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Molecular Docking Studies of Chemical Constituents *of Tinospora cordifolia* on Glycogen Phosphorylase

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Abstract

Tinospora cordifolia extract decreased the activity of glycogen phosphorylase in the liver and widely used in the treatment of diabetes mellitus. This *in silico* study aimed to screen the active compound(s) of *T. cordifolia* which play a role in its hypoglycemic activity as glycogen phosphorylase inhibitor by molecular docking analysis. Thirteen chemical constituents of *T. cordifolia* were used as ligands for docking study. The glycogen phosphorylase structure was obtained from Brookhaven protein databank (1LWO). Docking analysis was performed using Autodock Vina and PyRx 8. The inhibitory activity was analyzed by comparison the binding energy and binding mode of interaction of *T. cordifolia* chemical constituents on glycogen phosphorylase. The results indicate that magnoflorine, cordiofolioside A, and syringin exhibited good affinity to glycogen phosphorylase, by interacted at catalytic site of enzyme

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Keywords: Autodock Vina, diabetes mellitus, glycogen phosphorylase, molecular docking, *Tinospora cordifolia*

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1. Introduction

Malaria Liver is the major regulator of plasma glucose levels in the post absorptive state, and in type II diabetes hepatic glucose production is significantly elevated relative to nondiabetes. Glycogen phosphorylase, a key enzyme in the regulation of glycogen metabolism, catalyzes the degradative phosphorolysis of glycogen to glucose 1-phosphate. Glycogen phosphorylase has been exploited as a specific target of inhibitors that might prevent glycogenolysis under high glucose level conditions in type II diabetes[1,2].

Some anti-diabetes drugs have one or more adverse effects, so the management of diabetes mellitus is still a challenge in the medical field. Recently, the search for appropriate antihyperglycemic agents has been focused on

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traditional medicine product due to the lower side effect[3]. *Tinospora cordifolia* extract was reported can decrease the activity of glycogen phosphorylase in the liver and widely used in the treatment of diabetes mellitus[4,5]. A variety of chemical constituents have been isolated from *T. cordifolia* plant and their structures were elucidated. They belong to different classes such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides[6,7].

This *in silico* study aimed to screen the active compound(s) of *T. cordifolia* (figure 1), i.e. magnoflorine, cordiofolioside A, ecdysterone, syringin, palmatine, cordioside, tinocrisposide, berberine, tinocordiside, tembetarine, tinocordifolin, palmatoside as well as columbin, which play a role in its hypoglycemic activity as glycogen phosphorylase inhibitor by molecular docking using AutoDock Vina software. By molecular docking, the bound conformations and the binding affinity between chemical constituent of *T. cordifolia* and glycogen phosphorylase as target, could be predicted.

