

Identification of small-molecule inhibitors targeting the PD-1/PD-L1 interaction in colorectal cancer: insights from docking and molecular dynamics simulations

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Abstract

The PD-1/PD-L1 axis promotes tumour immune evasion and functions as a crucial immune checkpoint, highlighting its importance as a primary target for immunotherapy in colorectal cancer. Although there have been significant improvements in the use of antibodies to treat colorectal cancer, therapeutic antibodies still face several challenges, including immune responses and immunologically mediated toxicities. Natural compounds are emerging as attractive small-molecule inhibitors of PD-L1 and enhancers of anticancer T cell-mediated responses. We reviewed the marine natural products database to identify potential lead compounds for developing new small-molecule inhibitors targeting the PD-1/PD-L1 axis. Among the compounds examined, CMNPD3605 demonstrated the most powerful inhibition of PD-1/PD-L1 interaction and showed greater promise than current small-molecule inhibitors in clinical studies. In conclusion, this study identifies novel PD-L1 inhibitors and sets up a computational framework for making small-molecule immune checkpoint inhibitors that can be tested in living colorectal cancer models.

1. Introduction

The ability of cancer cells to evade the host immune response is one of the major key feature during cancer progression [1]. The major control of the immunological escape process is performed by the immunological checkpoints, interaction between PD-1 and PD-L1 is one of the main mechanisms that carries out the immune evasion process. [2, 3]. The PD-L1 is an crucial molecule and plays a significant part in the process of preserving immunological homeostasis inside the host [4]. Cancer cells expressed PD-L1 interact with PD-1 receptors expressed by T cytotoxic cells and initiate Programmed cell death in T cytotoxic cells resulting in decreased anticancer activity [5]. The PD-L1 is primarily expressed by cancer cells, neutrophils, natural killer (NK) cells, and macrophage-lineage cells and is absent from all other normal human tissues (Dong et al., 2002; Li et al., 2022; Yin et al., 2021). Yin et al reported that extracellular vesicles (EVs) released from colorectal cancer (CRC) cells that contain microRNAs reprogram macrophages towards M2 phenotype and enhance the expression of PD-L1 through the regulation of PTEN/AKT and SOCS1/STAT1 signaling pathways [6]. Consistent with this study, our results show that exosomes released from CRC cells are capable of inducing PD-L1 expression in macrophages, and these macrophages further upregulate PD-L1 levels in cancer cells, as confirmed by RT-PCR analysis. PD-1/PD-L1 checkpoint blockade immunotherapy for tumors showed promising results in patients with tumors, including lung cancer [7], breast cancer [8], and gastric cancer [9]. Currently PD-1/PD-L1-directed monoclonal antibodies (mAbs) are useful in clinical trials [10, 11]. Even though some cancer patients respond to monoclonal antibody as a cure, a few problems are still related to the therapy. For example, it is costly and not available in pill form, its production is arduous and expensive, it is not well absorbed, and it has the potential to produce issues with the

immune response at the location of the tumor [12, 13]. To deal with this issue, small molecule inhibitors offer numerous benefit over monoclonal antibodies. These advantages include the fact that they can be given orally, have a long half-life, can be easily reach the tumor site, have minimal immune-related adverse effects and easy to transport and store [14]. In order to enhance tumor immunotherapy, the substitution of monoclonal antibodies (mAbs) with small molecule inhibitors is necessary, which will allow for the elimination of the constraints that are associated with the former. Thus, there is a need to come up with better and safer PD-L1 inhibitors.

We analysed the inhibitor binding and complex dynamics by using molecular docking and MD simulations. Initially, molecules were sent through a series of tests to determine how well they could connect to each other and how they interacted with one another. Following the screening, a few compounds were shown to have exceptional promise. These molecules exhibited high affinity and stable interactions in the PD-L1 binding site. The top candidates were then comprehensively examined for their inhibitory capacity, biological characteristics, ADMET behavior, and interaction modes with PD-L1. Molecular dynamics simulations were performed on PD-L1 and its inhibitor-bound complexes for a duration of 200 nanoseconds. The purpose of these simulations was to strengthen the knowledge of conformational behavior by shedding light on features of complex stability and intermolecular interactions. In the context of cancer treatment, the findings collectively lend support to the use of small-molecule inhibitors of PD-L1 as potential therapeutic interventions.

2. Materials and methods

2.1. Cell lines and culture

The Colo 205 human colorectal carcinoma cell line and the THP-1 human monocytic cell line were obtained from the National Centre for Cell Science (NCCS), Pune, India. Both cell lines were maintained in RPMI-1640 medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco) and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin; Invitrogen). Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO₂. Differentiation of THP-1 monocytes into macrophages was induced by treatment with 25 ng/mL phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich) for 24 hours. Following differentiation, the PMA-containing medium was replaced with fresh complete RPMI medium before exposure to exosomes.

2.2. Exosome isolation and characterization

Colo-205 cultures were allowed to grow to 70–80% confluence, and the culture medium was replaced with serum-free media. The conditioned media were collected after 48 hours and centrifuged at 300×g for 5 minutes and 2,000×g for 30 minutes to remove cell debris.

Centrifuged supernatant was passed through a 0.22 µm PVDF filter, and then proceeded to ultracentrifugation at 100000×g for 2 h to isolate exosomes. Subsequently, isolated exosomes characterized through NTA and SEM.

2.2.1. Nanoparticle tracking analysis

To characterize the exosomes, nanoparticle tracking analysis (NTA) used to detect size as well as concentrations of the sample. The NTA analysis was performed with Nanosight (NTA Version: NTA 3.4 Build 3.4.003). The exosome samples were diluted 1000 times in Milli-Q water before analysis

2.2.2. Scanning electron microscopy (SEM)

To confirm the size and morphology of exosomes scanning electron microscope was utilized. 10 µL of the exosome suspension was diluted in PBS and then fixed with glutaraldehyde for 15 minutes. The sample was then rinsed with PBS, dehydrated using ethanol, and air-dried at room temperature for 24 hours. Finally, a coating of gold-palladium was applied to the samples, and imaging was conducted using a scanning electron microscope.

2.3. RNA isolation and quantitative real-time PCR (qRT-PCR)

THP-1 and Colo-205 cells were collected and washed with PBS after respective treatments. Total RNA was extracted using RNAiso Plus reagent (Takara Bio Inc., Japan) following the manufacturer's protocol. RNA concentration and purity were determined using a Nanodrop spectrophotometer. For cDNA synthesis, 1000 ng of RNA was reverse-transcribed using the Takara cDNA Synthesis Kit. qRT-PCR was performed with SYBR Green Master Mix on a QuantStudio 3 Real-Time PCR System (Applied Biosystems). Gene expression of **GAPDH** and **PD-L1** was quantified using the $2^{-\Delta\Delta C_t}$ method, with **GAPDH** as the internal reference. Primer sequences are **GAPDH**: F 5'-AATCCCATCACCATCTTCCA-3', R 5'-TGGACTCCACGACGTACTCA-3' and **PD-L1**: F 5'-ATGGTGGTGCCGACTACAAG-3' R 5'-GGAATTGGTGGTGGTGGTCT-3'

2.4. Target structure preparation

The PD-L1-PD-1 complex structure (PDB ID: 4ZQK; Resolution: 2.45 Å) [15] was initially inspected to define the protein-protein interaction interface. Using UCSF Chimera [16], residues at the PD-L1 interface located within 3 Å proximity to PD-1 were identified and catalogued through detailed inspection of the protein-protein complex. For all subsequent simulations, the PD-L1 protein was isolated by removing the PD-1 chain and any non-protein entities. The resulting PD-L1 structure was then prepared using the protein preparation wizard module [17] of Schrödinger, which performed all necessary

corrections including assignment of bond orders, addition of hydrogens, optimization of hydrogen bonding networks, and assignment of protonation states at pH 7.0. The prepared PD-L1 receptor, focused on its functional interface (F19, Y56, Q66, R113, A121, D122, Y123, K124, R125) [18], was then used for grid generation for molecular docking studies using the receptor grid generation module [19].

2.5. Ligand library processing

The ligand library was assembled from the Comprehensive Marine Natural Products Database (CMNPD) [20], a manually curated, open-access resource that catalogs over 31,000 marine natural products with associated 2D/3D structures, physicochemical and predicted ADMET properties, standardized bioactivity annotations, source-organism taxonomy and geographic metadata, and literature citations. CMNPD supports structure-based discovery workflows through downloadable bulk datasets and a web interface that enables structure, substructure and property queries, visualisation and network exploration, and provides compound-level report pages containing computed descriptors, bioactivity records and bibliographic links. For reproducibility, the CMNPD dataset (31,561 entries) was downloaded and a standardized LigPrep [21] workflow was applied, which involved desalting, generating possible tautomers, correcting protonation at physiological pH (7.0 ± 2.0), and generating all relevant stereoisomers and low-energy 3D conformers. Ligands were minimized using the OPLS3e [22] force field and stored in Maestro format for downstream virtual screening.

