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In silico docking evaluation of α-Amylase inhibitory activity of Butein and Tricetin

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ABSTRACT

The current objective of the study is to evaluate the α-amylase inhibitory activity of butein and tricetin using in silico docking studies. In this perspective, butein and tricetin ligands were prepared for the docking evaluation. Acarbose, a known α-amylase inhibitor was used as the standard. In silico docking studies were carried out using recent version of AutoDock 4.2, which has the basic principle of Lamarckian genetic algorithm. Three important docking evaluation parameters such as binding energy, inhibition constant and intermolecular energy were determined for the selected ligands. These results showed that all the selected flavonoids showed binding energy ranging between -6.73 kcal/mol to -6.63 kcal/mol when compared with that of the standard (-2.94 kcal/mol). Intermolecular energy (-8.52 kcal/mol to -8.72 kcal/mol) and inhibition constant (11.66 µM to 13.86 µM) of the ligands also coincide with the binding energy. Butein and tricetin contributed excellent α-amylase inhibitory activity because of its structural parameters. These molecular docking analyses of butein and tricetin could lead to the further development to identify the potent α-amylase inhibitors for the treatment of diabetes.

Key words: Binding energy, butein, Inhibition constant, Intermolecular energy, tricetin

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INTRODUCTION

Drug design is an central tool in the field of medicinal chemistry where new compounds are synthesized by chemical or molecular manipulation of the lead moiety in order to create highly active compounds with minimum steric effect [1]. There is a broad range of software packages available for the carry out the molecular docking simulations like, AutoDock, GOLD, FlexX etc. [2] AutoDock 4.2 is the most recent version which has been broadly used for virtual screening, due to its better docking speed [3]. 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 calculate the binding energy [4].

Diabetes has become a most important killer disease in current years. According to WHO, it is estimated that 3% of the world's population have diabetes and the occurrence is expected to double by the year 2025 to 6.3% [5]. Diabetes mellitus is a metabolic illness characterized by hyperglycemia resulting from fault in insulin action, insulin secretion or both. Type 1 diabetes is occurred by a deficiency of *β*-pancreatic cells insulin secretion. Type 2 diabetes is connected with obesity and is characterized by an early phase progressive insulin resistance, with result in the reduction of pancreatic hormone to promote peripheral glucose disposal and to falls in hepatic glucose output [6, 7].

Alpha-amylase (α-1,4 glucan-4- glucanohydrolase) initially change the starch into oligosaccharides by hydrolysing α-1,4-glucan bonds. Thus initial reaction in digestion of carbohydrates is begin by alpha amylase by developing oligosaccharides [8]. The α-amylases are a cluster of enzymes which divides many common characteristic properties. This class of enzymes has different specific sites for action on different glucose residues related through α-1-1, α-1-4 and α-1-6 glycosidic bonds [9].

Flavonoids belong to a set of natural substances with different benzopyran structures and are originates in flowers, fruit, vegetables, stems, tea, and wine. These natural products were recognized for their useful effects on health, long before flavonoids were isolated as the valuable compounds. Research on flavonoids established an added impulse with the discovery of the French paradox, the low cardiovascular mortality rate monitored in Mediterranean populations in association with red wine consumption and a high saturated fat ingestion. The flavonoids in red wine are accountable, at least in part, for this effect [10]. Flavonoidsexhibits various biological and pharmacological activities like anti-allergic, anti-bacterial, anti-mutagenic, anti-inflammatory, anti-oxidant, hepatoprotective, anti-thrombotic, and anti-viral effects and inhibition of several enzymes [11, 12].

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

MATERIALS AND METHODS

Softwares required

Python 2.7 - language was downloaded from www.python.com [13], Cygwin was downloaded from www.cygwin.com [14], Molecular graphics laboratory (MGL) tools and AutoDock4.2 was downloaded from www.scripps.edu [15], ChemSketch was downloaded from www.acdlabs.com [16], Discovery studio visualizer 2.5.5 was downloaded from www.accelrys.com [17]. Online smiles translation was carried out using cactus.nci.nih.gov/translate/ [18].

Docking Evaluation Methodology:

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 [19]. Crystal structure of α-amylase enzyme (target protein) was downloaded from the RCSB protein data bank (Fig. 1).

