Molecular Docking of Mangostin and Sinensetin Derivatives on SUR1-Pancreatic K_{ATP} Channel Target as Antidiabetic

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Abstract

Background*: Diabetes Mellitus (DM) is a complex chronic disease characterized by increased blood glucose. The incidence of this disease is rising, especially type 2 diabetes which is caused by insulin resistance in the body. SUR1-Pancreatic K_{ATP} Channel is a receptor as an antidiabetic target because its inhibition process can increase insulin production so that it can reduce blood glucose in people with type 2 diabetes.* **Objective***: This study aims to identify the in-silico activity of the SUR1-Pancreatic K_{ATP} Channel macromolecules.* **Methods***: Identification of macromolecular binding sites using Protein Plus software, then carried out molecular docking using AutoDock software, where the formed molecular interactions are further identified using the BIOVIA Discovery Studio software.* **Results***: After determining the macromolecular binding site, the RMSD value was 1.253, allowing for further molecular docking. Molecular docking showed that the Ligands of mangostin (α, β, γ-mangostin) and sinensetin derivatives had a good affinity, namely α-mangostin -6,31 kcal/mol; β-mangostin -5.78 kcal/mol; γ-mangostin -6.17 kcal/mol and sinensetin -4.75 kcal/mol.* **Conclusion***: The affinity sequence in the docking process for the SUR1 K_{ATP} channel macromolecules is α-mangostin > γ-mangostin > β-mangostin > sinensetin. The highest affinity for the docking process on the macromolecule SUR1 K_{ATP} channel was α-mangostin with a value of ΔG -6.31 kcal/mol Ki 23.65 µM.*

Keywords: mangostin derivatives, sinensetin, in-silico SUR1 K_{ATP} channel, antidiabetes

Abstrak

Pendahuluan: Penyakit Diabetes Melitus (DM) adalah penyakit kronik dan komplek ditandai dengan peningkatan glukosa darah. Angka kejadian penyakit ini semakin meningkat, terutama DM tipe 2 yang disebabkan karna resistensi insulin dalam tubuh. SUR1-Pancreatic K_{ATP} Channel merupakan reseptor sebagai target antidiabetes karena dengan proses penghambatannya mampu meningkatkan produksi insulin sehingga dapat menurunkan glukosa darah pada penderita DM tipe 2. **Tujuan**: Penelitian ini bertujuan untuk mengidentifikasi aktivitas secara *in silico* terhadap makromolekul SUR1-Pancreatic K_{ATP} Channel (reseptor sulfonilurea) dari turunan mangostin dan sinensetin. **Metode**: Identifikasi binding site makromolekul dengan menggunakan software Protein Plus kemudian dilakukan docking molekuler menggunakan software AutoDock dimana interaksi molekuler yang terbentuk selanjutnya diidentifikasi lebih lanjut menggunakan software BIOVIA Discovery Studio. **Hasil**: Berdasarkan identifikasi binding site makromolekuler didapatkan nilai RMSD 1,253 Å sehingga dapat dilakukan docking molekuler lebih lanjut. Docking molekuler menunjukkan bahwa Ligand dari turunan mangostin (α, β, γ-mangostin) dan sinensetin memiliki afinitas docking molekuler yang baik yaitu α-mangostin -6,31 kkal/mol; β-mangostin -5,78 kkal/mol; γ-mangostin -6,17 kkal/mol dan sinensetin -4,75 kkal/mol. **Kesimpulan**: Urutan afinitas

pada proses docking terhadap makromolekul SUR1 K_{ATP} channel adalah α-mangostin > γ-mangostin > β-mangostin > sinensetin. Afinitas tertinggi proses docking pada makromolekul SUR1 K_{ATP} channel adalah α-mangostin dengan nilai ΔG -6,31 kkal/mol K_i 23,65 μM.

