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Interaction of Singawalang Leaves (*Petiveria alliacea*) Compounds as a Reduction of Blood Glucose Levels in Treatment of Type 2 Diabetes Mellitus Disease

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AMPK-α2 is important in controlling glucose homeostasis, carbohydrate, fat, and protein metabolism, so AMPK-α2 plays an important role in the pathophysiology of type 2 diabetes. Therefore, AMPK-α2 is an important therapeutic target in managing type 2 diabetes. (*Petiveria alliacea*) is a type of plant in Indonesia and is empirically used by residents to treat diabetes mellitus. The purpose is to examine the interaction and affinity of compounds from singawalang to the AMPK-α2 receptor and its toxicity prediction so that it becomes an alternative treatment for type 2 diabetes mellitus with minimum risk of side effects. The methods of this study are molecular docking simulations and prediction toxicity. The results of the simulation molecular docking of the Singawalang isolate compound against the AMPK-α2 receptor obtained the best test ligands Benzyl 2-hydroxyethyl trisulphide, which have ΔG -5.16 kcal/mol and Ki 43.24 µM with the residues amino acids VAL A: 96, TYR A: 95, GLU A: 94. While the prediction of toxicity shows that the benzyl 2-hydroxyethyl trisulphide test ligand has the potential to be mutagenic but not carcinogenic. Therefore, it is necessary to modify the structure to be able to provide a lower toxicity effect. The conclusion is that benzyl 2-hydroxyethyl trisulphide is the most potential candidate compound with the highest affinity and low risk of toxicity.

Keywords: AMPK-α2 | Diabetes Type 2 | Molecular Docking | PreADMET | Singawalang

Diabetes mellitus (DM) is a chronic metabolic disorder with multiple etiologies characterized by high blood sugar levels that exceed normal limits. DM is caused by an increase in blood sugar due to decreased insulin secretion, so diabetes is classified as type 2 DM. Apart from being a cause of death, DM is also a cause of blindness, kidney failure, and heart disease. According to estimates by the International Diabetes Federation (IDF) in 2019, at least 483 million people in the age range 20-79 years in the world suffer from diabetes, equivalent to a prevalence rate of 9.3%. This figure is estimated to continue to increase in direct proportion to the addition of the age of the population to 19.9% or 111.2 million people aged 65-79 years. DM management in general aims to improve the quality of life of diabetic patients, which includes short-term goals, namely eliminating DM complaints, improving quality of life, and reducing the risk of acute complications; the long-term goal is to prevent and inhibit the progression of microangiopathy and macroangiopathy complications; and the ultimate goal of management is to reduce DM morbidity and mortality[1].

One of the actions that can be taken to control blood glucose is to improve the process of glucose metabolism through the regulation of the enzyme 5' adenosine monophosphate-activated protein kinase (AMPK). AMPK has an important role in controlling glucose homeostasis, carbohydrate, fat, and protein metabolism. AMPK consists of three subunits namely α1, α2, β, and γ. The α1 subunit is mostly located in the cytosol which functions to control signaling pathways for metabolic processes. The α2 subunit is located in the nucleus and functions to regulate transcription and gene expression[2]. Singawalang plant (*Petiveria alliacea*) belongs to the *Phytolaceae* family which is a plant that grows a lot in Indonesia but its use as a medicine has not been widely used. In the area where this plant originates, namely the Amazon forest, this plant has long been used as a traditional medicinal ingredient known as Anamu or Apacin[3]. Singawalang has been proven preclinically as a diabetes mellitus drug. According to the journal that became the reference for this study, Singawalang leaf extract can reduce blood glucose levels in type 2 DM rats at doses of 90 mg/kg bb and 360 mg/kg bb. The mechanism of action of Singawalang leaf extract in lowering blood glucose levels is through AMPK activation[2]. However, Singawalang leaf extract has more than one kind of active ingredient, It is suspected that each of these active ingredients has a different mechanism in providing pharmacological effects. These various active ingredients will bind to various receptors in various organs to produce signal transduction which results in improving blood glucose levels. These active ingredients include benzaldehyde, benzyl 2-hydroxyethyl trisulphide, coumarin, isoarborinol, isoarborinol acetate, isoarborinol cinnamate, isothiocyanates, polyphenols, senfols, tannins, and trithiolaniacine[2].

This study aims to predict the secondary metabolites in Singawalang leaf extract that interact with AMPK-α2 receptors. One of the methods that can be used is molecular docking then the prediction of ADME and the toxicity of the compounds obtained are carried out.

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Methods

Tools

Hardware: Laptop Rog-Strix Core i7 RAM 8 GB. Software: Chem3D Pro 12.0, ChemDraw Ultra 12.0 (https://chemistrydocs.com/chemdraw-ultra-12-0/), Biovia Discovery Studio 2020 (https://discover.3ds.com/discovery-studio-visualizer-download), and AutoDock Tools-1.5.6 (https://autodock.scripps.edu/downloads/).

