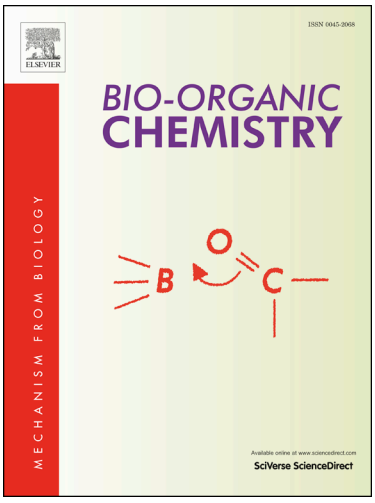

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Synthesis, *in-vitro* α-glucosidase inhibition, antioxidant, *in-vivo antidiabetic* and molecular docking studies of pyrrolidine-2,5-dione and thiazolidine-2,4-dione derivatives

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Abstract

α-Glucosidase is considered as a therapeutic target for the treatment of type 2 diabetes mellitus (DM2). In current study, we synthesized pyrrolidine-2,5-dione (succinimide) and thiazolidine-2,4-dione derivatives and evaluated for their ability to inhibit α-Glucosidase. Pyrrolidine-2,5-dione derivatives (**11a-o**) showed moderate to poor α-glucosidase inhibition. Compound **11o** with the IC_{50} value of 28.3 ±0.28 µM emerged as a good inhibitor of α-glucosidase. Thiazolidine-2,4-dione and dihydropyrimidine (TZD-DHPM) hybrids (**22a-c**) showed excellent inhibitory activities. The most active compound **22a** displayed IC_{50} value of 0.98 ±0.008 µM. Other two compounds of this series also showed activity in low micromolar range. The *in-vivo* antidiabetic study of three compounds **11n, 11o and 22a** were also determined using alloxan induced diabetes mice model. Compounds **11o and 22a** showed significant hypoglycemic effect compared to the reference drug. In-vivo acute toxicity study showed the safety of these selected compounds. *In-silico* docking studies were carried out to rationalize the *in-vitro* results. The binding modes and bioassay results of TZD-DHPM hybrids showed that interactions with important residues appeared significant for high potency.

Keywords: Pyrrolidine-2,5-dione; succinimide; cyanoacetate; thiazolidine-2,4-dione; α-Glucosidase

Introduction

Diabetes is a growing chronic metabolic disorder. It is characterized by hyperglycemia which can be detected by elevated blood glucose level. It is associated with long term dysfunction or failure of various body organs, like kidneys, eyes and heart. Generally, there are two types of diabetes; type-1 is due to deficiency of insulin production, while type-2 is due to insufficiency of insulin action or resistance [1-4]. By 2030, the frequency of diabetes is projected to be double with a 69% increase in developing countries and 20% increase between the grownups in developed countries. According to the reports, globally 300 million people will be affected with diabetes by 2025 and 90% of the affected people possess type 2 diabetes [5]. Currently, commonly used marketed oral hypoglycemic agents for type 2 are biguanides, sulfonylureas, thiazolidinediones, meglitinides and α-glucosidase inhibitors [6-7]. However, all these classes of the drugs are associated with some serious side or adverse effects such as increase food intake, cardiovascular mortality, gastrointestinal discomfort and weight gain *etc*. Traditional plants or their bioactive compounds are also currently used to treat hyperglycemia through various mechanism of actions [8]. These herbal remedies are often considered free from side effects.

Among other therapeutic targets, inhibition of the activities of the key hydrolyzing enzymes linked to type 2 diabetes is considered as a useful therapeutic approach [9-10]. The function of α-glucosidase, an intestinal cell membrane enzyme, is to hydrolyze polysaccharides. Its competitive inhibition facilitates the control of the blood sugar level. Hence, α-glucosidase inhibitors (synthetic or natural) are new class of the drugs that can reduce the type 2 diabetes. Up till now, three α-glucosidase inhibitors acarbose, miglitol and voglibose are in clinical use for the management of diabetes are obtained from natural sources [11-13]. Traditional antidiabetic therapies from medicinal plants can effectively control hyperglycemia. Majority of compounds obtained via

natural sources contain flavonoid, phenylpropanoid and terpene ring structures. However, recently published data revealed that α,β-unsaturated lactone ring with a hydroxy substituent is also considered important for α-glucosidase inhibition. Recently, a study carried out by Quan et al. discovered that Momilactones A and B are good inhibitors of α-glucosidase and α-amylase and can be further explored for antidiabetic therapy [14-15].

In diabetic condition, the electron transport chain and NADPH oxidase are activated producing the reactive oxygen species (ROS) in the body [13]. In hyperglycemia, the ROS reduce insulin level which control the gene transcription and ultimately cause apoptosis [14]. Overall, it is obvious that ROS are produced under hyperglycemic condition which further implicate various disorders [15-16]. The human immune system is constantly fighting to bring the free radicals to the controlled level. However, the immune system fails to bring it to the normal level especially under the condition of excessive production. Therefore, the use of antioxidant in combination with antidiabetic drug will greatly help the immune system to combat the hyperglycemic condition.

Nitrile (cyano) containing compounds have reported to show a substantial role in therapeutic drugs. The introduction of nitrile substituent is considered as important strategy to improve the pharmacokinetic properties, polar and π-π interactions and metabolic stability [20]. Cyanoacetate derivatives of succinimide (pyrrolidine-2,5-dione, **1**) are important building-blocks for many nitrogen-containing derivatives [21-22]. For asymmetric addition of cyanoacetates, efficient catalysts are required. Thiazolidinedione (TZD, **2**) is a known class of drugs for the management of a number of diseases including diabetes [23--27]. Antidiabetic drugs rosiglitazone (**3**) and pioglitazone (**4**) are the examples of thiazolidinedione containing drugs [28]. Structures of some potent TZD core containing α-glucose containing inhibitors (**5-6**) are shown in **Figure 1** [25].

Herein, we describe the synthesis of two closely related pyrrolidine-2,5-dione and thiazolidine-2,4-dione derivatives. These derivatives were then evaluated for *in-vitro* α-glucosidase inhibition, in-vivo cytotoxicity and blood glucose lowering and antioxidant potential.

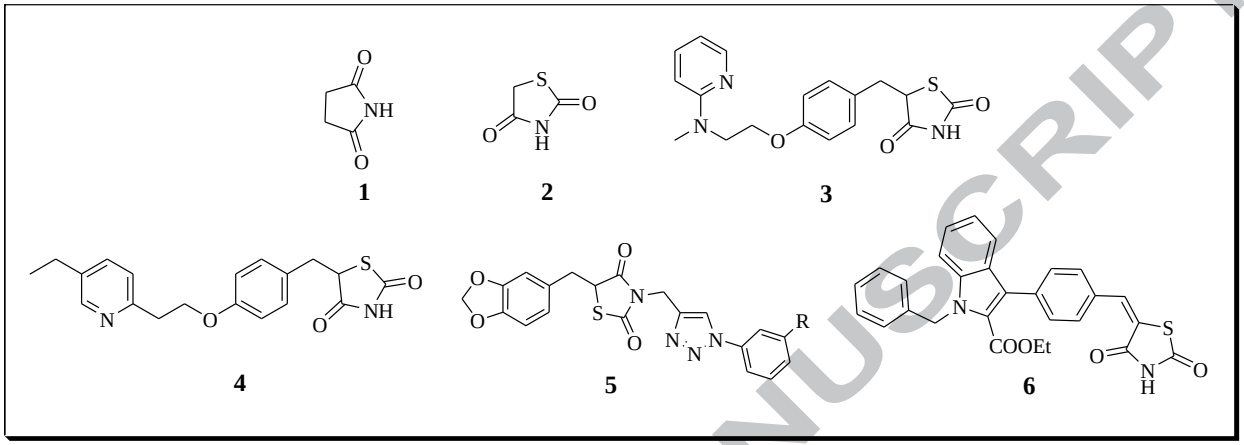


Figure 1: Structures of pyrrolidine-2,5-dione (**1**), thiazolidinedione (**2**), rosiglitazone (**3**), pioglitazone (**4**) and thiazolidinedione containing α-glucosidase inhibitors (**5-6**)

2. Results and discussion

2.1. Design strategy

Our design strategy involves the syntheses of cyanoacetate derivatives of pyrrolidine-2,5-dione and thiazolidine-2,4-dione-dihydropyrimidine (TDZ-DHPM) hybrids. For the better understanding of structure activity relationship (SAR), substitution at three different positions (R, R_1 and R_2) of pyrrolidine-2,5-dione core was planned (**7, Figure 2**). For thiazolidine-2,4-dione derivatives, we selected dihydropyrimidine (DHPM) scaffold (**8, Figure 2**).