Kata kunci: turunan mangostin, sinensetin, *in silico*, SUR1 KATP channel, antidiabetes

INTRODUCTION

K_{ATP} channel (ATP-sensitive potassium channels) is an ATP binding cassette (ABC) transporter complex consisting of 4 Kir6 channels and four sulfonylurea receptor (SUR1) subunits. It is important as an antidiabetic target because it has a good binding affinity with sulfonylurea and glinide inhibitors. In pancreatic beta cells, K_{ATP} channels are stimulated by the intracellular activity of ATP and ADP. When glucose enters the pancreatic beta cells through GLUT-1 (Glucose Transporter-1) there is glucose metabolism or an increase in the ratio of ATP and ADP so that, $K_{AT}P$ channels are closed, the membrane depolarizes and Ca^{2+} enters, which stimulates insulin granules and insulin release (Martin *et al*., 2017; Ding *et al*., 2019).

Diabetes mellitus (DM) is a complex chronic disease with hyperglycemia, glucose levels above average, and an increasing number of sufferers. This is characterized by insulin deficiency or reduced insulin sensitivity, followed by increased blood glucose and disorders of carbohydrate, protein, and lipid metabolism (Ibrahim *et al*., 2019). The potential of α-mangostin as an antidiabetic was proven by administering α-mangostin isolated from mangosteen pericarpium extract to streptozotocin (STZ) induced rats (Lee *et al*., 2018). The administration of γ-mangostin also showed reducing blood glucose in STZ-induced mice (Husen *et al*., 2019). Whereas β-mangostin, a mangostin derivative, also has the potential to reduce blood glucose. The potency of sinensetin as an antidiabetic can inhibit the activity of os-glucosidase and α-amylase enzymes. The inhibition of these two enzymes controls glucose absorption in type 2 diabetes mellitus (Mohamed *et al*., 2012).

Molecular docking is an in-silico study with computational methods to provide information about the intermolecular interactions of proteins, nucleic acids, lipids, and ligands. The aim is to optimize the formation of receptor and ligand bonds and obtain a picture of the orientation of the receptors and ligands with minimal free energy (Muchtaridi *et al.,* 2018). This study shows the potential of several Indonesian herbal compounds, namely magostin derivatives (α, β, mang-mangostin) and sinensetin through the molecular docking method on the target SUR1-Pancreatic K_{ATP} Channel. The performance of docking of these herbal compounds will be compared with the performance of Repaglinide docking as a cocrystal ligand, considering that its working mechanism is to stimulate and increase insulin production in patients with type 2 diabetes.

MATERIALS AND METHODS
Materials

2-dimensional (2D) structure of mangostin (α, β, γ-mangostin) and sinensetin (Figure 1) derivatives, 3-dimensional (3D) structure of mangostin derivatives (α, β, γ-mangostin), sinensetin, macromolecules (Figure 2) and Repaglinide.
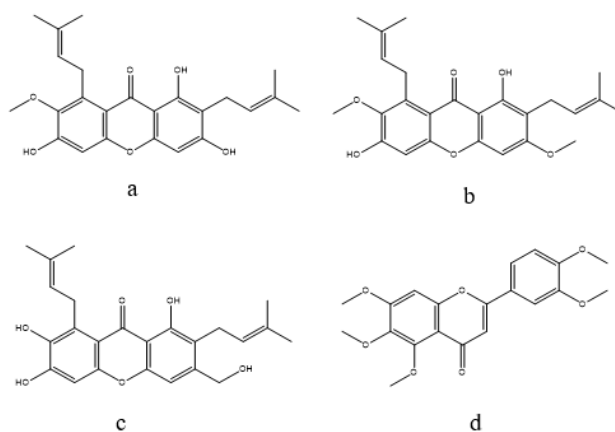
Figure 1. 2D structure of (a) α-mangostin, (b) β -mangostin, (c) γ-mangostin, (d) sinensetin

Figure 2. 3D structure of the SUR1-pancreatic K_{ATP} channel macromolecule which binds to repaglinide cocrystal ligands (PDB: 6JB3)

Tools

The hardware used is a computer with an Intel (R) Celeron (R) CPU 847 @ 1.10GHz processor specification, 64-bit operating system, 4.00 GB RAM with Windows® 8. The software used is ChemDraw 20.0, Chem3D 20.0, AutoDockTools 1.5.6 (The Scripps Research Institute, USA), ProteinPlus online tool (https://proteins.plus/), Protein Data Bank (https://www.rcsb.org), BIOVIA Discovery Studio 2020.

