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Evaluation of natural compounds as VEGFR-2 inhibitors for breast cancer therapy: insights from molecular docking and drug-likeness analysis

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Abstract: Breast cancer remains one of the most common cancers worldwide, with VEGFR-2 (KDR) playing a key role in tumor angiogenesis. Inhibiting VEGFR-2 is a promising therapeutic strategy. Natural compounds are increasingly studied for their potential to inhibit VEGFR-2. This study aims to assess the binding affinity of 11 natural compounds (andrographolide, alpha-mangostin, pinostrobin, pinocembrin, ethyl-p-methoxycinnamate (EPMC), xanthorrhizol, galangin, gamma-mangostin, curcumin, cinnamaldehyde, and alashanoid B) to the VEGFR-2 protein through molecular docking and Lipinski's rule analysis, identifying promising candidates for breast cancer treatment. Molecular docking simulations were performed for 11 compounds and sunitinib as a control, with binding energies and interactions analyzed. The compounds were also evaluated for drug-likeness using Lipinski's rule of five. Curcumin showed the highest binding affinity to VEGFR-2 with a binding energy of -9.9 kcal/mol, surpassing sunitinib (-9.4 kcal/mol). Key interactions were observed with active site residues Cys919 and Asp1046. All tested compounds met the criteria for oral bioavailability per Lipinski's rules. Curcumin demonstrates potential as a VEGFR-2 inhibitor due to its favorable binding affinity and drug-like properties. Enhancing curcumin's bioavailability is recommended for effective therapeutic application.

Keywords: angiogenesis, breast cancer, curcumin, molecular docking, VEGFR-2

Introduction

Breast cancer remains one of the most prevalent cancers globally, ranking as the second leading cause of cancer-related mortality. In 2020 alone, approximately 685,000 individuals died from breast cancer, and 2.3 million new cases were diagnosed worldwide, bringing the total number of diagnoses in the previous five years to 7.8 million [1]. A critical factor in breast cancer prognosis is the expression of Vascular Endothelial Growth Factor (VEGF), which correlates strongly with decreased overall survival and disease-free survival rates [2]. The kinase insert domain receptor (KDR), also known as Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2), is a key mediator of VEGF-driven endothelial functions, including cell proliferation, migration, survival, and vascular permeability [3].

While anticancer treatments targeting VEGFR-2—such as the monoclonal anti-VEGF antibody bevacizumab—have shown promise, their effectiveness has been inconsistent. Although bevacizumab combined with paclitaxel has demonstrated reduced metastatic progression in breast cancer, follow-up trials have not observed an improvement in overall survival [2]. Additionally, resistance mechanisms may limit drug penetration, exacerbate hypoxia, and stimulate VEGF production, thereby reducing treatment efficacy. Small-molecule inhibitors of VEGFR-2, such as sunitinib, have likewise yielded variable results; despite showing activity as a single agent against metastatic breast cancer in some studies, sunitinib has not consistently provided therapeutic benefits in either first-line or refractory settings [2]. These limitations underscore the need for continued exploration of alternative compounds capable of inhibiting the VEGFR-2 pathway in breast cancer treatment [4].

Plant-derived compounds are a promising area of research for anticancer therapies. Several such

compounds—including andrographolide, alpha mangostin, pinostrobin, pinocembrin, ethyl-p-methoxycinnamate (EPMC), xanthorrhizol, galangin, gamma-mangostin, curcumin, cinnamaldehyde, and alashanoid B—have shown potential in blocking the VEGFR-2 pathway. Experimental evidence supports their inhibitory effects on various breast cancer cell lines, with compounds like cinnamaldehyde and pinostrobin demonstrating inhibitory concentrations (IC_{50} values) of 12.2–34.953 µg/mL against MDA-MB-231 cells [5,6]. Similarly, andrographolide and α-mangostin have exhibited IC_{50} values of 4.36–32.4 µM in T47D cells [7,8], while pinocembrin and galangin inhibited MCF-7 cells with IC_{50} values between 39.61–108.36 µM [9,10]. Ethyl-p-methoxycinnamate (EPMC), xanthorrhizol, γ-mangostin, and curcumin have shown IC_{50} values ranging from 0.00215 to 360 µg/mL against MCF-7 cells [11-14].

