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Synthesis, *in vitro* evaluation and molecular docking studies of novel coumarin-isatin derivatives as α-glucosidase inhibitors

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Abstract.

This study synthesized a series of novel coumarin-isatin derivatives and evaluated them for α-glucosidase inhibitory activity. The majority of the screened compounds exhibited excellent inhibition activities with IC_{50} values of 2.56±0.08-268.79±3.04 μM, when compared to

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acarbose. Among the newly derivatives, compound **5p** was found to be the most active compound in the library of coumarin isatin derivatives. Furthermore, enzyme kinetic studies showed that compound **5p** is a non-competitive inhibitor with a K_i of 2.14 μM. Molecular docking analysis revealed the existence of hydrophobic and hydrogen interactions between compound **5p** and the active site of α-glucosidase. Our results indicate that coumarin-isatin derivatives as a new class of α-glucosidase inhibitors.

Keywords: Coumarin; Isatin; α-Glucosidase inhibitor; Enzyme kinetic study; Molecular docking

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia, which has become one of the most common non-communicable diseases of the globe [1]. In DM, chronic hyperglycemia causes serious damage to many of the body's organs, especially the nerves and blood vessels [2, 3].

α-Glucosidase enzyme is located in the brush-border surface membrane of intestinal cells, which catalyzes the cleavage of glucose from disaccharides, as human intestine only absorbs monosacharides for blood circulation. Thus, α-glucosidase has been recognized as a therapeutic target for modulation of postprandial hyperglycemia in type-2 diabetes. α-Glucosidase inhibitors, such as acarbose, miglitol, and voglibose, have been used in the clinical decrease the postprandial blood glucose levels in type-2 diabetic patients over the

past decade [4, 5]. However, these classic α-glucosidase inhibitors cause various side effects including bloating, flatulence, diarrhea, abdominal discomfort, and pain [6]. In addition, all known α-glucosidase inhibitors have low efficacy with high IC_{50} values against the enzyme. Therefore, safer and more efficient α-glucosidase inhibitors are still urgently needed.

Coumarin derivatives are an important class of naturally occurring compounds, widely present in plants, including edible vegetables and fruits. Coumarin derivatives are of great interest due to their diverse structural features and versatile biological properties, such as anticancer [7], antimalarial [8], antiinflammatory [9], antioxidant [10], antitubercular [11] and antimicrobial [12]. Despite numerous efforts have been focusing on the research and development of coumarin derivatives as potential drugs, reports on their anti-diabetic activity are scarce [13-17] (**Figure 1**). Isatin are reported to possess a wide variety of biological activities like antioxidant [18], antibacterial [19], antitumor [20] and anticonvulsant [21]. Recently, Schiff bases of isatin derivatives have been reported to show potent inhibitory activity against α-glucosidase [22].

Figure 1. Chemical structures of some α-glucosidase inhibitors containing coumarin moieties.

In our continued search for biologically active heterocyclic compounds, here we are reporting synthesis of a new serial of coumarin-isatin derivatives and evaluated for their α-glucosidase inhibitory activity. The molecular docking was also performed in order to study their binding affinity.

2. Chemistry

A general synthesis of coumarin-isatin derivatives **5a**-**5t** is exhibited in **Scheme 1**. Treatment of 7-hydroxycoumarin **1** with ethyl bromoacetate in the presence of anhydrous K_2CO_3 in dry acetone to afforded ethyl 2-((2-oxo-2H-chromen-7-yl)oxy) acetate **2** in good yield, which reacted with hydrazine hydrate to provide the key intermediate **3**. Finally, the new desired compounds **5a**-**5t** were obtained, in good yields (67.5%-89.1%), by condensing hydrazide **3** with the corresponding appropriate isatins **4a**-**4t** in the presence of glacial acetic acid. The structures of newly synthesized compounds were confirmed by their spectral analysis.

Scheme 1. Reagents and conditions: (a) K_2CO_3, acetone, reflux, 6h; (b) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 4h; (c) CH_3COOH, EtOH, reflux, 2h.

3. Biological results and discussion

3.1. α-Glucosidase inhibition assay

The α-glucosidase inhibitory activities of coumarin-isatin derivatives **5a**-**5t** were evaluated according to the literature procedure with minor modification [23]. Acarbose was used as a positive control for this assay. The results were summarized in **Table 1**. It was notable that compound **5m**, **5p**, **5q**, **5r**, **5s** and **5t** exhibited excellent inhibition activities with IC_{50} values of 4.07±0.08, 2.56±0.08, 2.88±0.05, 4.48±0.06, 2.85±0.06, and 3.98±0.09 μM, respectively, when compared to acarbose (817.38±6.27 μM). Among all the tested molecules, **5p** (IC_{50} = 2.56±0.08 μM) was found to be the most active compound in the library of coumarin-isatin derivatives.

