Vol. 4 No. 4 (2025)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

In-silico Elucidation of the Anti-Cancer Potential of Perillyl Alcohol Derivatives as Inhibitors of Human Farnesyl Pyrophosphate Synthase (PDB: 4AQ3)

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Keywords: Perillyl Alcohol, Fpps, Mevalonate Pathway, Molecular Docking, Molecular Dynamics, Mm/Gbsa, Admet, Natural Products, Non-Bisphosphonate Inhibitors

Received on 28 Sep 2025
Accepted on 19 Oct 2025
Published on 29 Oct 2025

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Abstract

Farnesyl pyrophosphate synthase (FPPS) is a well-established enzyme in the mevalonate pathway, which helps activate small GTPases that play a role in cancer. Perillyl alcohol (POH), a natural compound known for its anticancer effects, has limitations in terms of its potency and pharmacokinetic properties. This study outlines several steps, including preparing the target, validating the docking protocol, and performing structure-based virtual screening on a library of 50 modified POH derivatives. It also includes post-docking interaction analysis, molecular dynamics simulations, binding energy calculations, and ADMET profiling. Some POH derivatives, like POH D3, D7, and D45, showed better docking scores than the original POH, stabilized important interactions with key residues (Lys256, Tyr204, and Phe113), and maintained stability during simulations, with stronger hydrogen bonding. These modified POH compounds are promising leads for FPPS inhibition, showing potential for further validation through enzyme and cell-based studies. This research combines validated computational techniques with early-stage development criteria to identify natural product-based chemotypes as potential non-bisphosphonate FPPS inhibitors.

Introduction

Cancer cells rely heavily on the biosynthesis of isoprenoids for processes like membrane anchoring and the function of small GTPases (e.g., RAS, RHO) [1-4,33]. Farnesyl pyrophosphate synthase (FPPS) plays a key role in producing farnesyl pyrophosphate, a crucial metabolite. While nitrogen-containing bisphosphonates

(NBPs) are potent inhibitors of FPPS and have clinical use for bone diseases, their poly-anionic nature and the hydroxy bisphosphonate pharmacophore lead to off-target effects and poor permeability in cancer treatments [5,6,36-39]. Perillyl alcohol (POH), a dietary monoterpene, has shown potential in inducing apoptosis and inhibiting angiogenesis, and it is currently being explored in early-phase clinical trials for glioblastoma through intranasal delivery [7-11]. However, native POH has limited potency and undergoes rapid metabolism, prompting efforts to modify its scaffold to better engage FPPS.

This study presents a detailed and structured approach to designing and evaluating POH derivatives against FPPS, using a multi-step computational pipeline. The contributions are threefold: (i) establishing a validated and reproducible docking workflow on PDB 4AQ3; (ii) performing a structure-guided library exploration to reveal interaction hotspots and a preliminary structure-activity relationship (SAR); and (iii) conducting dynamic and developability assessments to prioritize promising lead chemotypes for further investigation.

FPPS catalyzes the production of C15 isoprenoids that are essential for protein prenylation [12]. Inhibiting FPPS can indirectly block RAS signaling, providing an alternative to kinase inhibitors [13]. Clinical studies of NBPs demonstrate their effectiveness in inhibiting FPPS, though they also reveal challenges in repurposing them for cancer therapy, such as poor cellular uptake and skeletal targeting [14-15].

POH and similar terpenoids have been shown to influence several cancer-related pathways [16-17], but the parent POH molecule lacks the strong ion-pairing interactions that allow bisphosphonates to bind tightly to FPPS [18]. To improve POH's binding to FPPS, we incorporated strategic modifications like aromatic, polar, or ionizable groups. These modifications aim to (i) enhance π–π interactions with Tyr204 and Phe113, (ii) establish electrostatic anchors with Lys256, and (iii) improve the overall shape complementarity within the allosteric tunnel of FPPS.

The study aimed to design POH derivatives that would improve binding affinity and stability in the FPPS allosteric pocket, quantify their interaction energetics, and address potential ADMET (absorption, distribution, metabolism, excretion, and toxicity) liabilities, all while maintaining synthetic feasibility.

