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RESEARCH ARTICLE

STUDY OF MOLECULAR DOCKING AND MOLECULAR DYNAMIC OF FLAVONOL COMPOUNDS AS A B-CELL LYMPHOMA-2 (BCL-2) RECEPTOR INHIBITOR IN SMALL LUNG CANCER

Fauzan Zein Muttaqin¹, Reri Herliana¹ and Fransiska Kurniawan²

1. Faculty of Pharmacy, Bhakti Kencana University, Bandung, Indonesia.
2. School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia.

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Abstract

Lung cancer is the most common cancer which causes deaths worldwide. Excessive expression of B-cell lymphoma (Bcl-2) will increase the survival of cancer cells. Thus, the Bcl-2 expression must be inhibited. Flavonoids are often known as antioxidant benefits, especially as free radical catchers. This research aims to study the interaction of several flavonol derivative compounds with Bcl-2 receptors. In this study, we performed computational studies, including molecular docking and molecular dynamics (MDs) simulations. Molecular docking simulations showed that six flavonol derivative compounds (ligand 3, 5, 7, 8, 9, and 11) had a good affinity to the Bcl-2 receptor with a low binding free energy (ΔG) and inhibition constant (K_i) lower than 1 μ M. Ligand 8 gave the lowest binding free energy and inhibition constant of -37.739 kJ/mol and 0.246 μ M respectively. But, MDs simulations results of those six flavonol derivative compounds showed that only two of them (ligand 7 and 11) could stabilize the protein conformation. It was concluded that several flavonol derivative compounds were predicted to be able to interact strongly with Bcl-2. The results in this study are useful for further studies in the development of novel Bcl-2 inhibitors as anti-lung cancer drugs.

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Introduction:-

Cancer is a leading cause of death in more developed and less economically developed countries. Lifestyle behaviors that are known to increase cancer risks, such as smoking, poor eating habits, and physical activity. Cancer is a disease that arises due to the growth of body tissue cells that are not normal and turn into cancer cells. Lung cancer and breast cancer are the most frequently diagnosed cancers and are the leading cause of cancer deaths in men and women (Torre, et al, 2015).

Generally, lung cancer is classified into four main subtypes, namely small cell lung cancer (SCLC), squamous cell cancer (SC), adenocarcinoma (AC), and large cell carcinoma (LC). Clinically, the last three are considered non-small cell lung cancer (NSCLC). The difference between the two is that SCLC has a higher aggressiveness compared to NSCLC (Kopper, et al., 2005).

From previous research, pharmacophore- and molecular docking-based virtual screening of B-cell lymphoma 2 (Bcl 2) inhibitor from ZINC natural database as anti-small cell lung cancer, it obtained 255 potential anti-lung cancer

Corresponding Author:- Fauzan Zein Muttaqin

Address:- Faculty of Pharmacy, Bhakti Kencana University, Bandung, Indonesia.

drug candidates, some of which are flavonoids (Muttaqin, et al., 2020). Flavonoid compounds are often known to have antioxidant benefits, especially free radical scavengers. The antioxidant ability of flavonoids can reduce the formation of free radicals and capture free radicals (Pietta, 2000).

SBDD (Structure-Based Drug design) is a drug design based on a target structure based on the receptor structure responsible for the activity and toxicity of a compound in the body. SBDD utilizes information from the structure of the target protein to find the active site of the protein that binds to the drug compound. One of the stages in the process of discovering new drug compounds is the study of interactions between drug candidate compounds and receptors. This can be done in *in-silico* through molecular docking and molecular dynamic simulations (Muttaqin, et al., 2017). Docking is a method for predicting the best orientation of a molecule when bound to one another to form stable complexes. In this case, molecular docking is an initial screening method used to describe the interaction between a molecule as a ligand with a receptor or protein. While molecular dynamics (MDs) simulation is a simulation of the movements of interacting molecules. MDs simulation is a simulation technique that allows us to see the movement of molecules in a material by calculating the movements of each atom one by one per unit time.

This study aims to determine the interaction between flavonol compounds and B-cell lymphoma-2 (Bcl-2) receptors as potential anti lung cancer drug candidates through molecular dynamics stability between flavonol compound and B-cell lymphoma-2 (Bcl-2) receptors from the results of molecular docking.

