Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supplementary Information

Molecular Modeling studies to characterize *N*-phenylpyrimidin-2-amines selectivity for CDK2 and CDK4 through 3D-QSAR and molecular dynamics simulations

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List of figures

List of tables

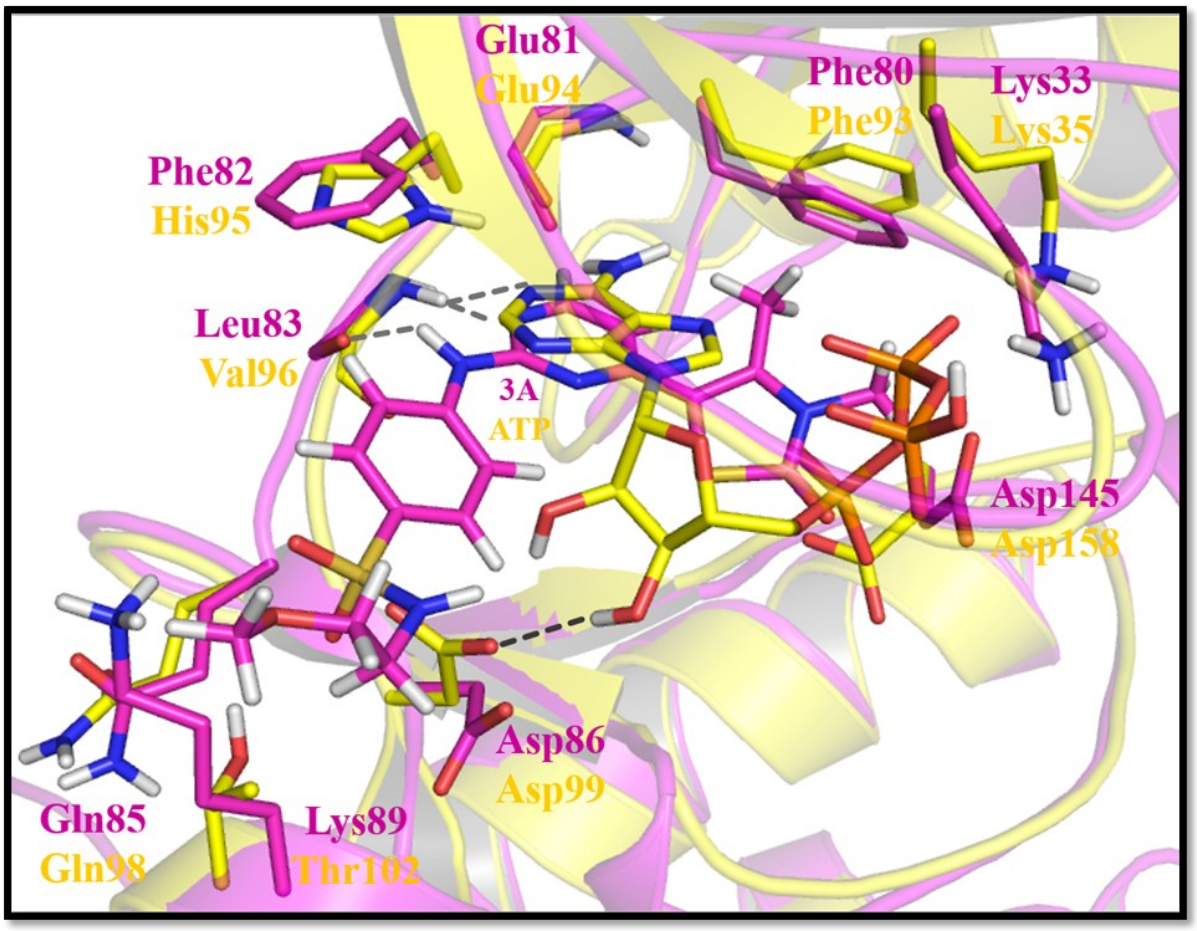
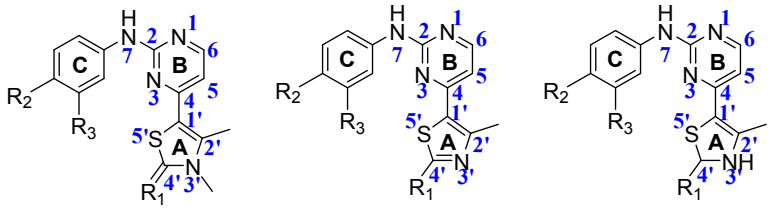


Figure. S1. Structure comparison between co-crystal structure of CDK4-ATP (yellow) and superposed over co-crystal structure of CDK2-3A (magenta).