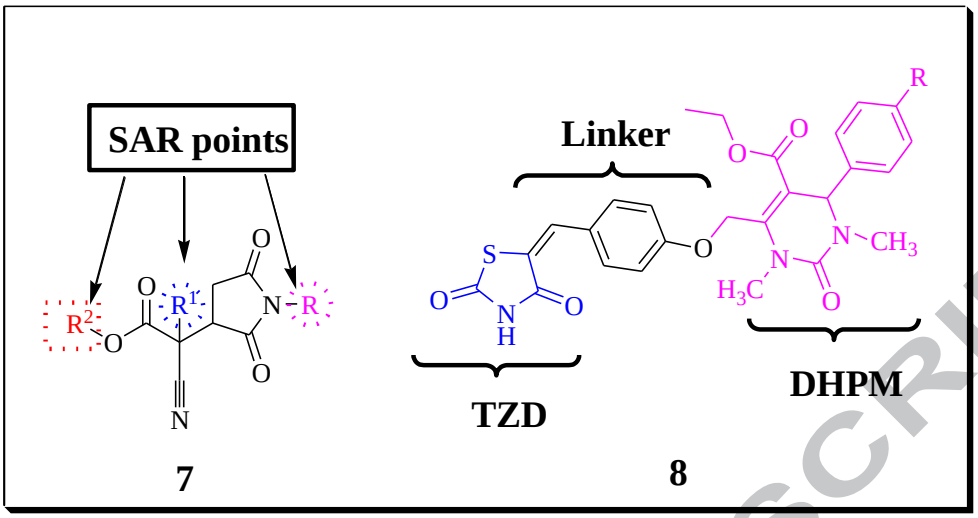
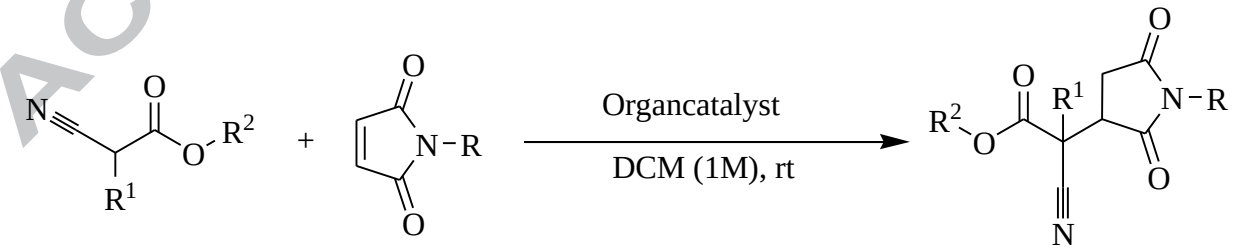


Figure 2: (A) Design strategy for the SAR studies of current research, **(B)** Generic structure of thiazolidinedione class of anti-diabetic drugs.

2.2. Chemistry

We planned to use maleimide in this study as an acceptor [29]. Interestingly, both Michael and cyclo-addition reactions are possible in the reaction of cyanoacetates and maleimides [30]. Both reactions are straightforward methods to synthesize chiral substituted pyrrolidine-2,5-dione [31-32]. Different cyanoacetate derivatives (**11a-o**, **Scheme 1**) of succinimides were prepared by adding cyanoacetates to the different *N*-substituted maleimides in the occurrence of appropriate organocatalyst, creatinine in combination with potassium hydroxide.

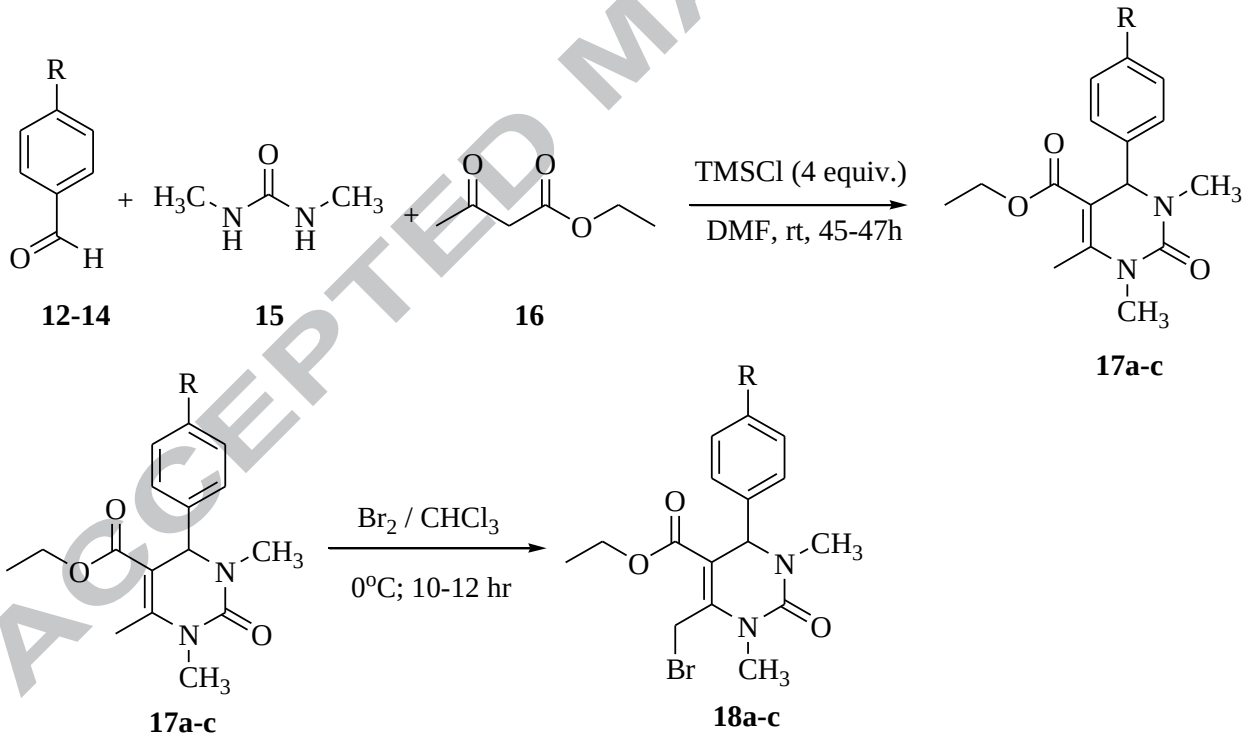


9 **10** **11a-o**

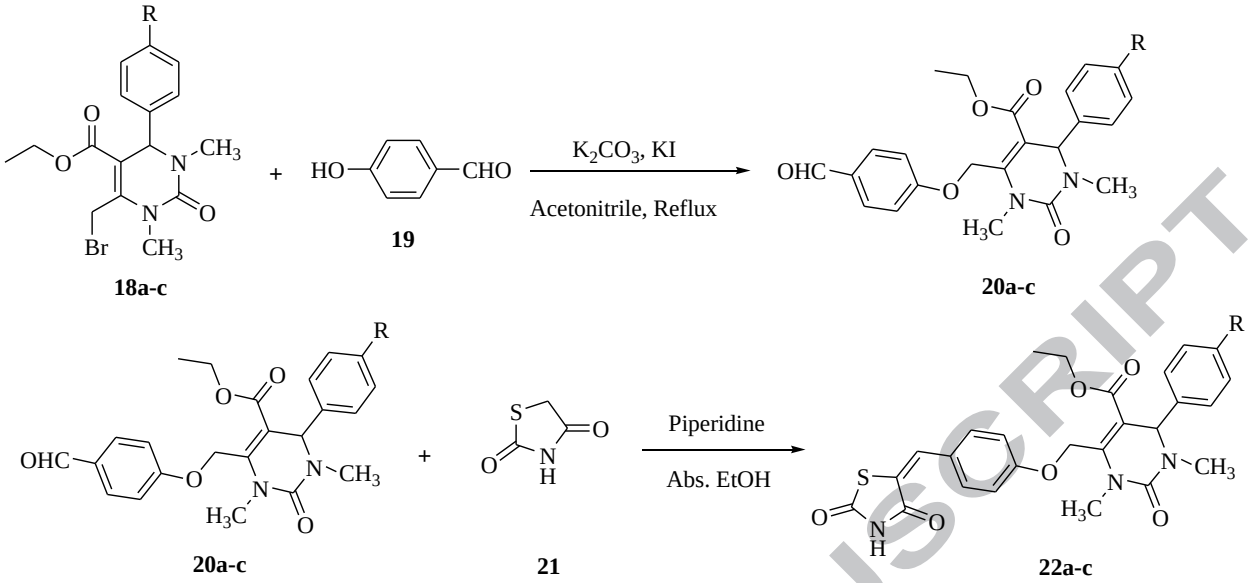
Scheme 1: Synthesis of cyanoacetate derivatives of succinimides (**11a-o**)

Thiazolidine-2,4-dione derivatives **22a-c** were obtained through a multistep protocol as shown in **Schemes 2** and **3**. Biginelli products **17a-c** were obtained by the reaction of 4-substituted aldehydes (**12-14**), 1,3-dimethylurea (**15**) and ethyl acetoacetate (**16**) in dimethylformamide (DMF) in the presence of trimethylsilyl chloride (TMSCl). These compounds were further reacted with bromine in chloroform to yield 6-bromo-dihydropyrimidine-2-ones (**18a-c**) (**Schemes 2)**.

Aldehydes **20a-c** were synthesized by the reaction of 6-bromoderivatives (**18a-c**) and 4-hydroxybenzaldehyde (**19**) in acetonitrile under basic conditions. These aldehydes undergo Knoevenagel-type condensation with thiazolidine-2,4-dione (**21**) in absolute ethanol using piperidine as base / condensing agent (**Schemes 3)**. The resulting thiazolidine-2,4-dione derivatives (**22a-c**) were obtained in fair yields.



Scheme 2: Synthesis of dihydropyrimidines (**17a-c**) and their 6-bromo derivatives **18a-c**.



Scheme 3: Synthesis of thizolidine2,4-dione and dihydropyrimidine hybrids (**22a-c**).

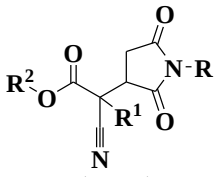
2.3. Inhibition of yeast α-Glucosidase

All the synthesized compounds were tested for their inhibitory potential against yeast α-glucosidase. Acarbose served as a control drug in this experiment. The IC_{50} values of compounds are tabulated in **Table 1** and **2**. Pyrrolidine-2,5-dione derivatives showed moderate activities. Apparently, phenyl substituted (R^1) and ethoxy (R^2) containing compounds showed better inhibition. Structure activity relationship (SAR) studies revealed that *N*-phenyl substituted compounds (**11e**, **11j** and **11o**) showed good inhibition. Compound **11o** with the IC_{50} of 28.3 ±0.28 µM emerged as a promising inhibitor of α-glucosidase. Cyclohexyl containing compounds also showed good inhibition.