Table 1. α-glucosidase inhibitory activities of coumarin-isatin derivatives

With respect to the study of structure-activity relationships (SARs), the importance of the substitution of the isatin ring was preferential discussed in this study. Introduction of electron-withdrawing group such as F (**5d**), Cl (**5e**, **5p** and **5q**), Br (**5f**, **5r**-**5t**) and NO_2 (**5g**) into the 5-position of isatin ring, results in a significant increase the inhibitory activity. It is important to point out the most of compounds with different substituted benzyl group (**5i-5t**) at the N1 position of isatin ring displayed potent activities, with IC_{50} values ranging from 2.56 to 77.79 μM. Additionally, the introduction of methyl group at N1 or C7 position of isatin ring (**5c**, **5h**) resulted in a remarkable decrease of the biological activity. These results indicated the pattern of substitution in the isatin ring is closely related to the biological activity of this class of compounds. In summary, the information of structure-activity relationships provided us a guideline to improve α-glucosidase inhibitory activity in the future structural modification.

3.2. Mode of α-glucosidase inhibition by coumarin-isatin derivatives

To study the mechanism of inhibition, the kinetic studies of the most active compound **5p** was performed using Lineweaver-Burk plot analysis. In the enzyme kinetic studies, different concentrations of test compound and substrate were used. The plot of velocity versus substrate *p*-nitrophenyl α-D-glucopyranoside (pNPG) concentration in the presence of different concentrations of **5p** gave a series of straight lines. The results of **Figure 2A** showed

that V_{max} of enzyme decreased without affecting the K_m of enzyme which indicated that **5p** is a non-competitive inhibitor of α-glucosidase. The secondary re-plots of Lineweaver-Burk plots were plotted to determine the K_i values (**Figure 2B**). The K_i value was calculated directly by plotting the slope of each line in the Lineweaver-Burk plots against the different concentrations of **5p**. These results were shown that **5p** is a noncompetitive inhibitor with a K_i of 2.14 μM.

Figure 2. (A) Lineweaver-Burke plot of the inhibition kinetics of α-glucosidase by **5p**. (B) Secondary re-plot of Lineweaver-Burk plot between the slopes of each line on Lineweaver-Burk plot *vs* various concentrations of **5p**.

3.3. 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 [22, 24]. 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) with 72.4% was selected as the template for modeling.

3.4. Molecular docking simulation

Molecular docking simulations was carried out to investigate the binding mode of these compound with *Saccharomyces cerevisiae* α-glucosidase. The theoretical binding mode between **5e** and *Saccharomyces cerevisiae* α-glucosidase was shown in **Figure 3A**. Compound **5e** adopted a hairpin conformation in the pocket of the α-glucosidase. The coumarin group of **5e** bind at the bottom of the α-glucosidase pocket and made a high density of vander Waals contacts, whereas the indole ring of **5e** was positioned near the entrance of the pocket and made only a few contacts. Detailed analysis showed that coumarin group of **5e** formed arene-cation interaction with the residue Arg-312. The indole ring of **5e** formed a π-π stacking with the residue Phe-157 and a CH-π interaction with the residue Phe-177. It was shown that Asn-347 (bond length: 2.2 and 3.2 Å) and His-279 (bond length: 2.6 Å) formed three hydrogen bonds with **5e**, which was the main interactions between **5e** and α-glucosidase.

In order to increase the activity of **5e**, 2-fluorobenzyl group was introduced to the indole ring of **5e** to obtain **5p**. Compound **5p** was docked to the binding pocket of the *Saccharomyces cerevisiae* α-glucosidase, and the theoretical binding mode between **5p** and *Saccharomyces cerevisiae* α-glucosidase was shown in **Figure 3B**. Compound **5p** adopted a compact conformation in the pocket of the α-glucosidase. The coumarin group of **5p** bind at the bottom of the α-glucosidase pocket and made a high density of vander Waals contacts, whereas the indole ring of **5p** was positioned near the entrance of the pocket and made only a

few contacts. Detailed analysis showed that coumarin group of **5p** formed arene-cation interaction with the residues Arg-312. The indole ring of **5p** formed a π-π stacking with the residue Phe-157 and a CH-π interaction with the residue Phe-177. In addition, the 2-fluorobenzyl group of **5p** located at the hydrophobic pocket, and maintained close hydrophobic contacts with the residues Ala-278, Val-277, Ala-216, Gly-217 and Leu-218. It was shown that Asn-347 (bond length: 2.1 and 3.1 Å), Tyr-344 (bond length: 3.5 Å) and His-279 (bond length: 2.1 Å) formed four hydrogen bonds with **5p**, which was the main interactions between **5p** and α-glucosidase.

