PROCEEDING
4th PLANAR: International Pharmacy Ulul Albab Conference & Seminar
Malang, Sept 4th, 2024

IN SILICO STUDY OF ACTIVE COMPOUNDS IN HIBISCUS FLOWER PLANT (*HIBISCUS ROSA SINENSIS* L.) ON ALPHA GLUCOSIDASE RECEPTOR (3A4A), DPP-4 RECEPTOR (1X70), AND PPAR-Γ RECEPTOR (5Y2O) AS POTENTIAL ANTIDIABETIC

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Abstract

The deficiency of insulin hormone or the body's inability to use insulin leads to uncontrolled blood glucose or sugar levels, which is the cause of diabetes mellitus. The ethanol extract of hibiscus flower plant (*Hibiscus rosa sinensis L.*) has the ability to reduce blood glucose, suppressing hyperglycemia that causes inflammation. This study aims to evaluate the antidiabetic activity against alpha-glucosidase receptor, DPP-4 (1X70), and PPAR-γ (5Y2O), perform physicochemical predictions, and predict the toxicity of compounds in the *Hibiscus rosa sinensis L.* flower in silico. Several target proteins in the body related to hyperglycemia diseases are alpha-glucosidase (3A4A), DPP-4 (1X70), and PPAR-γ (5Y2O). The SwissADME software, which uses Lipinski's Rule of Five parameters, was used for physicochemical predictions. The online software ProTox II and pkCSM were used to predict toxicity. This software refers to LD50 and classifies toxicity classes based on GHS, Ames toxicity, skin sensitivity, and hepatotoxicity. The Molegro Virtual Docker software was used to predict the activity of the compounds. The research results show that two compounds—taraxerol acetate and β-sitosterol xyloside—do not meet Lipinski's Rule of Five parameters. Three compounds are classified in toxicity class 5, three compounds in class 4, and three compounds in class 6. The activity prediction results indicate that β-sitosterol xyloside has the lowest Rerank score compared to the original ligand of alpha-glucosidase, DPP-4, and PPAR-γ receptors, as well as compared to its comparator drug. Therefore, β-sitosterol xyloside can be recommended for further research as an antidiabetic drug candidate.

Keywords: *antidiabetic, in silico, Hibiscus rosa-sinensis L.*

Introduction

Diabetes mellitus (DM), also known as diabetes, is a metabolic disorder caused by a deficiency of the insulin hormone or the body's inability to utilize insulin effectively. As a result, blood glucose or sugar levels cannot be controlled. Insulin is produced by beta cells in the pancreas and its primary role is to signal body cells to absorb glucose from the blood, maintaining blood glucose levels within normal limits (Nugroho & Prahutama, 2017). The World Health

Organization (WHO) states that diabetes is the sixth leading cause of death worldwide. Data show that approximately 1.3 million people die from diabetes, and about 4% of these deaths occur before reaching the age of 70. Compared to rural populations, the majority of diabetes-related deaths occur in the age group of 45 to 54 years. The International Diabetes Federation (IDF) estimates that by 2030, diabetes will become the seventh leading cause of death worldwide. The number of people with diabetes has doubled since 1980, rising from 4.7% to 8.5% of the adult population. Additionally, this increase reflects the growing number of obese individuals in recent decades (Huda *et al*., 2022).

Efforts to develop antidiabetic drugs have been driven by the increasing number of diabetes patients in Indonesia. One such medication comes from plants that have long been used in traditional medicine. Because they are considered safer, easier to obtain, cheaper, and more economical, people prefer using herbal plants. Hibiscus flowers (Hibiscus rosa sinensis L.) are among the plants that are frequently utilized and have proven to have numerous benefits. Hibiscus flowers have been shown to help with many ailments, including fever, cough, canker sores, diarrhea, hypertension, liver disorders, and diabetes mellitus. Hibiscus is also known to accelerate menstruation (Alya *et al*., 2020).

The most commonly used treatment for Type 2 Diabetes Mellitus (DM 2) is the use of α-glucosidase enzyme inhibitors. This enzyme plays a vital role in the breakdown of carbohydrates into glucose in the body, which can ultimately increase blood glucose levels. Therefore, an agent is needed to inhibit α-glucosidase enzyme activity in order to control the increase in blood sugar levels. α-glucosidase inhibitors work by blocking the activity of this enzyme, which is typically responsible for breaking down starch into glucose in the small intestine. As a result, the absorption of glucose from carbohydrate breakdown in the small intestine is delayed, which ultimately reduces postprandial blood glucose levels. Although acarbose and miglitol are recognized as standard α-glucosidase enzyme inhibitors by WHO and IDF, these drugs are also known to cause various side effects, such as bloating, nausea, diarrhea, and flatulence. Therefore, research continues to develop more effective treatments with minimal side effects (Weni *et al*., 2020).
Inhibiting the serine peptidase enzyme dipeptidyl peptidase-IV (also known as DPP-IV), which is responsible for converting incretins into inactive metabolites, is one effective method for controlling blood glucose levels. Incretins, or glucagon-like peptide-1 (GLP-1), stimulate insulin secretion that responds to glucose concentrations. By inhibiting dipeptidyl peptidase-IV, the levels of GLP-1 in circulation can be increased. In turn, insulin biosynthesis and secretion can be enhanced, which addresses hyperglycemia that often occurs in Type 2 diabetes.