2.6. Virtual screening and ADMET predictions

A tiered virtual screening workflow was applied to the full CMNPD library against the PD-L1 interface grid using Glide [23] HTVS, SP, and XP precision in sequence. High-throughput virtual screening (HTVS) was performed for initial filtering, then standard precision (SP) docking for pose refinement, and lastly, extra precision (XP) docking for final ranking. The docking grid was centred on the mapped PD-L1 interface residues, and van der Waals scaling was set at 0.8 for nonpolar atoms with a partial charge cutoff of 0.15 for diverse chemical sampling. Top hits from XP docking were ranked by GlideScore and manually inspected to ensure meaningful engagement with interface residues. Lead compounds: CMNPD16477, CMNPD21979 and CMNPD3605 were prioritized for further study alongside the benchmark inhibitor INCB086550 [24]. Subsequently three prioritized leads and the control were evaluated computationally for ADMET properties using pkCSM [25]. Predicted endpoints included Caco-2 permeability, blood-brain barrier penetration, CYP2D6 inhibition, AMES mutagenicity, hepatotoxicity, and skin sensitization. All three leads passed the predefined ADMET filters and were advanced, along with the control, to molecular dynamics simulations.

2.7. Molecular dynamics simulations

Molecular dynamics (MD) simulations were employed to assess the stability and dynamic behavior of the docked PD-L1–ligand complexes under explicit solvent conditions. The top-ranking complexes from virtual screening and ADMET analyses were prepared for simulation using the LEaP module of AmberTools19 [26]. Counterions were added as needed to neutralize each system, which was then solvated in a periodic octahedral box of TIP3P water molecules, providing a 10.0 Å buffer in all directions [27]. Parameterization was achieved by assigning the Amber ff14SB [28] force field to the protein, and the Antechamber-derived AM1-BCC [29] atomic charge, along with the GAFF2 [30] force field to ligand molecules. All simulations were conducted using the pmemd.cuda module of AMBER18 [26, 31–33]. Long-range electrostatic interactions were treated using the particle-mesh Ewald (PME) [34] method, and all covalent bonds involving hydrogen atoms were constrained using the SHAKE [35] algorithm. A 10 Å non-bonded cutoff was applied. Each simulation used a 2.0 fs time step. Energy minimization and multi-step heating to 300 K followed by equilibration were first performed, after which the systems were subjected to 200 ns of unrestrained MD in the NPT ensemble at 1 atm and 300 K. Pressure and Temperature during the simulations were controlled using the Berendsen barostat [36] and Langevin thermostat [37, 38], respectively. All trajectory post-processing and quantitative analyses, including RMSD, RMSF, hydrogen bonds, radius of gyration, and solvent-accessible surface area, were performed using the CPPTRAJ [39] module in AMBER18.

2.8. Trajectory analysis

Protein stability was evaluated by calculating the backbone root-mean-square deviation (RMSD) and root-mean-square fluctuation (RMSF), focusing on both global protein behavior and specifically on the PD-L1 interface region. Ligand stability was monitored by calculating the ligand RMSD relative to its initial docked conformation. To assess protein compactness and folding, the radius of gyration (Rg) was computed over the molecular dynamics trajectories, while solvent-accessible surface area (SASA) calculations provided insights into changes in surface exposure. Hydrogen bond analysis between the protein and the ligands was carried out with the CPPTRAJ module in AmberTools18. Standard geometric definitions were used, considering donor-acceptor distances within 3.5 Å and bond angles above 120°.

2.9. Binding free energy calculations and data visualization

Binding free energies (ΔG_{bind}) were estimated using the Molecular Mechanics Poisson–Boltzmann Surface Area (MM/PBSA) method [40, 41]. It was computed as:

$$\Delta G_{\text{bind}} = (G_{\text{complex}}) - (G_{\text{protein}}) - (G_{\text{ligand}})$$

where each G_x represents the average free energies of the respective species i.e., complex, protein, or ligand, obtained from equilibrated molecular dynamics snapshots. These total free energy values were further split into contributions from molecular mechanics and solvation energies:

$$G = E_{MM} + G_{solv}$$

Where

$$E_{MM} = E_{bonded} + \Delta E_{vdW} + \Delta E_{elec}$$

and

$$G_{solv} = \Delta G_{pol} + \Delta G_{np}$$

Here, ΔE_{vdW} and ΔE_{elec} are the van der Waals and electrostatic interaction energies, ΔG_{pol} is the polar solvation free energy estimated using the Poisson-Boltzmann equation, and ΔG_{np} is the non-polar solvation free energy calculated from solvent-accessible surface area (SASA) models. The entropic term ($-T\Delta S$) was excluded from the calculations because of its high computational demand and statistical variability.

To assess how individual residues contribute to binding affinity, MM/PBSA energy components were decomposed on a per-residue basis. Snapshots were taken every 100 ps during the production run, and the resulting profiles were averaged to pinpoint residues that play a central role in ligand binding and stabilisation of the PD-L1 interface. This approach offers a clear quantitative view of the thermodynamic forces driving the interaction. Data analysis and visualisation were carried out in Python (v3.13.5), using NumPy for array handling and numerical routines, and Matplotlib for generating publication-quality figures that highlight the key energetic trends across the dataset.

3. Results

3.1. Exosome-mediated upregulation of PD-L1 in macrophages and CRC cells

Colo-205-derived exosomes were successfully isolated by ultracentrifugation and subsequently characterized by nanoparticle tracking analysis (NTA), (Fig. 1A) and scanning electron microscopy (SEM) (Fig. 1B) and used to treat cells. The Size and morphology of the exosome are consistent with mentioned earlier in the research. To quantify the expression of PD-L1, RT-PCR was performed, and the results showed that exosomes derived from Colo 205 cells upregulate PD-L1 expression in macrophages. To extend these findings, colo-205 cells were treated with conditioned medium obtained from

exosome-treated macrophages for 48 hours, which resulted in a further elevation of PD-L1 expression relative to control cells. (Fig. 1C). Collectively, in TME, cancer cells increase PD-L1 expression on themselves and stimulate its upregulation in macrophages, resulting in a dual immunosuppressive mechanism that induces T-cell apoptosis and promotes tumor immune evasion. Based on these observations, we turned to an in-silico approach for the identification of potential PD-L1 inhibitors, aiming to design small molecules with favourable binding and therapeutic properties.

3.2. Site-directed docking reveals high-affinity marine natural products engaging the PD-L1- PD-1 interface

The PD-L1 receptor model used for in silico studies was derived from the crystal structure of the PD-1-PD-L1 complex (PDB ID: 4ZQK). Structural analysis performed in UCSF Chimera identified a prominent PD-1 binding interface on PD-L1, defined by nine key residues: F19, Y56, Q66, R113, A121, D122, Y123, K124, and R125 (Fig. 2). These residues were used to generate a focused Glide docking grid for all subsequent ligand screening steps. Upon screening the CMNPD ligand library, comprising 31,561 marine-derived compounds, via a hierarchical virtual screening workflow using the Glide module targeting the PD-L1 interface grid, three marine-derived compounds i.e. CMNPD16477, CMNPD21979, and CMNPD3605, were prioritized for further investigation based on docking scores, pose quality, and residue-level interactions (Fig. 3). INCB086550, a previously reported small-molecule PD-L1 inhibitor, was included as a control throughout the workflow to benchmark docking performance, dynamic behaviour, and energetic metrics. Prior to molecular dynamics simulations, all prioritized compounds and the control inhibitor were evaluated using pkCSM for in silico ADMET profiling (**Supplementary information Table S1**). CMNPD16477, CMNPD21979, and CMNPD3605 met the predefined thresholds for absorption, distribution, metabolism, and toxicity, and were subsequently advanced to explicit solvent molecular dynamics simulations. All four ligand-PD-L1 complexes were subjected to 200 ns all-atom explicit solvent molecular dynamics simulations under identical conditions. Trajectories were inspected to assess equilibration and production sampling. Each complex reached equilibrium within the initial 10 to 30 ns, and the remaining trajectory was treated as the production phase. Analysis of the trajectory mainly focused on overall structural stability, compactness, flexibility, and interaction profiles. Summary statistics for key structural and interaction parameters derived from the 200 ns production trajectories have been summarized in **Supplementary information Table S2**.

3.3. Marine database-derived ligands enhance PD-L1 structural stability and binding persistence

To check how each ligand influences PD-L1's structural behaviour, Root Mean Square Deviation (RMSD) analysis was done across the 200 ns simulation trajectory (Fig. 4A; **Supplementary information Table S2**). RMSD evaluation showed that all protein-ligand complexes reached stability rapidly and remained in equilibrium throughout the simulation. Backbone deviations averaged between 1.13 and 1.31 Å, with only brief, minor fluctuations that stayed below 1.5 Å. Compared to the control complex (1.31 ± 0.15 Å), all CMNPD ligand-bound systems exhibited lower or comparable RMSD values: CMNPD16477 showed the lowest mean RMSD (1.13 ± 0.13 Å), suggesting enhanced conformational rigidity, while CMNPD21979 (1.28 ± 0.18 Å) and CMNPD3605 (1.19 ± 0.21 Å) also maintained stable profiles. The RMSD profiles show that the CMNPD ligands stabilize the PD-L1 structure as well as, or better than, the reference compound. All ligand-bound complexes quickly reached equilibrium and remained stable throughout the simulation. Subtle variations in the RMSD traces highlight how each ligand uniquely influences the protein's conformational stability and flexibility.