Figure 3. Compound **5e** (A) and **5p** (B) was docked to the binding pocket of the *Saccharomyces cerevisiae* α-glucosidase.

The interaction between **5p** and α-glucosidase was nearly the same as **5e** (**Figure 4**). The only differences were that the 2-fluorobenzyl group of **5p** located at the hydrophobic pocket, and maintained close hydrophobic contacts with the residues Ala-278, Val-277, Ala-216, Gly-217 and Leu-218. Also, there were four hydrogen bonds between **5p** and α-glucosidase, while **5e** only had three. All these differences made **5p** was more active than **5e** against α-glucosidase.

Figure 4. Compounds **5e** and **5p** were docked to the binding pocket of the *Saccharomyces cerevisiae* α-glucosidase (overlaped).

To further clarify the activity order between **5p** and **5n**, compound **5n** was docked to the binding pocket of the *Saccharomyces cerevisiae* α-glucosidase, and the theoretical binding mode between **5n** and *Saccharomyces cerevisiae* α-glucosidase was shown in **Figure 5A**.

Compound **5n** adopted a compact conformation in the pocket of the α-glucosidase. The coumarin group of **5n** bind at the bottom of the α-glucosidase pocket and made a high density of vander Waals contacts, whereas the indole ring of **5n** was positioned near the entrance of the pocket and made only a few contacts. Detailed analysis showed that coumarin group of **5n** formed arene-cation interaction with the residues Arg-312. The indole ring of **5n** formed a π-π stacking with the residue Phe-157 and a CH-π interaction with the residue Phe-177. In addition, the 2-fluorobenzyl group of **5n** located at the hydrophobic pocket, and maintained close hydrophobic contacts with the residues Ala-278, Val-277, Ala-216, Gly-217 and Leu-218. It was shown that Asn-347 (bond length: 2.1 and 3.1 Å) and His-279 (bond length: 2.1 Å) formed three hydrogen bonds with **5n**, which was the main interactions between **5n** and α-glucosidase.

The compound **5n** shared the same conformation as **5p** in the pocket of the α-glucosidase (**Figure 5B**). The only difference was that there were four hydrogen bonds between **5p** and α-glucosidase, while **5n** only had three, which made **5p** was more active than **5n** against α-glucosidase. In summary, the above molecular simulations give us rational explanation of the interactions between **5e**, **5n**, **5p** and α-glucosidase, which provided valuable information for further development of α-glucosidase inhibitors.

Figure 5. (A) Compound **5n** was docked to the binding pocket of the *Saccharomyces cerevisiae* α-glucosidase. (B) Compounds **5n** and **5p** were docked to the binding pocket of the *Saccharomyces cerevisiae* α-glucosidase (overlaped).

4. Conclusion

This paper described the synthesis of a series of novel coumarin-isatin derivatives and evaluation of their α-glucosidase inhibitory activity. The majority of the screened compounds displayed potent biological activity. Among them, compound **5p** was found to be the most active compound with IC_{50} values of 2.56±0.08 μM. Meanwhile, enzyme kinetic analysis revealed that compound **5p** is a non-competitive inhibitor. Molecular docking study showed that compound **5p** has a high binding affinity with α-glucosidase due to the presence of hydrophobic and hydrogen interactions. This is the first report identifying the α-glucosidase inhibitory activities of coumarin-isatin derivatives and suggests that this type of compounds may serve as lead compounds for the development of novel 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 at 400 MHz on a JNM spectrometer (JEOL Ltd.) and reported in parts per million.).

5.1.1. Ethyl 2-((2-oxo-2H-chromen-7-yl)oxy)acetate (**2**)

To a solution of 7-hydroxycoumarin (1.62 g, 10 mmol, **1**) in acetone (100 mL) was added K_2CO_3 (4.15 g, 30 mmol), and the mixture was refluxed for 1 h. To this solution, ethyl bromoacetate (1.84 g, 11 mmol) was added, and the mixture was heated under refluxed for 6

h. After completion of reaction, the solid precipitate was removed by filtration. The filtrate was concentrated under reduced pressure, and recrystallization from ethanol afforded **2** as a white solid. Yield 86.4%; m.p. 107-110 ^{o}C.