Table S1. Structures, experimental and predicted inhibitory activities of the 3D-QSAR modeling data sets.



Lig.	R1	R2	R3	R4	CDK2						CDK4					
					Ki (μM)	pKi	CoMFA		CoMSIA		Ki	pKi	CoMFA		CoMSIA	
							Pred	Res	Pred	Res			Pre	Resd	Pred	Resid
Training Set Compounds																
2A	O	H	CN	H	0.0005	9.3	9.465	-0.185	9.037	0.243	0.1	7	7.015	-0.025	7.161	-0.171
3A	O	$SO_2NH(CH_2)_2OMe$	H	H	0.0001	10	9.969	-0.009	10.101	-0.141	NA	NA	NA	NA	NA	NA
4B	NHMe	H	SO_2Me	H	0.002	8.7	8.423	0.277	8.296	0.404	0.09	7	6.883	0.147	6.901	0.129
5B	NHMe	H	SO_2NH_2	H	0.002	8.7	8.524	0.176	8.694	0.006	0.01	8.1	8.181	-0.131	7.987	0.063
7B	NHMe	H	SO_2NHMe	H	0.033	7.5	7.515	-0.035	7.594	-0.114	0.02	7.7	7.621	0.079	7.682	0.018
8B	NH_2	H	SO_2NHMe	H	0.026	7.6	7.639	-0.049	7.492	0.098	0.16	6.8	6.805	-0.015	6.782	0.008
9B	NHEt	H	SO_2Me	H	0.008	8.1	8.091	0.009	8.244	-0.144	0.14	6.9	6.92	-0.06	6.871	-0.011
10B	NH_2	H	SO_2NHEt	H	0.047	7.3	7.141	0.189	7.435	-0.105	0.39	6.4	6.349	0.061	6.611	-0.201
11B	Me	H	H	H	0.08	7.1	6.733	0.367	6.692	0.408	2.6	5.6	5.728	-0.138	5.588	0.002
12B	Me	H	Cl	H	0.67	6.2	6.488	-0.318	6.537	-0.367	9.4	5	5.081	-0.051	5.289	-0.259
14B	Me	H	F	H	0.1	7	6.715	0.285	6.67	0.33	0.9	6.1	5.862	0.188	5.817	0.233
16B	Me	H	OH	H	0.06	7.2	7.383	-0.163	7.096	0.124	0.21	6.7	6.503	0.177	6.58	0.1
17B	NHMe	H	OH	H	0.11	7	7.176	-0.216	7.108	-0.148	0.37	6.4	6.412	0.018	6.225	0.205
18B	Me	OH	H	H	0.14	6.9	6.796	0.054	6.803	0.047	0.32	6.5	6.376	0.114	6.465	0.025
19B	NHMe	OH	H	H	0.07	7.2	7.085	0.065	7.207	-0.057	0.32	6.5	6.533	-0.043	6.544	-0.054
22B	NMe_2	H	NO_2	H	0.06	7.2	7.265	-0.045	7.43	-0.21	5.3	5.3	5.398	-0.118	5.459	-0.179

