should prevent T-cell activation and thus have broad application for the treatment of T-cell dependent processes such as autoimmune and inflammatory diseases as well as allogeneic organ transplant rejection.

Strategies toward the design and synthesis of tyrosine kinase inhibitors have been reviewed previously.[4,5] Pyrrolo[2,3-*d*]pyrimidines as c-src kinase inhibitors have also been described.[6–8] Early work from our laboratories described the synthesis and SAR of a series of pyrrolo[2,3-*d*]pyrimidines as lck inhibitors, exemplified by compounds **1** and **2**, containing a phenoxyphenyl group at C-5 and alkyl/cycloalkyl substituents at N-7.[9]

This communication describes the synthesis and SAR of a series of pyrrolo[2,3-*d*]pyrimidine analogues containing a variety of substituents at N-7. Since compound **2** did not exhibit the desired in vivo[10] and physicochemical profile our efforts focussed on improving these aspects whilst maintaining lck potency.

Inhibitors were initially screened at 1 mM ATP against a non-phosphorylated construct of human lck kinase, lck (64-509). The closely related kinase src and two receptor tyrosine kinases, kdr and tie-2 served as counterscreens.

Previous work had shown that the phenoxyphenyl moiety (occupying the lipophilic pocket in lck) was responsible for increased potency versus lck.[9] We envisaged improving solubility whilst maintaining potency by retaining this group and modifying the N-7 position. It was anticipated that additional binding interactions could be gained at the N-7 position (sugar pocket in ATP).



1, 2

Compound **2** is a potent inhibitor of IL-2 production in cellular assays with reasonable in vivo efficacy.[10] Although **2** inhibits IL-2 production (anti-CD3 mAb stimulation in mice) after ip dosing ($ED_{50}=4$ mg/kg), activity is reduced ($ED_{50}=25$ mg/kg) on po administration. The pharmacokinetic parameters after a single (25 mg/kg) oral dose (10% EtOH, 10% DMSO, 80% olive oil as vehicle) to female Balb/c mice are outlined in Table 1.

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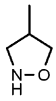
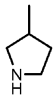
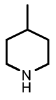
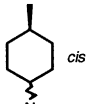
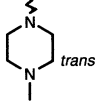
Table 1. Pharmacokinetic parameters for **2**

C_{max} (nM)	T_{max} (h)	AUC (ng h/mL)	$T_{1/2}$ (h)
28	0.25	34.3	7.7[a]

[a]Terminal half-life.

Table 2. Inhibition of lck (64–509), src, kdr and tie-2 for compounds **2**–**13** (IC_{50} μM) by HTRF [a] method

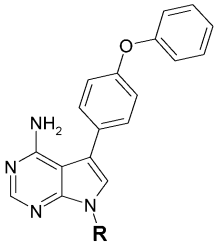
R		lck	src	kdr	tie-2
	**2**	0.10	0.56	4.44	9.07
	**3**[b]	0.042	0.32	NT	5.15
	**4**	0.12	1.12	NT	11.62
	**5**	0.13	1.47	NT	16.71
	**6**	0.89	32.34	NT	>50
	**7**[b]	0.046	0.39	NT	6.62
	**8**[b]	0.24	2.36	NT	NT
	**9**	0.17	1.78	NT	21.38
	**10**	0.52	5.46	NT	12.16
	**11**	0.082	0.298	NT	NT
	**12**	0.24	1.19	10.74	5.85
	**13**	0.015	0.042	1.19	0.25

[a]Mean of two experiments perfomed with seven concentrations of test compound.
[b]Racemic mixture.

The low peak plasma concentrations could be indicative of either poor absorption and/or extensive first pass metabolism. The aqueous solubility of **2** (0.44 μg/mL) precluded dosing in optimal aqueous vehicles for chronic studies and prevented further advancement of this series of compounds. Efforts were undertaken to improve the physicochemical properties of the compounds specifically the goal of improving the aqueous solubility and in vivo profile of this class of inhibitor. A variety of strategies were adopted to address solubility, including incorporation of alcohols, ethers, basic amines and low molecular weight heterocycles into our template.[11] Table 2 highlights certain aspects of the SAR derived from this strategy. Kinase Homogeneous Time Resolved Fluorescence (HTRF) data are shown at 1 mM ATP where noted.