Thus, inhibiting dipeptidyl peptidase-IV can elevate circulating GLP-1 levels, enhancing insulin biosynthesis and secretion, which ultimately helps manage the hyperglycemia often seen in Type 2 diabetes, enabling more effective and measurable blood glucose control (Fakih & Dewi, 2020). Ali *et al*. (2017) successfully isolated several compounds from hibiscus leaves and flowers (*H. rosa sinensis*), including n-tetracosanyl cyclopentylcarboxylate, taraxerol acetate, 26β-cyclopentyl n-hexacosan-5β-ol, β-sitosterol, stigmasterol, peltoboykinolic acid, maprounic acid, oleanolic acid, and β-sitosterol xyloside. Research on the compounds found in hibiscus flowers as antidiabetic agents is still limited, and therefore, it is necessary to test their activity and screen their physicochemical properties based on the Lipinski's Rule of Five parameters to determine whether these compounds have characteristics that align with pharmacokinetic parameters in the body. Furthermore, toxicity testing should also be conducted to ensure that these compounds do not have harmful effects on the human body. Thus, conducting this research can help in the development of new drugs as a therapy for diabetes mellitus from compounds derived from medicinal plants.

Material and Methods

This study uses a computer laptop with an Intel(R) Pentium(R) CPU B970 processor and 2 GB of RAM. Additionally, the software used includes Windows 7, SPSS for Windows, Chem Bio

Draw Ultra Version 12 (CambridgeSoft), Chem Bio 3D Ultra Version 12 (CambridgeSoft), Molegro Virtual Docker, SMILES Translator, pkCSM online tool, and ProTox-II online tool.

To create ligands, the Chem Bio Ultra Version 12 software is used to draw the 2D molecular structure, which is then converted into SMILES format. The 2D structure of the comparator drug is obtained from https://pubchem.ncbi.nlm.nih.gov/ and then copied into Chem Bio 3D Ultra Version 12. After obtaining the stereochemistry of the compound and the most stable form, the Chem Bio 3D Ultra Version 12 program is used to stabilize the structure by minimizing energy using methods such as MMFF94, MM2, MM3, OPLS, and others. The structure is then saved in the SYBYL.mol2 file for docking.

After downloading the protein structure of the receptor with the previously determined code in pdb format from the website https://www.rcsb.org, hydrogen atoms are added to the receptor (since the downloaded receptor does not contain hydrogen atoms), and the downloaded protein is corrected for any errors or missing amino acid components. This process is typically done automatically by the software.

To predict the physicochemical properties of a compound, Chem Bio Draw Ultra Version 12 is used to draw the 2D structure, which is then copied into SMILES format. Using the pkCSM online tool, the physicochemical parameters of the compound are predicted according to Lipinski's Rule of Five, such as molecular weight (MW), octanol/water partition coefficient (Log P), hydrogen bond acceptors (HBA), and hydrogen bond donors (HBD). The ProTox-II online tool is used to predict the toxicity (LD_{50}) per oral in rodents based on the Globally Harmonized System (GHS) requirements. The pkCSM online tool is used to measure toxicity based on skin sensitivity, Ames toxicity, and hepatotoxicity.

Receptor validation is performed by re-docking the original ligand with the intended receptor using the Molegro Virtual Docker 6.0 program. To use the validation parameters, the RMSD (Root Mean Square Deviation) value must be checked through the docking wizard menu. This procedure is similar to the one described for setting up Molegro Virtual Docker 6.0 above. Next, the reference ligand is selected for RMSD calculation to validate the RMSD value between the original ligand and the receptor. If the RMSD value $\leq$2 Å, the receptor is considered valid, and the docking process of the test compounds can proceed. Replication validation is done three times to test the precision of the assay.

Using the pkCSM online tool, the toxicity data of the compounds found in hibiscus are classified based on their LD_{50} value and the toxicity class is determined using ProTox-II. Then, hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), molecular weight (MW), and the octanol/water partition coefficient (Log P) can be used to predict the physicochemical properties of the compound.

Result and Discussion

Physicochemical Properties Prediction

Lipinski's Rule of Five typically explains how a compound, whether hydrophobic or hydrophilic, can dissolve or be solubilized so that a drug can penetrate the cell membrane through passive movement from high to low concentration. In Lipinski's Rule of Five, the first parameter is molecular weight (MW). A compound should not have a molecular weight greater than 500 g/mol. This is because if a compound has a molecular weight exceeding 500 g/mol, it will be difficult to penetrate the cell membrane (Narko *et al.*, 2017). Log P is the next parameter that indicates the lipophilicity and hydrophobicity of a molecule. A higher Log P value indicates that the molecule is more hydrophobic, as it will be retained longer in the lipid bilayer or the core structure of the cell membrane and will spread more widely in the body. A lower Log P value also indicates that the molecule is more toxic because it cannot cross the lipid membrane or the core structure of the cell membrane. High hydrophobicity of the molecule increases its toxicity as it will stay longer in the lipid bilayer or the core structure of the cell membrane and spread more

widely in the body. As a result, binding selectivity will decrease against the target enzyme. However, an extremely negative Log P value is also disadvantageous because the molecule will not be able to cross the lipid bilayer membrane, which consists of two layers (Kilo *et al*., 2019).

