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Pyrrole derivatives as potent inhibitors of lymphocyte-specific kinase: Structure, synthesis, and SAR

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ABSTRACT

We have described the synthesis, enzyme inhibitory activity, structure–activity relationships, and proposed binding mode of a novel series of pyrrole derivatives as lymphocyte-specific kinase (Lck) inhibitors. The most potent analogs exhibited good enzyme inhibitory activity (IC_{50}s <10 nM) for Lck kinase inhibition.

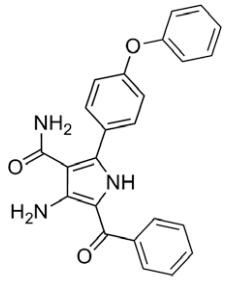
Lymphocyte-specific kinase (Lck), a Src family tyrosine kinase predominantly expressed in T lymphocytes, plays a critical role in development and activation of T-cells, including T-cell antigen receptor signaling.[1–3] Lck activity leads to production of cytokines such as interleukin-2 and IFNγ. Therefore, an inhibitor against Lck could be a potential immunosuppressive agent for treatment of inflammatory diseases such as rheumatoid arthritis, atopic dermatitis, asthma, and organ transplant rejection.

Several groups have previously reported the synthesis and characterization of Lck inhibitors.[4–12]

Our efforts identified compound **1** as a potent Lck inhibitor (Fig. 1). Molecular modeling was performed to understand the binding mode of the Lck binding site. In this study, we describe the synthesis, enzyme inhibitory activity,[13] cellular activity against mixed lymphocyte reaction (MLR[14]), and structure–activity relationships (SAR) of a series of pyrrole derivatives.

The synthesis of pyrrole derivatives is outlined in Scheme 1. Pyrrole derivatives were synthesized from commercially available 4-phenoxybenzoic acid **2**. The starting material was reacted with thionyl chloride under reflux to give the acid chloride analog. This compound was subsequently treated with malononitrile in the presence of diisopropylethylamine (DIPEA) to give compound **3**. Methylation of the hydroxyl group in compound **3** with dimethyl sulfate gave compound **4**. This compound was treated with 4-methoxybenzylamine to give compound **5**. The resulting intermediate **5** was reacted with the appropriate α-bromoacetophenone in the presence of K_2CO_3 to give compound **6**.[15] Hydrolysis of the cyano group in compound **6** with KOH gave the acid amide analog. Deprotection of the 4-methoxybenzyl group in this compound was achieved using trifluoroacetic acid (TFA)-triflic acid in the presence of dimethyl sulfide to give pyrrole derivative **1**. Alternatively, compound **6** was treated with polyphosphoric acid in the presence of thioanisole to give pyrrole derivatives.

With compound **1** as the initial lead, the effect of substituents on the benzoyl group at the 5-position of the pyrrole ring was studied, and these results are summarized in Table 1. Introduction of an *ortho* substituent on the benzoyl group resulted in an increase in potency (**7–11**) against Lck, whereas the *ortho*-methoxy substituent analog **12** showed similar potency compared to compound **1**. Introduction of *meta* or *para* substituent resulted in a slight decrease in potency compared to the corresponding *ortho* substituent



1
Lck IC_{50} = 11 nM

Figure 1. Activity for initial lead **1**.

