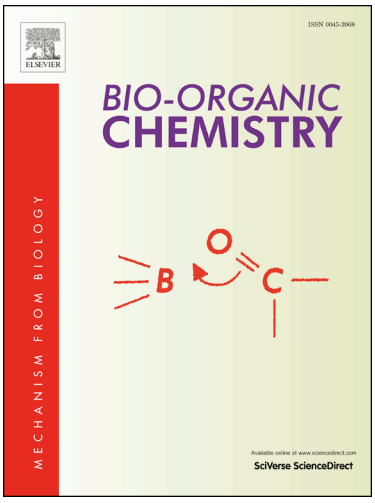

Design, synthesis and biological evaluation of theophylline containing variant acetylene derivatives as α-amylase inhibitors

Radhakrishnam Raju. Ruddarraju, Gangarapu. Kiran, Adharvana Chari. Murugulla, Ravichandar. Maroju, Devarakonda Krishna Prasad, Boyina Hemanth Kumar, Nukala Shravya Reddy

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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±0.03 μM respectively, as compared with standard, acarbose 1.37±0.26 μM. Further, molecular docking simulation studies were done to identify the interactions and binding mode of synthesized analogs at binding site of α-amylase enzyme (PBD 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.

Keywords: Theophylline, Acetylenes, α-Amylase, Molecular Docking, Anti-diabetic, High fat diet, Streptozotocin

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], 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 **(Figure-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.

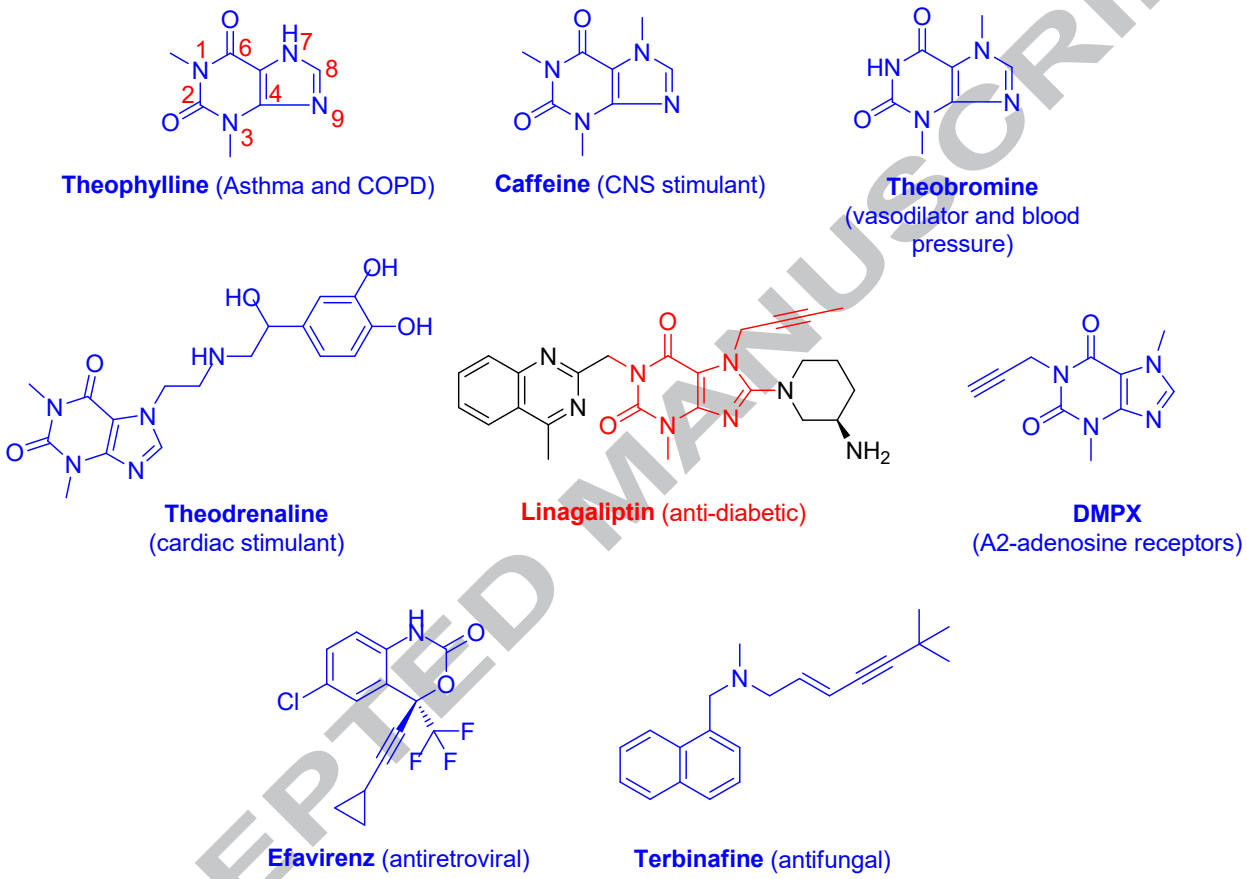


Figure-1. Multiple bioactive theophylline, acetylene, and theophylline containing acetylene moieties.

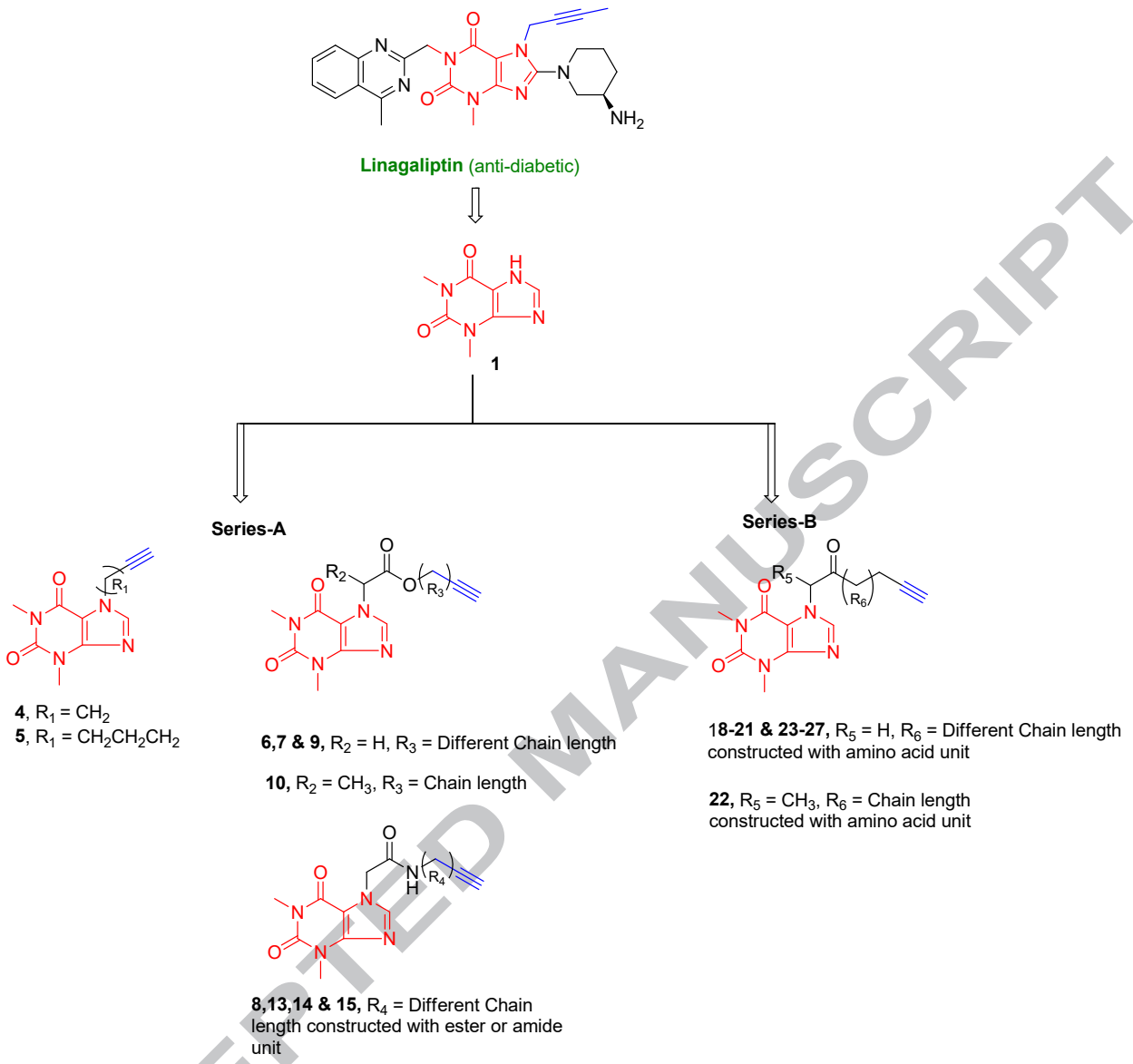
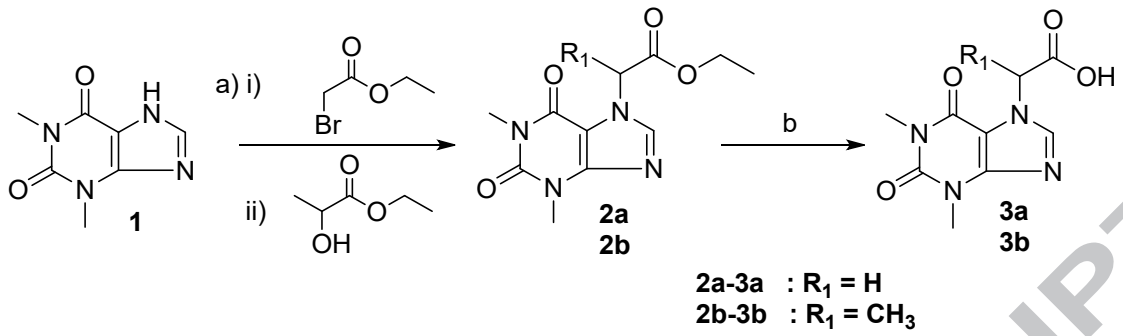


