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In silico study of the active compounds in bitter melon (*Momordica charantia* L) as antidiabetic medication

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ABSTRACT

Antidiabetic drugs are widely available in the market, but most medications have strong side effects that may cause toxicity. Therefore, a search for new drug compounds that are more potent but with lower side effects is necessary. Various research has shown that bitter melon (*Momordica charantia L*) exhibits antidiabetic activities. However, the compounds in this plant that actively acts as potent antidiabetic are not specifically known yet. This study aimed to identify these active compounds in silico through molecular docking, drug scan, PreADMET, and molecular dynamics simulation. The test results of the 26 active compounds in bitter melon showed one potential compound that was actively against the nuclear receptor RORα, i.e., Goyaglicoside-h, and more potent than Rosiglitazone.

Keywords: *M. charantia* L, Goyaglicoside-h, Antidiabetic, in silico

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INTRODUCTION

Diabetes Mellitus (DM) remains a very troubling health problem worldwide. The International Diabetes Federation (IDF) states that the prevalence of DM is 1.9%, which makes DM the seventh major cause of death in the world. In 2012, an estimated 371 people were living with DM. The global incidence of type 2 DM is 95%, whereas the remaining 5% of the world's population has type 1 DM (Fatimah, 2015).

Drug research and development have been vastly conducted, including the search for medicinal compounds from natural ingredients. One of them is a bitter melon, which is listed in the Materia Medika Indonesia Vol. VI/1995 as a medicinal plant whose fruit is efficacious as an antidiabetic medication (Indonesian Ministry of Health, 1995). Yuda *et al.* (2013) reveal that the administration of the ethanolic extract of bitter melon (*Momordica charantia*) can reduce blood glucose levels in white rats (*Rattus norvegicus*) subjected to alloxan-induced diabetes. Furthermore, Mulyanti *et al.* (2010) have successfully isolated two compounds of the triterpenoid group from the active fraction (n-hexane) of bitter melon as an antihyperglycemic agent.

Previous studies have identified several active compounds in bitter melon, namely the derivatives of cucurbitane triterpenoids (Murakani *et al*., 2001; Yoshikawa *et al.*, 2007; Nakamura *et al.*, 2006). Several in vitro studies also confirm the antidiabetic activity of cucurbitane triterpenoid through different types of screening and testing (Harinantenaina *et al.*, 2006; Jiang *et al*., 2016; Chawech *et al.*, 2015). Aside from in vitro, in silico research can be used to study the interactions between compounds and enzymes, receptors, or pharmacokinetic properties to predict the drug candidates for certain diseases (Ruswanto *et al*., 2018). Therefore, to predict and test the antidiabetic potential of the previously identified active compounds, this research employed in silico design through the identification of receptor target and other in silico tests.

RESEARCH METHOD

Tools

This research used computer hardware and software. The device was a personal computer with Intel (R) Core (TM) i3 and the following specifications: CPU M 380 @ 2.53GHz (4 CPUs), 2.5GHz 2048MB RAM, and software, such as Pendrivelinux, MarvinSketch v.5.2, YASARA v.9.10, PLANTS, and LigPlot (Ruswanto *et al.*, 2015).

Materials

This research processed the PDB (Protein Data Bank) files of the identified receptor, which were downloaded from http://www.rscb.org. and 26 active cucurbitane-type triterpene glycosides contained in bitter melon (see Table II).

Research Procedure

Ligand preparation

The ligands were prepared by drawing the chemical structures on Marvin sketch, protonated until pH 7.4 was achieved, and saved as .mrv files. The conformations were saved as .mol2 files. This procedure was performed on all ligands (Ruswanto *et al*., 2015; Ruswanto *et al*., 2017)

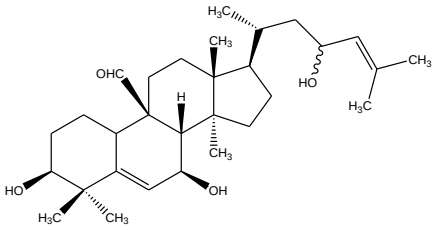
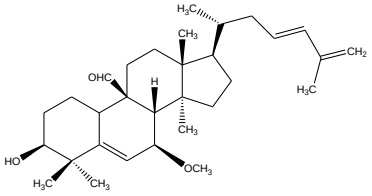
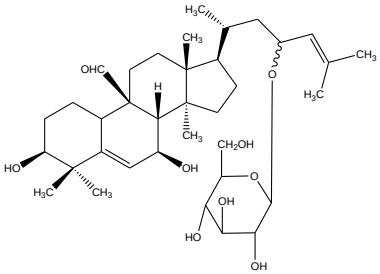
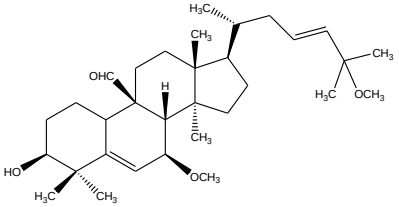
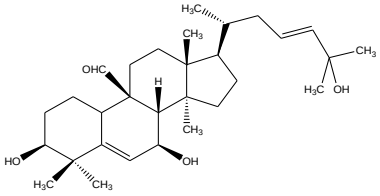
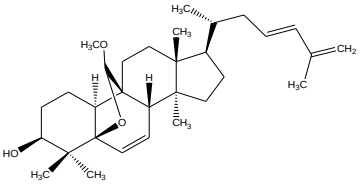
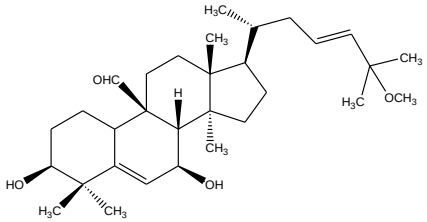
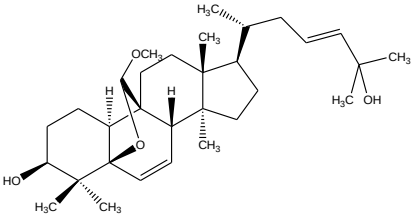
Receptor identification

