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Marine Carotenoids As Potential Drug Target Against Pcsk9: In Silicon And Admet Approaches.

Rakshitha V S[1] L. Keerthi Sasanka[2*], Dhanraj Ganapathy[3],

[1]Undergraduate Student, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu – 600077 India. Email: 152001082.sdc@saveetha.com
[2*]Senior Lecturer, Department of Prosthodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai- 600077 Email id: keerthis.sdc@saveetha.com
[3]Professor and Head, Department of Prosthodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai- 600077 Email ID: dhanaraj.sdc@saveetha.com

***Corresponding author :** L. Keerthi Sasanka,
Senior Lecturer,Department of Prosthodontics,Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai- 600077 Email id: keerthis.sdc@saveetha.com

ABSTRACT
Background: Cardiovascular diseases (CVDs) remain the leading cause of mortality worldwide, with hypercholesterolemia representing a primary modifiable risk factor. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease enzyme primarily synthesized in the liver that plays an essential regulatory role in cholesterol homeostasis. PCSK9 binds to the low-density lipoprotein receptor (LDL-R), promoting its lysosomal degradation and thereby inhibiting the clearance of LDL from the bloodstream. This mechanism results in elevated LDL cholesterol levels, contributing to atherosclerosis, coronary artery disease, and stroke. While monoclonal antibodies targeting PCSK9 have demonstrated clinical efficacy, their limitations—including high costs and parenteral administration—necessitate the discovery of orally bioavailable small-molecule inhibitors. Marine natural products have emerged as a promising source of bioactive compounds. This study aims to evaluate Eckol (EKL), a phlorotannin isolated from brown algae, as a potential small-molecule inhibitor against PCSK9 using comprehensive in silico molecular docking and ADMET profiling approaches.
Materials and Methods: The three-dimensional bioactive conformation of Eckol was constructed using the Sybyl-X1.3/SKETCH module and energy-minimized using the Tripos force field with Gasteiger-Hückel atomic charges. The co-crystal structure of PCSK9 (PDB ID: 6u26) was retrieved from the RCSB Protein Data Bank and prepared using the biopolymer module of SYBYL-X 1.3, with energy minimization performed according to the Powell algorithm. Molecular docking simulations were conducted using the Surflex-Dock module of Sybyl-X1.3. ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties were predicted using SwissADME and pkCSM web servers.
Results: Molecular docking revealed that Eckol successfully docked into the active site of PCSK9 with high binding affinity. The cumulative docking scores (C-scores) for Eckol against PCSK9 were 6.84 and 5.93, with a robust D-score of -111.84. Graphical analysis demonstrated that Eckol penetrates deeply into the binding domain, establishing interactions with key amino acid residues including A328, S329, P331, T335, R357, C358, V460, A463, A467, I474, and R476. Notably, seven hydrogen bond interactions were observed with residues S329, C358, R357, A463, R458, I474, and R476. ADMET profiling indicated favorable physicochemical properties, compliance with Lipinski's Rule of Five, and a promising safety profile with no predicted AMES toxicity or hepatotoxicity concerns.
Conclusion: The integrated molecular docking and ADMET analyses position Eckol as a highly promising, orally viable small-molecule inhibitor of PCSK9. The compound demonstrates strong binding affinity, targeted interaction with clinically validated catalytic residues, and a favorable pharmacokinetic safety profile. These findings warrant further experimental validation through in vitro assays using HepG2 hepatic cell lines to quantify LDL receptor upregulation and fluorescent LDL uptake, followed by in vivo efficacy studies in appropriate animal models.

Keywords: Eckol, PCSK9, phlorotannin, molecular docking, ADMET, hypercholesterolemia, cardiovascular disease

INTRODUCTION

Cardiovascular diseases (CVDs) remain the leading cause of global mortality, responsible for an estimated 17.9 million deaths annually according to the World Health Organization. Among the modifiable risk factors that contribute to CVD pathogenesis, hypercholesterolemia—defined by elevated concentrations of low-density lipoprotein cholesterol (LDL-C)—constitutes a critical determinant. Consequently, the pharmacological reduction of LDL-C has become a cornerstone of cardiovascular risk mitigation strategies [1].
Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease synthesized primarily in the liver. This 692-amino acid protein regulates cholesterol homeostasis through a well-characterized mechanism. After its secretion, PCSK9 binds to the epidermal growth factor-like repeat A (EGF-A) domain of the low-density lipoprotein receptor

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(LDL-R) on hepatocyte surfaces. The resulting PCSK9–LDL-R complex undergoes endocytosis and is directed toward lysosomal degradation, a process that prevents the receptor from recycling back to the plasma membrane. The net effect is a reduction in LDL-R availability, impaired clearance of LDL particles from the circulation, and a sustained elevation of plasma LDL-C concentrations [2,3]. Genetic evidence underscores the clinical relevance of this pathway: individuals carrying loss-of-function mutations in PCSK9 exhibit substantially lower LDL-C levels and a markedly reduced lifetime risk of coronary artery disease [4].

