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Biscoumarin-1,2,3-triazole hybrids as novel anti-diabetic agents: Design, synthesis, *in vitro* α-glucosidase inhibition, kinetic, and docking studies

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ABSTRACT

A novel series of biscoumarin-1,2,3-triazole hybrids **6a-n** was prepared and evaluated for α-glucosidase inhibitory potential. All fourteen derivatives exhibited excellent α-glucosidase inhibitory activity with IC_{50} values ranging between 13.0 ± 1.5 and 75.5 ± 7.0 μM when compared with the acarbose as standard inhibitor (IC_{50} = 750.0 ± 12.0 μM). Among the synthesized compounds, compounds **6c** (IC_{50} = 13.0 ± 1.5 μM) and **6g** (IC_{50} = 16.4 ± 1.7 μM) exhibited the highest inhibitory activity against α-glucosidase and were non-cytotoxic towards normal fibroblast cells. Kinetic study revealed that compound **6c** inhibits the α-glucosidase in a competitive mode. Furthermore, molecular docking investigation was performed to find interaction modes of the biscoumarin-1,2,3-triazole derivatives.

1. Introduction

α-Glucosidase is a digestive enzyme in the intestine which catalyzes the breakage of the carbohydrates into glucose and leading to postprandial hyperglycemia [1]. Given that the patients with type 2 diabetes have an abnormal postprandial increase in blood glucose level, inhibition of α-glucosidase can be a useful way to suppress postprandial hyperglycemia [2]. Several α-glucosidase inhibitors such as acarbose, voglibose, and miglitol are available in the market for treatment of type 2 diabetes. The synthesis of these drugs is tedious due to the presence of sugar moieties in their structures [3]. In addition, their usage has been associated with serious gastrointestinal side effects such as flatulence and diarrhea [4]. Thus, the development of potent α-glucosidase inhibitors with small structures and low side effects has attracted great attention in recent years [5–10].

Coumarin is oxygen containing bicyclic heterocycle that abundantly applied in the design of new potent therapeutic hybrid molecules [11]. Numerous hybrid structures of coumarin derivatives and various potentially bioactive pharmacophores with inhibitory activities against cholinesterase, monoamine oxidase, aldose reductase, alkaline phosphatase, urease, carbonic anhydrase, histone deacetylase, lipoxygenase, topoisomerase, tyrosinase, and cyclooxygenase and α-glucosidase have been introduced [12]. One of the coumarin derivatives which can be used as a pharmacophoric unit in the design of new α-glucosidase inhibitors is biscoumarin since simple derivatives of this structure exhibited strong inhibitory activity against α-glucosidase (Fig. 1, Compounds **A** and **B**) [13,14]. For example, biscoumarin-thiourea hybrids **C** exhibited excellent α-glucosidase inhibitory activity [15].

Another one of interest building blocks in the design bioactive hybrid structures is 1,2,3-triazole ring [16]. Hybrid molecules containing 1,2,3-traizole possess a wide variety of pharmacological properties including anti-bacterial, anticancer, anti-Alzheimer, anti-diabetics activities [17–20]. In this regards, our research team, using the 1,2,3-triazole ring and effective pharmacophores such as quinazolinone (Fig. 1, **D**)

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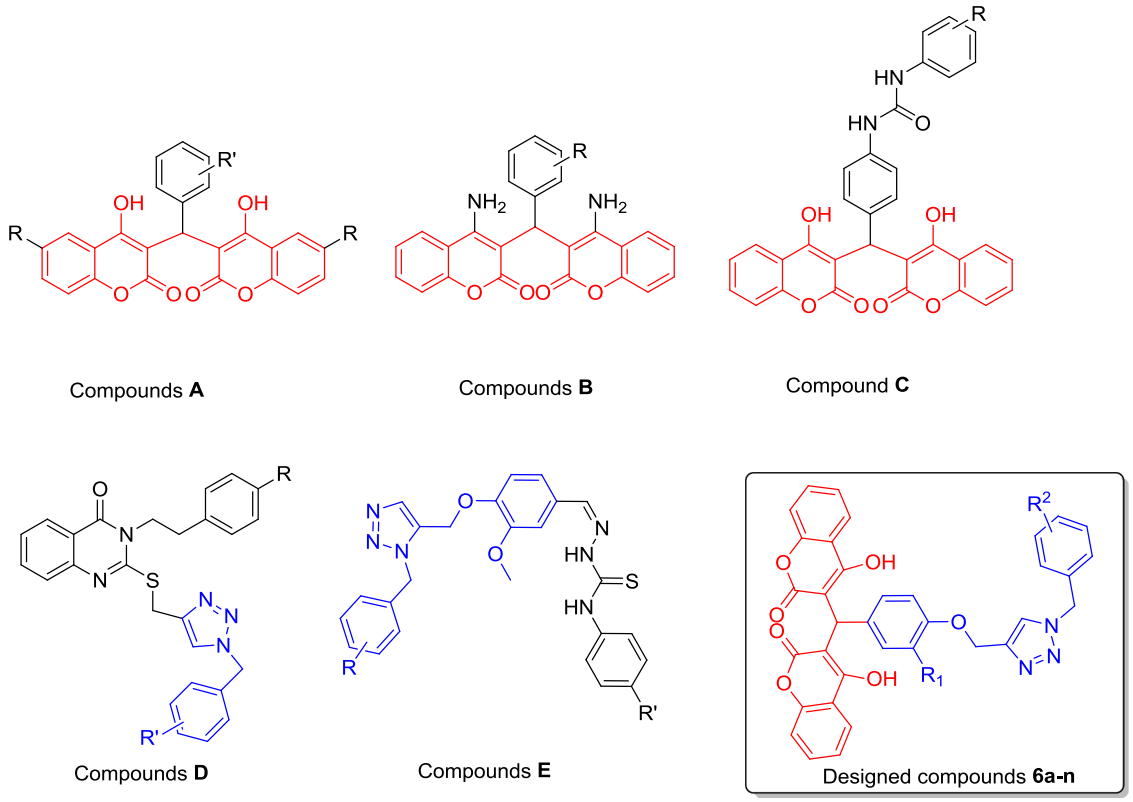


Fig. 1. Rational design of novel α-glucosidase inhibitors **6**; biscoumarin derivatives **A** and **B**, biscoumarin-thiourea hybrids **C**, biscoumarin-quinazolinone hybrids **D**, biscoumarin-thiosemicarbazide hybrids **E**, and new biscoumarin-1,2,3-triazole hybrids **6**.

and thiosemicarbazide (Fig. 1, **E**) introduced hybrid agents with high α-glucosidase inhibitory activity [21,22].