The activity results of derivatives of another five membered heterocyclic ring, thiazolidine-2,4-dione are tabulated in **Table 2**. The three synthesized hybrids of dihydropyrimidine and thiazolidine-2,4-dione (**22a-c**) have shown excellent inhibition of α-glucosidase. Unsubstituted

phenyl ring containing DHPM ring exhibited submicromolar activity (IC_{50} = 0.98 ±0.008 µM). Other two compounds also showed activity in low micromolar range (**Table 2**).

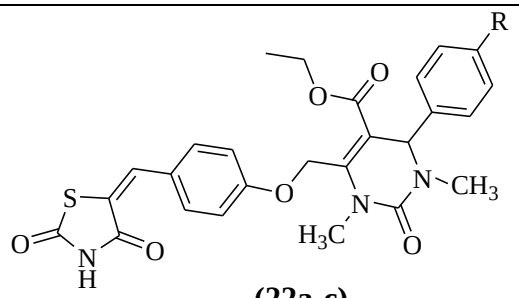
Table 1: α-Glucosidase inhibitory potential of the synthesized compounds (**11a-o**).



(11a-o)

Compound	R	R^1	R^2	IC_{50} (µM) ± SEM
11a	H	H	CH_3	213.4 ± 2.15
11b	CH_3	H	CH_3	164.2 ± 2.39
11c	CH_2CH_3	H	CH_3	154.5 ± 1.44
11d	Cyclohexyl	H	CH_3	77.2 ± 1.58
11e	C_6H_5	H	CH_3	43.5 ± 0.89
11f	H	H	CH_2CH_3	149.6 ± 2.32
11g	CH_3	H	CH_2CH_3	148.4 ± 2.67
11h	CH_2CH_3	H	CH_2CH_3	133.8 ± 1.99
11i	Cyclohexyl	H	CH_2CH_3	58.4 ± 1.05
11j	C_6H_5	H	CH_2CH_3	51.3 ± 1.45
11k	H	C_6H_5	CH_2CH_3	57.3 ± 1.08
11l	CH_3	C_6H_5	CH_2CH_3	39.7 ± 0.87
11m	CH_2CH_3	C_6H_5	CH_2CH_3	33.4 ± 0.91
11n	Cyclohexyl	C_6H_5	CH_2CH_3	31.9 ± 0.19
11o	C_6H_5	C_6H_5	CH_2CH_3	28.3 ± 0.28
	Acarbose (standard drug)			10.6 ± 0.10

Table 2: α-Glucosidase inhibitory potential of the synthesized compounds (**22a-c**)



(22a-c)

Compound	R	IC_{50} (μM) ± SEM
22a	H	0.98 ± 0.008
22b	OCH_3	1.09 ± 0.05
22c	Cl	6.10 ± 0.10
	Acarbose (standard drug)	10.6 ± 0.10

2.4. Antioxidant study of pyrrolidine-2,5-dione derivatives (11a-o)

Apart from α-glucosidase inhibitory potential, all the synthesized compounds were tested for their antioxidant potential by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) methods. From the pyrrolidine-2,5-dione derivatives (**11a-o**) series, only **11c** exhibited moderate activity in DPPH method. While, from TZD-DHPM hybrids (**22a-c**) series, only chloro-substituted **22c** showed moderate activity by DPPH method. The results of antioxidant assays are shown in **Table 3**.

Table 3: Antioxidant assays of the synthesized pyrrolidine-2,5-dione derivatives (**11a-o**) and TZD-DHPM hybrids (**22a-c**).

Compound	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)	Compound	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)
11a	147.81	65.69	**11i**	209.07	272.81
11b	114.22	110.43	**11j**	365.73	405.62
11c	92.50	149.24	**11k**	213.36	240.16
11d	258.62	221.61	**11l**	296.08	229.32
11e	256.04	71.15	**11m**	201.30	269.77
11f	535.65	746.48	**11n**	330.32	264.24
11g	351.23	176.59	**11o**	337.19	230.49
11h	209.07	272.20			
22a	>100	-	**22c**	89.59	-
22b	>100	-	**Standard Ascorbic acid**	0.035	12.50

In-vivo antidiabetic studies

Based on the *in-vitro* antidiabetic results, we selected compounds **11n, 11o and 22a** for the *in-vivo* antidiabetic assay. The selected compounds were initially screened for acute toxicity test. The compounds were administered orally in a dose range of 250-2000 mg/kg body weight. After 3 days, no unusual clinical sign was observed.

The results of *in-vivo* hypoglycemic study are presented in **Table 4**. All the three selected compounds (**11n, 11o and 22a**) revealed practical values in *in-vivo* results in comparison to the hypoglycemic control drug glibenclamide. We tested the compounds in five different doses, i.e.

500, 250, 125, 62.5 and 31.25 µg/kg body weight. Among the three compounds, **11o** and **22a** were successful in decreasing the blood glucose level better than reference drug glibenclamide.

Table 4: Result of *in-vivo* antidiabetic results of three most potent compounds.

Treatment	Conc/route	Glucose Level at varying time mmol/L					
		0 h	**2 h**	**4 h**	**6 h**	**8 h**	**24 h**
Group I Normal saline	Oral/IP	5.23	5.26	5.27	5.30	5.31.	5.33
Group II Tween 80	Oral/IP	23.1	23.6	23.9	24.1	24.2	24.3
Group III GB*	Oral/IP	23.4	20.3	17.1	14.2	10.6	**8.7****
Group IV Compound 11n	1 (500 µg) Oral/IP	22.1	20.3	18.6	16.7	13.3	11.5
	2(250 µg)	23.5	22.0	21.1	19.9	18.8	10.8
	3(125 µg)	22.8	21.8	21.1	20.7	19.8	18.4
	4(62.5 µg)	24.0	23.8	23.1	22.8	22.2	20.1
	5(31.25 µg)	23.4	23.1	22.9	22.6	22.1	21.8
Group V Compound 11o	1 (500 µg) Oral/IP	21.9	16.6	13.7	11.7	10.2	**7.2**
	2(250 µg)	20.5	17.4	13.7	11.4	10.2	8.1
	3(125 µg)	19.4	18.5	16.3	15.9	15.2	10.1
	4(62.5 µg)	21.5	20.7	20.1	19.3	18.5	11.2
	5(31.25 µg)	23.6	22.8	21.7	21.1	20.7	17.2
Group VI Compound 22a	1 (500 µg) Oral/IP	25.7	22.8	17.5	14.3	11.1	**7.6**
	2(250 µg)	25.1	23.7	20.6	18.7	13.2	10.3
	3(125 µg)	18.9	17.5	15.2	12.8	11.2	9.2
	4(62.5 µg)	20.8	19.7	17.8	15.3	12.3	8.1
	5(31.25 µg)	21.5	20.3	17.3	16.4	13.9	9.3

*Control drug Glibenclamide 500 µg/ml. ** Bold values shown are for reference drug and compounds with better results than GB

2.5. Docking studies

We performed docking simulations on our previously reported homology modelled α-glucosidase [33]. Molecular operating environment (MOE 2016.0802) software package was used for simulations. The active site of the model comprised of catalytic triad residues: Asp214, Glu276 and Asp349 (Red spheres in **Figure 1a**). The other important amino acid residues in the active site

are shown in yellow spheres (**Figure 1)**. First, we docked the standard acarbose into the binding site of the homology modelled α-glucosidase. The lowest-energy binding-pose of the standard α-glucosidase inhibitor acarbose is shown in **Figure 1b**.

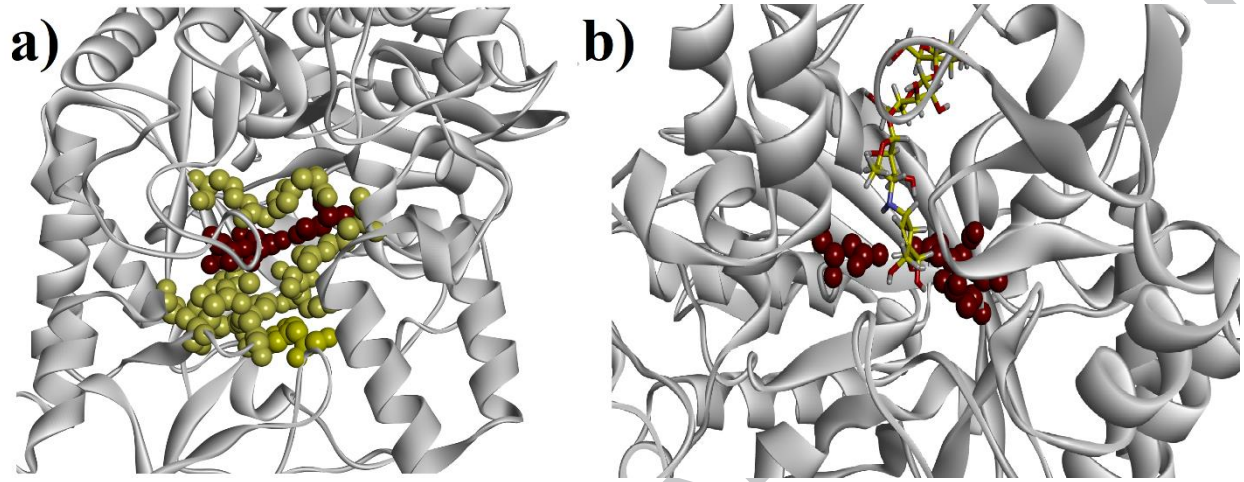


Figure 2: (a) Homology modelled α-glucosidase shown in ribbon rendering. Catalytically important residues (Asp214, Glu276 and Asp349) are shown as red spheres. While, the other active site residues are represented as yellow spheres. (b) Three-dimensional (3-D) docking pose of acarbose into the active site of homology modeled α-glucosidase.