Materials

The test ligands used were compounds isolated from Singawalang leaves, namely benzaldehyde, benzyl 2-hydroxyethyl trisulphide, myricitrin, isoarborinol acetate, enletin, astilbin, barbinervic acid, allantoin, oleic acid, coumarin, friedelinol, and isoarborinol. Native ligand coexisting with the AMPK-α2 receptor with the PDB code 3AQV. While the comparison ligand used was metformin.

Procedure

Receptor preparation

The receptor used is AMPK-α2, whose 3D structure was obtained from the Protein Data Bank with code 3AQV. Macromolecules or receptor proteins are then prepared by adding hydrogen atoms and Kollman charges using AutodockTools 1.5.6 to obtain files in the form of pdbqt.

Method Validation

Method validation was carried out by redocking native ligand compounds, 6-[4-(2-piperidine-1-ylethoxy)phenyl]-3-pyridine-4-ylpyrazolo[1,5-a]pyrimidineon the AMPK-α2 receptor. The parameter used in method validation is the Root Mean Square Deviation (RMSD) value. The method is declared valid if the RMSD value of the redocking results is ≤ 2 Å[4].

Preparation of Test Compounds

The 3D shape of the test compound is obtained by downloading the structure via Pubchem in sdf. Then the test compound format was changed to PDB using the Biovia Discovery Studio application. The structure of the test compound was then prepared with the help of AutodockTools-1.5.6 so that a file in pdbqt form was obtained.

Docking Simulation Process

Docking simulation was performed using AutoDockTools-1.5.6 software. The grid box set for the parameters of the ligand test compound and the receptor is adjusted according to the size of the ligand (fit to ligand). The grid box dimensions x have a value of 10, y has a value of 16, and z has a value of 10 with coordinates respectively -6.302, 44.128, and 7.231 with a distance of 1.000 Å. The parameter used is the Genetic Algorithm with 50 GA runs. Then the griding and docking processes were run through the Command Prompt program with the final result of docking being 50 poses[5].

Data analysis

Docking results were analyzed using Autodock 4.2.6. Parameters analyzed included amino acid residues, hydrogen bonds, predicted inhibition constants, and free energy Bonds. Determination of the best-docked ligand conformation is seen and considered based on these parameters[6].

Pharmacokinetic and Toxicity Prediction (Pre-ADMET)

Pharmacokinetic and Toxicity predictions are obtained through the website https://preadmet.bmdrc.kr/[7]. Initially, the structure of the test compound was drawn and then submitted to produce several parameters that indicated predictions of adsorption, distribution, metabolism, and excretion as well as predictions of toxicity[8]. Absorption parameters were obtained based on HIA and $Caco_2$ values, distribution parameters based on PPB (Plasma Protein Binding) and BBB (Blood-Brain Barrier) values, metabolic parameters based on CYP2C19, CYP2C9, CYP2D6, CYP3A4 activities, and toxicity based on the mutagenic and carcinogenic potential of each test compound[9].

Result

Table 1. Method Validation Results

PDB ID	GridBox (x,y,z)	Validation		Free Energy (kcal/mol)
		RMSD (Å)	Reference (Å) [10]	
3AQV	-6.302 44.128 7.231	1.45	≤ 2	-7.79

Table 2. Test Compound Docking Results and Comparison

No	Compound	ΔG(kcal / mol)	Ki (μM)	Amino acid residue	
				Hydrogen Bonds	Other Bonds
Native Ligand					
1	Pyrimidine	-7.79	1.94	VAL A:96	*LYS A:107, *GLY A:98, *GLY A:99, *GLU A:100, *TYR A:104, *ASP A:103, *GLY A:167, *MET A:164, *LYS A:45, *ASP A:157, ^^^ILE A:77, ^^^VAL A:30, ^^^ALA156, ^MET

