IN SILICO SCREENING FOR ALPHA GLUCOSIDASE INHIBITORS OF CHROMONE DERIVATIVES

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ARTICLE INFO	ABSTRACT
Key Words Binding affinity, alpha glucosidase, chromones, Molegro virtual docker 6.0	**Objective:** To evaluate the potent alpha glucosidase inhibitory activity of chromone derivatives using Molegro virtual docker 6.0 version. **Methods:** In this regard chromone derivatives were drawn using ACD chem. Sketch software. Acarbose a well known alpha glycosidase inhibitor chosen as standard chosen .Insilco docking studies were carried out using Molegro virtual docker version 6.0.The basic principle involved in Molegrodocker is inbuilt version MVD tools **Results**: Docking results showed all the selected Ligands has binding energy ranging between-14.5kcal/mol to-16.5.kcal/mol when compared with that of standard which has binding energy **Conclusion:** Chromone derivatives reported best alpha glucosidase inhibitory activity because of its structural parameters. Further study on chromone derivatives and invivo and invitro studyare necessary to develop target moieties for treatment of diabetes mellitus.

INTRODUCTION

Computer aided drug design (CADD) can be madein two phase: ligandbasedor structure-based. With the availability of the 3D structure of a biological target, it is feasible to use a Structurebased approach to evaluate and predict the binding mode of a ligand within the active site of the receptor with docking method[1-8]. Now it is a popular technique used for increasing the speed of drug designing process. This was made possible by the availability of many protein structures which helped in developing tools to understand the structure function relationships, automated docking and virtual screening. Diabetes Mellitus (DM) is a metabolic disorder characterized by thepresence of chronic hyperglycemia accompanied by greater or lesser impairment in the metabolism of carbohydrates, lipids and proteins.DM is probably one of the oldest diseases known to man. It was first reported in Egyptian manuscript about 3000 years ago [9]. In 1936, the distinction between type 1 and type 2 DM was clearly made [10]. Type 2 DM was first described as a component of metabolic syndrome in 1988 [11]. The origin and etiology of DM can vary greatly but always include defects in either insulin secretion or response or in both at some

point in the course of disease. Mostly patients with diabetes mellitus have either type 1 diabetes (which is immune-mediated or idiopathic) Type 2 DM (formerly known as non-insulin dependent DM) is the most common form of DM characterized by hyperglycemia, insulin resistance, and relative insulin deficiency [12]. Type 2 DM results from interaction between genetic, environmental and behavioral risk factors [13-14]. Diabetes also can be related to the gestational hormonal environment, genetic defects, other infections, and certain drugs [15].

In type 2 diabetes these mechanisms break down, with the consequence that the two main pathological defects in type 2 diabetes are impaired insulin secretion through a dysfunction of the pancreatic β-cell, and impaired insulin action through insulin resistance [16]. In situations where resistance to insulin predominates, the mass of β-cells undergoes a transformation capable of increasing the insulin supply and compensating for the excessive and anomalous demand. In absolute terms, the plasma insulin concentration (both fasting and meal stimulated) usually is increased, although "relative" to the severity of insulin resistance, the plasma insulin concentration is insufficient to maintain normal glucose homeostasis. Keeping in mind the intimate relationship between the secretion of insulin and the sensitivity of hormone action in the complicated control of glucose homeostasis, it is practically impossible to separate the contribution of each to the etiopathogenesis of DM2 [17]

Flavonoids are naturally occurring phenolic compounds thatare widely distributed in plants and some of them have beendescribed as glucosidase inhibitors. Flavonoids are potential antidiabetic agents because they exert multiple actions that are both hypoglycemic (insulinomimetic action) and antihyperglycemic (insulin secretagogue)[19-20]. Glucosidase inhibitorsare potential agents for diabetes therapy since glucosidases areinvolved in several important and relevant biological processes[21]. Acarbose, thefirsta-glucosidase inhibitor to be identified, iscurrently used for the treatment of type 2 diabetes[22]Flavonoids are potential antidiabetic agents because they exertmultiple actions that are both hypoglycemic (insulinomimeticaction) and antihyperglycemic (insulin secretagogue).