Given this background, the current study utilizes in silico molecular docking to assess the potential of selected anticancer compounds specifically targeting the KDR/VEGFR-2 protein, with the goal of identifying promising candidates for future therapeutic development.

Methods

Materials

This study examined 11 active compounds derived from natural sources: andrographolide, α-mangostin, pinostrobin, pinocembrin, ethyl-p-methoxycinnamate (EPMC), xanthorrhizol, galangin, γ-mangostin, curcumin, cinnamaldehyde, and alashanoid B (Figure 1). The chemical structures of these compounds were obtained through the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Compounds were optimized was using the MMFF94 force field with 100 steps per update. The MMFF94 force field is commonly applied for the optimization of organic compounds and drug-like molecules [15].

Molecular docking

The protein structure used for molecular docking was retrieved from the Protein Data Bank (PDB ID: 3WZE, 1.9 Å resolution) (https://rcsb.org). Protein and native ligand preparation was conducted using AutoDock Tools, with files saved in PDB format. Molecular docking validation was ensured by confirming an RMSD value of ≤ 2 Å [16]. Docking simulations were performed with AutoDock Vina, and molecular interactions were visualized using BIOVIA Discovery Studio.

Lipinski's rule of five

Lipinski's rule of five was applied to evaluate the drug-likeness of the 11 compounds. This analysis was conducted using SCFBio (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) with a pH value of 7.

Results

The molecular docking results for the 11 test compounds—andrographolide, α-mangostin, pinostrobin, pinocembrin, ethyl-p-methoxycinnamate (EPMC), xanthorrhizol, galangin, γ-mangostin, curcumin, cinnamaldehyde, and alashanoid B—alongside the control compound, sunitinib, are summarized in Table 1.

Curcumin exhibited a lower binding energy (-9.9 kcal/mol) compared to sunitinib (-9.4 kcal/mol), indicating a potentially stronger binding affinity to the target KDR protein. Visualization of the docking interactions between sunitinib and curcumin with the receptor is shown in Figure 2, while Table 2 provides detailed bond interactions for each compound.

The results of the Lipinski's rule of five assessment for the 11 test compounds and sunitinib are presented in Table 3.

Table 1. Binding energy values from molecular docking simulations

Compound	ΔG (kcal/mol)
Sunitinib	-9.4
Alashanoid B	-6.7
α-Mangostin	-2.5
Andrographolide	-4.8
Ethyl-p-methoxycinnamate (EPMC)	-7.2
Galangin	-9.3
γ-Mangostin	-2.5
Curcumin	-9.9
Pinocembrin	-9.3
Pinostrobin	-8.7
Cinnamaldehyde	-6.6
Xanthorrhizol	-8.1

