RESEARCH ARTICLE

Editorial Process: Submission:10/20/2025 Acceptance:04/16/2026 Published:05/19/2026

Selective Inhibition of VEGFR2 in Preference to Other Receptor Tyrosine Kinases by Diosgenin, a Natural Steroidal Sapogenin

Charmi Jyotishi[1], Mansi Patel[2], Suresh Prajapati[1], Reeshu Gupta[1,2]*

Abstract

Objective: This study aimed to assess the ability of diosgenin and its derivatives to suppress three angiogenic receptor tyrosine kinases-VEGFR2, FGFR1, and PDGFRA-through comprehensive in silico screening, molecular docking, and molecular dynamics simulations. Methods: We screened 1,525 plant-derived compounds, 20 sapogenins, and three diosgenin derivatives for drug-likeness and bioavailability using SwissADME. The top candidates were docked against the three receptor tyrosine kinases using PyRx. Diosgenin and 14 of its derivatives were then further analyzed. Molecular dynamics simulations were performed using NAMD3 with CHARMM force fields to assess the stability of the protein–ligand complexes. Parameters such as RMSD, RMSF, Rg, and ΔG were evaluated. Results: Diosgenin was among the top 10 hits for all three receptor tyrosine kinases. It showed the strongest binding and stable interactions with VEGFR2 (ΔG: –11.03 kcal/mol) compared to lenvatinib (ΔG: -7.52 kcal/mol) and sorafenib (ΔG: -4.01 kcal/mol). The FGFR1–diosgenin and PDGFRA-Diosgenin complexes displayed positive ΔG values, indicating less favorable thermodynamic binding. Three diosgenin derivatives (Formosanin C, Dioscin, and 2-Amin-5-(4-pyridyl)-1,3,4-thiadiazole DG-8d moiety) were also evaluated for their anticancer potential. While Formosanin C showed the highest binding affinities among all three derivatives, diosgenin uniquely interacted with the key catalytic residues of VEGFR2, suggesting functionally more relevant inhibition despite slightly lower docking scores. Similarly, Yamogenin, another sapogenin evaluated, exhibited a high binding affinity across all three angiogenic receptors. Although Yamogenin showed high affinity across all three receptors, molecular dynamics confirmed the superior stability of diosgenin with VEGFR2 (ΔG = –11.03 vs. –9.85 kcal/mol). Conclusion: Diosgenin represents a promising and selective VEGFR2 inhibitor with potential to have anti-angiogenic therapeutic activity.

Keywords: Diosgenin- Receptor tyrosine kinases- Lenvatinib- Sorafenib- Molecular Dynamics

Asian Pac J Cancer Prev, **27 (5)**, 1893-1904

Introduction

Angiogenesis, the formation of new blood vessels from pre-existing vasculature, plays a pivotal role in tumor growth, invasion, and metastasis. Several key RTKs, including the Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), Fibroblast Growth Factor Receptor 1 (FGFR1), and Platelet-Derived Growth Factor Receptor Alpha (PDGFRA), have been identified as central regulators of angiogenic signaling pathways. Dysregulation of these receptors has been implicated in the progression of various cancers, making them important therapeutic targets [1].

Multi-target tyrosine kinase inhibitors (TKIs), such as Lenvatinib and Sorafenib, have shown notable therapeutic effectiveness in targeting VEGFR2, FGFR1, and PDGFRA [2-5]. These agents are approved for use in advanced hepatocellular carcinoma (HCC), which is a leading cause of cancer-related mortality globally [6]. However, despite their initial benefits, acquired resistance to these TKIs remains a formidable barrier that limits long-term treatment success. Notably, resistance to Lenvatinib and Sorafenib has been reported in approximately 60% [7] and 70% [8] of HCC patients, respectively. The precise mechanisms underpinning this resistance are not fully elucidated, although they are likely multifactorial and include compensatory signaling via alternative pathways, receptor mutations, and microenvironmental factors [9]. This incomplete understanding of resistance pathways hinders the optimization of therapeutic regimens for HCC.

In this context, there is growing interest in exploring natural products, including plant-derived compounds and certain low-toxicity mycotoxins, which offer broader biological activity with lower toxicity profiles [10-12]. Natural products have been shown to be more effective in reducing HCC for several reasons, such as safety, minimal (or no) toxicity, fewer side effects, and better availability. Notable, 1/3rd FDA approved compounds

*[1]Parul Institute of Applied Sciences, Parul University, Post Limda, Waghodia Road, Vadodara, Gujarat, India. [2]Research and Development Cell, Parul University, Post Limda, Waghodia Road, Vadodara, Gujarat, India. *For Correspondence: reeshu.gupta25198@paruluniversity.ac.in*

are natural products and their derivatives [13]. Recent studies have demonstrated that many compounds from Traditional Chinese Medicine (TCM) are effective in the treatment of HCC (5, 6) and mouse mammary tumor models (7). Therefore, identifying natural compounds that can interact with multiple kinases offers a significant therapeutic advantage by potentially overcoming such resistance mechanisms.

Diosgenin, a steroidal sapogenin, is a major bioactive compound found in Dioscorea villosa, Dioscorea oppositifolia, Costus speciosus, and Trillium species [14-16]. Diosgenin is also a major component of the Fuzheng Jiedu Xiaoji (FZJDXJ) formulation, which has demonstrated efficacy in alleviating clinical symptoms in HCC patients and inhibiting tumor growth [17]. The anti-tumor effects of diosgenin are attributed to its involvement in multiple mechanisms with actions on different pathways such as mitogen-activated protein kinase (MAPK)/ERK pathway, phosphatidylinositol-3 kinase (PI3K)/Akt, nuclear factor kappa B (NF-κB)/STAT3 pathway, Wnt/β-catenin signal pathway, mitochondria/caspase-3-dependent pathway, and cAMP/PKA/CREB pathway [18, 19]. Notably, these signaling cascades are frequently dysregulated in HCC and other malignancies, contributing to enhanced proliferation, angiogenesis, immune evasion, and resistance to apoptosis [20, 21]. By targeting these critical pathways, diosgenin has the potential to interfere with multiple hallmarks of tumor progression, offering a multifaceted and promising therapeutic strategy for cancer treatment.

Given the complexity and redundancy of angiogenic signaling networks, targeting multiple angiogenic RTKs simultaneously is likely to yield more robust and sustained therapeutic responses than single-target inhibition. In this study, we adopted an integrative computational approach to identify potential multi-target inhibitors from a library of phytochemicals and their derivatives. Molecular docking, pharmacokinetic profiling (ADME), and molecular dynamics (MD) simulations were employed to comprehensively assess the binding affinities, structural stability, and drug-likeness of these compounds against VEGFR2, FGFR1, and PDGFRA. The goal was to evaluate whether phytochemicals could serve as viable, naturally derived alternatives or adjuncts to currently approved multi-target TKIs in the treatment of angiogenesis-driven malignancies such as HCC.

Materials and Methods

Acquisition and Preparation of Receptor Tyrosine Kinases and phytochemicals

The three-dimensional crystal structures of the target receptor tyrosine kinases (RTKs) were retrieved from the RCSB Protein Data Bank: VEGFR2 in complex with Lenvatinib (PDB ID: 3WZD) [22] and Sorafenib (3WZE) [23], FGFR1 in complex with Lenvatinib (PDB ID: 5ZV2) [24] and the wild-type kinase domain of PDGFRA with Lenvatinib (PDB ID: 8PQJ) [25]. Protein structures were prepared using UCSF Chimera by removing co-crystallized ligands, including drugs and water molecules (https://www.cgl.ucsf.edu/chimera/), adding polar hydrogen, and assigning Kollman charges. The cleaned structures were saved in the PDB format. No plant materials or chemical extracts were collected for this study. Additionally, the chemical structures of the top 20 phytochemicals, diosgenin derivatives, sorafenib, and sapogenins were downloaded in SDF format from PubChem for subsequent molecular docking with RTKs using PyRx (https://pyrx.sourceforge.io/). The atomic coordinates were converted to PDB format using Open Babel (an open-source chemical toolbox integrated into PyRx that allows conversion between various molecular file formats and basic structure editing). The research was performed solely through computational modeling.

