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Synthesis, in vitro evaluation and molecular docking studies of biscoumarin thiourea as a new inhibitor of α-glucosidases

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ABSTRACT

Biscoumarin analogs **1–18** have been synthesized, characterized by EI-MS and ^{1}H NMR and evaluated for α-glucosidase inhibitory potential. All compounds showed variety of α-glucosidase inhibitory potential ranging in between 13.5 ± 0.39 and 104.62 ± 0.3 μM when compared with standard acarbose having IC_{50} value 774.5 ± 1.94 μM. The binding interactions of the most active analogs were confirmed through molecular docking. The compounds showed very good interactions with enzyme. All synthesized compounds **1–18** are new. Our synthesized compounds can further be studied to developed lead compounds.

1. Introduction

Type-2 diabetes is characterized by chronic hyperglycemia and the development of microangiopathic complications such as retinopathy, nephropathy and neuropathy. Aggressive control of blood glucose level is preliminary and effective therapy for diabetic patients and reduces risk of complications [1].

α-Glucosidase (EC. 3.2.1.20) are membrane-bound enzymes located at the epithelium of the small intestine [2], and is the key enzymes of carbohydrate digestion [3]. It specifically hydrolyzed the α-glucopyranoside bond, thereby releasing α-D-glucose from the non-reducing end of the sugar. α-Glucosidase had been found to contribute to the glycosylation of human immunodeficiency virus type I [4], thus inhibitors of α-glucosidase can block the viral infection [5,6]. Clinical trials showed that the α-glucosidase inhibitor improved long-term glycemic control as measured by decreased hemoglobin A1c (HbA1c) in patients with type II diabetes and delay the development of type II diabetes in patients with impaired glucose tolerance [7].

Biscoumarin is a dimeric form of coumarin showed more potent biological activities. Biscoumarins have been reported as anti-urease agents [8]. Biscoumarin–chalcone hybrid molecules also showed anti-inflammatory and antioxidant activities [9]. A new dimeric biscoumarin, daphnoretin in which two coumarin linked by ether has property to inhibit DNA polymerase β-lyase, and protein kinase C activation [10] and also exhibit antifungal activity [11]. They were reported to show *in vivo* antineoplastic activity against the Ehrlich ascites carcinoma in mice and used as inhibitor in Ehrlich ascites cells to inhibit a number of enzymes involved in DNA synthesis [12].

Thiourea is a versatile reagent in synthetic chemistry [13]. Thioureas manifest important multiple biological effects and are the basis for target oriented synthesis. Moreover, ureas and thioureas evaluate their plant growth-regulating activity mainly on the herbicidal, root growth inhibitory and stimulatory and cytokinin-like activities [14]. Thiourea and urea have attracted much attention as drug candidates against a variety of diseases due to their bioactivities and broad spectrum as pesticides and in pharmacological activities [15]. A variety of thiourea derivatives and their metal complexes exhibit analgesic, anti-inflammatory [16], carbonic anhydrase inhibitors [17], β-glucuronidase [18], anti-urease [19] and antimicrobial activities [20,21]. Thiourea derivatives also possess anti-HCV [22], anti-HIV, antituberculosis, and

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antileukemic activity [23]. Fluorinated thioureas constitute a novel class of potent influenza virus neuraminidase inhibitors [24]. We have reported that sulfur and nitrogen containing compounds showed potent α-glucosidase inhibition [25] and we also reported biscoumarin as potent glucosidase inhibitors [26]. On the bases of that we synthesized hybrid molecules of both thiourea and biscoumarin and evaluated for potent α-glucosidase inhibition.

2. Result and discussion

2.1. Chemistry

Synthesis of target compounds was prompted by the reaction of 4-hydroxycoumarin and 4-nitrobenzaldehyde in catalytic amount of piperidine and product was further reduced to 4-amino-benzyli dene-bis-(4-hydroxycoumarin). The synthesis of the thiourea derivatives can be easily performed with good yield by condensation of 4-amino-benzylidene-bis-(4-hydroxycoumarin) with various phenyl isothiocyanate derivatives (Scheme 1).

2.2. Biological activity

In the continuation of our work on enzyme inhibition [27–32] we synthesized biscoumarin analogs **1–18** and evaluated for α-glucosidase inhibitory potential. All of the eighteen **(18)** analogs showed outstanding α-glucosidase inhibitory potential with IC_{50} values, 38.97 ± 0.99, 51.02 ± 1.41, 91.29 ± 0.27, 50.25 ± 1.43, 25.31 ± 0.73, 104.62 ± 0.3, 35.85 ± 0.91, 26.8 ± 0.76, 37.41 ± 0.95, 31.72 ± 0.83, 53.4 ± 1.58, 50.22 ± 0.86, 13.5 ± 0.39, 24.69 ± 0.68, 28.47 ± 0.54, 43.36 ± 0.59, 55.12 ± 1.63, 78.04 ± 0.23, μM respectively so many folds better than standard acarbose 774.5 ± 1.94.

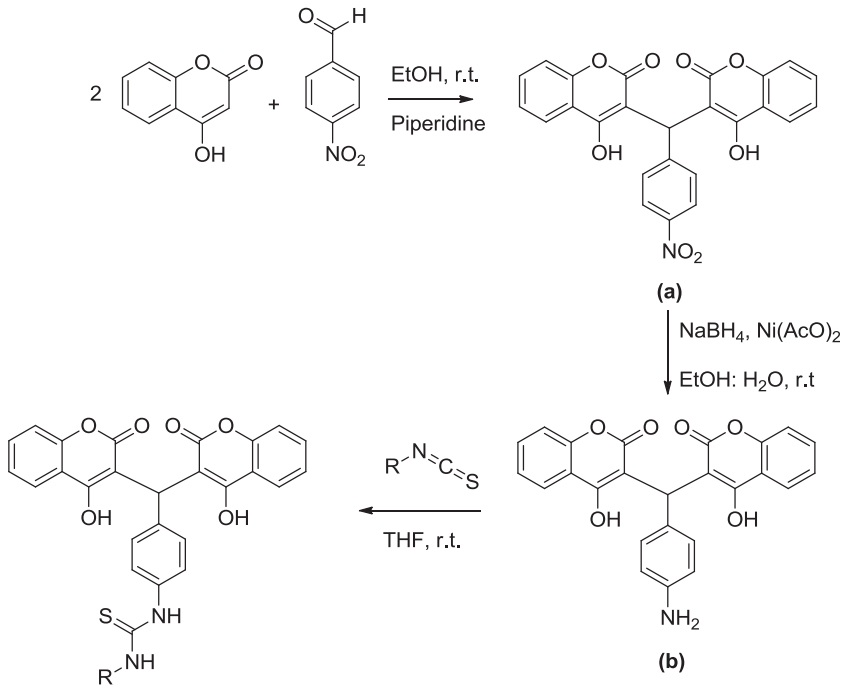
The structure activity relationship has been established. The activity difference among all the analogs is mainly due to the different substitution pattern on thiourea moiety. Compound **6** a *para*-methoxy analog was found to be the most active among the series with IC_{50} value 13.5 ± 0.39 μM many fold better than the standard acarbose. The greater potential of the compound might be due to electron donating group on aromatic ring attached with thiourea, if we compare compound **6** with **5** and **4** having *meta* and *ortho* methoxy group respectively. The activity difference among these analog might be due to the difference in position of substituents. Compound **18** a *para* nitro analog is found to be the next most active analog among the series with IC_{50} value 24.69 ± 0.68. The activity of this compound might be due to the electron withdrawing group on phenyl part of thiourea.

