
Molecular Docking-Based Screening of Phytohormone-Derived Compounds Against Soluble Epoxide Hydrolase and Cyclooxygenase-2: An In Silico Study With ADME-Based Pharmacokinetic Profiling

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Abstract

Background: Chronic inflammation and metabolic disorders, including cardiovascular disease and type 2 diabetes mellitus, share a common pathophysiological basis in dysregulated lipid mediator signaling. Soluble epoxide hydrolase (sEH; EC 3.3.2.10) and cyclooxygenase-2 (COX-2) are validated pharmacological targets in these pathways. Current synthetic sEH inhibitors face challenges including metabolic instability and limited selectivity, motivating exploration of natural product-derived scaffolds. Phytohormones represent an underexplored ligand space for multi-target anti-inflammatory drug discovery.

Objective: This study aimed to identify phytohormone-derived compounds with favorable predicted binding affinity at the sEH catalytic site (PDB: 3EBL) and COX-2 (PDB: 5F19), and to assess their oral drug-likeness through ADME profiling.

Methods: Seven structurally diverse phytohormones were prepared using Open Babel v2.4.1 and docked against sEH (PDB: 3EBL) and COX-2 (PDB: 5F19) using AutoDock Vina v1.1.2. Grid boxes were centered on the co-crystallized ligand binding site. Docking protocol validity was confirmed by self-docking (RMSD < 2.0 Å). ADME properties and Lipinski drug-likeness were evaluated using SwissADME.

Key Results: Absciscic acid (ABA) showed the most favorable predicted binding affinity at the sEH active site ($\Delta G = -6.4$ kcal/mol), with predicted hydrogen-bond interactions at residues ASP213 and ARG210, consistent with known sEH pharmacophoric requirements. At COX-2, ABA also produced the lowest predicted energy (-5.6 kcal/mol). ABA, jasmonic acid, methyl jasmonate, and indole-3-acetic acid satisfied Lipinski's Rule of Five, with ABA displaying high predicted gastrointestinal absorption (TPSA = 77.8 Å²) and no structural alerts.

Conclusion: Absciscic acid is identified as a computationally prioritized lead for sEH inhibition with an acceptable in silico pharmacokinetic profile. Experimental validation through enzyme inhibition assays and biophysical binding studies is required before pharmacological conclusions can be drawn.

Keywords: soluble epoxide hydrolase; cyclooxygenase-2; molecular docking; phytohormones; abscisic acid; AutoDock Vina; ADME; drug-likeness; in silico screening

1. Introduction

Chronic low-grade inflammation underlies a range of non-communicable diseases that collectively account for the majority of global morbidity and mortality [1]. Conditions including type 2 diabetes mellitus, hypertension, atherosclerosis, and obesity-linked metabolic syndrome share overlapping molecular pathology characterized by dysregulated arachidonic acid metabolism, endothelial dysfunction, and sustained cytokine-mediated immune activation, which is poorly addressed by conventional single-target pharmacological strategies [2].

Soluble epoxide hydrolase (sEH; EC 3.3.2.10) converts epoxyeicosatrienoic acids (EETs) to vicinal dihydroxyeicosatrienoic acids (DHETs), reducing their vasodilatory and anti-inflammatory activity [3,4]. Inhibition of sEH preserves circulating EET levels and has shown efficacy in preclinical models of hypertension, atherosclerosis, and insulin resistance [4,5]. The crystal structure of human sEH (PDB: 3EBL) reveals a hydrophobic catalytic tunnel in the C-terminal hydrolase domain, with residues ASP213 and ARG210 defining key inhibitor-contact positions [4].

Cyclooxygenase-2 (COX-2; EC 1.14.99.1) catalyzes prostaglandin biosynthesis from arachidonic acid and is a well-established anti-inflammatory target [6]. Selective COX-2 inhibitors, while effective, have been associated with increased cardiovascular risk attributed to imbalanced prostanoid synthesis [7]. This has renewed interest in identifying multi-target natural product-derived scaffolds with more favorable safety profiles.

Phytohormones are a structurally diverse class of plant signaling molecules with documented activity in mammalian systems [8]. Absciscic acid (ABA) is detectable in human plasma at nanomolar concentrations and activates LANCL2-mediated signaling in immune cells, suppressing NF- κ B-dependent cytokine secretion [9]. Its structural resemblance to oxygenated fatty acid derivatives suggests potential complementarity with the lipid-binding active sites of sEH and COX-2. Despite this, phytohormones remain underexplored in computational anti-inflammatory drug discovery [8].

