



A pyranonaphthoquinone from *Rhinacanthus nasutus* (L.) Kurz roots as a potential protein tyrosine phosphatase 1B allosteric binder: An *in silico* study

[Una piranonaftoquinona de las raíces de *Rhinacanthus nasutus* (L.) Kurz como potencial ligando alostérico de la proteína tirosina fosfatasa 1B: un estudio *in silico*]

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Abstract

Context: Protein tyrosine phosphatase 1B (PTP1B) is a validated therapeutic target for type 2 diabetes mellitus (DM) as it can deactivate insulin signalling.

Aims: To isolate the secondary metabolites from the roots of *Rhinacanthus nasutus* and assess their predicted binding affinity toward the PTP1B allosteric site using an *in silico* approach.

Methods: The isolated compounds were identified using spectroscopic techniques and the binding at the allosteric site of PTP1B was predicted by molecular docking. In addition, the most relevant complex was analyzed using molecular dynamics simulation and molecular mechanics-Poisson Boltzmann surface area (MM-PBSA) binding free-energy calculation.

Results: Compound **1** was identified as 4-oxo-rhinacanthin B, along with five known analogues of rhinacanthin obtained from the roots of *R. nasutus*. Docking indicated that compounds **1-6** may fit into the PTP1B allosteric pocket, with compound **1** forming contacts with Asn193, Phe196 and Arg199. The compound **1**-PTP1B complex remained associated with the allosteric pocket for most of the simulation period of 100 ns, although a larger deviation was observed towards the end of the trajectory. MM-PBSA suggested that compound **1** had slightly less favorable predicted binding free energy than ursolic acid, mainly because of a higher positive solvation penalty.

Conclusions: Pyranonaphthoquinones from *R. nasutus* showed plausible *in silico* accommodation within the PTP1B allosteric pocket and may serve as starting scaffolds for further optimization. However, compound **1** has not been evaluated in an enzymatic assay and should therefore be regarded as a computationally prioritized scaffold for future biochemical validation, not as a validated PTP1B inhibitor.

Keywords: molecular docking; molecular dynamics; PTP1B; pyranonaphthoquinone; *Rhinacanthus nasutus*.

Resumen

Contexto: La proteína tirosina fosfatasa 1B (PTP1B) es una diana terapéutica validada para la diabetes mellitus tipo 2 (DM), ya que puede desactivar la señalización de la insulina.

Objetivos: Aislar los metabolitos secundarios de las raíces de *Rhinacanthus nasutus* y evaluar su afinidad de unión predicha al sitio alostérico de PTP1B mediante un enfoque *in silico*.

Métodos: Los compuestos aislados se identificaron mediante técnicas espectroscópicas y se predijo la unión al sitio alostérico de PTP1B mediante acoplamiento molecular. Además, el complejo más relevante se analizó mediante simulación de dinámica molecular y cálculo de la energía libre de unión mecánica molecular-área superficial Poisson-Boltzmann (MM-PBSA).

Resultados: El compuesto **1** se identificó como 4-oxo-rinacantina B, junto con cinco análogos conocidos de rinacantina obtenidos de las raíces de *R. nasutus*. El acoplamiento molecular indicó que los compuestos **1-6** podrían encajar en el bolsillo alostérico de PTP1B, con el compuesto **1** estableciendo contactos con Asn193, Phe196 y Arg199. El complejo compuesto **1**-PTP1B permaneció asociado al bolsillo alostérico durante la mayor parte del período de simulación de 100 ns, aunque se observó una mayor desviación al final de la trayectoria. El análisis MM-PBSA sugirió que el compuesto **1** tenía una energía libre de unión predicha ligeramente menos favorable que la del ácido ursólico, principalmente debido a una mayor penalización de la solvatación positiva.

Conclusiones: Las piranonaftoquinonas de *R. nasutus* mostraron una acomodación *in silico* plausible en el bolsillo alostérico de PTP1B y podrían servir como andamios iniciales para una optimización posterior. Sin embargo, el compuesto **1** no se ha evaluado en un ensayo enzimático y, por lo tanto, debe considerarse un andamio priorizado computacionalmente para una futura validación bioquímica, no como un inhibidor de PTP1B validado.

Palabras Clave: acoplamiento molecular; dinámica molecular; piranonaftoquinona; PTP1B; *Rhinacanthus nasutus*.

ARTICLE INFO

Received: July 15, 2025.

Accepted: May 19, 2026.

Available Online: June 23, 2026.

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Abbreviations: ADMET: Absorption, distribution, metabolism, excretion, and toxicity; AMBER: Assisted model building with energy refinement; AM1-BCC: Austin Model 1-Bond Charge Correction; CC: Column chromatography; CDCl₃: Deuterated chloroform; CYP: Cytochrome P450; DM: Diabetes mellitus; DOCK6: Docking software version 6; EtOAc: Ethyl acetate; GBSA: Generalized born surface area; HPLC: High-performance liquid chromatography; IR: Infrared; MD: Molecular dynamics; MM-PBSA: Molecular mechanics-Poisson Boltzmann surface area; MTD: Maximum tolerated dose; NMR: Nuclear magnetic resonance; ODS: Octadecyl-bonded silica; OCT2: Organic cation transporter 2; OPLS-AA: Optimized potentials for liquid simulations-all atom; PDB: Protein data bank; PME: Particle mesh Ewald; PTP1B: Protein tyrosine phosphatase 1B; Rg: Radius of gyration; RMSD: Root mean square deviation; RMSF: Root mean square fluctuation; SASA: Solvent accessible surface area; SPC: Simple point charge; TFA: Trifluoroacetic acid; UV: Ultraviolet; WPD loop: Trp-Pro-Asp catalytic loop; YASARA: Yet another scientific artificial reality application.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia caused by impaired insulin secretion, impaired insulin action, or both (Dilworth et al., 2021). Protein tyrosine phosphatase 1B (PTP1B) has attracted attention as a negative regulator of insulin signalling because it dephosphorylates the activated insulin receptor and downstream substrates, thus dampening insulin responses. Increased PTP1B activity has been related to insulin resistance, supporting this enzyme as a validated target for improving insulin sensitivity in type 2 DM (Kanwal et al., 2022; Teimouri et al., 2022). Despite substantial efforts, clinical translation of PTP1B inhibitors was limited because of challenges in selectivity, cell permeability, and safety, showing the need for alternative scaffolds from chemically diverse natural sources.

This limitation is partly due to the catalytic pocket of PTP1B being highly conserved in classical protein tyrosine phosphatases, which contributes to the limited clinical progress of PTP1B inhibitors. Consequently, selective inhibition is difficult, and the risk of off-target effects may be increased (Mehlman et al., 2024; Woods et al., 2024). In this context, the allosteric pocket of PTP1B has been structurally validated by co-crystal studies and is functionally linked to the dynamics of the WPD-loop, providing a rationale for its use as a target for selective modulation.

Natural products are privileged scaffolds for enzyme modulation, due to their high chemical diversity and preorganized bioactive architectures. Several natural product classes reported to modulate PTP1B, such as flavonoids, terpenoids, and quinone-type metabolites, include some members that may be non-competitive inhibitors, consistent with allosteric binding (Liu et al., 2023). However, many natural-product studies rely mainly on enzyme screening or static docking snapshots, with little investigation of binding stability, conformational changes, and energetic contributions at the allosteric site. In scaffolds derived from quinones, polarity and solvent exposure can strongly influence binding energetics and should therefore be evaluated dynamically.

Against this background, *Rhinacanthus nasutus* (L.) Kurz (*Acanthaceae*) is generally applied in traditional medicine in Southeast Asia, and has been investigated for its structurally diverse secondary metabolites, particularly rhinacanthin-type naphthoquinones and pyranonaphthoquinones (Prasansuklab et al., 2020; Songserm et al., 2022). The importance of this plant as a source of oxidized pyranonaphthoquinone derivatives is supported by the previous phytochemical study that reported the presence of one of the new compounds 4-oxo-rhinacanthin A in the same species (Maarisit et al., 2017). The potential of *R. nasutus* as an antidiabetic has been demonstrated with extracts and isolated compounds in a number of models (Le et al., 2025), but the basis of the target level is still not clearly understood, especially at the PTP1B allosteric site.

In the present study, one pyranonaphthoquinone, 4-oxo-rhinacanthin B (**1**), along with five known congeners (**2-6**), were isolated from the roots of *R. nasutus* and identified spectroscopically. They were then subjected to a target-oriented computational workflow to investigate their potential interaction with PTP1B. Molecular docking at the validated allosteric site of PTP1B (PDB ID: 1T49), molecular dynamics simulation, and molecular mechanics-Poisson-Boltzmann surface area (MM-PBSA) analysis were then carried out to calculate the relative binding energy and stability of the complex. Because no enzymatic assay was performed, any proposed PTP1B-modulating activity should be regarded as a structure-based computational hypothesis requiring biological confirmation.

MATERIAL AND METHODS

Chemicals

All chemicals and reagents used in this study were of analytical grade. Ethanol, methanol, ethyl acetate, trifluoroacetic acid (TFA), and octadecyl-bonded silica (ODS) were purchased from Sigma-Aldrich (Merck Group, Germany). Deuterated chloroform (CDCl₃) for NMR analysis was purchased from Sigma-Aldrich (Merck Group, Japan).

Instrumentation

NMR (^1H 400 MHz and ^{13}C 100 MHz) spectra were recorded using a JEOL instrument. Chemical shifts were referenced to the residual solvent signals of CDCl_3 (δH 7.26, δC 77.0). A JMS-MS 700 mass spectrometer (JEOL, Tokyo, Japan) was used to obtain EIMS and High-resolution FAB mass spectra. Optical rotations were measured on a digital polarimeter (JASCO P-2300, JASCO, Ltd., Tokyo, Japan). IR spectra were recorded using a PerkinElmer Spectrum One Fourier transform infrared spectrometer (Waltham, MA, USA). Preparative HPLC was performed using a TOSOH-8020 system equipped with a TOYO SODA UV-8000 detector. Furthermore, column chromatography was conducted on ODS (Wakogel100C18). The three-dimensional structure of PTP1B with PDB ID 1T49 was retrieved from the Protein Data Bank (<https://www.rcsb.org/structure/1T49>) (Wiesmann et al., 2004).

Sample collection

R. nasutus roots were collected from Talaud Island, North Sulawesi Province, Indonesia, in February 2025 at 4°31'38" N and 126°48'28" E. Taxonomic identification and authentication were performed by Dr. Yessie Lengkey (Universitas Kristen Indonesia Tomohon) based on morphological characters and comparison with authenticated herbarium specimens. For future reference, a voucher specimen (W-0325) was deposited at the Herbarium of the Faculty of Mathematics and Natural Sciences, Universitas Kristen Indonesia Tomohon, Indonesia. The samples for this study were collected in accordance with local regulations and with permission from the Government of Talaud Island, Indonesia.

Extraction and isolation

Dried roots of *R. nasutus* (1.000 kg) were crushed and extracted by cold maceration using 70% ethanol (v/v) at room temperature over 72 hours in three successive 24-hour cycles with a solvent-to-solid ratio of 5:1 and intermittent agitation. The pooled filtrates were concentrated under low pressure at 40°C to obtain a crude extract, which was suspended in water and partitioned with EtOAc (ethyl acetate). ODS open-column chromatography (50 μm , 1.5 $\times$ 60 cm) with a MeOH:H₂O gradient (20:80 to 100:0 v/v) was used to fractionate the EtOAc fraction (1.520 g; 0.152% w/w of dried material). This process yielded eight fractions, which were pooled in 20 mL volumes, then analyzed by TLC (UV at 254 and 366 nm), concentrated, and weighed, as shown in Table 1.

Fraction 5 was purified by preparative HPLC (Pegasil ODS, i.d. 10 mm $\times$ 250 mm; 2.0 mL/min, UV 220 nm) using MeOH:H₂O (70:30, v/v) to afford compounds **6** (5.3 mg), **3** (15.0 mg), and **5** (1.2 mg). Fraction 6 (261.6 mg), purified under the same chromatographic conditions, afforded compounds **4** (3.4 mg), **6** (8.1 mg), and **3** (96.6 mg). Fraction 7 (300.9 mg), purified with 80% MeOH, afforded compounds **6** (1.5 mg), **3** (64.1 mg), and **5** (4.4 mg). Combined subfraction 7-2 (20.3 mg) was further purified to afford compound **1** (2.7 mg) and compound **2** (5.6 mg). Final purification was performed by preparative HPLC [column, PEGASIL ODS (Senshu Sci. Co. Ltd., Tokyo, Japan), i.d. 10 mm $\times$ 250 mm; solvent, MeOH:H₂O (80:20, v/v); flow rate, 2.0 mL/min; detection, UV 210 nm]. For structural characterization, the isolated compounds showed clean and well-resolved ^1H -NMR spectra, which supported their suitability for spectroscopic identification.

Table 1. Weight of fractions (1–8) obtained from the *R. nasutus* roots.

Fraction	Yield (mg)	Yield (% w/w to 1 kg sample)
1	628	0.0628
2	22.1	0.00221
3	33.8	0.00338
4	31.0	0.00310
5	85.0	0.00850
6	261.6	0.02616
7	300.9	0.03009
8	57.9	0.00579

Ligand and receptor preparation

Two-dimensional ligand structures were drawn in ChemDraw (PerkinElmer, USA) and converted into three-dimensional geometries using Chem3D. Ligand geometries were energy-minimized using the YASARA Minimisation Server, and the optimized structures were saved as .mol2 files. Hydrogen atoms and AM1-BCC partial charges were assigned in UCSF Chimera v1.18, and ligand protonation states were inspected at physiological pH (7.4). The human PTP1B crystal structure (PDB ID: 1T49) was selected because it contains a co-crystallized ligand in a structurally validated allosteric pocket located approximately 20 Å from the catalytic site. Chain A was used as the receptor. Missing residues were modelled with MODELLER v9.21 when required. Receptor protonation at pH 7.4 was assigned in UCSF Chimera with reference to standard residue protonation states, and H⁺⁺-based pK_a estimation was used to check ionizable residues where appropriate. Crystallographic waters were removed unless considered structurally conserved. Only non-polar hydrogen atoms were removed during generation of the distributed molecular surface (.dms) file, so polar hydrogens were retained for docking-based surface mapping and sphere generation in DOCK6 (Gordon et al., 2005; Maier et al., 2015).