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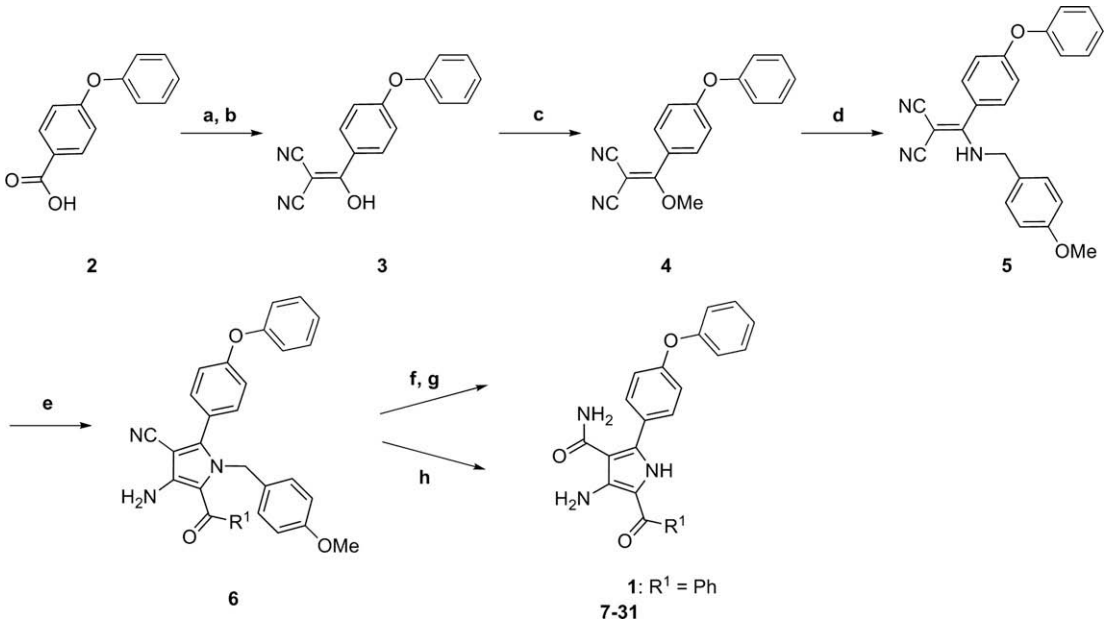
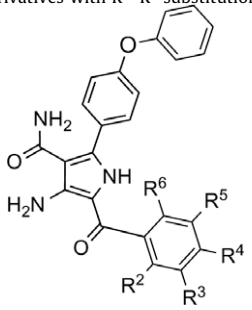


Scheme 1. Synthesis of pyrrole derivatives. Reagents and conditions: (a) $SOCl_2$, reflux; (b) malononitrile, DIPEA, toluene, THF, rt; (c) dimethyl sulfate, $NaHCO_3$, dioxane, H_2O, 100 °C; (d) 4-methoxybenzylamine, MeOH, rt; (e) R^1COCH_2Br, K_2CO_3, acetone, rt; (f) KOH, ^tBuOH, reflux; (g) dimethyl sulfide, TFA, CF_3SO_3H, 50 °C; (h) thioanisole, PPA, 100 °C.

Table 1
In vitro data of pyrrole derivatives with R^2–R^6 substitution modifications



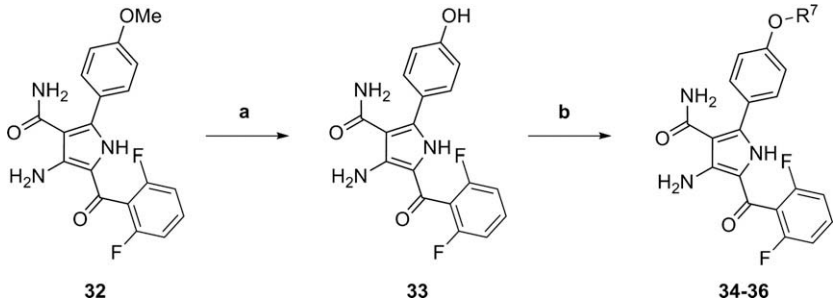
Compound	R^2	R^3	R^4	R^5	R^6	IC_{50} (nM)	
						Lck[a]	MLR[b]
1	H	H	H	H	H	11	—
7	F	H	H	H	H	1.5	—
8	Cl	H	H	H	H	1.8	69
9	Br	H	H	H	H	2.5	150
10	Me	H	H	H	H	2.7	180
11	CF_3	H	H	H	H	3.5	68
12	MeO	H	H	H	H	9.4	—
13	H	F	H	H	H	5.0	—
14	H	Cl	H	H	H	6.3	—
15	H	Br	H	H	H	6.2	—
16	H	Me	H	H	H	4.0	420
17	H	H	F	H	H	10	—
18	H	H	Cl	H	H	7.0	—
19	H	H	Br	H	H	5.9	—
20	H	H	Me	H	H	2.8	180
21	H	H	PhO	H	H	49	—
22	F	H	H	H	F	1.3	31
23	F	H	F	H	F	1.8	62
24	Cl	Cl	H	H	H	1.2	45
25	Me	Me	H	H	H	2.6	110
26	Cl	H	Cl	H	H	2.3	—
27	Me	H	Me	H	H	1.8	—
28	H	Cl	H	Cl	H	38	—
29	H	Me	H	Me	H	13	—
30	Cl	H	H	Cl	H	19	—
31	Me	H	H	Me	H	10	—

