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Original Article

DESIGN, SYNTHESIS, INSILICO MOLECULAR DOCKING STUDIES OF SOME NEW ANTI-DIABETIC AMINO ACID ESTERS AS POTENTIAL TARGETS FOR α– AMYLASE

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ABSTRACT

Objective: A series of amino acids including Glycine, Tryptophan, Arginine and Cysteine was reacted with alcohols like methanol, propanol, phenol, n-butanol was transformed to corresponding amino acid methyl ester hydrochlorides. **Methods:** Molegro Virtual Docker (MVD) was used, the 10 analogues were docked in the Acarbose bound active site of 3M07 using Acarbose and further compared between *in silico* and *in vitro* studies. **Results:** In this study, the results showed that T1, T2 proved to have better H-bonding in α - Amylase (3M07) which can be seen from Moldock score -108.711,-107.401 than Acarbose Moldock score was -93.1984 and it means that the energy was lower with more stable binding. Moldock Score of T1, T2, A1, A2, A3, C2, C1, G1, G3, and G2 were -108.711,-107.401,-92.685,-88.9983,-84.0515,-82.9936,-75.9834,-74.8734,-72.793 and -60.4139 respectively. The hydrogen bonds of T1, T2, and Acarbose with an amino acid of α - Amylase (3M07) were His, Tyr, Thr and Asp. All the compounds were studied for the in-vitro anti-diabetic activity by the alpha-amylase inhibition method. **Conclusion:** The compounds showed mild to moderate anti-diabetic activity. T1, T2 were found to be most active among the series of compounds in comparison with Acarbose. Therefore this class of compound could be a good starting point to develop new lead compounds in the treatment of anti-diabetic activity.

Keywords: Molecular docking, diabetes, lead compound, analogous design.

INTRODUCTION

Diabetes is a multifactorial disorder of the pancreas, in which the pancreas fails to perform its function to produce insulin hormone properly in the body. It involves multiple disorders like hyperglycemia, glycosuria, and abnormal metabolism of lipids, carbohydrates and proteins [1, 2]. There by affecting the human body at physiological, physical and social level. It has been known as a 3rd leading cause of death in humans along with other diseases such as cancer, cerebrovascular and heart diseases [3] of the major two types of diabetes: Type 1 also called as Insulin dependent diabetes mellitus, its cause is hereditary by nature and treated with insulin injections externally. The basis of Type 1 diabetes mellitus is the immunological destruction of pancreatic cells leading to deficiency of insulin in the body [4] and Type 2, “Adult type” known as Non-insulin dependent diabetes that mostly common in aged people. It is treated by diet control and oral hypoglycemic medicine. Hypoglycemic medication helps to lower the blood sugar level in the body or treat the other severe symptoms and complications of diabetes mellitus [2]. The basic mechanism of antidiabetic medications is stimulating insulin production from the pancreas or increasing the sensitivity of the body cells to insulin and is commonly used along with insulin. Different classes of anti-diabetic drugs available in the market that includes insulin secretagogue known as sulfonylureas and meglitinides. Insulin sensitizers are biguanides, thiazolidinediones and metformin, and important inhibitors are α-glucosidase inhibitors include acarbose and miglitol etc. The side-effects of these medications include extreme hypoglycemia, liver cell injury, lactic acidosis, digestive discomfort, permanent neurological deficit, headache, dizziness and even death [5, 6]. The basic challenge in curing diabetes is to maintain blood glucose level close to normal levels [7].These therapies are used as monotherapy or in combination for optimal control of glycemia [8]. As mentioned before that these drugs are normally expensive and come with side effects. These drugs have their limitations such as their pharmacokinetic properties, secondary failure rates and relative bad effects [9, 10]. Thus, the need for a new efficient class of compounds to reduce the side-effects Search for alternative drugs which will be most effective for diabetes is still at an on-going phase [11]. Mother Nature may prove to be a useful source of new oral hypoglycemic compounds for the progress of pharmaceutical entities or as a dietary adjunct to prevailing therapies which has fewer side effects [12-14]. With the rapid increase in biological and chemical information, CADD has been dramatically reshaping research and development pathways in drug candidate identification. Use of computational techniques in drug discovery and development process is widely appreciated in terms of implementation, time and money [15].Molecular docking is a competent tool for novel micro molecule drugs discovery for targeting protein [16]. Molecular Docking of protein structures involves various possibilities of association are tried and verified on the basis of energy value, and the conformation with the least energy value is titled “best match” i.e. having the best interaction of the protein with a ligand. Docking strategy plays a significant role in modern drug discovery. Kuntz et al contributed immensely in docking research to improve the computational speed and accuracy. One of the areas in molecular docking is protein-ligand docking, which is gaining fame due to its role in structure-based drug design [17-23]. Molecular docking is basically a computational method that predicts non-covalent association of macromolecules with a receptor and a small molecule (ligand) efficiently. The method starts unbound structures, structures acquired from MD simulations, or homology modeling, etc. The prediction of binding of small molecules to proteins has a huge impact on the prediction results are used to filter virtual libraries of drug-like molecules to identify leads for further drug development. Docking can even be used to calculate the bound conformation of known binders, in case the experimental holo structures are not available [24].

