Accepted Manuscript

Synthesis, *in vitro* evaluation and molecular docking studies of novel triazine-triazole derivatives as potential α-glucosidase inhibitors

Guangcheng Wang, Zhiyun Peng, Jing Wang, Xin Li, Juan Li

PII: S0223-5234(16)30803-0

DOI: 10.1016/j.ejmech.2016.09.067

Reference: EJMECH 8933

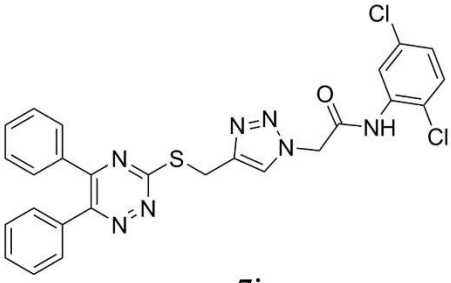
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 11 August 2016

Revised Date: 20 September 2016

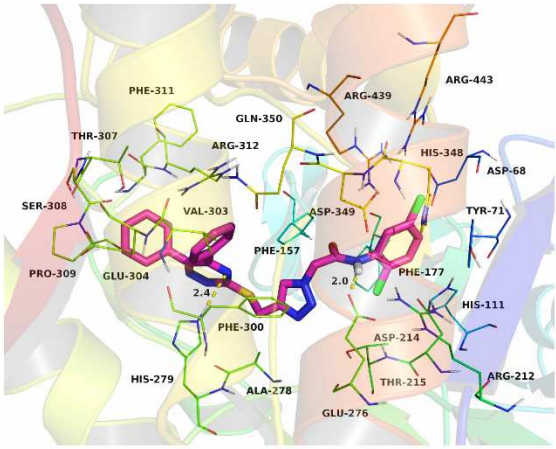
Accepted Date: 21 September 2016

Please cite this article as: G. Wang, Z. Peng, J. Wang, X. Li, J. Li, Synthesis, *in vitro* evaluation and molecular docking studies of novel triazine-triazole derivatives as potential α-glucosidase inhibitors, *European Journal of Medicinal Chemistry* (2016), doi: 10.1016/j.ejmech.2016.09.067.



7i

α-glucosidase IC_{50}= 11.63±0.15 μM



A novel series of triazine-triazole derivatives were synthesized and evaluated for their α-glucosidase inhibitory activity. Among these compounds, **7i** displayed the most potent α-glucosidase inhibitory activity with IC_{50} values of 11.63±0.15 μM as compared to the standard drug acarbose (817.38±6.27 μM).

Synthesis, *in vitro* evaluation and molecular docking studies of novel triazine-triazole derivatives as potential α-glucosidase inhibitors

Guangcheng Wang*, Zhiyun Peng, Jing Wang, Xin Li, Juan Li

College of Chemistry and Chemical Engineering, Jishou University, Jishou 416000, PR China

***Corresponding Author**

Tel.: +86 743 8563911

Fax: +86 743 8563911

E-mail address: wanggch123@163.com

Abstract.

A novel series of triazine-triazole derivatives **7a**-**7m** were synthesized, characterized by ^{1}H NMR and evaluated for their α-glucosidase inhibitory activity. All the synthesized compounds displayed potent α-glucosidase inhibitory activity with IC_{50} range of 11.63±0.15 to 37.44±0.35 μM, when compared to the standard drug acarbose (IC_{50} = 817.38±6.27 μM). Among the series, compound **7i** (IC_{50} = 11.63±0.15 μM) bearing 2,5-dichloro substitution at phenyl ring, represented the most potent α-glucosidase inhibitory activity. Molecular docking studies of the most active compounds with the homology modeled α-glucosidase were also performed to explore the possible inhibitory mechanism. Our studies shown that these triazine-triazole derivatives are a new class of α-glucosidase inhibitors.

Keywords: 1,2,4-Triazine; 1,2,3-Triazole; Click chemistry; α-Glucosidase inhibitor; Molecular docking

1. Introduction

Diabetes mellitus is a chronic metabolic disease which is characterized by high blood sugar levels over a prolonged period [1]. Uncontrolled hyperglycemia can lead to serious damage to many vital organs in the body, including kidney damage, heart disease, and nerve damage [2, 3]. The goal of treatment of diabetes mellitus is reduction of blood glucose levels and controlling subsequent complications. α-Glucosidase is a membrane-bound enzyme at the epithelium of the small intestine and play an important role in carbohydrate digestion [4]. Inhibition of α-glucosidase can significantly decrease the postprandial hyperglycemia [5]. Thus, α-glucosidase has been recognized as a therapeutic target for the treatment type-2 diabetes mellitus and several α-glucosidase inhibitors have been used in clinic [6]. Furthermore, α-glucosidase may also be used as therapeutic target for other carbohydrate mediated diseases including cancer [7], HIV [8, 9] and hepatitis [10].

1,2,4-Triazine is an important heterocyclic system, which is found in many biologically active natural products such as fervenulin, toxoflavin, and reurhycin [11]. 1,2,4-Triazine derivatives have been reported to exhibit a variety of biological activities such as antimalarial [12], anticonvulsant [13], antifungal [14], anti-inflammatory [15], anticancer [16], anti-HIV [17] and neuroprotective [18] activities. Furthermore, recent studies have shown that some 1,2,4-triazine derivatives have been identified to exhibit α-glucosidase inhibitory activity (**Figure 1**) [19, 20]. Such as, Rahim et al have reported that a novel series of triazinoindole derivatives as inhibitors of α-glucosidase [19]. In our previous work, we have found that a new

series of 1,2,4-triazine derivatives bearing carbazole or coumarin moieties show potent α-glucosidase inhibitory activity [20, 21].

1,2,3-Triazoles is an important class of heterocyclic compounds, which have attracted increasing attention in medicinal chemistry and drug discovery over the past decade [22-25]. Previous studies revealed that 1,2,3-triazole derivatives possess a wide variety of biological activities including antibacterial [26], antimalarial [27], anticancer [28], anti-HIV [29] and antitubercular activities [30]. In particular, several drugs containing 1,2,3-triazole group such as tazobactam, cephalosporin and cefatrizine have been clinically used for the treatment of bacterial infections [31, 32]. On the other hand, recent studies have shown that numbers of compounds containing the 1,2,3-triazole nucleus act as α-glucosidase inhibitors (**Figure 1**) [33, 34].

