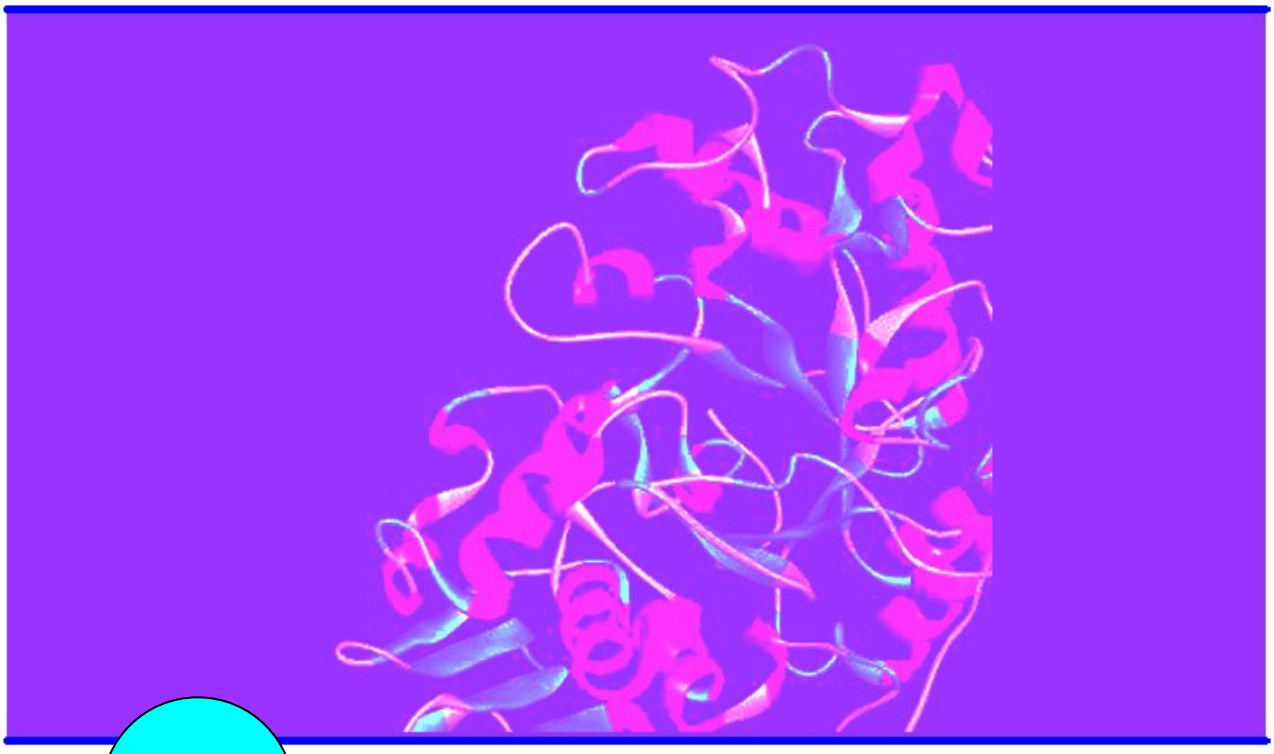

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Computational drug design of potential α-amylase inhibitors using some commercially available flavonoids

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Abstract

The primary objective of this study was to investigate the α-amylase inhibitory activity of flavonoids using *in silico* docking studies. In this perspective, flavonoids like biochanin, chrysin, hesperitin, morin, tricin and vitexycarpin were selected. Acarbose, a known α-amylase inhibitor was used as the standard. *In silico* docking studies were carried out using AutoDock 4.2, based on the Lamarckian genetic algorithm principle. The results showed that all the selected flavonoids showed binding energy ranging between -7.20 kcal/mol to -6.21 kcal/mol when compared with that of the standard (-2.94 kcal/mol). Inhibition constant (5.31 μM to 27.89 μM) and intermolecular energy (-8.99 kcal/mol to -7.41 kcal/mol) of the flavonoids also coincide with the binding energy. The α-amylase inhibitory activity of the selected flavonoids was in order of tricin > hesperitin > vitexycarpin > chrysin > morin > biochanin. These molecular docking analyses could lead to the further development of potent α-amylase inhibitors for the treatment of diabetes.

