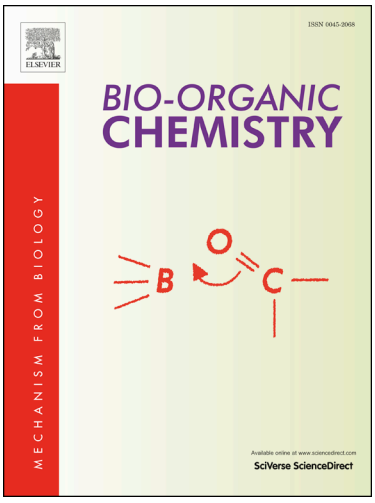

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Synthesis, *in vitro* α-glucosidase inhibitory potential and molecular docking study of thiadiazole analogs

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Abstract

α-Glucosidase is a catabolic enzyme that regulates the body's plasma glucose levels by providing energy sources to maintain healthy functioning. 2-Amino-thiadiazole (**1-13**) and 2-amino-thiadiazole based Schiff bases (**14-22**) were synthesized, characterized by ^{1}HNMR and HREI-MS and screened for α-glucosidase inhibitory activity. All twenty two (**22**) analogs exhibit varied degree of α-glucosidase inhibitory potential with IC_{50} values ranging between 2.30 ± 0.1 to 38.30 ± 0.7 μM, when compare with standard drug acarbose having IC_{50} value of 39.60 ± 0.70 μM. Among the series eight derivatives **1**, **2, 6**, **7**, **14**, **17, 19** and **20** showed outstanding α-glucosidase inhibitory potential with IC_{50} values of 3.30 ± 0.1, 5.80 ± 0.2, 2.30 ± 0.1, 2.70 ± 0.1, 2.30 ± 0.1, 5.50 ± 0.1,4.70 ± 0.2, and 5.50 ± 0.2μM respectively, which is many fold better than the standard drug acarbose. The remaining analogs showed good to excellent α-glucosidase inhibition. Structure activity relationship has been established for all compounds. The binding interactions of these compounds were confirmed through molecular docking.

Keywords: Synthesis, thiadiazole, α-glucosidase, molecular docking study, SAR.

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1. Introduction

Diabetes mellitus (DM) is one of the most serious and chronic metabolic diseases all over the world characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1, 2]. Normalization of blood glucose levels, especially postprandial hyperglycemia is important to prevent the complications of diabetes which include retinopathy, neuropathy, nephropathy, coronary heart disease, stroke, and peripheral arteriopathy [3]. α-Glucosidase catalyzes the final step in the digestive process of carbohydrates to effect postprandial blood glucose levels [4]. Patients with type-2 diabetes mellitus (T2DM) mostly suffer from hyperglycemia, need to maintain normal blood glucose level to control the development of type 2 diabetes [5, 6]. In this regard *α*-glucosidase inhibitors play a critical role to prevent or delay the digestion or absorption of carbohydrates and suppress postprandial hyperglycemia, making such inhibitors useful in management of type 2 diabetes [7]. In addition, α-glucosidase inhibitors have been thought to prevent postprandial endothelial dysfunction and cardio metabolic disorders, as well as tumor growth and metastasis [8-11]. Thus, the study of α-glucosidase inhibition has broad aspects and many potentially useful applications.

Heterocyclic compounds are of special interest to medicinal chemists because of their exceptional chemical and versatile biological profiles. Despite significant research progress on heterocyclic ring systems, efforts are on-going to identify novel heterocyclic compounds with potent bioactivities. The 1,3,4-thiadiazole scaffold has drawn special interest because of its inherent and diverse biological response [12].

1,3,4-Thiadiazole, a privileged structure, represents a key motif in heterocyclic chemistry and occupies a prime place in medicinal chemistry due its wide range of pharmacological activities [13]. Recently, derivatives of 1,3,4-oxadiazole/thiadiazole have been reported for their antibacterial [14, 15], antifungal [16], antiviral [17], anti-inflammatory [18], antianxiety [19], antimicrobial [20], anticonvulsant [21], anticancer [22], anti-depressant [23] and anti-tubercular activities [24]. Some other thiadiazole containing drugs present in the market include acetazolamide, methazolamide, cefazedone, timolol and xanomeline [25].

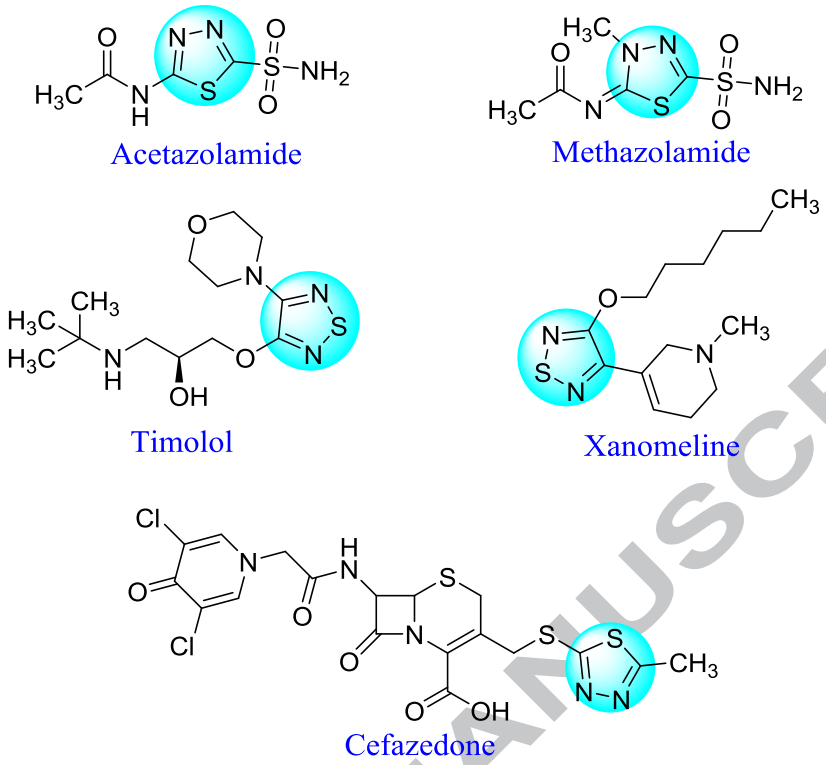


Fig-1: Thiadiazole containing drugs

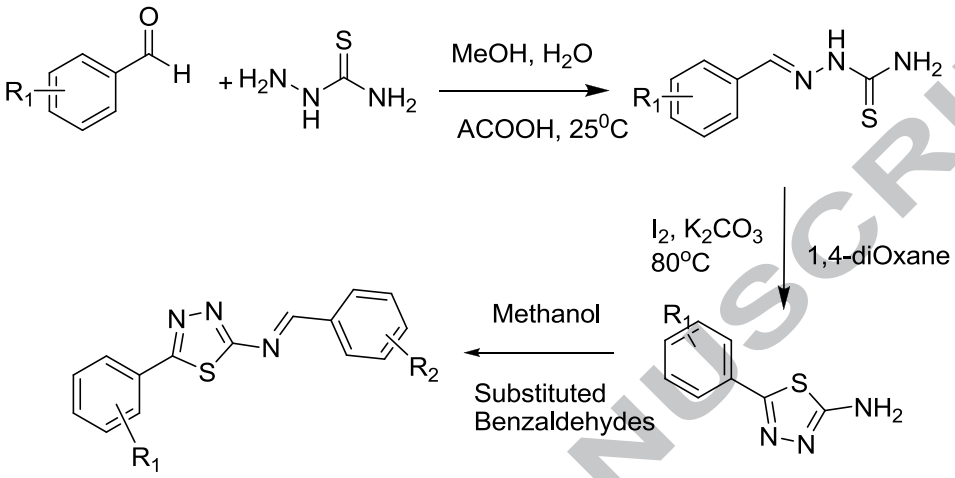
Our research group has been working on design and synthesis of heterocyclic compounds including thiadiazole, oxadiazole and oxazole derivatives in search of potential lead compounds since few years and had found promising results [26-35]. Here in this study we are reporting synthesis and α-glucosidase inhibition of thiadiazole derivatives.