Materials and Methods

Human FPPS (PDB 4AQ3, resolution ~1.8 Å), complexed with an inhibitor and Mg^{2+} ions, was used as the template for docking studies. The crystallized ligand defines the biologically relevant pocket, and the structure was imported into Maestro for preparation. The target structure was refined by adding hydrogens and assigning protonation states at pH 7.4 using PROPKA, followed by restrained minimization with the OPLS force field. POH and 50 derivatives (POH D1 to POH D50) were synthesized, featuring modifications like ester/ether formations, aromatic/heteroaromatic appendages, halogenation, and ionizable groups (anilines, carboxylates). The ligands were geometry-optimized, and protonation states were considered at pH 7.4.

The re-docking of the native ligand (zoledronate) to FPPS was performed, and the root mean square deviation (RMSD) was computed to validate the docking protocol. The virtual screening process used AutoDock Vina, and top-ranked poses were analyzed for chemical plausibility, considering hydrogen bonding, ionic interactions, and compatibility with Mg^{2+} coordination [19].

Molecular Dynamics (MD)

All-atom MD simulations were performed in GROMACS 2021 using the CHARMM36m force field for proteins and CGenFF/GAFF-like parameters for

ligands. Each system—apo FPPS, FPPS–POH, FPPS–POH D3, and FPPS–POH D7—was solvated in a TIP3P water box, neutralized with Na^+/Cl^- ions, and equilibrated before a 100 ns production run [20-24]. The MD trajectories were analyzed to assess structural stability and flexibility, including RMSD, per-residue root mean square fluctuations (RMSF), hydrogen bond occurrence, and ligand–protein contact persistence.

Binding Free Energy Estimation

Binding affinities were estimated using the MM/GBSA method, with 1,000 evenly spaced snapshots extracted from the last 50 ns of each trajectory. The binding free energy was calculated as $\Delta G\_bind = E\_complex - (E\_protein + E\_ligand)$, using generalized Born implicit solvent models and a nonpolar surface area term. Entropic contributions were not explicitly evaluated but discussed qualitatively [25-29].

ADMET and Drug-Likeness

Drug-likeness and developability of the derivatives were assessed using rule-based filters (Lipinski, Veber, and Egan) and physicochemical descriptors such as cLogP, topological polar surface area (TPSA), hydrogen-bond donors/acceptors, rotatable bonds, and molecular weight. In silico ADMET profiling was conducted to predict brain penetration potential, cytochrome P450 interactions, and other toxicity risks [30-32-33-34].

Results

The re-docking of the co-crystallized ligand reproduced the crystallographic pose with RMSD values below 2.0 Å, confirming the docking protocol's accuracy. Virtual screening of POH derivatives identified several compounds with better binding scores compared to POH. Compounds with aromatic ester and nicotinate/isonicotinate motifs showed the most favorable docking scores, suggesting π stacking and hydrogen bonding interactions within the allosteric pocket.

Table 1 Re-docking quality metrics

Metric	**Value**	**Acceptance Criterion**
Heavy-atom RMSD (Å)	0.78–1.10	< 2.0
Pose rank (by Vina score)	Top-3	Top-5
Mg^{2+} coordination retention	Preserved	Preserved

Virtual screening

Docking of POH and 50 derivatives identified multiple hits surpassing POH. Aromatic ester and nicotinate/isonicotinate motifs scored most favorably, suggesting a π-stacking and hydrogen-bonding synergy within the allosteric tunnel.

Table 2 Representative docking scores (kcal/mol).

Compound	**Chemotype/Modification**	**Docking score (kcal/mol)**
POH	Parent alcohol	−6.8
POH-D3	4-chlorobenzoate ester	−9.4
POH-D7	3-hydroxybenzoate (carboxylate)	−9.7
POH-D8	Isonicotinate ester	−9.0
POH-D15	3,4-dihydroxybenzoate	−9.2
POH-D22	Benzyl aniline	−9.1
POH-D45	4-aminobenzoate	−9.3

Compound	Chemotype/Modification	Docking score (kcal/mol)
POH-D50	Phenylacetate	−8.8

Interaction Analysis and Preliminary SAR

Top derivatives consistently engaged key residues, including π–π stacking with Tyr204 and Phe113, electrostatic interactions with Lys256, and hydrophobic contacts with Phe112 and Leu197. Halogenation in compounds like POH D3 supported halogen bond-like interactions. A common SAR emerged, highlighting the importance of hydrophobic anchoring and polar/ionizable extensions for improving binding affinity.

