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Triazinoindole analogs as potent inhibitors of α-glucosidase: Synthesis, biological evaluation and molecular docking studies

Fazal Rahim [a,*], Khadim Ullah [a], Hayat Ullah [a], Abdul Wadood [b], Muhammad Taha [c,d], Ashfaq Ur Rehman [b], Imad uddin [a], Muhammad Ashraf [e], Ayesha Shaukat [e], Wajid Rehman [a], Shafqat Hussain [f], Khalid Mohammed Khan [f]

[a] Department of Chemistry, Hazara University, Mansehra 21120, Khyber Pukhtunkhwa, Pakistan
[b] Department of Biochemistry, Computational Medicinal Chemistry Laboratory, UCSS, Abdul Wali Khan University Mardan, Pakistan
[c] Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA (UiTM), PuncakAlam Campus, 42300 Bandar PuncakAlam, Selangor DarulEhsan, Malaysia
[d] Faculty of Applied Science UiTM, 40450 Shah Alam, Selangor, Malaysia
[e] Department of Biochemistry and Biotechnology, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan
[f] H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan

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ABSTRACT

A new series of triazinoindole analogs **1**–**11** were synthesized, characterized by EI-MS and ^{1}H NMR, evaluated for α-glucosidase inhibitory potential. All eleven (**11**) analogs showed different range of α-glucosidase inhibitory potential with IC_{50} value ranging between 2.46 ± 0.008 and 312.79 ± 0.06 μM when compared with the standard acarbose (IC_{50}, 38.25 ± 0.12 μM). Among the series, compounds **1**, **3**, **4**, **5**, **7**, **8**, and **11** showed excellent inhibitory potential with IC_{50} values 2.46 ± 0.008, 37.78 ± 0.05, 28.91 ± 0.0, 38.12 ± 0.04, 37.43 ± 0.03, 36.89 ± 0.06 and 37.11 ± 0.05 μM respectively. All other compounds also showed good enzyme inhibition. The binding modes of these analogs were confirmed through molecular docking.

1. Introduction

Diabetes is a metabolic disease that controls the body's production and/or utilization of insulin. In Type-1 diabetes the body fails to produce insulin whereas in Type-2 diabetes the body produces insulin but due to various reasons the insulin does not function as it should. Glucose is essential for proper functioning of our brains as well as muscles. Mismanaged or uncontrolled diabetes can lead to blindness, circulatory problems, nerve conditions, kidney disease/failure, leg and foot amputations and even to death. Diabetes is also a major contributing risk factor for heart disease and stroke [1]. α-Glucosidase enzyme is responsible for catalytic cleavage of a glycosidic bond [2].

α-Glucosidase (EC 3.2.1.20) enzyme is involved in digestion of carbohydrates significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet and therefore can be an important strategy in the management of postprandial blood glucose level in type-2 diabetic patients and borderline patients [3]. The intestinal α-glucosidase hydrolyzes complex carbohydrates to glucose and monosaccharide in small intestine. Inhibition of this enzyme systems helps to reduce rate of digestion of carbohydrates [4]. In diabetics the short term effect of enzyme inhibitor drug therapies is to decrease high blood glucose levels [5].

α-Glucosidase has drawn a special interest of the pharmaceutical research community because in earlier studies it shows the inhibition of its catalytic activity resulted in the retardation of glucose absorption and decrease in postprandial blood glucose level [6]. α-Glucosidase inhibitors are expected to cause no hypoglycemic events or other life-threatening events, even at overdoses, and cause no weight gain [7]. Glucosidase inhibitors are potential bio-tools and highly useful for medical therapies, such as diabetes, obesity, hyperlipoproteinemia, cancer and HIV [8]. α-Glucosidase inhibitors have also been observed to block viral infections and proliferation in HIV-infections [9,10].

Triazinoindole analogs are known to possess important biological activities such as antiviral and antimalarial activities. Substituted triazinoindole compounds also showed an attractive antidepressant [11] and antihypertensive activities [12]. The triazinoindole derivatives have showed considerable interest due to its broad spectrum of antifungal, antibacterial [13], anti-inflammatory [14] and anti-hypoxic activities [15]. Some of the triazinoindole analogues act as potential drugs for the treatment of common cold [16–19].

* Corresponding author.
E-mail address: fazalstar@gmail.com (F. Rahim).

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Table 1
Synthesis of triazioindole analogs **1**–**11** and their α-glucosidase activity.