Molecular docking

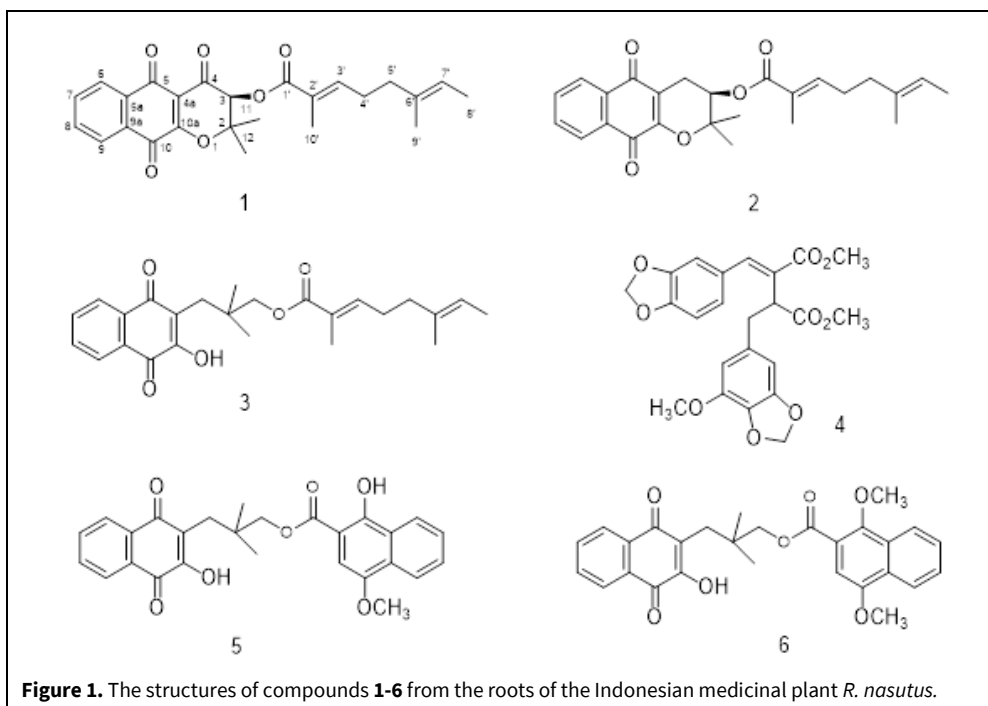
DOCK6 (Linux MPI version) was used to dock the ligands into the PTP1B allosteric pocket (PDB ID: 1T49). The co-crystallized ligand and non-biological heteroatoms were removed from the receptor, whereas structurally conserved crystallographic waters were retained when relevant. Polar hydrogens were retained, and the receptor protonation state at pH 7.4 was assigned in UCSF Chimera. Binding-site spheres were generated with sphgen, and spheres located within 10.0 Å of the co-crystallized ligand were selected with sphere selector to define the PTP1B allosteric site. This site was selected because the 1T49 co-crystal structure identifies a structurally validated non-catalytic pocket located approximately 20 Å from the catalytic site and widely used for selective PTP1B allosteric modulation studies (Wiesmann et al., 2004; Yang et al., 2021).

The grid center was set at $x = 21.21905$, $y = 16.30300$, and $z = 18.14000$, with box dimensions of $32 \times 42 \times 36$ Å and a grid spacing of 0.6 Å. Van der Waals and electrostatic potentials were used to generate the scoring grid. Flexible docking was carried out using the anchor-and-grow algorithm, and 50 poses were generated for each ligand and ranked using the Hawkins MM/GBSA scoring function in DOCK6 (Allen et al., 2015; Siswanto et al., 2019). Ligand partial charges

were assigned using the AM1-BCC model before docking of the prepared .mol2 structures. Receptor charges were assigned according to the selected force-field parameters. Final poses were selected using the minimum grid score, geometric plausibility, and consistency of interactions within the defined allosteric pocket. Native-ligand redocking validated the protocol with an RMSD ≤ 2.0 Å relative to the crystallographic pose (Allen et al., 2015; Alogheli et al., 2017). The DOCK6 sphere-generation, grid, scoring, and flexible-docking input parameters are provided in supplementary Table S1 to improve computational reproducibility.

Molecular dynamics simulation

Molecular dynamics simulations were carried out for the PTP1B-compound **1** and PTP1B-ursolic acid complexes to allow a focused comparative evaluation. Compound **1** was selected as the new scaffold of interest, whereas ursolic acid was retained only as a literature comparator for PTP1B modulation and not as a validated 1T49 allosteric reference ligand. The molecular dynamics runs were performed using GROMACS 2021, as documented by the NVT, NPT, production MD, and ligand hydrogen-bond command logs provided in Supplementary Table S2. The protein was described using the OPLS-AA force field, and ligand topologies were generated within a GROMACS-compatible OPLS-AA framework and applied consistently across the simulated complexes. Ionizable residues and ligands were assigned protonation states corresponding to physiological pH (7.0–7.4). Each system was solvated in a triclinic SPC water box and neutralized to an ionic strength of 0.15 M using Na⁺ and Cl[−] ions. To avoid steric clashes and unfavorable contacts, energy minimization was carried out using the steepest-descent algorithm for 5,000 steps. The systems were equilibrated for 500 ps with NVT ensemble and 500 ps with NPT ensemble at 300 K and 1 bar, respectively, followed by 100 ns production runs with periodic boundary conditions. Short-term equilibration, complex stability and conformational drift at late stages were examined in a single simulation window of duration 100 ns. Supplementary Table S2 improves transparency of the simulation execution and command environment. However, the exact external ligand-topology generation tool, software version, and charge-derivation workflow were not fully recoverable from the archived records and are therefore retained as a methodological limitation. Because the exact ligand-parameterization workflow could not be reconstructed, the MD and MM-PBSA results should be interpreted as qualitative support for binding stability rather than quantitative affinity estimates.



The trajectory analysis and MM-PBSA calculation

The backbone RMSD, ligand RMSD, RMSF, radius of gyration (Rg), solvent-accessible surface area (SASA), intramolecular protein hydrogen bonds and intermolecular protein-ligand hydrogen bonds were used to evaluate trajectory stability. The behavior of the compound **1**-PTP1B and ursolic acid-PTP1B complexes were descriptively compared during the 100 ns simulations and the following parameters were used. The binding free energy was calculated by the MM-PBSA approach following the equation [1].

$$\Delta G_{\text{bind}} = \Delta E_{\text{MM}} + \Delta G_{\text{solv}} \quad [1]$$

where: ΔE_{MM} describes the molecular mechanics energy in the gas phase, containing van der Waals and electrostatic contributions ($\Delta E_{\text{vdW}} + \Delta E_{\text{ele}}$). Negative values show favorable interactions. ΔG_{solv} represents the solvation free energy contribution, consisting of the polar solvation energy (measured applying the Poisson-Boltzmann model) and non-polar solvation energy (typically derived from SASA). More negative ΔG_{bind} values show stronger predicted binding affinities (Abraham et al., 2015).

ADMET prediction

The pkCSM server (<https://biosig.lab.uq.edu.au/pkcsml/>) was used to predict ADMET properties. The studied parameters were water solubility, intestinal absorption, permeability in Caco-2 cells, distribution volume, fraction unbound, blood brain barrier permeability, total clearance and selected toxicity endpoints such as AMES mutagenicity, hepatotoxicity and skin sensitisation.

Statistical analysis

The mean values of energy components (ΔG_{bind} , ΔG_{gas} , and ΔG_{solv}) from 11 frames of the equilibrated parts of the compound **1**-PTP1B and ursolic acid-PTP1B simulations were summarized as mean $\pm$ SD. Since these frames were taken from 1 MD trajectory and did not constitute replicate experiments, inferential statistical analysis was not carried out. The values were interpreted descriptively to compare the size and signs of the gas phase and solvation components. Descriptive summaries were made and figures prepared using GraphPad Prism 11 (GraphPad Software, La Jolla, CA, USA).

RESULTS

Isolation and structure elucidation

In this study, one compound was isolated along with five previously known rhinacanthin derivatives of the *R. nasutus* root. Fractionation of 1.0 kg dried root material with 70% ethanol provided a crude extract, which was subjected to liquid-liquid partitioning to provide an ethyl acetate fraction (1.520 g; 0.152, w/w, with respect to dried mass). Further chromatographic purification produced milligram quantities of six separated compounds. The isolated compounds were rhinacanthin B (**2**), rhinacanthin C (**3**), rhinacanthin E (**4**), rhinacanthin N (**5**), and rhinacanthin Q (**6**), as shown in Fig. 1. All isolates have been previously reported in the same species (Le et al., 2025; Kernan et al., 1997; Wu et al., 1988; 1998), along with one new compound (**1**).

Table 2. NMR data for compound **1** (CDCl₃).

Position	δ_c	δ_H mult. (J in Hz)	COSY	HMBC
1				
2	85.9			
3	74.9	5.51 s		C-2, C-4, C-11
4	185.9			
4a	112.3			
5	180.0			
5a	131.8			
6	126.2	8.12, d (8.0)	H-7	C-5, C-6a, C-7, C-8
7	135.5	7.83, t (8.0)	H-6	C-6, C-6a, C-8
8	133.5	7.75, t (8.0)	H-9	C-7, C9,
9	126.5	8.12, d (8.0)	H-8'	C-8, C9a, C10
9a	130.7			
10	179.5			
10a	161.3			
11-CH ₃	25.3	1.69, s		C-2, C-3, C-12
12-CH ₃	19.8	1.61, s		C-2, C-3, C-11
1'	166.1			
2'	127.1			
3'	144.7	6.85 t (8.0)	H-4'	C-2', C-4'
4'	38.0	2.34, dd (16, 8.0)	H-3', H-5'	C-3' C-5'
5'	27.4	2.13, t (8.0)	H-4'	C-4', C-6', C-7'
6'	134.4			
7'	119.4	5.24 d (8.0)	H-8'	
8'-CH ₃	13.3	1.57 d (8.0)	H-7'	C-6', C-7'
9'-CH ₃	12.4	1.52, s		C-6', C-7'
10'-CH ₃	15.5	1.88, s		C-1', C-2', C-3'

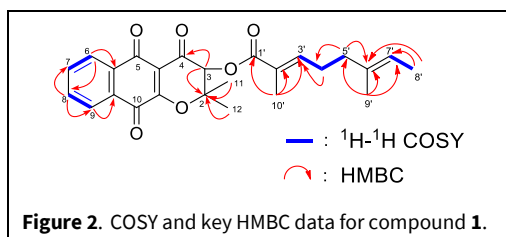
Compound **1**, isolated as pale-yellow needles, showed a C₂₅H₂₆O₆ molecular formula, as described by EIMS at m/z 422.1730 [M]⁺ (Δ + 0.1 mmu). The ¹³C-NMR spectra of **1** classified 25 carbon signals into four sp² methines signals (126.2, 135.5, 133.5, and 126.5), four carbonyls (180.0, 179.5, 185.9, and 166.1), six sp² quaternary carbons (112.3, 127.1, 130.7, 131.8, 134.4, and 161.1), two sp² methines (119.4 and 144.7), two sp³ methylenes (27.4 and 38.0), one sp³ oxygenated methine (74.9), and five methyls (12.4, 13.3, 15.5, 19.8, and 25.3). The ¹H-NMR spectrum of **1** indicated four aromatic protons at δ_H 8.12 (d, J = 8.0 Hz), 7.83 (t, J = 8.0 Hz), 7.75 (t, J = 8.0 Hz), and 8.12 (d, J = 8.0 Hz), two olefinic protons at δ_H 5.24 (d, J = 8.0 Hz) and 6.85 (t, J = 8.0 Hz), as well as two sp³ methylenes at δ_H 2.13 (t, J = 8.0 Hz) and 2.34 (dd, J = 16.0 and 8.0 Hz). The spectrum also showed one sp³ oxygenated methine at δ_H

5.51 (s) and five methyls at δ_H 1.52 (s), 1.57 (d, J = 8.0 Hz), 1.61 (s), 1.69 (s), and 1.88 (s) (Table 2).

The IR spectrum showed characteristic bands at 1719 and 1705 cm⁻¹ for ester and quinone carbonyl groups, respectively, and bands at 1690 and 1651 cm⁻¹, which confirmed the presence of a conjugated pyranoquinone system. Other absorptions observed were at 1545 cm⁻¹ (aromatic C=C stretching) and 1462-1379 cm⁻¹ (C-H bending). The UV spectrum showed absorption peaks at 210, 252, 259, 284 and 335 nm, which is further evidence for the presence of a pyranoquinone chromophore.

The ¹H and ¹³C NMR data of compound **1** were compared with rhinacanthin B (**2**), and the result showed a close structural resemblance. However, a carbonyl carbon (δ_c 185.9) substituted one sp³ methylene carbon in compound **2**. A closer examination of

the COSY and HMBC results showed that the planar arrangement of the structure was 4-oxo-rhinacanthin B (Fig. 2).



The relative configuration at C-3 was assigned by comparison with the reported optical rotation of rhinacanthin B rather than by direct absolute-configuration analysis. The observed optical rotation of compound **1** ($[\alpha]_D -46.7$, c 0.24, CHCl_3) was similar to that reported for rhinacanthin B ($[\alpha]_D -47.9$, c 0.24, CHCl_3), suggesting the same β -orientation/R configuration as a tentative stereochemical assignment (Wu et al., 1988). Further stereochemical confirmation by ECD, VCD, Mosher ester analysis, or X-ray crystallography would strengthen this assignment.

Molecular docking

RMSD of the co-crystallized allosteric ligand into PTP1B (PDB ID: 1T49) was found to be 0.87 Å, as shown in Fig. 3, which validated the docking protocol (< 2.0 Å). The docking grids for all compounds were determined using validated coordinates.

