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In-silico of Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) Inhibitors of Seven Bioactive Compounds from *Sonneratia alba*

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Abstract: Cancer is a disorder resulting from genetic changes, abnormal growth, and spread to other parts of the body. Cancer treatment is commonly administered through chemotherapy, which often results in adverse side effects. Therefore, research is essential to identify safer alternative cancer therapies. This study aims to identify potential bioactive compound ligand receptor targets from Sonneratia alba as anticancer candidates in silico by identifying VEGFR target proteins based on their pharmacophores and studying their interactions through the reverse docking method. Docking simulations between the native ligand (Tivozanib) and the receptor yielded promising results, with a free energy value of -12.76 kcal/mol and an inhibition constant of 440.70 µM. Among the seven bioactive compounds, Meperidine ($C_{15}H_{21}NO_2$) exhibited favorable outcomes, showing a free energy value of -7.39 kcal/mol and an inhibition constant of 3.83 µM. Additionally, the meperidine and gibberellin A7 compounds formed three and four hydrogen bonds, respectively, including one with Ala866, and interacted with 15 and 18 amino acid residues, such as Glu917, Cys919, Glu885, and Asp1046. The presence of active sites on the ligand or test compounds that bind to the target receptor indicates a potential for comparable affinity in inhibiting VEGFR-2 receptor activity.

Keywords: *in silico, Soneratia alba, VEGFR inhibitors*

Abstrak: *Kanker merupakan gangguan yang disebabkan oleh perubahan genetik, pertumbuhan abnormal, serta penyebarannya ke bagian tubuh lainnya. Pengobatan kanker umumnya dilakukan melalui kemoterapi, yang sering kali menimbulkan efek samping yang merugikan. Oleh karena itu, diperlukan penelitian untuk mengidentifikasi terapi alternatif kanker yang lebih aman. Penelitian ini bertujuan untuk mengidentifikasi target reseptor ligan senyawa bioaktif potensial dari Sonneratia alba sebagai kandidat antikanker secara in silico dengan mengenali protein target VEGFR berdasarkan farmakofornya, serta mempelajari interaksinya melalui metode reverse docking. Simulasi docking antara ligan asli (Tivozanib) dan reseptor menunjukkan hasil yang menjanjikan, dengan nilai energi bebas sebesar -12,76 kcal/mol dan konstanta inhibisi sebesar 440,70 µM. Di antara tujuh senyawa bioaktif, Meperidine ($C_{15}H_{21}NO_2$) menunjukkan hasil yang paling baik dengan nilai energi bebas sebesar -7,39 kcal/mol dan konstanta inhibisi sebesar 3,83 µM. Selain itu, senyawa meperidine dan gibberellin A7 masing-masing membentuk tiga dan empat ikatan hidrogen, termasuk satu dengan residu Ala866, serta berinteraksi dengan 15 dan 18 residu asam amino, seperti Glu917, Cys919, Glu885, dan Asp1046. Kehadiran situs aktif pada ligan maupun senyawa uji yang dapat berikatan dengan reseptor target menunjukkan potensi afinitas yang sebanding dalam menghambat aktivitas reseptor VEGFR-2.*

Kata kunci: *in silico, Sonneratia alba, inhibitor VEGFR*

INTRODUCTION

Cancer remains the second leading cause of death globally, responsible for approximately 9.6 million deaths annually. Developing countries, including Indonesia, bear a significant burden, with an estimated 70% of cancer-related deaths occurring in these regions. According to Globocan (Global Cancer Observatory) 2022, Indonesia reported 408,661 new cancer cases and 259,192 prevalent cases, with projections suggesting a rise to 29.4 million cases by 2040. Characterized by uncontrolled cell proliferation, cancer poses a severe threat to public health. Globally, in 2020 alone, there were an estimated 19.3 million new cancer cases (excluding 18.1 million non-melanoma skin cancer cases) and nearly 10 million cancer-related deaths (excluding

9.9 million non-melanoma skin cancer deaths), highlighting the urgency of effective prevention and treatment strategies (Putram *et al.* 2017). According to estimates by the World Health Organization (WHO) in 2019, the burden of cancer is expected to continue to increase until 2040. Cancer is the first or second cause of death before the age of 70 in 112 of 183 countries and ranks third or fourth in 23 countries. Most medicinal plants in Indonesia have a very important role, especially for people in rural areas where health facilities are still very limited. Communities around forest areas use medicinal plants as raw materials for medicines based on knowledge about the use of medicinal plants that has been passed down from generation to generation (Khafid *et al*. 2023). One of them is the Perepat plant (Sonneratia alba) which is a group of mangroves that grows abundantly on the east coast of Sumatra (Mandang *et al*. 2021).