The binding interactions of the most active analogs were confirmed through molecular docking studies.

2.3. Molecular docking calculations

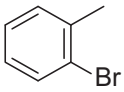
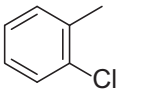
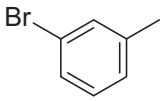
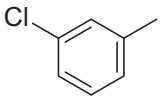
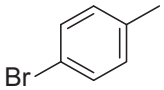
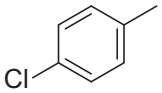
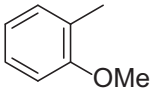
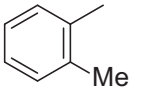
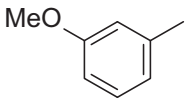
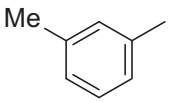
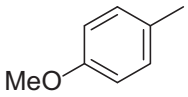
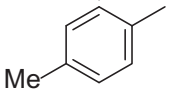
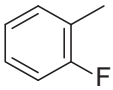
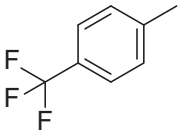
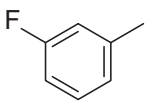
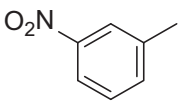
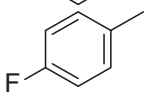
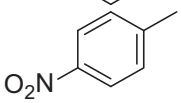
All the synthesized analogs of biscoumarin thiourea were docked into the binding pocket of α-glucosidase to find out the binding interactions, docking fitness scores and their specificity for enzyme. The docking scores and binding modes of most of the analogs are well correlated with the experimental results. In our docking study, all the docked compounds were analyzed from the two aspects. (1) Analogs which have different groups bonded to the same position (2) Analogs which have same groups on different positions. Inhibition values (IC50) of all the analogs except bromobenzene indicate that compounds with para substituted aryl moiety are more active than other compounds (Table 1). Compound **3** (IC_{50}38.97 ± 0.99 (μM)), with Br at para position of the substituted aryl group, showed docking score (S) −14.1155 and interactions with the residues Arg 312 and Glu 276. Arg 312 formed hydrogen bond with the oxygen (OH) of the compound while Glu 276 was in a polar interaction with the bromobenzene moiety of the ligand (Fig. 1a).

Compound **6** (IC_{50} 13.5 ± 0.39 (μM)), with methoxy group at para position of the substituted aryl group, showed docking score (S) −14.9010 and interactions with the residues Asn 241, His 279 and Ser 281. Asn 241 and Ser 281 showed polar bonds with Sulfur and oxygen of the compound respectively. His 279 was in a hydrogen bonding interaction with the lone pair of oxygen atom, as shown in (Fig. 1b). Compound **9** (IC_{50} 53.4 ± 1.58 (μM)), with F



Scheme 1. Synthesis of biscoumarin thiourea derivatives 1–18.

Table 1
Prepared various analogs of biscoumarin thiourea and their α-glucosidase Inhibition.

S. no.	R	IC_{50} (μM)	S	S. no	R	IC_{50} (μM)	S
1		37.41 ± 0.95	−14.2100	**10**		50.25 ± 1.43	−14.2773
2		35.85 ± 0.91	−14.3935	**11**		28.47 ± 0.54	−14.4123
3		38.97 ± 0.99	−14.1155	**12**		26.8 ± 0.76	−14.4468
4		104.62 ± 0.3	−13.0116	**13**		50.22 ± 0.86	−12.999
5		25.31 ± 0.73	−14.4225	**14**		78.04 ± 0.23	−12.6695
6		13.5 ± 0.39	−14.9010	**15**		43.36 ± 0.59	−13.2772
7		91.29 ± 0.27	−13.2324	**16**		31.72 ± 0.83	−14.3036
8		55.12 ± 1.63	−14.4613	**17**		51.02 ± 1.41	−13.2324
9		53.4 ± 1.58	−14.5199	**18**		24.69 ± 0.68	−14.5686

group at para position of the substituted aryl group, showed docking score (S) −14.5199 and interacts with the binding site residues His 239 and Asn 241 (Fig. 1c). A polar interaction was found between Asn 241 and the hydroxyl group of the compound and arene arene interaction between His 239 and compound.

Compound **12** (IC_{50} 26.8 ± 0.76 (μM)), with Cl at para position of the substituted aryl group, showed docking score (S) −14.4468 and interactions with the residues Arg 312, Lys 155 and Asn 412. Arg 312 and Lys 155 formed hydrogen bonds with the oxygen (OH) of the compound while Asn 412 was in a polar interaction with the benzene moiety of the ligand (Fig. 1d). In case of Compound **15** (IC_{50} 43.36 ± 0.59 (μM)), with methyl group at para position of the substituted aryl group, the docking score (S) observed was −13.2772 with two binding interactions to the active site residues His 239 and 279 (Fig. 1e). A polar interaction was found between His 279 and the hydroxyl group of the compound and arene arene interaction between His 239 and compound.

Some similar interactions were also found in compound **18**-protein docked complex (Fig. 1f). Compound **18** (IC_{50} 24.69 ± 0.68 (μM)) has a nitro (NO_2) at para position of the substituted aryl ring and showed the docking score (S) −14.5686. Here His 279 and Glu 276 were involved in binding interactions. His 279 showed arene–arene and a polar interaction with the compound and Glu 276 was found making a polar interaction with the benzene group of the compound.