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					A:93, ^^^LEU A:22, ^^^ALA A :43, ^^TYR A:95, ***LEU A:146, **GLU A:94
2	Metformin	-5.16	165.64	SER A: 165	GLU A:100, ASP A:103, ASP A:166, *LEU A:22, *GLY A:167, *GLU A:143, *MET A:164, *LEU A:146
			Test Compound		
1	Benzaldehyde	-3.90	1370	VAL A : 96	^^^ALA A : 43, ^^^ALA A : 156, ^^^VAL A : 30, ^MET A : 93, ^^^ILE A : 77, **GLU A : 94, **TYR A : 95
2	Benzyl 2-hydroxyethyl trisulphide	-5.95	43.24	VAL A: 96, TYR A: 95, GLU A: 94	***LEU A:146 , ^^^LEU A:22, ^^^ALA A: 43, **GLY A:99
3	Myricitrin	-2.49	14880	VAL A:96, GLY A:167, ASP A:103	*VAL A:30, *MET A:164, *GLU A:168, *GLU A:100, **GLY A:99, *GLY A:98,ASP A:166, ^^^LEU A:146, ^^^LEU A:22, ^^TYR A:95
4	Isoarborinol acetate	-7.57	2.84	-	*MET A:163, *LYS A:45, *MET A:93, *GLU A:94, *VAL A:96, *TYR A:95, *GLY A:99, *LYS A:107, *ASP A:103, *GLY A:167, *GLU A:100,VAL A:30, ALA A:43, ALA A:156, ILE A:77, LEU A:22, LEU A:146, MET A:164
5	Engeletin	-2.98	6530	ASP A:103, TYR A:95, GLY A:99, VAL A:96	***MET A:164, ***LEU A:146, ^^^ALA A:43, ^^^ALA A:156, ^^^ILE A:77
6	Astilbin	-4.43	562.11	GLU A:96, VAL A:96	^^^ALA A:43, ^^^LEU A:146, ***LEU A:22, **TYR A:95
7	Barbinervic acid	-2.37	18340	GLY A:167	*GLU A:168 *ASP A:103 *ASP A:166 *GLU A:100^^^LEU A:22 ^^^VAL

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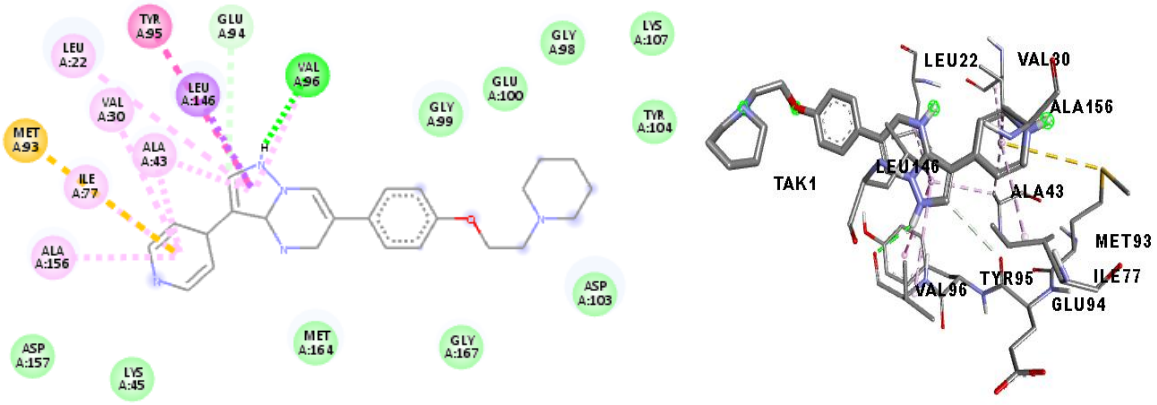
					A:30*ALA A:156 *ALA A:43^^^TYR A:95 ^^^LEU A:146*VAL A:96^^^MET A:164*GLY A:99
8	Allantoin	-3.18	4630	GLY A:99 VAL A:96	*GLU A:94, **TYR A:95 *LEU A:146 *SER A:97 *LEU A:22 *MET A:164 *VAL A:30 *ALA A:43
9	Oleic Acid	-3.90	1370	-	MET A:164, ALA A:156, LYS A:45, VAL A:30
10	Coumarins	-4.56	453.19	VAL A:96	^^^ALA A:43 ***LEU A:146 *MET A:164 *GLY A:99 ^^^LEU A:22 **TYR A:95 GLU A:94*ILE A:77
11	Friedelinol	-3.74	1810	-	*ASP A:103 *GLU A:100^^^LEU A:146 ^^^MET A:164 ^^^VAL A:30 ^^^TYR A:95 ^^^LEU A:22*GLU A:94^^^ALA A:43*ILE A:77 *VAL A:96 *GLY A:99 *SER A:97 *GLY A:98 *TYR A:104
12	Isoarborinol	-7.45	3.45	-	^^^VAL A:30 ^^^ALA A:156 ^^^LYS A:45 ^^^ALA A:43 ^^^ILE A:77 ^^^MET A:164 ^^^LEU A:146 ^ ^^LEU A:22 ^^^MET A:93 ^^^TYR A:95

Description:

*van der waals, **Carbon hydrogen bonds, ***Pi-sigma, ^Pi-sulfur, ^^Pi-pi stacked, ^^^Pi-alkyl, Salt Bridge and Attractive Charge, Unfavorable acceptor-acceptor, and Alkyl



(a)