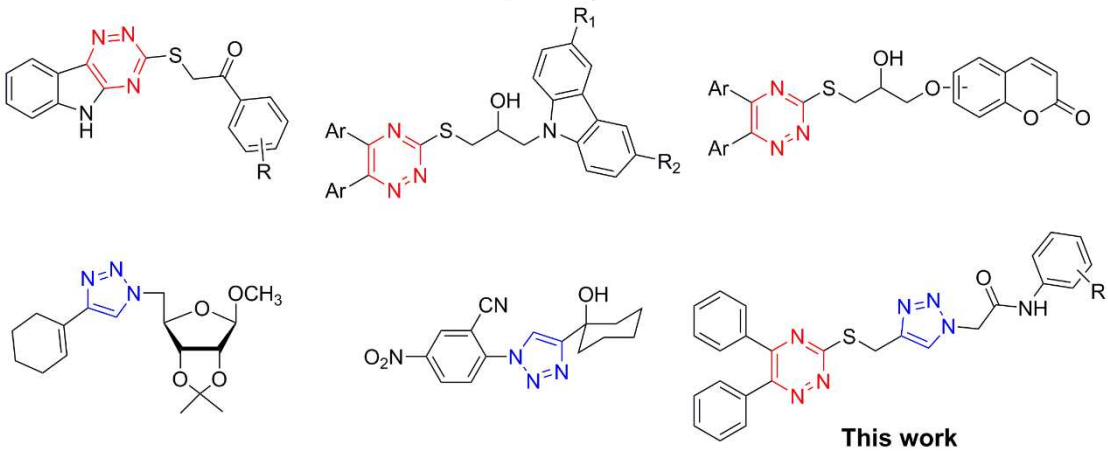


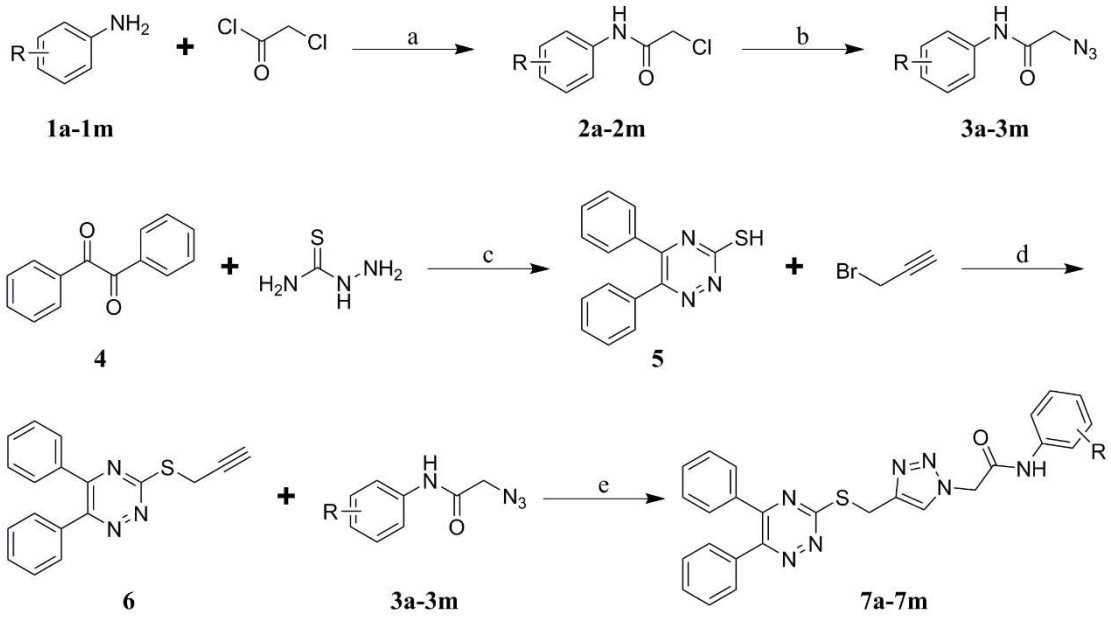
Figure 1. Chemical structures of some α-glucosidase inhibitors containing 1,2,4-triazine or 1,2,3-triazole rings.

In continuation of our interest in search of new α-glucosidase inhibitors [20], herein we reported the synthesis of a novel series of triazine-triazole derivatives. The synthesized compounds were tested for their *in vitro* α-glucosidase inhibitory activity.

Furthermore, the structure-activity relationship (SAR) and molecular docking studies of these compounds were also performed.

2. Chemistry

The triazine-triazole derivatives **7a-7m** were synthesized as showed in **Scheme 1**. Different substituted aniline **1a-1m** reacted with 2-chloroacetyl chloride to obtain compounds **2a-2m** in excellent yields. Reaction of **2a-2m** with NaN_3 in DMF at 30 ^oC for 24 h give the intermediates **3a-3m**. Condensation of benzil **4** with thiosemicarbazide in acetic acid at 120 ^oC for 3 h provided the 5,6-diphenyl-1,2,4-triazine-3-thiol **5** [35], which was then reacted with propargyl bromide in the presence of triethylamine as base to obtain compound **6**. Finally, triazine-triazole derivatives **7a-7m** were achieved by reaction of compound **6** with different intermediate **3** using sodium ascorbate and $CuSO_4 \cdot 5H_2O$ in DMF at room temperature. The structures of all the new synthesized compounds **7a-7m** were characterized by ^1H NMR spectra. For instance, the ^1H NMR spectrum of **7b** (R = 4-Me) shown a singlet at δ 2.28 ppm due to methyl protons of the phenyl ring. Two singlet signals at δ 4.69 and 5.01 ppm were corresponded to the methylene protons of –S–CH_2– and –CH_2–CO–, respectively. The fourteen aromatic protons were appeared as multiplet in the region of δ 7.06-7.51 ppm. The proton of –NH–CO– were appeared at δ 8.03 as a singlet signal. A singlet of C5-H of triazole ring were observed at δ 7.89 ppm. All these data are in agreement with the structure of compound **7b**.



Scheme 1. Reagents and conditions: (a) Et_3N, CH_2Cl_2, room temperature, 24 h; (b) NaN_3, DMF, 30 ^oC, 20 h; (c) AcOH, reflux, 3 h; (d) Et_3N, MeOH, room temperature, 24 h; (e) sodium ascorbate, $CuSO_4 \cdot 5H_2O$, DMF, r.t, 2 h.

