

**Discovery of *Caralluma*-Derived Pregnane Glycosides as Potent and Selective  
Cholinesterase Inhibitors: Integrated *In Silico* and *In Vitro* Evaluation**

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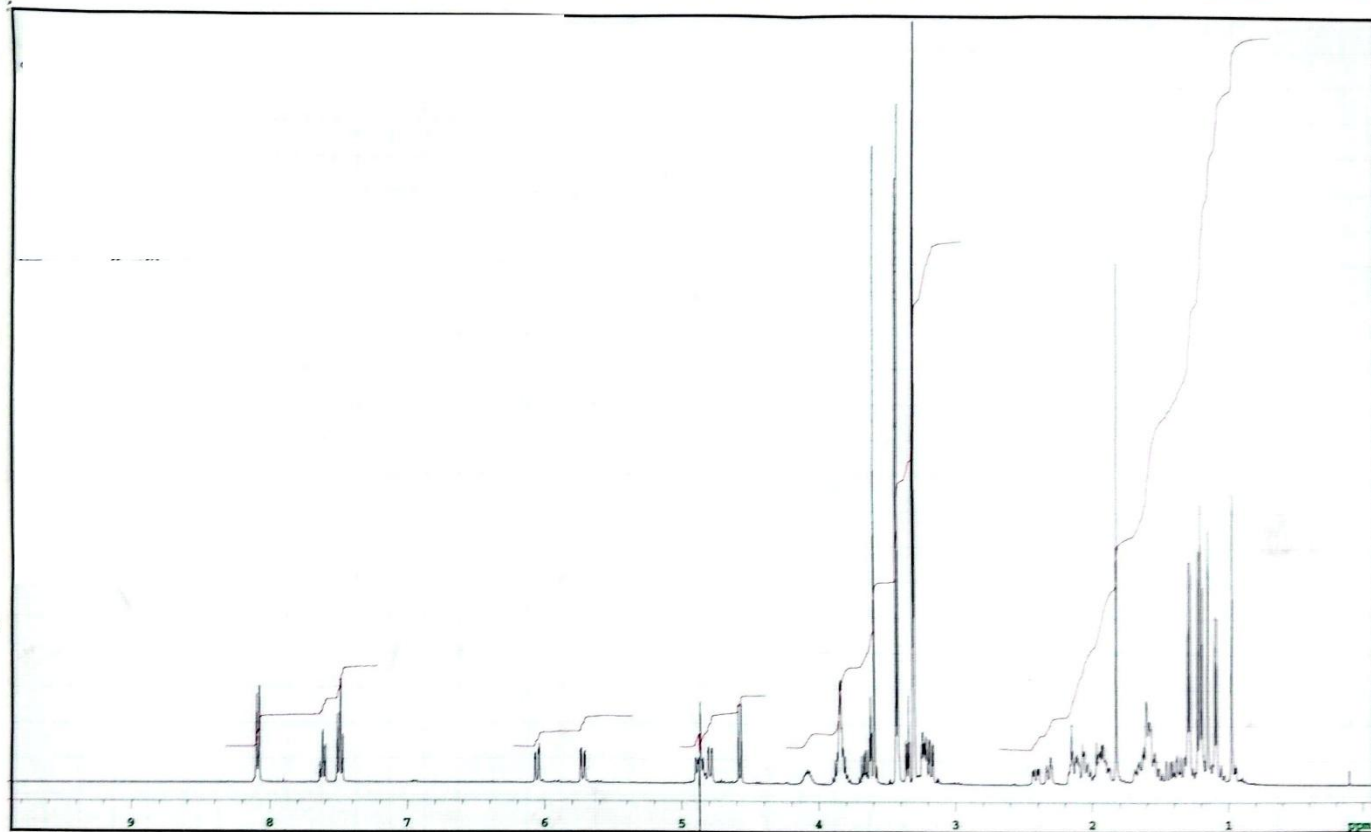
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# Spectral Data



RECEIVE	Nucleus _____	Freq _____ MHz	RECEIVE	Nucleus _____	Offset _____ Hz	PULSE PROCESSING	FN _____ K	PR _____ sec	CD _____ sec	EXPERIMENT	Pulse Sequence _____	SAMPLE	Number _____
	Spec. Width _____ Hz	Offset _____ Hz		Mode _____	Power _____ dB		LB _____ Hz	AF _____ sec	CCD _____		Tube O.D. _____ mm		File _____
	Acq. Time _____ sec	Delay _____ sec		Modulation Mode _____	Freq _____ Hz		Width _____ Hz/ppm	Start _____ Hz/ppm	Temp _____ °C		Date _____		
	Pulse Width _____ μsec	Transmits _____		Pulse Width _____ μsec	Power Mode _____		Reference _____	Solvent _____	XL _____				

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Fig. S1. <sup>1</sup>H NMR of Caratuberside E (10).