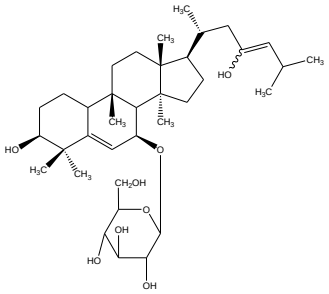
The structures of the compounds (.mol2 files) were uploaded to PharmMapper web server. This web server assigns a JOB ID to every modeling with which users can check the mapping results. The output was the list of the top 300 targets that were subjected to further evaluation to select the candidates for antidiabetics. Receptors with PDB codes were downloaded from http://www.rscb.org (Liu *et al*., 2010).

Protein analysis

The protein resulted from the identification of the receptor target was analyzed with the Ramachandran plots of PDB protein at http://www.ebi.ac.uk/pdbsum/. For protein target search, users can access the profiles using the four characters in PDB codes (Gunasekaran *et al*., 1996; Ruswanto *et al*., 2018).

Table I. The active compounds of bitter melon plants (Murakani *et al.*, 2001; Yoshikawa *et al.*, 2007; Nakamura *et al.*, 2006)

No	Senyawa	No	Senyawa
1	Momordicin I 	7	(23E)-3ß-Hidroksi-7ß-metoksi-kukurbita-5,23,25-trien-19-al 
2	Momordicin II 	8	(23E)-3ß-Hidroksi-7ß,25-dimetoksi-kukurbita-5,23-dien-19-al 
3	25-Hidroksi-23-dehidroksi-momordicin I 	9	(19R,28E)-5ß,19-Epoksi-19-metoksikukurbita-6,23,25-trien-3ß-ol 
4	25-metoksi-23-dehidroksi-momordicin I 	10	(19R,23E)-5ß,19-Epoksi-19-metoksikukurbita-6,23,dien-3ß,25-diol 
5	3ß,7ß,23-Trihidroksikukurbita-5,24-dien-7-O_ß-D-glukosida	11	(19R,23E)-5ß,19-Epoksi-19,25-dimetoksikukurbita-6,23-dien-3ß-ol



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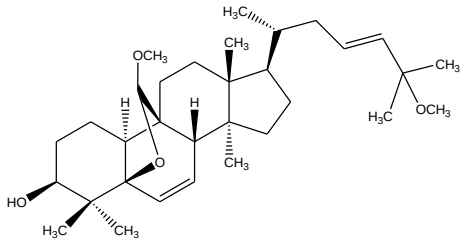

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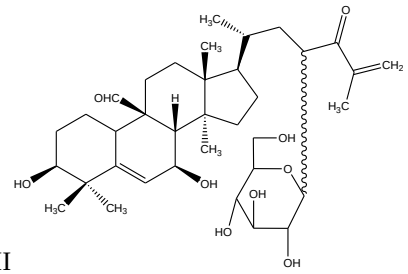

III

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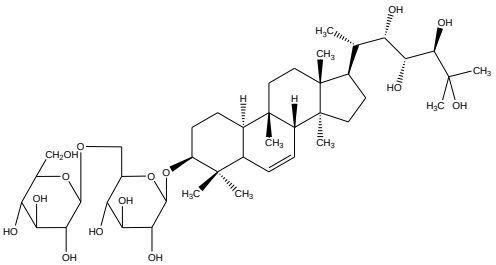

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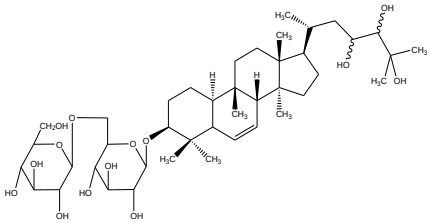

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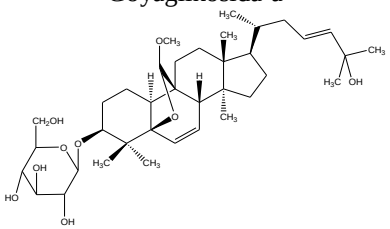

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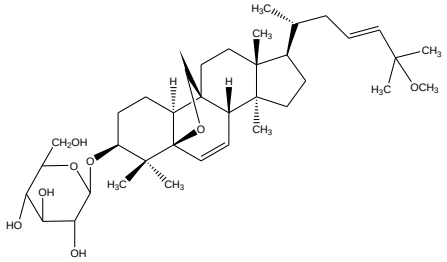

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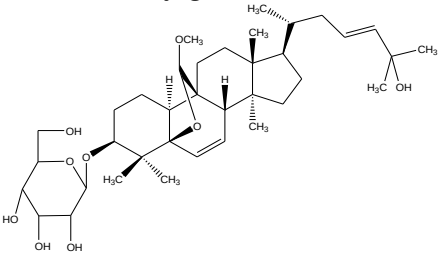

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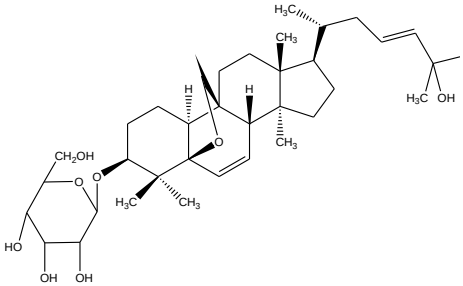