Phytochemical Library Compilation and Drug-Likeness Evaluation

A curated library of 1,525 phytochemicals, primarily plant-derived secondary metabolites, was obtained in the SDF format and prepared for virtual screening. The drug-likeness and physicochemical profiles of each compound were evaluated using the SwissADME web server. The assessed parameters included molecular weight, hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), molecular formula, and oral bioavailability score. The compounds were filtered based on established drug-likeness criteria, including Lipinski's Rule of Five, which assesses molecular properties predictive of good oral bioavailability. Additional filters, such as the Veber and Egan rules, were applied to evaluate molecular flexibility and polarity. To further assess pharmacokinetic suitability, predictions of P-glycoprotein (P-gp) substrate status were considered, as P-gp can influence drug efflux and absorption. Moreover, the compounds were screened for their potential to inhibit major cytochrome P450 (CYP450) enzymes, which are critical for drug metabolism. These combined parameters helped identify compounds with optimal physicochemical and ADME (absorption, distribution, metabolism, and excretion) profiles for further evaluation. Compounds meeting the following criteria were considered acceptable: molecular weight ≤500 g/mol, HBA ≤10, HBD ≤5, non-substrate to P-gp, and minimal CYP inhibition (not inhibiting more than one of the five major CYP isoforms). A bioavailability score ≥0.55 was considered favorable. Rule violations were scored as 0 (no violation) or 1 (single violation) to prioritize the most promising candidates.

Molecular Docking and Hydrogen Bond Analysis

Virtual screening was conducted using PyRx v0.9.7, which integrates the AutoDock Vina algorithm for structure-based molecular-docking. Each phytochemical was docked against VEGFR2, FGFR1, and PDGFRA. For molecular docking, the target site of each protein was defined using specific grid box coordinates to ensure accurate ligand placement and interaction analysis. For VEGFR2, the grid box was centered at X = 8.644, Y = –0.693, and Z = 17.069. For FGFR1, the coordinates were set to X = 12.709, Y = –24.551, Z = 12.478, whereas for PDGFRA, the grid box was positioned at X = 23.564, Y = 9.233, Z = 18.935. CASTp 3.0 was used to identify the binding pockets of Lenvatinib with VEGFR2 (available

online at http://cast.engr.uic.edu). These dimensions were selected to encompass the active binding pockets of each receptor, such as for VEGFR2 (KDR): LEU-840, GLY-841, VAL-848, ALA-866, VAL-867, LYS-868, GLU-885, ILE-888, LEU-889, VAL-899, VAL-914, GLU-917, PHE-918, CYS-919, LYS-920, GLY-922, ASN-923, ARG-1032, ASN-1033, LEU-1035, CYS-1045, ASP-1046, PHE-1047, LEU-1049, for FDGF-1: LEU-484, GLY-485, GLU-486, GLY-487, ALA-488,PHE-489, GLY-490, LYS-514, GLU-562, TYR-563, ALA-564, ASN- 568, ASP-623, ARG-627, ASP-641 and for PDGFRA: ASP-599, GLY-625, CYS-627, GLU-658, ASN-674, CYS-675, TYR-676, PHE-677, LEU-680, ALA-681, GLU-825, GLU=835, PHE-837 allowing optimal docking of the phytochemical compounds. Compounds with the lowest (most negative) binding affinities were selected for further analysis. Hydrogen bond interactions were visualized and identified using PyMOL, an open-source 3D molecular visualization tool (https://www.pymol.org/).

Molecular Dynamics Simulation and Free Energy Calculations

To evaluate the dynamic behavior and stability of the top-twenty ligand-protein complexes, molecular dynamics (MD) simulations were performed using NAMD v3 with the CHARMM force field, as described previously by our group [26]. Each complex was simulated for 50 ns under periodic boundary conditions. The structural stability of the protein–ligand complexes was evaluated using three key parameters: Root Mean Square Deviation (RMSD) to monitor overall conformational changes during the simulation, Root Mean Square Fluctuation (RMSF) to assess residue-level flexibility, and Radius of Gyration (Rg) to determine the compactness of the protein structures over time. These parameters were calculated and visualized using the Bio3D v2.3-0 package (http://thegrantlab.org/bio). In addition, the binding free energy (ΔG) of each ligand–protein complex was estimated using the NAMD Energy Plugin, providing insight into the thermodynamic favourability of ligand binding (https://www.ks.uiuc.edu/Research/vmd/plugins/namdplot/).

Results

Selection of Top Candidates via Docking Studies

Of the 1,525 phytochemicals screened, 258 displayed drug-like properties, indicating their potential as drug candidates. All selected compounds showed a bioavailability score of 0.55, which is considered a favorable threshold for oral bioavailability. A value of ≥ 0.55 suggests that the compound meets the essential criteria for physicochemical and pharmacokinetic behavior associated with oral drugs, including acceptable lipophilicity, solubility, molecular size, and permeability.

The top 20 phytochemicals were selected based on their binding affinities (Figure 1) and significant hydrogen bond interactions with VEGFR2 (Table 1), FGFR1 (Table 2), and PDGFRA (Table 3). Lenvatinib and Sorafenib was used as a reference compound to compare binding interactions and docking poses. A visual analysis of the docking results was performed to evaluate the binding affinity of each compound relative to both Lenvatinib and Sorafenib. Based on this evaluation, the twenty most promising phytochemicals were shortlisted. Among all the screened compounds, diosgenin uniquely demonstrated top-ranking binding affinities across VEGFR2, FGFR1, and PDGFRA, making it the most promising multi-target inhibitor. Other phytochemicals, including Caloxanthone A (active against VEGFR2 and FGFR1), G-Mangostin (VEGFR2 and PDGFRA), and Lutein (VEGFR2, FGFR1, and PDGFRA), also showed interactions with two or more targets (Table 1-3). However, only diosgenin was consistently ranked within the top ten across all three proteins, justifying its prioritization for

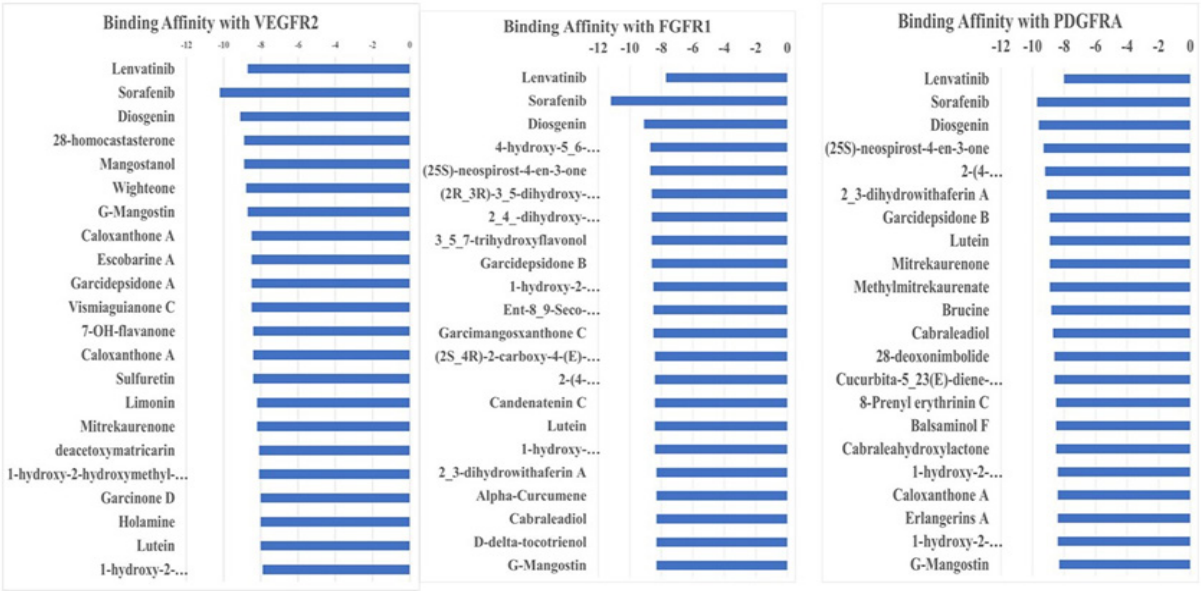

Figure 1. Binding Affinity Profiles of the Top Phytochemical Compounds against Three Angiogenic Receptor Tyrosine Kinases. Horizontal bar graphs show the molecular docking binding affinities (kcal/mol) of top 20 phytochmicals, compared against the reference tyrosine kinase inhibitors Lenvatinib and Sorafenib.