According to the predicted physicochemical properties of the compounds from the hibiscus plant, Table 1 shows that only the drug compounds acarbose, taraxerol acetate, and β-sitosterol xyloside do not meet the requirements of Lipinski's Rule of Five

Table 1. Result of Physicochemical Properties Prediction

Compounds	MW (g/mol)	Log P	HBD	HBA	TPSA	Lipinski RO5
n-tetracosanyl cyclopentylcarboxylate	450.78	9.85	0	2	26.30	Yes
Taraxerol acetate	507.79	7.66	0	3	38.66	No
6β-cyclopentyl n-hexacosan-5β-ol	450.82	10.37	1	1	20.23	Yes
β-sitosterol	414.71	7.24	1	1	20.23	Yes
Stigmasterol	412.69	6.98	1	1	20.23	Yes
Peltoboykinolic acid	456.70	5.96	2	3	57.53	Yes
Maprounic acid	456.70	6.14	2	3	57.53	Yes
Oleanolic acid	456.70	6.07	2	3	57.53	Yes
β-sitosterol xyloside	546.82	5.95	3	5	79.15	No
Acarbose	645.60	-6.03	14	19	321.17	No
Sitagliptin	407.31	2.51	1	10	77.04	Yes
Pioglitazone	356.44	3.09	1	4	93.59	Yes

Furthermore, since hydrogen bonding can influence various physicochemical properties of compounds, such as water solubility, boiling point, and melting point, the number of O-H and N-H groups that donate hydrogen bonds and the number of O and N atoms that accept hydrogen bonds each affect the biological activity of the compound (Narko *et al.*, 2017). The number of hydrogen bonds donated and accepted by the ligand can also determine the flexibility and ability of the ligand to bind to a specific protein or enzyme. The values for Hydrogen Bond Acceptors (HBA) and Donors (HBD) should not exceed five and ten, respectively. The Topological Polar Surface Area (TPSA) is a value that indicates a compound's ability to pass through the membrane and is used to predict drug transport properties. The TPSA value of the drug compound is <140 Å, and in the Lipinski SwissADME parameters, compounds that meet Lipinski's Rule of Five are displayed as "Yes; 0 violation" (Daina *et al*., 2017).

Toxicity Prediction

LD, or Lethal Dose, is the average amount of a substance that can cause death in 50% of a test animal group. It is a way to measure the potential short-term toxicity or acute toxicity of a substance. Based on the data above, it can be concluded that six compounds fall into class 4, three into class 5, and three into class 6. The compounds in class 4 include β-sitosterol, stigmasterol, peltoboykinolic acid, oleanolic acid, and pioglitazone. Compounds in class 5 include sitagliptin, taraxerol acetate, and n-tetracosanyl cyclopentylcarboxylate. On the other hand, compounds such as acarbose, maprounic acid, and β-sitosterol xyloside fall into class 6. According to the Globally Harmonized System (GHS), the six toxicity classes are divided based on the LD_{50} value. Classes 1 to 3 have high toxicity, making them quite dangerous; Classes 4 to 6 have low toxicity, making them less dangerous. The predicted toxicity results shown in the table above indicate that the compounds in the hibiscus plant have low toxicity, meaning they are less hazardous. If the LD50 value is lower, the compound is more toxic. Conversely, if the LD_{50} value is higher, the toxicity is lower.

The next parameter is the Ames test, also known as the mutagenic test, which is used to evaluate a compound's ability to cause mutagenic effects through bacteria. A positive test result indicates that the compound is mutagenic and may potentially be a carcinogenic agent (Kesuma *et al.*, 2018). Based on the results in the table above, it is known that none of the compounds are mutagenic.

Table 2. Result of Toxicity Prediction

Compound	LD_{50} (mg/kg)	Toxicity Class	Ames Toxicity	Hepatoxicity	Skin Sensitization
n-tetracosanyl cyclopentylcarboxylate	5000	5	No	No	Yes
Taraxerol acetate	5000	5	No	No	No
6β-cyclopentyl n-hexacosan-5β-ol	2000	4	No	No	Yes
β-sitosterol	890	4	No	No	No
Stigmasterol	890	4	No	No	No
Peltoboykinolic acid	750	4	No	Yes	No
Maprounic acid	6176	6	No	Yes	No
Oleanolic acid	2000	4	No	No	No
β-sitosterol xyloside	8000	6	No	No	No
Acarbose	24000	6	No	No	No
Sitagliptin	2500	5	No	Yes	No
Pioglitazone	1000	4	No	Yes	No

The next parameter is Hepatotoxicity, which is used to determine whether the tested compound is toxic to the liver. According to the data in the table above, there are 4 compounds that are toxic to the liver: Maprounic acid, Peltoboykinolic acid, and the reference compounds Sitagliptin and Pioglitazone. Meanwhile, the other compounds are predicted to be non-toxic to the liver (Pires *et al*., 2015). Skin Sensitivity, also known as the skin sensitivity test, occurs when susceptible individuals are exposed to a sufficient number of allergens that activate, develop, and expand T-cells responsive to the allergen. The last parameter in the toxicity prediction is this. The skin sensitivity test is a method used to determine whether the tested compounds can cause skin irritation. The two compounds that were tested and can cause skin irritation are 26β-cyclopentyl n-hexacosan-5β-ol and n-tetracosanyl cyclopentylcarboxylate (Pires *et al*., 2015).

Docking Validation

Using Molegro Virtual Docker 6.0 software, the original ligand is re-docked to the target receptor to validate the receptor. The RMSD value, or root mean square deviation, is used as the validation parameter. If the RMSD value is less than 2 Å, the docking process of the test compound can begin (Muttaqin *et al*., 2019). The average RMSD values for alpha-glucosidase (3A4A) is 0.660513 Å, dipeptidyl peptidase-4 (1X70) is 0.678937 Å, and Peroxisome Proliferator Activated Receptor (PPAR) (5Y2O) is 1.350087 Å. These values indicate that the receptors 3A4A, 1X70, and 5Y2O meet the receptor validation parameter because the RMSD values produced are ≤2 Å.