Materials and Methods

Preparation of molecules and ligands for docking: Molecular docking was performed using a crystallized yeast glucose-α-glucosidase. The 3D structure for yeast glucose-α-glucosidase was obtained from a protein data bank (http://www.rcsb.org/). Polar hydrogens were added to a macromolecule by usingMolegro, after which the structure was saved in file format that contains a protein structure with hydrogen in all polar residues. For ligands, the 3D structures of synthetic chromones were searched in the PubChem database (http://pubchem.ncbi.nlm.nih.gov/). An SDF file for the 3D structure was converted into a PDB file by ACD Chemsoftware. Six synthetic compound structures were minimized by computing gasteiger changes and the structures were saved in MVD format via Molegro.

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Virtual screening of α-glucosidase inhibitors

SELECTION OF TARGET PROTEIN FROM RCBS: The preparation of the target enzyme with molegro tool involved in the addition of hydrogen atom to the target enzymes which is a necessary step for the computation of partial atomic charges alpha amylase enzyme complexed with selective inhibitors with two chains with $3.88A^0$ and $3.13A^0$ respectively.

DESIGNING OF LIGANDS: Computational analysis was carried out in chain A of 5LRB .Six molecules were selected to study associated protein ligand interactions.All ligands were drawn by using chem. Sketch software.

MOLECULAR DOCKING ANALYSIS:

Mol Dock Score scoring function was employed to predict the binding energy for active site residue-ligand interactions and docking studies computed for all ligands using Molegro virtual docker program that predicted interactions in terms of Dock score.

MOLECULAR DYNAMICS: All calculations were done on a Intel core I5 laptop with windows seven configuration. Docking was performed by using Molegro Virtual Docker (MVD) software package. MVD files perform flexible ligand docking, so the optimal geometry of the ligand will be determined during the docking. To obtain better potential binding sites in the alpha amylase (PDB ID: 5LRB), a maximum of five cavities was detected using default parameters.

RESULTS

Study of Ligand-Substrate Interaction
The designed compounds were evaluated through docking techniques using MVD program. Designed compounds were docked on one of the crystal structures of alpha glucosidase available through the RCSB Protein Data Bank. The compounds were scored based on the minimized ligand protein complexes. New ligands were docked into the empty binding site of alpha glucosidase order to compare the binding affinity. ACR_1003 ligand with surrounding active site residues within 3.88 A°, hydrogen bonding interactions and the spatial orientation in binding pocket is given in Figure 3. The interacting residues surrounding the ligand within 3.13 A° distance are Glu167 ,Asn613, Lys704,His412.The SIX ligand molecules having minimum energy were screened out as the possible inhibitors for alpha amylase given in the (Table 1).

DISCUSSION:

The six ligand molecules having minimum energy were screened out as the possible inhibitors for 5LRB given in the (Table 1). 2-(2-chloro-5-hydroxyphenyl)-4*H*-chromen-4-one it had highest moldoc score of -40.20.It had one hydrogen bond. The Glu 167 of protein formed hydrogen bond with oxygen of chromone group of ligand.Thebond length was found to be 3.1 A^0.The active binding site of the alpha amylase inhibitors was found to have bond length of 2.2 A^O ,3.4 $A^{0,}$2.9 A^0 of Gly238 ,Thr237 ,Phe239 aminoacids respectively which has molecular docking score of -65.784 and its hydrogen bond energy was found to be -8.9. 2-(4-bromo-2-hydroxyphenyl)-4*H*-chromen-4-one had mol doc score -60.032.It had two hydrogen bond formed between Asn 12 and hyroxy group of chromonederivatives. The bond length was found to be 2.5 A^0.The aromatic aldehyde derivative formed between Ly13 and hydroxyl of aldehyde of chromones,the bond length was found to be 2.6 near aromatic substitution of chromone substitution. 2-(4-fluoro-2-hydroxyphenyl)-4*H*-chromen-4-one had mol doc score -200.575.It had two hydrogen bond formed between Asp 63 and hyroxy group of chromonederivatives.

General structure for ligand
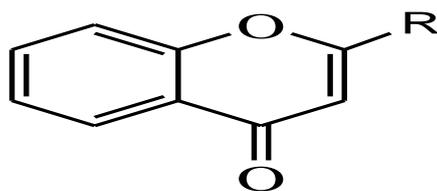
Lead moiety

TABLE1: Ligand with different substitution

S.NO:	R SUBSTITUENTS	IUPAC NAME
1.		2-(2-chloro-5-hydroxyphenyl)-4*H*-chromen-4-one
2.		2-[2-hydroxy-4-(nitromethyl)phenyl]-4*H*-chromen-4-one
3.		2-(4-fluoro-2-hydroxyphenyl)-4*H*-chromen-4-ol
4.		2-(4-bromo-2-hydroxyphenyl)-4*H*-chromen-4-one
5.		2-(4-fluoro-2-hydroxyphenyl)-4*H*-chromen-4-one
6.		2-(4-fluoro-2-hydroxyphenyl)-4*H*-chromen-4-one