Sonneratia alba (commonly known as Perepat) is a mangrove species with notable bioactivity, as demonstrated in several studies. It exhibits antimicrobial, antioxidant, and cytotoxic properties, making it a promising candidate for further exploration. The acetone and ethanol extracts of *S. alba* leaves have shown antioxidant activity, with IC_{50} values of 52.35 and 142.12 ppm, respectively (Latief *et al*. 2015; Latief *et al*. 2018). Additionally, its ethanol extract has demonstrated significant cytotoxic activity, with an LC_{50} value of 23.98 µg/ml (Latief *et al*. 2020), and has been effective in inhibiting the growth of both gram-negative and gram-positive bacteria (Manuhuttu *et al*. 2021). Given its diverse bioactive properties, *S. alba* holds potential as a natural source of compounds for therapeutic applications. Recent studies suggest that certain bioactive compounds from S. alba may target Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2), a key regulator in tumor angiogenesis. Therefore, investigating its potential as a VEGFR-2 inhibitor through in silico approaches could provide valuable insights into its role as a novel anti-cancer agent.

One of the existing drug development efforts is done by drug design. Drug design aims to obtain new drugs with better activity and lower toxicity through structural modification. Changes in the structure of a compound will change its physicochemical properties, including lipophilic, electronic, and steric properties, and changes in these physicochemical properties will cause changes in the biological activity of the compound (Handoyo *et al.* 2022). Drug development is a dynamic process that is rapidly evolving and facilitated by computers (Machine Learning). Modeling the interaction between chemical substances and biological targets facilitates the development of new pharmacophores. The result of this modeling is a study of ligand and receptor interactions to predict effects and processes in the body based on chemical structure (Putra *et al.* 2020). *In silico* tests have been widely used today and are popular in the world of computing. In silico studies, molecular docking techniques are used to predict the bioactivity of a compound before conducting experimental analysis in the laboratory. This method has advantages, including reducing the use of excessive equipment and materials and saving on experimental costs. The *in silico* method can also be used to predict compound activity, one of which is by looking at the amount of free binding energy formed in its interaction with the active site of the protein involved. (Dona *et al*. 2019).

In this study, in silico testing will be conducted on seven bioactive compounds from S. alba that have the potential to be anticancers by looking at the interaction of ligands and receptors/target proteins used. This study uses the VEGF/VEGFR2 receptor with PDB code 4ASE. Vascular endothelial growth factor receptor (VEGFR) tyrosine kinases are clinically validated drug targets for cancer therapy. VEGF/VEGFR2 is considered the most critical proangiogenic pathway to increase all stages of angiogenesis, including vascular permeability, endothelial cell survival, proliferation, migration or invasion into surrounding tissues, and capillary tube formation. Cancer development is often associated with VEGF expression, and the VEGF/VEGFR2 signaling pathway is generally considered the primary mediator of tumor angiogenesis, so VEGF/VEGFR2 is a target system for therapeutic intervention in cancer (Kesuma *et al*. 2018). This study aims to identify the target receptor of the ligand (quercetin) as an anticancer candidate in silico by identifying the VEGFR target protein based on its pharmacophore and studying its interaction through the reverse docking method. Based on this method, predictions of the interaction between the ligand and the test receptor can be generated.

MATERIALS AND METHOD

Materials

The materials used in this study include 3D test compounds, which were saved in PDB format, along with the structure of the VEGFR-2 receptor (PDB ID: 4ASE), obtained from the respective database web servers. The research utilized both hardware and software tools. The hardware used was a Lenovo PC IdeaCentre AIO 5i 24IAH7 F0GR006RID Storm Gray, equipped with an Intel Core i7-12700H processor, Windows 11 Home, 16GB DDR4 RAM, and an Intel ARC A370M 4GB GDDR6 GPU. The software employed included PyRx, ChemDraw Ultra version 22.0, Chem3D version 22.0, AutoDockTools, Discovery Studio Visualizer 2021, and UCSF Chimera. Additionally, the Research Collaboratory for Structural Bioinformatics (RCSB) database was utilized as the primary web server for structural data retrieval.

The materials employed consist of 3D test compounds stored in pdb format, along with the

structure of the receptor, VEGFR-2 (PDBid: 4ASE), also accessible in pdb format on the respective database web servers. The hardware utilized for this research includes a Lenovo PC IdeaCentre AIO 5i 24IAH7 F0GR006RID in Storm Gray, equipped with an Intel Core i7 12700H processor, running Windows 11 Home, and featuring 16GB DDR4 RAM and an Intel ARC A370M with 4GB GDDR6. The software tools involved are PyRx, ChemDraw Ultra version 22.0, Chem 3D version 22.0, AutoDockTools, Discovery Studio Visualizer 2021, and UCSF Chimera; RCSB (Research Collaboratory for Structural Bioinformatics) serves as the web server used.

Ligand Preparation

Ligand preparation is carried out by drawing the 2D structure of the test compound (ligand) using ChemDraw Ultra version 22.0 and then converting it into 3D form using Chem 3D version 22.0 and saving it in pdb format. The test ligands were optimized by minimizing energy using PyRx software with the open Babel feature and then saved in pdbqt format.

