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RESEARCH ARTICLE

Molecular Descriptor Enhancement of a Common Structure Towards the Development of α-Glucosidase and α-Amylase Inhibitors for Post-Prandial Hyperglycemia (PPHG).

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ABSTRACT

The most challenging goal in the management of diabetic patient is to achieve normal blood glucose levels caused by post-prandial hyperglycemia (PPHG) or hyperinsulinemia, the individual risk factor contributes to the development of macrovascular complications. Synthetic hypoglycemic agents are available which has its own limitations and serious side-effects. The present study deals about the development of a common small molecular structure by enhancing the molecular descriptors required for binding with α-glucosidase and α-amylase enzymes, the two major targets of PPHG and to develop a monosaccharide-type inhibitor with many insights derived from pharmacophore studies, molecular alignment and molecular docking studies of known inhibitors. A hypothesis was designed which suggest the essential and/or minimal requirement of molecular descriptors to be an efficient binder of these two hydrolytic enzymes and subsequently, molecules with naturally occurring flavonoid structural architecture obeying the hypothesis was developed and evaluated *in silico*.

KEYWORDS: Post-prandial hyperglycemia, Molecular descriptors, α-glucosidase, α-amylase, Pharmacophore features, Molecular docking, Hypothesis design.

INTRODUCTION

Diabetes mellitus type II, a metabolic disorder exemplified by chronic hyperglycemia or increased blood glucose levels with disturbances in carbohydrate, fat and protein metabolism resulting from absolute or lack of insulin secretion [1]. By 2025, this disorder is likely to hit 300 million people worldwide with India projected to have the largest number of diabetic cases [2]. In type 2 diabetic patients, non-fasting (post lunch and extended post lunch) plasma glucose levels are better correlated with glycated hemoglobin (HbA1c) than are fasting levels [3]. In addition, epidemiological studies revealed that post-prandial hyperglycemia (PPHG) or hyperinsulinemia is one of the independent risk factors which promote the development of macrovascular complications of diabetes mellitus. Diabetic management studies disclosed that even a mild post-prandial blood glucose elevation becomes a potential risk factor [4]. Human pancreatic α-amylase (E.C.3.2.1.1) is a key enzyme which catalyzes the initial step in the hydrolysis of dietary starch to a mixture of smaller oligosaccharides composed of maltose, maltotriose and a number of α-(1-6) and α-(1-4) oligoglucans [5]. These are then degraded by α-glucosidase (E.C. 3.2.1.20) to glucose by hydrolyzing terminal, non-reducing 1, 4 linked α-D-glucose residues. This causes rise in blood glucose thereby contributing PPHG [6]. Hence, inhibition of these enzymes can potentially control diabetes type II. Commercially available α-glucosidase inhibitors such as Acarbose, Miglitol and Voglibose shares some merits as well as pitfall. Acarbose can inhibit both α-glucosidase and to a lesser extent, α-amylase but is reported with gastrointestinal (GI) disturbance [7]. Miglitol and Voglibose inhibit α-glucosidase exclusively whereas the former molecule is systematically absorbed [8] and the latter one scores over in the side effect profile compared to Acarbose and Miglitol [9]. However, Miglitol is not metabolized and is rapidly excreted by the kidneys [8] and Voglibose is accounted for poor efficacy [9]. On the other hand, α-amylase inhibitors are expected to be a better suppressor of PPHG since it will stall the accumulation of maltose thereby preventing side effects such as abdominal pain, flatulence and diarrhea [10]

The principle objective of the present study is to develop a monosaccharide-type molecule which should inhibit both α-glucosidase and α-amylase enzymes (Figure 1). The pharmacophore features of known α-amylase inhibitors required for interaction was explored preliminarily. While retaining the molecular properties of α-glucosidase inhibitors, we developed strategies for enhancing molecular descriptors of α-amylase inhibitors so that it can inhibit both enzymes. Since we focused to develop monosaccharide-type inhibitors, Miglitol was considered as

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the reference molecule and Luteolin, a flavonoid of *Lonicera japonica* which inhibited α-glucosidase enzyme effectively and α-amylase enzyme less potent than Acarbose [11] was taken into account. As Acarbose is reported to be the only α-glucosidase inhibitor which can also inhibit α-amylase specifically, its molecular interaction with α-amylase at the active site cavity was further studied and designed a hypothesis which suggests the essential and/or minimal requirement of molecular descriptors in order to be an efficient binder of these two hydrolytic enzymes. Finally, molecules with Luteolin structural framework was developed and screened through molecular docking studies.

