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Article

Structure-Based Virtual Screening and Molecular Docking on the Indonesian Herbal Compound as a Promising Insulin Receptor (INSR) Inhibitor to Suppress Tumor Growth

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Veronica Hesti Candraningrum[1], Linda Erlina[1,2], Rafika Indah Paramita[1,2], Fadillah Fadillah[1,2], Surya Dwira[1,2], Jaka Fajar Fatriansyah[3]

[1]Master's Program in Biomedical Science, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.
[2]Department of Medical Chemistry, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.
[3]Metallurgical and Materials Engineering, Faculty of Engineering, Universitas Indonesia, Jakarta, Indonesia.

Abstract. A healthy cell maintains a homeostasis condition of glucose level, whereas cancer cells do not. Increased glucose uptake is a hallmark of cancer cells that helps them survive, proliferate, and spread. INSR is one of key feature that take part in glucose metabolism through insulin signaling. To block the entry of glucose into cells, researchers were aiming to disrupt the insulin signaling pathway as the upstream activation in glucose metabolism by inhibiting insulin receptor (INSR) using Indonesian herbal compounds. The approach during the screening was structure-based drug discovery (SBDD) method where INSR was determined as the macromolecules. Some parameters such as binding affinity, constant inhibition, drug-likeness, pharmacokinetics, and toxicity were applied to help the search of potential inhibitor. According to the test results, Heterophylin, Sanggenofuran A, and Epigallocatechin-3-O-caffeate had the strongest molecular binding activity against the INSR protein. Heterophylin is discovered in jackfruit fruit trees and Sanggenofuran A is present in mulberry trees. While Epigallocatechin-3-O-caffeate, is abundantly found in green tea plant.

Corresponding Author :
Department of Medical Chemistry, Faculty of Medicine,
Universitas Indonesia, Jakarta, Indonesia
Email : linda.erlina22@ui.ac.id

1. Introduction

Cancer is an uncontrollable cell growth and tends to spread to other parts of the body [1]. Cells can grow rapid because of the nutrients that enter the cell. Angiogenesis is crucial for the growth of tumors and the progression of cancer because it allows cancer cells to access enough nutrients and oxygen through a newly formed vascular system [2-3]. The vascular system then distributes the nutrients to the smallest level which is the cellular level. One of the most important nutrients is glucose.

Glucose enters the cells via GLUT4 protein which is activated by insulin signaling [4]. Insulin stimulates a protein phosphorylation signaling cascade through insulin receptor (INSR) activation that leads to the delivery of the glucose transporter GLUT4 to the cell surface, promoting the absorption of glucose into these tissues (**Fig. 1**) [5-6]. Normal cells provides a homeostasis state of glucose level but it tends to be different with cancer cells. They require more glucose as the main source of energy during the metastasis [7-8]. Cancer cells deploy a faster glucose metabolism that does not involved mitochondria as the increasing of energy needs in cellular level, known with Warburg Effect [9-11]. A number of studies state that there is a strong link between obesity in adults and a high chance of developing cancer [12-14].