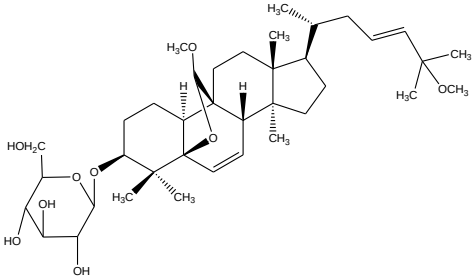

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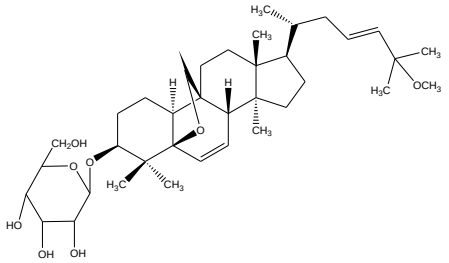

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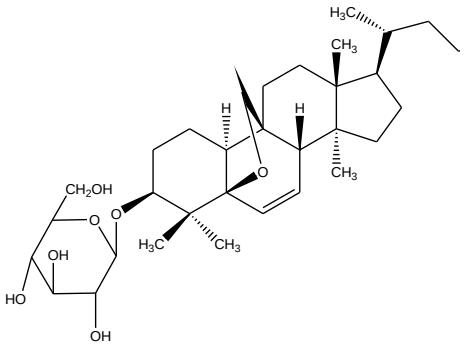

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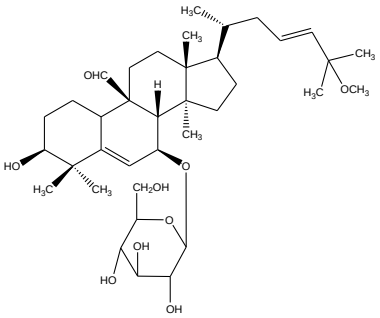

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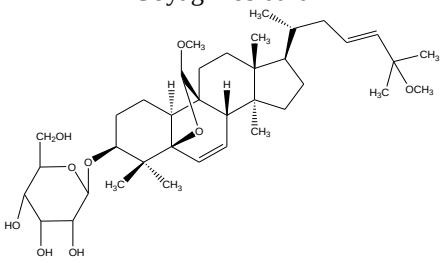

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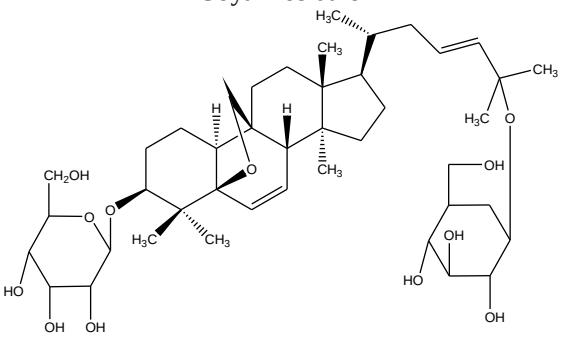

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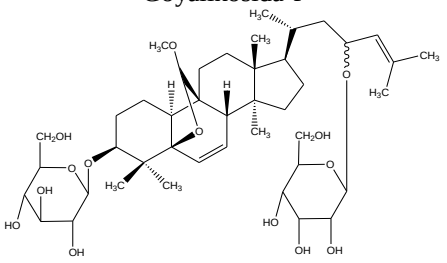
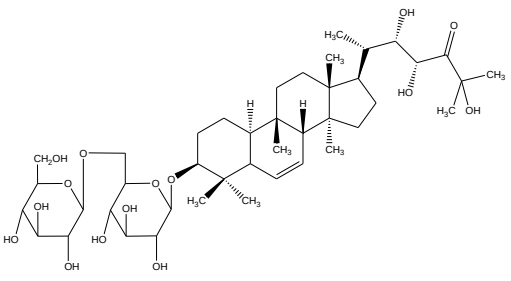
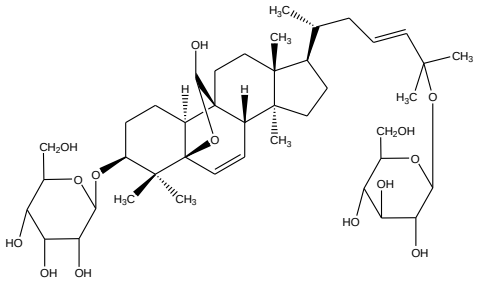


25 **Goyalikosida-g**

26 **Goyalikosida-h**





Molecular Docking

Receptor preparation

The protein (PDB) was downloaded from http/www./rscb.org/ and re-prepared in YASARA software. The results were saved in two files, namely protein.mol2 for the target receptor and ref_ligand.mol2 for the ligand comparator during the receptor validation process (Purnomo, 2013).

Receptor docking validation

The two files resulted from the preparation process, namely protein.mol2 and ref_ligand.mol2, were docked using PLANTS program that had been linked to Co-pendrivelinux. The complex of ref_ligand-protein with the lowest 'best score' was calculated with Root Mean Square Deviation (RMSD) value. A receptor was declared as valid and applicable as a test ligand if its RMSD was $\leq$ 2 (Purnomo, 2013).

Test ligand docking

The ligand docking of the active compounds of bitter melon was performed in PLANTS software that had been linked to Co-pendrivelinux. The results of the docking were the best scores of the test ligands that were compared with the best score of the ligand comparator obtained from the docking simulation. Furthermore, the protein-test ligand complex with a lower best score than the ligand comparator was visualized and interpreted to identify any molecular interactions (Purnomo, 2013). The position of each amino acid residue bound to the ligand was visualized in two dimensions (2D) in Ligplot (Wallace *et al.*, 1996).

