



Design, synthesis and biological evaluation of theophylline containing variant acetylene derivatives as α -amylase inhibitors



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ABSTRACT

A novel pharmacophore with theophylline and acetylene moieties was constructed by using a fragment-based drug design and a series of twenty theophylline containing acetylene conjugates were designed and synthesized, and all the compounds were evaluated by enzyme-based *in vitro* α -amylase inhibition activity. The *in vitro* evaluation revealed that most of the compounds displayed good inhibitory activities, and among them nine analogs **13–15**, **20**, **21** and **24–27** were exhibited more or nearly as equipotent inhibitory activity with IC_{50} values 1.11 ± 0.07 , 1.14 ± 0.17 , 1.07 ± 0.01 and 1.21 ± 0.03 , 1.33 ± 0.09 , 1.17 ± 0.01 , 1.05 ± 0.02 , 1.61 ± 0.04 , $1.02 \pm 0.03 \mu M$ respectively, as compared with standard, acarbose $1.37 \pm 0.26 \mu M$. Further, molecular docking simulation studies were done to identify the interactions and binding mode of synthesized analogs at binding site of α -amylase enzyme (PDB ID: 4GQR). Among the synthesized analogs, two compounds **25** and **27** were selected on the basis of α -amylase inhibition activity and evaluated for *in vivo* anti-diabetic activity by High Fat Diet-Streptozotocin (HFD-STZ) model in normal rats. At the dose of 10 mg/kg, *bw*, *po* these compounds have significantly reduced Plasma Glucose level in rats as compared to pioglitazone. The anti-diabetic activity results showed that the animal treated with the compounds **25** and **27** could better reverse and control the progression of the disease compared to the standard.

1. Introduction

Diabetes being a severe disease is the most raising problem in the world. Estimated data was collected in 2010 which stated that 285 million peoples were affected by this disease which may increase to 439 million by 2030 [1,2]. Almost 95% of diabetes cases are characterized by varying blood glucose level because of insulin resistance [3]. This leads to cardiovascular diseases, high blood pressure, stroke, blindness, and kidney failure [4]. Amylases are the group of enzymes namely, α -amylase, glucoamylase and β -amylase which hydrolyze α -1,4- and α -1,6-glucosidic bonds present in starch and glycogen [5]. Inhibition of α -amylase enzyme in the digestive system is one of the best options to maintain the postprandial glucose level [6,7]. One therapeutic approach for treating diabetes is to decrease post-prandial hyperglycemia which can be achieved by delaying the absorption of glucose and also by the inhibition of carbohydrate hydrolyzing enzyme α -amylase [8,9]. α -Amylase inhibitors significantly decrease the post-prandial

hyperglycemia in the treatment of type-II diabetes [7,10]. Examples of such inhibitors which are in clinical use are Acarbose, Miglitol, and Voglibose. The WHO recommends a search of safe, potent and less toxic natural anti-diabetic agent [11].

Unfortunately, most natural products themselves are not suitable for administration as drugs. But natural products served as an effective source of drug lead throughout the history [12–15]. In view of this background, theophylline was selected as lead which belongs to the class called xanthine family, mainly found in the beans of cocoa and it is highly present in *criollo cocoa* beans to an extent of 3.7 mg/kg and is also found in brewed tea [16]. It is reported to have a wide range of various biological activities such as treatment of respiratory diseases like asthma, chronic obstructive pulmonary disease (COPD) [17,18], and other airway diseases for more than 75 years. Theophylline containing acetylene derivatives have been reported as DPP-IV inhibitor with GPR119 agonist particularly in the treatment of type 2 diabetes mellitus [19–21]. Other biological activities such as anticancer [22],

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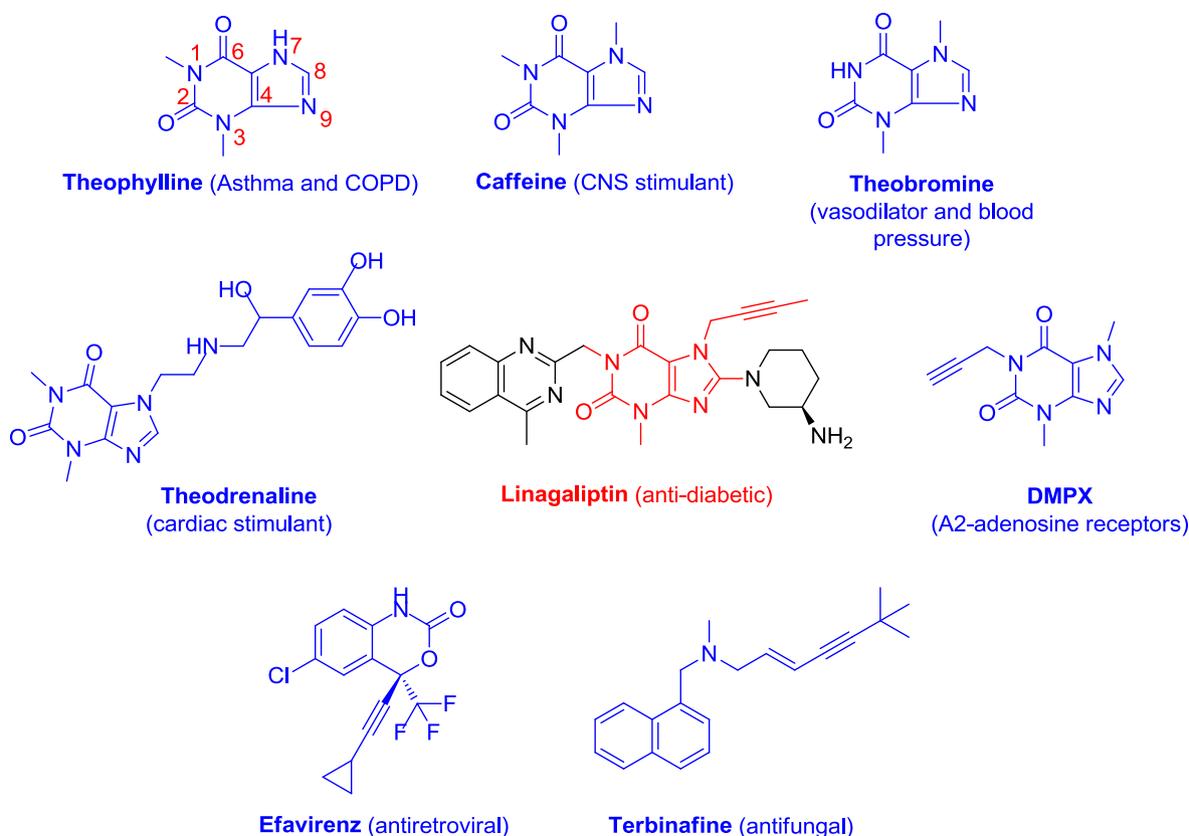


Fig. 1. Multiple bioactive theophylline, acetylene, and theophylline containing acetylene moieties.

anti-microbial [23] and antidepressant agents [24].

On the other hand, in medicinal chemistry and organic synthesis, acetylenes are a valuable and important class of compounds. The metabolites of acetylenes belong to a class of molecules having the triple bonds and they are found in fungi, plants, marine invertebrates and microorganisms [25–27]. Some of the acetylene, theophylline, and theophylline containing acetylene bioactive molecules are illustrated (Fig. 1).

So, in the present study, new Linagliptin based α -amylase inhibitors were designed by making suitable chemical modification in the theophylline nucleus. Thus, based on the above points it was thought worthwhile to prepare new theophylline containing acetylenes with an objective to obtain pharmacological active, safer, and less adverse effects anti-diabetic agents.

2. Results and discussion

2.1. Chemistry

The synthesis of key intermediate theophyllinoic acid compounds (3a–b) was described first in Scheme 1 and later, all novel theophylline containing acetylene derivatives were constructed as in Schemes 2–4. Commercially available theophylline 1 was used as the starting material to synthesize intermediate compounds (3a–b) and final analogs (4–10) by following literature procedure [28].

Now in Scheme 3, compound 3a was coupled with methyl 3-amino-2,2-dimethylpropanoate hydrochloride by using HATU and DIPEA at room temperature for 16 h gave compound 11 and then followed by hydrolysis with LiOH·H₂O in THF and H₂O (1:1) gave compound 12. In a similar way, compound 12 was treated with propargyl bromide in the presence of K₂CO₃ in DMF at room temperature to obtain compound 13 (60%) and treated with 5-chloro-1-pentyne at 80 °C for 12 h to get compound 14 (50%). But, compound 12 was coupled with

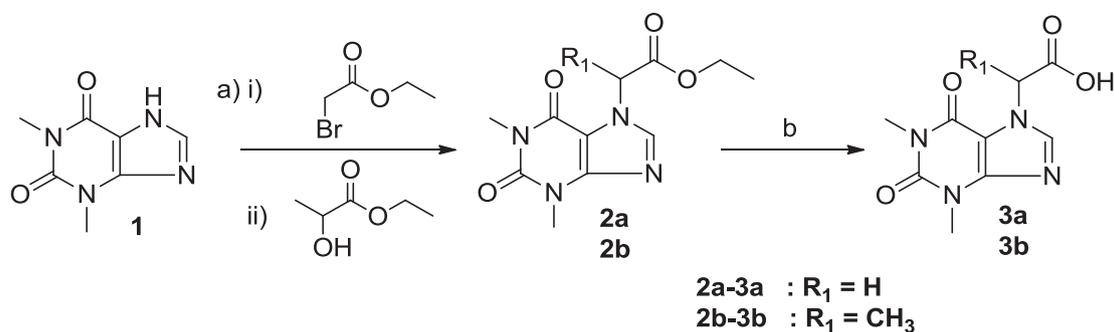
propargylamine by using reagents T₃P and triethylamine (TEA) in DMF at room temperature to get compound 15 with the yield 36%.

In the synthetic Scheme 4, compounds (3a–b) were coupled with various amino acids has been described in this section. Compound 3a was coupled with glycine methyl ester hydrochloride (a), alanine methyl ester hydrochloride (b), L-valanine ethyl ester hydrochloride (c), L-phenyl alanine benzyl ester hydrochloride (d) by using reagents HATU and DIPEA in dichloromethane at room temperature for 16 h which gave compounds 16a–d, while compound 3b was coupled with only alanine methyl ester hydrochloride (b) by using reagents HATU and DIPEA in dichloromethane at room temperature for 16 h gave compound 16e. The ester compounds 16a–c and 16e hydrolysis with LiOH·H₂O in THF and H₂O at room temperature for 4 h gave compounds 17a–c and 17e. Compound 16d hydrogenated with Pd/C in methanol at 60 Psi for 8 h to get compound 17d. Further, compounds 17a–b and 17e were coupled with propargyl alcohol by using DCC and DMAP in dichloromethane at room temperature for 16 h gave compounds 18, 19 (86% and 79%) and 22 with the yield 74%. Compounds 17c–d were treated with propargyl bromide in the presence of K₂CO₃ in DMF at room temperature to obtain compound 20 (54%), 21 (91%). But, compounds 17b–d were treated with 5-chloro-1-pentyne at 80 °C for 12 h to get compounds 23 (33%), 24 (72%) and 25 (47%), respectively. Compounds 17c–d were coupled with propargylamine by using reagents T₃P and triethylamine (TEA) in DMF at room temperature to get compounds 26 and 27 with yields 63%, 91% respectively.