Figure-2. Design strategy of the novel theophylline series **A** and **B.**

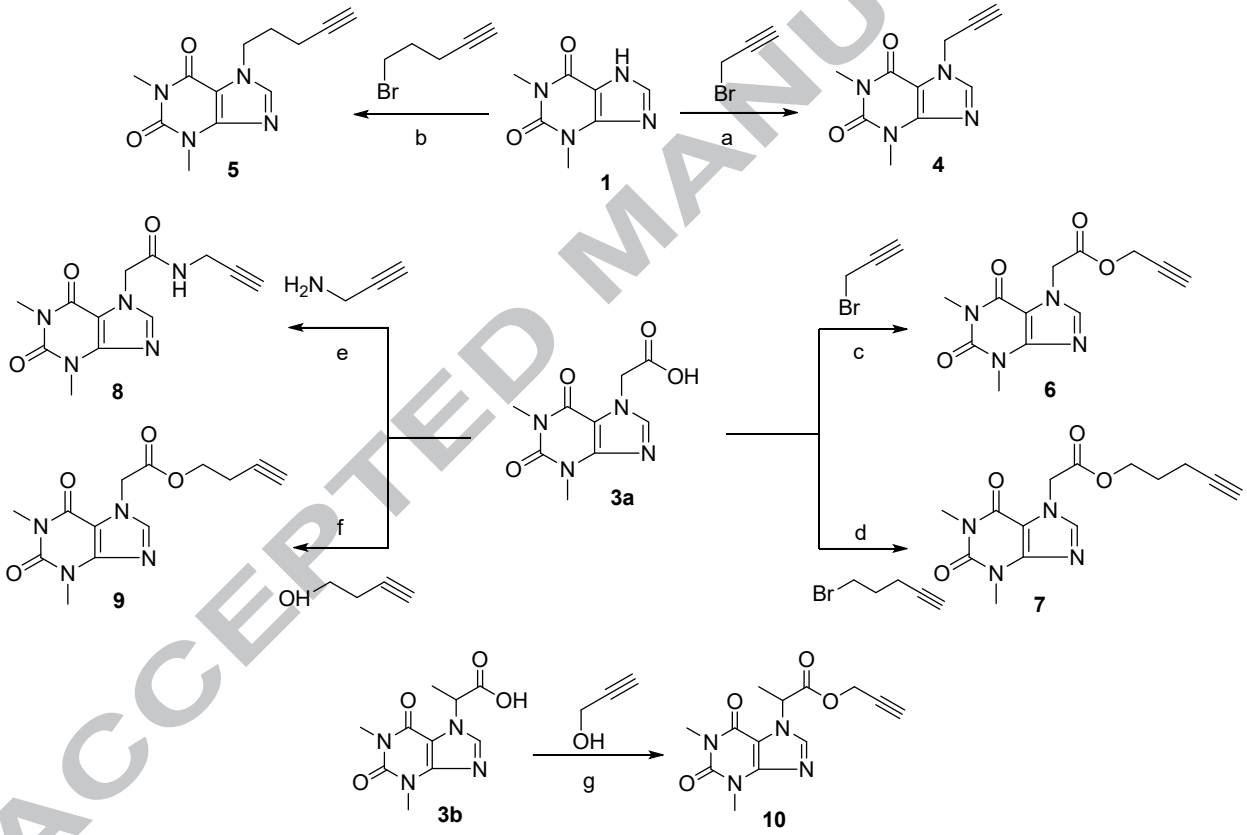
2 Results and Discussion

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 **Scheme-2, 3** and **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].

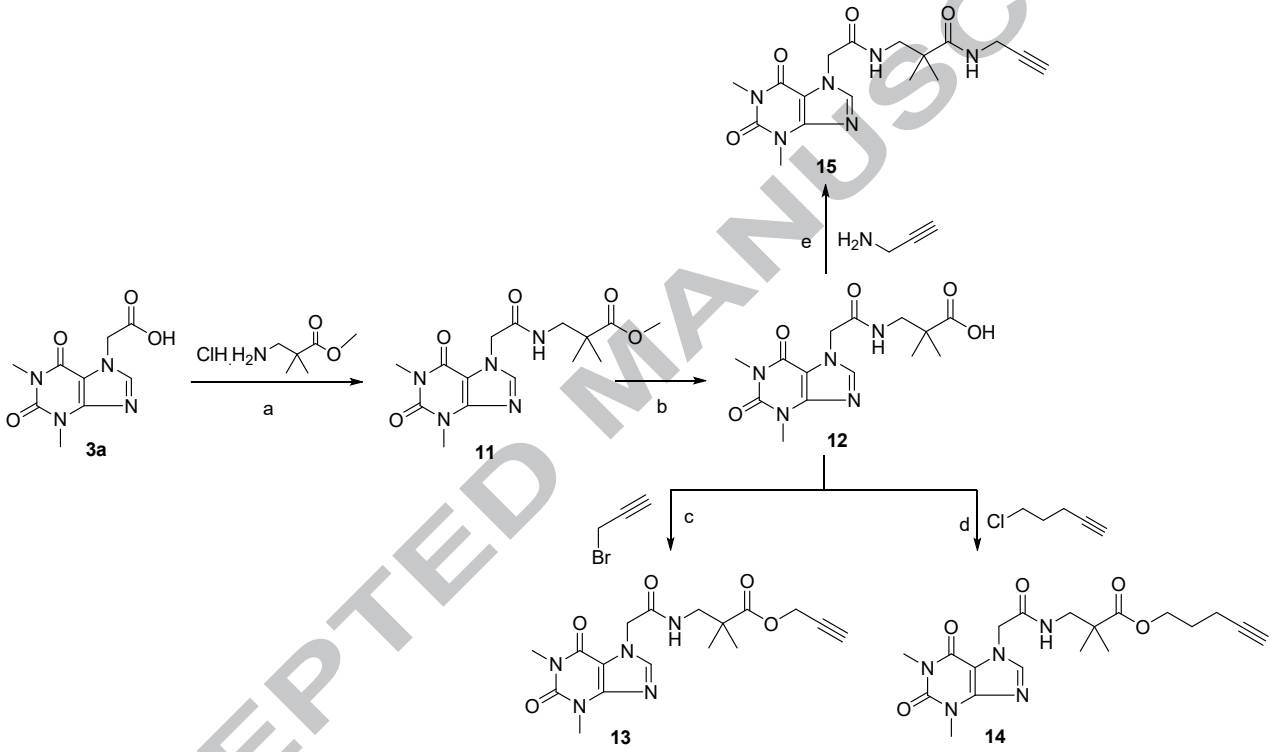


Scheme-1 Synthesis of theophylline acid compounds (**3a–3b)**. Reagents and conditions: (a) (i) K_2CO_3, DMF, 85°C, 12 h & (ii) TPP, DIAD, THF, rt, 5 h; (b) $LiOH.H_2O$, $THF:H_2O$, rt, 2 h.



