Supporting Information

Novel 2-Aminopyrimidine carbamates as Potent and Orally Active Inhibitors of Lck: Synthesis, SAR, and *In Vivo* Anti-inflammatory Activity

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Table S1. Combustion Analysis results

No.	Name	Formula	Calcd	Found
26	**2,6-dimethylphenyl 4-(methyloxy)phenyl(2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-4-pyrimidinyl)carbamate**	$C_{31}H_{34}N_6O_3$ ·0.9 H_2O	C, 67.10; H, 6.50; N, 15.15	C, 67.26; H, 6.87; N, 14.96
27	**2,6-dimethylphenyl 4-fluoro-2-((1-methylethyl)oxy)phenyl(2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-4-pyrimidinyl)carbamate**	$C_{33}H_{37}FN_6O_3$	C, 67.79; H, 6.38; N, 14.37	C, 68.07; H, 6.50; N, 14.23
28	**2,6-dimethylphenyl 2,4-bis(methyloxy)phenyl(2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-4-pyrimidinyl)carbamate**	$C_{32}H_{36}N_6O_4$ ·0.2 H_2O	C, 67.16; H, 6.41; N, 14.69	C, 67.10; H, 6.52; N, 14.41
29	**2,6-dimethylphenyl (2,5-bis(methyloxy)phenyl)methyl(2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-4-pyrimidinyl)carbamate**	$C_{33}H_{38}N_6O_4$	C, 68.02; H, 6.57; N, 14.42	C, 67.59; H, 6.77; N, 14.19
30	**2,6-dimethylphenyl 2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-4-pyrimidinyl(2-naphthalenyl)carbamate**	$C_{34}H_{34}N_6O_2$	C, 73.10; H, 6.13; N, 15.04	C, 72.09; H, 6.42; N, 14.21
31	**2,6-dimethylphenyl 1,1'-biphenyl-4-yl(2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-4-pyrimidinyl)carbamate**	$C_{36}H_{36}N_6O_2$	C, 73.95; H, 6.21; N, 14.37	C, 74.12; H, 6.34; N, 14.25
32	**2,6-dimethylphenyl 1,1'-biphenyl-3-yl(2-((4-(4-methyl-1-piperazinyl)phenyl)amino)-4-pyrimidinyl)carbamate**	$C_{36}H_{36}N_6O_2$ ·0.9 H_2O	C, 71.95; H, 6.34; N, 13.99	C, 72.09; H, 6.16; N, 13.70
35	**2,6-dimethylphenyl 2,4-bis(methyloxy)phenyl(2-((3-fluoro-4-(4-methyl-1-piperazinyl)phenyl)amino)-4-pyrimidinyl)carbamate**	$C_{32}H_{35}FN_6O_4$	C, 65.51; H, 6.01; N, 14.33	C, 65.53; H, 6.12; N, 14.13
36	**2,6-dimethylphenyl 2,4-bis(methyloxy)phenyl(2-((4-((2-(dimethylamino)ethyl)-oxy)phenyl)amino)-4-pyrimidinyl)carbamate**	$C_{31}H_{35}N_5O_5$	C, 66.77; H, 6.33; N, 12.56	C, 66.78; H, 6.42; N, 12.28
38	**2,6-dimethylphenyl 2,4-bis(methyloxy)phenyl(2-((3-fluoro-4-((2-(4-methyl-1-piperazinyl)ethyl)oxy)phenyl)amino)-4-pyrimidinyl)carbamate**	$C_{34}H_{39}FN_6O_5$ ·0.5 H_2O	C, 63.84; H, 6.30; N, 13.14	C, 63.89; H, 6.23; N, 12.83

