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In-Silico Analysis Of Maricaffeoylide From *Avicennia Marina* Using Molecular Docking With Tumor Necrosis Factor Receptor

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ABSTRACT

Introduction. Bioactive metabolites was isolated from *Avicennia marina* fruit belongs to a new caffeoyl derivative, maricaffeoylide. It was a class of phenolic compounds such as caffeic acid which had the potential as anticancer, anti-inflammatory, and antioxidant activity. One of proinflammatory cytokines that play a role in regulating immune cells against cancer is TNF-α. In-silico analysis is often used to molecular docking. It was carried out to determine the interaction molecule between bioactive compound and receptor. The purpose of this study is to analysed of interaction maricaffeoylide and TNF-α receptors using molecular docking. In this study, molecular docking will be carried out to determine the interaction and affinity of the bond energy. **Methods.** The method used in this research is experimental which is carried out in silico using molecular docking. Maricaffeoylide was prepared by using Marvin Skusing Marvin Sketch, and the downloaded from pdb. Molecular docking was carried out ligand and receptor preparation, validation, molecular tethering using *Pyrx-Autodock Vina,* and visualization by BIOVIA. **Result.** The result of the conformation of the maricaffeoylide has an energy of 69.77 kcal/mol. The gridbox is adjusted at center coordinates x = 14.85; y = -6.50; and z = -6.70 and at dimensions x = 31, y = 35, and z = 63 Å. The Lipinski's rule for drug-likeness was confirmed. **Conclusion.** Molecular docking of maricaffeoylide against TNF-α receptors was successfully performed with a docking score of -6.1 kcal/mol involving the interaction van der Waals, hydrogen, alkyl, and carbon hydrogen.

1. Introduction

Cilacap has many diverse mangrove forests, one of which is *Avicennia marina*. Plants of the genus *Avicennia* have shown the presence of iridoid glucosides, naftoquinone derivatives, flavonoids, and diterpenoids[1]. The bioactive metabolites of *A. marina* fruit have led to the isolation of a new caffeoyl derivative, maricaffeoylide, and a new megastigma derivative, diendiol maricyclohexene[2].

Maricaffeoylide are caffeic acid derivatives with antioxidant, free radical antidote, and anticancer properties[3]. Its structure belongs to the phenolic group, where the use of phenolic compounds, especially caffeine acid, is limited. Since the solubility of caffeine acid in hydrophilic and lipophilic media is poor, which can affect its absorption, it is necessary to modify the caffeoyl group using some more interesting molecules, for example alkyls and glycerides.

The caffeic acid derivative, maricaffeoylide, has a caffeoyl group. Based on research from Rezaei-Seresht[4], caffeic acid influences the apoptosis gene in breast cancer cells. In this study, ligand interactions and bindings were carried out on alpha-estrogen receptors by molecular docking. Caffeic acid has induced toxic effects, and there have been morphological changes in breast cancer cells that can indicate the possibility of being antitumor. Tumor necrosis factor is one of the agents that regulate immune cells against cancer.

Tumor necrosis factor is an inflammatory cytokine involved in the pathology of various human diseases such as autoimmune disorders, insulin resistance, and cancer. Tumor Necrosis Factor-alpha (TNF-α) helps regulate immune cell processes and cytokine activation. TNF-α is a family of membrane proteins that act as communication pathways that activate cell death pathways or induce the expression of genes involved in cellular differentiation and survival. These receptors work in many organ systems, especially the immune system. TNF-α regulates the development of the immune system and initiation of inflammatory reactions for effective host defense against pathogenic viruses and bacteria. Thus, TNF-α is an important molecular target for drug intervention[5].

The discovery or development of new drugs has become easier as technology has advanced. Advances

in science and technology can be used as opportunities in the development or design of new drugs so that they begin to limit the treatment of test animals because it is time-consuming and costly, and a code of ethics is needed for the use of test animals. In silico, because of its low cost and faster results, began to be considered. In silico is a research method that utilizes computational technology and databases to develop further research[6]. In-silico analysis is often used is the molecular docking. This method is carried out to determine the interaction of a compound with the target molecule, one of which is a receptor[7].

