Analysis of Absorption and Molecular Docking of Curcumin Compounds As Inhibitors Of NF-Kb For In Silico Anti-Breast Cancer Drug Candidates

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Abstract: Molecular docking of curcumin ligands and the NF-Kb protein, as well as understanding the Lipinski rule of five to describe these molecules, can allow them to move across cell membranes via passive diffusion. The goal of this research is to see if curcumin molecules may be employed as breast cancer treatments. The in silico method with molecular docking was used, as well as tethering curcumin ligands that have the potential to be NF-Kb inhibitors, using several programs such as Autodock Vina (PyRx), Yasara, PyMol, and Discovery Studio 2019, as well as tethering curcumin ligands that have the potential to be NF-Kb inhibitors. Gbind had the greatest value of -6.2 kcal/mol when docked with the target protein Nuclear Factor Kaffa Beta (2DBF). The Lipinski rule of five test was used to determine the absorption qualities of substances in the body using these potential ligands. Lipinski rule of five molecular weight 368 g/mol 500 g/mol, hydrogen bond donor 2 5, hydrogen bond acceptor 6 10, Log P 3.369898 > 5, and molar refraction 102.016571 in the 40-130 range. Curcumin satisfies Lipinski's criteria and hence has the potential to be classified as a drug.

Keywords: breast cancer; curcumin; in silico; lipinski; NF-Kb

1. Introduction

According to the annual increases in cancer morbidity and mortality rates, cancer cases occur all over the world. The prevalence of tumors/cancer in Indonesia grew from 1.4 per 1 million persons in 2013 to 1.79 per 1 million people in 2018, according to basic health research statistics (Riskesdas) [1].

Cancer is a broad term that refers to a set of diseases that affect humans and are characterized by the emergence of abnormal cells in the body that grow uncontrollably, eventually harming healthy cells and attacking other organs. Breast cancer is the most common cancer worldwide, according to GLOBOCAN 2020 data, and it is the second leading cause of cancer deaths [2].

With 42.1 incidences per 100 people, breast cancer is ranked 23rd in Asia and 8th in Southeast Asia. Breast cancer is the second leading cause of cancer-related death in Indonesia [3]. Breast cancer is a lump in the breast caused by a group of abnormal cells in the breast. Because of its complex character and inability to pinpoint its source, this disease has become the focus of global investigation. Observing the activation of NF-Kb can reveal one of the cancer parameters.

Kaffa Nuclear Factor Beta activation becomes a cancer apoptosis parameter, resulting in a variety of cellular functions such as increased cell preference and decreased apoptosis. NF-KB is a transcription factor that acts as a main integrator in the inflammatory response. It is found dormant in the cell cytoplasm and attached to cells [4].

NF-KB inhibitory drugs have been explored in breast cancers by a number of researchers. Curcumin is one of the chemicals with anti-cancer properties. Curcumin molecules have a variety of therapeutic actions and have long been used to cure illnesses [5].

Curcumin (diferuloylmethane) is a bioactive component of secondary metabolites found in Curcuma species of the Zingiberaceae family. It has anti-inflammatory and anticancer properties. Curcumin has been subjected to numerous modifications in order to produce stable molecules with particular activity against target proteins [6]. The growth of computers, which is the first step in developing and discovering novel medications, includes the modification of this molecule.

A computational method for identifying substances that could be employed as drug candidates is known as in silico. In silico research has a number of benefits, including lowering the number of experimental animals required in trials and serving as a value and thing alternative to in vivo and in vitro research [7]. Molecular docking is one of the in silico approaches [8].

The most optimal interaction pattern between the ligand molecule and the receptor is found using molecular docking [9]. In many parts of study in the biological and medical domains, computational methods are now increasingly important. This method's advantages can be seen in a variety of drug discovery and manufacturing processes [10]. The molecular anchoring analysis' results will demonstrate the value of the strength of the chemical's interaction with the protein structure, and will be utilized as a first step in determining a method or advantage of a compound.

It is envisaged that the results of this paper would shed light on the potential of curcumin molecules in suppressing NF-KB for breast cancer treatment.

2. Methods

2.1 Materials

The software is PyRx 0.8, Discovery Studio 2019, YASARA, and Pymol, while the hardware is a set of Lenovo laptops with Intel® InsideTM processors running Windows 10 Pro. The 2D structure of the curcumin (diferuloylmethane) ligand [11] and the 3D structure of the Nf-Kb protein [12] have been downloaded from the site.

2.2 Curcumin test ligand preparation

The curcumin (diferuloylmethane) ligand's 2D structure is downloaded as a "2D" file in the "SDF" format. The PyRx 0.8 application was used to make the ligands. Click "open babel," then "insert new item" in the right corner to add the ligand file. Click "minized chosen" to prepare the ligands, then convert to pdb*. pdbqt* has been saved.

2.3 Preparation of the NF-Kb receptor

The NF-Kb receptor is a "pdb" file with the protein code "2DBF" that can be downloaded. Keep an eye out for the Ramanchanran outlaner, the RMSD value, and the unique ligand. The YASARA application is used to prepare receptors. The prepared file is saved as a "pdb" file with the storage format "mol2-sybylmol2" and the protein code "2DBF.pdb" as the protein code.