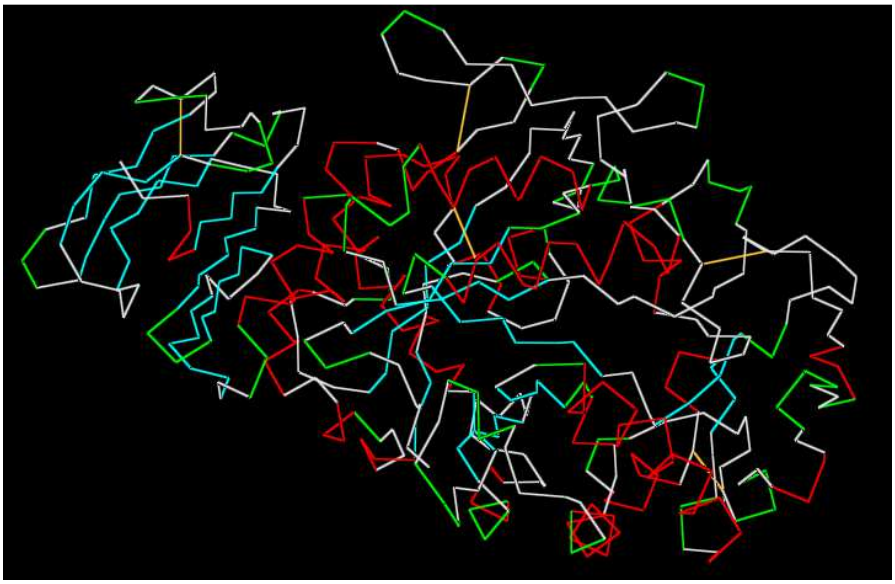

Fig. 1 α-amylase enzyme from RCSB protein data bank (1HNY)

The preparation of the target protein 1HNY 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 Å with 0.6 Å 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, a desolvation map and an electrostatic map were also calculated [20]. The sequence of the α-amylase enzyme was derived from the Accelrys photo studio viewer (Fig. 2). It represents the active sites or the binding mode of the α-amylase enzyme.

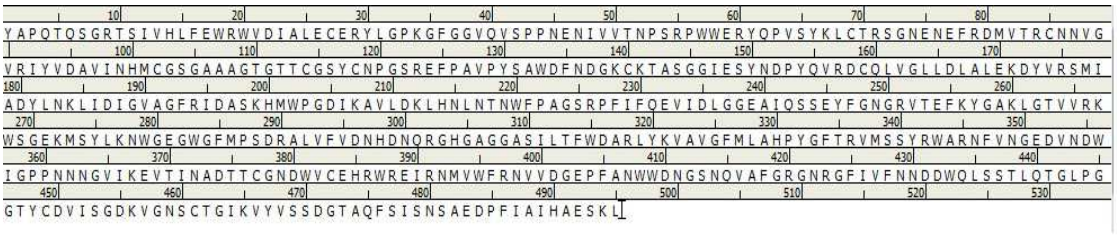

Fig. 2 Sequence of the α-amylase enzyme from RCSB protein data bank

The ligands such as butein, tricetin and the standard acarbose were built using ChemSketch and optimized using "Prepare Ligands" in the AutoDock 4.2 for docking studies (Fig. 3). The optimized ligand molecules were docked into refined α-amylase model using "LigandFit" in the AutoDock 4.2 [21]. 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 [22]. The energy of interaction of each atom in the ligand was encountered.

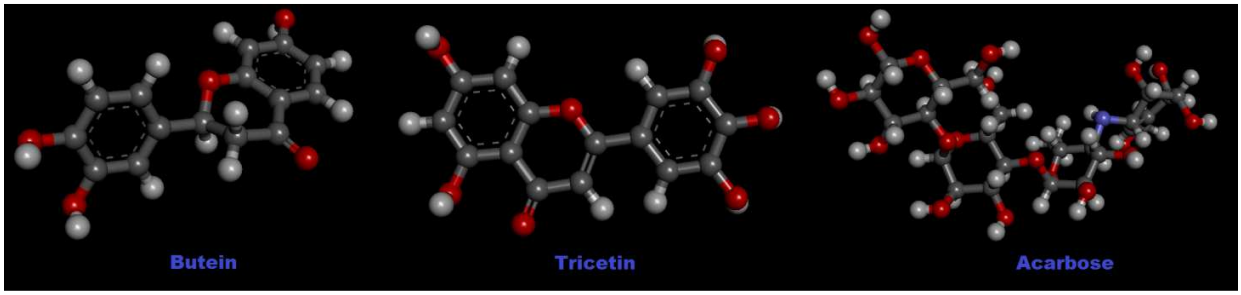

Fig. 3 The optimized ligand molecules

The following important docking parameters were selected for the LGA as follows: population size of 150 individuals, 2.5 million energy evaluations, maximum of 27000 generations, and 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 different docked conformations, and used to evaluate the predicted binding energy [23].

RESULTS AND DISCUSSION

Docking analysis

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 [24,25].