2.2. Anti diabetic activity

2.2.1. In vitro α -amylase inhibitory activity

This work is aimed at the biological evaluation of two novel series of theophylline derivatives as shown in Fig. 2. Our plan was based on using theophylline 1 and the simple acetylene as scaffolds (4–10 and 13–15). Although 4 and 5 have shown low activity, it can be used as a



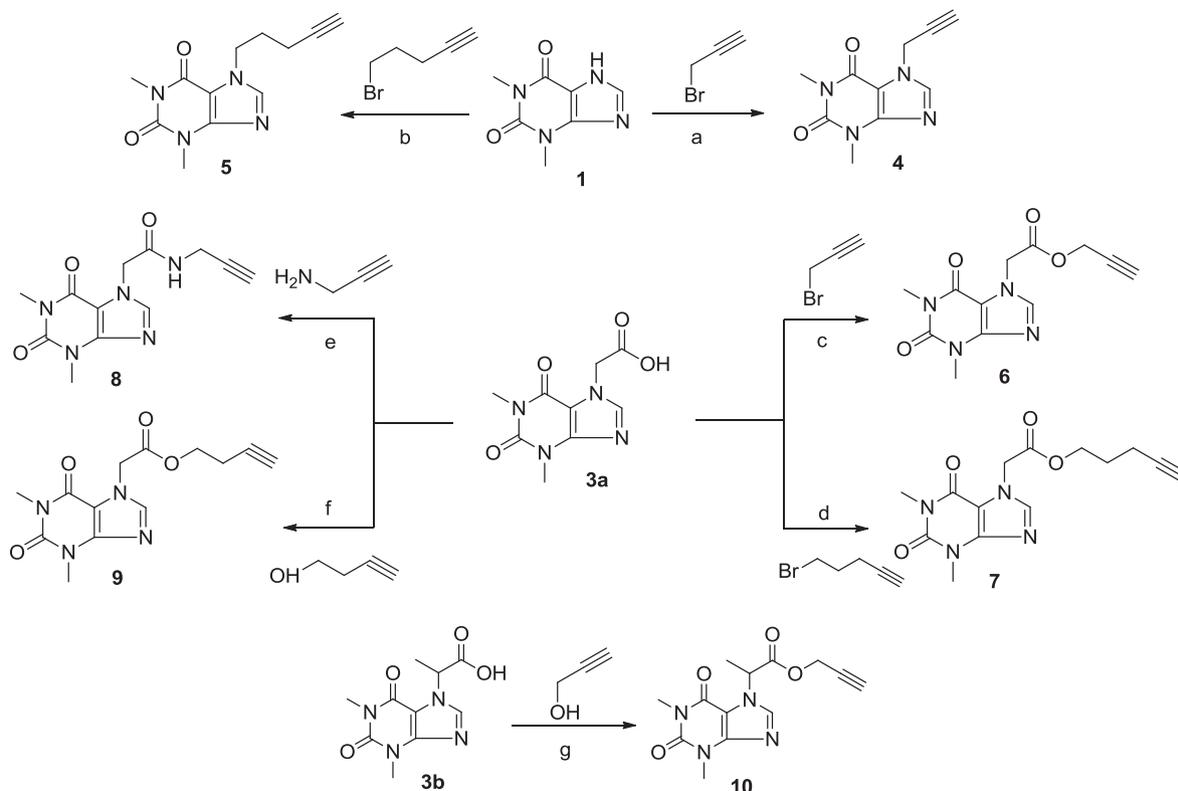
Scheme 1. Synthesis of theophylline acid compounds (**3a–3b**). Reagents and conditions: (a) (i) K₂CO₃, DMF, 85 °C, 12 h & (ii) TPP, DIAD, THF, rt, 5 h; (b) LiOH·H₂O, THF:H₂O, rt, 2 h.

starting point for the optimization of its biological activity by modifying its added carbonyl unit and length of chain. The simple acetylenes of series A (**4–10** and **13–15**) were planned by keeping the carbonyl scaffold of **1** and substituting it with different acetylenes in order to verify the importance of such subunits in the α -amylase activity. In the series B (**18–27**), the planned derivatives present the theophylline moiety protected by carbonyl and amino acid moieties aiming to observe the influence of these groups on the activity of the theophylline portion, as well as to verify the importance of the inclusion of the different amino acid units.

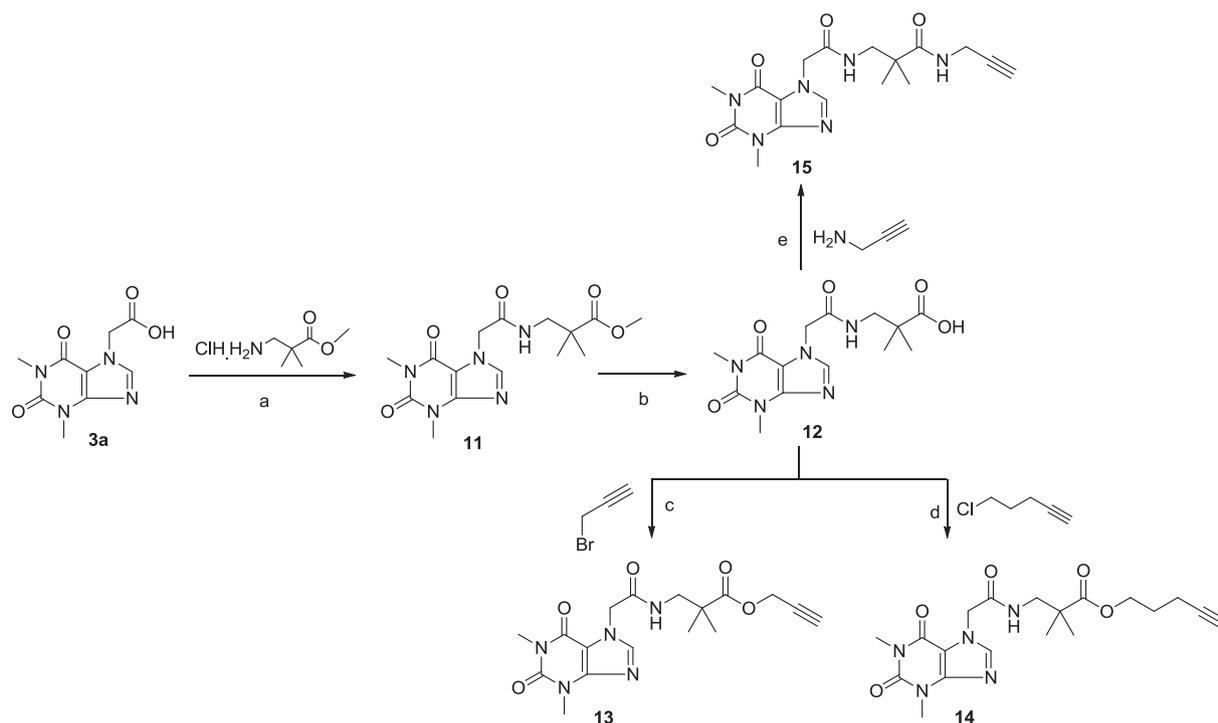
Importance from synthesized a novel series A and B of theophylline containing acetylene scaffolds (**4–10**, **13–15** and **18–27**) and were evaluated against the *in vitro* α -amylase inhibitory activity. All the evaluated analogs were found to be active and shown good inhibitory potentials with IC₅₀ values ranging between 14.8 ± 0.15 to 1.02 ± 0.03 μ M and compared with standard acarbose having IC₅₀ value 1.37 ± 0.26 μ M as shown in Table 1. Three analogs in series A: **13**, **14** and **15** shown excellent inhibitory potential with IC₅₀ values 1.11 ± 0.07, 1.14 ± 0.17 and 1.07 ± 0.01, and also six analogs in

the series B: **20**, **21**, **24**, **25**, **26**, and **27** shown excellent inhibitory potential with IC₅₀ values 1.21 ± 0.03, 1.33 ± 0.09, 1.17 ± 0.01, 1.05 ± 0.02, 1.61 ± 0.04 and 1.02 ± 0.03 μ M respectively, when compared with the standard acarbose with IC₅₀ value 1.37 ± 0.26 μ M. The remaining all other analogs showed good to moderate inhibitory potential. A limited structure–activity relationship could be established by analyzing at the different substituents on the theophylline ring part on 7th position.

The inactivity of the derivatives from series A compounds **4** and **5** highlights the importance of the theophylline moiety for the α -amylase activity IC₅₀ values 12.9 ± 0.17 and 14.8 ± 0.15 μ M such as introduced simple alkyl chain at 7th position. Then later, we introduced carbonyl unit along with different hydroxyl alkyl acetylene chains such as ester analogs **10**, **9** and **7** and shown increasing activity with IC₅₀ values 4.32 ± 0.04, 4.01 ± 0.14 and 3.27 ± 0.04 μ M having 4-fold potent than the **4** and **5** and although they are less active than standard drug. Next in that way, introduced carbonyl unit along with different amine alkyl acetylene chains such as amide analogs **8**, **14**, **13** and **15** shown excellent activity with IC₅₀ values 4.14 ± 0.09, 1.14 ± 0.17,



Scheme 2. Synthesis of theophylline containing acetylene compounds (**4–10**). Reagents and conditions: (a) K₂CO₃, DMF, 85 °C, 2 h; (b) K₂CO₃, DMF, 85 °C, 10 h; (c) K₂CO₃, DMF, rt, 10 h; (d) K₂CO₃, DMF, 80 °C, 12 h; (e) HATU, DIPEA, DMF, rt, 12 h; (f & g) DCC, DMAP, DCM, rt, 16 h.



Scheme 3. Synthesis of theophylline containing acetylene compounds (**13–15**). Reagents and conditions: (a) HATU, DIPEA, DCM, rt, 16 h; (b) LiOH·H₂O, THF:H₂O, rt, 4 h; (c) K₂CO₃, DMF, rt, 10 h; (d) K₂CO₃, DMF, 80 °C, 12 h; (e) T₃P, TEA, DMF, rt, 16 h.

1.11 ± 0.07 and 1.07 ± 0.01 μM, respectively, having more potent than the all compounds in this series-A but except compound **8** less active than standard. Finally, in this series we observed, carbonyl having oxygen or nitrogen introduced alkyl chains shown increasing activity and also here the length of the chain also plays a key role against the inhibitory activity.

Considering in the series **B**, the results for compounds **18–27** were proposed in order to evaluate the importance of the amino acid group in activity by adding it with a carbonyl group at the 7th position of theophylline moiety. Moreover, the conjugation of a carbonyl with the amino acid unit could change the conformation of these molecules, in which relative planarity could be achieved. In this series **B**, introduced amino acid unit by linking carbonyl is fixed at amine group and different acetylene chains at carboxyl group, and also shown variant groups at α-carbon. Firstly, introduced different hydroxyl alkyl acetylene chains having simple neutral non polar amino acids such as ester analogs glycine **18** (2.84 ± 0.13 μM), alanine **19**, **22** and **23** (2.62 ± 0.02, 2.09 ± 0.11 and 2.27 ± 0.09 μM), valine **20** and **24** (1.21 ± 0.03 and 1.17 ± 0.01 μM), and phenylalanine **21** and **25** (1.33 ± 0.09 and 1.05 ± 0.02 μM), respectively in which some of the analogs **20**, **21**, **24** and **25** shown excellent activity than the standard (1.37 ± 0.26 μM) and the other analogs **18**, **19**, **22** and **24** shown less active than standard. In contrast, amide analogs such as amino acid having different amine alkyl acetylene chains compounds **26** and **27** (1.61 ± 0.04 and 1.02 ± 0.03 μM) in which analog **27** shown highest activity and analog **26** shown good activity as compared with the standard. From these results, it is clearly suggesting that the α-amylase inhibition activity depends on the substitution of α-carbon and with an increasing order C–H < C–CH₃ < C–(CH₃)₂ < C–CH₂C₆H₅.

The substitutions of the carbonyl, amino acid unit and length of the acetylene chain are beneficial to the α-amylase activity, in this case, with the highest potency analogs are observed for **13–15** and **20**, **21** and **24–27** are more active than standard.