MATERIALS AND METHODS

LIGAND DATASET AND ITS PREPARATION:

Ligand dataset was comprised of Acarbose (CID 41774), Miglitol (CID 441314), Voglibose (CID 444020) and Luteolin (CID 5280445) and their respective 2D structure (wherever available 3D) were retrieved from NCBI PubChem in Structure Data Format (SDF) [12]. Ligand structures were then subjected to conformational analysis using Frog v1.01 hosted at Mobyle server [13] with the number of conformer generation limited to 100, the maximum energy threshold set to 100 Kcal/mol and the cycle of Monte Carlo simulation restricted to 100 steps. The conformer obtained for each ligand input was then geometrically optimized and energy minimized using molecular mechanics geometry optimization module implemented in HyperChem v8 (licensed version, HyperChem™) [14]. AMBER force field with distant dependent dielectric constant, scale factor for electrostatic and van der Waals forces set to 0.5 and without any cutoffs to bond types and its lengths were chosen to determine global minimum energy. This final step of geometric optimization and energy minimization of conformers were carried out only to attain global minimum energy as we had initially restricted Monte Carlo simulation to 100 steps in the Frog conformational analysis due to server overload. Subsequently, all the resultant structure was exported to hard disk in Tripos Mol2 format.

PROTEIN DATASET AND ITS PREPARATION:

The crystal structure of protein dataset consisted of α-glucosidase and α-amylase enzymes were retrieved from Protein Data Bank (PDB) [15]. The α-glucosidase protein complexed with Acarbose (PDB ID: 2QMJ) and with Miglitol (3L4W) while the α-amylase protein structure complexed with Acarviostatin (3OLD) were considered as targets for analysis. The side chains of the protein structures were initially fixed using "Quick and Dirty" method implemented in Swiss-Pdb Viewer 4.0.1 [16] which browses the rotamer library and selects the best rotamer combinations. It was ensured that amino acids residing in the active site were unselected during side chain fixation because it can potentially distort the molecular interactions made with the co-crystallized ligand. Afterward, the fixed structures were energy minimized using GROMOS96 utility (*in vacuo*; without reaction field) of Swiss-Pdb Viewer 4.0.1 [16].

PHARMACOPHORE FEATURES DETECTION AND ALIGNMENT:

Spatial pharmacophore features for the ligand dataset was detected and the best feature based pairwise alignment was executed using PharmaGist webserver [17] with no assignment over pivot (reference) molecule. This procedure provided an overview of available features and its counts as well as gave suggestion over the alignment made as the Acarbose atomic structure was superior to the rest of the molecules. In other words, an oligosaccharide alignment with the monosaccharide-type molecules posed a problem of concealing the prominent features of monosaccharide-type molecules such as Miglitol, Voglibose and Luteolin. Hence, search for a common pharmacophore was performed using Ligand Scout 2.0 (trial version) [18]. Initially, feature-based scheme of pharmacophore alignment was attempted using PharmaGist which provided no significant outcome. Thus, reference-point based 3D pharmacophore alignment was considered to get a clear picture of the alignment in Ligand Scout 2.0. In order to extract pharmacophore feature for α-glucosidase inhibitors, Acarbose was set to reference molecule with the rest opted to undergo superimposition. Although, for α-amylase inhibitors, Miglitol was selected as reference molecule and Voglibose and Luteolin were superimposed with the exclusion of Acarbose from the alignment step for the reason that our objective was to develop a monosaccharide-type inhibitors.

ACTIVE SITE EXPLORATION:

The active site of α-glucosidase and α-amylase enzymes were studied using Ligand Explorer integrated in PDB. Ligand Explorer (or LigPro), a component of Molecular Biology Toolkit (MBT) extensively uses Java-based application programming interface to visualize and manipulate the protein-ligand interactions [19]. However, the active sites residues-ligand interactions were also cross-referenced with the crystallographic information in the literature.

MOLECULAR DOCKING:

Due to the non-availability of α-glucosidase structure complexed with Voglibose in the PDB, molecular

docking was carried out with 3L4W as protein target using Molegro Virtual Docker (trial version) [20] to study its interaction with α-glucosidase. Luteolin was also docked with α-glucosidase (3L4W) and α-amylase (3OLD) enzymes. Cavity prediction was initially performed using "Detect Cavities" module of Molegro with expanded Van der Waals radii to find accessible region, maximum number of cavities set to 10 with probe size of 1.20 Å, minimum and maximum cavity volume of 10 $Å^3$ and 10000 $Å^3$. This module utilizes simple grid-based cavity prediction dependent on molecular surface and/or Van der Waals radii to detect regions of accessibility. Protein dataset was then imported using the "Protein Preparation" module with the settings as follow: the bond orders and its hybridization assignment, explicit hydrogens inclusion, atomic charges assignment and flexible torsions of co-crystallized ligand(s) detection. "Prepare Molecules" object was applied with the same parameters settings described above when ligand dataset was introduced. Subsequently, "Docking Wizard" was utilized to guide the docking process. "MolDock Score" scoring function was selected with the depiction of grid box (radius = 15 Å) centered to co-crystallized occupied cavity. The search algorithm was constrained to "MolDock Optimizer" with the following settings: population size of 50, maximum number of iterations to 2000 and cross-over rate of 0.90. MolDock uses guided differential evolution algorithm in which all the individuals are initialized and evaluated using a fitness function. During this step, an offspring is established by adding weighted difference of the randomly chosen parent solutions from the population. If the offspring is fitter than parent, then the offspring passes to next generation unless the fitter parent participates in next generation. This search is halted by a termination scheme in which the variance of the population scores below a certain threshold (default = 0.01).