Method

Ligand preparation

The ligands were drawn in 2D first in the ChemDraw 20.0 program which was then converted into 3D in the Chem3D 20.0 program and had undergone structural optimization. Then stored in the form of mol2. The ligands are converted to pdbqt format through the AutoDockTools 1.5.6 program and removed water molecules, increasing the charge and adjusting the torque.

Identify the active site areas of protein macromolecules

The macromolecule's active site is identified via the Protein Plus site (https://proteins.plus/) by entering the macromolecule in.pdb format, which is then automatically that the active site where the ligand is attached to the receptor can be identified. The active site of the attachment is in the form of a pocket that has the highest drug score.

Protein macromolecule preparation

Receptors are downloaded in .pdb format from the PDB website (https://www.rcsb.org). The SUR1-Pancreatic K_{ATP} Channel macromolecules are downloaded and then prepared in AutoDockTools1.5.6 to remove unused receptor parts, cocrystal ligands, water molecules, repair lost atoms, and additional charges. Then the file is saved in .pdbqt format.

Validation of the docking method

The docking process begins with the validation of the cocrystal ligand docking with the receptor. It is performed as a docking protocol used to predict the position between ligand and receptor interactions. The size and coordinates of the cocrystal ligand grid box position at the receptor are determined for further use in the ligand docking process. The RMSD value obtained is < 2Å, then the molecular docking protocol can be accepted or declared valid (Zubair *et al.*, 2016).

Molecular docking

Molecular docking between the test ligand and the receptor was carried out using the AutoDockTools 1.5.6 program. The docking process is carried out in the same way as in the docking method validation process. The molecular docking process can be carried out using the grid box's exact size and position coordinates as the cocrystal ligand.

Analysis of molecular docking results

The main parameters in the molecular docking process using AutoDockTools 1.5.6 are the Estimated Free Energy of Binding (ΔG) score, Inhibition Constant (Ki), amino acid residues, and the type of bond. The ΔG and Ki scores of the test ligands were compared with the cocrystal Repaglinide ligand; if the ΔG scores were more negative and the Ki scores were lower, they indicated higher ligand affinity. Amino acid residues and bond types were also compared using visualization with BIOVIA's Discovery Studio Visualizer program.

RESULTS AND DISCUSSION

Steps can be taken to design and develop herbal ingredients into antidiabetic dosage forms with specific targets and high selectivity for antidiabetic therapy by exploring the potential of natural ingredients by extracting and isolating methods from herbal ingredients whose benefits are known empirically, the molecular structure is known, and its biological activity. Compounds that have antidiabetic activity are mangostin derivatives (α, β, γ- mangostin) and

sinensetin which show a decrease in blood glucose in streptozotocin (STZ)-induced experimental animals (Lee *et al*., 2018; Husen *et al*., 2019; Mohamed *et al*., 2012). Research using a molecular doctoring simulation approach using a computer with a specific program can carry out drug discovery studies, where it can reduce the cost and time required for drug discovery in general as it is known that the drug discovery process is expensive and time-consuming (Geldenhuys *et al*., 2006).

Macromolecules are prepared by removing other unused receptor parts such as chains other than those used for ligand docking, removing all existing cocrystal ligands so as not to interfere with the molecular docking process, removing water molecules, adding polar hydrogen, repairing lost atoms, and adding a charge. These steps need to be prepared to ensure that the docking of molecules on macromolecules can produce good affinity and form stable interactions. In Figure 3 we can know the active site of the SUR1-pancreatic K$_{ATP}$ Channel macromolecular pocket. The results of the identification of the active site of macromolecules were carried out on the Protein Plus online tool by entering the macromolecule format. Å (Zubair *et al*., 2016).
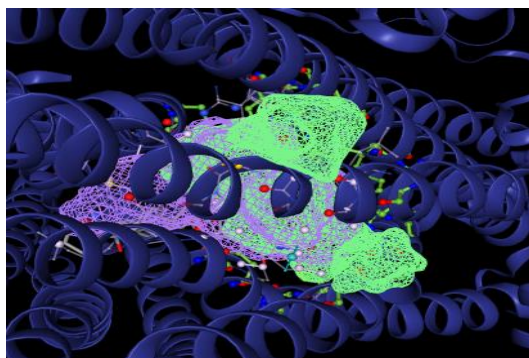
Figure 3. The identification results of the active site of the SUR1-pancreatic K$_{ATP}$ Channel macromolecular pocket on the protein plus online tool. The active site of the pocket (green color) of the native repaglinide ligand