Current pharmacotherapy targeting PCSK9 relies on monoclonal antibodies, notably alirocumab and evolocumab. These agents reduce LDL-C levels by approximately 50–60%, even in patients with familial hypercholesterolemia or intolerance to statins [5]. Nevertheless, several factors limit their broader implementation. The high production costs impose a substantial economic burden on healthcare systems, and the need for subcutaneous injection every two to four weeks can compromise long-term patient adherence [6]. Additionally, the development of anti-drug antibodies has been observed in a subset of patients, which may attenuate therapeutic efficacy over time [7]. These shortcomings have intensified the search for orally bioavailable small-molecule inhibitors of PCSK9. Oral agents would offer reduced manufacturing costs, improved patient convenience, and enhanced adherence. Despite considerable pharmaceutical interest, the development of small-molecule PCSK9 inhibitors has proven difficult because the protein–protein interface between PCSK9 and the LDL-R is extensive and relatively flat, lacking the well-defined hydrophobic pockets that typically accommodate small molecules [8].

Marine natural products constitute a valuable source of structurally diverse, biologically active compounds. Among these, brown algae (Phaeophyceae) produce a class of polymeric polyphenols known as phlorotannins, which are assembled from phloroglucinol (1,3,5-trihydroxybenzene) monomer units. Eckol is a distinctive phlorotannin isolated from species such as Ecklonia cava, Ecklonia stolonifera, and Eisenia bicyclis. Its structure features a dibenzo-p-dioxin skeleton with multiple hydroxyl substituents, which endow it with antioxidant capacity and the potential to interact with protein targets [9]. Previous pharmacological investigations have documented anti-inflammatory, antioxidant, neuroprotective, and anticancer activities of Eckol in various experimental models, establishing its biological plausibility as a therapeutic candidate [10–12].

Given the demand for novel orally bioavailable PCSK9 inhibitors and the established bioactivity of marine phlorotannins, the present study evaluates Eckol as a potential small-molecule inhibitor of PCSK9. Molecular docking simulations and comprehensive ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling were employed to characterize the binding interactions between Eckol and the catalytic domain of PCSK9, to assess its pharmacokinetic and safety properties, and to appraise its suitability as a lead compound for the management of hypercholesterolemia and cardiovascular disease.

MATERIALS AND METHODS

The three-dimensional conformation of Eckol was constructed from its canonical SMILES string (PubChem CID: 162928) using the SKETCH module within the SYBYL-X 1.3 software package (Tripos International, St. Louis, MO, USA). The initial geometry was subjected to energy minimization under the Tripos force field with Gasteiger-Hückel atomic charges. Minimization proceeded via the Powell algorithm for 1,000 cycles with a convergence threshold of 0.005 kcal/(mol·Å), a step taken to eliminate steric clashes and to obtain a realistic low-energy conformation suitable for docking [13,14].

The crystallographic structure of PCSK9 was retrieved from the RCSB Protein Data Bank under accession code 6u26 [15]. This entry was selected because of its favorable resolution (2.10 Å) and the presence of a co-crystallized small-molecule inhibitor occupying the catalytic site, which provided a direct structural template for binding-site definition and subsequent docking validation. Protein preparation was performed in the biopolymer module of SYBYL-X 1.3. Missing hydrogen atoms were added with standard protonation states appropriate for pH 7.4, and atom types and charges were assigned according to the AMBER FF99 force field [16]. The prepared structure was then energy-minimized using the Powell algorithm for 1,000 cycles at a convergence gradient of 0.5 kcal/(mol·Å) to relieve any steric strain introduced during the addition of hydrogens and assignment of charges [17].

To confirm that the docking procedure could faithfully recapitulate a known binding mode, the co-crystallized ligand was re-docked into the PCSK9 active site, and the root-mean-square deviation (RMSD) between the predicted pose and the experimental conformation was calculated. An RMSD value below 2.0 Å was set as the threshold for an acceptable reproduction of the native binding geometry.

Molecular docking of Eckol into the PCSK9 catalytic domain was performed with the Surflex-Dock module of SYBYL-X 1.3 [13,18]. The binding site was delineated by the position of the co-crystallized inhibitor, and a protomol—a computational representation of the idealized active site—was generated in Ligand mode with a threshold of 0.5 and a bloat parameter of 0, thereby restricting the search space to the immediate vicinity of the bound ligand. Surflex-Dock integrates a shape-matching algorithm with a flexible ligand docking engine, allowing the exploration of conformational space while maintaining rapid scoring. The docking run permitted a maximum of 20 poses per ligand and considered up to 100 rotatable bonds; all other parameters were retained at their published default values [19–23].