Hence, prompted by above observations and in continuation to our attempt in development of α-glucosidase inhibitors, herein we designed and synthesized for the first time a series of biscoumarin-1,2,3-triazole hybrids **6** [23–25]. These compounds were tested for their *in vitro* inhibitory activity against yeast α-glucosidase. Furthermore, kinetic and *in silico* studies were performed to gain an insight toward the interactions of title compounds with α-glucosidase.

2. Results and discussion

2.1. Chemistry

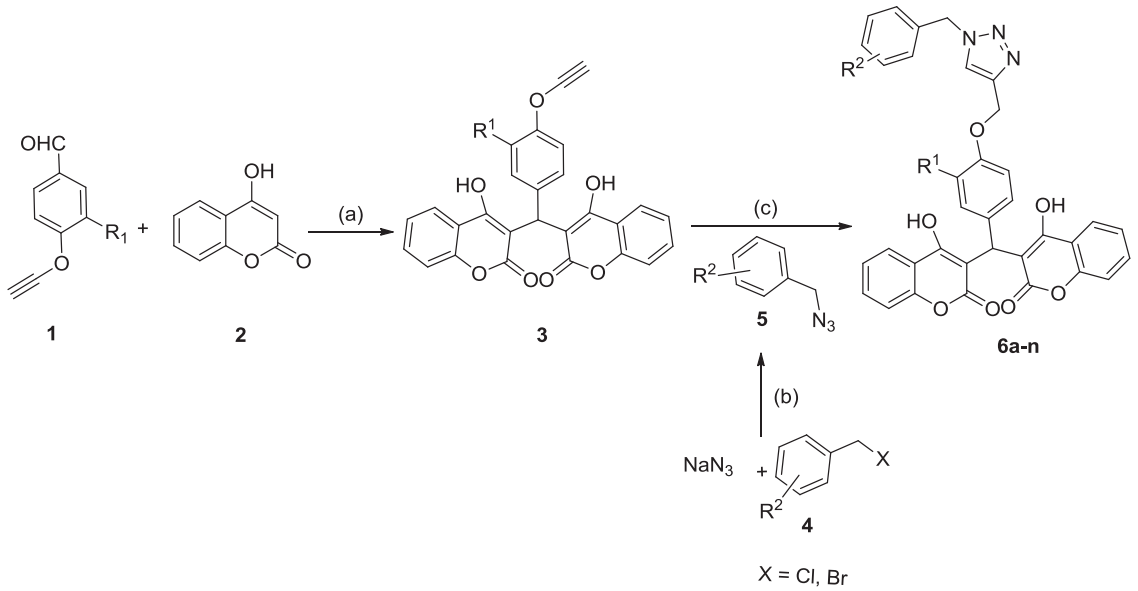
The synthetic route for the synthesis of biscoumarin-1,2,3-triazole hybrids **6a-n** has been depicted in Scheme 1. Firstly, a mixture of 4-(ethynyloxy)benzaldehyde derivatives **1** and 4-hydroxy-2H-chromen-2-one **2** in acetic acid was stirred at room temperature for 5 h, to afford biscoumarins **3**. On the other hand, various benzyl halide derivatives **4** and NaN_3 reacted in the mixture of H_2O/*t*-BuOH (1:1) in the presence of NEt_3 at room temperature for produce to benzyl azide derivatives **5**. Finally, click reaction between the latter compounds and biscoumarins **3** in presence of sodium ascorbate/$CuSO_4{\cdot}5H_2O$ at room temperature led to preparation of the biscoumarin-1,2,3-triazole hybrids **6a-n.**

2.2. In vitro α-glucosidase inhibitory activity

The biscoumarin-1,2,3-triazole hybrids **6a-n** were evaluated for their inhibitory activities against α-glucosidase in comparison with marketed α-glucosidase inhibitor acarbose. The *in vitro* α-glucosidase inhibition of two series of biscoumarin-1,2,3-triazole hybrids **6a-f** with phenoxy linker and **6g-n** with 4-methoxyphenoxy linker is shown in Table 1. The obtained IC_{50} values demonstrated that all the target compounds showed excellent inhibition against α-glucosidase at concentrations less than 75.5 ± 7.0 μM while acarbose exhibited IC_{50} value = 750.0 ± 12.0 μM. The most active compounds were 2-chlorophenyl derivative of phenoxy series and 2-methylphenyl derivative of 4-methoxyphenoxy series with IC_{50} values 13.0 ± 1.5 and 16.4 ± 1.7, respectively.

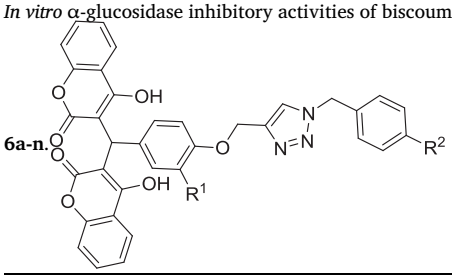
In the phenoxy series, compound **6c**, bearing 2-chloro phenyl moiety, was the most active against α-glucosidase. Replacement of 2-chloro substituent with 2-methyl group, as in compound **6a**, led to a decrease in activity. Moreover, changing the position of the chlorine atom in the pendant phenyl ring from C-2 to C-4, producing **6d** (49.0 ± 5.8 μM), led to a further reduction in inhibitory activity. As can be seen in the Table 1, inhibitory activities of the synthesized compounds with a substituent on 4-posotion of pendant phenyl ring is in order of Br > Cl > F > nitro (compounds **6e**, **6d**, **6b**, and **6f**, respectively). It seems that in the mentioned derivatives, inhibitory activity is dramatically dependent on the electron properties of the substitutions.

The observed IC_{50} values of 4-methoxyphenoxy derivatives **6g-n** against α-glucosidase revealed that the best result was obtained with 2-methyl substituent on the pendant phenyl ring (compound **6g**). Replacement of 2-methyl substituent with 2-fluoro substituent on the pendant phenyl ring led to a dramatically decrease in the inhibitory activity (compound **6g** vs. compound **6h**). As can be seen in Table 1, 2-fluorophenyl derivative **6h** showed inhibitory activity less than it 4-fluoro regioisomer. In contrast, 2-chlorophenyl derivative **6j** was slightly more active than 4-chlorophenyl analog **6k** and 2-nitrophenyl derivative **6m** was significantly more potent than 4-nitrophenyl analog **6n**. In this series, the compounds **6i**, **6k** and **6l** respectively with 4-fluoro, 4-chloro, and 4-bromo substituents on the pendant phenyl ring showed approximately same inhibitory activity against α-glucosidase (IC_{50} values in range of 29.3 ± 2.7 to 33.2 ± 4.1 μM). The 4-nitro derivative **6n** has the lowest inhibitory activity among all the



Scheme 1. Reagents and conditions for the synthesis of compounds **6a-n**: (a) Acetic acid, room temperature, 5 h; (b) NEt_3, H_2O/*t*-BuOH, 1 h (c) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, room temperature, 24–48 h.

