Multiple determinants for selective inhibition of apicomplexan calcium-dependent protein kinase CDPK1 - Supporting Information

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Table S1: Crystallographic data collection and refinement statistics for TgCDPK1 complexes.

	2a	**3a**	**2b**
Data collection			
Space Group	$P2_1$	$P2_1$	$P2_1$
a, b, c (Å)	47.97, 71.93, 66.62	47.45, 72.24, 65.82	47.63, 72.66, 66.10
α,β,γ (°)	90., 103.47, 90.	90., 99.18, 90.	90., 99.04, 90.
Wavelength (Å)	0.9762	0.9762	0.9795
Resolution (Å)	50-2.04 (2.11-2.04)	50-2.04 (2.09-2.04)	60-2.10 (2.16-2.10)
Unique reflections	28208	28166	26082
R_{merge}	0.076 (0.732)	0.064 (0.695)	0.095 (0.887)
mean $I/\sigma(I)$	17.45(1.7)	21.5 (1.9)	14.4 (1.5)
Completeness (%)	99.9 (99.9)	99.8 (98.9)	99.9 (99.4)
Redundancy	3.7 (3.7)	3.8 (3.7)	3.8 (3.8)
Wilson B factor (Å^2)	31	35	32
Refinement			
Resolution (Å)	39-2.04	39-2.04	39-2.10
Reflections	28140	28099	26055
Reflections (test set)	1470	1432	1328
R_{work}/R_{free}	0.197/0.240	0.201/0.234	0.194/0.237
No. atoms			
Protein	3632	3614	3629
Ligand	29	34	45
Water	96	76	111
TLS groups	6	5	7
mean B_{eq} (Å^2)	41	51	42
R.m.s. non-ideality			
Bond lengths (Å)	0.008	0.006	0.010
Bond angles (°)	0.966	0.850	1.078
PDB entry	3t3v	3sxf	3t3u

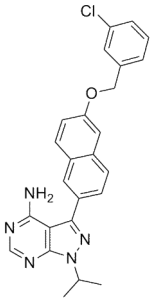
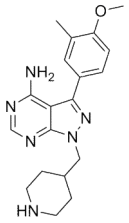
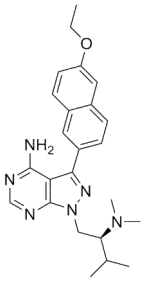
Table S1 (cont): Crystallographic data collection and refinement statistics for TgCDPK1 complexes.

	3b	**7b**	**10**
Data collection			
Space Group	$P2_1$	$P2_1$	$P2_1$
a, b, c (Å)	48.28, 72.48, 67.27	48.27, 72.79, 67.29	48.29, 72.93, 66.92
α,β,γ (°)	90. 103.81, 90.	90., 103.49, 90.	90., 101.02, 90.
Wavelength (Å)	0.9795	0.9795	0.9795
Resolution (Å)	47-2.65 (2.71-2.64)	37-2.20(2.32-2.20)	36-2.27 (2.39-2.27)
Unique reflections	13349	22898	20869
R_{merge}	0.145 (0.857)	0.084 (0.498)	0.089 (0.615)
mean $I/\sigma(I)$	9.8 (1.5)	9.5 (2.3)	6.9 (1.9)
Completeness (%)	99.5 (92.6)	99.1 (93.6)	98.6 (98.1)
Redundancy	3.8 (3.7)	4.0 (3.7)	4.1 (4.1)
Wilson B factor (Å^2)	50	33	40
Refinement			
Resolution (Å)	47-2.65	37-2.20	37-2.27
Reflections	13331	21708	19795
Reflections (test set)	677	1171	1073
R_{work}/R_{free}	0.197/0.244	0.191/0.251	0.204/0.264
No. atoms			
Protein	3664	3698	3528
Ligand	30	17	25
Water	26	65	36
TLS groups	0	7	6
mean B_{eq} (Å^2)	45	46	43
R.m.s. non-ideality			
Bond lengths (Å)	0.007	0.005	0.007
Bond angles (°)	0.907	0.890	1.093
PDB entry	3sx9	3upz	3upy

Table S2: Crystallographic data collection and refinement statistics for c-SRC complexes.