[a] Mean values from at least two independent experiments. IC_{50} values were determined from full eight-point, half-log concentration–response curves.
[b] Mean values from at least two independent experiments. IC_{50} values were determined from full nine-point, half-log concentration–response curves.

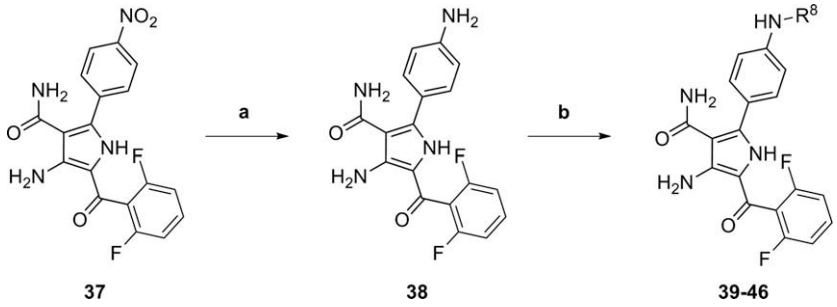
(**13–20**). In particular, the *para*-phenoxy substituent analog **21** exhibited decreased potency. As a general trend, *ortho* substitution was much more favored over *meta* or *para* substitution. Introduction of 2,3-, 2,4-, or 2,6-disubstituents showed similar potency to the corresponding *ortho* substituent analogs (**22**, **24–27** vs **7**, **8**, **10**). The 2,6-difluoro substituent analog **22** was identified as the most potent inhibitor against MLR in this series. On the other hand, both the 3,5-disubstituted analogs **28** and **29**, and the 2,5-disubstituted analogs **30 and 31** showed decreased potency.

Subsequently, we focused on developing SAR with respect to the phenoxy group. These compounds were prepared according to Schemes 2 and 3. Compound **32** was synthesized by a method similar to that described in Scheme 1, using commercially available 4-methoxybenzoic acid. Demethylation of the methoxy group in compound **32** with BBr_3 gave compound **33**. The resulting compound **33** was reacted with various halides in the presence of K_2CO_3 to give compounds **34–36**. Compound **37** was also prepared by a method similar to that described in Scheme 1, using commercially available 4-nitrobenzoic acid. Reduction of the nitro group with Fe in compound **37** gave compound **38**. This compound was treated with various halides to give compounds **40**, **42**, **44–46**. Alternatively, compound **38** was coupled to benzoic acid in the presence of WSC–HCl and HOBt–H_2O to give compound **39**. Compound **41** was prepared by reductive amination of compound **38** and cyclohexanal in the presence of $NaBH(OAc)_3$.

Table 2 outlines the SAR observed with a substituent on the phenyl group at the 2-position of the pyrrole ring. Modification of the substitution on the phenyl ring resulted in a dramatic change in potency. Replacement of the phenoxy group of compound **22** by a methoxy or pyrimidine-2-yloxy group resulted in a >100-fold loss of potency (**32** and **34**). Introduction of the benzyloxy group was tolerable (**35**); however, the 2,6-dichlorobenzyloxy analog **36** displayed significantly reduced activity. Both the amino analog **38** and the benzoylamino analog **39** were also significantly less potent. The benzylamino analog **40** was equipotent with the benzyloxy analog **35**. However, surprisingly, introduction of a 2,6-dichlorobenzylamino group resulted in an increase in potency, whereas the 2,6-dichlorobenzyloxy analog was significantly less potent (**42** vs **36**). Replacement of the benzylamino group by a cyclohexylamino group resulted in a ninefold loss of activity (**41**