3. Results and discussion

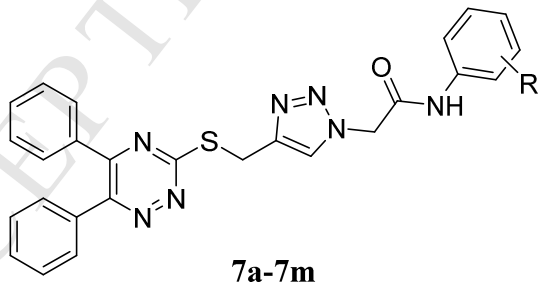
3.1. α-Glucosidase inhibition assay

The newly synthesized triazine-triazole derivatives **7a-7m** were tested for their α-glucosidase inhibitory activity by *in vitro* enzyme assay. The commercially available α-glucosidase inhibitors acarbose was used as a positive control for this assay. The results were shown in **Table 1**. All the tested compounds (**7a-7m**) displayed potent α-glucosidase inhibitory activity with IC_{50} values of 27.71±0.31, 18.22±0.28, 20.17±0.25, 37.44±0.35, 25.69±0.29, 15.97±0.17, 19.13±0.21, 21.00±0.18, 11.63±0.15, 16.94±0.19, 15.24±0.20, 17.02±0.25 and 14.45±0.17 μM, respectively, when compared to the standard drug acarbose (IC_{50} = 817.38±6.27 μM, The value of IC_{50} is similar to previous literature report [36, 37]). Among all the tested molecules, compound **7i** (11.63±0.15 μM) bearing 2,5-dichloro substitution at phenyl ring, represented the most potent α-glucosidase inhibitory activity. It was found to be seventy folds more active than the standard drug acarbose (817.38±6.27 μM).

Based on our results, the structure-activity relationship (SAR) of this class of

compounds can be summarized. Introduction of electron-donating groups such as methyl (**7b**, **7c** and **7e**), ethoxyl (**7j** and **7k**) and methoxyl (**7l**) into the phenyl ring, results in a slight increase the inhibitory activity, except the phenyl ring have ortho-methyl group (**7d**). Furthermore, compounds **7f** (4-Cl), **7g** (2,4-Cl_2), **7h** (4-F), **7i** (2,5-Cl_2) and **7m** (3-CF_3) with electron-withdrawing group also displayed potent inhibitory activity, with IC_{50} values of 15.97±0.17, 19.13±0.21, 21.00±0.18, 11.63±0.15 and 14.45±0.17 μM, respectively. Among them, compound **7i** (2,5-Cl_2, IC_{50} = 11.63±0.15 μM) was found to be the most active compound of the series. The activity of compound **7g** (2,4-Cl_2, IC_{50} = 19.13±0.21 μM) was lower than compound **7i** (2,5-Cl_2, IC_{50} = 11.63±0.15 μM), which indicates that the position of substituents on the phenyl ring influences inhibitory activity. Additionally, **7m** (IC_{50} = 14.45±0.17 μM) with strong electron-withdrawing 3-CF_3 substitution on the phenyl ring, was found to be the second most active compound. In summary, these results indicated the difference of biological activity among this class of compounds due to the pattern of substitution in the phenyl ring. The binding interactions of the most active analogs were confirmed through molecular docking studies.

Table 1. α-Glucosidase inhibitory activity of triazine-triazole derivatives **7a-7m**.



7a-7m

Compound	R	IC_{50} (μM)[a]
7a	H	27.71±0.31
7b	4-Me	18.22±0.28
7c	3-Me	20.17±0.25
7d	2-Me	37.44±0.35

7e	2,4-Me_2	25.69±0.29
7f	4-Cl	15.97±0.17
7g	2,4-Cl_2	19.13±0.21
7h	4-F	21.00±0.18
7i	2,5-Cl_2	11.63±0.15
7j	4-OEt	16.94±0.19
7k	2-OEt	15.24±0.20
7l	4-OMe	17.02±0.25
7m	3-CF_3	14.45±0.17
Acarbose		817.38±6.27

[a] Acarbose is standard for α-glucosidase inhibition activity

3.2. Homology model

The crystallographic structure of *Saccharomyces cerevisiae* α-glucosidase enzyme has not been published yet, a number of homology models of α-glucosidase have been reported in the literature [36, 38]. In order to expose the binding mode between the compounds and *Saccharomyces cerevisiae* α-glucosidase at the molecular level, the 3D structure of α-glucosidase was built by means of modeller 9.15 homology modeling software (http://salilab.org/modeller/). The sequence in FASTA format of α-glucosidase was retrieved from UniProt (access code P53341). The crystallographic structure of *Saccharomyces cerevisiae* isomaltase (PDB ID: 3AJ7) shows high sequence similarity (72.4%) with α-glucosidase, which was selected as the template for homology modeling.

The quality of homology model was validated by the Ramachandran plot using the PROCHECK (http://services.mbi.ucla.edu/PROCHECK/). The Ramachandran plot (**Figure 2**) of the modelled α-glucosidase enzyme provided further evidence of the model strength (92.3% of residues in most favored regions, 6.8% of residues in additional allowed region, 0.8% of residues in generously allowed region and only 0.2% of residues in disallowed regions). The good results obtained from the Psi/Phi Ramachandran plot suggested that the homology model could be used for the next phase of docking studies.