Table 1
In vitro α-glucosidase inhibitory activities of biscoumarin-1,2,3-triazole hybrids



Compound	R^1	R^2	IC_{50} (μM)[a]
6a	H	2-CH_3	26.1 ± 2.4
6b	H	4-F	54.7 ± 6.1
6c	H	2-Cl	13.0 ± 1.5
6d	H	4-Cl	49.0 ± 5.8
6e	H	4-Br	27.0 ± 2.5
6f	H	4-Nitro	61.8 ± 6.4
6g	OCH_3	2-CH_3	16.4 ± 1.7
6h	OCH_3	2-F	42.3 ± 5.2
6i	OCH_3	4-F	30.6 ± 4.0
6j	OCH_3	2-Cl	39.2 ± 4.9
6k	OCH_3	4-Cl	33.2 ± 4.1
6l	OCH_3	4-Br	29.3 ± 2.7
6m	OCH_3	2-Nitro	35.4 ± 4.5
6n	OCH_3	4-Nitro	75.5 ± 7.0
Acarbose	–	–	750.0 ± 12.0

[a] Values are the mean ± SD. All experiments were performed at least three times.

synthesized compounds (IC_{50} = 75.5 ± 7.0 μM).

The comparison of IC_{50} values of phenoxy derivatives with their corresponding 4-methoxyphenoxy analogs against α-glucosidase revealed that phenoxy analogs **6c** and **6n** (with 2-chloro and 4-nitro substituents, respectively) were more active than their analogs **6j** and **6f**. In contrast, the anti-α-glucosidase activitiy of **6a**, **6b**, and **6d** (with 2-methy, 4-fluoro, and 4-chloro substituents, respectively) was less than their 4-methoxyphenoxy analogs **6g**, **6i**, and **6k**. Moreover, the 4-bromo derivatives **6e** of phenoxy series and **6i** of 4-methoxyphenoxy series exhibited approximately same inhibitory activity against α-glucosidase.

2.3. Kinetic study

To study the inhibition mode of the synthesized compounds against α-glucosidase, the enzyme kinetic study of the most potent compound **6c** was performed. As can be seen in Fig. 2a, with increase of concentration of compound **6c**, V_m value remained unchanged while K_m value increased. Therefore, this finding revealed that the compound **6c** is a competitive inhibitor for α-glucosidase. The value of K_i was 11 μM that calculated directly by the secondary re-plot of the Lineweaver–Burk plots against the different concentrations of compound **6c**.

2.4. Docking study

Auto Dock Tools (version 1.5.6) was used to evaluate the binding modes of the synthesized compounds in the active site of modeled α-glucosidase exactly based on our previous report [25]. In this regard, acarbose as standard drug and most potent compounds **6c** and **6g** were docked in the active site of the enzyme. The superposed structure of acarbose and the most potent compound **6c** in the active site of α-glucosidase is shown in Fig. 3a. The calculated binding mode of acarbose revealed that this drug interacted with active site residues Gln322, Arg312, Pro309, Ser308, Thr307, Glu304, Thr301, His279, and Asn241 (Fig. 3b). The value of the binding energy of acarbose is −4.04 kcal/mol.

3D structures and the detailed binding modes of the compounds **6c** and **6g** are shown in Figs. 4 and 5. The most potent compound **6c** formed a hydrogen bond with residue Thr307 by one of its hydroxyl groups (Fig. 4b). One of the coumarin rings of this compound formed a π-π interaction with His279 and the other coumarin ring interacted with Val305 *via* hydrophobic interaction. The phenoxy linker established a π-anion interaction with Glu304. Furthermore, the 2-chlorophenyl group of the compound **6c** established a π-anion interaction with Asp408 through phenyl ring and two hydrophobic interactions with Tyr313 and Phe311 through 2-chloro substituent. The value of the binding energy of this compound is −9.9 kcal/mol.

One of the coumarin moieties of the compound **6g** by hydroxyl group formed two hydrogen bonds with Thr307 and Glu304 (Fig. 5b). The other coumarin ring established a hydrogen bond with His279 and a π-cation interaction with His239. The phenoxy of this compound, like to the compound **6c**, interacted with Glu304 *via* a π-anion interaction.

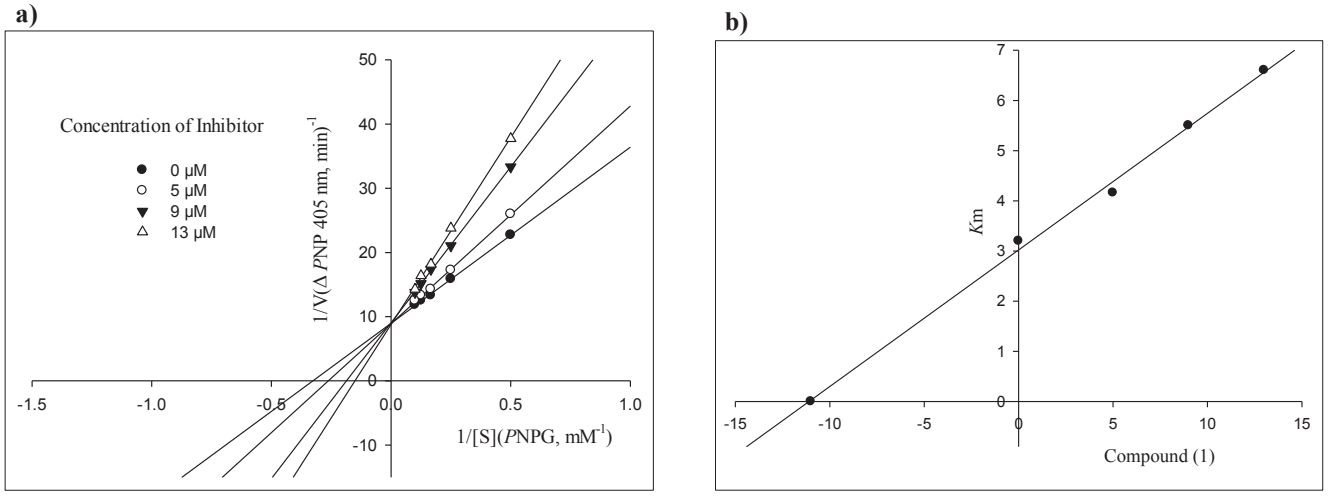


Fig. 2. Kinetic study of α-glucosidase inhibition by compound **6c**. (A) The Lineweaver–Burk plot in the absence and presence of different concentrations of compound **6c** (μM); (B) the secondary plot between 1/Vmax and various concentrations of compound **6c**.

