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Synthesis, molecular docking and α-glucosidase inhibition of 2-((5,6-diphenyl-1,2,4-triazin-3-yl)thio)-N-arylacetamides

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ABSTRACT

A novel series of 2-((5,6-diphenyl-1,2,4-triazin-3-yl)thio)-N-arylacetamides **5a**–**5q** have been synthesized and evaluated for their α-glucosidase inhibitory activity. All newly synthesized compounds exhibited potent α-glucosidase inhibitory activity in the range of IC_{50} = 12.46 ± 0.13–72.68 ± 0.20 μM, when compared to the standard drug acarbose (IC_{50} = 817.38 ± 6.27 μM). Among the series, compound **5j** (12.46 ± 0.13 μM) with strong electron-withdrawing nitro group on the arylacetamide moiety was identified as the most potent inhibitor of α-glucosidase. Molecular docking study was carried out to explore the binding interactions of these compounds with α-glucosidase. Our study identifies a novel series of potent α-glucosidase inhibitors for further investigation.

Diabetes mellitus (DM) is a group of metabolic diseases characterized by chronic hyperglycemia that leads to long-term macrovascular and microvascular complications.[1,2] Hence, one of the therapeutic approaches in treating diabetes is to reduce postprandial hyperglycemia by inhibiting major carbohydrate hydrolyzing enzymes. α-Glucosidase is the key carbohydrate hydrolyzing enzymes, located in the brush-border surface membrane of human intestinal cells, which plays an important role in the carbohydrate digestion.[3] Therefore, the inhibition of α-glucosidase is an effective approach in both preventing and treating diabetes through controlling the postprandial glucose levels and suppressing postprandial hyperglycemia.[4] Some α-glucosidase inhibitors like acarbose, voglibose, and miglitol, have been used in clinic for the treatment of type II diabetes mellitus.[4] Thus, discovery and development of new α-glucosidase inhibitors has attracted great attention in recent years.

1,2,4-Triazine is an important heterocyclic nucleus, which is present in many naturally occurring products such as fervenulin, toxoflavin, and reurhycin (Fig. 1).[5] In the last few decades, the chemistry of 1,2,4-triazines and their derivatives have received considerable attention owing to their broad spectrum of biological activities,[6,7] including antifungal,[8] anticonvulsant,[9] anti-HIV,[10] anticancer,[11] antiinflammatory,[12] neuroprotective,[13] antiviral,[14] antimalarial,[15] cyclin-dependent kinase inhibitors,[16] antimicrobial[17] activities. Recently, several compounds containing 1,2,4-triazine moiety have been reported as potent inhibitors of α-glucosidase (Fig. 1).[18–21]

Hence, prompted by these observations and in continuation to our interest in design and synthesis of novel heterocyclic compounds,[19–21] herein we report for the first time the synthesis of a series of 2-((5,6-diphenyl-1,2,4-triazin-3-yl)thio)-N-arylacetamides. All synthesized derivatives were tested for their *in vitro* α-glucosidase inhibitory activity. Furthermore, *in silico* molecular docking studies were performed to further investigate the interactions of these compounds with the active site of α-glucosidase.

A series of 2-((5,6-diphenyl-1,2,4-triazin-3-yl)thio)-N-arylacetamides (**5a**–**5q**) were synthesized according to the pathways described in Scheme 1. 5,6-Diphenyl-1,2,4-triazine-3-thiol **2** was prepared by condensation of benzil **1** with thiosemicarbazide under reflux in acetic acid. The commercially available anilines **3a**–**3q** was reacted with 2-chloroacetyl chloride in the presence of Et_3N as base at room temperature to give the corresponding 2-chloro-N-arylacetamides **4a**–**4q**. Finally, condensation of 5,6-diphenyl-1,2,4-triazine-3-thiol **2** with appropriate 2-chloro-N-arylacetamides **4a**–**4q** in the presence of Et_3N in CH_3OH to afford the title compounds (**5a**–**5q**) in high yields (68.7–96.3%). The chemical structures of the title compounds (**5a**–**5q**) were confirmed by ^1H NMR, ^{13}C NMR and HRMS. For instance, the ^1H NMR spectrum of compound **5b** shown a singlet at δ 2.27 ppm was attributed to the methyl group at the para position of the phenyl moiety. A two-proton singlet at δ 4.07 ppm was attributed to the methylene protons of —S—CH_2—CO—. The two-proton double peak at δ