Molecular Dynamics (MD) simulation

The molecular dynamics simulations of the protein-test ligand complexes, obtained from the docking, used MOE software v.2009. The simulation system involved the influence of temperature and solvent and used the Born solvation. The temperature of the simulation was 310 K (the normal temperature of a human body) (Kurniawan *et al*., 2018).

Drug scanning

The drug scan analysis was performed on ligands that had lower binding energy than the compound comparator. It also applied the Lipinski's parameter in MarvinSketch software (Tambunan *et al.*, 2012; Athar *et al.*, 2017; Choy and Prausnitz, 2011).

ADMET Study

The ADME (Absorption, Distribution, Metabolism, and Excretion) study was conducted in the PreADMET web-based program—accessible at http://preadmet.bmdrc.org/, using the Caco-2 cell (Carcinoma colon bilayer) permeability parameters, HIA (Human Intestinal Absorption), and

plasma protein binding (PPB). The toxicity parameters were observed from the carcinogenic and mutagenic properties of the compounds (Ruswanto *et al*., 2017).

RESULTS AND DISCUSSION

This study began with the stage of ligand preparation, which aimed to optimize the geometry of the test compounds contained in bitter melon plants using protonation until these compounds had the same pH as human blood, i.e., 7.4. Afterward, the protonated test compounds were subjected to further search for other conformations that were expected to be more stable when docked to the receptor. This conformation is very important in determining the drug-receptor interactions as it displays the spatial position of the atoms or groups relative to the receptor in the compound structures. It has a high affinity for forming stable bonds.

Receptor identification

The pharmacophore mapping approach on the PharmMapper web server resulted in the list of the top 300 targets of PDB (Protein Bank Data) IDs. The data were then evaluated to identify the suitable diabetes mellitus receptors. The evaluation results showed that there were two targets at the top 10 rankings, namely Nuclear receptor ROR alpha (PDB Code 1S0X) and Retinol-binding protein 4 (PDB Code 1RBP). Both targets are described in Table II.

Table II. Receptor targets

Ranks	PDB IDs	Receptor Targets	Fit Scores
1	1S0X	Nuclear receptor ROR alpha	6.013
7	1RBP	Retinol-binding protein 4	4.906

However, only one of the receptors with the highest fit score was selected, i.e., 1S0X. The higher the fit score, the higher the affinity of the pharmacophore group in the ligand and the more suitable the receptor. This research identified ten (10) pharmacophore groups that were suitable to the ligand and 1S0X receptor. They consisted of seven (7) hydrophobic groups, one (1) negative, and two (2) acceptors, as shown in Figure 1.

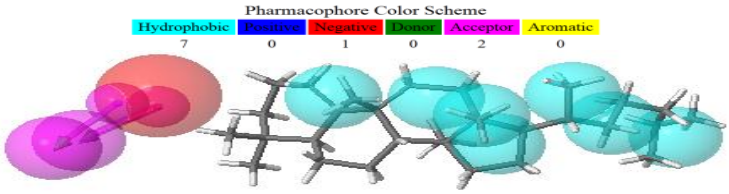


Figure 1. The pharmacophore of cucurbitacin compounds

ROR (Related Orphan Receptor) is one type of PPAR (Peroxisome Proliferator-Activated Receptors) receptor in humans which belongs to the nuclear receptor group. It is found in liver, muscle, and adipose tissue. It plays a role in several biological processes in the body, including glucose metabolism. Furthermore, it is indicated to have the potential as the therapeutic target in diabetes mellitus treatment (Kojetin and Burris, 2014).

Kojetin and Burris (2014) explain that one of the ligands bound to the receptor RORα is a diabetes mellitus drug from the thiazolidinedione group, namely Rosiglitazone. Therefore, this study used Rosiglitazone as a comparator in determining the effectivity of the test ligands.

Protein Analysis

1S0X was analyzed on the pdbSUM web server using the Ramachandran plot to identify the stability of the protein. Ramachandran plot is used to visualize the three-dimensional coordinates of proteins obtained from experiments in protein structure (using the internal coordinates). Every amino acid residue can be depicted as a region in the Ramachandran plot because it has one dihedral angle Φ (phi) and one dihedral angle ψ (psi), which constitute the internal coordinates in the three-dimensional coordinates of a protein (Torshin *et al.*, 2016). The results of the receptor analysis can be seen in Figure 2 and Table III.

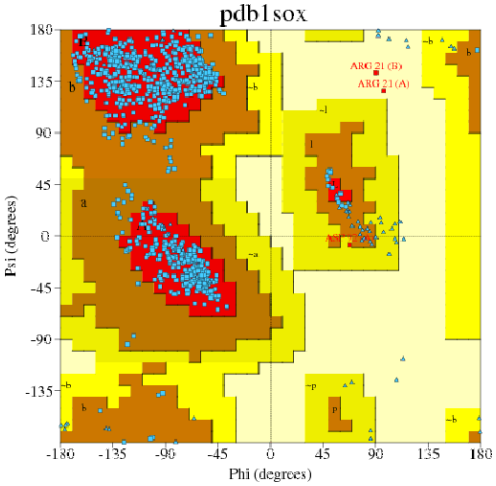


Figure 2. The Ramachandran plot of the receptor (1SOX) before molecular dynamics (MD) simulation

Table III. The ramachandran plot statistics

Categories		No. of Residues	Percentage
Most favored regions	[A,B,L]	659	89.3%
Additional allowed regions	[a,b,l,p]	76	10.3%
Generously allowed regions	[~a,~b,~l,~p]	1	0.1%
Disallowed regions	[XX]	2	0.3%

Based on the analysis results presented in Figure 2 and Table III, the disallowed region on the Ramachandran plot for non-glycine residues is smaller than 15%, indicating that the protein in 1S0X is stable and can be used for the next step in this study.