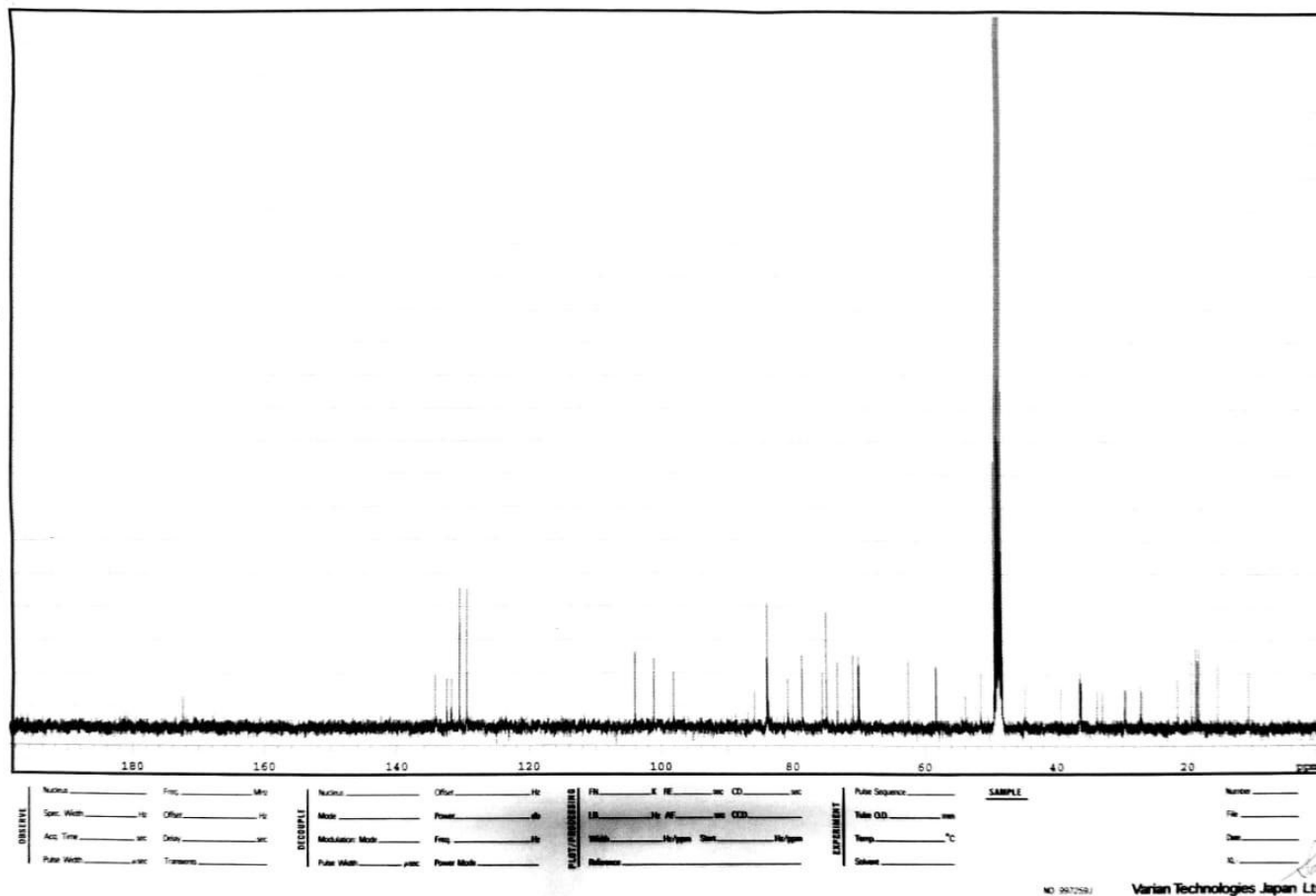
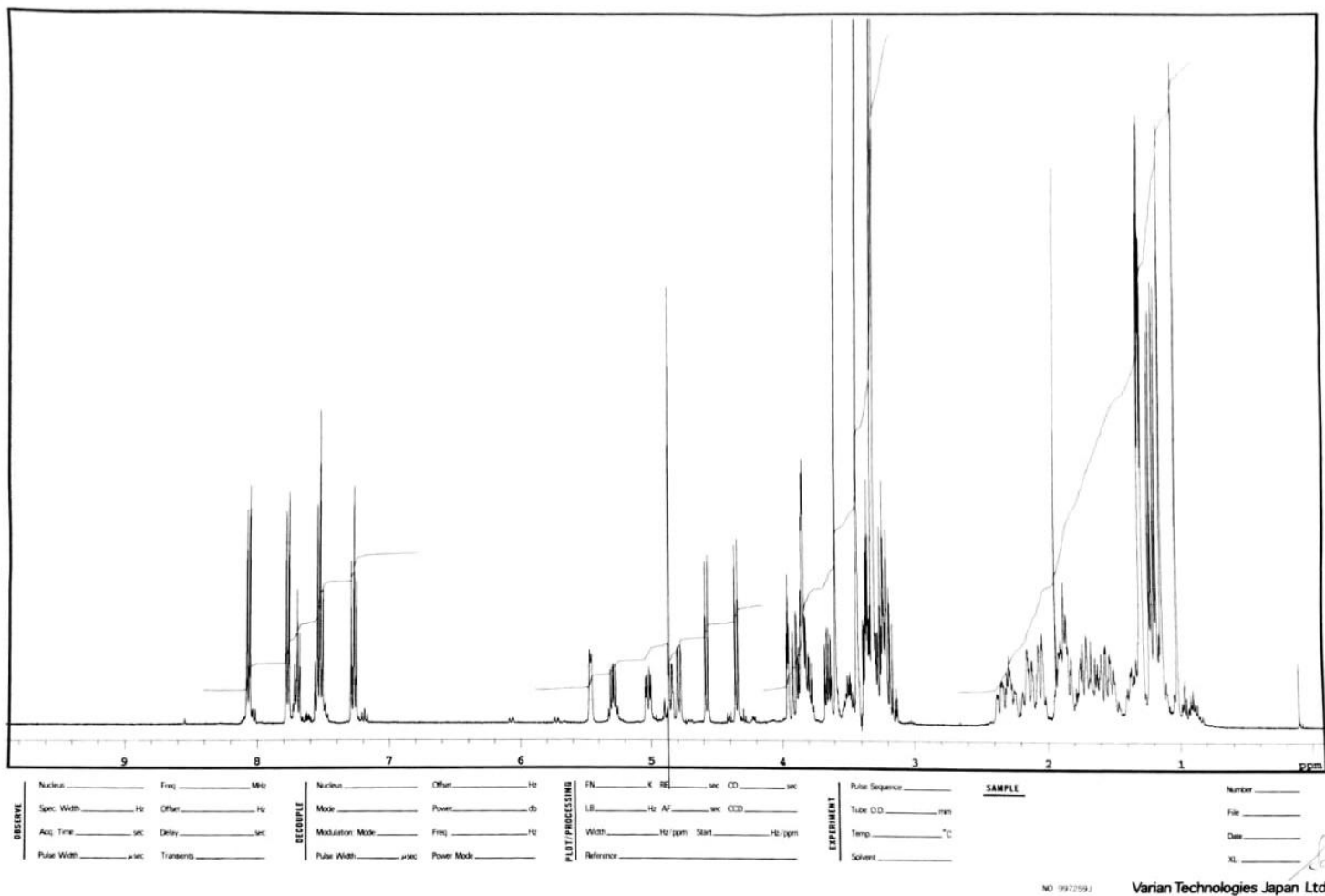