1,2,3-triazole ring of compound **6g** formed a π-π interaction with His239 and a hydrogen bond with Arg312 while this ring in the compound **6c** does not interact with the active site. Furthermore, 2-methylphenyl ring of compound **6g** *via* methyl group and phenyl ring established a hydrophobic interaction with Tyr313 and a π-π interaction with Phe158, respectively. The value of the binding energy of the compound **6g** is −9.49 kcal/mol.

The comparison of binding energy values of the compounds **6c** and **6g** with acarbose revealed that the synthesized biscoumarin-1,2,3-triazole hybrids **6c** and **6g** can be bind to the active site easier than the standard drug. This observation confirmed by the obtained results of *in vitro* screen (Table 1).

2.5. α-Amylase assay

The most active α-glucosidase inhibitors **6c**, **6g**, and **6a** were evaluated for their enzyme inhibition potential against α-amylase as another important carbohydrate hydrolyzing enzyme [26]. These compounds showed no inhibitory activity against α-amylase at 300 μM, and thus considered as inactive compounds when compared with standard drug acarbose (IC_{50} = 108 ± 0.71 μM).

2.6. Cytotoxicity studies

Cytotoxicity of the most potent compounds **6c**, **6g**, and **6a** was evaluated by using the human dermal fibroblasts [27]. Results revealed that at 60 μM concentration, all the selected compounds were non-cytotoxic against studied normal cell line.

3. Conclusion

In conclusion, we designed and synthesized a novel series of biscoumarin-1,2,3-triazole hybrids **6a-n** *via* click reaction. These compounds screened for *in vitro* α-glucosidase inhibition and demonstrated excellent α-glucosidase inhibitory activity when compared with the standard drug acarbose. Kinetic analysis revealed that the most potent compound **6c** inhibited α-glucosidase in a competitive inhibition manner. Consequently, docking simulation was performed to recognize the interaction model of the most potent analogs in the active site of α-glucosidase.

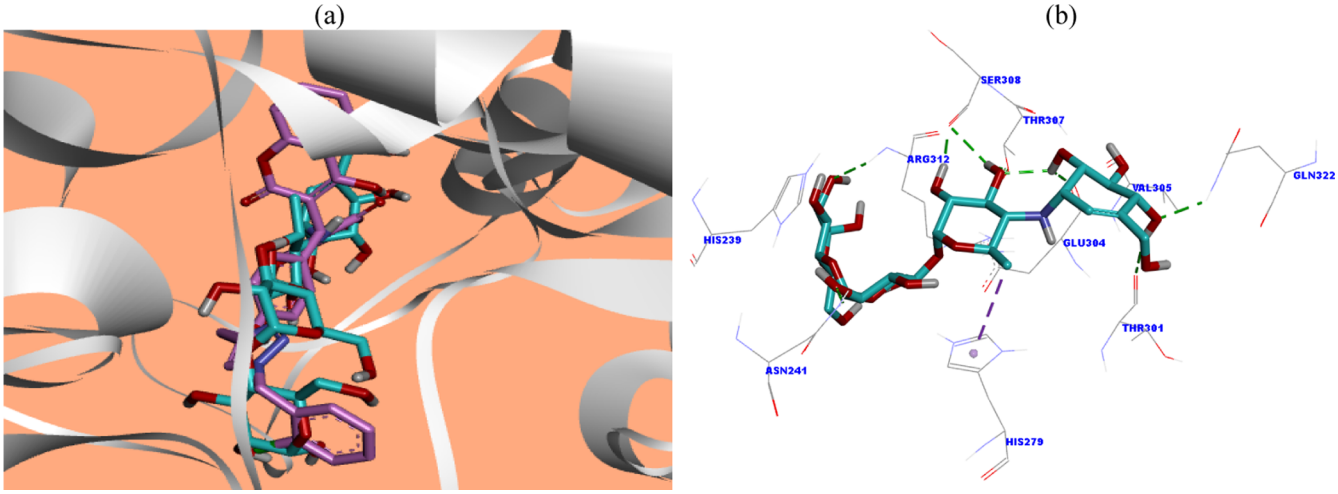


Fig. 3. (a) Acarbose (cyan) and most potent compound **6c** (pink) superimposed in the active site of modeled α-glucosidase and (b) the predicted binding mode of acarbose in the active site pocket.

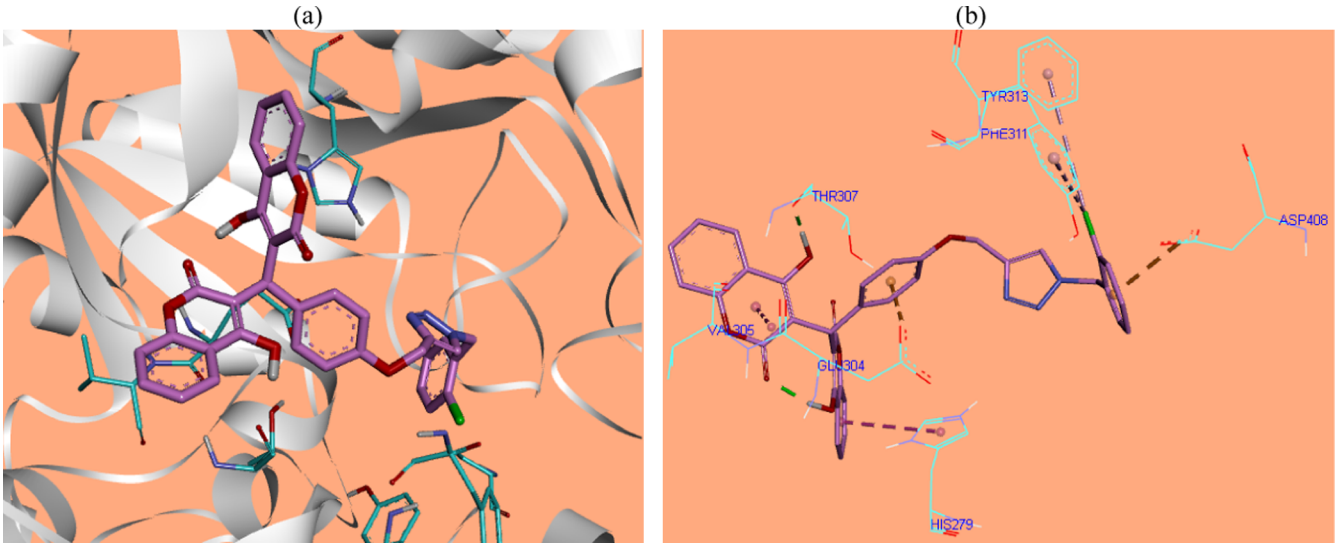


Fig. 4. (a) Compound **6c** in the active site of α-glucosidase and (b) binding mode of compound **6c** in the active site pocket.