Table 1. List of Top 20 Phytochemical Inhibitors Demonstrating Binding Affinity Scores with VEGFR2 and Hydrogen Bond Interactions

Phytochemical inhibitors	Binding affinity	Hydrogen bonds
Lenvatinib	-8.7	GLY-841, CYS-919
Sorafenib	-10.2	ASP-1046, GLU-885
Diosgenin	-9.1	GLY-841, LYS-868, VAL-848, LEU-840, LYS-920, ALA-866, CYS-919, CYS-1045, LEU-1035, ASN-923
28-homocastasterone	-8.9	GLY-841, GLU-917, LYS-920, CYS-919, PHE-918, ALA-866, VAL-916, VAL-899, LEU-1035, ARG-1032,LEU-840, LYS-838
Mangostanol	-8.9	GLU-917, GLY-841, LEU-840, VAL-916, PHE-918, CYS-919, ASN-900, LYS-1043
Wighteone	-8.8	ASN-923, CYS- 919, GLY-841, GLU-885
G-Mangostin	-8.7	ASP-1046, ASN-923, LYS- 868
Caloxanthone A	-8.5	GLY- 841, CYS-919, PHE-918
Escobarine A	-8.5	LYS- 868, CYS-919
Garcidepsidone A	-8.5	ASN-923
Vismiaguianone C	-8.5	CYS-919
7-OH-flavanone	-8.4	CYS-919
Caloxanthone A	-8.4	VAL-848, CYS-919
Sulfuretin	-8.4	ASP-1046, CYS- 919
Limonin	-8.2	ARG-863, THR-864, SER-1037
Mitrekaurenone	-8.2	LYS-868
deacetoxymatricarin	-8.1	GLY-841, CYS-919
1-hydroxy-2-hydroxymethyl-3-methoxyanthraquinone	-8.1	CYS-919
Garcinone D	-8	GLY-841, GLU-917
Holamine	-8	GLU-882, LYS-868
Lutein	-8	LYS-868
1-hydroxy-2-hydroxymethylanthraquinone	-7.9	CYS-919

further investigation.

Binding Affinity Comparison between Diosgenin and its derivatives

Additionally, three diosgenin derivatives reported in the literature for their anticancer potential were also evaluated [27-29]. The binding affinities of all three Diosgenin derivatives are presented in Figure 2. While Formosanin demonstrated stronger binding affinities across targets (VEGFR2: –9.6 kcal/mol, FGFR1: –9.6

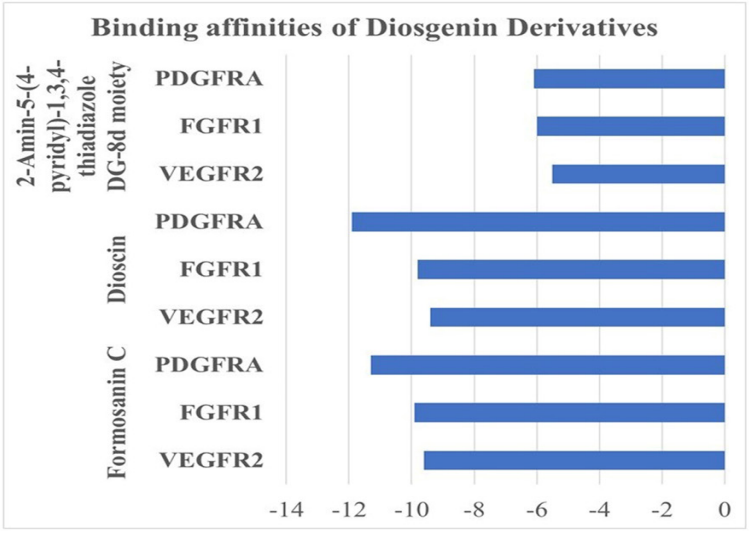

Figure 2. Molecular Docking–based Binding Affinities (kcal/mol) of Three Diosgenin Derivatives, 2-Amino-5(4-pyridyl)-1,3,4-thiadiazole DG-8d moiety, Dioscin, and Formosanin C, toward three angiogenic receptor tyrosine kinases.

Table 2. List of Top 20 Phytochemical Inhibitors Demonstrating Binding Affinity Scores with FGFR-1 and Hydrogen Bond Interactions

Phytochemical inhibitors	Binding affinity	Hydrogen bonds
Lenvatinib	-7.7	LEU-644, ARG-622, ARG-675
Sorafenib	-11.2	ASN-568
Diosgenin	-9.1	ARG-577, ASN-724, SER-723
4-hydroxy-5_6-dehydrokawain	-8.7	ALA-564
(25S)-neospirost-4-en-3-one	-8.7	ARG-577, ASP-720
(2R_3R)-3_5-dihydroxy-7-methoxy avanone	-8.6	ALA-564
2_4_-dihydroxy-4-methoxydihydrochalcone	-8.6	ALA-564
3_5_7-trihydroxyflavonol	-8.6	ALA-564
Garcidepsidone B	-8.6	
1-hydroxy-2-hydroxymethylanthraquinone	-8.5	ALA-5
Ent-8_9-Seco-7-alpha-acetoxykaura-8(14)_16-dien-9_15-dione	-8.5	LYS-514
Garcimangosxanthone C	-8.5	GLU-464, ASP-554, GLY-555
(2S_4R)-2-carboxy-4-(E)-p-coumaroyloxy-1_1-dimethylpyrrolidinium inner salt	-8.4	ASN-568, GLU-571
2-(4-Hydroxyphenyl)naphthalic anhydride	-8.4	GLU-562, ALA-564
Candenatenin C	-8.4	GLU-485, ALA564, LEU-630
Lutein	-8.4	ASN-568, ARG-627, ARG-622
1-hydroxy-2-hydroxymethyl-3-methoxyanthraquinone	-8.4	ALA-564, SER-565
2_3-dihydrowithaferin A	-8.3	ARG-577, LYS-721, SER-723
Alpha-Curcumene	-8.3	ALA-564
Cabraleadiol	-8.3	MET-515
D-delta-tocotrienol	-8.3	ALA-520, THR-521, GLU-522, LEU-465, LEU-525, TYR-463, LEU-557, LEU-516
G-Mangostin	-8.3	GLY-613

Table 3. List of Top 20 Phytochemical Inhibitors Demonstrating Binding Affinity Scores with PDGFRA and Hydrogen Bond Interactions

Phytochemical inhibitors	Binding affinity	Hydrogen bonds
Lenvatinib	-8	SER-972
Sorafenib	-9.7	SER-972, TYR-676, ASP-973
Diosgenin	-9.6	LEU-615, SER-972, PHE-969, LEU-661, GLU-675, MET-622, ASP-973, LYS-833, GLN-828
(25S)-neospirost-4-en-3-one	-9.3	LYS-833, GLU-675, MET-622, LEU-660, LEU-661, SER-616, LEU-615, TRP-586, PHE-969, SER-972
2-(4-Hydroxyphenyl)naphthalic anhydride	-9.2	LYS- 833, LYS-623, GLN-629
2_3-dihydrowithaferin A	-9.1	SER-961, LYS-779
Garcidepsidone B	-8.9	ILE-965, PHE-969
Lutein	-8.9	ARG-585, SER-972, LEU-615, TRP-586, LEU-661, PHE-969, GLU-675, LEU-782, LYS-964, ILE-965, LYS-779, SER-961
Mitrekaurenone	-8.9	TYR- 676
Methylmitrekaurenate	-8.9	TYR- 676
Brucine	-8.8	GLN-828
Cabraleadiol	-8.7	GLN-828
28-deoxonimbolide	-8.6	ARG-558, THR-855
Cucurbita-5_23(E)-diene-3-beta_7-beta_25-triol	-8.6	GLU-675
8-Prenyl erythrinin C	-8.5	GLN-828
Balsaminol F	-8.5	LYS-833, GLU-675, GLN-828, LEU-782
Cabraleahydroxylactone	-8.5	LEU-661
1-hydroxy-2-hydroxymethylanthraquinone	-8.4	GLU-556, ARG-554, ASP-836, THR-855
Caloxanthone A	-8.4	SER-854
Erlangerins A	-8.4	SER-972, GLN-619, LYS-833
1-hydroxy-2-hydroxymethyl-3-methoxyanthraquinone	-8.4	TYR-676