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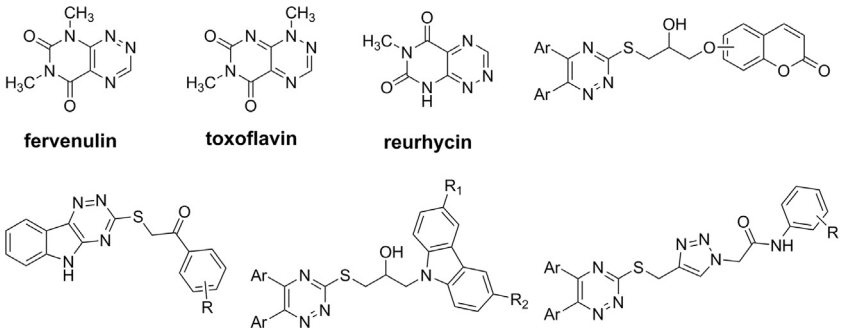
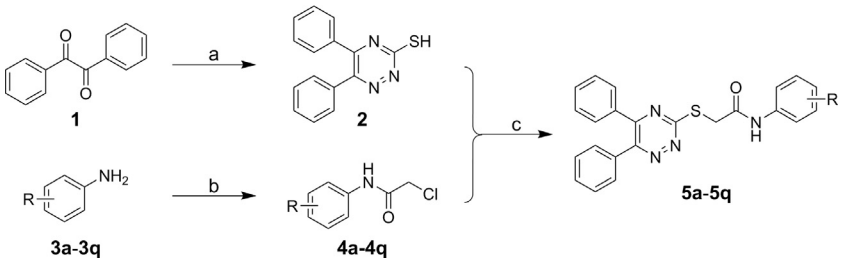


Fig. 1. Chemical structures of some 1,2,4-triazine natural products and α-glucosidase inhibitors containing 1,2,4-triazine moiety.



Scheme 1. Reagents and conditions: (a) thiosemicarbazide, AcOH, reflux, 3 h; (b) 2-chloroacetyl chloride, Et_3N, CH_2Cl_2, room temperature, 24 h; (c) Et_3N, CH_3OH, room temperature, 2 h.

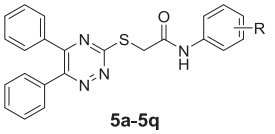
7.04 ppm (J = 7.6 Hz) was attributed to CH protons of the phenylacetamide. The ten aromatic protons of 5,6-diphenyl-1,2,4-triazine and two aromatic protons of phenylacetamide were appeared as multiplet in the region of δ 7.29–7.46 ppm and δ 7.51–7.56 ppm. The proton of —NH—CO— were appeared at 9.04 as a singlet signal. The above analysis clearly confirmed that the ^{1}H NMR spectrum of the synthesized compound **5b** was in agreement with the designed structure. Moreover, in ^{13}C NMR spectrum of compound **5b**, the number of signals equals the number of different carbons in the molecule.