Non-glycine residues are unfavorable because the presence of residues other than glycine in this region can cause a steric obstruction, which potentially interferes with the conformation of protein to create a stable bond with the ligand (Gunasekaran *et al.*, 1996).

Receptor validation

This step involved the redocking of the natif_ligand C3S (cholesterol sulfate) to the receptor 1S0X using the same procedure as the docking in this study, which was performed in PLANTS. Afterward, the comparison between the C3S obtained from the redocking and the C3S on the PDB file resulted in Root Mean Square Deviation (RMSD), and the results of the crystallography were downloaded from the Protein Data Bank.

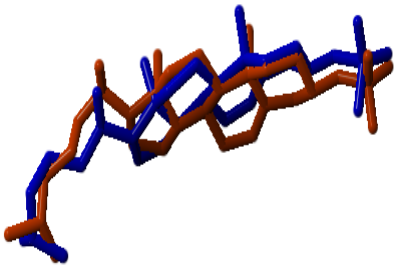


Figure 3. The comparison between the conformation of the crystal structure of C3S (red) and the docking results in the PLANTS program (blue)

The analysis in the YASARA program showed that the RMSD was 1.22 Å, indicating that the conformation of the redocking results is similar to the results of the x-ray crystallography. This RMSD also proves that the PLANTS method is valid for the docking of the test compound to the receptor 1S0X.

Test ligand docking

The free-energy of binding of the test compound-1S0X docking was compared with Rosiglitazone, which is an antidiabetic drug from the thiazolidinedione group. ΔG represents the strength of the affinity of the ligand-receptor binding. The smaller the value of ΔG, the higher the affinity. In other words, the ligand-receptor bond becomes more stable because the reaction occurs spontaneously (Levita and Mustarichie, 2012). The results of the docking between the test ligand and 1S0X are summarized in Table IV.

The affinity (ΔG) of the ligand comparator (i.e., Rosiglitazone) was -47.24 kcal/mol. Among the 26 test compounds in bitter melon plants, 14 of them had higher free-energy of binding than Rosiglitazone, namely Goyaglycoside-h, Goyaglycoside-g, Momordicoside F1, Momordicoside G, Goyaglycoside-d, Goyaglycoside-c, Momordicosida I, Momordicoside F2, Goyaglycoside-b, Goyaglycoside-a, Goyaglycoside-f, Goyaglycoside-e, Momordicoside K, and Momordicin II. Goyaglycoside-h had the least free energy (-90.4438 kcal/mol), meaning that the interaction between Goyaglycoside-h and 1S0X is the most stable among the other test ligands.

Visualization

To analyze the interactions occurring in the protein ligands, the docking results were visualized in two dimensions in the LigPlot program. Figures 4 and 5 are the visualizations of the binding of native ligand and Rosiglitazone to the receptor RORα (Laskowski and Swindells, 2011; Ruswanto *et al.*, 2015).