4. Experimental

Melting points of biscoumarin-1,2,3-triazole hybrids **6a-n** were determined with a Kofler hot stage apparatus. ^{1}H and ^{13}C NMR spectra of these compounds were obtained with a Bruker FT-500, using TMS as an internal standard. IR spectra recoded with a Nicolet Magna FTIR 550 spectrophotometer (KBr disks). Elemental analysis was performed by an Elementar Analysen system GmbH VarioEL CHNS mode. Compounds **1** were obtained according to described pathway in the our newly published article [22].

4.1. General procedure for the synthesis of biscoumarin derivatives *3*

A solution of 4-(ethynyloxy)benzaldehyde derivatives **1** (1 mmol) and 4-hydroxy-2H-chromen-2-one **2** (1 mmol) in acetic acid (15 mL) was stirred at room temperature for 5 h. Then, the reaction mixture was filtered off and obtained participates were washed with acetic acid to obtain pure biscoumarin derivatives **3**.

4.2. General procedure for the synthesis of biscoumarin-1,2,3-triazole hybrids *6a-n*

In order to do a click reaction, azide derivatives **5** were prepared in situ of reaction between benzyl halides **4** (1.1 mmol), sodium azide (0.9 mmol), and NEt_3 (1.3 mmol) in the mixture of H_2O plus *t*-BuOH (8 mL, 1:1) at room temperature for 1 h. Then, a mixture of biscoumarin derivatives **3** (1 mmol), sodium ascorbate, and $CuSO4{\cdot}5H_2O$ (7 mol %) was added to the benzyl azide derivatives **5**, and obtained mixture was stirred at room temperature for 24 h. Then, reaction mixture was poured into crushed ice and precipitated products **6a-n** were filtered off, washed with water, and purified by recrystallization in ethyl acetate.

4.2.1. 3,3′-((4-((1-(2-methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy) phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) *6a*

Yield: 73%; mp = 188–190 °C; IR (KBr): 3481, 3177, 1652, 1605, 1089, 766 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.15 (s, 1H), 7.81 (d,

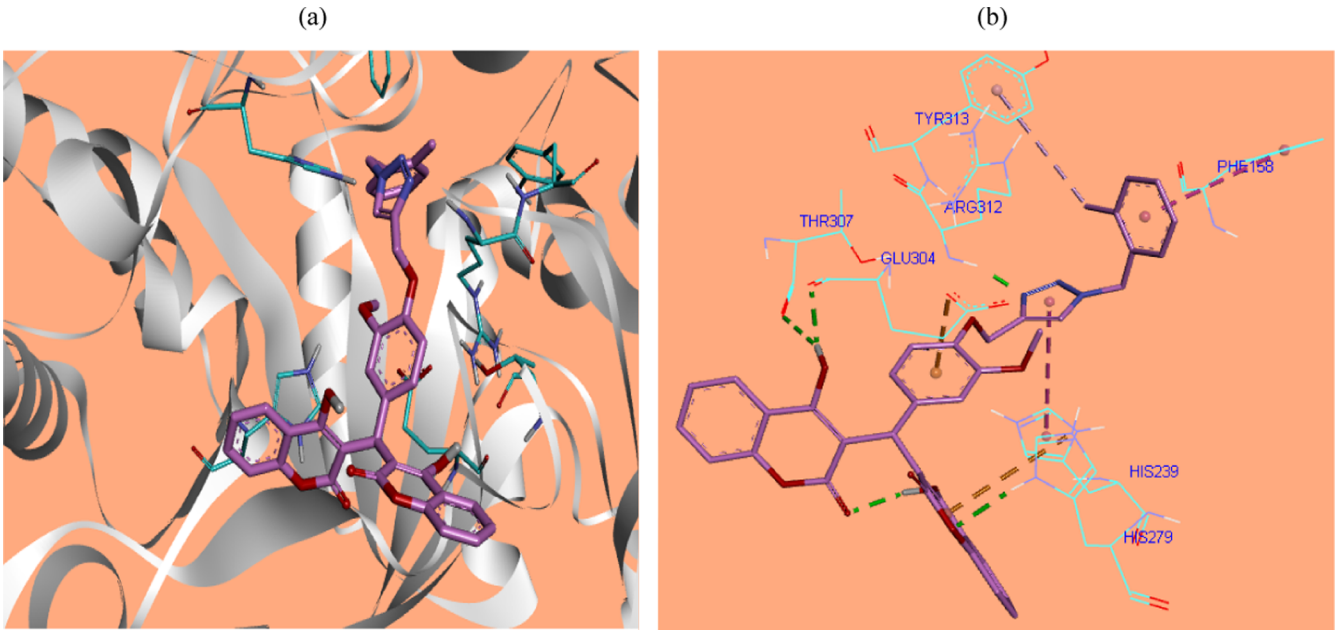


Fig. 5. (a) Compound **6g** in the active site of α-glucosidase and (b) binding mode of compound **6g** in the active site pocket.

J = 7.1 Hz, 2H), 7.50 (t, J = 8.2 Hz, 2H), 7.27–7.17 (m, 7H), 7.06 (d, J = 7.4 Hz, 1H), 6.99 (d, J = 8.6 Hz, 2H), 6.82 (d, J = 8.7 Hz, 2H), 6.20 (s, 1H), 5.60 (s, 2H), 5.05 (s, 2H), 2.29 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 168.1, 165.0, 156.0, 153.0, 136.7, 135.0, 134.6, 131.3, 130.8, 129.1, 128.7, 128.1, 126.7, 125.0, 124.5, 123.3, 120.4, 115.9, 114.4, 104.1, 61.5, 51.4, 35.8, 19.1. Anal. Calcd for $C_{36}H_{27}N_3O_7$: C, 70.47; H, 4.44; N, 6.85. Found: C, 70.59; H, 4.35; N, 6.71.