Using INCB086550 as a reference, the RMSD and distance profiles tracked over the 200 ns production runs highlighted unique interaction behaviours for each test compound. (Fig. 5; **Supplementary information Table S2**). Compared to the control's moderate ligand mobility (ligand RMSD 3.09 ± 0.22 Å) and shortest pocket separation (6.34 ± 0.67 Å), CMNPD16477 maintained the most conserved ligand pose (ligand RMSD 2.57 ± 0.17 Å) and a more stable pocket (pocket RMSD 0.61 ± 0.11 Å), despite sampling the largest ligand-pocket and ligand-protein separations (10.82 ± 0.67 Å and 17.86 ± 0.88 Å), indicating a stable but more external binding geometry. CMNPD21979 exhibited a broader ensemble of ligand conformations (ligand RMSD 3.50 ± 1.05 Å) and induced the greatest pocket flexibility (pocket RMSD 0.90 ± 0.09 Å), with intermediate ligand-pocket distance (8.75 ± 0.67 Å) and the shortest ligand-protein separation (13.39 ± 0.37 Å), reflecting a compact yet dynamically flexible placement. CMNPD3605 showed similar ligand heterogeneity (ligand RMSD 3.37 ± 1.19 Å), but achieved the greatest rigidity in the local pocket (pocket RMSD 0.45 ± 0.08 Å), with ligand-pocket and ligand-protein distances (8.30 ± 1.06 Å and 15.87 ± 0.98 Å) falling between the other ligands. Across the simulation, the binding pocket interaction with CMNPD3605 remained consistently stable, even with ligand movement. All the selected compounds maintained steady ligand-protein and ligand-pocket distances which indicate sustained binding with no signs of transient dissociation. However, the control ligand experienced a brief dissociation near the 50 ns time point. This consistent behaviour highlights robust interactions between the ligands and the PD-L1 binding site. To gain deeper insight how these interactions influence PD-L1's structural dynamics, the next step involved analysing residue-level flexibility.

3.4. Root-mean-square fluctuation analysis reveals PD-L1 stabilization by marine database-derived

ligands

Upon analysing the RMSF values of PD-L1 residues 18–132, two regions were found to show relatively increased fluctuation; one peak was around the residues 47–49 and another lay approximately at residues 63–83. It is to be noted that both these regions are located away from the key interacting surface consisting of residues F19, Y56, Q66, R113, A121, D122, Y123, K124, and R125 and thus represent flexible loop regions that are positioned far from the ligand-binding interface. Apart from the fluctuations seen in those two regions, all three molecules showed reduced mobility at the interface and interacted with the nine key residues, which reflects a stable binding surface (Fig. 4B;

Supplementary information Table S2). Minimum compound-specific fluctuations were observed, but they stayed confined to limited regions. CMNPD3605 showed a slight increase in flexibility at residue 66 (~ 0.8 Å compared to ~ 0.5 Å for the others), while CMNPD21979 exhibited a modest rise at residues 124–125 (~ 0.7 Å versus ~ 0.5 – 0.6 Å). Minor fluctuations did not compromise the stability of the interface. Overall, the findings indicate that all three ligands reinforce the PD-1 binding surface. To validate this effect further, global structural parameters were examined to evaluate protein integrity and solvent exposure.

3.5. Protein compactness and solvent exposure validate structural integrity of ligand-bound PD-L1 complexes

To evaluate how PD-L1 behaves when bound to the three ligands, the radius of gyration (R_g) was assessed throughout the 200 ns production runs. The results provide a view of the protein's overall folding and show that it consistently retained a compact, stable conformation in all ligand-bound states (Fig. 4C; **Supplementary information Table S2**). All ligand-bound PD-L1 systems remained structurally compact and well-folded throughout the simulation, with average radius of gyration (R_g) values showing minimal variation: INCB086550 (13.62 ± 0.08 Å), CMNPD16477 (13.60 ± 0.07 Å), CMNPD21979 (13.69 ± 0.09 Å), and CMNPD3605 (13.67 ± 0.08 Å). The close similarity in R_g values indicates that each ligand helps PD-L1 retain its native compact fold, with no evidence of loosening or unfolding. Among the tested compounds, CMNPD16477 showed the lowest average R_g , suggesting a modest increase in protein compaction compared with both the reference and the other ligand-bound systems. In all cases, the R_g stabilised early and remained steady throughout the trajectory, in line with the low RMSD values and the strong interface stability observed for these ligands. Taken together, these results confirm that all three compounds support the structural integrity of PD-L1 during extended simulations.

Consistent with this, Solvent-accessible surface area (SASA) analysis over the 200 ns trajectories revealed that all ligand-bound PD-L1 complexes maintained consistently low and tightly grouped SASA values. This demonstrates that none of the compounds induced significant unfolding or increased exposure of the protein surface (Fig. 4D; **Supplementary information Table S2**). CMNPD16477 and CMNPD21979 exhibited nearly identical SASA profiles, averaging 57.44 ± 1.58 and 57.27 ± 1.89 nm², respectively. CMNPD3605 displayed the lowest mean SASA (57.03 ± 1.94 nm²) suggesting slightly reduced solvent exposure at the binding interface. By contrast, the control compound INCB086550 recorded the highest average SASA (58.24 ± 1.96 nm²) reflecting a modest increase in surface accessibility. The small differences limited fluctuations across all systems indicate that structural adjustments did not disturb the overall fold of PD-L1. These results align with the compactness observed in the ligand-bound states and support the conclusion that each compound contributes to maintaining PD-L1's structural integrity. To further understand the molecular basis of this stability, hydrogen bonding analysis were carried out to evaluate how consistently and selectively each ligand engages the PD-L1 interface.

3.6. Hydrogen bonding patterns indicate stable and selective ligand anchoring at the PD-L1 interface

The hydrogen bonding pattern of the ligands at the PD-L1 interface played a central role in defining their stability and selectivity (Fig. 6; **Supplementary information Table S3**). CMNPD16477 consistently engaged Gln66 (16.45% occupancy) and Ser117 (19.45%), supporting a stable binding pose that may support its inhibitory effect and target specificity. In contrast, CMNPD21979 adopted a more flexible binding mode, with no hydrogen bond occupancy exceeding 10%. For most of the trajectory, its hydrogen bond count was close to zero, reflecting a tendency to assume multiple conformations and a less persistent inhibitory profile.

Among the compounds tested, CMNPD3605 showed the most robust interaction pattern. It formed durable hydrogen bonds with key interface residues, Arg113 and Arg125, with Arg113 reaching 50.87% occupancy. Over the course of the simulation, CMNPD3605 maintained an average of 4–6 hydrogen bonds and occasionally reached as many as 11, highlighting its strong anchoring and resistance to dissociation. By comparison, the control compound INCB086550 exhibited moderate hydrogen bond occupancy with several polar and charged residues, resulting in intermediate bond numbers and a more diffuse interaction profile.

Overall, CMNPD3605 emerged as the most effective ligand in terms of hydrogen bonding, surpassing the control compound by establishing a focused and long-lasting interaction network. This strong and persistent binding suggests its potential to act as a potent inhibitor of PD-L1 interactions, as summarized in **Supplementary information Table S3**. To

further investigate the energetic basis of these interactions, binding free energy calculations were performed.

3.7. End-state free energy decomposition identifies CMNPD3605 as the most thermodynamically favourable PD-L1 binder

To evaluate the binding strength of each compound with PD-L1, MM/PBSA calculations were performed. This well-established method [42–44] breaks down the total binding free energy into its individual components, as illustrated in Fig. 7. The accompanying bar chart and table present the mean values and standard deviations for each term, together with the overall ΔG_{bind} for every complex. The aim of this analysis was to clarify the energetic contributions that either support or oppose stable ligand-protein interactions. Among the compounds tested, CMNPD16477 exhibited the weakest binding affinity. CMNPD21979 contributed a strong van der Waals component ($\Delta E_{\text{vdW}} = -41.49 \pm 0.10$ kcal/mol) and achieved moderate overall binding strength. On the other hand, CMNPD3605 emerged as the top-performing ligand, with the most favourable total binding free energy ($\Delta G_{\text{bind}} = -35.95 \pm 0.16$ kcal/mol), outperforming even the reference compound INCB086550 ($\Delta G_{\text{bind}} = -31.28 \pm 0.23$ kcal/mol). This result positions CMNPD3605 as the most promising candidate among the marine database-derived ligands, offering a strong thermodynamic profile that is better than the control. In all systems, van der Waals and electrostatic interactions were the primary contributors to binding and solvation energies were important in shaping the net affinity. Complex formation was always favored by nonpolar solvation (ΔG_{np}) and inhibited by polar solvation (ΔG_{pol}) because of their positive terms. Interestingly, CMNPD21979 exhibited very favourable interactions between van der Waals, but the binding energy of the entire system was not optimum which highlights the significance of balancing between solvation and electrostatic potential. Taken together, these results, highlight CMNPD3605 as the most potent PD-L1 binder in the series and a promising candidate to be developed as a PD-L1/PD-1 interaction inhibitor. In order to identify the residues that are most important in this binding profile, a residue-level energy decomposition was performed subsequently.

3.8. Residue-level MM/PBSA analysis pinpoints key anchoring residues and confirms CMNPD3605 as the most stabilizing ligand

To gain a more detailed insight into the role of individual residues in the stability of these complexes, a residue-wise MM/PBSA decomposition was conducted on each of the ligand-PD-L1 complexes (**Supplementary information Table S4**). When comparing overall ligand contributions, the control compound INCB086550 showed the strongest stabilizing effect

(− 22.65 kcal/mol), followed by CMNPD3605 (− 15.63 kcal/mol), CMNPD21979 (− 8.10 kcal/mol), and CMNPD16477 (− 7.48 kcal/mol). CMNPD3605 was the most striking ligand of marine origin since its stabilization profile was closest to the control, and it was much stronger than either of the others. In all systems arginine residues proved to be important anchoring points. In the case of CMNPD3605, Arg113 and Arg125 were useful to complex stability, with ΔG_{total} values of − 8.49 and − 7.89 kcal/mol, respectively. Arg113 interacted with CMNPD21979 with less strength (− 2.43 kcal/mol). Tyr56 consistently provided favourable van der Waals interactions, contributing modest stabilization (− 1.54 kcal/mol for CMNPD16477 and − 3.07 kcal/mol for CMNPD21979). Additional support came from residues like Ala121, Tyr123, and Met115, which helped reinforce packing around the main anchors. For example, Tyr123 contributed − 1.98 kcal/mol for CMNPD3605, while Met115 showed stabilizing effects across all ligands, ranging from − 0.96 to − 2.83 kcal/mol. Some polar residues, including Asp122 and Lys124, introduced desolvation penalties when not fully compensated by new electrostatic contacts. For instance, Asp122 contributed + 0.97 kcal/mol in the CMNPD3605 complex, and Lys124 showed a G_{total} value of + 1.56 kcal/mol in the control, consistent with unfavourable polar solvation terms.

