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Molecular docking of hybrid coumarin-thiazole derivative compounds as anti-breast cancer on VEGFR-2 protein

Putri Liswatini, Sophia Rahma, Putri Mariska, Fibria Anggraeni, Desti Agustin, Desi Puspita Sari, Mahisa Shzara Afrian, Winni Nur Auli, Anjar Hermadi Saputro*

Departement of Phramacy, Faculty of Science, Institut Teknologi Sumatera, Indonesia

*Corresponding author: Jl. Terusan Ryacudu, Way Huwi, Jati Agung, South Lampung 35365, Lampung, Indonesia.
Email: anjar.saputro@fa.itera.ac.id

Abstract: VEGFR-2 is a tyrosine kinase receptor located on cell membranes, originally identified in endothelial cells but also expressed in tumor cells and various cancer types, including breast cancer. In breast cancer, VEGFR-2 expression is upregulated during early stages of primary tumors and invasive metastases, with elevated levels associated with lymph node metastasis and reduced survival outcomes. This computational study evaluated the potential of coumarin-thiazole derivative compounds against VEGFR-2 as anticancer agents using molecular docking analysis. Three coumarin-thiazole hybrid compounds (42a, 54a, and 54b) were assessed for their binding affinity to VEGFR-2, with sorafenib serving as the reference drug. The docking analysis utilized the three-dimensional structure of VEGFR-2 (PDB ID: 2OH4) downloaded from the RCSB Protein Data Bank. Ligand structures were prepared using molecular modeling software and converted to appropriate formats for analysis. Molecular docking was performed using AutoDockTools v.1.5.7, and protein-ligand interactions were visualized using BIOVIA Discovery Studio 2024 software.Method validation using the native GIG ligand yielded a binding energy of -10.88 kcal/mol. The binding energy values for the three test compounds were -9.81 kcal/mol for compound 42a, -12.71 kcal/mol for compound 54a, and -12.77 kcal/mol for compound 54b. Compound 54b demonstrated the strongest binding affinity to VEGFR-2, surpassing the native ligand GIG, the reference drug sorafenib, and the other test compounds. These results indicate that compound 54b represents the most promising candidate for anti-breast cancer therapy through VEGFR-2 targeting, warranting further experimental validation.

Keywords: breast cancer, coumarin-thiazole derivatives, VEGFR-2, molecular docking, anticancer agents

Introduction

Breast cancer is a malignant neoplasm originating from the epithelial cells of the breast ducts or lobules. Its pathogenesis is characterized by the uncontrolled proliferation of abnormal cells that have evaded standard regulatory mechanisms [1]. As the most frequently diagnosed cancer among women globally, it accounts for over 10% of all new cancer cases annually and represents the second leading cause of cancer-related deaths in this population. The frequently asymptomatic nature of early-stage disease underscores the importance of routine screening for early detection [2]. According to the International Agency for Research on Cancer (IARC), there were 65,858 new cases and 22,430 deaths from breast cancer among women in Indonesia in 2020 [3]. Etiological factors include genetic mutations in DNA repair pathways, hormonal influences, family history, lifestyle factors, and environmental exposures. Consequently, enhanced prevention and treatment strategies are critical for mitigating the burden of breast cancer in Indonesia.

Coumarin represents a privileged scaffold in medicinal chemistry and is a fundamental building block for numerous natural products with diverse pharmacological activities, including anticancer effects [4]. Coumarins and their derivatives are abundant in the seeds, roots, and leaves of various plant species, particularly within the *Rutaceae* and *Apiaceae* families. While most are of plant origin, certain coumarins such as novobiocin and coumermycin are derived from microbial sources [5]. Among synthetic derivatives, coumarin-thia(dia)zole hybrids have been investigated for their cytotoxic properties. Multiple variants of these hybrids have been synthesized and evaluated for activity against the MCF-7 breast cancer cell line, often

using the multi-tyrosine kinase inhibitor sorafenib as a reference compound [6]. Sorafenib inhibits tumor progression by targeting vascular endothelial growth factor (VEGF) and Raf kinase, thereby suppressing angiogenesis and proliferation while inducing apoptosis. However, its clinical utility is limited by side effects, including hypertension, and poor aqueous solubility [7]. These limitations necessitate the search for novel anticancer agents with improved efficacy and tolerability.