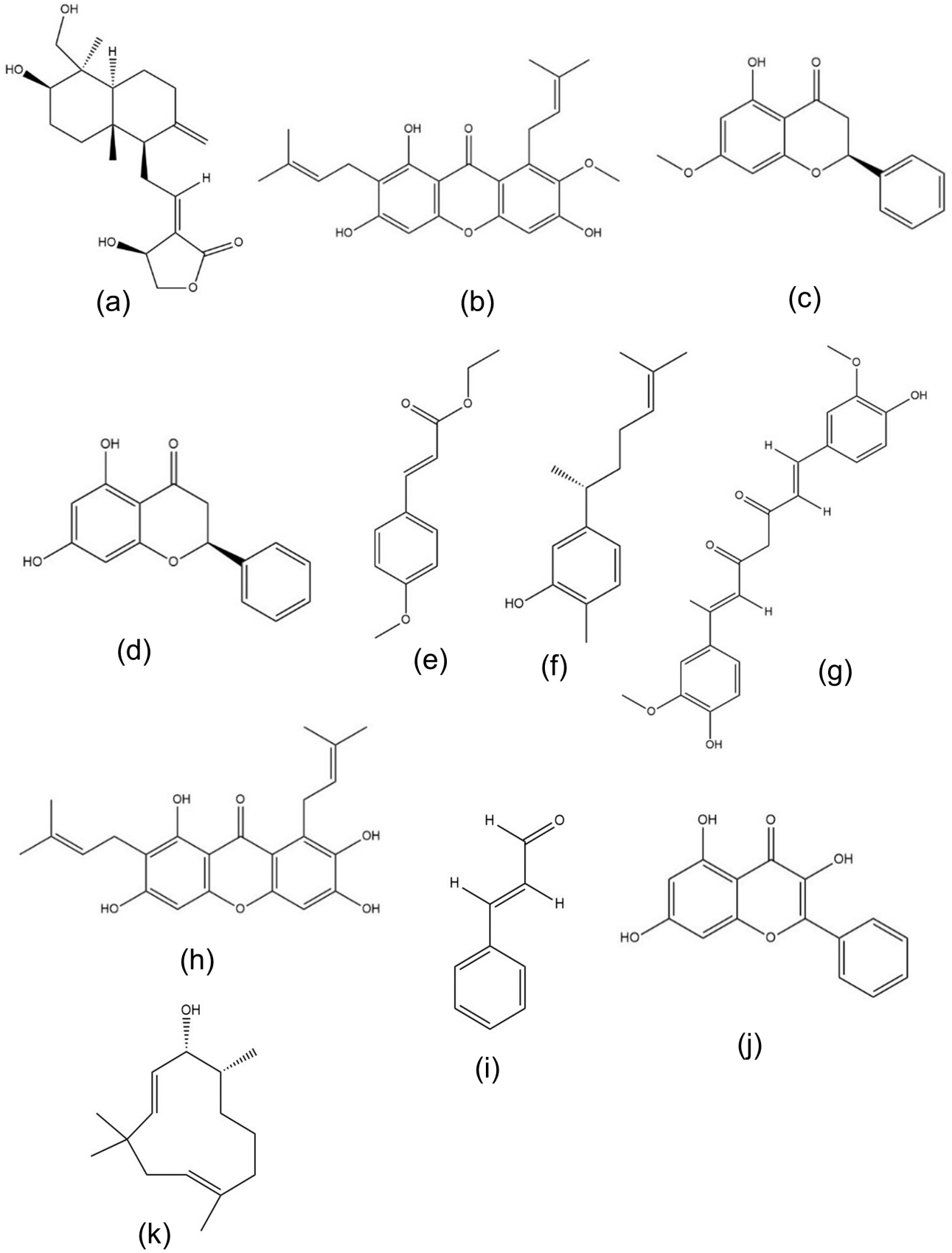

Figure 1. Chemical structure of test ligand. (a) andrographolide, (b) α-mangostin, (c) pinostrobin, (d) pinocembrin, (e) ethyl-p-methoxycinnamate (EPMC), (f) xanthorrhizol, (g) curcumin, (h) γ-mangostin, (i) cinnamaldehyde, (j) galangin, (j) alashanoid B