Fig. S2.  $^{13}\text{C}$ NMR of Caratuberside E (10).



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Fig. S3. <sup>1</sup>H NMR of Caratuberside G (11).

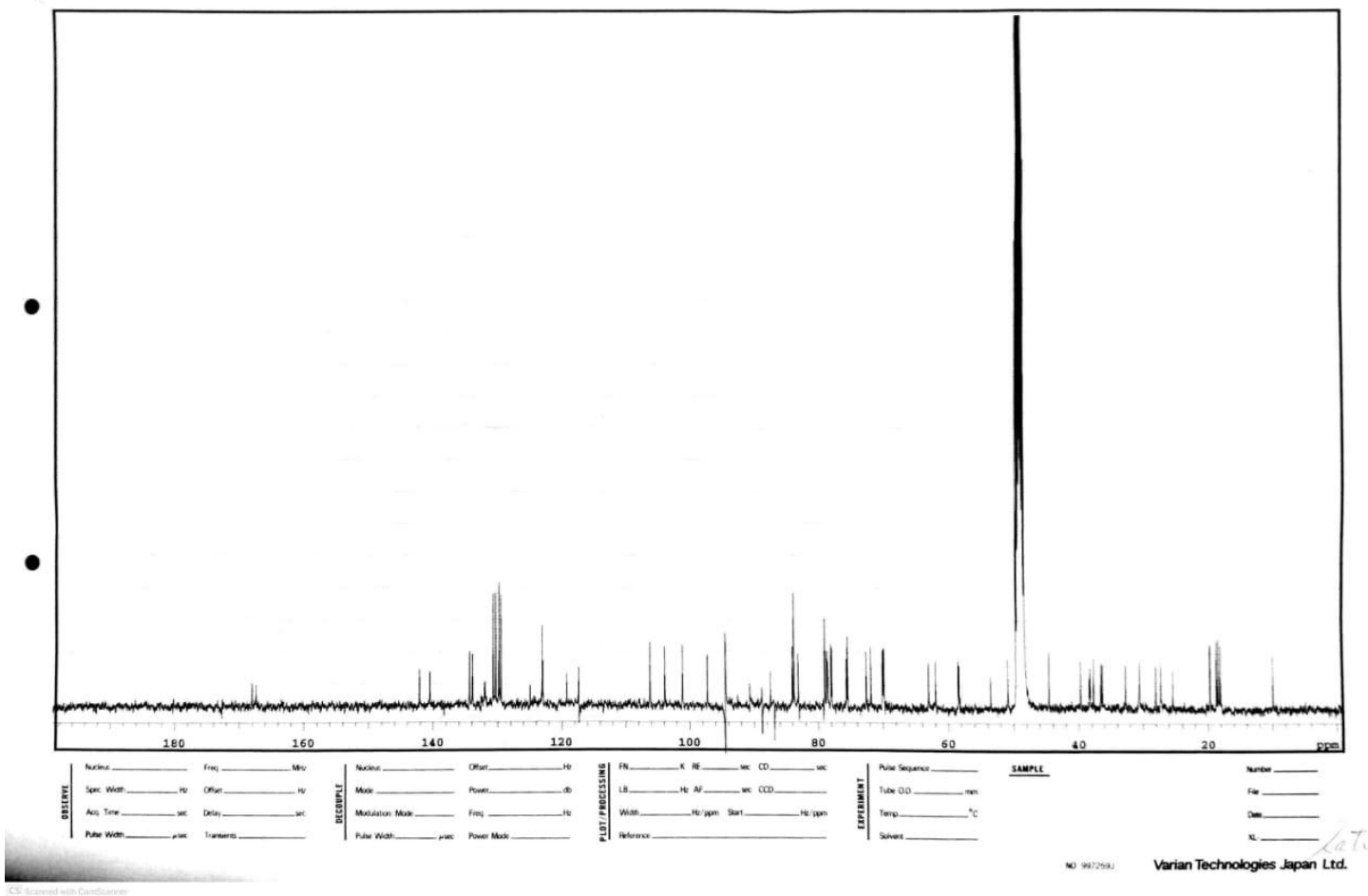
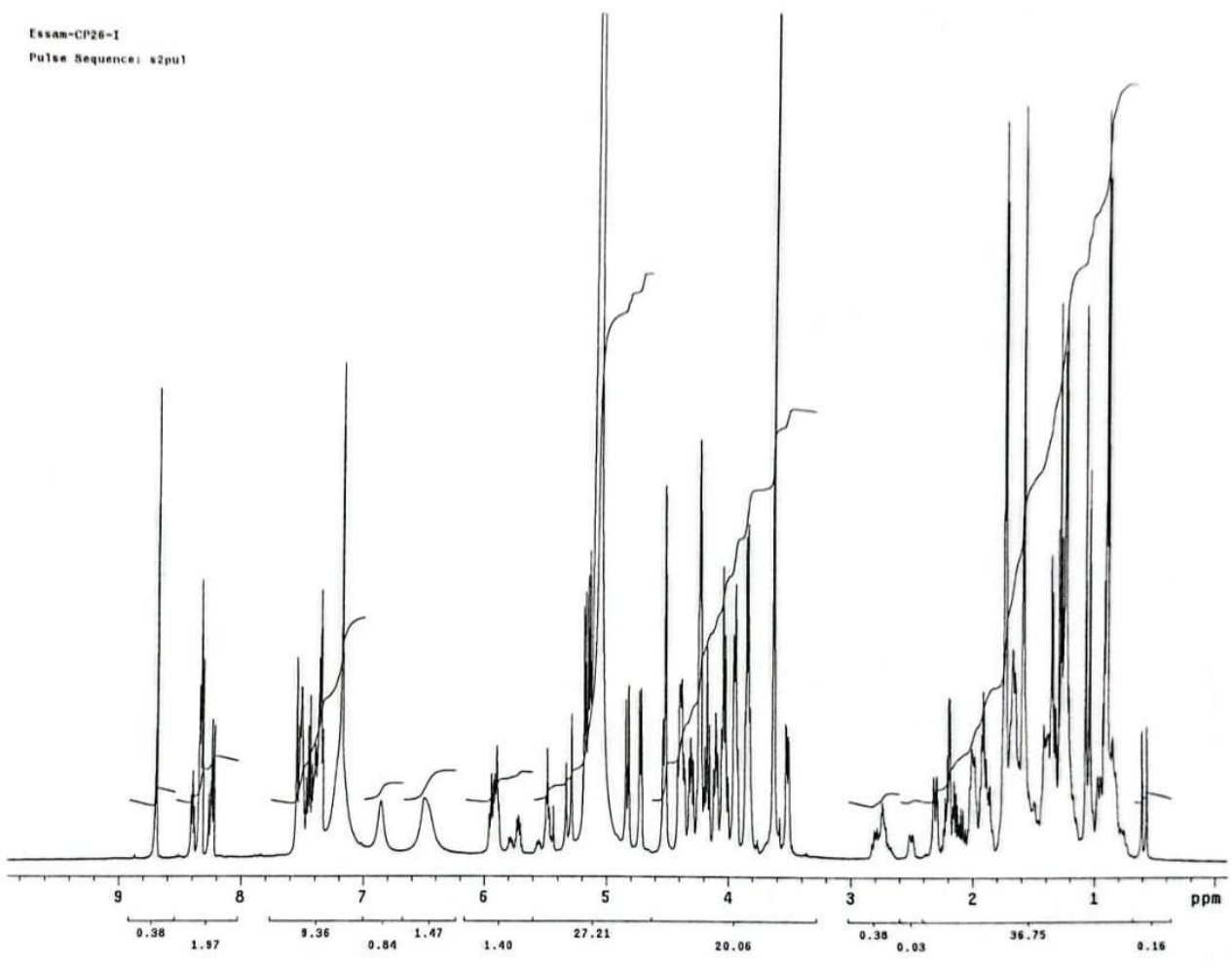


Fig. S4.  $^{13}\text{C}$ NMR of Caratuberside G (11).



