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Evaluation Of New Drug Candidature of Usnic Acid Derivatives As Selective Bcl-2 Inhibitors: Computational Designing And Molecular Docking

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***Abstract*— Breast cancer is malignant tumor that begun in the cells of breast. Abnormal or failed apoptosis consequences a malignant tumor can grow up then attack tissues around or metathesis to other tissue in the body. Apoptosis controlled by the Bcl-2 as anti-apoptotic. This situation happens on breast cancer MCF-7 cells. Therefore, at this study needed, molecular modification as a strategy in the design and development of drug analogs with better bioavailability, higher intrinsic activity and less toxicity. To be used the molecular docking method with Molegro Virtual Docker software and ADME-T properties of these compounds are calculated using ACD/ILab, Molinspiration, and preADMET calculator. The preparation of ligand and protein, and then need validation of research method that uses DRO ligand with the result RMSD (Root Mean Square Deviation) 0.180, and Moldock score -98,696 Kcal/Mol, prepare of ligand test ligand consideration, furthermore docking to the ligands. This process refers about interaction between ligand and protein which supervised then can be used as a new basic composition compound. New compound docked and inspected to know the reaction between ligand and protein. The Moldock score of the best five new compounds are -115.152, -106.454, -106.050, -105.185, and -104.964 Kcal/Mol. That percentage show us about these candidature compounds have better high binding energy percentage than the native DRO ligand. Interestingly, there are have good bioavailability and toxicity better than DRO was have bad and failed cathegory by ADMET approach.**

Keywords*— *Molecular docking, usnic acid, bcl-2, breast cancer.

I. Introduction

Cancer is one of the worldwide major causes of death. Based on the data compiled from Indonesian Ministry of Health in 2015 said that the breast cancer is the biggest cause of cancer deaths in each year with prevalence about 61.682. Breast cancer is a malignant tumor that starts from a group of cancer cells which can grow and invade surrounding tissues or metastasize to other tissues in the body as a result of abnormal apoptosis. Apoptosis or death programmed cell is the control mechanism of the elimination the dead cells in multicellular organisms [1].

The increase of cancer rates is not accompanied by prevention and treatment. It is shown from the growing of the amount of breast cancer patients. Hence, effort is needed to get through with this issue, which is to discover the new drugs. Previously new drug discovery was generally done by trial and error. It costed much and it took very long time. This prompted scientist in the development of new drug discovery in recent decades by utilizing advanced technology development in the field of medicinal chemistry with computational chemistry methods to learn about drug interactions with the receptor molecule or compound called molecular docking [2][3].

The death cell in the process of apoptosis has been given signal and mediated by several genes that encode proteins for digestion enzyme called caspase. Apoptotic events appearing after the disruption function of mitochondrial membrane barrier with the relinquish of cytochrome c through caspase-3 pathway in normal cells. Activation of caspase-3 is influenced by the Bcl-2 family protein. Protein is made up of anti-apoptotic such Bcl-2 and Bcl-X. Overexpression of Bcl-2 anti-apoptotic forestall the activation of caspase-3 at this stage to the release of cytochrome c, so that apoptosis does not occur. This is what happens in breast cancer cells MCF-7 [4].

Usnic acid (UA), a dibenzofuran originally hermit from lichens [5], In humans, it can act as antimitotic [6], antineoplasic [7,8], antibacterial [9], and antimycotic [10] agent. However,the potential benefits of UA therapeutic application are limited by its unfavorable physicochemical properties, particularly its very poor water solubility [11, 12].

In the study, as an addition to the molecular docking study for derivatives usnic acid and also this was followed by ADMET prediction and drug likeliness as well as drug score analysis of the docked compounds to evaluate the usage of some usnic acid as selective inhibitors of Bcl-2 Protein in MCF-7.

II. Materials and Methods

In this research, we simulated some derivatives usnic acid compounds based on their interactions with Bcl-2 breast cancer, using computer software applications (Molecular method [13] to determine the best compounds [14].