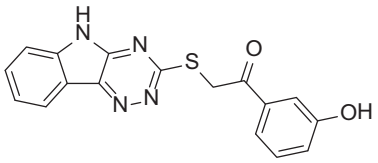
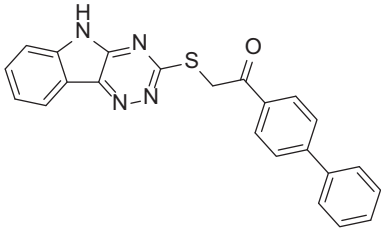
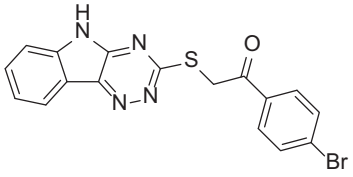
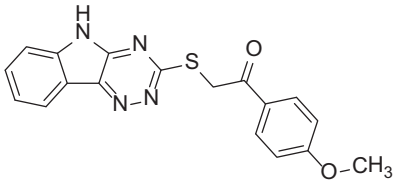
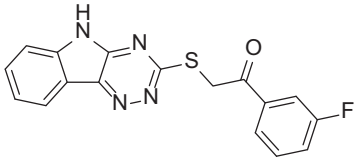
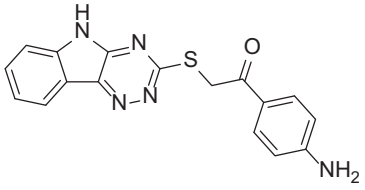
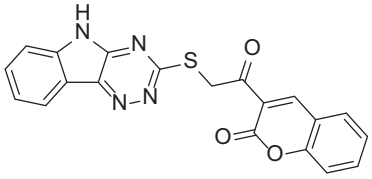
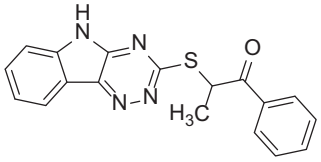
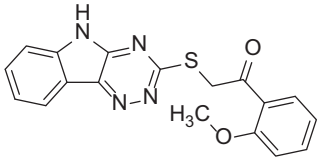
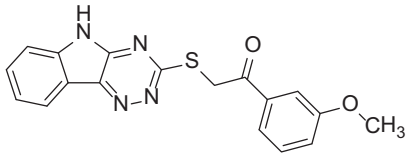
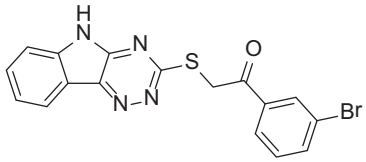
S. no.	Structures	%Yield	IC_{50} (μM)
1		70	2.46 ± 0.008
2		70	78.41 ± 0.02
3		73	37.78 ± 0.05
4		73	28.91 ± 0.01
5		68	38.12 ± 0.04
6		77	124.65 ± 0.09
7		71	37.43 ± 0.03
8		80	36.89 ± 0.06
9		73	51.57 ± 0.08

Table 1 (*continued*)

S. no.	Structures	%Yield	IC_{50} (μM)
10		65	312.79 ± 0.06
11		51	37.11 ± 0.05
	Acarbose		38.25 ± 0.12

We have planned to synthesize different substituted triazinoindole analogues in continuation of our research on enzyme inhibition [20]. In this study we are going on to report synthesis, α-glucosidase inhibitory potential and SAR of triazinoindole derivatives.

2. Result and Discussion

2.1. Chemistry

Synthesis of triazinoindole analogs were carried out in two steps. In first step isatin (10 mM) was mixed with thiosemicarbazide (10 mM) in distilled water in presence of potassium carbonate. The reaction mixture was refluxed for 6 h. Reaction completion was monitor by TLC. After completion of reaction the crude mixture was filtered and washed with ethanol and chloroform to yield pure triazino-indolthione. In second step triazinoindolthione (3 mM) was further treated and refluxed for 3 h with different phenacyl bromide (3 mM) in ethanol in the presence of triethylamine. The reaction completion was monitored by TLC. After reaction completion the mixture was filter and wash with hexane and chloroform to yield pure triazino-indole products (**1**–**11**). Different spectroscopic techniques such as EI-MS and ^{1}H NMR were used to determine the structure of all analogs.

2.2. Biological activity

Triazino-indole analogs (**1**–**11**) exhibited effective α-glucosidase inhibitory potential. Among the series analogs **1**, **3, 4, 5, 7, 8,** and **11** with IC_{50} values 2.46 ± 0.008, 37.78 ± 0.05, 28.91 ± 0.0, 38.12 ± 0.04, 38.12 ± 0.04, 36.89 ± 0.06 and 37.11 ± 0.05 showed excellent inhibitory potential as compared to standard acarbose (IC_{50} value = 38.25 ± 0.12 μM) (Table 1). All other analogs also exhibited good enzyme inhibition. The most active analog **1** having 3-hydroxyl substitution, analog **4** having methoxy substitution, analogs **11** and **3** having 3-bromo and 4-bromo substitution on the phenyl part of phenacyl bromide showed potent inhibitory activity as compared to standard acarbose. Analog **8** was unique having no substitution on phenyl group but exhibited potent inhibitory activity. Analog **5** having 3-flouro on the phenyl part while compound **7** having coumarin moiety exhibited potent inhibitory activity.