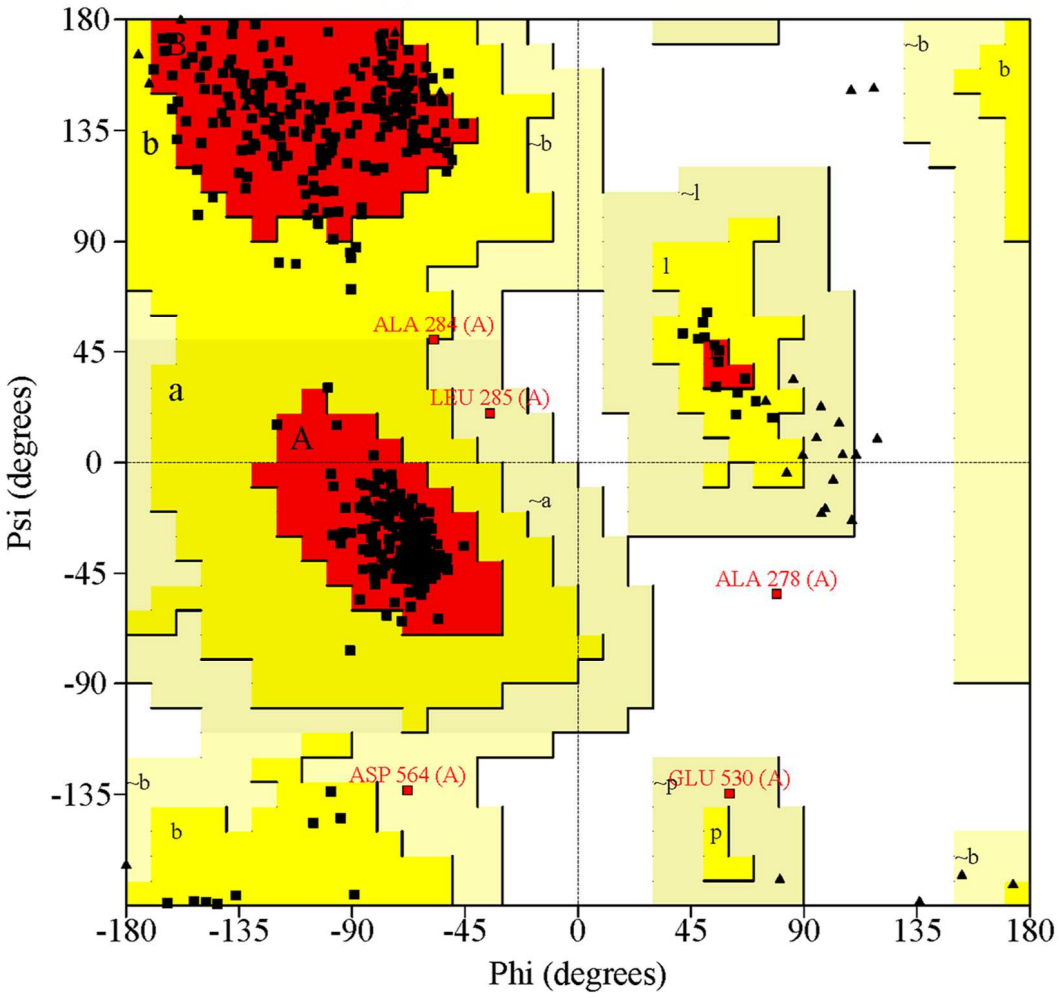


Figure 2. Ramachandran plot of the modelled α-glucosidase enzyme.

150 3.3. Molecular docking

151 Molecular docking simulations was carried out to investigate the binding mode of
152 these compound with *Saccharomyces cerevisiae* α-glucosidase. The theoretical
153 binding mode between **7a** and *Saccharomyces cerevisiae* α-glucosidase was shown in
154 **Figure 3A**. Compound **7a** adopted a compact conformation in the pocket of the
155 α-glucosidase. The mono-phenyl ring of **7a** bind at the bottom of the α-glucosidase
156 pocket and made a high density of van der Waals contacts, whereas the
157 diphenyltriazinyl group of **7a** was positioned near the entrance of the pocket and
158 made only a few contacts. Detailed analysis showed that the mono-phenyl group of **7a**
159 formed arene-cation interactions with the residue Arg-439. In addition, the
160 mono-phenyl group and the diphenyltriazinyl group of **7a** formed CH-π interactions
161 with the residues Tyr-71 and Phe-177, respectively. It was shown that Glu-276 (bond
162 length: 2.0 Å) and Asp-349 (bond length: 2.9 Å) formed two hydrogen bonds with **7a**,
163 which was the main interactions between **7a** and α-glucosidase.
164 On the other hand, molecular docking studies of the standard drug acarbose with
165 α-glucosidase was also performed. The result was shown in **Figure 3B**. Acarbose
166 adopted a U-shaped conformation in the pocket of the α-glucosidase. The the
167 pyranose and cyclohexenyl rings of acarbose bind at the bottom of the α-glucosidase
168 pocket and made a high density of van der Waals contacts, whereas the two pyranose
169 rings in the middle of acarbose were positioned near the entrance of the pocket and
170 made only a few contacts. It was shown that Asp-68 (bond length: 3.1 Å), Gln-181

(bond length: 2.0 Å) and Asp-349 (bond length: 1.6 Å) formed three hydrogen bonds with acarbose, which was the main interactions between acarbose and α-glucosidase. Results of molecular docking on α-glucosidase showed that compound **7a** has similar binding affinity as compared to standard drug.

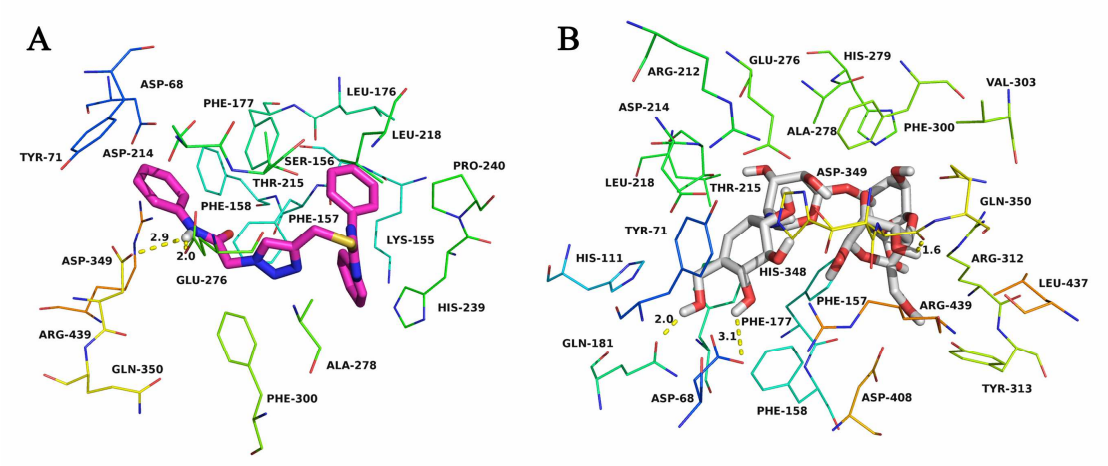


Figure 3. Compound **7a** (A) and acarbose (B) were docked to the binding pocket of the *Saccharomyces cerevisiae* α-glucosidase.

To increase the activity of **7a**, electron-withdrawing group (2,5-Cl_2) was introduced to the phenyl ring of **7a** to obtain **7i**. Compound **7i** was docked to the binding pocket of the *Saccharomyces cerevisiae* α-glucosidase, and the theoretical binding mode between **7i** and *Saccharomyces cerevisiae* α-glucosidase was shown in **Figure 4**. Compound **7i** adopted a compact conformation in the pocket of the α-glucosidase. The 2,5-dichlorophenyl ring of **7i** bind at the bottom of the α-glucosidase pocket and made a high density of van der Waals contacts, whereas the diphenyltriazinyl group of **7i** was positioned near the entrance of the pocket and made only a few contacts. Detailed analysis showed that one of the phenyl group and the 2,5-dichlorophenyl group of **7i** formed arene-cation interactions with the residues Arg-312 and Arg-439,

respectively. In addition, the dichlorophenyl group of **7i** formed a π-π stacking with the residue Tyr-71. It was shown that Glu-276 (bond length: 2.0 Å) and His-279 (bond length: 2.4 Å) formed two hydrogen bonds with **7i**, which was the main interactions between **7i** and α-glucosidase. In summary, the above molecular simulations give us rational explanation of the interactions between **7i** and α-glucosidase, which provided valuable information for further development of α-glucosidase inhibitors.