Table 3. Result Docking Validation

Receptor	RMSD (R1)	RSMD (R2)	RMSD (R3)	Average
3A4A	0.531149	0.921534	0.528855	0.660513
1X70	0.326394	0.510268	1.20015	0678937
5Y2O	1.28302	1.21724	1.55	1.350087

Docking Result

The results of the docking process between the ligands of compounds from hibiscus flowers and the comparator compounds with the receptors 3A4A, 1X70, and 5Y2O using Molegro Virtual Docker 6.0 software are displayed in Tables 4, 5, and 6. Three parameters are known: MolDock score, Rerank score, and H-bond. These three components can be used to measure the binding strength of the drug with the receptor (CLCbio, 2013). The first docking analysis result, on the alpha-glucosidase receptor (3A4A) with compounds from hibiscus flowers, shows that the average Rerank score for the test compound β-sitosterol xyloside is lower compared to other compounds from hibiscus flowers, which is -138.328 kcal/mol. The comparator drug, acarbose, has an average Rerank score of -137.633 kcal/mol, while the native ligand has an average Rerank score of -72.5425 kcal/mol. Based on these results, it can be concluded that the test compound β-sitosterol xyloside has the lowest average Rerank score on the alpha-glucosidase receptor (3A4A) compared to the native ligand, comparator drug, and other test compounds.

Table 4 Docking result on α-glucosidase receptor (3A4A) with acarbose as comparator

Compounds	Parameter	R1	R2	R3	Average
Native ligand	Rerank score	-73.1557	-71.3184	-73.1534	-72.5425
	MolDock Score	-75.049	-70.7749	-75.0483	-73.6241
	H-Bond	-17.7831	-14.4193	-17.8148	-16.6724
Acarbose	Rerank score	-142.949	-131.895	-138.056	-137.633
	MolDock Score	-147.75	-139.84	-142.329	-143.306
	H-Bond	-19.7692	-24.1923	-18.0316	-20.6644
n-tetracosanyl cyclopentylcarboxylate	Rerank score	-79.9224	-67.6999	-82.6352	-76.7525
	MolDock Score	-126.702	-117.482	-120.484	-121.556
	H-Bond	0	0	-0.609584	-0.20319
Taxerol acetate	Rerank score	-32.2402	-105.056	-104.052	-80.4494
	MolDock Score	-142.169	-141.122	-142.377	-141.889
	H-Bond	-0.418378	0	-2.32098	-0.91312
26β-cyclopentyl n-hexacosan-5β-ol	Rerank score	-94.5291	-96.9239	-36.1703	-75.8744
	MolDock Score	-146.856	-138.121	-118.345	-134.441
	H-Bond	0	0	0	0
β-sitosterol	Rerank score	-107.594	-100.294	-97.8058	-101.898
	MolDock Score	-130.919	-135.738	-125.683	-130.78
	H-Bond	0	0	0	0
Stigmasterol	Rerank score	-109.155	-99.4872	-104.139	-104.26
	MolDock Score	-134.823	-129.094	-135.525	-133.147
	H-Bond	-3.05679	0	-3.05921	-2.03867
Peltoboykinolic acid	Rerank score	-103.072	-102.951	-102.858	-102.96
	MolDock Score	-132.717	-132.697	-132.681	-132.698
	H-Bond	-2.51589	-2.50879	-2.5064	-2.51036
Maprounic acid	Rerank score	-16.3475	-16.1363	-16.2959	-16.2599
	MolDock Score	-106.719	-106.602	-106.724	-106.682
	H-Bond	-5.5945	-5.58727	-5.59322	-5.59166
Oleanolic acid	Rerank score	-63.4125	-63.4805	-63.4336	-63.4422
	MolDock Score	-111.732	-111.731	-111.733	-111.732
	H-Bond	-3.57712	-3.67923	-3.57711	-3.61115
β-sitosterol xyloside	Rerank score	-139.174	-131.787	-144.023	-138.328
	MolDock Score	-164.133	-158.256	-168.809	-163.733
	H-Bond	-6.63451	-6.41114	-6.01692	-6.35419

Next, the docking results on the DPP-4 receptor (1X70) show that the test compound β-sitosterol xyloside has a lower average Rerank score than other compounds from hibiscus flowers. The comparator compound, sitagliptin, has an average Rerank score of -88.3574 kcal/mol, and the native ligand has an average Rerank score of -121.03 kcal/mol. These results indicate that the native ligand has a lower Rerank score than the test compound β-sitosterol xyloside.

Table 5. Docking result on DPP-4 receptor (1X70) with sitagliptin as comparator

Compounds	Parameter	R1	R2	R3	Average
Native ligand	Rerank score	-126.151	-125.041	-111.897	-121.03
	MolDock Score	-150.041	-146.716	-138.877	-145.211
	H-Bond	-3.43108	-3.42368	-4.96422	-3.93966
Acarbose	Rerank score	-88.6823	-84.9871	-91.4029	-88.3574
	MolDock Score	-108.829	-111.003	-108.915	-109.582
	H-Bond	-5.61585	-1.12622	0	-2.24736
n-tetracosanyl cyclopentylcarboxylate	Rerank score	-97.408	-87.296	-89.1425	-91.2822
	MolDock Score	-134.101	-111.557	-118.755	-121.471
	H-Bond	0	-0.259573	0	-0.08652
Taxerol acetate	Rerank score	-92.4704	-84.4078	-85.5124	-87.4635
	MolDock Score	-124.235	-117.767	-123.254	-121.752
	H-Bond	0	-0.96647	-0.0161432	-0.32754
26β-cyclopentyl n-hexacosan-5β-ol	Rerank score	-106.718	-73.6831	-89.5907	-89.9973
	MolDock Score	-147.29	-115.099	-138.694	-133.694
	H-Bond	0	0	0	0
β-sitosterol	Rerank score	-87.9451	-75.7944	-75.8553	-79.8649
	MolDock Score	-112.366	-102.276	-99.1543	-104.599
	H-Bond	0	0	0	0
Stigmasterol	Rerank score	-105.503	-87.5255	-93.7014	-95.5766
	MolDock Score	-140.933	-112.036	-129.088	-127.352
	H-Bond	-2.88636	-3.10724	-3.45562	-3.14974