Recent studies indicate that coumarin-thia(dia) zole hybrid compounds exhibit potent inhibitory activity against the vascular endothelial growth factor receptor-2 (VEGFR-2) signaling pathway [8]. VEGFR-2 is highly expressed in both primary and metastatic invasive breast carcinomas, confirming the critical role of VEGF-mediated signaling in breast tumor angiogenesis and progression [6]. Therefore, the molecular integration of a thiazole moiety with a coumarin core presents a rational strategy for developing new anticancer candidates.

Given the limitations of existing therapies and the promising pharmacological profile of these hybrids, this study aims to computationally evaluate their therapeutic potential. Molecular docking simulation was performed using AutoDock software to analyze the binding interactions and stability of coumarin-thia(dia)zole hybrid compounds with the VEGFR-2 receptor, providing insights into their mechanism of action against breast cancer.

Methods

The computational studies were conducted using a computer with a 64-bit Windows 11 operating system. The software employed included AutoDockTools v.1.5.7, BIOVIA Discovery Studio 2024 Client, RCSB PDB database, PubChem, and Avogadro. The three-dimensional structure of VEGFR-2 was used as the target protein in this docking study and was downloaded from the RCSB Protein Data Bank (www.rcsb.org) with PDB ID 2OH4 [9]. The three-dimensional structure of the sorafenib ligand (CID216239) was retrieved from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/).

Protein preparation

The first step involved preparing the macromolecular structure and ligand. The macromolecular structure was retrieved from the Protein Data Bank website (http://www.rcsb.org/pdb/). The selected PDB code was 2OH4, representing a VEGFR-2 protein structure with a resolution of 1.90 Å, which was downloaded in PDB format.

Protein preparation was performed using AutoDockTools v.1.5.7. During this stage, the A chain (VEGFR-2 protein) and the GIG ligand were separated from the crystal structure. Optimization was performed on the selected A chain by removing water molecules and adding polar hydrogen atoms. The final preparation step involved saving the protein in PDBQT format. The GIG ligand was also saved in PDBQT format for use in subsequent docking simulations [10].

Validation procedure

Validation was conducted to rebind the GIG ligand to the VEGFR-2 protein using AutoDockTools v.1.5.7. The Root Mean Square Deviation (RMSD) parameter was used to validate the docking method. A method was considered valid when the RMSD value was less than 2 Å, confirming its suitability for molecular docking studies [10].

Ligand preparation

The ligands used were coumarin-thia(dia)zole hybrid compounds (42a, 54a, and 54b). Ligand structures were drawn using KingDraw or MolView software and subsequently converted to PDB format using BIOVIA Discovery Studio 2024.

Molecular docking of target compounds

Docking between the three ligands (42a, 54a, and 54b) and the VEGFR-2 protein was performed using AutoDockTools v.1.5.7 with the Lamarckian Genetic Algorithm. The docking procedure utilized ligand and protein files that had been previously converted to PDBQT format. The grid box size was configured based on validation results, with dimensions of 56 × 54 × 42 and coordinates set at X = 3.173, Y = 33.766, and Z = 17.175. The grid parameter file was generated and saved in GPF format.

Docking parameters were established by selecting the receptor macromolecule and ligand files in PDBQT format. Energy parameters were configured using the Genetic Algorithm search method, and the results were saved in DPF format. The final

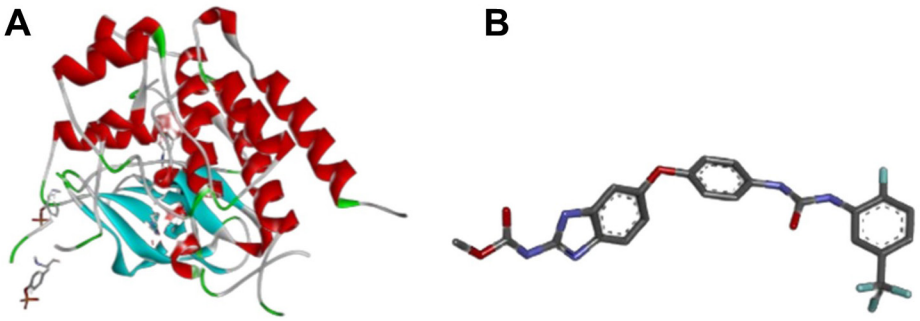


Figure 1. Three-dimensional structures of molecular docking components. (A) VEGFR-2 enzyme structure, and (B) GIG native ligand structure

docking stage involved executing the AutoDock4 and AutoGrid4 programs within AutoDockTools v.1.5.7. Upon completion, the results were generated as DLG files, which were analyzed using text editing software. The obtained results were compared with docking outcomes of reference compounds.