Methods:-

Macromolecule preparation:

The crystal structure of B-cell lymphoma-2 (Bcl-2) was obtained from the protein data bank <https://www.rcsb.org/structure/3spfw> with PDB ID: 3SPF. The small molecules (ligands) and water molecules were removed, and polar hydrogens and Kollman charges were added.

Ligand preparation:

The structures of 12 flavonol derivative compounds were built using the ChemOffice suite of programs (Table 1). Geometry optimization and density functional theory (DFT) calculations were performed in Gaussian using the Becke three-parameter Lee-Yang-Parr functional at the 6-21G basis set.

Molecular docking:

Each ligand molecule was prepared for docking using AutoDock Tools 4.2.3 (Morris, et al., 2009). Hydrogen atoms were added, and partial charges of each atom resulted from the DFT calculations were incorporated. Grid maps were created by centering the grid box at the position of the natural ligand of each macromolecule with a spacing of 0.375 Å and size covering the binding cavity of each target. Lamarckian genetic algorithm, 100 Number of GA Runs and medium Number of Evals were used for each simulation (Morris, et al., 1998).

Molecular Dynamics (MDs) simulations:

Five ligands with the best docking score for each target were chosen for further MDs study. MD simulations were carried out using the AMBER18 program (Case, et al., 2018). Energy minimization was carried out on the macromolecules in vacuum using the steepest descent algorithm. Then, the macromolecules were solvated with transferable intermolecular potential with 3 points water molecules in an octahedron box. Positive and negative ions were added to the system at a concentration of 0.15 N to neutralize all charges. Energy minimization was again performed on the macromolecule/solvent/ion system to release strains resulted from the solvation procedure; the steepest descent algorithm was used again. Next, the system was carefully heated to 310 K and pressurized to 1 atm. Then, equilibration was performed to ensure that the system was in a constant state. The stability of the system was evaluated by analyzing the root mean square deviation (RMSD) and root mean square fluctuation (RMSF) of the protein backbones. A production simulation run was carried out on each macromolecule for 50 nanoseconds (ns). Trajectory analysis of the stability of ligand-protein interactions was performed by calculating the RMSD and RMSF values of the atoms at the protein binding sites throughout the simulation (Case, et al., 2005).

Table 1:- The 12 flavonol derivatives as ligands.

Ligand No.	ZINC code	Test Compound
1	ZINC38991932	(2S,8R)-2-(3,4-dihydroxyphenyl)-5-hydroxy-8-(2-hydroxypropan-2-yl)-6-(3-methylbut-2-en-1-yl)-2,3,8,9-tetrahydro-4H-furo[2,3-h]chromen-4-one

2	ZINC44018332	7",4"-Dimethylamentoflavone
3	ZINC85491336	(2S,3S,4S,5R,6S)-5-(((S,E)-6-(2-ethylphenyl)-3-methylhex-5-en-1-yl)amino)methyl)-3,4,5-trihydroxy-6-((3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl)oxy)tetrahydro-2H-pyran-2-carboxylic acid
4	ZINC03984030	Amentoflavone
5	ZINC28462577	Ochnaflavone
6	ZINC14727604	3-(3,4-dihydroxyphenyl)-5,7-dihydroxy-6,8-bis(3-methylbut-2-en-1-yl)-4H-chromen-4-one
7	ZINC03979028	Bilobetin
8	ZINC70455591	2-(4-(3-(5,7-dihydroxy-4-oxo-4H-chromen-2-yl)-2-hydroxyphenoxy)phenyl)-5,7-dihydroxy-4H-chromen-4-one
9	ZINC70454501	6,8'-dihydroxy-2,2'-diphenethyl-4H,4'H-[5,5'-bichromene]-4,4'-dione
10	ZINC14610232	Ananixanthone
11	ZINC85569438	3,5,7-trihydroxy-2-(4-hydroxy-3-(3-hydroxybenzyl)-5-isobutylphenyl)-4H-chromen-4-one
12	ZINC26256601	(2S,2'S)-5,5',7,7'-tetrahydroxy-2,2'-bis(4-hydroxyphenyl)-[6,8'-bichromane]-4,4'-dione

Results and Discussion:-

Molecular docking:

Twelve flavonol derivative compounds were docked onto the macromolecular target (Bcl-2, PDB ID: 3SPF). The results showed that only six ligands (3, 5, 7, 8, 9, and 11) had a low binding free energy with inhibition constant lower than 1 μM (Table 2), showing a promising sign that these ligands have good affinities toward their target.

Table 2:- Binding free energies (ΔG) and inhibition constants (K_i) of the ligands.

