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In-Vitro α-Amylase Screening and Molecular Docking Studies of Theophylline Derivatives as Anti-Diabetic Agents

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Abstract**—***α-amylase of theophylline derivatives was rarely explored despite these agents have diverse pharmacological activities. The in vitro α-amylase inhibitory activity of the theophylline compounds Va-e were evaluated using acarbose as standard positive control. The IC50 values of these compounds were ranged from 26.89-60.82 µg/ml. All the compounds have exhibited good interactions with α-amylase target 4GQR and have good correlation with biological activity.*

Keywords**—** *α-amylase, theophylline, diabetes, acarbose, molecular docking, anti-diabetic agents.*

I. INTRODUCTION

Diabetes is a group of metabolic disorders which is associated with high blood glucose levels for a prolonged time [1]. It a chronic disorder which effect every cell of the body and occurs due to failure in production of insulin, sensitization of insulin toward its receptors and more synthesis of glucose in body [2]. The pathological symptoms associated with diabetes includes retinopathy, polyuria, ketonuria, neuropathy, nephropathy and various cardiovascular diseases [3]. According to WHO, prevalence of diabetes is more in middle and low-income countries. About 8.8% of worldwide population has suffering from diabetes and it could rise to 9.9% by 2045 [4]. There are various target proteins available for the treatment of diabetes among them is an α-amylase which is a hydrolase enzyme and which cleaves α -1,4-glycosidic linkage in starch into monosaccharides viz., glucose and maltose [5]. The α-amylase could be detrimental for rising of blood glucose levels and the inhibition of α -amylase can significantly decrease the postprandial blood glucose level which is important treatment management in Type 2 diabetes [6]. Several theophylline derivatives have a quest in medicinal chemistry having numerous biological and pharmacological activities. Theophylline known to have antiviral [7], antitumor [8], rheumatoid arthritis [9] CNS agents [10], bronchiectasis [11], and cardiovascular [12], activities. theophylline have wide scope for different pharmacological activities due to the presence of purine ring structure which is a core structure for several nucleotides [10, 13].

II. MATERIALS AND METHODS

Experimental Section

Theophylline derivatives were obtained from Dr Mac Biopharma Pvt Ltd, Hyderabad. α -amylase, DNS reagent were purchased from Sigma Aldrich. Starch and Sodium dihydrogen phosphate, disodium hydrogen phosphate was procured from Avira synthesis Labs, Hyderabad. Acarbose was procured from Krishna Pharmaceuticals Pvt Ltd., Hyderabad.

Theophylline Compounds

Theophylline containing acetylenes samples (**Va-Ve**) were gifted by Dr Mac Biopharma Pvt Ltd., Hyderabad and reported earlier in literature [14]. All the synthesized analogs were confirmed by their melting point and were compared with the reported literature (Fig 1).

In vitro α-Amylase Screening

The α-amylase inhibition was determined according to the assay method with modified as described by Shai et al. (2010) [15]. All the synthesized compounds and standard acarbose were prepared at different concentrations (50-200 µg mL-1). A total of 50 µL of compounds was incubated with 50 µL of porcine α-amylase (0.5 mg/mL in 0.02 M sodium phosphate buffer PH 6.9) at r.t for 30 min. After incubation 50 µL of 1% starch solution in sod phosphate buffer (pH 6.8) was added in each tube and further incubated at r.t for 20 min. 100 µL of DNS (Dinitro salicylic acid) reagent was then added and the reaction mixture was boiled for 10 min and cooled to r.t. After dilution the absorbance was measured at 540 nm and % inhibition was calculated by using formula as below and IC_{50} values were obtained as mean ± SD in triplicates.

$$\% \text{ Inhibition} = [A_{control} - A_{sample}] / [A_{control}] * 100$$

Where, $A_{control}$ = Absorbance of control
A_{sample} = Absorbance of Test compounds.

Molecular Docking Studies with α -Amylase Enzyme

Docking studies have been performed with α-amylase enzyme using Autodock Vina [16]. We have used PDB: ID 4GQR for docking studies. Both the protein and the ligand are prepared for docking with Autodock tools software and loaded on PyRx platform (which is used as graphical user interface) for docking. These processes are quite automated. The docking calculation generated ten poses. The selection of the best pose was done on the interaction energy between the ligand and the protein as well as on the interactions the ligand

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shows with experimentally proved important residues. Doted lines are showing hydrogen bond interaction with α-amylase enzyme. Apart from this, the cationic side chain of Asp 197, Trp 58, Gln 63 forms hydrogen bond interactions with ring of the standard.

