Comparative structural and functional studies of 4-(thiazol-5-yl)-2-(phenylamino)pyrimidine-5-carbonitrile CDK9 inhibitors suggest the basis for isotype selectivity

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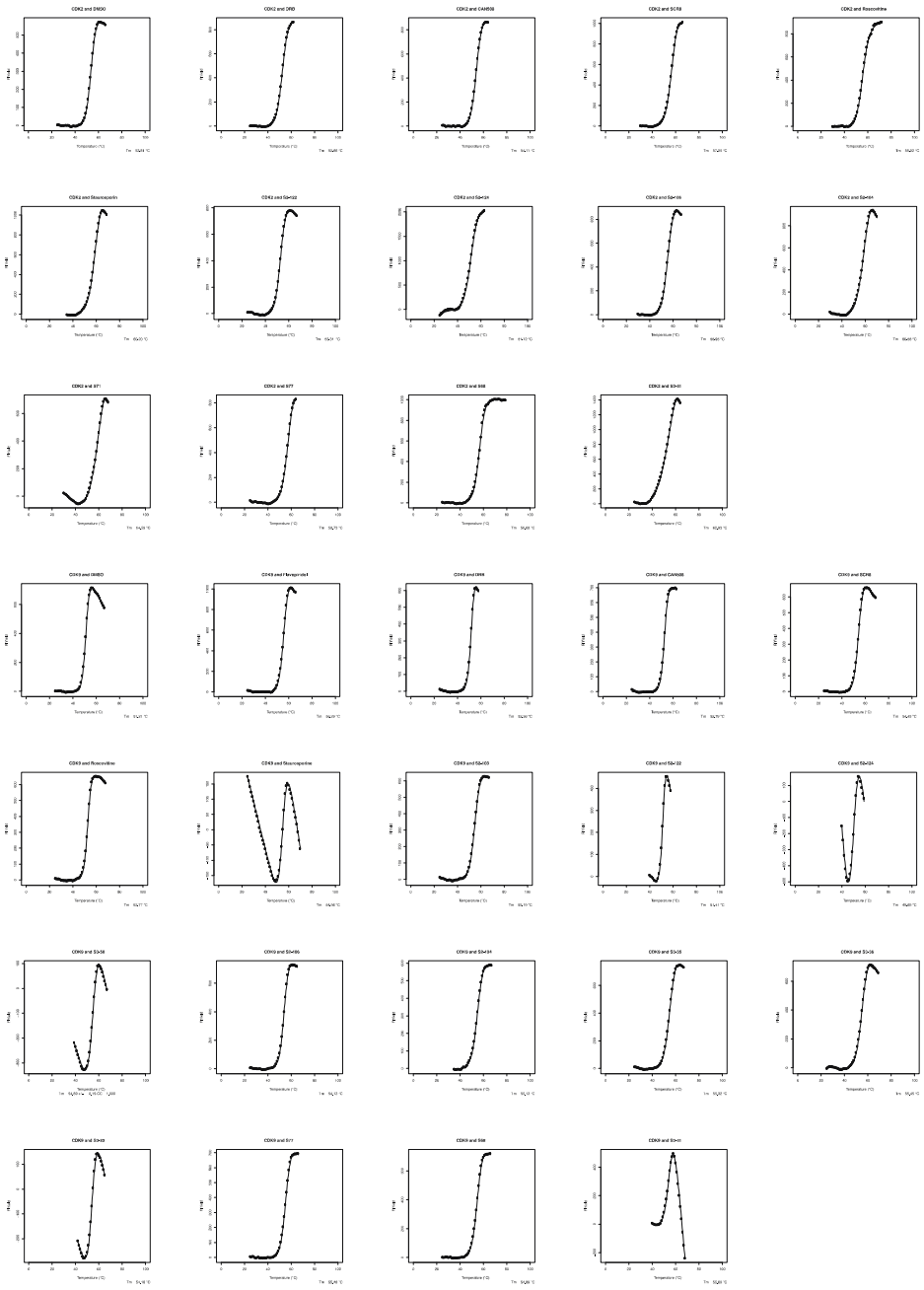
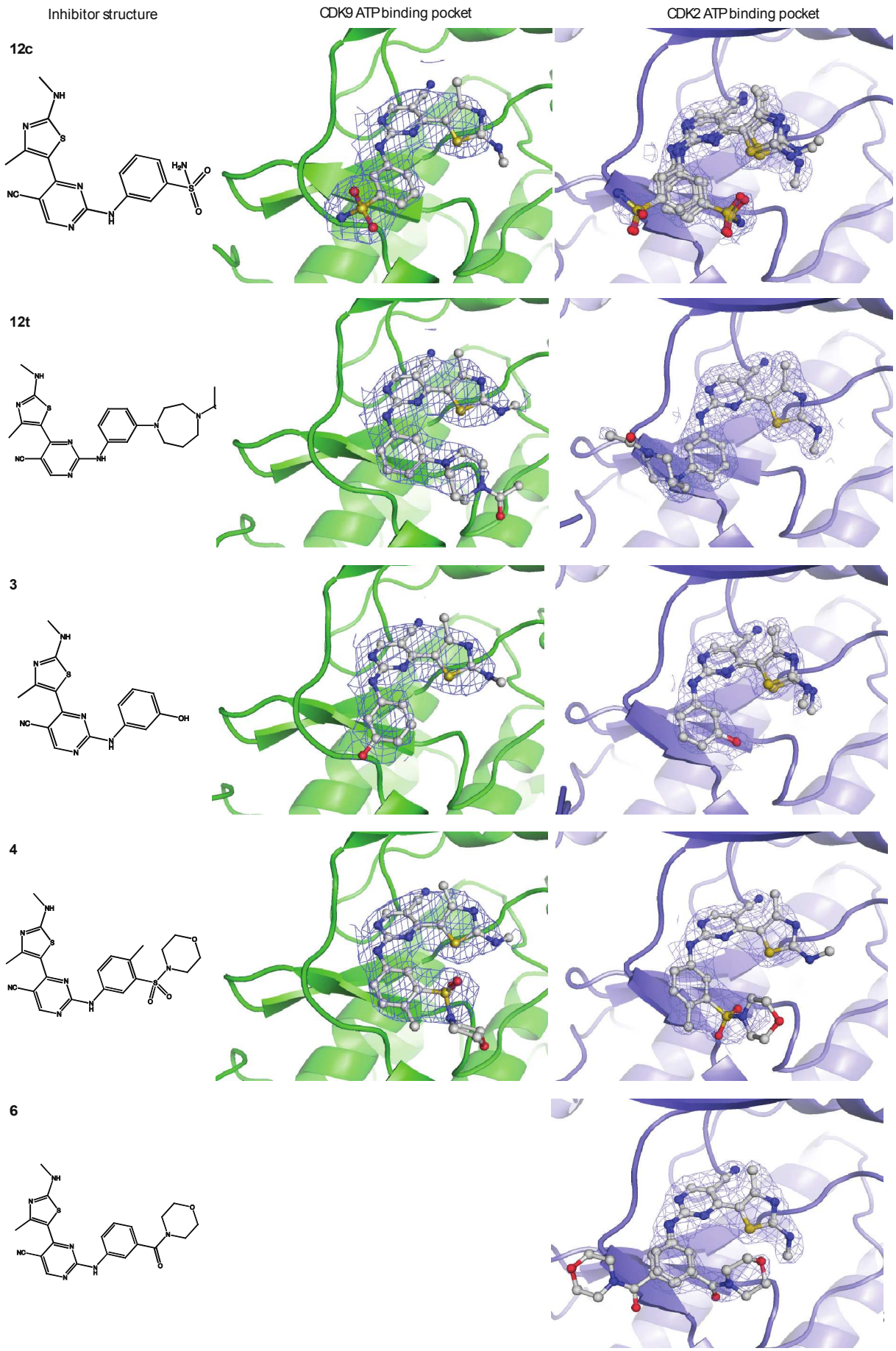
SUPPLEMENTARY FIGURES