The synthesized compounds were docked into the binding site of the homology modelled α-glucosidase. All the synthesized pyrrolidine-2,5-dione derivatives showed binding affinities in the range of -4 kcal/mol to -6 kcal/mol. The binding orientations of the active compound **11o** of this series is shown in **Figure 3a**. The 3-D interaction plot in **Figure 3b** shows that Phe157 forms π-π stacking interactions with the phenyl ring. While, Arg312 forms two hydrogen bond interactions with carbonyl oxygen and -NH of the pyrrolidine-2,5-dione ring (**Figure 3b**).

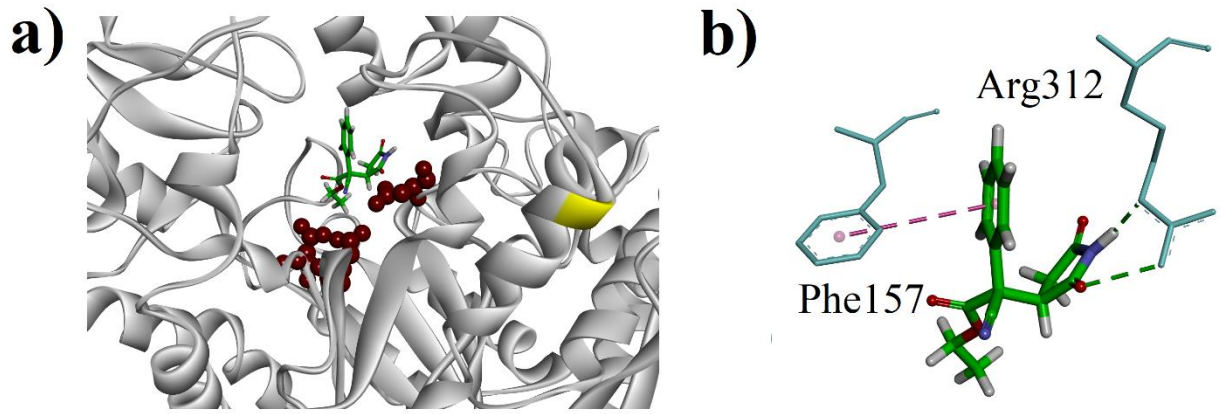


Figure 3: a) Three-dimensional (3-D) docking pose of compound **11o** into the active site of homology modeled α-glucosidase. Catalytic triad residues Asp214, Glu276 and Asp349 are shown in red spheres; (**b** Close-up depiction of the lowest-energy three-dimensional (3-D) docking interaction plot of **11o**.

The binding modes and bioassay results of DHPM-TZD hybrids (**22a-c**) show that interactions with important residues such as Phe157, Asp214, Glu276 and Arg312 appeared significant for high potency. These larger structures also fitted well in the broad cleft to enhance the binding affinities. The binding affinities of these three compounds are greater than -9 kcal/mol. The ribbon diagram and three-dimensional interaction diagram of compounds **22a** and **22b** is shown in **Figure 4a-d**. The compounds exhibited not only hydrogen bond and π-π stacking interactions, but also π-alkyl type of interactions also strengthen the ligand enzyme complex.

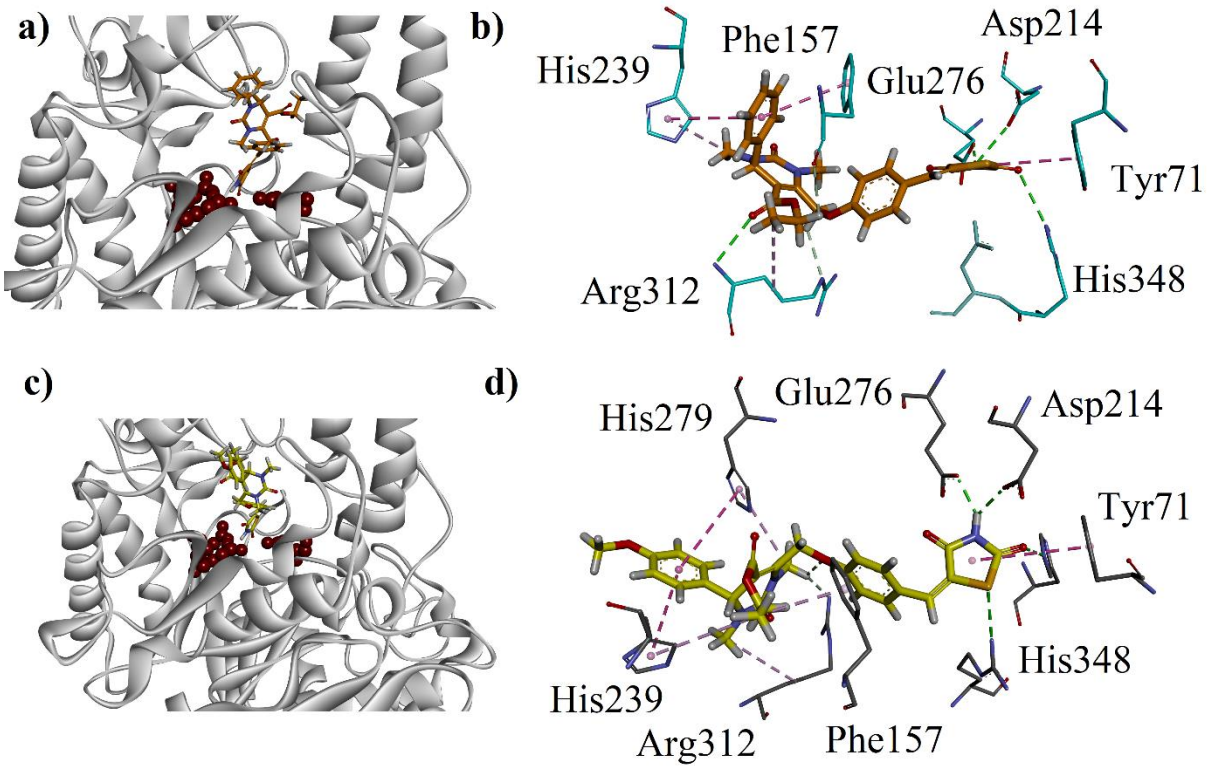


Figure 4: (a and **c)** Three-dimensional (3-D) docking pose of compound **15** into the active site of homology modeled α-glucosidase. Catalytic triad residues Asp214, Glu276 and Asp349 are shown in red spheres; (**b** and **d**) Close-up depiction of the lowest-energy three-dimensional (3-D) docking pose of **15**.

3. Conclusions

The identification of some new pyrrolidine-2,5-dione and thiazolidine-2,4-dione derivatives is reported herein. Some compounds of pyrrolidine-2,5-dione series showed moderate inhibition. Apparently, compounds with *N*-phenyl or *C*-phenyl substitution (R and R^1 respectively) and ethoxy (R^2) showed better inhibition. While, thiazolidine-2,4-dione and dihydropyrimidine (TZD-DHPM) hybrids (**22a-c**) showed excellent inhibitory activities. Moreover, we also subjected three of the most potent compounds (**11n, 11o and 22a**) to the *in-vivo* studies. Our selected compounds

showed significant hypoglycemic effects better than the standard drug glibenclamide. In the *in-vivo* acute toxicity study, no clinical unusual signs were observed. The *in-vitro* results were rationalized by using our previously reported homology modelled α-Glucosidase. The binding modes and bioassay results of active compounds showed that interactions with important residues such as Phe157, Asp214, Glu276 and Arg312 appeared significant for high potency. The larger structures also fitted well in the broad cleft to enhance the binding affinities. In future work, we planned to use medicinal chemistry approaches to design pyrrolidine-2,5-dione conjugates at R^2 ester with bioactive scaffolds. The chemical derivatization of thiazolidine-2,4-dione is also planned.

4. Material and methods

4.1. General

All the reagents and solvents were purchased from standard commercial vendors and were used without any further purification. ^{1}H and ^{13}C-NMR spectra were recorded in deuterated solvents on JEOL ECX 400 NMR/100 MHz (**for series 1**) and Bruker spectrometer at 300 and 75 MHz respectively (**for series 2**) using tetramethyl silane (TMS) as internal reference. Chemical shifts are given in δ scale (ppm). The progress of all the reactions was monitored by TLC on 2.0 x 5.0 cm aluminum sheets pre-coated with silica gel $60F_{254}$ with a layer thickness of 0.25 mm (Merck). LC-MS spectra were obtained using Agilent technologies 1200 series high performance liquid chromatography comprising of G1315 DAD (diode array detector) and ion trap LCMS G2445D SL. Final products were analyzed for their purity on Schimadzu system using C18 reversed phase column and isocratic solvent system of water/methanol/TFA (10:90:1) at room temperature.

Biologically screened compounds are > 95 % pure as determined by HPLC. Elemental analyses were conducted using LECO-932 CHNS Analyzer (LECO Corporation, USA).