HYPOTHESIS DESIGN AND NEW MOLECULE GENERATION:

The count of spatial pharmacophore features was employed as the base of designing hypothesis with manual inspection drew from standard structure visualizers. Luteolin, the inhibitor of both α-glucosidase and α-amylase enzymes was selected as the reference structure in which the chemical fragments obeying the hypothesis was connected with information pertained from molecular superimposition. The newly generated molecules were then individually docked with the protein dataset (docking protocol described above) and analyzed the binding efficiency.

RESULT AND DISCUSSION:

The complete work flow of the strategy to develop monosaccharide-type inhibitors was graphically presented in Figure 1. Ligand dataset under study was subjected to Monte Carlo simulation based conformational analysis using Frog v1.01 and the best generated conformation were then geometrically optimized and energy minimized using AMBER force field engineered in HyperChem v8. Protein dataset was recovered from PDB and their side chains were fixed and energy minimized (GROMOS96 force field) using Swiss-Pdb Viewer 4.0.1. The energy minimized α-glucosidase (2QMJ: -52118.984 KJ/mol; 3L4W: -52784.027 KJ/mol) and α-amylase (3OLD: -31212.363 KJ/mol) structures were saved in Brookhaven PDB (.pdb) format for further analysis.

Molecule	Aromatic Rings	Hydrophobic Points	Hydrogen Bond Donors	Hydrogen Bond Acceptors	Negative Ionizable Groups	Positive Ionizable Groups	Total Spatial Features
Acarbose	0	2	14	18	0	1	35
Miglitol	0	0	5	5	0	1	11
Voglibose	0	0	8	7	0	1	16
Luteolin	3	1	4	5	0	0	13

Table 1: Distribution of spatial pharmacophore features in the ligand dataset.