All compounds are potent, ATP competitive (data not shown) inhibitors of lck.

Incorporation of heteroatoms such as oxygen and nitrogen into the carbocyclic framework in general had little effect on lck activity as shown in compounds **3**, **4**, **7**, **8**, and **9**. Although aminoethyl analogue (**6**) allowed for easy salt formation, activity versus lck dropped 9-fold. The hydroxyethyl analogue (**5**) was equipotent but solubility was only marginally improved (data not shown). Selectivity for the receptor tyrosine kinases kdr (where shown) and tie-2 is >40-fold. However, src selctivity is generally <10-fold although the implications of this lack of selectivity are currently unclear.



We realized that a carbocyclic framework directly attached to N-7 was a motif that was beneficial for lck activity and therefore elected to append 'solubilizing substituents' to this motif. A cyclohexyl motif (as opposed to the cyclopentyl moiety in **2**) was chosen to avoid inherent stereochemical issues. Compounds **10**–**13** were inhibitors of lck with the *trans* diastereoisomers **11** and **13** being more potent than the corresponding *cis* diastereoisomers. The src family and csk data (HTRF data at 1 mM ATP) for compounds **12** and **13** are highlighted in Table 3.

Table 3. Src family and csk profile[a] for **12** and **13** (IC_{50} μM)

	blk	csk	fyn	lck	lyn
12	0.37	4.27	2.03	0.24	0.43
13	0.046	0.041	0.059	0.015	0.029

[a]Mean of two experiments performed with seven concentrations of test compound.

Table 4. Pharmacokinetic parameters for **13**

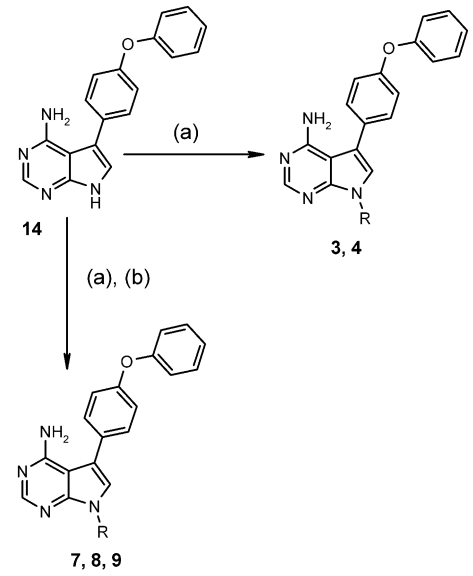
Oral dosing, 10 mg/kg			IV dosing, 5 mg/kg			
C_{max} (nM)	T_{max} (h)	AUC (ng h/mL)	$T_{1/2}$ (h)	V_z (L/kg)	Cl (L/h/kg)	F (%)
113	4	661	14.3	140	5.3	54

The overall src family selectivity for **13** is low and only ~3-fold selectivity is shown for the negative regulatory enzyme, csk. Compound **13** is inactive ($IC_{50} > 20$ μM, 1 mM ATP) against a panel of other kinases including ZAP-70, cMet, IGFR, INSR, PKC, cdc2 and PDGFR. Not withstanding the observed src family selectivity profile, compound **13** was further profiled primarily due to the aqueous solubility of the corresponding trimaleate salt, which allowed for effective in vivo screening. Compound **13** is a potent inhibitor of IL-2 production in Jurkat cells stimulated with anti-CD3 antibody ($IC_{50} = 25$ nM). In a whole blood assay, it inhibits IL-2 production with an $IC_{50} = 70$ nM. Significantly, compound **13** (trimaleate salt) inhibits TCR stimulated (anti-CD3 mAb) IL-2 production in mice (po administration, water vehicle) with an ED_{50} of 2.5 mg/kg, 10-fold more potent than **2** and utilizing a tolerable dosing regimen. Compound **13** inhibited antigen specific T-Cell immune response. After administering **13** for 3 days (po, q.d.) during the in vivo priming phase, inhibition of IFN-γ production was seen upon subsequent antigen-specific (KLH) challenge of lymphocytes from the draining lymph nodes in vitro ($ED_{50} = 2.2$ mg/kg). The pharmacokinetic parameters after a single (10 mg/kg) oral dose (water vehicle) and 5 mg/kg iv dose to male Sprague–Dawley rats for **13** are outlined in Table 4.

