Supplementary information

Structure-Based Discovery of Potent Small-Molecule Inhibitors Targeting the PD-1/PD-L1 Interaction

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S1.Supplementary Methods

Docking Parameter Details

Docking parameters were configured as follows:[1,2]

(a) The number of starting conformations per ligand and the maximum conformations per fragment were set to 10 and 20, respectively;

(b) The maximum number of rotatable bonds per molecule was set to 100;

(c) Flags were enabled for pre-dock minimization, post-dock minimization, molecular fragmentation, and soft grid treatment;

(d) The spin alignment method was activated with a search density of 9.0;

(e) The number of spins per alignment was set to 12.

To obtain hit compounds via virtual screening:[3]

(a) Ten starting conformations were generated for each in-house small molecule;

(b) Each was docked into the defined interfacial region of the PD-1/PD-L1 complex using SYBYL, and the top 20 poses were retained;

(c) Compounds were ranked by the best docking score;

(d) The top nine compounds were selected for experimental evaluation, among which two compounds showing measurable binding were advanced for further studies..

Table S1. Binding affinities and selection outcomes of nine candidate compounds

No.	Compound ID	KD (M)	Docking Score (kcal/mol)	SPR Result	Functional Test	Selection Outcome
1	30594145	NB	6.5104	Not detected	Not tested	Excluded
2	45941420	1.93×10^{-4}	6.5462	Weak binding	Not tested	Excluded
3	53820389	NB	6.5918	Not detected	Not tested	Excluded
4	57944596	4.53×10^{-5}	6.5114	Weak binding	Not tested	Excluded
5	66236437	1.07×10^{-4}	6.8512	Weak binding	Not tested	Excluded
6	7844239	1.51×10^{-6}	7.2694	Measurable binding	Significant activity	**Selected**
7	11313672	NB	7.2051	Not detected	Not tested	Excluded
8	17115039	NB	7.6046	Not detected	Not tested	Excluded
9	23468559	8.9×10^{-8}	7.2080	Measurable binding	Significant activity	**Selected**

KD values were determined by surface plasmon resonance (SPR). NB indicates no detectable binding under the experimental conditions. Representative KD values obtained from SPR fitting are shown. Only compounds showing robust and reproducible binding signals were selected for subsequent functional evaluation.

Fig.S1 Chemical structures of the nine candidate compounds identified from virtual screening