Overall, CMNPD3605 attains superior per-residue stabilization by engaging both R113 and R125 and by recruiting supportive van der Waals contacts (Met115, Tyr123), which together explain its more favourable ligand-level ΔG_{total} relative to the other screened compounds. These interactions explain its superior ligand-level ΔG_{total} compared to the other marine database-derived compounds. Full mean $\pm$ SD values for each residue are presented in **Supplementary information Table S2**. This comparative decomposition clarifies the molecular basis for differences in PD-L1 inhibition across the ligand series. CMNPD16477 contributes to structural stabilization and compactness, CMNPD21979 offers a flexible binding mode suited to dynamic targets, and CMNPD3605 excels in forming persistent, high-occupancy interactions with critical interface residues. CMNPD3605 demonstrated the most favorable thermodynamic profile among the novel ligands, closely aligning with the control compound and emerging as the strongest candidate for further development. Overall, all three ligands preserved PD-L1's global fold and compactness during the simulations, showing consistent binding and energetics that support their potential as effective modulators. Taken together, the combination of robust thermodynamic performance, specific binding interactions, and sustained structural stability highlights CMNPD3605 as a promising lead for future therapeutic exploration.

4. Discussion and conclusion

Three candidate pharmaceuticals were identified using in-silico docking as stable binders of the PD-L1 pocket, capable of inhibiting PD-1/PD-L1 interactions and preserving T cell-mediated antitumor efficacy. The PD-1/PD-L1 axis is crucial in the majority of

malignancies, and its inhibition is challenging due to its flat and hydrophobic surface. Even so, seven FDA-approved antibodies available since 2006 have shown notable anticancer effects [45]. Despite this progress, the adaptive nature and molecular heterogeneity of tumors still few patients failing to respond to these highly specific therapies [46]. The development of small-molecule inhibitors is essential to overcome the challenges associated with antibodies. Small-molecule inhibitors offer distinct advantages over antibody-based therapies, including suitability for oral administration, minimal immunogenicity and a relatively short half-life, which allows rapid management of any adverse reactions. Although PD-L1 is a challenging target due to its predominantly flat and hydrophobic interface, our screened compound achieves stronger predicted binding and stability than inhibitors currently available in clinical trials.

This study employed a comprehensive pipeline that included hierarchical virtual screening, ADMET evaluation, and molecular dynamics simulation. Through this integrated approach, marine natural product-derived ligands were identified as promising new PD-L1 inhibitors. The analysis has shown that all the shortlisted CMNPD compounds had some properties that were useful in stabilizing the PD-L1 structure and interact with critical binding residues. The study focused on defining and targeting the important PD-L1 interface site and comparing the best binders with a recognized small-molecule inhibitor. All selected compounds maintained the structural integrity and compactness of PD-L1 throughout the 200 ns molecular dynamics simulations. The stability of their binding was further supported by van der Waals and electrostatic interactions, as confirmed by per-residue MM/PBSA decomposition. The prominent interactions with Arg113 and Arg125 indicated that these residue sites are critical for effective inhibition.

The marine compounds were found to form more persistent and multivalent hydrogen interactions with the interface residues in comparison with INCB086550, and thereby increase more of the stabilization of the PD-L1 structure. Their unique chemical scaffolds and binding characteristics suggest that they may offer potential benefits over existing PD-L1 inhibitors. CMNPD16477 consistently reduced interface flexibility, promoted a more compact protein structure, and formed stable polar contacts, together supporting a persistent binding mode and the potential for prolonged inhibition. However, it had the lowest binding affinity compared to the compounds that were analysed. CMNPD21979 did not affect the overall compactness or interface integrity of the protein, but exhibited less hydrogen bond occupancy and more ligand RMSD. CMNPD3605 was the best ligand among the three test compounds in this study, as it demonstrated stable hydrogen bonding and high binding free energy, indicative of high anchoring and increased affinity, especially at the core residues of PD-L1. It showed long-lasting high-affinity binding with important interface residues with the most favourable thermodynamic profile among the marine database-derived compounds. CMNPD3605 was able to sustain a steady protein conformation with time and provided similar or even better results than the benchmark

inhibitor INCB086550. Based on these in silico results, CMNPD3605 represents a promising candidate for further preclinical and clinical development as a PD-L1 inhibitor for PD-L1-associated conditions.

To conclude, marine-derived ligands explored in this study show potential for further development as small-molecule immune checkpoint inhibitors. The in-silico analyses provided detailed insights into their binding mechanisms and stabilizing effects on the PD-L1 interface, offering a foundation for future experimental validation. CMNPD3605 is the most promising candidate for prompt preclinical and clinical assessment. Nonetheless, the remaining two ligands, CMNPD16477 and CMNPD21979, demonstrated encouraging molecular dynamics outcomes and could significantly benefit from focused optimization and modification to enhance their therapeutic efficacy and inhibitory strength.

Declarations

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Competing interests

No competing interests declared.

Data Availability Statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding authors.

Author Contributions

MSB: Conceptualization and investigation; KW, FS: Experiments; KW, FS: Data curation, formal analysis, and figure preparation; MSB, KW: Writing (Original Draft); MSB: Reviewing and editing. All authors have read and agreed to the published version of the manuscript.

References

1. McClanahan F, Hanna B, Miller S, Clear AJ, Lichter P, Gribben JG, Seiffert M (2015) PD-L1 checkpoint blockade prevents immune dysfunction and leukemia development in a mouse model of chronic lymphocytic leukemia. *Blood* 126:203–211. <https://doi.org/10.1182/blood-2015-01-622936>
2. Cha J-H, Chan L-C, Li C-W, Hsu JL, Hung M-C (2019) Mechanisms Controlling PD-L1 Expression in Cancer. *Mol Cell* 76:359–370. <https://doi.org/10.1016/j.molcel.2019.09.030>
3. Lee DY, Im E, Yoon D, Lee Y-S, Kim G-S, Kim D, Kim S-H (2022) Pivotal role of PD-1/PD-L1 immune checkpoints in immune escape and cancer progression: Their interplay with platelets and FOXP3 + Tregs related molecules, clinical implications and combinational potential with phytochemicals. *Sem Cancer Biol* 86:1033–1057. <https://doi.org/10.1016/j.semcancer.2020.12.001>
4. Lastwika KJ, Wilson W, Li QK, Norris J, Xu H, Ghazarian SR, Kitagawa H, Kawabata S, Taube JM, Yao S, Liu LN, Gills JJ, Dennis PA (2016) Control of PD-L1 Expression by Oncogenic Activation of the AKT-mTOR Pathway in Non-Small Cell Lung Cancer. *Cancer Res* 76:227–238. <https://doi.org/10.1158/0008-5472.CAN-14-3362>
5. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, Roche PC, Lu J, Zhu G, Tamada K, Lennon VA, Celis E, Chen L (2002) Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. *Nat Med* 8:793–800. <https://doi.org/10.1038/nm730>
6. Yin Y, Liu B, Cao Y, Yao S, Liu Y, Jin G, Qin Y, Chen Y, Cui K, Zhou L, Bian Z, Fei B, Huang S, Huang Z (2022) Colorectal Cancer-Derived Small Extracellular Vesicles Promote Tumor Immune Evasion by Upregulating PD-L1 Expression in Tumor-Associated Macrophages. *Adv Sci* 9:2102620. <https://doi.org/10.1002/advs.202102620>
7. Xia L, Liu Y, Wang Y (2019) PD-1/PD-L1 Blockade Therapy in Advanced Non-Small-Cell Lung Cancer: Current Status and Future Directions. *Oncologist* 24:S31–S41. <https://doi.org/10.1634/theoncologist.2019-IO-S1-s05>
8. Emens LA (2018) Breast Cancer Immunotherapy: Facts and Hopes. *Clin Cancer Res* 24:511–520. <https://doi.org/10.1158/1078-0432.CCR-16-3001>
9. Liu X, Choi MG, Kim K, Kim K-M, Kim ST, Park SH, Cristescu R, Peter S, Lee J (2020) High PD-L1 expression in gastric cancer (GC) patients and correlation with molecular features. *Pathol - Res Pract* 216:152881. <https://doi.org/10.1016/j.prp.2020.152881>
10. Tan S, Zhang CW-H, Gao GF (2016) Seeing is believing: anti-PD-1/PD-L1 monoclonal antibodies in action for checkpoint blockade tumor immunotherapy. *Sig Transduct Target Ther* 1:16029. <https://doi.org/10.1038/sigtrans.2016.29>
11. Zhan M-M, Hu X-Q, Liu X-X, Ruan B-F, Xu J, Liao C (2016) From monoclonal antibodies to small molecules: the development of inhibitors targeting the PD-1/PD-L1