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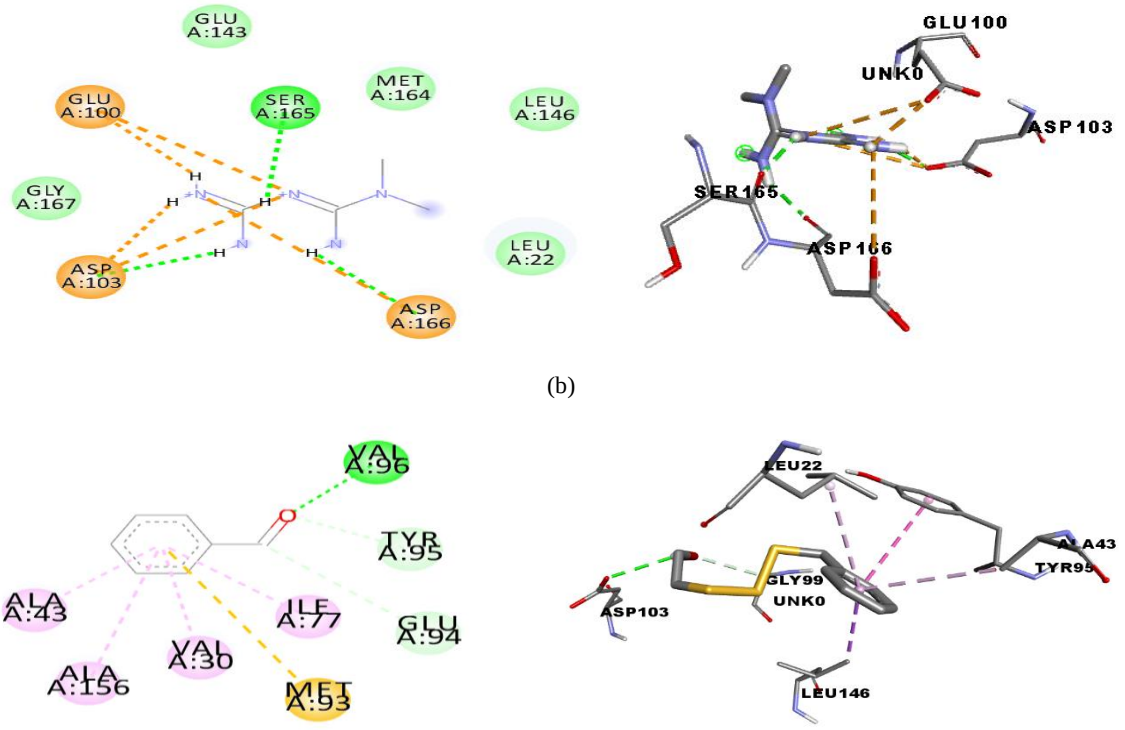
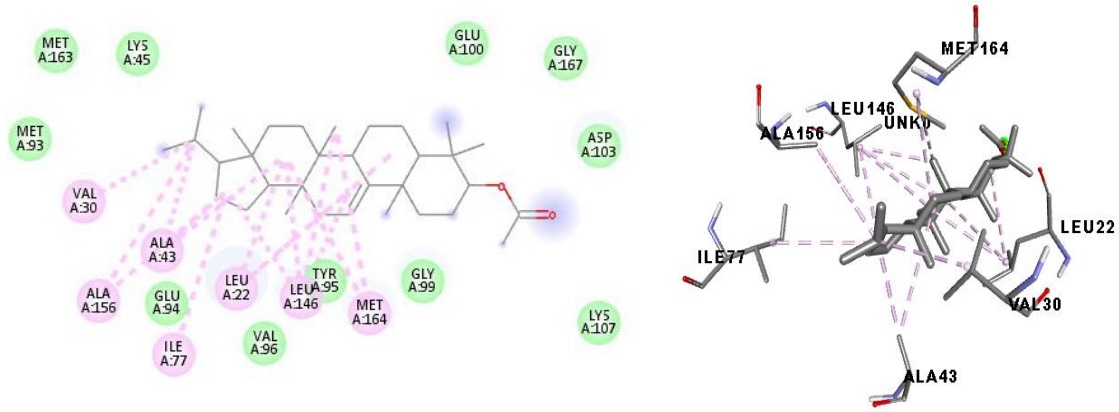
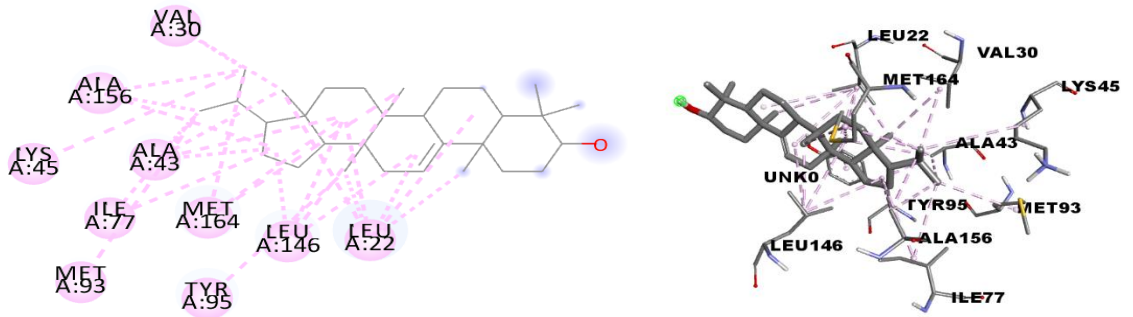