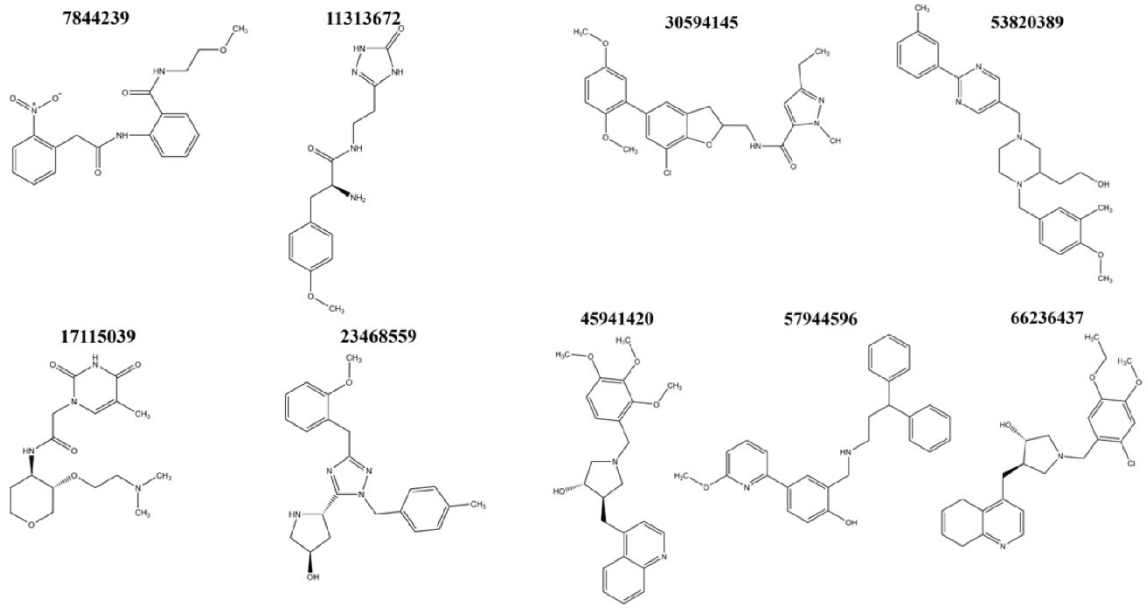
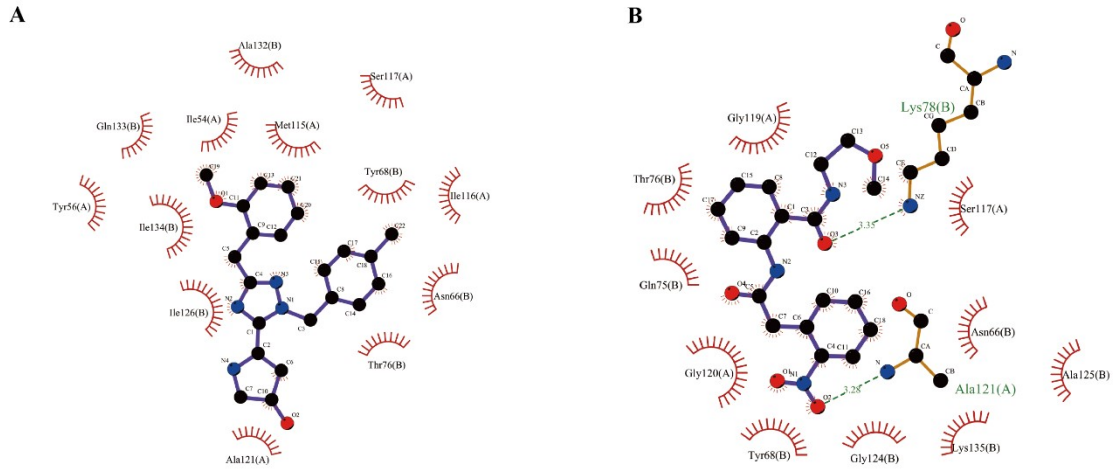


Fig.S2 Two-dimensional interaction diagrams of small molecules with the PD-1/PD-L1 interface



Two-dimensional interaction diagrams of (a) ID23468559 and (b) ID7844239 with the PD-1/PD-L1 complex generated using LigPlot. Hydrophobic contacts are represented by red arcs, and hydrogen bonds are shown as green dashed lines with corresponding distances.

Fig.S3 SPR analysis of the interaction between PD-1 and PD-L1.