MATERIALS AND METHODS

Chemicals

Tryptophan (Lobachemie PVT.LTD.107), Arginine (Lobachemie PVT.LTD.107), Cystein (Otto chem.LTD), Glycine (SD fine-chem.LTD), Concentrated H_2SO_4, N-butanol , water, acetic acid, Trimethylchlorosilane (TMSCl) Standard Drug used for activity comparsion: Acarbose.

Experimental Methodology

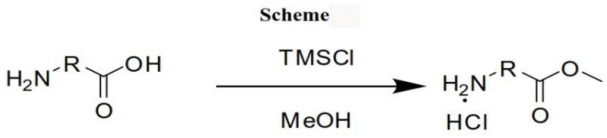
General procedure for Synthesis

The synthesis of acid methyl ester hydrochlorides is shown in Scheme [25].A series of amino acids including Glycine, Tryptophan, Arginine, Cysteine was reacted with alcohols like

methanol, propanol, phenol; n-butanol was transformed to corresponding amino acid methyl ester hydrochlorides by using below scheme. Trimethylchlorosilane (TMSCl) with methanol at room temperature is an efficient reagent for esterification of amino acids of all classes.

Preparation of amino acid ester hydrochlorides

Amino acid (0.1mol) was taken in a round bottom flask. Freshly distilled chlorotrimethylsilane (0.2mol) was added slowly and stirred with a magnetic stirrer. Then methanol (100 mL) was added and the resulting solution or suspension was stirred at room temperature. After the completion of reaction (as monitored by TLC), the reaction mixture was concentrated on a rotary evaporator to give the product amino acid ester hydrochloride. The above procedure was carried out with various amino acids and alcohols.

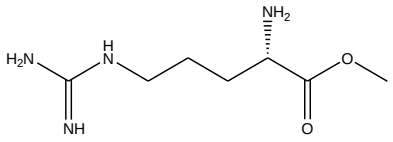


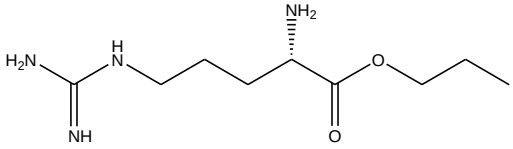
Scheme for synthesis of acid methyl ester hydrochlorides

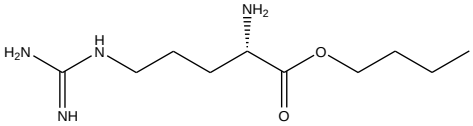
List of newly synthesized compounds

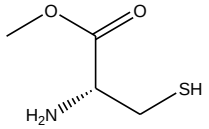
Table 1: Esterification of amino acids with methanol in the presence of TMSCl.

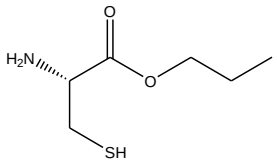
Code	Amino acid	Alcohol	Compound
A1	Arginine	Methanol	methyl 2-amino-5-guanidinopentanoate
A2	Arginine	propanol	propyl 2-amino-5-guanidinopentanoate
A3	Arginine	n-butanol	propyl 2-amino-5-guanidinopentanoate
C1	Cysteine	propanol	propyl 2-amino-3-mercaptopropanoate
C2	Cysteine	methanol	methyl 2-amino-3-mercaptopropanoate
G1	Glycine	Methanol	methyl 2-aminoacetate
G2	Glycine	propanol	propyl 2-aminoacetate
G3	Glycine	phenol	phenyl 2-aminoacetate
T1	Tryptophan	phenol	phenyl 2-amino-3-(1H-indol-3-yl)propanoate
T2	Tryptophan	propanol	propyl 2-amino-3-(1H-indol-3-yl)propanoate