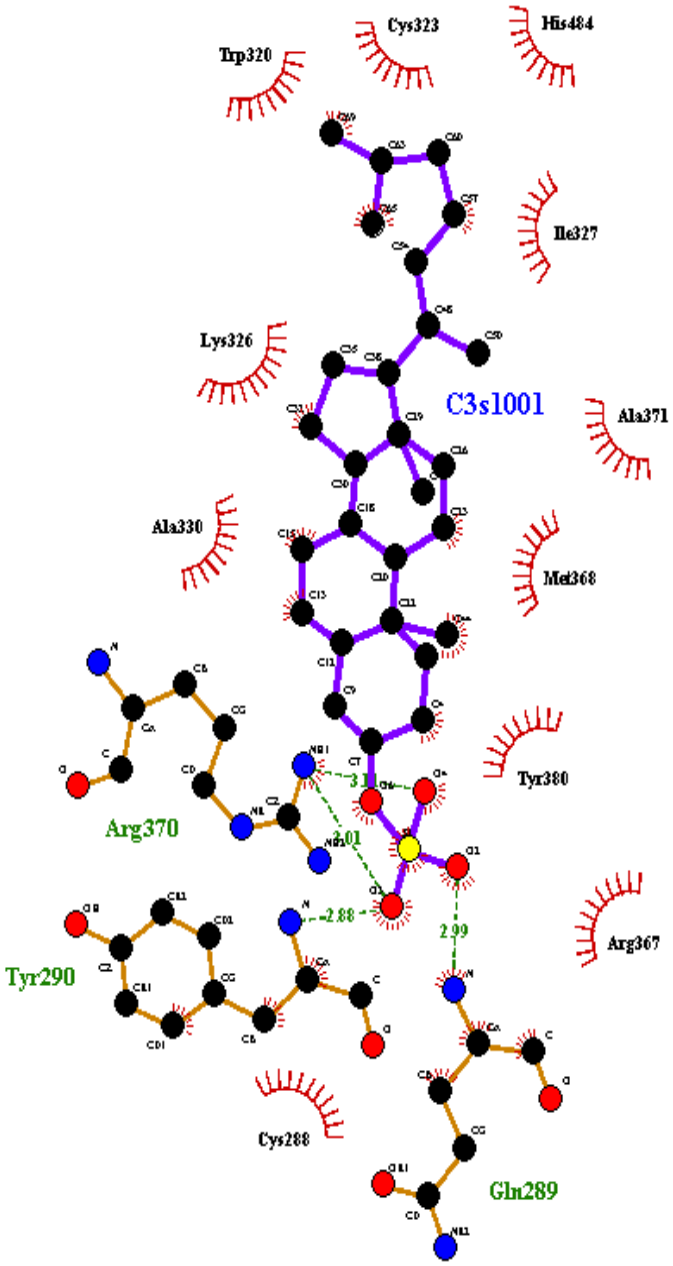


Figure 4. The visualization of the native ligand

Table IV. The bonding energy of the docking result

Ligands	ΔG (kcal/mol)
Rosiglitazone*	-47.24
Momordicin I	-42.65
Momordicin II	-49.00
Momordicin III	-44.96
25-Hydroxy-23-dihydroxy-momordicin I	-44.73
25-Methoxy-23- dihydroxy-momordicin I	-43.44
3B,7B,23-Trihydroxycucurbita-5,24-dien-7-O_B-D-glucoside	-46.21
(23E)-3B-Hydroxy-7B,25-dimethoxy-cucurbita-5,23-dien-19-al	-43.51
(23E)-3ß- Hydroxy-7ß-methoxy-cucurbita-5,23,25-trien-19-al	-41.12
(19R,23E)-5ß,19-Epoxy-19,25-dimethoxycucurbita-6,23-dien-3ß-ol	-41.77
(19R,23E)-5ß,19- Epoxy-19-methoxycucurbita-6,23,dien-3ß,25-diol	-44.89
(19R,28E)-5ß,19- Epoxy-19- methoxycucurbita -6,23,25-trien-3ß-ol	-40.85
Goyaglicoside-a	-59.59
Goyaglicoside-b	-59.80
Goyaglicoside-c	-60.31
Goyaglicoside-d	-60.49
Goyaglicoside-e	-49.34
Goyaglicoside-f	-58.48
Goyaglicoside-g	-63.93
Goyaglicoside-h	-90.44
Momordicoside A	-46.62
Momordicoside C	-46.60
Momordicoside F1	-60.59
Momordicoside F2	-59.97
Momordicoside G	-60.51
Momordicoside I	-60.11
Momordicoside K	-50.44

*Rosiglitazon= comparator

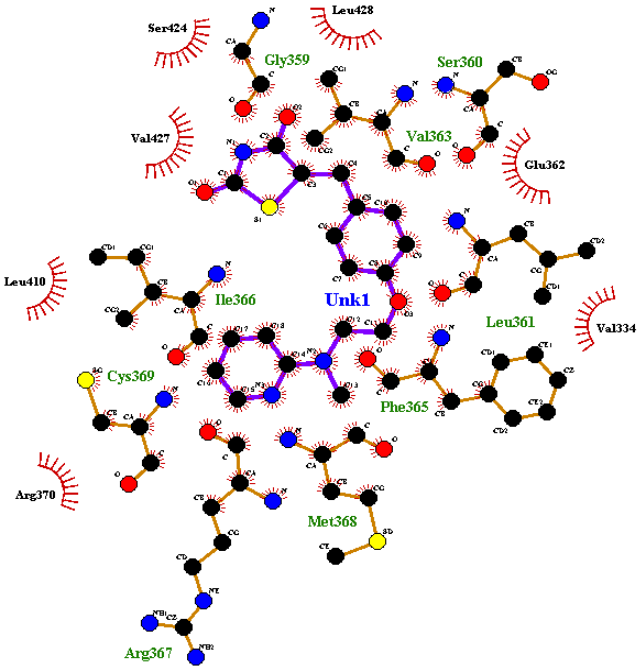


Figure 5. The visualization of Rosiglitazone

Notes: C= black, O= red, N= blue
Hydrogen bond = green dashed lines
Hydrophobic bod = eyebrow-shaped red lines

Figure 4 shows that the binding sites of the native ligand consist of hydrogen bonds with the amino acid residues Tyr290, Cys288, and Arg370 and hydrophobic bonds with Arg367, Gln289, Tyr380, Met368, Ala371, Ile327, His484, Cys323, Trp320, Lys326, and Ala330 (with hydrophobic bonds). Meanwhile, in Figure 5, hydrophobic interactions are found in the binding sites of Rosiglitazone near the amino acid residues Arg370, Leu410, Val427, Ser424, Leu428, Glu362, and Val334.

Some test ligands have similarities with the native ligand, i.e., bound to the residue Arg370 hydrophobically (such as Momordicoside K, Goyaglicoside-b, Goyaglicoside-c, Goyaglicoside-d and hydrophobically) and with hydrogen bonds (Momordicin II). Momordicin II and Goyaglicoside-g are also bound to Ala371 and Ile327 hydrophobically. Meanwhile, Momordicoside K is also bound to Met368 with hydrogen bonds. The binding of Goyaglycoside-h to Lys326 and Ile327, as well as Goyaglycoside-e to Lys326, also involve hydrogen bonds.

Furthermore, all test ligands occupy the same binding sites as Rosiglitazone, which is around Arg370, Leu410, Val427, Ser424, Leu428, Glu362, and Val334 either with hydrophobic or hydrogen bond. Such a similarity is very important for the generated pharmacological effects. The ligands are, thereby, predicted to exhibit activities on the receptor because they occupy the same binding sites as the native ligand and drug comparator.

Drug scan

The drug scan analysis aimed to examine the similarity of the properties of the test ligand and the existing drugs (drug-likeliness) and refer it to the similarity of a certain compound to oral

drugs. The Lipinski's rule can help to distinguish between drug-like and non-drug-like molecules by considering their level of absorption and permeability through the lipid bilayers in the human's body (Harganingtyas, 2011). This rule stipulates that molecules must have a molecular mass <500 g/mol, a partition coefficient (log P) <5, less than 5 hydrogen bond donors, less than 10 hydrogen donor acceptors, and molar refractivity between 40-130 (Wulandari, 2010).

Molecular mass and log P determine the ability of a compound to permeate the lipid bilayer of the epithelial tissue that lines the intestinal surface, which is penetrable only by small and sufficiently hydrophobic molecules. A greater log P indicates a more hydrophobic and fat-soluble molecule. In other words, the molecule can easily penetrate the membrane barrier. However, compounds that are too hydrophobic tend to have greater toxicity because the drug molecules are held within the lipid bilayers in a long time and cannot be excreted from the body (Wulandari, 2010).