The results showed that analogs **1, 4** with electron donating group's *i.e.* hydroxyl or methoxy are more potent than standard acarbose, while analogs **3, 5, 11** with electron withdrawing groups exhibit potent inhibition but comparatively low potential compared with analogs **1** and **4**. It was concluded from results that both electron donating groups as well as electron withdrawing groups play vital role but electron donating groups plays affective role in

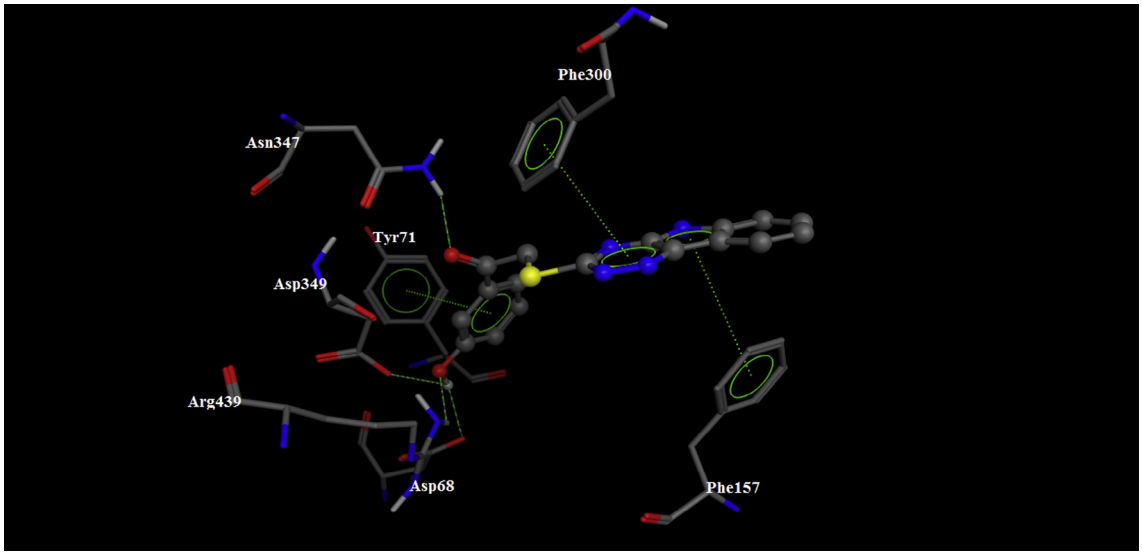


Fig. 1. Docking conformation of **Compound-1** (most active among all **11** compounds) properly accommodated into the binding cavity of α-glucosidase enzyme and developed four hydrogen bond interactions and three arene-arene bond interactions with catalytic residue Asn347, Asp349 and Arg347 and with other residues.

this assay. The SAR was established through molecular docking protocol.

2.3. Molecular docking simulation

From the docking simulation study, it was observed that the top ranked conformation of all the compounds were well accommodated inside the active site of the homology model of α-glucosidase. Computationally most of the synthesized compounds showed interaction with the hydroxyl group attach to benzene ring, carbonyl oxygen and 1, 2, 4-triazine moiety of the compounds with the important active site residues. In case of the most active compound-**1**, four hydrogen bond interactions and three π-interactions through arene-arene bond were found with the catalytically active residues Asp68, Asn347, Asp349, Arg439, Tyr71, Phe157 and Phe300 as shown in (Fig. 1). It was observed that both Asp68 and Asp349 established interaction with the hydrogen of hydroxyl group attach to benzene ring and same hydrogen interaction with the oxygen of OH group establish by Arg439, Asn347 was found in interaction with carbonyl oxygen of the compound-**1**. Out of three arene-arene interaction, one established with the 1H-pyrrole group another one was found in π-interaction with the benzene ring and the last third one with 1, 2,4-triazine group of the compound-**1**. The highest activity (IC_{50} value 2.46 ± 0.008) of compound-**1** is due to the presence of hydroxyl group attached to the benzene ring, and both oxygen and hydrogen of OH-group are involved in hydrogen bonding with the most important residue Asp349. The hydroxyl group is strong activating groups which polarize the molecule and enable it to make several interactions with other residues.

Similarly, Compound-**4** and **8** also exhibited the good biological activities against the enzyme, but if compare with the compound-**1**

