MOLECULAR DOCKING AND MOLECULAR DYNAMIC SIMULATION OF THE AGLYCONE OF CURCULIGOSIDE A AND ITS DERIVATIVES AS ALPHA GLUCOSIDASE INHIBITORS

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ABSTRACT

Diabetes mellitus (DM) is characterized by high blood sugar levels caused by insufficient insulin production in the pancreas or insulin resistance in the body. Alpha-glucosidase enzymes are therapeutic targets for type 2 diabetes treatment. Structurally, curculigoside has a resemblance to xathohumol (chalcone) which has a strong inhibitory activity against alpha glucosidase. The present study aims to determine the interaction of the aglycone of curculigoside A and its derivatives against alpha glucosidase (PDB ID 2QMJ) was evaluated based on interaction mode and binding stability by performing docking and molecular dynamic simulation by using AutoDock 4.2 and AMBER 18, respectively. All ligands can interact with alpha glycosidase and ligand **34**, **36**, **43**, and **56** have a best binding mode with free bonding energy of -6.30, -5.67, -5.16, and -5.92 kcal/mole, respectively. The hydrogen bonds formed in MD are different from the docked pose because of a large movement of alpha glucosidase receptor and ligand during the MD process. In conclusion, ligand **34**, **36**, **43**, and **56** are candidates for lead compounds as alpha glucosidase inhibitors.

Keywords: Aglycone Curculigoside A, Alpha Glucosidase, Chalcone, Docking, MD

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INTRODUCTION

Diabetes mellitus (DM) currently affects 10.3 million Indonesian people and this number is predicted to increase to 16.7 million by 2045.¹ DM occurs due to deficiency of insulin secretion by β -pancreatic cells, increased insulin resistance or damage to insulin action in the target tissue. Type 2 diabetes (T2D) is the most common form of the disease, accounting for approximately 90 % of cases.² The magnitude and time of the peak plasma glucose (PG) depends on a variety of factors, including the timing, composition and quantity of the meal. PG peaks for diabetic individuals about ≥ 200 mg/dL but in non-diabetic individuals rarely exceed 140 mg/dL (about 60 minutes after the start of a meal). Thus, controlling PPHG to achieve blood glucose levels such as non-diabetic individuals becomes one of the therapeutic strategies for T2D.³ The α -amylase inhibitor is a compound that inhibits carbohydrates breakdown into glucose by amylase enzyme. The α -amylase is a carbohydrate hydrolase located on the border of the small intestinal epithelial brush⁴ and acts as a catalyst in the final stages of carbohydrate digestion which only monosaccharides such as glucose and fructose can be absorbed by the intestine.⁵

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This enzyme breaks down oligosaccharides through the hydrolysis reaction by breaking the 1,4- α glycosidic linkage between glucosyl residues and glycosidic oxygen (C1-O) accompanied by proton exchange between water and glucosyl residues and resulting D-glucose as a final product that is easily absorbed by the intestine and will cause an increase in postprandial blood glucose levels.⁶ The inhibitory action of this enzyme can effectively reduce the digestion of complex carbohydrates and their absorption which causes decreased postprandial glucose levels in diabetics.⁴

One of the chalcone derivatives which has a strong inhibitory activity against α glucosidase is xanthohumol with IC_{50} of 8.8 μ M.⁷ The aglycone of curculigoside A has a structural similarity to the chalcone. The aglycone of curculigoside A is a phenolic glycoside isolated from the rhizome of *Curculigo orchoides*.⁸ Its aglycone was predicted good in absorption, moderate in permeability, and weak binding into plasma proteins.⁹

In silico, one of the common approaches to determine the mechanism of action of a compound by considering the similarity of chemical structures. The similarity aspect of chemical structure refers to the similarity of chemical elements, molecules as well as substructure compounds. The basic principle is assumed that the similarity of a chemical structure compound will have similar biological properties and compounds with similar structures will tend to bind to the same protein.¹⁰ Therefore, several studies have been carried out to modify the structure of the lead compound to obtain a more active compound but less toxic both chemically¹¹ and *in silico*¹².