A. *Hardware and software*

Molegro Virtual Docker 5.0, Chem Sketch 12.00, Marvin Beans Suite Version 15.9.7, preADMET, Molinspiration, ACD / I- Prediction Lab and YASARA 15.9, Intel ® Core i7-2670 QM TM® CPU @ 2.20GHz, 8GB RAM.

B. *Software Methodology*

In the present molecular docking study, software Molegro Virtual Docker (MVD) v 5.0, MVD tools was utilized to generate grid, calculate dock score and evaluate conformers. Molecular docking was performed using MolDock docking engine of software. The scoring function used by MolDock

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is derived from the Piecewise Linear Potential (PLP) scoring functions. The active binding site region was defined as a spherical region which encompasses all protein within 15.0 A o of bound crystallographic ligand atom with selected coordinates of X, Y and Z axes, respectively. Default settings were used for all the calculations. Docking was performed using a grid resolution of 0.30 A and for each of the 10 independent runs; a maximum number of 1500 iterations were executed on a single population of 50 individuals. The active binding site was considered as a rigid molecule, whereas the ligands were treated as being flexible, i.e. all non-ring torsions were allowed [15].

C. *Methods*

Preparation of Target Protein: The selection of protein for docking studies is based upon several factors i.e. structure should be determined by X-ray diffraction, and resolution should be between 2.0-2.5A°, it should contain a co crystallized ligand. The crystal structure of Bcl-2 Protein in complex with DRO (PDB code: 2W3L) was selected as the protein target model in this virtual screening study [16]. Water molecules were removed from the protein molecule using Molegro Virtual Docker version 5.0.

Preparation of Ligand: The ligand molecule structures of usnic acid (2,6-diacetyl-7,9-dihydroxy-8,9 dimethyldibenzofuran-1,3-dione) (**Fig.1**), and its derivatives were made design form Marvin Beans Suite 15.9.7 (**Tab. 1**) and The three dimensional models of all the compounds were generated using ChemSketch 12.00 Software. Each ligand structure was optimized by ChemSketch 12.00.

Validation Methods: Before screening the ligands, the docking protocol was validated by redocking DRO ligand into its binding pocket within the Bcl-2 crystal structure to obtain the docked pose and RMSD (Root Mean Square Deviation) by YASARA Program. The result showed that the optimized native DRO almost exactly superimposed with the experimental crystal structure of DRO (**Fig. 2**). Thus, the protocol is good in reproducing the X-ray crystal structure and can be applied for further docking experiments.

Protein-Ligand Docking: The docking of the target protein with the ligand was performed using the Molegro Virtual Docker. Docking was performed to obtain a population of possible conformations and orientations for the ligand at the binding site. The binding site was defined as a spherical region which encompasses all protein atoms within 15.0 A° of bound crystallographic ligand atom (dimensions X (38.8691 A°), Y (26.8816 A°), Z (-12.5203 A°) axes, respectively). Default settings were used for all the calculations. The best conformation was chosen with the lowest docked energy, after the docking search was completed. The interactions of complex protein-ligand conformations, including hydrogen bonds and the bond lengths were analyzed (**Fig. 3**).