2. Results and Discussion

2.1. Chemistry

Synthesis of 2-amino-1,3,4-thiadiazole analogs was carried out in two steps. In step-1, equimolar thiosemicarbazide was mixed with different substituted benzaldehyde in ethanol in the presence of 2-3ml of HCl as a catalyst and refluxed for 3-4 hrs. In step-2, these Schiff base intermediate was cyclized by treating it with iodine and potassium carbonate using 1,4-dioxane as a solvent and refluxed for 4 hrs. After reaction completion it was cooled at room temperature then reacted with $Na_2S_2O_3$ and extracts the desired organic compound from the reaction mixture using ethyl acetate to get the corresponding solid product of 2-amino-1,3,4-thiadiazole analogs.

In step-3, equimolar 2-amino-1,3,4-thiadiazole was further reacted with substituted benzaldehydes in methanol in the presence of few drops of acetic acid to give us the desired thiadiazole based Schiff bases analogs (**14-22**).



Scheme-1: Synthesis of 2-amino-1,3,4-thiadiazole based Schiff bases analogs

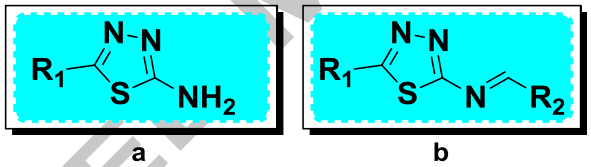
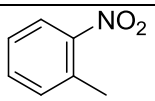
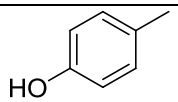
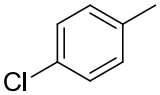
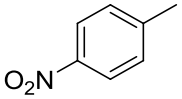
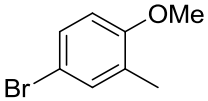
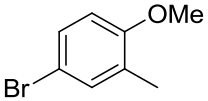
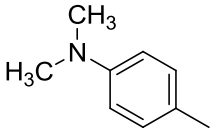
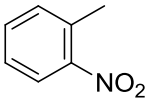
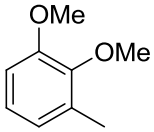
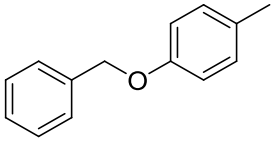
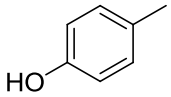
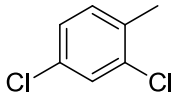
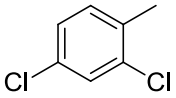
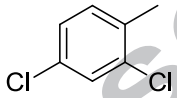
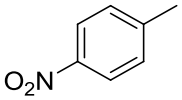
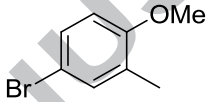
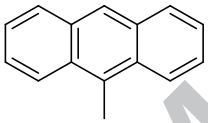
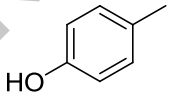
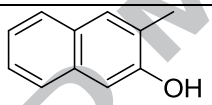
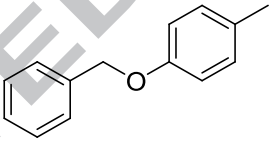
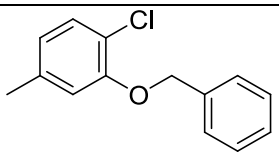
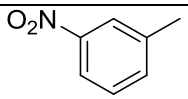


Fig-2: General structure of (a) 2-amino-1,3,4-thiadiazole analogs (**1-13)** and (b) 2-amino-1,3,4-thiadiazole based Schiff bases analogs (**14-22)**

Table-1: Different substituents of 2-amino-1,3,4-thiadiazole analogs (**1-13)** and 2-amino-1,3,4-thiadiazole based Schiff bases analogs (**1-22**)

Sr. No.	R_1	R_2
1		
2		
3		

4		
5		
6		
7		
8		
9		
10		
11		
12		
13		

2.2. *α*-Glucosidase activity

2-Aminothiadiazole (**1-13**) and thiadiazole based Schiff bases (**14-22**) were illustrated for α-glucosidase inhibitory potential. All the synthesized analogs showed potent inhibitory potential ranging between (2.70 ± 0.1μM to 38.30 ± 0.7μM) against standard drug acarbose with IC_{50} value of (38.45 ± 0.80 μM). Structure activity relationship shows that the activity of synthesized

analogs depends upon substituents attached to phenyl ring. 1,3,4-thiadiazole analogs having substituent with phenyl ring i.e, 4-hydroxy analog (**6**) (IC_{50} = 2.30 ± 0.1 μM), 2,4-dichloro analog (**7**) (IC_{50} = 2.70 ± 0.1 μM) and 2-nitro analog (**1**) (IC_{50} = 3.30 ± 0.1 μM) showed potent α-glucosidase inhibitory potential. 4-hydroxy analog shows the best activity then that of 2,4-dichloro and 2-nitro analogs this may be due to the hydroxy substituent. 2-nitro analog having less IC_{50} value then 4-hydroxy and 2,4-dichloro analogs.

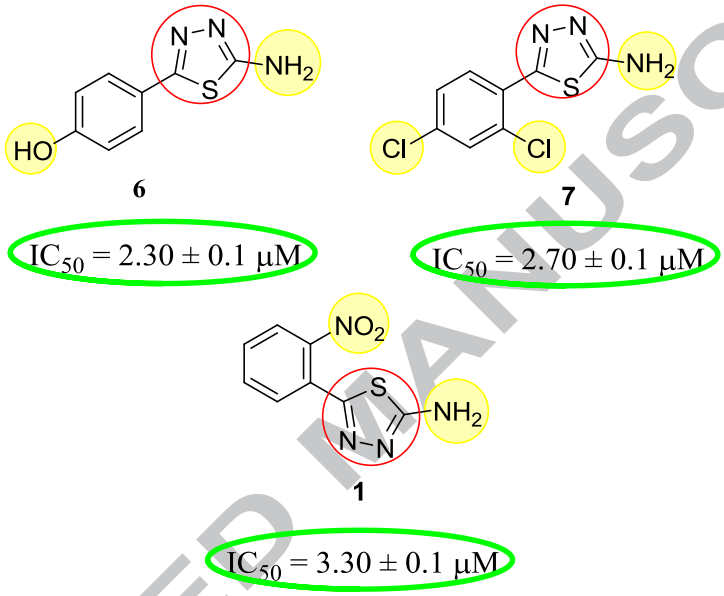


Figure 3: Most potent analogs**6, 7** and **1**

1,3,4-thiadiazole analogs having 4-chloro analog (**2**) (5.80 ± 0.2), 4-nitro analogue (**8**) (6.40 ± 0.2),3-nitro analog (**13**) (8.40 ± 0.2) and naphthalen-2-ol analog (**10**) (9.30 ± 0.3) showed outstanding inhibitory potential due to the different substituents on phenyl part. Other 1,3,4-thiadiazole analogs having substituents as 4-N,N-dimethylamine analog (**4**), 2-methoxy-5-bromo analog (**3**), 2,3-dimethoxy analog (**5**), 3-benzyloxy-4-chloro analog (**12**), 4-benzyloxy analog (**11**), anthracenyl analog (**9**) with IC_{50} values of 11.70 ± 0.3, 15.90 ± 0.3, 20.10 ± 0.4, 25.30 ± 0.4, 35.30 ± 0.6 and 38.30 ± 0.7µM, respectively showed excellent inhibitory potential.