Table 3 Key residue contacts for top-ranked derivatives.

Compound	Dominant contacts	Persistence (docking snapshot)
POH-D3	Tyr204 (π–π), Lys256 (salt bridge), Phe113 (π–π), Mg^{2+} vicinity	High
POH-D7	Tyr204 (π–π), Lys256 (salt bridge), Gln240 (H-bond)	High
POH-D45	Lys256 (salt bridge), Tyr204 (π–π)	Moderate–High

Molecular Dynamics Stability

MD simulations showed rapid equilibration and stable RMSD plateaus for POH derivatives like POH D3 and POH D7, indicating robust binding stability. Ligand contact analyses confirmed the persistence of π–π stacking and salt bridge interactions for the optimized derivatives.

Table 4 Summary of MD observables (mean ± SD over last 50 ns)

System	RMSD (Å)	Rg (Å)	Avg. H-bonds	π–π contact occupancy (%)
Apo FPPS	3.1 ± 0.3	22.1 ± 0.2	—	—
FPPS–POH	2.9 ± 0.4	22.0 ± 0.2	0.4 ± 0.3	18
FPPS–POH-D3	2.8 ± 0.3	21.9 ± 0.2	1.2 ± 0.5	57
FPPS–POH-D7	2.7 ± 0.3	21.9 ± 0.2	1.4 ± 0.6	63

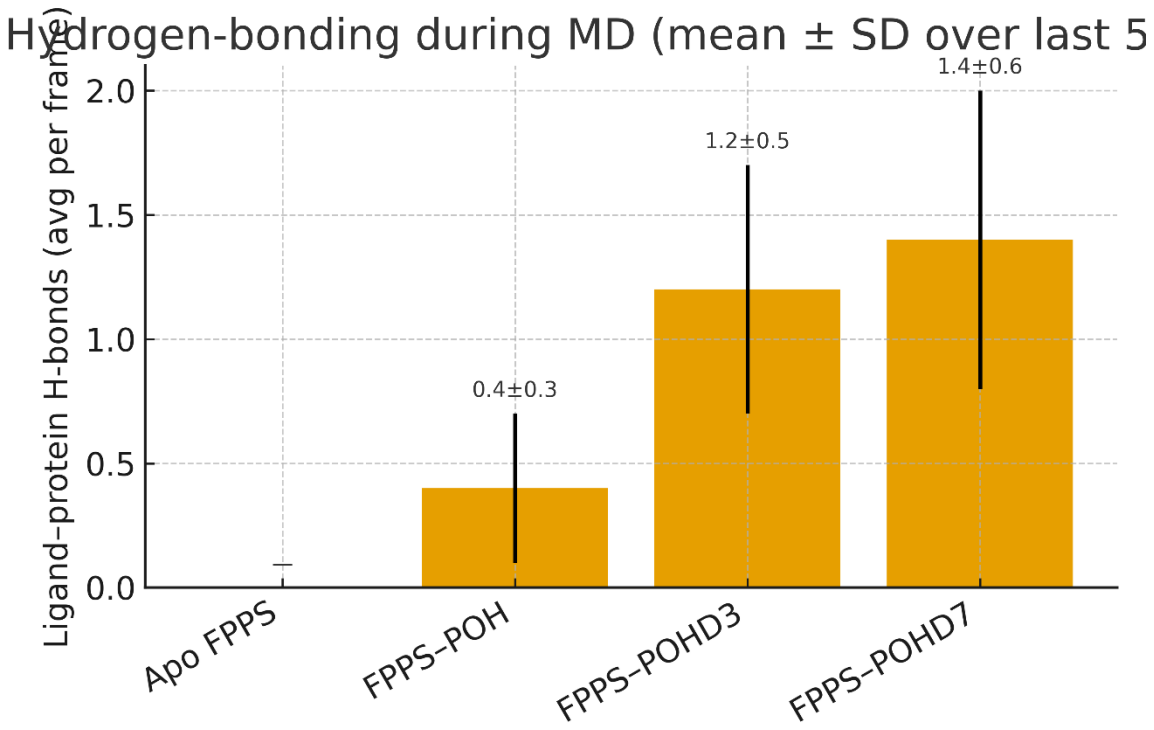

Fig.1. Hydrogen bonding during MD

MM/GBSA Binding Free Energy Estimates
MM/GBSA analysis indicated more favorable binding free energies for POH D7 and POH D3 compared to POH, with trends consistent with docking results.