Macromolecule Preparation

Macromolecule preparation is carried out by downloading the receptor in the https://www.rcsb.org database with the receptor code VEGFR-2 (PDBid: 4ASE). Macromolecules are separated from solvents and native ligands or nonstandard residues using the UCSF Chimera application. Macromolecular (receptor) files are saved in pdb format. Next, the macromolecules were optimized using AutoDockTools by adding hydrogen ions and Kollman charges and saved in pdbqt file format.

Analysis of Drug Candidate Compound Properties

The drug similarity profiles of the test and reference compounds were determined using Lipinski prediction. This analysis was performed using the SwissADME website (http://www.swissadme.ch/). While the ADMET profile analysis used the pkCSM pharmacokinetics website (https://biosig.lab.uq.edu.au/pkcsm/).

Analysis of Protein Properties

Protein analysis aims to see the protein profile that will be used. The analysis was carried out using the PDBsum website (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/).

Molecular Validation and Docking

Validation of the molecular docking method was carried out using AutoDockTools software. This was done by re-docking the natural ligands of each macromolecule (receptor). The parameter used is Root Mean Square Deviation (RMSD). The results obtained in this process are the grid box parameters and RMSD values. The docking method is valid if it has an RMSD value ≤ 3 Å (Dewi *et al*. 2021). The molecular docking process is carried out using Autoodock Tools software. The macromolecule (receptor) and ligand structures that have been optimized separately are stored in one folder. The molecular docking process uses a grid box and energy minimization parameters according to validation results. Grid box parameter settings are carried out using grid box coordinates, which are determined based on the ligand coordinates of the receptor used in the docking validation process. Next, mooring is carried out using Autoodock Tools software. The docking data displayed is in the form of binding affinity values and amino acid residue interactions. Docking results are saved in pdbqt format.

Visualization and Analysis of Docking Results

The visualization process is carried out to see the interactions that occur in the docking results between the receptor and the ligand. Visualization of docking results was carried out using Discovery Studio Visualizer 2021 software.

RESULT AND DISSCUSION

Lipinski's Rule Bioactive Compounds

Analysis of candidate drug compounds needs to be done to see if the compound can be used as an alternative in drug therapy. This analysis is based on the Lipinski rule. The Lipinski rule is a rule for evaluating drug similarity or determining the properties of a chemical compound with certain pharmacological or biological activities with properties that make it a drug that may be orally active in humans. The parameters used are molecular weight ≤ 500; hydrogen bond donor ≤ 5; hydrogen bond acceptor ≤ 10; and Log P ≤ 4.15 [1]. The analysis was carried out using the SwissADME website (http://www.swissadme.ch/). From the results of the analysis that has been carried out on seven test compounds, it is known that all compounds have fulfilled the Lipinski rule. The results of the analysis can be seen in Table 1.

ADMET Prediction

ADMET profile analysis includes the adsorption, distribution, excretion, metabolism, and toxicity of drugs or compounds when entering the body. The analysis was carried out using the pkCSM pharmacokinetics website (https://biosig.lab.uq.edu.au/pkcsm/).

The Caco-2 permeability value indicates the absorption of a compound or drug in the intestine. The Caco-2 prediction value > 0.90 means that the compound or drug is likely to be well absorbed through the intestinal wall when consumed orally (Pires *et al*. 2015). The analysis results showed that the compounds meperidine ($C_{15}H_{21}NO_2$) and gibberellin ($C_{19}H_{22}O_5$) had good intestinal absorption values with values > 0.90. While the other five

Table 1. Lipinski's Rule of the seven potential-bioactive compounds

No	Bioactive Compounds	Formula	Molar Mass <500	Hydrogen Donor <5	Hydrogen Acceptors<10	Log P<5	Drug-likeness
1	Meperidine	$C_{15}H_{21}NO_2$	247.338	0	3	2.213	Yes
2	Apigenin-7-O-glucoside	$C_{21}H_{20}O_{10}$	432.381	6	10	0.049	Yes
3	(S)-2-(3-(1-((4-Isopropylbenzyloxy)carbonyl)piperidin-3-yl)phenoxy)-2-methylpropanoic acid	$C_{26}H_{33}NO_5$	439.552	1	4	5.568	Yes
4	2-ketobenzothiazole 54	$C_{29}H_{38}N_8O_3$	546.676	4	7	1.116	Yes
5	Salicyloylaminotriazole	$C_9H_8N_4O_2$	204.189	3	4	0.762	Yes
6	Gibberellin A7	$C_{19}H_{22}O_5$	330.380	2	4	1.912	Yes
7	N-Cyclopentyl-N-[2-(dimethylamino)ethyl]-2-(5-{[isopropyl(methyl) amino]methyl}-1-tetrazolidinyl)acetamid	$C_{17}H_{37}N_7O$	355.531	3	7	-0.185	Yes