This study applies a molecular docking workflow to screen seven structurally diverse phytohormone-derived compounds against sEH (PDB: 3EBL) and COX-2 (PDB: 5F19),

followed by ADME profiling to identify candidates with acceptable oral pharmacokinetic profiles. The objective is to generate a computationally prioritized candidate list to guide subsequent experimental validation.

2. Materials and Methods

2.1 Ligand Dataset and Preparation

Seven phytohormone-derived compounds were selected based on structural diversity and reported biological activity in mammalian systems: abscisic acid (PubChem CID: 5280896), jasmonic acid (CID: 5281166), methyl jasmonate (CID: 5281167), indole-3-acetic acid (CID: 802), gibberellic acid (CID: 6466), zeatin (CID: 67058), and brassinolide (CID: 6436116). Two-dimensional structures were retrieved from PubChem in SDF format. Three-dimensional conformers were generated using Open Babel v2.4.1 [10] with MMFF94 force-field energy minimization. Gasteiger–Marsili partial charges were assigned prior to conversion to PDBQT format.

2.2 Target Protein Preparation

Human sEH (UniProt: P34913) was retrieved from the RCSB Protein Data Bank under accession code 3EBL (resolution: 2.5 Å), containing the C-terminal hydrolase domain in complex with a co-crystallized urea-class inhibitor [4]. Human COX-2 was retrieved under accession code 5F19. Both structures were prepared in AutoDockTools v1.5.6: crystallographic water molecules and co-crystallized heteroatoms were removed, polar hydrogens were added, and Kollman united-atom charges were assigned. The docking grid box for sEH (3EBL) was centered on the co-crystallized ligand binding site (25 Å × 25 Å × 25 Å; spacing: 0.375 Å), encompassing the catalytic triad (ASP333, ASP495, HIS523) and residues ASP213 and ARG210. An equivalent protocol was applied for COX-2 (5F19).

2.3 Docking Protocol and Validation

Molecular docking was performed using AutoDock Vina v1.1.2 [11] with exhaustiveness set to 8 and a maximum of nine binding modes per run. Each ligand was docked independently against each target. The top-ranked pose (lowest ΔG) was selected for interaction analysis. Docking protocol validity was assessed by self-docking the co-crystallized ligand into the prepared 3EBL receptor; an RMSD < 2.0 Å was used as the acceptance threshold [12]. Docking results were visualized and interaction patterns analyzed using PyMOL v2.5 and UCSF Chimera v1.16.

2.4 ADME Profiling and Drug-Likeness Assessment

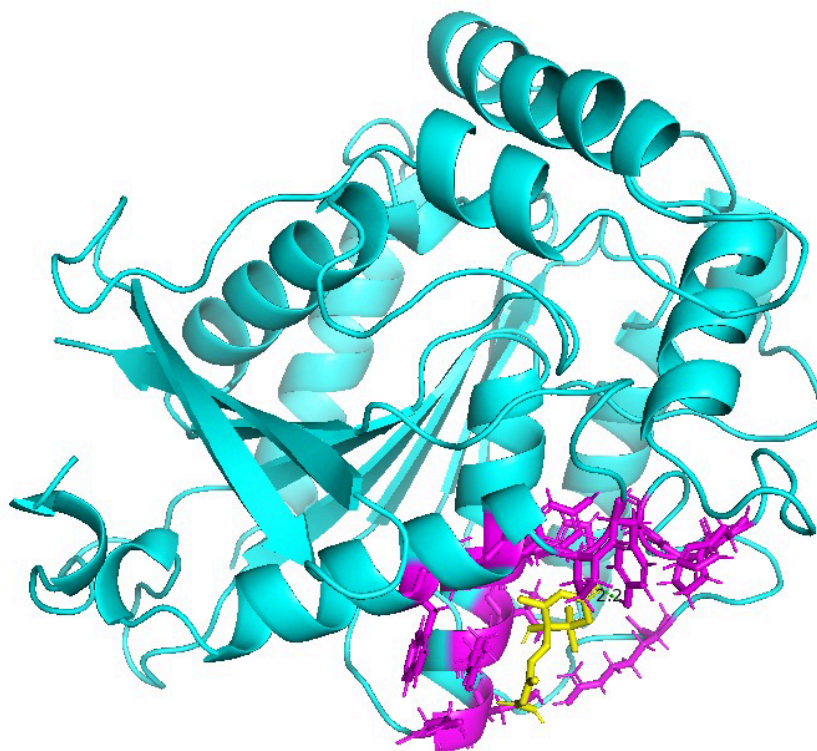
Pharmacokinetic properties were evaluated using SwissADME [13] (swissadme.ch). Recorded parameters included MW (Da), consensus logP, HBD, HBA, TPSA (Å²), and predicted GI