5.1.2. 2-((2-Oxo-2H-chromen-7-yl)oxy)acetohydrazide (**3**)

To a solution of **2** (2.48 g, 10 mmol) in ethanol (100 mL) was added hydrazine (1.00 g, 20 mmol) and the mixture was heated to reflux for 4 hours. After cooling, the precipitate was collected by filtration and washed with ethanol to give **3** as a white solid. Yield 65.3%; m.p. 171-173 ^{o}C.

5.1.3. General procedure for the synthesis of **5a**-**5t**

A mixture of **3** (1 mmol), different isatin **4a**-**4t** (1 mmol) were refluxed in ethanol for 2 h in the presence of few drops of glacial acetic acid. After the resulting mixture was cooled at room temperature, the product precipitated is filtered off and then washed with petroleum ether to give **5a-5t** as a solid.

5.1.3.1.(*Z*)-2-((2-Oxo-2H-chromen-7-yl)oxy)-N'-(2-oxoindolin-3-ylidene)acetohydrazide (**5a**)

Yield 87.4%. Red solid; m.p. 237-240 ^{o}C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 5.01 (s, 2H), 6.32 (d, 1H, J = 9.6 Hz), 6.94 (d, 1H, J = 8.0 Hz), 7.05-7.10 (m, 3H), 7.37 (d, 1H, J = 8.4 Hz), 7.58-7.68 (m, 2H), 8.00 (d, 1H, J = 9.6 Hz), 10.23 (s, 1H), 11.32 (s, 1H).

5.1.3.2.(*Z*)-N'-(5-Methyl-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chromen-7-yl)oxy)acetohydr azide (**5b**)

Yield 81.3%. Yellow solid; m.p. 253-255 °C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 2.30 (s, 3H), 5.00 (s, 2H), 6.32 (d, 1H, J = 9.6 Hz), 6.83 (d, 1H, J = 8.0 Hz), 7.05-7.10 (m, 2H), 7.19 (d, 1H, J = 8.0 Hz), 7.41 (s, 1H), 7.69 (s, 1H), 8.00 (d, 1H, J = 9.6 Hz), 10.21 (s, 1H), 11.18 (s, 1H).

5.1.3.3.(*Z*)-N'-(5,7-Dimethyl-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chromen-7-yl)oxy)aceto hydrazide (**5c**)

Yield 75.5%. Yellow solid; m.p. 231-232 °C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 2.21 (s, 3H), 2.67 (s, 3H), 5.02 (s, 2H), 6.32 (d, 1H, J = 9.6 Hz), 7.04-7.11 (m, 2H), 7.21-7.28 (m, 2H), 7.70 (s, 1H), 8.01 (d, 1H, J = 9.6 Hz), 10.22 (s, 1H), 11.24 (s, 1H).

5.1.3.4.(*Z*)-N'-(5-Fluoro-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chromen-7-yl)oxy)acetohydr azide (**5d**)

Yield 79.3%. Brown solid; m.p. 241-244 °C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 5.02 (s, 2H), 6.33 (d, 1H, J = 9.6 Hz), 6.94-6.97 (m, 1H), 7.06 (d, 1H, J = 8.8 Hz), 7.11 (s, 1H), 7.22-7.27 (m, 1H), 7.42 (s, 1H), 7.69 (s, 1H), 8.01 (d, 1H, J = 9.6 Hz), 10.25 (s, 1H), 11.31 (s, 1H).

5.1.3.5.(*Z*)-N'-(5-Chloro-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chromen-7-yl)oxy)acetohydr azide (**5e**)

Yield 89.1%. Brown solid; m.p. 215-217 °C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 5.04 (s, 2H), 6.30 (d, 1H, J = 9.6 Hz), 6.35-6.38 (m, 2H), 6.97-7.08 (m, 2H), 7.43 (dd, 1H, J = 2.4, 8.8 Hz), 7.68 (s, 1H), 8.01 (d, 1H, J = 9.6 Hz), 10.23 (s, 1H), 11.42 (s, 1H).

5.1.3.6.(*Z*)-N'-(5-Bromo-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chromen-7-yl)oxy)acetohydr azide (**5f**)

Yield 69.2%. Brown solid; m.p. 205-206 °C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 5.04 (s, 2H), 6.34 (d, 1H, J = 9.6 Hz), 6.91 (d, 1H, 8.8 Hz), 7.06 (d, 1H, J = 8.8 Hz), 7.12 (d, 1H, J = 2.0 Hz), 7.56 (dd, 1H, J = 2.0, 8.8 Hz), 7.69-7.81 (m, 2H), 8.02 (d, 1H, J = 9.6 Hz), 10.24 (s, 1H), 11.40 (s, 1H).