- pathway. *Drug Discovery Today* 21:1027–1036.
<https://doi.org/10.1016/j.drudis.2016.04.011>
12. Konstantinidou M, Zarganes-Tzitzikas T, Magiera-Mularz K, Holak TA, Dömling A (2018) Immune Checkpoint PD-1/PD-L1: Is There Life Beyond Antibodies? *Angew Chem Int Ed* 57:4840–4848. <https://doi.org/10.1002/anie.201710407>
 13. Naidoo J, Page DB, Li BT, Connell LC, Schindler K, Lacouture ME, Postow MA, Wolchok JD (2015) Toxicities of the anti-PD-1 and anti-PD-L1 immune checkpoint antibodies. *Ann Oncol* 26:2375–2391. <https://doi.org/10.1093/annonc/mdv383>
 14. Wu Q, Jiang L, Li S, He Q, Yang B, Cao J (2021) Small molecule inhibitors targeting the PD-1/PD-L1 signaling pathway. *Acta Pharmacol Sin* 42:1–9.
<https://doi.org/10.1038/s41401-020-0366-x>
 15. Zak KM, Dubin G, Holak TA (2015) Structure of the complex of human programmed death-1 (PD-1) and its ligand PD-L1.: 4zqk. <https://doi.org/10.2210/pdb4zqk/pdb>
 16. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE (2004) UCSF Chimera—A visualization system for exploratory research and analysis. *J Comput Chem* 25:1605–1612. <https://doi.org/10.1002/jcc.20084>
 17. Madhavi Sastry G, Adzhigirey M, Day T, Annabhimoju R, Sherman W (2013) Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. *J Comput Aided Mol Des* 27:221–234. <https://doi.org/10.1007/s10822-013-9644-8>
 18. Bojko M, Węgrzyn K, Sikorska E, Kocikowski M, Parys M, Battin C, Steinberger P, Kogut MM, Winnicki M, Sieradzan AK, Spodzieja M, Rodziewicz-Motowidło S (2022) Design, synthesis and biological evaluation of PD-1 derived peptides as inhibitors of PD-1/PD-L1 complex formation for cancer therapy. *Bioorg Chem* 128:106047.
<https://doi.org/10.1016/j.bioorg.2022.106047>
 19. Schrödinger (2025) Release 2025-3: Glide, Schrödinger. LLC, New York, NY. (n.d.)
 20. Lyu C, Chen T, Qiang B, Liu N, Wang H, Zhang L, Liu Z (2021) CMNPD: a comprehensive marine natural products database towards facilitating drug discovery from the ocean. *Nucleic Acids Res* 49:D509–D515.
<https://doi.org/10.1093/nar/gkaa763>
 21. Schrödinger (2025) Release 2025-3: LigPrep, Schrödinger. LLC, New York, NY. (n.d.)
 22. Roos K, Wu C, Damm W, Reboul M, Stevenson JM, Lu C, Dahlgren MK, Mondal S, Chen W, Wang L, Abel R, Friesner RA, Harder ED (2019) OPLS3e: Extending Force Field Coverage for Drug-Like Small Molecules. *J Chem Theory Comput* 15:1863–1874.
<https://doi.org/10.1021/acs.jctc.8b01026>
 23. Yang Y, Yao K, Repasky MP, Leswing K, Abel R, Shoichet BK, Jerome SV (2021) Efficient Exploration of Chemical Space with Docking and Deep Learning. *J Chem Theory Comput* 17:7106–7119. <https://doi.org/10.1021/acs.jctc.1c00810>

24. Koblisch HK, Wu L, Wang L-CS, Liu PCC, Wynn R, Rios-Doria J, Spitz S, Liu H, Volgina A, Zolotarjova N, Kapilashrami K, Behshad E, Covington M, Yang Y, Li J, Diamond S, Soloviev M, O'Hayer K, Rubin S, Kanellopoulou C, Yang G, Rupar M, DiMatteo D, Lin L, Stevens C, Zhang Y, Thekkat P, Geschwindt R, Marando C, Yeleswaram S, Jackson J, Scherle P, Huber R, Yao W (2022) Hollis, Characterization of INCB086550: A Potent and Novel Small-Molecule PD-L1 Inhibitor. *Cancer Discov* 12:1482–1499. <https://doi.org/10.1158/2159-8290.CD-21-1156>
25. Pires DEV, Blundell TL, Ascher DB (2015) pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. *J Med Chem* 58:4066–4072. <https://doi.org/10.1021/acs.jmedchem.5b00104>
26. Case DA, Cheatham TE, Darden T, Gohlke H, Luo R, Merz KM, Onufriev A, Simmerling C, Wang B, Woods RJ (2005) The Amber biomolecular simulation programs. *J Comput Chem* 26:1668–1688. <https://doi.org/10.1002/jcc.20290>
27. Price DJ, Brooks CL (2004) A modified TIP3P water potential for simulation with Ewald summation. *J Chem Phys* 121:10096–10103. <https://doi.org/10.1063/1.1808117>
28. Maier JA, Martinez C, Kasavajhala K, Wickstrom L, Hauser KE, Simmerling C (2015) ff14SB: Improving the Accuracy of Protein Side Chain and Backbone Parameters from ff99SB. *J Chem Theory Comput* 11:3696–3713. <https://doi.org/10.1021/acs.jctc.5b00255>
29. Jakalian A, Jack DB, Bayly CI (2002) Fast, efficient generation of high-quality atomic charges. AM1-BCC model: II. Parameterization and validation. *J Comput Chem* 23:1623–1641. <https://doi.org/10.1002/jcc.10128>
30. Wang J, Wolf RM, Caldwell JW, Kollman PA, Case DA (2004) Development and testing of a general amber force field. *J Comput Chem* 25:1157–1174. <https://doi.org/10.1002/jcc.20035>
31. Götz AW, Williamson MJ, Xu D, Poole D, Le Grand S, Walker RC (2012) Routine Microsecond Molecular Dynamics Simulations with AMBER on GPUs. 1. Generalized Born. *J Chem Theory Comput* 8:1542–1555. <https://doi.org/10.1021/ct200909j>
32. Lee T-S, Cerutti DS, Mermelstein D, Lin C, LeGrand S, Giese TJ, Roitberg A, Case DA, Walker RC, York DM (2018) GPU-Accelerated Molecular Dynamics and Free Energy Methods in Amber18: Performance Enhancements and New Features. *J Chem Inf Model* 58:2043–2050. <https://doi.org/10.1021/acs.jcim.8b00462>
33. Salomon-Ferrer R, Götz AW, Poole D, Le Grand S, Walker RC (2013) Routine Microsecond Molecular Dynamics Simulations with AMBER on GPUs. 2. Explicit Solvent Particle Mesh Ewald. *J Chem Theory Comput* 9:3878–3888. <https://doi.org/10.1021/ct400314y>
34. Darden T, York D, Pedersen L (1993) Particle mesh Ewald: An $N \cdot \log(N)$ method for Ewald sums in large systems. *J Chem Phys* 98:10089–10092.

<https://doi.org/10.1063/1.464397>

35. Krüutler V, Van Gunsteren WF, Hünenberger PH (2001) A fast SHAKE algorithm to solve distance constraint equations for small molecules in molecular dynamics simulations. *J Comput Chem* 22:501–508. [https://doi.org/10.1002/1096-987X\(20010415\)22:5%3C501::AID-JCC1021%3E3.0.CO;2-V](https://doi.org/10.1002/1096-987X(20010415)22:5%3C501::AID-JCC1021%3E3.0.CO;2-V)
36. Berendsen HJC, Postma JPM, Van Gunsteren WF, DiNola A, Haak JR (1984) Molecular dynamics with coupling to an external bath. *J Chem Phys* 81:3684–3690. <https://doi.org/10.1063/1.448118>
37. Loncharich RJ, Brooks BR, Pastor RW (1992) Langevin dynamics of peptides: The frictional dependence of isomerization rates of *N*-acetylalanyl- *N*'-methylethylamide. *Biopolymers* 32:523–535. <https://doi.org/10.1002/bip.360320508>
38. Pastor RW, Brooks BR, Szabo A (1988) An analysis of the accuracy of Langevin and molecular dynamics algorithms. *Mol Phys* 65:1409–1419. <https://doi.org/10.1080/00268978800101881>
39. Roe DR, Cheatham TE (2013) *J Chem Theory Comput* 9:3084–3095. <https://doi.org/10.1021/ct400341p>. PTRAJ and CPPTRAJ: Software for Processing and Analysis of Molecular Dynamics Trajectory Data,
40. Kollman PA, Massova I, Reyes C, Kuhn B, Huo S, Chong L, Lee M, Lee T, Duan Y, Wang W, Donini O, Cieplak P, Srinivasan J, Case DA, Cheatham TE (2000) Calculating Structures and Free Energies of Complex Molecules: Combining Molecular Mechanics and Continuum Models, *Acc. Chem. Res.* 33 889–897. <https://doi.org/10.1021/ar000033j>
41. Miller BR, McGee TD, Swails JM, Homeyer N, Gohlke H, Roitberg AE (2012) *MMPBSA.py*: An Efficient Program for End-State Free Energy Calculations. *J Chem Theory Comput* 8:3314–3321. <https://doi.org/10.1021/ct300418h>
42. Başar Y, Demirtaş İ, Yenigün S, İpek Y, Özen T, Behçet L (2025) Molecular docking, molecular dynamics, MM/PBSA approaches and bioactivity studies of nepetanudoside B isolated from endemic *Nepeta aristata*. *J Biomol Struct Dynamics* 43:5486–5499. <https://doi.org/10.1080/07391102.2024.2309641>
43. El Rhabori S, Alaqrbeh M, Naanaai L, El Allouche Y, El Aissouq A, Bouachrine M, Zaitan H, Chtita S, Khalil F (2025) Integrative computational strategy for anticancer drug discovery: QSAR-ANN modeling, molecular docking, ADMET prediction, molecular dynamics and MM-PBSA simulations, and retrosynthetic analysis. *New J Chem* 49:14748–14768. <https://doi.org/10.1039/D5NJ02417J>
44. Hajipasha A, Cherati NG, Darzi M, Nateghi SS, Mohsenian SAS, Noorzaei M, Dinaki FK, Halimi M (2025) Structure-based identification of novel FAK1 inhibitors using pharmacophore modeling, molecular dynamics, and MM/PBSA calculations. *Sci Rep* 15:39506. <https://doi.org/10.1038/s41598-025-23203-8>