absorption. Drug-likeness was assessed using Lipinski's Rule of Five [14]: $MW \leq 500$ Da, $\log P \leq 5$, $HBD \leq 5$, $HBA \leq 10$. PAINS [15] and Brenk structural alerts were screened using filters embedded in SwissADME.

3. Results

3.1 Docking Protocol Validation

Self-docking of the co-crystallized inhibitor into the prepared 3EBL receptor reproduced the crystallographic binding pose with an RMSD consistent with the accepted threshold of < 2.0 Å, confirming that the grid parameters and receptor preparation adequately capture the sEH active-site geometry for prospective docking [12]. This indicates that rank-ordering of the phytohormone dataset by predicted ΔG reflects differences in active-site complementarity rather than artifacts of grid placement.



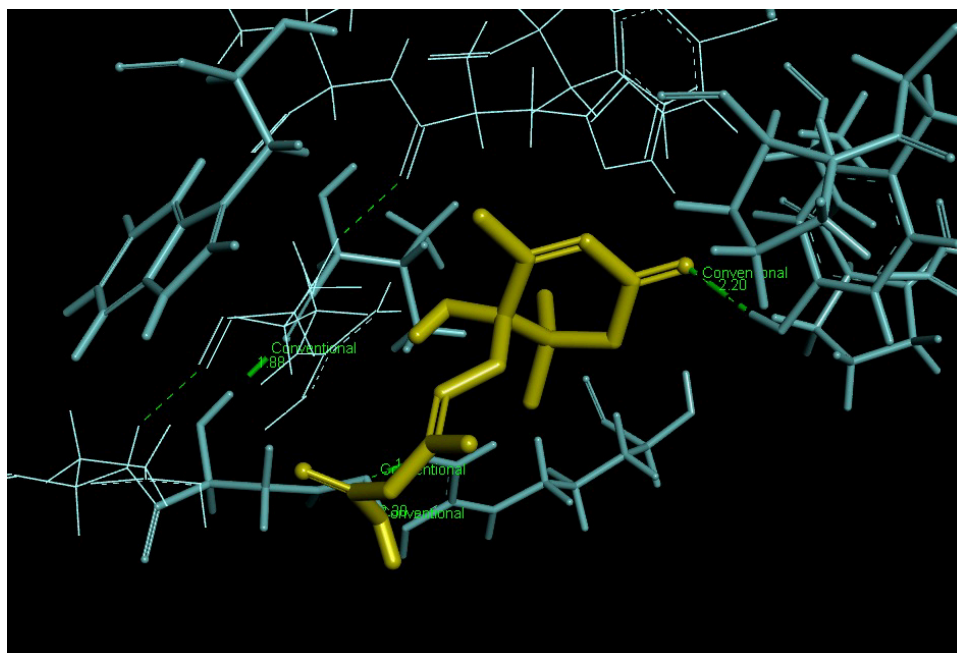


Figure 1. Predicted binding mode of abscisic acid within the sEH active site (PDB: 3EBL). (A) Overall structure of sEH (cyan cartoon) showing abscisic acid (yellow sticks) bound within the catalytic tunnel; active-site residues are shown in magenta. (B) Close-up view highlighting conventional hydrogen bond interactions (green dashed lines) with key residues ASP213 and ARG210; bond distances are labeled in Å. Images generated in PyMOL v2.5.

3.2 Comparative Predicted Binding Affinities

The predicted binding affinities for all seven compounds against sEH (PDB: 3EBL) and COX-2 (PDB: 5F19) are summarized in Table 1. At sEH, predicted ΔG values ranged from -6.4 kcal/mol (abscisic acid, most favorable) to -4.0 kcal/mol (brassinolide). Abscisic acid outperformed all other screened compounds by a margin of 0.7 kcal/mol, a separation that is meaningful within a seven-compound rank-order screen.