2.2.2. Molecular docking

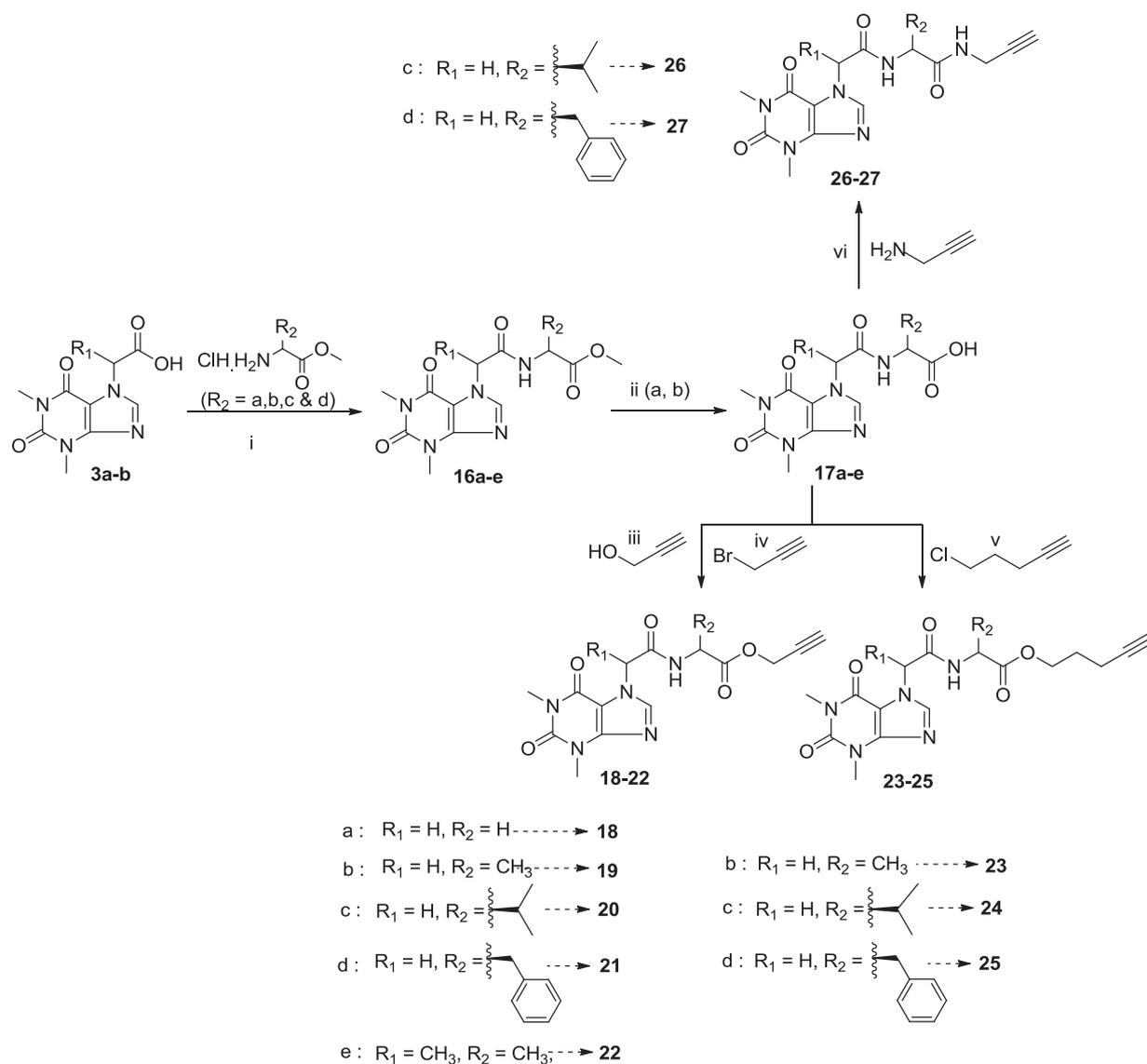
The molecular docking studies were carried out of these synthesized

compounds against α-amylase enzyme (PDB ID: [4GQR](#)) in order to predict the possible binding interactions between the ligands and enzymes. Compound **25** (docking score = –6.49) the most potent compound in the series-**B**, purine ring of 5 and 6-rings showed pi-pi interactions with Trp59 (3.13 Å) and formed H-bond interactions between the carbon atom and carboxylate oxygen atom of Asp300 (3.13 Å) and side chain carbon atom and oxygen atom of Glu233 (3.2 Å). Whereas, other residues Trp58, His101, Thr163, Leu165, Asp197, and Ala307 showed hydrophobic interactions. The acetylene chain in compound **25** showed Vander Waals clashes with Ile235 and Glu233 residues.

In series-**B**, compound **27** (docking score = –6.46) showed good binding interactions with the active site of 4GQR of α-amylase protein. The compound shows strong H-bond interactions as a donor with His305 with distance 2.68 Å between the carbon atom of acetylene side chain with the oxygen atom of Histidine residue. Trp59 formed H-pi interactions with N-CH₃ of purine ring and pi-H interactions with Leu165, Ala307, and Gly308 residues. The hydrophobic interactions are shown with Asp300, Tyr62, and Trp58 residues.

Compound **14** (docking score –6.86) in the series-A showed good binding interactions with the active site of amino acid residues. The compound forms pi-H interactions with 5-ring of purine with Trp59 residue. It also forms H-donor interactions with Tyr62 (3.15) and H-acceptor interactions with Gln63. The hydrophobic interactions are formed with residues Asp197, Leu165, Ala198, Trp58 and Ala307. In case of compound **21** also showed similar pi-pi interaction with Trp59 with distance 3.71 but additional hydrogen bond interaction with NH of the ligand with ASP300 with distance 2.78 and exhibits hydrophobic interactions with Trp58, Leu162, Leu165, and Ala198. In addition, compound **21** has dock score and binding energy values are –6.51 and –3.6 kcal/mol. The acetylene group shows Vander Waals clashes with Arg195 and Glu233 residues.

Similarly, all the compounds have exhibited binding interactions with the active site residues. From the docking studies, it could be revealed that acetylene group has formed Vander Waals interactions, side chain containing amide or carboxylate groups –NH and C=O forms H-bond interactions and purine containing 6 and 5 membered rings shows pi-H or pi-pi interactions.



Scheme 4. Synthesis of theophylline containing amino acid acetylene compounds (18–27). Reagents and conditions: (i) HATU, DIPEA, CH_2Cl_2 , rt, 16 h; (ii) (a) $LiOH \cdot H_2O$, $THF:H_2O$, rt, 4 h & (b) Pd/C, MeOH, H_2 , 60 Psi, rt, 8 h; (iii) DCC, DMAP, CH_2Cl_2 , rt, 16 h; (iv) K_2CO_3 , DMF, rt, 10 h; (v) K_2CO_3 , DMF, $80^\circ C$, 12 h; (vi) T_3P , TEA, DMF, rt, 16 h.

The co-crystal ligand, Myricetin assumed to occupy the α -amylase and established hydrogen bonding interactions with Gln63, Asp197, and Asp300. All the docked compounds superimposed well with the co-crystal ligand Myricetin and all common interactions (Gln63, Asp197, Asp300 and Leu162, Leu165, Trp59, and Trp58) were consistent with the ones in the crystal complexes of ligand binding domain (LBD) of α -amylase, as shown in Fig. 3. The ligand-protein interactions were illustrated in Table 2.

2.2.3. In vivo anti-diabetic activity

2.2.3.1. HFD-STZ induced diabetic model [29]. The *in vitro* α -amylase inhibitory activity and docking studies have revealed that the compounds 25 and 27 showed potential inhibition activity against the enzyme. Hence, these two compounds 25 and 27 were evaluated for their *in vivo* anti-diabetic effect using HFD-STZ-induced diabetic model. HFD along with the low dose of STZ produced impairment of insulin secretion which was an important characteristic of human insulin-deficient type 2 Diabetes Mellitus (T2D). Fig. 4 showed the significant increase in plasma glucose level (PGL) in HFD-STZ-Control treated group compared to the NDC group. The pioglitazone and compounds 25 and 27 were administered at the dose of 10 mg/kg, *b.w p.o* in 1% Sod

CMC in HFD-STZ rats. The PGL levels on the 7th day were significantly decreased in pioglitazone-treated rats when compared to HFD-STZ-Control group ($P < 0.01$) interestingly, compound 25 has shown moderate significance ($P < 0.05$) and whereas compound 27 has shown any significant effect when compared to diabetic control. However, the PGL levels recorded on 14th day showed that treatment with pioglitazone and compound 25 has significant ($P < 0.001$; $P < 0.01$) effect evident by the subsequent decrease in the PGL levels compared to HFD-STZ-Control group. But compound 27 has shown a mild significant effect ($P < 0.05$) when compared to diabetic control on the 14th day. Nevertheless, the antidiabetic activity of selected compound 25 and 27 have shown moderate activity when compared with known standard drug pioglitazone. Thus, administration of selected compounds 25 and 27 have shown significant improvement in the PGL levels.

2.2.4. Histological analysis of pancreas and liver samples

The haematoxylin and eosin staining were used to analyze the histological aberrations in pancreas and liver samples. The islets of the pancreas were observed for the appearance of acinar cells, apoptosis, and degeneration of β -cells in control, diabetic control, standard,

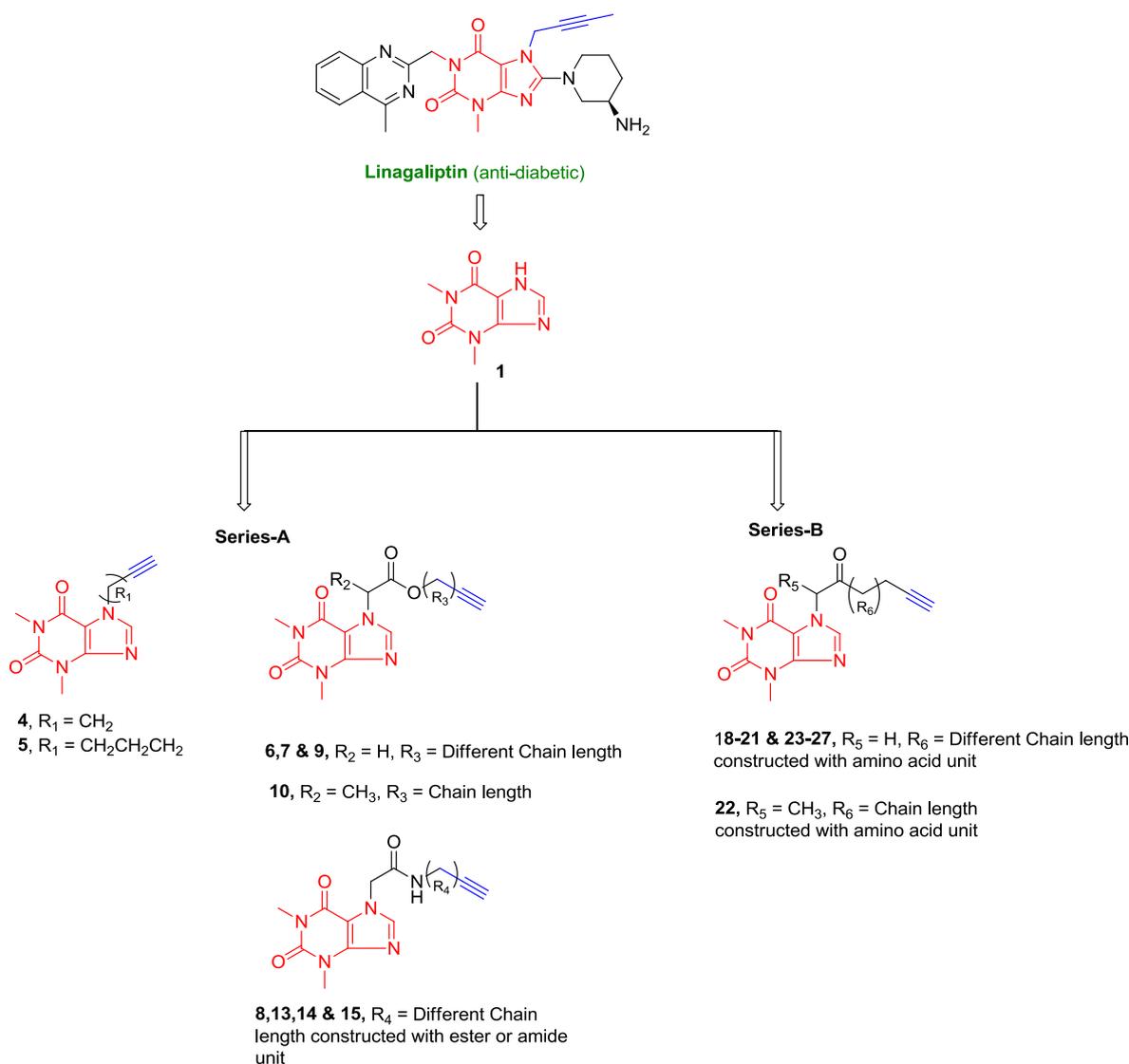


Fig. 2. Design strategy of the novel theophylline series A and B.

compound **25** and **27** groups respectively (Fig. 5A–D). The photomicrographs of pancreas tissue samples of diseased animals showed degeneration and reduced number of pancreatic islet cells. In non-diabetic control and pioglitazone group showed normal pancreatic islet cells compared with HFD-STZ-control group (shown in Fig. 5C). Treatment with compound **25** has shown normal histology and proliferation of pancreatic islet cells is seen interestingly, compound **27** treated rat pancreas showed recovery and moderate degenerative changes in pancreatic islets compared with HFD-STZ-Control group (shown in Fig. 5D and E).