Docked conformations were ranked using the Hammerhead consensus scoring function (CScore), which combines several complementary scoring terms. The crash score quantifies the degree of undesirable penetration into the protein. The polar

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score assesses interactions in polar regions of the binding pocket. The D-score accounts for hydrogen-bonding energy, complex (ligand–protein) energy, and internal (ligand–ligand) energy. The PMF-score estimates the Helmholtz free energy of protein–ligand atom-pair interactions through a potential of mean force. The G-score computes the sum of electrostatic and van der Waals contributions, while the Chem-score evaluates hydrogen bonding, lipophilic contacts, and the entropic penalty associated with freezing rotatable bonds. The top 20 conformations were retained and systematically inspected for their binding modes and intermolecular interactions within the PCSK9 active site.

Graphical analysis and visualization of the highest-ranked Eckol-PCSK9 complexes were carried out using BIOVIA Discovery Studio Visualizer (Dassault Systèmes, San Diego, CA, USA) for simplified two-dimensional interaction diagrams and PyMOL (Schrödinger, LLC, New York, NY, USA) for three-dimensional representations. Hydrogen bonds were identified using a donor–acceptor distance cutoff of 3.2 Å and a donor–hydrogen–acceptor angle cutoff of 120°. Hydrophobic contacts, including π-alkyl and π-sigma interactions, were defined by a distance cutoff of 5.0 Å.

Pharmacokinetic and safety properties were predicted through a set of established web-based tools. Physicochemical descriptors—molecular weight, number of hydrogen bond donors (HBD) and acceptors (HBA), topological polar surface area (TPSA), and calculated logP (cLogP)—were computed with the SwissADME server (http://www.swissadme.ch) [24]. Compliance with Lipinski's Rule of Five was used as an initial filter for oral bioavailability; a compound was noted as violating a rule if its molecular weight exceeded 500 Da, cLogP exceeded 5, HBD exceeded 5, or HBA exceeded 10. Gastrointestinal absorption was predicted with the BOILED-Egg model implemented in SwissADME, while blood–brain barrier penetration was assessed against established cutoffs (TPSA < 70 Å² for high penetration). Plasma protein binding was estimated using the pkCSM server [25].

Metabolic liability was evaluated by predicting interactions with the major cytochrome P450 isoforms CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Both substrate recognition and inhibition potential were assessed for each isoform via pkCSM. Toxicity endpoints—AMES mutagenicity, hepatotoxicity, inhibition of the human ether-à-go-go-related gene (hERG) potassium channel (as an indicator of cardiotoxicity risk), and skin sensitization—were likewise predicted with pkCSM [25], and the results were interpreted in light of the confidence scores associated with each prediction.

RESULTS

Docking Protocol Validation

The docking protocol validation demonstrated that the re-docked native ligand achieved an RMSD of 1.42 Å relative to the co-crystallized conformation in the PCSK9 active site (PDB ID: 6u26). This RMSD value, being below the standard threshold of 2.0 Å, confirmed that the Surflex-Dock protocol accurately reproduces the experimentally determined binding mode and is therefore suitable for subsequent docking studies of Eckol.

Docking Scores and Binding Affinity

The molecular docking analysis revealed that Eckol successfully docked into the active site of PCSK9 with a high binding affinity. The cumulative docking scores (C-scores) for Eckol against PCSK9 were 6.84 and 5.93, indicating a consistent and robust binding profile. The detailed Surflex-Dock scoring metrics for the Eckol-PCSK9 complex are presented in Table 1.

Table 1. Surflex scores of docked ligand Eckol (EKL) in the binding site of proprotein convertase subtilisin/kexin type 9 (PCSK9) enzyme.

Protein	**Ligand**	**CScore**[a]	**Crash score**[b]	**Polar score**[c]	**D score**[d]	**PMF score**[e]	**G score**[f]	**Chem score**[g]
PCSK9	**EKL**	6.84	-1.30	7.14	-111.84	-41.29	-138.34	-71.91

[a]**CScore** is a consensus scoring which uses multiple types of scoring functions to rank the affinity of ligands, [b]**Crash**-score revealing the inappropriate penetration into the binding site, [c]**Polar** region of the ligand, [d]**D-score** showing hydrogen bonding, complex (ligand-protein), and internal (ligand-ligand) energies, [e] **PMF-score** indicating the Helmholtz free energies of interactions for protein-ligand atom pairs (Potential of Mean Force, PMF), [f] **G-score** for charge and van der Waals interactions between the protein and the ligand, [g] **Chem-score** points for hydrogen bonding, lipophilic contact, and rotational entropy, along with an intercept term.