One method used is molecular docking which is the process of docking a molecule into the active site of the target macromolecule through noncovalent bonds. It is important to know the basic structure of the drug that will be designed to optimize the ligand binding interaction in macromolecules.¹³ Docking is used to predicting the interaction and orientation of ligand binding to the target protein as well as in the virtual screening of several candidate compounds to obtain the best hit for specific targets.¹⁴

Docking is more focused on poses and interactions of ligands on an active site, to obtain more comprehensive information, it is necessary to simulate through molecular dynamics that provide information about the stability of ligand-protein binding. Although an acceptable binding mode can be provided, the solvent effect and protein flexibility were not fully considered.

Therefore, MD simulations were carried out on the best-docked interaction to further explore the ligand-receptor interactions. In this study, the docking mode and interaction stability of the aglycone of curculigoside A and its derivatives on α -glucosidase was carried out by using docking and molecular dynamic simulation.

EXPERIMENTAL

Hardware and Technical Specifications

Computers are equipped with an Intel® Core™ i5-7200U CPU @2.50 GHz (4 CPUs, 2.7 GHz), 8GB of RAM, OS: Windows 10 Home Single Language 64-bit (10.0 Build 17134) and Intel® Xeon® CPU E-2620 v4 @2.10 GHz, 64 GB of RAM, OS: Linux Ubuntu 16.04 64-bit. Chemical structures of the molecules were drawn using ChemDraw in ChemOffice 8.0. Preparation of macromolecule and ligand were using AutoDock 4.2 and visualization was carried out by using Discovery Studio Visualizer.

Ligand Preparation

The molecular structures of the aglycone of curculigoside A and its derivatives⁹ were sketched using ChemDraw Ultra software¹⁵ of ChemOffice and subjected to energy minimization technique using Allinger's molecular Mechanics (MM2) force field followed by geometry optimization using semi-empirical Quantum Mechanics based on AM-1 (Austin Model-1).

Preparation of Alpha Glucosidase Macromolecules

The protein structure of 2QMJ was obtained from Protein Data Bank (PDB) with the resolution of 1.9 Å bound with the ligand 1,4-deoxy-4-((5-hydroxymethyl-2,3,4-trihydroxycyclohex-5,6 enyl)amino) fructose and prepared using Discovery Studio Visualizer¹⁶ and was used for the optimization and minimization until the root mean square deviation reached 2.0 Å. Then, Grid was generated using Grid generation Wizard for docking studies.

Molecular Docking

Molecular docking was carried out by using the AutoDock 4.2.¹⁷ In this program, docking was chosen according to default. Dimension of grid box was $(60 \times 60 \times 60)$ Å and X= 18.738 Å, Y= -7.053 Å, and Z= -4.05 Å as coordinate.

MD Simulations

The MD simulation was carried out by using The AMBER 18 software package.¹⁸ The initial structures of **34**, **36**, **43**, and **56** complexes from the docked results were used for the MD simulations. The FF14SB AMBER force field was taken from the protein, and charges were added to the protein by using the software database. The general AMBER force field (GAFF2) was taken for ligands¹⁹, and AM1-BCC method was applied to assign their partial charges because of the lack of partial charge parameters for ligands in GAFF2 force field.²⁰ The Antechamber suite (AMBER 18 package) was used to produce the atomic charges and topology files of ligands.¹⁹ The Tleap module of the AMBER 18 was used to produce the topology and coordinate files of the whole system.

The whole system was dipped into a water box of TIP3P with a margin distance of 10 Å.²¹ To neutralize the charge of the system, a proper number of sodium ions were added. The particle mesh Ewald (PME) was adopted during the MD simulations to deal with the long-range electrostatic interactions²², and the cut-off distance of nonbonded interactions was set to 10 Å. The SHAKE algorithm was used to constrain the bonds involving hydrogen.²³

Firstly, two-stage energy minimizations were performed on each system: the algorithms (1,000 steps of the steepest descent and 1,000 steps of the conjugate gradient) with restrain were performed in the first stage; the same algorithms without restrain were further used in the second stage. Secondly, each system was heated from 0 to 300 K within 20 picoseconds (ps), gradually.

Then, the system was equilibrated up to 100 ps at 300 K and constant pressure. Finally, a production process of 50 ns was performed in the constant temperature and pressure (NTP) with a step of 2 fs. The trajectories were recorded every 10 ps and the stability of the system was checked by the RMSD of the backbone. Trajectory analysis was carried out by using the CPPTRAJ.²⁴

Calculation of Binding Free Energy

The MM-GBSA method in AMBER 18 was used to compute the binding free energies of the receptor-ligand complexes.²⁵ All the 100 snapshots of the simulated structures within the last 1 ns trajectory of MD simulations were extracted to perform the binding free energies calculations.