Scheme-2 Synthesis of theophylline containing acetylene compounds (**4-10**). Reagents and conditions: (a) K_2CO_3, DMF, 85°C, 2 h; (b) K_2CO_3, DMF, 85°C, 10 h; (c) K_2CO_3, DMF, rt, 10 h; (d) K_2CO_3, DMF, 80°C, 12 h; (e) HATU, DIPEA, DMF, rt, 12 h; (f & g) DCC, DMAP, DCM, rt, 16h.

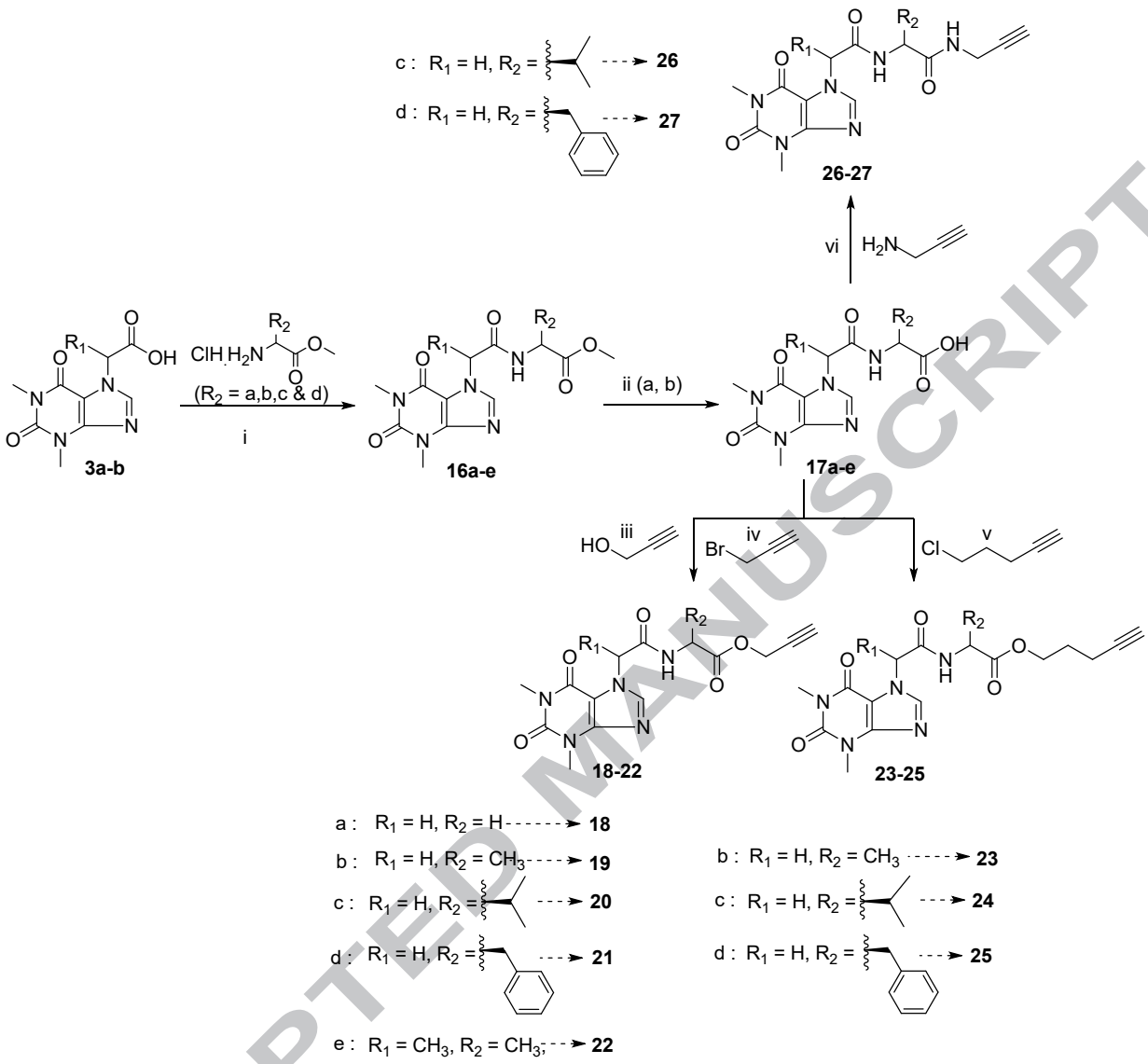
Now in **Scheme-3**, compound **3a** was coupled with methyl 3-amino-2,2-dimethylpropanoate hydrochloride by using HATU and DIPEA at room temperature for 16 hours gave compound **11** and then followed by hydrolysis with $LiOH.H_2O$ in THF and H_2O (1:1) gave compound **12**. In a similar way, compound **12** was treated with propargyl bromide in the presence of K_2CO_3 in DMF at room temperature to obtain compound **13** (60%) and treated with 5-chloro-1-pentyne at 80°C for 12 hours to get compound **14** (50%). But, compound **12** was coupled with propargylamine by using reagents T_3P and triethylamine (TEA) in DMF at room temperature to get compound **15** with the yield 36%.



Scheme-3 Synthesis of theophylline containing acetylene compounds (**13-15**). Reagents and conditions: (a) HATU, DIPEA, DCM, rt, 16 h; (b) $LiOH{\cdot}H_2O$, THF:H_2O, rt, 4 h; (c) K_2CO_3, DMF, rt, 10 h; (d) K_2CO_3, DMF, 80°C, 12 h; (e) T_3P, TEA, DMF, rt, 16 h.

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 hours 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 hours gave compound **16e**. The ester compounds **16a-c** and **16e** hydrolysis with $LiOH.H_2O$ in THF and H_2O 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 hours 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 K_2CO_3 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 hours to get compounds **23** (33%), **24** (72%) and **25** (47%), respectively. Compounds **17c-d** were coupled with propargylamine by using reagents T_3P and triethylamine (TEA) in DMF at room temperature to get compounds **26** and **27** with yields 63%, 91% respectively.



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) $LiOH.H_2O$, $THF:H_2O$, rt, 4h & (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°C, 12 h; (vi) T_3P, TEA, DMF, rt, 16 h.

2.2 Anti diabetic activity

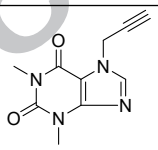
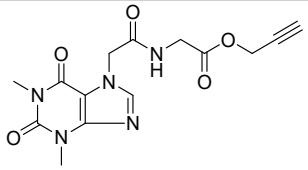
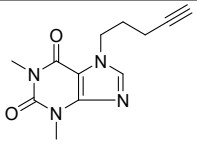
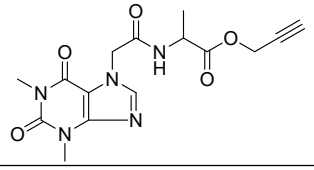
2.2.1 In vitro a-amylase inhibitory activity