The hydrogen bond donors and acceptors are the major contributors to the polar surface area of a molecule. The wider the area, the lower the permeability. Therefore, molecules with many H-donors and H-acceptors hardly infiltrate the membrane barrier (Wulandari, 2010).

Table V. The results of the Lipinski's rule application

Ligands	The Lipinski's Rule of Five				
	Molecular Mass	Log P	H-Donors	H-Acceptors	Molar Refractivity
Goyaglicoside-h	814.99	0.81	10	15	204.21
Goyaglicoside-g	811.00	2.22	8	14	207.20
Momordicoside F1	632.86	4.35	4	8	173.75
Momordicoside G	632.86	4.35	4	8	173.75
Goyaglicoside D	662.89	4.63	4	9	179.53
Goyaglicoside C	662.89	4.63	4	9	179.53
Momordicoside I	618.84	3.71	5	8	169.00
Momordicoside F2	618.84	3.71	5	8	169.00
Goyaglicoside-b	648.86	3.99	5	9	174.78
Goyaglicoside-a	648.86	3.99	5	9	174.78
Goyaglicoside-f	811.00	2.26	8	14	206.76
Momordicoside K	648.86	3.23	5	10	176.15
Goyaglicoside-e	779.00	1.89	8	12	205.15
Momordicin II	664.90	2.63	6	10	170.97
Rosiglitazon*	357.42	3.08	1	7	97.79

*Rosiglitazon= comparator

Based on the analysis results presented in Table V, all test compounds do not meet with the Lipinski's rule because the molecular mass is greater than 500 g/mol and the molar refractivity is not within the range of 40-130.

ADMET STUDY

The compounds resulted from the docking process were analyzed pharmacokinetically to assess their absorption, distribution, metabolism, and excretion abilities in the body. Absorption is the process of transferring the drug from its site of application to the systemic circulation and leaving a sufficient concentration to produce the desired drug effect. Because the drug is administered orally, it can experience a first pass effect, i.e., metabolism in several organs like the small intestines, blood, and liver, even before reaching the systemic circulation (Nugroho, 2012).

Distribution is the transfer of drugs from the systemic circulation to an area (fluid and tissues) in the body with the help of blood plasma proteins. However, in this case, only the free

compounds (not protein-bound) in the drugs can penetrate the body's tissues. Drugs that are strongly bound to proteins have a larger molecular size that prevents them from infiltrating the membrane pores to reach the body tissues (Nugroho, 2012).

Table VI lists the data of permeability, HIA absorption, and the percentage of plasma protein binding for each test ligand. Drug compounds must have good permeability and absorption ability in the intestines and not be bound strongly to plasma proteins to reach the target receptors and produce the desired biological activity. Based on the evaluation results, five test ligands met the criteria, namely Goyaglicoside-a, Goyaglicoside-b, Goyaglicoside-c, Goyaglicoside-d, and Momordicoside K.

In addition to ADME study, the pharmaceutical candidate compounds were subjected to toxicity test. This test can ensure that these compounds are safe for use. The drug toxicity can be acute (related to the administration of toxic substance) or chronic because the human's body is exposed to a small amount of substance over a long period—where this substance accumulates and reaches a toxic concentration that leads to the symptoms of poisoning (Mutschler, 1999).

Table VI. The ADME analysis results

Ligands	Caco-2 Cells Permeability		HIA Absorption		Plasma Protein Binding (%PPB)	
Rosiglitazone	28.61	S	97.45	B	91.09	K
Goyaglicoside-h	18.55	S	7.19	k	59.32	L
Goyaglicoside-g	18.07	S	43.15	S	68.36	L
Momordicoside F1	28.80	S	90.25	B	91.36	K
Momordicoside G	28.80	S	90.25	B	91.36	K
Goyaglicoside-d	28.80	S	90.11	B	89.08	L
Goyaglicoside-c	28.80	S	90.11	B	89.08	L
Momordicoside I	20.50	S	85.12	B	93.13	K
Momordicoside F2	20.50	S	85.12	B	93.13	K
Goyaglicoside-b	21.81	S	84.75	B	89.54	L
Goyaglicoside-a	21.81	S	84.75	B	89.54	L
Goyaglicoside-f	18.48	S	43.19	S	76.93	L
Momordicoside K	20.83	S	84.03	B	87.59	L
Goyaglicoside-e	17.51	S	53.89	S	75.85	L
Momordicin II	20.16	S	74.14	B	91.12	K

Notes: B (Good), S (Fair), k (Poor); K (Strong), L (Weak), *(Comparator)

In this study, the toxic effects could only be ascertained after a fairly long (chronic) latent period through mutagenic and carcinogenic properties. Mutagens are compounds that cause changes in basic DNA sequences and, subsequently, in proteins encoded by genes (Mutschler, 1999), while carcinogens are the ones that transform DNA and change normal cells to tumor cells, initiating tumor growth. Therefore, the effects of carcinogens are closely related to mutagenic properties, interpreted as mutagenic effects (Mutschler, 1999).

The mutagenicity of the compounds was predicted using the preADMET program based on the presence of structure alerts—which, according to Pratiwi *et al*. (2014), shows that the compounds have functional groups that can cause mutations in *S. typhimurium* in the Ames Test. Based on the results summarized in Table VII, the 14 test compounds are non-mutagenic but positive for carcinogens when administered to mice, except for Goyaglycoside-h, Goyaglycoside-f, and Momordicoside K.