Thiadiazole based Schiff bases analogs (**14-22**) were showed potent α-glucosidase inhibitory potential. Thus these analogs having substituents on phenyl ring i.e, 4-hydroxy with 4-chloro (**14**) (2.30 ± 0.1μM), 2,4-dichloro with 4-chloro analog (**19**) (4.70 ± 0.2μM), 4-chloro with 2-

nitro analog (**17**) (5.50 ± 0.1μM) and 3-nitro with 2,4-dichloro (**20**) (5.50 ± 0.2μM) showed important α-glucosidase inhibitory activity.

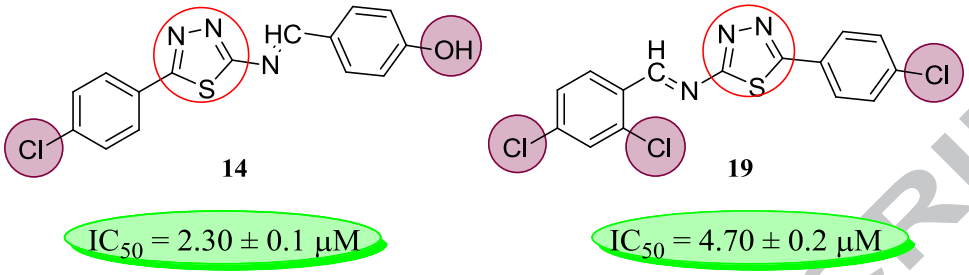


Figure-4: Most potent analogs **14** and **19**

Other thiadiazole based Schiff bases analogs having 4-chloro with 4-nitro analog (**15**) (11.60 ± 0.3), 5-bromo-2-methoxy with 3-nitro analog (**21**) (15.30 ± 0.3), 4-benzyloxy with 4-chloro analog (**18**) (17.6 ± 0.4), 4-benzyloxy-3-methoxy with 4-hydroxy analog (**22**) (10.20 ± 0.2)5-bromo-2-methoxy with 4-chloro analog (**16**) (25.30 ± 0.5), substituents on phenyl ring showed excellent inhibitory potential.

The synthesized analogs are more active than the standard acarbose. Analogs with nitro, chloro and hydroxy substituents possess outstanding activity.

Table-2: α-Glucosidase inhibition of thiadiazole derivatives (**1-22**)

Comp. No.	**IC_{50} ± SEM[a]**	**Comp. No.**	**IC_{50} ± SEM[a]**
1	3.30 ± 0.1	13	8.40 ± 0.2
2	5.80 ± 0.2	14	2.30 ± 0.1
3	15.90 ± 0.3	15	11.60 ± 0.3
4	11.70 ± 0.3	16	25.30 ± 0.5
5	20.10 ± 0.4	17	5.50 ± 0.1
6	2.30 ± 0.1	18	17.6 ± 0.4
7	2.70 ± 0.1	19	4.70 ± 0.2
8	6.40 ± 0.2	20	5.50 ± 0.2
9	38.30 ± 0.7	21	15.30 ± 0.3

10	9.30 ± 0.3	22	10.20 ± 0.2
11	35.30 ± 0.6		
12	25.30 ± 0.4	**Acarbose**	**39.60 ± 0.70**

2.3. Molecular docking analysis

In order to get insight into the binding interactions of the 2-amino-1,3,4-thiadiazole derivatives with the active site amino acids of α-glucosidase the docking study was carried out. Although the crystal structure of all eukaryotic α-glucosidase enzyme is still unavailable, so the 3D homology model was used in this work for docking study. All the optimized compounds were docked into the binding pocket of α-glucosidase. All the compounds showed good docking results as experimental results. The top ranked conformations of the most active compounds were selected for further studies and visual inference. Interaction detail of compounds and protein

To discuss the interaction detail of compounds and α-glucosidase, all the twenty-two synthesized compounds were classified into two groups on the basis of their IC_{50} value.

i. Interactions of the most active compounds

The compounds which have IC_{50} values between 2.30 ± 0.1 and 9.30 ± 0.3 exhibit varying degree of α-glucosidase inhibitory activity. From the molecular docking study (docking score -5.231) it was observed that the top ranked conformation of the most active compound **06** (IC_{50} 2.30 ± 0.1mM) (**Fig. 5a**) established three hydrogen bonds through amine and hydroxyl group with the binding site residues Glu276 and Asp408. The sulfur atom of thiadiazole group of compound formed a hydrogen bond with Asn347 residues. Where as the compound shows an arene-hydrogen interaction with the Arg439 and shows some hydrophobic interactions towards Phe300 and His348 residues.

The docking results, of second most active compound 14 which have the same experimental results as compound 6 with IC_{50} 2.30 ± 0.1 μM (docking score -5.743) (**Fig. 5b**) revealed that the chlorobenzene moiety of the compound establishes a hydrogen donor interaction with the oxygen atom of Pro309 residue. Whereas three arene-H bonds were observed with Tyr71, Phe157 and Arg312 and hydrophobic interactions with Tyr313 residue.

Compound **07** (2.70 ± 0.1) can be seen clearly in **Figure 5c** forming four interactions with the residues Asp68, Asp214 and Asp349. Asp68 was involved in side chain hydrogen donor interaction with the sulfur of thiadiazol group and amine group. The Asp214 shows a side chain

hydrogen donor interaction with the chlorine atom of chlorophenyl moiety and Asp349 also involved in hydrogen bond with the sulfur of thiadiazol group of the compound.

Compound **1** which the third most active compound with IC_{50} value 3.30 ± 0.1 (docking score -5.783) (**Fig. 5d**) established six hydrogen bonds with the binding site residues of enzyme. Arg212 and His348 formed hydrogen bonds with the nitro group of nitrobenzene moiety. Glu276 and Arg439 show hydrogen bonds with the thiadiazol moiety and Asp214 makes a hydrogen bond with the benzene. Where as Phe177 and Phe300 involved in hydrophobic interactions.

The interaction of the compound with the active site of the enzyme may be due to the strong electron withdrawing group like halogen and nitro (NO_2) groups and electron donating groups hydroxyl and amine (OH and NH_2) groups in the compound.