In the diabetic liver histopathology multifocal necrosis in the central lobular region along with peribiliary inflammation with infiltration of inflammatory cells was noticed in diabetic control. In non-diabetic control and standard groups, proper arrangement and normal structure of hepatic cells were noticed. After treatment with test compounds **25** and **27**, the inflammation hepatocytes were significantly alleviated, and liver histology gradually returns to the normal state, especially in standard and compound **25** groups (Fig. 6A–E).

From these observations, it indicates that the administration compound **25** and **27** showed partial restoration in the liver and pancreatic cells. Collectively, these results suggest that the investigation of the anti-hyperglycemic mechanisms of compound **25** and **27** compared to standard drug and ameliorated the degree of disease and decreased

proliferation is seen in pancreas and liver tissues.

This study has demonstrated that in the combination of HFD and the low dose of STZ is sufficient to induce type-2 diabetic in rats. The present work is the comprehensively study the effect of theophylline containing acetylene derivatives on T2DM. The experimental study has demonstrated that the theophylline containing acetylene derivatives have anti-hyperglycemic property and further optimization of these leads could discover a novel anti-diabetic agent.

3. Conclusion

In conclusion, two series of novel theophylline containing acetylene compounds were synthesized and evaluated for their *in vitro* α -amylase inhibitory activity and *in vivo* anti-diabetic activity by HFD-STZ induced diabetic model. Molecular docking simulation study of the compounds has been done to study interactions and binding mode of synthesized compounds on α -amylase enzyme (PDB ID: 4GQR). Compounds **13–15**, **20**, **21**, and **24–27** have shown excellent α -amylase inhibitory activity. Structure-activity relationships showed that the enzyme α -amylase inhibition of compounds was dependent on residues on α -carbon and exhibited with increasing order of activity C–H < C–CH₃ < C–(CH₃)₂ < C–CH₂C₆H₅. Further *in vivo* HFD-STZ-induced diabetic models showed that compound **25** showed significant anti-diabetic

Table 1
 α -Amylase activity of synthesized theophylline acetylene analogs.

| No | Structure | IC ₅₀ (μ M) | No | Structure | IC ₅₀ (μ M) |
|----------|-----------|-----------------------------|----------|-----------|-----------------------------|
| 4 | | 12.9 \pm 0.17 | 18 | | 2.84 \pm 0.13 |
| 5 | | 14.8 \pm 0.15 | 19 | | 2.62 \pm 0.02 |
| 6 | | - | 20 | | 1.21 \pm 0.03 |
| 7 | | 3.27 \pm 0.04 | 21 | | 1.33 \pm 0.09 |
| 8 | | 4.14 \pm 0.09 | 22 | | 2.09 \pm 0.11 |
| 9 | | 4.01 \pm 0.14 | 23 | | 2.27 \pm 0.09 |
| 10 | | 4.32 \pm 0.04 | 24 | | 1.17 \pm 0.01 |
| 13 | | 1.11 \pm 0.07 | 25 | | 1.05 \pm 0.02 |
| 14 | | 1.14 \pm 0.17 | 26 | | 1.61 \pm 0.04 |
| 15 | | 1.07 \pm 0.01 | 27 | | 1.02 \pm 0.03 |
| Acarbose | | 1.37 \pm 0.26 | Acarbose | | 1.37 \pm 0.26 |

*All the values are expressed as mean \pm SD, (n = 3).

activity and compared with a standard drug. Thus, based on *in silico* molecular docking and experimental studies, it can be suggested that theophylline containing acetylenes should be further explored for the anti-hyperglycemic mechanism.

4. Experimental section

4.1. Chemistry and chemical methods

Starting materials were obtained from commercial suppliers and used without further purification. The ¹H NMR and ¹³C NMR spectra were taken on a VNMRS 400 MHz spectrometer using the solvent (CDCl₃ 7.26 ppm and 77.0 ppm, DMSO-*d*₆ 2.49 ppm 39.7 ppm) and TMS used as an internal standard. Chemical shifts are given in δ ppm and

coupling constant (J) is given in Hz. IR spectra were recorded on a Perkin Elmer FTIR 1600 spectrometer for samples in KBr discs. The mass spectra were recorded on QSTAR XL hybrid MS/MS system (Applied Biosystems, USA) under ESI. All compounds were purified by flash chromatography (FC) was performed using on silica gel (100–200 mesh) and all the reactions were monitored by Thin-Layer Chromatography (TLC) on Silica Gel 60 F254 plates (VWR, Darmstadt); visualization by UV detection at 254 nm.

4.2. Synthesis

The synthesis of intermediate compounds (2a-b), (3a-b) and final analogues (4–10) reported by the following literature [28].

4.2.1. Methyl 3-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)-2,2-dimethylpropanoate (11)

To a stirred solution of compound **3a** (3 g, 12.60 mmol) and 3-amino-2,2-dimethylpropanoate hydrochloride (3.1 g, 18.90 mmol) in dichloromethane (40 mL), HATU (7.18 g, 18.90 mmol) and DIPEA

(6.5 mL, 37.81 mmol) were added at room temperature. The reaction mixture was stirred for 16 h. On completion of the reaction as monitored by TLC, the reaction mixture was poured into water and extracted with dichloromethane. The organic extracts were washed with aq. NaHCO₃ solution and aq. KHSO₄ solution, dried over anhydrous

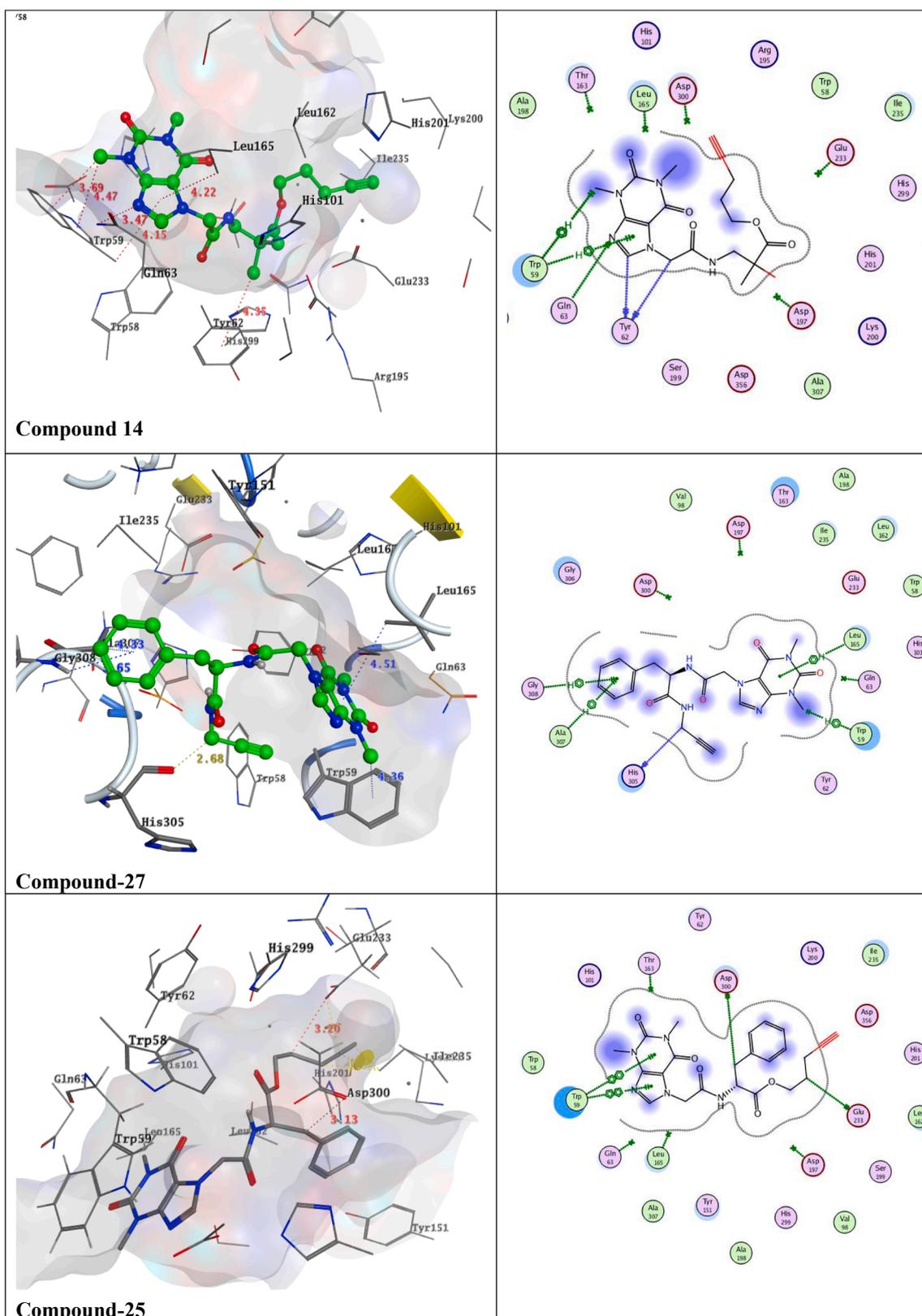


Fig. 3. Binding interactions of compounds with α -amylase enzyme (PDB ID: 4GQR).