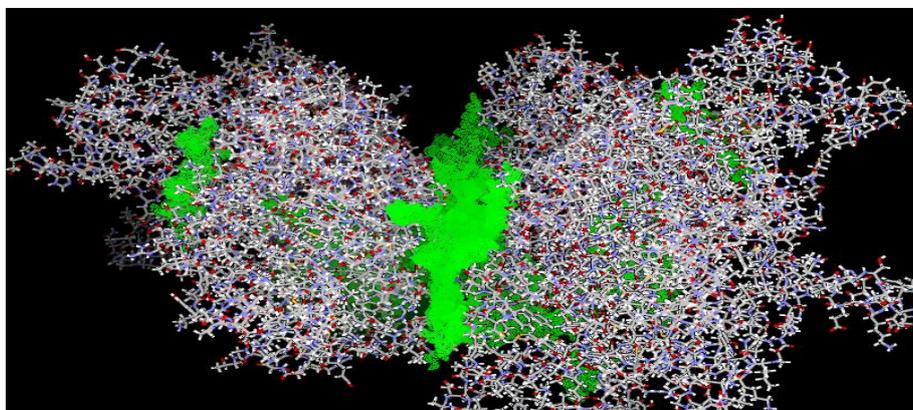**FIG 2:2-(2-chloro-5-hydroxyphenyl)-4*H*-chromen-4-one binds with 5LRB**

Table 2: docking score for alpha glucosidase inhibitor pdb code: 5LRB

Name of the Ligands	Mol. Dock score	Reranking score	H BOND ENERGY	H BOND IN A^0	RESIDUES
ACR_1003	-90.7002	-72.6867	-16.16	3.88	Tyr474
2-(2-chloro-5-hydroxyphenyl)-4*H*-chromen-4-one	-104.186	-72.6867	-16.67	3.88	Tyr 747
2-[2-hydroxy-4-(nitromethyl)phenyl]-4*H*-chromen-4-one	-79.8826	-11.45	-13.46	3.13	Glu167
2-(2-fluoro-5-hydroxyphenyl)-4*H*-chromen-4-one	-58.25	-61.66	-13.36	3.13 3.19 2.4 2.8	Glu167 Asn613 Lys704 His412
2-(4-chloro-2-hydroxyphenyl)-4*H*-chromen-4-ol	-61.96	-65.16	-13.8	3.3 3.1 2.4	Glu167 Glu173 Arg140
2-(4-bromo-2-hydroxyphenyl)-4*H*-chromen-4-one	-72.77	-89.44	-14.6	3.6	Glu176
2-(4-hydroxy-2-hydroxyphenyl)-4*H*-chromen-4-one	-77.56	-90.64	-15.4	3.5	Lys34

FIG 3:2-(2-chloro-5-hydroxyphenyl)-4*H*-chromen-4-one bind with 5LRB surface view
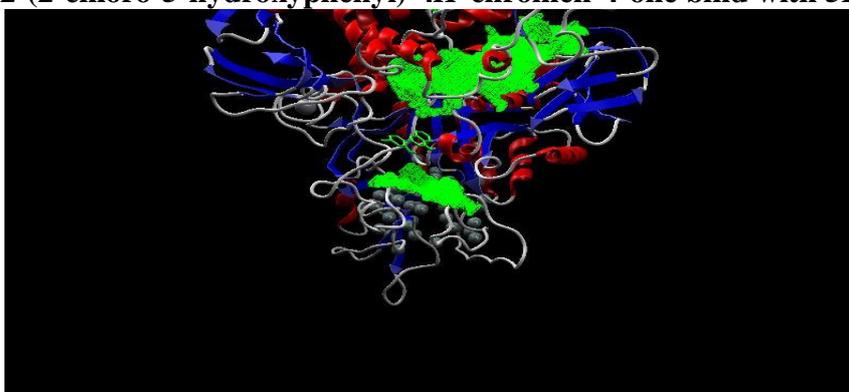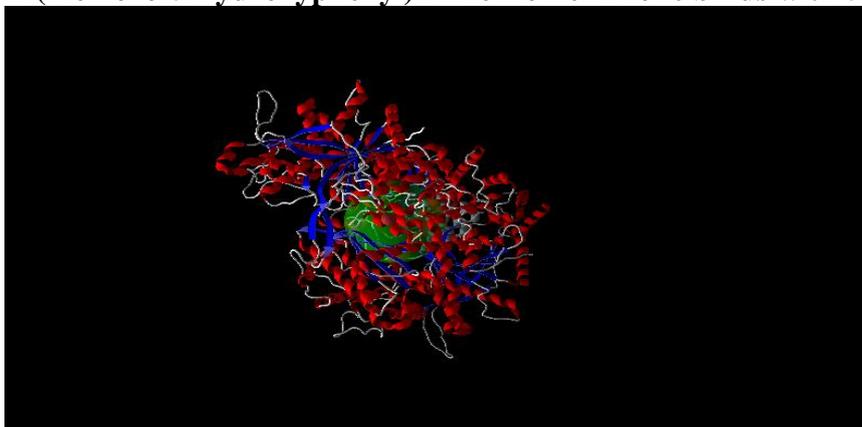