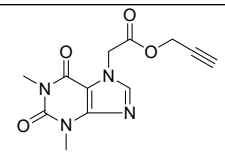
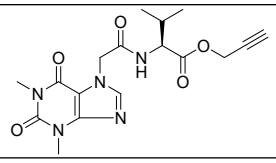
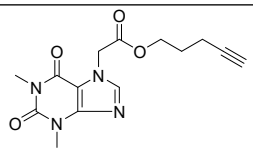
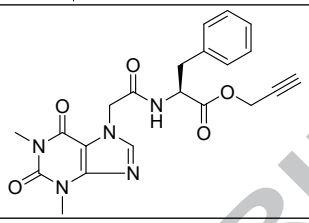
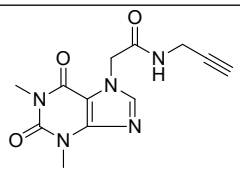
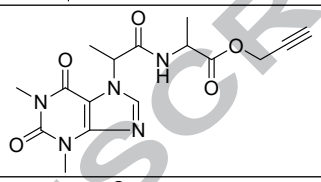
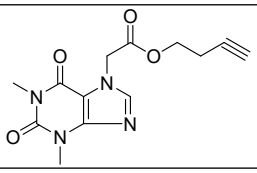
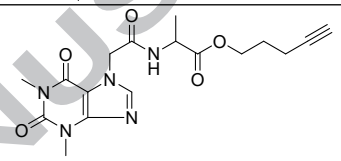
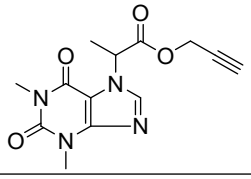
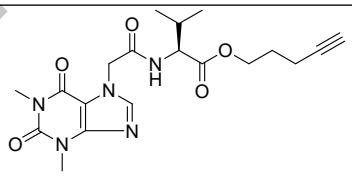
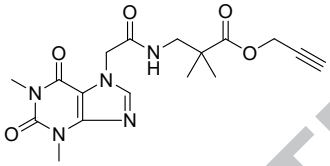
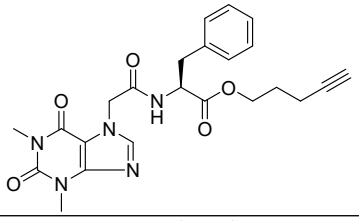
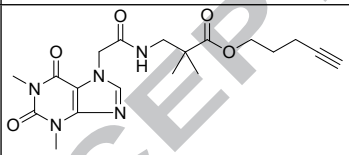
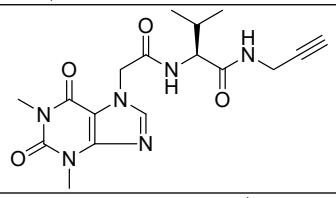
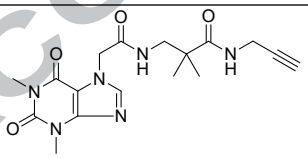
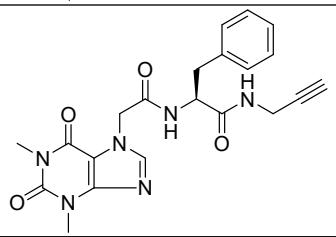
This work is aimed at the biological evaluation of two novel series of theophylline derivatives as shown in **Figure-2**. Our plan was based on using theophylline **1** and the simple acetylene as scaffolds (**4-10** and **13-15**). Although **4** and **5** have shown low activity, it can be used as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

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 IC_{50} values ranging between 14.8±0.15 to 1.02±0.03 μM and compared with standard acarbose having IC_{50} value 1.37±0.26 μM as shown in **Table 1**. Three analogs in series **A**: **13**, **14** and **15** shown excellent inhibitory potential with IC_{50} 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 IC_{50} 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 μM respectively, when compared with the standard acarbose with IC_{50} value 1.37±0.26 μM. 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.

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

No	Structure	IC_{50} (μM)	No	Structure	IC_{50} (μM)
4		12.9±0.17	**18**		2.84±0.13
5		14.8±0.15	**19**		2.62±0.02

6		-	**20**		1.21±0.03
7		3.27±0.04	**21**		**1.33±0.09**
8		4.14±0.09	**22**		2.09±0.11
9		4.01±0.14	**23**		2.27±0.09
10		4.32±0.04	**24**		**1.17±0.01**
13		**1.11±0.07**	**25**		**1.05±0.02**
14		**1.14±0.17**	**26**		**1.61±0.04**
15		**1.07±0.01**	**27**		**1.02±0.03**
Acarbose		1.37±0.26	Acarbose		1.37±0.26

*All the values are expressed as mean ± SD, (n=3);

The inactivity of the derivatives from series **A** compounds **4** and **5** highlights the importance of the theophylline moiety for the α-amylase activity IC_{50} values 12.9±0.17 and 14.8±0.15 μM 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 IC_{50} values 4.32±0.04, 4.01±0.14 and 3.27±0.04 μM 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 IC_{50} values 4.14±0.09, 1.14±0.17, 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 liking 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-CH_3<C-$(CH_3)_2$<C-$CH_2C_6H_5$.

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 (3.13A^o) and formed H-bond interactions between the carbon atom and carboxylate oxygen atom of Asp300 (3.13A^o) and side chain carbon atom and oxygen atom of Glu233 (3.2A^o). 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.

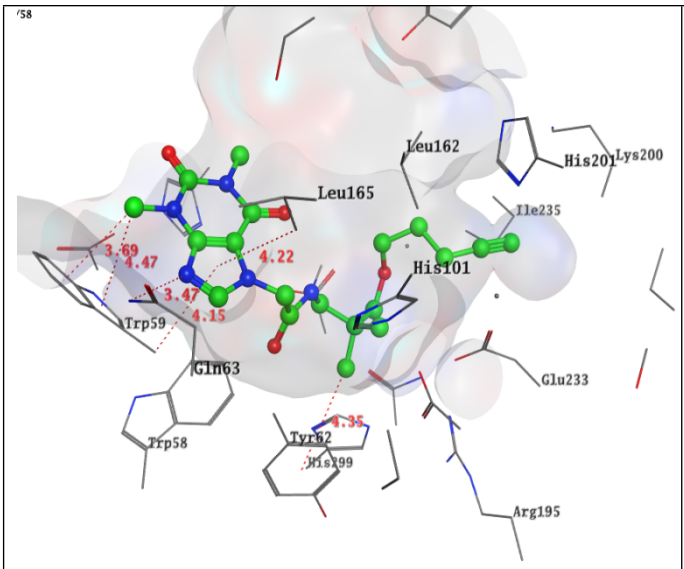
In series-**B**, compound **27 (**docking score = -6.46) showed good binding interactions with the active site of 4GQR of α-amylase protein. The compound shows strong H-bond interactions as a donor with His305 with distance 2.68 A^o between the carbon atom of acetylene side chain with the oxygen atom of Histidine residue. Trp59 formed H-pi interactions with N-CH_3 of purine ring and pi-H interactions with Leu165, Ala307, and Gly308 residues. The hydrophobic interactions are shown with Asp300, Tyr62, and Trp58 residues.

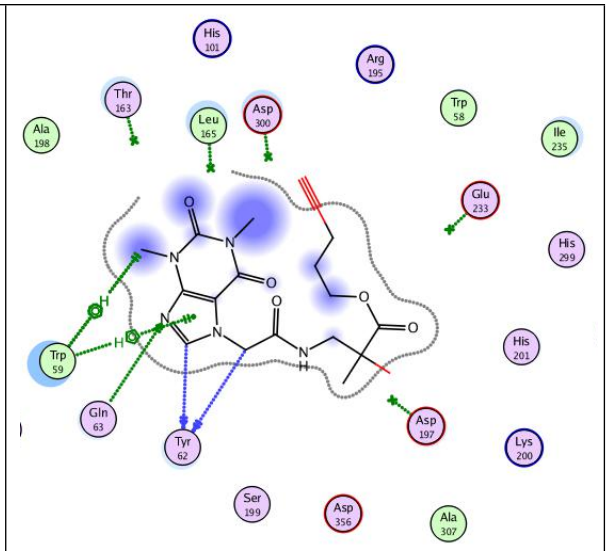
Compound **14** (docking score -6.86) in the series-**A** showed good binding interactions with the active site of amino acid residues. The compound forms pi-H interactions with 5-ring of purine with Trp59 residue. It also forms H-donor interactions with Tyr62 (3.15) and H-acceptor interactions with 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

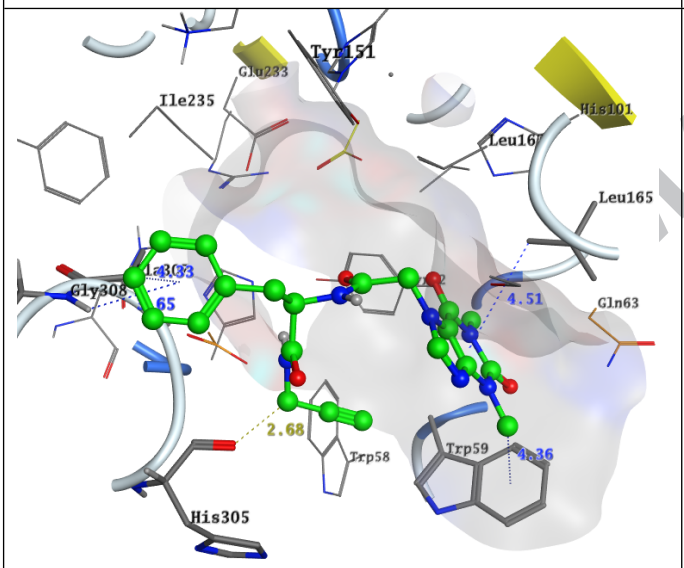
Waals interactions, side chain containing amide or carboxylate groups -NH and C=O forms H-bond interactions and purine containing 6 and 5 membered rings shows pi-H or pi-pi interactions.

