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Apoptosis Potency Prediction of Steroid Saponin from Gembili (*Dioscorea esculenta*) as Bcl-2 and Bcl-xL Inhibitors in Colon Cancer

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Abstract

Steroid saponins from gembili tuber (*Dioscorea esculenta*) are natural compounds that are known to have anticancer activity by inducing apoptosis through inhibition of Bcl-2 dan Bcl-xL. This study was determine the potency, mechanism and binding affinity of steroid saponin compounds as Bcl-2 and Bcl-xL inhibitor of colon cancer *in silico* using molecular docking. *In silico* assay was carried out using VEGAZZ (use for Bcl-2 protein preparation), Open Babel (use for minimizing the 3D structure of steroid saponin compounds), Autodock Vina in the PyRx 0.8 program (use for molecular docking), and PyMOL, Discovery Studio Visualizer (use for 2D and 3D visualization). The result of molecular docking was a binding affinity value based on the formation of hydrogen and hydrophobic interactions between ligand and receptor, more smaller binding affinity value, more stronger the bond. Data were compared with navitoclax, ABT-737 and obatoclax. The binding affinity between Bcl-2 and navitoclax, ABT-737, obatoclax, prosapogenin A, dioscin, gracillin, diosgenin, protogracillin, dichotomin and protodioscin were –9,8; –8,9; –7,2; –9,0; –8,8; –8,0; –7,9; –8,4; –8,2 and –7,7 kcal/mol. Then the binding affinity between Bcl-xL and navitoclax, ABT-737, obatoclax, prosapogenin A, dioscin, gracillin, diosgenin, protogracillin, dichotomin, and protodioscin were –10,6; –9,3; –8,3; –9,7; –9,4; –8,9; –9,7; –9,1; –9,9 and –8,8 kcal/mol. Based on the results obtained, the steroid saponin compounds of gembili tuber are possible have potential as anti-colon cancer through inhibition of Bcl-2 and Bcl-xL protein in order to increase the regulation of apoptosis.

Keywords: *In silico* prediction, Steroid saponin, Gembili tuber, Bcl-2 inhibitor, Bcl-xL inhibitor

Introduction

Human life in various fields is increasingly complex along with the development of science and technology, including in the field of science. One of the uses of technology in science is bioinformatics. Bioinformatics is often used as problem solving using an *in silico* approach. *In silico* is defines as a simulation using a computer that predicts actual conditions to facilitate a study by shortening time and costs [1].

Cancer is a group of diseases characterized by changes in the cells in the body, in which cells continue to grow and spread uncontrollably [2]. Cells that undergo changes continuously proliferate to suppress normal cell growth. The National Cancer Institute says that in cancer, cells become abnormal and continue to divide unnecessarily and form growths called tumors [3].

The Indonesian Ministry of Health reports that cancer is the 2nd leading cause of death worldwide by 13 % [4]. In Indonesia, cancer cases are quite high, namely around 14.1 million new cases with 8.2 million deaths in 2012 [5]. Then in 2018, cancer cases increased to 18.1 million new cases with 9.6 million deaths [6]. One of the most common types of cancer is colon cancer. Colon cancer is a tumor that grows in the large intestine. This cancer ranks 3rd in men and 2nd in women [7].

A process that plays an important role in cancer is apoptosis. Apoptosis is programmed cell death or better known as Programmed Cell Death (PCD) [8]. Lindsay *et al.* [9] stated that apoptosis is very important for multicellular organisms to maintain development and homeostasis. The apoptosis mechanism occurs through 2 pathways, namely the intrinsic pathway in the mitochondria and the extrinsic pathway on the cell surface [10]. The main process of apoptosis in the intrinsic pathway is

regulated by the Bcl-2 protein family. In cancer there is often overexpression of the anti-apoptotic proteins Bcl-2 and Bcl-xL which allows inhibition of the intrinsic apoptotic pathway in the mitochondria. Bcl-2 and Bcl-xL inhibit apoptosis by maintaining the integrity of the mitochondrial membrane [11].

Currently, 3 methods have been developed for cancer treatment, there are surgery, radiation therapy and chemotherapy [12]. Some drugs for medical therapy often have side effects for cancer sufferers. This encourages the development of new conventional drugs that have good therapeutic effects. This therapeutic effect is commonly found in herbal medicines that contain natural compounds from plants as anti-cancer. One of the natural compounds that can be developed is steroid saponins derived from the gembili tuber (*Dioscorea esculenta*).

Gembili (*Dioscorea esculenta*) is a type of tuber from the family *Dioscoreacea* [13]. Gembili has various bioactive compounds that are useful for treatment such as steroid saponins. Saponin steroids are known to have roles as insecticides, anti-inflammatory, anti-tumor, antidiabetic, anti-fungal, antibacterial, anti-parasitic, antihyperlipidemic and antioxidant. As an anti-tumor agent, saponin steroids have various molecular mechanisms. Saponin steroids can induce apoptosis through inhibition of anti-apoptotic proteins Bcl-2 and Bcl-xL [14]. Several types of steroidal saponins have been known to have apoptotic activity against colon cancer cell lines, such as diosgenin which can induce apoptosis in HT-29 cell lines [15]. In addition, prosapogenin A also has cytotoxic activity on colon cancer cell line LS174T with IC_{50} 4.0 ± 0.3 [16].

Based on the mechanism of cancer occurrence and the potency of saponin steroid compounds from gembili tubers as anticancer that can induce apoptosis, it is necessary to explore steroid saponins as potential anticancer agents. Therefore, activity prediction was carried out through the interaction of certain proteins with ligands (steroid saponins) using *in silico* method. One of *in silico* method is molecular docking which is used to model molecular bonds by attaching ligands to the active side of the protein or receptor [17]. In this study, was carried out molecular docking activity on the steroid compound of gembili tubers (*Dioscorea esculenta*) as an inhibitor of the anti-apoptotic protein Bcl-2 and Bcl-xL in colon cancer.

Materials and methods

Materials

Tools: A set of Laptop ACER Aspire specific processor ES14 with Intel (R) Celeron (R) CPU @ 1.60GHz N3050 1.92 GB of RAM memory that comes with the program VegaZZ, PyRx 0.8, Discovery Studio Visualizer and PyMOL. As well as the PubChem web server, PDB (Protein Data Bank), CLC Pred and PASS Online.