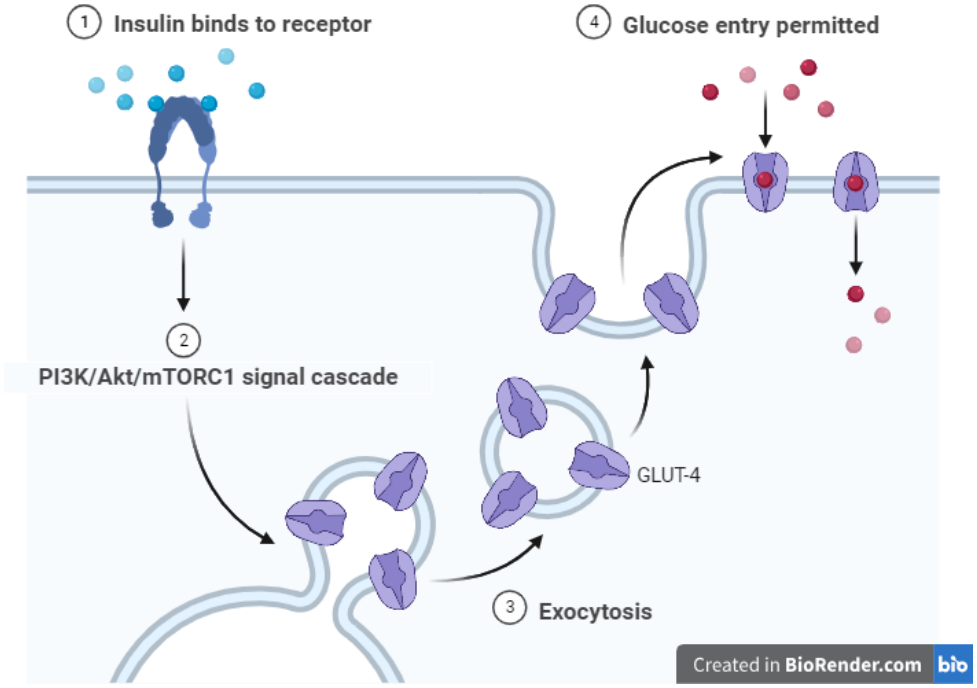

Figure 1. Activated insulin receptor (INSR) allows glucose to enter the cells

The INSR protein has been investigated for many years and has been revealed to be involved in a variety of chronic diseases, including Type 2 Diabetes Mellitus (T2DM)[15], Alzheimer's disease (AD)[16], numerous malignancies, neurological disorders, and metabolic syndromes [17-18]. The INSR has recently arisen as an attractive therapeutic target in human cancers owing to its overexpression in various cancers. Several studies have stated that there are inhibitor compounds that have been successfully synthesized as tumor kinase inhibitors (TKI) that target INSR [19]. One of them is the substance BI885578 which will be used as the native ligand in this research. BI885578 was created by Sanderson *et al* (2015) and had been proven for its potential ability to inhibit insulin receptors and to reduce the activity of tumor cell development [20].

Natural resources abound in tropical country Indonesia. Several plants that have been known as cancer chemoprevention are green tea, tomatoes, garlic, *Curcuma longa*, *etc* [21-22]. However, there's only few exploratory investigations or applications of herbal substances as tumor growth suppressor. Through this *in silico* research, a protein model of INSR was used as the macromolecule to screen

potential herbal compound to inhibit INSR through structure-based approach. The top potential herbal compound showing highest binding affinity and fit to ADME-Tox parameters will be chosen as the most potential compound to inhibit INSR.

2. Experimental Section

2.1. Materials

The search for candidate test compounds was executed using LigandScout v.4.4 by Structure-based drug discovery (SBDD) methods. The INSR protein structure (PDB ID: 5E1S) was downloaded from RCSB Protein Data Bank (https://ww.rcsb.org). Features and characteristics of the selected protein are designated according to the number of chains they have, the presence of native ligand, and the root-mean-square-deviation (RMSD) value in Angstroms (Å). Macromolecules obtained in .pdb format are then separated from the native ligand using AutoDock and both are stored in .pdbqt format. A number of ligands that appear as the best hit compounds values against the HerbalDB dataset from the SBDD test results are downloaded from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). Other software such as MarvinSketch, AutoDock, and LigPlot are used to help the docking visualization. The research was mainly proceeded following the flowchart below **(Fig.2)**.