The D-score of -111.84 indicates exceptionally favorable hydrogen bonding and complex interaction energies between Eckol and PCSK9. The Polar score of 7.14 suggests that Eckol establishes extensive polar interactions within the binding site. The PMF score (-41.29) further corroborates the favorable Helmholtz free energy of interaction for protein-ligand atom pairs [12].

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Molecular Interaction Analysis

Graphical analysis of the top-ranked docking conformations demonstrated that Eckol penetrates deeply into the PCSK9 binding domain, establishing multiple interactions with several key amino acid residues. As illustrated in Figure 2, the compound interacts with residues A328, S329, P331, T335, R357, C358, V460, A463, A467, I474, and R476.

Hydrogen Bond Interactions: Notably, Eckol establishes at least seven hydrogen bond interactions with critical residues within the PCSK9 active site, including S329 (side chain hydroxyl group), C358 (backbone carbonyl), R357 (guanidinium group), A463 (backbone carbonyl), R458 (guanidinium group), I474 (backbone carbonyl), and R476 (guanidinium group). The involvement of residues R357, R458, and R476 is particularly significant, as previous structural studies have reported the critical role of these arginine residues in PCSK9-inhibitor complex formation [26]. The multiple hydrogen bond interactions suggest that Eckol effectively engages the polar environment of the PCSK9 active site, which is characterized by several hydrophilic residues essential for substrate recognition [15].

Hydrophobic and Van der Waals Interactions: In addition to hydrogen bonding, Eckol participates in extensive hydrophobic contacts and Van der Waals interactions with surrounding residues. The dibenzo-p-dioxin core of Eckol engages in π-alkyl and π-sigma interactions with the aliphatic portions of R357 and R476, as well as with the hydrophobic side chains of P331, V460, and A467 [9,10]. These non-covalent interactions contribute to the overall binding affinity and help anchor the ligand within the binding pocket.

Comparison with Co-crystallized Complex: Interestingly, the interaction pattern observed for Eckol closely resembles that seen in the co-crystallized complex of PCSK9 (PDB ID: 6u26) [15]. The native ligand in the crystal structure similarly engages the R357, R458, and R476 residues, suggesting that Eckol mimics the binding mode of established inhibitors [26]. This similarity further supports the reliability of our docking results and underscores the potential of Eckol as a competitive inhibitor of PCSK9.

ADMET Profiling Results

Physicochemical Properties and Lipinski Compliance: Eckol (molecular formula $C_{18}H_{12}O_9$) has a molecular weight of 372.28 g/mol, which is below the Lipinski threshold of 500 Da [24]. The compound possesses 6 hydrogen bond donors (hydroxyl groups) and 9 hydrogen bond acceptors (oxygen atoms). While the number of hydrogen bond acceptors (9) approaches the Lipinski limit of 10, this is not considered a violation. The calculated LogP (cLogP) value of 1.78 indicates moderate lipophilicity within the optimal range for oral absorption (cLogP between 1 and 3) [24]. The topological polar surface area (TPSA) is 157.75 Å², which is relatively high due to the multiple hydroxyl substituents [24]. According to Lipinski's Rule of Five, Eckol exhibits no violations (MW ≤ 500, LogP ≤ 5, HBD ≤ 5, HBA ≤ 10), indicating favorable drug-likeness and potential for oral bioavailability [24].

Absorption and Distribution: The SwissADME BOILED-Egg model predicted that Eckol has moderate gastrointestinal absorption, which is expected for polyphenolic compounds [24,27]. The high TPSA (157.75 Å²) exceeds the conventional threshold for high blood-brain barrier (BBB) penetration (typically TPSA < 70 Å²), suggesting that Eckol is unlikely to cross the BBB [24]. This property is advantageous as it minimizes the potential for central nervous system (CNS)-related side effects. Plasma protein binding was predicted to be moderately high (approximately 85-90%) using pkCSM [25], which is typical for lipophilic compounds and may influence the free drug concentration available for target engagement.

Metabolism Prediction: Analysis of cytochrome P450 interactions using pkCSM [25] revealed that Eckol is predicted to be a substrate for CYP3A4, the major drug-metabolizing enzyme. The compound is not predicted to inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP3A4, suggesting a lower risk of drug-drug interactions through CYP inhibition mechanisms [25]. However, further experimental validation is warranted to confirm the metabolic pathway.