(b)

(c)



(d)



(e)

Figure 1. Interaction Amino Acids Residue of (a) pyrimidine, (b) metformin, (c) Benzyl 2-hydroxyethyl trisulphide, (d) Isoarborinol acetate, and (e) Isoarborinol

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Table 3. ADME Prediction Results and Toxicity of Test Compounds and Comparisons

Compound	Absorption		Distribution		Metabolism				Toxicity	
	HIA (%)	Caco2	PPB (%)	BBB (%)	CYP2C19	CYP2C9	CYP2D6	CYP3A4	Mutagen	Carcinogen
Metformin (Comparison)	45.66	45.66	3.95	0.22	Yes	No	No	No	Mutagens	Positive / Positive
Benzyl 2- hydroxyethyl trisulphide	97.63	51.56	84.61	0.1	Yes	Yes	No	Yes	Mutagens	Negative / Negative
Isoarborinol	100	47.1	100	20.56	No	Yes	No	Yes	Non-Mutagenic	Positive / Positive
Isoarborinol acetate	100	51.61	100	16.28	No	Yes	No	Yes	Non-Mutagenic	Positive / Positive
Benzaldehyde	100	21.87	4.5	1.39	Yes	Yes	No	Yes	Mutagens	Negative / Negative
Myricitrin	11.64	6.14	65.37	0.03	Yes	Yes	No	Yes	Non-Mutagenic	Negative / Negative
Engeletin	41.98	8.17	74.48	0.04	Yes	Yes	No	Yes	Non-Mutagenic	Negative / Negative
Astilbin	9:55 p.m	7.36	76.44	0.03	Yes	Yes	No	Yes	Non-Mutagenic	Negative / Negative
Barbinervic acid	91.23	20.92	95.45	0.62	No	Yes	No	Yes	Non-Mutagenic	Positive / Positive
Allantoin	35.76	15.73	12	0.12	Yes	No	No	No	Mutagens	Negative / Positive
Oleic Acid	98.43	28.19	100	7.48	Yes	Yes	No	Yes	Mutagens	Positive / Positive
Coumarins	100	32.12	43.39	1.56	Yes	Yes	No	No	Mutagens	Positive / Positive
Friedelinol	100	45.94	100	20.76	No	Yes	No	Yes	Non-Mutagenic	Negative / Positive

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Discussion

Receptor and Ligand Preparation

The test protein used is the AMPK-α2 protein with PDB code 3AQV. This protein complex consists of the native ligand 6-[4-(2-piperidin-1-ylethoxy)phenyl]-3-pyridine-4-pyrazole [1,5-a]pyrimidine. The 3D structure of AMPK-α2 was obtained from the Protein Data Bank with code 3AQV on the site https://www.rcsb.org/pdb/[11]. The receptor protein was prepared by adding hydrogen atoms and Kollman charges using AutodockTools 1.5.6 until a pdbqt file was obtained. Test ligands totaling 12 compounds derived from Singawalang leaves obtained from several research journals. All of the tested ligands were modeled in 2 and 3 dimensions to determine the molecular docking simulation and ADMET prediction parameters[12].

Method Validation

Validation of the analytical method is an act of evaluating certain parameters, based on experiments, in this case, the in silico test, to prove that these parameters meet the requirements for their use 6-[4-(2-piperidine-1-ylethoxy)phenyl]-3-pyridine-4-ylpyrazolo[1,5-a] pyrimidine ($C_{24}H_{25}N_5O_2$) on the AMPK-α2 receptor.

Validation was carried out using the Biovia Discovery Studio 2020 application and AutoDock Tools-1.5.6. The parameter used in method validation is the Root Mean Square Deviation value (RMSD). RMSD is a parameter that describes how much the protein-ligand interaction changes in the crystal structure before and after docking. The method is declared valid if the RMSD value of the redocking results is ≤ 2 Å[4]. The validation of this method uses an optimal grid that is made to have the same size as the native ligand with details of the grid box dimensions x is worth 10, y is worth 16, and z is worth 10 with coordinates respectively, namely -6.302, 44.128, and 7.231 and a distance of 1,000 Å. From the simulation results of native ligand docking, the RMSD value was 1,450 Å, which indicated that the selected receptor, AMPK-α2 (3AQV), could be used to perform in silico molecular docking tests. More details can be seen in Table 1.