Structures

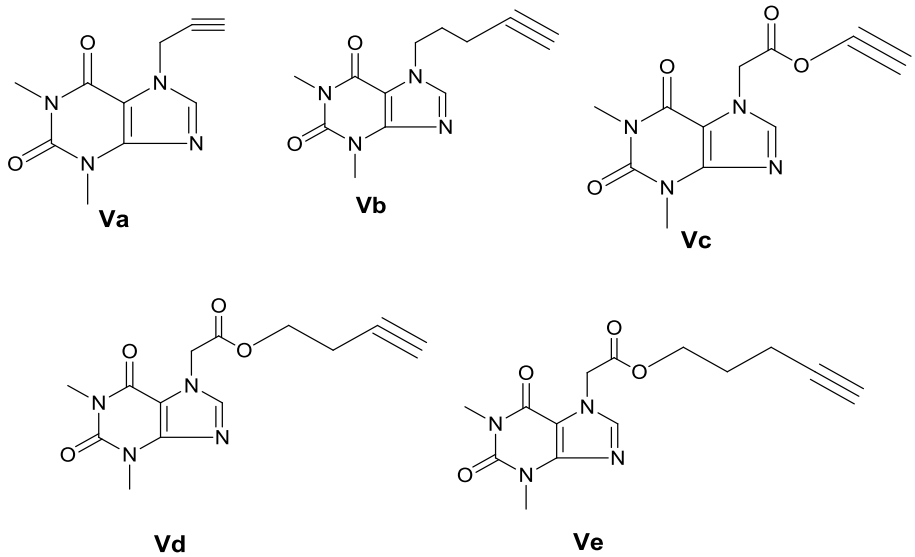

Fig. 1. The synthetic theophylline containing acetylenes compounds were obtained from Dr Mac Biopharma Pvt Ltd., Hyderabad.

III. RESULTS

In vitro α-Amylase Inhibitory Activity

In vitro α-amylase inhibitory activity of the Theophylline compounds **Va-e** were evaluated using acarbose as standard positive control. The IC_{50} values of the compounds were ranged from 26.89-60.82 µg/ml . Compounds (**Vc-e**) exhibit most potent α-amylase inhibitory activity with IC_{50} values ≤ 50. Further compounds **Va & Vb** have shown weak inhibitory potency (IC_{50} ≥50) against the enzyme α-amylase. In this study, the standard compound acarbose exhibited the IC_{50} value 32.69 µg/ml and compounds **Ve** and **Vd** exhibited most significant potency against α-amylase as compared- to the positive control acarbose with IC_{50} values 26.89 and 38.30 µg/ml respectively.

Docking Studies

In order to predict the putative binding mode tested compounds (**Va-e**) with the target protein (PDB ID: 4GQR), docking studies were carried out. The value of RMSD obtained between X-ray 63333pose and re-docked pose (Fig. 2) for Myricetin (Co-*crystallized ligand in target protein) was found to be 0.74 Å, suggesting that docking protocol could be relied on for the docking studies. The analysis of best docked pose of compound **Ve** (Fig. 3) revealed that, purine moiety of compound **Ve**, exhibited prominent hydrophobic interaction with amino acid Trp-59, while its alkylene moiety showed similar interactions with Lys-200, His-201 and Ilu-235.

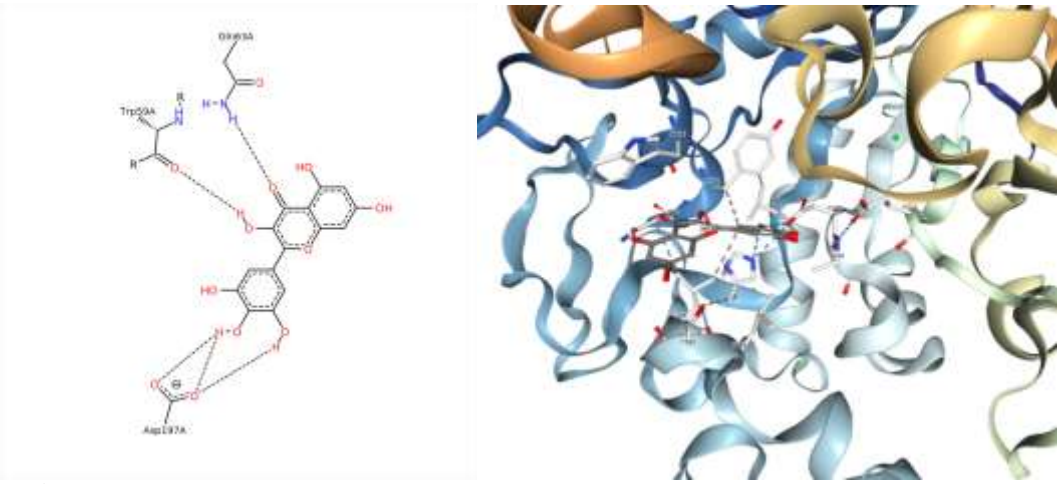

Fig. 2. Interaction of best compound with 4GQR. Here doted lines showing hydrogen bond interaction with enzyme, solid lines show pie-pie and pi-cation interactions.