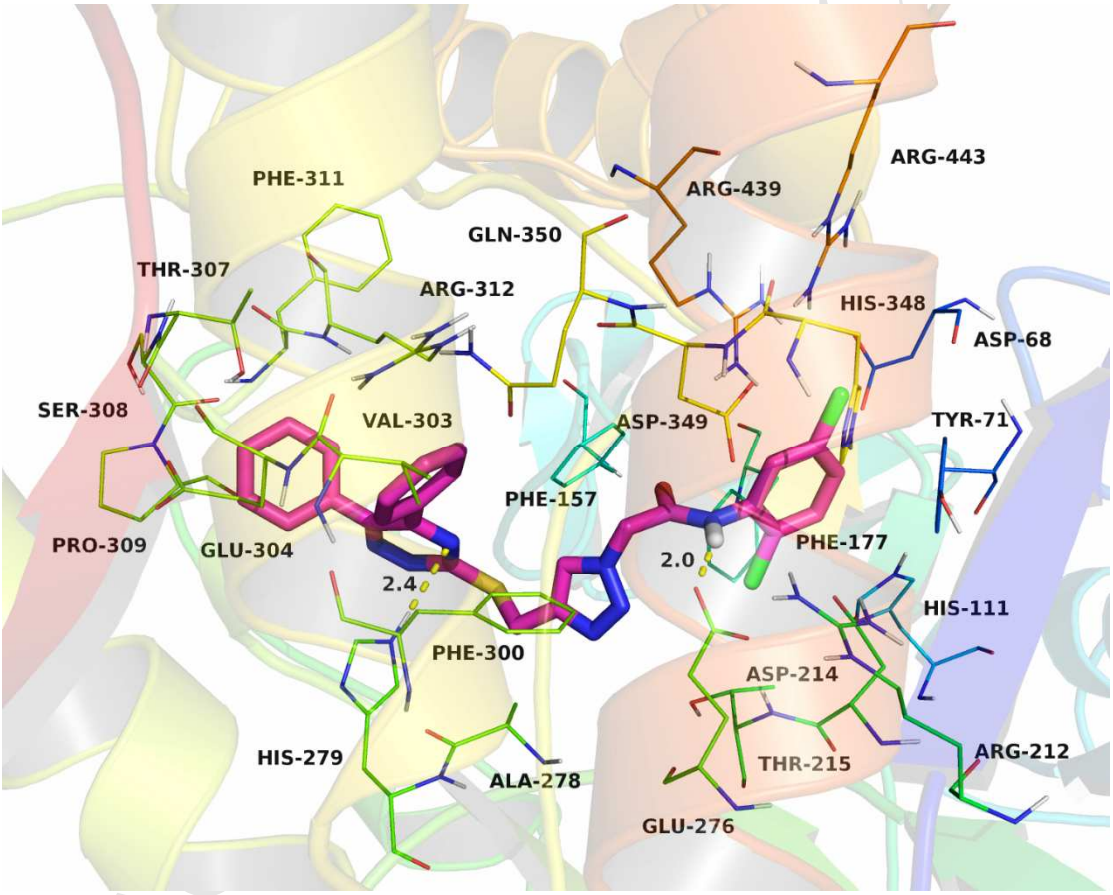


Figure 4. Compound **7i** was docked into the binding pocket of the *Saccharomyces cerevisiae* α-glucosidase.

4. Conclusion

In conclusion, we designed and synthesized a novel series of triazine-triazole derivatives **7a-7m**. All the synthesized compounds were tested for their α-glucosidase

inhibitory activity. Among them, compound **7i** (IC_{50} = 11.63±0.15 μM) having 2,5-dichloro substitution at phenyl ring was found to be the most active compound, with seventy folds more active than the standard drug acarbose (IC_{50} = 817.38±6.27 μM). Molecular docking studies showed that these triazine-triazole derivatives were binding to the active site of α-glucosidase enzyme with the hydrophobic interactions, arene-cation interactions, π-π interactions and hydrogen bonds interactions. Hence, this study identified a new structural type of α-glucosidase inhibitors, which could be used as lead molecules for further research and development of potent α-glucosidase inhibitors.

5. Experimental section.

5.1. Chemistry.

All starting materials and reagents were purchased from commercial suppliers. TLC was performed on 0.20 mm Silica Gel 60 F_{254} plates (Qingdao Ocean Chemical Factory, Shandong, China). Nuclear magnetic resonance spectra (NMR) were recorded on a Bruker spectrometer (400 MHz) with TMS as an external reference and reported in parts per million. The Supplemental Materials contain sample ^{1}H NMR spectrum for **7a-7m** (**Figures S1**–**S13**).

5.1.1. General procedures for the synthesis of triazine-triazole derivatives (**7a-7m**)

A mixture of **6** (303 mg, 1.0 mmol), **3** (1.0 mmol), $CuSO_4 \cdot 5H_2O$ (0.025 g; 0.1 mmol) and sodium ascorbate (0.10 g, 0.5 mmol) in DMF (20 mL) was stirred at room temperature for 4 h. After the completion of the reaction, the mixture was poured into 100 mL of ice-cold water and the precipitate was filtered. The crude product was purified by chromatography to give the title product **7a-7m**.

5.1.1.1.

2-(4-(((5,6-Diphenyl-1,2,4-triazin-3-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-N-phenyla

cetamide (7**a**)

Yellow solid, yield 33.8 %, m.p. 197-199 ^{o}C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.69 (s, 2H, SCH_2), 5.12 (s, 2H, CH_2CO), 7.11 (t, 1H, J = 7.2 Hz, ArH), 7.27 (d, 2H, J = 8.4 Hz, ArH), 7.32 (d, 2H, J = 7.6 Hz, ArH), 7.36 (d, 2H, J = 7.2 Hz, ArH), 7.40-7.43 (m, 4H, ArH), 7.47(d, 2H, J = 8.0 Hz, ArH), 7.51 (d, 2H, J = 7.6 Hz, ArH), 7.88 (s, 1H, CH-triazole), 8.12 (s, 1H, NH).

5.1.1.2.

2-(4-(((5,6-Diphenyl-1,2,4-triazin-3-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-N-(p-tolyl) acetamide (7**b**)

Yellow solid, yield 63.1 %, m.p. 173-174 ^{o}C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.28 (s, 3H, CH_3), 4.69 (s, 2H, SCH_2), 5.01 (s, 2H, CH_2CO), 7.06 (d, 2H, J = 8.4 Hz, ArH), 7.28-7.38 (m, 6H, ArH), 7.40-7.43 (m, 2H, ArH), 7.47(d, 2H, J = 8.4 Hz, ArH), 7.51 (d, 2H, J = 8.4 Hz, ArH), 7.89 (s, 1H, CH-triazole), 8.03 (s, 1H, NH).

5.1.1.3.