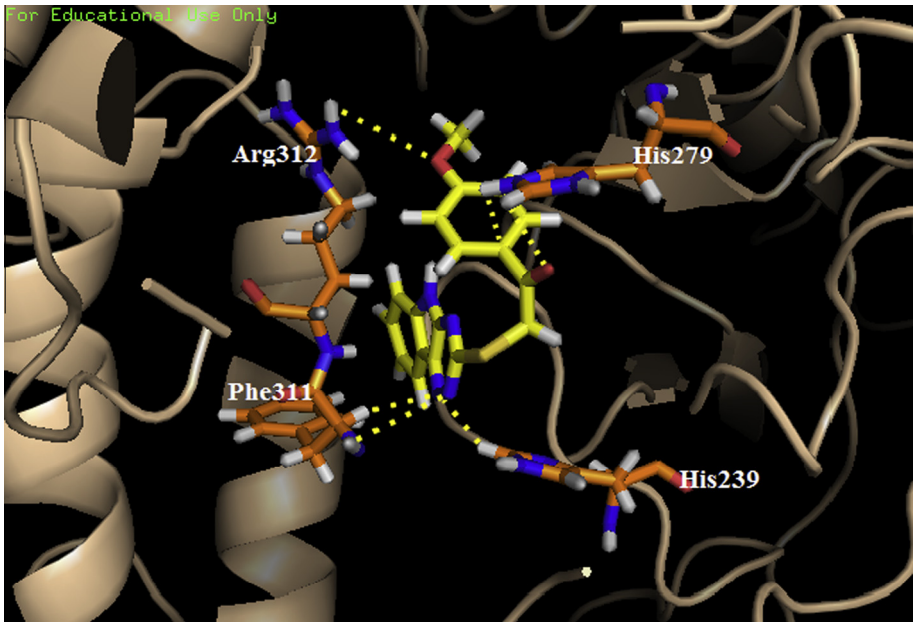


Fig. 2. Binding mode of compound-**4** in the active site of α-glucosidase enzyme.

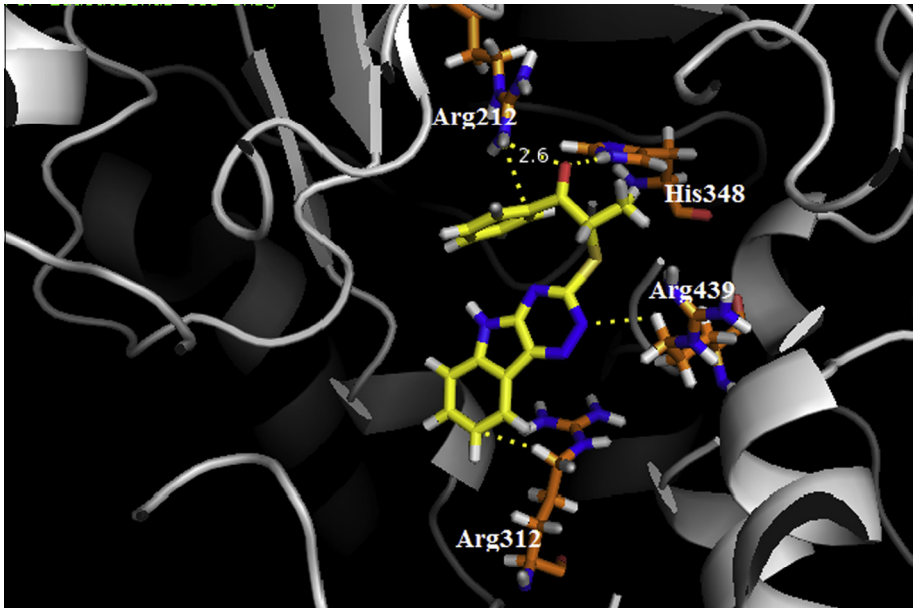


Fig. 3. Binding mode of compound-**8** in the active site of α-glucosidase enzyme.

these compounds show less activity against the enzyme, due to compound-**1** having OH-group instead of other groups at the same position. In case of compound-**4** having methoxy moiety instead of hydroxyl groups attached to benzene ring and compound-**8** have not any group at that position. The docked conformation of compound-**4** showed five hydrogen bonds and one arene-arene interaction with active site residues Phe311, His239, Arg312 and His279 which made it the second active compound in series (Fig. 2).

In case of compound-**8**, the hydroxyl group not exist as in compound-**1**, similarly several interactions was observed with active site residues, three hydrogen bonds and two arene-cation interactions with the Arg439, His348, Arg212 (H-interaction), Arg212 (arene-cation) and Arg312 which made it the third active compound in series (Fig. 3).

Similarly compound-**11** and compound-**7** also have not OH group as seem in compound-**1** which made these compound fourth and fifth active compounds in series of eleven synthesized compounds. In case of compound-**11**, two hydrogen, one arene-arene interaction and one arene-cation interaction were found with the active site residue Tyr313 (two hydrogen interactions), Arg312 and Phe300 (Fig. 4).

Similarly in compound-**7**, two arene-cation and one arene-arene interaction were found with residues His279 and Tyr71 (Fig. 5).

Similar interaction were found in compound-**3**, **5** and compound-**9** with residue Tyr71, Arg312, Arg439 and Asn347, these interactions made these compounds 5th, 6th and 7th active compounds in the series. On the other hand, least active compound-**2**, **6** and compound-**10** show less potent against enzyme and no interactions were found between these compounds and binding residues of α-glucosidase.