24B	NHAll	H	NO_2	H	0.16	6.8	6.929	-0.129	6.61	0.19	7.1	5.2	5.083	0.067	4.94	0.21
26B	Me	NO_2	H	H	4.1	5.4	5.451	-0.061	5.312	0.078	NA	NA	NA	NA	NA	NA
27B	Me	H	NH_2	H	0.4	6.4	6.141	0.259	6.326	0.074	10.6	5	4.923	0.047	4.876	0.094
28B	Me	H	CN	H	0.3	6.5	6.766	-0.246	6.83	-0.31	0.48	6.3	6.461	-0.141	6.292	0.028
29B	Me	NMe_2	H	H	0.22	6.7	7.21	-0.55	7.045	-0.385	0.96	6	6.272	-0.252	6.203	-0.183
30B	Me	NMe_2	NO_2	H	0.02	7.7	7.326	0.374	7.463	0.237	0.27	6.6	6.305	0.265	6.433	0.137
31B	Me	NMe_2	Cl	H	0.1	7	7.072	-0.072	6.985	0.015	1	6	6.108	-0.108	6.175	-0.175
34C	CN	H	NO_2	H	0.14	6.9	6.887	-0.037	7.021	-0.171	0.96	6	6.331	-0.311	6.327	-0.307
35C	CN	Me	NO_2	H	0.05	7.3	7.133	0.167	7.182	0.118	0.18	6.7	6.542	0.198	6.465	0.275
37C	CN	Me	OH	H	0.13	6.9	7.014	-0.124	7.142	-0.252	0.22	6.7	6.654	0.006	6.646	0.014
38C	CN	F	Me	H	0.09	7.1	7.001	0.049	7.059	-0.009	0.3	6.5	6.429	0.091	6.479	0.041
40C	Cl	F	H	H	0.36	6.4	6.553	-0.113	6.541	-0.101	1.91	5.7	5.66	0.06	5.739	-0.019
41C	Br	F	H	H	0.27	6.6	6.48	0.09	6.424	0.146	1.6	5.8	5.898	-0.098	5.8	0
Test Set Compounds																
1A	O	Me	NO_2	H	0.018	7.7	7.911	-0.171	8.737	-0.997	0.06	7.2	7.167	0.053	6.841	0.379
6B	NHEt	H	SO_2NH_2	H	0.304	6.5	6.825	-0.305	7.48	-0.96	0.15	6.8	6.641	0.189	6.397	0.433
13B	Me	H	H	F	1.2	5.9	6.045	-0.125	6.513	-0.593	1.2	5.9	5.594	0.526	5.451	0.469
15B	Me	CF_3	H	H	0.29	6.5	7.043	-0.503	7.075	-0.535	NA	NA	NA	NA	NA	NA
20B	NH_2	OH	H	H	0.038	7.4	7.494	-0.074	7.134	0.286	0.32	6.5	5.966	0.524	6.218	0.272
21B	Me	H	NO_2	H	0.11	7	7.055	-0.095	7.083	-0.123	NA	NA	NA	NA	NA	NA
23B	NHMe	H	NO_2	H	0.8	6.1	6.184	-0.084	7.016	-0.916	1.6	5.8	5.8	0	5.651	0.149
25B	NH_2	H	NO_2	H	0.002	8.7	8.196	0.504	7.961	0.739	0.05	7.3	6.987	0.293	6.493	0.787
32B	NH_2	NMe_2	H	H	0.7	6.2	6.354	-0.204	6.566	-0.416	0.9	6.1	6.122	-0.072	6.16	-0.11
33C	H	H	NO_2	H	0.05	7.3	7.638	-0.338	6.755	0.545	4.17	5.4	6.182	-0.802	5.942	-0.562
36C	CN	H	OH	H	0.03	7.5	6.801	0.719	7.126	0.394	0.18	6.7	6.931	0.809	6.675	0.065
39C	CN	F	H	H	0.1	7	6.487	0.513	6.814	0.186	0.9	6.1	5.971	0.079	6.109	-0.059

*Compounds in the test set for 3D-QSAR models. Ki stands for inhibition constant values. Pred stands for predicted Ki values, Res stands for difference between actual and predicted Ki values.