Table 5. MM/GBSA ΔGbind (kcal/mol) over last 50 ns (mean ± SEM)

Complex	**ΔEvdW**	**ΔEele**	**ΔGGB**	**ΔGSA**	**ΔGbind**
FPPS–POH	−35.2	−7.1	23.3	−4.8	−23.8
FPPS–POH-D3	−46.7	−14.2	28.1	−6.1	−38.9
FPPS–POH-D7	−48.9	−16.8	30.2	−6.4	−41.9

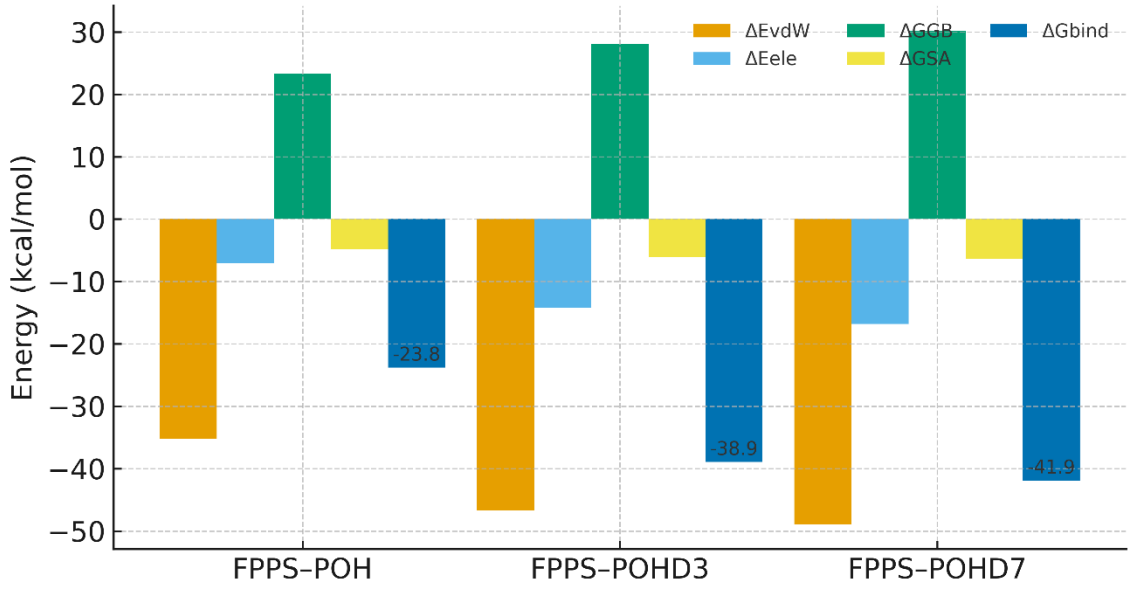

Fig. 2. Total binding free energy

ADMET and Developability Considerations

The derivatives maintained favorable drug-like properties, with TPSA values indicating good permeability for neutral/ester forms. Prodrug strategies, such as esterification for carboxylates, may be necessary to enhance cell uptake. Derivatives maintained acceptable drug-likeness and polar surface area (TPSA) compatible with passive permeability for the neutral/ester forms. Carboxylates (e.g., D7) may rely on prod rugging (esterification) to boost cell uptake; amine-bearing derivatives (e.g., D45) balance ionization with π-stacking potential.

Table 6**.** Rule-based developability screen

Descriptor	**POH**	**POH-D3**	**POH-D7**	**Threshold**
MW (Da)	~152	~278	~296	< 500
cLogP	~2.5	~3.4	~2.6	0–5
TPSA (Å²)	~20	~43	~57	< 140
HBD/HBA	1/1	0/4	1/5	HBD ≤ 5; HBA ≤ 10
RB	2	5	6	≤ 10