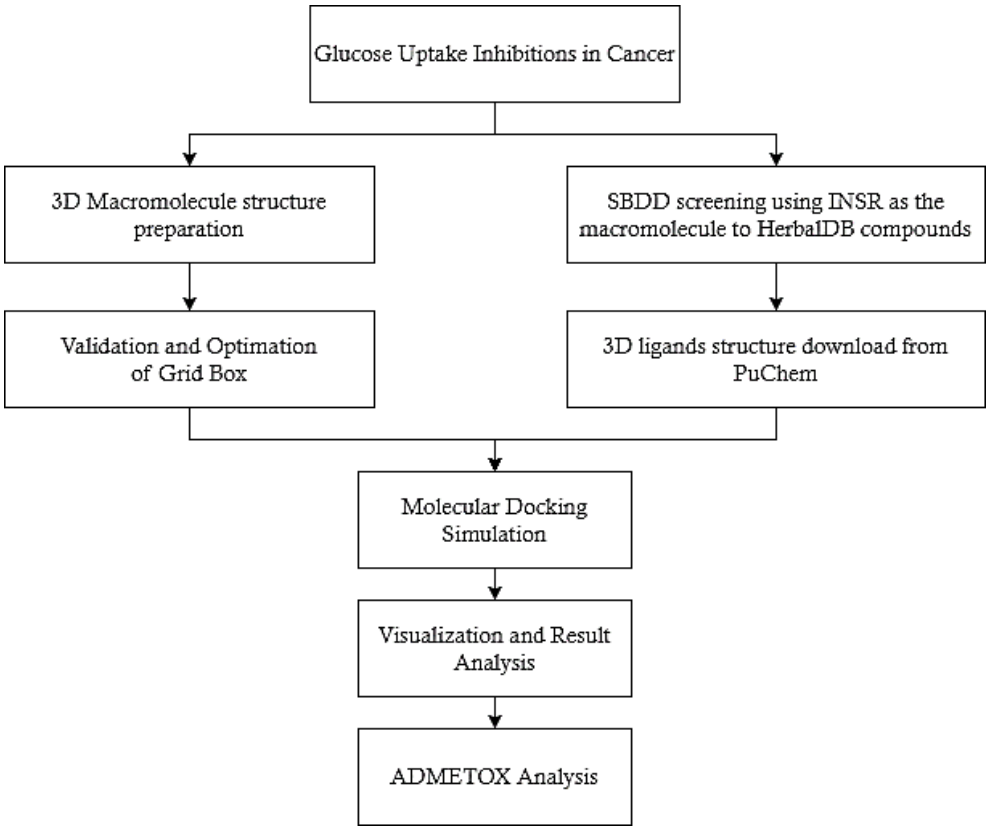

Figure 2. Schematic works in this study by applying SBDD method

2.2. Methods

2.2.1. Structure-based Drug Discovery

The Structure Based Drug-Discovery (SBDD) test is one of the methods used in the search for drugs based on a previously known protein molecular structure. These proteins have a direct link to the mechanism of a disease or specific metabolic pathways in the body. This is in accordance with Batool

et al (2019) [23] which states that the basic steps in the SBDD process are identification and validation of target proteins. The INSR protein (ID: 5E1S) gained from PDB are imported to LigandScout software, along with the HerbalDB database provided by Prof. Dr. Arry Yanuar, M.Si., Apt. The top hit compounds which were resulted from the SBDD test are recorded, and the 3D structure of each compound then gained from PubChem.

2.2.2. Validation and Optimization of Molecular Docking

Validation and optimization are the initial stage before starting the docking by analyzing the position of the grid box binding macromolecules to the native ligand [24]. This is intended to obtain precise molecular docking coordinates and obtain protein hydrogen binding interactions with specific ligands and not too far from the native ligand. The grid box areas used in the validation are 40x40x40, 50x50x50, and 60x60x60.

2.2.3. Molecular Docking Simulation

Molecular docking simulations were carried out using AutoDock version 4.2.6 software against 31 test ligands. The grid box used in the molecular docking simulation with the test ligand is the grid box that has the most optimal conditions from the previous validation and optimization stages. Recording was carried out on the Binding Energy and Inhibition Constant values for each of the test ligand molecular docking results [25].

2.2.4. Visualization and Analysis of Molecular Docking Results

This step aims to provide a visual description of the specific interaction pattern of the protein with each tested ligand that has the lowest Binding Energy value. Visualization of chemical features such as hydrogen bond donor acceptor, hydrogen bond donor, hydrophobic interaction, amino acid specific bond [26-27] were analyzed using LigandScout, AutoDock, and LigPlot.