Visualization

Ligand-protein interactions were analyzed using BIOVIA Discovery Studio 2024 software. The docked complexes between ligands and protein were generated in AutoDockTools v.1.5.7 and subsequently visualized in BIOVIA Discovery Studio 2024 to examine binding interactions and molecular recognition patterns.

Results

Molecular docking was performed on coumarin compounds against the VEGFR-2 protein to determine the binding affinity of the compounds to the target protein. This in silico study involved method validation and molecular docking of target compounds. Method validation consisted of the initial simulation docking of the VEGFR-2 target protein against its native ligand, GIG (Figure 1). Molecular docking of the target compounds was subsequently performed using compounds 42a, 54a, and 54b.

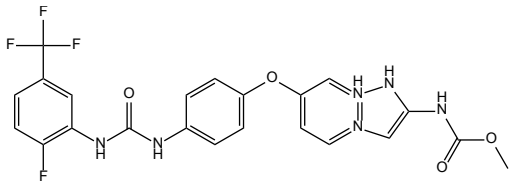
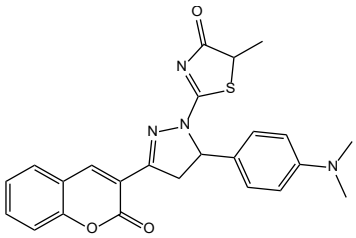
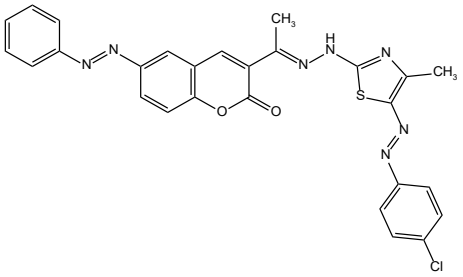
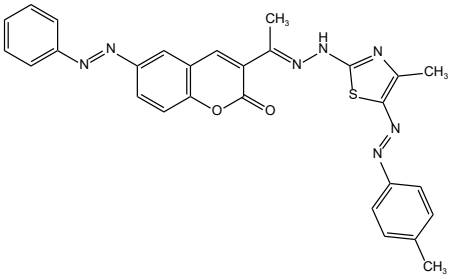
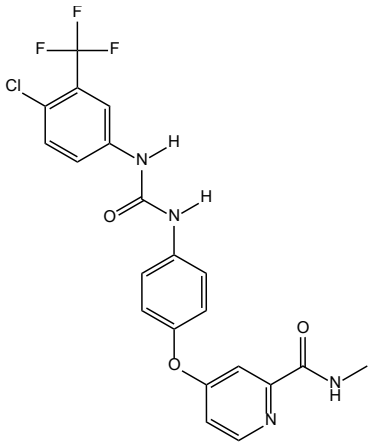
The docking results demonstrated varying binding affinities across all tested compounds. The sorafenib drug showed a binding energy value of -11.91 kcal/mol. The binding energy values for the three test ligands were as follows: 42a (-9.81 kcal/mol), 54a (-12.71 kcal/mol), and 54b (-12.77 kcal/mol) (Table 2). These data indicated that compounds 54a and 54b had lower binding energy values than sorafenib, suggesting stronger binding affinity to the VEGFR-2 protein.

Hydrogen atoms were added to complement amino acid residues that may have lost hydrogen atoms during X-ray crystallography structure determination. Additionally, Kollman charges were applied to the receptor to ensure that amino acid residues possessed appropriate electrostatic potential energy based on quantum mechanical calculations. Polar hydrogen atoms played a crucial role in molecular docking as they participated in hydrogen bonding interactions. Similar to the receptor preparation, Gasteiger charges were added to the ligands. This charge assignment aimed to optimize conditions within the molecular docking environment to achieve accurate calculation results [11].

The 2D visualization analysis revealed varied binding mechanisms between VEGFR-2 and the four different ligands. The native GIG ligand exhibited multiple interaction types including conventional hydrogen bonds with Asn921, Arg1049, Asp1044, Glu883, and His1024, halogen bonds with Cys1043 and Ile1042, and pi-sigma interactions with Leu1033 and Val914.

In contrast, ligands 42a and 54a demonstrated predominantly hydrophobic interactions through alkyl and pi-alkyl bonds involving Val914, Val897, Leu1033, and Ala864, along with pi-pi interactions with aromatic residues Phe1045 and Phe916. Ligand 54b displayed unique electrostatic interactions through salt bridges and attractive charges with Lys869 and Arg840, complemented by conventional hydrogen bonds with multiple residues including Ala1048, Ile1051, and Lys1053. The diverse interaction patterns across different ligands highlighted the flexibility of the VEGFR-2 binding site in accommodating structurally distinct compounds.