4.2. Synthetic procedure for the synthesis of cyanoacetate derivatives of succinimides

To a stirred solution of cyano-acetates (2 mmol) in chloroform, different N-substituted maleimides (1 mmol), creatinine and 20 mol% KOH were added at room temperature. After the completion of the reaction (TLC), the reaction was quenched by adding sufficient amount of water (15 ml). The chloroform portion was separated by using a separating funnel. The separation of the organic layer was repeated three times (each 15 ml). After separation, the organic layer was dried by low vacuum using rotary evaporator apparatus. The reaction mixture was then adsorbed at silica gel for loading into the column for purification [34]. In column chromatography *n*-hexane and ethyl acetate were used as solvent. The yield of the final product was calculated from the obtained pure product.

4.2.1. Methyl 2-cyano-2-(2,5-dioxopyrrolidin-3-yl)acetate (11a)

The reaction was completed in 19 hours and the color of the product was white with 92% isolated yield. R_f = 0.43 (DCM/MeOH 5:1). ^{1}H-NMR (400MHz, $CDCl_3$): δ 8.72 (brs, 1H, NH), 4.66-4.41 (m, 2H, $H_{\alpha\text{-}\beta}$), 3.91 (s, 3H, -OMe), 2.77 (dd, 1H, *J* = 5.93, 16.90 Hz, one proton of CH_2 of pyrrolidinedione ring), 2.52 (dd, 1H, *J* = 9.15, 16.90 Hz, one proton of CH_2 of pyrrolidinedione ring); ^{13}C-NMR (100MHz, $CDCl_3$): 175.21, 173.69, 164.74, 115.90, 60.38, 51.95, 47.85, 31.25; HPLC purity = 98.5 %, T_R = 6.2 min. Analysis calculated for $C_8H_8N_2O_4$**:** C, 48.98; H, 4.11; N, 14.28. Found**:** C, 48.92; H, 4.13; N, 14.30. LC-MS found (m/z) = 197.2 [$M+H^+$].

4.2.2. Methyl 2-cyano-2-(1-methyl-2,5-dioxopyrrolidin-3-yl)acetate (11b)

The reaction was completed 24 hours and the color of the product was white with 92% isolated yield. R_f = 0.48 (DCM/MeOH 5:1). ^{1}H-NMR (400MHz, $CDCl_3$): δ 4.63-4.37 (m, 2H, $H_{\alpha\text{-}\beta}$), 3.97

(s, 3H, -OMe), 3.04 (s, 3H, -NCH_3), 2.80 (dd,1H, *J* = 6.90, 16.50 Hz, one proton of CH_2 of pyrrolidinedione ring), 2.44 (dd, 1H, *J* = 5.50, 16.50 Hz, one proton of CH_2 of pyrrolidinedione ring); ^{13}C-NMR (100MHz, $CDCl_3$): 175.18, 173.11, 163.41, 113.77, 61.21, 51.80, 48.28, 30.74, 25.65; HPLC purity = 97.7 %, T_R = 5.5 min. Analysis calculated for $C_9H_{10}N_2O_4$**:** C, 51.43; H, 4.80; N, 13.33. Found: C, 51.37; H, 4.81; N, 13.35; O, 30.46; LC-MS (m/z) = 211.2 [$M+H^+$].

4.2.3. Methyl 2-cyano-2-(1-ethyl-2,5-dioxopyrrolidin-3-yl)acetate (11c)

The reaction was completed 21 hours and the color of the product was yellowish with 92% isolated yield. R_f = 0.42 (DCM/MeOH 5:1). ^{1}H-NMR (400MHz, $CDCl_3$): δ 4.62-4.38 (m, 2H, $H_{\alpha\text{-}\beta}$), 3.97 (s, 3H, -OMe), 3.04-3.02 (m, 1H, one proton of NCH_2-), 2.87-2.84 (m,1H, one proton of NCH_2-), 2.75 (dd, 1H, *J* = 5.40, 17.20 Hz, one proton of CH_2 of pyrrolidinedione ring), 2.44 (dd, 1H, *J* = 8.30, 17.20 Hz, one proton of CH_2 of pyrrolidinedione ring), 1.45(t,3H, *J* = 4.90 Hz, -NCH_2CH_3); ^{13}C-NMR (100MHz, Chloroform-D): 174.91, 173.44, 164.07, 114.30, 62.48, 54.03, 47.05, 31.61, 25.73, 16.28; HPLC purity = 98.9 %, T_R = 8.3 min. Analysis calculated for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.39; N, 12.49. Found: C, 53.61; H, 5.37; N, 12.46. LC-MS (m/z) = 225.3 [$M+H^+$].

4.2.4. Methyl 2-cyano-2-(1-cyclohexyl-2,5-dioxopyrrolidin-3-yl)acetate (11d)

The reaction was completed 32 h and the color of the product was Yellowish with 93% isolated yield. R_f = 0.51 (DCM/MeOH 5:1). ^{1}H-NMR (400MHz, $CDCl_3$): δ 4.57-4.34 (m,2H, $H_{\alpha\text{-}\beta}$), 3.95 (s, 3H, -OMe), 3.08-2.94 (m,1H, NCH), 2.70 (dd,1H, *J* = 5.70, 16.80 Hz, one proton of CH_2 of pyrrolidinedione ring), 2.50 (dd, 1H, *J* = 9.10, 16.80 Hz, one proton of CH_2 of pyrrolidinedione ring), 1.98-1.84 (m, 2H, CH_2 of cyclohexyl ring), 1.79-1.74 (m, 2H, CH_2 of cyclohexyl ring), 1.63-1.34 (m, 6H, - $CH_2CH_2CH_2$- of cyclohexyl ring); ^{13}C-NMR (100MHz, $CDCl_3$): 175.48, 173.10, 164.27, 114.21, 62.93, 52.91, 48.12, 30.95, 27.92, 23.80, 23.61, 14.21, 7.73, 7.11; HPLC purity =

97.7 %, T_R = 10.6 min. Analysis calculated for $C_{14}H_{18}N_2O_4$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.31; H, 6.54; N, 10.09; LC-MS found for $C_{14}H_{18}N_2O_4$ (m/z) = 279.2 [$M+H^+$].

4.2.5. Methyl 2-cyano-2-(2,5-dioxo-1-phenylpyrrolidin-3-yl)acetate (11e)

The reaction was completed 17 hours and the color of the product was white with 92% isolated yield. R_f = 0.41 (DCM/MeOH 5:1). ^{1}H-NMR (400MHz, $CDCl_3$): δ 7.38-7.32 (m, 2H, ArH), 7.33-7.24(m, 3H, ArH), 4.57.4.39 (m, 2H, $H_{\alpha\text{-}\beta}$), 3.97 (s, 3H, -OMe), 2.78 (dd, 1H, *J* = 4.70, 17.10 Hz, one proton of CH_2 of pyrrolidinedione ring), 2.50 (dd, 1H, *J* = 9.10, 17.10 Hz, one proton of CH_2 of pyrrolidinedione ring); ^{13}C-NMR (100MHz, $CDCl_3$): 174.83, 172.35, 164.63, 129.62, 129.56, 128.34, 127.84, 64.32, 52.73, 46.81, 31.24; HPLC purity = 95.1 %, T_R = 11.8.2 min. Analysis calculated for $C_{14}H_{12}N_2O_4$: C, 61.76; H, 4.44; N, 10.29; Found: C, 61.70; H, 4.46; N, 10.31. LC-MS found for $C_{14}H_{12}N_2O_4$ (m/z) = 273.1 [$M+H^+$].

4.2.6. Ethyl 2-cyano-2-(2,5-dioxopyrrolidin-3-yl)acetate (11f)

The reaction was completed 25 hours and the color of the product was white with 92% isolated yield. R_f = 0.42 (DCM/MeOH 5:1). ^{1}H-NMR (400MHz, $CDCl_3$): δ 8.77 (brs, 1H, -NH), 4.63-4.33 (m, 4H, $2H_{\alpha\text{-}\beta}$, 2x OCH_2), 2.74(dd,1H, *J* = 5.90, 16.77 Hz, one proton of CH_2 of pyrrolidinedione ring), 2.42 (dd, 1H, *J* = 4.40, 16.80 Hz, one proton of CH_2 of pyrrolidinedione ring), 1.14(t,3H, *J* = 3.7 Hz, CH_2CH_3); ^{13}C-NMR (100MHz, Chloroform-D): 174.88, 172.95, 164.52, 115.90, 60.38, 51.95, 47.85, 31.25; LC-MS found for $C_9H_{10}N_2O_4$ (m/z) = 211.2 [M+H+]. HPLC purity = 96.2 %, T_R = 5.1 min. Analysis calculated for $C_9H_{10}N_2O_4$: C, 51.43; H, 4.80; N, 13.33. Found: C, 51.45; H, 4.82; N, 13.31.

4.2.7. Ethyl 2-cyano-2-(1-methyl-2,5-dioxopyrrolidin-3-yl)acetate (11g)

The reaction was completed 29 hours and the color of the product was yellowish with 92% isolated yield. R_f = 0.46 (DCM/MeOH 5:1). ^{1}H-NMR (400MHz, $CDCl_3$): δ 4.68-4.31 (m, 4H, $2H_{\alpha-\beta}$, 2x OCH_2), 3.06 (s, 3H, -NCH_3), 2.72(dd,1H, *J* = 5.56, 18.20 Hz, one proton of CH_2 of pyrrolidinedione ring), 2.54(dd,1H, *J* = 5.30, 18.20 Hz, one proton of CH_2 of pyrrolidinedione ring), 1.11(t,3H, *J* = 5.60 Hz, -CH_2CH_3); ^{13}C-NMR (100MHz, Chloroform-D): 175.10, 173.54, 165.95, 114.31, 62.05, 56.23, 50.62, 46.96, 30.95; Analysis calculated for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.39; N, 12.49. Found: C, 53.51; H, 5.41; N, 12.51.