The Ramachandran plot and Hydrophobicity plot were drawn for the α-amylase enzyme using Accelrys photo studio viewer (Fig. 4). It provides the information about the conformational similarity, structural similarity, visualizing the

binding site and the nature of hydrophobicity. The nature of the target enzyme was analyzed using the plot and it showed the higher affinity towards its active site. Therefore, the predicted structural similarity it resembles with the actual structure of the α-amylase.

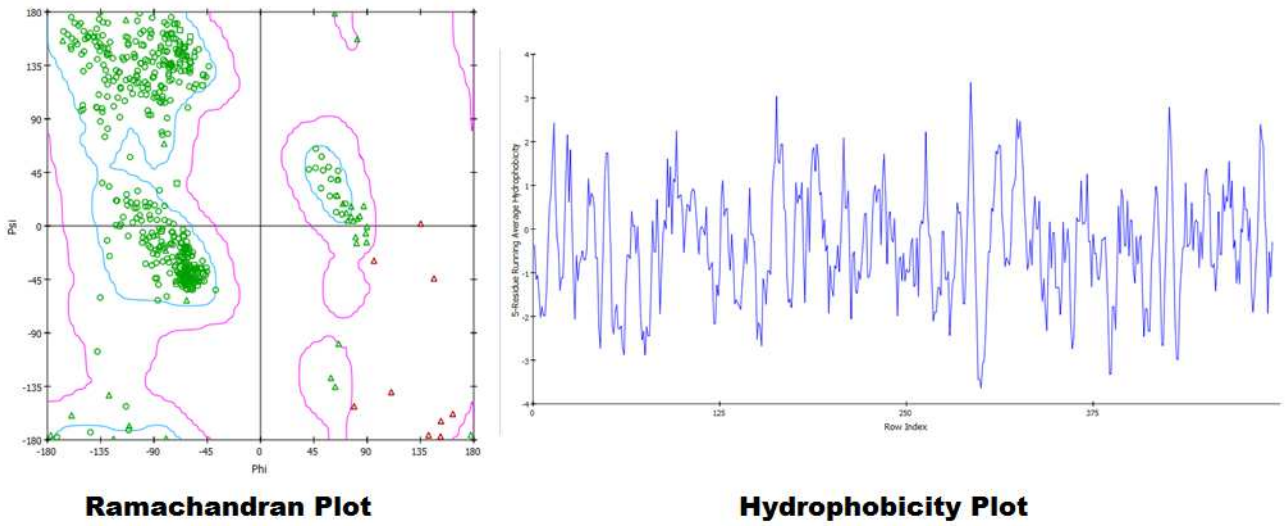

Fig. 4 Ramachandran and Hydrophobicity Plot of α-amylase enzyme

The docking poses were ranked according to their docking scores and both the ranked list of docked ligands and their corresponding binding poses [26]. The binding sites of the acarbose was found to be Trp 58, Trp 59, Tyr 62, His 101, Leu 162, Arg 195, Asp 197, Ala 198, Ser 199, Lys 200, His 201, Glu 233, Asp 300 [27]. In Fig. 5, docked pose of α-amylase enzyme with the ligands butein and tricetin clearly demonstrated the binding positions of the ligand with the enzyme.

