Supporting Information

Insights into DFG-in and DFG-out JAK2 binding modes for a rational strategy of type Ⅱ inhibitors combined computational study

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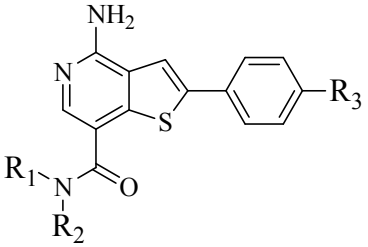
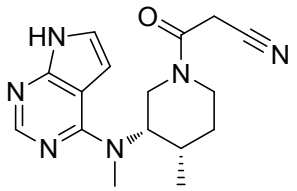
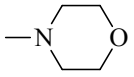
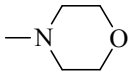
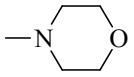
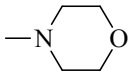
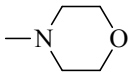
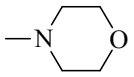
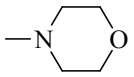
Table S1. The structures, pIC_{50} values and the corresponding docking scores for the type I JAK2 inhibitors.





20

Compound	R_1	R_2	R_3	pIC_{50}	Docking score
3	H	H		7.72	-9.437
8	H	Me		7.51	-9.112
9	H	Et		7.32	-6.901
10	H	Ph		7.14	-8.267
11	H	CH_2Ph		7.00	-6.977
12	H	^{t}Bu		5.93	-8.364
13	Me	Me		5.93	-5.237
14	H	Me	H	7.24	-9.436
15	H	Me	Me	7.57	-10.036
16	H	Me	^{t}Bu	8.00	-9.838
17	H	Me	OCF_3	7.92	-9.476

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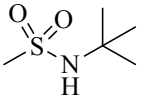
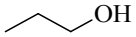
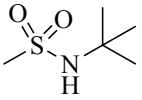
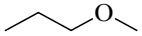
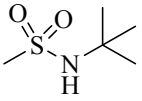
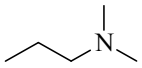
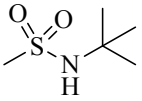
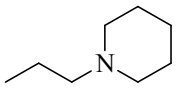
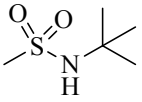
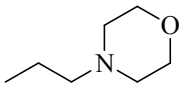
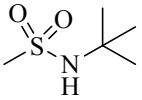
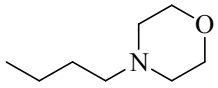
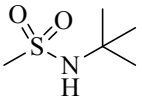
18	H	Me		8.40	-9.859
19	H	Me		9.00	-10.298
20				8.40	-9.069
21		H		8.10	-11.149
22		H		8.52	-10.368
23		H		8.10	-10.521
24		H		8.30	-11.151
25		H		8.70	-11.304
26		H		8.30	-10.917

Table 2S Hydrogen bonds analysis for the complexes **25**-JAK2-in and **12**-JAK2-in from MD simulation.

Complex	Donor	Acceptor	Distance (Å)	Angle (°)	Occupancy (%)
25-JAK2-in	**25**(N16-H43)	Glu930(O)	2.870	19.08	73.73
	25(N24-H47)	Asp994(OD1)	2.874	17.36	59.13
	Leu932(N-H)	**25**(N1)	2.921	16.35	52.27
	25(N18-H44)	Leu932(O)	2.913	33.04	16.53
12-JAK2-in	Leu932(N-H)	**12**(N1)	2.932	15.70	30.36
	Arg980(NH1-HH12)	**12**(O22)	2.880	16.83	24.38

Table 3S

Complex	Donor	Acceptor	Distance (Å)	Angle (°)	Occupancy (%)
BC1-JAK2-out	Asp994(N-H)	**BC1**(O23)	2.872	21.24	60.60
	BC1(N31-H54)	Glu898(OE2)	2.818	18.11	51.72
	BC1(N7-H35)	Leu932(O)	2.890	33.06	35.36
	BC1(N24-H48)	Glu898(OE2)	2.901	15.90	28.52
	Leu932(N-H)	**BC1**(N2)	2.931	22.00	27.72
C1-JAK2-out	**C1**(N21-H40)	Glu898(OE2)	2.854	15.82	74.55
	Leu932(N-H)	**C1**(N2)	2.917	20.13	56.35
	C1(N7-H34)	Leu932(O)	2.900	32.23	36.50
	Asp994(N-H)	**C1**(N20)	2.928	16.37	29.60

Fig. 1S Time evolution of the RMSD values for four complexes of JAK2-in with type I inhibitors (**12**, **13**, **22** and **25**). (a) all protein backbone atoms; (b) C_α atoms for the residues around 5 Å of the ligand; (c) the heavy atoms for the ligand.