2.4. α-glucosidase assay

The inhibitory activities of all the isatin derivatives were measured by using the methods similar to those described previously [21,22]. Typically, α-glucosidase activity was assayed in 50 mM phosphate buffer (pH 6.8) containing 5% v/v dimethylsulfoxide

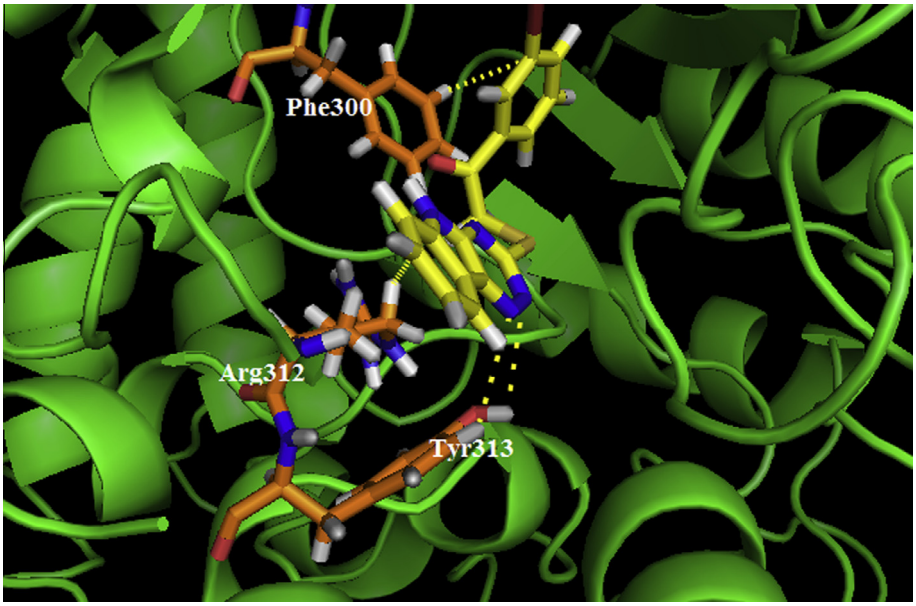


Fig. 4. Binding mode of the compound-**11** in the active site of α-glucosidase enzyme.

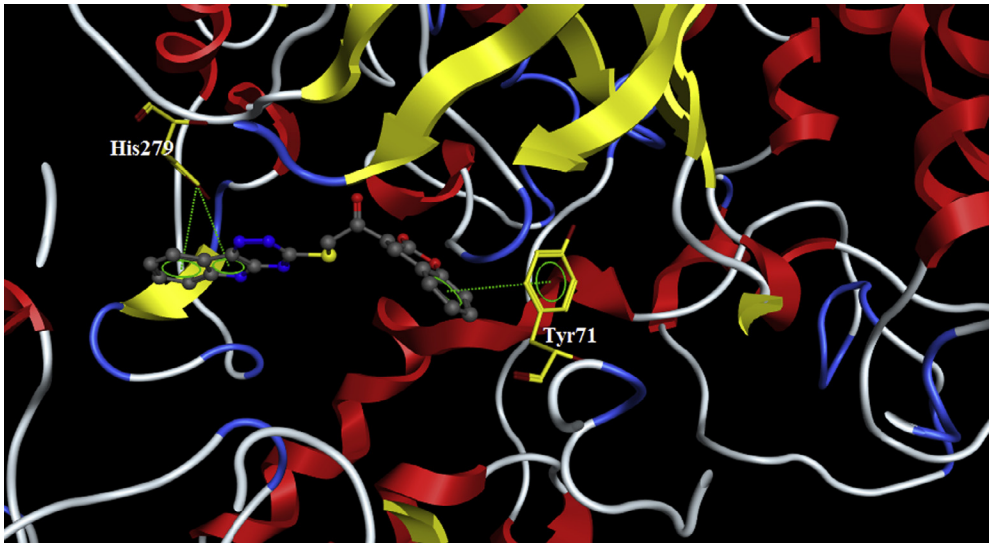


Fig. 5. Binding mode of compound-**7** in the active site of α-glucosidase enzyme.

and PNP glycoside was used as a substrate. The inhibitors were pre-incubated with enzyme at 37 °C for 0.5 h. The substrate was then added and the enzymatic reaction was carried out at 37 °C for 60 min. The reaction was monitored spectrophotometrically by measuring the absorbance at 400 nm. The assay was performed in triplicate with five different concentrations around the IC_{50} values that were roughly estimated in the first round of experiments, and the mean values were adopted.

3. Conclusion

A new sequence of eleven analogs were synthesized and evaluated for α-glucosidase inhibitory potential. Seven analogs exhibited much potent α-glucosidase inhibitory potential with IC_{50} value ranging between 2.46 ± 0.008 and 312.79 ± 0.06 μM when compared with the standard acarbose (IC_{50} 38.25 ± 0.12 μM). Compounds **1**, **3**, **4**, **5**, **7**, **8**, **11** with IC_{50} values 2.46 ± 0.008, 37.78 ± 0.05, 28.91 ± 0.0, 38.12 ± 0.04, 38.12 ± 0.04, 36.89 ± 0.06 and 37.11 ± 0.05 showed excellent inhibitory potential. All other compounds also showed excellent to good enzyme inhibition.