Introduction

Diabetes has become a leading killer disease in recent years. According to WHO, it is estimated that 3% of the world's population have diabetes and the prevalence is expected to double by the year 2025 to 6.3% (Li et al., 2011). Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin action, insulin secretion or both. Type 1 diabetes is caused by a deficiency of β-pancreatic cells insulin secretion. Type 2 diabetes is associated with obesity and is characterized by an initial phase progressive insulin resistance, with ensuing reduction in the ability of pancreatic hormone to promote peripheral glucose disposal and to decreases hepatic glucose output (Vianna et al., 2011; Lamba et al., 2011).

The α-amylases are a group of enzymes which shares many common characteristic properties. This class of enzymes has a variety of different specific sites for action on various glucose residues linked through α-1-1, α-1-4 and α-1-6 glycosidic bonds (Wolfenden et al., 1998). Amylases are mainly classified into two categories, endoamylases and exoamylases. Endoamylases produces linear and branched oligosaccharides of different chain lengths catalysing hydrolysis in a random manner in the interior of the starch molecule. Exoamylases produces short end products by acting from the non-reducing end (Gupta et al., 2003).

Alpha-amylase (α-1,4 glucan-4-glucanohydrolase) at first converts starch to oligosaccharides by hydrolyzing α-1,4-glucan bonds. Thus first reaction in digestion of carbohydrates is initiated by alpha amylase by forming oligosaccharides. Unabsorbed carbohydrates (disaccharides and oligosaccharides) will then get bound to alpha glucosidase enymes in the brush border of small intestine (Sarikaya et al., 2000).

In the field of molecular modeling, docking is a method

which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex (Sandeep et al., 2011). Currently, the use of computers to predict the binding of libraries of small molecules to known target structures is an increasingly important component in the drug discovery process (Koppen, 2009).

AutoDock 4.2 is the most recent version which has been widely used for virtual screening, due to its enhanced docking speed (Collignon et al., 2011; Prakhov et al., 2010). Its default search function is based on Lamarckian Genetic Algorithm (LGA), a hybrid genetic algorithm with local optimization that uses a parameterized free-energy scoring function to estimate the binding energy. Docking of small molecules in the receptor binding site and estimation of binding affinity of the complex is a vital part of structure based drug design (Cosconati et al., 2010; Seeliger and Groot, 2010).

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruit, vegetables, stems, flowers, tea, and wine. These natural products were known for their beneficial effects on health long before flavonoids were isolated as the effective compounds. Research on flavonoids received an added impulse with the discovery of the French paradox, the low cardiovascular mortality rate observed in Mediterranean populations in association with red wine consumption and a high saturated fat intake. The flavonoids in red wine are responsible, at least in part, for this effect (Groot and Rauen, 1998).

Flavonoids and their related compounds are low molecular weight substances, which are a group of natural products which exhibits various biological and pharmacological activities like antibacterial, antiviral, antioxidant, antiinflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral and antimutagenic effects and inhibition of several enzymes (Madeswaran et al., 2012; Formica and Regelson, 1995).

However there is no conclusive report as to whether the α-amylase activity of the flavonoids. The stereochemistry of binding of the flavonoids on α-amylase has not yet been characterized. In the present study, the structural models of the ligands in the α-amylase binding sites has been carried out, which may facilitate further development of more potent α-amylase inhibitory agents.