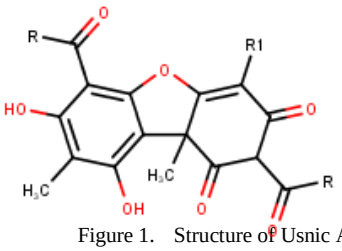

Figure 1. Structure of Usnic Acid

TABLE I. THE SUBTITUENTS OF USNIC ACID

No	*R*		*R1*
1	CH_3	1	H
2	C_3H_7	2	Cl
3	Siklo-C_5H_9	3	Cl_2
4	Siklo-C_6H_{11}	4	CF_3
5	CH_2-C_6H_5	5	NO_2
6	$(CH_2)_2 – C_6H_5$	6	Br
7	Siklo-C_4H_9	7	I
8	CH_2-Siklo-C_3H_5	8	NO_2
9	C_4H_9	9	CH_3
10	C_2H_5	10	$N(CH_3)$
11	$CHCl_2$	11	OCH_3
12	CF_3	12	F
13	CH_2-CF_3	13	OH
14	CH_2-SCH_3	14	OCH(CH3)
15	C_6H_5		
16	H		
17	CH_2-OCH_3		
18	CH_2-SO_2CH_3		
19	C_5H_9		

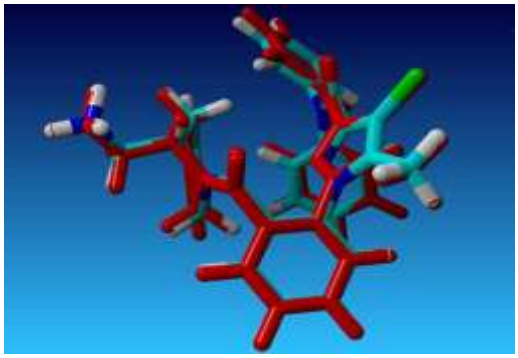

Figure 2. The superimposition of the docking structure of DRO experimental (red) with the X-ray structure (blue) within the active binding site region of 2W3L

III. Result and Discussion

Preparation of proteins and ligands used to prepare before the docking process. The purpose of this process is to prepare the ligand and protein docking process. Repair and remove water of the target protein used in the process is a protein docking results of X-Ray. The result of this preparation is a protein without a ligand and a ligand. Preparation of protein and ligand is done directly using software Molegro Virtual Docker.

Optimization performed to correct existing deficiencies of protein data downloaded, among others improvement cargo and residues as well as the addition of hydrogen. Optimization performed using Molegro program with facilities protein preparation serves to repair residues proteins imperfect such as Arg12, Lys17, Gln25, Lys54, Arg26, Arg65, Ser76.

Each docking process begins with a genuine validation of the target protein ligand. This validation is performed to determine the proximity or similarity of the results between the ligand crystallography results with a ligand that has been optimized thus docking method. Coordinates a ligand interactions and protein for docking process is X = 38.8691, Y = 26.8816, Z = -12.5203. The results of this validation should be worth 0.180 with binding energy -98.696 kcal/mol. Data shows less than 2.0 indicates the method can be used.

There are one hundred two compounds outcome from thirty-three subtituents combination of modifications on

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usnic acid compounds based on the Topliss Decision Tree [16], were simulated using molecular docking on target protein of Bcl-2 breast cancer. In this study served with the five ligands smallest based on binding energy. The results are displayed in **Table 2**.

TABLE II. THE SUBTITUENTS OF USNIC ACID

Compounds	*R*	*R1*	*R2*	**Binding Energy** *Kcal/Mol*
AU3	Cyclo-C_5H_9	-	-	-115.152
AU57	C_5H_9	C_5H_9	-	-106.454
AU53	C_6H_5	C_6H_5	-	-106.050
AU25	-	$(CH_2)_2$-C_6H_5	-	-105.185
AU19	C_5H_9	-	-	-104.964
DRO				-98,696

As shown in **Table 2**, compared to DRO (1-(2-{[(3S)-3 (aminomethyl)-3,4-dihydroisoquinolin-2(1H)-yl]carbonyl} phenyl)-4-chloro-5-methyl-N,N-diphenyl-1H-pyrazole-3 carboxamide) that act as selective inhibitors Bcl-2 protein, exhibited higher binding energy, affinity, and hydrogen bond, steric interaction on Bcl-2 breast cancer cells, indicating that five compounds has a stronger inhibitory activity against Bcl-2 protein targets.