2-(4-(((5,6-Diphenyl-1,2,4-triazin-3-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-N-(m-tolyl)acetamide (7**c**)

Yellow solid, yield 51.8 %, m.p. 156-158 ^{o}C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.29 (s, 3H, CH_3), 4.69 (s, 2H, SCH_2), 5.11 (s, 2H, CH_2CO), 6.92 (d, 1H, J = 7.2 Hz, ArH), 7.15 (t, 1H, J = 8.0 Hz, ArH), 7.21 (d, 1H, J = 8.0 Hz, ArH), 7.26-7.30 (m, 1H, ArH), 7.32 (d, 2H, J = 7.6 Hz, ArH), 7.35 (d, 2H, J = 7.6 Hz, ArH), 7.39-7.42 (m, 2H, ArH), 7.47(d, 2H, J = 8.4 Hz, ArH), 7.51 (d, 2H, J = 8.4 Hz, ArH), 7.88 (s, 1H, CH-triazole), 8.13 (s, 1H, NH).

5.1.1.4.

2-(4-(((5,6-Diphenyl-1,2,4-triazin-3-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-N-(o-tolyl) acetamide (7**d**)

Yellow solid, yield 57.2 %, m.p. 110-112 ^{o}C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.06 (s, 3H, CH_3), 4.70 (s, 2H, SCH_2), 5.15 (s, 2H, CH_2CO), 7.06 (t, 1H, J = 7.2 Hz, ArH), 7.11 (d, 1H, J = 7.2 Hz, ArH), 7.17 (t, 1H, J = 8.0 Hz, ArH), 7.30-7.38 (m, 4H),

7.40-7.44 (m, 2H, ArH), 7.47 (d, 2H, *J* = 8.4 Hz, ArH), 7.51 (d, 2H, *J* = 8.4 Hz, ArH), 7.77 (d, 1H, *J* = 8.0 Hz, ArH), 7.81 (s, 1H, NH), 7.89 (s, 1H, CH-triazole).

5.1.1.5.

N-(2,4-Dimethylphenyl)-2-(4-(((5,6-diphenyl-1,2,4-triazin-3-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)acetamide (7**e**)

Yellow solid, yield 59.7 %, m.p. 171-173 ^{o}C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.01 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 4.70 (s, 2H, SCH_2), 5.14 (s, 2H, CH_2CO), 6.93 (s, 1H, ArH), 6.95 (d, 1H, *J* = 8.0 Hz, ArH), 7.30-7.38 (m, 4H), 7.40-7.43 (m, 2H, ArH), 7.47 (d, 2H, *J* = 8.0 Hz, ArH), 7.51 (d, 2H, *J* = 8.4 Hz, ArH), 7.57 (d, 1H, *J* = 8.0 Hz, ArH), 7.68 (s, 1H, NH), 7.89 (s, 1H, CH-triazole).

5.1.1.6.

N-(4-Chlorophenyl)-2-(4-(((5,6-diphenyl-1,2,4-triazin-3-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)acetamide (7**f**)

Yellow solid, yield 42.7 %, m.p. 184-186 ^{o}C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.67 (s, 2H, SCH_2), 5.12 (s, 2H, CH_2CO), 7.19 (d, 2H, *J* = 8.0 Hz, ArH), 7.29-7.35 (m, 3H, ArH), 7.37-7.42 (m, 4H, ArH), 7.45 (d, 2H, *J* = 8.4 Hz, ArH), 7.50 (d, 2H, *J* = 7.2 Hz, ArH), 7.88 (s, 1H, CH-triazole), 8.62 (s, 1H, NH).

5.1.1.7.

N-(2,4-Dichlorophenyl)-2-(4-(((5,6-diphenyl-1,2,4-triazin-3-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)acetamide (7**g**)

Yellow solid, yield 42.3 %, m.p. 195-197 ^{o}C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.71 (s, 2H, SCH_2), 5.17 (s, 2H, CH_2CO), 7.20 (dd, 1H, *J* = 8.8 Hz, 2.0 Hz, ArH), 7.30-7.32 (m, 2H, ArH), 7.34-7.38 (m, 3H, ArH), 7.40-7.43 (m, 2H, ArH), 7.46 (d, 2H, *J* = 8.4 Hz, ArH), 7.51 (d, 2H, *J* = 8.4 Hz, ArH), 7.90 (s, 1H, CH-triazole), 8.20 (d, 1H, *J* = 8.0 Hz, ArH), 8.22 (s, 1H, NH).

5.1.1.8.

2-(4-(((5,6-Diphenyl-1,2,4-triazin-3-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-fluorophenyl)acetamide (7**h**)

Yellow solid, yield 75.7 %, m.p. 200-201 ^{o}C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.69 (s, 2H, SCH_2), 5.11 (s, 2H, CH_2CO), 6.96 (t, 2H, J = 8.8 Hz, ArH), 7.30-7.34 (m, 2H), 7.36-7.45 (m, 6H), 7.47 (d, 2H, J = 8.4 Hz, ArH), 7.51 (d, 2H, J = 8.4 Hz, ArH), 7.88 (s, 1H, CH-triazole), 8.16 (s, 1H, NH).

5.1.1.9.

N-(2,5-Dichlorophenyl)-2-(4-(((5,6-diphenyl-1,2,4-triazin-3-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)acetamide (7**i**)

Yellow solid, yield 46.0 %, m.p. 160-162 ^{o}C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.71 (s, 2H, SCH_2), 5.18 (s, 2H, CH_2CO), 7.02 (dd, 1H, J = 8.8 Hz, 2.4 Hz, ArH), 7.21 (d, 1H, J = 8.8 Hz, ArH), 7.30-7.38 (m, 4H), 7.40-7.43 (m, 2H, ArH), 7.46 (d, 2H, J = 8.4 Hz, ArH), 7.51 (d, 2H, J = 8.4 Hz, ArH), 7.90 (s, 1H, CH-triazole), 8.28 (s, 1H, NH), 8.35 (d, 1H, J = 2.4 Hz, ArH).

5.1.1.10.

2-(4-(((5,6-Diphenyl-1,2,4-triazin-3-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-ethoxyphenyl)acetamide (7**j**)

Yellow solid, yield 12.5 %, m.p. 175-176 ^{o}C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.38 (t, 3H, J = 7.2 Hz, $OCH_2\underline{CH_3}$), 3.94 (q, 2H, J = 7.2 Hz, $O\underline{CH_2}CH_3$), 4.69 (s, 2H, SCH_2), 5.10 (s, 2H, CH_2CO), 6.77 (d, 2H, J = 8.8 Hz, ArH), 7.29-7.32 (m, 3H, ArH), 7.34-7.38 (m, 3H, ArH), 7.40-7.44 (m, 2H, ArH), 7.47 (d, 2H, J = 8.4 Hz, ArH), 7.51 (d, 2H, J = 8.4 Hz, ArH), 7.88 (s, 1H, CH-triazole), 7.95 (s, 1H, NH).