Materials and Methods

Software required

Python 2.7- language was downloaded from www.python.com, Cygwin (a data storage) c:\ program and Python 2.5 were simultaneously downloaded from www.cygwin.com, Molecular graphics laboratory (MGL) tools and AutoDock4.2 was downloaded from www.scripps.edu, Discovery studio visualize 2.5.5 was downloaded from www.accelerys. com, Molecular orbital package (MOPAC), ChemSketch was downloaded from www.acdlabs.com. Online smiles translation was carried out using cactus.nci.nih. gov/translate/.

Docking methodology

Lamarckian genetic algorithm (LGA) is employed for the ligand conformational searching, which is a hybrid of a genetic algorithm and a local search algorithm. This algorithm first builds a population of individuals (genes), each being a different random conformation of the docked molecule. Each individual is then mutated to acquire a slightly different translation and rotation and the local search algorithm then performs energy minimizations on a userspecified proportion of the population of individuals. The individuals with the low resulting energy are transferred to the next generation and the process is then repeated. The algorithm is called Lamarckian because every new generation of individuals is allowed to inherit the local search adaptations of their parents (Madeswaran et al., 2012).

An extended PDB format, termed as PDBQT file was used for coordinate files which includes atomic partial charges. AutoDock Tools was used for creating PDBQT files from traditional PDB files (Khairallah et al., 2008). Crystal structure of α-amylase enzyme was downloaded from the Brookhaeven protein data bank (Figure 1).

In Figure 2, the flavonoid ligands like biochanin,

Figure 1: α-amylase enzyme from Brookhaeven protein data bank (1HNY)

chrysin, hesperitin, morin, tricin, vitexycarpin, and acarbose were built using ChemSketch and optimized using "Prepare Ligands" in the AutoDock 4.2 for docking studies (Bikadi and Hazai, 2009).

Lead optimization of the selected compounds was done by computation of drug-likeness properties. The drug-likeness scores of the compounds were evaluated with the help of Lipinski's rule. The preparation of the target protein 1HNY (unbound target) with the AutoDock Tools software involved adding all hydrogen atoms to the macromolecule, which is a step necessary for correct calculation of partial atomic charges. Gasteiger charges are calculated for each atom of the macromolecule in AutoDock 4.2 instead of Kollman charges which were used in the previous versions of this program. Three-dimensional affinity grids of size 277 × 277 × 277 A with 0.6 A spacing were centered on the geometric center of the target protein and were calculated for each of the following atom types: HD, C, A, N, OA, and SA, representing all possible atom types in a protein. Additionally, an electrostatic map and a desolvation map were also calculated (Konc et al., 2011).

Rapid energy evaluation was achieved by precalculating atomic affinity potentials for each atom in the ligand molecule. In the AutoGrid procedure, the target enzyme was embedded on a three dimensional grid point (Madeswaran et al., 2013). The energy of interaction of each atom in the ligand was encountered.

Important docking parameters for the LGA as follows: population size of 150 individuals, 2.5 million energy evaluations, maximum of 27,000 generations, number of top individuals to automatically survive to next generation of 1, mutation rate of 0.02, crossover rate of 0.8, 10 docking runs, and random initial positions and conformations. The probability of performing local search on an individual in the population was set to 0.06. AutoDock was run several times to get various docked conformations, and used to analyze the predicted docking energy. The binding sites for these molecules were selected based on the ligand-binding pocket of the templates. AutoDock Tools provide various methods to analyze the results of docking simulations such as, conformational similarity, visualizing the binding site and its energy and other parameters like intermolecular energy and inhibition constant. For each ligand, ten best poses were generated and scored using AutoDock 4.2