4. Materials and methods

NMR spectra were obtained with AVANCE AV 300 MHZ spectrometers. NMR spectra were recorded by using DMSO and acetone as solvent. TMS was used as internal standard. Finnegan MAT-311A spectrometer was used for electron impact mass spectra (EI-MS) analysis. Cesium iodide was used as an internal standard for mass measurement. All the above characterizations were performed at H.E.J Research Institute of Chemistry, Karachi University Pakistan. Column chromatography was performed on silica gel (E. Merck, type 60, 70-230 mesh). Pre-coated silica gel aluminum plates (Kieselgel 60, 20 × 20 and 0.5 mm thick, E. Merck, Germany) were used for TLC analysis. Light of wavelength 254 and 365 nm were used to visualize the chromatogram.

4.1. Computational methods

The crystallographic structure for α-glucosidase enzyme has not been solved up-to yet. However, only few homology models have been reported [23–25] so, we build the 3D structure for α-glucosidase by comparative homology modeling technique using the same propriety as described by Burke et al [26]. The primary sequence of α-glucosidase was retrieved from UniProt protein resource data bank (http://www.uniprot.org/) under the access code **P53341.** Template search was performed by means of MOE-Search tools against the PDB-database implemented in MOE 2010.11. The 1.30 Å resolving crystallographic structure of Saccharomyces cerevisiae isomaltase (SCI) (PDB code: 3aj7) [27] with 72.4% of sequence identity with the target was selected as the template for modeling. The 3D structure of α-glucosidase for SCI was built by means of MOE homology modeling tools. The constructed 3D model was subjected to energy minimization up to 0.05 gradients. Before docking simulation, ligands and protein were prepared using MOE 2010.11 software. 3D structure of all synthesized compounds was built by mean of Molecular Builder program implemented in MOE software. Finally, a database was created in which all the ligands were converted into their particular 3D structures and this database was used as input file MOE-docking. Subsequently, the energy of compounds present in the database was minimized up to 0.05 Gradient using MMFF94x force field. Energy minimization of the database was followed by the preparation of protein for docking purposes. Most macromolecular crystal structures contain little or no hydrogen coordinate data due to limited resolution and thus protonation was accomplish prior to docking using Protonate 3D tools implemented in MOE. Protonation was followed by energy minimization up to 0.05 Gradient using Amber99 force field. The database was docked into the active site of protein using the Triangular Matching docking method and 30 conformations of each Ligand protein complex were generated with docking score (S). Each complex was analyzed for interactions and their 3D pose was taken.

4.2. General procedure for synthesis of triazinoindole derivatives

Isatin (4.41 g, 30 mmol) was reacted with thiosemicarbazide (2.73 g, 30 mmol) in 50 mL distilled water in the presence of potassium carbonate (2.73 g, 30 mmol) which afforded triazinoindolthione. The triazinoindolthione was recystalized in methanol afforded 92% 5.5 g yield. The triazinoindolthione (1 mmol, 0.202 g) was treated with different phenacyl bromide (1 mmol) in ethanol in the presence triethylamine (1 mmol) to obtained different substituted derivatives of triazinoindole moiety. They were purified by recrystalization in methanol. The structures of all compounds were confirmed through EI MS and ^{1}H NMR.

4.2.1. 2-(5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-1-(3-hydroxyphenyl)-ethanone (1)

M.p. 315 °C, *Yield*: 70%; ^{1}H NMR: (DMSO-d_6, 300 MHz): δ 12.5 (s, 1H,NH), 9.8 (s, 1H, OH), 8.2(d, $J_{4,5}$ = 7.8 Hz, 1H, H-4), 7.6 (t, $J_{7/6,5}$ = 7.8 Hz, 1H,H-7), 7.5 (t, $J_{6/7,5\ 5/4,6}$ = 9.6 Hz, 2H, H-5/6), 7.4 (m, 3H, H-2′/6′/5′), 7.0 (d, $J_{4',5'}$ = 8.4 Hz 1H, H-4′), 4.9 (s, 2H, SCH_2); EI-MS: *m/z* (rel. int.%): 336 (M^+, 45), 303 (27), 215 (51), 202 (45), 121 (100).