Table 4. Molecular Docking Analysis of Diosgenin Derivatives: Binding Affinities and Hydrogen Bond Interactions with Target Proteins

SN	Diosgenin derivative	RTIs	Binding affinity	Hydrogen bonds
1	Formosanin C	VEGFR2	-9.6	LYS-1043, GLU-917, HIS-894, LYS-1039, THR-864, ARG-863
		FGFR1	-9.9	LYS-510, LYS-638, ASN-546
		PDGFRA	-11.3	TYR-676
2	Dioscin	VEGFR2	-9.4	ARG-863, GLY-855, GLU-828
		FGFR1	-9.8	SER-723, THR-695, ARG-577
		PDGFRA	-11.9	SER-972, GLN-828
3	2-Amin-5-(4-pyridyl)-1,3,4-thiadiazole DG-8d moiety	VEGFR2	-5.5	GLU-917
		FGFR1	-6	ASP-614
		PDGFRA	-6.1	ASP-818, ASP-836, GLU-556

kcal/mol, PDGFRA: –11.3 kcal/mol), diosgenin showed moderately high affinities (VEGFR2: –9 .1 kcal/mol, FGFR1: –9.1 kcal/mol, PDGFRA: –9.6 kcal/mol). For VEGFR2, Diosgenin bound to crucial catalytic residues, which are part of the receptor's active site. Although Formosanin had a slightly better binding energy, it interacted mainly with peripheral residues such as LYS-1043, GLU-917, HIS-894, and THR-864, indicating that Diosgenin binding may be functionally more relevant for VEGFR2 inhibition. Notably, key FGFR1 active site residues include LEU-484 to PHE-489, GLY-490, and LYS-514, which were not directly contacted by Diosgenin, Dioscin or Formosanin, indicating all of three compounds may bind at an allosteric or extended site. This study demonstrated that Formosanin binds to the canonical active site residue Tyr-676 of PDGFRA, indicating its possible involvement in functional modulation. However, molecular dynamics simulations revealed a positive free binding energy of 0.192 kcal/mol, suggesting weak and possibly unstable interactions. In conclusion, diosgenin interacts with more canonical active site residues in VEGFR2, indicating possible functional inhibition (Table 4). Further in vitro validation is required to correlate these findings with biological activity.

Structural Stability of Diosgenin–Protein Complexes

The stability and compactness of the diosgenin-bound protein complexes (VEGFR2, FGFR1, and PDGFRA) were evaluated using Root Mean Square Deviation (RMSD), Radius of Gyration (Rg), and Root Mean Square Fluctuation (RMSF) (Figure 3; Table 5). Among the three complexes, FGFR1-Diosgenin displayed the lowest RMSD (2.81 Å), indicating greater structural stability over the simulation period. The VEGFR2-Diosgenin complex showed a moderate RMSD (3.16 Å), whereas PDGFRA-diosgenin exhibited a slightly higher deviation (3.54 Å), suggesting relatively less structural rigidity. The Rg values were consistent across all complexes, with

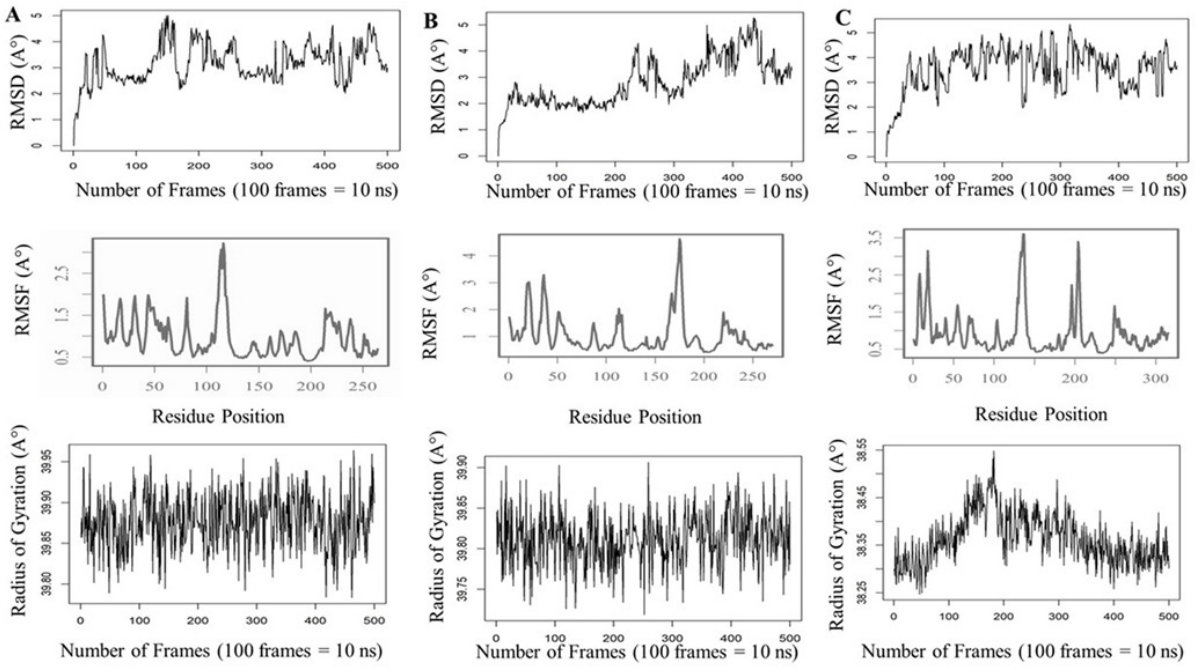

Figure 3. Molecular Dynamics Simulation Analysis Showing RMSD, RMSF, and Rg Profiles of Diosgenin Bound to (A) VEGFR2, (B) FGFR1, and (C) PDGFRA.

Table 5. Structural Stability Parameters (RMSD, Radius of Gyration, and RMSF) of Diosgenin and Lenvatinib with VEGFR2, FGFR1, and PDGFRA

Drug-RTKs complex	RMSD (A°)	RMSF (A°)	Rg (A°)
VEGFR2- Diosgenin	3.16± 0.032 (SEM)	1.14± 0.135(SEM)	39.87±0.001(SEM)
FGF-1- Diosgenin	2.81±0.040(SEM)	1.16±0.112(SEM)	39.81±0.001(SEM)
PDGFRA- Diosgenin	3.54±0.038(SEM)	1.05±0.13(SEM)	38.36±0.002(SEM)
VEGFR2- Lenvatinib	2.06 ± 0.020 (SEM)	1.22 ± 0.045 (SEM)	39.93 ± 0.002 (SEM)
FGF-1- Lenvatinib	2.35 ± 0.016 (SEM)	1.10 ± 0.042 (SEM)	39.82 ± 0.001 (SEM)
PDGFRA- Lenvatinib	1.82 ± 0.011 (SEM)	0.77 ± 0.026 (SEM)	38.33 ± 0.001 (SEM)
VEGFR2- Sorafenib	2.007±0.016(SEM)	1.13±0.051(SEM)	39.89±0.001(SEM)
FGF-1- Sorafenib	1.7±0.009(SEM)	1.02±0.035(SEM)	38.87±0.004(SEM)
PDGFRA- Sorafenib	1.36±0.08	0.75±0.023	38.35±0.02

minor variations, indicating stable overall compactness. Similarly, RMSF values were within an acceptable range (1.05–1.16 nm), indicating limited residue-level fluctuations and supporting the structural integrity of the protein-ligand complexes.