Molecular Simulation of Test Compound Docking

A docking simulation of the test compound was carried out to determine the interaction between the test compound and the active site of the receptor and to predict the test compound which has the best conformation of interaction with the receptor[4]. There are several parameters obtained from the docking of the test compounds, including the value of free energy bonds (ΔG), Inhibition Constant (Ki), and the interaction that occurs between the ligand and the amino acid on the receptor[13]. The interaction is predicted through hydrogen bonds, van der Waals bonds, and other bonds. The parameters resulting from the docking of the test compounds were then compared with the comparison[14]. The comparisons used are pyrimidine compounds as native ligands and metformin as diabetes drugs which are available in the market today and Metformin is a drug that induces the formation of the hormone insulin[15].

Molecular docking results show that the smallest Gibbs free energy value of the interaction between the test compounds originating from Singawalang and the receptors, namely isoarborinol acetate, isoarborinol, benzyl 2-hydroxyethyl trisulphide, coumarin, and astilbin each has an energy value of -7.57, -7.45, -5.95, -4.56, and -4.43. Meanwhile, the Gibbs free energy value of the native ligand was -7.79 and -5.16 for metformin. The Gibbs energy value indicates the strength of the bond and conformational stability between the tested ligand and the 3AQV receptor. A lower Gibbs energy value indicates a more stable conformation[16]. This value is influenced by various interactions that occur between the ligand and the receptor, such as hydrogen bonds, electrostatic interactions, and hydrophobic interactions.

Another parameter seen from the molecular docking results is the inhibition constant. The inhibition constant is a parameter that shows the interaction that occurs between the ligand and the receptor[17]. The value of the inhibition constant is directly proportional to the Gibbs free energy, the smaller value of inhibition constant, the more stable interactions that occur. So the compounds that have the best inhibition constants are isoarborinol acetate, isoarborinol, and benzyl 2-hydroxyethyl trisulphide. However, when viewed from the type of interaction that occurs between the three test compounds with the AMPK-α2 receptor, it is known that isoarborinol acetate and isoarborinol do not have hydrogen bonds. This makes it easy for the ligand to lose interaction with the receptor.

Important amino acid residues at the AMPK-α2 receptor (PDB code: 3AQV) are Ala43, Lys45, Tyr95, Val96, Leu146, Ala156, and Met164[18]. Which correspond to amino acid residues that are hydrogen bonded to the ligands, namely pyrimidine, metformin, benzaldehyde, Benzyl 2 -hydroxyethyl trisulphide, myricitrin, engeletin, astilbin, allantoin, and coumarins. Benzyl 2-hydroxyethyl trisulphide has hydrogen bonds with VAL A: 96 and TYR A: 95 which are important amino acid residues on the AMPK-α2 receptor whereas pyrimidine standard ligand only binds VAL A: 96. Therefore, although pyrimidine has free binding energy larger than Benzyl 2-hydroxyethyl trisulphide but in terms of binding to the active site of the receptor, Benzyl 2-hydroxyethyl trisulphide binds more strongly to the receptor. The complex shape of the bonds and amino acid residues involved in the interaction between the native ligand, the reference compound, and the test compound originating from Singawalang and the AMPK-α2 receptor can be seen in Figures 1.

ADMET prediction

The next stage in the procedure for selecting potential compounds for drug candidates is the analysis of ADME and its toxicity properties to reduce the possibility of failure in drug development. ADME review and its toxicity properties were carried out using the PreADMET program. There are several parameters used in this ADMET review, including the HIA score, Caco2 as an absorption aspect, then PPB and BBB as a distribution aspect, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 as a metabolism and excretion aspect as well as carcinogenic and mutagenic potential for toxicity aspects. The prediction results of ADME and the toxicity of the tested and comparator compounds can be seen in Table 3[19].

Based on the Gibbs energy and inhibition constant, 3 compounds were selected, namely benzyl 2-hydroxyethyl trisulphide, isoarborinol acetate, and isoarborinol for comparison with metformin (the comparator drug). The HIA or Human Intestinal Absorption score is a parameter that can predict the absorption of active substances in the human intestine. Where the HIA score has 3 categories, namely HIA 0-20% is classified as low, 20-70% is classified as moderate, and 70-100% is classified as high. Judging from the results obtained, the HIA score of the three tested compounds is a good value because if the HIA score is in the range of 70-100% it indicates that the compound can be absorbed properly in the intestine. The HIA scores of the three test compounds also had higher HIA score than metformin[20,21].

In addition to assessing the absorption of candidate drug compounds, Caco2 cell testing can also be performed. Caco2 cell assay is recommended as an in vitro model to predict the absorption of active drug substances/transport of drugs administered orally through the intestinal epithelium. Judging from the results, the three test compounds have moderate permeability or absorption ability in Caco2 cells because they are in the range of 4-70 nm/sec and have a better value than metformin[22].

