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Original article

Synthesis and molecular docking studies of potent α-glucosidase inhibitors based on biscoumarin skeleton

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ABSTRACT

In our effort directed toward the discovery of new anti-diabetic agent for the treatment of diabetes, a library of biscoumarin derivative **1**–**18** was synthesized and evaluated for α-glucosidase inhibitory potential. All eighteen (**18**) compounds displayed assorted α-glucosidase activity with IC_{50} values 16.5 –385.9 μM, if compared with the standard acarbose (IC_{50} = 906 ± 6.387 μM). In addition, molecular docking studies were carried out to explore the binding interactions of biscoumarin derivatives with the enzyme. This study has identified a new class of potent α-glucosidase inhibitors.

1. Introduction

Mammalian α-glucosidases inhibitors, which impede with enzymatic action in small intestine, might slow the release of D-glucose from oligosaccharides and disaccharides, causing in delaying glucose absorption and decreasing postprandial blood glucose levels [1]. The acarbose [2] and voglibose [3] from microorganisms, and nojirimycin [4] and 1-deoxynojirimycin [5] from plants have been reported as potent α-glucosidase inhibitors. The effects of all these compounds on blood glucose levels after food uptake have been reported [6–9].

Glucosidases are liable for the catalytic cleavage of α-glycosidic bond with specificity depending on the position of cleavage site, the number of monosaccharides, and the configuration of the hydroxyl groups on the substrate [10]. The pharmaceutical research community has a special interest in α-glucosidases (EC 3.2.1.20) because the inhibition of its catalytic activity caused in the impedance of glucose absorption and decreases the postprandial blood glucose level. Thus, effective α-glucosidases inhibitors may help as chemotherapeutic agents for clinical use in the treatment of obesity and diabetes [11–13]. The α-glucosidase inhibitors have also been well appreciated as a therapeutic target for the other carbohydrate mediated diseases including viral infections [14,15], cancer [16] and hepatitis [17].

In the course of our efforts in the development of biologically important synthetic compounds, we observed α-glucosidase inhibitory potential in substituted new biscoumarin derivatives. It is obvious that polyphenols well intermingle with proteins and lead to inhibit enzyme activities [18]. Natural as well as synthetic biscoumarin have become an important class of oxygenated heterocycle mainly due to their wide variety of biological activities such as urease inhibition, anti-inflammatory, antioxidant, CYP3A and antifungal activities [19–21].

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There is few reported synthetic and natural coumarin derivatives showed potent α-glucosidase inhibition [22–25]. Surprisingly no biscoumarin was tested for α-glucosidase inhibition. Therefore, we designed our project to synthesize biscoumarin and test them for α-glucosidase inhibitory properties.

In the continuation of our work, on biological potent small molecules [26] and their evaluation for enzyme inhibition [27], we herein report synthesis of substituted biscoumarin analogs, their α-glucosidase inhibitory potentials. The molecular docking was also performed in order to study their binding affinity.

2. Results and discussion

2.1. Chemistry

In synthesis of biscoumarin analogs **1**–**18** (Scheme 1), to a stirred mixture of coumarin derivatives (4.0 mmol) and substituted aromatic aldehydes (2.0 mmol) in water and a catalytic amount of tetraethylammonium bromide (TEAB) was added. The reaction mixture was stirred at 60 °C for 1–2 h. Completion of the reaction was monitored by periodic TLC. After completion of reaction, it was filtered and washes with distilled water affording a pure product in high yields. In some cases the compounds purified through column chromatography using 3:7 acetone and *n*-hexane as eluent afforded pure products in good yield. The structures of compounds **1**–**18** were deduced by using different spectroscopic techniques such as ^{1}H NMR and EI mass spectroscopy. All compounds gave a satisfactory elemental analysis.