The structural differences among these compounds are based on different groups at para position of the substituted aryl group. The biological activity (Table 1) revealed better inhibitory activity for the compounds having electron withdrawing groups at para position except for Flourine and almost the same results were observed in docking analysis. On the basis of IC_{50} value, docking score and binding interaction compound **6** demonstrated high inhibitory potential among the compounds of this series. The electron withdrawing capability of the oxygen of methoxy group might be a reason for the high activity of this compound and the same cause is reflected in case of compound **18** (NO_2 group) followed by compound **12** (Cl group) and Compound 3 (Br group). In case of compound **9** the electron withdrawing capability of F did not provide the results as given away by the above mentioned groups, although it showed good docking score (S) −14.5199. However, it may be attributed to the smaller size of F and its low electron affinity. The substituted methyl group with electron donating inductive effect in compound **15** was also observed with mild activity and docking score.

The biological activity and docking score (Table 1) of the analogs which have same groups on different positions showed that substitution at para position in the aryl group resulted in better inhibition than ortho and meta position except in case of Br. From the knowledge of chemistry it is apparent that para substituted group may directly transfer their electronic effect over the rest of the compound and might be a reason for the high activity of para substituted compounds. However, the bromoaryl substituted compounds **1**, **2** and **3**, contrast, showed better results at ortho and meta position than para.

In the docking study it was observed that most of the compounds in this series showed good agreement between the docking and experimental results. The good correlation between

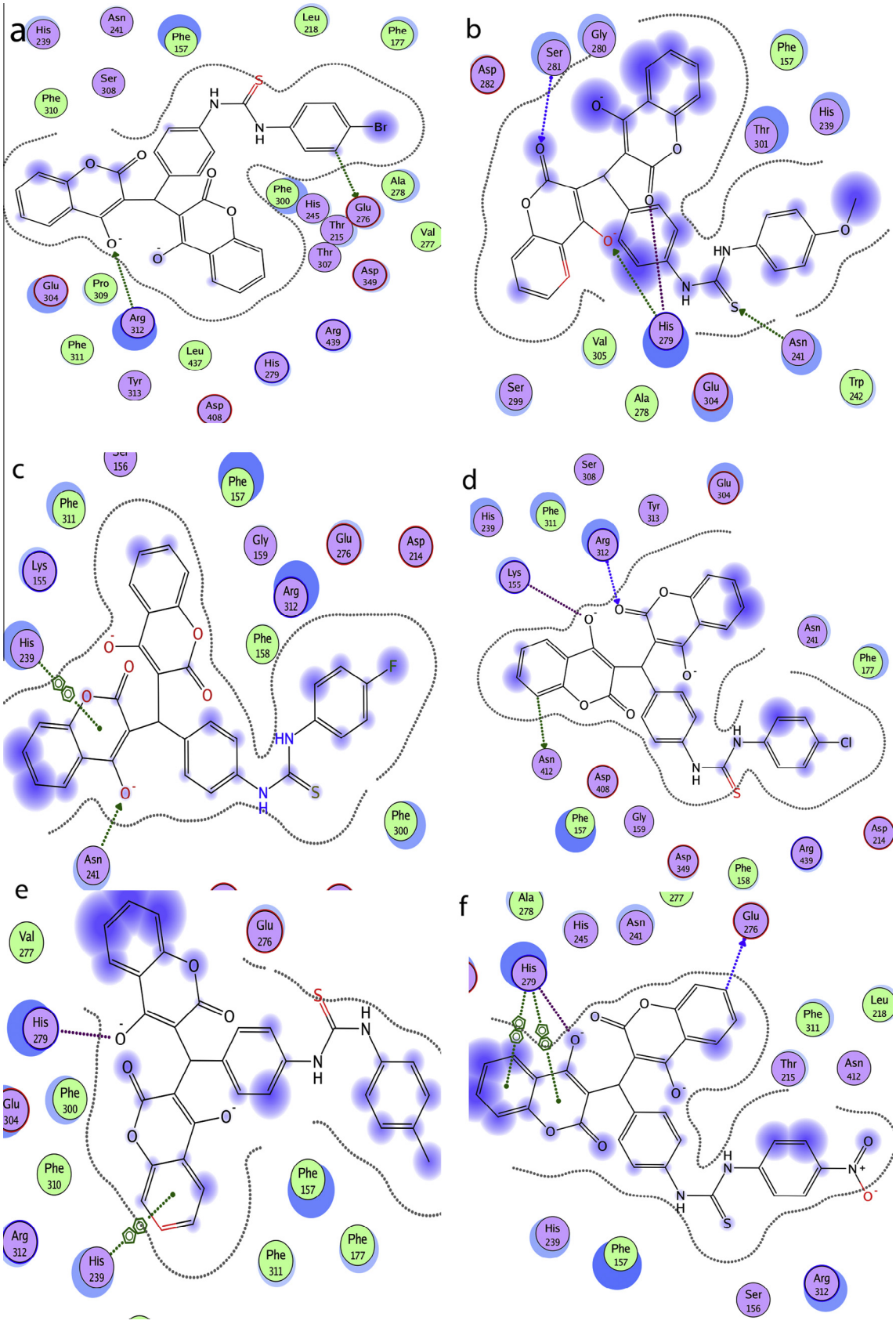


Fig. 1. Predicted binding mode of (a) compound **3**, (b) compound **06**, (c) compound **09**, (d) compound **12**, (e) compound **15** and (f) compound **18** within the binding pocket of predicted homology model of α-glucosidase enzyme.