Material: 3-dimensional structure of the prosapogenin A compounds, dioscin, gracillin, diosgenin, protodioscin, dichotomin, protogracillin and parrisaponin downloaded from http://pubchem.ncbi.nlm.nih.gov. The structure of the Bcl-2 PDB-ID 6GL8 and Bcl-xL PDB-ID 3QKD target proteins were downloaded from http://www.rcsb.org. The control compounds include navitoclax, ABT-737 and Obatoclax.

Collection of 3D Ligand

Structure 3D ligand structure, namely saponin steroid compounds of gembili tubers, including prosapogenin A CID 11061578, dioscin CID 119245, gracillin CID 159861, diosgenin CID 99474, protodioscin CID 441891, dichotomin CID 3085030, protogracillin CID 441892 downloaded from Pubchem database (https://pubchem.ncbi.nlm.nih.gov/) in *sdf format and converted to *pdbqt format using Open Babel. Positive control of the anti-apoptotic protein inhibitor Bcl-2 using navitoclax CID 24978538, ABT-737 CID 11228183 and Obatoclax CID 16681698.

Prediction of activity in cytotoxic compound against cancer cell line

Prediction was carried out using the CLC-Pred (web-serverCell Line Cytotoxicity Predictor) via http://way2drug.com/Cell-line/ in a way entered the SMILES ligand obtained from the Pubchem database.

Prediction of target protein

Prediction is done using web-werver PASS Online (Prediction of Activity Spectra for Substance) through http://www.pharmaexpert.ru/passonline/predict.php by way incorporated the ligand SMILES obtained from Pubchem database. The 3D receptor structures, namely the anti-apoptotic protein Bcl-2 and Bcl-xL were downloaded from the RCSB Protein Data Bank (http://www.rcsb.org) in *pdb format and

prepared using the VEGA ZZ application. Protein preparation is done to remove water molecules and residues that are not needed.

Molecular docking and visualization

Molecular docking between ligands and receptors was carried out based on the mode of action using the Autodock vina application in the PyRx 0.8 program. Discovery Studio Visualizer software is used to visualize ligand-receptor interactions in 2D and PyMOL for visualization of interactions in 3D.

Data analysis

The result of molecular docking is the energy of the bonds and the types of bonds formed. The bond energy is used to show the strength of the bond between the ligand and the target protein . The lower the bond energy value, the stronger and more stable the bonds are. The type of bond formed is used to analyze the interaction mechanism between ligands and proteins.

Results and discussion

3D ligand collection

The 3D structure of ligand compounds was obtained from PubChem database in Sybil Data Files (*.sdf) format and protein receptors were obtained from RCSB database in Protein Data Bank (*.pdb) format. The ligands test include prosapogenin A, dioscin, gracillin, diosgenin, protodioscin, dichotomin, protogracillin, and parrisaponin (**Figure 1**). Meanwhile, the comparison ligands included navitoclax, ABT-737 and Obatoclax (**Figure 2**).

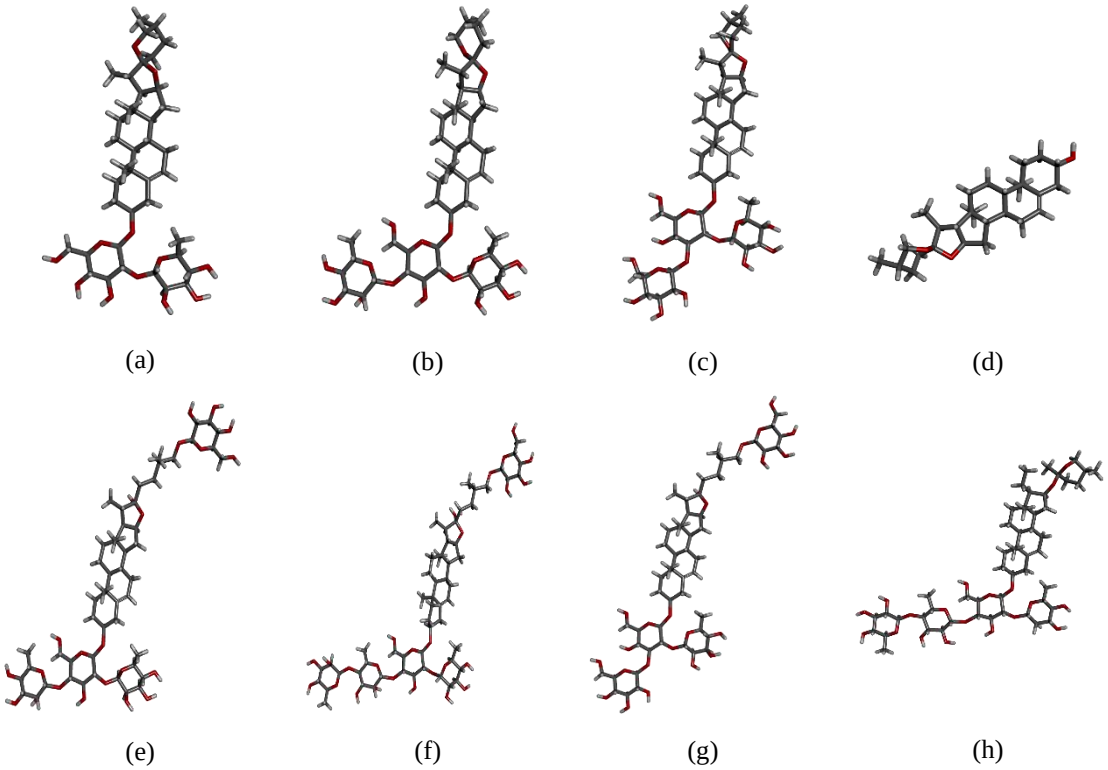
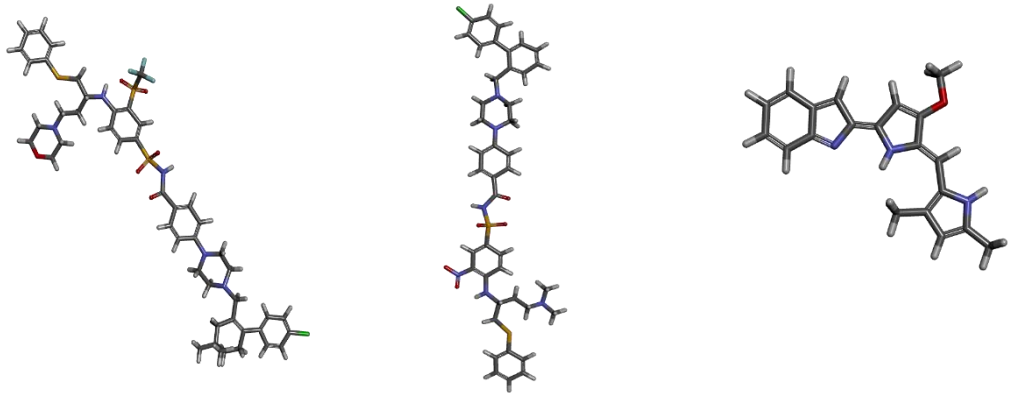