45. Upadhaya S, Neftelinov ST, Hodge J, Campbell J (2022) Challenges and opportunities in the PD1/PDL1 inhibitor clinical trial landscape. *Nat Rev Drug Discov* 21:482–483. <https://doi.org/10.1038/d41573-022-00030-4>
46. Sartori GR, Albuquerque ADO, Santos-Costa AH, Andrade LM, Almeida DDS, Gaieta EM, Sampaio JV, Albuquerque VTDML (2022) Martins Da Silva, In silico mapping of the dynamic interactions and structure-activity relationship of flavonoid compounds against the immune checkpoint programmed-cell death 1 pathway. *Front Drug Discov* 2:1032587. <https://doi.org/10.3389/fddsv.2022.1032587>

Figures

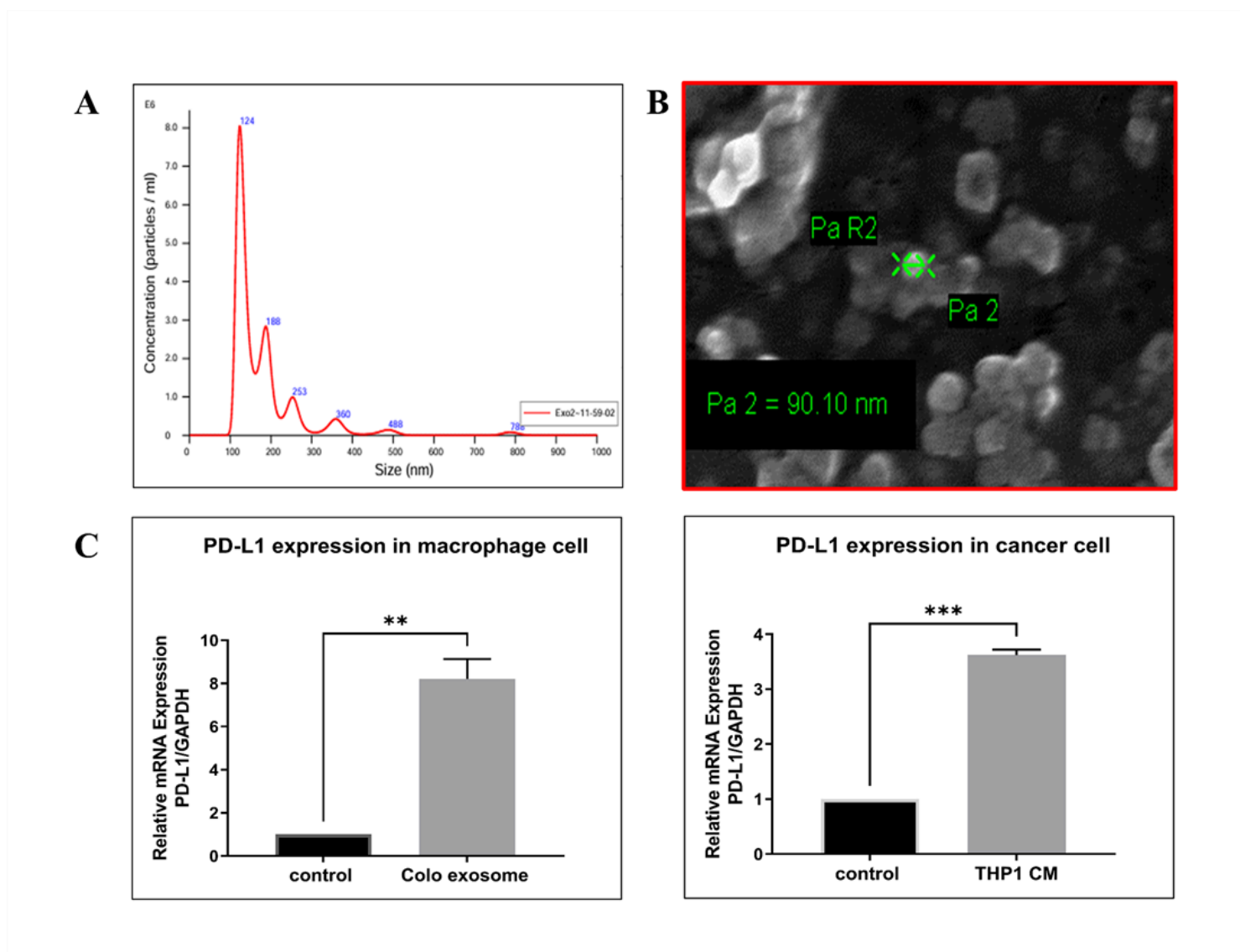


Figure 1

Exosome characterization and PD-L1 expression analysis. Colo-205-derived exosomes were isolated by ultracentrifugation and characterized by (A) nanoparticle tracking

analysis (NTA) and (B) scanning electron microscopy (SEM) (C) CRC cells-derived exosomes induced PD-L1 expression in macrophages and conditioned media (CM) obtained from exosome-stimulated macrophages subsequently elevated PD-L1 expression in CRC cells was quantified by RT-PCR $*p<0.05$, $**p<0.005$, $***p<0.0005$, $****p<0.00005$.

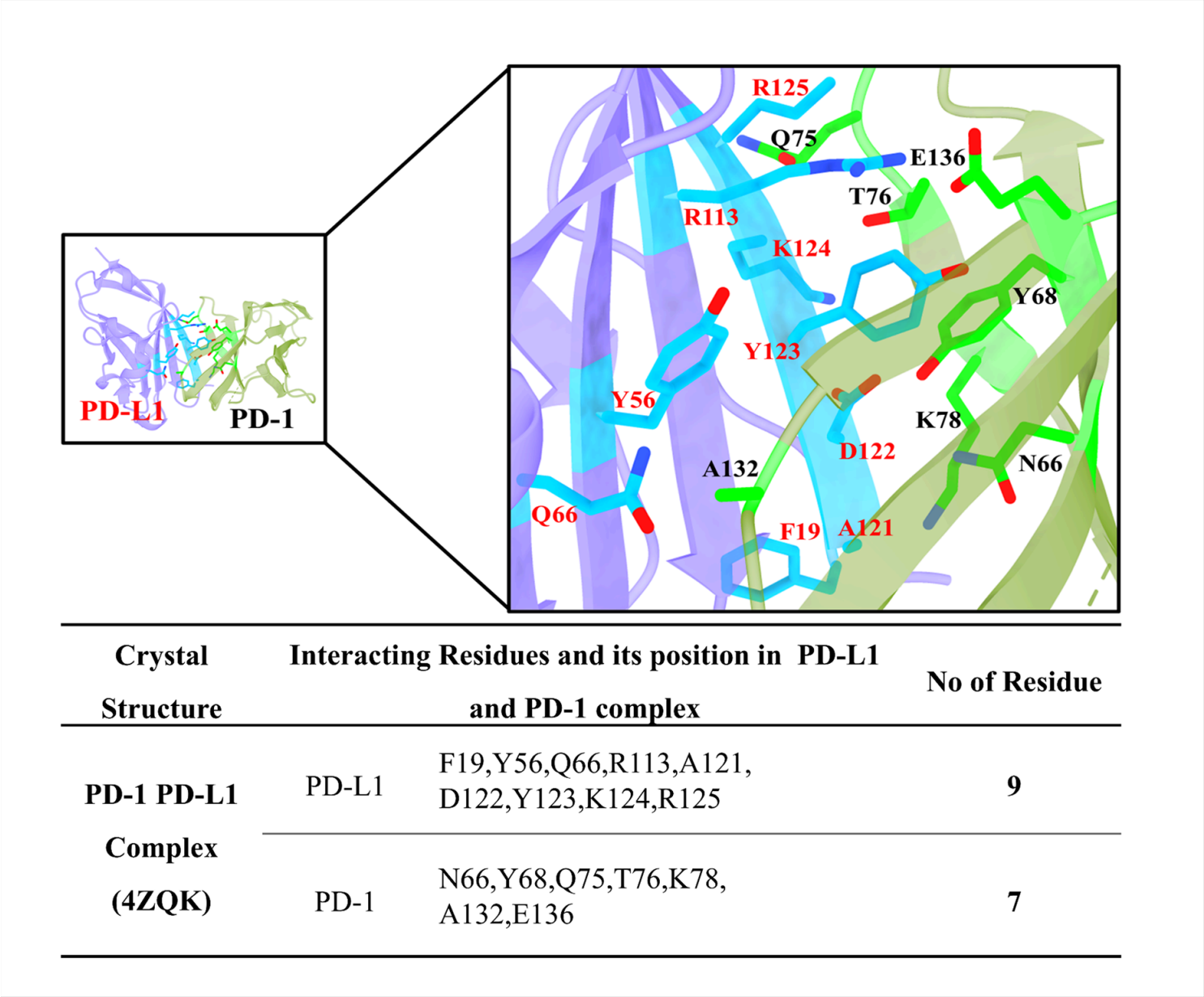


Figure 2

Structural visualization and residue-level analysis of the PD-1/PD-L1 complex (PDB: 4ZQK), highlighting interface residues within 3Å proximity. Identified contact residues were used to define the PD-L1 binding grid for virtual screening.