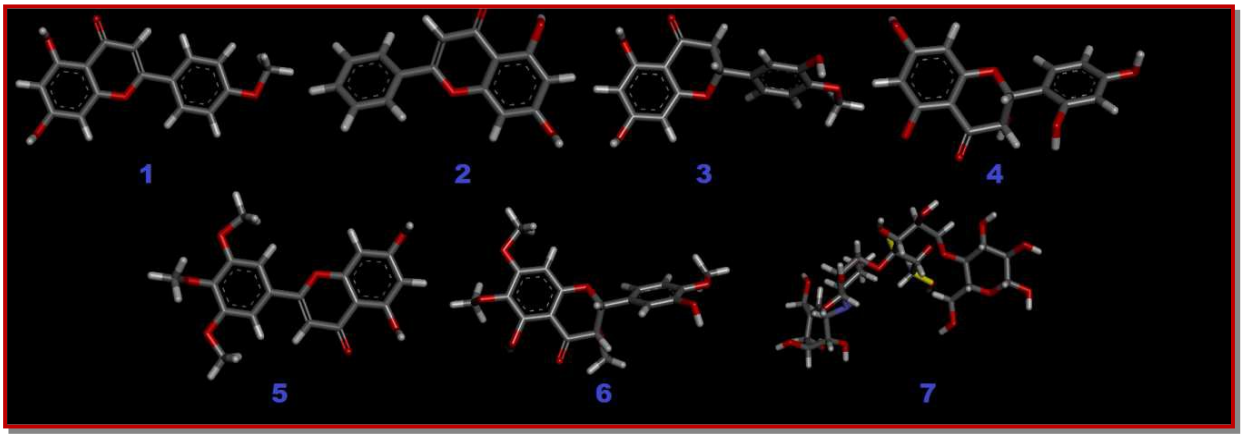

Figure 2: The optimized ligand molecules (**1** biochanin, **2** chrysin, **3** hesperitin, **4** morin, **5** tricin, **6** vitexycarpin, and **7** acarbose)

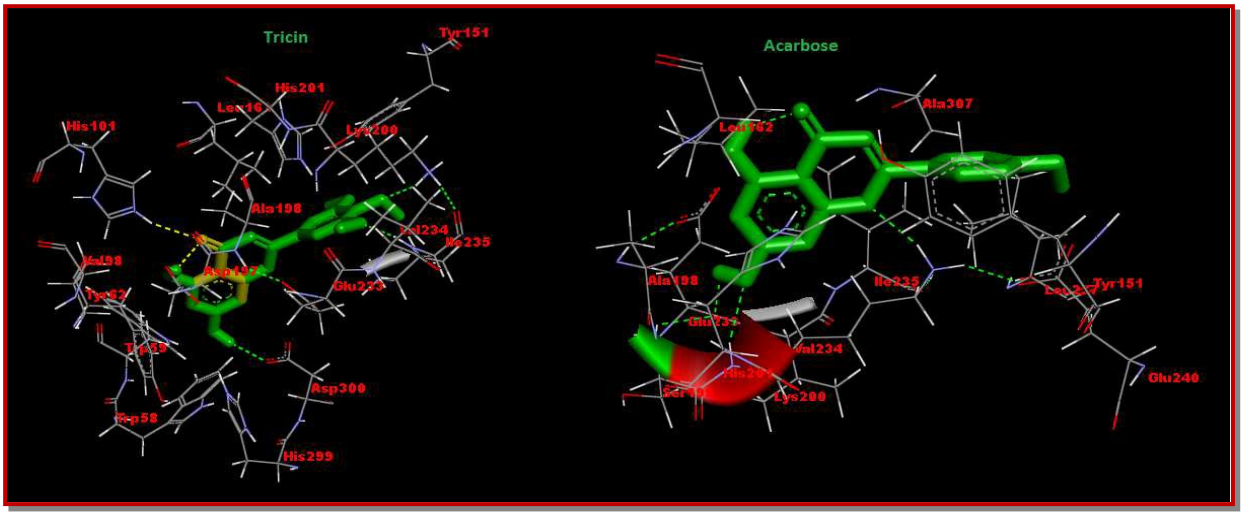

Figure 3: Docked pose of α-amylase enzyme (1HNY) with tricin and acarbose

scoring functions (Madeswaran et al., 2011).