Comparative Stability and Binding Energy Analysis of Diosgenin and Reference TKIs

In the case of Lenvatinib and Sorafenib (reference drugs), the RMSD values across all three targets were lower (1.82–2.35 Å), indicating higher structural rigidity in the presence of these drugs (Figure 4-5; Table 5).

However, a contrasting trend was observed when evaluating the binding free energies (ΔG) (Supplementary Table 1). VEGFR2–Diosgenin exhibited a favorable ΔG of –11.03 kcal/mol, indicating a stable and spontaneous binding interaction. In contrast, the FGFR1–Diosgenin and PDGFRA–diosgenin complexes showed positive binding energies (+24.23 and +16.40 kcal/mol, respectively), suggesting thermodynamically unfavorable binding under the simulated conditions. For comparison, Lenvatinib and Sorafenib exhibited a free binding energy of –7.52 kcal/mol and -4.01 Kcal/mol, respectively, with VEGFR2. However, it was much higher (and even positive) for FGFR1 and PDFGRA, indicating inconsistencies or possibly different binding environments during the simulation (Supplementary Table 2). Despite their lower RMSD values, neither drug outperformed diosgenin in terms of binding energy, particularly against VEGFR2. This highlights Diosgenin's potential as a more energetically favorable multi-target inhibitor.

Diosgenin Outperforms Yamogenin in Stability and Binding Energy

In addition to diosgenin, 20 sapogenin compounds were screened to explore their potential as multi-target inhibitors of VEGFR2, FGFR1, and PDGFRA. The sapogenin with the highest binding affinity and significant hydrogen bond interactions with VEGFR2 (Supplementary Table 1), FGFR1 (Supplementary Table 3), and PDGFRA

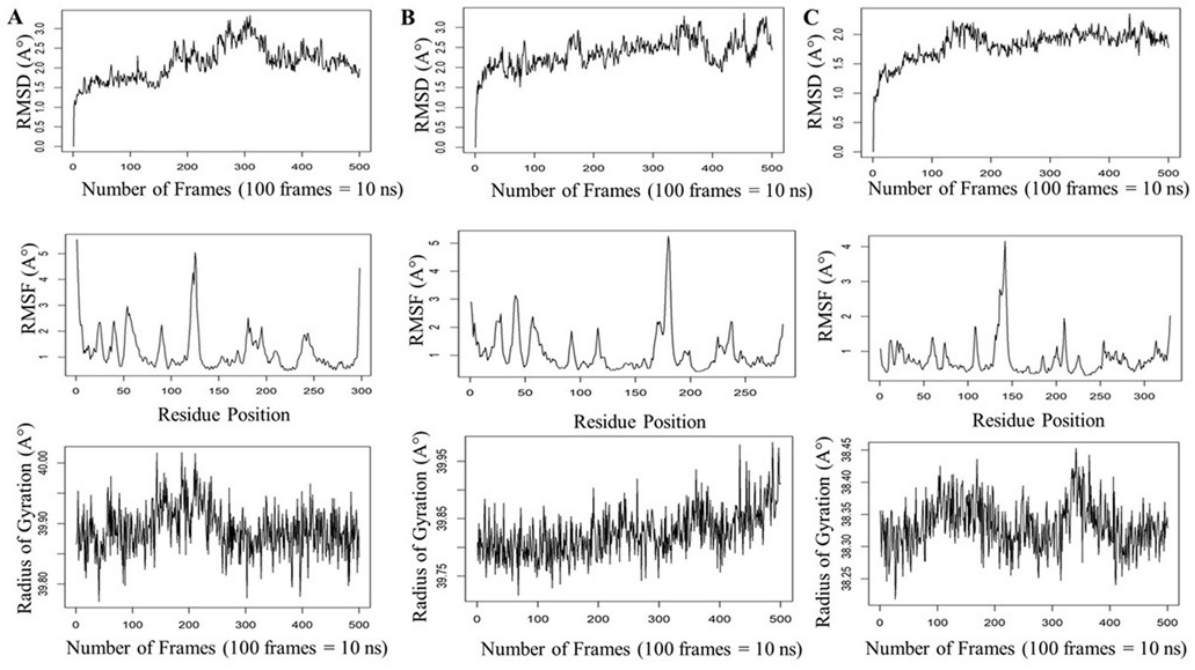

Figure 4. Molecular Dynamics Simulation Analysis showing RMSD, RMSF, and Rg Profiles of Lenvatinib Bound to (A) VEGFR2, (B) FGFR1, and (C) PDGFRA.

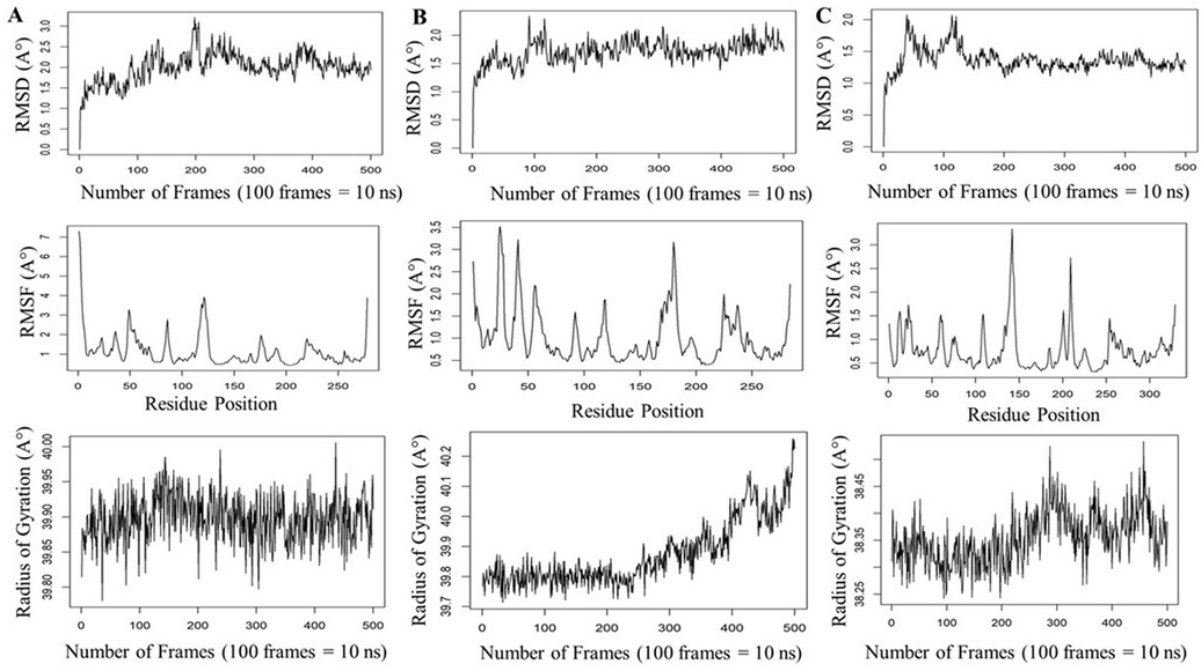

Figure 5. Molecular Dynamics Simulation Analysis Showing RMSD, RMSF, and Rg Profiles of Sorafenib Bound to (A) VEGFR2, (B) FGFR1, and (C) PDGFRA.

(Supplementary Table 4) was selected.