The purpose of this study is to analyzed the interaction of maricaffeoylide and TNF-α receptor using molecular docking. In this study, molecular docking will be carried out to determine the interaction and affinity of the bond energy so that the chemical content of the active compound can be used as a basis for the discovery of drugs that are predicted to have potential anticancer candidates.

2. Methods

This research is experimental research using *the in-silico method.* The tests carried out are ligand preparation, receptor preparation, validation, molecular tethering using *Pyrx-Autodock Vina,* and visualization by BIOVIA *Discovery Studio Visualizer*.

The hardware used in this study is an Asus-X441M laptop with an Intel® insideTM N-4000 CPU running at 2.6 GHz and 4.00 GB of RAM. The software used is Pyrx-Autodock and Vina, Marvin Sketch, and BIOVIA *Discovery Studio Visualizer*.

The molecule used in the *in-silico* study are the structures of natural compounds that have the potential to be anticancer in the *A. marina* plant, namely maricaffeoylide prepared using the Marvins Sketch and macromolecules downloaded from the *Protein Data Bank* (https://www.rcsb.org/). Infliximab (INF) is a chemical compound which downloaded from the *Protein Data Bank* (https://www.rcsb.org/). TNF-α inhibitor compounds were also downloaded from Pubchem (https://pubchem.ncbi.nlm.nih.gov/).

3. Results

The result of the conformation of the maricaffeoylide has an energy of 69.77 kcal/mol. The 3D structure of the macromolecules downloaded on the *Protein Data Bank* has bound chains or ligands. Validation of tethering is done with *the gridbox* settings. *The gridbox* is adjusted at center coordinates x = 14.85; y = -6.50; and z = -6.70 and at dimensions x = 31, y = 35, and z = 63 Å. It was followed by a bolt using the *Pyrx-Autodock Vina* so that an RMSD (*Root Mean Square Deviation*) was generated. The RMSD value itself is the main indicator of the docking validation process, where the smaller the RMSD shows the position of the atom which is getting closer to the original position before the docking process is carried out[8].

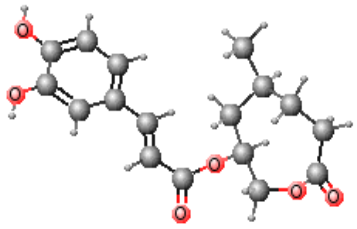

Figure 1. Maricaffeoylide Conformation

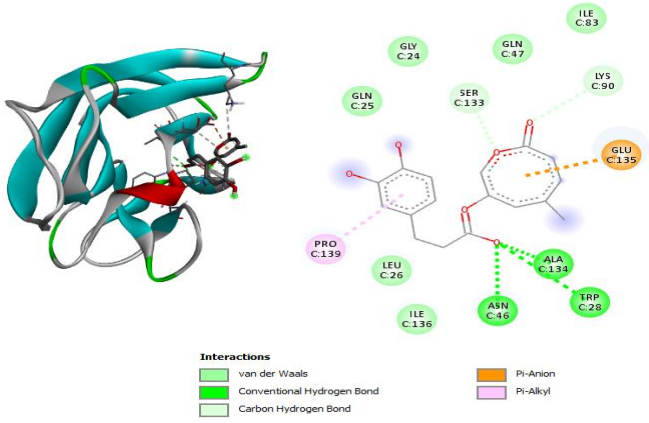

Figure 2. Interaction Of Maricaffeoylide With TNF Receptors

Table1. Results Of Maricaffeoylide Molecular Tethering To TNF Receptors

Chemical compound	Docking score	Bond	Residue
Maricaffeoylide	-6,1	Van der waals, hydrogen bond, hydrogen carbon bond, pi anion, pi alkyl	**GLN C:25**; **GLN C:47**; GLY C:24; **SER C: 133**; LYS C:90; **GLU C:135**; **PRO C:139**; **LEU C:26**; **ILE C:136**; **ASN C:46; ALA C:134**; TRP C:28