Figure 1 3D Structure of the ligand test (a) Prosapogenin A, (b) Dioscin, (c) Gracillin, (d) Diosgenin, (e) Protodioscin, (f) Dichotomin, (g) Protogracillin and (h) Parrisaponin.



(a) (b) (c)

Figure 2 3D Structure of the comparison ligands (a) Navitoclax, (b) ABT-737 and (c) Obatoclax.

Saponin steroids are triterpenoid modifications commonly found in Dioscoreacease plants including *Dioscorea* esculenta [18,19]. In the *dioscorea esculenta* (gembili) tuber, there are several steroid saponin compounds, including prosapogenin, dioscin, gracillin, diosgenin, protodioscin, dichotomin, protogracillin and parrisaponin (**Figure 1**). In this study it was proven that steroid saponin ha potential as an anticancer through inhibition of anti-apoptotic proteins Bcl-2 and Bcl-xL *in silico* using molecular docking.

Steroid saponin of gembili tuber as ligand. Meanwhile, the comparison ligands included navitoclax, ABT-737 and Obatoclax (**Figure 2**). Navitoclax is a protein inhibitor of Bcl-2 family that has high affinity for Bcl-2, Bcl-xL and Bcl-w < 1 nmol/L [20]. Obatoclax is a small molecule that functions as a Bcl-2 inhibitor including Bcl-2, Bcl-xL, Bcl-w and Mcl-1 [21]. And ABT-737 is a small molecule that functions as an inhibitor of the antiapoptosis protein family Bcl-2, this molecule is mimetic BH3 (Bcl-2 homology region 3) which can bind Bcl-2, Bcl-xL and Bcl-w with high affinity [22].

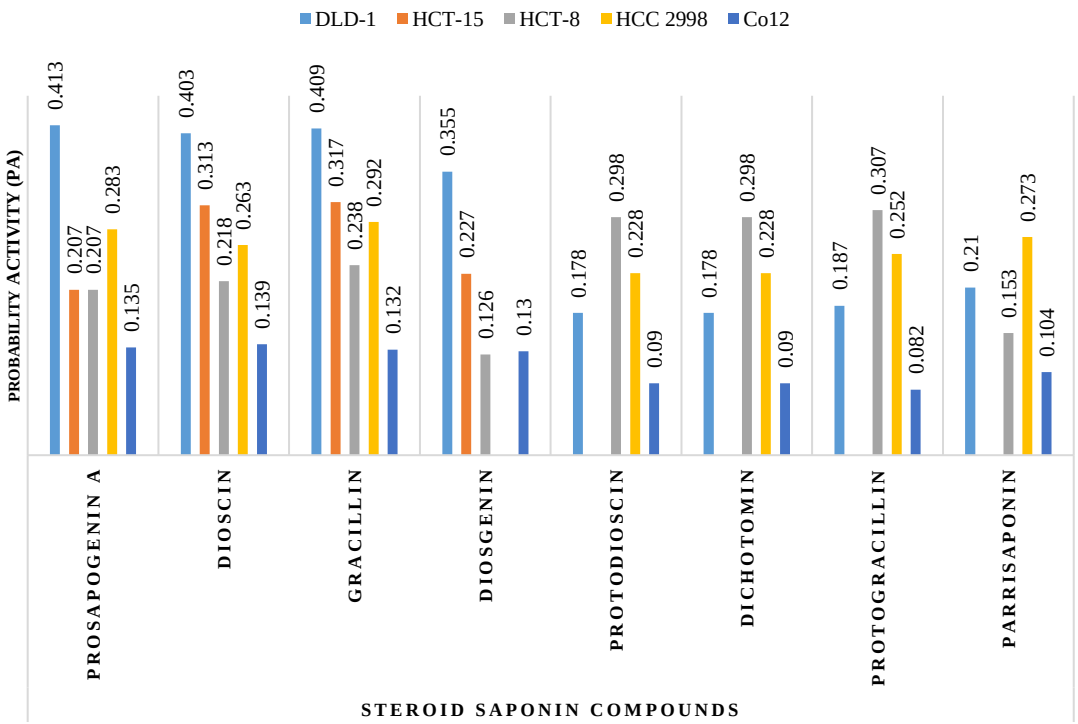


Figure 3 Cytotoxic activity of the tuber saponin steroid compounds against several types of cancer cell lines.

Prediction of cytotoxic activity of compounds against cancer cell line

The prediction results of cytotoxic activity of steroid saponin compounds of gembili tuber against cancer cell line were obtained through the web server CLC-Pred (Cell Line Cytotoxicity Predictor). CLC-Pred was performed to predict cytotoxicity based on the cytotoxic relationship of cancer cell line structures that had been designed in the training sets PASS. The results of CLC-Pred have an accuracy of 96 % from results of *in vivo* research [23]. With the prediction of CLC, it can facilitate the development of potential anticancer drugs *in silico*. The results of the CLC-Pred are indicated by the values of Pa (Possibility to be active) and Pi (Possibility to be inactive).

Based on the results of CLC predictions (**Figure 3**), it shows that the steroid saponin compounds from the gembili tuber (*Dioscorea esculenta*) have cytotoxic potential in several types of cancer cell lines. Some examples of these cancer cell lines include DLD-1 (*Colon adenocarcinoma* in the colon), HCT-15 (*Colon adenocarcinoma* in the colon), HCT-8 (*Ileocecal adenocarcinoma* in the colon), HCC 2998 (*Colon adenocarcinoma* in the colon) and Co12 (*Colon carcinoma* of the colon). Compounds that have Pa > 0.5 have a high potential for cytotoxic activity and the results will not be significantly different when tested on a laboratory scale, a value of 0.3 < Pa < 0.5 has a moderate potential for cytotoxic activity, and a value of Pa < 0.3 has a potential for low cytotoxic activity if tested on a laboratory scale.