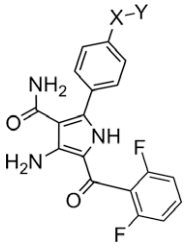
Scheme 2. Reagents and conditions: (a) BBr_3, 1,2-dichloroethane, reflux; (b) various R^7-halide, K_2CO_3, DMF, rt to 80 °C.



Scheme 3. Reagents and conditions: (a) Fe, NH_4Cl aq, 2-PrOH, 80 °C; (b) preparation of **39**: benzoic acid, WSC–HCl, HOBt–H_2O, DMF, 80 °C; preparation of **40**,**42**,**44**–**46**: various R^8-halide, Et_3N, DMF, 100 °C; preparation of **41**: cyclohexanal, AcOH, $NaBH(OAc)_3$, THF, rt.

vs **40**), and N-methylation of the aniline moiety of compound **42** led to a 100-fold loss of activity (**43**). The importance of 2,6-disubstitution in the phenyl ring of the benzylamino moiety was demonstrated by the finding that the 2,5-disubstituted analog **44** was 100-fold less potent than the 2,6-disubstituted analog **42**.

Table 2
In vitro data of pyrrole derivatives with X and Y modifications



Compound	X	Y	IC_{50} (nM)	
			Lck[a]	MLR[b]
22	O	Ph	1.3	31
32	O	Me	>100	—
34	O	2-Pyrimidinyl	>100	—
35	OCH_2	Ph	6.1	>1000
36	OCH_2	2,6-Dichlorophenyl	>100	>1000
38	NH	H	>100	>1000
39	NHCO	Ph	>100	—
40	$NHCH_2$	Ph	6.4	—
41	$NHCH_2$	Cyclohexyl	74	—
42	$NHCH_2$	2,6-Dichlorophenyl	0.92	31
43	$NMeCH_2$	2,6-Dichlorophenyl	92	>1000
44	$NHCH_2$	2,6-Dichlorophenyl	93	—
45	$NHCH_2$	2,6-Dichlorophenyl	2.7	140
46	$NHCH_2$	2-Chloro-6-methylphenyl	3.6	56

[a] Mean values from at least two independent experiments. IC_{50} values were determined from full eight-point, half-log concentration–response curves.
[b] Mean values from at least two independent experiments. IC_{50} values were determined from full nine-point, half-log concentration–response curves.

The most potent analog **22** was docked into the molecular model, which was developed based on the published X-ray co-crystal structure of Lck and imatinib (PDB code: 2PL0).[16,17] As shown in Figure 2, several hydrogen bonding interactions are thought to be involved: the 4-NH_2 of the pyrrole ring is in H-bond contact with the Met319 main-chain carbonyl, the carbonyl of the 3-carboxamide is in H-bond contact with the Met319 main-chain NH, and the NH of the 3-carboxamide is in H-bond contact with the Glu317 main-chain carbonyl. We believe that *ortho* substitution of the phenyl ring at the 5-position of pyrrole enables the phenyl ring to adopt a preferred orientation wherein the ring is skewed out of plane with respect to the pyrrole ring. This conformation allows the *ortho* substituent of the phenyl ring to fit into the hydrophobic pocket consisting of Leu251, Gly252, and Val259. Furthermore, this

