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In vitro anti-diabetic activity and molecular docking studies of theophylline containing acetylene derivatives

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ABSTRACT

α–Glucosidase of the synthetic Theophylline containing acetylene derivatives was rarely explored despite these agents have wide range of biological activities. The *In vitro* α–glucosidase inhibitory activity of the synthetic motifs Va-e were evaluated using acarbose as standard positive control. The IC_{50} values of these compounds were ranged from 22.84-45.43 µg/ml and standard acarbose is 25.35 µg/ml. All the motifs have exhibited good interactions with α–glucosidase target 3TOP and have good correlation with *In vitro* α–glucosidase activity.

Keywords: *α–glucosidase, theophylline, molecular docking, acarbose.*

1. INTRODUCTION

Diabetes is a group of metabolic (carbohydrates, lipids and proteins) disorders which is associated with high blood glucose levels for a prolonged time [1]. It has become major challenges health problem in the world based on the global scale. The IDF (International Diabetes Federation) estimated that 451 million people were suffering from diabetes worldwide especially in between age of 18-99 years in 2017 and this number was speculated to rise to 693 million by 2045[2]. According to W.H.O. 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[3]. It is a chronic disorder which effect every cell of the body and occurs due to failure in synthesis as well as release of insulin, sensitization of insulin toward its receptors and more synthesis of glucose in body [4]. The pathological symptoms associated with diabetes includes ketonuria, polyuria, retinopathy, neuropathy and various CVS diseases [5]. 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[6]. The α--Glucosidase could be detrimental for rising of blood glucose levels and the inhibition of α--Glucosidase can significantly decrease the postprandial blood glucose level which is important treatment management in Type 2 diabetes [7].

Several Theophylline derivatives have a quest in medicinal chemistry having numerous biological and pharmacological activities. Theophylline known to have antiviral [8], antitumor [9], rheumatoid arthritis [10] CNS agents [11], immunodeficiency [12], and antimicrobial [13], activities. Theophylline's have wide scope for different pharmacological activities due to the presence of purine ring structure which is a core structure for several nucleotides [14].

2. EXPERIMENTAL SECTION

Theophylline derivatives were obtained (Dr Mac Biopharma Pvt Ltd, Hyderabad). α-glucosidase (Sigma Aldrich, Mumbai). Starch and Sodium dihydrogen phospahate, disodium hydrogen phosphate, phosphate buffer was procured (Avira synthesis Labs, Hyderabad). Acarbose was procured (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[15]. All the synthesized analogs were confirmed by their melting point and were compared with the reported literature (Fig 1).

In vitro α-Glucosidase Screening

The α-glucosidase inhibition was determined according to the assay method with modified as described by Juan Zhang et al. (2014) [16, 17]. The solution of α-glucosidase enzyme was prepared by dissolve in 10mM (pH6.8) of phosphate buffer solution. All the synthesized compounds and standard acarbose were prepared at different concentrations (50-200 µg mL-1). After that different concentration of α-glucosidase enzyme solution (10µL) was added to synthetic compound (60 µL) solution and further the mixture was allowed to stand for 20min at 370C. Acarbose used as positive control and buffer solution (instead of substrate) used as blank, after 15 min, the absorbance was measured at 405 nm and % inhibition was calculated by using formula as below and IC_{50} values were obtained as mean ± SD in triplicates.

% Inhibition = [Acontrol – Asample] / [Acontrol] * 100

Where Acontrol = Absorbance of control; Asample = Absorbance of Test compound.

Structures.

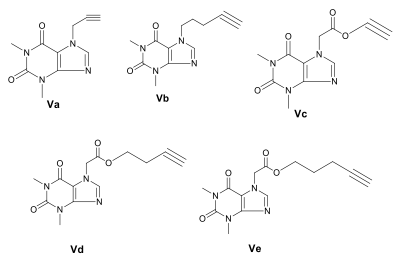

Fig. 1. The synthetic Theophylline containing acetylenes compounds were obtained from Dr Mac Biopharma Pvt Ltd., Hyderabad.

Molecular docking studies with α-glucosidase enzyme. Docking studies have been performed with α-glucosidase enzyme using FlexX module in LeadIT (BioSolveIT GmbH, 2014). We have used PDB: ID 3TOP for docking studies which is extracted from protein database (www.rscb.org) . Both the protein and the ligands are prepared for docking with Chimera tools software and loaded on LeadIT 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 shows with experimentally proved important residues. Dotted lines are showing hydrogen bond interaction with α-glucosidase enzyme. The docking studies were carried out on LeadIT software using the default settings. The results of the docking scores for each ligand are shown in Table-1. Interactions of 3D and 2D poses between protein and ligands were obtained.

3. RESULTS SECTION

In vitro a-Glucosidase Inhibitory Activity.