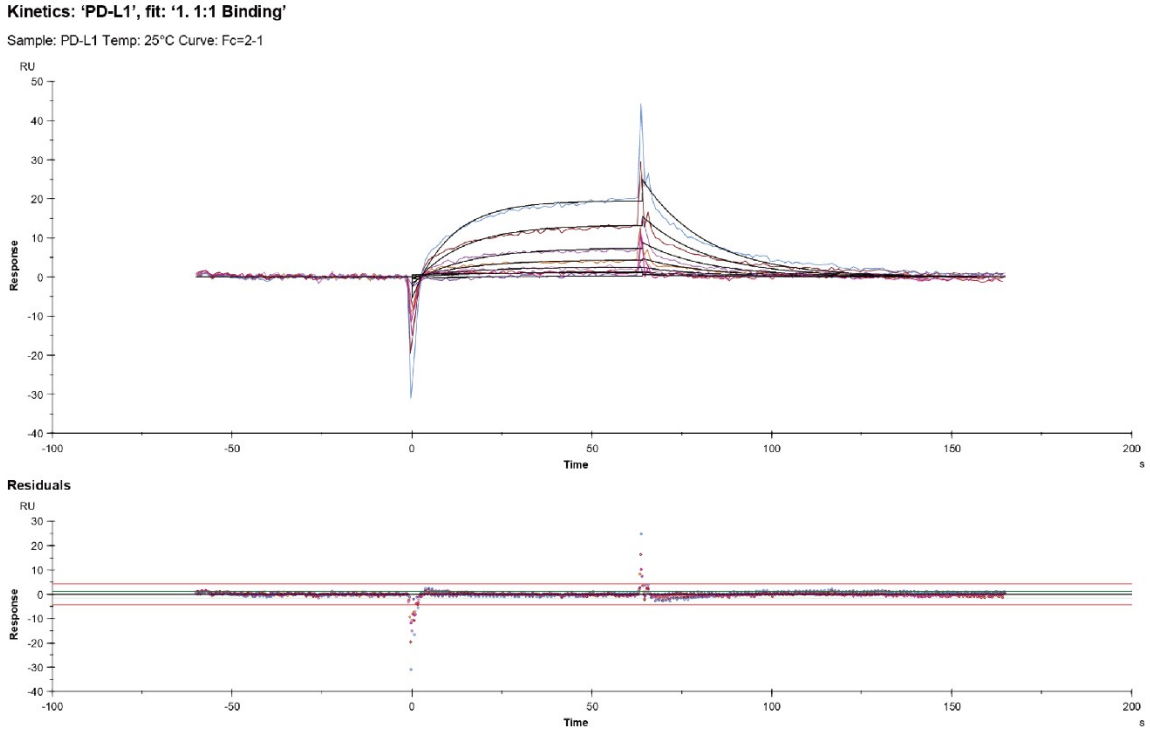
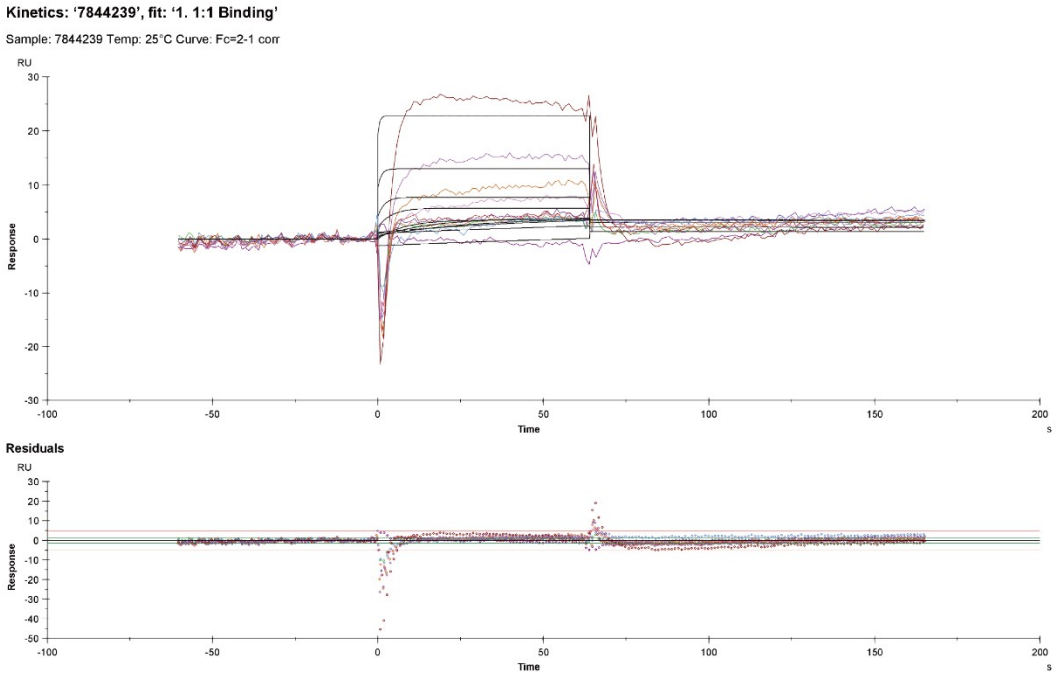