39	**2,6-dimethylphenyl 2-((3,5-bis(methyloxy)-4-((2-(4-methyl-1-piperazinyl)ethyl)oxy)phenyl)amino)-4-pyrimidinyl(2,4-bis(methyloxy)phenyl)carbamate**	$C_{36}H_{44}N_6O_7$ ·0.7 H_2O	C, 63.09; H, 6.68; N, 12.26	C, 63.00; H, 6.71; N, 12.20
40	**2,6-dimethylphenyl 2-((3,5-bis(methyloxy)-4-((2-(4-morpholinyl)ethyl)oxy)phenyl)amino)-4-pyrimidinyl(2,4-bis(methyloxy)phenyl)carbamate**	$C_{35}H_{41}N_5O_8$ ·0.7 H_2O	C, 62.52; H, 6.36; N, 10.42	C, 62.57; H, 6.32; N, 10.37
41	**2,6-dimethylphenyl 2,4-bis(methyloxy)phenyl(2-((4-((3-(1-piperidinyl)propyl)oxy)phenyl)amino)-4-pyrimidinyl)carbamate**	$C_{35}H_{41}N_5O_5$	C, 68.72; H, 6.76; N, 11.45	C, 68.78; H, 7.09; N, 11.10
43	**2,6-dimethylphenyl 2-((3,5-bis(methyloxy)-4-((3-(4-methyl-1-piperazinyl)propyl)-oxy)phenyl)amino)-4-pyrimidinyl(2,4-bis(methyloxy)phenyl)carbamate**	$C_{37}H_{46}N_6O_7$ ·0.3 H_2O	C, 64.20; H, 6.79; N, 12.14	C, 64.19; H, 6.89; N, 12.25
44	**2,6-dimethylphenyl 2,4-bis(methyloxy)phenyl(2-((3-fluoro-4-((3-(4-methyl-1-piperazinyl)propyl)oxy)phenyl)amino)-4-pyrimidinyl)carbamate**	$C_{35}H_{41}FN_6O_5$ ·0.5 H_2O	C, 64.30; H, 6.48; N, 12.86	C, 64.27; H, 6.59; N, 12.77
45	**2,6-dimethylphenyl 2,4-bis(methyloxy)phenyl(2-((3-fluoro-4-((3-(1-piperidinyl)propyl)oxy)phenyl)amino)-4-pyrimidinyl)carbamate**	$C_{35}H_{40}FN_5O_5$ ·0.25 H_2O	C, 66.28; H, 6.44; N, 11.04	C, 66.27; H, 6.54; N, 10.95

Crystal structure determination. The kinase domain of human Lck (residues 225-501) with a C-terminal His6 tag was expressed in insect cells and purified by immobilized metal affinity, anion exchange, and size exclusion chromatographies. The protein was then phosphorylated by incubation with of 5 mM Mg^{++}·ATP for 10 min at room temperature. Phosphorylated Lck was further purified by anion exchange chromatography and concentrated to 10 mg/ml. Crystals were grown by vapor diffusion at 4 ºC. Equal volumes of protein were mixed with a well solution consisting of 25-35 % (w/v) PEG 4000, 0.2 or 0.4 M lithium sulfate, and 0.1 M Tris (pH 8.5). These crystals belong to space group $P2_12_12_1$ with unit cell dimensions a = 42.43 Å, b = 73.53 Å, and c = 92.81 Å. There is one molecule per asymmetric unit. Diffraction data of the crystals of Lck with compound **43** were collected on Raxis IV^{++} mounted on FR-E generator and processed using DENZO and SCALEPACK. (Otwinowski & Minor, 1997). The structure was solved by molecular replacement using AMORE (Navaza, 1994). Refinements were performed with CNX and model building was done in QUANTA (Accelrys, San Diego, CA). Data collection and refinement statistics are presented in Table S2.

References

Otwinowski, Z.; Minor, W. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.* **1997**, *276*, 307-326.

Navaza, J. *AMoRe*: an automated package for molecular replacement. *Acta Crystallogr. A* **1994**, *50*, 157-163.

Table S2. Data collection and refinement statistics

	Lck + Compound **43**
Data Collection	
Resolution (Å)	30-2.00 (2.07-2.00)
Total reflections	57666
Unique reflections	20131
Completeness (%)	99.3 (99.6)
R_{merge}	0.042 (0.111)
I/σ(I)	21.0 (11.3)
Refinement	
Reflections used	20293
R_{cryst}	0.228
R_{free}	0.291
Average B-value ($Å^2$)	16.1
Number of protein atoms	2208
Number of ligand atoms	51
Number of sulfate ions	1
Number of solvent atoms	312
r.m.s.d. bonds (Å)	0.012
r.m.s.d. angles (º)	1.57