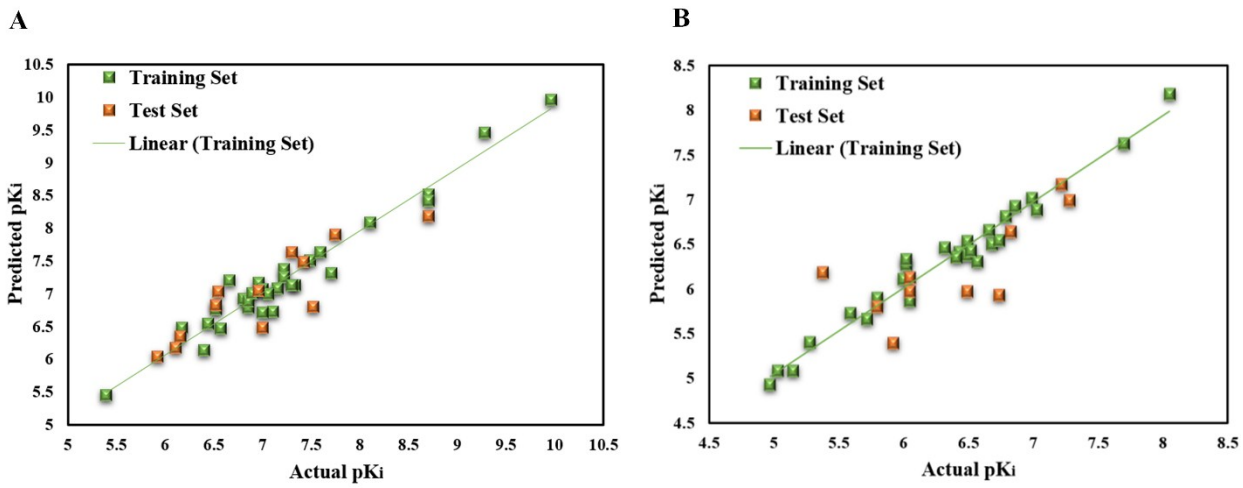


Figure S2. Correlation between actual and predicted activities of training (green) and test (orange) set compounds (CoMFA Model) associated with CDK2 (A) and CDK4 (B).