**Fig. S5.**  $^1\text{H}$ NMR of penicilloside C (15).



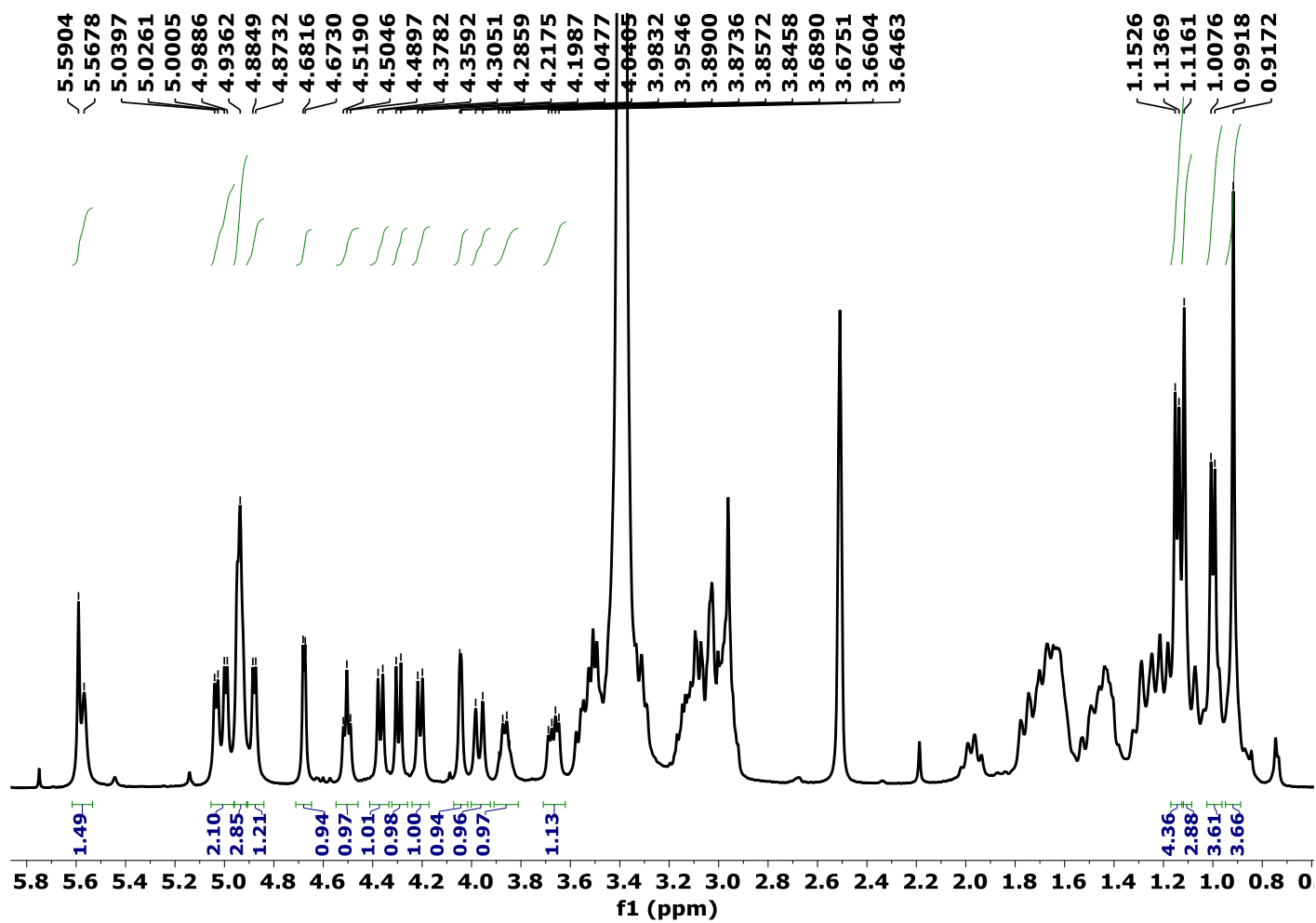


Fig. S7. <sup>1</sup>H NMR of awdelioside B (25).