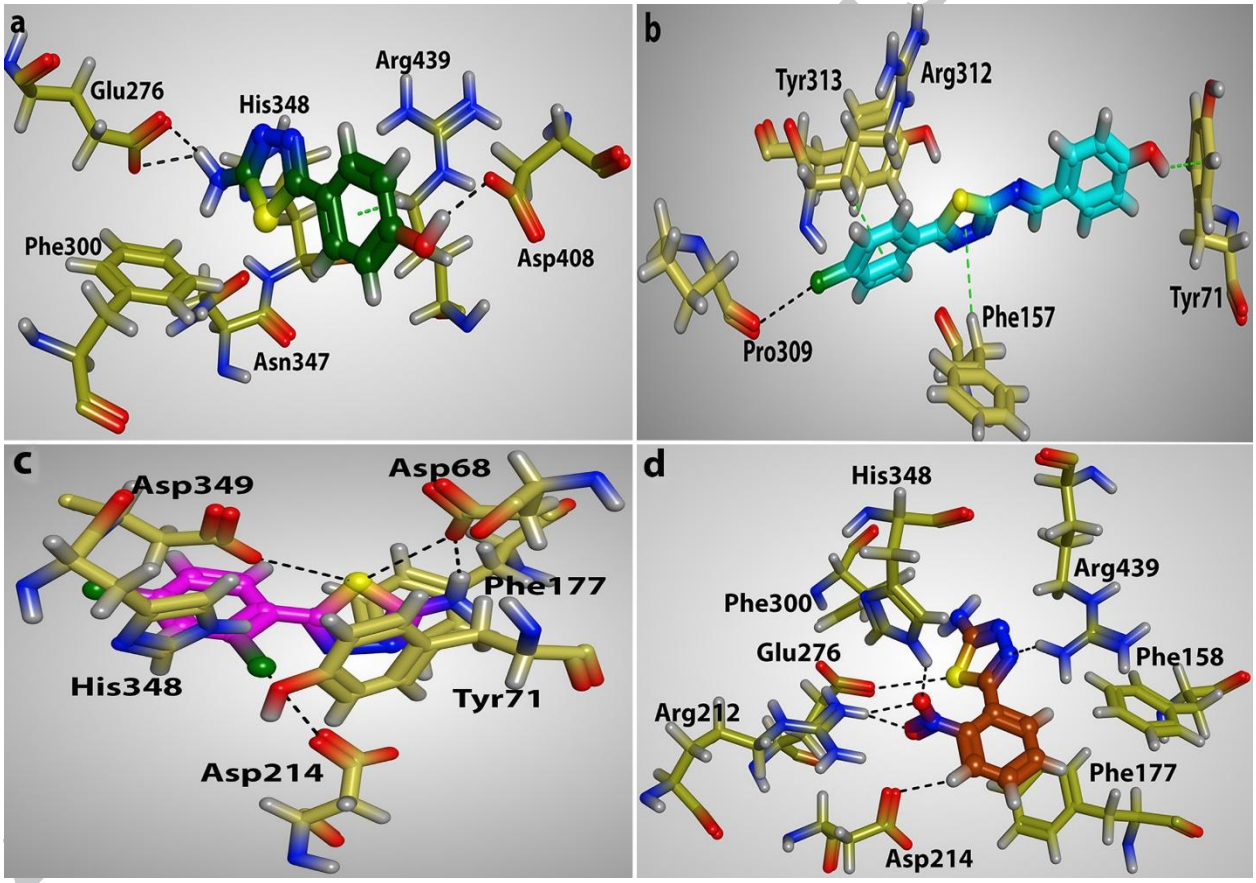


Figure 5: Representation of most active compounds (green, cyan, pink and brown) in the binding site (lemon yellow) of α-glucosidase (a) compound **6** (b) compound **14** (c) compound **7** (d) compound **1**. Interactions are represented as dotted lines, H-bonds black, arene-H bonds green.

ii. Binding interactions of moderate active compounds

Compounds having IC_{50} value between 10.20 ± 0.2 μM and 38.30 ± 0.7 μM exhibit varying degree of α-glucosidase inhibitory activity and good docking results. The molecular docking study of the top ranked compound of this series shows various interactions with the binding site residues. Compound **22** with IC_{50} value 10.20 ± 0.2 μM (docking score -6.126) shows four interactions. The benzene hydrogen formed one hydrogen bond with Gln181 and the phenol group and benzene ring establishes three arene-hydrogen interactions with Phe177, Phe311 and Arg312 residues of the enzymes (**Fig. 6a**).

Compound **15** with IC_{50} value of 11.60 ± 0.3 μM (docking score -6.492) also showed considerable interactions with the binding site residues. The binding mode of compound 15 shown in figure (**15C**). The nitrophenyl moiety of the compound formed two hydrogen bonds with Arg212 and His348. While the chlorophenyl moiety forms a hydrogen bond with Pro309 and two arene-hydrogen interactions with Phe157 and Arg312 residues (**Fig. 6b**).

The thiadiazol-2-amine moiety of compound **4** shows two hydrogen bonds (docking score -5.207) with the Phe157 residue of enzyme. Where as the Arg439 shows arene-hydrogen bond with the thiadiazole ring of the compound (**Fig. 6c**). While compound **21** with IC_{50} value 15.30 ± 0.3 (docking score -6.216) exhibited two hydrogen bonds with the Arg212 and His348 residues. The compound also shows few hydrophobic interactions with residues Tyr71, Phe177 and Phe300 (**Fig. 6d**). The moderate inhibitory activity of this group of compound may be due to the presence of bulky methoxy groups. The brief interaction detail of the compounds is given in **Table-3.**

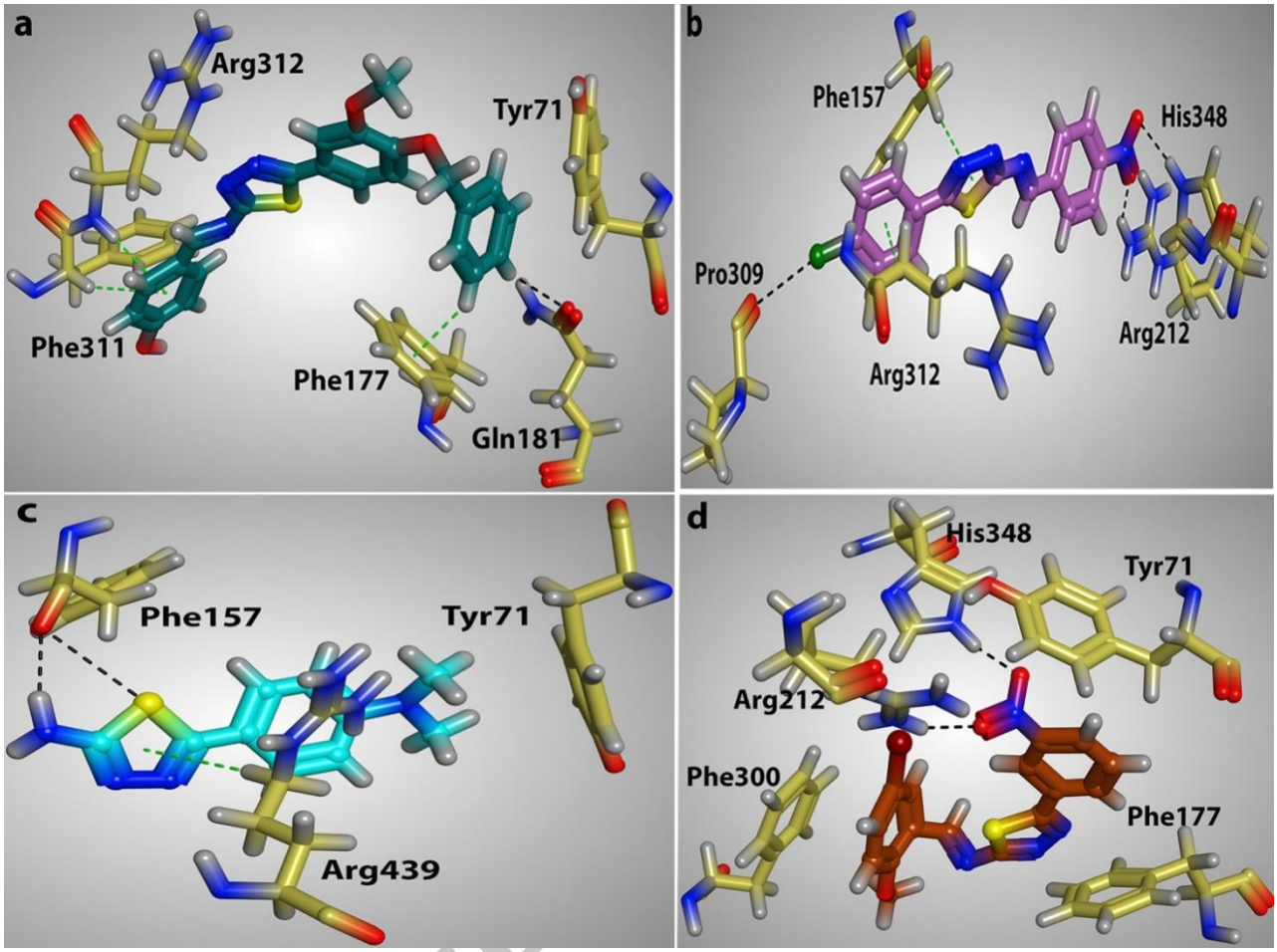


Figure 6: Predicted binding interactions of moderately active compounds (green, pink, cyan and brown) in the binding site (lemon yellow) of α-glucosidase. (a) compound**22** (b) compound **15** (c) compound **4** (d) compound **21**. Interactions are represented as dotted lines, H-bonds black, arene-H bonds green.

Table-3: Interaction detail of all twenty-two compounds.