4.2.2. 3,3′-((4-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy) phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) ***6b***

Yield: 69%; mp = 207–209 °C; IR (KBr): 3478, 3174, 1654, 1605, 1089, 761 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.27 (s, 1H), 7.83 (d, J = 7.8 Hz, 2H), 7.55–7.47 (m, 2H), 7.39 (dd, J = 8.4, 5.6 Hz, 2H), 7.28–7.18 (m, 6H), 7.02 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 8.7 Hz, 2H), 6.24 (s, 1H), 5.59 (s, 2H), 5.06 (s, 2H).^{13}C NMR (126 MHz, DMSO) δ 167.9, 165.1, 156.1, 152.9, 143.7, 134.7, 131.4, 130.8, 130.7, 128.1, 124.9, 124.5, 123.4, 120.2, 116.1, 115.9, 114.4, 104.1, 61.5, 52.5, 35.8. Anal. Calcd for $C_{35}H_{24}FN_3O_7$: C, 68.07; H, 3.92; N, 6.80. Found: C, 68.17; H, 3.78; N, 6.78.

4.2.3. 3,3′-((4-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy) phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) ***6c***

Yield: 72%; mp = 196–198 °C; IR (KBr): 3482, 3175, 1654, 1606, 1088, 760 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.23 (s, 1H), 7.82 (dd, J = 7.8, 1.4 Hz, 2H), 7.54–7.47 (m, 3H), 7.41–7.31 (m, 2H), 7.29–7.21 (m, 4H), 7.18 (dd, J = 7.3, 1.8 Hz, 1H), 7.00 (d, J = 8.7 Hz, 2H), 6.83 (d, J = 8.8 Hz, 2H), 6.22 (s, 1H), 5.70 (s, 2H), 5.06 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 167.6, 165.0, 156.1, 152.9, 133.7, 131.5, 130.8, 130.6, 130.0, 128.2, 128.1, 125.4, 124.5, 123.4, 120.1, 116.0, 114.5, 104.2, 61.4, 51.0, 35.8. Anal. Calcd for $C_{35}H_{24}ClN_3O_7$: C, 66.30; H, 3.82; N, 6.63. Found: C, 66.47; H, 3.75; N, 6.74.

4.2.4. 3,3′-((4-((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy) phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) ***6d***

Yield: 71%; mp = 213–215 °C; IR (KBr): 3486, 3172, 1657, 1604, 1086, 763 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.27 (s, 1H), 7.85 (dd, J = 7.9, 1.6 Hz, 2H), 7.52 (ddd, J = 8.5, 7.2, 1.7 Hz, 2H), 7.44 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 8.5 Hz, 2H), 7.29–7.23 (m, 4H), 7.03 (d, J = 7.1 Hz, 2H), 6.84 (d, J = 8.2 Hz, 2H), 6.25 (s, 1H), 5.61 (s, 2H), 5.07 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 167.7, 165.1, 156.1, 152.9, 143.8, 135.4, 134.5, 133.3, 131.5, 130.3, 129.2, 128.1, 125.0, 124.5, 123.5, 120.1, 116.0, 114.4, 104.2, 61.5, 52.5, 35.8. Anal. Calcd for $C_{35}H_{24}ClN_3O_7$: C, 66.30; H, 3.82; N, 6.63. Found: C, 66.19; H, 3.65; N, 6.82.

4.2.5. 3,3′-((4-((1-(4-bromobenzyl)-1H-1,2,3-triazol-4-yl)methoxy) phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) ***6e***

Yield: 68%; mp = 215–217 °C; IR (KBr): 3482, 3176, 1653, 1601, 1085, 769 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.26 (s, 1H), 7.82 (d, J = 7.9 Hz, 2H), 7.58 (d, J = 8.3 Hz, 2H), 7.51 (t, J = 8.4 Hz, 2H), 7.29–7.22 (m, 6H), 7.00 (d, J = 8.6 Hz, 2H), 6.83 (d, J = 8.7 Hz, 2H), 6.21 (s, 1H), 5.59 (s, 2H), 5.05 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 167.9, 165.0, 156.1, 152.9, 135.7, 134.8, 132.1, 131.4, 130.7, 128.1, 125.0, 124.5, 123.3, 121.8, 120.3, 115.9, 114.4, 104.1, 61.5, 52.5, 35.8. Anal. Calcd for $C_{35}H_{24}BrN_3O_7$: C, 61.96; H, 3.57; N, 6.19. Found: C, 62.09; H, 3.64; N, 6.31.

4.2.6. 3,3′-((4-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl) methylene)bis(4-hydroxy-2H-chromen-2-one) ***6f***

Yield: 65%; mp = 221–223 °C; IR (KBr): 3489, 3172, 1653, 1601, 1090, 765 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.36 (s, 1H), 7.91–7.84 (m, 3H), 7.58–7.51 (m, 5H), 7.32–7.22 (m, 4H), 7.09 (d, J = 8.5 Hz, 2H), 6.91 (d, J = 8.7 Hz, 2H), 6.34 (s, 1H), 5.59 (s, 2H), 5.12 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 166.9, 165.3, 156.3, 152.8, 132.2, 131.8, 130.3, 129.5, 129.5, 128.2, 125.83, 125.4, 124.3, 123.8, 119.3, 116.1, 115.6, 114.6, 104.5, 61.5, 52.4, 35.8. Anal. Calcd for $C_{35}H_{24}N_4O_9$: C, 65.22; H, 3.75; N, 8.69. Found: C, 65.31; H, 3.84; N, 8.56.

4.2.7. 3,3′-((3-methoxy-4-((1-(2-methylbenzyl)-1H-1,2,3-triazol-4 yl) methoxy)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) ***6g***

Yield: 69%; mp = 178–180 °C; IR (KBr): 3485, 3175, 1659, 1606, 1088, 760 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.13 (s, 1H), 7.84 (d, J = 9.3 Hz, 2H), 7.52 (t, J = 8.5 Hz, 2H), 7.29–7.16 (m, 7H), 7.06 (d, J = 7.6 Hz, 1H), 6.91 (d, J = 8.5 Hz, 1H), 6.69 (s, 1H), 6.63 (d, J = 8.5 Hz, 1H), 6.23 (s, 1H), 5.61 (s, 2H), 5.04 (s, 2H), 3.50 (s, 3H), 2.29 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 167.8, 165.0, 152.9, 149.1, 145.6, 143.6, 136.7, 135.8, 134.6, 131.4, 130.8, 129.1, 128.7, 126.7, 125.2, 124.5, 123.4, 120.1, 119.3, 116.0, 114.2, 112.0, 104.2, 62.4, 55.9, 51.4, 36.2, 19.1. Anal. Calcd for $C_{37}H_{29}N_3O_8$: C, 69.04; H, 4.54; N, 6.53. Found: C, 68.92; H, 4.68; N, 6.39.