Table 3 summarizes the docking results for isolated compounds **1-6**, the co-crystallized allosteric ligand from PDB 1T49, and ursolic acid. More negative grid scores indicate more favorable docking scores within the applied scoring function. Rhinacanthin N (**5**) showed the most favorable grid score (-37.474 kcal/mol), followed by rhinacanthin Q (**6**) (-36.573 kcal/mol) and rhinacanthin C (**3**) (-35.214 kcal/mol). Compound **1** showed a favorable grid score (-29.896 kcal/mol), whereas the co-crystallized allosteric ligand showed a grid score of -30.785 kcal/mol. Ursolic acid showed a weaker docking score (-18.022 kcal/mol) and was retained only as a non-allosteric/literature comparator, not as a structurally validated allosteric reference for the 1T49 pocket.

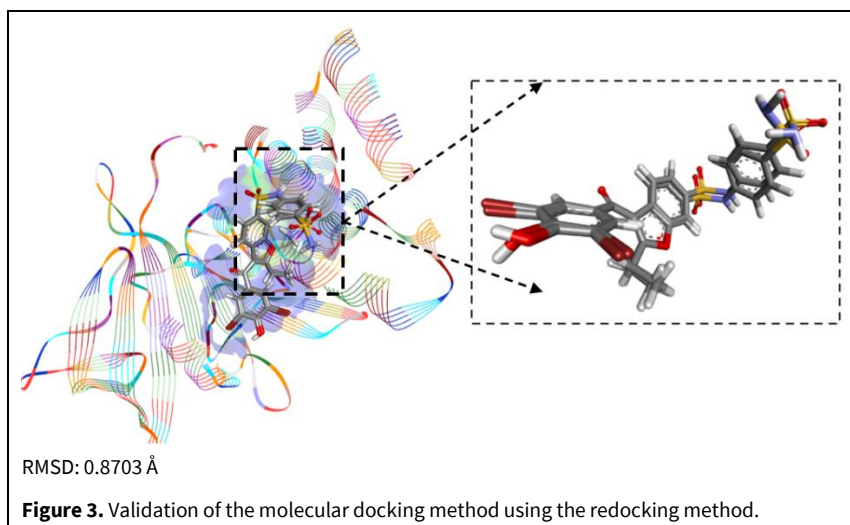
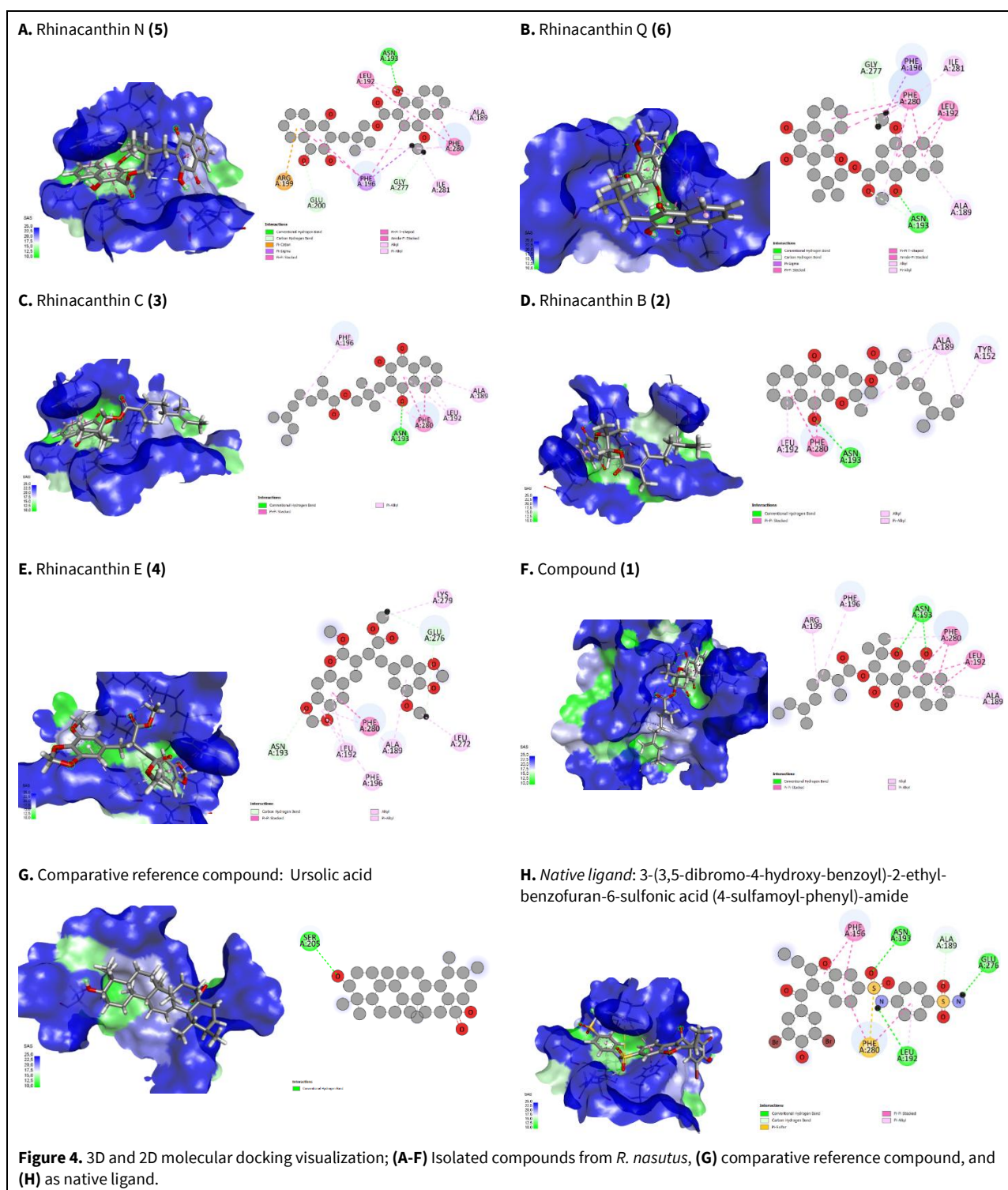


Table 3. Grid score value (Molecular Mechanics Hawkins GBSA Score) of the molecular docking results of the target compound against the PTP1B enzyme.

No.	Compound	Grid score (kcal/mol) through flexible docking
1.	Compound 1	-29.896
2.	Rhinacanthin B (2)	-32.358
3.	Rhinacanthin C (3)	-35.214
4.	Rhinacanthin E (4)	-32.137
5.	Rhinacanthin N (5)	-37.474
6.	Rhinacanthin Q (6)	-36.573
7.	3-(3,5-dibromo-4-hydroxy-benzoyl)-2-ethyl-benzofuran-6-sulfonic acid (4-sulfamoyl-phenyl)-amide (<i>native ligand</i>)	-30.785
8.	Ursolic acid (comparative reference compound)	-18.022



The docking poses of the six isolated compounds are shown in Fig. 4A-F. The binding orientation was evaluated based on interactions within the validated PTP1B allosteric pocket, including Leu192, Phe196, Phe280, Trp179, Pro180, Asp181, Asn193, and Glu276.

Rhinacanthin N (5) formed hydrogen-bonding interactions with Asn193 and π - π stacking interactions with Phe196 and Phe280, as shown in Fig. 4A. Rhinacanthin Q (6) showed hydrogen bonding to Asn193

and hydrophobic interactions with Phe280 (Fig. 4B). Furthermore, rhinacanthin C (3) interacted with Asn193 via hydrogen bonding and formed π - π stacking with Phe280, and additional hydrophobic contacts with Phe196 were observed (Fig. 4C). Rhinacanthin B (2) showed a similar interaction profile, with additional alkyl and π -alkyl contacts including Ala189 (Fig. 4D). Rhinacanthin E (4) formed π - π stacking interactions with Phe196 and Phe280 and hydrogen bonds

with Asn193 and Glu276 (Fig. 4E). Compound **1** adopted a binding orientation within the same pocket, forming hydrophobic interactions between its geranyl-derived substituent and Arg199 and Phe196, as well as hydrogen-bonding interactions with Asn193 (Fig. 4F). Ursolic acid primarily interacted with Ser205 and did not establish contact with key residues defining the validated allosteric region (Fig. 4G). The native co-crystallised ligand formed characteristic interactions with Phe196 and Asn193, as shown in Fig. 4H.

Molecular dynamics simulation

Backbone RMSD analysis (Fig. 5A) showed that both systems reached an initial equilibration phase

within approximately the first 20 ns. The ursolic acid-PTP1B complex remained comparatively stable throughout the simulation, while the compound **1**-PTP1B complex showed broader fluctuations and a more evident increase in deviation after approximately 90 ns (> 0.3 nm). Ligand RMSD analysis (Fig. 5B) showed lower positional fluctuation for ursolic acid (around 0.08 nm) than for compound **1**, which fluctuated within an approximately 0.15–0.25 nm range during the 100 ns simulation. RMSF analysis (Fig. 5C) showed generally similar residue-level fluctuation profiles for the two systems, although relatively higher flexibility was observed in the regions around residues 30–50, 110–130, and 180–190.

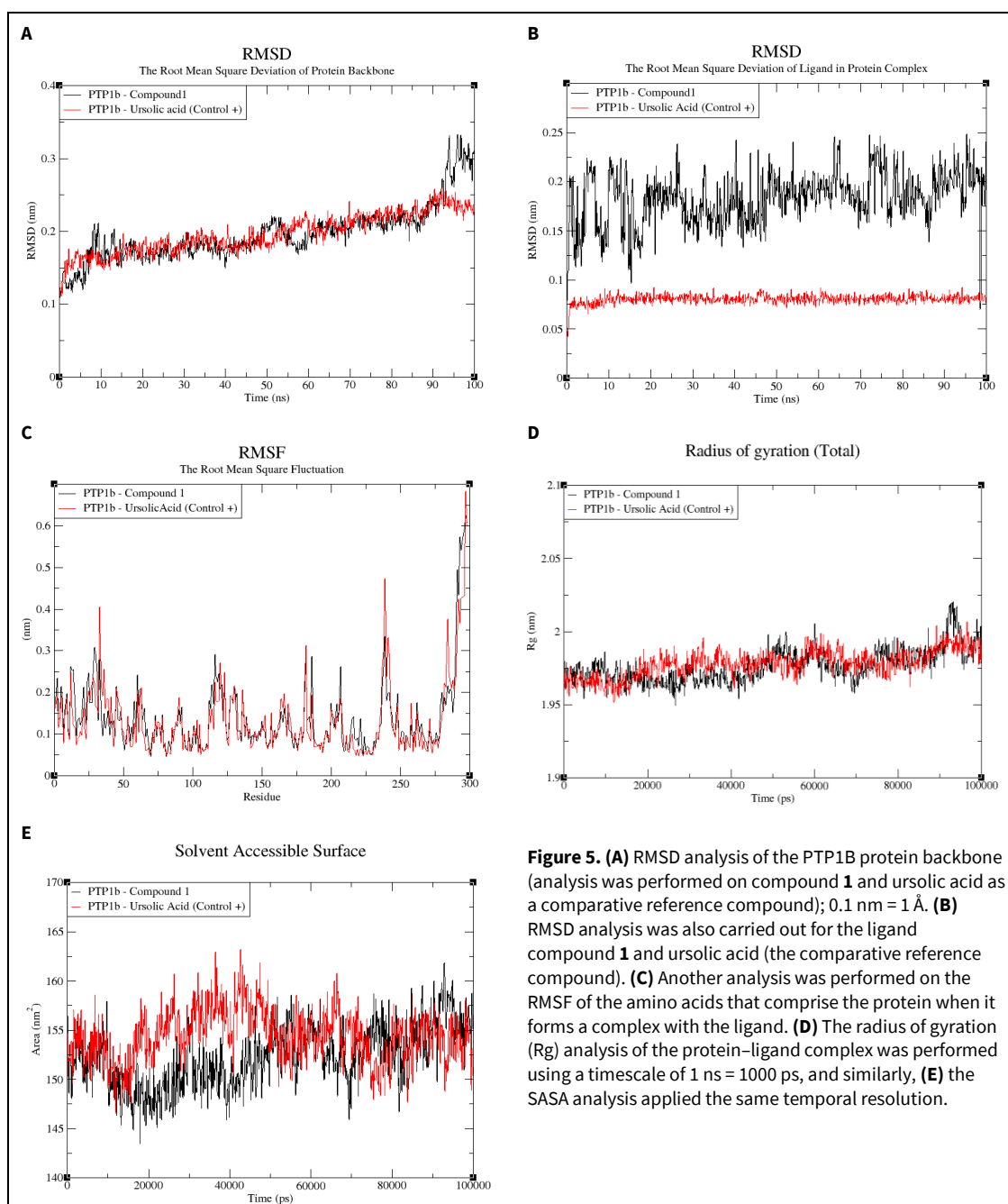


Figure 5. (A) RMSD analysis of the PTP1B protein backbone (analysis was performed on compound **1** and ursolic acid as a comparative reference compound); 0.1 nm = 1 Å. (B) RMSD analysis was also carried out for the ligand compound **1** and ursolic acid (the comparative reference compound). (C) Another analysis was performed on the RMSF of the amino acids that comprise the protein when it forms a complex with the ligand. (D) The radius of gyration (Rg) analysis of the protein-ligand complex was performed using a timescale of 1 ns = 1000 ps, and similarly, (E) the SASA analysis applied the same temporal resolution.

Table 4. MM-PBSA binding free-energy components.

Component	Protein tyrosine phosphatase 1B (PTP1B)	
	Compound 1	Ursolic acid
Delta Energy Terms with MM-PBSA (Calculation based on Equation 2)		
$\bar{x} \Delta G_{Gas} / \Delta E_{MM}$	-56.6673	-51.3882
$\bar{x} \Delta G_{Solv}$	25.58909	16.78455
$\bar{x} \Delta G_{bind}$	-31.08	-34.6018

MM-PBSA binding free-energy components (ΔG_{bind} , kcal/mol) calculated as the mean ($\bar{x}$) of 11 frames extracted from the 100 ns production trajectory.

Table 5. Analysis of metabolism, excretion and toxicity of compounds.