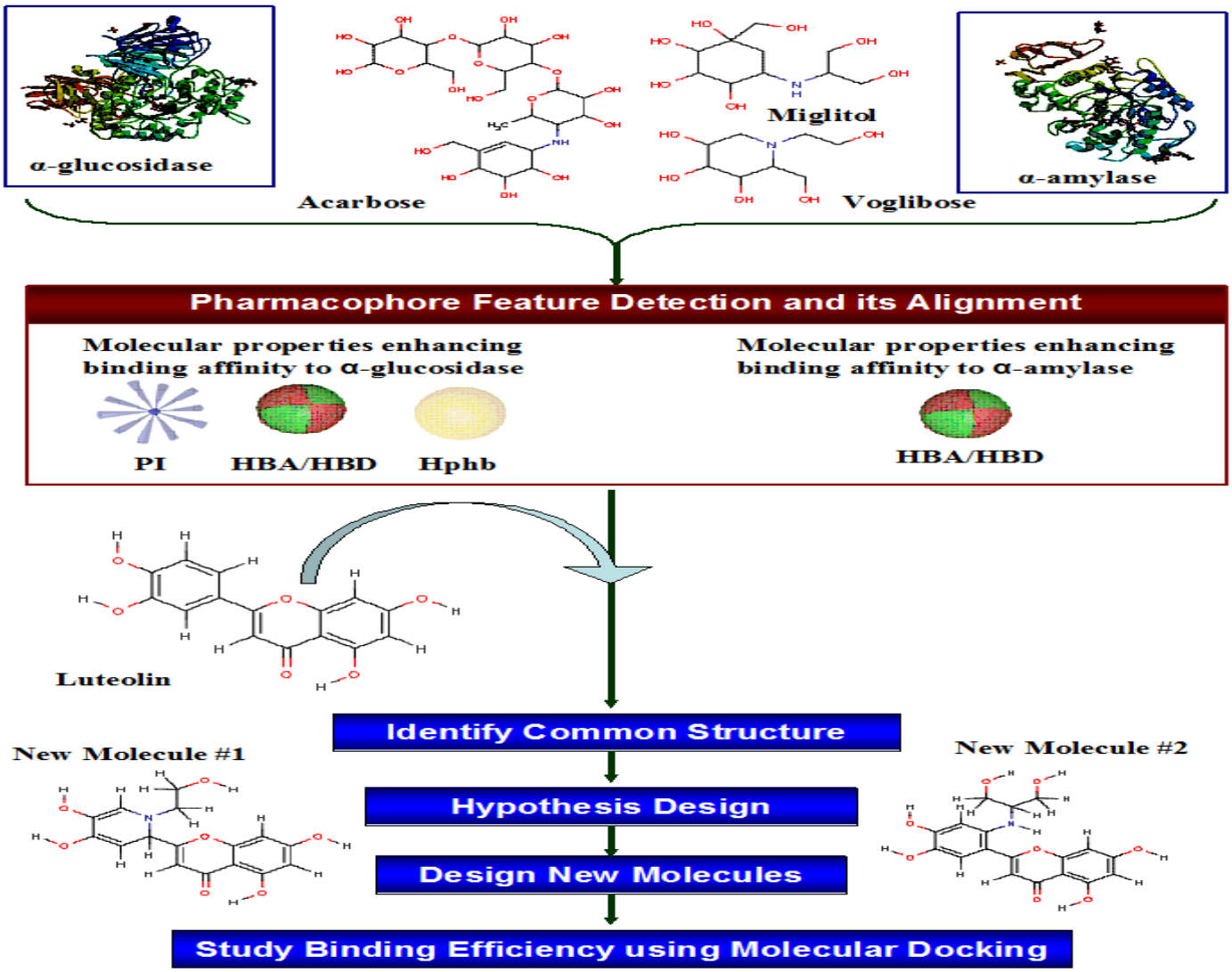

Figure 1: Workflow of the strategy to develop monosaccharide-type inhibitors

The numerical estimation of spatial pharmacophore features mapped over the ligand dataset was analyzed (Table 1) to generate a consensus of features overlaid in the inhibitors. Feature-based pharmacophore alignment yielded no significant alignment as the molecules were conformationally regulated. The fact that Acarbose is superior in its atomic structure compared to the rest of the molecules in the dataset is predicted to be the reason for this insignificant alignment. Superimposition of Acarbose with Miglitol, Voglibose and Luteolin showed that the root mean squared deviation (RMSD) values were 5.0617 Å, 5.3142 Å and 5.1903 Å while Miglitol, Voglibose and Luteolin alignment gave 1.7795 Å. This calculation was performed using "Superpose" utility of YASARA View [21]. It is predictable from RMSD values (>5 Å) that the incorporation of Acarbose in pharmacophore alignment yielded no significant information whereas exclusion gave value equal to 1.7795 Å. Hence, reference-point based 3D pharmacophore alignment was executed using Ligand Scout 2.0. The pharmacophore feature extraction of α-glucosidase inhibitors was carried out with Acarbose represented as reference molecule (Figure 2A). The count of hydrogen bond acceptor and donor (HBA & HBD) revealed that it is the greatest feature which plays a vital role in making H bonding with the α-glucosidase active site residues. Beside, hydrophobic point was observed both in Acarbose (count = 2) and Luteolin (count = 1) whereas positive ionizable group was located in all the molecules except Luteolin. It should also be noticed the count of positive ionizable group was equal to 1 in all the ligands (Table 1). The feature extraction of α-amylase inhibitors was achieved using the pharmacophore alignment of Miglitol, Voglibose and Luteolin (Figure 2B) with the intention of identifying the subtle differences of this alignment with Acarbose's own descriptors (excluded in the alignment process as we had focused on developing monosachharide-type inhibitors). The individual pharmacophore of Acarbose was compared with the

alignment produced and cross-checked with the crystallographic data published in literature which furnished more insights. Acarbose makes hydrogen bonding with active site waters (frequency = 5 contribution = 27.73 %) and with amino acids (frequency = 13 contribution = 72.22%) [22]. Another α-amylase inhibitor, Luteolin (although less potent than Acarbose in inhibition [11]) possessed only 4 and 5 as its HBD and HBA count interacted with α-amylase specifically. Thus, the frequency of HBD and HBA can be attributed to the hydrogen bonding ability with the amino acid residues along with crystallographic waters.