2.2.5. ADME-Tox Analysis

The ADME-Tox profile of the test ligand was calculated using the tools of the SwissADME website (http://www.swissadme.ch/) and admetSTAR (http://lmmd.ecust.edu.cn/admetsar1/predict/). The results of the ADME-Tox analysis include adequate absorption, distribution and excretion values, features related to bioavailability [28-29]. The drug-likeness aspect of the tested ligands was projected according to Lipinski's "Rule of Five" who states that good adsorption of compounds is more likely to occur if the molecular weight (MW) is <500 Da, the number of hydrogen bond donors (HBD) is <5, the LogP value is <5, and the number of hydrogen bond acceptors (HBA) is <10 [30-31]. CYP Inhibition and Toxicity analysis are also applied. Mutagenicity Ames is used to evaluate potential teratogenicity and genotoxicity in the early stages of drug discovery [32]. The acute oral toxicity (AOT) and carcinogenicity are the toxicological end points that pose the highest concern to human health [26][30]. Compounds who follow the mentioned rules will be selected as the potential inhibitor to INSR and regarded as safe for usage.

3. Results and Discussion

3.1. Structure-based Drug Discovery

The research was applying the SBDD method yielded 31 hits compounds to the HerbalDB dataset (**Table 1**). The pharmacophore features found in protein compounds with native ligands consist of 3 hydrogen bond acceptors, 1 hydrogen bond donor, and 3 hydrophobic interactions. The amino acids that were observed to have bonds with the native ligand were VAL1060A, MET1076A, ALA1028A, MET1139A, and LEU1002A **(Fig. 3)**. Thirty-one ligands that appear as the best hit compounds values against the HerbalDB dataset from the SBDD test results are downloaded from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). MarvinSketch, AutoDock, and LigPlot were utilized to help the docking visualization.

Table 1. Top hits compounds yielded from SBDD test

No	Ligands
1	Mirabijalone B
2	(R)-(-)-Xanthorrhizol
3	Dehydrogingerdione
4	Geraniol
5	Gartanin
6	Heteroartonin A
7	Heterophyllin
8	Sanggenofuran A
9	trans,trans-Farnesol
10	(Z,Z,Z)-3,6,9-Dodecatrien-1-ol
11	beta-Tocopherol
12	trans-p-Feruloyl-beta-D-glucopyranoside
13	Isoscoparin 4'-O-glucoside
14	Epigallocatechin 3-O-caffeate
15	Arbortristoside D
16	Kaempferol 4'-glucoside
17	Quercetin 4'-glucuronide
18	Miraxanthin-V
19	Apigenin 5,7-dimethyl ether 4'-galactoside
20	Isoscutellarein 4'-methyl ether 8-(6"-n-butylglucuronide)
21	DIMBOA glucoside
22	Demethylmedicarpin
23	Benzoic acid
24	Quinamine
25	Nicotinic acid
26	Vanillic acid
27	Angolensin
28	3'-Deoxysappanone B
29	Isoshinanolone
30	Isoliquiritigenin 4'-methyl ether
31	Skullcapflavone I 2'-glucoside