4.2.2. 2-(5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-1-(biphenyl-4-yl)-ethanone (2)

M.p. 310 °C, *Yield*: 70%; ^{1}H NMR: (DMSO-d_6, 300 MHz): δ 12.5 (s, 1H,NH), 8.2 (d,$J_{7,6}$ = 7.8 Hz,1H, H-7), 8.1(d, $J_{4,5}$ = 8.4 Hz, 1H, H-4), 7.8 (d, $J_{2',3'}$ = 8.4 Hz, 1H,H-2'), 7.7 (d, $J_{6',5'}$ = 7.2 Hz, 1H, H-5'), 7.6 (t, $J_{6/7,5\ 5/4,6}$ = 9.6 Hz, 2H, H-5/6), 7.5 (t, $J_{2'''/3'',6''/2'',5''}$ = 6.9 Hz, 2H, H-2″/6″), 7.4 (m, 5H, H-3′/5′/3″/4″/5″), 5.0 (s, 2H, CH_2); EI-MS: *m/z* (rel. int.%): 396 (M^+, 25), 363 (43), 335 (34), 181 (100), 153 (52).

4.2.3. 2-(5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-1-(4-bromophenyl)-ethanone (3)

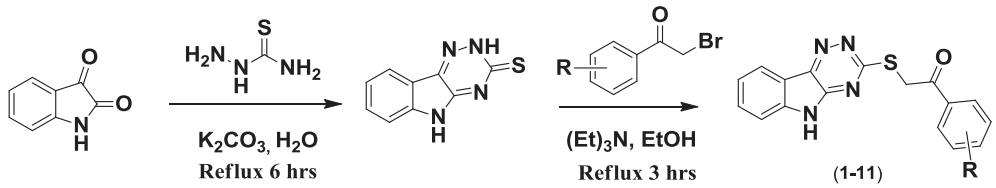
M.p. 298 °C, *Yield*: 73%; ^{1}H NMR: (DMSO-d_6, 300 MHz): δ 12.5 (s, 1H,NH), 8.2 (d,$J_{7,6}$ = 7.8 Hz,1H, H-7), 8.0(d, $J_{4,5}$ = 8.4 Hz, 1H, H-4), 7.8 (d, $J_{2',3'/6',5'}$ = 8.4 Hz, 2H, H-2′/6′), 7.6 (t, $J_{6/5,7}$ = 7.5 Hz, 1H, H-6), 7.5 (d, $J_{3',2'/5',6'}$ = 8.1 Hz, 1H, H-3′/5′), 7.4 (t, $J_{5/4,6}$ = 7.5 Hz 1H, H-5), 4.9 (s, 2H, SCH_2); EI-MS: *m/z* (rel. int.%): 399 (M^+, 30), 366 (42), 338 (24), 214 (100), 184 (85).

4.2.4. 2-(5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-1-(4-methoxyphenyl)-ethanone (4)

M.p. 306 °C, *Yield:* 73%; ^{1}H NMR: (DMSO-d_6, 300 MHz): δ 12.5 (s, 1H,NH), 8.2 (d,$J_{7,6}$ = 7.5 Hz,1H, H-7), 8.0(d, $J_{4,5}$ = 8.4 Hz, 1H, H-4), 7.6 (t, $J_{6/5,7}$ = 7.8 Hz, 1H, H-6), 7.5 (d, $J_{2',3'/6',5'}$ = 7.8 Hz, 2H, H-2′/6′), 7.4 (m, 1H, H-5), 7.1 (d, $J_{3',2'/5',6'}$ = 8.4 Hz, 2H, H-3′/5′), 4.9 (s, 2H, SCH_2); EI-MS: *m/z* (rel. int.%): 350 (M^+, 95),322 (29), 317 (63), 215 (29), 134 (100).

4.2.5. 2-(5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-1-(3-flourophenyl)-ethanone (5)

M.p. 290 °C, *Yield:* 68%; ^{1}H NMR: (DMSO-d_6, 300 MHz): δ 12.5 (s, 1H,NH), 8.2 (d,$J_{2',6'}$ = 1.5 Hz,1H, H-2′), 7.9(d, $J_{4',5'}$ = 7.5 Hz, 1H, H-4′), 7.8 (d, $J_{6',5'}$ = 8.7 Hz, 1H, H-6′), 7.6 (t, $J_{4/5,6/\ 7/6,5}$ = 7.2 Hz, 2H, H-4/7), 7.5 (m, 2H, H-5/6), 7.4 (t, $J_{5'/6',5'}$ = 7.5 Hz, 1H, H-5′), 4.9 (s, 2H, SCH_2); EI-MS: *m/z* (rel. int.%): 338 (M^+, 21), 215 (100), 173 (27), 123 (88), 95 (32).



Scheme 1. Protocol for synthesis of triazioindole analogs **1–11**.

4.2.6. 2-(5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-1-(4-nitrophenyl)-ethanone(6)

M.p. 320 °C, *Yield*: 77%; ^{1}H NMR: (DMSO-d_6, 300 MHz): δ 12.5 (s, 1H,NH), 8.4 (d,$J_{3',2'/5',6'}$ = 8.7 Hz,2H, H-3′/5′), 8.3(d, $J_{2',3'/6',5'}$ = 9 Hz, 2H, H-2′/6′), 8.2 (d, $J_{4,5}$ = 7.8 Hz, 1H, H-4), 7.6 (t, $J_{5/4,6}$ = 7.5 Hz, 1H, H-5), 7.5 (d, $J_{7,6}$ = 8.1 Hz, 1H, H-7), 7.4 (t, $J_{6/5,7}$ = 7.5 Hz, 1H, H-6), 5.0 (s, 2H, SCH_2); EI-MS: *m/z* (rel. int.%): 365 (M^+, 26),332 (41), 215 (100), 173 (37), 150 (67).