5.1.3.7.(*Z*)-N'-(5-Nitro-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chromen-7-yl)oxy)acetohydrazide (**5g**)

Yield 77.9%. Yellow solid; m.p. 228-229 °C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 5.04 (s, 2H), 6.32 (d, 1H, J = 9.6 Hz), 7.08-7.17 (m, 3H), 7.68-7.70 (m, 1H), 8.00 (d, 1H, J = 9.6 Hz), 8.30 (dd, 2H, J = 2.0, 8.8 Hz), 10.21 (s, 1H), 11.91 (s, 1H).

5.1.3.8.(*Z*)-N'-(1-Methyl-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chromen-7-yl)oxy)acetohydrazide (**5h**)

Yield 68.3%. Yellow solid; m.p. 193-194 °C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 3.23 (s, 3H), 5.04 (s, 2H), 6.33 (d, 1H, J = 9.6 Hz), 7.01-7.18 (m, 3H), 7.49-7.51 (m, 1H), 7.62-7.73 (m, 2H), 8.02 (d, 1H, J = 9.6 Hz), 10.24 (s, 1H), 11.40 (s, 1H).

5.1.3.9.(*Z*)-N'-(1-Benzyl-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chromen-7-yl)oxy)acetohydrazide (**5i**)

Yield 85.4%. Yellow solid; m.p. 247-248 °C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 5.03 (s, 2H), 5.04 (s, 2H), 6.34 (d, 1H, J = 9.6 Hz), 7.08-7.19 (m, 3H), 7.29-7.44 (m, 7H), 7.68-7.71 (m, 2H), 8.03 (d, 1H, J = 9.6 Hz), 10.25 (s, 1H).

5.1.3.10.(*Z*)-N'-(1-(2-Fluorobenzyl)-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chromen-7-yl)oxy)acetohydrazide (**5j**)

Yield 87.9%. Yellow solid; m.p. 252-255 °C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 5.03 (s, 2H), 5.04 (s, 2H), 6.33 (d, 1H, J = 9.6 Hz), 6.99-7.08 (m, 2H), 7.12-7.19 (m, 3H), 7.23-7.28 (m, 1H), 7.36-7.45 (m, 3H), 7.68-7.70 (m, 2H), 8.02 (d, 1H, J = 9.6 Hz), 10.25 (s, 1H).

5.1.3.11.(*Z*)-N'-(1-(3-Fluorobenzyl)-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chromen-7-yl)oxy)acetohydrazide (**5k**)

Yield 71.4%. Yellow solid; m.p. 248-250 °C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 5.03 (s, 2H), 5.04 (s, 2H), 6.33 (d, 1H, J = 9.6 Hz), 7.05-7.19 (m, 5H), 7.22 (d, 1H, J = 8.8 Hz), 7.26 (d, 1H, J = 8.8 Hz), 7.38-7.43 (m, 2H), 7.68-7.73 (m, 2H), 8.02 (d, 1H, J = 9.6 Hz), 10.24 (s, 1H).

5.1.3.12.(*Z*)-N'-(1-(2-Chlorobenzyl)-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chromen-7-yl)ox y)acetohydrazide (**5l**)

Yield 82.0%. Brown solid; m.p. 185-188 °C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 5.04 (s, 2H), 5.06 (s, 2H), 6.33 (d, 1H, J = 9.6 Hz), 6.93 (d, 1H, J = 8.0 Hz), 7.04 (dd, 1H, J = 2.0, 8.0 Hz), 7.18 (t, 1H, J = 8.0 Hz), 7.24-7.40 (m, 5H), 7.52 (d, 1H, J = 8.8 Hz), 7.68-7.69 (m, 2H), 8.02 (d, 1H, J = 9.6 Hz), 10.22 (s, 1H).

5.1.3.13.(*Z*)-N'-(1-(4-Bromobenzyl)-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chromen-7-yl)ox y)acetohydrazide (**5m**)

Yield 74.6%. Yellow solid; m.p. 255-257 °C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 5.00 (s, 2H), 5.05 (s, 2H), 6.33 (d, 1H, J = 9.6 Hz), 7.05-7.19 (m, 4H), 7.35 (d, 2H, J = 8.0 Hz), 7.42 (t, 1H, J = 8.0 Hz), 7.55 (d, 2H, J = 8.0 Hz), 7.65-7.67 (m, 2H), 8.02 (d, 1H, J = 9.6 Hz), 10.23 (s, 1H).