2.2. Pharmacology

Glucosidase are responsible for the catalytic cleavage of a glycosidic bond of complex molecules of carbohydrates. Enzyme inhibition is one of the most significant tools in pharmaceutical research as well as in the field of drug discovery. During this study, we have synthesized eighteen (**18**) derivatives and evaluated for α-glucosidase enzyme inhibitory activity. All compounds showed a potent inhibition superior to the standard inhibitor of α-glucosidase.

Compounds **1**–**18** exhibited a varying degree of α-glucosidase inhibitory activity with IC_{50} values between 16.54 ± 0.36–385.99 ± 0.65 μM when compared with standard acarbose (IC_{50} = 906 ± 6.387 μM). Although the main skeleton for all compounds is same, the slight difference in their inhibitory potential might be due to the different substitution pattern on benzaldehyde.

Compound 3,3′-((2-4-Dichlorophenyl)methylene)*bis*(6-chloro-4-hydroxy-2*H*-chromen-2-one) (**12**) showed an excellent activity (IC_{50} = 16.54 ± 0.36 μM) 54-fold more active than the standard acarbose (IC_{50} = 906 ± 6.387 μM). Similarly, compound 3,3′-((3-nitrophenyl) methylene)*bis*(6-chloro-4-hydroxy-2*H*-chromen-2-one) (**13**) showed an excellent activity with an IC_{50} value of 27.07 ± 0.13 μM, 33-fold better than the standard. The remaining all compounds also exhibited potent inhibitory activities.

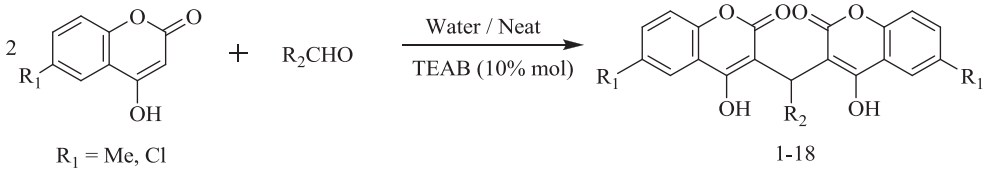
To know the mechanism of α-glucosidase inhibition and binding mode of biscoumarin analogs inside the binding pocket of α-glucosidases, molecular docking studies were performed.

2.3. Molecular docking studies

The molecular docking study was carried out to explore the binding mode of biscoumarin derivatives within the binding pocket of α-glucosidase and to understand their structure activity relationship using MOE-Dock as docking software (www.chemcomp.com). As a means of testing the adopted protocols, the known inhibitor acarbose (the first α-glucosidase inhibitor approved for type 2 diabetes treatments) was docked into the binding pocket of a developed homology model, the acarbose fit well in the binding pocket and showed interaction to the important active site residues (Fig. 1).

Although the X-ray crystallographic structures of α-glucosidase have been reported from some bacteria, the three-dimensional structural information is still not available for the eukaryotic α-glucosidase enzyme from *Saccharomyces species* (the enzyme use in our biological assay). However, only a few homology models have been reported for this enzyme previously [28–30]. The three dimensional coordinates of none of these model are publically available. Therefore, we construct the 3D structure of α-glucosidase by homology modeling using the same protocol as described by Burke et al. [31], based on the crystal structure of *Saccharomyces cerevisiae* (3AJ7.pdb) [32].

All the compounds were docked into the binding pocket of a developed homology model of α-glucosidase enzyme. From the molecular docking it was observed that the top ranked conformation of the most active compound **12** (IC_{50} = 16.54 ± 0.36 μM) (Fig. 2) established six hydrogen bonds between the hydroxyl group on coumarin ring of the compound and the active site residues (Asp 214, Glu 276, Arg 312, Asp 408 and Arg 439). Additionally the aryl group of the compound formed an arene–cation interaction with the Arg 439. Furthermore, several hydrophobic interactions were observed between the compound and the active site residues, *e.g.*, Val 108, Phe 157, Phe 177 and Phe 300 are the other residues that stabilized the binding of the compound **12** in the active site of α-glucosidase. The strong hydrogen bonding network observed for compound **12** by the hydroxyl groups attached to the coumarin ring might be due to chloro groups, particularly dichlorobenzene in which the chloride moiety has strong electron withdrawing inductive effect. This effect of chloride moieties increases the ionizing ability of hydroxyl group that might be one of the reasons for its highest activity showed in the series (Table 1). These observations can be verified in case of compounds **2** (Fig. 3), **15** (Fig. 4) and **17** (Fig. 5) as they have low biological activities as well as less interaction with active site residue as compared to compound **12**. As these compounds have groups with electron donating inductive effect instead of chloride present as in compound **12**. Similarly compounds **6** to **11** have two side chain chloride groups, but lacking the dichlorobenzene group that makes them slightly less active as compare to compound **12**. From the