Yamogenin showed a high affinity for all three angiogenin receptors and was therefore subjected to free binding energy calculations and molecular dynamics simulations to evaluate its interaction stability and structural compactness. The RMSD, RMSF, and radius of gyration (Rg) profiles of the Yamogenin–protein complexes are presented in Figure 6. It showed slightly lower RMSD values than those of diosgenin when complexed with VEGFR2, suggesting greater stability. For instance, the VEGFR2-Yamogenin complex had an RMSD of 2.97 Å versus 3.16 Å for Diosgenin, while for FGF-1, Yamogenin exhibited 3.21 Å versus 2.81 Å for Diosgenin. In terms of binding energy, Diosgenin displayed stronger binding energy to VEGFR2 (ΔG = -11.03 kcal/mol) than Yamogenin (-9.85 kcal/mol), indicating a more stable complex in the case of Diosgenin (Supplementary Table 5). This increased stability could be explained by the optimal conformation of diosgenin, more profound insertion of a hydrophobic pocket, and more

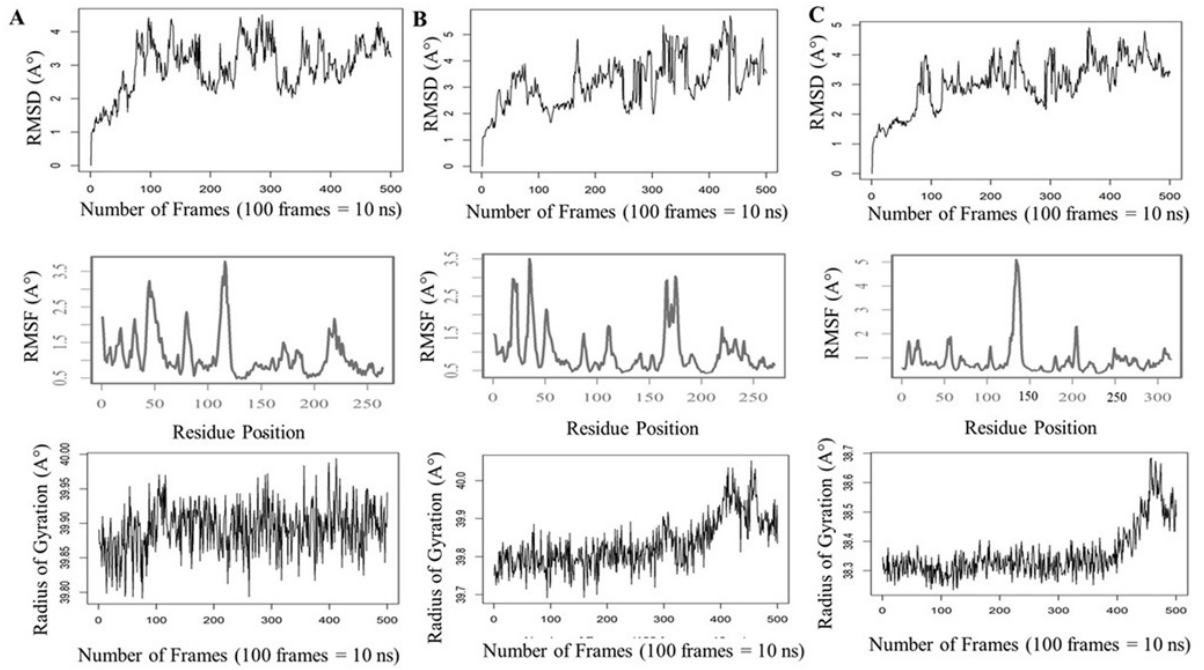

Figure 6. Molecular Dynamics Simulation Analysis Showing RMSD, RMSF, and Rg Profiles of Yamogenin Bound to (A) VEGFR2, (B) FGFR1, and (C) PDGFRA.

stable hydrogen bonding with catalytic residues in the active site of VEGFR2. These structural characteristics are likely responsible for the reduced fluctuations during MD simulations and complementarity with the ATP-binding cleft. These mechanistic observations underlie the greater binding capacity of diosgenin as a lead sapogenin-based angiogenic RTK inhibitor.

Discussion

The current study investigated the potential of diosgenin as potential VEGFR2 inhibitor. VEGFR2 is central to the angiogenic signaling pathways that sustain tumor growth, invasiveness, and metastasis [1]. VEGFR-2 is overexpressed in a wide range of malignant tumors and plays a critical role in tumor initiation, progression, growth, and development of drug resistance. To date, the U.S. The FDA has approved nine VEGFR-2 inhibitors for clinical use as anticancer agents [30]. However, the therapeutic benefits of these inhibitors are often limited by their suboptimal efficacy and associated toxicities. Therefore, there is a growing need to explore alternative approaches to enhance the clinical performance and safety profiles of VEGFR-2-targeted therapies. These strategies may circumvent the compensatory mechanisms often responsible for the therapeutic resistance observed with TKIs such as Sorafenib and Lenvatinib [1]. In the initial phase, 1,525 phytochemicals were screened, of which 278 were identified with favorable drug-like properties, including a bioavailability score ≥0.55. Among these, Diosgenin emerged as a prime candidate, satisfying Lipinski, Veber, and Egan filters, and showing no inhibitory interactions with cytochrome P450 enzymes or P-gp efflux transporters. These properties indicate a good pharmacokinetic profile, supporting its potential as an orally available therapeutic compound.

Although Diosgenin has been shown to suppress angiogenesis in various cancers by downregulating VEGF, FGF, MMP-2, and MMP-9 at the expression level, its direct molecular interaction with VEGFR2 or other angiogenic RTKs has not been well characterized in previous molecular docking studies. Thus, while functional studies support its anti-angiogenic effects, the binding specificity of diosgenin toward VEGFR2 remains to be elucidated at the structural level [18, 31, 32, 33]. The current study demonstrated that diosgenin showed a notably favorable ΔG of –11.03 kcal/mol with VEGFR2, suggesting a strong binding interaction with the VEGFR2 active site. Conversely, Diosgenin complexes with FGFR1 and PDGFRA yielded positive ΔG values, implying less thermodynamically favorable binding. These differences may stem from variations in receptor-binding site environments or limitations in docking precision, such as rigid protein conformations or solvent exclusion. To the best of our knowledge, no study has comprehensively evaluated the molecular interactions of Diosgenin with PDGFRA or FGFR1, either in silico or in vitro, making this multi-target comparative analysis a novel contribution.

Several diosgenin derivatives, including (25R)-5, benzoic acid mustard hybrids, and fluorophore-appended analogues, have demonstrated potent anti-HCC effects by inducing apoptosis, autophagy, and cell cycle arrest via pathways such as p38α MAPK and ER stress [18, 32, 34, 35]. Notably, certain derivatives, such as L-isoleucine-conjugated analogues, have exhibited pro-angiogenic and neuroprotective effects in non-cancer models, including SH-SY5Y cells and the CAM assay. The current study observed that Formosanin exhibited stronger overall binding compared to Diosgenin; however, Diosgenin interacted with key catalytic residues of VEGFR2, indicating a potentially more functional mode of inhibition. In contrast, Formosanin mainly engaged peripheral residues, which may limit its inhibitory potential despite its higher affinity. Notably, Diosgenin outperformed its known derivatives, which exhibited weaker binding and less consistent interactions across targets. These findings highlight that minor modifications in the steroidal backbone or attached groups may compromise essential interactions, possibly by introducing steric hindrance or reducing the number of hydrogen bond donor/acceptor sites [36]. Therefore, retaining the native diosgenin scaffold may be more favourable for drug development.

In addition to diosgenin, other sapogenins, such as Lanostane, Dammarane, Lupane, and Hederagenin, have demonstrated antitumor activities [37]. For example, sarsasapogenin induces a distinct dose- and time-dependent reduction in cell viability in HepG2 cells [38]. Therefore, a broader screening of sapogenins was undertaken to explore whether other compounds within the same phytochemical class could act as multi-target inhibitors. While some compounds showed promising docking scores against one or two targets, none demonstrated superior or comparable activity to diosgenin across all three angiogenic receptors. Additionally, when subjected to molecular dynamics and free energy calculations, these sapogenins did not surpass diosgenin in terms of binding energy with VEGFR2, reinforcing diosgenin's unique position as a lead candidate.