Table 2. ADMET analysis profile of the seven potential-bioactive compounds

Chemical Formula	Absorption			Distribution			Excretion	Toxicity	
	Caco-2 (log Papp in 10^{-6} cm/s)	Intestinal Absorption (% absorbed)	Skin Permeability (log Kp)	VDess (log L/kg)	BBB Permeability (log BBB)	CNS Permeability (log PS)	Total Clearance (log mL/min/kg)	Oral Rat Acute Toxicity (LD_{50} = mol/kg)	Oral Rat Chronic Toxicity (LOAEL = log mg/kg_bw/day)
$C_{15}H_{21}NO_2$	1.671	95.134	-2.575	0.666	0.310	-2.119	0.911	2.737	1.559
$C_{21}H_{20}O_{10}$	0.330	37.609	-2.735	0.342	-1.391	-3.746	0.547	2.595	4.359
$C_{26}H_{33}NO_5$	0.666	88.283	-2.701	-0.584	-0.005	-1.973	0.49	2.889	0.909
$C_{29}H_{38}N_8O_3$	-0.315	55.327	-2.735	-0.006	-0.741	-3.769	0.566	2.481	3.346
$C_9H_8N_4O_2$	0.302	88.842	-2.735	0.384	-1.467	-5.254	0.054	2.622	3.596
$C_{19}H_{22}O_5$	1.146	100	-2.735	-0.970	-0.084	-2.458	0.752	2.087	2.379
$C_{17}H_{37}N_7O$	0.808	64.162	-2.859	-0.059	0.023	-4.43	0.884	2.403	1.72

compounds showed unfavorable results with values < 0.90, indicating that the absorption of the drug compound is not good. In intestinal absorption, if the value is < 30%, it indicates that the absorption of the drug compound is not good (Pires *et al*. 2015). The analysis showed that the seven compounds have a value of >30% which means that the compound has good absorption. However, one of the compounds, apigenin-7-O-glucoside ($C_{21}H_{20}O_{10}$) showed a value of 37% which is close to 30% so that the compound has poor absorption properties compared to other compounds. The log kP value of > -2.5 indicates good skin permeability, where the drug compound is likely to easily penetrate the skin layer (Kesuma *et al*. 2018). From the results of the analysis, all compounds showed good absorption in the intestine and skin with values > -2.5.

The VDess (Volume of Distribution at Steady State) value is a parameter used to indicate drug distribution in the body, with parameters including low < 0.71 L/kg; medium 0.71 - 2.81 L/kg, and high > 2.81 L/kg (Pires *et al*. 2015). From the analysis, it was found that three compounds, including meperidine ($C_{15}H_{21}NO_2$), apigenin-7-O-glucoside ($C_{21}H_{20}O_{10}$) and salicyloylaminotriazole ($C_9H_8N_4O_2$) showed low VDess with values smaller than 0.71 (<0.71). While the rest showed a very low VDess value with a value of minus. The BBB (Blood-Brain Barrier) value is the ability of a compound or drug to penetrate the blood-brain barrier, where logBB > 0.3 indicates good penetration and logBB < -1 indicates poor penetration (Pires *et al*. 2015; Nursanti *et al*. 2022). From the analysis, it is known that of the seven compounds, only the meperidine ($C_{15}H_{21}NO_2$) compound shows good results with a value > 0.310. While other compounds showed unfavorable results so that the compound could not penetrate the blood-brain barrier. The CNS (Confident Number System) value is similar to the BBB value, with the condition that the logPS value > -2 indicates good permeability, and logPS < -3 indicates poor permeability (Pires *et al*. 2015). From the analysis, it is known that of the seven compounds only three compounds showed good logPS results > -2 including the compounds meperidine ($C_{15}H_{21}NO_2$), (S)-2-(3-(1-((4-Isopropylbenzyloxy) carbonyl)piperidin-3-yl)phenoxy)-2-methylpropanoic acid ($C_{26}H_{33}NO_5$) and gibberellin A7 ($C_{19}H_{22}O_5$), while the rest showed unfavorable results.

Clearance is a pharmacokinetic parameter used to measure the ability of an organism to clear or eliminate a drug. Total clearance indicates that an organ or tissue can permanently clear the compound or drug from the organism either through the liver or kidneys. Normal total clearance is in the range of 0.5 - 0.2 mL/min/kg (Talevi & Bellera 2021) and from the results of the analysis it is known that all compounds show total clearance which is within the normal range. In addition, oral rat acute toxicity displays the lethal dose (LD_{50}), a parameter commonly used to evaluate the acute toxicity of a compound. LD_{50} is the amount of compound administered at once that causes 50% mortality of a group of test animals. he average LD_{50} value is about 2.5 mol/kg and the higher the value of LD_{50}, the lower the toxicity of the compound (Pires *et al*. 2015). From the analysis, the gibberellin A7 ($C_{19}H_{22}O_5$) compound has the lowest LD_{50} value of 2.087 mol/kg, indicating that the compound has higher acute toxicity than other compounds. Whereas chronic toxicity (LOAEL) is done to identify the lowest dose of a compound that can produce adverse effects. This dose can be done in the low-medium range over a long period of time. Higher values are generally safer at around > 3.0 mg/kg bw/day for long-term exposure (Pires *et al.* 2015). From the analysis, the compound apigenin-7-O-glucoside ($C_{21}H_{20}O_{10}$) showed the most favorable results because it showed adverse effects that only appeared at higher doses.