All newly synthesized compounds **5a**–**5q** were evaluated for their *in vitro* α-glucosidase inhibitory activity. The results were shown that all compounds exhibited potent α-glucosidase inhibitory activity in the range of IC_{50} = 12.46 ± 0.13–72.68 ± 0.20 μM, when compared to the standard drug acarbose (IC_{50} = 817.38 ± 6.27 μM[22,23]) (Table 1). Among all the tested molecules, compound **5i** (14.09 ± 0.14 μM), **5j** (12.46 ± 0.13 μM) and **5n** (15.99 ± 0.18 μM) displayed outstanding inhibitory activity than the standard drug acarbose. Compounds **5k**, **5l**, **5m**, **5o** and **5p** also displayed potent α-glucosidase inhibitory activity with IC_{50} values of 36.79 ± 0.15, 28.03 ± 0.18, 26.27 ± 0.23, 34.94 ± 0.19, 25.25 ± 0.20, respectively. Furthermore, all other tested compounds shown moderate inhibitory activity with IC_{50} value of 71.09 ± 0.22, 52.01 ± 0.19, 42.54 ± 0.21, 72.68 ± 0.20, 67.24 ± 0.24, 45.65 ± 0.17, 46.98 ± 0.19, 50.63 ± 0.21 and 49.44 ± 0.19 μM, respectively.

Among the series, compound **5j** (12.46 ± 0.13 μM) with strong electron-withdrawing nitro group on the arylacetamide moiety was identified as the most potent inhibitor of α-glucosidase. Additionally, **5i** (14.09 ± 0.14 μM) with *para*-chloro substitution on the phenyl ring, was found to be the second most active compound. In summary, it was observed that the difference of substitution on the arylacetamide ring greatly influence the inhibitory activity of this class of compounds. In order to verify these observations, molecular docking study was performed.

The X-ray crystal structure of *Saccharomyces cerevisiae* α-glucosidase has never been reported. To understand the ligand-enzyme interactions, the 3D structure of α-glucosidase was built by homology modeling methods using modeller 9.15 homology modeling software (http://salilab.org/modeller/). The protein sequence of α-glucosidase in FASTA format was retrieved from Uniprot (Uniprot id: P53341). The crystallographic structure of *Saccharomyces cerevisiae* isomaltase (PDB ID: 3AJ7, Resolution 1.30 Å) with 72.4% of sequence identity with the target was selected as the template for homology modeling.[24] The quality of homology model

Table 1
α-Glucosidase inhibitory activity and binding energies of 2-((5,6-diphenyl-1,2,4-triazin-3-yl)thio)-N-arylacetamides (**5a**–**5q**).



Compound	R	IC_{50} (μM)[a]	Binding energy (kcal/mol)
5a	H	71.09 ± 0.22	−8.5
5b	4-Me	52.01 ± 0.19	−8.9
5c	3-Me	42.54 ± 0.21	−9.1
5d	2-Me	72.68 ± 0.20	−8.3
5e	2,4-Me_2	67.24 ± 0.24	−9.2
5f	2-NO_2-4-Me	45.65 ± 0.17	−9.0
5g	2-NO_2-4-MeO	46.98 ± 0.19	−9.2
5h	4-F	50.63 ± 0.21	−8.8
5i	4-Cl	14.09 ± 0.14	−10.3
5j	4-NO_2	12.46 ± 0.13	−10.7
5k	2,4-Cl_2	36.79 ± 0.15	−9.6
5l	2,5-Cl_2	28.03 ± 0.18	−9.2
5m	4-EtO	26.27 ± 0.23	−9.5
5n	2-EtO	15.99 ± 0.18	−10.2
5o	3-CF_3	34.94 ± 0.19	−9.4
5p	4-PhO	25.25 ± 0.20	−9.8
5q	4-MeO	49.44 ± 0.19	−9.0
Acarbose		817.38 ± 6.27	−6.8

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

was verified by PROCHECK (http://services.mbi.ucla.edu/PROCHECK/). The result was shown that the model could be used to study the interactions between this class of compounds and the active site of α-glucosidase.[21]