	3a	**7b**
Data collection		
Space Group	*P*1	*P*1
a, b, c (Å)	41.79, 63.24, 73.30	42.54, 63.72, 74.98
α,β,γ (°)	79.32, 89.35, 89.88	78.17, 89.34, 90.08
Wavelength (Å)	0.9795	0.9795
Resolution (Å)	41-2.27 (2.39-2.27)	62-2.20 (2.32-2.20)
Unique reflections	32963 (4766)	38121 (5485)
R_{merge}	0.101 (0.666)	0.114 (0.893)
mean $I/\sigma(I)$	8.3 (2.0)	7.2 (1.3)
Completeness (%)	96.8 (95.7)	97.5 (96.7)
Redundancy	3.9 (3.9)	4.0 (4.0)
Wilson B factor (Å^2)	38	36
Refinement		
Resolution (Å)	36-2.27	53-2.20
Reflections	31291	36215
Reflections (test set)	1672	1905
R_{work}/R_{free}	0.227/0.256	0.229/0.259
No. atoms		
Protein	4058	4132
Ligand	52	34
Water	70	28
TLS groups	5 per chain	1 per chain
mean B_{eq} (Å^2)	35	56
R.m.s. non-ideality		
Bond lengths (Å)	0.008	0.009
Bond angles (°)	1.194	1.231
PDB entry	3uqf	3uqg