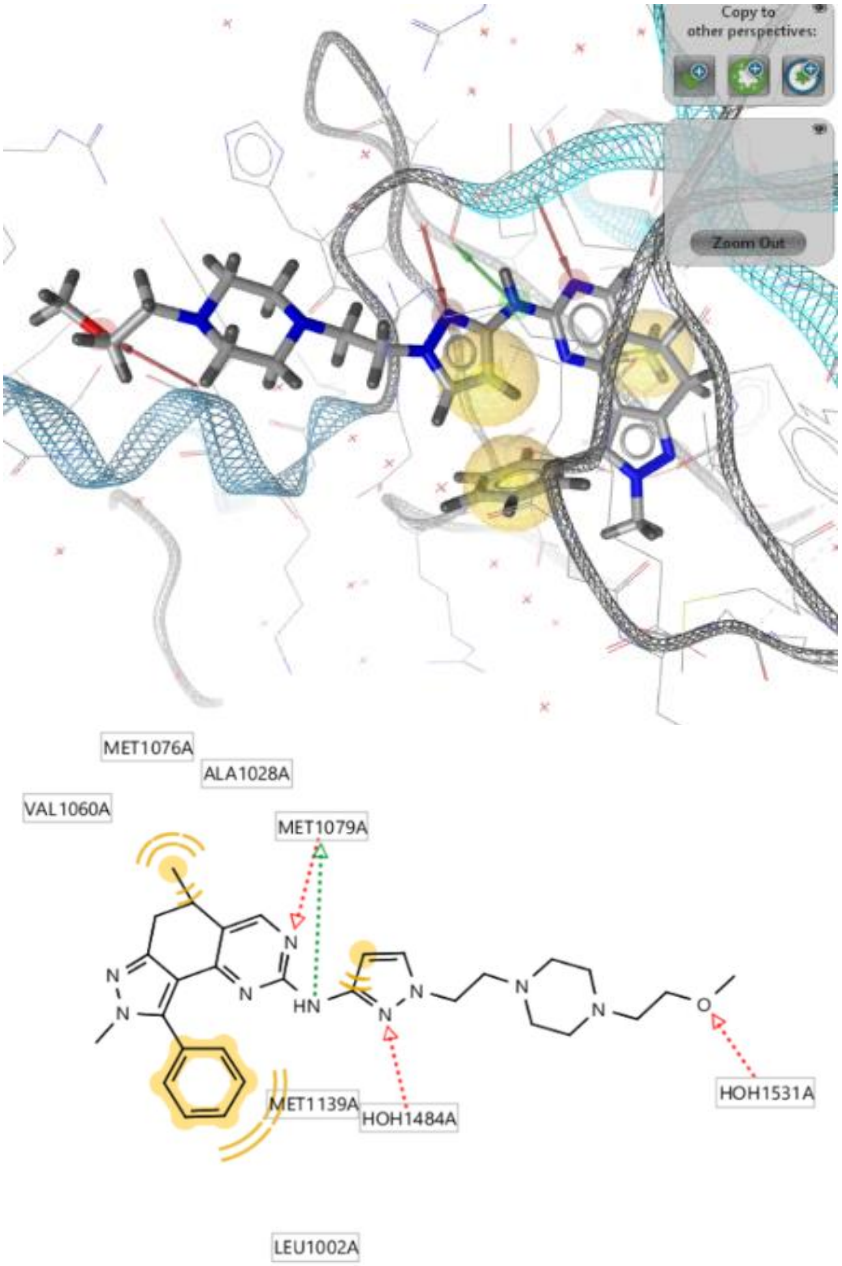

Figure 3. 3D (up) and 2D (down) visualization of amino acids bonding with native ligand

3.2. Validation and Optimization of Molecular Docking

Pre-processing of INSR, a target macromolecule that has been dissociated from the native ligand, as well as 31 candidate compounds as ligands is required prior to docking analysis. The 3D structure of the INSR protein (ID: 5E1S) was retrieved from the PDB (Protein Data Bank) database in .pdb format. Additionally, preparations for the native ligand (BI885578 or 5JA) were made using the AutoDock application. This included splitting the ligand, removing the water groups, adding polar hydrogen groups, combining the non-polar molecules, and adding *gasteiger* charges. This preparation was done to aid in the ligand and macromolecule's ability to attach to one another.

In molecular docking analysis, it is necessary to know the potential binding position by optimizing and validating grid boxes on macromolecules. It is expected that by validating the grid box it would facilitate drug candidates in the right binding pocket to produce specific interactions with the active sites belonging to macromolecules [33]. Grid boxes with the following parameters were optimized and validated in this study: 40x40x40, 50x50x50, and 60x60x60. The 50x50x50 grid box has the potential for docking, according to subsequent study, because it has the lowest binding energy (-10.59 kcal/mol), the smallest RMSD distance (1.488 Å), and the lowest inhibition constant (17.15 nM). A grid box with parameters 50x50x50 and coordinates x=3.538; y=19.827; z=21.984 is used to get validation as a result **(Table 2)**.

Table 2. Grid box validation result in this study

Grid Box	40x40x40	50x50x50	60x60x60
Binding Energy	-10.49 run 7	-10.59 run 3	-9.23 run 4
X coordinate	3.538	3.538	3.538
Y coordinate	19.827	19.827	19.827
Z coordinate	21.984	21.984	21.984
RMSD	1.603 Å	1.488 Å	1.972 Å
Inhibition Constant	20.43 nM	17.15 nM	172.60 nM

3.3. Molecular Docking Simulation

All 31 candidate ligand compounds (**Table 1**) and a positive control compound were used for docking analysis of INSR using AutoDock. The results obtained are the binding energy and constant inhibition values that vary in each compound. Three compounds with the lowest binding energy and constant inhibition value were selected to be visualized and used for ADME-Tox analysis.