Prediction of target protein

Target protein is obtained through prediction from the web-server PASS Online and data mining from PubChem base on the similarity structure compound. The saponin steroids of gembili tubers used, showed different prediction of antagonistic apoptotic activity, namely prosapogenin A, dioscin and gracillin had Pa > 0.7, while diosgenin, protodioscin, dichotomin and protogracillin had 0.3 < Pa < 0.7 (**Figure 4**).

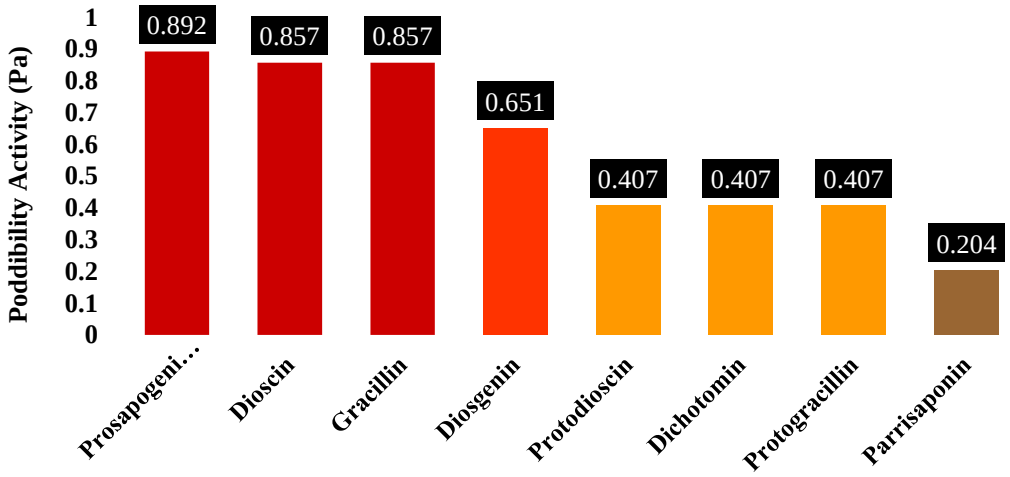


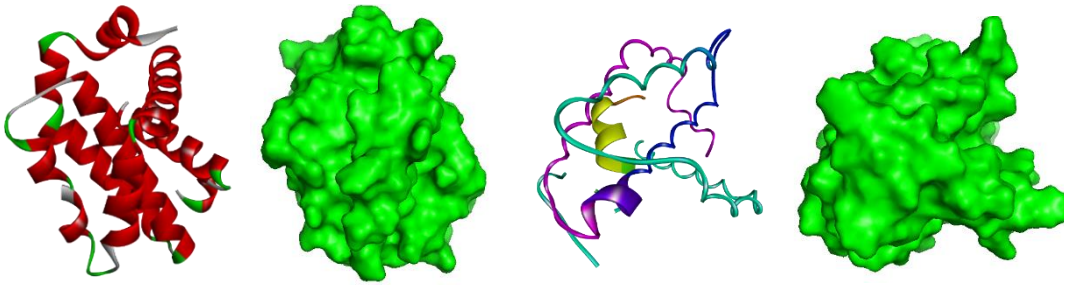
Figure 4 Apoptotic activity of steroid saponins for gembili tubers.

Target protein is obtained through prediction from the web-server PASS Online (Prediction of Activity Spectra for Substance) and data mining from PubChem base on the similarity structure compound. The activity spectrum of chemical compounds is set from different types of biological activity resulting from the interaction of a compound with various biological entities [24]. PASS results are indicated by the value of probability activity (Pa) and probability inactivity (Pi).

Chellian interpret the Pa value as follows: If the value is more than 0.7 (Pa > 0.7), then the compound has a very high biological activity and the results are not significantly different from the test on a laboratory scale. If the Pa value is more than 0.5 but less than 0.7 (0.5 < Pa < 0.7), the compound has a fairly high biological activity on a laboratory scale Pa values greater than 0.3 but less than 0.5 (0.3 < Pa < 0.5), the compounds have biological activity, but lack the potential to be tested on a laboratory scale. And if the Pa value is less than 0.3 (Pa < 0.3), the compound has very low biological activity and does not

have the potential to be tested on a laboratory scale, unless the compound is a new chemical entity whose biological activity has not been identified much [25].

The apoptotic antagonist activity referred to in this research is that steroid saponin compounds from gembili tubers can prevent and inhibit the apoptotic receptor response by binding to these receptors. Sanchez *et al.* [14] stated that saponin steroid compounds can induce apoptosis through inhibition of the anti-apoptotic proteins Bcl-2 and Bcl-xL (**Figure 5**).



(a) (b)

Figure 5 Prepared receptor protein (a) Bcl-2 and (b) Bcl-xL.

Molecular docking and visualization

Molecular docking and visualization of ligands - Bcl-2 receptors

The aim of this study was to determine the role of the gembili tuber steroid compound as an inhibitor of the anti-apoptotic protein Bcl-2 and Bcl-xL *in silico*. Molecular docking is done using autodock vina which is integrated in the software PyRx 0.8. The results were docking indicated by the lowest binding affinity of the interaction between the ligands and the receptor protein. The smaller or negative the binding affinity, the greater the ability of the ligands to bind to the receptors [26].

Based on the docking results, the binding affinity highest value was navitoclax as the comparison ligand, which was –9.8 kcal/mol. Then the prosapogenin A compound has the highest binding affinity value after navitoclax, which is –9.0 kcal/mol. Furthermore, the comparison ligands ABT-737 –8.9 kcal/mol, dioscin –8.8 kcal/mol, protogracillin –8.4 kcal/mol, dichotomin –8.2 kcal/mol, gracillin –8.0 kcal/mol, diosgenin –7.9 kcal/mol, protodioscin –7.7 kcal/mol and the comparative ligand for obatoclax –7.2 kcal/mol (**Figure 6**).