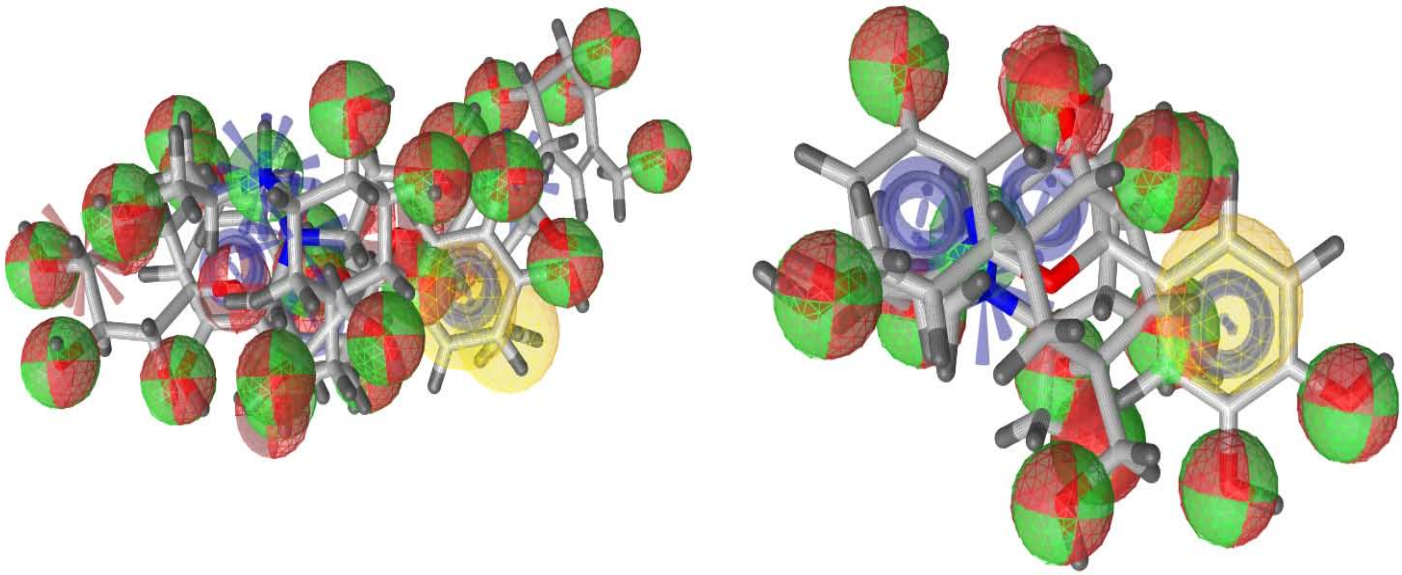

Figure 2: Overlaid pharmacophore features. A. α-glucosidase inhibitors and B. α-amylase inhibitors. Legends: Spheres in red: H bond acceptors, green: H bond donors and yellow: hydrophobic point; Blue color spikes: positive ionizable group; Blue color donut: aromatic ring.

The bibliographic information was merged with the computationally predicted ligand interaction with protein dataset (using Ligand Explorer). Structural analysis of the α-glucosidase-Acarbose complex showed that Acarbose makes extensive use of side-chains to interact with active sites and almost no interaction was observed with its glycone rings [23]. It was demonstrated that Asp443 plays a role of catalytic nucleophile by which Acarbose unable to make interaction and Miglitol succeeds in making contact as its ring nitrogen falls within the range of hydrogen bonding distance (2.8 Å) [24]. The protonation of nitrogen in the α-glucosidase active site makes the molecule to mimic the shape and/or charge of the presumed transition state for enzymatic glycoside hydrolysis [25]. Fortunately, the presence of nitrogen for α-amylase inhibition was found to be due to the participation in N-linked glycosidic bond which cannot be cleaved by α-amylase [22]. Studies indicated the role of nitrogen atom in Acarviosin moiety of Acarbose renders them to bind tighter than other α-amylase inhibitors (1-3 orders of magnitude) [26]. There are many subsites ranging from -4 to +3 in the active site of α-amylase. Crystallographic data confirmed that acarbose bounds to -3 to +2 subsites of α-amylase (Table 2) [23]. These critical findings led to the design of a hypothesis which suggests the essential and/or minimal requirement of molecular descriptors in order to be an efficient binder of these two hydrolytic enzymes. The minimum count of positive ionizable group should be 1 as it is required for protonation and for N-glycosidic linkage formation. Hydrophobic points if introduced, it should be near positive ionizable group due to the cause that hydrolysis step occurs in -1 and +1 subsites of α-amylase and if placed somewhere, it will potentially distort the hydrogen bonding ability of the molecule. The frequency of HBD/HBA in the molecular structure can be better correlated to the hydrogen bonding capability of the molecule and increases the opportunity of making interactions with water as we had studied the inability of Acarbose to interact with catalytic residue, Asp443 of α-glucosidase. Hence, the choice of HBD/HBA is dependent upon the atomic structure. To develop monosaccharide-type inhibitors, the HBD and HBA count (=5) of Miglitol was considered as the minimum requirement for a binder.

α-glucosidase active site interaction						
Active Site Residues	Acarbose	Miglitol	Voglibose	Luteolin	Molecule #1	Molecule #2
Asp203	+		+	+	+	+
Thr205	+					
Asn207	+					
Asp327	+	+	+	+	+	+
Trp406					+	+
Asp443		+	+		+	+
Arg526	+	+	+			
Asp542	+	+	+	+	+	+
His600	+	+	+	+	+	+
Water	+	+	+	+	+	+
Other Contacts					Trp539	Ser448
α-amylase active site interaction						
Active Site's Subsite	Active Site Residues		Acarbose	Luteolin	Molecule #1	Molecule #2
-3	Gln63, Thr163, Asp433, Water		+	+	+	+
-2	Trp59, His305, Water		+	+	+	+
-1	His101, Arg195, Asp197, His299, Asp300		+	+	+	+
+1	His201, Glu233 Water		+			+
+2	Lys200, Glu240 Water		+			
+3	Water					
Other Contacts						Gly306

Table 2: Active site interaction of ligand dataset with α-glucosidase and α-amylase