Compounds	Metabolism			Excretion		Toxicity			
	CYP2D6	CYP3A4	CYP2C9	Total clearance	Renal OCT2 substrate	AMES	Max tolerated dose	Hepato-toxicity	Skin sensitisation
1	No	Yes	No	1.241	No	No	0.833	No	No
2	No	Yes	No	1.252	No	No	0.601	No	No
3	No	Yes	No	1.241	No	No	0.556	No	No
4	No	Yes	Yes	0.205	No	No	0.674	No	No
5	No	Yes	Yes	0.319	No	No	0.187	No	No
6	No	Yes	Yes	0.449	No	No	0.494	No	No

In general, the C-terminal region exhibited the largest fluctuations. The radius of gyration (Fig. 5D) remained broadly stable for both complexes until approximately 90 ns, after which the compound **1** complex showed a slight increase. This suggests modest late-stage expansion. SASA values in Fig. 5E remained generally constant at around 155 nm² for both systems. Collectively, these data suggested that the compound **1**-PTP1B complex was associated with the allosteric region during most of the simulation, but showed lower late-stage conformational persistence than the ursolic acid reference complex. Therefore, the MD and MM-PBSA findings should be interpreted qualitatively as supportive evidence for complex stability rather than as quantitative estimates of binding affinity.

MM-PBSA analysis (Table 4) indicated that compound **1** and ursolic acid had predicted total binding free energies of the same order of magnitude. Compound **1** showed a mean ΔG_{bind} of -31.08 kcal/mol, whereas ursolic acid showed a mean ΔG_{bind} of -34.60 kcal/mol. The less favorable total value for compound **1** was mainly associated with a higher positive solvation penalty, despite favorable gas-phase contributions. Because the values were obtained from frames of single trajectories, these data were interpreted descriptively rather than as statistically independent evidence of affinity differences.

ADMET prediction

The compounds (**1–6**) were all predicted to be non-inhibitors of CYP2D6 but inhibitors of CYP3A4. CYP2C9 inhibition was also predicted in compounds **4–6**. The estimated total clearance values from 0.205 (**4**) to 1.252 (**2**). None of the compounds was expected to be substrates of the renal OCT2 transporter. Furthermore, the AMES model predicted that most compounds were non-mutagenic. The highest dose tolerance was estimated to range from 0.833 (**1**) to 0.187 (**5**). Hepatotoxicity predictions were predominantly negative, and the general assessment showed that the compounds were non-sensitising (Table 5).

DISCUSSION

The allosteric pocket of PTP1B (PDB ID: 1T49) was selected for docking and simulation because it is structurally distinct from the catalytic site. Structural studies define this pocket as a ligand-binding site located approximately 20 Å from the catalytic center, where ligand binding can regulate WPD-loop dynamics through allosteric coupling without occupying the catalytic pocket (Wiesmann et al., 2004). The use of allosteric targeting can also increase the selectivity and drug-likeness of PTP1B inhibitors, as those targeted to the catalytic pocket tend to be highly polar and interact with conserved residues (Maccari & Ottanà, 2023).

The rhinacanthin series docks into the validated allosteric pocket, where the amino acids Asn193, Phe196, Phe280, Leu192, Glu276, and Arg199 are involved in ligand recognition (Wiesmann et al., 2004). Interestingly, the most promising docking scores were obtained for rhinacanthin N (**5**) and rhinacanthin Q (**6**), while compound **1** was still found to interact with the residues Asn193, Arg199 and Phe196. The docking scores do not necessarily indicate activity, selectivity or mechanism, and should not be used to prove, but rather to support, a plausible fit in the pocket.

Molecular dynamics simulation provided time-dependent information beyond static docking. The global protein fold was preserved in both simulated systems with generally stable SASA and intramolecular hydrogen-bond profiles. In the case of compound **1**, the molecule stayed bound to the allosteric pocket for the entire 100 ns trajectory. Higher RMSD and Rg values were observed after approximately 90 ns. This pattern indicates lower binding persistence at the end of the simulation than was seen for the ursolic acid comparator in the same simulation configuration. The observation is more consistent with a local rearrangement in the allosteric pocket than with global protein destabilization. However, this interpretation remains limited because only a single trajectory per complex was available, and replicate simulations would be required to quantify trajectory-to-trajectory variability.

MM-PBSA analysis indicated that compound **1** and ursolic acid showed predicted binding free energies of the same order of magnitude. Compound **1** showed more favorable gas-phase interactions, suggesting favorable non-bonded contacts within the complex. However, this contribution was counterbalanced by a higher positive solvation penalty, resulting in a slightly less favorable total ΔG_{bind} than that of ursolic acid. Because the MM-PBSA values were calculated from 11 frames extracted from single MD trajectories, they were interpreted only as descriptive intra-trajectory indicators and not as confirmatory statistical evidence of binding differences.

In silico ADMET profiling suggested heterogeneous pharmacokinetic behavior across the series. The absence of predicted CYP2D6 inhibition represents a favorable feature. Recurrent prediction of CYP3A4 inhibition and CYP2C9 inhibition for compounds **4-6** shows potential metabolic interaction liabilities that require experimental confirmation. The toxicity outputs were generally acceptable in the applied models, but these predictions remain preliminary and were not a substitute for laboratory evaluation.

Several limitations should be noted. First, docking was performed with a semi-rigid receptor model and therefore could not fully capture the conformational

plasticity of the allosteric pocket. Second, only one 100 ns trajectory was available for each complex, so slower conformational events and replicate-to-replicate variability could not be assessed. Third, MM-PBSA provides approximate relative energetics and is sensitive to sampling, dielectric assumptions, and solvation treatment. Fourth, although the addition of DOCK6 and GROMACS input/command details improves reproducibility, the exact ligand-topology generation workflow remains unavailable. Fifth, the stereochemical assignment of compound **1** was based on optical-rotation comparison rather than direct absolute-configuration analysis. Most importantly, no PTP1B enzymatic inhibition assay, selectivity assay, kinetic analysis, or cell-based insulin-signaling assay was performed. Therefore, the proposed PTP1B-modulating activity of compound **1** remains a computational hypothesis.

CONCLUSION

Molecular docking and molecular dynamics simulations suggested that 4-oxo-rhinacanthin B (**1**) may be accommodated within the validated allosteric pocket of PTP1B. MM-PBSA indicated that the predicted binding of compound **1** was slightly less favorable than that of ursolic acid, mainly because of a higher solvation penalty. Because no enzymatic assay was performed, compound **1** should not be considered a confirmed PTP1B inhibitor. Instead, it should be regarded as a computationally prioritized scaffold requiring biochemical validation.

CONFLICT OF INTEREST

The research was conducted entirely free from any involvement in commercial, financial, or personal matters that might be perceived as a possible conflict of interest or that could have influenced the outcomes or interpretation of the study.

ACKNOWLEDGMENTS

The gratitude is expressed to DIKTISAINTEK (Indonesia Ministry of Higher Education, Science, and Technology) for funding this study through the Regular Fundamental Research scheme, appropriate to the contract No. 137/C3/DT.05.00/PL/2025 in the name of WM.

GENERATIVE ARTIFICIAL INTELLIGENCE (AI)

The authors used Grammarly Inc. to improve the language and readability of the manuscript. The authors reviewed and edited the content and take full responsibility for its accuracy. No images were generated or manipulated using AI tools.

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AUTHOR CONTRIBUTION:

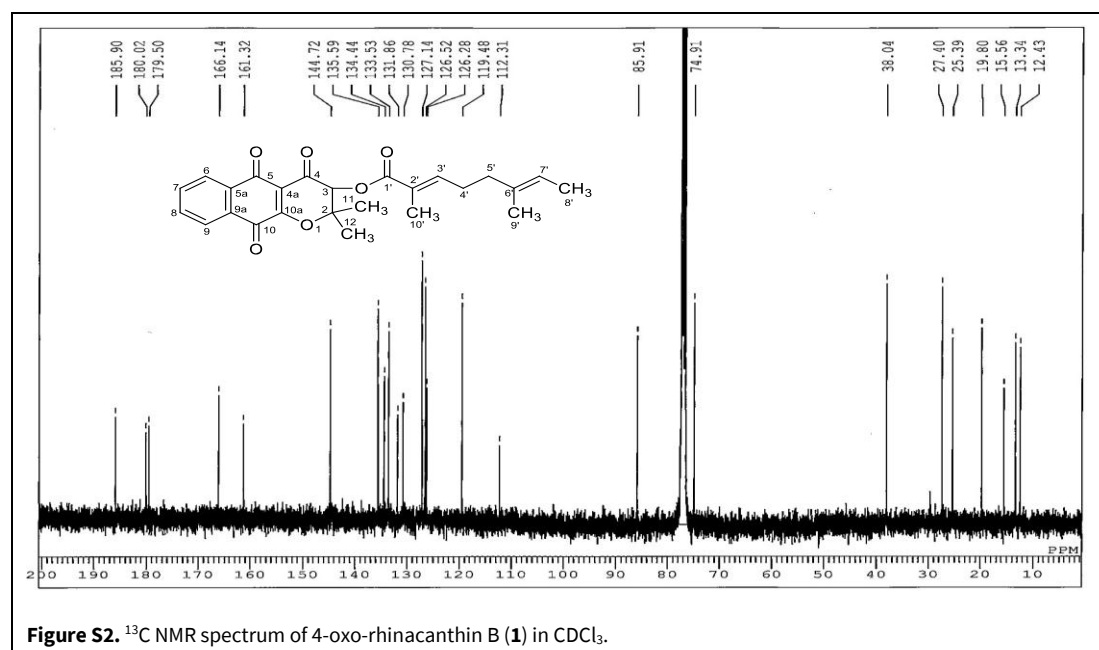
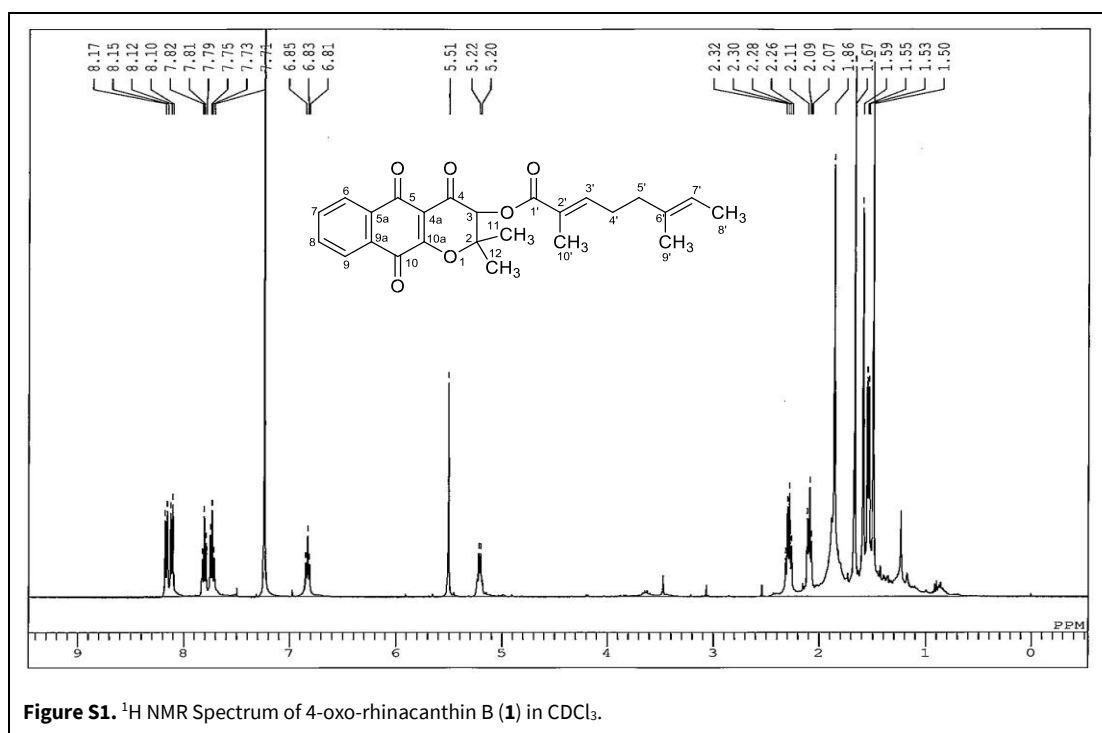
Contribution	Maarisit M	Mongi J	Tumbel SL
Concepts or ideas	x		
Design	x		
Definition of intellectual content	x		
Literature search	x	x	x
Experimental studies	x	x	x
Data acquisition	x		
Data analysis	x	x	x
Statistical analysis	x		
Manuscript preparation	x	x	x
Manuscript editing	x	x	x
Manuscript review	x	x	x

Citation Format: Maarisit M, Mongi J, Tumbel SL (2026) A pyranonaphthoquinone from *Rhinacanthus nasutus* (L.) Kurz roots as a potential protein tyrosine phosphatase 1B allosteric binder: An *in silico* study. J Pharm Pharmacogn Res 13(3): 2526. https://doi.org/10.56499/jppres_14.3.2526

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Supplementary data



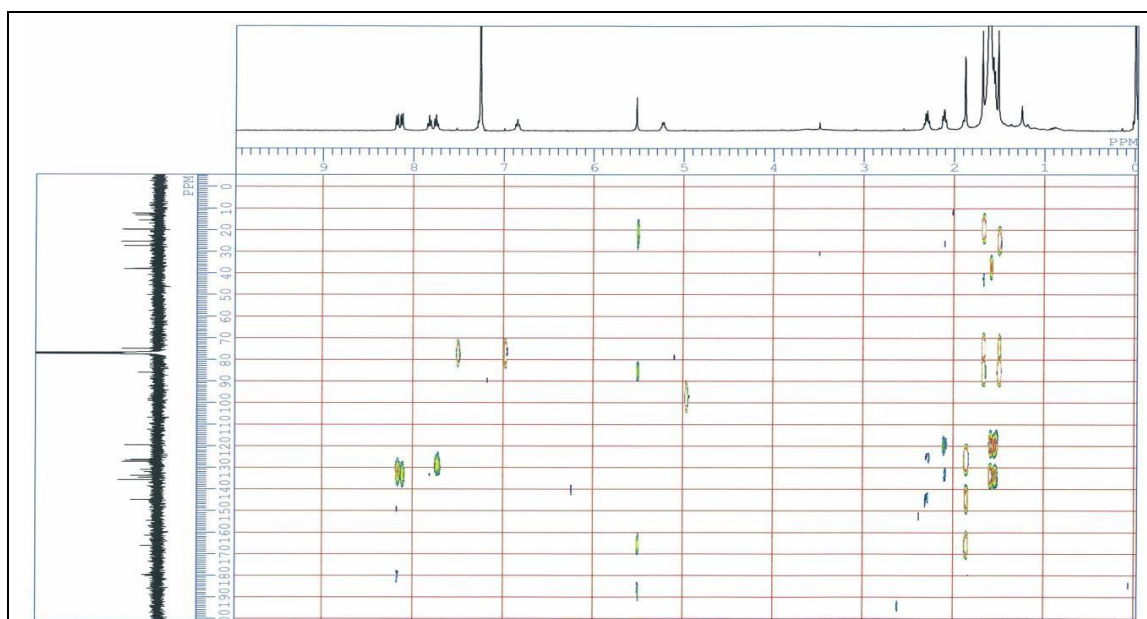


Figure S3. HMQC spectrum of 4-oxo-rhinacanthin B (**1**) in CDCl₃.