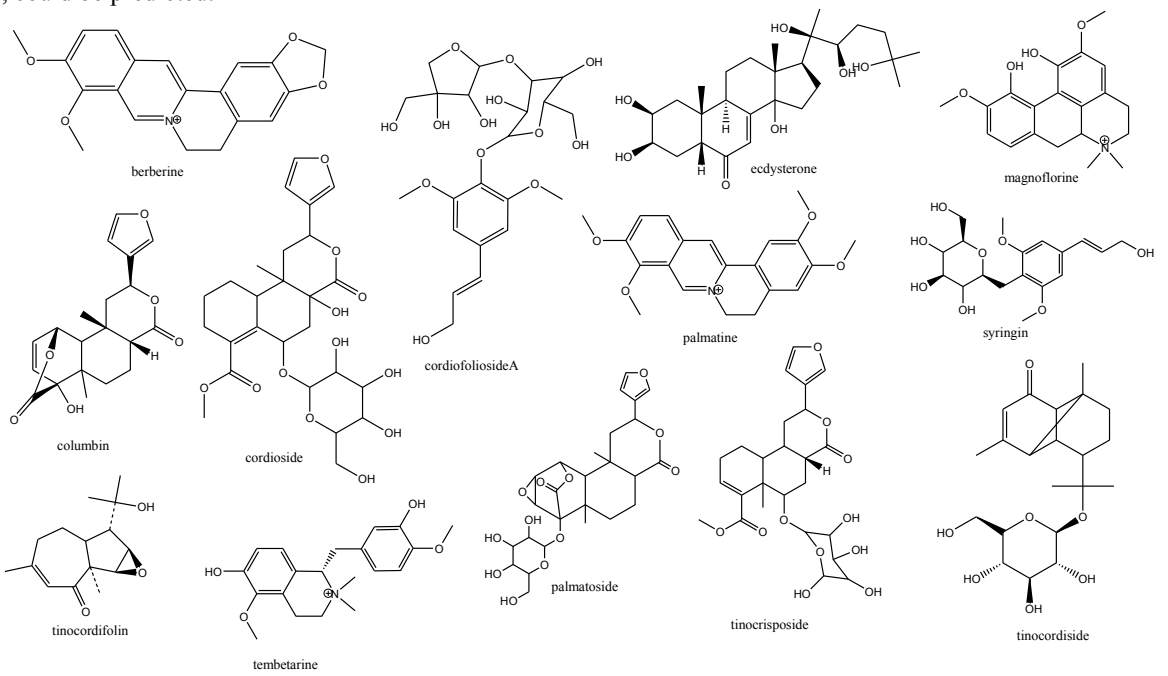

Figure 1. Chemical structures of *T. cordifolia* constituents.

2. Experiments

2.1. Trizol Ligands Preparation

The thirteen structures of chemical constituents of *T. cordifolia* i.e. magnoflorine, cordiofolioside A, ecdysterone, syringin, palmatine, cordioside, tinocrisposide, berberine, tinocordiside, tembetarine, tinocordifolin, palmatoside as well as columbin (figure 1) were collected from published literatures[6,7]. The two-dimensional (2D) chemical structures of the ligands were sketched using ChemDraw Ultra 2008, and the energy minimizations of the prepared ligands were carried out with Chem3D Ultra and were saved in pdb format.

2.2. Target Preparation and Validation of Docking Method

The three dimensional structure of glycogen phosphorylase was obtained from Brookhaven protein databank (PDB ID: 1LWO). The docking study was started with the definition of a binding site, in general a restricted region of the protein. The size and location of this binding site was visualized in PyMOL. The protein target were further validated with AutoDock Vina in PyRx 0.8 by RMSD value determination[8].

2.3. Molecular Docking Analysis

Binding mode and interaction of glycogen phosphorylase with individual chemical constituent of *T. cordifolia*, was performed using AutoDock Vina software. Docking was performed to obtain a population of possible conformations and orientations for the ligand at the binding site. The protein was loaded in PyRx software, creating a PDBQT file that contains a protein structure with hydrogens in all polar residues. All bonds of ligands were set to be rotatable. All calculations for protein-fixed ligand-flexible docking were done using the Lamarckian Genetic Algorithm (LGA) method. The docking site on protein target was defined by establishing a grid box with the dimensions of X: 38.0729 Y: 33.3208 Z: 25.0000 Å, with a grid spacing of 0.375 Å, centered on X: 20.2892 Y: 10.3219 Z: 32.3218 Å. The best conformation was chosen with the lowest docked energy, after the docking search was completed. Ten runs with AutoDock Vina were performed in all cases per each ligand structure, and for each run the best pose was saved. The average affinity for best poses was taken as the final affinity value. The interactions of complex glycogen phosphorylase protein-ligand conformations, including hydrogen bonds and the bond lengths were analyzed using PyMol.

3. Results and Discussion

Docking of small molecule compounds into the binding site of a receptor and estimating the binding affinity of the complex is an important part of the structure based drug design process. AutoDock Vina is a open-source program for drug discovery, molecular docking and virtual screening, offering multicore capability, high performance and enhanced accuracy and ease tof use. The parameters chosen for the docking can be judged by the docking tool's ability to reproduce the binding mode of a ligand to protein, when the structure of the ligand–protein complex is known. The criterion usually used is the all atom root mean square deviation (RMSD) between the docked position and the crystallographically observed binding position of the ligand, and success is typically regarded as being less than 2Å[9].

In this study, the native ligand was CP320626 (5-chloro-1H-indole-2-carboxylic acid [1-(4-fluorbenzyl)-2-(4-hydroxypiperidin-1yl)-2-oxoethyl] amide). This compound has been identified as a potent inhibitor of human liver glycogen phosphorylase. CP320626 is also a potent inhibitor of human muscle glycogen phosphorylase. Oikonomakos et al. elucidated the structural basis of the inhibition mechanism of CP320626, by determination the complex of rabbit muscle glycogen phosphorylase with CP320626[1]. Figure 2 showed the model of interaction between CP320626 and binding site of glycogen phosphorylase. Amino acids residues of glycogen phosphorylase which involved in interaction with CP320626 were Glu190A, Arg60A and Lys191A. A hydrogen bond was occured between carbonyl group of Glu190A as hydrogen bond acceptor and –NH of indole ring as hydrogen bond donor. Between cationic center of Arg60A and anionic center of CP320626, an electrostatic interaction was formed. Prathipati et al. reported that the mayor interaction between CP320626 and the enzyme are hydrophobic interactions between the indole-aromatic ring and hydrophobic residues such as Pro229, Trp189, and Trp67 which is represented by a hydrophobic feature on the indole-ring[1].