Compounds	Parameter	R1	R2	R3	Average
Peltoboykinolic acid	Rerank score	-71.3343	-71.3695	-71.3794	-71.3611
	MolDock Score	-96.212	-96.1706	-96.1924	-96.1917
	H-Bond	-1.14774	-1.20588	-1.20984	-1.18782
Maprounic acid	Rerank score	-71.8057	-71.59	-71.8074	-71.7344
	MolDock Score	-95.8053	-95.8476	-95.809	-95.8206
	H-Bond	-2.5	-2.5	-2.5	-2.5
Oleanolic acid	Rerank score	-74.3709	-75. 1147	-72.9411	-73.656
	MolDock Score	-102.823	-89.8651	-102.949	-98.5457
	H-Bond	-4.48063	-1.59309	-4.66485	-3.57952
β-sitosterol xyloside	Rerank score	-112.973	-91.4286	-92.5635	-98.9884
	MolDock Score	-130.816	-114.863	-113.323	-119.667
	H-Bond	-8.47312	-5.56737	-4.08269	-6.04106

The final docking results on the PPAR-γ receptor (5Y2O) show that the test compound β-sitosterol xyloside has an average Rerank score of -104.873 kcal/mol. The comparator compound, pioglitazone, has an average Rerank score of -116.776 kcal/mol, and the native ligand has -105.448 kcal/mol. Based on these results, the comparator compound pioglitazone has a lower average Rerank score compared to the test compound β-sitosterol xyloside and the native ligand.

Table 6. Docking result on PPAR-γ receptor (5Y20) with pioglitazone as comparator

Compounds	Parameter	R1	R2	R3	Average
Native ligand	Rerank score	-103.8	-108.247	-104.296	-105.448
	MolDock Score	-122.075	-126.58	-123.116	-123.924
	H-Bond	-2.32921	-2.23511	-4.71143	-3.09192
Acarbose	Rerank score	-109.583	-123.452	-117.294	-116.776
	MolDock Score	-134.418	-153.177	-151.678	-146.424
	H-Bond	-0.358398	-2.31168	-1.65986	-1.44331
n-tetracosanyl cyclopentylcarboxylate	Rerank score	-97.0177	-86.6833	-89.4677	-91.0562
	MolDock Score	-145.349	-129.888	-124.98	-133.406
	H-Bond	0	0	0	0
Taxerol acetate	Rerank score	-75.0169	-63.7691	-51.9776	-63.5879
	MolDock Score	-112.581	-128.791	-133.814	-125.062
	H-Bond	0	-1.35001	-2.24931	-1.19977
26β-cyclopentyl n-hexacosan-5β-ol	Rerank score	-105.722	-106.19	-95.0753	-102.329
	MolDock Score	-156.257	-140.835	-137.54	-144.877
	H-Bond	0	0	0	0
β-sitosterol	Rerank score	-62.3756	-86.6412	-83.8937	-77.6368
	MolDock Score	-112.064	-112.998	-110.747	-111.936
	H-Bond	0	0	0	0
Stigmasterol	Rerank score	-100.137	-84.5678	-92.6082	-92.4377
	MolDock Score	-130.282	-125.399	-119.453	-125.045
	H-Bond	-3.03527	-0.972757	-2.5	-2.16934
Peltoboykinolic acid	Rerank score	-79.6443	-79.8678	-79.8628	-79.7916
	MolDock Score	-106.755	-106.941	-106.829	-106.842
	H-Bond	0	0	0	0
Maprounic acid	Rerank score	-59.9214	-62.3766	-71.6064	-64.6348
	MolDock Score	-106.163	-105.75	-103.495	-105.136
	H-Bond	-2.5	-2.20943	-2.5	-2.40314
Oleanolic acid	Rerank score	-36.2986	-66.5874	-29.1778	-44.0213
	MolDock Score	-79.4427	-90.5806	-110.134	-93.3858
	H-Bond	-2.5	-1.57545	-0.41695	-1.49747
β-sitosterol xyloside	Rerank score	-106.594	-101.71	-106.315	-104.873
	MolDock Score	-112.609	-126.125	-124.454	-121.063
	H-Bond	-1.73133	-7.0863	-1.6286	-3.48208

Ligand-Receptor Interaction

There are 3 types of bonds in the amino acid interactions between hibiscus flower compounds, the reference drug, and the receptor: hydrogen bonds, electrostatic bonds, and steric interactions. Only the hydrogen bonds and steric interactions are marked with blue and red dashed lines in visualization. The binding process of the test compound with fifteen amino acid residues (Arg 315, Glu 277, Phe 159, Phe 303, Lys 156, Ser 240, Glu 411, Asp 352, Asp 215, Asp 69, Arg

442, Gln 279, Gln 353, Ser 241, and Lys 156) was studied. Several test compounds from the hibiscus flower have amino acid residues similar to the native ligand and acarbose; the compound β-sitosterol xyloside has the lowest Rerank score among the ligands with the 3A4A receptor. Sitosterol xyloside binds to amino acids such as His 280, Lys 156, Ser 157, Glu 411, Arg 442, Asp 215, and Asp 69. The amino acid residues of this compound are similar to the native ligand and the reference drug acarbose. Figure 1 shows how the amino acids in the 3A4A receptor interact with the ligand of the test compound.