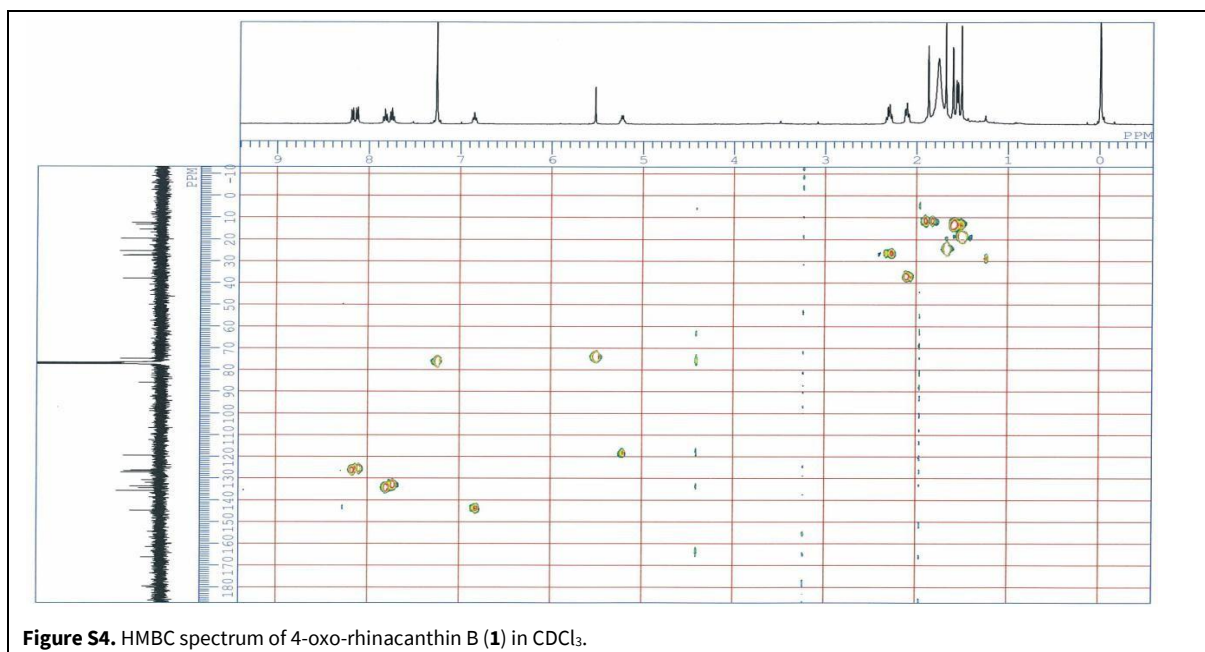
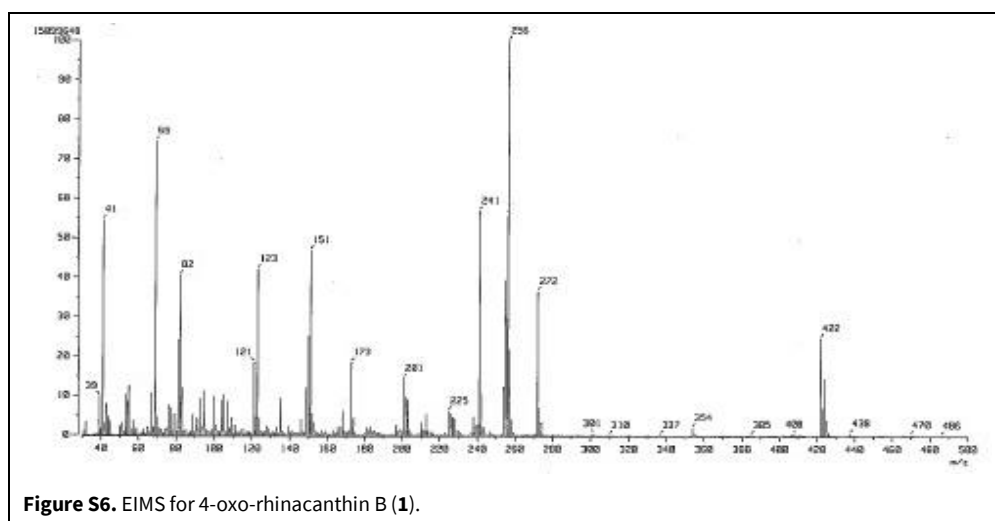
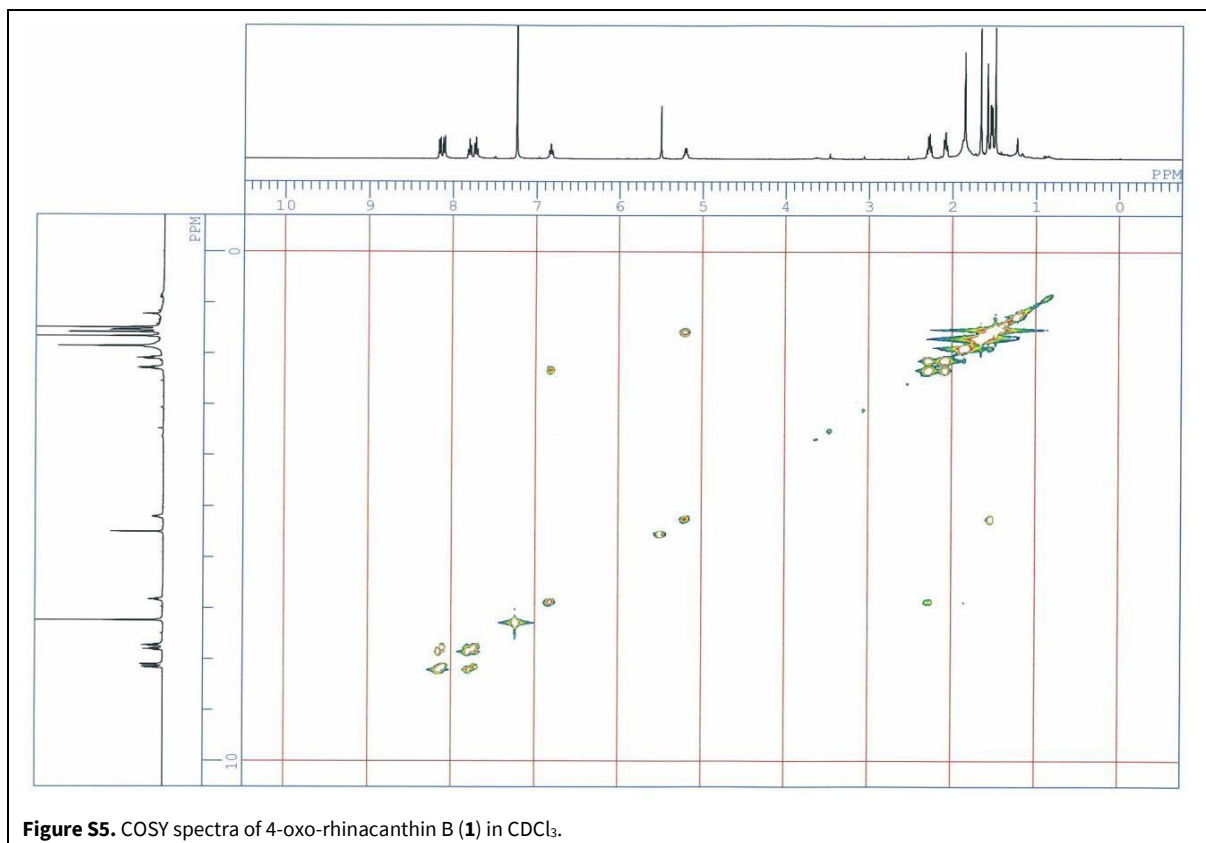
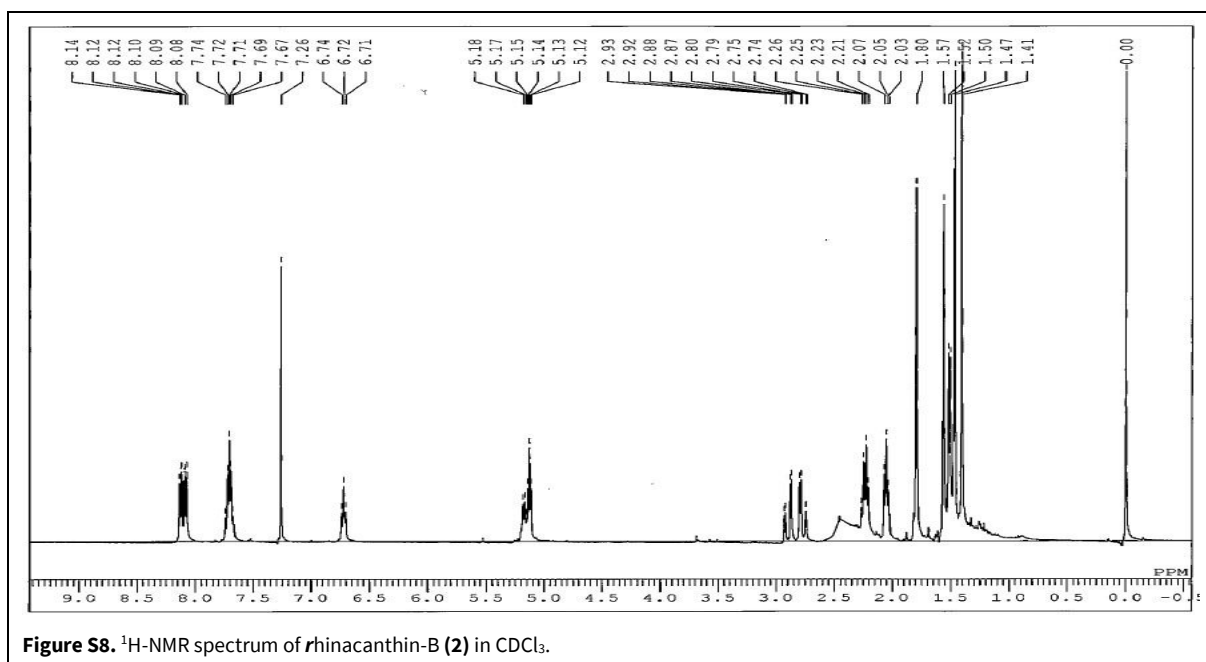
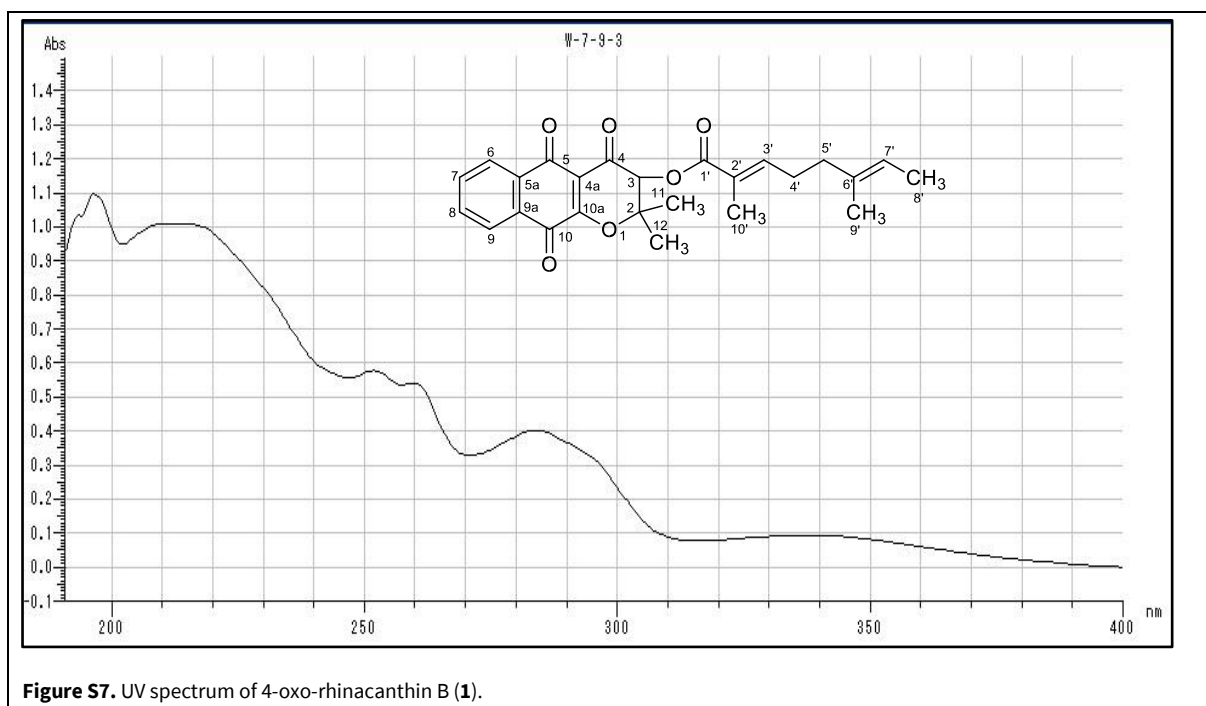


Figure S4. HMBC spectrum of 4-oxo-rhinacanthin B (**1**) in CDCl₃.