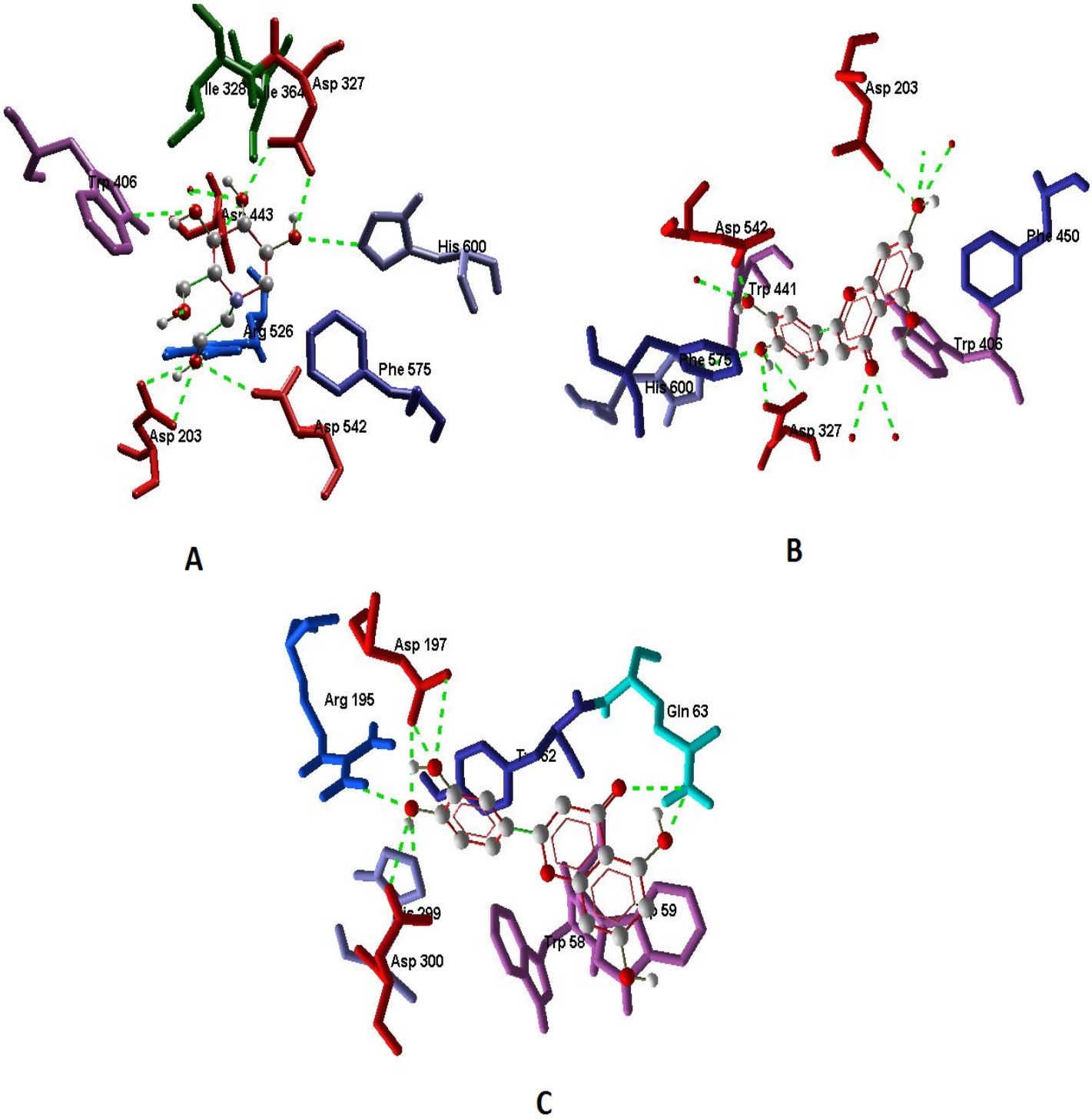

Figure 3: Docked conformations. (A) Voglibose docked on α-glucosidase (B) Luteolin docked on α-glucosidase and (C) Luteolin docked on α-amylase.