5.1.1.11.

2-(4-(((5,6-Diphenyl-1,2,4-triazin-3-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-N-(2-ethoxyphenyl)acetamide (7**k**)

Yellow solid, yield 28.1 %, m.p. 178-179 ^{o}C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.38 (t, 3H, J = 7.2 Hz, $OCH_2\underline{CH_3}$), 3.94 (q, 2H, J = 7.2 Hz, $O\underline{CH_2}CH_3$), 4.69 (s, 2H, SCH_2), 5.10 (s, 2H, CH_2CO), 6.77 (d, 2H, J = 8.8 Hz, ArH), 7.29-7.32 (m, 3H, ArH), 7.34-7.38 (m, 3H, ArH), 7.40-7.44 (m, 2H, ArH), 7.47 (d, 2H, J = 8.0 Hz, ArH), 7.51 (d, 2H, J = 8.4 Hz, ArH), 7.88 (s, 1H, CH-triazole), 7.96 (s, 1H, NH).

5.1.1.12.

2-(4-(((5,6-Diphenyl-1,2,4-triazin-3-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-N-(4-methoxyphenyl)acetamide (7**l**)

Yellow solid, yield 41.4 %, m.p. 185-187 ^{o}C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.75 (s, 3H, OCH_3), 4.69 (s, 2H, SCH_2), 5.10 (s, 2H, CH_2CO), 6.78 (d, 2H, J = 8.8 Hz, ArH), 7.30-7.34 (m, 4H, ArH), 7.36 (d, 2H, J = 7.2 Hz, ArH), 7.40-7.44 (m, 2H, ArH), 7.47 (d, 2H, J = 7.6 Hz, ArH), 7.51 (d, 2H, J = 7.6 Hz, ArH), 7.89 (s, 1H, CH-triazole), 8.09 (s, 1H, NH).

5.1.1.13.

2-(4-(((5,6-Diphenyl-1,2,4-triazin-3-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)-N-(3-(trifluoromethyl)phenyl)acetamide (7**m**)

Yellow solid, yield 36.5 %, m.p. 202-204 ^{o}C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.69 (s, 2H, SCH_2), 5.15 (s, 2H, CH_2CO), 7.29-7.36 (m, 6H, ArH), 7.40-7.44 (m, 2H, ArH), 7.46 (d, 2H, J = 8.0 Hz, ArH), 7.50 (d, 2H, J = 8.0 Hz, ArH), 7.58 (d, 1H, J = 7.2 Hz, ArH), 7.84 (d, 1H, J = 7.2 Hz, ArH), 7.90 (s, 1H, CH-triazole), 8.71 (s, 1H, NH).

5.2. *In vitro* assay of α-glucosidase inhibitory activity

α-Glucosidase inhibitory activity was assayed by using 0.1 M phosphate buffer (pH 6.8) at 37 ^{o}C. The enzyme (0.1 U/mL) in phosphate buffer saline was incubated with various concentrations of test compounds at 37 ^{o}C for 15 min. Then 1.25 mM *p*-nitrophenyl α-D-glucopyranoside was added to the mixture as a substrate. After further incubation at 37 ^{o}C for 30 min. The absorbance was measured spectrophotometrically at 405 nm. The sample solution was replaced by DMSO as a control. Acarbose was used as a positive control. All experiments were carried out in triplicates. The% inhibition has been obtained using the formula: Inhibition (%) = (1-ΔAsample/ΔAcontrol) * 100%. IC_{50} value is defined as a concentration of samples

inhibiting 50% of α-glucosidase activity under the stated assay conditions.

5.3. Molecular docking

Molecular docking studies were performed to investigate the binding mode between the compound **7a**, **7i** and acarbose with α-glucosidase using Autodock vina 1.1.2. The 3D structures of **7a**, **7i** and acarbose were obtained by ChemBioDraw Ultra 14.0 and ChemBio3D Ultra 14.0 softwares. The AutoDockTools 1.5.6 package was employed to generate the docking input files. The search grid of α-glucosidase was identified as center_x: -19.676, center_y: -7.243, and center_z: -21.469 with dimensions size_x: 15, size_y: 15, and size_z: 15. The value of exhaustiveness was set to 20. For Vina docking, the default parameters were used if it was not mentioned. The best-scoring poses as judged by the Vina docking score were chosen and visually analyzed using PyMOL 1.7.6 software (http://www.pymol.org/).

Conflict of Interest

The authors confirm that this article content has no conflict of interest.

Acknowledgments

This work was supported by National Natural Science Foundation of China (Grant No. 81660574), Hunan Provincial Natural Science Foundation of China (2015JJ3099), Scientific Research Fund of Hunan Provincial Education Department (15B194).

SUPPLEMENTARY DATA

Supplemental data for this article can be accessed on the publisher's website at

References and notes

355 [1] R.A. DeFronzo, Med. Clin. North Am., 88 (2004) 787-835.

356 [2] A. Lopez-Candales, Journal of Medicine (Westbury), 32 (2001) 283-300.

357 [3] A.D. Deshpande, M. Harris-Hayes, M. Schootman, Phys. Ther., 88 (2008)
358 1254-1264.

359 [4] A.J. Hirsh, S.Y.M. Yao, J.D. Young, C.I. Cheeseman, Gastroenterology, 113 (1997)
360 205-211.

361 [5] S.A. Ross, E.A. Gulve, M.H. Wang, Chem. Rev., 104 (2004) 1255-1282.

362 [6] S.R. Joshi, E. Standl, N. Tong, P. Shah, S. Kalra, R. Rathod, Expert Opin.
363 Pharmacother., 16 (2015) 1959-1981.

364 [7] R. Pili, J. Chang, R.A. Partis, R.A. Mueller, F.J. Chrest, A. Passaniti, Cancer Res.,
365 55 (1995) 2920-2926.

366 [8] A. Mehta, N. Zitzmann, P.M. Rudd, T.M. Block, R.A. Dwek, FEBS Lett., 430
367 (1998) 17-22.

368 [9] A.J. Rawlings, H. Lomas, A.W. Pilling, M.J.R. Lee, D.S. Alonzi, J.S.S. Rountree,
369 S.F. Jenkinson, G.W.J. Fleet, R.A. Dwek, J.H. Jones, T.D. Butters, Chembiochem, 10
370 (2009) 1101-1105.

371 [10] N. Zitzmann, A.S. Mehta, S. Carrouée, T.D. Butters, F.M. Platt, J. McCauley, B.S.
372 Blumberg, R.A. Dwek, T.M. Block, Proc. Natl. Acad. Sci. U. S. A., 96 (1999)
373 11878-11882.

374 [11] F. Yoneda, T. Nagamatsu, Tetrahedron Lett., 14 (1973) 1577-1580.