Table 2. *Lipinski Rule of Five* Test Results

No	Parameters	Score	Condition
1	Molecular weight	368	< 500 Da
2	Log P	0,54	Log P <5
3	Donor H	0	< 5
4	Acceptor H	5	< 10
5	Molar Refractivity	87,8	40 - 130

The Lipinski's rule for drug-likeness was originally proposed in response to the large number of randomly made compound libraries[9]. The test is used to determine the physicochemical properties of ligands and determine the hydrophobic/ hydrophilic character of a compound through cell membranes. Log P is related to lipophilic or hydrophobicity of drug molecules, namely the ability of a chemical compound to dissolve in fat solvents, oils or non-polar solvents because when the compound is easily soluble in lipids, it makes it easier for the compound to penetrate the biological membranes of the bod[10]. The greater the value of Molar Refractivity, the better the permeability of the compound. In general, *Lipinski's Rule of Five* describes the solubility of certain compounds to penetrate cell membranes by passive diffusion[11].

4. Discussion

The chemical structure of a drug generally consists of the structure of the core and the side chains. The core structure can be cyclic, heterocyclic, or polycyclic ring-shaped. The side chain (R) is aliphatic, cyclic, or heterocyclic. The side chain acts as a minor group for determining the biological activity and chemical physical properties of the drug. The biological activity of drugs is also influenced by the physical properties of drug molecules such as in the process of drug distribution and drug interactions with receptors. The process of drug distribution with biological membrane penetration is influenced by the lipophilic properties of drug molecules, solubility, degree of ionization, and pH. Cell-penetrating peptide (CPP) can directly penetrate the cytosol (cytolysis) and is expected to be a potent vector for a drug delivery system (DDS). Although there is general agreement that CPP cytolysis is related to dynamic membrane deformation, a distinctive process has yet to be established. Here, we report the key process and factors controlling CPP cytolysis[12].

Previous chemical investigations in the genus Avicennia have shown the presence of iridoid glucosides, naftoquinone derivatives, flavonoids, and diterpenoids[1]. Further chemical investigations for bioactive metabolites of the mangrove fruit *Avicennia marina* led to the isolation of new caffeic acid derivatives, maricaffeolyide and maricyclohexene. The structure of the maricaffeoylide contains an 8-membered ring lactone saturated with two chiral centers[2].

Macromolecules as tethering targets are searched through the Protein Data Bank (PDB). The identity structure downloaded on PDB is a 4G3Y crystal structure. 4G3Y is a structure crystal of TNF-alpha downloaded bound to an infliximab fragment or TNF inhibitor ligand. The choice of 4G3Y identity is because the TNF-alpha structure has bound to the monoclonal antibody drug, namely infliximab which has been widely used to treat tumor necrosis factor (TNF) related diseases during more than 10 years. For resolution, 4G3Y has a considerable resolution value (2.60Å) and has three subunits[13].

The original ligand used was TNF-α inhibitor which is a drug that suppresses the physiological response to TNF-α which is part of the inflammatory response. TNF-α is not only produced by activated macrophages, but also in monocytes, fibroblasts, *mast* cells, and *NK cells*, which are responsible for inducing signals in the immune formation pathway related to the formation of inflammatory factors[14].

The three-dimensional structure of the macromolecules downloaded on the *Protein Data Bank* has bound chains or ligands. The macromolecule that will be used in the molecular docking process is the *Tumor Necrosis Factor*. Macromolecules are cleared of ligand structures tethered to those macromolecules when downloaded from the PDB site. Optimization of the structure needs to be done because of the presence of several characters that can interfere with the molecular tethering process. Optimization is carried out by removing solvent molecules or solvents, namely water so as not to interfere with the molecular

tethering process. After the removal of water molecules, the addition of hydrogen atoms is necessary because the presence of hydrogen atoms can affect the results of molecular interactions.