CompNo.	**Docking score**	**Interaction detail (Ligands/α-glucosidase)**				
01	-5.783	Ligand	Receptor	Interaction	Distance	E(kcal/mol)
		S 11	OE1 GLU 276	H-donor	3.55	-1.2
		N 8	NH1 ARG 439	H-acceptor	3.26	-1.6
		O 14	NH2 ARG 212	H-acceptor	3.22	-2.6
		O 15	NH2 ARG 212	H-acceptor	3.33	-1.3

		O 15	NE2 HIS 348	H-acceptor	3.05	-1.8
02	-4.505	Ligand	Receptor	Interaction	Distance	E(kcal/mol)
		S 11	OD2 ASP 408	H-donor	3.70	-0.7
		N 12	OD2 ASP 408	H-donor	2.95	-3.7
		CL 13	OD1 ASP 214	H-donor	3.30	-1.0
		5-ring	CB PHE 157	pi-H	4.25	-0.8
03	-5.895	Ligand	Receptor	Interaction	Distance	E(kcal/mol)
		S 11	OD2 ASP 68	H-donor	3.61	-4.1
		S 11	OD2 ASP 349	H-donor	3.62	-1.8
		N 12	OD2 ASP 68	H-donor	3.12	-4.9
		O 14	NH2 ARG 212	H-acceptor	3.09	-1.2
		5-ring	6-ring PHE 177	pi-pi	3.93	-0.0
04	-5.207	Ligand	Receptor	Interaction	Distance	E(kcal/mol)
		S 11	O PHE 157	H-donor	3.58	-0.4
		N 12	O PHE 157	H-donor	3.30	-1.7
		5-ring	CD ARG 439	pi-H	4.62	-0.4
05	-5.695	Ligand	Receptor	Interaction	Distance	E(kcal/mol)
		N 12	O PHE 157	H-donor	3.12	-0.9
		N 12	OD2 ASP 408	H-donor	2.97	-0.8
06	-5.231	Ligand	Receptor	Interaction	Distance	E(kcal/mol)
		O 8	OD2 ASP 408	H-donor	2.83	-2.3
		S 12	OD1 ASN 347	H-donor	4.02	-0.5
		N 13	OE1 GLU 276	H-donor	3.03	-2.7
		N 13	OE2 GLU 276	H-donor	3.08	-0.6
		6-ring	CD ARG 439	pi-H	3.64	-0.8
07	-5.446	Ligand	Receptor	Interaction	Distance	E(kcal/mol)
		S 11	OD2 ASP 68	H-donor	3.45	-2.9
		S 11	OD2 ASP 349	H-donor	4.00	-0.6
		N 12	OD2 ASP 68	H-donor	3.08	-4.7
		CL 14	OD1 ASP 214	H-donor	3.19	-0.2
		5-ring	6-ring PHE 177	pi-pi	3.78	-0.0

08	-5.327	Ligand	Receptor	Interaction	Distance	E(kcal/mol)
		S 11	OD2 ASP 408	H-donor	4.02	-0.8
		N 12	OD2 ASP 408	H-donor	2.96	-2.0
		O 14	NH2 ARG 212	H-acceptor	3.37	-0.9
		O 15	NH2 ARG 212	H-acceptor	3.01	-1.9
09	-6.115	Ligand	Receptor	Interaction	Distance	E(kcal/mol)
		S 6	OD2 ASP 68	H-donor	3.87	-3.6
		N 7	OD2 ASP 68	H-donor	3.43	-2.3
		6-ring	CD ARG 439	pi-H	4.29	-0.8
		5-ring	6-ring PHE 177	pi-pi	3.86	-0.0
10	-5.477	Ligand	Receptor	Interaction	Distance	E(kcal/mol)
		S 11	OD2 ASP 68	H-donor	3.54	-4.8
		S 11	OD2 ASP 349	H-donor	3.76	-0.5
		N 12	OD2 ASP 68	H-donor	3.41	-1.8
		6-ring	CD ARG 439	pi-H	3.95	-0.6
		5-ring	6-ring PHE 177	pi-pi	3.93	-0.0
11	-6.222	Ligand	Receptor	Interaction	Distance	E(kcal/mol)
		S 11	OD2 ASP 408	H-donor	3.55	-0.4
		N 12	OD2 ASP 408	H-donor	2.99	-4.1
		5-ring	CB PHE 157	pi-H	4.36	-1.2
12	-6.121	Ligand	Receptor	Interaction	Distance	E(kcal/mol)
		S 1	OD1 ASP 214	H-donor	3.88	-3.0
		S 1	OE1 GLU 276	H-donor	3.99	-1.4
		N 6	OD1 ASP 214	H-donor	3.10	-3.9
13	-5.529	Ligand	Receptor	Interaction	Distance	E(kcal/mol)
		S 11	OD1 ASP 214	H-donor	3.92	-1.7
		N 12	OE1 GLN 181	H-donor	3.53	-0.8
		O 15	ND2 ASN 347	H-acceptor	3.21	-0.8
14	-5.743	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
		CL 13	O PRO 309	H-donor	3.48	-0.5
		O 21	6-ring TYR 71	H-pi	3.86	-0.2

		5-ring	CB PHE 157	pi-H	4.36	-0.8
		6-ring	CB ARG 312	pi-H	3.72	-0.5
15	-6.492	Ligand	Receptor	Interaction	Distance	E(kcal/mol)
		CL 13	O PRO 309	H-donor	3.22	-0.8
		O 22	NH2 ARG 212	H-acceptor	2.96	-3.2
		O 23	NE2 HIS 348	H-acceptor	3.24	-0.7
		5-ring	CB PHE 157	pi-H	4.60	-1.2
		6-ring	CB ARG 312	pi-H	3.53	-0.5
16	-6.579	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
		BR 20	OD2 ASP 68	H-donor	3.51	-0.6
		CL 21	O PRO 309	H-donor	3.72	-0.4
		5-ring	CB PHE 157	pi-H	4.71	-1.2
17	-6.621	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
		O 22	NH2 ARG 212	H-acceptor	3.45	-0.9
		O 23	NH2 ARG 212	H-acceptor	3.23	-1.9
		O 23	NE2 HIS 348	H-acceptor	3.17	-1.1
18	-7.851	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
		CL 20	O PRO 309	H-donor	2.99	-0.4
		C 27	6-ring PHE 177	H-pi	3.57	-0.5
		6-ring	N ARG 312	pi-H	3.84	-0.4
		6-ring	CB ARG 312	pi-H	3.56	-0.3
19	-6.216	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
		CL 20	O PRO 309	H-donor	3.43	-0.7
		CL 21	NH2 ARG 212	H-acceptor	3.70	-0.4
		5-ring	CB PHE 157	pi-H	4.61	-1.2
		6-ring	CB ARG 312	pi-H	3.53	-0.5
20	-6.751	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
		C 7	OD2 ASP 408	H-donor	3.61	-0.4
		S 11	O PHE 157	H-donor	3.30	-0.1
		S 11	OD2 ASP 408	H-donor	3.48	-0.2
		5-ring	CB PHE 157	pi-H	4.84	-0.3

21	-6.216	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
		O 24	NH2 ARG 212	H-acceptor	3.20	-3.3
		O 25	NE2 HIS 348	H-acceptor	2.87	-2.9
22	-6.126	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
		H 11	OE1 GLN 181	H-donor	2.77	-0.8
		C 25	6-ring PHE 177	H-pi	3.54	-0.5
		6-ring	CA PHE 311	pi-H	4.51	-0.6
		6-ring	N ARG 312	pi-H	3.97	-1.3

2.4. Conclusion

In conclusion we have synthesized and evaluated α-glucosidase inhibition of 2-Aminothiadiazole (**1-13**) and 2-Aminothiadiazole based Schiff base analogs (**14-22**). All compounds showed varied degree of α-glucosidase inhibition ranging between 1.73 ± 0.001 to 69.65 ± 0.12 μM when compared with the standard inhibitor acarbose having IC_{50} value 21.25 ± 0.15 μM. Among the series, analogs **1**, **2, 6**, **7**, **14**, **17, 19** and **20** showed outstanding α-glucosidase inhibitory potential which were more active than standard acarbose. The remaining analogs also showed good to excellent α-glucosidase inhibition. SAR has been established for all compounds.