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

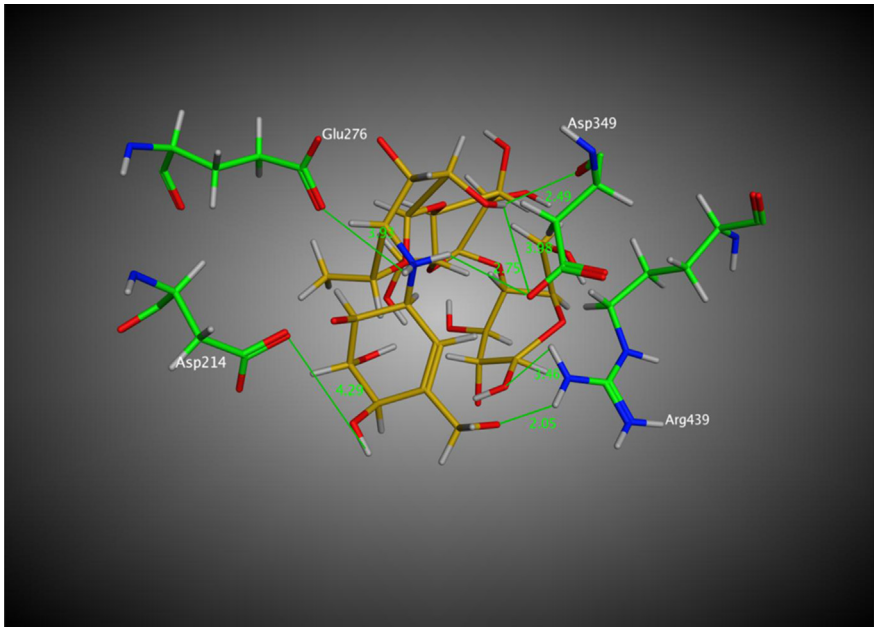


Fig. 1. Binding mode of acarbose (known inhibitor) in the binding pocket of a developed homology model of α-glucosidase.

docking conformation of compounds **13** it was observed that its *m*-nitrobenzene showed almost similar behavior as dichlorobenzene in compound **12** because the nitro group has also electron withdrawing inductive effect.

2.4. Molecular docking

Homology modeling of α-glucosidase for *S. cerevisiae* was performed to predict its three dimensional (3D) structure. The amino acid sequence of α-glucosidase from *S. cerevisiae* was retrieved from UniProt protein resource data bank (http://www.uniprot.org/) under the access code P53341. Similarity search was carried out in MOE-2010.11 using default parameter against PDB Databank implemented in MOE2010.11. The crystallographic structure of *S. cerevisiae* isomaltase (PDB code 3AJ7, 1.30 Å resolution) with 72.4% of sequence identity with the target was selected as the template. 3D structure was built using homology modeling tool implemented in MOE. The developed structure was subjected to energy minimization up to 0.05 RMS gradients and the minimized structure was then refined by MD simulation up to 500 ps. The final refine structure was then used for the molecular docking purpose.