4.2.8. 3,3′-((4-((1-(2-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) ***6h***

Yield: 70%; mp = 162–164 °C; IR (KBr): 3482, 3179, 1654, 1606, 1078, 763 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.22 (s, 1H), 7.84 (d, J = 9.3 Hz, 2H), 7.54–7.50 (m, 2H), 7.42–7.37 (m, 3H), 7.32 (t, J = 6.9 Hz, 1H), 7.28–7.23 (m, 4H), 6.92 (d, J = 8.5 Hz, 1H), 6.69 (s, 1H), 6.63 (d, J = 8.4 Hz, 1H), 6.23 (s, 1H), 5.67 (s, 2H), 5.04 (s, 2H), 3.80 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 167. 8, 165.0, 152.9, 145.7, 143.7, 142.6, 135.8, 131.4, 131.3, 131.1, 126.2, 125.7, 125.3, 124.5, 123.4, 120.1, 119.3, 116.0, 114.0, 113.1, 112.0, 110.1, 104.1, 62.1, 55.9, 47.3, 36.2. Anal. Calcd for $C_{36}H_{26}FN_3O_8$: C, 66.77; H, 4.05; N, 6.49. Found: C, 66.84; H, 3.96; N, 6.57.

4.2.9. 3,3′-((4-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) ***6i***

Yield: 75%; mp = 169–171 °C; IR (KBr): 3477, 3174, 1653, 1606, 1089, 761 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.24 (s, 1H), 7.83 (dd, J = 7.8, 1.4 Hz, 2H), 7.54–7.47 (m, 2H), 7.41 (q, J = 7.5, 6.7 Hz, 1H), 7.34–7.30 (m, 1H), 7.28–7.21 (m, 5H), 7.01 (d, J = 8.2 Hz, 2H), 6.83 (d, J = 8.8 Hz, 2H), 6.22 (s, 1H), 5.66 (s, 2H), 5.06 (s, 2H), 3.44 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 167.8, 165.0, 156.1, 152.9, 143.7, 134.7, 131.4, 131.2, 131.1, 128.1, 125.3, 125.1, 124.5, 123.4, 120.2, 116.1, 116.0, 114.4, 104.1, 61.4, 47.3, 47.3, 35.8. Anal. Calcd for $C_{36}H_{26}FN_3O_8$: C, 66.77; H, 4.05; N, 6.49. Found: C, 66.65; H, 3.91; N, 6.57.

4.2.10. 3,3′-((4-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) ***6j***

Yield: 73%; mp = 172–174 °C; IR (KBr): 3481, 3175, 1656, 1601, 1093, 765 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.22 (s, 1H), 7.83 (dd, J = 7.8, 1.4 Hz, 2H), 7.52 (d, J = 6.5 Hz, 2H), 7.40–7.34 (m, 2H), 7.30–7.22 (m, 5H), 7.18 (dd, J = 7.4, 1.8 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.69 (s, 1H), 6.63 (d, J = 8.2 Hz, 1H), 6.22 (s, 1H), 5.71 (s, 2H), 5.05 (s, 2H), 3.51 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 167.8, 165.0, 152.9, 149.1, 145.7, 143.62, 143.6, 135.8, 133.7, 133.0, 131.4, 130.8, 130.6, 130.0, 128.2, 125.5, 124.5, 123.4, 120.1, 119.3, 116.0, 114.2, 112.1, 104.16, 62.4, 55.9, 51.0, 36.2. Anal. Calcd for $C_{36}H_{26}ClN_3O_8$: C, 65.11; H, 3.95; N, 6.33. Found: C, 65.21; H, 4.16; N, 6.27.

4.2.11. 3,3′-((4-((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) ***6k***

Yield: 66%; mp = 177–179 °C; IR (KBr): 3481, 3178, 1654, 1606, 1083, 762 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.24 (s, 1H), 7.83 (dd, J = 7.8, 1.3 Hz, 2H), 7.54–7.49 (m, 2H), 7.44 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 7.30–7.22 (m, 4H), 6.91 (d, J = 8.4 Hz, 1H), 6.69 (s, 1H), 6.63 (d, J = 8.4 Hz, 1H), 6.22 (s, 1H), 5.61 (s, 2H), 5.04 (s, 2H), 3.51 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 167.9, 165.00, 152.9, 149.1, 145.6, 143.8, 135.9, 135.4, 133.3, 131.4, 130.3, 129.2, 125.1, 124.5, 123.4, 120.2, 119.3, 115.9, 114.1, 112.0, 104.1, 62.3, 55.9, 52.4, 36.2. Anal. Calcd for $C_{36}H_{26}ClN_3O_8$: C, 65.11; H, 3.95; N, 6.33.

Found: C, 65.27; H, 3.79; N, 6.48.

4.2.12. 3,3′-((4-((1-(4-bromobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-3-methoxyphenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) ***6l***

Yield: 67%; mp = 186–188 °C; IR (KBr): 3481, 3176, 1654, 1601, 1088, 765 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ 8.24 (s, 1H), 7.82 (d, J = 9.3 Hz, 2H), 7.57 (d, J = 8.3 Hz, 2H), 7.50 (t, J = 7.7 Hz, 2H), 7.28–7.20 (m, 6H), 6.90 (d, J = 8.4 Hz, 1H), 6.68 (s, 1H), 6.63 (d, J = 8.5 Hz, 1H), 6.21 (s, 1H), 5.59 (s, 2H), 5.03 (s, 2H), 3.50 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 168.1, 165.0, 152.9, 149.1, 145.6, 143.8, 136.2, 135.9, 132.1, 131.3, 130.6, 125.1, 124.5, 123.3, 121.84, 120.4, 119.3, 115.9, 114.1, 112.0, 104.0, 62.4, 55.9, 52.5, 36.2. Anal. Calcd for $C_{36}H_{26}BrN_3O_8$: C, 61.03; H, 3.70; N, 5.93. Found: C, 59.93; H, 3.84; N, 6.07.