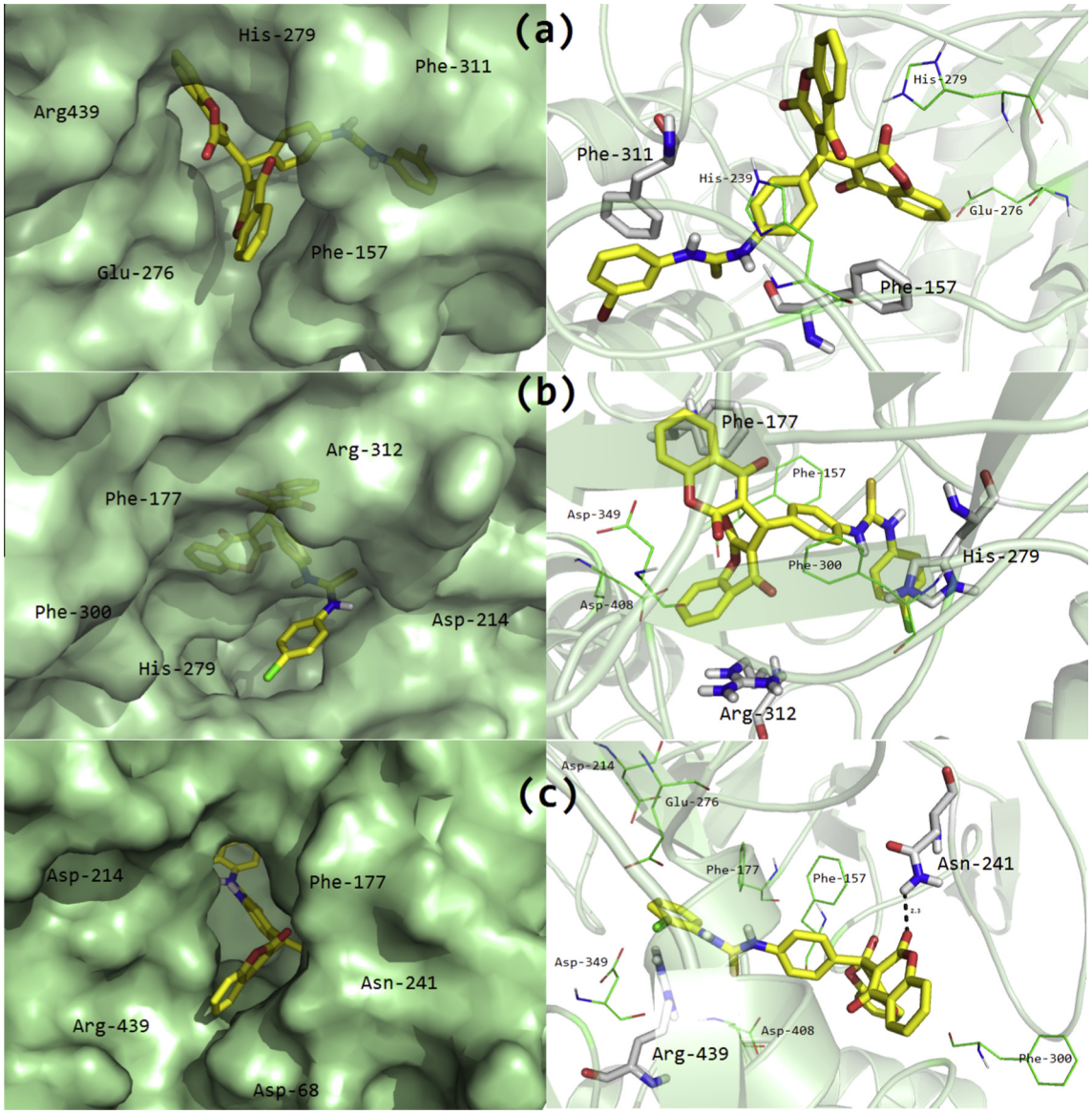


Fig. 2. Predicted binding mode of (a) compound **02**, (b) compound **12** and (c) compound **10** within the binding pocket of predicted homology model of α-glucosidase enzyme.

experimental findings and docking results can be analyzed from the IC_{50} values and docking scores given in Table 1.

As compared to compound **02** having Br attachment at *ortho* position showed slightly poor interactions with the active site residues (Fig. 2a). In case of compound **12** having Chlorine (Cl) attachment at *para* position over the phenyl ring also showed good interaction pattern (Fig. 2b) as compared to compound **10** which have Cl attachment at *ortho* position showed poor interaction (Fig. 2c).

Overall the docking results showed that the compounds having *para* substituted phenyl ring have good interactions with active site residues and good inhibitory activities as compared to compounds having either *meta* or *ortho* substituted phenyl ring. The *para* position attachment might be one of the best clues for good interaction network and good IC_{50} value.

3. Conclusion

Synthesis of biscoumarin thiourea derivatives and their α-glucosidase inhibitory potential was evaluated. Compound **6** showed the most potent α-glucosidase inhibitory potential with IC_{50} 13.5 ± 0.39 μM. On the basis of in-vitro testing, we demonstrated that biscoumarin derivatives are potential α-glucosidase inhibitors that exert stronger inhibitory effects than does acarbose.

4. Materials and method

4.1. General

Melting point was taken on Buchi M-560 melting point instrument and was uncorrected. IR spectra were recorded on a Spectrum One FT-IR spectrometer (Perkin Elmer), using KBr discs and values were signified in cm^{-1}. The ^{1}H NMR and ^{13}C NMR spectra were measured on Bruker 500 Ultrashield Plus NMR (500 MHz) in DMSO-*d*6 as solvent, using tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed as ppm. HR-ESI-MS were determined on Agilent 6224 TOF-LC/MS using negative mode at Faculty of Pharmacy, UiTM Puncak Alam, Malaysia

4.2. Synthesis of 3,3′-((4-nitrophenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) (*a*)

Compound **a** was synthesized by stirring the mixture of 4-hydroxycoumarin (26 mmol) and 4-nitrobenzaldehyde

(13 mmol) in EtOH and catalytic amount of piperidine overnight. Completion of reaction was monitored by periodic TLC. After completion of reaction, it was filtered and then washed with distilled water affording a pure product in high yields. Yield 93%. m.p. 235.6 °C; IR(KBr) (ν_{max}, cm^{-1}): 3445, 3083, 1649, 1596, 1536, 1441, 1357, 1133, 1054. ^{1}H NMR (500 MHz, DMSO) δ 8.23 (s, 1H), 8.07 (d, *J* = 8.8 Hz, 2H), 7.82 (dd, *J* = 7.8, 1.5 Hz, 2H), 7.53 (m, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.29 (d, *J* = 7.9 Hz, 2H), 7.25 (m, 2H), 6.36 (s, 1H). EI-MS: 456.0724 (M^-).

*4.3. Synthesis of 3,3′-((4-aminophenyl)methylene)bis(4-hydroxy-2H-chromen-2-one) (**b**)*

In a round-bottomed flask (500 ml) equipped with a magnetic stirrer, a solution of compound **a** (11.425 g, 25 mmol) in EtOH–H_2O (375:0.375 ml) was prepared. To the resulting solution, Ni(OAc)·$4H_2O$ (1.225 g, 5 mmol) was added and the mixture was then stirred for 5 min. Afterwards, $NaBH_4$ (3.775 g, 100 mmol) was added to the reaction mixture and a fine black precipitate was immediately deposited. The mixture continued to be stirred for 45 min and the progress of the reaction was monitored by TLC. At the end of reaction, distilled water (125 ml) was added to the reaction mixture and stirred for 10 min. The mixture was extracted with CH_3Cl (3 × 300 ml) and the CH_3Cl extract was evaporated under reduced pressure to give compound **b**. Yield 82%. m.p. 176.2 °C; IR(KBr) (ν_{max}, cm^{-1}): 3358,3027, 1665, 1605, 1536, 1509, 1399, 1182, 1032. ^{1}H NMR (500 MHz, DMSO) δ 7.82 (dd, *J* = 7.7, 1.1 Hz, 2H), 7.52–7.46 (m, 2H), 7.23 (dd, *J* = 12.7, 7.7 Hz, 4H), 6.76 (d, *J* = 8.1 Hz, 2H), 6.39 (d, *J* = 8.4 Hz, 2H), 6.12 (s, 1H), 4.64 (s, 2H). EI-MS: 426.0988 (M^-).