Prior to docking ligand and protein were prepared using MOE2010.11. All synthetic compounds were modeled using Builder program implemented in MOE, finally a database was created in which all the compound structures were present in 3D format. Subsequently, their energies were minimized up to 0.05 Gradient, using the MMFF94x force field. Energy minimization of compound database was followed by the preparation of protein for docking purposes. Most of macromolecular crystal structures contain little or no hydrogen coordinate data due to limited resolution, thus

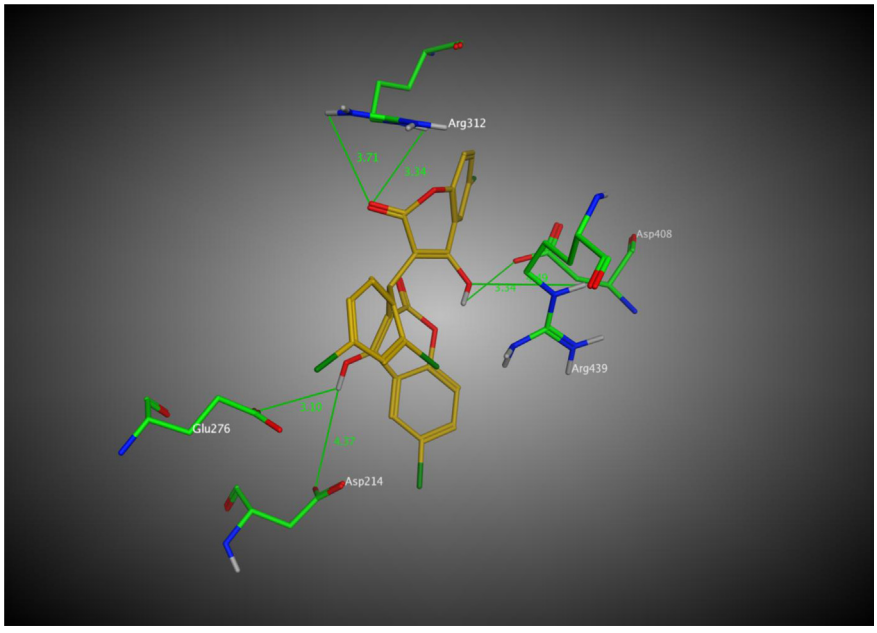


Fig. 2. Binding mode of compound **12** in the binding pocket of developed homology model of α-glucosidase.

Table 1
α-Glucosidase inhibitory potential of biscoumarin analogs **1**–**18**.

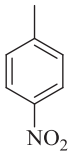
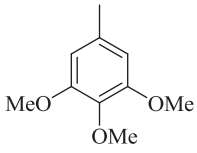
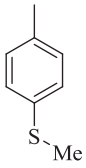
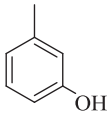
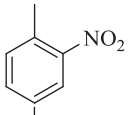
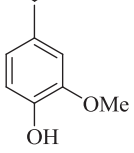
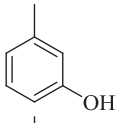
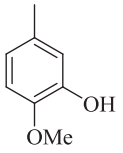
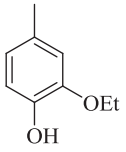
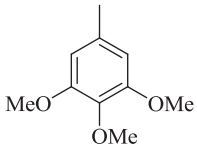
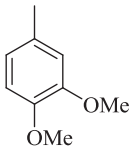
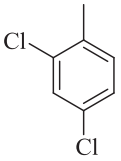
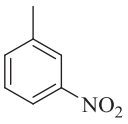
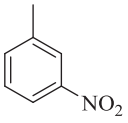
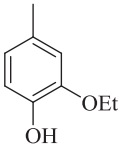
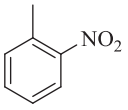
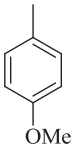
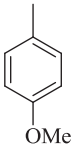
Compound no.	R_1	R_2	$IC_{50} \pm SEM^a$ (μM)
1	6-Me		79.18 ± 2.7
2	6-Me		385.99 ± 0.65
3	6-Me		37.38 ± 0.69
4	6-Me		52.6 ± 0.21
5	6-Cl		80.94 ± 0.62
6	6-Cl		113.05 ± 3.43
7	6-Cl		84.06 ± 5.7
8	6-Cl		83.64 ± 3.39
9	6-Cl		57.14 ± 0.35
10	6-Cl		128.14 ± 2.04
11	6-Cl		91.36 ± 1.16