3. Experimental

3.1. Materials and Methods

The NMR spectra of concerned analogs of thiadiazole were recorded by on Avance Av-500 MHz NMR spectrometers in which DMSO-$d_6$were used as a solvent. Chemical shift values of spectra are assigned in δ (ppm). Tetramethylsilane act as internal standard. Jeol JMS-600H instrument were used to obtained Electron Ionization Mass Spectra. The probability of reactions was checked by TLC on pre-coated silica gel aluminum plates (kieselgel 60,254, E. Merck, Germany). UV lamp at range of 254 and 365nm were used to observe the spots on TLC plate.

3.2. Reaction procedure for the synthesis of 2-amino-1,3,4-thiadiazole

Synthesis of 2-amino-1,3,4-thiadiazole analogs was carried out in two steps. In first step various benzaldehyde (1 mmol) was mixed with thiosemicarbazide (1 mmol) in ethanol. 2-3ml of HCl was added as a catalyst and refluxes the reaction mixture for 3-4 hrs. The color change indicates

reaction progress. Reaction completion was monitored by TLC. On completion, reaction mixture was filtered, washed with hexane followed by ethanol to get Schiff base intermediate.

In step-2, these Schiff base intermediate was cyclized by treating it with iodine and potassium carbonate at 80^0C using 1,4-dioxane as a solvent. The reaction mixture was refluxed for 4 hrs. Reaction completion was monitored by TLC. After reaction completion it was cooled at room temperature then reacted with 5% $Na_2S_2O_3$ (20 mL) and extract the desired organic compound from the reaction mixture using ethyl acetate. Upper layer with desired organic compound will be collected and on evaporation get the corresponding solid product of 2-amino-1,3,4-thiadiazole analogs.

3.2.1. 5-(2-nitrophenyl)-1, 3, 4-thiadiazol-2-amine

Pale Yellow solid , Yield: 75%; ^{1}H-NMR: (500 MHz, DMSO-d_6): δ 10.2 (s, 2H, -NH_2), 8.2 (d, J = 6.4 HZ, 1H, Ar), 8.0(d, J= 8.2 HZ, 1H,Ar), 7.9 (dd, J = 7.2 HZ, J = 7.8 HZ, 1H,Ar), 7.6 (m, 1H, Ar); ^{13}C-NMR (125 MHz, DMSO-d_6):δ 171.4, 158.2, 146.2, 135.0, 131.1, 129.2, 128.0, 123.9; HR-EI-MS: *m/z* calcd for $C_8H_6N_4O_2S$, $[M]^+$ 222.0201; found 222.0200.

3.2.2. 5-(4-chlorophenyl)-1,3,4-thiadiazole-2-amine

White solid, Yield: 85%; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 10.1 (s, 2H, -NH_2), 7.82 (d, J = 8.2 Hz, 2H, Ar), 7.60 (d, J = 8.6Hz, 2H, Ar); ^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.4, 161.2, 134.0, 131.2, 128.7, 128.7, 128.1, 128.1; HR-EI-MS: m/z calcd for $C_8H_6ClN_3S$, $[M]^+$ 210.9971; found 210.9973.

3.2.3. 5-(5-bromo-2-methoxyphenyl)-1,3,4-thiadiazol-2-amine

White solid, Yield: 70%; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 10.0 (s, 2H, -NH_2), 7.5 (d, J= 2.3 Hz, 1H, Ar), 7.2 (dd, J = 2.9 Hz, J = 8.9 Hz 1H, Ar), 7.1 (d, J = 8.8 Hz, 1H, Ar), 6.4(s, 3H, -OCH3); ^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.6, 161.1, 156.0, 133.5, 132.1, 124.0, 116.4, 114.0, 55.8; HR-EI-MS:m/z calcd for $C_9H_8BrN_3OS$, $[M]^+$ 284.9571; found 284.9575.

3.2.4. 5-(4-(dimethylamino)phenyl)-1,3,4-thiadiazol-2-amine

White solid, Yield: 83%., ^{1}H-NMR (500 MHz, DMSO-d_6): δ 9.7 (s, 2H, -NH2), 7.4 (d, J= 8.2 Hz, 2H, Ar), 6.6 (d, J = 8.8 Hz, 2H, Ar), 3.14 (s, 6H, -CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.5, 161.3, 155.0, 129.1, 129.1, 122.4, 112.2, 112.2, 40.7, 40.7; HR-EI-MS: m/z calcd for $C_{10}H_{12}N_4S$, $[M]^+$ 220.0784; found 220.0781.

3.2.5. 5-(2,3-dimethoxyphenyl)-1,3,4-thiadiazol-2-amine

Pale yellow, Yield: 80%. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 10.1 (s, 2H, -NH2), 7.5 (dd, J = 1.7 Hz, J = 7.7Hz, 1H, Ar), 7.2 (t, J = 8.2 Hz, J = 7.0 Hz, 1H, Ar), 7.0 (dd, J = 1.7 Hz, J = 6.2 Hz, 1H, Ar), 3.5 (s, 3H, -OCH_3), 3.3 (s, 3H, -OCH_3);^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.7, 161.2, 151.8, 147.6, 122.7, 122.2, 120.3, 114.8, 60.1, 55.4; HR-EI-MS: m/z calcd for $C_{10}H_{11}N_3O_2S$, $[M]^+$ 237.0571; found 237.0570.

3.2.6. 5-(4-hydroxy phenyl)-1,3,4-thiadiazole-2-amine

White, Yield: 75%; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 10.0 (s, 1H, -OH) 8.5 (s, 2H, -NH2), 8.3 (d, J = 7.7 Hz, 2H, Ar), 7.6 (d, J = 8.5 Hz, 2H, Ar); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 173.4, 161.2, 158.1, 128.4, 128.4, 125.6, 116.0, 116.0; HR-EI-MS:m/z calcd for $C_8H_7N_3OS$, $[M]^+$ 193.0311; found 193.0313.

3.2.7. 5-(2,4-dichlorophenyl)-1,3,4-thiadiazole-2-amine

Solid white, Yield: 78%, ^{1}H-NMR (500 MHz, DMSO-d_6):δ 10.3 (s, 2H, -NH2), 8.1 (d, J= 8.2 Hz, 1H,Ar), 7.5 (d, J = 1.6 Hz, 1H, Ar), 7.3 (dd, J = 1.5 Hz, J = 8.3 Hz, 1H, Ar); ^{13}C-NMR (125 MHz, DMSO-d_6):δ 174.0, 161.1, 135.4, 134.3, 133.1, 130.4, 129.7, 127.0; HR-EI-MS: m/z calcd for $C_8H_5Cl_2N_3S$, $[M]^+$ 244.9581; found, 244.9584.

3.2.8. 5-(4-nitrophenyl)-1,3,4-oxadiazol-2-amine

Yellow. Yield: 70%,^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.431−8.267 (m, 2H, Ar), 8.132−8.044 (m, 2H, Ar), 7.5 (s, 2H, -NH2);^{13}C-NMR (125 MHz, DMSO-d_6):δ 169.0, 164.2, 147.3, 131.6, 130.4, 130.4, 128.2, 128.2; HR-EI-MS: m/z calcd for $C_8H_6N_4O_2S$, $[M]^+$ 206.0440; found, 206.0438.