*4.3.1. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(2-bromophenyl)-thiourea (**1**)*

Yield 81%. m.p. 219.2 °C. IR(KBr) (ν_{max}, cm^{-1}): 3348, 1671, 1615, 1523, 1185, 1047.^{1}H NMR (500 MHz, DMSO) δ 9.88 (s, 1H), 9.16 (s, 1H), 7.83 (dd, *J* = 7.8, 1.3 Hz, 2H), 7.61 (ddd, *J* = 15.6, 8.0, 1.0 Hz, 2H), 7.51 (m, 2H), 7.35 (m, 1H), 7.30 (d, *J* = 8.5 Hz, 2H), 7.24 (dd, *J* = 16.7, 8.0 Hz, 4H), 7.15 (td, *J* = 8.0, 1.4 Hz, 1H), 7.08 (d, *J* = 8.1 Hz, 2H), 6.26 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 180.5 (C), 168.2 (C), 165.0 (C), 153.0 (C), 139.8 (C), 138.5 (C), 136.3 (C), 132.9 (CH), 131.4 (CH), 130.4 (CH), 128.1 (CH), 127.4 (CH), 124.6 (CH), 124.0 (CH), 123.3 (CH), 121.3 (C), 120.4 (CH), 115.9 (CH), 103.9 (C), 36.3 (CH). HREI-MS: 641.0217 (M^-).

*4.3.2. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(3-bromophenyl)-thiourea (**2**)*

Yield 79%. m.p. 252 °C. IR(KBr) (ν_{max}, cm^{-1}): 3360, 1664, 1615, 1527, 1180, 1028.^{1}H NMR (500 MHz, DMSO) δ 9.82 (s, 1H), 9.72 (s, 1H), 7.86–7.81 (m, 3H), 7.54–7.49 (m, 2H), 7.43 (dd, *J* = 6.7, 2.2 Hz, 1H), 7.29–7.21 (m, 8H), 7.07 (d, *J* = 8.3 Hz, 2H), 6.26 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 179.9 (C), 168.2 (C), 165.0 (C), 153.0 (C), 141.9 (C), 139.7 (C), 136.4 (C), 131.4 (CH), 130.6 (CH), 127.3 (CH), 127.1 (CH), 126.3 (CH), 124.6 (CH), 123.9 (CH), 123.3 (CH), 122.8 (CH), 121.2 (C), 120.4 (C), 115.9 (CH), 103.8 (C), 36.3 (CH). HREI-MS: 641.0215 (M^-).

*4.3.3. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(4-bromophenyl)-thiourea (**3**)*

Yield 81%. m.p. 236.3 °C. IR(KBr) (ν_{max}, cm^{-1}): 3366, 1668, 1533, 1186, 1012. ^{1}H NMR (500 MHz, DMSO) δ 9.74 (s, 1H), 9.67 (s, 1H), 7.83 (dd, *J* = 7.8, 1.2 Hz, 2H), 7.51 (dd, *J* = 10.0, 3.2 Hz, 2H), 7.47–7.46 (m, 3H), 7.27–7.23 (m, 7H), 7.06 (d, *J* = 8.2 Hz, 2H), 6.26 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 179.9 (C), 168.2 (C), 165.1 (C), 153.0 (C), 139.6 (C), 139.6 (C), 136.5 (C), 131.5 (CH), 131.4 (CH), 127.3 (CH), 126.1 (CH), 124.6 (CH), 123.9 (CH), 123.3 (CH), 120.4 (C), 116.6 (C), 115.9 (CH), 103.9 (C), 36.3 (CH). HREI-MS: 641.0220 (M^-).

*4.3.4. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(2-chlorophenyl)-thiourea (**4**)*

Yield 82%. m.p. 195.4 °C. IR(KBr) (ν_{max}, cm^{-1}): 3358, 1664, 1608, 1530, 1186, 1059. ^{1}H NMR (500 MHz, DMSO) δ 9.89 (s, 1H), 9.23 (s, 1H), 7.85–7.79 (m, 2H), 7.64 (d, *J* = 7.4 Hz, 1H), 7.49 (ddd, *J* = 11.0, 9.0, 4.0 Hz, 3H), 7.25 (ddd, *J* = 12.2, 11.6, 6.4 Hz, 8H), 7.08 (d, *J* = 8.3 Hz, 2H), 6.26 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 180.5 (C), 168.2 (C), 165.0 (C), 153.0 (C), 139.8 (C), 137.0 (C), 136.4 (C), 131.4 (CH), 130.0 (CH), 129.7 (CH), 127.6 (CH), 127.4 (CH), 127.4 (CH), 124.6 (CH), 124.0 (CH), 123.3 (CH), 120.4 (C), 115.9 (CH), 103.9 (C), 36.3 (CH). HREI-MS: 595.0742 (M^-).

*4.3.5. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(3,4-dichlorophenyl) thiourea (**5**)*

Yield 83%. m.p. 207.3 °C. IR(KBr) (ν_{max}, cm^{-1}): 3357, 1657, 1608, 1531, 1185, 1031. ^{1}H NMR (500 MHz, DMSO) δ 9.89 (s, 1H), 9.79 (s, 1H), 7.89 (d, *J* = 2.4 Hz, 1H), 7.83 (dd, *J* = 7.8, 1.3 Hz, 3H), 7.55–7.49 (m, 4H), 7.44 (dd, 1H), 7.27–7.22 (m, 8H), 7.07 (d, *J* = 8.2 Hz, 2H), 6.26 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 179.9 (C), 168.2 (C), 165.0 (C), 153.0 (C), 140.5 (C), 139.9 (C), 136.3 (C), 131.4 (CH), 130.8 (CH), 130.5 (CH), 127.4 (CH), 126.2 (C), 125.3 (CH), 124.6 (CH), 124.1 (CH), 124.0 (CH), 123.3 (CH), 120.4 (C), 115.9 (CH), 103.8 (C), 36.3 (CH). HREI-MS: 629.0350 (M^-).