Table 1 (*continued*)

Compound no.	R_1	R_2	$IC_{50} \pm SEM^a$ (μM)
12	6-Cl		16.54 ± 0.36
13	6-Cl		27.07 ± 0.13
14	6-Me		67.96 ± 2.44
15	6-Me		221.6 ± 2.47
16	6-Me		128.6 ± 1.16
17	6-Me		106.63 ± 1.61
18	6-Cl		75.74 ± 1.11
Acarbose	–	–	906 ± 6.387

[a] SEM is the standard error of the mean, Acarbose is standard inhibitor for α-glucosidase.

protonation should be done prior to docking using the Protonate 3D Option. The protonation was followed by energy minimization up to 0.05 gradient, using Amber99 force field. The database was docked into the binding pocket of a protein using the Triangular Matching docking method and 30 conformations for each ligand protein complex were generated with docking score. Each complex was analyzed for interactions and the 3D image was taken.

2.5. α-Glucosidase inhibitory assay

Rat intestinal acetone powder in typical saline (100:1; w/v) was sonicated appropriately and the supernatant was used as a source of basic intestinal α-glucosidase after centrifugation. In short, 10 mL of test samples of 5 mg/mL in DMSO solution were reconstituted in 100 mL of 100 mM-phosphate buffer at pH 6.8 in 96-well microplate and incubated with 50 mL of basic intestinal α-glucosidase for 5 min before 50 mL substrate (5 mM, *p*-nitrophenyl-α-D-

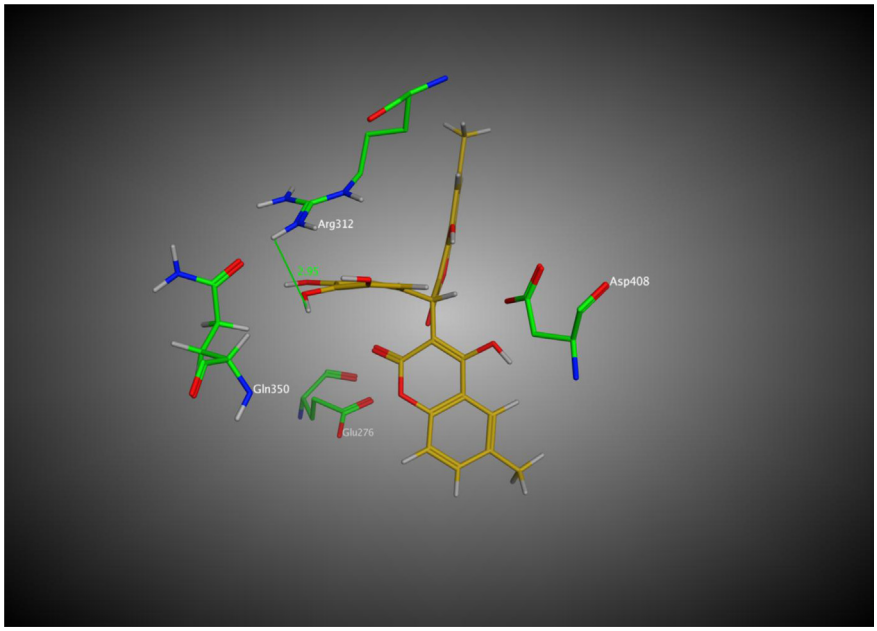


Fig. 3. Binding mode of compound **2** in the binding pocket of developed homology model of α-glucosidase.

glucopyranoside prepared in same buffer) was added. *p*-Nitrophenol released was measured at 405 nm spectrophotometrically (SpectraMax plus384, Molecular Devices Corporation, Sunnyvale, CA, USA) 5 min after incubation with the substrate. Individual blanks for test samples were prepared to accurate background absorbance where the substrate was changed with 50 mL of buffer. Control sample contained 10 mL DMSO beside test samples. Percentage of enzyme inhibition was measured as $(1 - B/A) \times 100$ where [A] represents absorbance of control exclusive of test samples, and [B] corresponding to absorbance in presence of test samples [33].