5.1.3.14.(*Z*)-N'-(1-(2-Fluorobenzyl)-5-methyl-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chrome n-7-yl)oxy)acetohydrazide (**5n**)

Yield 73.9%. Yellow solid; m.p. 214-216 ^{o}C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 2.32 (s, 3H), 5.04 (s, 2H), 5.05 (s, 2H), 6.34 (d, 1H, J = 9.6 Hz), 6.93 (d, 1H, J = 8.0 Hz), 7.01 (d, 1H, J = 8.8 Hz), 7.13 (s, 1H), 7.18 (t, 1H, J = 8.0 Hz), 7.23 (d, 1H, J = 8.8 Hz), 7.26 (d, 1H, J = 8.0 Hz), 7.34-7.38 (m, 2H), 7.51 (s, 1H), 7.66-7.72 (m, 1H), 8.02 (d, 1H, J = 9.6 Hz), 10.25 (s, 1H).

5.1.3.15.(*Z*)-N'-(1-(3-Fluorobenzyl)-5-methyl-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chrome n-7-yl)oxy)acetohydrazide (**5o**)

Yield 80.1%. Yellow solid; m.p. 215-217 ^{o}C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 2.32 (s, 3H), 5.02 (s, 2H), 5.04 (s, 2H), 6.34 (d, 1H, J = 9.6 Hz), 6.37 (dd, 1H, J = 2.4, 8.8 Hz), 6.43 (d, 1H, J = 2.4 Hz), 6.94 (d, 1H, J = 8.0 Hz), 7.08-7.16 (m, 2H), 7.20-7.28 (m, 2H), 7.40 (d, 1H, J = 8.0 Hz), 7.51 (s, 1H), 7.66-7.72 (m, 1H), 8.03 (d, 1H, J = 9.6 Hz), 10.25 (s, 1H).

5.1.3.16.(*Z*)-N'-(5-Chloro-1-(2-fluorobenzyl)-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chromen -7-yl)oxy)acetohydrazide (**5p**)

Yield 83.2%. Yellow solid; m.p. 240-243 ^{o}C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 5.07 (s, 2H), 5.08 (s, 2H), 6.33 (d, 1H, J = 9.6 Hz), 6.35 (dd, 1H, J = 2.4, 8.8 Hz), 6.41 (d, 1H, J = 2.4 Hz), 7.06-7.10 (m, 2H), 7.13 (d, 1H, J = 2.4 Hz), 7.18 (d, 1H, J = 8.8 Hz), 7.25 (t, 1H, J = 8.8 Hz), 7.35-7.38 (m, 1H),7.49 (dd, 1H, J = 2.0, 8.0 Hz), 7.69-7.71 (m, 1H), 8.02 (d, 1H, J = 9.6 Hz), 10.23 (s, 1H).

5.1.3.17.(*Z*)-N'-(5-Chloro-1-(3-fluorobenzyl)-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chromen-7-yl)oxy)acetohydrazide (**5q**)

Yield 67.5%. Yellow solid; m.p. 230-233 ^{o}C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 5.04 (s, 2H), 5.05 (s, 2H), 6.33 (d, 1H, J = 9.6 Hz), 7.06-7.09 (m, 2H), 7.11-7.16 (m, 2H), 7.21(d, 1H, J = 8.0 Hz), 7.26 (d, 1H, J = 8.8 Hz), 7.39 (d, 1H, J = 8.8 Hz), 7.46 (dd, 1H, J = 2.4, 8.0 Hz), 7.69-7.71 (m, 2H), 8.01 (d, 1H, J = 9.6 Hz), 10.23 (s, 1H).

5.1.3.18.(*Z*)-N'-(1-Benzyl-5-bromo-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chromen-7-yl)oxy)acetohydrazide (**5r**)

Yield 72.8%. Yellow solid; m.p. 241-244 ^{o}C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 5.02 (s, 2H), 5.03 (s, 2H), 6.33 (d, 1H, J = 9.6 Hz), 7.02 (d, 1H, J = 8.0 Hz), 7.06 (dd, 1H, J = 2.0, 8.8 Hz), 7.13 (d, 1H, J = 2.0 Hz), 7.27-7.39 (m, 6H), 7.58 (dd, 1H, J = 2.4, 8.0 Hz), 7.69 (d, 1H, J = 8.8 Hz), 8.01 (d, 1H, J = 9.6 Hz), 10.23 (s, 1H).