Table 2. Results of docking between ligands and VEGFR-2 protein

Ligands	Binding energy (kcal/mol)
 **Native ligand (GIG)**	-10.88
 **42a**	-9.81
 **54a**	-12.71
 **54b**	-12.77
 **Sorafenib (control drug)**	-11.91

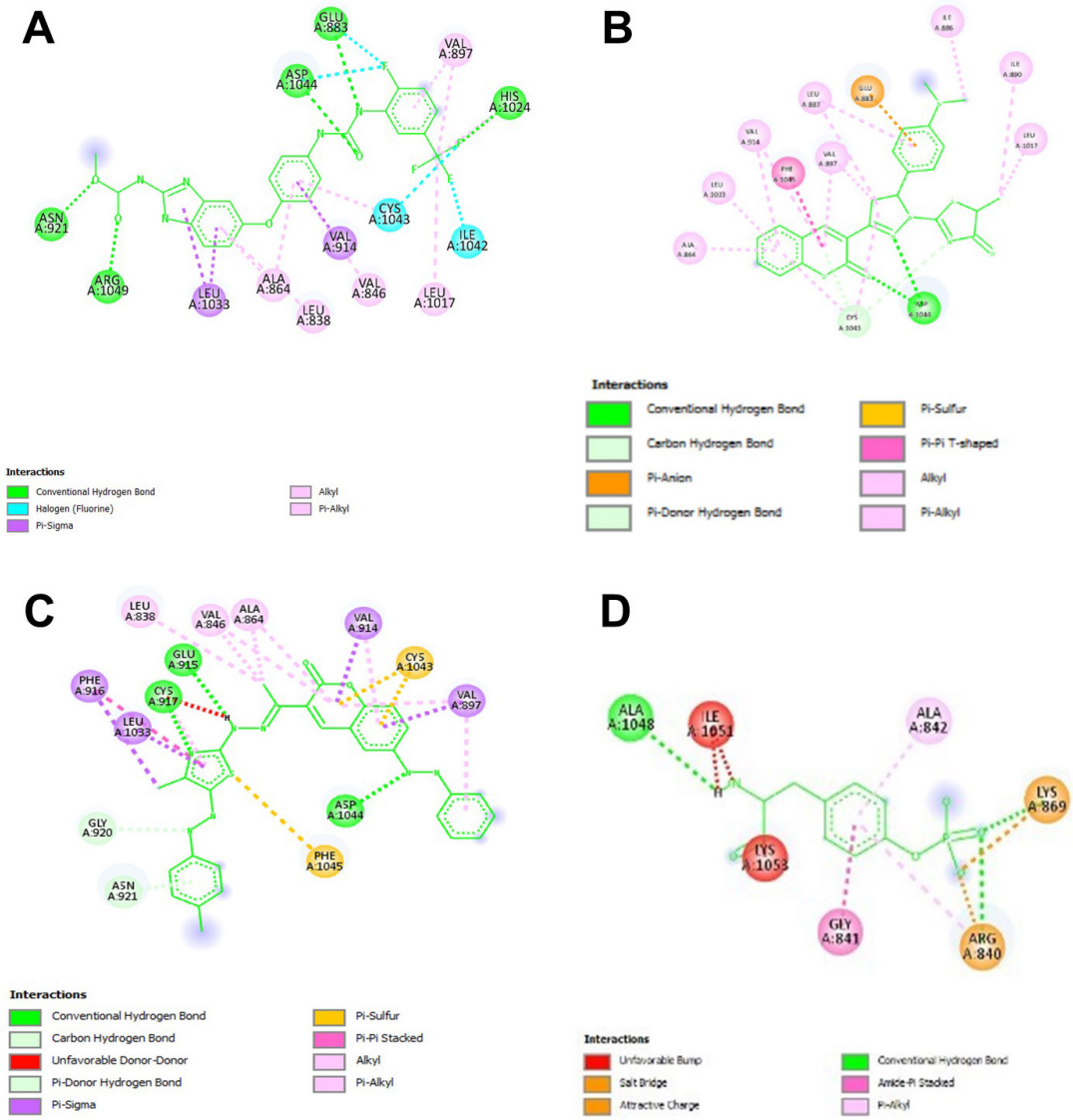


Figure 2. 2D visualization of protein-ligand interactions between VEGFR-2 protein and test ligands: (A) GIG (native ligand), (B) Ligand 42a, (C) Ligand 54a, and (D) Ligand 54b.