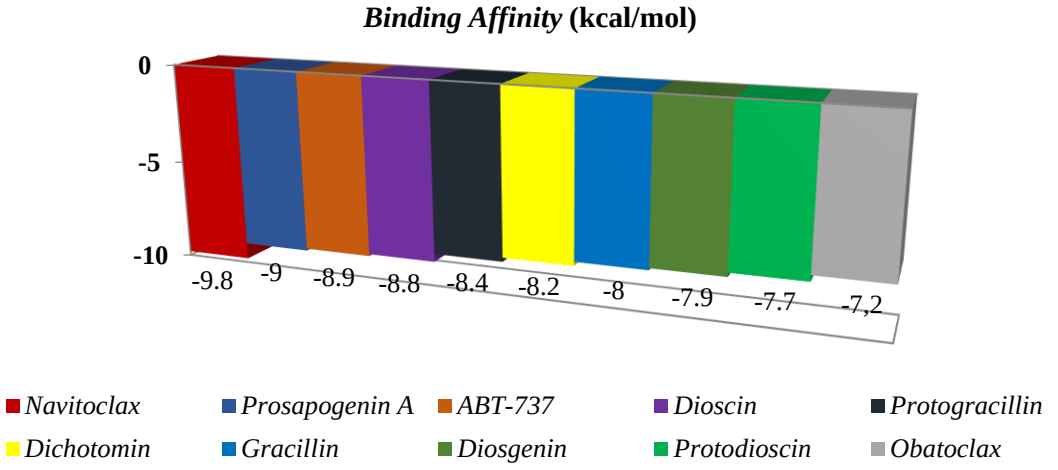


Figure 6 The binding affinity value resulting from docking of steroid saponin compounds of gembili tubers with receptors Bcl-2.

Figure 6 shows that saponin steroid compound of gembili tubers at the receptor Bcl-2 has different binding affinity values, where the compound with the smallest binding affinity prosapogenin A. Syahputra *et al.* [27] stated that the more negative the binding affinity is, the stronger and more stable the bond between the ligand and the receptor. Supported by Saputri *et al.* [28], the smaller the value of binding affinity, the higher the bond affinity between the receptor and the ligand, and conversely, the greater the value, the lower binding affinity the bond affinity between the receptor and the ligand.

The different values of binding affinity for each compound are influenced by the interactions and bonds that are formed intermolecularly. The bonds formed can be in the form of hydrogen bonds or hydrophobic bonds. Hydrogen bonds play an important role in determining the value of binding affinity because they have a greater energy than hydrophobic bonds. Hydrogen bonds have a higher energy 1 - 7 kcal/mol compared to hydrophobic bonds [29]. However, hydrophobic bonds are also important in maintaining the stability of the bonds.

Based on the results of the visualization, both the ligands test and the comparison ligands docking with the receptor Bcl-2 have hydrogen bonds and hydrophobic bonds at different amino acid residues as follows (**Figure 7**).

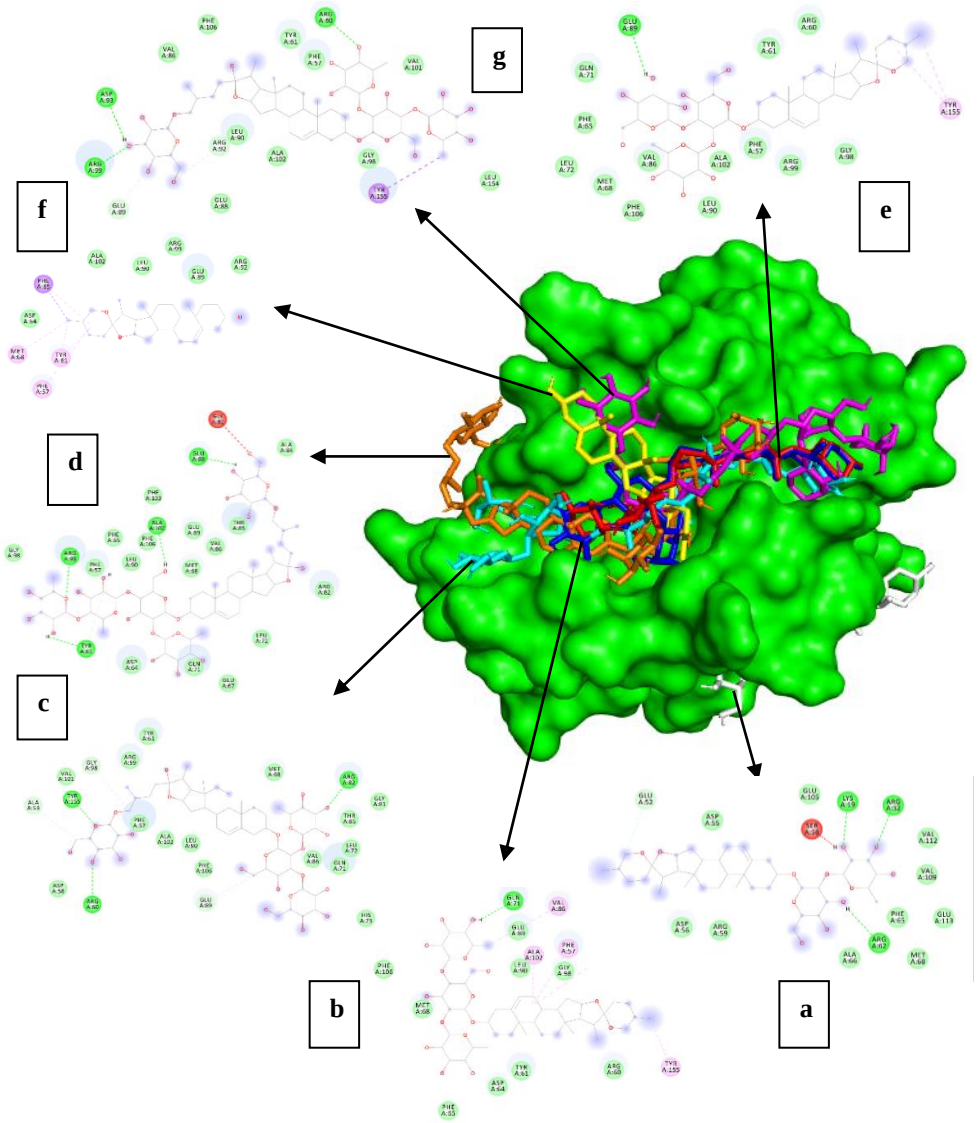


Figure 7 Visualization of docking ligand test - receptor Bcl-2 (a) Prosapogenin A, (b) Dioscin, (c) Protogracillin, (d) Dichotomin, (e) Gracillin, (f) Diosgenin and (g) Protodioscin.