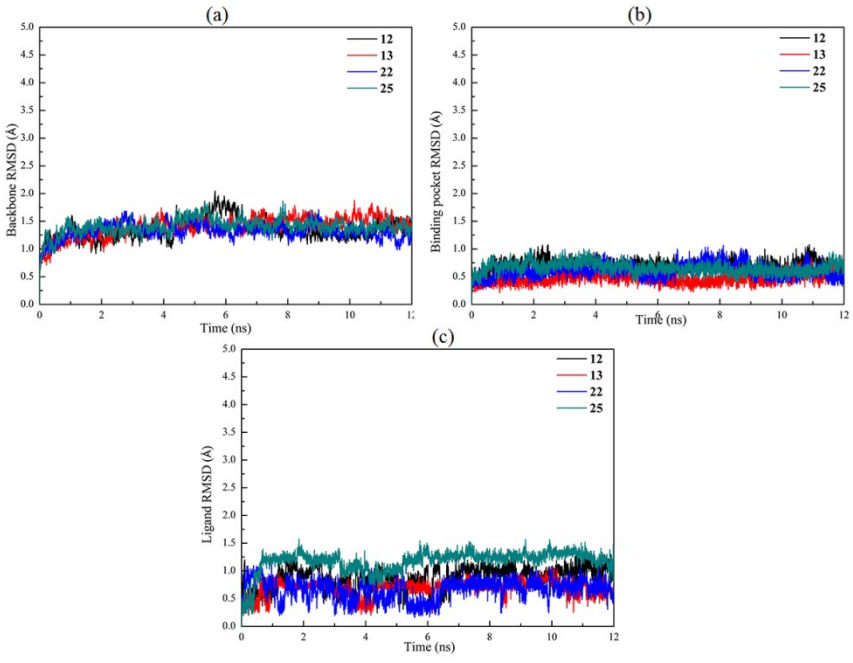


Fig. 2S Time series of the RMSDs of all protein backbone atoms, C_α atoms for the residues around 5 Å of the ligand and the heavy atoms for the ligand for the complexes (a) **BBT594**-JAK2-

out and (b) **CHZ868**-JAK2-out.

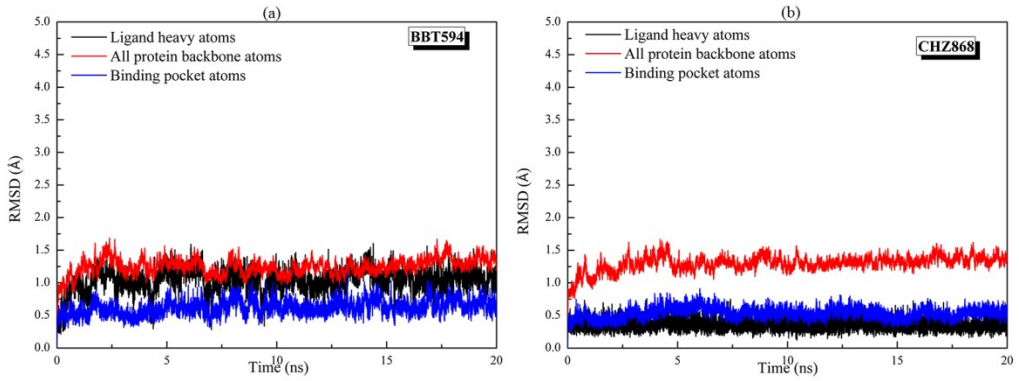


Fig. 3S (a) The comparison of the averaged structures for the **25**-JAK2-in and **12**-JAK2-in complexes (carbon atoms are colored in red and green, respectively); (b) energy difference of each residue to the binding of inhibitors **25** and **12**; (c) the contributions of the individual energy terms for the key residues (ΔG_{polar} is marked in red and blue, and $\Delta G_{nonpolar}$ is marked in magenta and navy in **25**-JAK2-in and **12**-JAK2-in, respectively).