Discussion

This computational study employed in silico molecular docking methods to evaluate the binding potential of coumarin-thiazole hybrid compound against the VEGFR-2 protein. Molecular docking represents a standard computational chemistry technique used to predict binding patterns and affinities between proteins (receptors) and compounds (ligands). This method serves as a fundamental tool for drug modeling, particularly in compound optimization through virtual screening for new drug discovery. The pharmaceutical industry extensively utilizes this approach for designing new therapeutic agents and optimizing existing drug candidates [12].

Method validation was conducted by calculating the RMSD between the target protein and its native ligand following re-docking procedures. The RMSD parameter determines the similarity between the docked ligand conformation and the original crystallographic structure. RMSD values serve as critical indicators for validating docking program accuracy, with values ≤ 2 Å generally considered acceptable for reliable predictions [10]. Higher RMSD values indicate greater prediction errors in ligand-protein interactions [13]. AutoDock employs two RMSD variations: the lower bound (rmsd/lb) and upper bound (rmsd/ub), with rmsd/ub pairing each atom in one conformation with its corresponding

atom in another conformation regardless of symmetry considerations [14].

Based on the molecular docking results, the native GIG ligand demonstrated a binding energy of -10.88 kcal/mol with the VEGFR-2 protein. The three test compounds exhibited binding energies of -9.81 kcal/mol (42a), -12.71 kcal/mol (54a), and -12.77 kcal/mol (54b). Among these results, compound 54b achieved the most favorable binding energy at -12.77 kcal/mol, while compound 42a showed the least favorable binding energy at -9.81 kcal/mol. More negative binding energy values indicate stronger protein-ligand interactions, positioning compound 54b as the most potent candidate among the evaluated compounds.

Sorafenib functions as a multi-kinase inhibitor targeting Raf-1, B-Raf, and various kinases within the Ras/Raf/MEK/ERK signaling pathway to prevent tumor cell proliferation. The drug inhibits angiogenesis by targeting multiple receptors including vascular endothelial growth factor receptors (VEGFR-2 and VEGFR-3), hepatocyte factor receptors (c-Kit), Fms tyrosine kinase (FLT-3), and platelet-derived growth factor receptor (PDGFR-β). Clinical studies have demonstrated sorafenib's effectiveness against various tumor cell lines, including LOX melanoma and MDA-MB-231 breast cancer cells containing B-Raf gene mutations [15].

The hydrogen bonding analysis revealed distinct interaction patterns among the evaluated compounds. Compound 42a formed hydrogen bonds with Asp, Cys, and other residues at distances ranging from 2.79 Å to 4.02 Å. Compound 54a established hydrogen bonding interactions with Cys, Asp, Gly, and Asn residues at distances between 2.13 Å and 3.12 Å. Compound 54b demonstrated the most extensive hydrogen bonding network, interacting with Arg, Lys, Cys, Asp, and additional residues at distances ranging from 1.58 Å to 3.07 Å. The analysis indicates that compounds 54a and 54b form more stable binding complexes compared to the reference compound, as evidenced by their multiple close-contact hydrogen bonding interactions and overall binding energy profiles.

Conclusion

The molecular docking analysis demonstrated that coumarin-thiazole hybrid compound 54a and 54b exhibit significantly enhanced binding affinities toward VEGFR-2 compared to both the reference drug sorafenib and the native ligand. Compound 54b emerged as the most promising candidate with a binding energy of -12.77 kcal/mol, followed closely by compound 54a at -12.71 kcal/mol. Both compounds substantially outperformed sorafenib (-11.91 kcal/mol) and the native GIG ligand (-10.88 kcal/mol). The superior binding affinity of compound 54b, coupled with its extensive hydrogen bonding network within the VEGFR-2 active site, suggests enhanced potential for therapeutic efficacy. These computational findings indicate that compound 54b warrants further investigation through experimental validation studies to confirm its viability as a potential anti-cancer therapeutic targeting VEGFR-2-mediated pathways.

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None.

Declaration of Interest

The authors declare that none of them has any conflict of interest with any private, public or academic party related to the information contained in this manuscript.

Author Contributions

Conceptualization, AHS, PL, WNA; Methodology, SR, PM, FA, DA, DPS, MSA, WNA; Investigation, WNA, AHS; Writing – Original Draft, PL, SR, PM, FA, DA, DPS, MSA, AHS; Writing – Review & Editing, AHS.

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