*4.3.6. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(4-chlorophenyl)-thiourea (**6**)*

Yield 81%. m.p. 197.2 °C. IR(KBr) (ν_{max}, cm^{-1}): 3319, 1675, 1607, 1541, 1183, 1043. ^{1}H NMR (500 MHz, DMSO) δ 9.74 (s, 1H), 9.67 (s, 1H), 7.85–7.82 (m, 2H), 7.53–7.51 (m, 3H), 7.35 (m, 2H), 7.27–7.23 (m, 7H), 7.07 (d, *J* = 8.3 Hz, 2H), 6.26 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 180.0 (C), 168.2 (C), 165.1 (C), 153.0 (C), 140.0 (C), 139.2 (C), 136.6 (C), 131.4 (CH), 128.8 (CH), 128.6 (CH), 127.3 (CH), 125.9 (CH), 125.8 (CH), 124.6 (CH), 123.9 (CH), 123.4 (CH), 120.4 (C), 115.9 (CH), 103.9 (C), 36.31 (CH). HREI-MS: 595.0742 (M^-).

*4.3.7. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(2-fluorophenyl)-thiourea (**7**)*

Yield 81%. m.p. >400 °C. IR(KBr) (ν_{max}, cm^{-1}): 3363, 1668, 1603, 1533, 1270, 1185, 1042. ^{1}H NMR (500 MHz, DMSO) δ 9.85 (s, 1H), 9.31 (s, 1H), 7.84 (dd, *J* = 7.8, 1.3 Hz, 2H), 7.64 (t, *J* = 7.9 Hz, 1H), 7.51 (m, 2H), 7.25 (ddd, *J* = 19.9, 9.8, 5.7 Hz, 8H), 7.15 (ddd, *J* = 8.5, 5.5, 3.2 Hz, 1H), 7.08 (d, *J* = 8.0 Hz, 2H), 6.27 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 180.8 (C), 168.2 (C), 165.1 (C), 157.7 (C), 153.0 (C), 139.6 (C), 136.5 (C), 131.4 (CH), 129.0 (CH), 127.8 (C), 127.7 (C), 127.5 (CH), 127.4 (CH), 127.3 (CH), 124.6 (CH), 124.4 (2CH), 123.9 (CH), 123.4 (CH), 120.4 (C), 116.1 (CH), 115.9 (CH), 103.9 (C), 36.3 (CH). HREI-MS: 579.1031 (M^-).

*4.3.8. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(3-fluorophenyl)-thiourea (**8**)*

Yield 83%. m.p. 311 °C. IR(KBr) (ν_{max}, cm^{-1}): 3366, 1671, 1611, 1530, 1180, 1035. ^{1}H NMR (500 MHz, DMSO) δ 9.78 (d, *J* = 12.4 Hz, 2H), 7.83 (dd, *J* = 7.8, 1.4 Hz, 2H), 7.53 (ddd, *J* = 13.9, 8.2, 4.5 Hz, 3H), 7.32 (dd, *J* = 15.0, 8.1 Hz, 1H), 7.24 (dd, *J* = 8.3, 6.6 Hz, 7H), 7.06 (d, *J* = 8.1 Hz, 2H), 6.91 (td, *J* = 8.4, 2.1 Hz, 1H), 6.25 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 179.8 (C), 168.2 (C), 165.1 (C), 163.2 (C), 153.0 (C), 139.7 (C), 136.5 (C), 131.4 (CH), 130.3 (CH), 130.2 (CH), 127.3 (CH), 124.6 (CH), 124.0 (CH), 123.4 (CH), 120.4 (C), 119.5 (CH), 115.9 (CH), 111.1 (CH), 110.9 (CH), 110.5 (CH), 110.3 (CH), 103.8 (C), 36.3 (CH). HREI-MS: 579.1033 (M^-).

*4.3.9. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(4-fluorophenyl)-thiourea (**9**)*

Yield 80%. m.p. 205.2 °C. IR(KBr) (ν_{max}, cm^{-1}): 3358, 1671, 1607, 1530, 1213, 1183, 1042. ^{1}H NMR (500 MHz, DMSO) δ 9.67 (s, 1H), 9.57 (s, 1H), 7.83 (dd, *J* = 7.8, 1.0 Hz, 2H), 7.54–7.48 (m, 2H), 7.46 (dd, *J* = 8.8, 5.0 Hz, 2H), 7.25 (dd, *J* = 16.8, 8.2 Hz, 6H), 7.13 (t, *J* = 8.8 Hz, 2H), 7.06 (d, *J* = 8.2 Hz, 2H), 6.26 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 180.3 (C), 168.1 (C), 165.0 (C), 160.5 (C), 158.6 (C), 153.0 (C), 139.5 (C), 136.6 (C), 136.4 (C), 136.4 (C), 131.4 (CH), 127.3 (CH), 126.7 (2CH), 124.6 (CH), 123.9 (CH), 123.4 (CH), 120.4 (C), 115.9 (CH), 115.4 (CH), 115.2 (CH), 103.9 (C), 36.3 (CH). HREI-MS: 579.1028 (M^-).

*4.3.10. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(4-(trifluoromethyl)-phenyl) thiourea (**10**)*

Yield 83%. m.p. 270.7 °C. IR(KBr) (ν_{max}, cm^{-1}): 3363, 1660, 1615, 1533, 1324, 1167, 1111, 1067. ^{1}H NMR (500 MHz, DMSO) δ 9.95, 9.992 (s, 2H), 7.84 (dd, *J* = 7.8, 1.3 Hz, 2H), 7.76 (d, *J* = 8.5 Hz, 2H), 7.65 (d, *J* = 8.6 Hz, 2H), 7.54–7.49 (m, 2H), 7.35–7.13 (m, 6H), 7.09 (d, *J* = 8.1 Hz, 2H), 6.28 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 179.9 (C), 168.3 (C), 165.1 (C), 153.0 (C), 144.1 (C), 139.8 (C), 136.5 (C), 131.4 (CH), 127.3 (CH), 125.9 (CH), 125.8 (CH), 124.6 (CH), 123.9 (CH), 123.4 (CH), 123.3 (CH), 120.4 (C), 115.9 (CH), 113.5 (CH), 103.9 (C), 36.3 (CH). HREI-MS: 629.1 (M^-).