The docking of compounds outcome produced the five best ranked compounds, namely, compounds AU3, AU57, AU53, AU25, and AU19, which showed lower moldock score as binding energy value and a higher number of hydrogen bonding interaction than the others compounds. The binding energy values of compounds AU3, AU57, AU53, AU25, and AU19 are -115.152, -106.454, -106.050, -105.185, and -104.964 kcal/mol, respectively, which are better than DRO with binding energy value of -98,696 kcal/mol.These results showed that, compared to DRO as selective Bcl-2 protein, those five top-ranked compounds will form more stable complex and selective with Bcl-2, as well as, be better able to inhibit and reduce the activity of Bcl-2. The highest binding energy has shown AU3 compounds.

The interaction between the best five compounds and amino acid of the Bcl-2 breast cancer cells are Lys22, Ala59, Ser64, Arg66, Tyr67, Arg68, and Tyr161. If a compound interacts with the amino acid of the protein target, it will reduce the activity of the target protein, and change the protein conformation. Generally, the interaction of the compound with the complex protein target is the hydrogen bond and steric interactions. The quantities of hydrogen bond interactions of the compound with the amino acid of the target protein indicate Its ability to inhibit the protein target. **Figure 3** displays the ligand complex interaction of the best five compounds AU3, AU57, AU53, AU25, and AU19 and DRO with the receptor target Bcl-2 consisting of hydrogen bond and steric interaction.

As shown, all the best five compounds could change the conformation of the receptor target cavity, and were able to enter the binding site of the Bcl-2 protein target. In addition, compared to DRO , those best five compounds showed more hydrogen binding interaction within Bcl-2. Hydrogen bond interactions between amino acid residues of Bcl-2 breast cancer with AU3, AU57, AU53, AU25, and AU19 and DRO are summarized in **Table 3**.

As shown in **Table 3**, the best compounds AU3, AU57, AU53, AU25 have a higher number of hydrogen bonds and steric interactions to the protein target Bcl-2 than that DRO.

TABLE III. THE HIDROGEN BOND AND STERIC INTERACTION OF COMPOUNDS WITH BCL-2 PROTEIN

Compounds	*Hidrogen Bond*	*Steric Interaction*
AU3	Lys22, Ala59, Ser64, Arg66, Tyr67, Arg68	Lys 22, Ala59, Tyr67, Tyr161
AU57	Ser64, Arg68, Tyr67, Arg66, Gln25	Arg66
AU53	Arg66, Ser75, Phe71, Arg65	Ser64, Arg68
AU25	Asp62, Tyr161, Pro63, Gly162,	Ala59, Glu58, Leu160
AU19	Ser64, Arg66, Arg68,	Arg65, Arg68, Tyr67
DRO	Glu199	-

The red color represents the amino acid of Bcl-2

In this study also has successfully qualified Lipinski's Rules, CMC like rule, MDDR like rule and WDI like rule but we only show 5 best compounds were have highest activity based on docking result in **Table 4.** Ligands outcome tested in this study were predicted to have good oral bioavailability in **Table 5**. The best five of the compounds have shown excellent permeability, while the DRO have poor permeability indicated by violated on Rule of Five and failed on CMC like rule or WDI like rule (**Table 4**). The physical properties like ionization potential, electronic energy and dipole plays an important role inactivity of compounds.

Molecular descriptor properties

The selected compounds used in this study were evaluated as selective inhibitor Bcl-2 protein target by comparis. The oral bioavailability of the compounds projected as potential drugs were evaluated by determining the molecular weight, number of rotatable bonds , number of hydrogen bonds (nON and nOHNH), and drug's polar surface (TPSA). Since the individual molecular weights of all the compounds were less than 500, the number of the rotatable bond were <10, the number of hydrogen bond donors and acceptors were < 12, and TPSA values being <140, they qualified to be an ideal oral drug. Ligands tested in this study were also predicted to have good oral bioavailability.