3.2.9. 5-(anthracen-9-yl)-1,3,4-oxadiazol-2-amine

Green, Yield: 68%,^{1}H-NMR (500 MHz, DMSO-d_6): δ 10.4 (s, 2H, -NH2), 8.4 (d, J = 10.6 Hz, 2H, Ar), 7.7 (d, J = 10.1 Hz, 2H, Ar), 7.4 (t, J = 7.1 Hz, J = 7.6 Hz, 2H,Ar), 7.548-7.227 (m, 2H, Ar), 7.1 (s, 1H, Ar); ^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.5, 161.3, 133.8, 131.7, 131.7, 130.0, 130.0, 129.3, 127.7, 127.7, 125.2, 125.2, 125.1, 125.1, 123.5, 123.5; HR-EI-MS: m/z calcd for $C_{16}H_{11}N_3S$, $[M]^+$ 261.0902; found, 261.0905.

3.2.10. 3-(5-amino-1,3,4-thiadiazol-2-yl)naphthalen-2-ol

Yellow, Yield: 65%,^{1}H-NMR (500 MHz, DMSO-d_6): δ 10.2 (s, 1H, -OH), 8.6 (s, 2H, -NH2), 8.1 (dd, J = 10.0 Hz, J = 11.2Hz, 1H, Ar), 7.91 (d,J = 9.6 Hz, 1H, Ar), 7.7 (dd, J = 3.5 Hz, J = 9.4 Hz, 1H, Ar), 7.305-7.217 (m, 1H, Ar), 7.1 (t, J = 10.6 Hz, J = 10.0 Hz, 1H, Ar), 7.0 (d, J = 10.4 Hz, 1H, Ar);^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.7, 161.2, 151.3, 134.0, 128.1, 127.3, 127.0,

126.2, 126.0, 125.3, 123.3, 109.6; HR-EI-MS: m/z calcd for $C_{12}H_9N_3OS$, $[M]^+$ 243.0466; found, 243.0465.

3.2.11. 5-(4-(benzyloxy)phenyl)-1,3,4-thiadiazol-2-amine

White, Yield: 75%, ^{1}H-NMR (500 MHz, DMSO-d_6): δ 10.0 (s, 2H, -NH2), 7.5 (d, J = 10.1 Hz, 2H, Ar), 7.4 (d, J = 10.1 Hz, 2H, Ar), 7.454-7.367 (m, 5H, Ar), 5.0 (s, 2H, -OCH_2); ^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.4, 161.3, 158.5, 136.2, 128.5, 128.5, 128.1, 128.1, 127.2, 126.7, 126.7, 125.3, 114.3, 114.3, 70.3; HR-EI-MS:m/z calcd for $C_{15}H_{13}N_3OS$, $[M]^+$ 283.0779; found, 283.0777.

3.2.12. 5-(3-(benzyloxy)-4-chlorophenyl)-1,3,4-thiadiazol-2-amine

White, Yield: 88%, ^{1}H-NMR (500 MHz, DMSO-d_6): δ 10.4 (s, 2H, -NH2), 7.5 (s, 1H, Ar), 7.563-7.221 (m, 5H, Ar), 7.2 (dd, J = 1.80 Hz, J = 8.3Hz, 1H, Ar), 7.1 (d, J = 7.5 Hz, 1H, Ar), 2.5 (s, 2H, -OCH_2);^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.2, 161.1, 154.6, 136.2, 132.2, 130.0, 128.4, 128.4, 127.1, 126.5, 126.5, 122.1, 120.7, 112.3, 70.0; HR-ESI-MS: m/z calcd for $C_{15}H_{12}ClN_3OS$, $[M]^+$ 317.0390; Found 317.0393.

3.2.13. 5-(3-nitrophenyl)-1, 3, 4-thiadiazol-2-amine

Yellow solid, Yield: 79%, ^{1}H-NMR (500 MHz, DMSO-d_6):δ 10.2 (s, 2H, -NH2) 8.22 (t, J =2.0 Hz, 1H, Ar), 8.24 (m, J = 8.2, J =2.2, J =0.7 Hz, 1H, Ar), 8.22−8.10 (m, 1H, Ar), 7.44 (t, J = 8.0 Hz, 1H, Ar); ^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.4, 161.1, 148.0, 136.5, 134.0, 129.5, 123.4, 122.3; HR-EI-MS:m/z calcd for $C_8H_6N_4O_2S$ $[M]^+$ 222.0211; Found, 222.0210.

3.3. Synthetic procedure of (*E*)-4-((5-(4-chlorophenyl)-1,3,4-thiadiazol-2-ylimino)methyl)phenol

In step-3, equimolar 2-amino-1,3,4-thiadiazole was further reacted with substituted benzaldehydes in the presence of methanol and 2-3 drops of acetic acid. The reaction mixture was refluxed for 2-3 hrs. Reaction completion was monitored by TLC. After reaction completion desired organic compound was collected and washed with hexane. On evaporation get the corresponding solid product of thiadiazole based Schiff bases analogs.

3.3.14. (E)-4-((5-(4-chlorophenyl)-1,3,4-thiadiazol-2-ylimino)methyl)phenol

White solid, Yield: 71%, (^{1}H-NMR (500 MHz, DMSO-d_6), δ 9.76 (s, 1H, -CH=N), 7.82 (d, J= 8.2 Hz, 2H, Ar), 7.75 (d, J = 8.1 Hz, 2H, Ar), 7.51 (d, J = 7.8, 2H, Ar), 6.91 (d, J = 7.1, 2H, Ar),

5.41 (s, 1H, -OH).^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.7, 161.1, 160.3, 159.5, 133.8, 131.1, 113.4, 130.1, 130.1, 128.8, 128.8, 128.5, 128.3, 128.3, 115.5;HR-EI-MS:m/z calcd for $C_{15}H_{10}ClN_3OS$ [M] $^+$ 315.0233; Found, 315.0235.

3.3.15. (E)-5-(4-chlorophenyl)-N-(4-nitrobenzylidene)-1,3,4-thiadiazol-2-amine

Pale yellow solid, Yield: 74%, ^{1}H-NMR (500 MHz, DMSO-d_6), δ 10.15 (s, 1H, -CH=N), 8.68 (d, J = 8.8, 2H, Ar), 8.53 (d, J = 8.7, 2H, Ar), 8.34 (d, *J* = 8.5 Hz, 2H, Ar), 7.91 (d, J = 8.1, 2H, Ar); ^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.5, 161.2, 159.5, 149.6, 142.0, 133.7, 131.0, 129.0, 129.0, 128.5, 128.5, 127.3, 127.3, 123.6, 123.6;HR-EI-MS:m/z calcd for $C_{15}H_9N_4ClN_4O_2S$ [M] $^+$ 344.0135; Found, 344.0133.

3.3.16. (E)-N-(5-bromo-2-methoxybenzylidene)-5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine

Light yellow solid, Yield: 77%, ^{1}H-NMR (500 MHz, DMSO-d_6), δ10.27 (s,1H, -CH=N), 7.84 (d, J = 8.91, 2H, Ar), 7.74 (s, 1H, Ar), 7.51 (d, J = 7.8, 1H, Ar), 7.25 (d, J = 7.5, 2H, Ar), 7.23 (d, J = 7.4, 1H, Ar), 3.92 (s, 3H, -CH_3).^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.6, 161.0, 159.5, 156.1, 134.5, 134.0, 131.1, 131.1, 128.8, 128.8, 128.4, 128.4, 118.6, 112.5, 109.7, 55.6;HR-EI-MS:m/z calcd for $C_{16}H_{11}BrClN_3OS$ [M] $^+$ 406.9495; Found, 406.9493.