RESULTS AND DISCUSSION

Molecular Docking

Molecular docking is done to predict the orientation of one molecule into the receptor and its interaction is evaluated based on conformation and electrostatic properties.²⁶ Native ligand, *1,4-deoxy-4-((5hydroxymethyl-2,3,4-trihydroxycyclohex-5,6-enyl)amino) fructose* (acarbose) was docked into the binding site of alpha-glucosidase (PDB: 2QMJ) receptor²⁷ for validating the docking method. The root-mean-square deviation (RMSD) between the docked structure (red color) and the X-ray crystal structure (green color) of acarbose was 1.52 Å (less than 2 Å) (Fig.-1), which is satisfactory. The smaller RMSD shows the position of the re-docking ligand which is getting closer to the position of the crystallographic ligand.¹³

Fig.-1: Overlay of Docked Structure (Red Color) and the X-ray Crystal Structure (Green Color) of Acarboseare Quite Similar.

Also, all the 57 curculigosides A⁹ were docked into the binding pocket of alpha glucosidase (Table-1). To illustrate the interaction between ligands and alpha glucosidase, all docking mode were formed bonding to amino acid residues Asp327, Asp203, Arg526, Asp542, and His600; obtained the four best compounds, i.e. **34** (3,5-dihydroxybenzyl-4-chlorobenzoate), **36** (3,5-dihydroxybenzyl-3-bromobenzoate), **43** (3,5-dihydroxybenzyl-4-(tert-butyl)benzoate), and **56** (4-hydroxybenzyl-4-(tert-butyl)benzoate) with free binding energy (kcal/mole) of –6.74, –6.69, –6.81, and –6.68, respectively.

Table-1: Interaction Properties between Ligands and Alpha Glucosidase

Ligands	ΔG (Kcal/mole)	H-Bond			Amino Acid Residues
		Donor	Acceptor	Length (Å)	
Native Ligand (Acarbose)	-4,88	OH	O (Asp203)	2.05179	Asp203, Thr205, Asn207, Tyr299, Asp327, Ile328, Trp406, Trp441, Asp443, Met444, Ser448, Phe450, Arg526, Trp535, Asp542, Asp571, Phe575, Arg598, His600
		OH	O(Asp203)	2.10537	
		NH	O(Asp542)	2.40579	
		NH	O(Asp542)	1.92847	
		OH	O(Asp542)	2.02195	
		OH	O(Asp327)	2.1414	
		NH(Arg526)	O(OH)	1.78717	
		NH(Arg526)	O(OH)	2.18891	
		NH(His600)	O(OH)	2.07274	
34	-6.74	NH(His600)	O(OH)	2.18926	Tyr299, Asp327, Trp406, Trp441, Asp443, Met444, Arg526, Trp539, Asp542, Phe575, His600, Gln603, Tyr605
		OH	O(Asp443)	1.94482	
		OH	O(Asp327)	1.89379	
		NH(Arg526)	O(OH)	2.02255	
36	-6.69	NH(His600)	O(OH)	1.90908	Asp203, Tyr299, Asp327, Ile364, Asp366, Trp406, Trp441, Asp443, Met444, Phe450, Arg526, Asp542, Phe575, His600
		OH	O(Asp443)	1.99091	
		OH	O(Asp327)	1.8032	
		NH(Trp406)	O(H)	2.42645	
		NH(Arg526)	O(CO)	1.86545	
43	-6.81	NH(His600)	O(OH)	1.96354	Arg202, Asp203, Thr204, Tyr299, Asp327, Ile364, Trp406, Trp441, Asp443, Met444, Ser448, Asn449, Phe450, Lys480, Arg526, Asp542, His600
		OH	O(Ser448)	1.90706	
		OH	O(Asp203)	1.78573	
		NH(Arg202)	O(OH)	2.36903	
56	-6.68	O(Asp327)	CH ₃	3.276	Arg202, Asp203, Thr204, Tyr299, Asp327, Ile364, Trp406, Trp441, Asp443, Met444, Ser448, Phe450, Lys480, Arg526, Asp542, Phe575, His600
		NH(Arg202)	O(OH)	2.13038	

Native ligand has a docking mode for amino acid residues on the active site of the receptor. These ligand-receptor interactions are formed through hydrogen bonds, Van der Waals bonds and or electrostatic

interactions (Fig.-2) and ligands **34**, **36**, **43**, and **56** have docking modes similar to native ligand (acarbose). Aglycone curculigoside A has a planar-shaped aromatic ring (Table-2) which able to form van der Waals interaction and π -interactions. Asp542 residue formed π -anion interaction to **34**, **36**, **43**, and **56**. The π -anion interaction plays a role in the stability of the binding interaction.²⁸

Fig.-2: Docking Mode of **34**, **36**, **43**, **56**, and Native Ligand (Acarbose) into the Active Site of Alpha Glucosidase.