4.2.8. Ethyl 2-cyano-2-(1-ethyl-2,5-dioxopyrrolidin-3-yl)acetate (11h)

The reaction was completed 13 hours and the color of the product was white with 94% isolated yield. R_f = 0.53 (DCM/MeOH 5:1). ^{1}H-NMR (400MHz, $CDCl_3$): δ 4.70-4.35 (m, 4H, $2H_{\alpha-\beta}$, 2x OCH_2), 3.04-3.02 (m, 1H, NCH_2), 2.98-2.92 (m, 1H, NCH_2), 2.75(dd, 1H, *J* = 8.30, 17.20 Hz, one proton of CH_2 of pyrrolidinedione ring), 2.44 (dd,1H, *J* = 5.40, 17.20 Hz, one proton of CH_2 of pyrrolidinedione ring), 1.45(t,3H, *J* = 3.60 Hz, NCH_2CH_3), 1.23(t,3H, *J* = 3.90 Hz, OCH_2CH_3); ^{13}C-NMR (100MHz, Chloroform-D): 174.24, 172.21, 165.32, 113.28, 62.64, 57.95, 51.54, 47.32, 26.65, 17.41, 14.52; LC-MS found for $C_{11}H_{14}N_2O_4$ (m/z) = 239.1 [M+H+]. Analysis calculated for $C_{11}H_{14}N_2O_4$: C, 55.46; H, 5.92; N, 11.76. Found: C, 55.39; H, 5.94; N, 11.78.

4.2.9. Ethyl 2-cyano-2-(1-cyclohexyl-2,5-dioxopyrrolidin-3-yl)acetate (11i)

R_f = 0.49 (n-hexane/ethyl acetate, 4:1). ^{1}H-NMR (400MHz, Chloroform-D): δ 4.45-4.10 (m, 4H, (4H, $2H_{\alpha-\beta}$, 2x OCH_2), 3.05-2.98(m, 1H NCH-), 2.74(dd,1H, *J* = 5.70, 16.80 Hz, one proton of CH_2 of pyrrolidinedione ring), 2.52 (dd, 1H, *J* = 9.10, 16.80 Hz, one proton of CH_2 of pyrrolidinedione ring), 2.04-1.97 (m, 1H, from CH_2 of cyclohexyl ring), 1.96-1.79 (m, 2H, CH_2 of cyclohexyl ring), 1.76-1.40 (m, 7H, CH_2 of cyclohexyl ring), 1.38-0.94 (m, 3H, - $CH_2CH_2CH_2$- of

cyclohexyl ring); ^{13}C-NMR (100MHz, $CDCl_3$): 174.17, 172.43, 165.08, 113.63, 60.35, 51.26, 46.36, 31.94, 30.58, 29.69, 29.60, 29.52, 29.36, 14.13, 9.22, 8.54; HPLC purity = 97.3 %, T_R = 14.1 min. Analysis calculated for $C_{15}H_{20}N_2O_4$: C, 61.63; H, 6.90; N, 9.58. Found: C, 61.70; H, 6.88; N, 9.56. LC-MS found for $C_{15}H_{20}N_2O_4$ (m/z) = 293.2 [$M+H^+$].

4.2.10. Ethyl 2-cyano-2-(2,5-dioxo-1-phenylpyrrolidin-3-yl)acetate (11j)

R_f = 0.55 (n-hexane/ethylacetate, 4:1). ^{1}H-NMR (400MHz, $CDCl_3$): δ 7.38-7.31 (m,2H, ArH), 7.27-7.21 (m, 3H, ArH), 4.57-4.32 (m, 2H, $2H_{\alpha\text{-}\beta}$), 4.16 (q, 2H, *J* = 6.9 Hz, -$COCH_2$), 2.79 (dd, 1H, *J* = 4.70, 17.10 Hz, one proton of CH_2 of pyrrolidinedione ring), 2.50 (dd, 1H, *J* = 9.10, 17.10 Hz, one proton of CH_2 of pyrrolidinedione ring); 1.08 (t,3H, *J* = 6.7 Hz, -CH_2CH_3). ^{13}C-NMR (100MHz, $CDCl_3$): 177.3, 175.4, 165.2, 130.2(2), 125.6, 123.4 (2), 114.7, 62.3, 45.0, 34.6, 31.8, 30.5, 28.1, 15.6, 14.2; LC-MS (m/z) = 286.1 [M+H+]. Analysis calculated for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.93; N, 9.79. Found: C, 62.88; H, 4.94; N, 9.81.

4.2.11. Ethyl 2-cyano-2-(2,5-dioxopyrrolidin-3-yl)-2-phenylacetate (11k)

R_f = 0.47 (n-hexane/ethylacetate, 4:1). ^{1}H-NMR (400MHz, $CDCl_3$): δ 8.79 (brs, 1H, NH), 7.60-7.57 (m, 2H, ArH), 7.46-7.41 (m, 3H, ArH), 4.39-4.20 (m, 3H, one proton of CH of pyrrolidinedione ring and two OCH_2CH_3), 2.67 (dd, 1H, *J* = 5.93, 16.40 Hz, one proton of CH_2 of pyrrolidinedione ring), 2.43 (dd, 1H, *J* = 4.30, 16.30 Hz, one proton of CH_2 of pyrrolidinedione ring), 1.27 (t, 3H, *J* = 6.90 Hz, OCH_2CH_3). ^{13}C-NMR (100MHz, $CDCl_3$): 174.72, 173.55, 165.12, 131.05, 129.72, 129.62, 125.89, 115.62, 63.85, 54.56, 47.76, 32.56, 13.52; HPLC purity = 96.8 %, T_R = 13.6 min. Analysis calculated for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.93; N, 9.79. Found: C, 63.00; H, 4.91; N, 9.77.

4.2.12. Ethyl 2-cyano-2-(1-methyl-2,5-dioxopyrrolidin-3-yl)-2-phenylacetate (11l)

R_f = 0.49 (n-hexane/ethylacetate, 4:1). ^{1}H-NMR (400MHz, $CDCl_3$): δ 7.65-7.60 (m, 2H, ArH), 7.51-7.44 (m, 3H, ArH), 4.40-4.22 (m, 3H, one proton of CH of pyrrolidinedione ring and two OCH_2CH_3), 3.04 (s, 3H, NCH_3), 2.70 (dd, 1H, *J* = 8.70, 17.10 Hz, one proton of CH_2 of pyrrolidinedione ring), 2.43 (dd, 1H, *J* = 4.30, 17.10 Hz, one proton of CH_2 of pyrrolidinedione ring), 1.30(t,3H, *J* = 7.60 Hz, OEt). ^{13}C-NMR (100MHz, $CDCl_3$): 174.72, 173.55, 165.12, 131.05, 129.72, 129.62, 125.89, 115.62, 63.85, 56.42, 50.12, 47.76, 32.56, 13.52; Analysis calculated for $C_{16}H_{16}N_2O_4$: C, 63.99; H, 5.37; N, 9.33; O, 21.31; Found: C, 63.94; H, 5.36; N, 9.36; O, 21.34. LCMS (m/z) = 301.2 [M+H+].

4.2.13. Ethyl 2-cyano-2-(1-ethyl-2,5-dioxopyrrolidin-3-yl)-2-phenylacetate (11m)

R_f = 0.57 (n-hexane/ethylacetate, 4:1). ^{1}H-NMR (400MHz, $CDCl_3$): δ 7.60-7.54 (m, 2H, ArH), 7.53-7.43 (m, 3H, ArH), 4.42-4.19 (m, 3H, one proton of CH of pyrrolidinedione ring and two OCH_2CH_3), 3.15(q,2H *J* = 6.8 Hz), 2.66 (dd, 1H, *J* = 7.6, 15.90 Hz, one proton of CH_2 of pyrrolidinedione ring), 2.38 (dd, 1H, *J* = 4.70, 15.90 Hz, one proton of CH_2 of pyrrolidinedione ring), 1.32 (t, 3H, *J* = 6.80 Hz, NCH_3), 1.20 (t, 3H, *J* = 7.30 Hz, OCH_2CH_3); ^{13}C-NMR (100 MHz, Chloroform-D): 174.72, 173.55, 165.12, 131.05, 129.72, 129.62, 125.89, 115.62, 63.85, 56.42, 50.12, 47.76, 32.56, 13.52; ^{13}C NMR (100 MHz, $CDCl_3$): 174.32 172.98 165.44 131.65 129.65129.10 126.87 114.98 63.55 56.32 47.65 32.65 14.02 13.57; LC-MS found for C17H18N2O4 (m/z) = 315.1 [M+H+]. Analysis calculated for $C_{17}H_{18}N_2O_4$: C, 64.96; H, 5.77; N, 8.91; O, 20.36; Found: C, 65.01; H, 5.79; N, 8.88; O, 20.32.

4.2.14. Ethyl 2-cyano-2-(1-cyclohexyl-2,5-dioxopyrrolidin-3-yl)-2-phenylacetate (11n)

R_f = 0.55 (n-hexane/ethylacetate, 6:1). ^{1}H-NMR (400MHz, $CDCl_3$): δ 7.59-7.56 (m, 2H, ArH), 7.51-7.40 (m, 3H, ArH), 4.42-4.19 (m, 3H, one proton of CH of pyrrolidinedione ring and two OCH_2CH_3), 3.12 (m, 1H, NCH), 2.72(dd, *J* = 8.10, 16.20 Hz, one proton of CH_2 of pyrrolidinedione ring, 1H), 2.48(dd, *J* = 5.60, 16.20 Hz, one proton of CH_2 of pyrrolidinedione ring, 1H), 2.08-2.05 (m, 2H), 1.99-1.92 (m, 3H, cyclohex ring), 1.79-1.35(m, 5H), 1.21-0.94 (m, 3H, OCH_2CH_3); ^{13}C-NMR (100MHz, $CDCl_3$): 174.55, 172.97, 164.87, 131.64, 129.98, 129.66, 127.02, 114.94, 62.56, 52.23, 46.66, 30.88, 22.54, 20.55, 16.55, 12.88, 9.88; Analysis calculated for $C_{21}H_{24}N_2O_4$: C, 68.46; H, 6.57; N, 7.60; O, 17.37; Found: C, 68.53; H, 6.55; N, 7.58; O, 17.34.