Fig.S3. A series of PD-L1 concentrations (9.375–600 nM) were injected over immobilized PD-1, and the binding kinetics were analyzed using a 1:1 binding model. The equilibrium dissociation constant (KD) was determined to be 8.8×10^{-7} M. The fitting χ^2 value was 2.61 RU^2

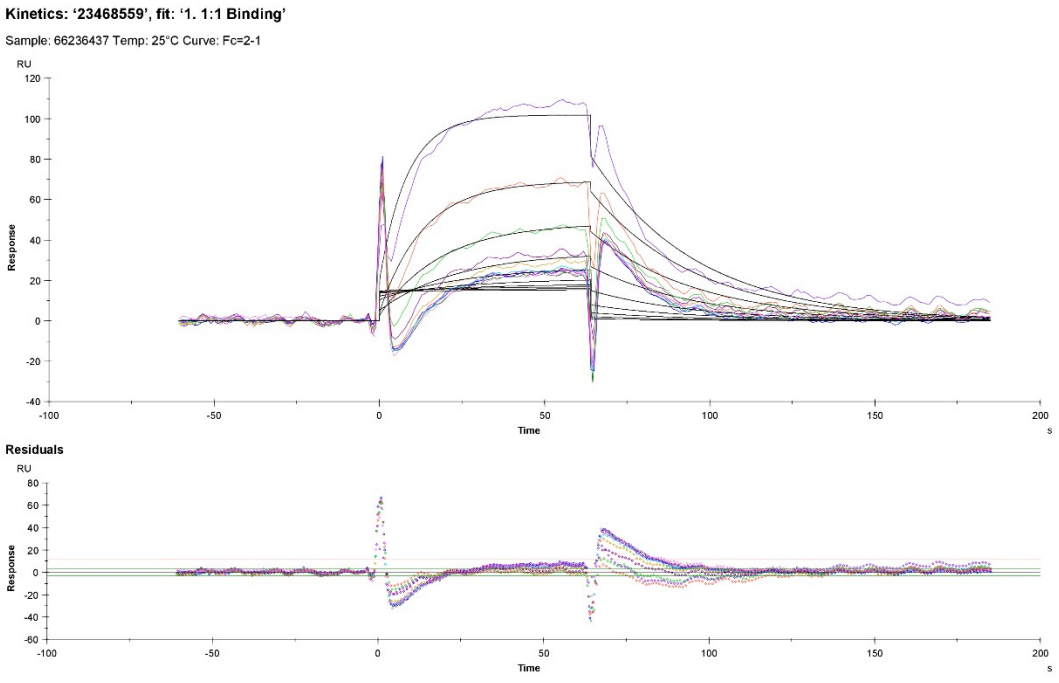
Fig.S4. Representative SPR sensorgrams showing the binding of ID7844239 (A) and ID23468559 (B) to human PD-1

A



(A)7844239

B



(B)23468559

Fig.S4 Representative SPR sensorgrams showing the binding of ID7844239 (A) and ID23468559 (B) to human PD-1. The data were fitted using a 1:1 binding model. The reported KD values represent the mean of three independent experiments. The fitting χ^2 values were 7.17 RU^2 and 14.70 RU^2 for ID7844239 and ID23468559, respectively.