Table 3. The molecular docking results of 31 test ligands to INSR

No	Ligand Name	Binding Energy (kcal / mol)	Constant Inhibition
1	Positive Control (Native Ligand, BI885578)	-10.59	17.15 nM
2	Heterophyllin	-10.23	31.72 nM
3	Sanggenofuran A	-9.12	204.88 nM
4	Epigallocatechin 3-O-caffeate	-8.66	451.96 nM
5	Apigenin 5,7-dimethyl ether 4'-galactoside	-8.65	454.31 nM

Based on the analysis results, it was found that Heterophyllin, Sanggenofuran A, and Epigallocatechin 3-O-caffeate appeared as compounds with the lowest binding energy values with constant inhibition values of 972.11 nM, 204.88 nM, and 451.96 nM, respectively. Low binding energy interprets that the compound only requires a small amount of energy to be able to bind to the target protein. The lower the binding energy value, the more stable and stronger the bonds formed.[24] The inhibition constant (Ki) value shows that the drug can occupy half of those receptors even at low dosage s[34], so in this case it is interpreted as the easiness to inhibit INSR activity at low drug concentrations.

3.4. Visualization and Analysis of Molecular Docking Results

It is necessary to observe the specific binding position between the ligand and the target protein in order to view and evaluate the specific binding position of the amino acid that results in interaction with the ligand. The interaction between the ligand and the protein is influenced by the functional groups contained in the ligand and the amino acids making up the protein which results in interactions such as hydrogen bond acceptor bonds, hydrogen bond donors, electrostatic interactions, van der Walls interactions, hydrophobic interactions, and others [27][31][32]. The 3D and 2D visualization of the three best compounds resulting from molecular docking was performed using AutoDock and LigPlot.