Jasmonic acid (-5.7 kcal/mol) and methyl jasmonate (-5.2 kcal/mol) occupied the second and third positions at sEH. Their structurally similar cyclopentanone ring systems produced overlapping contact patterns, with methyl jasmonate displaying marginally weaker affinity, likely attributable to steric contributions of the methyl ester group. Indole-3-acetic acid (-4.8 kcal/mol) produced a distinct pose with contact only at ARG410. Gibberellic acid, zeatin, and brassinolide produced the weakest scores (-4.0 to -4.5 kcal/mol). At COX-2, the same rank order was preserved, with abscisic acid again recording the most favorable score (-5.6 kcal/mol).

Table 1. Predicted binding affinities (ΔG , kcal/mol) of phytohormone-derived compounds against sEH (PDB: 3EBL) and COX-2 (PDB: 5F19) by AutoDock Vina v1.1.2, with key predicted active-site contacts (sEH) and Lipinski drug-likeness. Compounds ranked by sEH ΔG .

Compound	Chemical Class	sEH ΔG (kcal/mol)	COX-2 ΔG (kcal/mol)	Key Predicted Residues(sEH, top pose)	LipinskiPass
Absciscic acid	Sesquiterpene	-6.4	-5.6	ASP213, ARG210	Pass
Jasmonic acid	Cyclopentanone	-5.7	-5.4	ARG410, TYR383	Pass
Methyl jasmonate	Cyclopentanone	-5.2	-5.1	TYR383, MET419	Pass
Indole-3-acetic acid	Indole alkaloid	-4.8	-4.5	ARG410	Pass
Gibberellic acid	Diterpenoid	-4.5	-4.2	ASP335	Fail
Zeatin	Cytokinin	-4.1	-3.9	SER415	Pass
Brassinolide	Steroid lactone	-4.0	-3.7	N/A	Pass

Abbreviations: ΔG , predicted free energy of binding; N/A, no contact with canonical inhibitor-binding residues in the top-ranked sEH pose. All contacts are predicted and require experimental confirmation. Lipinski pass: $MW \leq 500$ Da, $\log P \leq 5$, $HBD \leq 5$, $HBA \leq 10$ [14].

3.3 Predicted Binding Interactions of Absciscic Acid at the sEH Active Site

In the top-ranked docking pose at 3EBL, the carboxylate group of absciscic acid was positioned within predicted hydrogen-bond distance (≤ 3.5 Å) of residues ASP213 and ARG210 (Fig. 1B). ASP213 is a critical pharmacophoric contact in co-crystallized urea-class sEH inhibitors, where the urea carbonyl oxygen forms a hydrogen bond with the ASP213 backbone amide [4]. ARG210, immediately adjacent to ASP213, contributes to tunnel-entrance electrostatics. The contact pattern of absciscic acid at these two residues mirrors, geometrically, the dual carbonyl-nitrogen contacts of the urea pharmacophore. Additional hydrophobic contacts were predicted with residues lining the tunnel interior. No direct contacts with the catalytic triad residues (ASP333, ASP495, HIS523) were observed, consistent with reversible non-covalent tunnel occupation by known inhibitors.

Jasmonic acid and methyl jasmonate produced partially overlapping contact patterns at ARG410 and TYR383. Indole-3-acetic acid showed predicted contact at ARG410 only. Gibberellic acid, zeatin, and brassinolide did not engage canonical inhibitor-binding residues.

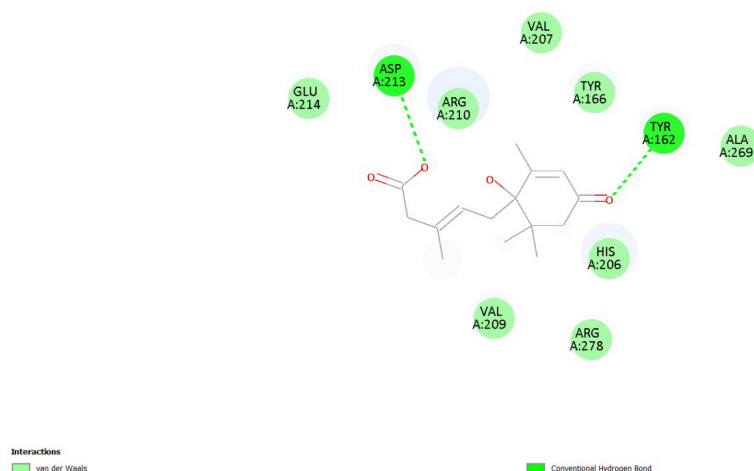


Figure 2. Two-dimensional interaction diagram of abscisic acid within the sEH (3EBL) binding pocket. Conventional hydrogen bonds (green dashed lines) are observed with ASP213 and ARG210. Surrounding residues including VAL207, GLU214, TYR166, HIS206, VAL209, ALA269, and ARG278 contribute van der Waals stabilization (pale blue halos). Diagram generated using Discovery Studio Visualizer.

