MOLECULAR DOCKING AND MOLECULAR DYNAMIC SIMULATION OF THE AGLYCONES 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 glucoside 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

INTRODUCTION
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 glucoside receptor and ligand during the MD process. In conclusion, ligand 34, 36, 43, and 56 are candidates for lead compounds as alpha glucosidase inhibitors.

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This enzyme breaks down oligosaccharides through the hydrolysis reaction by breaking the 1,4-α glycosidic linkage between glucosil residues and glycosidic oxygen (C1-O) accompanied by proton exchange between water and glucosil 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. 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.

One of the chalcone derivatives which has a strong inhibitory activity against alpha glucosidase is xathohumol with IC$_{50}$ of 8.8 µM. 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 orchioides*. Its aglycone was predicted good in absorption, moderate in permeability, and weak binding into plasma proteins.

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. 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 chemically and *in silico*.

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. 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. Docking is more focused on poses and interactions of ligands on an active side, 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 curculigodise A and its derivatives were sketched using ChemDraw Ultra software 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 1.9Å bound with the ligand 1,4-deoxy-4-((5-hydroxymethyl-2,3,4-trihydroxycyclohex-5,6 enyl)amino) fructose and prepared using *Discovery Studio Visualizer* 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. In this program, docking was chosen according to default. Dimension of grid box was (60 × 60 × 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. 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 ligands, 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. The Antechamber suite (AMBER 18 package) was used to produce the atomic charges and topology files of ligands. 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. To neutralize the charge of the system, a proper number of natrium ions were added. The particle mesh Ewald (PME) was adopted during the MD simulations to deal with the long-range electrostatic interactions, and the cut-off distance of nonbonded interactions was set to 10 Å. The SHAKE algorithm was used to constrain the bonds involving hydrogen.

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.

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. 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. Native ligan, 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) receptor 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.

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 A\(^9\) 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.

| Ligands     | \(\Delta G\) (Kcal/mole) | H-Bond | Amino Acid Residues                  |
|-------------|-------------------------|--------|-------------------------------------|
| Native Ligand (Acarbose) | -4.88              | OH O (Asp203) 2.05179  | Asp203, Thr205, Asn207, Tyr299, Asp327, Ile328, Trp406, Trp441, Asp443, Met444, Ser448, Phe450, Arg526, Trp539, Asp542, Asp571, Phe577, Arg598, His600 |
| 34          | -6.74                  | OH O (Asp327) 1.94482  | Tyr299, Asp327, Trp406, Trp441, Asp443, Met444, Arg526, Trp539, Asp542, Phe577, His600, Gln603, Tyr605 |
| 36          | -6.69                  | OH O (Asp327) 1.89379  | Asp203, Thr204, Tyr299, Asp327, Ile364, Asp366, Trp406, Trp441, Asp443, Met444, Phe450, Arg526, Asp542, Phe577, His600 |
| 43          | -6.81                  | O(Asp327) CH\(_3\) 3.276 | Arg202, Asp203, Thr204, Tyr299, Asp327, Ile364, Trp406, Trp441, Asp443, Met444, Ser448, Asn449, Phe450, Lys480, Arg526, Asp542, His600 |
| 56          | -6.68                  | NH(Asp203) 2.13038  | Arg202, Asp203, Thr204, Tyr299, Asp327, Ile364, Trp406, Trp441, Asp443, Met444, Ser448, Asn449, Phe450, Lys480, Arg526, Asp542, Phe577, His600 |

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. 

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. 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 receptors. 

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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.

**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

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

| 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 an 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.

| 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   |

**Table-3: Hydrogen Bonds Analysis for Acarbose, 34, 36, 43, and 56**
Binding Free Energies
The calculated $\Delta G_{\text{bind}}$ of the five complexes was carried out by using MMGBSA method (Table-3), involved $\Delta E_{\text{vdw}}$, $\Delta E_{\text{ele}}$, $\Delta G_{\text{GB}}$, $\Delta G_{\text{SA}}$, $\Delta E_{\text{gas}}$, $\Delta G_{\text{sol}}$, and $\Delta G_{\text{bind}}$. 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 ($\Delta G_{\text{GB}}$) (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 $\Delta G_{\text{GB}}$ whereas non-polar solvation contributions ($\Delta G_{\text{SA}}$) 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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