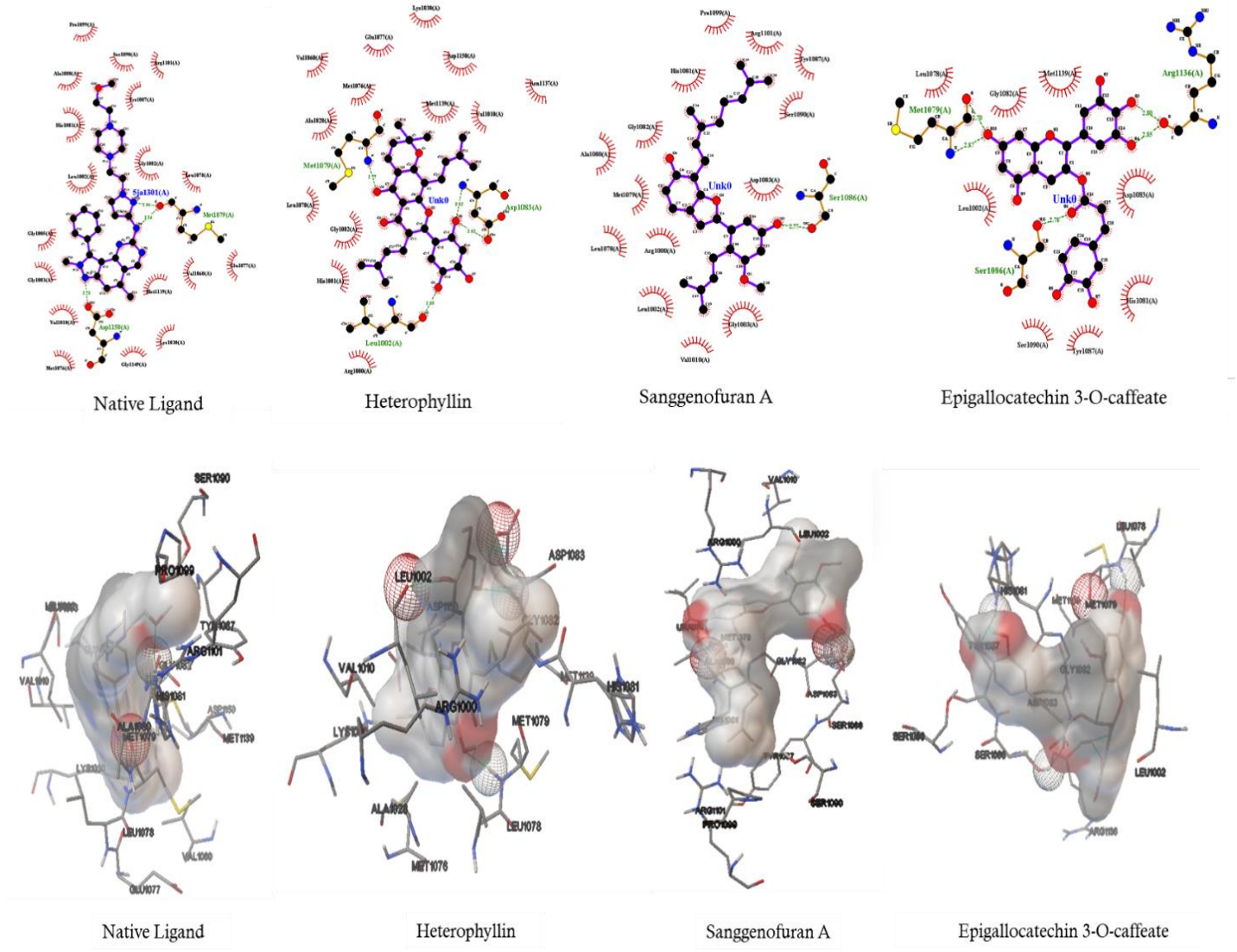

Figure 4. The 2D and 3D visualization of docking results reveal the amino acids interaction with each top selected ligand

The specific interactions of the ligands with amino acids are summarized in **Table 4**. Based on these data, the amino acids that show specific interactions between the three candidate herbal compounds with INSR are Met1079, Leu 1002, His1081, Gly1082, and Leu1078. These amino acids show specific binding patterns to the native ligand and the three candidate herbal compounds. Heterophyllin has 4 hydrogen bonds with INSR protein amino acids, 2 Sanggenofuran A, and 5

Epigallocatechin 3-O-caffeate. The herbal compound Epigallocatechin 3-O-caffeate possesses the greatest number of hydrogen bonds compared to the other 2 compounds.

Table 4. Ligand-amino acids specific interaction

Amino Acid	Native Ligand (Pos. Control)	Heterophyllin	Sanggenofuran A	Epigallocatechin 3-O-caffeate
Met1079	+ (H) (2.86 and 3.14)	+ (H) (2.77)	+	+ (H) (2.7 and 2.87)
Asp1150	+ (H) (2.73)	+	-	-
Asp1083	-	+ (H) (3.02 and 2.82)	+	+
Ser1086	-	-	+ (H) (2.77)	+ (H) (2.78)
Arg1136	-	-	-	+ (H) (2.8 and 2.85)
Leu1002	+	+ (H) (2.85)	+	+
Pro1099	+	-	+	-
Ala1080	+	-	+	-
Ser1090	+	-	+	+
Arg1101	+	-	+	
Tyr1087	+	-	+	+
His1081	+	+	+	+
Gly1082	+	+	+	+
Leu1078	+	+	+	+
Gly1005	+	-	-	-
Gly1003	+	-	+	-
Glu1077	+	+	-	-
Val1060	+	+	-	-
Met1139	+	+	-	+
Val1010	+	+	+	-
Met1076	+	+	-	-
Gly1149	+	-	-	-
Lys1030	+	+	-	-
Asn1137	-	+	-	-
Ala1028	-	+	-	-
Arg1000	-	+	+	-

* *+ : present, - : absent*

3.5. ADME-Tox Analysis

Characteristics and physicochemical properties of drug candidates can be determined through the ADME-Tox test [26][29][30][33]. This analysis can assist in various subsequent approaches to the drug discovery and development process, such as providing guidance in the synthesis of derivative compounds and the development of dosage and formulation materials. The analysis in this study was carried out using the SwissADME and AdmetSTAR websites by uploading canonical smiles. The results of the ADME-Tox analysis are summarized in **Table 5**.