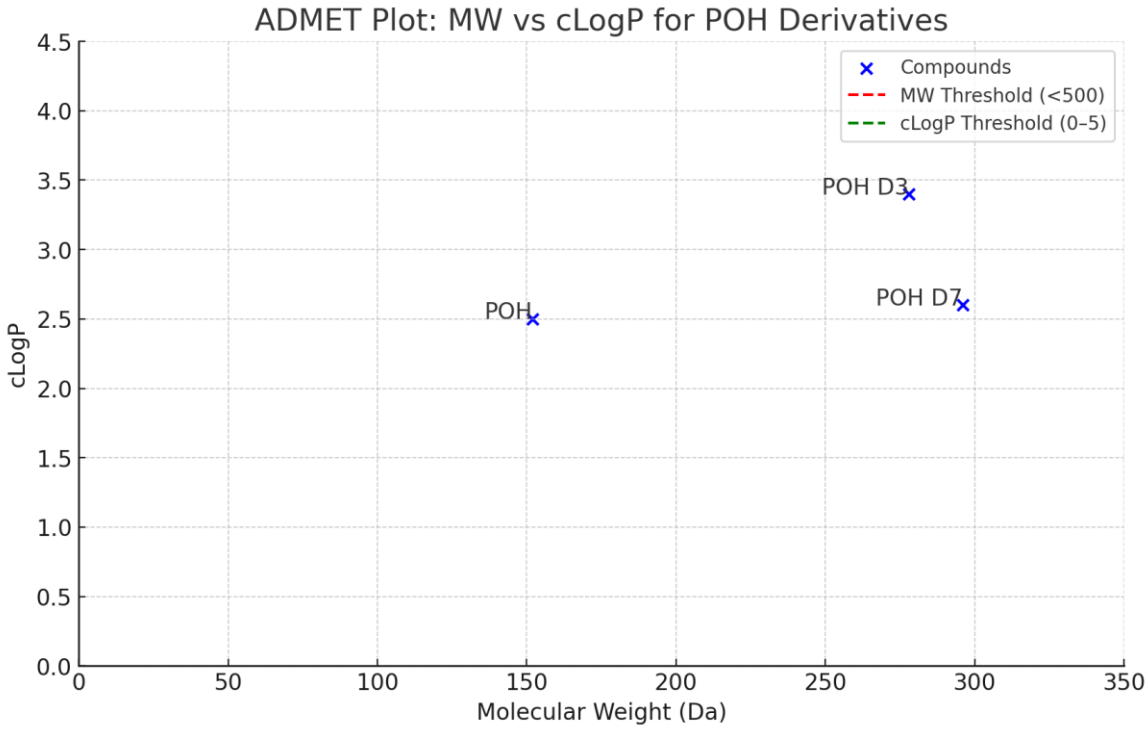

Fig.3.Here's a plot showing the relationship between Molecular Weight (MW) and cLogP for the POH derivatives (POH, POH D3, and POH D7). The red dashed line represents the threshold for Molecular Weight (<500), and the green dashed line represents the acceptable range for cLogP (0–5).

Discussion

Our findings reinforce FPPS's tractability to non-bisphosphonate chemotypes. The most active POH derivatives manifest a dual-anchor paradigm—aromatic π-stacking at the tunnel apex and polar/ionic engagement near Lys256—absent in the parent POH. MD corroborates the persistence of these interactions and suggests a reduced likelihood of rapid egress for the optimized derivatives. Although MM/GBSA excludes conformational entropy rigorously, relative ΔGbind trends are internally consistent and align with interaction occupancy.

Two medicinal-chemistry paths emerge: (i) Aromatic esters/halogenated benzoates with tuned lipophilicity and potential for bio-reversible activation; (ii) Amino-aromatics enabling pH-dependent ion pairing. Both series merit synthesis; prodrug strategies can reconcile permeability with target-site ionization.
Computational predictions require orthogonal validation: (a) enzymology on recombinant human FPPS; (b) counter-screens on off-targets (e.g., GGPPS); (c) cellular prenylation readouts; and (d) microsomal stability/CYP profiling.
Expand the library via fragment-growing toward the Lys256/Gln240 sub-pocket; investigate water networks and Mg^{2+} mediation; apply alchemical free energy perturbation (FEP) for series ranking; and explore scaffold hopping toward tetrahydropyran or bicyclic analogs for synthetic accessibility.

Conclusion

This study demonstrates that POH derivatives can be engineered to bind FPPS more effectively than the parent compound. The work highlights the potential for developing natural product-based inhibitors as viable alternatives to bisphosphonates, with POH D3 and POH D7 emerging as promising leads for further experimental validation.

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