Table.2

Docking score and Binding interactions of compounds with α -amylase enzyme into the active site of 4GQR.

| Interactions report | | | | | |
|---------------------|---------------|------------|----------------|-------------|-------------|
| Comp. | Docking score | Ligand | Receptor | Interaction | Distance |
| 04 | -3.99 | C 13 | OE2 GLU 233 | H-donor | 3.15 |
| | | C 14 | OE1 GLU 233 | H-donor | 3.30 |
| | | O 11 | NE2 HIS 299 | H-acceptor | 2.79 |
| | | C 13 | 5-ring HIS 299 | H-pi | 4.41 |
| 05 | -4.59 | C 18 | OE1 GLU 233 | H-donor | 3.56 |
| | | O 15 | NE2 HIS 101 | H-acceptor | 2.85 |
| 06 | -4.54 | C 13 | OE1 GLU 233 | H-donor | 3.35 |
| | | O 10 | NE2 HIS 101 | H-acceptor | 2.90 |
| 07 | -4.64 | C 19 | 6-ring TRP 59 | H-pi | 4.15 |
| | | C 19 | 6-ring TRP 59 | H-pi | 4.21 |
| 08 | -5.51 | C 13 | OE2 GLU 233 | H-donor | 3.29 |
| | | C 14 | OD2 ASP 197 | H-donor | 2.75 |
| | | 5-ring | NE2 HIS 101 | pi-cation | 3.86 |
| 09 | -4.93 | C 13 | O TYR 62 | H-donor | 3.06 |
| | | O 10 | NE2 HIS 101 | H-acceptor | 3.55 |
| | | 6-ring | CB TYR 62 | pi-H | 3.93 |
| 10 | -4.90 | C 8 | OD2 ASP 197 | H-donor | 3.32 |
| | | C 12 | O TYR 62 | H-donor | 2.66 |
| | | 6-ring | CD1 LEU 165 | pi-H | 4.41 |
| 13 | -6.01 | C 38 | 6-ring TRP 59 | H-pi | 3.63 |
| | | 5-ring | CB TRP 59 | pi-H | 4.20 |
| 14 | -6.86 | C 5 | O TYR 62 | H-donor | 3.30 |
| | | C 39 | O TYR 62 | H-donor | 3.15 |
| | | N 41 | NE2 GLN 63 | H-acceptor | 3.47 |
| | | C 44 | 5-ring TRP 59 | H-pi | 4.47 |
| | | C 44 | 6-ring TRP59 | H-pi | 3.69 |
| | | 5-ring | CB TRP 59 | pi-H | 4.15 |
| 15 | -5.88 | C 5 | O TYR 62 | H-donor | 3.30 |
| | | C 39 | O TYR 62 | H-donor | 3.15 |
| | | N 41 | NE2 GLN 63 | H-acceptor | 3.47 |
| | | C 44 | 5-ring TRP 59 | H-pi | 4.47 |
| | | C 44 | 6-ring TRP 59 | H-pi | 3.69 |
| | | 5-ring | CB TRP 59 | pi-H | 4.15 |
| 18 | -5.55 | 6-ring | 5-ring TRP 59 | pi-pi | 3.82 |
| | | 6-ring | 5-ring TRP 59 | pi-pi | 3.94 |
| 19 | -5.15 | O 12 | NE2 HIS 201 | H-acceptor | 3.07 |
| | | O 31 | NE2 HIS 201 | H-acceptor | 3.28 |
| 20 | -4.97 | O 11 | NE2 HIS 201 | H-acceptor | 2.89 |
| 21 | -6.12 | N 13 | OD2 ASP 300 | H-donor | 2.78 |
| | | 6-ring | 5-ring TRP 59 | Pi-pi | 3.71 |
| 22 | -5.05 | 5-ring | CD1 LEU 162 | pi-H | 4.21 |
| | | 6-ring | CD1 LEU 162 | pi-H | 4.08 |
| | | 5-ring | CD1 LEU 162 | pi-H | 4.25 |
| 23 | -5.13 | 6-ring | NE2 HIS 201 | pi-cation | 4.58 |
| 24 | -5.58 | C 18 | 5-ring TRP 59 | H-pi | 4.21 |
| 25 | -6.49 | C 17 | OD1 ASP 300 | H-donor | 3.13 |
| | | C 36 | OE1 GLU 233 | H-donor | 3.20 |
| | | 5-ring | 5-ring TRP 59 | pi-pi | 3.54 |
| | | 6-ring | 5-ring TRP 59 | pi-pi | 3.94 |
| 26 | -5.54 | C 27 | 6-ring TRP 59 | H-pi | 4.38 |
| | | C 27 | 6-ring TRP 59 | H-pi | 4.38 |
| 27 | -6.46 | C 38 | O HIS 305 | H-donor | 2.68 |
| | | C 25 | 6-ring TRP 59 | H-pi | 4.36 |
| | | 6-ring | CD1 LEU 165 | pi-H | 4.51 |
| | | 6-ring | CA ALA 307 | pi-H | 4.33 |
| | | 6-ring | N GLY 308 | pi-H | 4.51 |
| | | MYC | -14.1194 | O 23 | OD2 ASP 300 |
| O 24 | OD2 ASP 197 | H-donor | 3.15 | | |
| O 26 | OD2 ASP 197 | H-donor | 2.78 | | |
| O 21 | NE2 GLN 63 | H-acceptor | 3.19 | | |
| 6-ring | CD1 LEU 165 | pi-H | 4.40 | | |

4.3. General procedure for the synthesis of compounds (16a-e)

To a stirred solution of acid compounds **3a-b** (1.0 equiv) and their corresponding glycine methyl ester hydrochloride (**a**), D,L-alanine methyl ester hydrochloride (**b**), L-valanine ethyl ester hydrochloride (**c**) and L-phenyl alanine benzyl ester hydrochlorid (**d**) (1.1 to 1.2 equiv) in dichloromethane was added HATU (1.2 to 1.5 equiv), DIPEA (3.0 equiv) at room temperature. The reaction mixture was stirred at room temperature for 16 h. On completion of the reaction as monitored by TLC, the reaction mixture was poured into water and extracted with dichloromethane. The organic extracts was washed with aq. NaHCO₃ solution and aq. KHSO₄ solution, dried over anhydrous Na₂SO₄, filtered and evaporated under reduced pressure to afford the desired products (**16a-e**) as off-white solids.

4.3.1. Methyl 2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H-yl)acetamido) acetate (**16a**)

Compound **3a** (2 g, 8.40 mmol) and glycine methyl ester hydrochloride (1.26 g, 10.081 mmol), HATU (4.78 g, 12.60 mmol) and DIPEA (4.4 mL, 25.21 mmol) in dichloromethane (40 mL) were taken to afford 2.1 g (81%) of **16a** as a white solid. ¹H NMR (400 MHz, DMSO-d₆): δ 8.69 (t, $J = 5.2$ Hz, 1H), 8.03 (s, 1H), 5.06 (s, 2H), 3.91 (d, $J = 5.6$ Hz, 2H), 3.63 (s, 3H), 3.44 (s, 3H), 3.20 (s, 3H); m/z (ES)⁺: 310.03 [M + H]⁺.

4.3.2. Methyl 2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H-yl)acetamido)propanoate (**16b**)

Compound **3a** (3 g, 12.60 mmol) and D,L-alanine methyl ester hydrochloride (2.11 g, 15.12 mmol), HATU (7.18 g, 18.90 mmol) and DIPEA (6.6 mL, 37.81 mmol) in dichloromethane (45 mL) gave 3.96 g (97%) of **16b** as an off-white solid. ¹H NMR (400 MHz, DMSO-d₆): δ 8.74 (d, $J = 7.6$ Hz, 1H), 8.01 (s, 1H), 5.03 (s, 2H), 4.33–4.29 (m, 1H), 3.62 (d, 3H), 3.43 (s, 3H), 3.20 (s, 3H), 1.30 (d, $J = 7.6$ Hz, 3H); m/z (ES)⁺: 324.21 [M + H]⁺.

4.3.3. (S)-ethyl 2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H-yl)acetamido)-3-methylbutanoate (**16c**)

Compound **3a** (1.7 g, 7.14 mmol) and L-valanine ethyl ester hydrochloride (1.42 g, 7.85 mmol), HATU (3.2 g, 8.57 mmol) and DIPEA (2.5 mL, 14.28 mmol) in dichloromethane (42 mL) to afford 2.4 g (92%) of **16c** as an off-white solid. ¹H NMR (400 MHz, DMSO-d₆): δ 8.64 (d, $J = 8.28$ Hz, 1H), 8.01 (s, 1H), 5.08 (s, 2H), 4.20–4.04 (m, 3H), 3.43 (s, 3H), 3.19 (s, 3H), 2.09–2.01 (m, 1H), 1.19 (t, $J = 7.27$ Hz, 3H), 0.90 (t, $J = 7.02$ Hz, 6H); m/z (ES)⁺: 366.12 [M + H]⁺.

4.3.4. (S)-benzyl-2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H-yl)acetamido)-3-phenylpropanoate (**16d**)

Compound **3a** (2.0 g, 8.40 mmol) and L-phenyl alanine bezyl ester hydrochloride (2.6 g, 9.24 mmol), HATU (3.8 g, 10.08 mmol) and DIPEA (2.9 mL, 16.80 mmol) in dichloromethane (60 mL) to afford 3.8 g (95%) of **16d** as an off-white solid. ¹H NMR (400 MHz, DMSO-d₆): δ 8.87 (d, $J = 7.52$ Hz, 1H), 7.97 (s, 1H), 7.33–7.19 (m, 10H), 5.10–4.98 (m, 4H), 4.55 (q, $J = 7.78$ Hz, 1H), 3.43 (s, 3H), 3.18 (s, 3H), 3.06–2.95 (m, 2H); m/z (ES)⁺: 476.20 [M + H]⁺.

4.3.5. Methyl-2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H-yl)propanamido) propanoate (**16e**)

Compound **3b** (2 g, 7.933 mmol) and D,L-alanine methyl ester hydrochloride (1.32 g, 9.520 mmol), HATU (4.52 g, 11.90 mmol) and DIPEA (4.1 mL, 23.80 mmol) in dichloromethane (40 mL) gave 1.82 g (68%) of **16e** as a white solid. ¹H NMR (400 MHz, DMSO-d₆): δ 8.75 (d, $J = 5.2$ Hz, 1H), 8.25–8.19 (m, 1H), 5.62–5.57 (m, 1H), 4.29–4.23 (m, 1H), 3.61 (d, $J = 12.8$ Hz, 3H), 3.43 (d, $J = 2.0$ Hz, 3H), 3.21 (d, $J = 6.4$ Hz, 3H), 1.72–1.67 (m, 3H), 1.29 (dd, $J = 1.2$ Hz, 7.2 Hz, 3H); m/z (ES)⁺: 338.4 [M + H]⁺.

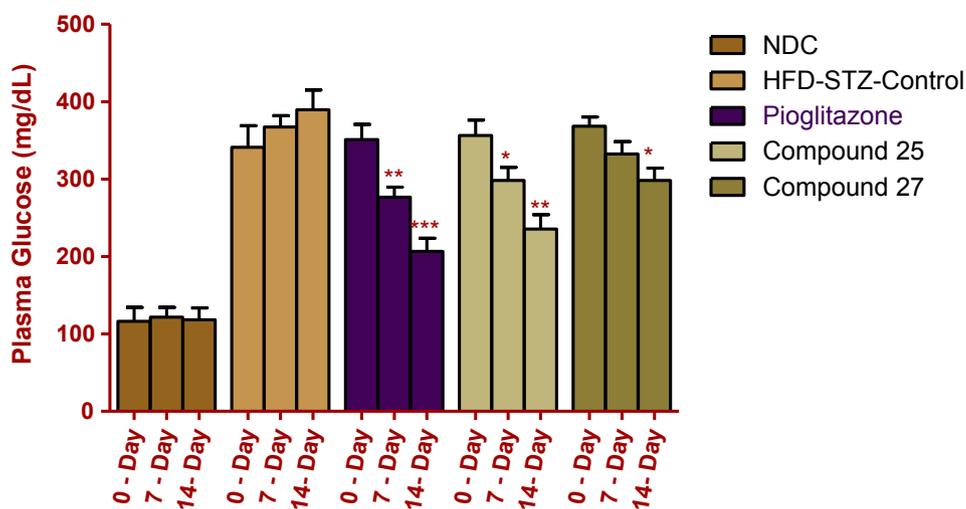


Fig. 4. In vivo anti-diabetic activity of compounds 25, 27 and standard Pioglitazone in HFD-STZ induced diabetic rats. All Data were expressed as Mean \pm SEM (n = 6) using One-Way ANOVA followed by Dunnett's multiple comparison test. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs HFD-STZ-Control. NDC: Normal rats with normal diet; HFD-STZ-Control is Diabetic Control.