5.1.3.19.(*Z*)-N'-(5-Bromo-1-(2-chlorobenzyl)-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chromen-7-yl)oxy)acetohydrazide (**5s**)

Yield 82.4%. Brown solid; m.p. 187-189 ^{o}C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 5.05 (s, 2H), 5.06 (s, 2H), 6.32 (d, 1H, J = 9.6 Hz), 6.92 (d, 1H, J = 8.8 Hz), 7.05 (dd, 1H, J = 2.4, 8.8 Hz), 7.12 (d, 1H, J = 2.4 Hz), 7.26-7.35 (m, 4H), 7.52 (d, 1H, J = 8.0 Hz), 7.58 (dd, 1H, J = 2.4, 8.0 Hz), 7.68 (d, 1H, J = 8.8 Hz), 8.01 (d, 1H, J = 9.6 Hz), 10.23 (s, 1H).

5.1.3.20.(*Z*)-N'-(5-Bromo-1-(4-bromobenzyl)-2-oxoindolin-3-ylidene)-2-((2-oxo-2H-chromen-7-yl)oxy)acetohydrazide (**5t**)

Yield 81.2%. Yellow solid; m.p. 238-240 ^{o}C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ: 5.00 (s, 2H), 5.01 (s, 2H), 6.33 (d, 1H, J = 9.6 Hz), 7.01 (d, 1H, J = 8.8 Hz), 7.06 (dd, 1H, J = 2.4, 8.8 Hz), 7.13 (d, 1H, J = 2.4 Hz), 7.34 (d, 2H, J = 8.0 Hz), 7.54 (d, 2H, J = 8.0 Hz), 7.59 (dd, 1H, J = 2.4, 8.8 Hz), 7.69 (d, 1H, J = 8.0 Hz), 7.76-7.81 (m, 1H), 8.01 (d, 1H, J = 9.6 Hz), 10.23 (s, 1H).

5.2. Biological Assay

α-Glucosidase from *Saccharomyces cerevisiae* and *p*-nitrophenyl α-D-glucopyranoside (pNPG) were purchased from Sigma Aldrich Chemical Company. DPPH radical was purchased from Aladdin Industrial Inc., Shanghai, China.

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

The test compounds were dissolved in DMSO to prepare the required distributing concentration. 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:

$$\text{Inhibition (\%)} = (1-\Delta A_{sample}/\Delta A_{control}) * 100\%$$

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 study was performed to investigate the binding mode between the compounds **5e**, **5n**, **5p** and α-glucosidase using Autodock vina 1.1.2 [25]. The 3D structure of **5p** was obtained by ChemBioDraw Ultra 14.0 and ChemBio3D Ultra 14.0 softwares. The AutoDockTools 1.5.6 package [26, 27] 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 pose as judged by the Vina docking score was 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.

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References and Notes

1. Kitabchi A.E., Umpierrez G.E., Miles J.M., Fisher J.N. (2009) Hyperglycemic crises in adult patients with diabetes. *Diabetes Care;***32**: 1335-43.
2. Lopez-Candales A. (2001) Metabolic syndrome X: A comprehensive review of the pathophysiology and recommended therapy. *J Med;***32**: 283-300.
3. Deshpande A.D., Harris-Hayes M., Schootman M. (2008) Epidemiology of diabetes and diabetes-related complications. *Phys Ther;***88**: 1254-64.
4. Martin A.E., Montgomery P.A. (1996) Acarbose: an α-glucosidase inhibitor. *Am J Health Syst Pharm;***53**: 2277-90.
5. Toeller M. (1994) α-Glucosidase inhibitors in diabetes-efficacy in niddm subjects. *Eur J Clin Invest;***24**: 31-5.
6. Hollander P. (1992) Safety profile of acarbose, an α-glucosidase inhibitor. *Drugs;***44 Suppl 3**: 47-53.
7. Belluti F., Fontana G., Dal Bo L., Carenini N., Giommarelli C., Zunino F. (2010) Design, synthesis and anticancer activities of stilbene-coumarin hybrid compounds: identification of novel proapoptotic agents. *Bioorg Med Chem;***18**: 3543-50.
8. Sashidhara K.V., Kumar A., Dodda R.P., Krishna N.N., Agarwal P., Srivastava K., et al. (2012) Coumarin-trioxane hybrids: synthesis and evaluation as a new class of antimalarial scaffolds. *Bioorg Med Chem Lett;***22**: 3926-30.