Table 1 The amino acid residue ligand-receptor Bcl-2.

No	Ligan name	Van der Waals interanctions	Hydrofobic bond			Hydogen bond	
			Pi bond	Alkyl bond	Carbon Hidrogen bond	Residu	Distance (Å)
1	Navitoclax (–9,8 kcal/mol)	Arg A: 99 Val A: 101 Gly A: 98 Arg A: 60 Ala A: 53 Asp A: 53 Arg A: 59 Leu A: 90 Glu A: 105 Tyr A: 61 Phe A: 106 Val A: 86 Leu A: 72	Phe A: 57 Tyr A: 155 Phe A: 65 Asp A: 64 Ala A: 102	Leu A: 154 Met A: 68	Gln A: 71	-	-
2	Prosapogenin A (–9 kcal/mol)	Asp A: 55 Glu A: 105 Val A: 112 Val A: 109 Glu A: 113 Phe A: 65 Met A: 68 Ala A: 66 Arg A: 59 Asp A: 56	-	-	Glu A: 52	Lys A: 19 (O – H) Arg A: 32 (O – H) Arg A: 62 (OH – O)	2,26 2,28 2,30
3	ABT-737 (–8,9 kcal/mol)	Val A: 86 Tyr A: 61 Phe A: 106 Trp A: 97 Asn A: 96 Tyr A: 155 Gly A: 98 Leu A: 72 Met A: 68 Asp A: 64	Phe A: 65 Leu A: 154 Phe A: 57 Leu A: 90 Ala A: 102	-	Gln A: 71	Arg A: 99 (O – H)	2,74
4	Dioscin (–8,8 kcal/mol)	Glu A: 89 Leu A: 90 Gly A: 98 Arg A: 60 Tyr A: 61 Asp A: 64 Phe A: 65 Met A: 68 Phe A: 106 Leu A: 72	Phe A: 57 Tyr A: 155	Val A: 86 Ala A: 102 Arg A: 99	-	Gln A: 71 (OH – O)	1,98
5	Protogracillin (–8,4 kcal/mol)	Val A: 101 Arg A: 99 Tyr A: 61 Met A: 68 Gly A: 81 Thr A: 85 Leu A: 72 Gln A: 71 Val A: 86 His A: 71 Phe A: 106 Leu A: 90	-	-	Ala A: 53 Glu A: 89 Gly A: 98	Tyr A: 155 (O – H) Arg A: 60 (O – H) Arg A: 82 (O – H)	2,05 2,87 3,69

No	Ligan name	Van der Waals interanctions	Hydrofobic bond			Hydogen bond	
			Pi bond	Alkyl bond	Carbon Hidrogen bond	Residu	Distance (Å)
		Ala A: 102 Phe A: 57 Asp A: 56					
6	Dichotomin (–8,2 kcal/mol)	Gly A: 98 Phe A: 57 Phe A: 65 Phe A: 106 Leu A: 90 Phe A: 103 Met A: 68 Glu A: 89 Val A: 86 Thr A: 85 Ala A: 84 Arg A: 82 Leu A: 72 Glu A: 67 Gln A: 71 Asp A: 64	Tyr A: 61	-	-	Arg A: 99 (O – H) Ala A: 102 (OH – O) Glu A: 88 (OH – O) Tyr A: 61 (OH – OH)	1,98 2,43 1,93 2,48
7	Gracillin (–8 kcal/mol)	Tyr A: 61 Arg A: 60 Gly A: 98 Arg A: 99 Phe A: 57 Ala A: 102 Leu A: 90 Val A: 86 Phe A: 106 Met A: 68 Leu A: 72 Phe A: 65 Gln A: 71	Tyr A: 155	-	-	Glu A: 89 (OH – O)	2,69
8	Diosgenin (–7,9 kcal/mol)	Asp A: 64 Ala A: 102 Leu A: 90 Arg A: 99 Glu A: 89 Arg A: 92	Phe A: 65 Tyr A: 61 Phe A: 57	Met A: 68	-	-	-
9	Protodioscin (–7,7 kcal/mol)	Val A: 86 Phe A: 106 Tyr A: 61 Phe A: 57 Val A: 102 Leu A: 154 Gly A: 98 Ala A: 102 Leu A: 90 Glu A: 88	Tyr A: 155	-	Glu A: 89 Arg A: 92	Arg A: 99 (O – H) Asp A: 93 (OH – O) Arg A: 60 (O – H)	2,28 2,76 2,20
10	Obatoclax (–7,2 kcal/mol)	Gln A: 71 Leu A: 72 Asp A: 64 Ala A: 102 Leu A: 90	Phe A: 106 Phe A: 57 Phe A: 65 Tyr A: 61 Met A: 68 Val A: 86	-	-	-	-

Molecular docking and visualization of ligand - Bcl-2 receptors

The results of molecular docking between the receptor protein Bcl-xL with the ligand test and the comparison ligand were shown to be binding affinity. Based on the docking results, the binding affinity highest value was navitoclax –10.6 kcal/mol. Then the dichotomin compounds had the highest binding affinity value after navitoclax, namely –9.9 kcal/mol. Then followed by diosgenin compounds –9.7 kcal/mol, prosapogenin A –9.77 kcal/mol, dioscin –9.4 kcal/mol, comparative ligand ABT-737 –9.3 kcal/mol, protogracillin –9.1 kcal/mol, gracillin –8.9 kcal/mol, protodioscin –8.8 kcal/mol and the comparative ligand for obatoclax –8.3 kcal/mol (**Figure 8**).