Supplemental Figure S1. Representative thermal denaturation profiles. Thermal denaturation curves of CDK2/cyclin A and CDK9/cyclin T in the presence of inhibitors. Original data points and the fitted curve are shown for one representative assay. Figure associated with Figure 1.


Inhibitor structure
CDK9 ATP binding pocket
CDK2 ATP binding pocket
12c
12t
3
4
6

Supplemental Figure S2. Inhibitor binding to CDK9$_{330}$/cyclin T and CDK2/cyclin A. For each inhibitor the chemical structure is drawn together with their structures as bound within the ATP binding site of CDK9$_{330}$/cyclin T (middle panels) and CDK2/cyclin A (RHS panels). CDK9$_{330}$/cyclin Tand CDK2/cyclin A are drawn in ribbon representation and coloured green and blue respectively. The inhibitor electron density in the final $2mF_o$-DF_c maps is contoured at 1σ. The same view is taken of each co-complex structure. The electron density maps of **12c** and **6** bound to CDK2/cyclin A support a model in which the inhibitors adopt two alternative conformations within the CDK2 active site.

SUPPLEMENTARY TABLES

Supplementa1 Table S1. Crystallographic data collection and refinement statistics.

	CDK2/cyc A/**12c**	CDK2/cyc A/**12t**	CDK2/cyc A/**3**
Data collection			
Beam line	Diamond I-04	Diamond I-03	Diamond I-02
Space group & unit cell (Å)	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
	a=77.20; b=140.37; c=155.17 α=β=γ=90°	a=73.90; b=133.81; c=148.38 α=β=γ=90°	a=74.05; b=135.08; c=148.19 α=β=γ=90°
Resolution (highest resolution shell) (Å)	29.86-2.05	52.36-2.05	49.91-2.10
	(2.16-2.05)	(2.16-2.05)	(2.21-2.10)
Total observations	381071 (37285)	296524 (43881)	350905 (51105)
Unique	101828 (12695)	92057 (13331)	87330 (12605)
R_{merge}	0.044 (0.544)	0.080 (0.537)	0.085 (0.549)
Multiplicity	3.7 (2.9)	3.2 (3.3)	4.0 (4.1)
Mean I/s_I	17.9 (2.0)	8.6 (2.0)	11.9 (2.5)
Completeness (%)	96.5 (83.7)	99.2 (99.4)	99.9 (99.9)
Refinement Statistics			
(highest resolution shell) (Å)	(2.08-2.05)	(2.07-2.05)	(2.12-2.10)
Total number of atoms	9877	9356	9678
Number of waters	629	519	687
R	19.32 (32.17)	18.87 (24.92)	18.38 (25.19)
R_{free}	23.58 (37.47)	22.60 (28.48)	21.91 (28.26)
Rms bonds (Å)	0.023	0.005	0.004
Rms angles (°)	1.675	0.902	0.774

	CDK2/cyc A/**4**	CDK2/cyc A/**6**	CDK9/cyc T/**12c**
Data collection			
Beam line	Diamond I-02	Diamond I-04	Diamond I-02
Space group & unit cell (Å)	$P2_12_12_1$	$P2_12_12_1$	H3
	a=74.07; b=135.41; c=148.63 α=β=γ=90°	a=77.08; b=141.16; c=155.52 α=β=γ=90°	a=b=174.11; c=99.26 α=β=90°; γ=120°
Resolution (highest resolution shell) (Å)	59.54-2.45 (2.58-2.45)	52.26-2.40 (2.53-2.40)	60.04-3.10 (3.27-3.10)
Total observations	191491 (28077)	209450 (25810)	73375 (10822)
Unique	55186 (7979)	65528 (8880)	20202 (2943)
R_{merge}	0.107 (0.534)	0.127 (0.464)	0.064 (0.593)
Multiplicity	3.5 (3.5)	3.2 (2.9)	3.6 (3.7)
Mean I/s_I	7.3 (2.1)	5.1 (1.7)	10.8 (2.0)
Completeness (%)	99.3 (99.7)	97.9 (93.0)	99.3 (99.5)
Refinement Statistics			
(highest resolution shell) (Å)	(2.51-2.45)	(2.43-2.40)	(3.18-3.10)
Total number of atoms	9111	9271	4597
Number of waters	287	331	8
R	19.59 (32.07)	21.52 (32.05)	17.77 (32.6)
R_{free}	25.80 (37.14)	25.18 (37.48)	22.13 (42.9)
Rms bonds (Å)	0.006	0.004	0.009
Rms angles (°)	0.964	0.800	1.125

Supplemental Table S1

	CDK9/cyc T/**12t**	CDK9/cyc T/**3**	CDK9/cyc T/**4**
Data collection			
Beam line	Diamond I-03	ESRF ID14-EH4	Diamond I-03
Space group & unit cell (Å)	H3	H3	H3
	a=b=172.32; c=98.82	a=b=174.59; c=99.42	a=b=174.18; c=98.30
	α=β=90°; γ=120°	α=β=90°; γ=120°	α=β=90°; γ=120°
Resolution (highest resolution shell) (Å)	82.39-3.01 (3.17-3.01)	50.40-3.16 (3.33-3.16)	87.09-2.96 (3.12-2.96)
Total observations	61068 (8985)	69409 (9897)	121625 (18367)
Unique	20836 (3057)	19246 (2800)	23220 (3407)
R_{merge}	0.049 (0.386)	0.104 (0.548)	0.068 (0.581)
Multiplicity	2.9 (2.9)	3.6 (3.5)	5.2 (5.4)
Mean I/s_I	13.5 (2.4)	8.2 (2.1)	16.3 (2.8)
Completeness (%)	96.2 (96.6)	99.6 (99.0)	99.9 (99.9)
Refinement Statistics			
(highest resolution shell) (Å)	(3.01-3.09)	(3.24-3.16)	(2.96-3.04)
Total number of atoms	4597	4598	4617
Number of waters	14	6	13
R	16.65 (25.7)	15.94 (29.8)	16.67 (28.2)
R_{free}	22.44 (25.2)	20.73 (33.2)	20.97 (31.8)
Rms bonds (Å)	0.011	0.010	0.011
Rms angles (°)	1.556	1.379	1.566