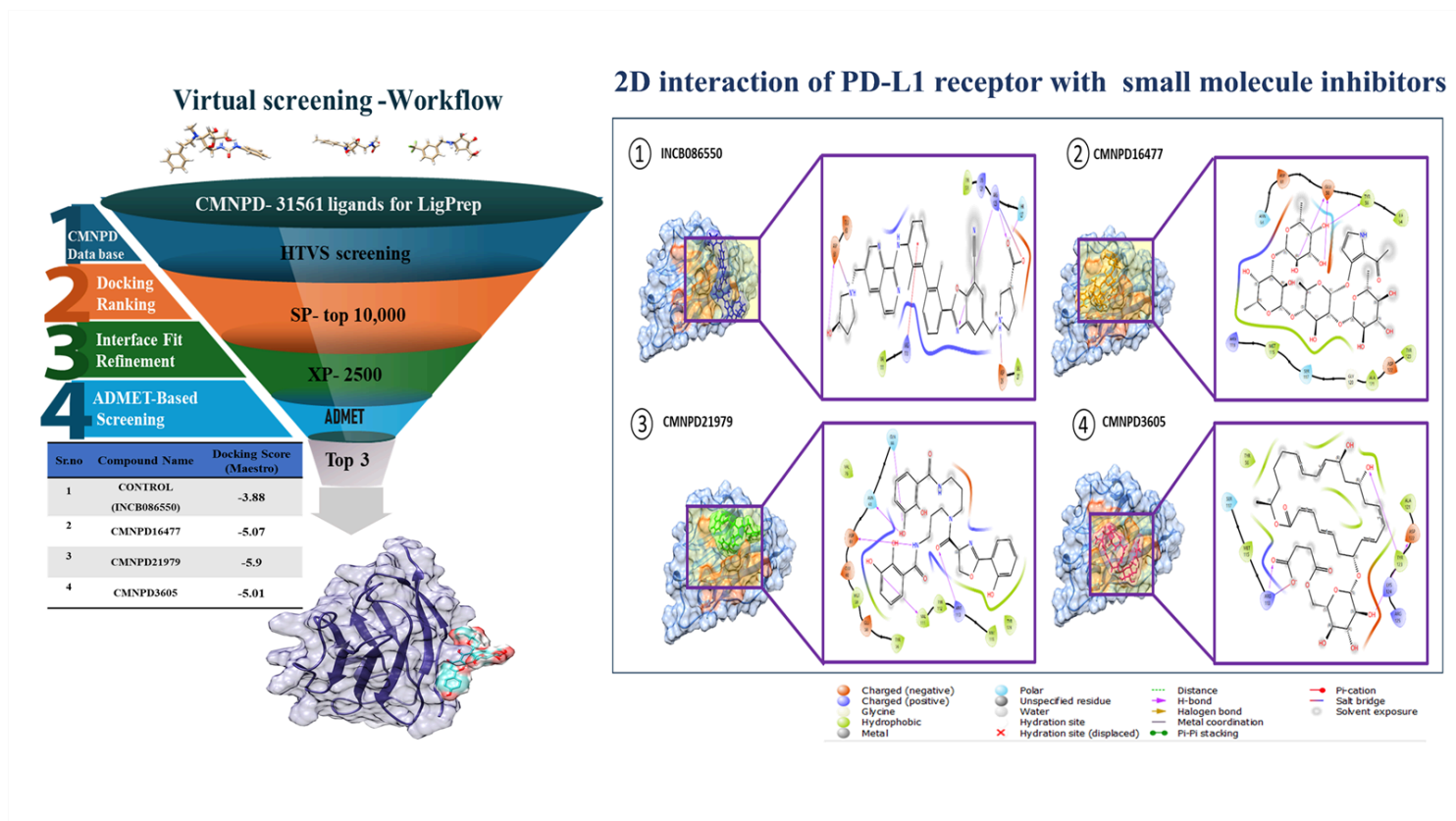


Figure 3

Virtual screening workflow and molecular interaction of small-molecule inhibitors with PD-L1. (A) Schematic representation of the virtual screening workflow used to identify potential PD-L1 inhibitors, including ligand selection, docking, and pharmacokinetic evaluation steps. (B) Two-dimensional interaction diagrams illustrating the binding modes of selected small-molecule inhibitors within the PD-L1 binding pocket, highlighting key residues involved in hydrogen bonding and hydrophobic interactions.

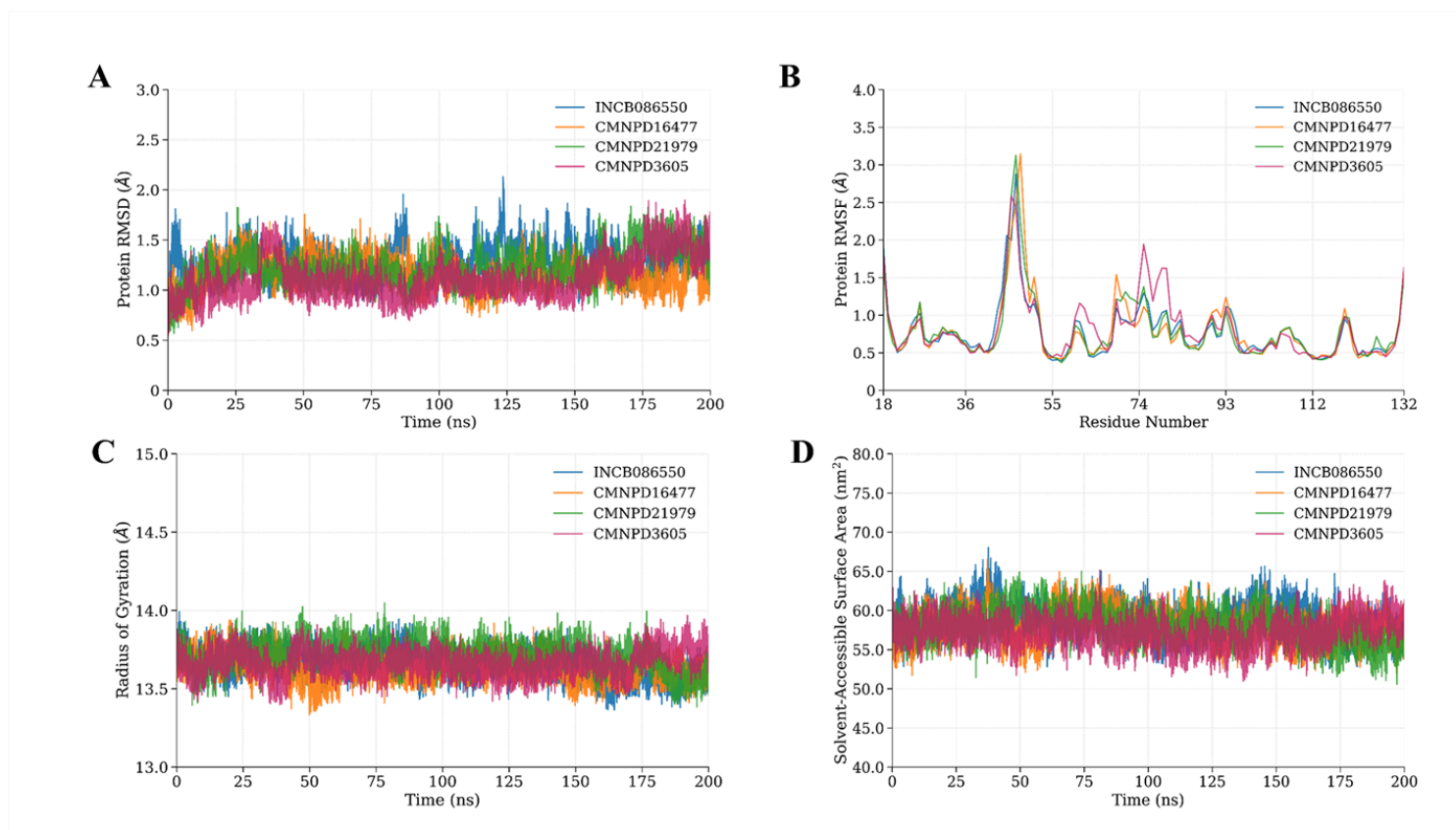


Figure 4

Molecular dynamics trajectory analysis. (A) RMSD showing overall structural stability during the simulation. (B) RMSF depicting residue-wise flexibility. (C) The radius of gyration (Rg) indicates protein compactness. (D) Solvent-accessible surface area (SASA) reflects changes in solvent exposure over time.