2.4 Ligand-receptor docking has been the process of binding ligands to receptors

The PyRx 0.8 program on the Vina Wizard menu is used to dock receptor ligands. Click "Select Molecules" – "Add Macromolecule" to enter the target protein, then "open" and "add ligand" – "open" to add a ligand. The next step is to click "advance" - if you don't have active site information, select "Maximize." The "pdbqt" format is used to save the file.

2.5 Visualization of the docking result

Pymol visualization for more detailed prediction. By pressing "Open" and inserting the test ligand and target protein, the PyRx output is converted to "pdb" format along with the target protein. By selecting "Color" in the right corner, users can change the color of the ligands and proteins. To see a 3D shape, select "Surface." PDB and MOL2 formats are used to save the files.

The Biovia Discovery studio 2019 application allows for the visualization of ligand interactions. Click "open file" or (Ctrl+O) to open the PDB complex file. In the windows window, the protein will be in 3D. Click "Scripts" > "Visualization" > "Publication Quality" to edit the automatic script visualization. Protein-ligand interactions in 3D and 2D are illustrated.

3. Result

Proteins of interest : NF-kappa B p105 (ID 2DBF)
Ligand : Curcumin (diferuloylmenthane)

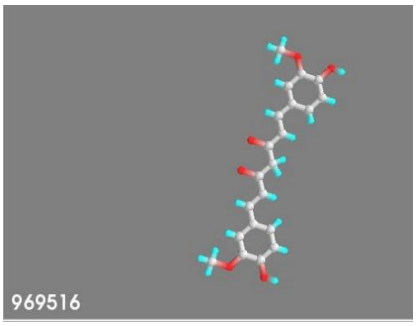

Figure a. Curcumin ligand assay preparation

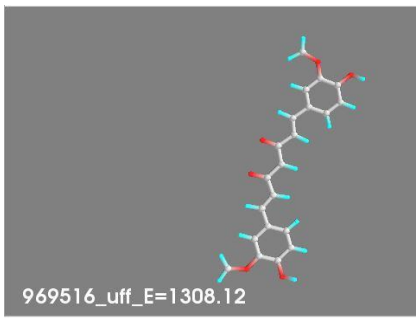

Figure b. after production of curcumin test ligand

Figure 1. Curcumin Test Ligand Visualization PyRx

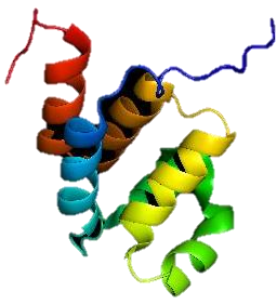

Figure a. Before preparation, the macromolecular structure of nuclear factor Kaffa Beta (2DBF).

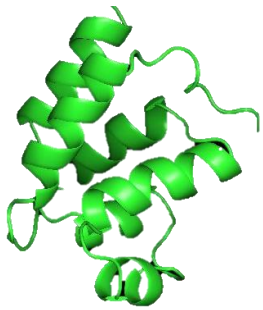

Figure b. The macromolecular structure of nuclear factor Kaffa Beta (2DBF.pdb) after processing.

Figure 2. Factor nuclear The macromolecular structure of Kaffa Beta (2DBF): visualization with PyMOL

Table 1. Results of Affinity Binding

No.	Protein	Ligand	Binding affinity	RMSD
1.	NF-kappa B p105	Curcumin	-6.2 kkal/mol	<2.00

Table 2. Lipinski rule of five as a result

No.	Parameter	Curcumin
1.	Mass	368
2.	Hydrogen bond donor	2
3.	Hydrogen bond acceptors	6
4.	Log P	3.369898
5.	Molar Refractivity	102.016571

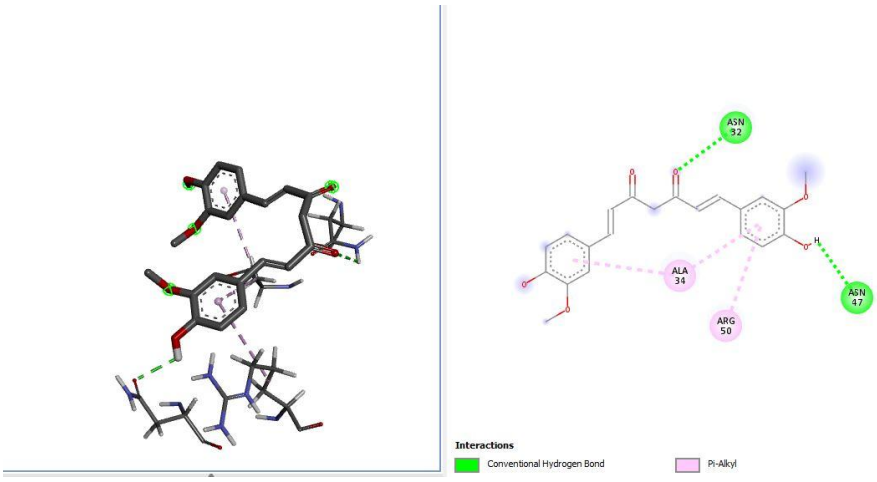

Figure 3. Visualization of interactions between receptor ligands in 3D and 2D

4. Discussion

Breast cancer is the most prevalent invasive cancer in women, and it develops from tissue in the breast. Changes in the contour of the breast, lumps in the breast, nipple discharge, and a few red scales on the skin are all signs of this cancer. Women, obesity, infrequent physical activity, alcohol, hormone release therapy during menopause, ionizing radiation, and menstrual cycles that are too fast or too slow are all risk factors for breast cancer [13].