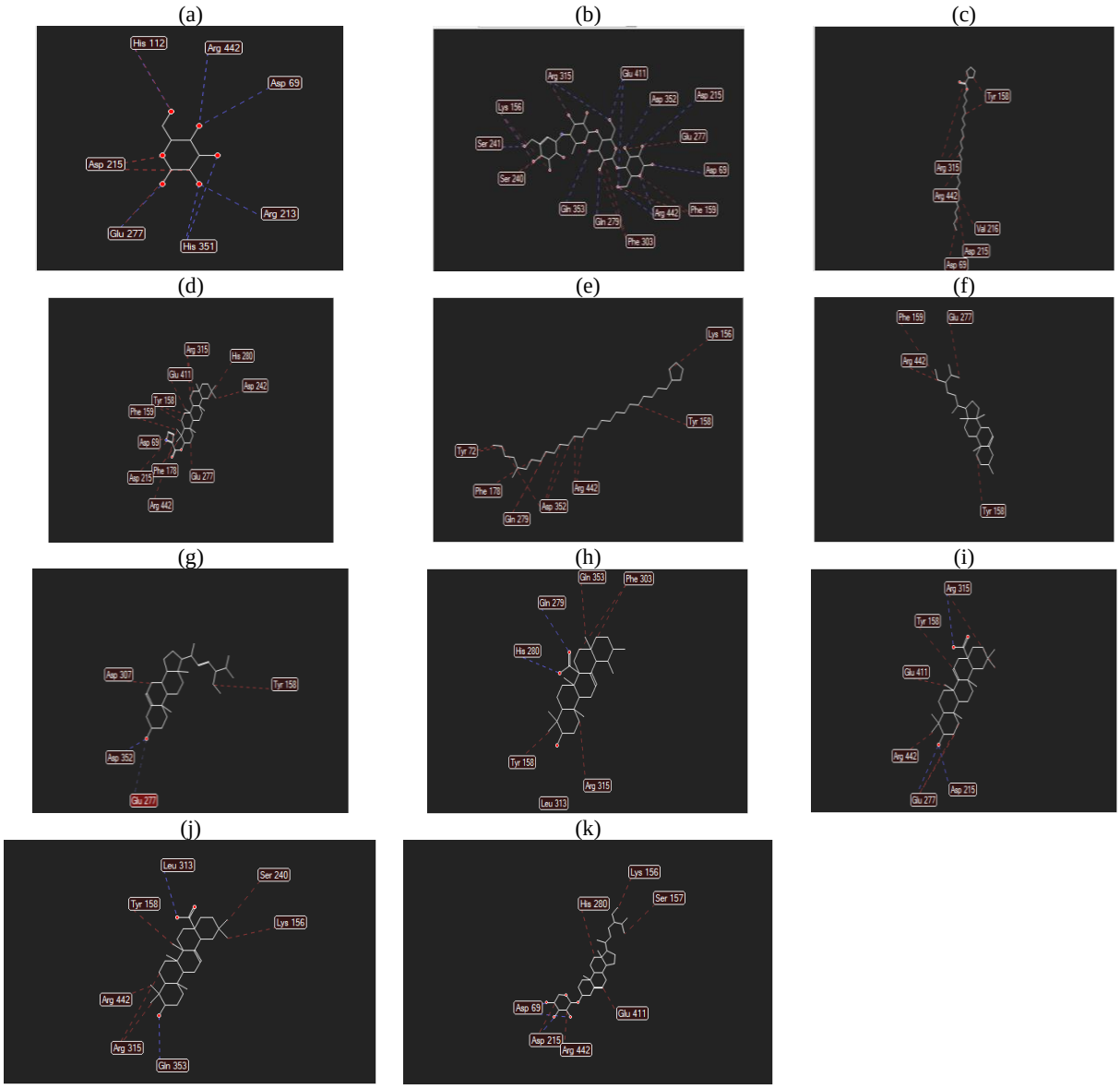

Figure 1: Interaction of ligand with amino acid from α-glucosidase receptor (3A4A). a). *Native ligand, b*). Acarbose c). n-tetracosanyl cyclopentylcarboxylate d). Taxerol acetate e). 26β-cyclopentyl n-hexacosan-5β-ol f). β-sitosterol g). Stigmasterol h). Peltoboykinolic acid i). Maprounic acid j). Oleanolic acid k). β-sitosterol xyloside

The native ligand of the DPP-4 receptor (1X70) binds with ten residues: Ser 209, Arg 358, Tyr 662, Asn 710, Arg 125, Val 711, Val 656, Glu 206, Glu 205, and Tyr 661. In contrast, the reference drug sitagliptin binds with four amino acid residues: Arg 356, Ser 360, Glu 206, and Arg 358. The amino acid residues of the hibiscus flower compounds share some similarities with the native ligand and reference drug binding. The compound β-sitosterol xyloside has the amino acid residue Arg 125, similar to the native ligand. The similarity of the compound's residues with the

reference drug or native ligand suggests that these compounds may exhibit similar activity. This illustrates how the amino acids in the DPP-4 receptor (1X70) interact with the ligand of the test compound.