9. Kontogiorgis C.A., Hadjipavlou-Litina D.J. (2005) Synthesis and antiinflammatory activity of coumarin derivatives. *J Med Chem;***48**: 6400-8.
10. Kostova I., Bhatia S., Grigorov P., Balkansky S., Parmar V.S., Prasad A.K., et al. (2011) Coumarins as antioxidants. *Curr Med Chem;***18**: 3929-51.
11. Cardoso S.H., Barreto M.B., Lourenco M.C.S., Henriques M.d.G.M.d.O., Candea A.L.P., Kaiser C.R., et al. (2011) Antitubercular activity of new coumarins. *Chem Biol Drug Des;***77**: 489-93.
12. Shi Y., Zhou C.-H. (2011) Synthesis and evaluation of a class of new coumarin triazole derivatives as potential antimicrobial agents. *Bioorg Med Chem Lett;***21**: 956-60.
13. Qi L.-W., Liu E.H., Chu C., Peng Y.-B., Cai H.-X., Li P. (2010) Anti-diabetic agents from natural products-an update from 2004 to 2009. *Curr Top Med Chem;***10**: 434-57.
14. Khan K.M., Rahim F., Wadood A., Kosar N., Taha M., Lalani S., et al. (2014) Synthesis and molecular docking studies of potent α-glucosidase inhibitors based on biscoumarin skeleton. *Eur J Med Chem;***81**: 245-52.
15. Raju B.C., Tiwari A.K., Kumar J.A., Ali A.Z., Agawane S.B., Saidachary G., et al. (2010) α-Glucosidase inhibitory antihyperglycemic activity of substituted chromenone derivatives. *Biorg Med Chem;***18**: 358-65.
16. Shen Q., Shao J., Peng Q., Zhang W., Ma L., Chan A.S.C., et al. (2010) Hydroxycoumarin derivatives: novel and potent α-glucosidase inhibitors. *J Med Chem;***53**: 8252-9.
17. Wang S., Yan J., Wang X., Yang Z., Lin F., Zhang T. (2010) Synthesis and evaluation of the α-glucosidase inhibitory activity of 3-[4-(phenylsulfonamido) benzoyl]-2H-1-benzopyran-2-one derivatives. *Eur J Med Chem;***45**: 1250-5.
18. Andreani A., Burnelli S., Granaiola M., Leoni A., Locatelli A., Morigi R., et al. (2010) New isatin derivatives with antioxidant activity. *Eur J Med Chem;***45**: 1374-8.
19. Zhang X.-M., Guo H., Li Z.-S., Song F.-H., Wang W.-M., Dai H.-Q., et al. (2015) Synthesis and evaluation of isatin-beta-thiosemicarbazones as novel agents against antibiotic-resistant Gram-positive bacterial species. *Eur J Med Chem;***101**: 419-30.

20. Nguyen-Hai N., Tran Lan H., Do Thi Mai D., Phan Thi Phuong D., Dao Thi Kim O., Do Q., et al. (2013) Novel isatin-based hydroxamic acids as histone deacetylase inhibitors and antitumor agents. *Eur J Med Chem;***70**: 477-86.
21. Xie C., Tang L.-M., Li F.-N., Guan L.-P., Pan C.-Y., Wang S.-H. (2014) Structure-based design, synthesis, and anticonvulsant activity of isatin-1-N-phenylacetamide derivatives. *Med Chem Res;***23**: 2161-8.
22. Rahim F., Malik F., Ullah H., Wadood A., Khan F., Javid M.T., et al. (2015) Isatin based schiff bases as inhibitors of α-glucosidase: synthesis, characterization, *in vitro* evaluation and molecular docking studies. *Bioorg Chem;***60**: 42-8.
23. Taha M., Ismail N.H., Imran S., Rokei M.Q.B., Saad S.M., Khan K.M. (2015) Synthesis of new oxadiazole derivatives as α-glucosidase inhibitors. *Bioorg Med Chem;***23**: 4155-62.
24. Barakat A., Islam M.S., Al-Majid A.M., Ghabbour H.A., Fun H.-K., Javed K., et al. (2015) Synthesis, *in vitro* biological activities and in silico study of dihydropyrimidines derivatives. *Bioorg Med Chem;***23**: 6740-8.
25. Trott O., Olson A.J. (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem;***31**: 455-61.
26. Sanner M.F. (1999) Python: a programming language for software integration and development. *J Mol Graph Model;***17**: 57-61.
27. Morris G.M., Huey R., Lindstrom W., Sanner M.F., Belew R.K., Goodsell D.S., et al. (2009) AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J Comput Chem;***30**: 2785-91.