Results and Discussion

In silico docking study, was carried out to identify the inhibiting potential of selected flavonoids against α-amylase enzyme. The docking studies were performed by the use of AutoDock4.2. In the docking studies, if a compound shows lesser binding energy compared to the standard it proves that the compound has higher activity (Chang et al., 2010).

Table I

Docking parameters of the selected flavonoids

Com-pound	Binding energy (kcal/mol)	Inhibition constant (μM, mM[a])	Intermolecu-ladr energy (kcal/mol)
Biochanin	-6.2	27.9	-7.4
Chrysin	-6.4	21.9	-7.3
Hesperitin	-7.2	5.4	-8.7
Morin	-6.4	22	-8.1
Tricin	-7.2	5.3	-9
Vitexycar-pin	-6.6	15.6	-8.7
Acarbose	-2.9	7[a]	-9.5

Analysis of the receptor/ligand complex models generated after successful docking of the flavonoids was based on the parameters such as hydrogen bond interactions, π – π interactions, binding energy, RMSD of active site residues and orientation of the docked compound within the active site (Madeswaran et al., 2012).

The docking poses were ranked according to their docking scores and both the ranked list of docked ligands and their corresponding binding poses. This ranking of the compounds were based on their binding energy with the enzyme. If the binding energy of the compound is less, then the particular compound has more active in nature. In Figure 3, docked pose of α-amylase enzyme with the ligands tricin and acarbose clearly demonstrated the binding positions of the ligand with the enzyme.

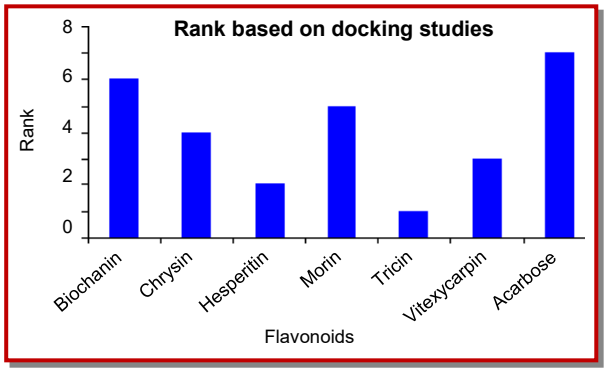

Figure 4: α-amylase inhibitory activity of flavonoids based on docking studies

The binding sites of the acarbose was found to be Tyr151, Leu162, Ala198, Ser199, Lys200, His201, Glu233, Val234, Ile235, Leu237, Glu240, Ala307. The potential binding sites of the tricin was found that, Trp58, Trp59, Tyr62, Val98, His101, Tyr151, Leu162, Asp197, Ala198, Lys200, His201, Glu233, Val234, Ile235, His299, Asp300. This proves that the effective binding sites are present in the selected flavonoid tricin when compared with the standard acarbose.

Binding energy of the individual compounds were calculated using the following formula,

Binding energy = A + B + C - D

Where, A denotes final intermolecular energy + van der Walls energy (vdW) + hydrogen bonds + desolvation energy + electrostatic energy (kcal/mol), B denotes final total internal energy (kcal/mol), C denotes torsional free energy (kcal/mol), D denotes unbound system's energy (kcal/mol)

Based on the docking studies, the α-amylase inhibitory activity of the selected compounds was found to be decreased in the order of tricin, hesperitin, vitexycarpin, chrysin, morin and biochanin (Table I). Further investigations on the above compounds and *in vivo* studies are necessary to develop potential chemical entities for the prevention and treatment of diabetes. On the basis of the above study, tricin and hesperitin possess potential α-amylase inhibitory binding sites similar to that of the standard (Figure 4). This may be attributed due to the differences in the position of the functional groups in the compounds.

Conclusion

These results clearly indicate that from the selected flavonoids, tricin and hesperitin have better binding sites and interactions with α-amylase enzyme.

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Conflict of Interest

Authors declare no conflict of interest

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