Receptor Analysis

In this molecular docking study, the VEGFR-2 protein with the PBD code 4ASE was used. The protein was obtained from the RCSB PBD database (https://www.rcsb.org/). The protein used is a protein derived from the Homo sapiens with a resolution of 1.83 Å. Then, the protein was analyzed using the PDBsum website (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/). A Ramachandran plot will be produced from the analysis's findings. The Ramachandran plot is a two-dimensional figure with four quadrants that shows the amino acid residues in the structure of the enzyme. The x-axis has an angle φ (phi) and the y-axis has an angle Ψ (psi). The protein model's stereochemical quality is evaluated using the φ (phi) and Ψ (psi) angles. The presentation of amino acids in the protein model's most favored and prohibited regions (disallowed regions) from the Ramachandan plots serves as the basis for this type of protein evaluation (Laskowski *et al*. 1993).

From the analysis results on the Ramachandan plot, it can be seen that the protein used is good. Seen in quadrant I there are 91.7% amino acid residues, in quadrant II 8.3%, in quadrant III 0.0% and in quadrant IV as much as 0.0% (Figure 1).

Molecular Docking

Molecular docking validation was performed using the principle of re-docking between protein receptors and the materials used in this study include 3D test compounds, which were saved in PDB format, along with the structure of the VEGFR-2 receptor (PDB ID: 4ASE), obtained from the respective database web servers (Figure 2). The research utilized both hardware and software tools. The hardware used was a Lenovo PC IdeaCentre AIO 5i 24IAH7 F0GR006RID Storm Gray, equipped with an Intel Core i7-12700H processor, Windows 11 Home, 16GB DDR4 RAM, and an Intel ARC

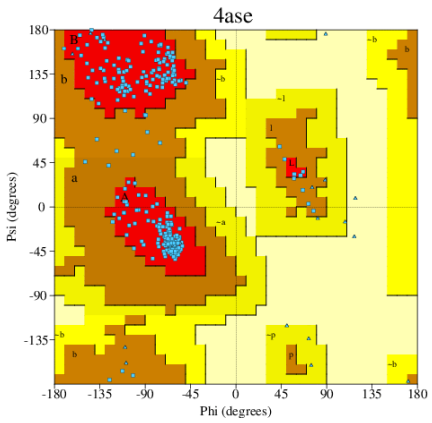
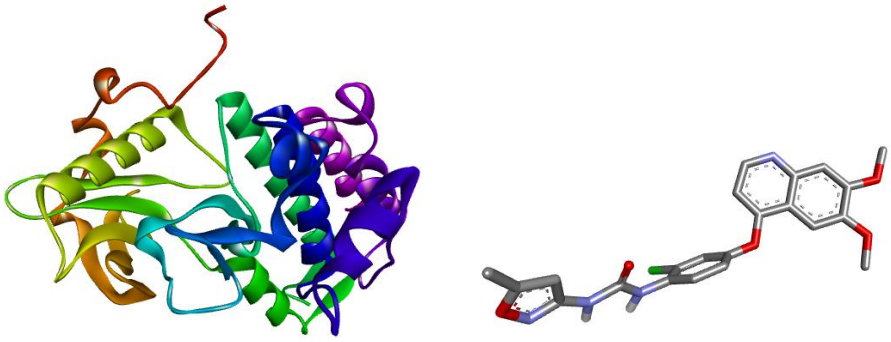


Figure 1. Plot ramachandan protein VEGFR-2 (code PDB: 4ASE)



a b

Figure 2. (a) Receptor VEGFR-2 (kode PDB: 4ASE) and (b) Native ligan (Tivozanib)

A370M 4GB GDDR6 GPU providing sufficient computational power for molecular docking and network pharmacology analyses. The software employed included PyRx, ChemDraw Ultra version 22.0, Chem3D version 22.0, AutoDockTools, Discovery Studio Visualizer 2021, and UCSF Chimera. Additionally, the Research Collaboratory for Structural Bioinformatics (RCSB) database was utilized as the primary web server for structural data retrieval., the aim of which is to obtain the appropriate grid box size in order to obtain an RMSD value of < 2 Å. This validation process was carried out using the Autodock Tools software. The results of re-docking validation obtained grid box sizes x, y, and z are 40, 30, and 40 with x, y, and z coordinates of -22.651, 0.651, and -10.261. From the results of the grid box size and coordinates used, the RMSD value of the redocking results is 0.305 Å; this RMSD value is smaller than 2 Å, which means that the validation results are valid (Nursanti *et al*. 2022).

The VEGFR-2 (Vasecular Endothelial Growth Factor Receptor 2) protein receptor is a tyrosine kinase receptor consisting of 1356 amino acids and has a very important role in the development and spread of cancer cells, one of which is in breast cancer cells. This protein is a transmembrane receptor that can bind to growth factors that play a role in the angiogenesis process in tumor cells. VEGFR is part of the tyrosine kinase receptor superfamily consisting of 3 subtypes, namely, VEGFR-1 (Flt-1), VEGFR-2 (KDR), and VEGFR-3 (Flt-4) (Modi & Kulkarni 2019).