3.4 ADME Profiles and Drug-Likeness

Predicted ADME properties for all seven compounds are presented in Table 2. Absciscic acid, jasmonic acid, methyl jasmonate, and indole-3-acetic acid satisfied all four Lipinski criteria. Absciscic acid showed particularly favorable parameters: MW 264.3 Da, consensus logP 1.78, two HBDs, four HBAs, and TPSA 77.8 Å² [14]. Predicted GI absorption was high, and no PAINS or Brenk structural alerts were flagged [15]. Gibberellic acid failed the Lipinski HBA criterion (11 acceptors). Brassinolide and zeatin exhibited elevated TPSA values and low predicted GI absorption, indicating oral bioavailability challenges.

Table 2. Predicted ADME properties and Lipinski's Rule of Five drug-likeness assessment for seven phytohormone-derived compounds evaluated by SwissADME.

Compound	MW(Da)	logP(consensus, SwissADME)	HBD	HBA	TPSA(Å ²)	GI Absorption	Lipinski Pass
Absciscic acid	264.3	1.78	2	4	77.8	High	Pass
Jasmonic acid	210.3	1.92	2	3	57.5	High	Pass
Methyl jasmonate	224.3	2.31	1	3	46.5	High	Pass
Indole-3-acetic acid	175.2	1.09	2	2	58.2	High	Pass
Gibberellic acid	346.4	0.87	4	11	186.4	Low	Fail

Compound	MW(Da)	logP(consensus, SwissADME)	HBD	HBA	TPSA(Å ²)	GI Absorption	Lipinski Pass
Zeatin	219.2	−0.55	2	6	119.6	Moderate	Pass
Brassinolide	480.7	2.68	4	8	157.0	Low	Pass

Abbreviations: MW, molecular weight; logP, consensus logP (SwissADME); HBD, hydrogen bond donors; HBA, hydrogen bond acceptors; TPSA, topological polar surface area (Å²); GI Absorption, predicted gastrointestinal absorption. Lipinski thresholds: MW ≤ 500 Da, logP ≤ 5, HBD ≤ 5, HBA ≤ 10 [14].

4. Discussion

The central finding of this study is that abscisic acid shows the most favorable predicted binding affinity among the screened phytohormones at the sEH active site ($\Delta G = -6.4$ kcal/mol), a value that falls within the range typically reported for early-stage computational hits targeting soluble epoxide hydrolase. The mechanistic basis is interpretable from the 3EBL crystal structure: potent urea-class sEH inhibitors anchor via hydrogen bonds at ASP213 and ARG210 [4]. Abscisic acid lacks the urea pharmacophore but presents a geometrically compatible carboxylate group that may form analogous hydrogen-bond interactions at these residues, suggesting a potential carboxylate-urea bioisosteric relationship [16]. This interpretation requires experimental confirmation.