Essam AbdElSattar\_C\_CW-D.10.fid  
Essam AbdElSattar\_C\_CW-D

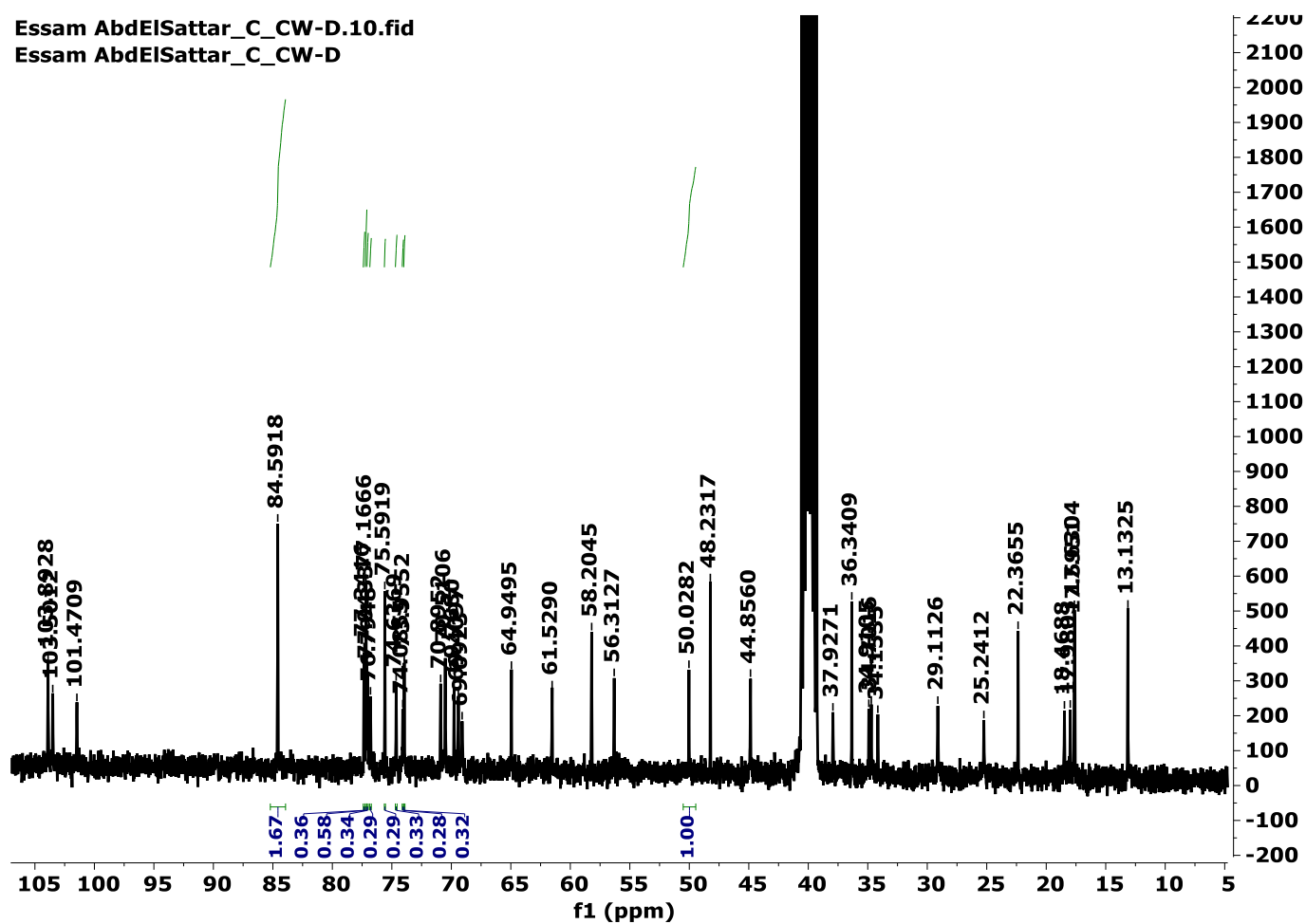
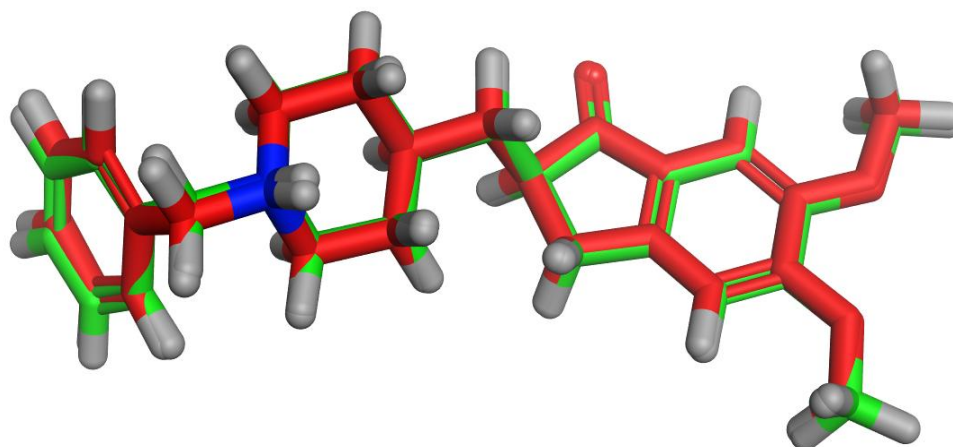
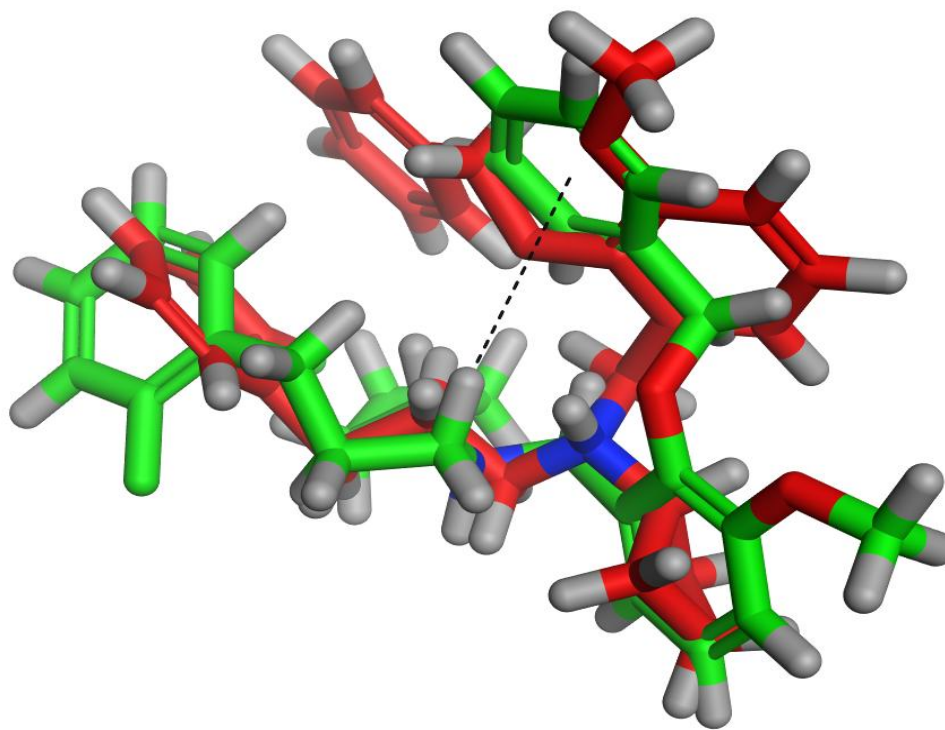


Fig. S8.  $^{13}\text{C}$  NMR of awdelioside B (25).