Ligand No.	Binding free energy (ΔG)	Inhibition constant (K_i)
	kJ/mol	μM
1	-32.259	2.25
2	-33.054	1.62
3	-36.861	0.346
4	-33.514	1.34
5	-36.903	0.343
6	-31.045	3.61
7	-35.062	0.725
8	-37.739	0.246
9	-35.815	0.528
10	-30.752	4.07
11	-34.476	0.911
12	-28.869	8.78

Ligand eight is the best ligand which binds the active site of Bcl-2 receptor with a binding free energy of -37.739 kJ/mol and the inhibition constant of 0.246 μM . It forms four hydrogen bonds towards TYR101, GLN111, LEU112, and SER122 amino acid residues; five van der Waals interactions toward GLN125, LEU130, ASN136, GLY138, and PHE146 amino acid residues; and seven pi interactions toward PHE97, ALA104, LEU108, VAL126, ARG139, and ALA142 amino acid residues (Fig. 1).

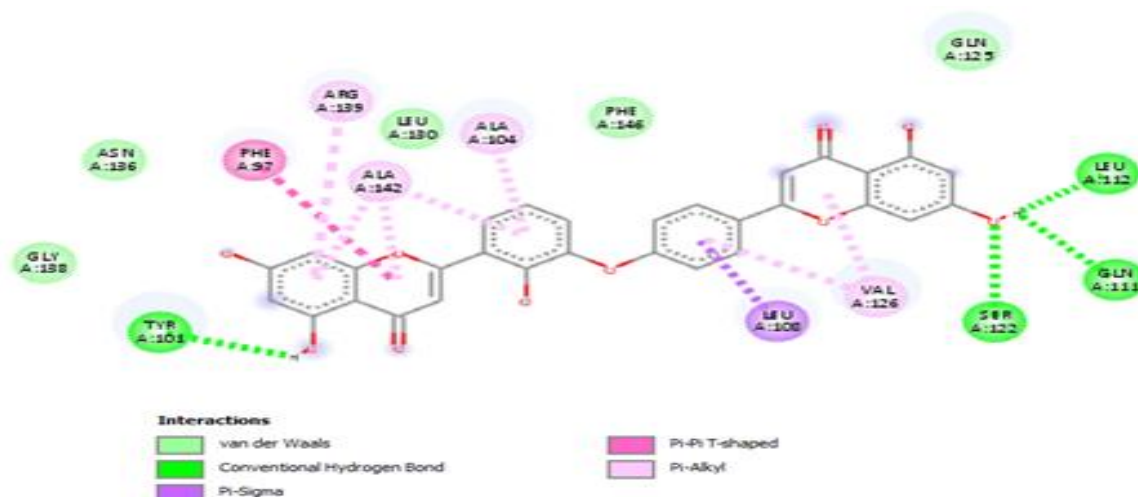


Figure 1:- Visualization of the ligand 8 interaction toward Bcl-2 receptor.

Interactions between the six ligands and the active site of the macromolecular targets comprise mainly of hydrogen bonds, with the ligands mainly acting as hydrogen bond donors and the protein residues as hydrogen bond acceptors.

Interaction dynamics:

Further studies were conducted on the six ligands with the lowest binding free energy and inhibition constant. The interaction dynamics between these ligands and their target were studied using MDs simulations with explicit solvent. The purpose of such simulations was to examine the effects of ligand binding on the residues of the protein targets, especially at the binding regions. Strong binders tend to lower the movements of the atoms they bind to, and generally stabilize the binding region of the protein. This was analyzed by calculating the RMSD of the protein binding site atoms throughout the 50 ns simulation. In addition, the overall fluctuation of each atom in the binding site was also analyzed by calculating the RMSF.

The RMSD values of the Bcl-2 protein backbone were compared to those of the Bcl-2 in complex with ligand 3, 5, 7, 8, 9, and 11. In figures 2 and 3, it can be observed that overall the system had increased RMSD backbone which showed that the structure of the protein started to open at about 1 ns at a distance of about 1 Å. An increase in the value of RMSD showed that the structure of the protein started to open and the test compound began to look for the suitable bonding site to the protein.