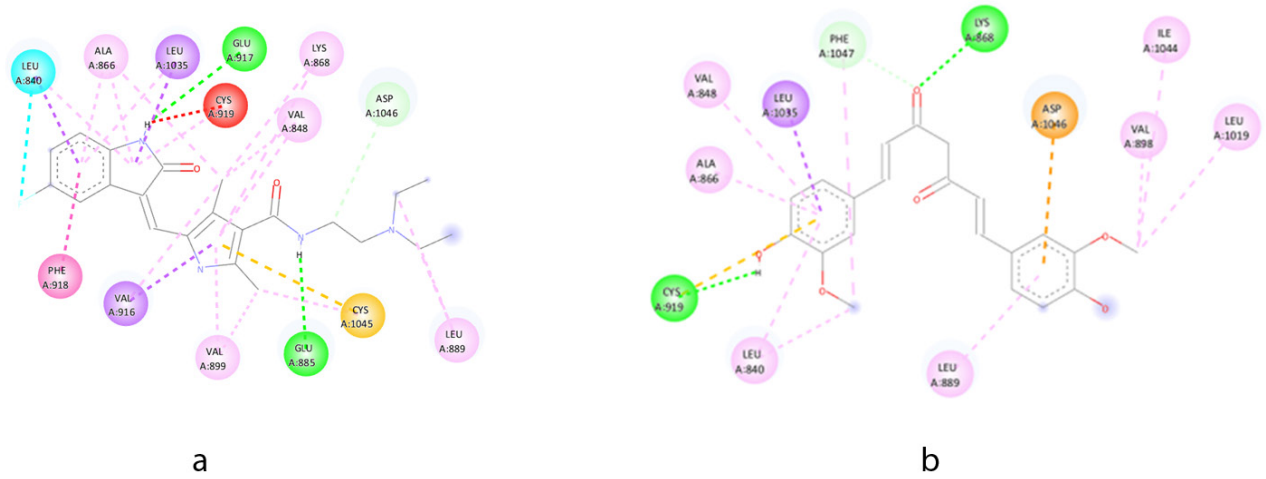

Figure 2. Docking visualization of test compound against KDR protein. (A) sunitinib, (B) curcumin

Table 2. Binding interactions for each compound against KDR

Compound	Interaction	Amino acid residues (bond distance)
Sunitinib	Hydrogen	**Glu917 (3.06 Å); Glu885 (2.14 Å), Asp1046 (3.31 Å)**
	Halogen	Leu840 (3.24 Å)
	π-sigma	Leu840 (3.91 Å); Val916 (3.71 Å); Leu1035 (3.85 Å)
	π-sulfur	Cys1045 (5.08 Å)
	π-π stacked	Phe918 (5.52 Å)
	Alkyl	Ala866 (4.02 Å); Val899 (3.40 Å); Cys1045 (3.42 Å); Val848 (3.23 Å); Lys868 (3.98 Å); Val916 (4.49 Å); Leu889 (5.13 Å); Leu889 (4.70 Å)
	π-alkyl	Leu840 (5.48 Å); Ala866 (3.80 Å); **Cys919 (5.02 Å)**; Val848 (4.96 Å); Lys868 (5.33 Å); Val899 (4.90 Å); Ala866 (4.97 Å); Leu1035 (5.27 Å)
Curcumin	Hydrogen	Lys868 (3.39 Å); **Cys919 (2.39 Å)**, Phe1047 (3.07 Å)
	π-anion	**Asp1046 (4.94 Å)**
	π-sigma	Leu1035 (3.97 Å)
	π-sulfur	**Cys919 (5.55 Å)**
	Alkyl	Val898 (4.43 Å); Leu1019 (4.50 Å); Ile1044 (5.06 Å); Leu840 (4.37 Å)
	π-alkyl	Phe1047 (4.97 Å); Leu889 (4.67 Å); Leu840 (5.43 Å); Val848 (5.23 Å); Ala866 (3.70 Å)

Discussion

This study employed in silico molecular docking and Lipinski's rule assessment to evaluate the binding interactions between the KDR/VEGFR-2 protein and 11 natural compounds. KDR/VEGFR-2 is crucial in tumor angiogenesis, integrating pro-angiogenic signals necessary for tumor growth and vascularization [4]. The docking results identified three compounds with binding energy values close to that of the control compound, sunitinib: curcumin (-9.9 kcal/mol), galangin (-9.3 kcal/mol), and pinocembrin (-9.3 kcal/mol). Among these, curcumin displayed the lowest binding energy, surpassing sunitinib's binding energy (-9.4 kcal/mol), suggesting a more stable interaction with KDR/VEGFR-2 [17]. The negative binding energy values indicate spontaneous reactions and suggest a high affinity of curcumin for KDR/VEGFR-2, making it a promising inhibitor candidate [18].