## Molecular Docking Validation



A



B

**Fig. S9. Superimposition of the docked co-crystal (green) over the native co-crystal (red) for (A) AChE (PDB ID: 4EY7 and RMSD = 0.13 Å) and (B) BuChE (PDB ID: 8CGO and RMSD = 1.32 Å).**

## Molecular Dynamics Simulation Validation

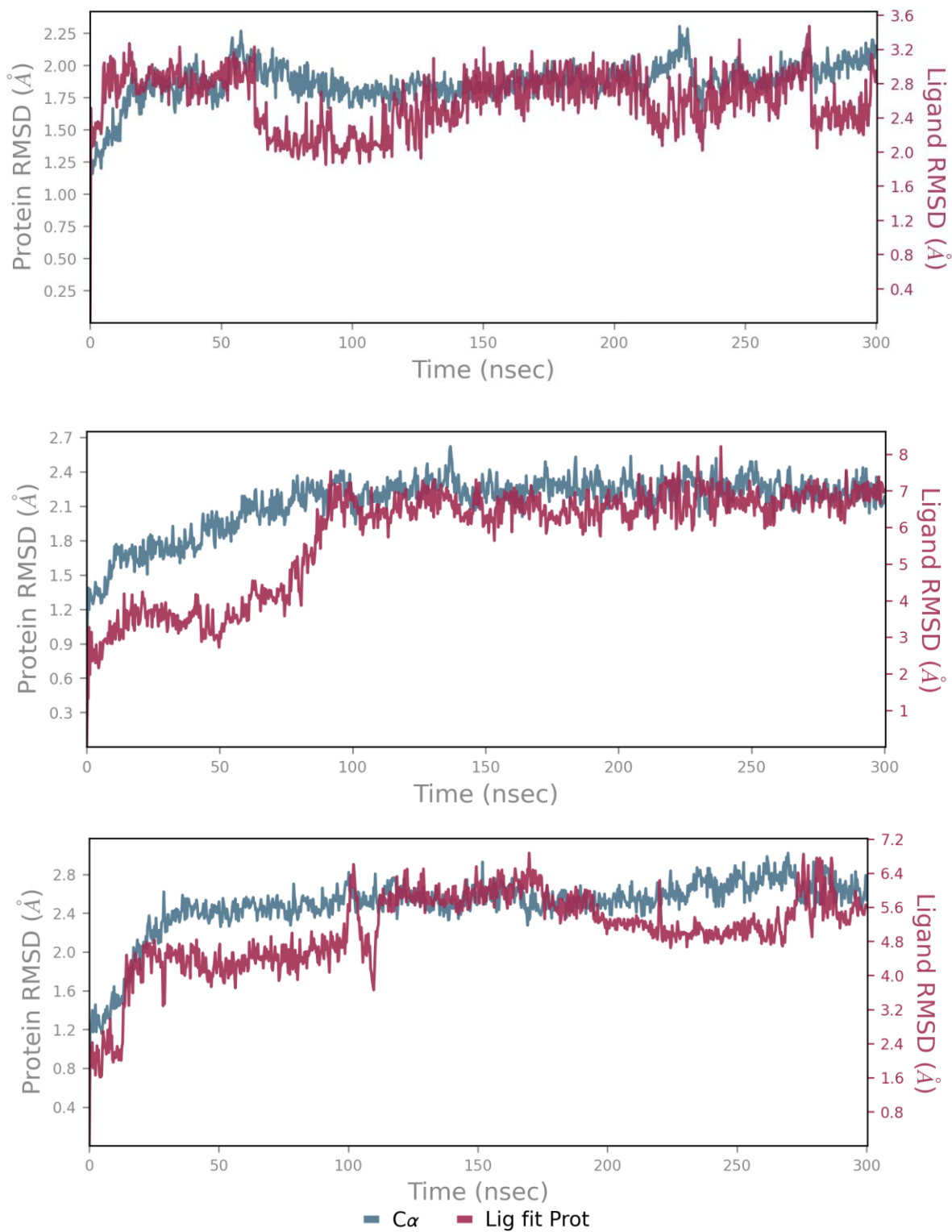
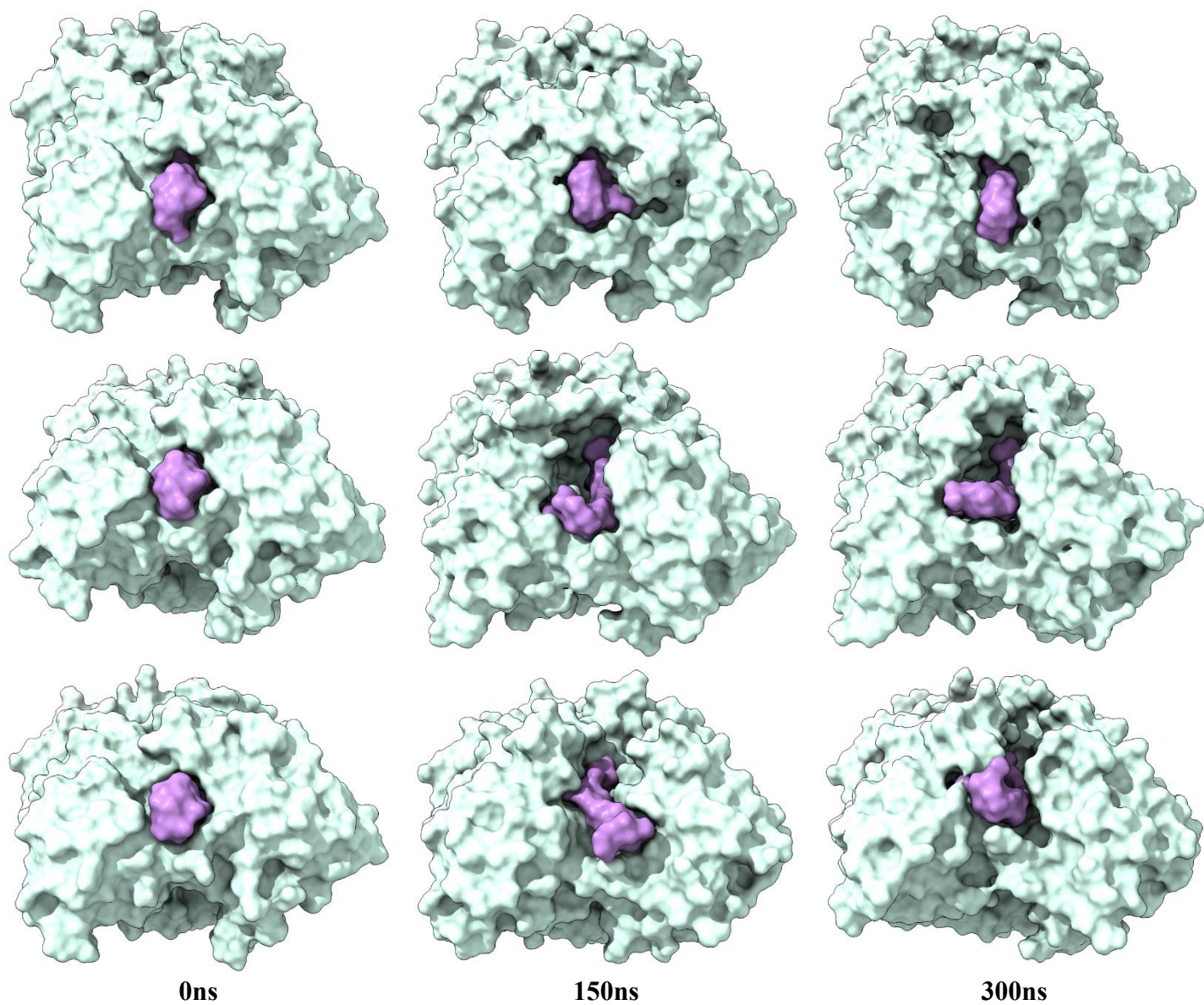