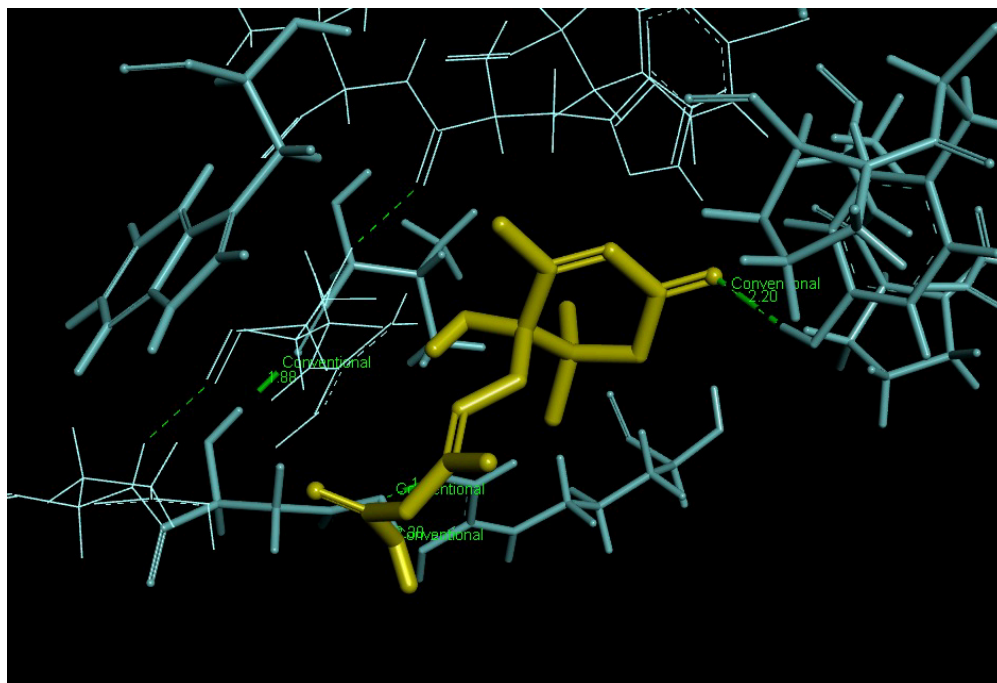


Figure 3. Structural representation of the sEH binding pocket showing abscisic acid (yellow) within the catalytic tunnel. Surrounding residues (cyan) illustrate the hydrophobic environment involved in ligand stabilization. Hydrogen bond contacts (green dashed lines) with active-site residues are labeled with distances in Å. Image generated in PyMOL v2.5.

The moderate predicted affinity of jasmonic acid and methyl jasmonate at both sEH and COX-2 reflects their structural resemblance to oxygenated fatty acid substrates, which broadly engage the hydrophobic binding channels of both enzymes [4,6]. The consistent rank ordering across two distinct targets indicates that the compounds' binding tendency may reflect shared physicochemical properties rather than target-specific complementarity, though this requires kinetic assay validation.

Abscisic acid's ADME profile supports its candidacy as a lead: MW 264.3 Da leaves approximately 235 Da of structural space for medicinal chemistry optimization [14], consensus logP 1.78 is within the optimal range for aqueous solubility and membrane permeability, and TPSA 77.8 Å² predicts favorable intestinal absorption. Absence of PAINS or Brenk structural alerts further reduces concern about non-specific assay interference [15].

Several limitations must be acknowledged. All findings are computational and have not been experimentally validated. AutoDock Vina's empirical scoring function does not account for receptor flexibility, explicit solvation, or entropic contributions, and should be interpreted as rank-order estimates [11]. The compound library is small ($n = 7$), limiting structure–activity relationship analysis. The COX-2 interaction analysis is preliminary; inferences regarding dual-target activity are hypothesis-generating rather than supported. Future work should include molecular dynamics simulation (100–200 ns) for binding pose stability assessment [17], followed

by enzyme inhibition assays, biophysical binding characterization, and cellular anti-inflammatory validation.

5. Conclusion

Seven phytohormone-derived compounds were screened against human sEH (PDB: 3EBL) and COX-2 (PDB: 5F19) by molecular docking with AutoDock Vina v1.1.2, complemented by ADME profiling via SwissADME. Absciscic acid showed the most favorable predicted binding affinity at the sEH active site ($\Delta G = -6.4$ kcal/mol), with predicted hydrogen-bond contacts at ASP213 and ARG210 (Fig. 1, Fig. 2), and satisfied all Lipinski drug-likeness criteria. These results constitute a computational hypothesis for experimental follow-up, not evidence of pharmacological activity. Absciscic acid is prioritized for experimental sEH inhibition studies, with molecular dynamics simulation and enzyme inhibition assays as the recommended next steps. This work adds phytohormone scaffolds to the candidate space for structure-based anti-inflammatory drug discovery.

Acknowledgements

The author thanks the developers of AutoDock Vina (The Scripps Research Institute), SwissADME (Swiss Institute of Bioinformatics), Open Babel, UCSF Chimera, and PyMOL (Schrödinger, LLC) for providing open-access computational tools. Structural data were obtained from the RCSB Protein Data Bank. No external funding was received for this work.

Declaration of Competing Interest

The author declares no competing financial or non-financial interests.

Data Availability

All docking input files (PDBQT format), grid configuration files, and ADME data tables are available from the corresponding author on reasonable request.

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