*4.3.11. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(o-tolyl)thiourea (**11**)*

Yield 81%. m.p. 202 °C. IR(KBr) (ν_{max}, cm^{-1}): 3354, 1668, 1605, 1525, 1182, 1037. ^{1}H NMR (500 MHz, DMSO) δ 9.55 (s, 1H), 9.15 (s, 1H), 7.83 (d, *J* = 7.0 Hz, 2H), 7.53–7.49 (m, 2H), 7.25 (dd, *J* = 17.9, 7.8 Hz, 8H), 7.17–7.13 (m, 2H), 7.07 (d, *J* = 8.3 Hz, 2H), 6.26 (s, 1H), 2.22 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 180.7 (C), 168.2 (C), 165.1 (C), 153.0 (C), 139.4 (C), 138.5 (C), 136.7 (C), 135.3 (C), 131.4 (CH), 130.7 (CH), 128.5 (CH), 127.3 (CH), 126.8 (CH), 126.4 (CH), 124.6 (CH), 123.9 (CH), 123.4 (CH), 120.4 (C), 115.9 (CH), 103.9 (C), 36.3 (CH), 18.3 (CH_3). HREI-MS: 575.1279 (M^-).

*4.3.12. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(m-tolyl)thiourea (**12**)*

Yield 86%. m.p. 222 °C. IR(KBr) (ν_{max}, cm^{-1}): 3356, 1668, 1608, 1538, 1186, 1042. ^{1}H NMR (500 MHz, DMSO) δ 9.60 (s, 1H), 9.54 (s, 1H), 7.83 (dd, *J* = 7.8, 1.3 Hz, 2H), 7.56–7.45 (m, 2H), 7.22 (ddd, *J* = 26.2, 14.8, 7.9 Hz, 9H), 7.05 (d, *J* = 8.2 Hz, 2H), 6.92 (d, *J* = 7.5 Hz, 1H), 6.25 (s, 1H), 2.28 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 179.9 (C), 168.2 (C), 165.1 (C), 153.0 (C), 139.9 (C), 139.4 (C), 138.1 (C), 136.8 (C), 131.4 (CH), 128.6 (CH), 127.2 (CH), 125.5 (CH), 124.6 (CH), 123.9 (CH), 123.3 (CH), 121.3 (CH), 120.4 (C), 115.9 (CH), 103.9 (C), 36.3 (CH), 21.5 (CH_3). HREI-MS: 575.1282 (M^-).

*4.3.13. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(p-tolyl)thiourea (**13**)*

Yield 80%. m.p. 218.5 °C. IR(KBr) (ν_{max}, cm^{-1}): 3356, 1668, 1607, 1534, 1184, 1040. ^{1}H NMR (500 MHz, DMSO) δ 9.57 (s, 1H), 9.53 (s, 1H), 7.84 (d, *J* = 7.6 Hz, 2H), 7.51 (t, *J* = 7.1 Hz, 2H), 7.34 (d, *J* = 8.2 Hz, 2H), 7.25 (dd, *J* = 16.4, 8.5 Hz, 6H), 7.11 (d, *J* = 8.2 Hz, 2H), 7.06 (d, *J* = 8.2 Hz, 2H), 6.26 (s, 1H), 2.27 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 180.0 (C), 168.3 (C), 165.1 (C), 153.0 (C), 139.3 (C), 137.4 (C), 136.8 (C), 134.0 (C), 131.4 (CH), 129.3 (CH), 127.2 (CH), 124.6 (CH), 124.4 (CH), 123.9 (CH), 123.4 (CH), 120.4 (C), 115.9 (CH), 103.9 (C), 36.3 (CH), 21.0 (CH_3). HREI-MS: 575.1283 (M^-).

*4.3.14. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(2-methoxyphenyl)-thiourea (**14**)*

Yield 88%. m.p. 186.2 °C. IR(KBr) (ν_{max}, cm^{-1}): 3344, 1664, 1609, 1538, 1234, 1100. ^{1}H NMR (500 MHz, DMSO) δ 9.85 (s, 1H), 8.96 (s, 1H), 8.04 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.83 (dd, *J* = 7.8, 1.4 Hz, 2H), 7.51 (td, 1H), 7.29–7.22 (m, 6H), 7.11 (td, 1H), 7.08 (d, *J* = 8.0 Hz, 2H), 7.02 (d, 1H), 6.90 (t, 1H), 6.26 (s, 1H), 3.81 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 179.5 (C), 168.2 (C), 165.1 (C), 153.0 (C), 151.8 (C), 139.7 (C), 136.4 (C), 131.4 (CH), 128.4 (CH), 127.3 (CH), 125.8 (CH), 125.4 (CH), 124.6 (CH), 124.1 (CH), 123.3 (CH), 120.4 (CH), 120.2 (C), 115.9 (CH), 111.7 (CH), 103.9 (C), 56.2 (OCH_3), 36.3 (CH). HREI-MS: 591.123 (M^-).

*4.3.15. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(3-methoxyphenyl)-thiourea (**15**)*

Yield 84%. m.p. 300 °C. IR(KBr) (ν_{max}, cm^{-1}): 3333, 1671, 1610, 1538, 1180, 1043. ^{1}H NMR (500 MHz, DMSO) δ 9.64 (s, 1H), 9.61 (s, 1H), 7.84 (dd, *J* = 7.8, 1.3 Hz, 2H), 7.51 (dt, *J* = 11.9, 2.5 Hz, 2H), 7.23 (ddd, *J* = 18.1, 16.9, 8.3 Hz, 9H), 7.07 (d, *J* = 8.3 Hz, 1H), 7.03 (d, *J* = 7.4 Hz, 1H), 6.68 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.27 (s, 1H), 3.73 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 179.8 (C), 168.3 (C), 165.2 (C), 159.7 (C), 153.0 (C), 141.2 (C), 139.4 (C), 136.7 (C), 131.4 (CH), 129.6 (CH), 127.2 (CH), 124.6 (CH), 124.0 (CH), 123.4 (CH), 120.4 (C), 116.1 (CH), 115.9 (CH), 110.2 (CH), 109.6 (CH), 103.9 (C), 55.5 (OCH_3), 36.3 (CH). HREI-MS: 591.1232 (M^-).