Asp327 residues form hydrogen bonds to O atoms in hydroxyl groups substituted in benzene in **34**, **36**, **43**, and **56** as well as in Asp327, His600, and Arg526 residues. Based on these results, ligand **34**, **36**, **43**, and **56** were able to interact with amino acid residues (key residues) in the binding pocket of alpha glucosidase via hydrogen bonds to the Asp327, Asp542, Arg526 and His600 residues which are important amino acid residues on the active side of alpha glucosidase.²⁷

The **34**, **36**, **43**, and **56** have a value of free bonding energy higher than the value in the native ligand (–4.88 kcal/mole). These results indicate that compound **34**, **36**, **43**, and **56** have a good affinity for alpha glucosidase receptors. Free bonding energy (ΔG) shows the stability of the ligand (bond) interaction to the

alpha glucosidase enzyme into the binding site. The higher the value of free bonding energy, the more stable interaction of ligand-receptor.²⁹

MD Simulations

MD Simulations Features

Molecular dynamics simulations were carried out to explore receptor-ligand interactions by considering protein flexibility. To observe the stability of the complexes, the properties of each complex (such as pressure, temperature, structure, and energy) were examined during the entire MD trajectory (Fig.-3). This simulation was carried out on protein-ligands complexes (acarbose, **34**, **36**, **43**, and **56**). The RMSD value of backbone atom referring to the starting structure of the protein-ligand complex was used to monitor the dynamic stability of the MD trajectories. The average RMSD fluctuations for the protein and ligand (acarbose, **34**, **36**, **43**, and **56**) are 1.36 Å and 1.91 Å; 1.60 Å and 1.57 Å; 1.28 Å and 1.71 Å; 1.33 Å and 1.72 Å; and 1.30 Å and 1.28 Å, respectively. These results reveal the average RMSD fluctuations of the five ligands: **56** < **34** < **43** < **36** < acarbose.

Fig-3: RMSD Versus Time for the Acarbose, **34**, **36**, **43**, and **56**.

RMSF (Stability of the Binding Pocket)

The root-mean-squared (RMSF) was used to explore the stability of the binding pocket during the MD simulation process. The RMSF of all residues around the acarbose, **34**, **36**, **43**, and **56** complexes were computed within the last 10 ns trajectory of MD simulations by using Discovery Studio. The residues around the ligand and their RMSF values compared to the initial complexes can be seen in Table 2. In all

the complexes, the RMSF for each residue surrounding the ligand is lower than 1.5 Å (Table-2), which means that the binding pocket is stable during the MD simulation.

Table-2: RMSF Values (Å) and Residues of the Binding Pocket

Residues	Mol 1	Mol 2	Mol 3	Mol 4	Mol 5
LEU 401	0.575	0.787	0.644	0.610	0.565
ILE 402	0.563	0.804	0.650	0.613	0.564
GLY 403	0.553	0.782	0.635	0.590	0.570
GLU 404	0.584	0.807	0.662	0.635	0.624
GLN 409	0.695	0.755	0.757	0.677	0.666
PHE 412	0.512	0.662	0.537	0.498	0.504
ASN 442	0.496	0.743	0.601	0.523	0.535
PHE 450	0.583	0.831	0.658	0.627	0.633
VAL 451	0.587	0.823	0.665	0.652	0.643
ASP 452	0.567	0.797	0.680	0.650	0.592
GLY 453	0.542	0.781	0.654	0.613	0.554
SER 454	0.591	0.865	0.703	0.647	0.617
VAL 455	0.695	0.990	0.816	0.772	0.742
SER 456	0.918	1.256	0.948	1.053	1.051
GLY 457	0.897	1.236	0.905	0.990	1.043
CYS 458	0.769	1.065	0.851	0.872	0.846
SER 459	0.805	1.012	0.950	0.974	0.857
ASN 464	0.560	0.748	0.635	0.644	0.567
PHE 478	0.497	0.783	0.610	0.599	0.536
THR 481	0.446	0.724	0.546	0.497	0.476
LEU 482	0.458	0.719	0.553	0.494	0.472
CYS 483	0.478	0.747	0.587	0.548	0.502
ASP 484	0.516	0.771	0.617	0.594	0.542