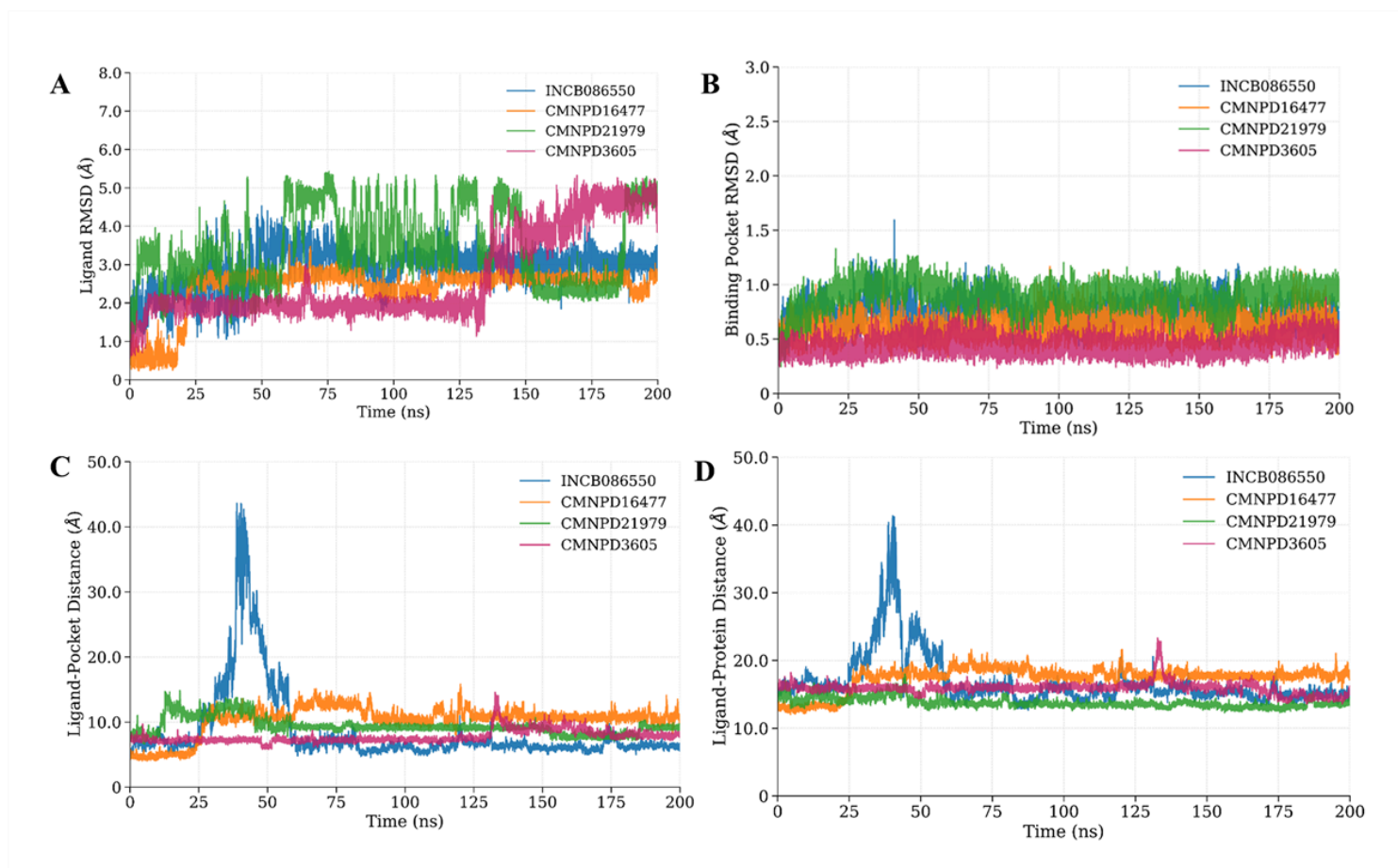


Figure 5

Molecular dynamics analysis of PD-L1 complexes with INCB086550, CMNPD16477, CMNPD21979, and CMNPD3605. **(A)** Ligand RMSD **(B)** Binding pocket RMSD **(C)** Ligand-pocket distance and **(D)** Ligand-protein distance plotted as a function of simulation time. These traces illustrate the degree of ligand stability, pocket rigidity, and spatial fluctuations for each system, allowing for direct comparison of binding persistence and conformational dynamics.

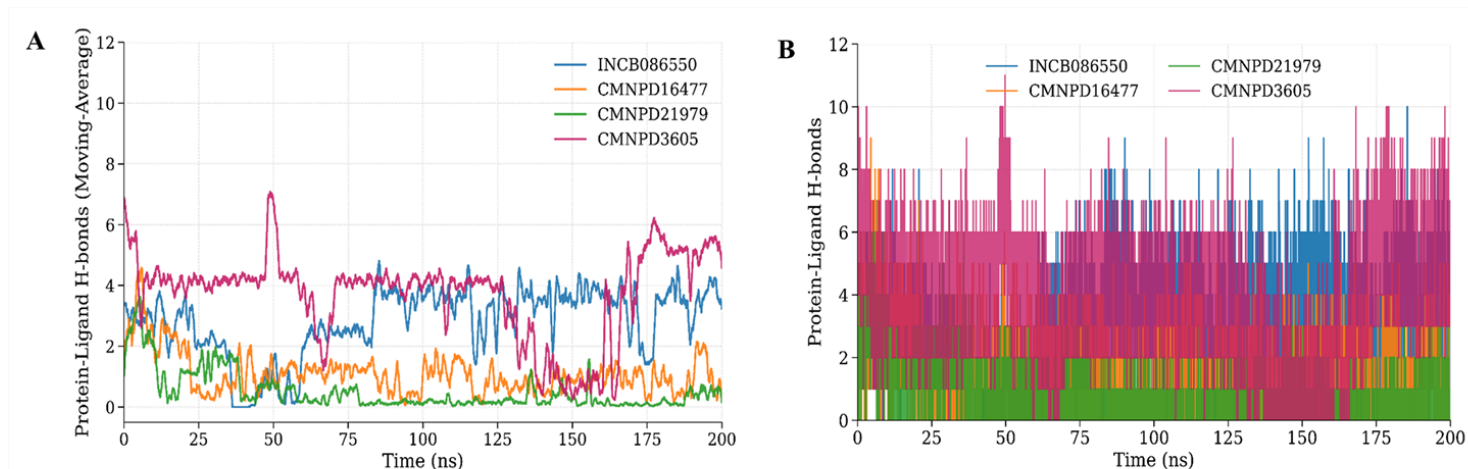


Figure 6

Protein-ligand hydrogen bond formation during 200 ns molecular dynamics simulations for PD-L1 complexes with INCB086550, CMNPD16477, CMNPD21979, and CMNPD3605. (A) Moving-average number of hydrogen bonds quantifies persistence and fluctuation of polar contacts, and (B) total hydrogen bonds at each timestep reveal the overall temporal pattern and differences in anchoring strategy among ligands.

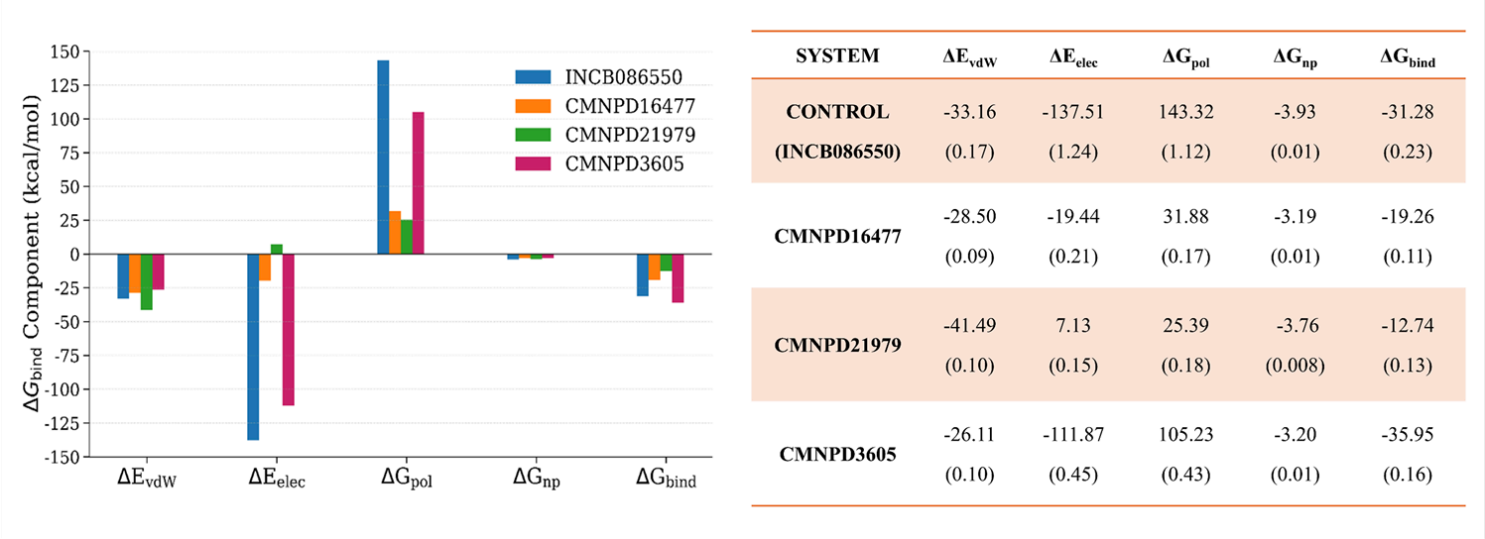


Figure 7

MM/PBSA binding free energy decomposition for PD-L1 complexes with INCB086550, CMNPD16477, CMNPD21979, and CMNPD3605. Bars depict the contributions (kcal/mol) of van der Waals (ΔE_{vdW}), electrostatic (ΔE_{elec}), polar solvation (ΔG_{pol}), and nonpolar (ΔG_{np}) terms, as well as total binding free energy (ΔG_{bind}), highlighting differences in the thermodynamic drivers of binding across ligand systems.

Supplementary Files

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