VEGFR-2 has a role as the primary receptor that regulates the VEGF (Vascular Endothelial Growth Factor) process that occurs in endothelial cells, namely cells that line blood vessels. The activity of the VEGFR-2 receptor by VEGF will trigger endothelial cell proliferation and increase the permeability of blood vessels, which is an essential process in angiogenesis (Guo *et al*. 2010). In addition, another role of VEGFR-2 is to regulate the development of blood vessels from precursors during the embryogenesis process, can help the formation of blood vessels, and is able to increase vascular permeability in the angiogenesis process (Wang *et al*. 2020).

Molecular Docking Visualization and Analysis

From the results of the docking analysis on the test compounds, one compound was obtained that had quite good interactions. The docking results between the native ligand (Tivozanib) and the receptor showed promising results with free energy values and inhibition constants of -12.76 kcal/mol and 440.70 µM. The docking results on the seven test compounds obtained one compound with quite good results, namely the meperidine compound, which has

Table 3. The result of molecular *docking*

Bioactive Compounds	Free Energy (kcal/mol)	Inhibition Constant (μM)	Hydrogen Bond	Amino Acids Residu
Native Ligan (Tivozanib)	-12.76	440.70	Ala866	Lys920, **Phe918, Val848, Cys919,** Phe1047, Ala866, Glu917, Val914, Val867, Ile915, **Lys868, Val916, Leu889,** Glu885, Asp1046, **Val899, Cys1045, Leu1035,** Leu840, Gly922
Meperidine	-7.39	3.83	Val914, Ala866, Asp1046	Val867, **Val899, Cys919,** Ala866, Val916, Val914, **Cys1045, Leu1035,** Phe918, Glu917, Asp1046, **Phe1047, Ala1050, Lys868, Val848**
Apigenin-7-O-glucoside	-6.50	17.18	Cys919, Asp1046	Phe918, Ala866, Leu840, **Phe1047, Val848,** Lys868, Val914, Glu885, **Leu889, Val899, Cys1045,** Ile1044, Val916, Asp1046, Leu1035, Gly922, Cys919
(S)-2-(3-(1-((4-Isopropylbenzyloxy)carbonyl) piperidin-3-yl)phenoxy)-2-methylpropanoic acid	-5.72	64.29	Lys868	Asp1046, Lys868, **Ala1050, Val848,** Gly841, **Leu1035, Leu840, Ala866, Val899, Val916, Cys1045,** Phe918, Glu917, Cys919, Gly922, **Phe1047**
2-ketobenzothiazole 54	-6.85	9.46	Ile1044, Glu885, Asp1046	**Ala866, Lys868, Val848,** Val914, Val898, Leu1019, His1026, Ile1044, Glu885, Val899, Val867, **Val916,** Leu889, Leu901, Cys1045, Asp1046, Phe1047, Leu11035, Leu840, Cys919
Salicyloylaminotriazole	-1.34	104.96	Glu917, Cys919, Asp1046	Lys868, Asp1046, **Val899, Cys1045,** Phe1047, Val916, Glu917, **Cys919,** Phe918, **Val848, Leu840, Ala866,** Leu1035
Gibberellin A7	-5.10	182.49	Ala866, Val914, Glu885, Lys868	Phe918, Glu917, Ile915, Val867, Ala866, Val914, Glu885, Lys868, **Val848, Leu840, Leu1035, Phe1047, Val916, Cys1045, Val899, Cys919,** Asp1046, Leu889
N-Cyclopentyl-N-[2-(dimethylamino)ethyl]-2-(5-{[isopropyl(methyl)amino] methyl}-1-tetrazolidinyl)acetamid	-6.36	21.68	Asp1046	Ala1050, Glu885, Asp1046, **Lys868, Cys1045,** Phe1047, **Val848, Ala866, Leu840, Leu1035,** Val867, Glu917, Gly922, Phe918, Cys919, Val899, Val916, Leu889