Hydrogen Bonds Interaction

Hydrogen bonds interaction plays important role in the complexes between receptor and ligand. Hydrogen bonds were computed within the last 10 ns trajectory. All the possible hydrogen acceptors were taken into consideration, such as protein, ligands, and water molecules. The results of hydrogen bonds analysis for the five systems are listed in Table-3.

There were five, three, six, three, and one hydrogen bonds formed in acarbose, **34**, **36**, **43**, and **56**, respectively (Table-2). The hydrogen bonds formed in complexes (MD simulation) are quite different compared to the binding mode (docking simulation), because of a large movement of ligand and receptor during the MD simulation.

Table-3: Hydrogen Bonds Analysis for Acarbose, **34**, **36**, **43**, and **56**

Complex	Acceptor	Donor	Length (Å)	Angle (°)
Mol 1	Lig C=O	LYS 195 N-H1	2.90	144.97
	Lig C=O	ASP474 N-H2	2.83	107.67
	ARG 471 C=O	Lig N1-H	2.06	167.68

	Lig C=O	LYS 195 N-H3	2.17	115.70
	Lig C=O	ASP474 N-H4	1.84	96.45
Mol 2	ASP443 C=O	Lig N1-H	1.94	159.91
	ASP327 C=O	Lig N2-H	1.89	126.99
	Lig C=O	HIS 600 N-H1	1.91	140.19
Mol 3	Lig C=O	ARG 526 N-H1	2.88	165.93
	ASP 443 C=O	Lig N1-H	1.99	137.88
	ASP 327 C=O	Lig N2-H	1.80	174.54
	Lig C=O	TRP 406 N-H2	2.43	118.34
	Lig C=O	ARG 526 N-H3	1.87	171.42
	Lig C=O	HIS 600 N-H4	1.96	145.07
Mol 4	SER 448 C=O	Lig N1-H	1.91	176.76
	ASP 203 C=O	Lig N2-H	1.79	151.67
	Lig C=O	ARG 202 N-H1	2.37	120.70
Mol 5	ASP 203 C=O	Lig N1-H	1.72	158.13

Binding Free Energies

The calculated ΔG_{bind} of the five complexes was carried out by using MMGBSA method (Table-3), involved ΔE_{vdw} , ΔE_{ele} , ΔG_{GB} , ΔG_{SA} , ΔE_{gas} , ΔG_{sol} , and ΔG_{bind} . The van der Waals interactions occur when adjacent atoms come close enough that their outer electron clouds just barely touch. The **56** complex has the lowest van der Waals energy of -18.38 kcal/mole which is influenced by hydrophobic interactions between *t*-butoxy benzyl with residues surrounding **56**. The **43** complex has a van der Waals energy of -15.36 kcal/mole which is influenced by the hydrophobic interaction between methoxy benzyl with residues surrounding **43**. The **36** complex has a van der Waals energy of -12.50 kcal/mole which is influenced by the hydrophobic interaction between dihydroxy benzyl with residues surrounding **36**. The **34** complex has a van der Waals energy of -10.88 kcal/mole which is influenced by hydrophobic interactions between dihydroxy benzyl with residues surrounding **34**. Acarbose complex has the highest van der Waals energy that is -6.01 kcal/mole which is influenced by hydrophobic interactions between dihydroxy benzyl with residues surrounding acarbose.

The electrostatic also affects free binding energy because each complex shows the presence of hydrogen bonds with the receptor as well as unfavorable polar solvation (ΔG_{GB}) (e.g. **34** and **36** complexes). The **34** complex has a low electrostatic value, but the free binding energy is higher than **36** complex because it has a high in ΔG_{GB} whereas non-polar solvation contributions (ΔG_{SA}) do not affect bond free energy.

CONCLUSION

In conclusion, the binding modes of four inhibitors (**34**, **36**, **43**, and **56**) in docking simulation are similar; and RMSD fluctuations (MD simulation) of the four complexes are consistent with their inhibitory activities. Therefore, these ligands can be considered as a lead compound for alpha-glucosidase inhibitors.

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