Taken together, these findings strongly advocate for further exploration of diosgenin as a potential anti-angiogenic agent targeting VEGFR2. Its strong binding affinity, conformational stability, and favourable pharmacokinetic properties suggest therapeutic relevance, particularly in HCC, where current first-line TKIs suffer from significant resistance. The documented involvement of diosgenin in regulating multiple cancer-related signaling pathways, such as PI3K/Akt, MAPK/ERK, and Wnt/β-catenin, further strengthens its candidacy [18, 32]. Moreover, given the high incidence of acquired resistance to Sorafenib and Lenvatinib in HCC, Diosgenin may serve as a complementary or alternative therapy. Its natural origin, safety profile, and multi-pathway engagement offer substantial benefits over synthetic single-target TKIs. This study underscores the importance of natural compounds in developing next-generation anti-angiogenic therapies and highlights diosgenin as a rational and promising lead molecule for further experimental validation.

The strength of this study lies in its multiparameter evaluation approach. Rather than relying solely on binding affinity or single-target docking, we integrated pharmacokinetic profiling, hydrogen bonding interaction

mapping, molecular dynamics simulation, and free-binding energy analysis. This layered framework provides a more nuanced understanding of ligand behavior, capturing not only the initial docking poses but also the dynamic and energetic feasibility of ligand retention within the binding pocket over time. Such a holistic assessment is essential for prioritizing candidates for downstream in vitro and in vivo validations. These findings have practical implications for drug discovery pipelines that focus on natural product libraries. This study reaffirms that nature-derived compounds can offer structurally diverse, biologically potent, and pharmacokinetically viable scaffolds for the inhibition of angiogenesis. Diosgenin, a known sapogenin found in several medicinal plants including Dioscorea species, fits this profile well and warrants further preclinical development. The development of advanced delivery systems, including nanoparticle-based formulations, and the facilitation of properly designed preclinical and clinical trials should also be among the priorities of future studies to assess the safety, pharmacokinetics, and efficacy of diosgenin [39, 40].

Although the current study presents good computational data that substantiate the use of diosgenin as a potential VEGFR2 inhibitor, there are several weaknesses that should be considered. Although the current study presents good computational data that substantiate the use of diosgenin as a potential VEGFR2 inhibitor, there are several weaknesses that should be considered. First, all observations are based on in silico studies, which, although predictive, are not capable of modeling intricate biological interactions; thus, they should be verified by experiments. Second, docking experiments are based on largely rigid protein structures and are not necessarily accurate representations of dynamic receptor conformations or solvent effects, which could affect the predicted affinities. Third, although MD simulations provide information about ligand stability, long-term conformational events may not be well represented by the simulation timescale. Lastly, despite the number of sapogenins/diosgenin derivatives screened, only a few were analyzed, and further exploration of the chemical space might uncover more promising candidates. These limitations highlight the need for comprehensive biochemical assays, kinase inhibition studies, and in vivo models to validate the anti-angiogenic potential of diosgenin.

In conclusion, overall, the evidence presented supports Diosgenin as a lead compound with promising VEGFR2 inhibitory potential. Its superior binding performance, dynamic stability, and favorable drug-likeness characteristics make it a viable candidate for future antiangiogenic drug development. Subsequent studies, including in vitro kinase inhibition assays, cell-based functional validation, and in vivo efficacy models, are necessary to confirm these computational insights and establish their translational relevance in oncology, specifically for HCC.

Author Contribution Statement

CJ: Conceptualization, methodology, writing original draft preparation, MP and SP: making tables, figures and literature search, RG: Supervision, editing the manuscript. All the authors have read and approved the manuscript.

Acknowledgements

None.

Funding

The study is supported by the intramural funding from Parul University (RDC/IMSL/213).

Data availability

Data is available on the reasonable request to the corresponding author.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Liu ZL, Chen HH, Zheng LL, Sun LP, Shi L. Angiogenic signaling pathways and anti-angiogenic therapy for cancer. Signal Transduct Target Ther. 2023;8(1):198. https://doi.org/10.1038/s41392-023-01460-1.
2. Chen X, Yang G, Guo X, Zhang J, Sun W, Liu D, et al. Dj-1/fgfr-1 signaling pathway contributes to sorafenib resistance in hepatocellular carcinoma. Oxid Med Cell Longev. 2022;2022:2543220. https://doi.org/10.1155/2022/2543220.
3. Babu M, Sadasivan S, TP A, Van Gelder T, Thomas S, Aravindkumar CT,et al. Therapeutic Drug Monitoring of Sorafenib and Lenvatinib in Hepatocellular Carcinoma Patients–A Key to Personalised Pharmacotherapy. F1000Research. 2024;13:1334.
4. Mou L, Tian X, Zhou B, Zhan Y, Chen J, Lu Y, et al. Improving outcomes of tyrosine kinase inhibitors in hepatocellular carcinoma: New data and ongoing trials. Front Oncol. 2021;11:752725. https://doi.org/10.3389/fonc.2021.752725.
5. Zhao B, Zhou Y, Cheng N, Zheng X, Chen G, Qi X, et al. Targeted inhibition of pdgfra with avapritinib, markedly enhances lenvatinib efficacy in hepatocellular carcinoma in vitro and in vivo: Clinical implications. J Exp Clin Cancer Res. 2025;44(1):139. https://doi.org/10.1186/s13046-025-03386-8.
6. Luo J, Gao B, Lin Z, Fan H, Ma W, Yu D, et al. Efficacy and safety of lenvatinib versus sorafenib in first-line treatment of advanced hepatocellular carcinoma: A meta-analysis. Front Oncol. 2022;12:1010726. https://doi.org/10.3389/fonc.2022.1010726.
7. Qin Y, Han S, Yu Y, Qi D, Ran M, Yang M, et al. Lenvatinib in hepatocellular carcinoma: Resistance mechanisms and strategies for improved efficacy. Liver Int. 2024;44(8):1808-31. https://doi.org/10.1111/liv.15953.
8. Cabral LKD, Tiribelli C, Sukowati CHC. Sorafenib resistance in hepatocellular carcinoma: The relevance of genetic heterogeneity. Cancers (Basel). 2020;12(6). https://doi.org/10.3390/cancers12061576.
9. Zou Y, Wan X, Zhou Q, Zhu G, Lin S, Tang Q, et al. Mechanisms of drug resistance in hepatocellular carcinoma. Biol Proced Online. 2025;27(1):19. https://doi.org/10.1186/s12575-025-00281-6.
10. Antonius Y, Kharisma VD, Widyananda MH, Ansori ANM,