4.2.15. Ethyl 2-cyano-2-(2,5-dioxo-1-phenylpyrrolidin-3-yl)-2-phenylacetate (11o)

R_f = 0.59 (n-hexane/ethylacetate, 6:1). ^{1}H-NMR (400Mz, $CDCl_3$): δ 7.70-7.61 (m, 2H, ArH), 7.47-7.34 (m, 6H, ArH), 7.35-7.30 (m, 2H, ArH), 4.46-4.22 (m, 3H, one proton of CH of pyrrolidinedione ring and two OCH_2CH_3), 4.82 (dd, 1H, *J* = 6.0 Hz, 16.40 Hz, one proton of CH_2 of pyrrolidinedione ring), 2.52(dd,1H, *J* = 8.0 Hz, 16.40 Hz, one proton of CH_2 of pyrrolidinedione ring), 1.31(t,3H, *J* = 6.9 Hz, OCH_2CH_3); ^{13}C-NMR (100MHz, $CDCl_3$): 174.63, 173.59, 165.41, 129.17, 129.04, 128.90, 128.70, 128.68, 128.65, 128.00, 127.91, 115.07, 64.58, 54.99, 46.63, 31.12, 13.49; Analysis calculated for $C_{21}H_{18}N_2O_4$: C, 69.60; H, 5.01; N, 7.73. Found: C, 69.66; H, 5.00; N, 7.71. LC-MS found for $C_{21}H_{18}N_2O_4$ (m/z) = 363.1 [$M+H^+$].

4.3. General method for the synthesis of N-substituted dihydropyrimidine-2-ones (17a-c)

To a solution of 4-substituted aldehydes (20 mmol), ethyl acetoacetate (20 mmol) and 1,3-dimethyl urea (22 mmol) in DMF, trimethylsilyl chloride was added drop-wise. The solution was heated for 24 h at 70-80 ^{o}C. At complete TLC conversion, the solution was transferred into ice cold water and stir for further 20-25 min. The resulting precipitates were washed with EtOH. Finally, the crude product was recrystallized from ethyl acetate-n-hexane.

4.3.1. Ethyl 1,3,6-trimethyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (17a)

Yield = 79 %, ^{1}H-NMR (300MHz, DMSO-d_6): δ 7.10 (t,1H, ArH), 6.68-6.61 (m,4H, ArH), 5.04 (s, 1H, CH), 4.00(q, J = 7.2 Hz, -$COCH_2$, 2H), 3.23 (s, 3H, Me), 3.05 (s, 3H, Me), 2.24 (s, 3H, Me), 1.12 (t, J = 7.2 Hz, -CH_2CH_3, 3H).

4.3.2. Ethyl 4-(4-methoxyphenyl)-1,3,6-trimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (17b)

Yield = 77.0 %, ^{1}H-NMR (300MHz, DMSO-d_6): δ 6.98 (d, J = 7.8 Hz, ArH, 2H), 6.79 (d, J = 7.8 Hz, ArH, 2H), 5.03(s, CH, 1H), 4.08 (q, J = 6.9 Hz, -$COCH_2$, 2H), 3.84 (s, 3H, OMe), 3.23 (s, 3H, Me), 3.04 (s, 3H, CH_3), 2.25 (s, 3H, Me), 1.13(t, 3H, J = 7.1 Hz, -CH_2CH_3).

4.3.3. Ethyl 4-(4-chlorophenyl)-1,3,6-trimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (17c)

Yield = 83.0 %, ^{1}H-NMR (300MHz, DMSO-d_6): δ 7.24 (d, J = 8.1 Hz, ArH, 2H), 7.01(d, J = 8.1 Hz, ArH, 2H), 5.02 (s, 1H, CH), 4.06 (q, 2H, J = 5.6Hz, -$COCH_2$), 3.24 (s, 3H, Me), 3.06 (s, 3H, Me), 2.26 (s, 3H, Me), 1.11 (t, J = 6.9 Hz, -CH_2CH_3, 3H).

4.4. General method for the synthesis 6-bromo-dihydropyrimidine-2-one derivatives (18a-c)

For the synthesis of target derivatives (**18a-c**), we first synthesized 6-bromoderivatives according to our previously reported method [35].

To a suspension of N-substituted DHPMs (**17a-c**) in chloroform, bromine was added dropwise and then stirred with TLC monitoring time to time. When the reaction completed, the solvent was evaporated and was used without purification.

4.5. General method for the synthesis dihydropyrimidine-2-one derivatives (20a-c)

A solution of 4-hydroxybenzaldehyde (10 mmol), 6-bromo DHPMs (10 mmol), K_2CO_3 and few crystals of KI were refluxed in acetonitrile for 4-6 h. The reaction completed and then the solvent was evaporated. The product was suspended in water and extracted with ethyl acetate (2 x 20 mL). Finally, the product was recyrstlized from DMF/EtOH.

4.5.1. Ethyl 6-((4-formylphenoxy)methyl)-1,3-dimethyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (20a)

Yield = 71.3 %, ^{1}H-NMR (300MHz, DMSO-d_6): δ 9.86(s, 1H, -CHO), 7.90 (d, J = 8.1 Hz, ArH, 2H), 7.13 (d, J = 8.4 Hz, ArH, 2H), 7.01-6.87 (m, 5H, Ar-H), 5.12 (s, 1H, CH), 4.90 (s, 2H, OCH_2), 3.98 (q, J = 6.9 Hz, -$COCH_2$, 2H), 3.23 (s, 3H, Me), 3.04 (s, 3H, Me), 1.10 (t, J = 7.2 Hz, -CH_2CH_3, 3H).

4.5.2. Ethyl 6-((4-formylphenoxy)methyl)-4-(4-methoxyphenyl)-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (20b)

Yield = 68.7 %, ^{1}H-NMR (300MHz, DMSO-d_6): δ 9.88 (s, 1H, -CHO), 7.91(d, *J* = 6.7 Hz, ArH, 2H), 7.15(d, *J* = 6.5 Hz, ArH, 2H), 6.96 (d, *J* = 7.8 Hz, ArH, 2H), 6.80(d, *J* = 5.9 Hz, ArH, 2H), 5.13 (s, 1H, CH), 4.87 (s, 2H, OCH_2), 4.06 (q, *J* = 7.0 Hz, -$COCH_2$, 2H), 3.82 (s,3H, OCH_3), 3.23 (s, 3H, Me), 3.04 (s, 3H, CH_3), 1.13 (t, 3H, *J* = 6.9 Hz, -CH_2CH_3).

4.5.3. Ethyl 4-(4-chlorophenyl)-6-((4-formylphenoxy)methyl)-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (20c)

Yield = 74.1 %, ^{1}H-NMR (300MHz, DMSO-d_6): δ 9.79 (s,1H, -CHO), 7.91 (d, *J* = 7.6 Hz, ArH, 2H), 7.25 (d, *J* = 7.6 Hz, ArH, 2H), 7.14 (d, *J* = 6.9 Hz, ArH, 2H), 7.02 (d, *J* = 7.0 Hz, ArH, 2H), 5.14 (s, 1H, CH), 4.89 (s, 2H, OCH_2), 3.98 (q, *J* = 6.9 Hz, -$COCH_2$, 2H), 3.20 (s, 3H, Me), 3.00 (s, 3H, Me), 1.11(t, *J* = 6.9 Hz, -CH_2CH_3, 3H).

4.6. General method for the synthesis thiazolidine-2,4-dione derivatives (22a-c)

To a hot solution of DHPM aldehyde (5 mmol) in 20 mL of absolute ethanol, thiazolidine-2,4-dione and few drops of piperidine were added. The mixture was refluxed for 24 h. The resulting precipitates formed were recrystallized from DMF/EtOH.

4.6.1. Ethyl 6-((4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)methyl)-1,3-dimethyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (22a)

Yield = 59.9 %, ^{1}H-NMR (300MHz, DMSO-d_6): δ 12.57 (s, 1H, CO*NH*CO), 7.72 (s, 1H, CH), 7.18 (d, 2H, *J* = 8.4 Hz, ArH), 7.10-6.95 (m, 5H, ArH), 6.72 (d, 2H, *J* = 8.4 Hz, ArH), 5.12 (s, 1H, CH), 4.85 (dd, 1H, *J* = 9.9 Hz, 6 Hz, OCH_2), 4.02 (q, *J* = 7.0 Hz, -$COCH_2$, 2H), 3.21 (s, 3H, Me),

3.03 (s, 3H, Me), 1.10 (t, *J* = 6.9 Hz, -CH_2CH_3, 3H). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 170.4, 169.3, 168.1, 161.0, 151.3, 145.1, 144.8, 139.1, 129.1 (2), 128.6 (2), 128.2 (2), 127.8, 127.1, 116.7, 115.3 (2), 100.5, 73.1, 68.9, 59.6, 35.4, 34.3, 14.6. HPLC purity = 98.4 %, T_R = 17.0 min. LC-MS found for $C_{26}H_{25}N_3O_6S$ (m/z) = 508.2 [M+H+]. Analysis calculated for $C_{26}H_{25}N_3O_6S$: C, 61.53; H, 4.96; N, 8.28; S, 6.32; Found: C, 61.45; H, 4.94; N, 8.31; S, 6.35.