4.2.7. 3-(2-(5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)acetyl)-2H-chromen-2-one (7)

M.p. 320 °C, *Yield:* 71%; ^{1}H NMR: (DMSO-d_6, 300 MHz): δ 14.5 (s, 1H,NH), 8.7 (s, 1H, H-4′), 8.2 (d, $J_{7,6}$ = 7.8 Hz, 1H, H-7), 7.9 (d, $J_{4,5}$ = 7.2 Hz, 1H, H-4), 7.7 (t, $J_{5'/6',7'}$ = 7.2 Hz, 1H, H-5′), 7.6 (m, 1H, H-8′), 7.5 (t, $J_{6/5,7}$ = 5.1 Hz, 1H, H-6), 7.4 (m, 2H, H-6′/7'), 7.3 (t, $J_{5/4,6}$ = 7.8 Hz, 1H, H-5), 4.9 (s, 2H, SCH_2);; EI-MS: *m/z* (rel. int.%): 387 (M^+, 38), 214 (66), 201 (43), 187 (43), 173 (100).

4.2.8. 2-(5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-1-phenylpropane-1-one (8)

M.p. 295 °C, *Yield*: 80%; ^{1}H NMR: (DMSO-d_6, 300 MHz): δ 12.6 (s, 1H,NH), 8.2 (d,$J_{4,5}$ = 7.2 Hz,1H, H-4), 8.1(d, $J_{2',3'/\ 6',5'}$ = 7.5 Hz, 2H, H-2′/6′), 7.6 (m, 2H, H-4′/7), 7.5 (t, $J_{3'/2',4'\ 5'/4',6'\ 6/5,7}$ = 7.5 Hz, 3H, H-3′/5′/6), 7.4 (t, $J_{5/4,6}$ = 7.5 Hz, 1H, H-5), 5.8 (s, 2H, SCH_2); EI-MS: *m/z* (rel. int.%): 334 (M^+, 9), 317 (16), 229 (100), 105 (62), 70 (27).

4.2.9. 2-(5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-1-(2-methoxyphenyl)-ethanone (9)

M.p. 327 °C, *Yield*: 73%; ^{1}H NMR: (DMSO-d_6, 300 MHz): δ 7.4 (d,$J_{4,5/7,6}$ = 7.5 Hz, 2H, H-4/7), 7.3(d, $J_{3',4'/6',5'}$ = 7.2 Hz, 2H, H-3′/6′), 7.0 (m, 4H, H-4′/5′/5/6), 3.8 (s, 2H, SCH_2), 3.1(s, 3H, OCH_3),;EI-MS: *m/z* (rel. int.%): 350 (M^+, 100), 322 (54), 289 (43), 131 (54), 118 (39).

4.2.10. 2-(5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-1-(3-methoxyphenyl)-ethanone(10)

M.p. 302 °C, *Yield*: 65%; ^{1}H NMR: (DMSO-d_6, 300 MHz): δ 12.5 (s, 1H,NH), 8.2 (d,$J_{4,5}$ = 8.4 Hz, 1H, H-4), 8.0(d,$J_{7,6}$ = 7.5 Hz,1H, H-7), 7.6 (t, $J_{6/5,7}$ = 7.8 Hz, 1H, H-6), 7.5 (d, $J_{4',5'/6',5'}$ = 7.3 Hz, 2H, H-4′/6′), 7.4 (m, 1H, H-5), 7.1 (*m*, 2H, H-2′/5′), 4.9 (s, 2H, SCH_2); EI-MS: *m/z* (rel. int.%): 350 (M^+,81), 329 (35), 310 (45) 249 (33), 122 (100).

4.2.11. 2-(5H-[1,2,4]triazino[5,6-b]indol-3-ylthio)-1-(3-bromophenyl)-ethanone (11)

M.p. 305 °C, *Yield*: 51%; ^{1}H NMR: (DMSO-d_6, 300 MHz): δ 12.5 (s, 1H,NH), 8.0 (d,$J_{4',5'}$ = 7.5 Hz,1H, H-4′), 7.9(d, $J_{6',5'}$ = 7.5 Hz, 1H, H-6′), 7.6 (s, 1H, H-2′), 7.6 (t, $J_{4/5,\ 7/6}$ = 7.2 Hz, 2H, H-4/7), 7.5 (m, 2H, H-5/6), 7.4 (m, 1H, H-5′), 4.9 (s, 2H, SCH_2); EI-MS: *m/z* (rel. int.%): 399 (M^+, 34), 243 (49), 169 (100), 91 (70) (see Scheme 1).

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