*4.3.16. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(4-methoxyphenyl)-thiourea (**16**)*

Yield 85%. m.p. 225.1 °C. IR(KBr) (ν_{max}, cm^{-1}): 3359, 1664, 1519, 1180, 1032. ^{1}H NMR (500 MHz, DMSO) δ 9.49 (s, 1H), 9.42 (s, 1H), 7.84 (d, *J* = 7.7 Hz, 2H), 7.54–7.49 (m, 1H), 7.32 (d, *J* = 8.6 Hz, 1H), 7.25 (dd, *J* = 16.7, 8.0 Hz, 2H), 7.06 (d, *J* = 8.2 Hz, 2H), 6.88 (d, *J* = 8.9 Hz, 2H), 6.26 (s, 1H), 3.74 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 180.3 (C), 168.2 (C), 165.1 (C), 157.0 (C), 153.0 (C), 139.3 (C), 136.8 (C), 132.8 (C), 131.4 (CH), 127.2 (CH), 126.5 (CH), 124.6 (CH), 123.9 (CH), 123.4 (CH), 120.4 (C), 115.9 (CH), 114.1 (CH), 103.9 (C), 55.7 (OCH_3), 36.3 (CH). HREI-MS: 591.1233 (M^-).

*4.3.17. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(3-nitrophenyl)-thiourea (**17**)*

Yield 85%. m.p. 193.7 °C. IR(KBr) (ν_{max}, cm^{-1}): 3357, 1660, 1609, 1532, 1347, 1186, 1039. ^{1}H NMR (500 MHz, DMSO) δ 10.00 (s, 2H), 8.57 (s, 1H), 7.93 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.91–7.87 (m, 1H), 7.84 (dd, *J* = 7.8, 1.4 Hz, 2H), 7.58 (t, *J* = 8.2 Hz, 1H), 7.54–7.49 (m, 2H), 7.29–7.21 (m, 7H), 7.10 (d, *J* = 8.2 Hz, 2H), 6.28 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 180.1 (C), 168.2 (C), 165.1 (C), 153.0 (C), 147.9 (C), 141.6 (C), 140.0 (C), 136.2 (C), 131.4 (CH), 130.1 (CH), 129.9 (CH), 127.4 (CH), 124.6 (CH), 124.1 (CH), 123.4 (CH), 120.4 (C), 118.9 (CH), 118.1 (CH), 115.9 (CH), 103.8 (C), 36.3 (CH). HREI-MS: 606.0985 (M^-).

*4.3.18. 1-(4-(bis(4-Hydroxy-2-oxo-2H-chromen-3-yl)methyl)phenyl)-3-(4-nitrophenyl) thiourea (**18**)*

Yield 72%. m.p. 225.6 °C. IR(KBr) (ν_{max}, cm^{-1}): 3358, 1664, 1608, 1509, 1335, 1183, 1043. ^{1}H NMR (500 MHz, DMSO) δ 10.25 (s, 1H), 10.15 (s, 1H), 8.18 (d, *J* = 9.0 Hz, 2H), 7.91–7.78 (m, 4H), 7.51 (t, *J* = 7.7 Hz, 2H), 7.35–7.17 (m, 6H), 7.09 (d, *J* = 8.4 Hz, 2H), 6.27 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 179.6 (C), 168.2 (C), 165.1 (C), 153.0 (C), 146.9 (C), 142.7 (C), 140.0 (C), 136.3 (C), 131.6 (C), 131.4 (CH), 127.4 (CH), 126.8 (CH), 124.7 (CH), 124.6 (CH), 123.9 (CH), 123.4 (CH), 122.0 (CH), 120.4 (C), 115.9 (CH), 112.9 (CH), 103.8 (C), 36.3 (CH). HREI-MS: 606.0973 (M^-).

4.4. Baker's Yeast α-glucosidase inhibition assay

The enzyme inhibition was evaluated according to the method previously reported by Taha et al. [25] with slight modification. Various concentration of test compounds (10 μL) were dissolved in DMSO (ranging from 200 to 6.25 μg/mL) and premixed with 95 μL of 50 mM phosphate buffer (pH 6.8). Then, 25 μL of enzyme (0.0625 U/mL) in phosphate buffer saline was added into each well and the plate was incubated at 37 °C for 10 min. Afterward, 25 μL of PNPG in phosphate buffer saline (5 mM) were added and pre-read of the plate was taken by using a microplate reader (Spectrostar Nano BMG Labtech, Germany). The reaction mixture was then incubated at 37 °C for 30 min and change in absorbance at 405 nm was monitored up to 30 min. For negative control, the test samples were replaced with 10 μL of DMSO and acarbose was used as positive control. All experiments were triplicated and the results were expressed as the mean ± S.E.M of three determinations. The percentage (%) inhibition of α-glucosidase inhibitory activity was calculated using the equation: where $\Delta A_{control}$ and ΔA_{sample} are the different absorbances of control, sample at time t_{30} and t_0, respectively.

$$\% \text{ Inhibition} = \frac{\Delta A_{control} - \Delta A_{sample}}{\Delta A_{control}} \times 100$$

4.5. Molecular docking calculation

The study was designed to dock Biscoumarin derivatives against α-glucosidase enzyme with the following communications; Intel(R) xenon(R) CPU E5620@2.40 GHz system having 3.8 GB RAM with the open 11.4 (X 86_64) operating platform. Protein–Ligand docking was carried out using the Molecular Operating Environment (MOE 2010.11) software package. The three dimensional structure for α-glucosidase of *Saccharomyces cerevisiae* has not been solved up-to yet. Only a few homology models have been reported [33–36]. In the current study we predict 3D structure for α-glucosidase of *S. cerevisiae* by using same protocol as described by (Burke et al.) of homology modeling [37]. The pasta sequence was retrieved from UniProt (Access code P53341). Template search was performed against the PDB. The crystallographic structure of *S. cerevisiae* isomaltase (PDB code 3AJ7; Resolution 1.30 Å) with 72.4% of sequence identity with the target was selected as a template [37]. The 3D structure of α-glucosidase for *S. cerevisiae* was predicted using MOE homology modeling tools. The predicted model was then subjected to energy minimization up to 0.05 gradients.

Before docking, ligands and protein were prepared using MOE v2010.11. 3D structure of all nineteen compounds were built by using Molecular Builder Module program implemented in MOE and save as a (.mdb) file for molecular docking. Subsequently, the energy of all compounds was minimized up to 0.05 Gradient using MMFF 94x force field. *Energy minimization* of all compounds was followed by the preparation of protein for docking purposes. Most macromolecular crystal structures contain little or no hydrogen coordinate data due to limited resolution and thus protonation was done prior to docking using Protonate 3D tools. *Protonation* was followed by energy minimization up to 0.05 Gradient using Amber 99 force field. All the synthesized compounds was docked into the active site of protein using the Triangular Matching docking method and 30 conformations of all the compounds and protein complex were generated with docking score (S). The complex was analyzed for interactions and their 3D images were taken by using visualizing tool PyMol.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2015.09.004.

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