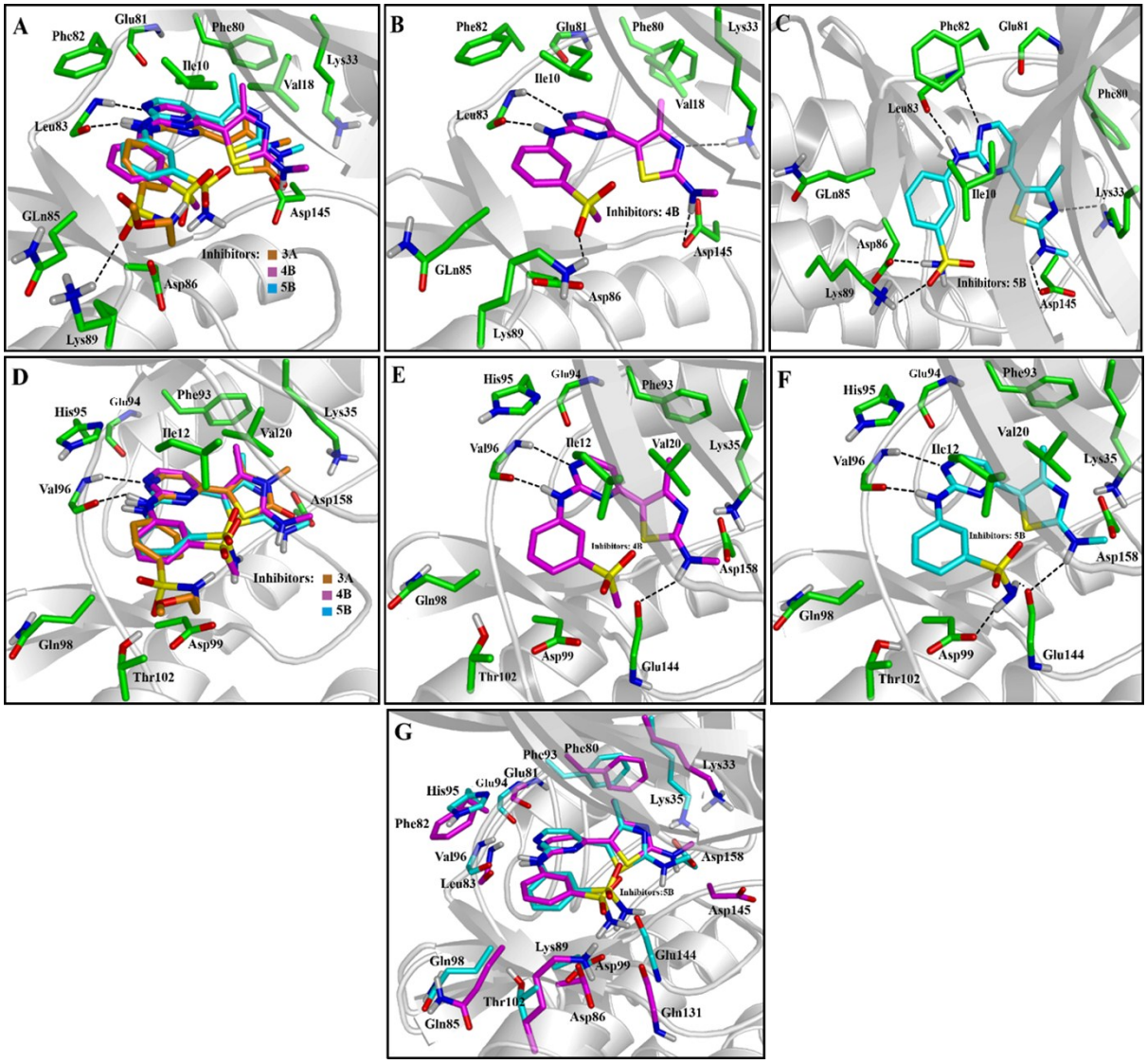


Figure S3. Obtained binding modes of ligands in the ATP binding domain of CDK2 and CDK4. (A) Superimposing of compounds **3A** (orange), **4B** (magenta) and **5B** (cyan) docked to CDK2. (B) Binding mode of compound 4B in CDK2 ATP binding site. (C) Binding mode between compound 5B and CDK2. (D) Superimposing of compounds **3A** (orange), **4B** (magenta) and **5B** (cyan) docked to CDK4; (E) 4B-CDK4 complex; (F) 5B-CDK4 complex. (G) Superimposing of CDK2 (magenta) and CDK4 (cyan) backbones with compound 5B.

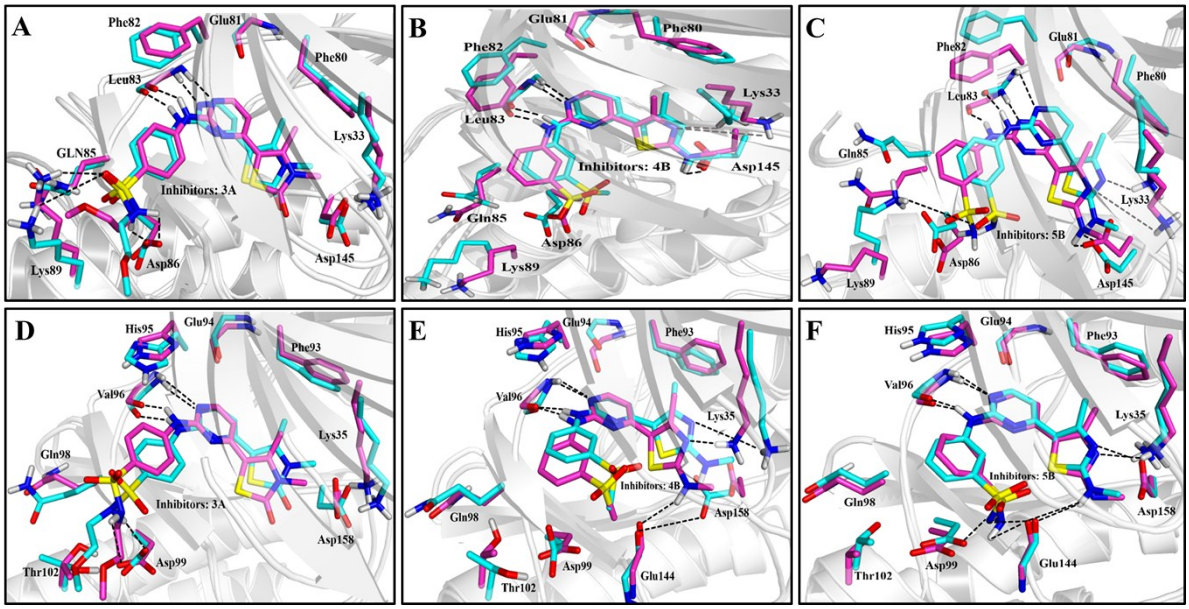


Figure. S4. Structural comparison between initial (magenta) and representative snapshots from MD (cyan) of: **(A)** CDK2-3A, **(B)** CDK2-4B, **(C)** CDK2-5B, **(D)** CDK4-3A, **(E)** CDK4-4B, **(F)** CDK4-5B.