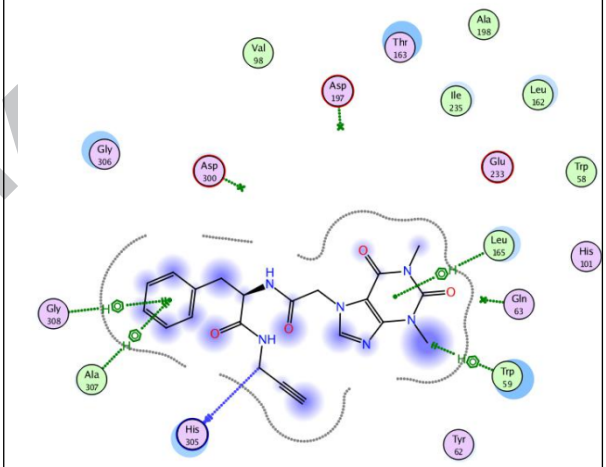
The co-crystal ligand, Myricetin assumed to occupy the α-amylase and established hydrogen bonding interactions with Gln63, Asp197, and Asp300. All the docked compounds superimposed well with the cocrystal ligand Myricetin and all common interactions (Gln63, Asp197, Asp300 and Leu162, Leu165, Trp59, and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





Compound 14





Compound-27

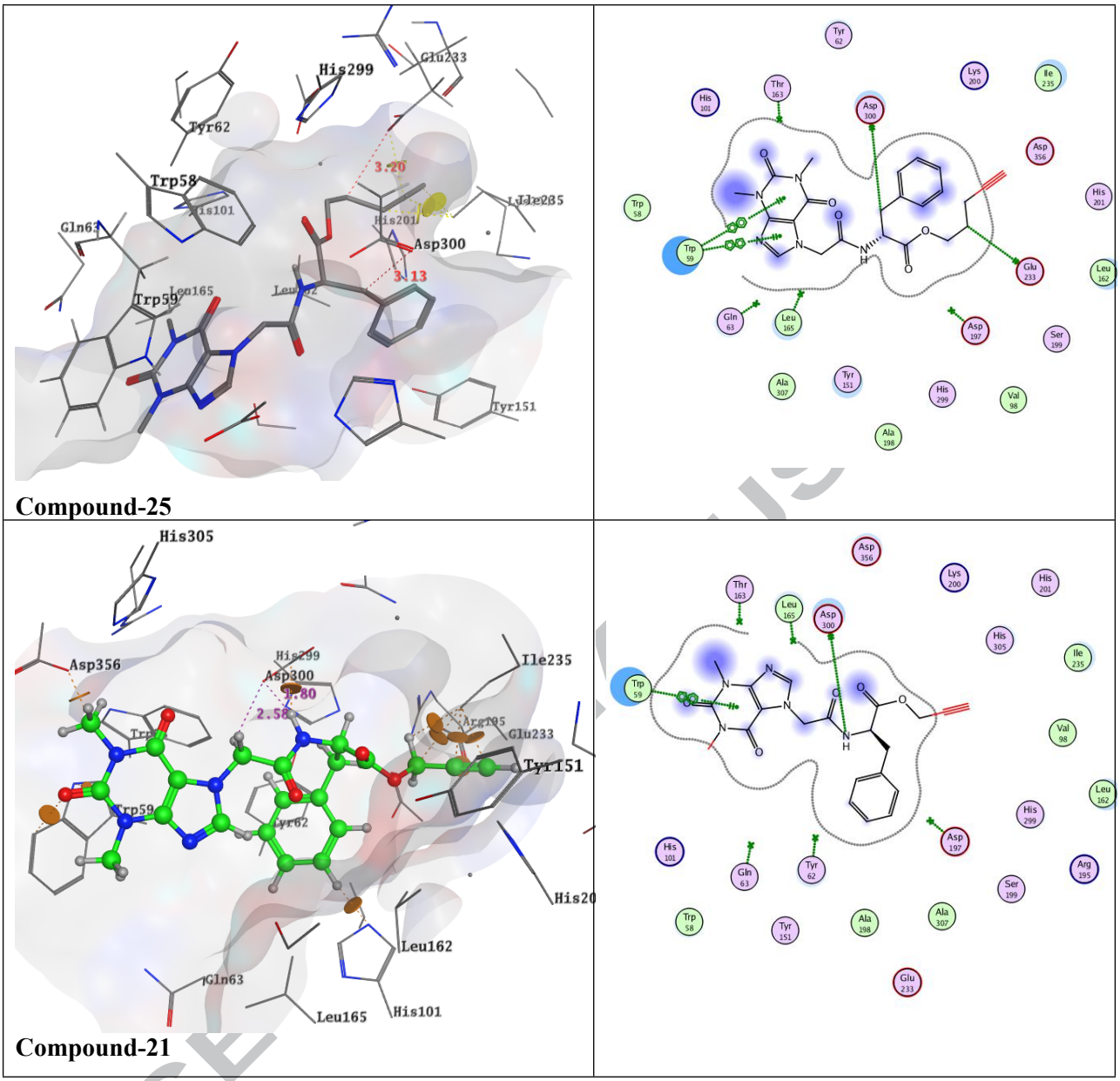


Figure-3: Binding interactions of compounds with α-amylase enzyme (PDB ID: 4GQR)

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 C 14 O 11 C 13	OE2 GLU 233 OE1 GLU 233 NE2 HIS 299 5-ring HIS 299	H-donor H-donor H-acceptor H-pi	3.15 3.30 2.79 4.41
05	-4.59	C 18	OE1 GLU 233	H-donor	3.56

		O 15	NE2 HIS 101	H-acceptor	2.85
		C 13	OE1 GLU 233	H-donor	3.35
06	-4.54	O 10	NE2 HIS 101	H-acceptor	2.90
		C 19	6-ring TRP 59	H-pi	4.15
07	-4.64	C 19	6-ring TRP 59	H-pi	4.21
		C 13	OE2 GLU 233	H-donor	3.29
		C 14	OD2 ASP 197	H-donor	2.75
08	-5.51	5-ring	NE2 HIS 101	pi-cation	3.86
		C 13	O TYR 62	H-donor	3.06
		O 10	NE2 HIS 101	H-acceptor	3.55
09	-4.93	6-ring	CB TYR 62	pi-H	3.93
		C 8	OD2 ASP 197	H-donor	3.32
		C 12	O TYR 62	H-donor	2.66
10	-4.90	6-ring	CD1 LEU 165	pi-H	4.41
		C 38	6-ring TRP 59	H-pi	3.63
13	-6.01	5-ring	CB TRP 59	pi-H	4.20
		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
14	-6.86	5-ring	CB TRP 59	pi-H	4.15
		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
15	-5.88	5-ring	CB TRP 59	pi-H	4.15
		6-ring	5-ring TRP 59	pi-pi	3.82
18	-5.55	6-ring	5-ring TRP 59	pi-pi	3.94
		O 12	NE2 HIS 201	H-acceptor	3.07
19	-5.15	O 31	NE2 HIS 201	H-acceptor	3.28
20	-4.97	O 11	NE2 HIS 201	H-acceptor	2.89
		N 13	OD2 ASP 300	H-donor	2.78
21	-6.12	6-ring	5-ring TRP 59	Pi-pi	3.71
		5-ring	CD1 LEU 162	pi-H	4.21
		6-ring	CD1 LEU 162	pi-H	4.08
22	-5.05	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
		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
25	-6.49	6-ring	5-ring TRP 59	pi-pi	3.94
		C 27	6-ring TRP 59	H-pi	4.38
26	-5.54	C 27	6-ring TRP 59	H-pi	4.38
		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
27	-6.46	6-ring	N GLY 308	pi-H	4.51

		O 23	OD2 ASP 300	H-donor	3.19
		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
MYC	-14.1194	6-ring	CD1 LEU 165	pi-H	4.40

2.2.3 In vivo anti-diabetic activity

2.2.3.1. HFD-STZ induced diabetic model[30]

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). **Figure-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 administrated 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.