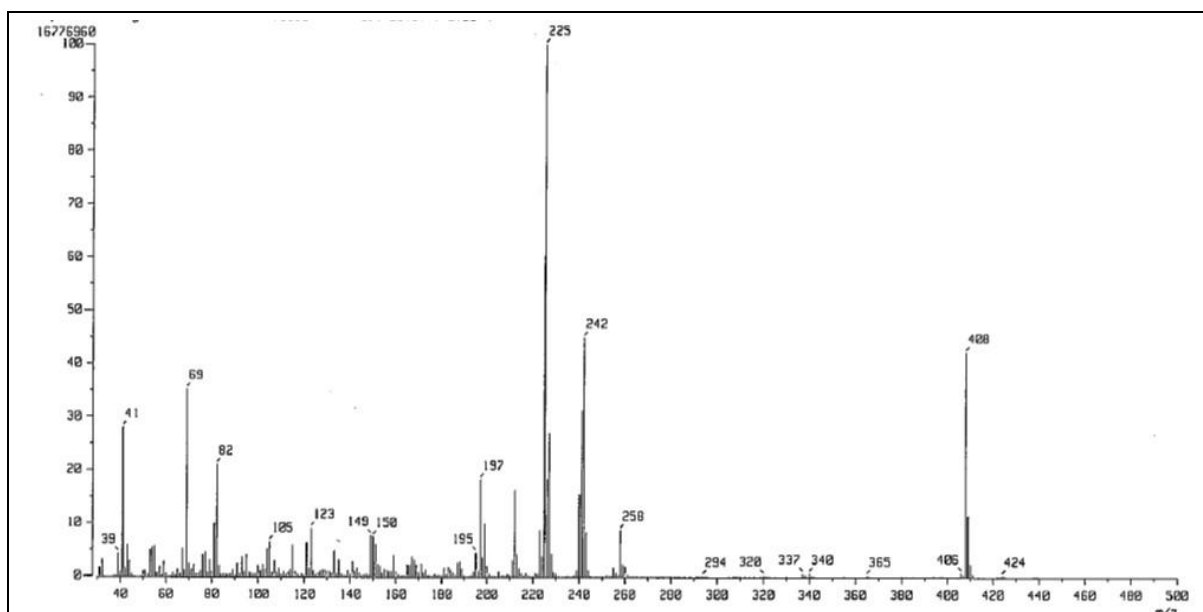


Figure S9. EIMS of Rhinacanthin-B (**2**).

Compound 2 (rhinacanthin B)

Yellow needles; $[\alpha]_D^{25}$ -135.9 (c 0.24, CHCl_3); UV (MeOH) λ_{max} (Log ϵ): 201 (4.55), 245 (4.37), 250 (4.37), 281 (4.10), and 331 (3.37); EI-MS m/z 408 (calcd for $\text{C}_{25}\text{H}_{28}\text{O}_5$, 408.1934). $^1\text{H-NMR}$ (CDCl_3) 8.14 (1H, d, $J = 8.0$ Hz), 8.10 (1H, d, $J = 8.0$ Hz), 7.73 (1H, t, $J = 8.0$ Hz), 7.71 (1H, t, $J = 8.0$ Hz), 6.72 (1H, t, $J = 8.0$ Hz), 5.20 (1H, t, $J = 8.0$ Hz), 5.14 (1H, t, $J = 4.0$ Hz), 2.93 (1H, dd, $J = 4.0, 16.0$ Hz), 2.80 (1H, dd, $J = 4.0, 16.0$ Hz), 2.26 (2H, dt, $J = 8.0$ Hz), 2.07 (2H, dt, $J = 8.0$ Hz), 1.80 (3H, s), 1.57 (3H, s), 1.52 (3H, d, $J = 8.0$ Hz), 1.47 (3H, s), 1.41 (3H, s).

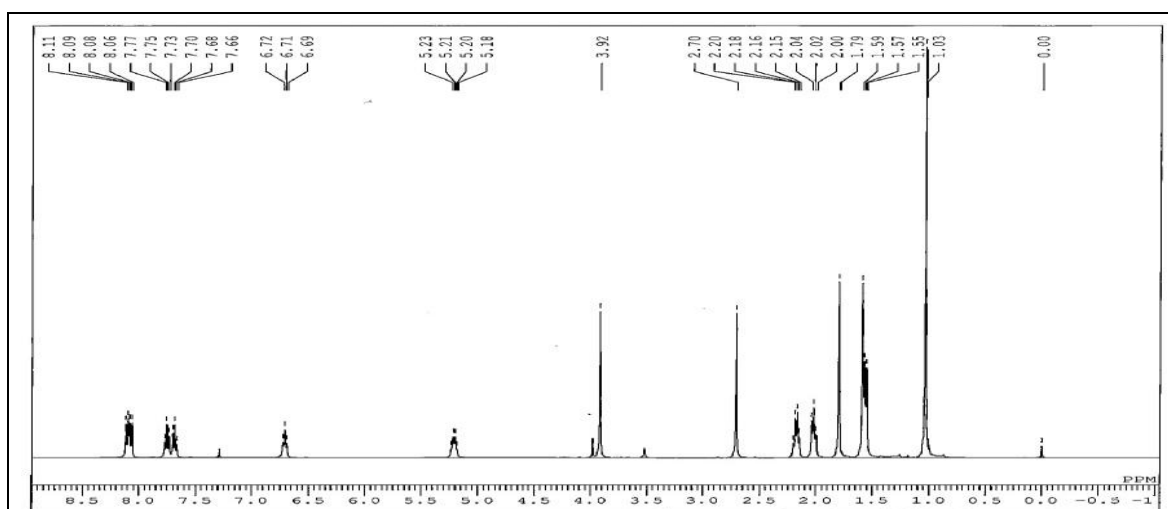


Figure S10. $^1\text{H-NMR}$ spectrum of rhinacanthin-C (**3**) in CDCl_3 .

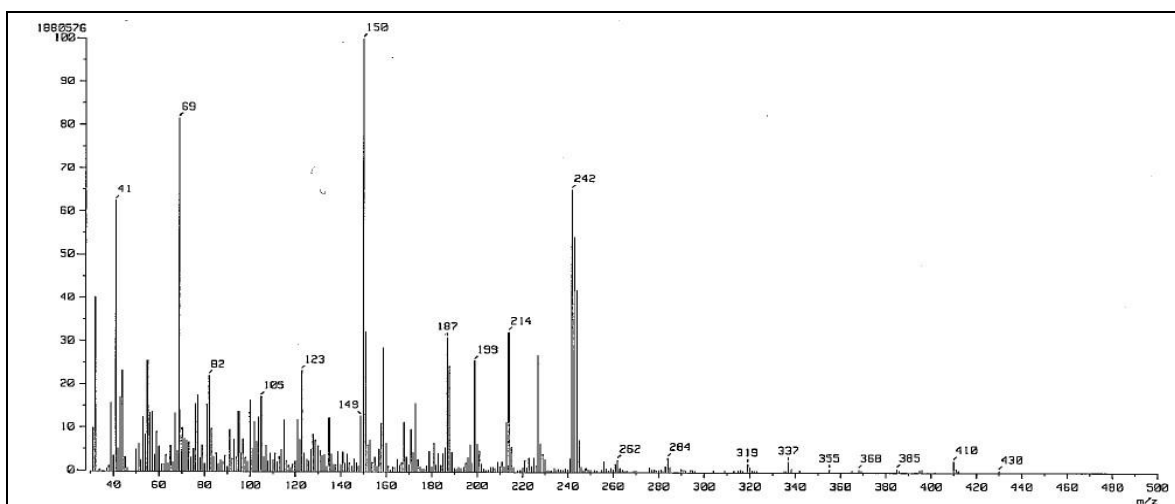


Figure S11. EIMS for rhinacanthin-C (**3**).

Compound **3** (rhinacanthin C)

Yellow oil: UV (MeOH) λ_{max} (Log ϵ): 201 (3.86), 251 (3.53), 274 (3.47), 311 (3.00), and 323 (2.94); EI-MS m/z 410 (calcd for $\text{C}_{25}\text{H}_{30}\text{O}_5$, 410.2903); $^1\text{H-NMR}$ (CDCl_3) 8.11 (1H, d, $J=8.0$ Hz), 8.08 (1H, d, $J=8.0$ Hz), 7.77 (1H, t, $J=8.0$ Hz), 7.70 (1H, t, $J=8.0$ Hz), 6.72 (1H, t, $J=8.0$ Hz), 5.23 (1H, d, $J=8.0$ Hz), 3.90 (2H, s), 2.70 (2H, s), 2.20 (1H, dd, $J=4.0, 16.0$ Hz), 2.06 (2H, dt, $J=8.0$ Hz), 1.79 (6H, s), 1.59 (6H, s), 1.57 (3H, d, $J=8.0$ Hz).

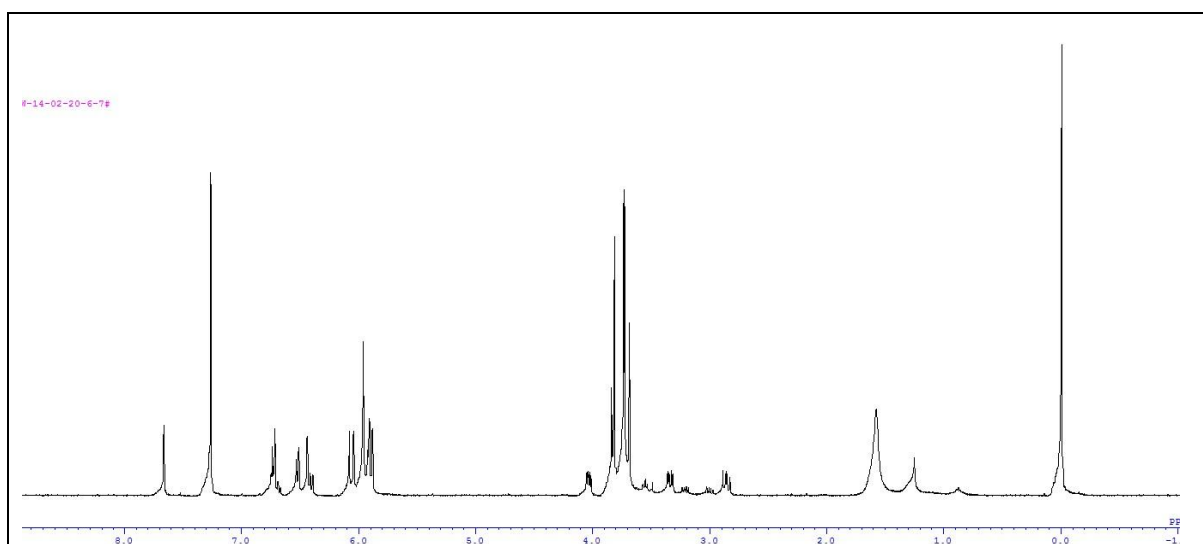
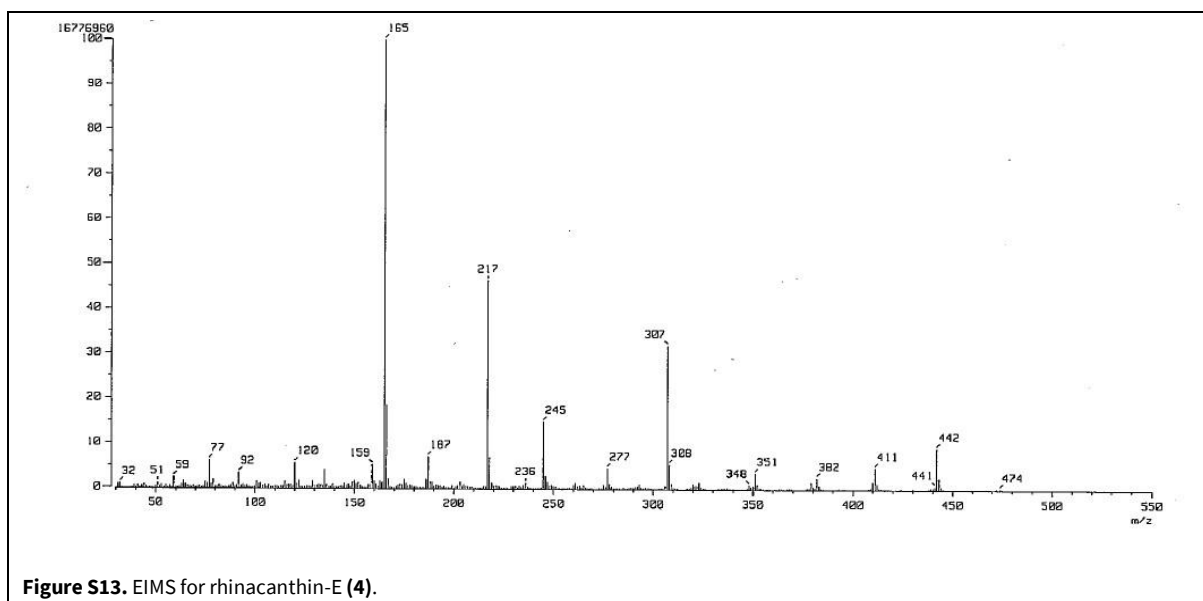
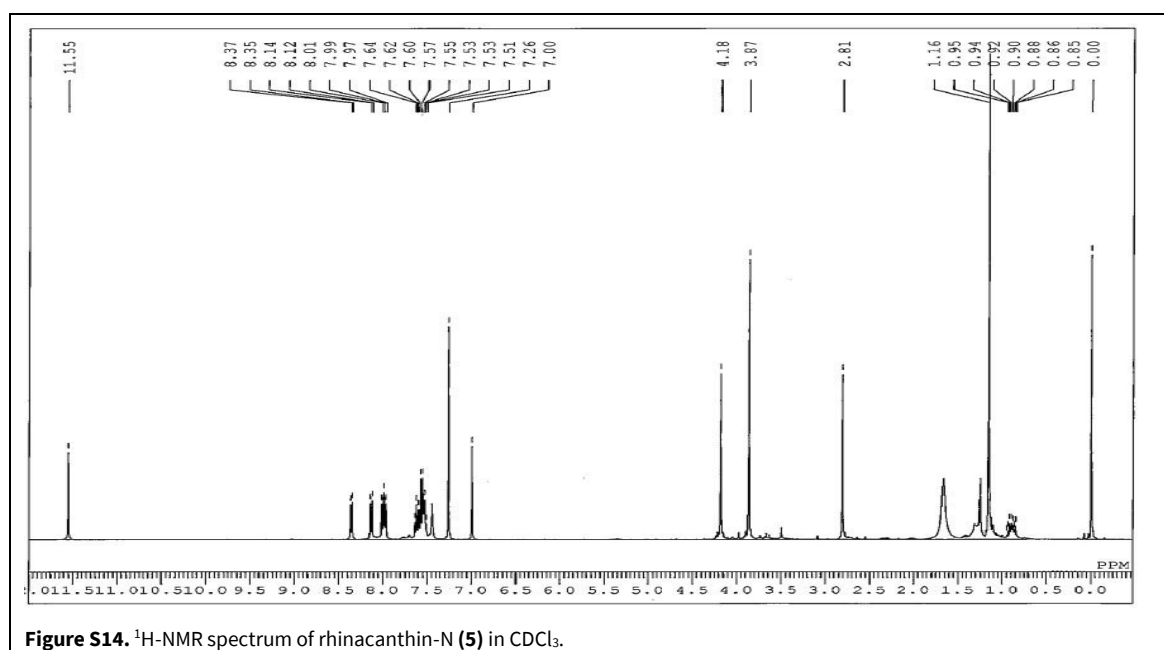


Figure S12. $^1\text{H-NMR}$ spectrum of rhinacanthin-E (**4**) in CDCl_3 .