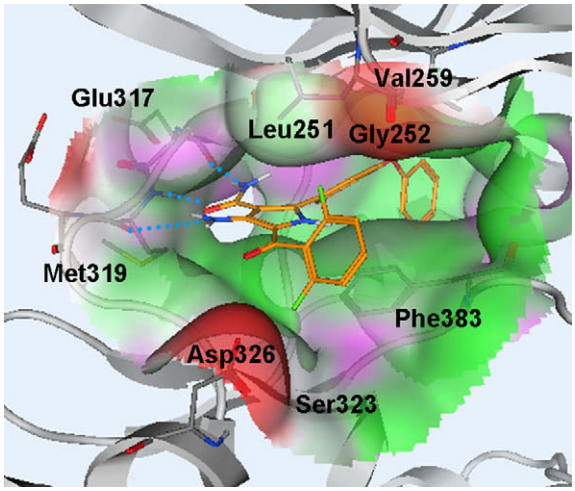


Figure 2. Model of **22** (orange) bound to the ATP-binding site of Lck. Oxygen atoms are shown in red and nitrogen atoms in blue. Hydrogen bonds are shown as cyan dotted lines.

Table 3
Kinase selectivity IC_{50} values (nM)

	LcK	Src	Csk	ZAP-70	MEK1	PKA	PKB	PKC	Abl	CAMK2	CDK1
22	1.3	23	4.5	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000

phenyl ring is presumed to be in contact with the phenyl group in the side chain of Phe383 from the DFG motif by a π–π interaction.

Compound **22**, the most potent analog, was tested for selectivity against several kinases, and these results are summarized in Table 3.[18] Compound **22** exhibited >17-fold selectivity versus the Src family member Src and >3-fold versus Csk. Compound **22** was inactive for other kinases, including ZAP-70, MEK-1, PKA, PKB, PKC, Abl, CAMK2 and CDK1 (IC_{50} >10 μM).

In summary, we have described the synthesis, enzyme inhibitory activity, SAR, and proposed Lck binding mode of a novel class of pyrrole derivatives, as represented by **1**. Optimization of **1** led to compound **22** with excellent enzymatic activity against Lck (IC_{50} = 1.3 nM).

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13. Inhibition of Lck activity was measured using a recombinant human Lck kinase domain, expressed as a GST fusion in insect cells. Lck (5 ng/well) was incubated in kinase buffer (50 mM Hepes, 50 mM KCl, 25 mM $MgCl_2$, 5 mM $MnCl_2$, 100 μM Na_3VO_4, 0.01% CHAPS, 1 mM DTT) with test compound. The final concentration of 5 μM ATP, 0.1 μCi/well γ^{33}P-ATP, and 10 μM poly (L-glutamic acid-L-tyrosine, 4:1) were added and incubated at 25 °C for 60 min and stopped with 100 μL of 100 mM phosphoric acid. The mixture was transferred to a MultiScreen-HA mixed cellulose ester membrane plate and harvested by filtration. Scintillation cocktail was added, and radioactivity was measured on a Packard Topcount instrument.
14. To isolate na T-cells from C57BL/6 mice (Charles River Japan), spleen and lymph node cell suspensions were passed through nylon wool columns. Nonadherent T-cells (3×10^5 cells/well) were cocultured with mitomycin-C treated spleen cells from BALB/c mice (Charles River Japan) (2×10^5 cells/well). These cultures were incubated at 37 °C in 5% CO_2 for 72 h. Cell proliferation was assayed by pulsing the cells with ^{3}H-thymidine for the last 4 h. ^{3}H-Thymidine incorporation into DNA was measured by Topcount. Compounds were added at the start of the culture.
15. General procedure for preparation of pyrrole derivatives (**1**, **7**–**31**). To a mixture of **5** (1 mmol) and K_2CO_3 (3 mmol) in acetone (7.5 ml) was added R^1COCH_2Br (1.2 mmol), stirred at room temperature for 15 h. H_2O was added, and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography to give **6**. Compound **6** (1 mmol) was treated with thioanisole (10 mmol) and PPA (3 g), stirred at 100 °C for 2 h, then cooled to room temperature. H_2O was added, and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography to give **1**, **7**–**31**.
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18. Lck, Src, and Csk were purchased from Upstate (NY, USA). Kinase assays for ZAP-70, MEK1, PKA, PKB, PKC, Abl, CAMK2 and CDK1 were run by Cerep (Paris, France) using the Kinase profiler service according to the manufacturer's procedures.