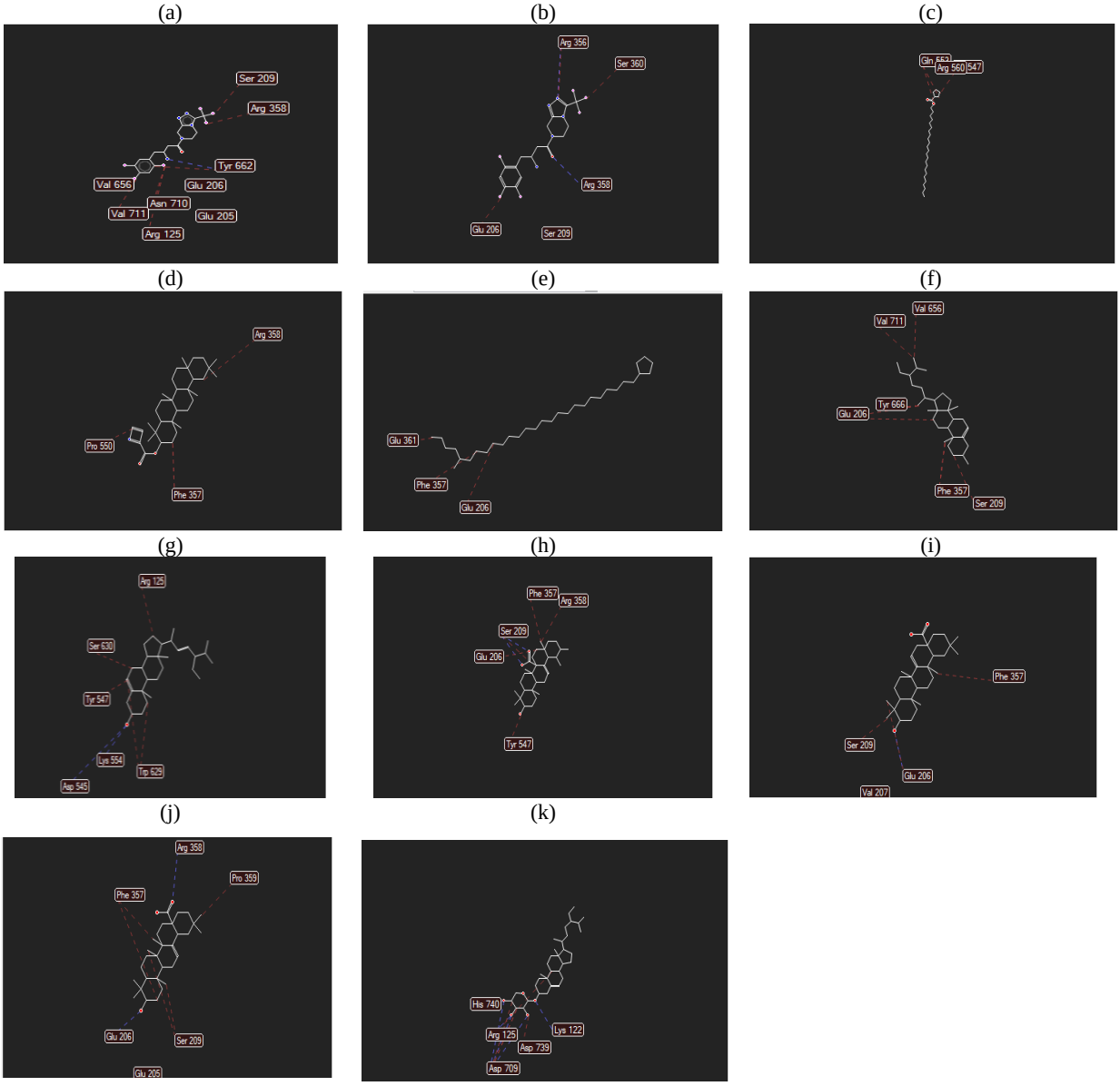

Figure 2. Interaction of ligand with amino acid from DPP-4 receptor (1X70). a). *Native ligand, b*). Acarbose c). n-tetracosanyl cyclopentylcarboxylate d). Taxerol acetate e). 26β-cyclopentyl n-hexacosan-5β-ol f). β-sitosterol g). Stigmasterol h). Peltoboykinolic acid i). Maprounic acid j). Oleanolic acid k). β-sitosterol xyloside

In the PPAR-γ receptor (5Y2O), it is known that the native ligand of this receptor binds with six residues: Ser 289, His 323, Phe 282, Ser 289, Tyr 473, and His 449. The reference drug (pioglitazone) binds with eight amino acid residues: Phe 282, Cys 285, Phe 226, Met 329, Ala 292, Ile 326, Ser 289, and Arg 288. Several test compounds from the hibiscus plant have amino acid residues similar to pioglitazone, one of which is the compound β-sitosterol xyloside, which has the lowest Rerank score among the ligands with the PPAR-γ receptor (5Y2O). The compound β-sitosterol xyloside binds with amino acids Gln 283, Gly 284, Cys 285, Ile 281, Cys 285, and Leu 340, where the amino acid residue Cys 285 is common with the reference drug pioglitazone. The

interaction results between the amino acids in the PPAR-γ receptor (5Y2O) and the ligand of the test compound are illustrated in Figure 3.