The docking process begins with using a cocrystal ligand, in this case, Repaglinide, which is also used as a standard type-2 antidiabetic drug at the SUR1-Pancreatic KATP Channel receptor. The results of the interaction between Repaglinide and the receptor produce energy of binding (ΔG) -7.63 kcal/mol and an estimated inhibition constant (Ki) 2.54 μM, the interaction with the receptor produces 2 hydrogen bonds to the amino acid residue Asn1245 (1.99 Å) and Arg1246 (1.84 Å) with van der Waals bonds totaling 5 bonds with amino acid residues Arg306, Asn437,

Met441, Ser 595, and Thr1242. The energy of binding (ΔG) is used to predict the ligand affinity to the receptor, while the estimated inhibition constant (Ki) is used to predict the in-vitro analysis process in the next process (Natesan *et al*., 2012). The ΔG and Ki scores determine the ligand affinity to the receptor in the docking method. More negative ΔG and lower Ki indicate higher ligand affinity for the active site of the receptor used (Neshich *et al*., 2015). Hydrogen bonds are bonds between H atoms and O, N, and F atoms. These bonds can affect the physicochemical properties of compounds, such as boiling point, melting point, solubility in water, chelating ability, and acidity. Van der Waal's bonds are bonds of attraction between molecules or atoms that are not charged and occur because of the nature of polarity (Siswandono, 2016). The amino acid residue of the test ligand on the receptor as compared with the cocrystal ligand to assess the interaction equation, the same amino acid residue shows the higher the probability that the test ligand will have the same activity as the cocrystal ligand (Miller *et al*., 2015).

Table 1 shows that the value of the energy of binding (ΔG) and the estimated inhibition constant (Ki) of α-mangostin is the most negative and low compared to the other test ligands. The energy of binding (ΔG) α-mangostin is -6.31 kcal/mol, and the estimated inhibition constant (Ki) is 23.65μM with 1 hydrogen bond with the same amino acid residue as the amino acid residue of the cocrystal ligand, namely Arg1246 (2.19 Å), and the van der Waals bonds amount to 1 with the amino acid Asn437 residue. This shows that compared to other test ligands, α-mangostin has the highest affinity for the active site of the receptor because ΔG is the most negative and Ki is the lowest, so it is predicted that the potential of α-mangostin as an antidiabetic is higher than the γ-mangostin compound, β- mangostin and sinensetin. The energy of binding (ΔG) value of α-mangostin -6.31 kcal/mol was not too far from the cocrystal Repaglinide ligand -7.63 kcal/mol, it was predicted that the affinity of the α-mangostin test ligand as an antidiabetic did not differ significantly too significant with the cocrystal repaglinide ligand.

Table 1. Free energy from the energy of binding (ΔG) and the estimated inhibition constant (Ki)

Component	ΔG (kcal/mol)	K$_i$ (μM)
α-mangostin	-6,31	23,65
β-mangostin	-5,78	58,44
γ-mangostin	-6,17	30,13
Sinensetin	-4,75	331,18
Repaglinide	-7,63	2,54

In Table 2 shows the equation of the amino acid residues of the hydrogen bonds and the van der Waals bonds that have between the test ligand and the cocrystal ligand. There are 1 hydrogen bond Asn1245 (2.16 Å) and 3 van der Waals bonds (Thr1242, Arg306, and Asn437) in β-mangostin, 1 hydrogen bond with the amino acid residue Arg1246 (3.08 Å) and van der Waals bonds amounting to 2 (Asn1245 and Asn437) on γ-mangostin, 1 hydrogen bond Asn437 (2.84 Å) and 2 van der Waals bonds (Thr1242 and Asn 1245) on sinensetin. The amino acid equation formed between the test ligand and the cocrystal ligand shows that the test ligand has a high probability of having the same activity as the cocrystal ligand (Miller *et al*., 2015).