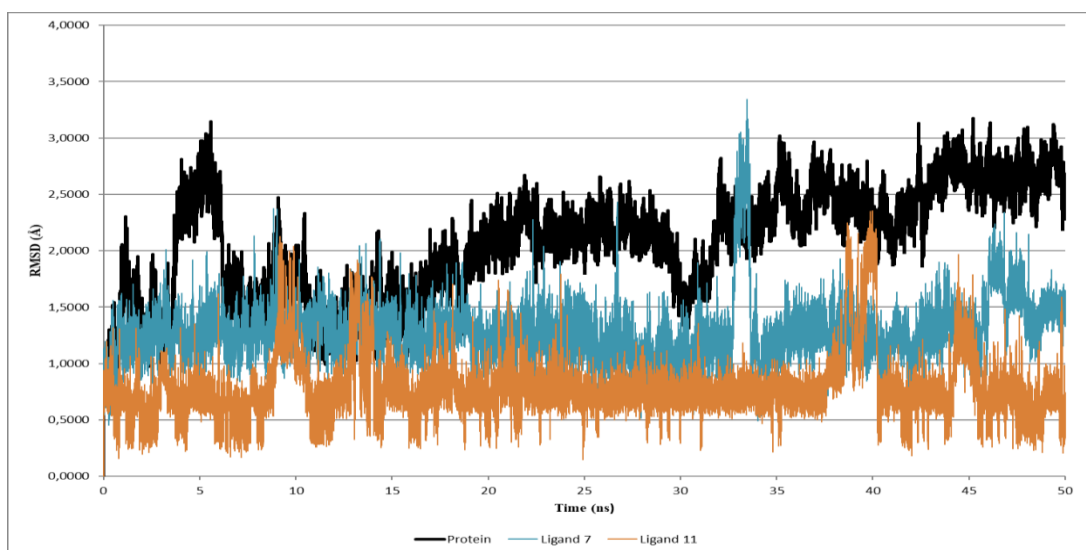


Figure 2: Backbone root mean square deviation variation of the B-cell lymphoma-2 (Bcl-2) sole protein (black), and in complex with ligand 7 (blue) and 11 (brown).

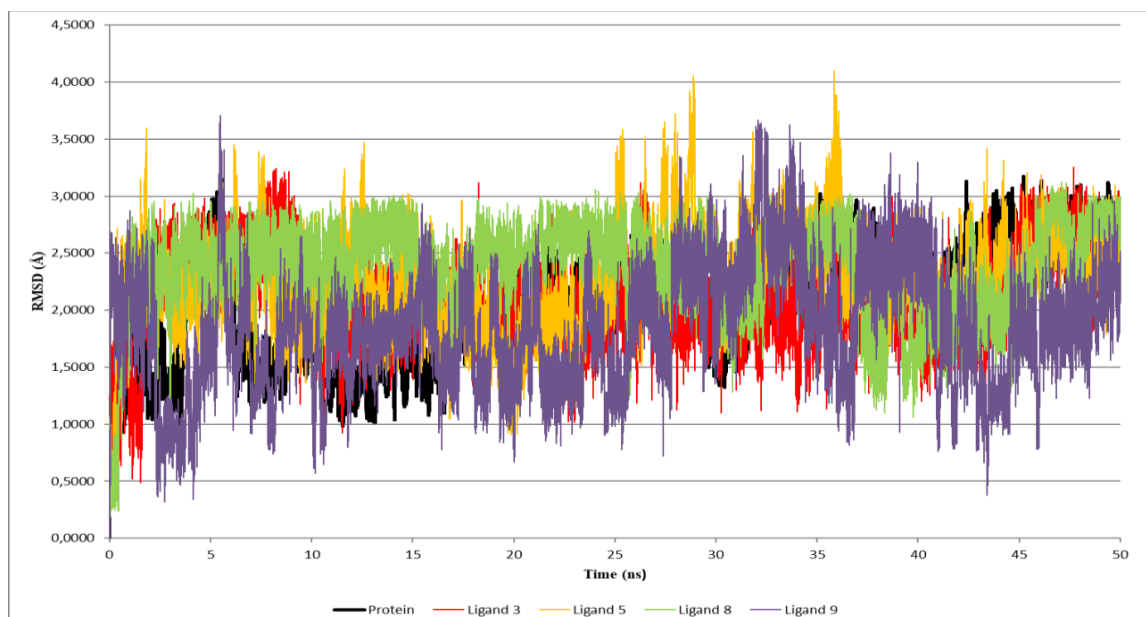


Figure 3:- Backbone root mean square deviation variation of the B-cell lymphoma-2 (Bcl-2) sole protein (black), and in complex with ligand 3 (red), 5 (orange), 8 (green), and 9 (purple).