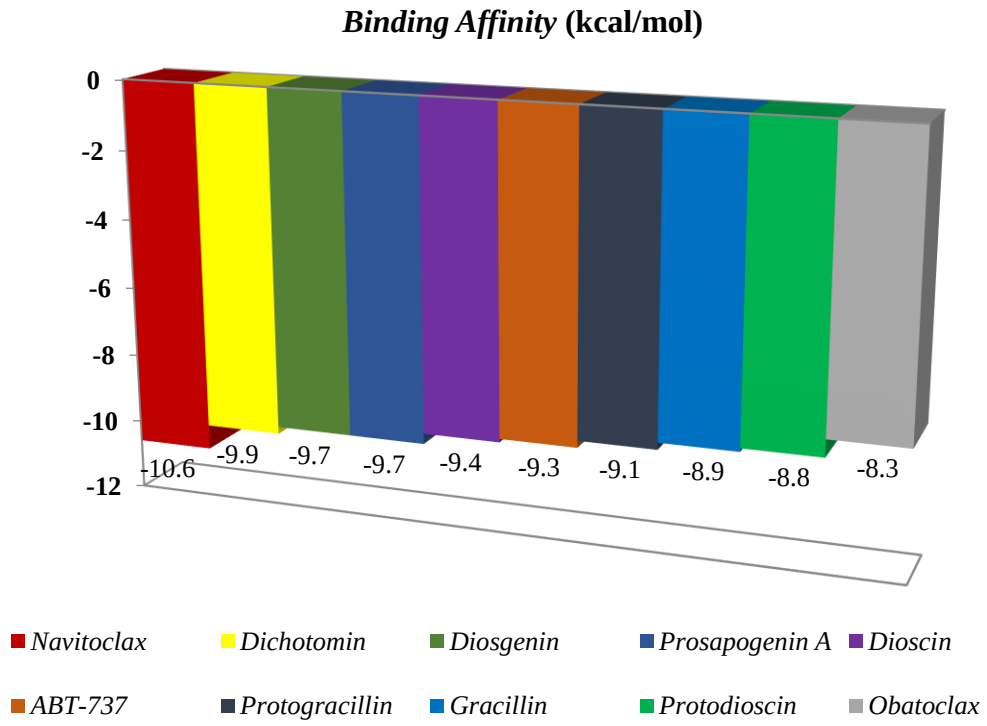


Figure 8 The binding affinity value resulting from docking of steroid saponin compounds of gembili tubers with receptors Bcl-xL.

Figure 8 shows that the saponin steroid compound of gembili tubers at the receptor Bcl-xL has different binding affinity values, where the saponin steroid compound that has the lowest binding affinity is dichotomin. The lower or negative the value of binding affinity, the stronger and more stable the bond is formed. Based on this, prosapogenin A has strogest bonds among other steroid saponin to bind Bcl-2 receptors and dichotomin compounds has the strongest bonds to bind Bcl-xL receptors.

The size of binding affinity value obtained is influenced by the interactions formed between the ligand and the receptor Bcl-xL. These interactions can take the form of hydrogen bonds and hydrophobic bonds at different amino acid residues as follows (**Figure 9**).

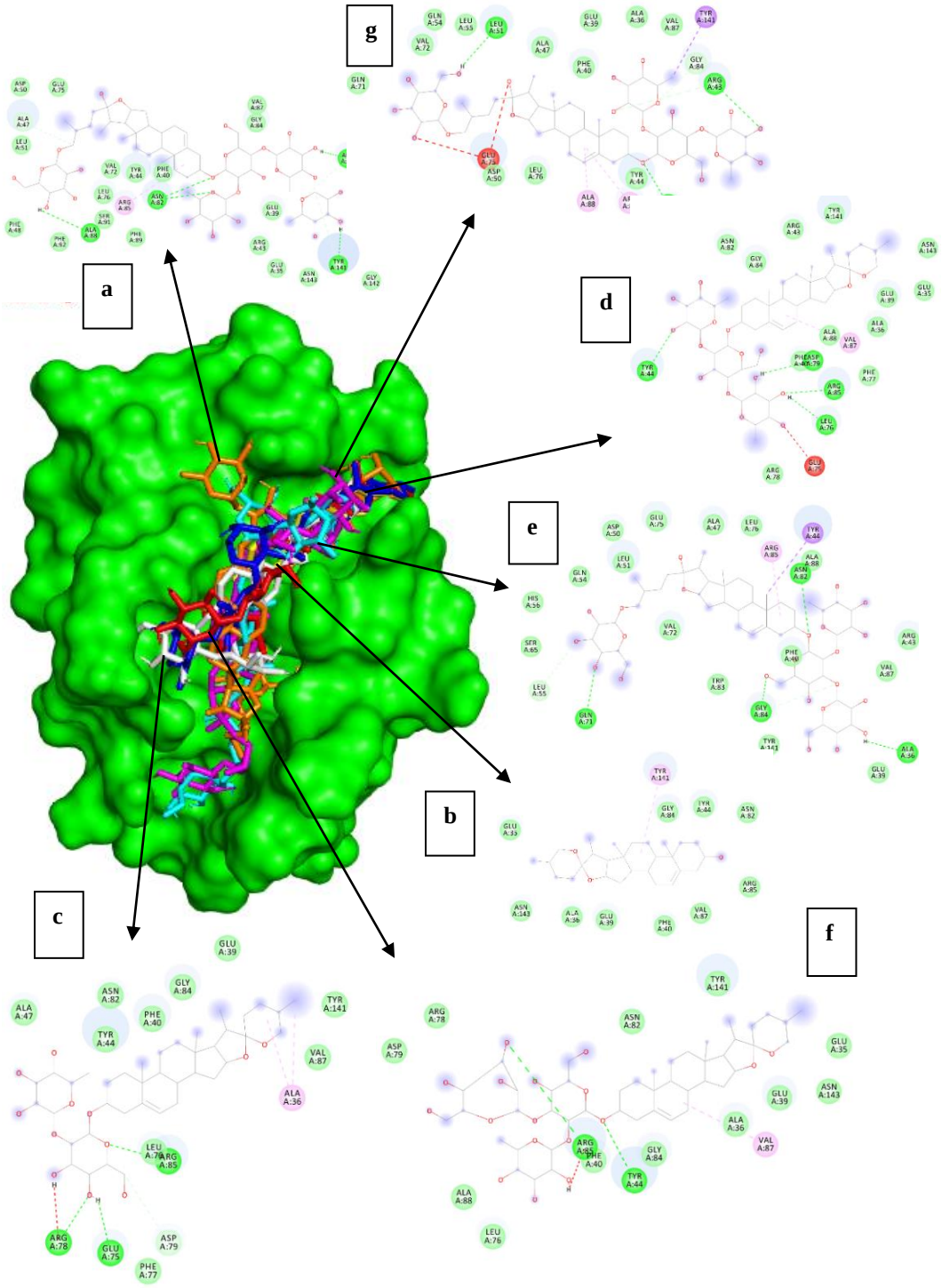


Figure 9 Visualization of the test ligand-receptor Bcl-xL (a) Dichotomin, (b) Diosgenin, (c) Prosapogenin A, (d) Dioscin, (e) Protogracillin, (f) Gracillin and (g) Protodioscin.