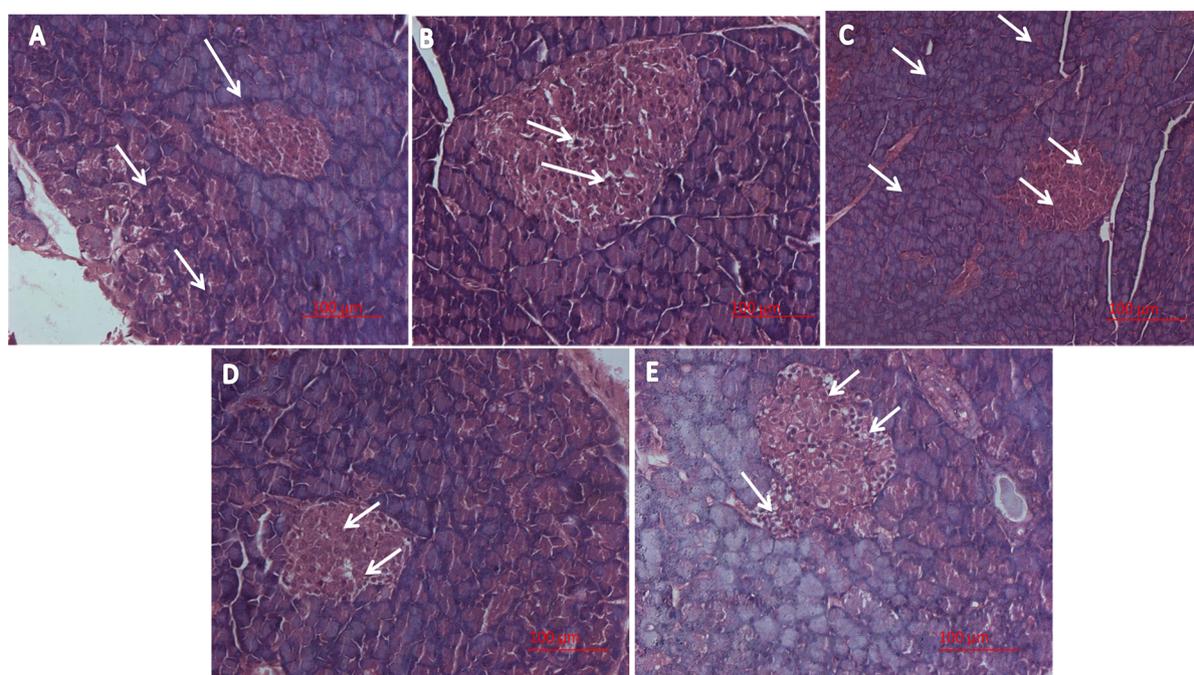


Fig. 5. Histopathological changes observed in pancreas tissue samples of HFD-STZ induced diabetic rats treated with compound-25 and compound-27. (A) Normal diabetic control group—arrows shows normal proliferation of β -cells and acinar cells; (B) Diabetic control group—arrows indicates degeneration of pancreas islet cells; (C) Pioglitazone treated group—arrow shows recovered pancreas islet cells; (D) Compound-25 treated group—arrows indicates recovered pancreas islet cells without any degeneration; (E) Compound-27 treated group—arrows indicates showing partly recovered pancreas islet cells with moderate degeneration.

4.4. General procedure for the synthesis of compounds (17a-c & 17e)

LiOH·H₂O (1.2 to 1.5 equiv) was added portion wise to a stirred solution of ester compounds 16a-c and 16e (1.0 equiv) in THF (10 vol) and H₂O (10 vol) at room temperature and the reaction mixture was stirred for 2–4 h. After completion, from the reaction mixture THF was concentrated and acidified with aqueous KHSO₄ solution and extracted with 10% methanol in dichloromethane. The combined organic extracts were dried over anhydrous Na₂SO₄, filtered and evaporated under reduced pressure to afford the desired products.

4.4.1. 2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)acetic acid (17a)

Compound 16a (2.1 g, 6.79 mmol) and LiOH·H₂O (342 mg, 8.15 mmol) in 42 mL of THF:H₂O (1:1) to afford 1.6 g (80%) of 17a as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.59 (brs, 1H), 8.57 (t,

$J = 5.2$ Hz, 1H), 8.03 (s, 1H), 5.05 (s, 2H), 3.81 (d, $J = 5.6$ Hz, 2H), 3.44 (s, 3H), 3.20 (s, 3H); m/z (ES)⁺: 296.10 [M+H]⁺.

4.4.2. 2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)propanoic acid (17b)

Compound 16b (3.96 g, 12.25 mmol) and LiOH·H₂O (610 mg, 14.70 mmol) in 78 mL of THF:H₂O (1:1) to afford 2.6 g (68%) of 17b as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.64 (brs, 1H), 8.60 (d, $J = 7.6$ Hz, 1H), 8.01 (s, 1H), 5.02 (d, $J = 2.8$ Hz, 2H), 4.26–4.19 (m, 1H), 3.43 (s, 3H), 3.20 (s, 3H), 1.29 (d, $J = 7.6$ Hz, 3H); m/z (ES)⁺: 310.03 [M+H]⁺.

4.4.3. (S)-2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)-3-methylbutanoic acid (17c)

Compound 16c (2.4 g, 6.57 mmol) and LiOH·H₂O (610 mg, 14.70 mmol) in 40 mL of THF:H₂O (1:1) to afford 1.8 g (81%) of 17c as

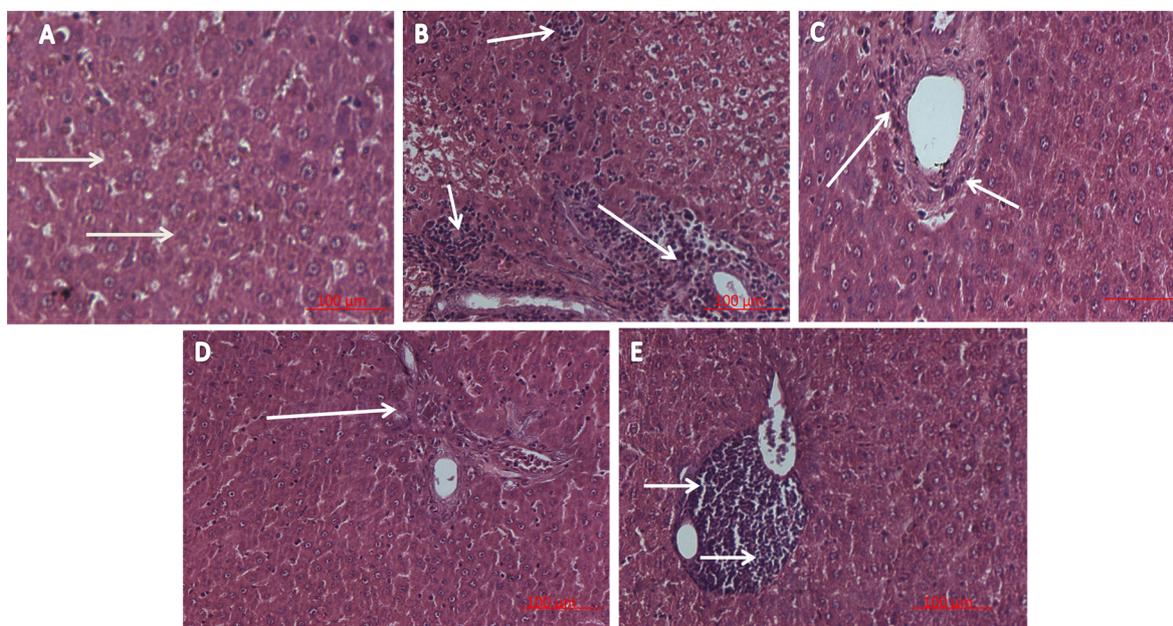


Fig. 6. Histopathological changes observed in liver tissue samples of HFD-STZ induced diabetic rats treated with compound-25 and compound-27. (A) Normal diabetic control—arrows indicating normal histological structure liver cells; (B) Diabetic control group—arrows showing Multi focal necrosis in centrilobular region along with peribiliary inflammation with infiltration of inflammatory cells; (C) Pioglitazone treated group—arrows indicate mild infiltration of inflammatory cells noticed in peribiliary region; (D) Compound-27 treated group—arrows indicating the improved structure with mild periportal infiltration of inflammatory cells; (E) Compound-25 treated group—arrows showing moderate periportal infiltration of inflammatory cells.

a white solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.51 (d, $J = 8.8$ Hz, 1H), 8.02 (s, 1H), 5.08 (s, 2H), 4.19–4.16 (m, 1H), 3.43 (s, 3H), 3.19 (s, 3H), 2.11–2.02 (m, 1H), 0.91–0.88 (m, 6H); m/z (ES) $^+$: 338.14 [M+H] $^+$.

4.4.4. 2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)propanamido)propanoic acid (17e)

Compound **16e** (1.4 g, 4.152 mmol) and $\text{LiOH}\cdot\text{H}_2\text{O}$ (200 mg, 4.983 mmol) in 28 mL of $\text{THF}:\text{H}_2\text{O}$ (1:1) to afford 1 g (74%) of **17e** as a white solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 12.59 (brs, 1H), 8.65–8.60 (m, 1H), 8.22 (d, $J = 17.2$ Hz, 1H), 5.64–5.55 (m, 1H), 4.24–4.14 (m, 1H), 3.43 (s, 3H), 3.22 (s, 3H), 1.71–1.66 (m, 3H), 1.28 (d, $J = 7.2$ Hz, 3H); m/z (ES) $^+$: 324.06 [M+H] $^+$.

4.5. General procedure for the synthesis of compounds (17d)

4.5.1. (S)-2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)-3-phenylpropanoic acid (17d)

Pd/C (500 mg) was added portion wise to a stirred solution of compound **16d** (2 g, 4.20 mmol) in EtOH (100 mL) under H_2 atmosphere at 60 Psi for 8 h. After completion, reaction mixture was filtered on a celite bed and filtrate was evaporated under reduced pressure to afford 1.5 g (92%) of **17d** as an off-white solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.65 (d, $J = 7.78$ Hz, 1H), 7.97 (s, 1H), 7.31–7.19 (m, 5H), 5.0 (d, $J = 6.02$ Hz, 2H), 4.44 (q, $J = 8.03$ Hz, 1H), 3.43 (s, 3H), 3.20 (s, 3H), 3.06–3.01 (m, 1H), 2.94–2.88 (m, 1H); m/z (ES) $^+$: 386.20 [M+H] $^+$.

4.6. General procedure for the synthesis of compounds (18, 19 & 22)

DCC (1.5 equiv) was added to a stirred and cooled (0°C) solution of acid compounds **17a-b** and **17e** (1.0 equiv) and propargyl alcohol (2.0 equiv) in dichloromethane (30 vol). After 5 min, DMAP (0.4 equiv) was added and the solution was allowed to stirred at room temperature for 16 h. After completion, the reaction mixture was added to water and extracted with 10% methanol in dichloromethane. The combined organic extracts was dried with anhydrous Na_2SO_4 , filtered and evaporated under reduced pressure. The crude product was triturated with

acetone and diethyl ether, filtered to afford the desired products.