In the distribution aspect, PPB and BBB were reviewed. Where PPB or Plasma Protein Binding is a parameter used to predict the distribution of a drug based on the drug's attachment to plasma proteins. The PPB value is classified into two, namely if it has a value of more than 90% then it binds strongly to plasma protein whereas if the value is less than 90% then it binds weakly to plasma protein. A drug is said to be efficient if it can freely cross the membrane and reach the target rather than binding to plasma

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proteins. From the results obtained, the benzyl 2-hydroxyethyl trisulphide and metformin compounds have weak binding activity with plasma proteins because the PPB value is less than 90%[23].
While BBB or Blood Brain Barrier is a parameter used to see the ability of a drug to penetrate the brain barrier area or not. BBB is one of the most important parameters because test compounds that have activity in the central nervous system must have the ability to penetrate the brain barrier area. Conversely, if the target of the drug is not related to the activity of the central nervous system, it should not penetrate the brain barrier area because it can cause side effects on the central nervous system. A BBB review was carried out and the results showed that the BBB values of metformin and benzyl 2-hydroxyethyl trisulphide were in the middle range, namely 0.1 to 2, indicating that these two compounds have a moderate ability to penetrate the brain barrier area[24].
Aspects that need to be considered next are metabolism and excretion. This aspect can be reviewed by seeing whether the test compound has the same activity as its comparator compound/drug in terms of inhibition of CYP2C19, CYP2C9, CYP2D6, and CYP3A4. From the results of the review, it was found that the compound benzyl 2-hydroxyethyl trisulphide has activity as an inhibitor on CYP2C19, CYP2C9, and CYP3A4. The compounds isoarborinol and isoarborinol acetate have activity as inhibitors on CYP2C9 and CYP3A4. Whereas metformin only showed activity as an inhibitor on CYP2C19. So the test compound that has an inhibitory activity similar to metformin is the compound benzyl 2-hydroxyethyl trisulphide.
The last aspect to note is the aspect of toxicity. Testing the toxicity aspect can be carried out with the Ames Test which is used to see whether the tested compound has potential mutagenic properties or not, and tests are carried out on rats and mice to determine the carcinogenic potential of the drug compound. carcinogenic, the compounds isoarborinol and isoarborinol acetate have the potential to be carcinogenic, while the tested compound benzyl 2-hydroxyethyl trisulphide has the potential to be mutagenic[25].

Conclusion

The best compound from Singawalang leaf isolate (*Petiveria alliacea*) which has the potential to reduce blood glucose levels in the treatment of diabetes mellitus is the compound benzyl 2-hydroxyethyl trisulphide because it has the best interaction and affinity for the AMPK-α2 receptor. The Gibbs energy and Ki of benzyl 2-hydroxyethyl trisulphide are lower than the comparator compounds with Gibbs energy values of -5.95 and Ki 43.24µM. While the prediction of ADME activity and toxicity with the ADMET Predictor showed that the benzyl 2-hydroxyethyl trisulphide compound could be said to be quite good based on several parameters. However, it should be noted that this compound is mutagenic, so it is necessary to modify the structure to reduce the effect of the mutagen.

References

1. Ministry of Health of the Republic of Indonesia. National Guidelines for Medical Services Management of Diabetes Mellitus Type 2 Adult. Jakarta: Ministry of Health of the Republic of Indonesia; 2020.

2. Mustika A, Indrawati R, and Sari GM. Effect of Singawalang Leaf Extract (*Petiveria alliacea*) in Reducing Daraj Glucose Levels through Increasing AMPK-α1 Expression in Diabetes Mellitus Model Rats. Indonesian Journal of Clinical Pharmacy. 2017;6(1) :22-31.

3. Tropical Plant Database. Tropical Plant Database Entry for Anamu-*Petiveria alliacea* [accessed 27 May 2021]. Available from :http://raintree.com/anamu.htm

4. Muttaqin FZ, Pratama MF, and Kurniawan F. Molecular Docking and Molecular Dynamic Studies of Stilbene Derivative Compounds As Sirtuin-3 (Sirt3) Histone Deacetylase Inhibitor on Melanoma Skin Cancer and Their Toxicities Prediction. Pharmacopolium. 2019;2(2) :112-121.

5. Ravi L, Krishnan K. a Handbook on Protein-Ligand Docking Tool: Autodock4. 2016;4(3):1–6.

6. Harmita H. Instructions for Implementing Method Validation and Calculation Methods. Pharmaceutical Sciences and Research (PSR). 2012;1(3) : 117-135.