In vitro α-glucosidase inhibitory activity of the synthetic Theophylline compounds **Va-e** were evaluated using acarbose as a standard positive control for the evaluation of antidiabetic activity. The IC_{50} values of the compounds were ranged from 22.84-45.43 μg/ml. Compounds (**Vc & Ve**) exhibit the most potent α-glucosidase inhibitory activity with IC_{50} values ≤ 50. Further compounds **Va & Vb** have shown weak inhibitory potency against the enzyme α-glucosidase. In this study, the standard compound acarbose has shown the IC_{50} value 25.35 μg/ml and compounds **Vc** and **Ve** exhibited most significant potency against α-glucosidase as compared to the positive control acarbose with IC_{50} values 22.84 and 27.94 μg/ml respectively.

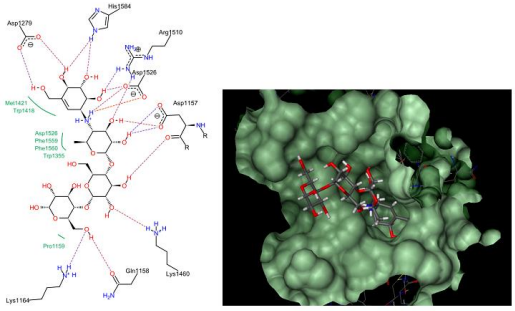

Fig. 2. Interaction of acarbose with 3TOP. Here doted lines showing hydrogen bond interaction with enzyme. solid lines show hydrophobic interactions.

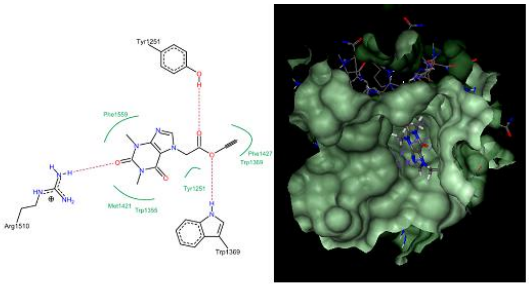

Fig. 3. Compound Vc with 2D and 3D interactions with 3TOP.

Molecular Docking studies. In order to predict the putative binding mode tested compounds (**Va-e**) with the target protein (PDB ID: 3TOP), docking studies were carried out. The value of RMSD obtained between X-ray pose and re-docked pose (Fig. 2) for Acarbose (Co-crystallized ligand in target protein) was found to be 0.96 Å, suggesting that docking protocol could be relied on for the docking studies.

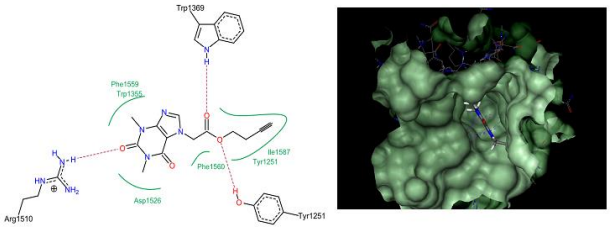

Fig. 4. Compound Vd with 2D and 3D interactions with 4GQR.

Table 1. IC_{50} Values and Docking results of all The Test Compounds Against α-Glucosidase Enzyme.

S.NO	COMPOUNDS	IC_{50} (μg/ml)	BINDING ENERGY (kcal/mole)
1	Acarbose	25.35	-31.14
2	Va	45.43	-16.75
3	V_b	35.13	-14.99
4	V_c	22.84	-21.16
5	V_d	31.51	-19.25
6	V_e	27.94	-18.98

The analysis of best docked pose of compound **Vc** (Fig. 3) revealed that oxygen groups of compounds **Vc**, have shown hydrogen bond interactions with Tyr 1251, Arg 1510 and Trp 1369 and purine ring forms hydrophobic interaction with amino acid Phe 1559, Met 1421, Trp 1355, while its acetylene moiety showed similar interactions with Phe 1427 and Trp 1369. Further, the oxygen groups of **Vd** showed hydrogen interactions with residues Trp 1369, Arg 1510, Tyr 1251 and hydrophobic interactions with Phe 1559, Phe 1560, Asp 1526 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 **Ve** (Fig. 4), its purine moiety displayed weak hydrophobic interaction with amino acids. Furthermore, it was noteworthy that, compound **Va** does not exhibit hydrogen bonding interaction with the receptor, so overall weak binding affinity of compound **Va** may be responsible for its less potency against α-glucosidase in the *in vitro* assay.

4. CONCLUSIONS

The IC_{50} of the theophylline compounds is compared with the standard positive control Acarbose. The compounds **Vc** and **Vd** have shown significant α-glucosidase inhibition with IC_{50} values ≤50μg/ml when compared to standard positive control

acarbose. Hence sample **Vc** and **Vd** compounds could be the potential anti-diabetic agents.

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