ADME prediction

In the sophisticated drug designing process, computational draw on like preADMET prediction; MDCK and Caco-2 cell permeability, etc. serve as computational screening model for the prediction of intestinal drug absorption. All the compounds under study have qualified HIA%, *in vitro* plasma% (>90% in all the cases) and Caco-2 cell permeability (>25 nm/Sec) to be a commonsense drug candidate. Some of the compounds have shown excellent permeability, while others have relatively less or poor permeability in relation to qualify as CNS drug and MDCK permeability as shown in Table 4. Less permeability is predicted because of the lesser solubility; and solubility, to a certain extent, depends on the arrangement of molecules in the crystal. It is to be noted that the topological aspects cannot be predicted via atom types or substructure fragments.

TABLE IV. THE DATA REPRESENTING THE QUALIFICATION OF THE SUBSTITUENTS FOR DRUG LIKELINESS USING CMC LIKE RULE, MDDR LIKE RULE AND WDI LIKE RULE ALONG WITH RULE OF FIVE AS PREDICTED USING PREADMET SERVER

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Compounds	*CMC like rule*	*MDDR like rule*	Rule of five	WDI like rule
AU3	Qualified	Mid-structure	Suitable	90%
AU57	Qualified	Mid-structure	Suitable	90%
AU53	Qualified	Mid-structure	Suitable	90%
AU25	Qualified	Mid-structure	Suitable	90%
AU19	Qualified	Mid-structure	Suitable	90%
DRO	Failed	Mid-structure	Violated	Failed

TABLE V. PREADME PREDICTION OF LIGANDS

Comp ounds	*In vitro blood barrier*	*Caco-2 nm/sec*	HIA%	MDCK nm/se	In vitro plasma %
AU3	0.2070	19.5653	94.6202	0.0480	94.5751
AU57	0.1193	19.3162	93.5592	0.1536	95.9790
AU53	0.0121	18.1399	85.1093	4.7135	72.2935
AU25	0.0121	18.1399	85.1093	4.7135	72.2935
AU19	0.0997	18.8938	89.8190	19.4418	87.3994
DRO	0.1514	34.1907	95.9112	0.0464	100.0000

TABLE VI. TOXICITY PREDICTION AS OUTPUT OF PREADMET

Compounds	*Ames_test*	*Carcino_Rat*	in vitro hERG inhibition
AU3	non-mutagen	negative	low_risk
AU57	non-mutagen	negative	low_risk
AU53	non-mutagen	negative	low_risk
AU25	non-mutagen	negative	low_risk
AU19	non-mutagen	negative	ambiguous
DRO	non-mutagen	negative	high_risk

All the five parameters of Lipinski's rule of five are qualified by This pharmacophore and thus could be considered as a lead molecule to generate conformations or virtual screening library along with more modifications which could enhance its therapeutic index by upgrading the kind of interactions it could possibly make with the target protein. Docking process that has been carried out, generating the value energy ligand binding to plasma proteins and shows the interaction between the ligand and the target protein. These interactions involve various amino acids and kind of bond, bonding both hydrophobic and hydrogen bonding. The number of amino acids that interact with ligand, will affect the value of the binding energy of protein ligands.

The fate of a promising drug depends on its toxicity. The therapeutic index of a drug would be higher when it shows low toxicity or adverse effects. Based on this we have performed toxicity predication using pre ADMET calculator. Results amest test is the best five compounds (AU3, AU57, AU53, AU25, and AU19) revealed that the compounds are negative, it is indicated no metabolic activation and clear evidence of carcinogenic activity. Moreover, hERG inhibition *in vitro* shows low risk. Meanwhile, The DRO ligand on hERG inhibition of *in vitro* shown high risk as represent evidence to cardio toxic