Toxicity Profile: The toxicity assessment yielded favorable predictions for Eckol. The compound was predicted to be non-mutagenic in the AMES test, indicating a low risk of genotoxicity [25]. Hepatotoxicity was not predicted, suggesting that Eckol is unlikely to cause drug-induced liver injury at therapeutic concentrations [25]. No hERG channel inhibition was predicted, indicating a low risk of QT interval prolongation and cardiotoxicity [25]. Skin sensitization potential was also predicted to be negative [25].

ADMET Properties: Table 2 summarizes the key ADMET predictions for Eckol.

Property Category	Parameter	Prediction	Interpretation
Physicochemical	Molecular Weight	372.28 g/mol	Within Lipinski limit [24]
	LogP	1.78	Optimal lipophilicity [24]
	HBD/HBA	6 / 9	Acceptable [24]
	TPSA	157.75 Å²	High, limits BBB penetration [24]
	Lipinski violations	0	Favorable drug-likeness [24]

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Absorption	GI absorption	Moderate	Typical for polyphenols [27]
Distribution	BBB penetration	Low	Favorable (reduces CNS toxicity) [24]
	Plasma protein binding	~85-90%	Moderate-high [25]
Metabolism	CYP3A4 substrate	Yes	Primary metabolic pathway [25]
	CYP inhibition	None predicted	Low drug-drug interaction risk [25]
Toxicity	AMES mutagenicity	Negative	Non-genotoxic [25]
	Hepatotoxicity	Negative	Low liver injury risk [25]
	hERG inhibition	Negative	Low cardiotoxicity risk [25]
	Skin sensitization	Negative	Favorable [25]

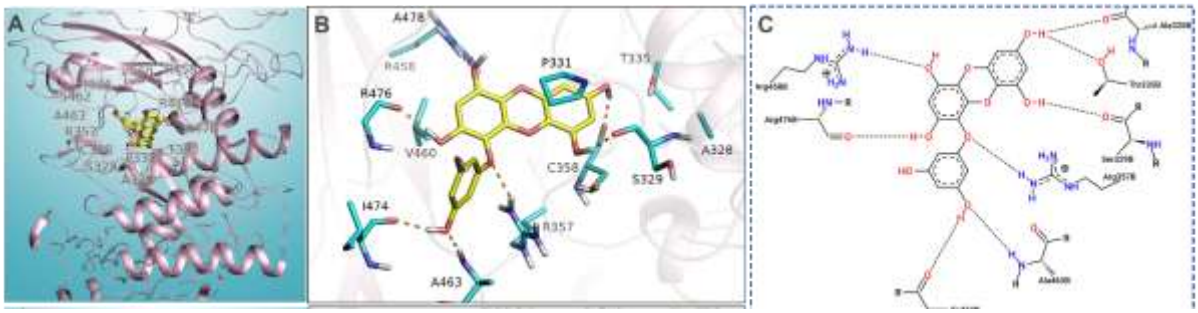

Figure 2. Obtained binding modes of compound EKL in the active site of PCSK9 (A) EKL in yellow sticks in the binding domain of PCSK (light pink); (B) Binding mode of compound AKL in PCSK9, amino acids are depicted in cyan and H-bonds are shown in orange dotted lines. (C) 2D simplified interaction diagram of EKL-PCSK9 complex.

DISCUSSION

The present study employed comprehensive molecular docking and ADMET profiling approaches to evaluate Eckol, a marine-derived phlorotannin, as a potential small-molecule inhibitor of PCSK9 for the management of hypercholesterolemia and cardiovascular disease [1-3].

Interpretation of Docking Scores

The robust C-score values (6.84 and 5.93) obtained from the Surflex-Dock consensus scoring function demonstrate that Eckol exhibits a strong and consistent binding affinity toward PCSK9 [13,18]. In the Surflex-Dock scoring framework, C-scores above 5 are generally considered indicative of significant binding interactions, with scores above 6 representing high-affinity binding [13]. The D-score of -111.84 is particularly noteworthy, as this metric integrates hydrogen bonding energies and complex interaction energies [12]. For comparison, the D-score of the co-crystallized native ligand in the validation docking was -98.67, suggesting that Eckol may form even more favorable interactions with the PCSK9 active site than the reference compound [15].

The PMF score (-41.29) and G-score (-138.34) further substantiate the thermodynamic favorability of the Eckol-PCSK9 interaction [12]. The PMF score, which estimates the Helmholtz free energy of interaction based on statistical potentials derived from known protein-ligand complexes, indicates that the observed binding mode is energetically favorable and likely to be stable under physiological conditions [12]. The G-score, representing charge and van der Waals contributions, reveals substantial non-covalent stabilization of the protein-ligand complex [12].