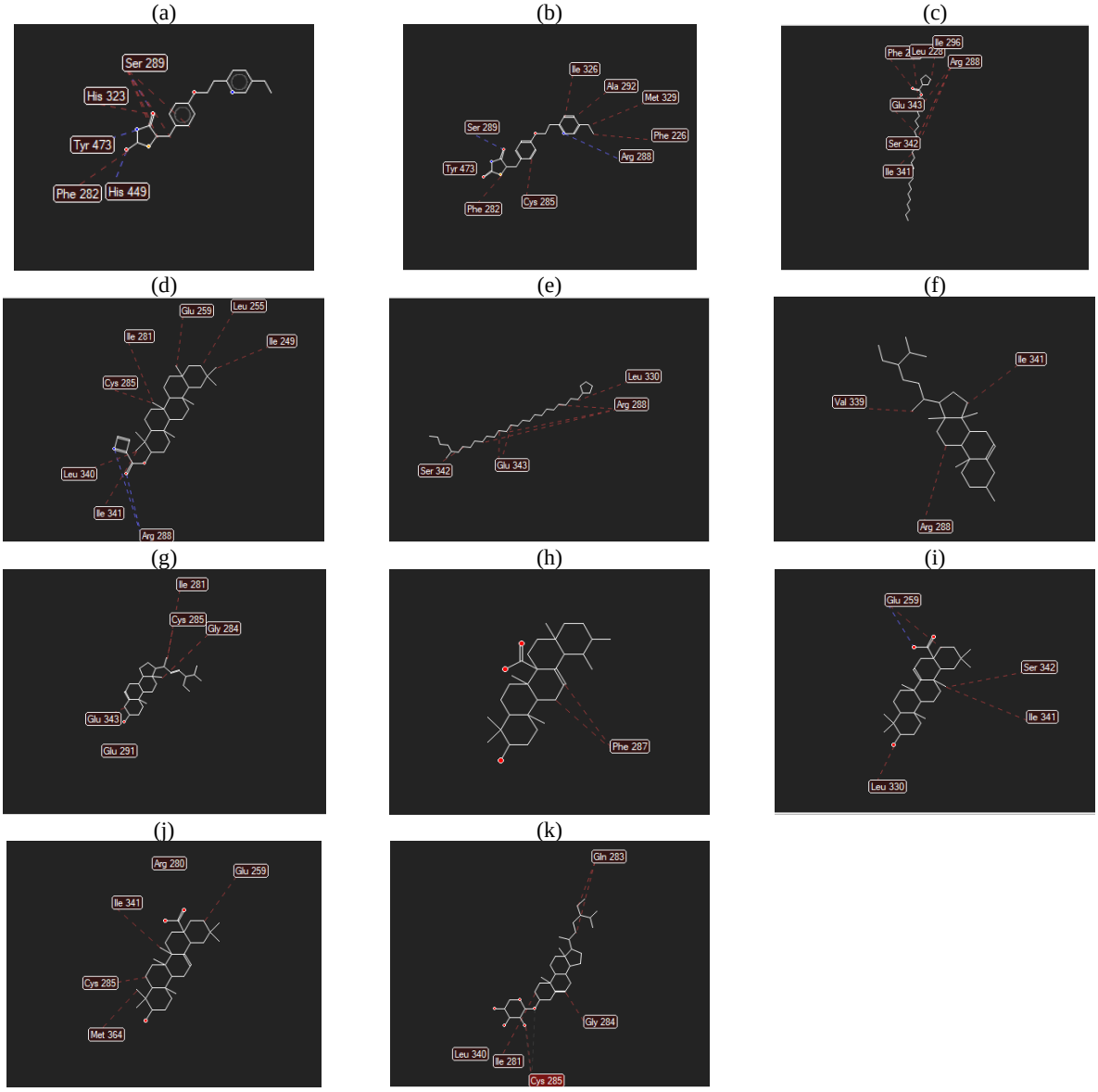

Figure 3. Interaction of ligand with amino acid from DPP-4 receptor (1X70). a). *Native ligand, b*). Acarbose c). n-tetracosanyl cyclopentylcarboxylate d). Taxerol acetate e). 26β-cyclopentyl n-hexacosan-5β-ol f). β-sitosterol g). Stigmasterol h). Peltoboykinolic acid i). Maprounic acid j). Oleanolic acid k). β-sitosterol xyloside

The hibiscus plant compound, β-sitosterol xyloside, shows the highest potential activity among the other test compounds based on amino acid interactions and docking results. The lowest Rerank score indicates that its binding is more stable. Additionally, the compound β-sitosterol xyloside binds with amino acids similar to the reference drug. Due to its comparable activity with the native ligand and the reference compounds on glucosidase, Dipeptidyl peptidase-4 (DPP-4), and Peroxisome Proliferator Activated Receptor - gamma (PPAR-gamma) receptors, it can be concluded that the compound β-sitosterol xyloside found in hibiscus plants has potential as an antihypertensive. As a result, further testing of the hibiscus plant compound on these receptors is necessary to determine the outcome.

Conclusion

The compounds found in hibiscus flowers plant show good activity predictions against the α-glucosidase receptor, Dipeptidyl peptidase-4 (DPP-4), and Peroxisome Proliferator Activated Receptor -γ (PPAR-γ). Among these compounds, β-sitosterol xyloside stands out as the most effective anti-diabetic candidate, based on the Rerank score and amino acid interactions. The compounds found in hibiscus flowers are predicted to have physicochemical properties that align with Lipinski's Rule of Five, except for two compounds, β-sitosterol xyloside and taraxerol acetate, which do not meet the Lipinski's Rule of Five parameters. Six compounds fall under toxicity class 4; three compounds fall under toxicity class 5, three other compounds under toxicity class 6.

Acknowledgement

Researcher would like to thank Prof. Dr. Siswandono, Apt., M.S., to have license to the Molegro Virtual Docker 6.0 program.

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