Fig. S10. Three runs were conducted on Caratuberside E for 300 ns as a proof-of-concept.

## Molecular Dynamics Simulation Validation



**Fig. S11. Snapshots of the triplicate run at 0, 150, and 300 ns.**

### **SI1. Molecular dynamics simulation**

The molecular dynamics simulations were carried out using the Desmond simulation package of Schrödinger LLC.<sup>1-3</sup> The NPT ensemble with the temperature 300 K and a pressure of 1.01 bar was applied in all runs. The simulation length was 200 ns with a relaxation time of 1 ps. The OPLS4 force field parameters were used in all simulations.<sup>4</sup> The cutoff radius in Coulomb interactions was 9.0 Å. The orthorhombic periodic box boundaries were set 10 Å away from the protein atoms. The water molecules were explicitly described using the transferable intermolecular potential with the three-point (TIP3P) model.<sup>5</sup> Salt concentration was set to 0.15 M NaCl and was built using the System Builder utility of Desmond. The Martyna–Tuckerman–Klein chain coupling scheme with a coupling constant of 2.0 ps was used for the pressure control, and the Nosé–Hoover chain coupling scheme for the temperature control.<sup>6, 7</sup> Nonbonded forces were calculated using a RESPA integrator where the short-range forces were updated every step, and the long-range forces were updated every three steps. The trajectories were saved at 300 ps intervals for analysis. The behavior and interactions between the ligands and protein were analyzed using the Simulation Interaction Diagram tool implemented in the Desmond MD package. The stability of MD simulations was monitored by looking at the RMSD of the ligand and protein atom positions as a function of simulation time.

### **SI2. MD trajectory analysis and prime MM-GBSA calculations**

The simulation interactions diagram panel of the Maestro software was used to monitor the contribution to the ligand-protein stability. The molecular mechanics generalized born/solvent accessibility (MM – GBSA) was performed to calculate the ligand binding free energies and ligand strain energies for docked compounds over the last 50 ns with `thermal_mmgbsa.py` python script provided by Schrodinger which takes a Desmond trajectory file, splits it into individual snapshots, runs the MM-GBSA calculations on each frame, and outputs the average computed binding energy.

## References

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