Table 2. Comparison of the interaction between the amino acid residues of the test ligand and the cocrystal ligand. Hydrogen bonds are spaced (Å), van der walls bonds are marked √

Component	Asn1245	Arg1246	Arg306	Asn437	Met441	Ser595	Thr1242
α-mangostin	-	2,19	-	√	-	-	-
β-mangostin	2,16	-	√	√	-	-	√
γ-mangostin	√	3,08	-	√	-	-	-
Sinensetin	√	-	-	2,84	-	-	√
Repaglinide	1,99	1,84	√	√	√	√	√

Figure 4 and Table 3 show the predicted amino acid residues of hydrogen bonds and van der Waals bonds formed due to ligand and receptor interactions. These results indicate that the interaction formed between the test ligand and the receptor has a stable bond in the active site area of the SUR1-Pancreatic K_{ATP} Channel macromolecular pocket (Norel *et al*., 2001).
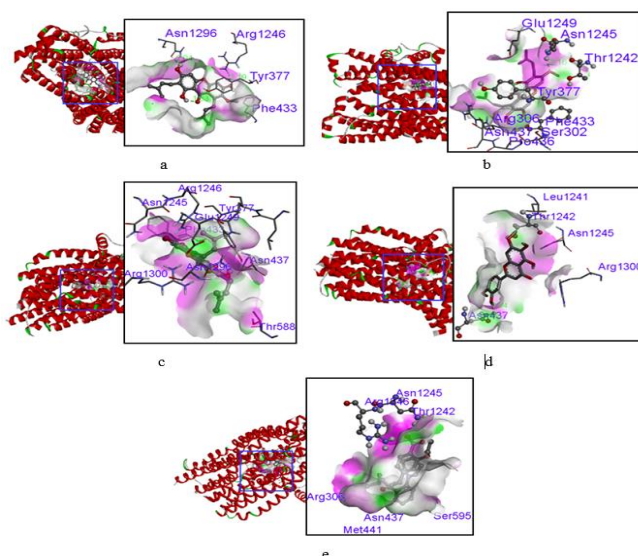
Figure 4. Bonding of (a) α-mangostin, (b) β-mangostin, (c) γ-mangostin, (d) sinensetin, and (e) repaglinide with SUR1-pancreatic K_{ATP} channel macromolecules

Table 3. Total interactions of amino acid residues resulting from molecular docking between the test ligand and the receptor

Component	Number ofinteractions	Amino acid residues
α-mangostin	4	Arg 1246, Asn1296, Tyr377, Phe433
β-mangostin	9	Glu1249, Asn1245, Thr1242, Tyr377, Phe433, Arg306, Asn437, Ser302, Pro436
γ-mangostin	9	Arg 1246, Asn1245, Tyr377, Glu1249, Phe433, Arg1300, Asn1296, Asn437, Thr588
sinensetin	5	Arg1300, Asn1245, Leu1241, Thr1242, Asn437

CONCLUSION

Mangostin (α, β, γ-mangostin) and sinensetin derivatives are predicted to have potential antidiabetic activity because there are similarities in the amino acid residues formed between the test ligand and cocrystal ligand against the SUR1 K_{ATP} channel receptor through

the in-silico test. The affinity sequence in the docking process for the SUR1 K_{ATP} channel macromolecules is α-mangostin > γ-mangostin > β-mangostin > sinensetin. The highest affinity for the docking process on the macromolecule SUR1 K_{ATP} channel was α-mangostin with a value of ΔG -6.31 kcal/mol Ki 23.65 μM.

ACKNOWLEDGMENT

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