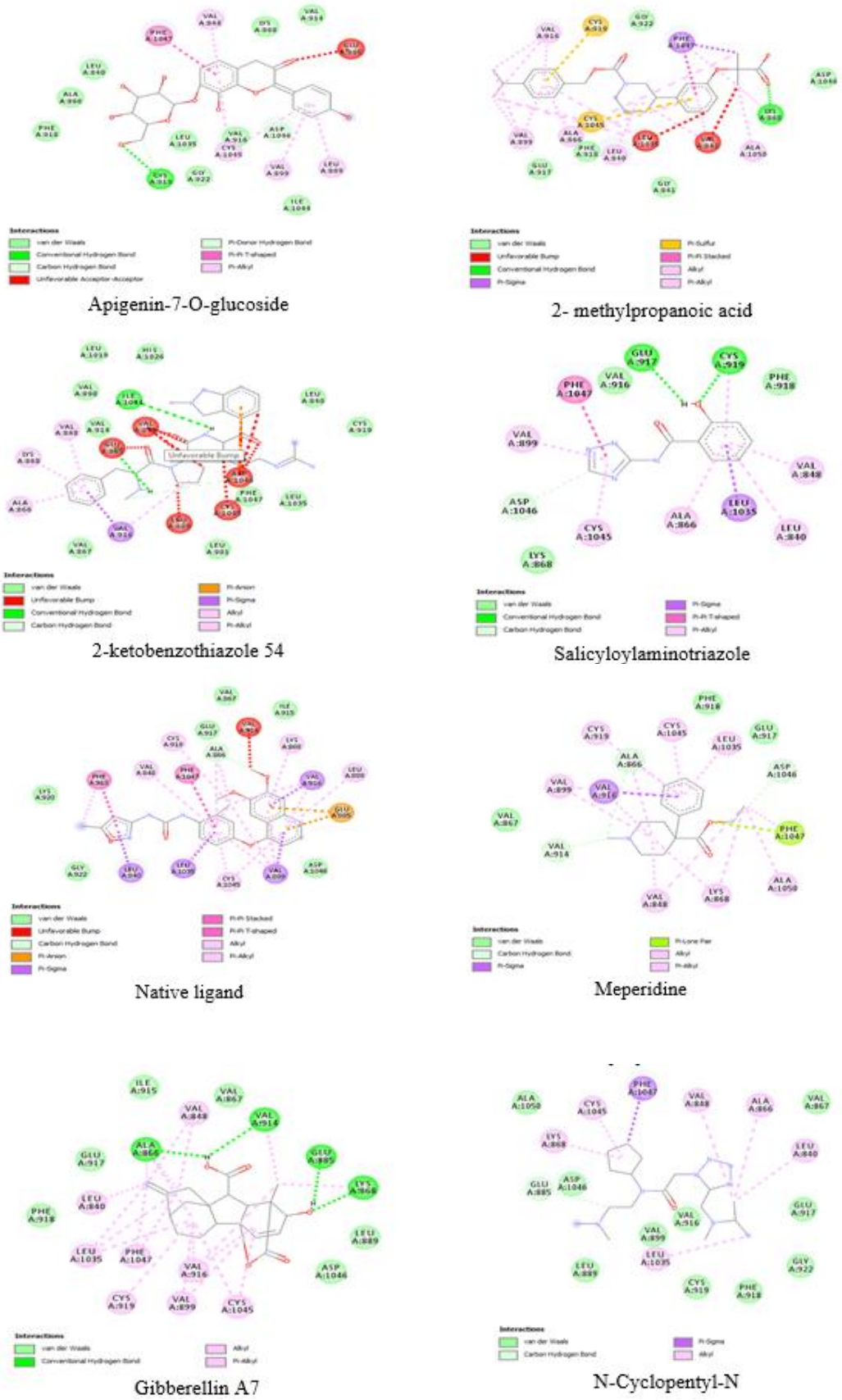


Figure 3. 2D visualization of docking results of native ligands and test compounds on the VEGFR2 receptor