FIG 4: 2-(2-chloro-5-hydroxyphenyl)-4*H*-chromen-4-one binds with 5LRB

The bond length was found to be 2.5 A^0.The aromatic aldehyde derivative formed between Asp 63 and hydroxyl of aldehyde of chromones,the bond length was found to be 2.5 near aromatic substitution of chromone substitution. The other hydrogen was found between Ser199 and hyroxy group of chromonederivatives.The bond length was found to be 2.9 A^0.The aromatic aldehyde derivative formed between Ser199and hydroxyl of aldehyde of chromones,the bond length was found to be 2.9 A^0 near aromatic substitution of chromone derivatives. 2-(4-chloro-2-hydroxyphenyl)-4*H*-chromen-4-one had mol doc score -45.23Kcal/mol.It had four hydrogen bond formed between Thr 237 and hyroxy group of chromonederivatives.The bond length was found to be -4.5 A^0.The aromatic aldehyde derivative formed between Thr 237 and hydroxyl of aldehyde of chromones,the bond length was found to be -4.5 A^0 near aromatic substitution of chromone substitution. The other hydrogen was found between Gly238 andhyroxy group of chromonederivatives.The bond length was found to be-1.1A^0.The aromatic aldehyde derivative formed between Gly238 and hydroxyl of aldehyde of chromones,the bond length was found to be -1.1 near aromatic substitution of chromone substitution. The aromatic aldehyde derivative formed between Phe239 and hydroxyl of aldehyde of chromones,the bond length was found to be 1.4A^0 near aromatic substitution of chromone substitution. The other hydrogen was found between Ly2 andhyroxy group of chromonederivatives.The bond length was found to be—4.8 A^0 aromatic substitution of chromone derivatives. The active site is present in A ring of the protein. The residue bind with the ligand is same as that of the standard compounds docked. The residues found in the active sites are as follows are Glu167, Asn613, Lys704, His412.

CONCLUSION:

By using computational approaches derivatives designed showed good interactions withalpha amylase inhibitors protein. 2-(4-fluoro-2-hydroxyphenyl)-4*H*-chromen-4-one- 200.575 kcal/mol against 5LRB (PDB ID) in docking analysis. Docking studies confirm that the main interaction of IVWI inhibitors with enzyme is Hydrogen bond and Hydrophobic interactions with the binding pockets made by OH group of chromones and aromatic aldehyde substitution of the ligands. This information has potential implications to understand the mechanism of IVWI related enzymatic inhibition reactions, and also applicable in the prediction of more effective inhibitors and engineering 3D structures of other enzymes as well.Hence, it is concluded that that 2-(4-fluoro-2-hydroxyphenyl)-4*H*-chromen-4-one,2-(4-hydroxy-2-hydroxyphenyl)-4*H*-chromen-4-one, 2-(4-chloro-2-hydroxyphenyl)-4*H*-chromen-4-olcould be a potent anti-diabetic target molecule against IVWI which may be worth for further clinical trials. In this study, computations on the interactions at the active site of I were carried out for Six ligands. In future, it may be necessary to explore the development of potential new alpha amylase inhibitors for treating diabetis The present study shall help in rational drug design and synthesis of new selective alphaamylase inhibitors with predetermined affinity and activity and provides valuable information for the understanding of interactions between 5LRB and the novel 4 hydroxychromones.

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