Table 5. ADME-TOX analysis result of the three ligands test

Ligands			Heterophyllin (C30H32O7)	Sanggenofuran A (C30H36O4)	Epigallocatechin 3-O-caffeate (C24H20O10)
Drug Likeness	MW (gr/mol)		504.57	460.60	468.41
	HBA		7	4	10
	HBD		4	2	7
	Log P		5.39	6.84	1.71
Pharmacokinetics	GI Absorption		Low	Low	Low
	CYP Inhibitor	CYP1A2	-	-	-
		CYP2C19	✓	-	-
		CYP2C9	-	-	-
		CYP2D6	-	✓	-
		CYP3A4	-	-	-
Toxicities	AMES		-	-	-
	Carcinogenesis		-	-	-
	AOT		III	I	IV

**MW: Molecular Weight, HBA: Hydrogen Bond Acceptor, HBD: Hydrogen Bond Donor, GI Abs: Gastrointestinal Absorption, CYP: Cytochrome P450, AMES: potential carcinogenic effect of chemicals by using the bacterial strain, AOT: Acute Oral Toxicity*

Information on the pharmacokinetics, toxicities, and drug similarity of each ligand is provided in **Table 5**. For the drug likeness, compounds should follow the Lipinski "Rules of Five" where the molecular weight must be 500 Da or less, an HBA <10, an HBD <5, and a logP value <5.[31][32] Heterophyllin appears to have two violation of Lipinski "Rules of 5", since it has more than 500 Da of molecular weight and has more than 5 of log P value. The other two compounds only have one violation of the rules. When there are more than five H-bond donors, more than ten H-bond acceptors, the molecular weight is larger than 500, and the computed Log P (cLog P) is greater than five, poor absorption or penetration is more likely to occur according to the Ro5 [32][35].

Inhibition of cytochrome P450 (CYP) enzymes by new chemical entities can reduce drug metabolism [36]. It is also feasible to draw the conclusion that the most anticipated category is metabolism, and within this area, the potential of a medicine to interact with CYP450 enzymes as a substrate or inhibitor. However, the early stages of drug development are when researchers are most interested in learning about metabolic stability and the site of metabolism [32]. Epigallocatechin 3-O-caffeate appears to have no interaction with any of CYP inhibitors, so it is regarded that its metabolism will not be reduced.

The physicochemical qualities of a drug candidate can be utilized to understand and predict its physiological absorption, which improves the likelihood of having a biological effect following oral administration. These physicochemical qualities can be predicted based on their chemical structures to predict the likelihood of gastrointestinal (GI) absorption following oral ingestion [37]. The synergistic effect of phytonutrient extracts, on the other hand, may make phytochemicals with projected poor GI absorption nonetheless interesting to examine [38]. Based on the analysis result, all of three subjected compounds appear with low GI absorption result.

It is identified through Toxicities summary analysis that Epigallocatechin 3-O-caffeate occupies the safest AOT level. Based on US EPA toxicity classification, level IV includes safe chemical consisted of compounds with LD50 values > 5000mg/kg, consequently described as "Practically non-toxic and not an irritant". Sanggenofuran A compound has the highest level of AOT which is categorized as "Highly toxic and seriously irritating" [39-40]. The three produced compounds must, however, be tested for pharmacokinetic efficacy and *in vitro* toxicity.

The three compounds are naturally easy to be found in Indonesia. Heterophyllin is identified in jackfruit fruit trees and Sanggenofuran A is present in mulberry trees. Epigallocatechin-3-O-caffeate, known as one of green tea catechins, is abundantly present in green tea plant [41-43] or *Camellia sinensis* plant based on a search of the LOTUS database (https://lotus.naturalproducts.net/). Green tea catechin disrupts cell signaling pathways that promote cell growth, angiogenesis, metastasis, and acceleration of apoptotic pathways, among other cancer-fighting mechanisms [44-45].

4. Conclusion

The highest molecular binding activity against the INSR protein was shown by Heterophylin, Sangenofuran A and Epigallocatechin-3-o-Caffeine. All three compounds are potential as INSR inhibitor based on their binding affinity values and other aspects to bind to insulin receptor proteins. Lipinski's "Rule of Five" has not been strictly adhered to, despite epigallocatechin-3-O-caffeate having the best pharmacokinetic characteristics and level of acute oral toxicity. However, *in vitro* examination must be done on the three produced compounds.

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