Table S3: *In vitro* activity for additional compounds shown in Figure 1d

			
	11	**12**	**13**
TgCDPK1 K_i(μM)	0.0028	0.0011	0.006

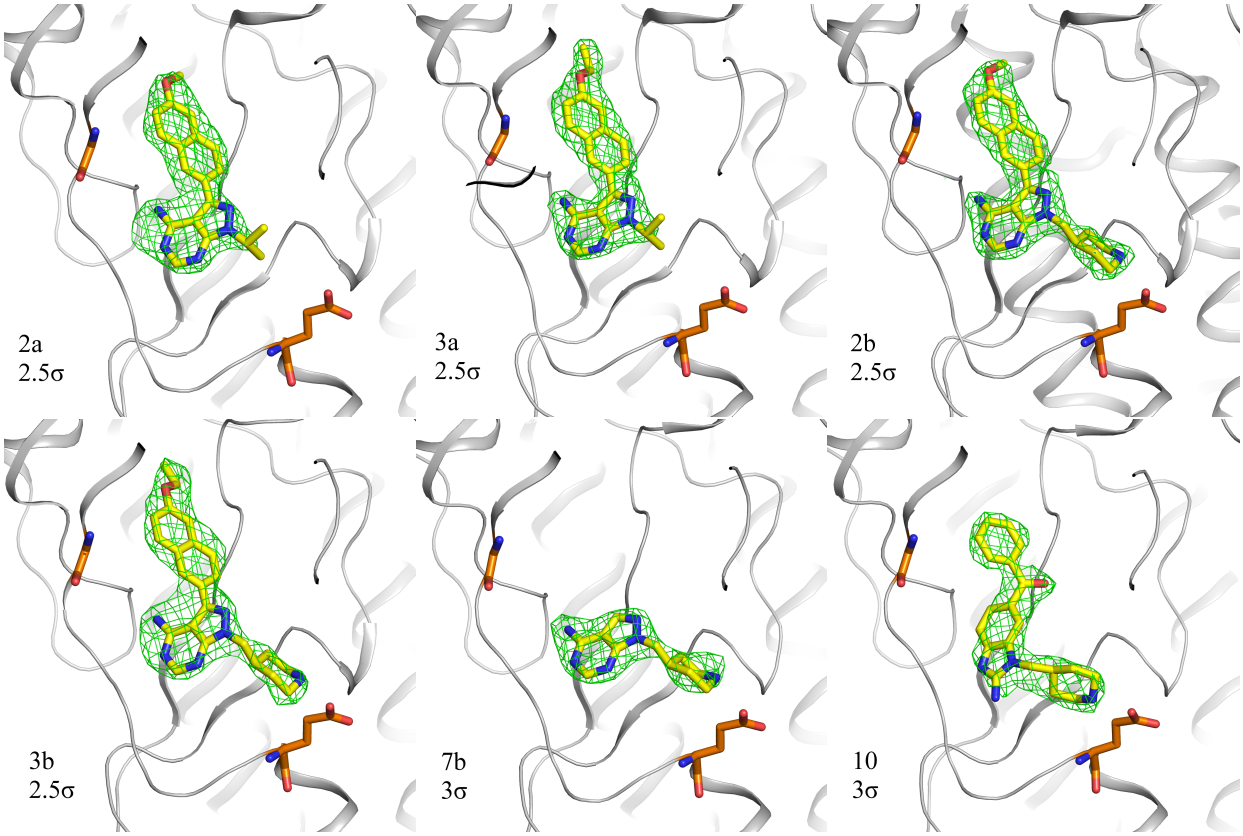


Figure S1: Difference electron density for inhibitors in *Tg*CDPK1:inhibitor complexes. In each case the density contours are from an $(mF_{obs} - F_{calc})$ Fourier synthesis after refinement of the model with no inhibitor atoms contributing to F_{calc}.

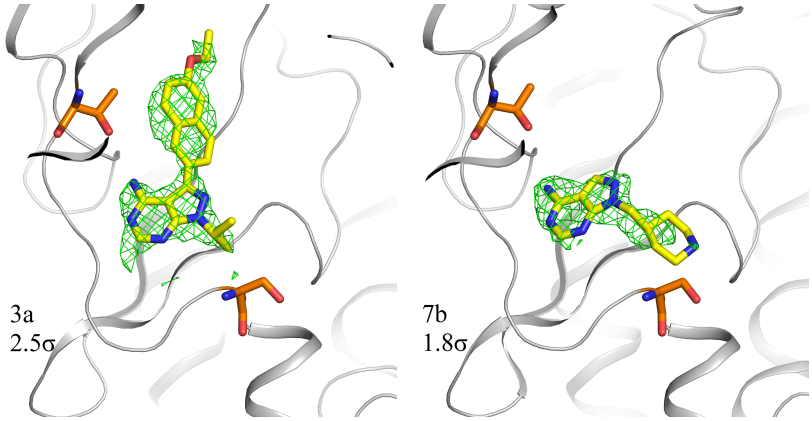


Figure S2: Difference electron density for inhibitors in SRC:inhibitor complexes. In each case the density contours are from an $(mF_{obs} - F_{calc})$ Fourier synthesis after refinement of the model with no inhibitor atoms contributing to F_{calc}.

Experimental

Compound **8** was purchased from SigmaAldrich. Compound **9** was synthesized according to the literature.[1] ^{1}H NMR (500 MHz, DMSO) δ 7.64 (d, J = 5.7 Hz, 2H), 7.61 – 7.54 (m, 1H), 7.54 – 7.46 (m, 3H), 7.36 (dd, J = 8.2, 1.6 Hz, 1H), 7.15 (d, J = 8.2 Hz, 1H), 6.60 (s, 2H). MS (ESI) 238.3 *m/z* [MH^+]. Compound **10** was synthesized according to the literature by using (3-fluoro-4-nitrophenyl)(phenyl)methanone and tert-butyl 4-(aminomethyl)piperidine-1-carboxylate.[2] ^{1}H NMR (500 MHz, MeOD) δ 7.98 (s, 1H), 7.79 (d, J = 7.1 Hz, 2H), 7.77 – 7.72 (m, 1H), 7.68 (t, J = 7.3 Hz, 1H), 7.61 – 7.50 (m, 3H), 4.20 (d, J = 9.4 Hz, 2H), 3.44 (d, J = 12.6 Hz, 2H), 2.98 (t, J = 11.1 Hz, 2H), 2.40 – 2.26 (m, 1H), 1.95 (m, 2H), 1.67 (m, 2H). MS (ESI) 335.4 *m/z* [MH^+].

References

(1) Ohemeng, K. A.; Roth, B. Receptor-based design of novel dihydrofolate reductase inhibitors: benzimidazole and indole derivatives. *J. Med. Chem.* **1991**, *34*, 1383–1394.

(2) McGuinness, B. F.; Cole, A. G.; Dong, G.; Brescia, M. R.; Shao, Y.; Henderson, I.; Rokosz, L. L.; Stauffer, T. M.; Mannava, N.; Kimble, E. F.; Hicks, C.; White, N.; Wines, P. G.; Quadros, E. Discovery of 2-aminoimidazopyridine adenosine A(2A) receptor antagonists. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6845–6849.