3. Conclusions

Synthesis of biscoumarin analogs and their α-glucosidase inhibitory potential was evaluated. All these eighteen (18) derivatives showed potent α-glucosidase inhibitory potential. Consequently, *in silico* studies were performed to recognize the binding mode of these compounds. The planned scaffold of α-glucosidase inhibitors offers the possibility of expedient additional modifications that could give rise to lead structures with enhanced inhibitory activity and selectivity towards the enzyme.

4. Material and methods

NMR experiments were performed on an Avance Bruker AM 300 MHz machine. CHN Analyses were carried out a Carlo Erba Strumentazion-Mod-1106, Italy. Electron impact mass spectra (EI MS) were recorded on a Finnigan MAT-311A (Germany) mass spectrometer. Thin layer chromatography (TLC) was carried out on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck,

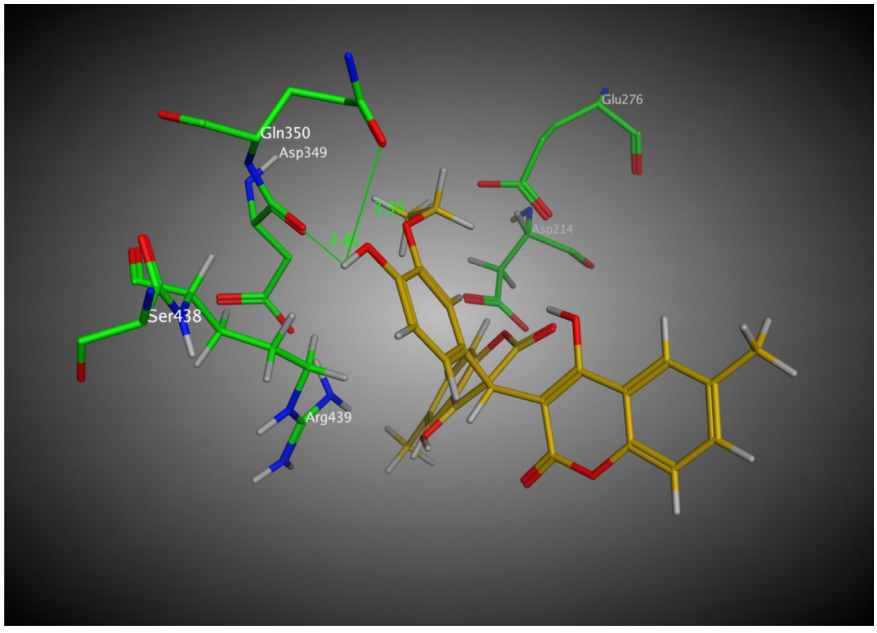


Fig. 4. Binding mode of compound **15** in the binding pocket of developed homology model of α-glucosidase.