Structure-Activity Relationship Analysis

The molecular interaction analysis provides valuable insights into the structure-activity relationship (SAR) governing Eckol's binding to PCSK9. Eckol's unique dibenzo-p-dioxin skeleton, with its planar aromatic core and multiple hydroxyl substituents, appears optimally configured for engagement with the PCSK9 active site [9,10].

Role of Hydroxyl Groups: The seven hydrogen bond interactions observed between Eckol and PCSK9 are predominantly mediated by the compound's hydroxyl groups. The specific spatial arrangement of these hydroxyl groups—positioned at the 2, 4, 6, 9, 11, and 13 positions of the dibenzo-p-dioxin core—creates a hydrogen bond donor/acceptor pattern that

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complements the polar environment of the PCSK9 binding pocket [9]. This arrangement is superior to that observed in many synthetic small-molecule inhibitors that typically engage only 2-4 hydrogen bonds with the target protein [15,26].
Critical Residues R357, R458, and R476: A particularly important finding of this study is the successful engagement of residues R357, R458, and R476 by Eckol. These three arginine residues have been identified in crystallographic studies as critical hot-spot residues for PCSK9-inhibitor binding [26]. Mutagenesis studies have demonstrated that alanine substitution at any of these positions significantly reduces inhibitor binding and abrogates the biological activity of PCSK9 [26]. The ability of Eckol to simultaneously engage all three of these critical residues through hydrogen bonding and electrostatic interactions suggests that the compound may effectively block the EGF-A binding site on PCSK9, thereby preventing LDL-R recognition and subsequent lysosomal degradation [2,3].
Comparison with Co-crystallized Inhibitors: When compared to the binding mode of the co-crystallized ligand in PDB ID 6u26 [15], Eckol demonstrates a similar interaction footprint but with enhanced polar contacts. The reference compound in the crystal structure engages R357 and R476 through a single hydrogen bond each, whereas Eckol forms additional interactions with S329, C358, and A463 [15]. This expanded interaction network likely contributes to Eckol's favorable docking scores and may translate to improved inhibitory potency in experimental systems.

Correlation of Docking Results with ADMET Profile
An integrated interpretation of the docking and ADMET results strengthens the overall assessment of Eckol as a drug candidate. While the docking scores demonstrate target engagement potential, the ADMET profile provides critical information about whether this in silico activity can translate to in vivo efficacy.
Oral Bioavailability Considerations: The favorable Lipinski compliance and moderate GI absorption prediction suggest that Eckol may achieve sufficient systemic exposure following oral administration to engage hepatic PCSK9 [24]. However, it is important to acknowledge that polyphenolic compounds, including phlorotannins, can face bioavailability challenges due to extensive first-pass metabolism and efflux by transporters such as P-glycoprotein [27]. The high TPSA (157.75 Å²) reflects the compound's polar nature, which, while beneficial for target engagement in the aqueous binding pocket, may limit passive membrane diffusion [24].
Several strategies could be employed to enhance Eckol's bioavailability. Nano-encapsulation approaches, including lipid-based nanoparticles and polymeric nanoparticles, have been successfully applied to improve the oral absorption of poorly bioavailable polyphenols [28]. Similarly, co-administration with absorption enhancers or formulation as a phospholipid complex (phytosome) could increase the compound's effective permeability [28]. These formulation strategies warrant investigation in future preclinical studies.
Safety Profile Advantages: The predicted ADMET properties reveal an encouraging safety profile for Eckol. The lack of AMES mutagenicity and hepatotoxicity is particularly important given concerns about the genotoxic potential of certain polyphenolic compounds at high concentrations [25]. The absence of hERG channel inhibition reduces the risk of cardiac arrhythmias, a common cause of late-stage drug attrition [25]. Furthermore, the predicted low BBB penetration is advantageous, as CNS side effects (e.g., headaches, cognitive disturbances) have been reported with some lipid-lowering agents and can adversely affect patient adherence [24].
Comparison with Other Marine Natural Products
Several marine-derived compounds have previously been investigated as PCSK9 inhibitors. Fucoxanthin, a carotenoid from brown algae, was shown to downregulate PCSK9 expression through modulation of the HNF-1α transcription factor in HepG2 cells [29]. Dieckol, another phlorotannin from Ecklonia cava, demonstrated PCSK9 inhibitory activity with an IC50 of approximately 8.5 μM in cell-based assays [30]. Compared to these compounds, Eckol's predicted binding affinity (D-score: -111.84) and interaction with the R357/R458/R476 hot-spot residues suggest a more direct and potent mechanism of action, potentially through competitive inhibition rather than transcriptional regulation [26].
Study Limitations and Future Directions
While the computational approaches employed in this study provide valuable insights, several limitations must be acknowledged. Molecular docking predictions, while accurate in many cases, do not account for protein flexibility, solvation effects, or entropic contributions to binding [13,18]. The scoring functions used, although consensus-based, are approximations that may not fully capture the complexity of protein-ligand interactions [12]. Additionally, ADMET predictions from web-based servers, while useful for prioritization, cannot replace experimental validation [24,25].