As the α-glucosidase-Voglibose complex was unavailable in the PDB, molecular docking studies were performed to identify its association with α-glucosidase. Voglibose made hydrogen bonding interactions with Asp203, Asp327, Asp443, Arg526, Asp542, His600 and crystallographic waters (Figure 3A). Docked conformations revealed that its interaction closely resembles Miglitol. Luteolin, the inhibitor of both enzymes were individually docked and found that it did not made hydrogen bonding through the catalytic nucleophile of α-glucosidase, Ap443 but had contacts over other catalytic residues (Table 2; Figure 3B). Furthermore, Luteolin successfully bound to -1 and +1 subsites residues of α-amylase procured for hydrolysis (Figure 3C). Thus, it is clear that Luteolin inability to bind tighter as accomplished by Acarbose is principally due to the unavailability of positive ionizable area where nitrogen resides. With Luteolin structural framework as template, positive ionizable groups of Miglitol and Voglibose were introduced which led to the generation of two molecules complying with the hypothesis (Figure 4A and B). On docking with the protein dataset, the binding efficiency in terms of MolDock score and interaction energy was evaluated. Finally, the co-crystallized ligands of the protein dataset, Acarbose and Miglitol were redocked with their proteins (2QMJ, 3L4W) while Acarbose was docked with α-amylase (3OLD) for comparison with docking scores and energy. The docking results of α-glucosidase demonstrated that MolDock Score of Acarbose (-157.853) was very close to Molecule #2 (-146.088) and the interaction energy when compared to Miglitol, Voglibose and Luteolin was found to be the lowest (-145.395 Kcal/mol) (Table 3, Figure 5A and B). α-amylase docking results suggested that Acarbose scored lowest when compared to Molecule #2 in terms of MolDock Score (-155.591) and interaction energy (-196.914 Kcal/mol) (Table 3, Figure 5C and D). The best scores of Acarbose are predominantly due to higher counts of HBA/HBD and the interaction made with crystallographic waters. We believe that the addition of HBA/HBD in Molecule #2 will promote interaction with water molecules. An important insight was that Molecule #2 made exclusive interaction with active site residues not with waters. Hence, it has the molecular descriptors required for specifically interacting with active site residues and can induce a change in pKa value of the enzyme-inhibitor complex. It should also be noticed that Molecule #2 bound more efficiently compared to its template structure, Luteolin. The clinical value of Molecule #2 needs to be evaluated *in vitro*. We expect that further structure optimization of generated molecules will enhance its geometrical and molecular descriptor and will emerge as an efficient binders. Interacting amino acids for generated molecules were shown for its ability to make multiple H bonding.

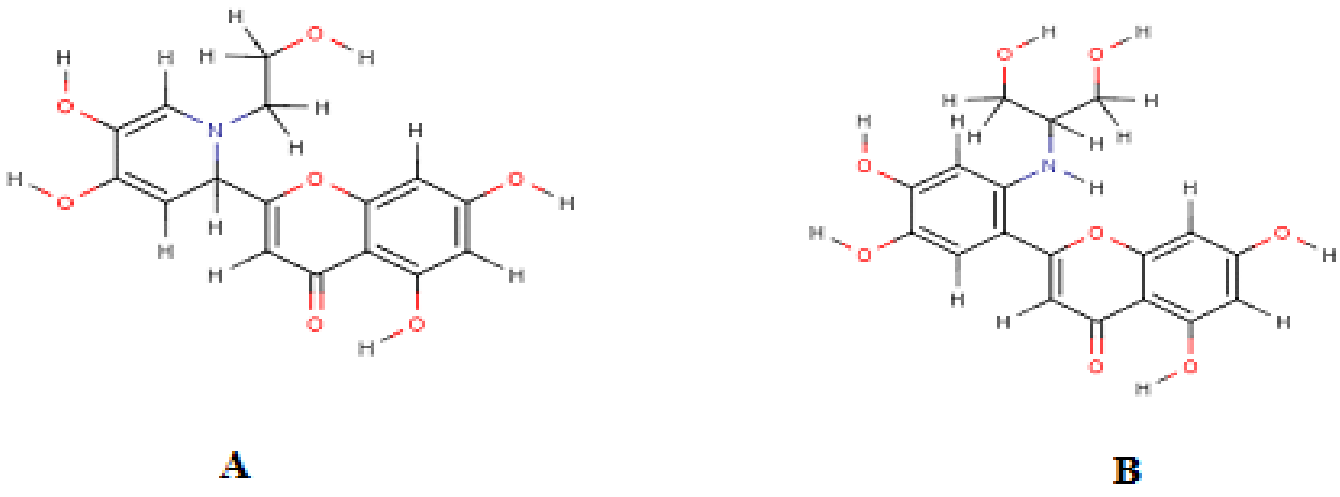

Figure 4: 2D structure of generated molecule (complied with hypothesis). (A) Molecule #1 and (B) Molecule #2.

Docking result of α-glucosidase		
Molecule	MolDock Score	Interaction Energy (Kcal/mol)
Acarbose	-157.853	-201.144
Miglitol	-94.612	-107.458
Voglibose	-84.899	-100.458
Luteolin	-102.126	-121.721
Molecule #1	-124.597	-139.49 Asp203(3), Asp327(2), Trp406, Asp443, Trp539, Asp542,His600, H_2O(5)
Molecule #2	-146.088	-145.395 Asp203, Asp327(2), Trp406, Asp443, Ser448, Asp542(2), His600, H_2O (7)
Docking result of α-amylase		
Acarbose	-155.591	-196.914
Luteolin	-110.309	-130.182
Molecule #1	-122.318	-128.442 Gln63, Arg195, Asp197(2) His299, Asp300(3), His305, H_2O (5)
Molecule #2	-139.116	-135.511 Trp59, Gln63, Thr163, Arg195(2), Asp197(2), Glu233(2), His299, Asp300, His305, Gly306