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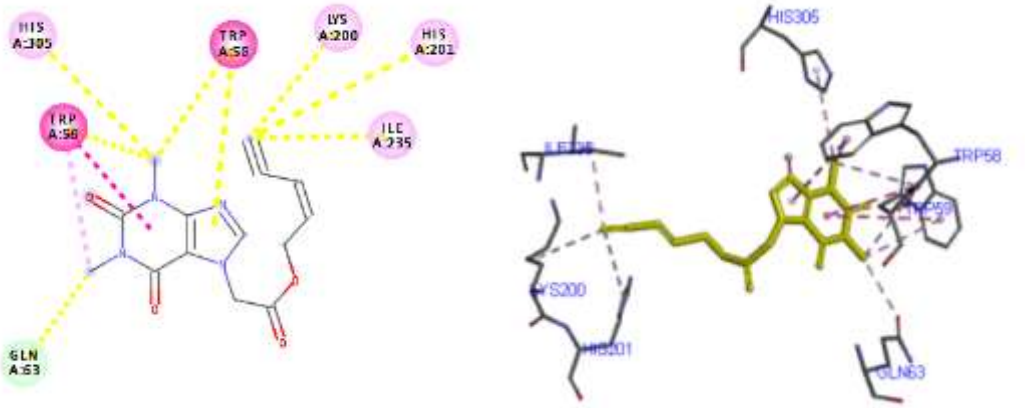

Fig. 3. Compound Ve with 2D and 3D interactions with 4GQR.

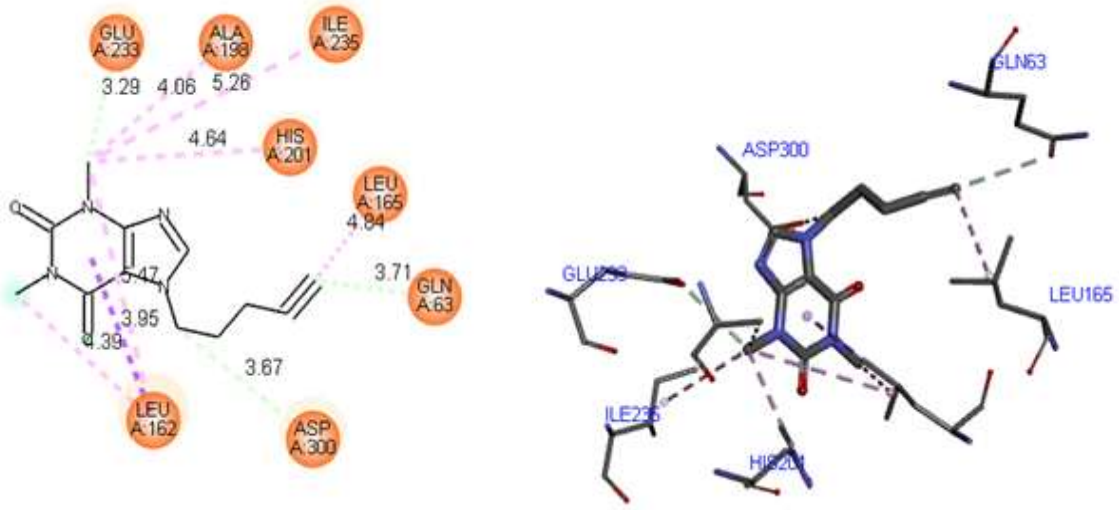

Fig. 4. Compound Vb with 2D and 3D interactions with 4GQR.

TABLE I. IC50 values and docking results of all the test compounds against α-amylase enzyme.

Sl. No.	Compounds	IC_{50} (µg/ml)	Binding Energy (kcal/mole)
1	Acarbose	32.69	-6.9 (Myricetin)
2	Va	60.82	-6
3	V_b	49.31	-6.4
4	V_c	44.85	-6.4
5	V_d	38.30	-6.2
6	V_e	26.89	-6.6

Further, methyl group of **Ve** showed hydrogen as well as hydrophobic interaction with residues His-305, Trp-58 and Trp-59 respectively that stabilize its binding affinity with the target receptor, consequently may be responsible for its significant *in vitro* activity. While, in the best docked pose of compound **Vb** (Fig. 4), its purine moiety displayed weak hydrophobic interaction with amino acids Tyr-62 and Leu-162, while its methyl moiety showed similar interaction of moderate intensity with Glu-233, Ala-198, Ile-235 and His-201. Furthermore, it was noteworthy that, compound **Va** does not exhibit hydrogen bonding interaction with receptor, so overall weak binding affinity of compound **Va** may be responsible for its less potency against α-amylase in the *in vitro* assay.

IV. Conclusion

The IC_{50} of the theophylline compounds is compared with the standard positive control acarbose. The compounds Ve and Vd has significant α-amylase inhibiton with IC_{50} values ≤ 50µg/ml when compared to standard positive control acarbose. Hence sample Vd and Ve compounds could be the potential anti-diabetic agents.

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Conflict of Interest

Author declares that there is no conflict of interest to disclose.

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