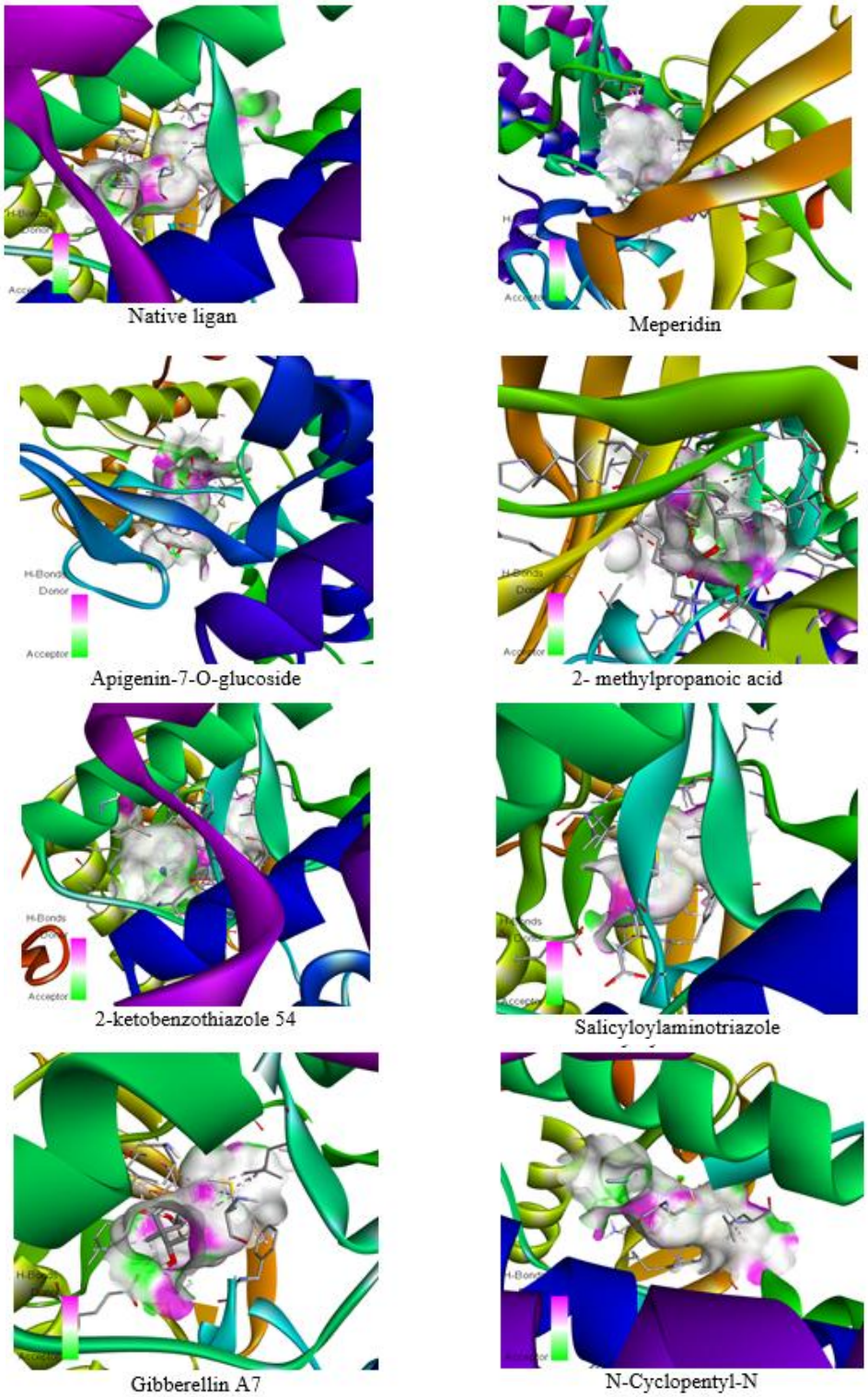


Figure 4. 3D visualization of docking results of native ligands and test compounds on the VEGFR2 receptor

free energy values and inhibition constants of -7.39 kcal/mol and 3.83 µM (Figure 3, Table 3). The free energy value in the docking results shows the strength of the bond between the ligand and the protein receptor, where the lower the free energy value, the more stable the bond between the ligand and the receptor will be. This is directly proportional to the inhibition constant value; with the smaller the inhibition constant value, the better the docking results obtained (Thafar *et al*. 2019).

In addition to free energy and inhibition constants, the results of molecular docking visualization also show hydrogen bonds and amino acid residues (Figure 4). The docking results on the

native ligand and VEGFR-2 receptor show one hydrogen bond, namely Ala866, and 20 bound amino acid residues. Based on previous research (Kajal *et al.* 2019), it was stated that amino acid residues that play a role in the design of a drug candidate must be targeted with ATP and VEGFR-2 inhibitor activity, such as hydrogen bonds with Glu917 and Cys919 residues and hydrophobic interactions (Glu885 and Asp1046). Meanwhile, in the test compound, two compounds show the presence of hydrogen bonds and amino acid residues that are the same as the native ligand, namely meperidine and gibberellin A7 compounds. In the meperidine and gibberellin A7 compounds, there are 3 and 4 hydrogen bonds, one of which is Ala866, and they have 15 and 18 amino acid residues, including Glu917, Cys919, Glu885, and Asp1046. The presence of an active site on the ligand that binds to a target receptor plays a crucial role in determining its inhibitory potential against VEGFR-2 (Izzaturahmi *et al*. 2023). In this context, refers to the strength of the interaction between the test compound and the VEGFR-2 receptor, which should ideally be comparable to that of its native ligand, Tivozanib. A high-affinity interaction suggests that the test compound can effectively compete with Tivozanib for the same binding site, potentially leading to a similar inhibitory effect on VEGFR-2 activity. This inhibition occurs through interactions with key amino acid residues within the receptor's active site, which are essential for its function. Therefore, if the test compound exhibits strong binding affinity and engages with the same critical residues as Tivozanib, it may serve as an effective VEGFR-2 inhibitor, contributing to the development of novel anti-cancer therapeutic.

CONCLUSION

Docking simulations between the native ligand (Tivozanib) and the receptor yielded promising results, with a free energy value of -12.76 kcal/mol and an inhibition constant of 440.70 μM. Among the seven test compounds, meperidine demonstrated favorable outcomes, showing a free energy value of -7.39 kcal/mol and an inhibition constant of 3.83 μM. Additionally, the meperidine and gibberellin A7 compounds formed three and four hydrogen bonds, respectively, including one with Ala866, and interacted with 15 and 18 amino acid residues, such as Glu917, Cys919, Glu885, and Asp1046. The presence of active sites on the ligand or test compounds that bind to the target receptor indicates a potential for comparable affinity in inhibiting VEGFR-2 receptor activity.

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