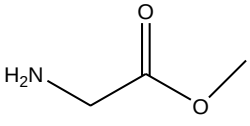

(*S*)-methyl 2-amino-5-guanidinopentanoate

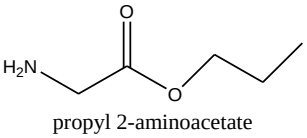

(*S*)-propyl 2-amino-5-guanidinopentanoate


(*S*)-butyl 2-amino-5-guanidinopentanoate


(*R*)-methyl 2-amino-3-mercaptopropanoate


(*R*)-propyl 2-amino-3-mercaptopropanoate


methyl 2-aminoacetate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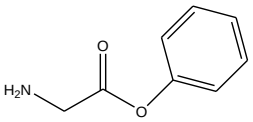
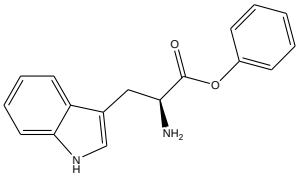
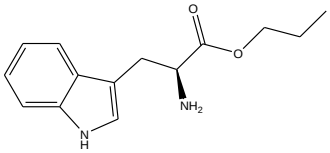

phenyl 2-aminoacetate


(*S*)-phenyl 2-amino-3-(1*H*-indol-3-yl)propanoate


(*S*)-propyl 2-amino-3-(1*H*-indol-3-yl)propanoate

DOCKING STUDY

Preparation of Ligand

To investigate the detailed intermolecular interactions between the analogues we carried out docking of molecules using Molegro Virtual Docker (MVD), Ligand structures were drawn and optimized using MM2 force field by using Chem3D Ultra 8.0 and saved in mol format.

The ligands are imported to the workspace and preparation of them is done. The docking scores of the active constituents are compared against the standard drugs (Acarbose) obtained from the drug bank in .mol format.

Preparation of Protein target

The target for docking studies is selected as α - Amylase. Docking analysis is done by initially selecting the target for the disease and followed by obtaining the 3D structure of α - Amylase (3M07) D from protein data bank in the .pdb format [26]. It is well known that PDB files often have poor or missing assignments of explicit hydrogens, and the PDB file format cannot accommodate bond order information. Therefore, proper bonds, bond orders, hybridization, and charges were assigned using the MVD. The potential binding sites of both the targets were calculated using the built-in cavity detection algorithm implemented in MVD. The search space of the simulation exploited in the docking studies was studied as a subset region of 25.0 Angstroms around the active side cleft. The water molecules are also taken into consideration and the replaceable water molecules were given a score of 0.50.

Molegro Virtual Docker's docking search algorithms and scoring functions

Ligand docking studies were performed by which has recently been introduced and gained attention among medicinal chemists. MVD is a fast and flexible docking program that gives the most likely conformation of ligand binding to a macromolecule. Mol-Dock software is based on a new heuristic search algorithm that combines differential evolution with a cavity prediction algorithm [27]. It has an interactive optimization technique inspired by Darwinian Evolution Theory (Evolutionary Algorithms - EA), in which a population of individuals is exposed to the competitive selection that weeds out poor solutions. Recombination and mutation are used to generate new solutions. The scoring function of Mol-Dock is based on the Piecewise Linear Potential (PLP), which is a simplified potential whose parameters are fit to protein-ligand structures and a binding data scoring function [28,29] that is further extended in GEMDOCK (Generic Evolutionary Method for molecular DOCK) with a new hydrogen bonding term and charge schemes.

α - amylase inhibition assay

α - amylase activity was carried out by the starch-iodine method. 10 µL of a-amylase solution (0.025 mg/mL) was mixed with 390 µL of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) containing different concentration of extracts. After incubation at 37 °C for 10 min, 100 µL of a starch solution (1%) was added, and the mixture was re-incubated for 1 h. Next, 0.1 mL of 1% iodine solution was added, and after adding 5 mL distilled water, the absorbance was taken at 565 nm. Sample, substrate and α - amylase blank determinations were carried out under the same reaction conditions. Inhibition of enzyme activity was calculated as (%) = (A-C) X100/ (B-C), where, A= absorbance of the sample, B= absorbance of blank (without a-amylase), and C= absorbance of control (without starch) [30].