4.6.1. Prop-2-yn-1-yl-2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)acetate (18)

Compound **17a** (1.6 g, 5.42 mmol), propargyl alcohol (0.62 mL, 10.84 mmol), DCC (1.67 g, 8.13 mmol) and DMAP (264 mg, 2.168 mmol) in 42 mL of dichloromethane to afford 1.56 g (86%) of **18** as a white solid. IR (KBr): $\nu_{\text{max}}/\text{cm}^{-1} = 3281, 3111, 2953, 2130, 1754, 1708, 1659, 1548, 1474, 1457, 1384, 1376, 1217, 1190, 1029, 947, 763, 748$; ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.73 (t, $J = 5.6$ Hz, 1H), 8.03 (s, 1H), 5.07 (s, 2H), 4.73 (d, $J = 2.4$ Hz, 2H), 3.95 (d, $J = 6.0$ Hz, 2H), 3.57 (t, $J = 2.4$ Hz, 1H), 3.44 (s, 3H), 3.20 (s, 3H); ^{13}C NMR (400 MHz, $\text{DMSO}-d_6$): δ 169.37, 167.27, 154.80, 151.44, 148.33, 144.14, 106.79, 78.57, 78.47, 52.66, 48.26, 33.79, 29.88, 27.90; HRMS (ESI): calcd for $\text{C}_{14}\text{H}_{16}\text{N}_5\text{O}_5$ [M+H] $^+$: 334.1151, found: 334.1157; Anal. calcd for $\text{C}_{14}\text{H}_{15}\text{N}_5\text{O}_5$: C, 50.45; H, 4.54; N, 21.01%; Found: C, 50.48; H, 4.56; N, 21.02%; m/z (ES) $^+$: 334.11 [M+H] $^+$.

4.6.2. Prop-2-yn-1-yl 2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)propanoate (19)

Compound **17b** (1.8 g, 5.82 mmol), propargyl alcohol (0.67 mL, 11.64 mmol), DCC (1.80 g, 8.73 mmol) and DMAP (284 mg, 2.33 mmol) in 54 mL of dichloromethane to afford 1.6 g (79%) of **19** as a white solid. Mp: $150\text{--}156^\circ\text{C}$; IR (KBr): $\nu_{\text{max}}/\text{cm}^{-1} = 3289, 3119, 2949, 2138, 1750, 1702, 1649, 1542, 1476, 1467, 1376, 1352, 1214, 1192, 1023, 942, 748$; ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.79 (d, $J = 6.8$ Hz, 1H), 8.01 (s, 1H), 5.04 (d, $J = 3.6$ Hz, 2H), 4.71 (d, $J = 2.8$ Hz, 2H), 4.35–4.32 (m, 1H), 3.56 (s, 1H), 3.43 (s, 3H), 3.20 (s, 3H), 1.31 (d, $J = 7.2$ Hz, 3H); Anal. calcd for $\text{C}_{15}\text{H}_{17}\text{N}_5\text{O}_5$: C, 51.87; H, 4.93; N, 20.16%; Found: C, 51.89; H, 4.98; N, 20.15%; m/z (ES) $^+$: 348.3 [M+H] $^+$.

4.6.3. Prop-2-yn-1-yl-2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)propanamido)propanoate (22)

Compound **17e** (1 g, 3.094 mmol), propargyl alcohol (0.35 mL, 6.189 mmol), DCC (0.95 g, 4.64 mmol) and DMAP (151 mg, 1.237 mmol) in 30 mL of dichloromethane to afford 0.82 g (74%) of **22**

as a white solid. Mp: 155–160 °C; IR (KBr): $\nu_{\max}/\text{cm}^{-1} = 3324, 2928, 2850, 1746, 1705, 1666, 1625, 1573, 1473, 1449, 1435, 1311, 1242, 1213, 1153, 1087, 994, 748$; $^1\text{H NMR}$ (400 MHz, DMSO- d_6): δ 8.80 (d, $J = 6.8$ Hz, 1H), 8.25–8.19 (m, 1H), 5.61–5.58 (m, 1H), 4.72–4.68 (m, 2H), 4.31–4.27 (m, 1H), 3.58–3.53 (m, 1H), 3.43 (s, 3H), 3.22 (s, 3H), 1.72–1.67 (m, 3H), 1.31 (d, $J = 7.6$ Hz, 3H); $^{13}\text{C NMR}$ (400 MHz, DMSO- d_6): δ 171.83, 169.85, 154.94, 151.34, 148.54, 142.24, 106.36, 78.42, 78.34, 54.92, 52.74, 48.23, 29.87, 28.01, 18.50, 16.95; HRMS (ESI): calcd for $\text{C}_{16}\text{H}_{19}\text{N}_5\text{O}_5\text{Na}$ [M + Na] $^+$: 384.1284, found: 384.1283; Anal. calcd for $\text{C}_{16}\text{H}_{19}\text{N}_5\text{O}_5$: C, 53.18; H, 5.30; N, 19.38%; Found: C, 53.20; H, 5.37; N, 19.39%; m/z (ES) $^+$: 362.4 [M + H] $^+$.

4.7. General procedure for the synthesis of compounds (20 & 21)

To a stirred solution of compounds **17c-d** (1.0 eq) in DMF, K_2CO_3 (1.2 to 1.3 eq) was added at room temperature. After 20 min, propargyl bromide (2.0 eq) was added to the reaction mixture at room temperature and stirring was continued at room temperature for 12 h. As monitored by TLC, the reaction mass was added to water and a solid precipitate was formed that was filtered and dried to afford the desired solid compounds.

4.7.1. (S)-prop-2-yn-1-yl 2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)-3-methylbutanoate (20)

Compound **17c** (150 mg, 0.44 mmol), propargyl bromide (0.076 mL, 0.89 mmol), K_2CO_3 (71 mg, 0.57 mmol) in 5 mL of DMF to afford 70 mg (54%) of **20** as an off-white solid. Mp: 165–170 °C; IR (KBr): $\nu_{\max}/\text{cm}^{-1} = 3284, 3246, 3117, 2964, 2127, 1741, 1649, 1553, 1466, 1372, 1224, 1200, 1142, 1027, 964, 746, 691$; $^1\text{H NMR}$ (400 MHz, DMSO- d_6): δ 8.72 (d, $J = 6.72$ Hz, 1H), 8.01 (s, 1H), 5.09 (s, 2H), 4.76 (s, 2H), 4.26–4.23 (m, 1H), 3.59 (s, 1H), 3.43 (s, 3H), 3.19 (s, 3H), 2.08 (brs, 1H), 0.92 (brs, 6H); $^{13}\text{C NMR}$ (400 MHz, DMSO- d_6): δ 170.52, 166.53, 154.30, 150.96, 147.79, 143.67, 106.36, 77.84, 57.27, 52.08, 47.75, 30.34, 29.37, 27.40, 18.72, 17.99; HRMS (ESI): calcd for $\text{C}_{17}\text{H}_{22}\text{N}_5\text{O}_5$ [M + H] $^+$: 376.1621, found: 376.1636; Anal. calcd for $\text{C}_{17}\text{H}_{22}\text{N}_5\text{O}_5$: C, 54.39; H, 5.64; N, 18.66%; Found: C, 54.42; H, 5.68; N, 18.70%; m/z (ES) $^+$: 376.42 [M + H] $^+$.

4.7.2. (S)-prop-2-yn-1-yl 2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)-3-phenylpropanoate (21)

Compound **17d** (200 mg, 0.51 mmol), propargyl bromide (0.093 mL, 1.03 mmol), K_2CO_3 (93 mg, 0.61 mmol) in 5 mL of DMF to afford 200 mg (91%) of **21** as an off-white solid. Mp: 178–180 °C; IR (KBr): $\nu_{\max}/\text{cm}^{-1} = 3310, 3227, 3104, 2951, 2127, 1739, 1706, 1650, 1546, 1471, 1368, 1208, 1230, 1032, 969, 719, 698$; $^1\text{H NMR}$ (400 MHz, DMSO- d_6): δ 8.81 (d, $J = 7.2$ Hz, 1H), 7.94 (s, 1H), 7.27–7.19 (m, 5H), 5.0 (d, $J = 6.0$ Hz, 2H), 4.68 (s, 2H), 4.49 (q, $J = 6.0$ Hz, 1H), 3.55 (t, $J = 2.0$ Hz, 1H), 3.41 (s, 3H), 3.18 (s, 3H), 3.04–3.01 (m, 1H), 2.99–2.94 (m, 1H); $^{13}\text{C NMR}$ (400 MHz, DMSO- d_6): δ 170.76, 166.74, 154.75, 151.44, 148.27, 144.08, 137.0, 129.64, 128.72, 127.09, 106.76, 78.50, 78.40, 54.11, 52.84, 48.27, 37.10, 29.86, 27.89; HRMS (ESI): calcd for $\text{C}_{21}\text{H}_{22}\text{N}_5\text{O}_5$ [M + H] $^+$: 424.1621, found: 424.1629; Anal. calcd for $\text{C}_{21}\text{H}_{21}\text{N}_5\text{O}_5$: C, 59.57; H, 5.00; N, 16.54%; Found: C, 59.62; H, 5.08; N, 16.58%; m/z (ES) $^+$: 424.16 [M + H] $^+$.

4.8. General procedure for the synthesis of compounds (23, 24 & 25)

To a stirred solution of compounds **17b-d** (1.0 eq) in DMF, K_2CO_3 (1.3 eq) was added at room temperature. After 20 min, 5-chloro-1-pentyne (2.0 eq) was added to the reaction mixture at room temperature and was heated to stirred at 80 °C for 12 h. As monitored by TLC, reaction mass was cooled to 0 °C, water was added and a solid precipitate was formed that was filtered and dried to get desired products (or) the reaction mass was added to water and extracted with ethyl acetate, dried with Na_2SO_4 , filtered and concentrated under reduced

pressure. The crude product was purified by column chromatography silica gel (100–200 mesh) eluted in 3% MeOH in dichloromethane to afford the desired solid compounds.

4.8.1. Pent-4-yn-1-yl 2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)propanoate (23)

Compound **17b** (200 mg, 0.64 mmol), 5-chloropent-1-yne (0.36 mL, 1.29 mmol), K_2CO_3 (116 mg, 0.84 mmol) in 5 mL of DMF to afford 80 mg (33%) of **23** as an off-white solid. Mp: 90–93 °C; IR (KBr): $\nu_{\max}/\text{cm}^{-1} = 3275, 3094, 2940, 2122, 1738, 1698, 1650, 1556, 1461, 1370, 1264, 1217, 1150, 1023, 973, 845, 753, 688$; $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 7.69 (s, 1H), 7.33 (d, $J = 6.8$ Hz, 1H), 4.91 (s, 2H), 4.53–4.49 (m, 1H), 4.22 (t, $J = 6.4$ Hz, 2H), 3.60 (s, 3H), 3.42 (s, 3H), 2.27–2.23 (m, 2H), 1.97 (t, $J = 2.4$ Hz, 1H), 1.84 (p, $J = 6.8$ Hz, 2H), 1.42 (d, $J = 7.6$ Hz, 3H); HRMS (ESI): calcd for $\text{C}_{17}\text{H}_{22}\text{N}_5\text{O}_5$ [M + H] $^+$: 376.1621, found: 376.1634; Anal. calcd for $\text{C}_{17}\text{H}_{22}\text{N}_5\text{O}_5$: C, 54.39; H, 5.64; N, 18.66%; Found: C, 54.41; H, 5.68; N, 18.68%; m/z (ES) $^+$: 376.26 [M + H] $^+$.