7. pre-ADMET. Pre-Admet [Internet]. 2020 [cited 2020 Mar 20]. Available from: https://preadmet.bmdrc.kr

8. Leelananda SP, Lindert S. Computational methods in drug discovery. Beilstein J Org Chem. 2016;12:2694–718.

9. Prasetiawati R, Zamri A, Barliana MI, Muchtaridi M. In silico predictive formodification of chalcone with pyrazole derivatives as a novel therapeutic compound for targeted breast cancer treatment. J Appl Pharm Sci. 2019;9(2):20–8.

10. Lestari, D., Sari, R. P., Musfiroh, I., Megantara, S., Praceka, M. S., Ikram, N. K. K., & Muchtaridi. (2023). Interactions of Xanthone Compounds From the Mangosteen (Garcinia Mangostana L) Pericarps Against Inos, Cox-1, and Cox-2 Enzyme Receptors As Anti-Inflammatory. International Journal of Applied Pharmaceutics, 15(1), 186–194. https://doi.org/10.22159/ijap.2023v15i1.45861

11. Burley SK, Berman HM, Christie C, Duarte J, Feng Z, Westbrook J, et al. RCSB Protein Data Bank: Sustaining a living digital data resource that enables breakthroughs in scientific research and biomedical education. 2017

12. Bhowmik R, Roy S, Sengupta S, Sharma S. Biocomputational and pharmacological analysis of phytochemicals from zingiber officinale (Ginger), allium sativum (garlic), and murrayakoenigii (curry leaf) in contrast to type 2-diabetes. Int J Appl Pharm. 2021;13(5):280–6.

13. Yu W, Mackerell AD. Computer-aided drug design methods. In: Methods in Molecular Biology. Humana Press Inc.; 2017.p. 85–106.

14. Praceka MS, Megantara S, Mustarichie R. Molecular Modeling of Sauropus Androgynus Compounds. J Global Pharma Technol. 2020;12(02):939–47.

15. Jiang B, Zhou X, Yang T, Wang L, Feng L, Wang Z, et al. The role of autophagy in cardiovascular disease: Cross-interference of signaling pathways and underlying therapeutic targets. Front Cardiovasc Med. 2023;10(March).

16. Purnomo H. Computational Chemistry of In Silico Tests for Anti-Cancer Compounds. Yogyakarta: Student Library; 2013.

17. Ruswanto, Aimi R, and Lilis T. Synthesis of Compound N'-(3,5-Dinitrobenzoyl)- Isonicotinohydrazide and Study of Its Interaction in Mycobacterium Tuberculosis Enoyl Acyl Carrier Protein Reductase (INHA). Bakti Tunas Husada Health Journal. 2015;14(1).

18. Ramesh M, Vepuri SB, Oosthuizen F, Soliman ME. Adenosine Monophosphate-Activated Protein Kinase (AMPK) as a

Diverse Therapeutic Target: A Computational Perspective. Appl Biochem Biotechnol. 2016;178(4):810–30.

19. Mustarichie R, Warya S, Moektiwardoyo M, Megantara S, Saputri FA. Docking, absorption, distribution, metabolism and toxicity prediction of anticancer compounds found in plants. World J Pharm Pharm Sci [Internet]. 2014;3(10):72–90, 19 pp. Available from: http://isindexing.com/isi/papers/1415967853.pdf

20. Praceka MS, Yunita EN, Semesta CD, Putri RN, Tenggara A. Molecular Docking and Toxicity from Temulawak Rhizome (Curcuma xanthorrhiza Roxb.) against COX-2 Molecular Docking and Toxicity from Temulawak Rhizome (Curcuma xanthorrhiza Roxb.) against COX-2. 2022;1(1).

21. Cheng J, Palva AM, de Vos WM, and Satokari R. Contribution of the Intestinal Microbiota to Human Health : From Birth to 100 Years of Age. Curr Top Microbiol Immunol. 2013;323-46.

22. Yazdanian M, Glynn SL, Wright JL, and Hawi A. Correlating Partitioning and Caco-2 Cell Permeability of Structurally Diverse Small Molecular Weight Compounds. Pharm. Res. 1998;15 : 1490-1494.

23. Kumar R, Giri A, and Nadendha RR. In Silico ADME Profiling of CDK9 Inhibitors. Journal of Scientific Research in Pharmacy. 2018;7(3) : 30-34.

24. Hardjono S. Prediction of Pharmacokinetic Properties, Toxicity and Cytotoxic Activity of N-Benzoyl-N'-(4-fluorophenyl)thiourea Derivatives as Prospective Anticancer Drugs through Molecular Modeling. Journal of Indonesian Pharmaceutical Sciences. 2016;14(2) : 246-255.

25. Holik HA, Ibrahim FM, Wianatalie E, Achmad A, Faried A, Kartamihardja AHS. the Molecular Interaction and Admet Prediction of Modified Jph203 As a Potential Radiopharmaceutical Kit for Molecular Imaging of Cancer: an in Silico Research. Int J Appl Pharm. 2021;13(special issue 4):205–9.

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