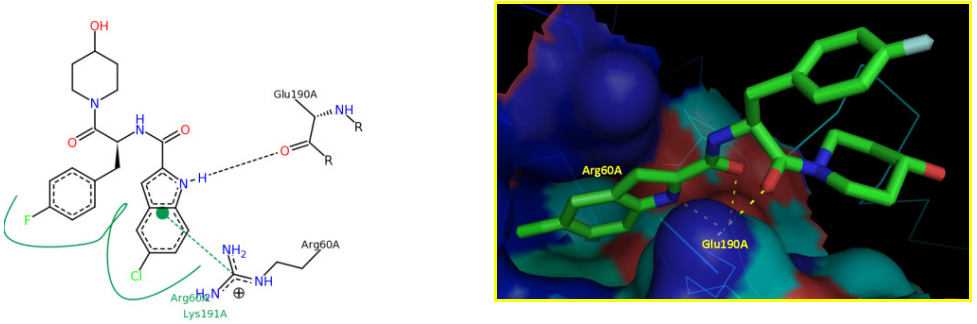

Figure 2. Model of interaction of CP320626 and glycogen phosphorylase binding site

Figure 3 showed the superimposition of CP320626 from the X-ray crystal structure and from the docking result. The RMSD value was 1.765 Å, indicated that Autodock-Vina's algorithms used in this research were valid. In addition, both of ligands (native ligand and that from the docking simulation) interacted to the same amino acid residues of glycogen phosphorylase, i.e. Arg60A and Glu190A, so this docking method then was used to perform the docking calculation of the chemical constituents of *T. cordifolia*

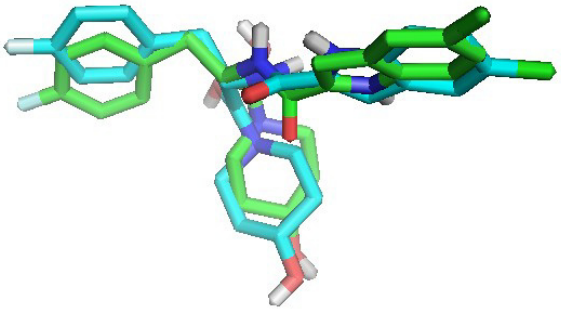

Figure 3. Superimposition of CP320626 from The X-Ray Crystal Structure (atom C in blue) and from the Docking Result (atom C in cyan)

Structures of selected chemical constituents of *T. cordifolia* were used as ligands for molecular docking to glycogen phosphorylase binding site, and the binding affinity was characterized by binding energy (ΔG) value. Among 13 chemical constituents of *T. cordifolia*, magnoflorin, cordiofolioside A and syringin showed the best binding energy (ΔG) value, i.e. -8.80, -8.830, and -8.20 kcal/mole, respectively (table 1).

Table 1. Binding Energy of *T. cordifolia* Chemical Constituents

Ligands	Binding Energy (kcal/mole)
magnoflorine	-8.80 ± 0.95
cordiofolioside-A	-8.30 ± 0.87
syringin	-8.20 ± 0.91
palmatine	-7.90 ± 0.76
cordioside	-7.30 ± 0.84
tinocrisposide	-7.10 ± 0.66
columbin	-7.00 ± 0. 76
tinocordiside	-7.00 ± 0.98
ecdysterone	-6.90 ± 0.79
palmatoside	-6.70 ± 0.80
tembetarine	-6.70 ± 0.54
tinocordifolin	-6.10 ± 0.92
berberine	-3.00 ± 0,71

Glycogen phosphorylase has at least six different ligand binding sites, i.e. (1) the Ser14-phosphate recognition site, (2) the catalytic site that binds glycogen, glucose-1-P, glucose and glucose analogues, (3) the AMP allosteric site, (4) the purine nucleoside site, (5) the glycogen storage site, and (6) the dimer interface site, which binds indole derivatives. The catalytic site, the AMP allosteric site, the inhibition site and the dimer interface site are known to be inhibitor binding sites[10]. The allosteric site is made up of helices α2 (residues 47-78), α8 (residues 289-314), β4 (residues 153-160), β10 (residues 222-232), β11 (residues 237-247), β7 (residues 191-193) and the loop (residues 195-197). Ligands occupying this site are able to inhibit this enzyme by either direct and/or indirect inhibition of AMP binding. CP320626 acted as inhibitor by interacted to to the new allosteric (or indole) site, located inside the central cavity of the dimeric enzyme of the enzyme[11].