4.6.2. Ethyl 6-((4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)methyl)-4-(4-methoxy-phenyl)-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (22b)

Yield =63.2%, ^{1}H-NMR (300MHz, DMSO-d_6): δ 12.55 (s, 1H, CO*NH*CO), 7.73 (s, 1H, CH), 7.16 (d, *J* = 7.8 Hz, ArH, 2H), 6.98 (d, *J* = 7.5 Hz, ArH, 2H), 6.85 (d, *J* = 7.8 Hz, ArH, 2H), 6.73 (d, *J* = 7.7 Hz, ArH, 2H), 5.11 (s, 1H, CH), 4.93 (d, 1H, *J* = 9.9 Hz, OCH_2), 4.78 (d, 1H, *J* = 9.9 Hz, OCH_2), 4.01 (q, *J* = 7.0 Hz, -$COCH_2$, 2H), 3.84 (s, 3H, OMe), 3.24 (s, 3H, Me), 3.02(s, 3H, Me), 1.12(t, *J* = 7.3 Hz, -CH_2CH_3, 3H). ^{13}C-NMR (75MHz, DMSO-d_6): δ 170.3, 169.3, 168.0, 161.3, 158.9, 151.5, 145.2, 144.7, 132.2, 130.1 (2), 128.3 (2), 127.8 (2), 116.8, 115.3 (2), 114.5 (2),100.4, 72.9, 69.0, 59.7, 56.2, 35.4, 34.2, 14.7. HPLC purity = 99.4 %, T_R = 16.5 min. LC-MS found for $C_{27}H_{27}N_3O_7S$ (m/z) = 538.1 [M+H+]. Analysis calculated for $C_{27}H_{27}N_3O_7S$: C, 60.32; H, 5.06; N, 7.82; S, 5.96; Found: C, 60.41; H, 5.04; N, 7.79; S, 5.99.

4.6.3. Ethyl 4-(4-chlorophenyl)-6-((4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy) methyl)-1,3-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (22c)

Yield = 67.4%, ^{1}H-NMR (300MHz, DMSO-d_6): δ 12.51 (s, 1H, CO*NH*CO), 7.75 (s, 1H, CH), 7.31 (d, *J* = 7.5 Hz, ArH, 2H), 7.16(d, *J* = 609 Hz, ArH, 2H), 7.01(d, *J* = 6.9 Hz, ArH, 2H), 6.77(d, *J* = 7.1 Hz, ArH, 2H), 5.15 (s, 1H, CH), 4.92 (d, 1H, *J* = 9.3 Hz, OCH_2), 4.79 (d, 1H, *J* = 9.3 Hz, OCH_2), 3.97 (q, *J* = 6.9 Hz, -$COCH_2$, 2H), 3.20 (s, 3H, Me), 3.01 (s, 3H, CH_3), 1.12 (t, 3H, *J* = 6.9 Hz, -CH_2CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 170.2, 169.1, 168.2, 161.2, 151.4, 145.2, 144.6,

137.5, 134.1, 129.6 (2), 129.0 (2), 127.5, 127.0 (2), 116.6, 115.0 (2), 100.3, 73.0, 68.8, 60.0, 35.5, 34.3, 14.5. HPLC purity = 98.3%, T_R = 19.6 min. LC-MS found for $C_{26}H_{24}ClN_3O_6S$ (m/z) = 542.1 [M+H+]. Analysis calculated for $C_{26}H_{24}ClN_3O_6S$: C, 57.62; H, 4.46; N, 7.75; S, 5.92; Found: C, 57.70; H, 4.44; N, 7.74; S, 5.95.

4.7. *In vitro* α-Glucosidase inhibition study of synthesized compounds

The antidiabetic role of synthesized compounds was established by using α glucosidase inhibition assay. Briefly the reaction mixture was comprised of 200 µL α-glucosidase solution, 1200 µL phosphate buffer and 100 µL of various concentrations ranging from 62.5 to 1000 µg/ml of synthesized compounds. Allowed to stand for 20 min at 37 centigrade. Added 800 µL of sodium carbonate solution to the reaction mixture after cooling. Checked absorption of the reaction mixture at 405 nm using UV visible spectrophotometer. Glucopyranoside was used as standard in the experiment. The given formula was used to find out the percent inhibition of the αglucosidase enzyme:

$$\% \text{ Inhibition} = \frac{\text{Abs of Control} - \text{Abs of sample}}{\text{Abs of control}} \times 100$$

4.7. Antioxidant assays of the compounds

4.7.1. DPPH assay

The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was used to determine the antioxidant assay. Serial dilutions of the compounds were prepared, i.e. 1000, 500, 250, 125, 62.5, 31.25, 15.60 and 7.80 µg/ml. The compounds' solution (0.1 ml) was added to the DPPH solution. After half an hour, the absorbance was determined with double beam spectrophotometer at 517 nm. Ascorbic acid

was used as a positive control as per the protocols [36]. The percent DPPH activity was calculated by the given formula;

$$\text{Percent DPPH activity} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

4.7.2. ABTS assay

The antioxidant activity was also determined with ABTS free radicals as per the proceure [37]. In this assay, 7 mM of ABTS solution was prepared and was mixed with already prepared 2.45 mM K2S2O4 solution. The mixture was stored for around 16 h in dark. The absorbance was measured with double beam spectrophotometer at 734 nm. Ascorbic acid served as positive control in ABTS assay also. The percent ABTS activity was calculated by the given formula;

$$\text{Percent ABTS activity} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Estimation of IC_{50} values:

Concentrations of the synthesized compounds at which 50% of inhibition is observed (IC_{50}), were calculated among the inhibition percentages against the tested concentrations using the MS-Excel program.

Statistical analysis:

All the assays were performed in triplicate and values were expressed as means$\pm$ standard error means (SEM). Statistical analysis was performed by two-way analysis of difference (ANOVA), followed by Bonferroni tests. The difference was measured to be statistically significant when the p value < 0.05.

4.8. *In-vivo* hypoglycemic study

4.8.1. Experimental animals

Albino mice (3-4 weeks old, weighing 20-27 gm) were used in the in-vivo part of experiment. The experimental animals were treated as per the standard protocols of food, water, shelter, temperature, day and night cycle. The animals were used as per the approval of the Ethical Committee, Department of Pharmacy, University of Malakand, Pakistan [38].

4.8.2. Acute toxicity

The experimental animals were divided into test and control groups with five animals in each group. The compounds were administered orally in a dose range of 250-2000 mg/kg body weight. After the compounds' administrations, the animals were checked for 3 days for any unusual response [39].

4.8.3. Induction of diabetes

The diabetes was induced by giving 150 mg/kg weight of 10% alloxan monohydrate intraperitoneally. The control group was normal saline intraperitoneally. After induction of diabetes, the blood glucose level was measured with glucometer. Only diabetic mice were proceeded for further experiments as per the procedure [40].

4.8.4. *In-vivo* experiment

For the *in-vivo* antidiabetic assay, the mice were divided into six groups with five mice in each. The group I was the normal group having no diabetes and was given normal saline. The group II was diabetic animals but were given Tween 80 solution. The group III was under the treatment of standard drug glibenclamide 500µg/kg. The remaining three groups, i.e. IV, V, VI were under the treatment of the synthesized compounds and were given the selected three compounds [40].

4.9. Docking Studies

Docking studies were carried our using our previously reported homology modelled α-glucosidase using Molecular Operating Environment (MOE 2016.0208) software package [41]. Preparation of ligand and enzyme, active site determination was carried out according to our previously reported procedure [35]. 3-D visualization of docking poses was carried out by using Discovery Studio Visualizer [42].

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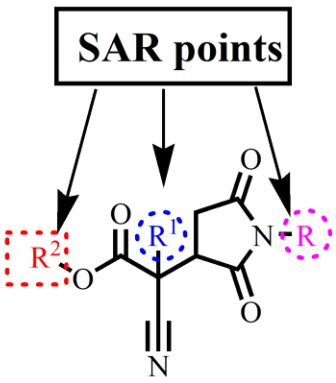
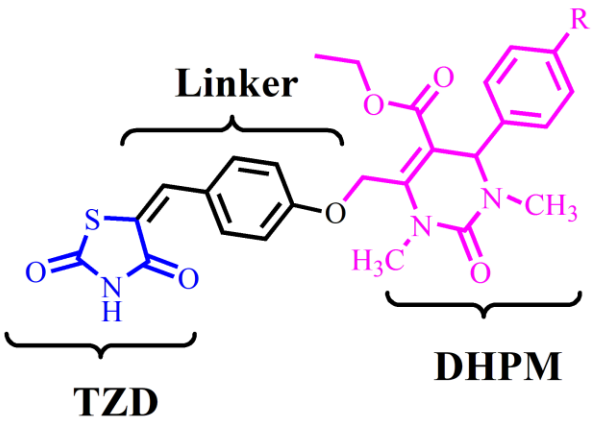
Graphical abstract



(11a-o)



(22a-c)

α-Glucosidase **11o** = 28.3 ± 0.28 **µM** **22a** = 0.98 ± 0.008 **µM**

Highlights

- Fifteen pyrrolidine-2,5-dione and three thiazolidine-2,4-dione derivatives were synthesized
- Tested for α-Glucosidase inhibition and antioxidant potential
- Compound **22a** exhibited IC_{50} value 0.98 + 0.008 µM against α-Glucosidase
- Compounds **11o** and **22a** showed significant in-vivo hypoglycemic effect compared to the reference drug
- Molecular docking studies were also carried out