Table 3: Docking result of ligand dataset with α-glucosidase and α-amylase

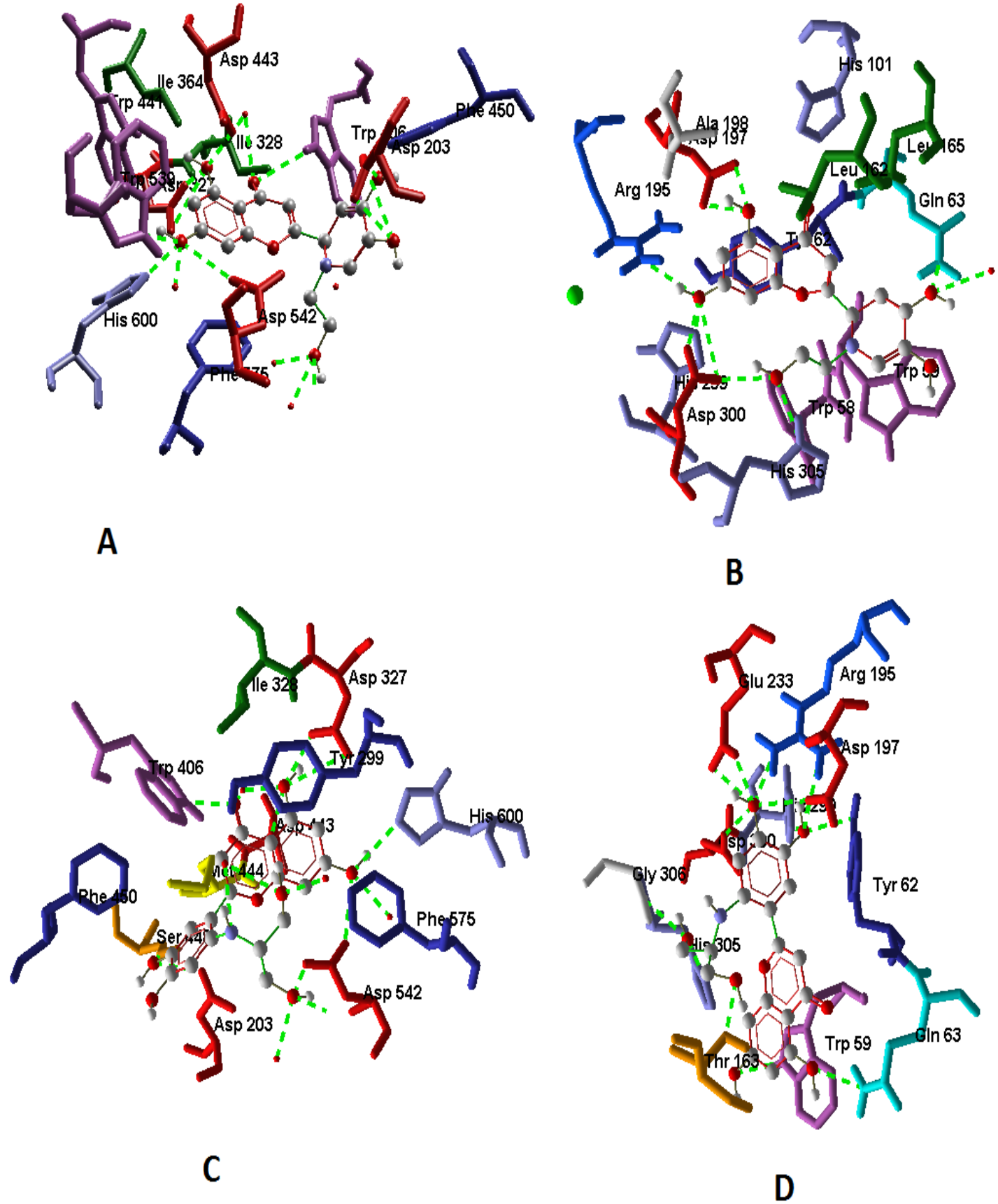

Figure 5: Docked Conformation of Molecule #1 and #2 with α-glucosidase (A and C) and Molecule #2 with α-amylase (B and D).

CONCLUSION

Considerable attention has been given for clinical management of PPHG due to its role in promoting risk over cardiovascular disease in people with impaired glucose tolerance and to achieve optimal glycemic control in type 2 diabetic patients. Here, we developed a strategy for the computational development of potent α-glucosidase and α-amylase inhibitors. With the input of knowledge from pharmacophore features, its alignments and docking studies, the molecular descriptors required for binding with both the hydrolytic enzymes were deciphered which helped us design a hypothesis to propose the essential and/or minimal requirement of molecular descriptors for an efficient binder. The positive ionizable group and the count of HBA and HBD along with the ring structure (either glycan or aromatic) projecting these features forms the backbone of an efficient inhibitor. Molecules complied with the hypothesis were computationally designed and confirmed their interaction with enzymes using docking procedure. We achieved better MolDock score and interaction energy compared to its parent structure, Luteolin. Plants extracts containing non-proteinaceous molecules with glycosidic or flavone architecture has shown *in vitro* inhibition. There arises a need of developing potent α-glucosidase and α-amylase inhibitors from plants to control PPHG.

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