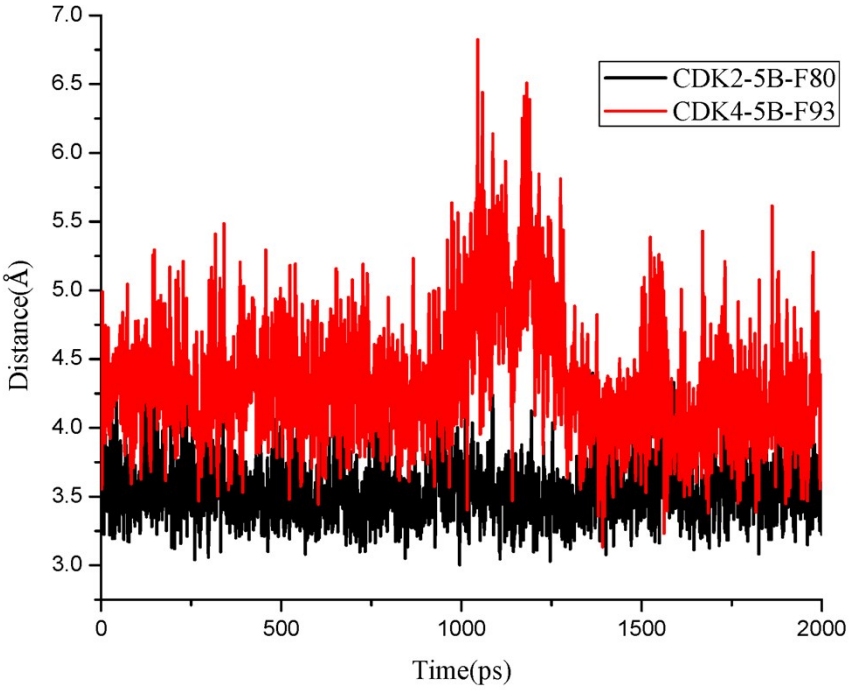


Figure S5. The distance of CDK2-Phe80 and CDK4-Phe93 from 2′-CH_3 group of inhibitor 5B, plotted as a function of time in CDK2-5B and CDK4-5B.

Table S2. Summary of Mulliken Population Analysis

Inhibitor 3A		Inhibitor 26B		Inhibitor 27B	
Atom No	**Mulliken Charge**	**Atom No**	**Mulliken Charge**	**Atom No**	**Mulliken Charge**
C1	-0.21	C1	0.20	C1	-0.28
C2	-0.19	C2	-0.15	C2	-0.18
C3	-0.19	C3	-0.20	C3	-0.28
C4	0.15	C4	0.20	C4	0.16
C5	-0.18	C5	-0.21	C5	-0.25
C6	-0.29	C6	-0.14	C6	0.16
S7	2.25	H7	0.24	N7	-0.80
N8	-1.08	H8	0.23	H8	0.40
H9	0.42	N9	-0.59	H9	0.40
H10	0.42	C10	0.61	H10	0.21
O11	-0.94	N11	-0.56	N11	-0.61
O12	-0.95	C12	0.24	C12	0.61
H13	0.24	C13	-0.30	N13	-0.56
N14	-0.60	S14	0.51	C14	0.23
C15	0.61	C15	0.05	C15	-0.30
N16	-0.57	C16	-0.61	S16	0.51
C17	0.24	H17	0.23	C17	0.04
C18	-0.34	H18	0.23	C18	-0.61
S19	0.47	H19	0.23	H19	0.22
C20	0.24	N20	-0.53	H20	0.22
N21	-0.61	C21	0.19	H21	0.23
C22	-0.36	C22	-0.61	N22	-0.53
H23	0.21	H23	0.23	C23	0.19
H24	0.19	H24	0.22	C24	-0.61
H25	0.20	H25	0.23	H25	0.22
H26	0.42	C26	-0.29	H26	0.22
N27	-0.60	C27	0.11	H27	0.22
C28	0.20	N28	-0.55	C28	-0.30
C29	-0.60	H29	0.21	C29	0.10
H30	0.22	H30	0.23	N30	-0.56
H31	0.22	H31	0.44	H31	0.20
H32	0.22	H32	0.24	H32	0.23
C33	-0.29	H33	0.24	H33	0.43
C34	0.09	N34	0.49	H34	0.21
N35	-0.56	O35	-0.43	H35	0.21
H36	0.20	O36	-0.43	H36	0.21
H37	0.22	NA	NA	NA	NA
H38	0.43	NA	NA	NA	NA
H39	0.22	NA	NA	NA	NA
H40	0.22	NA	NA	NA	NA
H41	0.23	NA	NA	NA	NA