Location of interaction site of the ligands in the enzyme, which were different from the interaction site of CP320626. Magnoflorin, cordiofolioside A, syringin, palmatine, as well as tinocordiofoline interacted at the catalytic sites of the enzyme. Somsák et al. reported that the catalytic site is buried at the centre of the enzyme, consists of residues α6 (134-150), β13 (371-376), and β18 (478-484) in domain 1 and from β 19 (562-570), the loop (571-574)

between β 19 (562-570) and β 18 (575-593), and the loop (666-675) between β 22 (661-665) and β 21 (676-684) in domain 2[11]. Other ligands did not locate at any potential regulatory sites.

Magnoflorine, an isoquinoline alkaloid, as well as cordifolioside A and syringin (phenylpropanoid compounds), showed the lowest binding energy that indicated the highest affinity to the enzyme. Figure 5 showed the binding modes of these compounds in the catalytic site pocket of GP and the hydrogen bonds contributed for the binding. Catalytic site of GP binds glycogen and glucose-1-P as well as glucose and glucose analogues. Cordifolioside A and syringin are glycoside compounds; this enabled their interaction with catalytic site of GP. Large compounds with glucopyranosylamine structure have synthesized and showed the GP inhibition activity[11].

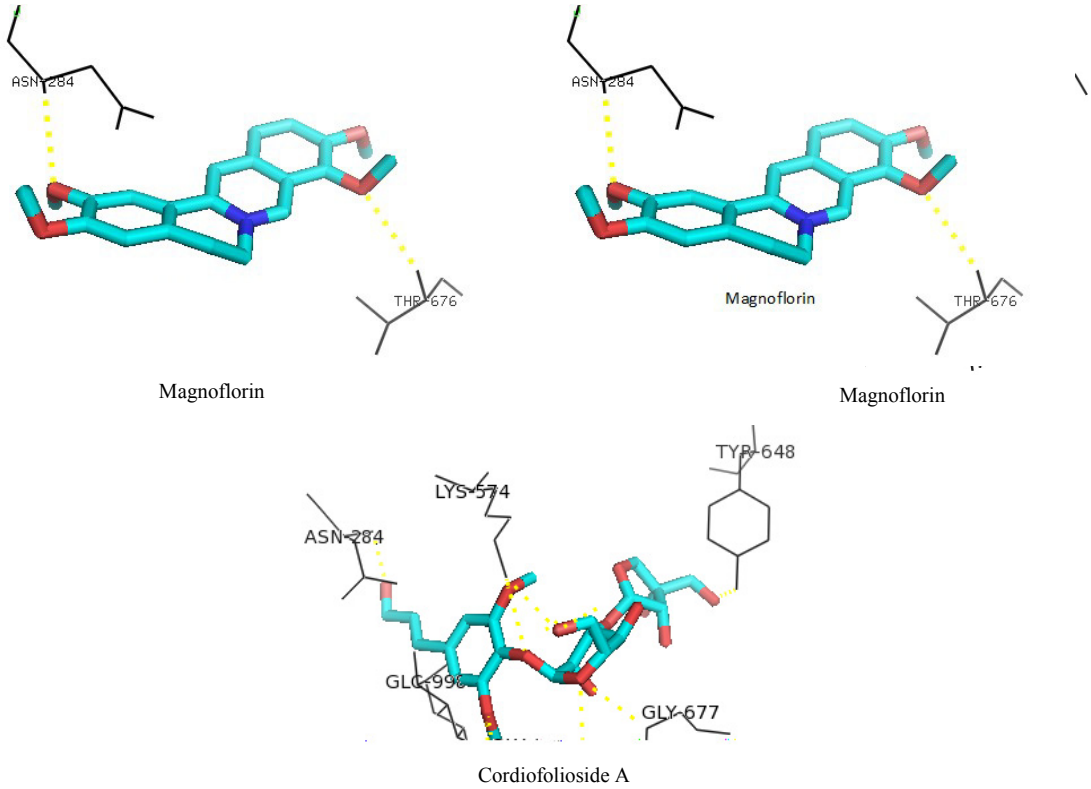

Figure 4. The binding mode of magnoflorine, syringin and cordiofolioside A in the catalytic site pocket of GP. The hydrogen bonds between ligands and residues are indicated as broken lines

4. **Conclusion**

The results indicated that Autodock-Vina's algorithms were valid, as re-docking the native ligand to binding site of glycogen phosphorylase showed a RSMD value less than 2 A 1.765. The docking result revealed that magnoflorin, cordiofolioside A, and syringin exhibited good binding interaction to catalytic site of glycogen phosphorylase

Acknowledgements

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