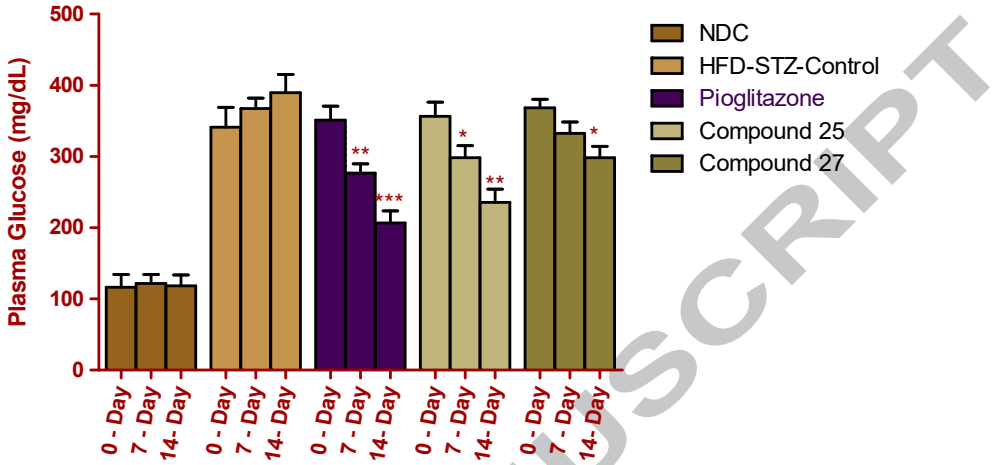


Figure-4. *In vivo* anti-diabetic activity of compounds **25**, **27** and standard Pioglitazone in HFD-STZ induced diabetic rats. All Data were expressed as Mean ± SEM (n=6) using One-Way ANOVA followed by Dunnett's multiple comparison test. $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ vs HFD-STZ-Control.

NDC: Normal rats with normal diet; HFD-STZ-Control is Diabetic Control.

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, compound **25** and **27** groups respectively (**Figure 5A-5D**). 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 **Figure 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 **Figure 5D and 5E**).

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 (**Figure 6A-6E**).

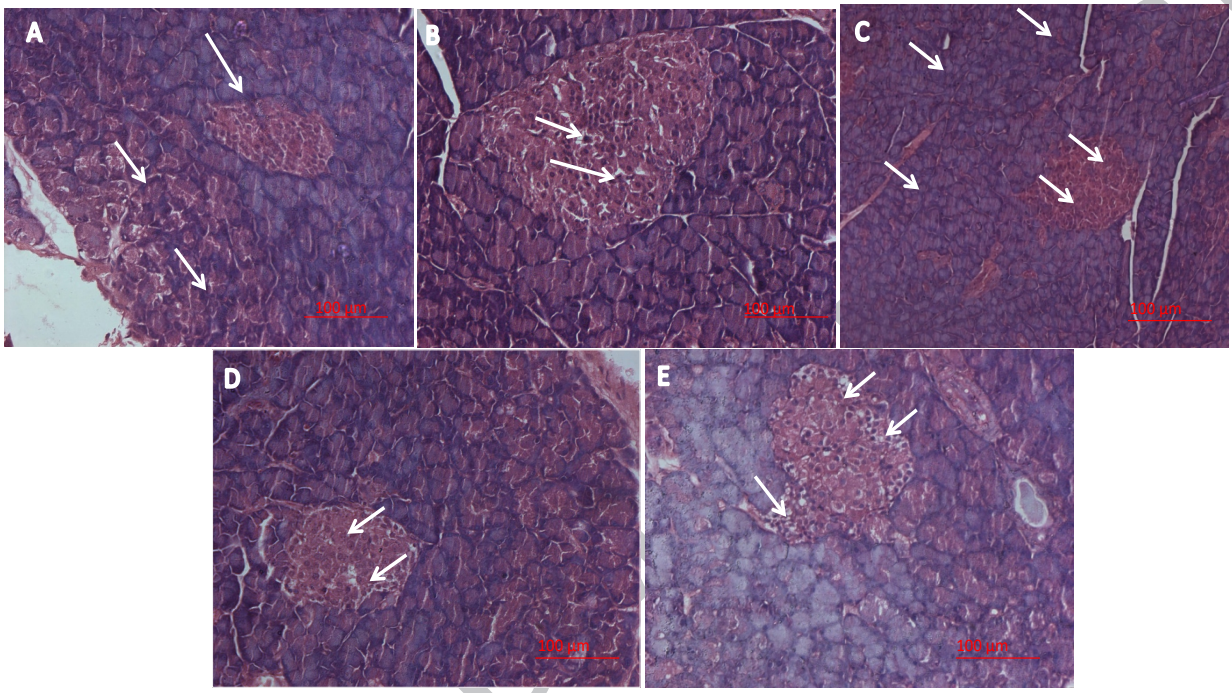


Figure-5. Histopathological changes observed in pancreas tissue samples of HFD-STZ induced diabetic rats treated with compound-**25** and compound-**27**.
(**A**) Normal diabetic control group-arrows shows normal proliferation of β-cells and acinar cells; (**B**) Diabetic control group–arrows indicates 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.

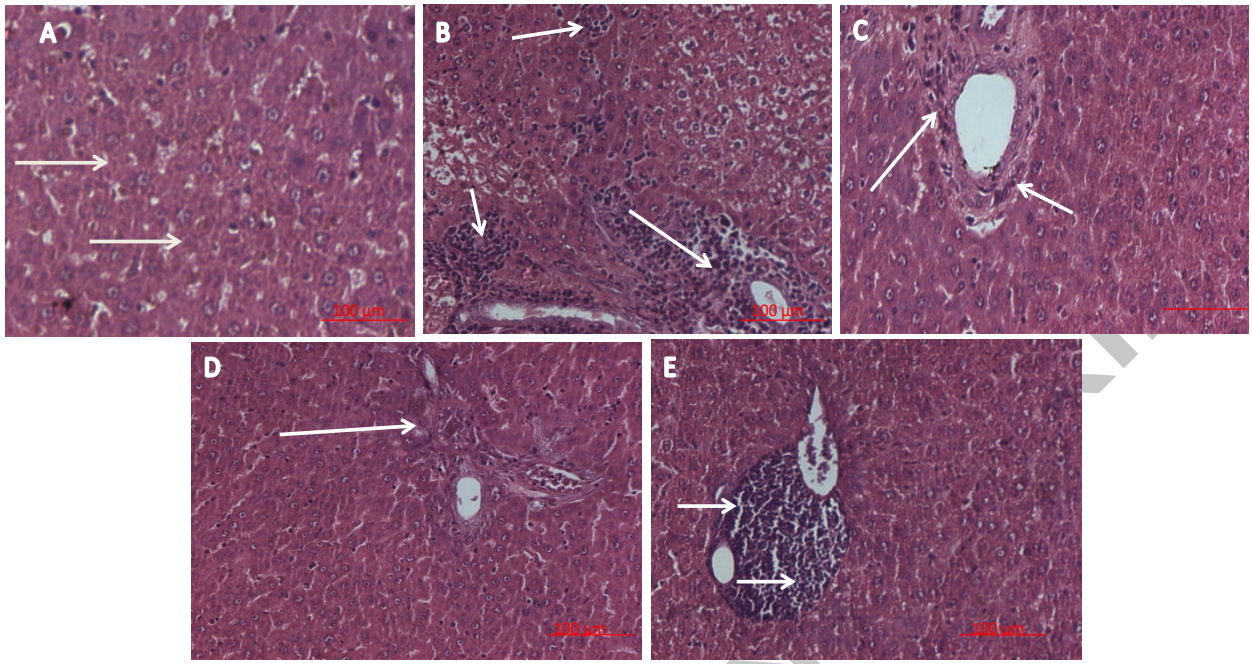


Figure-6. Histopathological changes observed in liver tissue samples of HFD-STZ induced diabetic rats treated with compound-**25** and compound-**27**.
(**A**) Normal diabetic control–arrows indicating normal histological structure liver cells; (**B**) Diabetic control group–arrows showing Multi focal 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 peribillary 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.