Compound **4** (rhinacanthin E)

Amorphous powder: UV (MeOH) λ_{max} (Log ϵ): 201 (4.01), 285 (3.24), 311 (3.26), 383 (1.20), and 394 (1.07); EI-MS m/z 442 (calcd for $C_{23}H_{22}O_9$, 442.1264); $^1\text{H-NMR}$ (CDCl_3) 7.66 (1H, s), 6.73 (1H, d, $J = 8.0$ Hz), 6.53 (1H, br d, $J = 8.0$, Hz), 6.44 (1H, br s), 6.08 (1H, s), 6.04 (1H, br s), 5.96 (2H, s), 5.92 (1H, d, $J = 4.0$ Hz), 5.88 (1H, br s), 4.05 (1H dd, $J = 4.8, 10.4$ Hz) 3.81 (3H, s), 3.73 (3H, s), 3.68 (3H, s), 3.63 (1H, dd, $J = 4.8, 9.2$ Hz), 2.89 (1H, dd, $J = 4.0, 10.0$ Hz).



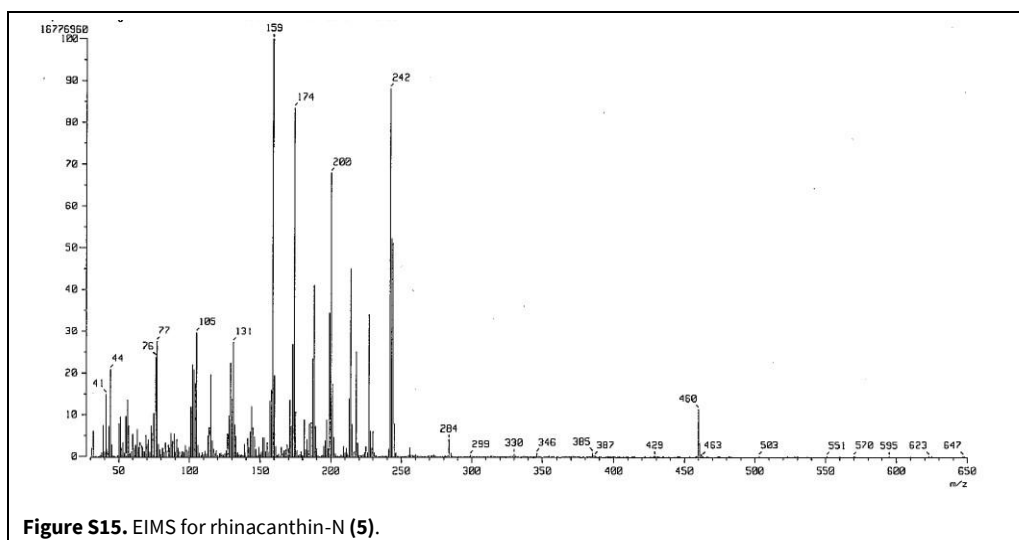


Figure S15. EIMS for rhinacanthin-N (**5**).

Compound 5 (rhinacanthin N)

Orange needles: UV (MeOH) λ_{max} (Log ϵ): 200 (4.00), 214 (3.94), 265 (3.80), 275 (3.67) and 359 (2.80); EI-MS m/z 460 (calcd for $\text{C}_{27}\text{H}_{24}\text{O}_7$, 460.4805); $^1\text{H-NMR}$ (CDCl_3) 11.55 (1H, s), 8.37 (1H, d, $J = 4.0$ Hz), 8.14 (1H, t, $J = 8.0$ Hz), 8.01 (1H, d, $J = 8.0$ Hz), 7.99 (1H, d, $J = 8.0$ Hz), 7.64 (1H, d, $J = 8.0$ Hz), 7.60 (1H, d, $J = 12$ Hz), 7.55 (1H, d, $J = 12$ Hz), 7.53 (1H, d, $J = 12$ Hz), 7.00 (1H, s), 4.18 (2H, s), 3.87 (3H, s), 2.81 (2H, s), 1.16 (6H, s).

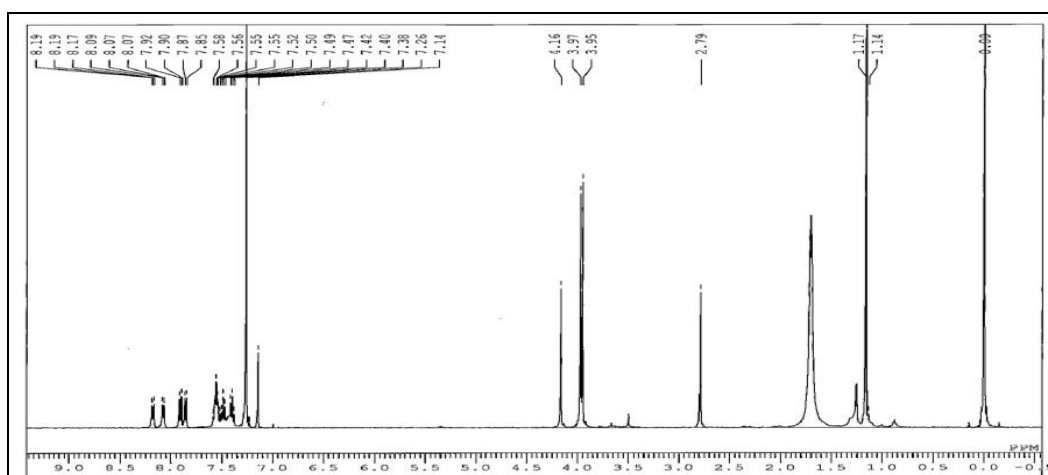


Figure S16. $^1\text{H-NMR}$ spectrum of rhinacanthin-Q (**6**) in CDCl_3 .

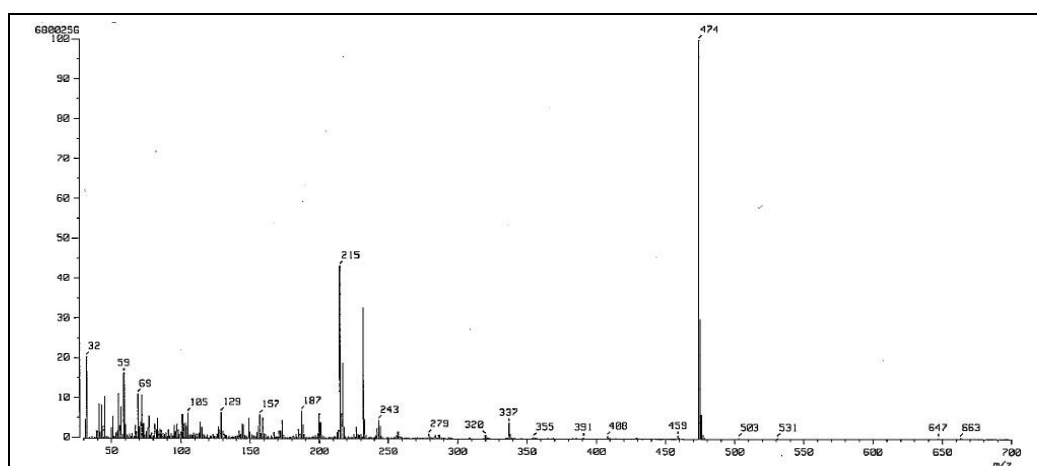


Figure S17. EIMS for rhinacanthin-Q (**6**).

Compound **6** (rhinacanthin Q)

Orange needles: UV (MeOH) λ_{max} (Log ϵ): 216 (4.41), 249 (4.33), 276 (3.08), 334 (3.45), and 382 (1.65); EI-MS m/z 474 (calcd for $\text{C}_{28}\text{H}_{26}\text{O}_7$, 474.1679); ^1H -NMR (CDCl_3) 8.19 (1H, d, $J = 8.0$ Hz), 8.09 (1H, d, $J = 8.0$ Hz), 7.92 (1H, d, $J = 8.0$ Hz), 7.87 (1H, d, $J = 8.0$ Hz), 7.56 (1H, t, $J = 4$ Hz), 7.52 (1H, m), 7.50 (1H, m), 7.47 (1H, m), 7.40 (1H, m), 7.14 (1H, s), 4.16 (2H, s), 3.97 (3H, s), 3.95 (3H, s), 2.79 (2H, s), 1.17 (6H, s).

Hardware and software environment: The computations were performed on a Lenovo LOQ 15IAX9 laptop equipped with a 12th Gen Intel(R) Core(TM) i5-12450HX processor (2.40 GHz), 12.0 GB RAM, and a 64-bit operating system. The software environment included Ubuntu 24.04.1 LTS, YASARA Minimisation Server, DOCK6, UCSF Chimera 1.18 (University of California, San Francisco, USA), PyMOL (Schrodinger LLC, USA), BIOVIA Discovery Studio 2024 (Dassault Systèmes BIOVIA, USA), GROMACS 2021 with the OPLS-AA force field as documented in Table S2, and GraphPad Prism 11 (GraphPad Software, La Jolla, CA, USA).

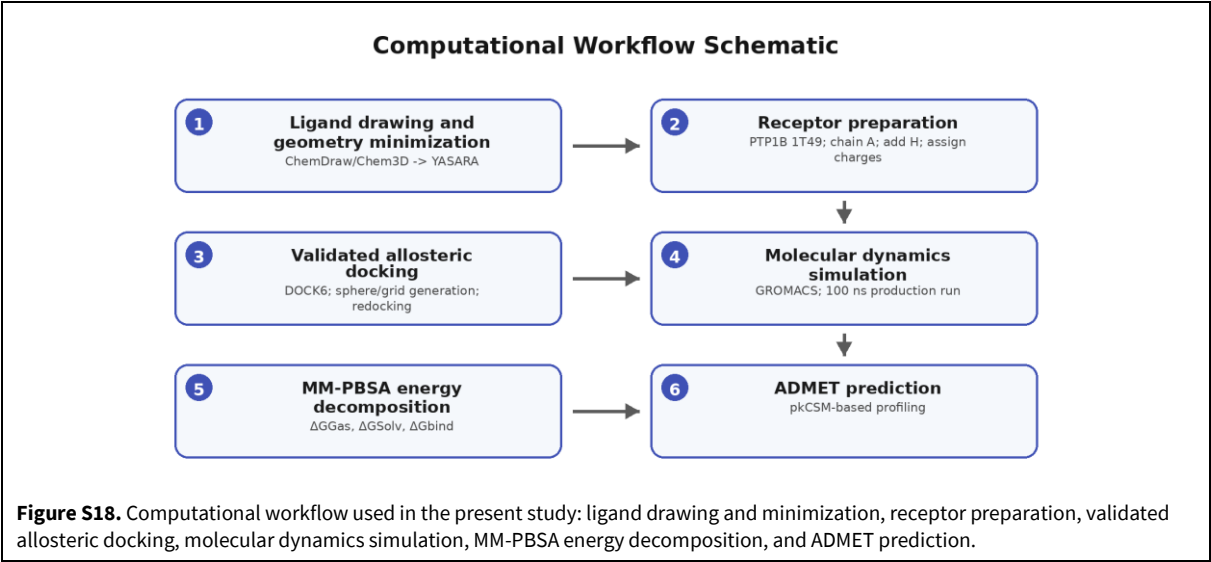


Table S1. DOCK6 file input.

Spheres		
	rec_noH.dms	
	R	
	X	
	0.0	
	4.0	
	1.4	
	rec.sph	
Grid		
	compute_grids	yes
	grid_spacing	0.6
	output_molecule	no
	contact_score	no
	energy_score	yes
	energy_cutoff_distance	9999
	atom_model	a
	attractive_exponent	6
	repulsive_exponent	12
	distance_dielectric	yes
	dielectric_factor	4

bump_filter	yes
bump_overlap	0.75
receptor_file	/1.dockprep/rec_withH.mol2
box_file	box.pdb
vdw_definition_file	vdw_AMBER_parm99.defn
score_grid_prefix	grid
allow_non_integral_charges	no
Docking Input	
conformer_search_type	flex
write_fragment_libraries	no
user_specified_anchor	no
limit_max_anchors	no
min_anchor_size	5
pruning_use_clustering	yes
pruning_max_orients	1000
pruning_clustering_cutoff	100
pruning_conformer_score_cutoff	100.0
pruning_conformer_score_scaling_factor	1.0
use_clash_overlap	no
write_growth_tree	no
use_internal_energy	yes
internal_energy_rep_exp	12
internal_energy_cutoff	100.0
ligand_atom_file	/1.dockprep/LigUji.mol2
limit_max_ligands	no
skip_molecule	no
read_mol_solvation	no
calculate_rmsd	yes
use_rmsd_reference_mol	yes
rmsd_reference_filename	native_lig.min_scored.mol2
use_database_filter	no
orient_ligand	yes
automated_matching	yes
receptor_site_file	/2.surface_spheres/selected_spheres.sph
max_orientations	1000
critical_points	no
chemical_matching	no
use_ligand_spheres	no
bump_filter	no
score_molecules	yes
contact_score_primary	no
contact_score_secondary	no
grid_score_primary	yes

grid_score_secondary	no
grid_score_rep_rad_scale	1
grid_score_vdw_scale	1
grid_score_es_scale	1
grid_score_grid_prefix	/3.boxgrid/grid
multigrid_score_secondary	no
dock3.5_score_secondary	no
continuous_score_secondary	no
footprint_similarity_score_secondary	no
pharmacophore_score_secondary	no
descriptor_score_secondary	no
gbsa_zou_score_secondary	no
gbsa_hawkins_score_secondary	no
SASA_score_secondary	no
amber_score_secondary	no
minimize_ligand	yes
minimize_anchor	yes
minimize_flexible_growth	yes
use_advanced_simplex_parameters	no
simplex_max_cycles	1
simplex_score_converge	0.1
simplex_cycle_converge	1.0
simplex_trans_step	1.0
simplex_rot_step	0.1
simplex_tors_step	10.0
simplex_anchor_max_iterations	500
simplex_grow_max_iterations	500
simplex_grow_tors_premin_iterations	0
simplex_random_seed	0
simplex_restraint_min	no
atom_model	all
vdw_defn_file	vdw_AMBER_parm99.defn
flex_defn_file	flex.defn
flex_drive_file	flex_drive.tbl
ligand_outfile_prefix	ligan.flex
write_orientations	no
num_scored_conformers	10
write_conformations	yes
cluster_conformations	yes
cluster_rmsd_threshold	2.0
rank_ligands	no

Table S2. GROMACS file input and command log.