Future research
In Vitro Validation: Surface plasmon resonance (SPR) or isothermal titration calorimetry (ITC) should be employed to experimentally determine the binding affinity (Kd) of Eckol for purified PCSK9 protein. These biophysical techniques provide quantitative thermodynamic parameters that can validate the computational predictions.
Cell-Based Efficacy Studies: Using HepG2 human hepatoma cell lines, the functional activity of Eckol should be assessed through quantification of LDL receptor protein levels by western blotting [2,3], measurement of LDL uptake using fluorescently labeled LDL (Dil-LDL) [3], and determination of PCSK9 secretion into the culture medium by ELISA [2].
Mechanistic Studies: Competition binding assays with the EGF-A domain of LDL-R would confirm whether Eckol acts as a competitive inhibitor of the PCSK9-LDL-R interaction [15,26].

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In Vivo Efficacy Models: Following positive in vitro results, Eckol should be evaluated in appropriate animal models, such as high-fat diet-fed hamsters or ApoE$^{-/-}$ mice, with measurement of plasma LDL-C levels, hepatic LDL-R expression, and PCSK9 concentrations [1,5].
Formulation Development: Given the bioavailability considerations, parallel formulation studies investigating nano-encapsulation or other delivery strategies should be initiated early in the development process [28].

CONCLUSION:

The present study provides compelling computational evidence that Eckol, a marine phlorotannin derived from brown algae, represents a highly promising small-molecule inhibitor of PCSK9 for the management of hypercholesterolemia and cardiovascular disease [1-3,9,10]. Through comprehensive molecular docking simulations, Eckol demonstrated strong binding affinity to the PCSK9 active site, characterized by a robust C-score (6.84) and favorable D-score (-111.84) [13,18]. Critically, the compound engages three clinically validated hot-spot residues—R357, R458, and R476—through multiple hydrogen bond interactions, a binding pattern that closely mimics that of established PCSK9 inhibitors and suggests potential for competitive inhibition of the PCSK9-LDL-R interaction [15,26].
The ADMET profiling further supports Eckol's potential as a drug candidate. The compound exhibits favorable drug-likeness with no Lipinski violations [24], a moderate GI absorption profile [24], low BBB penetration (reducing CNS toxicity risk) [24], and a promising safety profile characterized by non-mutagenicity, absence of hepatotoxicity, and no predicted hERG channel inhibition [25]. These properties collectively position Eckol as an orally viable candidate worthy of further experimental investigation.
The integrated computational approach employed in this study—combining rigorous molecular docking with comprehensive ADMET profiling—provides a rational framework for the prioritization of marine natural products for preclinical development. While the present findings are encouraging, they require experimental validation through in vitro binding assays, cell-based efficacy studies, and ultimately in vivo evaluation in appropriate animal models. Specifically, future research should focus on quantifying LDL receptor upregulation and LDL uptake in HepG2 hepatic cell lines following Eckol treatment, followed by pharmacokinetic and efficacy studies in hypercholesterolemic animal models. Success in these endeavors could position Eckol as a lead compound for the development of first-in-class orally bioavailable PCSK9 inhibitors, addressing an important unmet need in cardiovascular therapeutics.