375 [12] K. Ban, S. Duffy, Y. Khakham, V.M. Avery, A. Hughes, O. Montagnat, K.

376 Katneni, E. Ryan, J.B. Baell, Bioorg. Med. Chem. Lett., 20 (2010) 6024-6029.

377 [13] P. Ahuja, N. Siddiqui, Eur. J. Med. Chem., 80 (2014) 509-522.

378 [14] J.N. Sangshetti, D.B. Shinde, Bioorg. Med. Chem. Lett., 20 (2010) 742-745.

379 [15] H. Irannejad, A. Kebriaieezadeh, A. Zarghi, F. Montazer-Sadegh, A. Shafiee, A.

380 Assadieskandar, M. Amini, Biorg. Med. Chem., 22 (2014) 865-873.

381 [16] F. Krauth, H.-M. Dahse, H.-H. Ruettinger, P. Frohberg, Biorg. Med. Chem., 18

382 (2010) 1816-1821.

383 [17] P. Zhan, X. Li, Z. Li, X. Chen, Y. Tian, W. Chen, X. Liu, C. Pannecouque, E. De

384 Clercq, Bioorg. Med. Chem. Lett., 22 (2012) 7155-7162.

385 [18] H. Irannejad, M. Amini, F. Khodagholi, N. Ansari, S.K. Tusi, M. Sharifzadeh, A.

386 Shafiee, Biorg. Med. Chem., 18 (2010) 4224-4230.

387 [19] F. Rahim, K. Ullah, H. Ullah, A. Wadood, M. Taha, A.U. Rehman, I. Uddin, M.

388 Ashraf, A. Shaukat, W. Rehman, S. Hussain, K.M. Khan, Bioorg. Chem., 58 (2015)

389 81-87.

390 [20] G. Wang, J. Wang, D. He, X. Li, J. Li, Z. Peng, Bioorg. Med. Chem. Lett., 26

391 (2016) 2806-2809.

392 [21] G. Wang, J. Wang, D. He, X. Li, J. Li, Z. Peng, Heterocycles, 92 (2016)

393 1430-1439.

394 [22] H.C. Kolb, K.B. Sharpless, Drug Discovery Today, 8 (2003) 1128-1137.

395 [23] G.C. Tron, T. Pirali, R.A. Billington, P.L. Canonico, G. Sorba, A.A. Genazzani,

396 Med. Res. Rev., 28 (2008) 278-308.

397 [24] S.G. Agalave, S.R. Maujan, V.S. Pore, Chemistry-an Asian Journal, 6 (2011)
398 2696-2718.
399 [25] P. Thirumurugan, D. Matosiuk, K. Jozwiak, Chem. Rev., 113 (2013) 4905-4979.
400 [26] K.D. Thomas, A.V. Adhikari, N.S. Shetty, Eur. J. Med. Chem., 45 (2010)
401 3803-3810.
402 [27] S. Manohar, S.I. Khan, D.S. Rawat, Chem. Biol. Drug Des., 78 (2011) 124-136.
403 [28] A. Kamal, N. Shankaraiah, V. Devaiah, K.L. Reddy, A. Juvekar, S. Sen, N.
404 Kurian, S. Zingde, Bioorg. Med. Chem. Lett., 18 (2008) 1468-1473.
405 [29] H.B. Lazrek, M. Taourirte, T. Oulih, J.L. Barascut, J.L. Imbach, C. Pannecouque,
406 M. Witrouw, E. De Clercq, Nucleosides Nucleotides & Nucleic Acids, 20 (2001)
407 1949-1960.
408 [30] N. Boechat, V.F. Ferreira, S.B. Ferreira, M.d.L.G. Ferreira, F.d.C. da Silva, M.M.
409 Bastos, M.d.S. Costa, M.C.S. Lourenco, A.C. Pinto, A.U. Krettli, A.C. Aguiar, B.M.
410 Teixeira, N.V. da Silva, P.R.C. Martins, F.A.F.M. Bezerra, A.L.S. Camilo, G.P. da
411 Silva, C.C.P. Costa, J. Med. Chem., 54 (2011) 5988-5999.
412 [31] V.F. Ferreira, D.R. da Rocha, F.C. da Silva, P.G. Ferreira, N.A. Boechat, J.L.
413 Magalhães, Expert Opin. Ther. Pat., 23 (2013) 319-331.
414 [32] F. de Carvalho da Silva, M.F.d.C. Cardoso, P.G. Ferreira, V.F. Ferreira, Biological
415 Properties of 1H-1,2,3- and 2H-1,2,3-Triazoles, in: W. Dehaen, A.V. Bakulev (Eds.)
416 Chemistry of 1,2,3-triazoles, Springer International Publishing, Cham, 2015, pp.
417 117-165.

418 [33] S.B. Ferreira, A.C.R. Sodero, M.F.C. Cardoso, E.S. Lima, C.R. Kaiser, F.P. Silva,
419 V.F. Ferreira, J. Med. Chem., 53 (2010) 2364-2375.
420 [34] F. Jabeen, S.A. Shehzadi, M.Q. Fatmi, S. Shaheen, L. Iqbal, N. Afza, S.S. Panda,
421 F.L. Ansari, Bioorg. Med. Chem. Lett., 26 (2016) 1029-1038.
422 [35] M. Khoshneviszadeh, M.H. Ghahremani, A. Foroumadi, R. Miri, O. Firuzi, A.
423 Madadkar-Sobhani, N. Edraki, M. Parsa, A. Shafiee, Biorg. Med. Chem., 21 (2013)
424 6708-6717.
425 [36] F. Rahim, F. Malik, H. Ullah, A. Wadood, F. Khan, M.T. Javid, M. Taha, W.
426 Rehman, A.U. Rehman, K.M. Khan, Bioorg. Chem., 60 (2015) 42-48.
427 [37] H. Niaz, H. Kashtoh, J.A.J. Khan, A. Khan, W. Atia tul, M.T. Alam, K.M. Khan,
428 S. Perveen, M.I. Choudhary, Eur. J. Med. Chem., 95 (2015) 199-209.
429 [38] F. Rahim, H. Ullah, M.T. Javid, A. Wadood, M. Taha, M. Ashraf, A. Shaukat, M.
430 Junaid, S. Hussain, W. Rehman, R. Mehmood, M. Sajid, M.N. Khan, K.M. Khan,
431 Bioorg. Chem., 62 (2015) 15-21.

Highlights

►We designed and synthesized a new series of triazine-triazole derivatives.

► All the synthesized compounds displayed potent α-glucosidase inhibitory activity.

►Compound **7i** was found to be the most active compound.

►The structure-activity relationship has been discussed.

►Molecular docking study was carried out to reveal the interaction between enzyme and inhibitors.