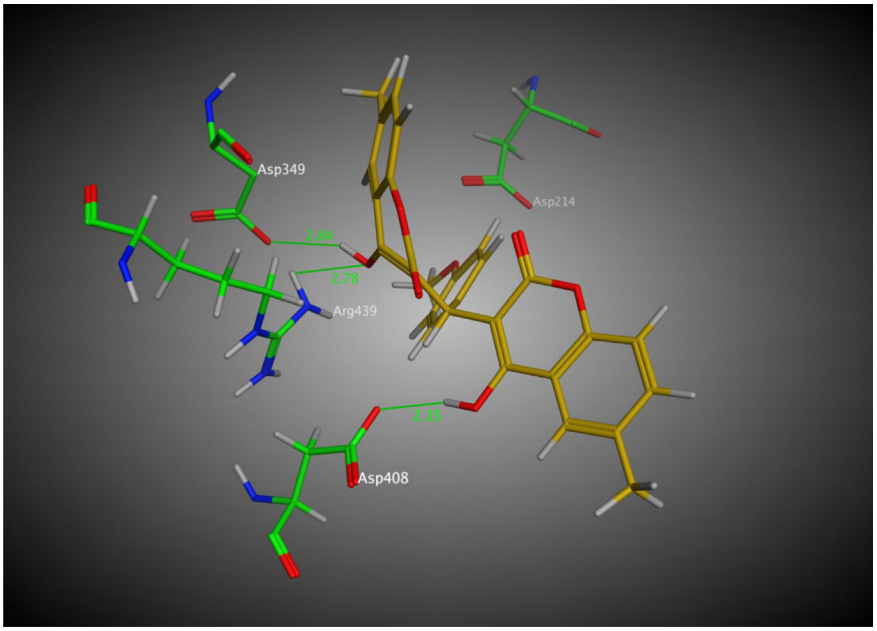


Fig. 5. Binding mode of compound **17** in the binding pocket of developed homology model of α-glucosidase.

Germany). Chromatograms were visualized by iodine vapors or UV at 254 and 365 nm.

4.1. General procedure for the synthesis of compounds *1*–*18*

In synthesis of biscoumarin analogs **1**–**18** (Scheme 1), to a stirred mixture of coumarin derivatives (4.0 mmol) and substituted aromatic aldehyde (2.0 mmol) in water and 10 mol% triethylammonium bromide (TEAB) was added. The reaction mixture was stirred at 60 °C for 1–2 h. 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. In some cases pure products were obtained through column chromatography (silica gel) using 3:7 acetone and *n*-hexane as eluent. The structures of compounds **1**–**18** were deduced by using different spectroscopic techniques, including ^{1}H NMR and EI mass spectroscopy. All synthetic compounds **1**–**18** gave satisfactory elemental analyses.

4.1.1. 3,3′-((4-Nitrophenyl)methylene)bis(4-hydroxy-6-methyl-2H-chromen-2-one) (*1*)

Yield: 0.23 g (84%); ^{1}H NMR: (DMSO-d$_6$, 300 MHz): δ 8.05 (d, 2H, $J_{3'',2''/6'',5''}$ = 8.7 Hz, H-3″/5″), 7.59 (br s, 2H, H-5/5′), 7.47 (d, 2H, $J_{2'',3''/6'',5''}$ = 8.6 Hz, H-2″/6″), 7.34 (dd, 1H, $J_{7,8/7',8'}$ = 8.1, $J_{7,5/7',5'}$ = 2.6 Hz, H-7/7′), 7.17 (d, 1H, $J_{8,7/8',7'}$ = 8.2 Hz, H-8/8′), 6.31 (s, 1H, Ar$_3$CH), 2.32 (s, 6H, 2 × CH$_3$); Anal. Calcd for $C_{27}H_{19}NO_8$, C = 66.80, H = 3.95, N = 2.89, Found C = 66.78, H = 3.97, N = 2.91; EI-MS: *m/z* (rel. int. %): 485 (M$^+$, 42), 308 (15), 292 (45), 134 (100), 106 (20).

4.1.2. 3,3′-((3,4,5-Trimethoxyphenyl)methylene)bis(6-methyl-4-hydroxy-2H-chromen-2-one) (*2*)

Yield: 0.22 g (82%); ^{1}H NMR: (DMSO-d$_6$, 300 MHz): δ 7.64 (br, s, 2H, H-5/5′), 7.35 (dd, 2H, $J_{7,8/7',8'}$ = 8.4, $J_{7,5/7',5'}$ = 1.8 Hz, H-7/7′), 7.19 (d, 2H, $J_{8,7/8',7'}$ = 8.1 Hz, H-8/8′), 6.39 (s, 2H, H-2″/6″), 6.21 (s, 1H, Ar$_3$CH), 3.61 (s, 3H, OCH$_3$), 3.54 (s, 6H, OCH$_3$), 2.33 (s, 6H, 2 × CH$_3$); Anal. Calcd for $C_{30}H_{26}O_9$, C = 67.92, H = 4.94, Found C = 67.94, H = 4.96; EI-MS: *m/z* (rel. int. %): 530 (M$^+$, 65), 353 (63), 322 (100), 175 (28), 134 (13).