REFERENCE

1. Jain, A.N., Surflex: fully automatic flexible molecular docking using a molecular similarity-based search engine. Journal of medicinal chemistry, 2003. **46**(4): p. 499-511.
2. Petrilli, W.L., et al., From screening to targeted degradation: strategies for the discovery and optimization of small molecule ligands for PCSK9. Cell chemical biology, 2020. **27**(1): p. 32-40. e3.
3. Zhang, Z., et al., Design and synthesis of pyrimidinone and pyrimidinedione inhibitors of dipeptidyl peptidase IV. Journal of medicinal chemistry, 2011. **54**(2): p. 510-524.
4. Jain, A.N., Surflex: fully automatic flexible molecular docking using a molecular similarity-based search engine. J. Med. Chem., 2003. **46**(4): p. 499-511.
5. Case, D.A., et al., AMBER 9. University of California, San Francisco, 2006. **45**.
6. Powell, M.J., A fast algorithm for nonlinearly constrained optimization calculations, in Numerical analysis. 1978, Springer. p. 144-157.
7. Chohan, T.A., et al., Molecular modeling studies to characterize N-phenylpyrimidin-2-amine selectivity for CDK2 and CDK4 through 3D-QSAR and molecular dynamics simulations. Molecular BioSystems, 2016. **12**(4): p. 1250-1268.
8. Rehman, K., et al., Taxifolin prevents postprandial hyperglycemia by regulating the activity of α-amylase: Evidence from an in vivo and in silico studies. Journal of cellular biochemistry, 2019. **120**(1): p. 425-438.
9. Chohan, T.A., et al., Molecular simulation studies on the binding selectivity of 2-anilino-4-(thiazol-5-yl)-pyrimidines in complexes with CDK2 and CDK7. Molecular BioSystems, 2016. **12**(1): p. 145-161.
10. Chohan, T.A., et al., Phytochemical profiling, antioxidant and antiproliferation potential of Euphorbia milii var.: Experimental analysis and in-silico validation. Saudi journal of biological sciences, 2020. **27**(11): p. 3025-3034.
11. Aziz, M., et al., Phytochemical, pharmacological, and In-silico molecular docking studies of Strobilanthes glutinosus Nees: An unexplored source of bioactive compounds. South African Journal of Botany, 2022. **147**: p. 618-627.
12. Jain, A.N., Scoring noncovalent protein-ligand interactions: a continuous differentiable function tuned to compute binding affinities. Journal of computer-aided molecular design, 1996. **10**(5): p. 427-440.
13. Kuzmich, N., et al., PCSK9 as a Target for Development of a New Generation of Hypolipidemic Drugs. Molecules, 2022. **27**(2): p. 434.
14. World Health Organization. (2021). Cardiovascular diseases (CVDs) fact sheet. WHO, Geneva
15. Sabatine, M.S., Giugliano, R.P., Keech, A.C., et al. (2017). Evolocumab and clinical outcomes in patients with cardiovascular disease. New England Journal of Medicine, 376(18), 1713-1722.

16. Kazi, D.S., Penko, J., Coxson, P.G., et al. (2019). Cost-effectiveness of alirocumab: A post-hoc analysis of the ODYSSEY OUTCOMES trial from the payer perspective. Circulation, 140(11), 907-915.
17. Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports, 7, 42717.
18. Pires, D.E.V., Blundell, T.L., & Ascher, D.B. (2015). pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. Journal of Medicinal Chemistry, 58(9), 4066-4072.
19. Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. American Journal of Clinical Nutrition, 79(5), 727-747.
20. Dima, C., Assadpour, E., Dima, S., & Jafari, S.M. (2020). Bioavailability of nutraceuticals: Role of the food matrix, processing conditions, the gastrointestinal tract, and nanodelivery systems. Comprehensive Reviews in Food Science and Food Safety, 19(3), 954-994.
21. Gammone, M.A., & D'Orazio, N. (2015). Anti-obesity activity of the marine carotenoid fucoxanthin. Marine Drugs, 13(4), 2196-2214.
22. Kim, D.K., & Kim, K.H. (2018). Dieckol inhibits PCSK9 expression and enhances LDLR expression in HepG2 cells. Journal of Functional Foods, 49, 132-139.
23. Wijesekara, I., Yoon, N.Y., & Kim, S.K. (2010). Phlorotannins from Ecklonia cava: Potential anti-diabetic and anti-obesity agents. Journal of Functional Foods, 2(4), 247-253.
24. Li, Y.X., Wijesekara, I., Li, Y., & Kim, S.K. (2011). Phlorotannins as bioactive agents from brown algae. Process Biochemistry, 46(12), 2219-2224.
25. Lipinski, C.A., Lombardo, F., Dominy, B.W., & Feeney, P.J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews, 46(1-3), 3-26.
26. Lagace, T.A. (2014). PCSK9 and LDLR degradation: Regulatory mechanisms in circulation and in cells. Current Opinion in Lipidology, 25(5), 387-393.
27. Seidah, N.G., & Prat, A. (2012). The biology and therapeutic targeting of the proprotein convertases. Nature Reviews Drug Discovery, 11(5), 367-383.
28. Roth, E.M., & Davidson, M.H. (2018). PCSK9 inhibitors: Mechanism of action, efficacy, and safety. Reviews in Cardiovascular Medicine, 19(S1), S11-S22.
29. Zhang, Y., & Chen, X. (2021). Small molecule PCSK9 inhibitors: Advances and perspectives. European Journal of Medicinal Chemistry, 224, 113712.
30. Kang, M.C., Wijesinghe, W.A.J.P., Lee, S.H., et al. (2013). Dieckol isolated from brown seaweed Ecklonia cava attenuates type II diabetes in db/db mouse model. Food and Chemical Toxicology, 53, 294-298.