To investigate the binding mode of these compounds with α-glucosidase, molecular docking study was carried out by using Autodock VINA 1.1.2.[25] The theoretical binding mode between **5a** and *Saccharomyces cerevisiae* α-glucosidase was shown in Fig. 2A. Compound **5a** adopted a F-shaped conformation in the pocket of the α-glucosidase. One of the *o*-diphenyl group of **5a** bind at the bottom of the α-glucosidase pocket and made a high density of van der Waals contacts, whereas the phenylamino group of **5a** was positioned near the entrance of the pocket and made only a few contacts. Detailed analysis showed that *o*-diphenyl group of **5a** formed arene-cation interaction with the residue Arg-312, and CH-π interactions with the residues Tyr-71 and Phe-157. It was shown that the Glu-276 (bond length: 3.3 Å) formed a hydrogen bond with **5a**, which was the main interaction between **5a** and α-glucosidase. On the other hand, molecular docking study of the standard drug acarbose with α-glucosidase was also performed (Fig. 2B). Acarbose adopted a U-shaped conformation in the pocket of the α-glucosidase. The results were shown that compound **5a** (binding energy was about −8.5 kcal mol^{-1} and a hydrogen bond with Glu-276) has similar binding affinity as compared to standard drug acarbose (binding energy was about −6.8 kcal mol^{-1} and three hydrogen bonds with Asp-68, Gln-181 and Asp-349).

In order to increase the activity of **5a**, a nitro group was introduced to 4-position of the phenylamino group of **5a** to obtain **5j**. Compound **5j** was docked to the binding pocket of the *Saccharomyces cerevisiae* α-glucosidase, and the theoretical binding mode

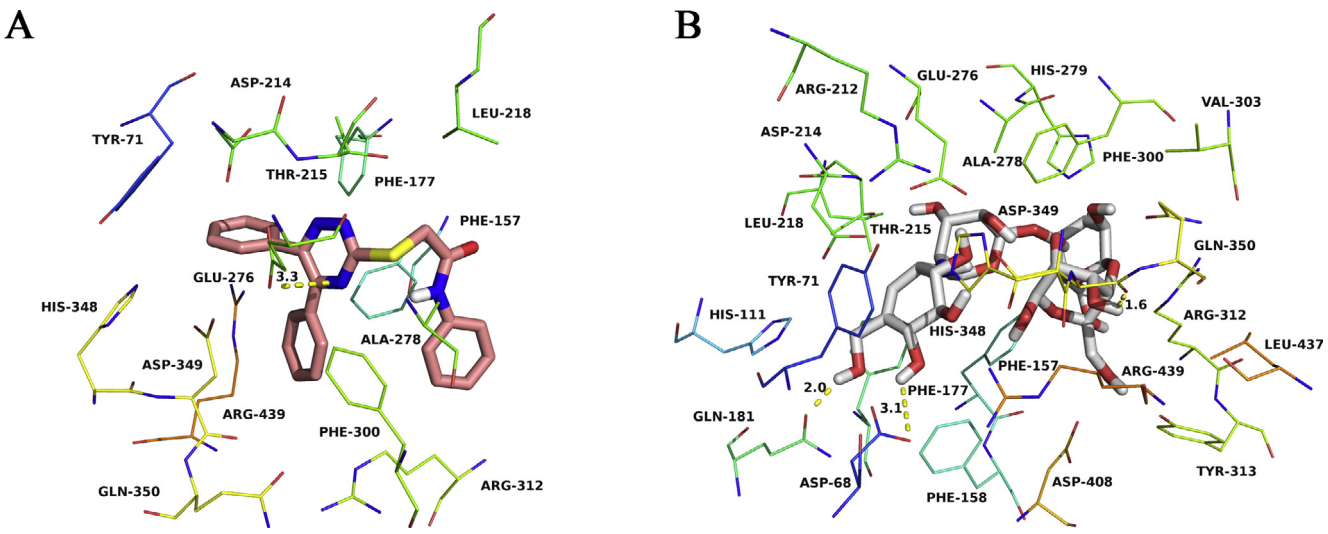


Fig. 2. Compound **5a** (A) and acarbose (B) were docked to the binding pocket of the *Saccharomyces cerevisiae* α-glucosidase.