The use of macromolecules was determined based on earlier research that found that NF-Kb is a transcription factor that regulates the production of various genes that repress apoptosis and promote tumor cell development [14]. Protein selection based on the value of the outlaner scatter as well as past research. Protein data was downloaded using the code "2DBF" from the RSCB Protein Data Bank. The structure of the nuclear factor molecule NF-kaffa-B p105 subunit in humans (Homo sapiens) found using the solution NMR (Nuclear Magnetic Resonance) method is the identity of the protein 2DBF.

The ligands selected identified based on the benefits of turmeric, which were based on many sources relating to the presence of curcuminoid molecules, specifically curcumin. Curcumin inhibits a number of inflammatory chemicals, including tumor necrosis factor. The curcumin molecule was downloaded in 2D and saved in SDF format from PubChem [15].

The Preparation ligand Figure 1. with the PyRx application on the open babel feature is used to prepare ligands with the objective of minimizing the ligand to obtain a structural conformation with lower energy than before minimization, making the anchoring process easier [16]. Because control ligands and water molecules bound to macromolecules can interfere with the tethering process, Figure 2. The protein preparation with the yasara application aims to remove them [17].

Tethering is done using the PyRx program on the Autodock vina functionality, which includes establishing proteins and ligands prepared in the "pdbqt" format. The tethering process yielded binding affinity values, bond affinity, and conformation of the prepared ligands, after which the ligands with the lowest binding affinity and the best binding affinity were saved in "sdf" format and opened later in the pymol application to view their position and orientation. The amino acids attached to the ligands, as well as the amino acid bond distances, were visible in 3D and 2D in the discovery studio.

The RMSD (Root Mean Square Deviation) of the docking data between the receptor ligands was 2.00, with a binding affinity of -6.2kcal/mol. The RMSD value was 2.00, showing strong docking conformational precision when comparing the position of the attached ligand with the native co-crystal ligand in the protein [16]. The RMSD value is used to show the compatibility between an atom in one atomic conformation of the same element in another conformation, as well as to indicate the success of the docking process and the validation of the docking program [18]. The ability of a drug to bind to a receptor is measured by binding affinity. The stronger the affinity between the receptor and the ligand, the smaller the binding affinity value; conversely, the higher the binding affinity value, the lower the affinity between the receptors [18].

The mass was 368 Da, the hydrogen bond donor was 2, the hydrogen bond acceptor was 6, the log P was 3.369898, and the molar refractivity was 102.016571, according to Lipinski test results. And based on these findings, Lipinski's [19] rule holds that if the molecular weight is 500 Da, the drug can diffuse across the cell membrane, and the larger the size of the large molecule, the slower it can diffuse across the molecular membrane, so the molecular weight is limited to 500 g/mol [19]. Membrane permeability is affected by hydrogen bond donors 5 and acceptors 10 where the drug must cross the membrane and the membrane is passed passively by the drug that diffuses across the membrane is nonpolar, therefore oral medications do not need to cross the membrane.

Figure 3. shows the results of the binding of curcumin ligands to the NF-Kb receptor seen with Discovery Studio 2019. In ASN 32 and ASN 47, hydrogen bonds are shown in green, while pi-alkyl bonds, which are marked in light purple and categorized as hydrophobic bonds, are created. ARG 50, ALA 34 [17]. The presence of hydrophobic bonds permits hydrophobic interactions between ligands and receptors to occur during the binding process, increasing the number of hydrophobic interactions between ligands in the receptor binding pocket [20].

5. Conclusion

Based on the findings of the investigation, the following conclusions can be drawn:

a) The Lipinski test findings show that the compound curcumin can be utilized as a breast cancer treatment since it meets the conditions for a molecular weight of 369 Da ≥ 500 Da, HBD 2 ≥ 5 and HBA 6 ≥ 10, log P 3.369898 ≥ 5.
b) The curcumin compound has a molecular weight of 369 Da ≥ 500 Da, indicating that the curcumin molecule can cross cell membranes; HBD 2 ≥ 5 and HBA 6 ≥ 10 indicate that the drug moves passively across the membrane; log P 3.369898 ≥ 5 determines the

hydrophobicity of the molecule; the more hydrophobic the molecule, the more toxic; log P 3.369898 ≥ 5 determines the hydrophobicity of the molecule

Conflicting Interest

All authors declare no conflict of interest.

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