Results

***In-silico* evaluation of physicochemical properties**

Table 2: The below table shows the *in-silico* physicochemical properties of the newly synthesized compounds

Compounds	Molecular Formula	Molecular Weight	ClogP	Log S	Dock score
A1	$C_7H_{16}N_4O_2$	188.12	-1.86	-0.284	-15.782
A2	$C_9H_{20}N_4O_2$	216.24	-1.00	-0.840	-13.574
A3	$C_{10}H_{22}N_40_2$	233.31	-0.55	-1.124	-18.296
C1	$C_6H_{13}NO_2S$	163.24	-0.04	-1.761	-16.351
C2	$C_4H_9NO_2S$	135.18	-0.90	-1.191	-18.601
G1	$C_3H_7NO_2$	89.09	-1.19	-0.156	-3.375
G2	$C_5H_{11}NO_2$	151.16	0.32	-1.485	-1.131
G3	$C_8H_9NO_2$	117.147	-0.33	-0.726	-1.141
T1	$C_{17}H_{16}N_2O_2$	280.3	-2.16	-3.523	-14.870
T2	$C_{14}H_{18}N_2O_2$	246.3	0.61	-2.520	-16.360

***In-silico* evaluation of Toxicological properties**

Table 3: The below given table shows the *in-silico* toxicity profile of the newly synthesized compounds.

Compounds	Mutagenicity	Tumorigenicity	Irritating effects	Reproductive effects
A1	Green	Green	Green	Green
A2	Green	Green	Green	Green
A3	Green	Green	Green	Green
C1	Red	Green	Green	Green
C2	Red	Green	Green	Green
G1	Green	Green	Green	Green
G2	Green	Green	Green	Green
G3	Green	Green	Green	Green
T1	Green	Green	Green	Green
T2	Green	Green	Green	Green

Table 4: In-silico docking analysis of designed molecules on α - Amylase (3M07) ranking based on MolDock Score and H-Bond Interaction.

Ligand	Mol-Dock Score	H-Bond Interaction
T1	-108.711	-19.1187
T2	-107.401	-15.0872
Acarbose	-93.1984	-12.9581
A1	-92.685	-12.3217
A2	-88.9983	-11.058
A3	-84.0515	-10.8187
C2	-82.9936	-10.2517
C1	-75.9834	-9.63825
G1	-74.8734	-9.399
G3	-72.793	-8.90358
G2	-60.4139	-8.22987

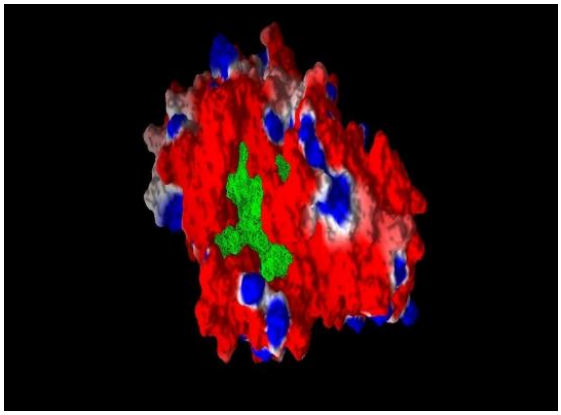


Fig.1: Binding pockets in α - Amylase (3M07)

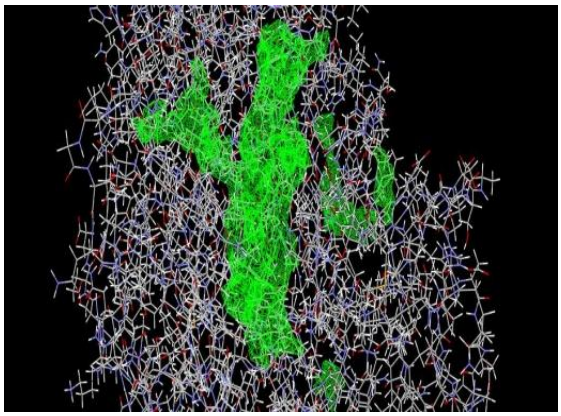


Fig.2: Cavities in Alpha- amylase.

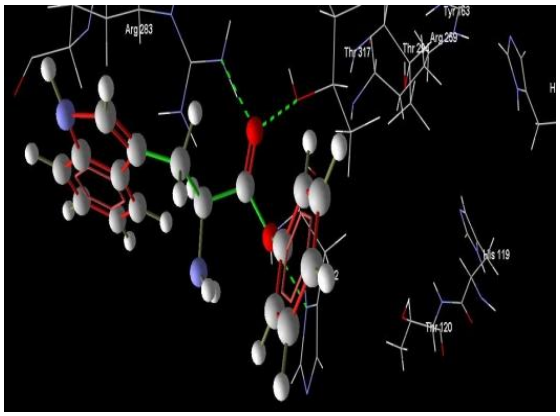


Fig.3: shows the structure of the protein α - Amylase (3M07) the bonding of the target with the ligand T2.