Validation is carried out in the absence of water because water will block the ligand with its receptors, water can form hydrogen bonds with receptors[15]. Root Mean Square Deviation (RMSD) indicates the distance of atoms in a conformation, the smaller the RMSD value, the better the ligand position because it is close to the conformation of the ligand. The value of RMSD depends on the interaction of bonds and energy between proteins and ligands, the smaller the RMSD value, the more similar the structure of the reacted ligand. The RMSD value received is less than two[8].

In this study, testing of *maricaffeoylide* and TNF-alpha inhibitor was carried out by tethering these compounds with tumor *necrosis factor* (TNF) receptors. The file format of *maricaffeoylide* and TNF-α inhibitor will be processed automatically by the open babel program to protein data bank, partial charge, and atomic type (pdbqt) format, then by setting the *gridbox* equal to validation, the tethering process is carried out on whole test ligand. The result of tethering is the value of RMSD and gibbs free energy (ΔG), as well as ligand conformation with the file format, pdbqt.

Infliximab is a chimeric antibody found in the human IgG region. Infliximab is known for its ability to neutralize the biological activity of TNF[13]. The interaction between TNF-Infliximab interacts with only one TNF molecule in a complex structure with a high affinity between infliximab and TNF is 1.977. The interaction that occurs between alpha TNF and infliximab is hydrogen bond, and van der waals bond, which connects the alpha TNF molecule with infliximab, this indicates a strong and stable interaction between the two proteins and may explain their high binding affinity.

The docking score or bond-free energy describes the strength or affinity of the bond resulting from the interaction of ligands and receptors, in the form of low energy in the formation of receptor drug complexes. The binding energy of the *scoring* results is in the form of Gibbs free energy (ΔG). These data demonstrate the stability of ligand and receptor interactions at the *binding site*. If $\Delta G < 0$ the reaction runs spontaneously (the reaction goes to the product). $\Delta G = 0$ reversible running reactions. If $\Delta G > 0$ the reaction does not occur (the reaction goes towards the reactants). The smaller the ΔG value, the stronger the bond that occurs between the ligand and the receptor, and the more stable it is. Because the compounds maricaffeoylide and TNF-alpha Inhibitors are negative which means that the reaction can occur and run spontaneously (the reaction goes towards the product) so that the bond between *the* maricaffeoylide test ligand and TNF-α receptor becomes stable. The value of ΔG indicates the magnitude of the energy released by a compound to interact or form bonds with its receptors. The smaller the number or the larger the minus, the more energy is used to form the bond so that the bond is stronger.

Table 3. Interaction TNF-A And Infliximab With Receptor

TNF-α		INFLIXIMAB	
Residue	**Atom**	**Residue**	**Atom**
Gln-67	C^8	Ile-56	C
	O	Ser-53	O
	N	Ser-55	O
Pro-70	C	Ser-105	O
Ser-71	C	Tyr-50	OH
His-73	C	Ser-105	C
Thr-105	O	Tyr-103	O
Glu-107	N	Tyr-50	C
	C	Tyr-102	OH
Ala-109	O	Tyr-103	OH
Glu-110	C	Asn-31	N
Asn-137	O	Trp-94	N
Asn-137	C	Ser-93	C
	N		O
Arg-138	C	His-92	O
	NH_1		C
Asp-140	O		N
Tyr-141	C	Ser-91	O
	OH	Trp-94	C

Visualization and analysis of the interaction of docking results was carried out to see the results of tethering between the comparison ligand and the test ligand used. The result of this visualization is the interaction of amino acid residues against ligands. The presence of amino acid interactions involved allows for contact between the comparison ligand and the test ligand used. The result of this visualization is the interaction of amino acid residues with ligands. The existence of these interactions allows contact between ligands and receptors so that they have inhibitory activity.

5. Conclusion

Molecular docking of maricaffeoylide against TNF-α receptors was successfully performed with a docking score of -6.1 kcal/mol involving the interaction van der Waals, hydrogen, alkyl, and carbon hydrogen.

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