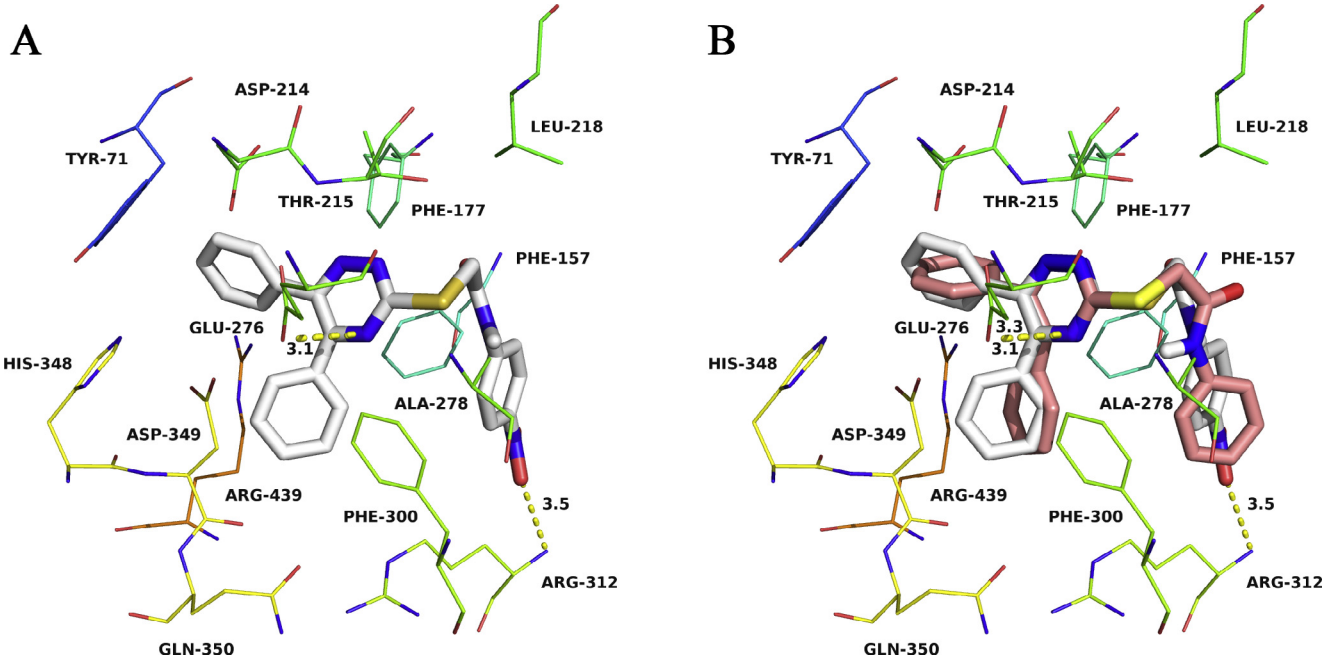


Fig. 3. (A) Compound **5j** was docked to the binding pocket of the *Saccharomyces cerevisiae* α-glucosidase. (B) Compounds **5a** and **5j** were docked to the binding pocket of the *Saccharomyces cerevisiae* α-glucosidase (overlapped).