Table 2 The amino acid residue ligand-receptor Bcl-xL.

No	Ligan name	Van der Waals interactions	Hydrofobic bond			Hydrogen bond	
			Pi bond	Alkyl bond	Carbon Hidrogen bond	Residu	Distance (Å)
1	Navitoclax (–10,6 kcal/mol)	Val A: 72 Glu A: 75 Asn A: 143 Trp A: 83 Glu A: 39 Phe A: 40 Asn A: 82 Ser A: 91 Phe A: 92	Tyr A: 44 Val A: 87 Arg A: 85 Ala A: 88 Leu A: 51 Ala A: 47 Phe A: 48 Tyr A: 141	Leu A: 51 Ala A: 95 Ala A: 36 Leu A: 76	Glu A: 35	Gly A: 84 (O – H) Tyr A: 141 (O – H)	2,00 2,99
2	Dichotomin (–9,9 kcal/mol)	Leu A: 51 Asp A: 50 Glu A: 75 Val A: 87 Gly A: 84 Gly A: 143 Asn A: 143 Glu A: 35 Arg A: 43 Glu A: 39 Phe A: 40 Tyr A: 44 Val A: 72 Leu A: 76 Ser A: 91 he A: 89 Phe A: 92 Phe A: 48	-	Arg A: 85 Tyr A: 141 Ala A: 36	Ala A: 47	Ala A: 88 (OH – O) Asn A: 82 (O – H) Ala A: 36 (HO – H) Tyr A: 141 (HO – H)	2,29 2,54 2,22 2,00
3	Diosgenin (–9,7 kcal/mol)	Glu A: 35 Gly A: 84 Tyr A: 44 Asn A: 82 Arg A: 85 Val A: 87 Phe A: 40 Glu A: 39 Ala A: 36 Asn A: 143	Tyr A: 141	-	-	-	-
4	Prosapogenin A (–9,7 kcal/mol)	Ala A: 47 Asn A: 82 Tyr A: 44 Phe A: 40 Gly A: 84 Glu A: 39 Tyr A: 141 Val A: 87 Leu A: 76 Phe A: 77	-	Ala A: 36	Asp A: 76	Arg A: 85 (O – H) Arg A: 78 (O – H) Glu A: 75 (OH – O)	2,47 2,37 2,03
5	Dioscin (–9,4 kcal/mol)	Asn A: 82 Gly A: 84 Arg A: 43 Tyr A: 141 Asn A: 143 Glu A: 35 Glu A: 39	-	Val A: 87	-	Tyr A: 44 (O – H) Asp A: 79 (HO – H) Arg A: 85 (O – H)	2,67 2,86 2,40

No	Ligan name	Van der Waals interactions	Hydrofobic bond			Hydrogen bond	
			Pi bond	Alkyl bond	Carbon Hidrogen bond	Residu	Distance (Å)
		Ala A: 36 Ala A: 88 Phe A: 40 Phe A: 77 Arg A: 78				Leu A: 76 (HO – H)	2,18
6	ABT-737 (–9,3 kcal/mol)	Arg A: 43 Phe A: 40 Gln A: 54 Glu A: 75 Phe A: 92 Asn A: 82 Ala A: 88 Phe A: 137 Trp A: 83	Glu A: 39 Ala A: 36 Val A: 87 Ala A: 47 Asp A: 50 Leu A: 51 Tyr A: 44	Val A: 72 Leu A: 76	Asn A: 143 Tyr A: 141 Leu A: 140	Tyr A: 44 (OH – OH) Gly A: 84 (O – HN)	2,70 2,77
7	Protogracillin (–9,1 kcal/mol)	Ser A: 65 His A: 56 Gln A: 54 Leu A: 51 Asp A: 50 Glu A: 75 Ala A: 47 Leu A: 76 Ala A: 88 Arg A: 43 Val A: 87 Glu A: 39 Phe A: 40 Tyr A: 141 Trp A: 83 Val A: 72	Tyr A: 44	Arg A: 85	Leu A: 55 Gly A: 84	Gln A: 71 (O – H) Asn A: 82 (O – (H) Ala A: 36 (HO – H) Gly A: 84 (O – HN)	2,73 2,42 2,29 2,13
8	Gracillin (–8,9 kcal/mol)	Asp A: 79 Arg A: 78 Asn A: 82 Tyr A: 141 Glu A: 35 Asn A: 143 Glu A: 39 Ala A: 36 Gly A: 84 Phe A: 40 Leu A: 76 Ala A: 88	-	Val A: 87	-	Arg A: 85 (O – H) Tyr A: 44 (O – H)	2,60 2,89
9	Protodioscin (–8,8 kcal/mol)	Gln A: 71 Val A: 72 Gln A: 54 Leu A: 55 Ala A: 47 Phe A: 40 Glu A: 39 Ala A: 36 Val A: 87 Tyr A: 44 Leu A: 76 Asp A: 50	Tyr A: 141	Arg A: 85 Ala A: 88	Gly A: 84 Arg A: 43	Leu A: 51 (OH – O) Arg A: 43 (O – H) Asn A: 82 (O – H)	2,54 2,43 2,30

No	Ligan name	Van der Waals interactions	Hydrofobic bond			Hydrogen bond	
			Pi bond	Alkyl bond	Carbon Hidrogen bond	Residu	Distance (Å)
10	Obatoclax (–8,3 kcal/mol)	Glu A: 39 Phe A: 40 Leu A: 76 Tyr A: 44 Phe A: 137 Arg A: 43 Tyr A: 141	Val A: 87 Ala A: 88 Arg A: 85 Ala A: 36	-	-	Ala A: 36 (NH – O) Gly A: 84 (NH – O)	2,86 2,29

Conclusions

The conclusions that can be drawn from this study are as he steroid saponins compounds of gembili tuber include prosapogenin A, dioscin, gracillin, diosgenin, protogracillin, dichotomin and protodioscin has potential as an anti-colon cancer through inhibition of anti-apoptotic proteins Bcl-2 and Bcl-xL *in silico* using molecular docking and Prosapogenin A compound has the most effective potential as an anticancer through inhibition of receptors Bcl-2 and dichotomin has the most effective potential as an anticancer through inhibition of receptors Bcl-xL.

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