However, it can be observed that only ligand 7 and 11 which were able to stabilize the protein, marked by lower RMSD values compared to the lone protein (Fig.2). While ligand 3, 5, 8, and 9 were still not able to stabilize the protein until 50 ns MDs simulation (Fig.3), but at the end of the simulation, the fourth complex's RMSD curve tended to decrease.

Most enzyme inhibitors work by binding strongly to the active sites of the enzymes and competing with their natural substrates, and also stabilizing the enzyme structure and prevents conformation changes that are required for the enzyme to catalyze reactions. Hence, by reducing the atomic deviations of the protein target, ligands 7 and 11 have shown the potential to act as Bcl-2 inhibitors. It was also supported by examining the RMSF values of the binding site atoms of the enzyme (Fig. 4).

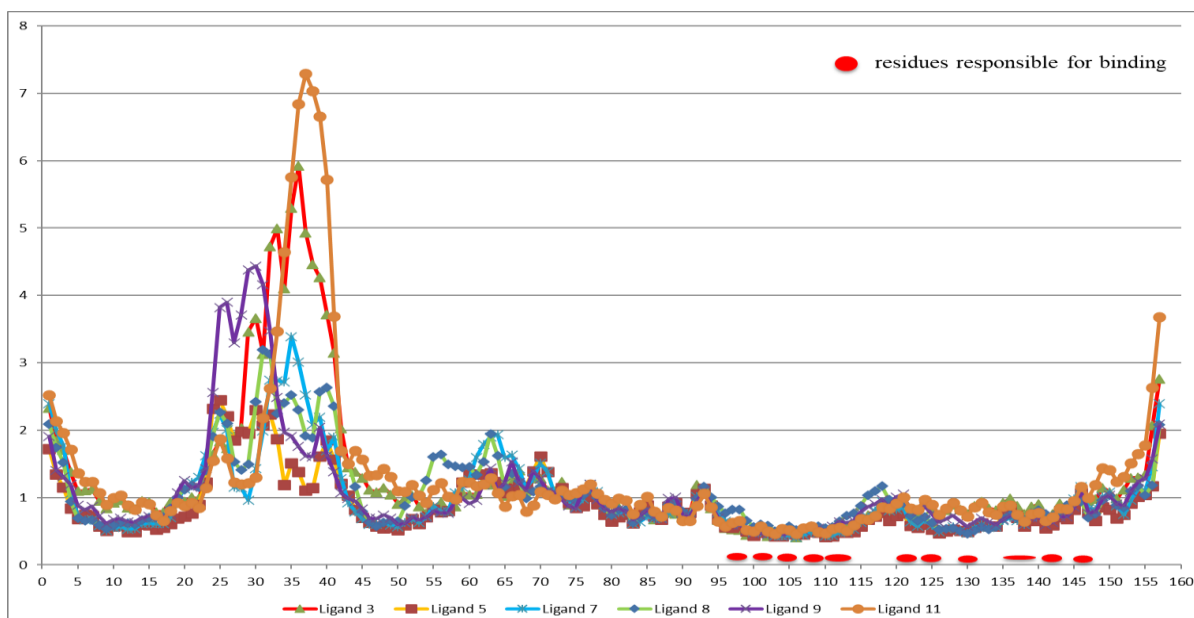


Figure 4:- Root mean square fluctuation variation of the B-cell lymphoma-2 (Bcl-2) sole protein (black), and in complex with ligand 3 (red), 5 (orange), 8 (green), and 9 (purple).

Generally, all ligandshad low-fluctuation (RMSF value) atoms belong to the residues responsible for binding with the ligand, namely, TYR101, GLN111, LEU112, SER122, GLN125, LEU130, ASN136, GLY138, PHE146, PHE97, ALA104, LEU108, VAL126, ARG139, and ALA142.

Conclusions:-

Several derivative compounds of flavonol are able to bind strongly to lysine-specific histone demethylase. The compounds Bilobetin (ligand 7) and 3,5,7-trihydroxy-2-(4-hydroxy-3-(3-hydroxybenzyl)-5-isobutylphenyl)-4H-chromen-4-one (ligand 11) were able to form very stable interactions with the active site of B-cell lymphoma-2. The results suggest that these compounds have the potential to be developed further as Bcl-2 inhibitors in small lung cancer therapy.

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