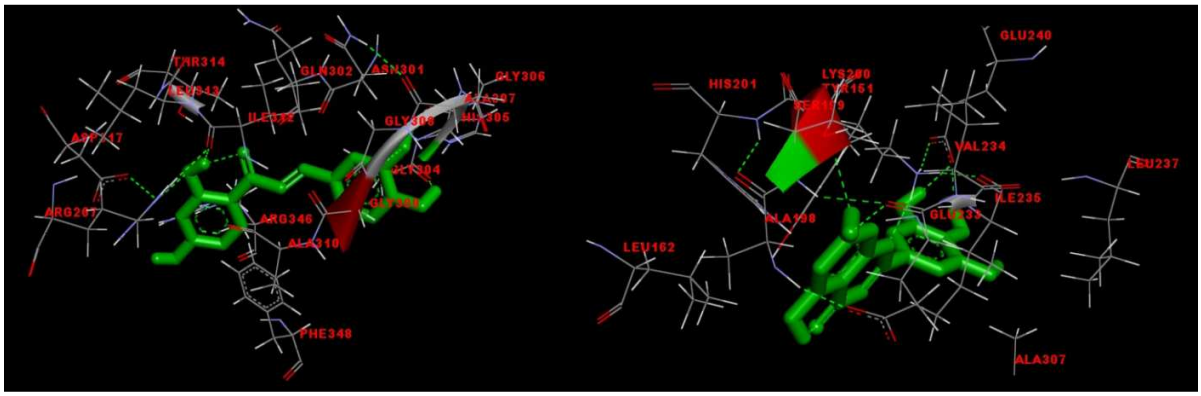

Fig. 5 Docked pose of α-amylase enzyme with butein and tricetin

The potential binding sites of the butein was found that, Leu 162, Arg 195, Asp 197, Ala 198, Lys 200, His 201, Glu 233, Arg 267, Asp 300, Gln 302, His 305, Gly 304, Gly 306, Gly 308, Gly 308, Gly 309, Ala 310, Ile 312, Leu 313,Thr 314,Asp 317, Arg 346, Phe 348. The potential binding sites of the tricetin was found that, Tyr 151, Leu 162, Ala 198, Ser 199, Lys 200, His 201, Glu 233, Ile 235, Val 234, Leu 237, Glu 240, Ala 307. This proves that the effective binding sites are present in the selected flavonoids butein and tricetin when compared with the standard.

As shown in table 1, flavonoids showed binding energy ranging between -6.63 kcal/mol to -6.73 kcal/mol. All the selected flavonoids had showed binding energy compared to that of standard acarbose (-2.94 kcal/mol). This proves that flavonoids consist of potential α-amylase inhibitory binding sites similar to that of the standard.

Table 1. Binding energies of the compounds based on their rank

COMPOUNDS	Binding energies of the compounds based on their rank (kcal/mol)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Butein	-6.63	-6.43	-5.64	-6.29	-5.2	-5.43	-5.42	-5.19	-5.16	-4.91
Tricetin	-6.73	-5.34	-6.73	-6.6	-5.86	-5.82	-5.76	-5.75	-5.74	-5.71
Acarbose	-2.94	-2.92	-2.79	-2.82	-2.38	-2.22	-1.54	-1.57	-1.41	-1.28

In addition, two other parameters like inhibition constant (K_i) and intermolecular energy were also determined. As shown in table 2, butein showed inhibition constant ranging from 13.86 µM to 250.29 µM and tricetin showed 11.66 µM to 65.09 µM. Both the compounds had lesser inhibition constant when compared to the standard (6.98 mM). Inhibition constant is directly proportional to binding energy. Thus, the α-amylase inhibitory activity of the butein and tricetin were proved.

Table 2. Inhibition Constant of the compounds based on their rank

COMPOUNDS	Inhibition Constant of the compounds based on their rank (µM, mM*)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Butein	13.86	19.45	73.06	24.58	153.26	105.29	106.44	156.14	166.13	250.29
Tricetin	11.66	121.13	11.69	14.65	50.74	54.25	60.34	60.67	61.53	65.09
Acarbose	6.98*	8.76*	12.22*	15.32*	22.14*	36.92*	47.32*	69.45*	82.66*	135.98*

As shown in table 3, butein showed intermolecular energy ranging from -8.72 kcal/mol to -7.00 kcal/mol and tricetin showed intermolecular energy ranging from -8.52 kcal/mol to -7.50 kcal/mol which was lesser when compared to the standard (-9.50 kcal/mol). This result further proved the α-amylase inhibitory activity of all the selected flavonoids.

Table 3. Intermolecular energies of the compounds based on their rank

COMPOUNDS	Inter molecular energies of the compounds based on their rank (kcal/mol)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Butein	-8.72	-8.52	-7.73	-8.38	-7.29	-7.51	-7.51	-7.28	-7.24	-7
Tricetin	-8.52	-7.13	-8.52	-8.38	-7.65	-7.61	-7.55	-7.54	-7.53	-7.5
Acarbose	-9.50	-9.43	-9.34	-9.32	-9.28	-9.22	-9.20	-9.16	-9.12	-9.11

Based on the docking studies, the α-amylase inhibitory activity of the selected compounds was found to be decreased in the order of tricetin, butein and acarbose. On the basis of the above study, butein and tricetin possess potential α-amylase inhibitory binding sites similar to that of the standard. This may be attributed due to the differences in the position of the functional groups in the compounds.

CONCLUSION

In conclusion, the results of the present study clearly demonstrated the *in silico* molecular docking studies of acarbose and selected flavonoids with α-amylase enzyme exhibited binding interactions and warrants further studies needed for the development of potent α-amylase inhibitors for the treatment of inflammation. These results clearly indicate that, butein and tricetin have similar binding sites and interactions with α-amylase compared to the standard. This *in silico* studies is actually an added advantage to screen the α-amylase inhibition. Flavonoids may serve as useful leads in the development of clinically useful α-amylase inhibitors. Further investigations on the above compounds and *in vivo* studies are necessary to develop potential chemical entities for the prevention and treatment of diabetes.

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