Table VII. The toxicity test results

Ligands	Toxicity		
	Mutagenic	Carcinogenic	
	Ames_test	Carcino_ Mouse	Carcino_Rat
Rosiglitazone*	Mutagenic	-	-
Goyaglycoside-h	Non-mutagenic	+	-
Goyaglycoside-g	Non-mutagenic	+	+
Momordicoside F1	Non-mutagenic	+	+
Momordicoside G	Non-mutagenic	+	+
Goyaglycoside-d	Non-mutagenic	+	+
Goyaglycoside-c	Non-mutagenic	+	+
Momordicoside I	Non-mutagenic	+	+
Momordicoside F2	Non-mutagenic	+	+
Goyaglycoside-b	Non-mutagenic	+	+
Goyaglycoside-a	Non-mutagenic	+	+
Goyaglycoside-f	Non-mutagenic	+	-
Momordicoside K	Non-mutagenic	+	-
Goyaglycoside-e	Non-mutagenic	+	+
Momordicin II	Non-mutagenic	+	+

*Rosiglitazone= comparator

Molecular Dynamics (MD)

The molecular dynamics simulation was performed on one of the best candidate test compounds based on the previous analysis results, namely Goyaglycoside-h ligand that forms a complex with the receptor RORα, the output of the docking process. This simulation was carried out using the dynamic MOE (Molecular Operating Environment) program v.2009. The protein was positioned in a flexible state and influenced by solvents. The simulation used Born solvation, which is an explicit system. Proteins are naturally surrounded by solvents; therefore, the generated energy is influenced by their interaction with the solvation system.

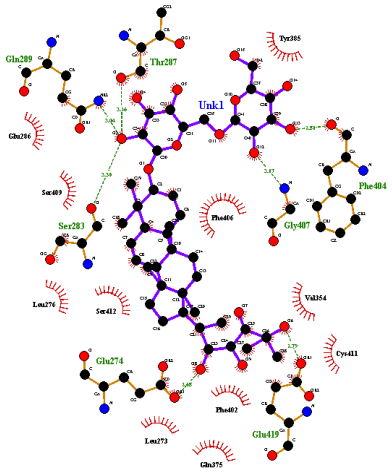


Figure 6. The visualization of the Molecular Dynamics (MD) simulation results

Before the simulation, the minimization stage was performed to relax the system by adjusting the geometric position of the atoms to obtain the lowest energy for the system (Nurbaiti,

2009). Furthermore, the simulation was run for 2,000 ps at 310 K. The addition of temperature as an influencing factor aimed to create a simulation system that resembled the normal condition of a human body. Temperature can affect the stability of the drug-receptor complex.

Figure 6 shows that after the MD simulation, the glycoside-h is attached to the amino acid residues Gln289, Thr287, Ser283, Gly407, Phe404, Gln274, and Glu419 by forming hydrogen bonds and to the residues Glu286, Ser409, Leu276, Ser412, Leu273, Gln375, Phe402, Cys411, Val354, Phe406, and Tyr385 hydrophobically.

Based on the changes in the type and amount of contact residues, the binding sites of Goyaglycoside-h has shifted due to the conformation of the protein. One residue remains interacting hydrophobically, namely Phe406. However, the ligand does not emerge from the protein, which shows that the complex formed between the ligand and the receptor is stable at 310 K.

The stability of the complex produced from the docking process is observable from the stability of the protein conformation by analyzing the generated Ramachandran Plot. The plot of the PDB files of the complex was analyzed on the Rampage web server. The stability of the protein was determined from the number of non-glycine residues in the disallowed region. The results are presented in Figure 7.

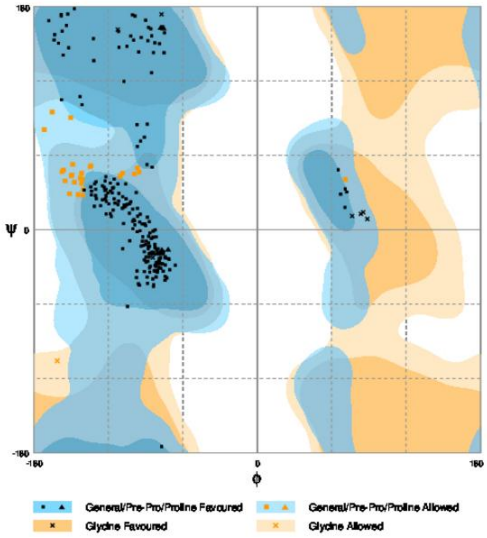


Figure 7. The Ramachandran Plot for 1S0X after MD Simulation

The plot depicts the position of amino acid residues at the dihedral angle Φ (phi) and Ψ (psi) of the peptide bond. The total number of the residues in the entire area is shown in Table VIII.

Table VIII. The ramachandran plot Statistics after MD simulation

Categories		No. of Residues	Percentage
Most favored regions	[A,B,L]	222	89.2%
Generously allowed regions	[~a,~b,~l,~p]	27	10.8%
Disallowed regions	[XX]	0	0.0%

Table VII shows that the number of non-glycine residues in the disallowed regions is 0.0%. Because it is not larger than 15%, the protein after being subjected to molecular dynamics simulation is considered stable.

CONCLUSION

Based on the pharmacophoric properties, the cucurbitacin compounds are concluded as active against the receptor RORα (Related Orphan Receptor alpha). Furthermore, the free-energy of binding from the molecular docking of the 26 cucurbitacin compounds in bitter melon plants (*M. charantia*) shows that Goyaglycoside-h is the best ligand. The molecular dynamics simulation results at a temperature of 310 K for 2,000 ps confirmed the stable interaction of the complex of Goyaglycoside-h and nuclear receptor RORα. Based on the in silico study, the active compound in bitter melon plants (*M. charantia*) that is a potential candidate for antidiabetic medication is Goyaglycoside-h.

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