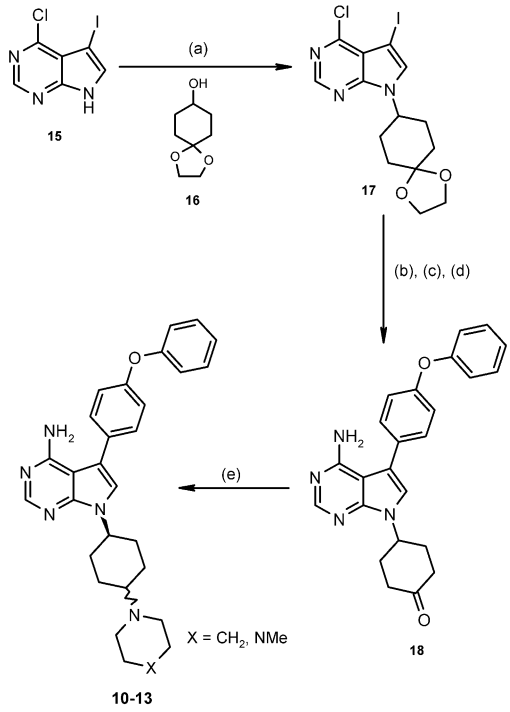
As seen in Table 4, compound **13** has improved exposure and a long half-life after oral dosing. The volume of distribution is very high with clearance slightly above hepatic blood flow. Bioavailability of **13** amounted to 54% with maximum plasma levels of 113 nM (above the whole blood assay IC_{50} for inhibition of IL-2) at a dose of 10 mg/kg po.

Further profiling of compounds related to **13** in T-cell dependent disease models will be reported subsequently.

Two routes were used to prepare the pyrrolo[2,3-*d*]pyrimidines described above. Scheme 1 starts from a previously described intermediate (**14**)[9] and involves nucleophilic displacement of the appropriate *tert*-butoxycarbonyl protected tosylate (**7**, **8** and **9**) followed by deprotection. The tosylate starting materials were prepared by standard methods. This sequence allowed access to **3**, **4**, **7**, **8**, and **9**. Compound **5** was prepared from the same intermediate by alkylation with ethylene carbonate (NaOH, DMF) in 47% yield. Compound **6** was obtained by alkylation of bromoethylphthalimide (NaH, DMF) followed by hydrazinolysis (17% over two steps). Compounds **10**–**13** were prepared by a convergent route (Scheme 2) which allows access to a more structurally diverse set of analogues.[12,13] Thus, Mitsunobu coupling of **15**[14] with the ketal-alcohol **16** (readily



Scheme 1. Reagents and conditions: (a) ROTs, NaH, DMF, 95 °C, 30–60%; (b) TFA, CH_2Cl_2, 0 °C, 90–95%.



Scheme 2. Reagents and conditions: (a) Ph_3P, DEAD, THF, 70%; (b) 4-phenoxyphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, 75%; (c) NH_4OH, dioxan, Parr pressure vessel, 120 °C, 80%; (d) acetone, 5 N HCl (aq), 92%; (e) amine, $Na(OAc)_3BH$, $ClCH_2CH_2Cl$, AcOH, 65–72%.

prepared by $NaBH_4$ reduction of the commercially available ketone) followed by a Suzuki coupling, amination and deprotection protocol furnished the intermediate ketone **18**.

Reductive amination with the appropriate amine provided a 3:1 mixture of *cis* and *trans* diastereoisomers, which were easily separated by flash silica gel column chromatography.

Conclusion

Pyrrolo[2,3-*d*]pyrimidines with a variety of substituents at N-7 are shown to be potent inhibitors of lck. One of these inhibitors, compound **13**, inhibits IL-2 production in cellular and whole blood assays. Significantly, it is bioavailable and shows oral efficacy in mice after dosing in water vehicle. Further efforts to improve overall src family selectivity and more detailed in vivo analysis of compounds related to **13** will be disclosed in due course.

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