4.2.13. 3,3′-((3-methoxy-4-((1-(2-nitrobenzyl)-1H-1,2,3-triazol-4-yl) methoxy)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) ***6m***

Yield: 65%; mp = 192–194 °C; IR (KBr): 3483, 3179, 1654, 1605, 1088, 761 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ 8.27 (s, 1H), 8.14 (d, J = 8.1 Hz, 1H), 7.85 (d, J = 7.8 Hz, 2H), 7.71 (t, J = 6.4 Hz, 1H), 7.62 (t, J = 7.8 Hz, 1H), 7.54 (d, J = 8.2 Hz, 2H), 7.30–7.25 (m, 3H), 7.03 (d, J = 8.4 Hz, 2H), 6.99 (d, J = 7.7 Hz, 1H), 6.86 (d, J = 8.8 Hz, 2H), 6.25 (s, 1H), 5.98 (s, 2H), 5.11 (s, 2H); ^{13}C NMR (126 MHz, DMSO) δ 167.4, 165.1, 156.2, 152.8, 147.9, 143.8, 134.8, 134.2, 131.6, 131.3, 130.4, 130.0, 128.1, 125.8, 125.5, 124.5, 123.6, 119.9, 116.0, 114.6, 104.3, 61.5, 50.4, 35.8. Anal. Calcd for $C_{36}H_{26}N_4O_{10}$: C, 64.09; H, 3.88; N, 8.31. Found: C, 64.18; H, 3.69; N, 8.48.

4.2.14. 3,3′-((3-methoxy-4-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl) methoxy)phenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) ***6n***

Yield: 67%; mp = 184–186 °C; IR (KBr): 3481, 3175, 1654, 1605, 1088, 769 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ 8.33 (s, 1H), 8.25–8.22 (m, 2H), 7.89 (d, J = 9.1 Hz, 2H), 7.57–7.53 (m, 4H), 7.35–7.27 (m, 4H), 6.97 (d, J = 8.5 Hz, 1H), 6.74 (s, 1H), 6.68 (d, J = 8.4 Hz, 1H), 6.28 (s, 1H), 5.80 (s, 2H), 5.09 (s, 2H), 3.54 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 166.4, 165.1, 153.2, 149.3, 147.7, 146.0, 143.9, 134.3, 132.0, 129.5, 126.0, 125.5, 124.3, 123.9, 119.3, 119.0, 116.2, 114.1, 113.1, 112.0, 104.6, 55.9, 52.4, 49.1, 36.2. Anal. Calcd for $C_{36}H_{26}N_4O_{10}$: C, 64.09; H, 3.88; N, 8.31. Found: C, 64.17; H, 3.74; N, 8.49.

4.3. In vitro α-glucosidase inhibition assay

The *in vitro* α-glucosidase inhibitory activities of biscoumarin-1,2,3-triazole hybrids **6a-n** were obtained according to the previously reported method [21–25]. For this purpose, a mixture of α-glucosidase solution 20 μL (Saccharomyces cerevisiae), test compounds dissolved in DMSO (10% final concentration) in the various concentrations (20 μL), potassium phosphate buffer (135 μL) was incubated for 10 min at 37 °C. Then, p-nitrophenyl-α-glucopyranoside (substrate, 25 μL, 4 mM) was added to the mixture and the final mixture was incubated at 37 °C for 20 min. Finally, absorbance was measured at 405 nm using a spectrophotometer (Gen5, Power wave xs2, BioTek, USA), and IC_{50} values of the target compounds were calculated using the nonlinear regression curve (logit method).

4.4. Kinetic study of α-glucosidase inhibition

Kinetic study was carried out to determine the mechanism of inhibition of the most potent compound **6c**. The enzyme solution (0.2 U/mL) was incubated with different concentrations (0, 5, 9, and 13 μM) of the compound **6c** for 15 min at 30 °C. The reaction was then initiated by adding substrate in the various concentrations (1–4 mM) and change in absorbance was measured for 20 min at 405 nm on microtitre plate reader (Spectra Max M2, Molecular Devices, CA, USA).

4.5. Docking study

The docking studies of the most potent compounds **6c** and **6g** in the active site of α-glucosidase were carried out by AutoDock Tools, using a previously described method [23].

4.6. In vitro α-amylase inhibition assay

The α-amylase inhibitory activity of the compounds **6c**, **6g**, and **6a** was determined based on the colorimetric method using acarbose as the reference drug [26]. In the first step, 40 μL of test compound (dissolved in DMSO, 10% final concentration), 40 μL of α-amylase (Porcine pancreatic α-amylase) (0.5 mg/ml) that prepared in the sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride, 0.02 M) were added to the proper tubes and incubated at 25 °C for 10 min. In the second step, 40 μL of a 1% starch solution in 0.02 M sodium phosphate buffer was added to each tube at 5 s intervals and the obtained reaction mixtures were incubated at 25 °C for 10 min. In the third step, these reactions were stopped with 100 μL of dinitrosalicylic acid as color reagent. In the fourth step, the test tubes plate was incubated in a boiling water bath for 5 min and cooled to room temperature. Finally, the reaction mixture was diluted after adding 900 μL distilled water and the absorbance was measured at 540 nm. IC_{50} values of the selected inhibitors were calculated from non-linear regression curve using the Logit method.

4.7. In vitro cytotoxicity assay

Dermal fibroblasts were isolated from fresh human foreskins of donors aged from 1 to 1.5 months who received a routine circumcision. Enzymatic method using dispase and collagenase enzymes (Sigma, Germany) was used to isolate fibroblasts [27]. The isolated fibroblasts were incubated in a complete culture medium containing Dulbecco's modified eagle's medium (DMEM, Biowest, France), supplemented with 10% fetal bovine serum (FBS, Sigma, Germany), streptomycin (100 μg/mL) and Penicillin (100 IU/mL).

Dermal fibroblast cells (5 × 104 cells/mL in 96-well culture plates) were incubated for 48 h with different concentrations of the selected compounds **6c**, **6g**, and **6a** dissolved in DMSO (the final volume of DMSO/medium was less than 1% in all experiments). After treatment, the medium was removed and 50 μL of a PBS containing MTT (5 mg/mL, final concentration) was added to all wells. After 4 h of incubation, the culture medium was replaced with 150 μL of DMSO to each well. The absorbance was measured at 573 nm with a multi-well plate reader (Rayto, China). All experiments were performed three times and the IC_{50} values for all compounds were calculated by nonlinear regression analysis.

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