4.8.2. (S)-pent-4-yn-1-yl 2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)-3-methylbutanoate (24)

Compound **17c** (150 mg, 0.44 mmol), 5-chloropent-1-yne (0.094 mL, 0.89 mmol), K_2CO_3 (71 mg, 0.57 mmol) in 5 mL of DMF to afford 130 mg (72%) of **24** as an off-white solid. Mp: 101–108 °C; IR (KBr): $\nu_{\max}/\text{cm}^{-1} = 3279, 3088, 2965, 2126, 1733, 1705, 1653, 1549, 1473, 1373, 1229, 1201, 1028, 975, 748, 694$; $^1\text{H NMR}$ (400 MHz, DMSO- d_6): δ 8.64 (d, $J = 8.28$ Hz, 1H), 8.01 (s, 1H), 5.09 (s, 2H), 4.21–4.06 (m, 3H), 3.43 (s, 3H), 3.19 (s, 3H), 2.81 (t, $J = 2.51$ Hz, 1H), 2.23 (td, $J = 2.51$ Hz, 7.02 Hz, 2H), 2.10–2.02 (m, 1H), 1.75 (p, $J = 7.02$ Hz, 2H), 0.91 (t, $J = 7.02$ Hz, 6H); $^{13}\text{C NMR}$ (400 MHz, DMSO- d_6): δ 171.12, 166.47, 154.30, 150.97, 147.79, 143.67, 106.34, 83.25, 71.61, 63.04, 57.59, 47.77, 30.14, 29.38, 27.40, 18.82, 18.03, 14.38; HRMS (ESI): calcd for $\text{C}_{19}\text{H}_{26}\text{N}_5\text{O}_5$ [M + H] $^+$: 404.1934, found: 404.1937; Anal. calcd for $\text{C}_{19}\text{H}_{25}\text{N}_5\text{O}_5$: C, 56.57; H, 6.25; N, 17.36%; Found: C, 56.60; H, 6.29; N, 17.38%; m/z (ES) $^+$: 404.32 [M + H] $^+$.

4.8.3. (S)-pent-4-yn-1-yl 2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)-3-phenylpropanoate (25)

Compound **17d** (200 mg, 0.51 mmol), 5-chloropent-1-yne (0.11 mL, 1.03 mmol), K_2CO_3 (93 mg, 0.67 mmol) in 5 mL of DMF to afford 110 mg (47%) of **25** as an off-white solid. Mp: 120–128 °C; IR (KBr): $\nu_{\max}/\text{cm}^{-1} = 3295, 3115, 2953, 1732, 1705, 1655, 1548, 1474, 1373, 1224, 1198, 1028, 973, 748, 701$; $^1\text{H NMR}$ (400 MHz, DMSO- d_6): δ 8.77 (d, $J = 7.2$ Hz, 1H), 7.95 (s, 1H), 7.26–7.19 (m, 5H), 5.0 (d, $J = 5.2$ Hz, 2H), 4.45 (q, $J = 6.4$ Hz, 1H), 4.02 (t, $J = 6.0$ Hz, 2H), 3.41 (s, 3H), 3.18 (s, 3H), 3.02–2.95 (m, 2H), 2.76 (brs, 1H), 2.08 (brs, 2H), 1.64 (brs, 2H); $^{13}\text{C NMR}$ (400 MHz, DMSO- d_6): δ 171.43, 166.67, 154.78, 151.45, 148.29, 144.13, 137.24, 129.58, 128.72, 127.05, 106.76, 83.87, 71.98, 63.72, 54.41, 48.26, 37.27, 29.86, 27.88, 27.45, 14.08; Anal. calcd for $\text{C}_{23}\text{H}_{25}\text{N}_5\text{O}_5$: C, 61.19; H, 5.58; N, 15.51%; Found: C, 61.23; H, 5.65; N, 15.56%; m/z (ES) $^+$: 452.15 [M + H] $^+$.

4.9. General procedure for the synthesis of compounds (26 & 27)

To a stirred solution of compound **17c-d** (1.0 eq) in DMF were added T_3P (2.0 eq) and TEA (3.0 eq) were added at room temperature. Then, propargyl amine (2.0 eq) was added to the reaction mixture and stirred at room temperature for 16 h. On completion of the reaction as monitored by TLC, diluted with water and extracted with ethyl acetate, dried over anhydrous Na_2SO_4 , filtered and evaporated under reduced pressure. The crude compound was purified by silica gel (100–200 mesh) eluted in 2% MeOH in dichloromethane to afford desired solid compounds.

Table 3
Composition of High Fat Diet.

| Ingredients | Diet (g/kg) |
|-------------------------|-------------|
| Powdered NPD | 365 |
| Lard | 310 |
| Casein | 250 |
| Cholesterol | 10 |
| Vitamin and mineral mix | 60 |
| dl-Methionine | 03 |
| Yeast powder | 01 |
| Sodium chloride | 01 |

4.9.1. (S)-2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)-3-methyl-N-(prop-2-yn-1-yl)butanamide (26)

Compound **17c** (200 mg, 0.59 mmol), propargyl amine (0.076 mL, 1.18 mmol), T₃P (0.38 mL, 1.18 mmol) and TEA (0.24 mL, 1.78 mmol) in 5 mL of DMF to afford 140 mg (63%) of **26** as an off-white solid. Mp: 210–212 °C; IR (KBr): $\nu_{\max}/\text{cm}^{-1}$ = 3274, 3118, 3087, 2967, 2124, 1740, 1706, 1645, 1549, 1474, 1455, 1373, 1229, 1028, 974, 748, 694; ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.47–8.43 (m, 2H), 8.01 (s, 1H), 5.06 (d, *J* = 4.51 Hz, 2H), 4.14 (t, *J* = 6.77 Hz, 1H), 3.87–3.85 (m, 2H), 3.43 (s, 3H), 3.20 (s, 3H), 3.11 (t, *J* = 2.25 Hz, 1H), 2.03–1.96 (m, 1H), 0.86 (dd, *J* = 2.0 Hz, 6.77 Hz, 6H); ¹³C NMR (400 MHz, DMSO-*d*₆): δ 170.38, 166.19, 154.39, 150.94, 147.87, 143.67, 106.40, 80.86, 72.89, 57.69, 48.04, 30.71, 29.40, 27.74, 27.44, 19.02, 17.94; HRMS (ESI): calcd for C₁₇H₂₃N₆O₄ [M+H]⁺: 375.1781, found: 375.1784; Anal. calcd for C₁₇H₂₃N₆O₄: C, 54.54; H, 5.92; N, 22.45%; Found: C, 54.56; H, 5.98; N, 22.46%; *m/z* (ES)⁺: 375.16 [M+H]⁺.

4.9.2. (S)-2-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)-3-phenyl-N-(prop-2-yn-1-yl)propanamide (27)

Compound **17d** (200 mg, 0.51 mmol), propargyl amine (0.066 mL, 1.03 mmol), T₃P (0.33 mL, 1.03 mmol) and TEA (0.21 mL, 1.55 mmol) in 5 mL of DMF to afford 200 mg (91%) of **27** as an off-white solid. Mp: 220–226 °C; IR (KBr): $\nu_{\max}/\text{cm}^{-1}$ = 3269, 3081, 2954, 2125, 1705, 1648, 1551, 1476, 1412, 1376, 1227, 1230, 1183, 1030, 978, 745, 701, 679; ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.64 (d, *J* = 8.4 Hz, 1H), 8.36 (brs, 1H), 7.91 (s, 1H), 7.23–7.16 (m, 5H), 4.94 (s, 2H), 4.44 (q, *J* = 6.2 Hz, 1H), 3.85 (brs, 2H), 3.41 (s, 3H), 3.20 (s, 3H), 3.09 (s, 1H), 3.05–3.01 (m, 1H), 2.80–2.74 (m, 1H); ¹³C NMR (400 MHz, DMSO-*d*₆): δ 170.66, 166.48, 154.97, 151.39, 148.43, 144.09, 137.96, 129.56, 128.55, 126.75, 106.78, 81.17, 73.59, 54.58, 48.61, 37.96, 29.88, 28.46, 27.93; Anal. calcd for C₂₁H₂₂N₆O₄: C, 59.71; H, 5.25; N, 19.89%; Found: C, 59.74; H, 5.30; N, 19.91%; *m/z* (ES)⁺: 423.17 [M+H]⁺.

4.10. Biological activity protocol

4.10.1. In vitro α -Amylase inhibitory activity

The α -amylase inhibition was determined according to the assay method with modified as described by Shetty et al. [30]. All the synthesized compounds and standard acarbose were prepared at different concentrations. A total of 50 μ L of compounds was incubated with 50 μ L of porcine α -amylase (0.5 mg/mL in 0.02 M sodium phosphate buffer pH 6.9) at room temperature for 30 min. After incubation 50 μ L of 1% starch solution in sodium phosphate buffer (pH 6.8) was added in each tube and further incubated at room temperature for 20 min. 100 μ L of DNS (Dinitro salicylic acid) reagent was then added and the reaction mixture was boiled for 10 min and cooled to room temperature. After dilution, the absorbance was measured at 540 nm and % inhibition was calculated by using formula as below and IC₅₀ values were obtained as mean \pm SD in triplicates as shown in Table 1.

$$\% \text{Inhibition} = \frac{[A_{\text{control}} - A_{\text{sample}}]}{[A_{\text{control}}]} \times 100$$

where A_{control} = Absorbance of control; A_{sample} = Absorbance of Test

compounds.

4.10.2. Molecular docking protocol

In this molecular docking, study was carried out to examine the possible interactions with the target enzyme using Dock Methodology of Molecular Operating Environment (MOE) software [31,32]. The docking methodology consists of many parameters such as target selection and preparation, isolation of binding cavity with site finder, preparation of ligands, and finally docking to its receptor. The crystal structure of α -amylase was retrieved from Protein Data Bank (PDB ID: 4GQR) having a co-crystal ligand MYC and water molecules are removed and protein structure was energy minimized using default settings. The ligands are built using builder in MOE and energy minimized using MMFF94x force field. The docking protocol was carried out with ligand mbd file, Triangle Matcher as Placement, Rescoring using London dG scoring and finally optimized poses are ranked using GBVI/WSA DG score. The docking poses were browsed visually and best interactions were isolated and computed with ligand interactions.

4.10.3. In vivo anti-diabetic activity

Streptozotocin was purchased from Sigma-Aldrich, USA and carboxymethyl cellulose, pioglitazone, sucrose, and feed (High Fat Diet) ingredients were procured from the commercial source. The composition of HFD is as follows (see Table 3).

Animals

Adult Wistar albino rats of male sex weighing 150–170 g were used and the animals were maintained on the suitable nutritional and environmental condition throughout the experiment. The animals were housed in polypropylene cages with paddy house bedding under standard laboratory condition for an acclimatization period of 7 days prior to performing the experiment. The animals had access to laboratory chow, water, and high-fat diet.

All the experimental procedures were carried out accordance with the committee for the purpose of control and supervision of experiments on animal (CPCSEA) guidelines. All the experimental procedures were approved by the institutional animal ethical committee (IAEC). (Protocol No: I/IAEC/AGI/022/2018WR).

4.10.4. HFD-STZ induced diabetic model (HFD-STZ)

The *in vivo* anti-diabetic activity of the two most potent α -amylase inhibitors **25** and **27** were screened by High Fat Diet-Streptozotocin (HFD-STZ) model as per the published protocol [29]. Briefly, Adult Wistar albino rats of male sex weighing 150–170 g were used for the study. Rats were fed with high-fat diet (HFD) as the specified composition for the period of 2 weeks *ad libitum*. The HFD rats were administered intraperitoneally with a low dose of STZ (35 mg/kg, *b.w*) in citrate buffer pH \sim 4.4 and the control group containing rats were given citrate buffer with a dose volume of 1 mg/kg, *i.p*. The rats with the non-fasting plasma glucose level (PGL) of \geq 300 mg/dl were considered diabetic and selected for further studies.

Study design: All the rats were divided into five groups of each six (*n* = 6).

Group I: Normal rats with normal diet and treated with (2 mL/kg, *p.o*) of 1% sodium carboxymethyl cellulose (Sod CMC). (NDC)

Group II: Served as diabetic/disease control and received 1% Sod CMC (1 mL/kg, *b.w*, *p.o*) (HF-STZ-Diabetic Control). (HFD-STZ-Control)

Group III: Diabetic Rats treated with standard pioglitazone .at a dose of 10 mg/kg, (*b.w*, *p.o*).

Group IV: Diabetic Rats treated with compound **25** at a dose of 10 mg/kg, (*b.w* *p.o*).

Group V: Diabetic Rats treated with compound **27** at a dose of 10 mg/kg, (*b.w*, *p.o*).

The treatment was given for 14 days and blood samples were

collected at different time intervals.

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