Table S3. Hydrogen bonds analysis from MD trajectories [a]

System	Donor[a]	Acceptor[a]	Occupancy(%)[b]	Distance(Å)[c]	Angle(°)[d]
CDK2-3A	Leu83 N-H	3A N1	99.48	2.999 ± 0.17	27.90 ± 8.12
	3A N1-H9	Leu83 O	98.16	3.099 ± 0.14	24.15 ± 13.12
	Gln85 NE2-HE21	3A O1	56.11	3.021 ± 0.12	20.90 ± 9.96
	Lys89 NZ-HZ3	3A O2	51.69	3.234 ± 0.45	33.05 ± 15.12
	Asp86 N-H	3A O2	15.05	4.910 ± 0.07	27.91 ± 6.60
CDK4-3A	3A N1-H9	Val96 O	99.20	2.987 ± 0.16	23.04 ± 11.23
	Val96 N-H	3A N3	96.30	3.023 ± 0.12	32.38 ± 13.01
	3A N2-H14	Asp99 OD2	62.00	4.031 ± 0.64	39.77 ± 13.88
	Gln98 NE2-HE22	3A O1	23.96	4.661 ± 0.08	33.23 ± 12.67
CDK2- 4B	4B N7-H9	Leu83 O	99.96	3.019 ± 0.16	17.62 ± 9.35
	Leu83 N-H	4B N1	96.70	3.142 ± 0.18	32.86 ± 13.1
	4B N27-H6	Asp145 OD1	82.20	3.003 ± 0.23	31.17 ± 13.60
	Lys33 NZ-HZ3	4B N2A	14.30	3.198 ± 0.18	53.66 ± 5.66
CDK4- 4B	4B N7-H9	Val96 O	99.75	3.069 ± 0.15	23.22 ± 11.53
	Val96 N-H	4B N1	96.35	3.035 ± 0.14	32.45 ± 10.86
	4B N27-H6	Asp158 OD2	90.25	3.220 ± 0.41	28.12 ± 13.81
	Lys35 NZ-HZ3	4B N2A	28.21	4.142 ± 0.72	37.56 ± 13.74
CDK2-5B	4B N7-H9	Leu83 O	99.50	2.899 ± 0.12	25.45.9 ± 11.40
	Leu83 N-H	5B N1	97.75	3.131 ± 0.17	32.31 ± 12.13
	5B N37-H8	Asp86 OD1	97.30	2.938 ± 0.19	22.73 ± 11.54
	Lys33 NZ-HZ2	5B N2A	26.95	3.854 ± 0.62	40.91 ± 13.08
	Lys89 NZ-HZ2	5B O35	17.55	2.873 ± 0.15	41.53 ± 13.92
	5B N27-H6	Asp145 OD2	15.55	4.195 ± 0.47	51.35 ± 9.86
CDK4-5B	5B N7-H9	Val96 O	99.95	2.95 ± 0.15	16.34 ± 8.76
	Val96 N-H	5B N1	97.35	3.140 ± 0.16	32.55 ± 11.63
	5B N37-H8	Asp99 OD2	37.40	2.851 ± 0.13	19.70 ± 19.70
	5B N37-H8	Glu144 OE2	11.45	4.586 ± 0.46	36.49 ± 10.14
	5B N27-H6	Asn158 OD2	6.95	4.178 ± 0.31	50.14 ± 9.51

[a] The listed donor and acceptor pairs satisfy the criteria for the hydrogen bond over 30.0% of the time during the 30 ns of simulation. [b] Occupancy is in unit of percentage of the investigated time period. [c] The average distance with standard error (SE = standard deviation/$N_{1/2}$) between hydrogen acceptor atom and proton on hydrogen donor atom in the investigated time period. [d] The average angle with standard error (SE = standard deviation/$N_{1/2}$) in parentheses for hydrogen bond in the investigated time period.