Curcumin exhibited key interactions with amino acid residues, particularly Cys919 and Asp1046, which are also involved in the binding of sunitinib (Figure 1 and Table 2). This similarity in binding sites suggests that curcumin could exert inhibitory effects on KDR/VEGFR-2 similar to those of sunitinib. Additionally, galangin and pinocembrin formed hydrogen bonds with the Cys919 residue, essential for ATP binding

Table 3. Lipinski's rule of five analysis results for test compounds and control

Compound	Molecular weight (Da)	LogP	Hydrogen bond donor	Hydrogen bond acceptor	Molar refractivity
Sunitinib	398	3.334939	3	5	113.288574
Alashanoid B	222	4.086098	1	1	70.316772
α-Mangostin	410	5.166101	3	6	114.206856
Andrographolide	350	1.962600	3	5	93.560364
Ethyl-p-methoxycinnamate (EPMC)	206	2.271500	0	3	58.660984
Galangin	270	2.599699	3	5	70.720879
γ-Mangostin	396	4.863101	4	6	109.319656
Curcumin	368	3.369898	2	6	102.016571
Pinocembrin	256	2.804299	2	4	68.530083
Pinostrobin	270	3.107298	1	4	73.417282
Cinnamaldehyde	132	1.898700	0	1	41.539997
Xanthorrhizol	218	4.550519	1	1	69.923782

within the receptor. However, as these compounds interact with fewer active site residues, they exhibit slightly higher binding energy values than curcumin and sunitinib. Binding at Cys919 is significant as it positions these compounds as Type I ATP-competitive inhibitors, maintaining the receptor in an inactive conformation and hindering VEGFR-2 activity [19].

Curcumin's inhibitory mechanism on VEGFR-2 involves the suppression of angiogenesis, a critical process in cancer progression, as it supplies nutrients to cancer cells. By inhibiting VEGF production, curcumin can disrupt the nutrient flow, thereby inhibiting cancer cell growth. This aligns with previous findings showing that VEGF inhibition can effectively slow breast cancer progression [20].

The Lipinski analysis (Table 3) indicated that all test compounds met the criteria for good oral bioavailability, as each satisfied at least two of Lipinski's rules [21]. Curcumin, galangin, and pinocembrin, with a molecular weight of 368 Da, are likely to diffuse efficiently through cell membranes, in contrast to larger compounds (>500 Da) [22]. Curcumin's LogP value of 3.36 indicates optimal lipophilicity, facilitating membrane permeability while avoiding excessive hydrophobicity, which could lead to high toxicity and prolonged lipid retention. Additionally, curcumin possesses two hydrogen bond donors and six hydrogen bond acceptors, contributing to its membrane permeability via passive diffusion. The presence of multiple hydrogen bonds enhances biological activity but may increase the energy required for absorption [23].

Despite its potential, curcumin's low bioavailability limits its therapeutic application; nearly 80% of an orally administered dose is excreted, with significant metabolism occurring in the intestinal mucosa and liver. This highlights the need for formulation strategies to enhance curcumin's bioavailability for it to reach its full therapeutic potential as a KDR/VEGFR-2 inhibitor [20].

Conclusion

Curcumin demonstrates strong potential as a KDR/VEGFR-2 protein inhibitor, with binding energy lower than sunitinib and other tested compounds. Its binding interactions include the Cys919 residue, a key site within the KDR protein's active region. Curcumin also satisfies Lipinski's rule requirements, suggesting good oral bioavailability. However, optimizing its formulation to improve bioavailability will be essential for effective clinical application.

Acknowledgments

None.

Declaration of interest

None.

Author contributions

VA: formal analysis, writing—original draft preparation, visualization. S: methodology, supervision,

writing—review & editing, funding acquisition; MSF: software, data curation, formal analysis, visualization; HNB: resources, data curation, validation. NAC: conceptualization, project administration, supervision, writing—review & editing.

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