NVT	GROMACS: gmx mdrun, version 2021 Executable: /home/gmx2021/bin//gmx Data prefix: /home/gmx2021 Working dir: /home/sibiolead/biolead/9362 Process ID: 3960072 Command line: gmx mdrun -nt 2 -v -deffnm nvt GROMACS version: 2021 Verified release checksum is 3e06a5865d6ff726fc417dea8d55afd37ac3cbb94c02c54c76d7a881c49c5dd8 Precision: mixed Memory model: 64 bit MPI library: thread_mpi OpenMP support: enabled (GMX_OPENMP_MAX_THREADS = 64) GPU support: CUDA SIMD instructions: AVX2_256 FFT library: fftw-3.3.8-sse2-avx-avx2-avx2_128 RDTSCP usage: enabled TNG support: enabled Hwloc support: disabled Tracing support: disabled C compiler: /usr/bin/cc GNU 8.4.0 C compiler flags: -mavx2 -mfma -Wno-missing-field-initializers -fexcess-precision=fast -funroll-all-loops -O3 -DNDEBUG C++ compiler: /usr/bin/c++ GNU 8.4.0 C++ compiler flags: -mavx2 -mfma -Wno-missing-field-initializers -fexcess-precision=fast -funroll-all-loops -fopenmp -O3 -DNDEBUG CUDA compiler: /usr/bin/nvcc nvcc: NVIDIA (R) Cuda compiler driver; Copyright (c) 2005-2019 NVIDIA Corporation; Built on Sun_Jul_28_19:07:16_PDT_2019; Cuda compilation tools, release 10.1, V10.1.243 CUDA compiler flags: -std=c++14;-gencode;arch=compute_30,code=sm_30;-gencode;arch=compute_35,code=sm_35;-gencode;arch=compute_37,code=sm_37;-gencode;arch=compute_50,code=sm_50;-gencode;arch=compute_52,code=sm_52;-gencode;arch=compute_60,code=sm_60;-gencode;arch=compute_61,code=sm_61;-gencode;arch=compute_70,code=sm_70;-gencode;arch=compute_35,code=compute_35;-gencode;arch=compute_50,code=compute_50;-gencode;arch=compute_52,code=compute_52;-gencode;arch=compute_60,code=compute_60;-gencode;arch=compute_61,code=compute_61;-gencode;arch=compute_70,code=compute_70;-gencode;arch=compute_75,code=compute_75;-use_fast_math;-D_FORCE_INLINES;-mavx2 -mfma -Wno-missing-field-initializers -fexcess-precision=fast -funroll-all-loops -fopenmp -O3 -DNDEBUG CUDA driver: 11.40 CUDA runtime: 10.10 Running on 1 node with total 8 cores, 16 logical cores, 1 compatible GPU Hardware detected: CPU info: Vendor: AMD Brand: AMD Ryzen 7 5800X 8-Core Processor Family: 25 Model: 33 Stepping: 2 Features: aes amd apic avx avx2 clflush cmov cx8 cx16 f16c fma htt lahfs misalignsse mmx msr nonstop_tsc pclmuldq pdep1gb popcnt pse rdrnd rdtscp sha sse2 sse3 sse4a sse4.1 sse4.2 ssse3 x2apic Hardware topology: Basic Sockets, cores, and logical processors: Socket 0: [0 8] [1 9] [2 10] [3 11] [4 12] [5 13] [6 14] [7 15] GPU info: Number of GPUs detected: 1 #0: NVIDIA NVIDIA GeForce RTX 3050, compute cap.: 8.6, ECC: no, stat: compatible
NPT	GROMACS: gmx mdrun, version 2021 Executable: /home/gmx2021/bin//gmx Data prefix: /home/gmx2021 Working dir: /home/sibiolead/biolead/9362 Process ID: 3960144 Command line:

	<pre> gmxdrun -nt 2 -v -deffnm npt GROMACS version: 2021 Verified release checksum is 3e06a5865d6ff726fc417dea8d55afd37ac3cbb94c02c54c76d7a881c49c5dd8 Precision: mixed Memory model: 64 bit MPI library: thread_mpi OpenMP support: enabled (GMX_OPENMP_MAX_THREADS = 64) GPU support: CUDA SIMD instructions: AVX2_256 FFT library: fftw-3.3.8-sse2-avx-avx2-avx2_128 RDTSCP usage: enabled TNG support: enabled Hwloc support: disabled Tracing support: disabled C compiler: /usr/bin/cc GNU 8.4.0 C compiler flags: -mavx2 -mfma -Wno-missing-field-initializers -fexcess-precision=fast -funroll-all-loops -O3 -DNDEBUG C++ compiler: /usr/bin/c++ GNU 8.4.0 C++ compiler flags: -mavx2 -mfma -Wno-missing-field-initializers -fexcess-precision=fast -funroll-all-loops -fopenmp -O3 -DNDEBUG CUDA compiler: /usr/bin/nvcc nvcc: NVIDIA (R) Cuda compiler driver; Copyright (c) 2005-2019 NVIDIA Corporation; Built on Sun_Jul_28_19:07:16_PDT_2019; Cuda compilation tools, release 10.1, V10.1.243 CUDA compiler flags: -std=c++14;-gencode;arch=compute_30,code=sm_30;-gencode;arch=compute_35,code=sm_35;-gencode;arch=compute_37,code=sm_37;-gencode;arch=compute_50,code=sm_50;-gencode;arch=compute_52,code=sm_52;-gencode;arch=compute_60,code=sm_60;-gencode;arch=compute_61,code=sm_61;-gencode;arch=compute_70,code=sm_70;-gencode;arch=compute_35,code=compute_35;-gencode;arch=compute_50,code=compute_50;-gencode;arch=compute_52,code=compute_52;-gencode;arch=compute_60,code=compute_60;-gencode;arch=compute_61,code=compute_61;-gencode;arch=compute_70,code=compute_70;-gencode;arch=compute_75,code=compute_75;-use_fast_math;-D_FORCE_INLINES;-mavx2 -mfma -Wno-missing-field-initializers -fexcess-precision=fast -funroll-all-loops -fopenmp -O3 -DNDEBUG CUDA driver: 11.40 CUDA runtime: 10.10 Running on 1 node with total 8 cores, 16 logical cores, 1 compatible GPU Hardware detected: CPU info: Vendor: AMD Brand: AMD Ryzen 7 5800X 8-Core Processor Family: 25 Model: 33 Stepping: 2 Features: aes amd apic avx avx2 clflush cmov cx8 cx16 f16c fma htt lahfs misalignsse mmx msr nonstop_tsc pclmuldq pdep1gb popcnt pse rdnd rdtscp sha sse2 sse3 sse4a sse4.1 sse4.2 ssse3 x2apic Hardware topology: Basic Sockets, cores, and logical processors: Socket 0: [0 8] [1 9] [2 10] [3 11] [4 12] [5 13] [6 14] [7 15] GPU info: Number of GPUs detected: 1 #0: NVIDIA NVIDIA GeForce RTX 3050, compute cap.: 8.6, ECC: no, stat: compatible </pre>
MD Simulation	<pre> GROMACS: gmxdrun, version 2021 Executable: /home/gmx2021/bin//gmxdrun Data prefix: /home/gmx2021 Working dir: /home/sibiolead/biolead/9362 Process ID: 3961116 Command line: gmxdrun -nt 1 -v -deffnm mdsim -pme gpu -nb gpu -bonded gpu -update gpu GROMACS version: 2021 Verified release checksum is 3e06a5865d6ff726fc417dea8d55afd37ac3cbb94c02c54c76d7a881c49c5dd8 Precision: mixed Memory model: 64 bit MPI library: thread_mpi OpenMP support: enabled (GMX_OPENMP_MAX_THREADS = 64) </pre>

	GPU support: CUDA SIMD instructions: AVX2_256 FFT library: fftw-3.3.8-sse2-avx-avx2-avx2_128 RDTSCLP usage: enabled TNG support: enabled Hwloc support: disabled Tracing support: disabled C compiler: /usr/bin/cc GNU 8.4.0 C compiler flags: -mavx2 -mfma -Wno-missing-field-initializers -fexcess-precision=fast -funroll-all-loops -O3 -DNDEBUG C++ compiler: /usr/bin/c++ GNU 8.4.0 C++ compiler flags: -mavx2 -mfma -Wno-missing-field-initializers -fexcess-precision=fast -funroll-all-loops -fopenmp -O3 -DNDEBUG CUDA compiler: /usr/bin/nvcc nvcc: NVIDIA (R) Cuda compiler driver; Copyright (c) 2005-2019 NVIDIA Corporation; Built on Sun_Jul_28_19:07:16 PDT_2019; Cuda compilation tools, release 10.1, V10.1.243 CUDA compiler flags: -std=c++14; -gencode; arch=compute_30, code=sm_30; -gencode; arch=compute_35, code=sm_35; -gencode; arch=compute_37, code=sm_37; -gencode; arch=compute_50, code=sm_50; -gencode; arch=compute_52, code=sm_52; -gencode; arch=compute_60, code=sm_60; -gencode; arch=compute_61, code=sm_61; -gencode; arch=compute_70, code=sm_70; -gencode; arch=compute_35, code=compute_35; -gencode; arch=compute_50, code=compute_50; -gencode; arch=compute_52, code=compute_52; -gencode; arch=compute_60, code=compute_60; -gencode; arch=compute_61, code=compute_61; -gencode; arch=compute_70, code=compute_70; -gencode; arch=compute_75, code=compute_75; -use_fast_math; -D_FORCE_INLINES; -mavx2 -mfma -Wno-missing-field-initializers -fexcess-precision=fast -funroll-all-loops -fopenmp -O3 -DNDEBUG CUDA driver: 11.40 CUDA runtime: 10.10 Running on 1 node with total 8 cores, 16 logical cores, 1 compatible GPU Hardware detected: CPU info: Vendor: AMD Brand: AMD Ryzen 7 5800X 8-Core Processor Family: 25 Model: 33 Stepping: 2 Features: aes amd apic avx avx2 clflush cmov cx8 cx16 f16c fma htt lahfs misalignsse mmx msr nonstop_tsc pclmuldq pdp10b popcnt pse rdrnd rdtsclp sha sse2 sse3 sse4a sse4.1 sse4.2 sse3 x2apic Hardware topology: Basic Sockets, cores, and logical processors: Socket 0: [0 8] [1 9] [2 10] [3 11] [4 12] [5 13] [6 14] [7 15] GPU info: Number of GPUs detected: 1 #0: NVIDIA NVIDIA GeForce RTX 3050, compute cap.: 8.6, ECC: no, stat: compatible
Ligand H-bond analysis	Specify 2 groups to analyze: Selected 1: 'Protein' Selected 13: 'UNL' Checking for overlap in atoms between Protein and UNL Calculating hydrogen bonds between Protein (4829 atoms) and UNL (57 atoms) Found 445 donors and 884 acceptors Will do grid-search on 20x18x15 grid, rcut=0.34999999 Frame loop parallelized with OpenMP using 16 threads. Average number of hbonds per timeframe 0.458 out of 196690 possible

Table S3. Exploratory frame-based descriptive comparison of ΔG_{bind} , ΔG_{gas} , and ΔG_{solv} between the ursolic acid-PTP1B and compound 1-PTP1B trajectories.

n=11	Ursolic Acid			Compound 1		
	GGAS	GSOLV	TOTAL	GGAS	GSOLV	TOTAL
1	-35.13	22.66	-12.47	-48.42	34.23	-14.19
2	-50.5	14.79	-35.71	-52.01	19.78	-32.24
3	-43.73	12.13	-31.59	-59.19	28.21	-30.99
4	-43.49	12.99	-30.49	-59.56	26.2	-33.35
5	-51.61	12.44	-39.17	-62.51	27.14	-35.37
6	-60.3	16.16	-44.15	-60.37	27.76	-32.61
7	-53.66	17.23	-36.43	-57.59	24.45	-33.14
8	-59.42	23.06	-36.36	-60.77	24.8	-35.97
9	-50.73	16.56	-34.17	-58.72	27.52	-31.2
10	-49.6	15.91	-33.69	-60.61	22.66	-37.95
11	-67.1	20.7	-46.39	-43.59	18.73	-24.87

Component	Mean ursolic acid	SD ursolic acid	Mean compound 1	SD compound 1	t-value	p-value	Interpretation
ΔG_{Gas}	-51.38818182	2.672723098	-56.66727273	1.810939029	-1.635173484	0.1198	Not significant
ΔG_{Solv}	16.78454545	1.166009016	25.58909091	1.293503667	5.055803136	0.0001	significant
ΔG_{bind}	-34.60181818	2.655135955	-31.08	1.293503667	1.192439949	0.3004	Not significant

These frame values are not independent biological or simulation replicates and are provided only to support descriptive interpretation.