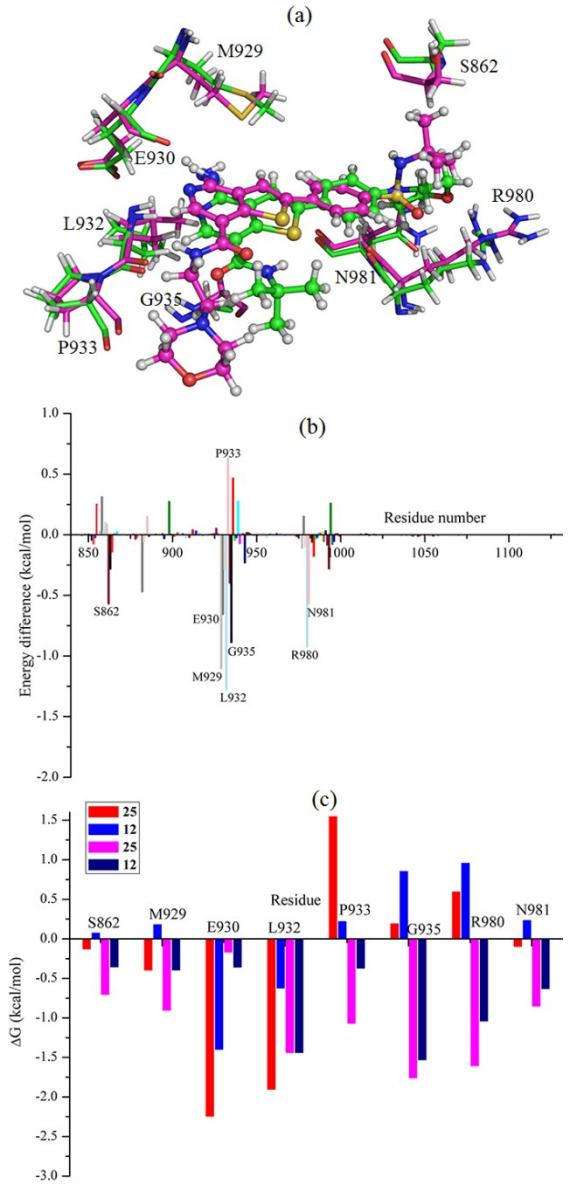
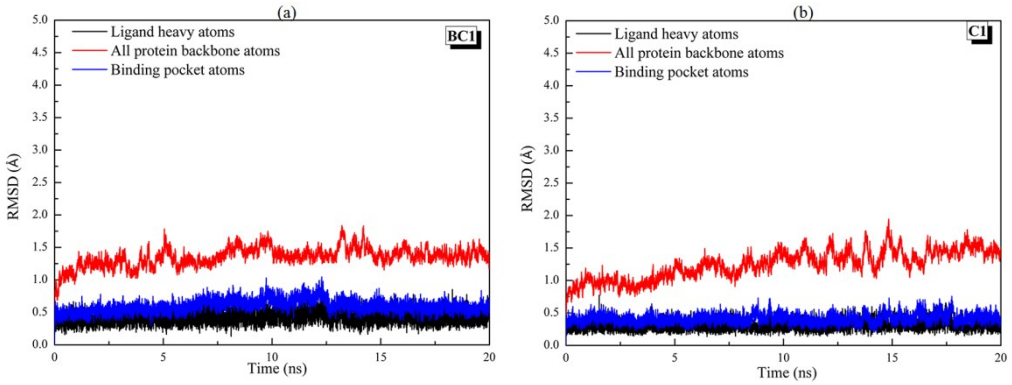


Fig. 4S Time series of the RMSDs of all protein backbone atoms, C_α atoms for the residues around 5 Å of the ligand and the heavy atoms for the ligand for the complexes (a) **BC1**-JAK2-out and (b) **C1**-JAK2-out.


(a)
Ligand heavy atoms
All protein backbone atoms
Binding pocket atoms
BC1
RMSD (Å)
Time (ns)
(b)
Ligand heavy atoms
All protein backbone atoms
Binding pocket atoms
C1
RMSD (Å)
Time (ns)