Trinugroho JP, Ullah ME, et al. Prediction of aflatoxin-b1 (afb1) molecular mechanism network and interaction to oncoproteins growth factor in hepatocellular carcinoma. J Pure Appl Microbiol. 2022;16(3):1844-54. https://doi.org/10.22207/jpam.16.3.29
11. Aini NS, Ansori AN, Widyananda MH, Kharisma VD, Murtadlo AA, Herdiansyah MA,et al. Molecular Docking Analysis of Flavonoids from Syzygium cumini (L.) Skeels: Proapoptotic Potential as an Anticancer Mechanism. Borneo Journal of Pharmacy. 2025;8(3):252-62. https://doi.org/10.33084/bjop.v8i3.9843
12. Widyananda MH PS, Samoedra RS, Sari FN, Kharisma VD, Ansori ANM, Antonius Y. Molecular docking study of sea urchin (arbacia lixula) peptides as multi-target inhibitor for non-small cell lung cancer (nsclc) associated proteins. J Pharm Pharmacogn Res. 2021;9(4):484–96. https://doi.org/10.56499/jppres21.1047_9.4.484
13. Choi Y, Kim Y, Boo HJ, Yoon D, Cha JS, Yoo J. Natural product-derived drugs: Structural insights into their biological mechanisms. Biomolecules. 2025;15(9). https://doi.org/10.3390/biom15091303.
14. Pandey P, Ramniwas S, Baldaniya L, Lakhanpal S, Mahmood D, Chaudhary K, et al. An updated review of molecular mechanisms implicated with the anticancer potential of diosgenin and its nanoformulations. Drug Des Devel Ther. 2025;19:2205-27. https://doi.org/10.2147/DDDT.S502322.
15. Semwal P, Painuli S, Abu-Izneid T, Rauf A, Sharma A, Dastan SD, et al. Diosgenin: An updated pharmacological review and therapeutic perspectives. Oxid Med Cell Longev. 2022;2022:1035441. https://doi.org/10.1155/2022/1035441.
16. Kumari R, Thakur A, Thakur P, Sharma V, Sharma R, Upmanyu S, et al. An update on the nutritional and therapeutic potential of dioscorea oppositifolia. Food Sci Nutr. 2025;13(5):e70179. https://doi.org/10.1002/fsn3.70179.
17. Yang X, Feng Y, Liu Y, Ye X, Ji X, Sun L, et al. Fuzheng jiedu xiaoji formulation inhibits hepatocellular carcinoma progression in patients by targeting the akt/cyclind1/p21/p27 pathway. Phytomedicine. 2021;87:153575. https://doi.org/10.1016/j.phymed.2021.153575.
18. Ren QL, Wang Q, Zhang XQ, Wang M, Hu H, Tang JJ, et al. Anticancer activity of diosgenin and its molecular mechanism. Chin J Integr Med. 2023;29(8):738-49. https://doi.org/10.1007/s11655-023-3693-1.
19. Ge Y, Ding S, Feng J, Du J, Gu Z. Diosgenin inhibits wnt/beta-catenin pathway to regulate the proliferation and differentiation of mg-63 cells. Cytotechnology. 2021;73(2):169-78. https://doi.org/10.1007/s10616-021-00454-7.
20. Sun EJ, Wankell M, Palamuthusingam P, McFarlane C, Hebbard L. Targeting the pi3k/akt/mtor pathway in hepatocellular carcinoma. Biomedicines. 2021;9(11). https://doi.org/10.3390/biomedicines9111639.
21. Rascio F, Spadaccino F, Rocchetti MT, Castellano G, Stallone G, Netti GS, et al. The pathogenic role of pi3k/akt pathway in cancer onset and drug resistance: An updated review. Cancers (Basel). 2021;13(16). https://doi.org/10.3390/cancers13163949.
22. Fouad MA, Osman AA, Abdelhamid NM, Rashad MW, Nabawy AY, El Kerdawy AM. Discovery of dual kinase inhibitors targeting vegfr2 and fak: Structure-based pharmacophore modeling, virtual screening, and molecular docking studies. BMC Chem. 2024;18(1):29. https://doi.org/10.1186/s13065-024-01130-5.
23. Wang Z, Sun L, Xu Y, Huang J, Yang F, Chang Y. Discovery of novel vegfr2 inhibitors against non-small cell lung cancer based on fingerprint-enhanced graph attention convolutional network. J Transl Med. 2024;22(1):1097. https://doi.org/10.1186/s12967-024-05893-2.
24. Spahn S, Kleinhenz F, Shevchenko E, Stahl A, Rasen Y, Geisler C, et al. The molecular interaction pattern of lenvatinib enables inhibition of wild-type or kinase-mutated fgfr2-driven cholangiocarcinoma. Nat Commun. 2024;15(1):1287. https://doi.org/10.1038/s41467-024-45247-6.
25. Teuber A, Schulz T, Fletcher BS, Gontla R, Muhlenberg T, Zischinsky ML, et al. Avapritinib-based sar studies unveil a binding pocket in kit and pdgfra. Nat Commun. 2024;15(1):63. https://doi.org/10.1038/s41467-023-44376-8.
26. Patel MS, Pratik; Gandupalli, Lithip; Gupta, Reeshu. . Identification and evaluation of survival-associated common chemoresistant genes in cancer. Biomed Biotechnol Res J. 2024;8(3):320-7. https://doi.org/10.4103/bbrj.bbrj_227_24.
27. Dai YW, Wu ZX, Cheng Y, Wu HD, Chen JW, Lv LX, et al. Formosanin c inhibits triple-negative breast cancer progression by suppressing the phosphorylation of stat3 and the polarization of m2 macrophages. Breast Cancer Res Treat. 2025;211(1):71-89. https://doi.org/10.1007/s10549-025-07623-8.
28. Xie Y, Chen G. Dioscin induces ferroptosis and synergistic cytotoxicity with chemotherapeutics in melanoma cells. Biochem Biophys Res Commun. 2021;557:213-20. https://doi.org/10.1016/j.bbrc.2021.04.024.
29. Yatoo GN, Banday JA. Synthesis, antioxidant, antiproliferative activity, molecular docking and dft studies of novel isoxazole derivatives of diosgenin, a steroidal sapogenin from dioscorea deltoidea. Fitoterapia. 2023;170:105621. https://doi.org/10.1016/j.fitote.2023.105621.
30. Liu XJ, Zhao HC, Hou SJ, Zhang HJ, Cheng L, Yuan S, et al. Recent development of multi-target vegfr-2 inhibitors for the cancer therapy. Bioorg Chem. 2023;133:106425. https://doi.org/10.1016/j.bioorg.2023.106425.
31. Khanal P, Patil VS, Bhandare VV, Patil PP, Patil BM, Dwivedi PSR, et al. Systems and in vitro pharmacology profiling of diosgenin against breast cancer. Front Pharmacol. 2022;13:1052849. https://doi.org/10.3389/fphar.2022.1052849.
32. Arya P, Kumar P. Diosgenin a steroidal compound: An emerging way to cancer management. J Food Biochem. 2021;45(12):e14005. https://doi.org/10.1111/jfbc.14005.
33. Liao AM, Cai B, Huang JH, Hui M, Lee KK, Lee KY, et al. Synthesis, anticancer activity and potential application of diosgenin modified cancer chemotherapeutic agent cytarabine. Food Chem Toxicol. 2021;148:111920. https://doi.org/10.1016/j.fct.2020.111920.
34. Maimaitiming M, Lv L, Zhang X, Xia S, Li X, Wang P, et al. Semi-synthesis and biological evaluation of 25(r)-26-acetoxy-3beta,5alpha-dihydroxycholest-6-one. Mar Drugs. 2023;21(3). https://doi.org/10.3390/md21030191.
35. Wang W, Li C, Chen Z, Zhang J, Ma L, Tian Y, et al. Novel diosgenin-amino acid-benzoic acid mustard trihybrids exert antitumor effects via cell cycle arrest and apoptosis. J Steroid Biochem Mol Biol. 2022;216:106038. https://doi.org/10.1016/j.jsbmb.2021.106038.
36. Ding Y, Xue X. Medicinal chemistry strategies for the modification of bioactive natural products. Molecules. 2024;29(3). https://doi.org/10.3390/molecules29030689.
37. Dycha N, Michalak-Tomczyk M, Jachula J, Okon E, Jarzab A, Tokarczyk J, et al. Chemopreventive and anticancer activity of selected triterpenoids in melanoma. Cancers (Basel). 2025;17(10). https://doi.org/10.3390/cancers17101625.
38. Peng J, Zhao K, Zhu J, Wang Y, Sun P, Yang Q, et al. Sarsasapogenin suppresses rankl-induced osteoclastogenesis

in vitro and prevents lipopolysaccharide-induced bone loss in vivo. Drug Des Devel Ther. 2020;14:3435-47. https://doi.org/10.2147/DDDT.S256867.
39. Muhammad S, Fai M, Aththar A, Zulfi Zakaria M, Kharisma V, Affan A, et al. Nano transdermal delivery potential of fucoidan from sargassum sp. (brown algae) as chemoprevention agent for breast cancer treatment. Pharmacogn J. 2022;14:789-95. https://doi.org/10.5530/pj.2022.14.169
40. Prajapati S, Patel M, Jyotishi C, Gupta R. Phytochemicals in overcoming venetoclax resistance in acute myeloid leukemia: Mechanistic insights and therapeutic potential. Ann Hematol. 2025;104(11):5573-91. https://doi.org/10.1007/s00277-025-06710-6.