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-CH_3<C-$(CH_3)_2$<C-$CH_2C_6H_5$. Further *in vivo* HFD-STZ-induced diabetic models showed that compound **25** showed significant anti-diabetic 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 ^{1}H NMR and ^{13}C NMR spectra were taken on a VNMRS 400 MHz spectrometer using the solvent ($CDCl_3$ 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 Perkine 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 was washed with aq. $NaHCO_3$ solution and aq. $KHSO_4$ solution, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to afford **11** (3.0 g, 68%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (s, 1H), 7.14 (brs, 1H), 4.87 (s, 2H), 3.66 (s, 3H), 3.60 (s, 3H), 3.41 (s, 3H), 3.37 (d, J = 6.4 Hz, 2H), 1.14 (s, 6H); m/z (ES)$^+$: 352.2 $[M+H]^+$.

4.2.2 3-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)-2,2-dimethylpropanoic acid (12). $LiOH{\cdot}H_2O$ (717 mg, 17.09 mmol) was added portion wise to a stirred solution of ester compound **11** (3 g, 8.547 mmol) in THF (15 mL, 5 vol) and H_2O (15 mL, 5 vol) at room temperature and the reaction mixture was stirred for 4 h. After completion, from the reaction mixture THF was concentrated, diluted with water and acidified with aqueous $KHSO_4$ solution, extracted with 10% methanol in dichloromethane (2 x 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to afford **12** (2.0 g, 69%) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.33 (s, 1H), 8.13 (t, J = 6.02 Hz, 1H), 8.00 (s, 1H), 5.0 (s, 2H), 3.43 (s, 3H), 3.24 (d, J = 6.27 Hz, 2H), 3.19 (s, 3H), 1.06 (s, 1H); *m/z* (ES)$^+$: 338.16 $[M+H]^+$.

4.2.3 prop-2-yn-1-yl 3-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)-2,2-dimethylpropanoate (13). To a stirred solution of compound **12** (150 mg, 0.44 mmol) in 5 mL of DMF, was added K_2CO_3 (79 mg, 0.57 mmol) at room temperature. After 20 min, propargyl bromide (0.76 mL, 0.89 mmol) was added to the reaction mixture at room temperature and was stirred for 10 h. As monitored by TLC, to the reaction mixture water was added and a solid precipitate was formed that was filtered and dried to afford **13** (166 mg, 60%) as an off-white solid. Mp: 145–148^{o}C; IR (KBr): υ_{max}/ cm^{-1} = 3306, 3233, 3111, 2945, 2123, 1722, 1697, 1676, 1649, 1573, 1550, 1472, 1433, 1373, 1261, 1230, 1134, 1029, 979, 747, 698; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.19 (t, J = 6.0 Hz, 1H), 7.97 (s, 1H), 4.97 (s, 2H), 4.65 (d, J

= 2.4 Hz, 2H), 3.52 (s, 1H), 3.41 (s, 3H), 3.25 (d, *J* = 6.0 Hz, 2H), 3.17 (s, 3H), 1.08 (s, 6H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 175.39, 167.02, 154.82, 151.47, 148.34, 144.11, 106.88, 78.93, 78.09, 52.57, 48.50, 46.65, 43.54, 29.88, 27.90, 23.02; HRMS (ESI): calcd for $C_{17}H_{22}N_5O_5$ $[M+H]^+$: 376.1621, found: 376.1631; Anal. calcd for $C_{17}H_{21}N_5O_5$: C, 54.39; H, 5.64; N, 18.66%; Found: C, 54.40; H, 5.68; N, 18.67%; *m/z* $(ES)^+$: 376.14 $[M+H]^+$.

4.2.4 pent-4-yn-1-yl 3-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)-2,2-dimethylpropanoate (14). To a stirred solution of compound **12** (150 mg, 0.44 mmol) in 5 mL of DMF, K_2CO_3 (79 mg, 0.57 mmol) was added at room temperature. After 20 min, 5-chloropent-1-yne (0.094 mL, 0.89 mmol) was added to the reaction mixture at room temperature and was stirred at 80°C for 12 h. As monitored by TLC, reaction mass was cooled to 0°C, diluted with water and extracted with ethyl acetate, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude compound was triturated with DCM and *n*-pentane to afford **14** (90 mg, 50%) as an off-white solid. Mp: 78–80°C; IR (KBr): υ_{max}/ cm^{-1} = 3298, 3226, 3093, 2971, 2946, 2116, 1732, 1700, 1655, 1552, 1474, 1432, 1370, 1231, 1144, 1029, 976, 754, 709; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.14 (t, *J* = 6.0 Hz, 1H), 7.97 (s, 1H), 4.97 (s, 2H), 4.04 (t, *J* = 6.0 Hz, 2H), 3.41 (s, 3H), 3.25 (d, *J* = 6.0 Hz, 2H), 3.17 (s, 3H), 2.78 (s, 1H), 2.20 (t, *J* = 6.8 Hz, 1H), 1.75–1.71 (m, 2H), 1.07 (s, 6H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 176.10, 166.96, 154.80, 151.45, 148.31, 144.10, 106.85, 83.92, 72.0, 63.40, 48.51, 46.75, 43.54, 40.60, 29.86, 27.87, 27.49, 23.20, 14.94; HRMS (ESI): calcd for $C_{19}H_{26}N_5O_5$ $[M+H]^+$: 404.1934, found: 404.1938; Anal. calcd for $C_{19}H_{25}N_5O_5$: C, 56.57; H, 6.25; N, 17.36%; Found: C, 56.59; H, 6.28; N, 17.38%; *m/z* $(ES)^+$: 404.34 $[M+H]^+$.

4.2.5 3-(2-(1,3-dimethyl-2,6-dioxo-2,3-dihydro-1H-purin-7(6H)-yl)acetamido)-2,2-dimethyl-N-(prop-2-yn-1-yl)propanamide (15). To a stirred solution of compound **12** (150 mg, 0.44 mmol) in DMF, T_3P (0.26 mL, 0.89 mmol) and TEA (0.18 mL, 1.33 mmol) were added at room temperature. Propargyl amine (0.056 mL, 0.089 mmol) 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 **15** (60 mg, 36%) as an off-white solid.

Mp: 157–160°C; IR (KBr): υ_{max}/ cm^{-1} = 3321, 3066, 2969, 2123, 1740, 1704, 1654, 1558, 1530 1476, 1418, 1369, 1229, 1031, 977, 755, 668; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.06–8.0 (m, 3H), 4.99 (s, 2H), 3.85–3.83 (m, 2H), 3.43 (s, 3H), 3.22–3.19 (m, 5H), 3.06 (t, J = 2.25 Hz, 1H), 1.05 (s, 6H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 175.51, 166.49, 154.32, 150.97, 147.84, 143.61, 106.39, 81.52, 72.50, 48.11, 46.48, 42.71, 29.38, 28.21, 27.41, 22.90; HRMS (ESI): calcd for $C_{17}H_{23}N_6O_4$ $[M+H]^+$: 375.1781, found: 375.1791; Anal. calcd for $C_{17}H_{22}N_6O_4$: C, 54.54; H, 5.92; N, 22.45%; Found: C, 54.56; H, 5.98; N, 22.47%; *m/z* $(ES)^+$: 375.12 $[M+H]^+$.

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. $NaHCO_3$ solution and aq. $KHSO_4$ solution, dried over anhydrous Na_2SO_4, 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. ^{1}H NMR (400 MHz, DMSO-d_6): 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. ^{1}H NMR (400 MHz, DMSO-d_6): 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. ^{1}H NMR (400 MHz, DMSO-d_6): 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. ^{1}H NMR (400 MHz, DMSO-d_6): 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. ^{1}H NMR (400 MHz, DMSO-d_6): 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 $[M+H]^+$.