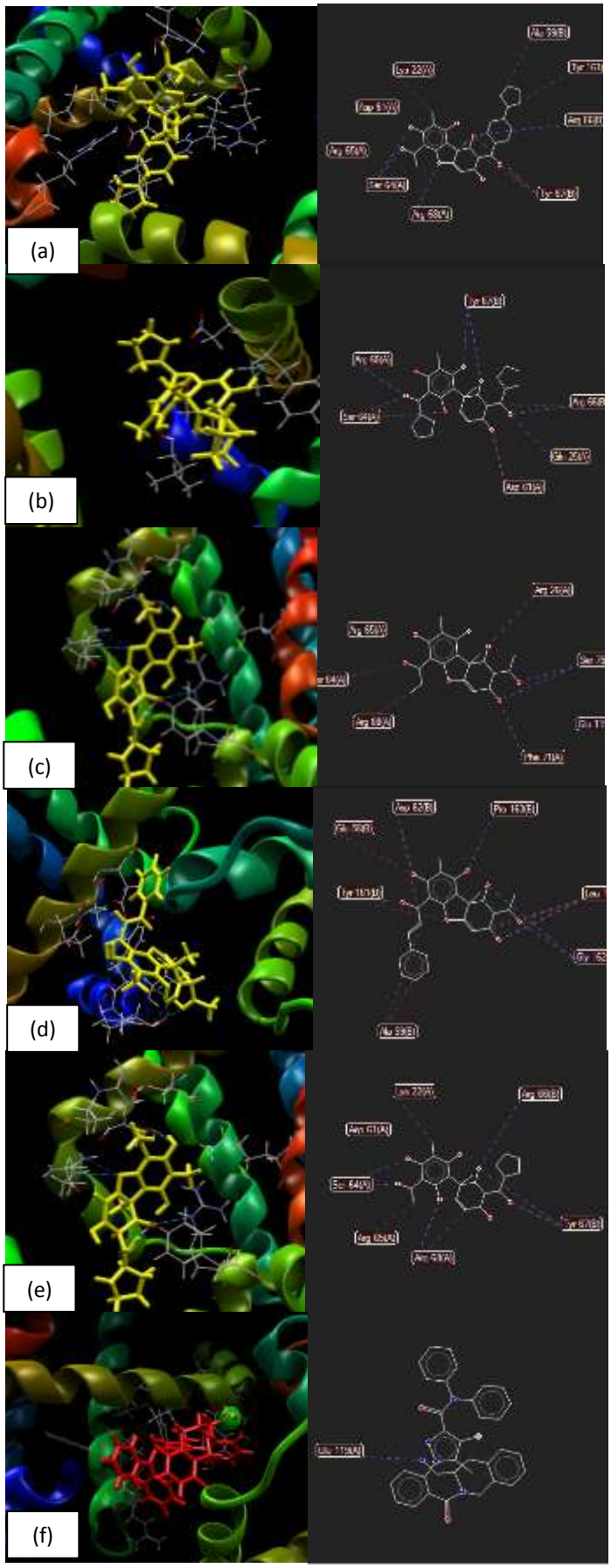

Figure 3. Binding Interctions mode of (a)AU3, (b)AU57, (c)AU53, (d)AU25, (e)AU19, (f)DRO compounds with Bcl-2, blue dots (-------) indicates H bonding and red dots (.....) indicates steric interactions.

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IV. Conclusion

In conclusion, we have simulated one hundred and two designed compounds from thirty three subtituents by molecular docking and ADME-Toxicity approach. Among them, there are the best five compunds derivatives such as AU3, AU57, AU53, AU25, and AU19, in particular, to show stronger inhibitory activity and greater interaction with amino acid residues in the binding site of Bcl-2 breast cancer compared to the original DRO as selective inhibit Bcl-2 protein breast cancer. Interestingly, there are have good oral bioavailability better than DRO was have bad and failed cathegory by ADMET approach. Moreover, there compounds have low toxicity value. The compounds were predicted to be safe (nonmutagenic as well as non-carcinogenic).

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