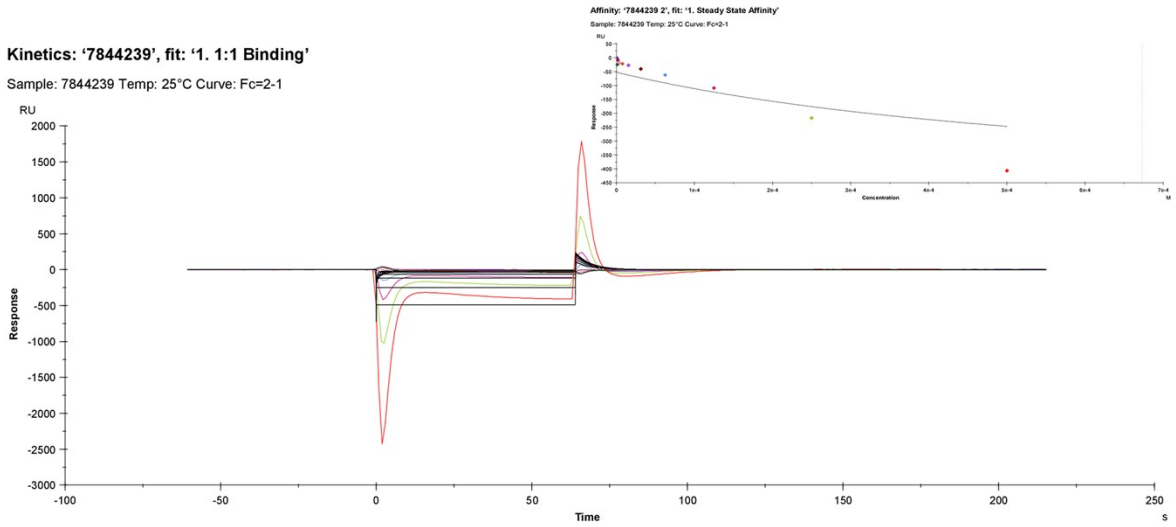
Table.S2 SPR-based competitive binding assay

Table.s2 Summary of small-molecule inhibitors and their competitive inhibition with PD-1/PD-L1

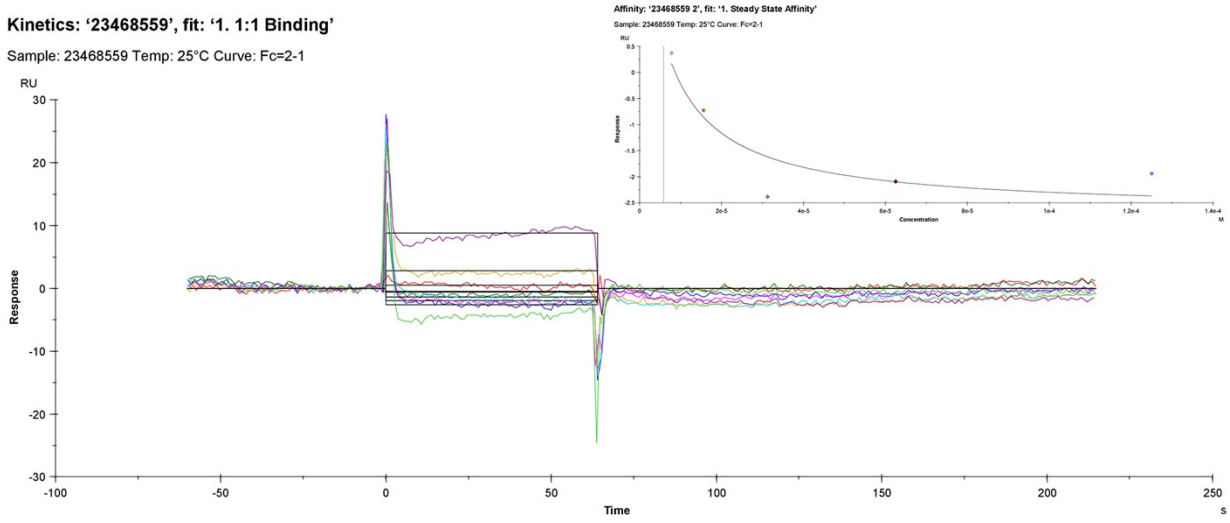
No.	Small Molecule ID	Competitive Inhibition	Figure
1	7844239	Y	Fig. S5a
2	23468559	Y	Fig. S5b

Note: “Y” indicates confirmed competitive inhibition between the small molecule and PD-1/PD-L1.

Fig.S5 Competitive inhibition of small-molecule on PD-1/PD-L1



(a)7844239



(b)23468559

Fig.S5 Competitive inhibition of small-molecule on PD-1/PD-L1.(7844239/23468559)