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

$LiOH{\cdot}H_2O$ (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 H_2O (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 $KHSO_4$ solution and extracted with 10% methanol in dichloromethane. The combined organic extracts were dried over anhydrous Na_2SO_4, 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 $LiOH{\cdot}H_2O$ (342 mg, 8.15 mmol) in 42 mL of THF:H_2O (1:1) to afford 1.6 g (80%) of **17a** as a white solid. ^{1}H 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 $LiOH{\cdot}H_2O$ (610 mg, 14.70 mmol) in 78 mL of THF:H_2O (1:1) to afford 2.6 g (68%) of **17b** as a white solid. ^{1}H 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 $LiOH{\cdot}H_2O$ (610 mg, 14.70 mmol) in 40 mL of THF:H_2O (1:1) to afford 1.8 g (81%) of **17c** as a white solid. ^{1}H NMR (400 MHz, 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 $LiOH.H_2O$ (200 mg, 4.983 mmol) in 28 mL of THF:H_2O (1:1) to afford 1 g (74%) of **17e** as a white solid. ^{1}H NMR (400 MHz, 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. ^{1}H NMR (400 MHz, 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): υ_{max}/ cm^{-1} = 3281, 3111, 2953, 2130, 1754, 1708, 1659, 1548, 1474, 1457, 1384, 1376, 1217, 1190, 1029, 947, 763, 748; ^{1}H NMR (400 MHz, 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, 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 $C_{14}H_{16}N_5O_5$ $[M+H]^+$: 334.1151, found: 334.1157; Anal. calcd for $C_{14}H_{15}N_5O_5$: 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: 150–156^{o}C; IR (KBr): υ_{max}/ cm^{-1} = 3289, 3119, 2949, 2138, 1750, 1702, 1649, 1542, 1476, 1467, 1376, 1352, 1214, 1192, 1023, 942,748; ^{1}H NMR (400 MHz, 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 $C_{15}H_{17}N_5O_5$: 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^{o}C; IR (KBr): υ_{max}/ cm^{-1} = 3324, 2928, 2850, 1746, 1705, 1666, 1625, 1573, 1473, 1449, 1435, 1311, 1242, 1213, 1153, 1087, 994, 748; ^{1}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}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 $C_{16}H_{19}N_5O_5Na$ $[M+Na]^{+}$: 384.1284, found: 384.1283; Anal. calcd for $C_{16}H_{19}N_5O_5$: 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): υ_{max}/ cm^{-1} = 3284, 3246, 3117, 2964, 2127, 1741, 1649, 1553, 1466, 1372, 1224, 1200, 1142, 1027, 964, 746, 691; ^{1}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}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 $C_{17}H_{22}N_5O_5$ $[M+H]^+$: 376.1621, found: 376.1636; Anal. calcd for $C_{17}H_{21}N_5O_5$: 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): υ_{max}/ cm^{-1} = 3310, 3227, 3104, 2951, 2127, 1739, 1706, 1650, 1546, 1471, 1368, 1208, 1230, 1032, 969, 719, 698; ^{1}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}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 $C_{21}H_{22}N_5O_5$ $[M+H]^+$: 424.1621, found: 424.1629; Anal. calcd for $C_{21}H_{21}N_5O_5$: 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^oC; IR (KBr): υ_{max}/ cm^{-1} = 3275, 3094, 2940, 2122, 1738, 1698, 1650, 1556, 1461, 1370, 1264, 1217, 1150, 1023, 973, 845, 753, 688; ^1H 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 $C_{17}H_{22}N_5O_5$ $[M+H]^+$: 376.1621, found: 376.1634; Anal. calcd for $C_{17}H_{21}N_5O_5$: 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^oC; IR (KBr): υ_{max}/ cm^{-1} = 3279, 3088, 2965, 2126, 1733, 1705, 1653, 1549, 1473, 1373, 1229, 1201, 1028, 975, 748, 694; ^1H 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}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 $C_{19}H_{26}N_5O_5$ $[M+H]^+$: 404.1934, found: 404.1937; Anal. calcd for $C_{19}H_{25}N_5O_5$: 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^oC; IR (KBr): υ_{max}/ cm^{-1} = 3295, 3115, 2953, 1732,

1705, 1655, 1548, 1474, 1373, 1224, 1198, 1028, 973, 748, 701; ^{1}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}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 $C_{23}H_{25}N_5O_5$: 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.

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), T_3P (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^{o}C; IR (KBr): υ_{max}/ cm^{-1} = 3274, 3118, 3087, 2967, 2124, 1740, 1706, 1645, 1549, 1474, 1455, 1373, 1229, 1028, 974, 748, 694; ^{1}H 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); ^{13}C 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 $C_{17}H_{23}N_6O_4$ $[M+H]^+$: 375.1781, found: 375.1784; Anal. calcd for $C_{17}H_{22}N_6O_4$: 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), T_3P (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): υ_{max}/ cm^{-1} = 3269, 3081, 2954, 2125, 1705, 1648, 1551, 1476, 1412, 1376, 1227, 1230, 1183, 1030, 978, 745, 701, 679; ^{1}H 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); ^{13}C 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 $C_{21}H_{22}N_6O_4$: 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*.,[31]. All the synthesized compounds and standard acarbose were prepared at different concentrations. 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 room temperature for 30 min. After incubation 50 µL 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 µL 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 IC_{50} values were obtained as mean ± SD in triplicates as shown in **Table 1**.

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

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 [32,33]. 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.

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

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[30]. 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 P^H~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 ≥ 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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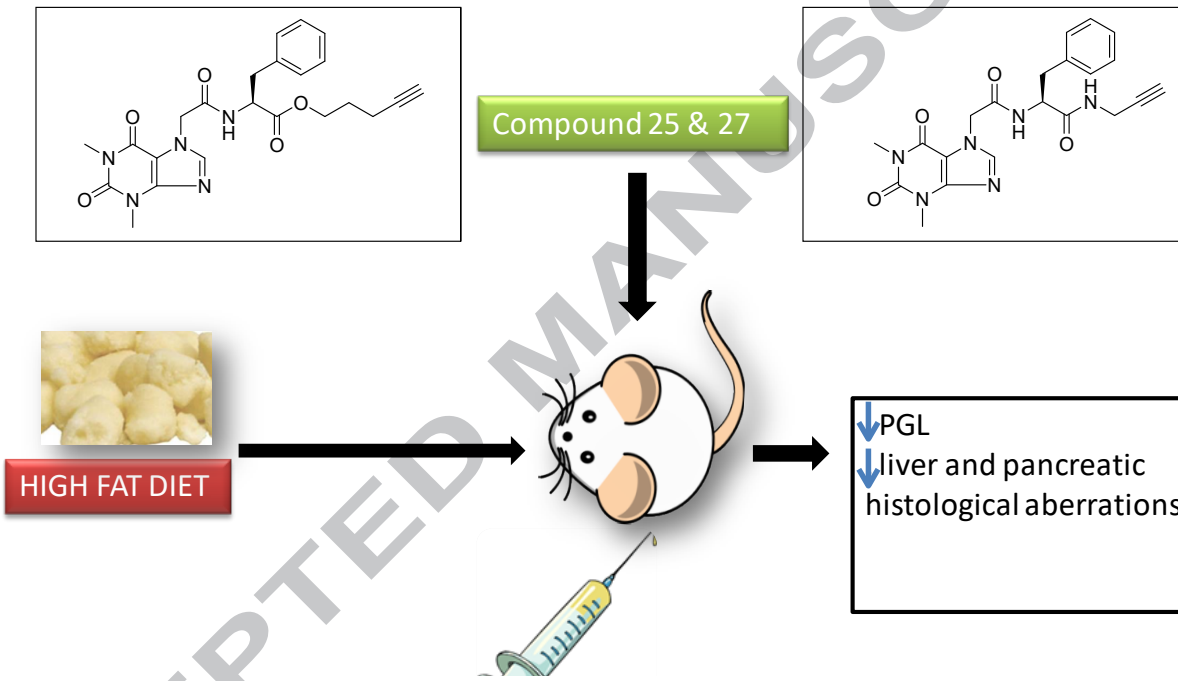
Graphical Abstract



Highlights

- Theophylline containing variant acetylene derivatives were designed and synthesized.
- All synthesized compounds were evaluated for *in vitro* α-amylase inhibitory activity and molecular docking studies were performed on α-amylase enzyme (4GQR)
- The potent two α-amylase inhibitors were further screened for *in vivo* anti-diabetic activity by High Fat Diet-Streptozotocin (HFD-STZ) model in normal rats.
- Structure-activity relationship was established.
- The *in vivo* anti-diabetic and histological studies reveled these compounds have the potential analogs in the treatment of type-2 diabetes.