3.3.17. (E)-5-(4-chlorophenyl)-N-(2-nitrobenzylidene)-1,3,4-thiadiazol-2-amine

White solid,Yield: 78%, ^{1}H-NMR (500 MHz, DMSO-d_6), δ 10.24 (s,1H, -CH=N), 8.07 (d, J = 8.3, 2H, Ar), 7.85 (d, J = 8.1, 1H, Ar), 7.75 (d, J = 7.8, 1H, Ar), 7.72 (d, J = 7.7, 2H, Ar), 7.63 (t, 1H, Ar), 7.45 (t, 1H, Ar);^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.5, 161.1, 159.4, 147.3, 134.4, 133.9, 131.4, 131.1, 129.5, 128.8, 128.8, 128.4, 128.4, 127.8, 123.5;HR-EI-MS:m/z alcd for $C_{15}H_9ClN_4O_2S$ [M] $^+$ 344.0136; Found, 344.0135.

3.3.18. (E)-N-(4-(benzyloxy)benzylidene)-5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine

Greenish solid, Yield: 72%, ^{1}H-NMR (500 MHz, DMSO-d_6), δ 10.27 (s,2H, -O-CH_2), 9.59 (s, 1H, -HC=N), 7.85 (d, J = 7.6, 2H, Ar), 7.83 (d, *J* =7.5 Hz, 2H, Ar), 7.75 (d, J = 7.3, 2H, Ar), 7.51 (d, J = 7.6, 2H, Ar), 7.25 (t, 3H, Ar), 7.08 (d, J = 6.8, 2H, Ar);^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.6, 161.0, 160.8, 159.5, 136.2, 133.8, 131.1, 129.7, 129.7, 129.0, 129.0, 128.4, 128.4, 128.4, 128.4, 128.2, 127.1, 126.7, 126.7, 114.0, 114.0, 70.3;HR-EI-MS:m/z calcd for $C_{22}H_{16}ClN_3OS$ [M] $^+$ 405.0704; Found, 405.0702.

3.3.19. (E)-5-(4-chlorophenyl)-N-(2,4-dichlorobenzylidene)-1,3,4-thiadiazol-2-amine

Light grey solid,Yield: 68%, ^{1}H-NMR (500 MHz, DMSO-d_6), δ 10.27 (s,1H, -HC=N), 7.88 (d, J = 7.7, 2H, Ar), 7.84 (d, J = 7.6, 1H, Ar), 7.72 (s, 1H, Ar), 7.64 (d, J = 7.4, 2H, Ar), 7.62 (d, J =

7.3 Hz, 1H, Ar); ^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.5, 161.1, 159.4, 133.8, 131.1, 131.0, 130.5, 128.8, 128.8, 128.6, 128.5, 128.4, 128.4, 127.8, 126.5;HR-EI-MS:m/z calcd for $C_{15}H_8Cl_3N_3S$ [M] $^+$ 366.9505; Found, 366.9503.

3.3.20. (E)-N-(2,4-dichlorobenzylidene)-5-(3-nitrophenyl)-1,3,4-thiadiazol-2-amine

White solid,Yield: 76%, ^{1}H-NMR (500 MHz, DMSO-d_6), δ 10.28 (s,1H, -CH-N), 8.66 (s, 1H, Ar), 8.33 (d, J = 8.5 Hz, 1H, Ar), 8.22 (d, J = 8.3 Hz, 1H, Ar), 7.85 (d, J = 8.1 Hz, 1H, Ar), 7.70 (s, 1H, Ar), 7.64 (t, 1H, Ar), 7.53 (d, J = 7.6 Hz, 1H, Ar); ^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.4, 161.1, 159.5, 148.0, 136.5, 133.8, 131.0, 130.5, 129.6, 128.6, 128.5, 127.8, 127.5, 123.4, 122.3;HR-EI-MS:m/z calcd for $C_{15}H_8Cl_2N_4O_2S$ [M] $^+$ 377.9745; Found, 377.9743.

3.3.21. (E)-N-(5-bromo-2-methoxybenzylidene)-5-(3-nitrophenyl)-1,3,4-thiadiazol-2-amine

Light green solid, Yield: 68%, ^{1}H-NMR (500 MHz, DMSO-d_6), δ 10.26 (s,1H, -CH=N), 8.65 (s, 1H, Ar), 8.33 (d, J = 8.4 Hz, 1H, Ar), 8.23 (d, J = 8.3 Hz, 1H, Ar), 7.83 (s, 1H, Ar), 7.73 (d, J = 7.8 Hz, 1H, Ar), 7.67 (d, J = 7.7 Hz, 1H, Ar), 7.24 (d, J = 7.3 Hz, 1H, Ar), 3.92 (s, 3H, -OCH_3);^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.5, 161.2, 159.5, 156.1, 147.9, 136.5, 134.4, 134.0, 131.1, 129.6, 123.4, 122.2, 118.6, 112.7, 109.6, 55.2;HR-EI-MS:m/z calcd for $C_{16}H_{11}BrN_4O_3S$ [M] $^+$ 417.9734; Found, 417.9736.

3.3.22. (E)-4-((5-(4-(benzyloxy)-3-methoxyphenyl)-1,3,4-thiadiazol-2-ylimino)methyl)phenol

Light brown solid,Yield: 79%, ^{1}H-NMR (500 MHz, DMSO-d_6), δ 9.78 (s,1H, -CH=N), 7.76 (d, J = 7.9 Hz, 2H, Ar), 7.50 (d, J = 7.7, 2H, Ar), 7.41 (t, 3H, Ar), 7.29 (s, 1H, Ar), 7.27 (d, J = 7.4 Hz, 1H, Ar), 7.12 (d, J = 7.2 Hz, 1H, Ar), 6.92 (d, J = 7.1 Hz, 2H, Ar), 5.39 (s, 1H,-OH), 5.19 (s, 2H, -CH_2), 3.93 (s, 3H, -OCH_3);^{13}C-NMR (125 MHz, DMSO-d_6):δ 173.6, 161.1, 160.3, 159.5, 149.7, 149.2, 136.2, 130.1, 130.1, 128.5, 128.3, 128.3, 127.1, 126.6, 126.6, 126.4, 120.3, 115.6, 115.6, 111.8, 110.5, 70.6, 55.6;HR-EI-MS:m/z calcd for $C_{23}H_{19}N_3O_3S$ [M] $^+$ 417.1147; Found, 471.1145.

3.4. α-Glucosidase assay

α-Glucosidase inhibitory activities was determined as per reported methods [36].10 μL of test samples (5 mg/mL DMSO solution)were reconstituted in 100 μL of 100 mM-phosphate buffer (pH6.8) in 96-well microplate and incubated with 50 μL of crude intestinal α-glucosidase for 5 min before 50 μL substrate (5 mM, p-nitrophenyl-α-D-glucopyranoside prepared in same buffer) was added. Release of p-nitrophenol was measured at 405 nm spectrophotometrically (SpectraMax® plus384) for5 min after incubation with substrate. Individual blanks for test

samples were prepared to correct background absorbance where substrate was replaced with 50μL of buffer. Control sample contained 10 μL DMSO in place of test samples. Percentage of enzyme inhibition was calculated as (1-B/A) x 100 whereA represents absorbance of control without test samples, and B represents absorbance in presence of test samples.

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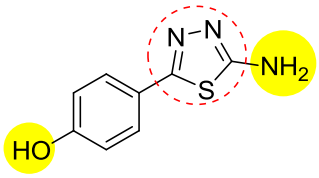
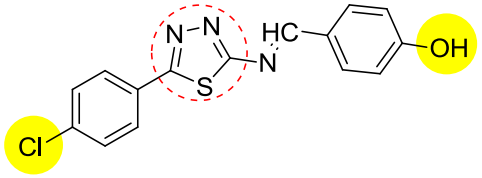
Highlights:

- Synthesis of Thiadiazole derivatives
- *In vitro* α-Glucosidase activity
- Identification of a new class of α-Glucosidase inhibitor
- Structure Activity Relationship established
- Molecular docking



Compound 6
(IC_{50} = 2.30 ± 0.1 *u*M)



Compound 14
(IC_{50} = 2.30 ± 0.1 *u*M)

Potent α-glucosidase Inhibitor

Standard Drug Acarbose
(IC_{50} = 53.02 ± 0.12 *u*M)