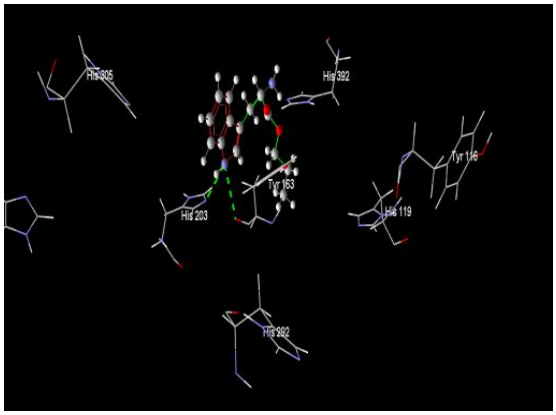


Fig.4: shows the structure of the protein *α* - Amylase (3M07) the bonding of the target with the ligand T1.

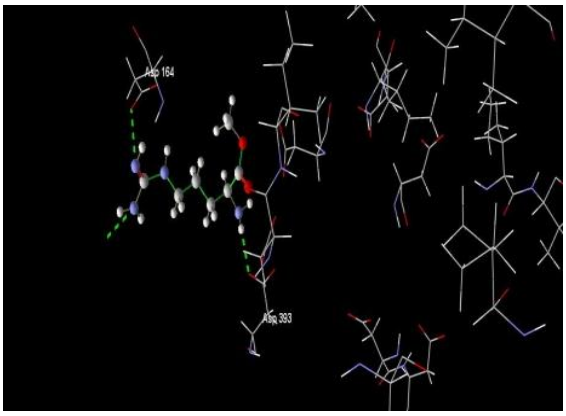


Fig.5: shows the structure of the protein α - Amylase (3M07) the bonding of the target with the ligand A1

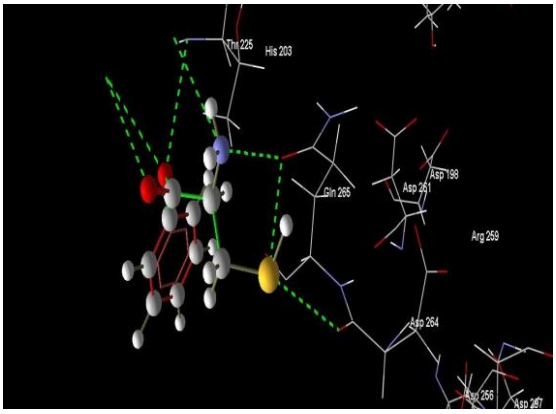


Fig.6: shows the structure of the protein *α* - Amylase (3M07) shows the bonding of the target with the ligand Acarbose

Table 5: Docking score and Hydrogen binding interactions derivatives.

S.No	Compounds	Docking scores	Binding interactions
1	T2	-107.401	His & Tyr
2	T1	-108.711	Thr
3	A1	-92.685	Asp
4	Acarbose	-93.1984	Thr

Table 6; Comparison of Invitro activity of compounds with Acarbose IC_{50} in µg/ml against Alpha-amylase activity.

COMPOUND	IC_{50}
A1	33.68±.25
A2	39.50±.97
A3	22.48±2.6
C1	55.55±1.1
C2	68.22±1.7
G1	102±5.6
G2	76.15±3.5
G3	64.12±3.2
T1	8.22±.68
T2	8.42±.72
Acarbose	8.48±1.02

All values reported as Mean ± S.E.M (n=3)

DISCUSSION

The ability of the phytoconstituents to bind with the targets is given in terms of MolDock Score. The MolDock Score is used as the parameter for analyzing the docking results. The phytoconstituents are ranked accordingly. The ligand possessing the highest mol dock score shows a strong affinity towards its target. All compounds showed non-toxic but C1 and C2 showed mutagenic nature. Among all compounds T2, T1 and A1 were showed best docking score.

CONCLUSION

The current study was aimed at finding novel drug-like molecules as antidiabetic compounds using *in-silico* approach. Intermolecular interactions between the target protein binding site of the target and synthesized ester molecules as antidiabetic compounds were observed. We propose in this study that Compounds T1 (phenyl 2-amino-3-(1H-indol-3-yl) propanoate), T2 (propyl 2-amino-3-(1H-indol-3-yl) propanoate), A1 (methyl 2-amino-5-guanidino pentanoate) possess good antidiabetic activity. In short, this study reveals that *in-silico* approaches were used to discover lead compound and its analogues that lend a hand in inhibiting diabetes mellitus.

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