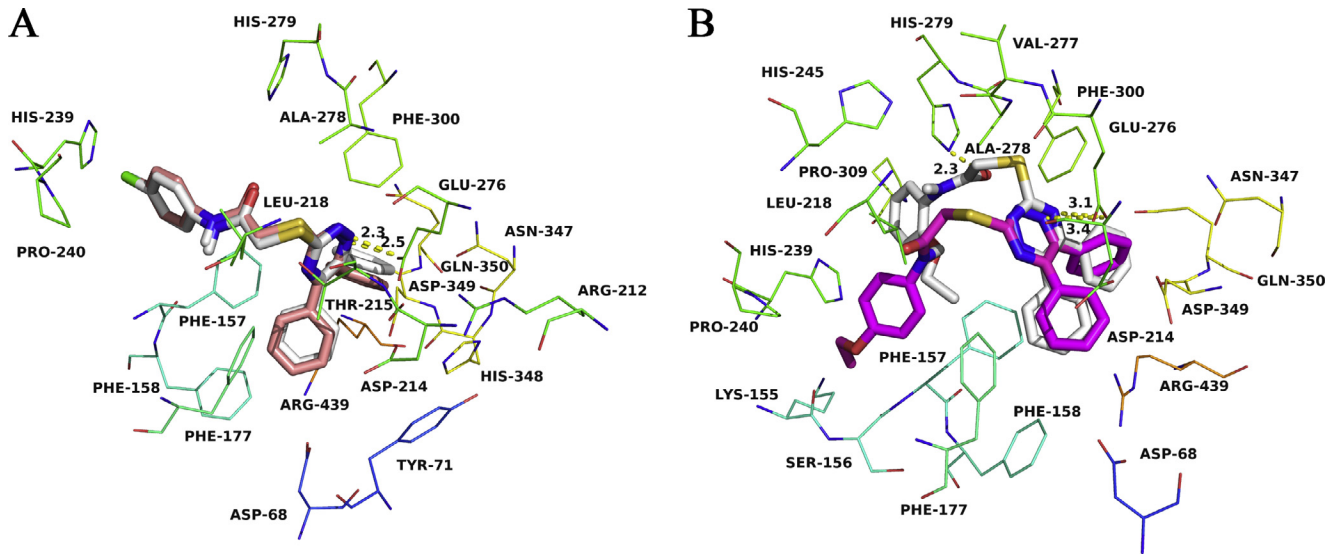


Fig. 4. (A) Compounds **5h** and **5i** were docked to the binding pocket of the *Saccharomyces cerevisiae* α-glucosidase (overlapped). (B) Compounds **5m** and **5n** were docked to the binding pocket of the *Saccharomyces cerevisiae* α-glucosidase (overlapped).

between **5j** and *Saccharomyces cerevisiae* α-glucosidase was shown in Fig. 3A. The interaction between **5j** and α-glucosidase was nearly the same as the precursor **5a**. The only difference was that the introduced nitro group formed a hydrogen bond with the residue Arg-312, with the length of 3.5 Å, which made **5j** was more active than **5a** against α-glucosidase (Fig. 3B).

To explain the activity order of **5h**, **5i**, **5m** and **5n** against α-glucosidase, these compounds were docked into the binding site of α-glucosidase. The theoretical binding modes of **5h**, **5i**, **5m** and **5n** were nearly the same as **5a** (Fig. 4). The only difference between **5h** and **5i** was that the chlorine atom of **5i** formed a Cl-π interaction with residue His-239, which made **5i** was more active than **5h** against α-glucosidase (Fig. 4A). The estimated binding energies were -8.8 kcal mol^{-1} for **5h** and -10.3 kcal mol^{-1} for **5i**, respectively, which was consistent with the results of the *in vitro* anti-α-glucosidase assay (Table 1). In addition, the difference between **5m** and **5n** was that the C=O group of **5n** formed an extra hydrogen bond with the residue His-279, with the length of 2.3 Å, which made **5n** was more active than **5m** against α-glucosidase (Fig. 4B). The estimated binding energies were -9.5 kcal mol^{-1} for **5m** and -10.2 kcal mol^{-1} for **5n**, respectively, which was consistent with the results of the *in vitro* α-glucosidase inhibitory activity (Table 1). In summary, the above molecular simulations give us rational explanation of the interactions between this class of compounds and α-glucosidase, which provided valuable information for further development of α-glucosidase inhibitors.

A novel series of 2-((5,6-diphenyl-1,2,4-triazin-3-yl)thio)-N-arylacetamides **5a–5q** have been synthesized and evaluated for their α-glucosidase inhibitory activity. All newly synthesized compounds exhibited potent α-glucosidase inhibitory activity in the range of IC_{50} = 12.46 ± 0.13–72.68 ± 0.20 μM, when compared to the standard drug acarbose (IC_{50} = 817.38 ± 6.27 μM). Molecular docking study was performed to understand the interaction pattern of these compounds at the binding site of α-glucosidase enzyme. Hence, this study identified compound **5j** could be used as lead molecules for further research to discover more potent α-glucosidase inhibitors.

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A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.01.094.

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