4.1.3. 3,3′-((4-(Methylthio)phenyl)methyl)(6-methyl-2H-chromen-2-one) (*3*)

Yield: 0.23 g (84%); ^{1}H NMR: (DMSO-d$_6$, 300 MHz): δ 7.61 (br. s, 2H, H-5/5′), 7.35 (dd, 2H, $J_{7,8/7',8'}$ = 8.4 Hz, $J_{7,5/7',5'}$ = 1.8 Hz, H-7/7′), 7.19 (d, 2H, $J_{8,7/8',7'}$ = 8.4 Hz, H-8/8′), 7.09 (m, 4H, H-2″/3″/5″/6″), 6.21 (s, 1H, Ar$_3$CH), 2.39 (s, 3H, −SCH$_3$), 2.33 (s, 6H, CH$_3$); Anal. Calcd for $C_{28}H_{22}O_6S$, C = 69.12, H = 4.56, S = 6.59, Found C = 69.14, H = 4.55, S = 6.57; EI-MS: *m/z* (rel. int. %): 486 (M$^+$, 42), 308 (59), 263 (100), 176 (42), 134 (61).

4.1.4. 3,3′-((3-Hydroxyphenyl)methylene)bis(6-methyl-4-hydroxy-2H-chromen-2-one) (*4*)

Yield: 0.24 g (86%); ^{1}H NMR: (DMSO-d$_6$ 300 MHz): δ 7.58 (br.s, 2H, H-5/5′), 7.30 (dd, 2H, $J_{7,8/7',8'}$ = 8.7, $J_{7,5/7',5'}$ = 2.1 Hz, H-7/7′), 7.13 (d, 2H, $J_{8,7}$ = 8.1 Hz, H-8/8′), 6.92 (t, 1H, $J_{5''(4'',6'')}$ = 7.8 Hz, H-5″), 6.52 (m, 3H, H-2″/4″/6″), 6.14 (s, 1H, Ar$_3$CH), 2.32 (s, 6H, 2 × CH$_3$); Anal. Calcd for $C_{27}H_{20}O_7$, C = 71.05, H = 4.42, Found C = 71.06, H = 4.40; EI-MS: *m/z* (rel. int. %): 456 (M$^+$, 42), 345 (18), 279 (37), 176 (52), 134 (100).

4.1.5. 3,3′-((2-Nitrophenyl)methylene)bis(6-chloro-4-hydroxy-2H-chromen-2-one) (*5*)

Yield: 0.21 g (80%); ^{1}H NMR (DMSO-d$_6$, 300 MHz); δ 7.69 (d, 2H, $J_{5,7/5',7'}$ = 2.7 Hz, H-5/5′), 7.52 (m, 4H, H-7/7′/3″/6″), 7.33 (m, 4H, H-8/8′/4″/5″), 6.42 (s, 1H, Ar$_3$CH); Anal. Calcd for $C_{25}H_{13}Cl_2NO_8$, C = 57.05, H = 2.49, Found C = 57.07, H = 2.51; EI-MS *m/z* (rel. int. %): 526 (M$^+$, 42), 298 (67), 283 (58), 196 (27), 154 (100).

4.1.6. 3,3′-((3-Methoxy,4-hydroxyphenyl)methylene)bis(6-chloro-4-hydroxy-2H-chromen-2-one) (*6*)

Yield: 0.23 g (84%); ^{1}H NMR (DMSO-d$_6$, 300 MHz); δ 7.73 (d, 2H, $J_{5,7/5',7'}$ = 2.7 Hz, H-5/5′), 7.54 (dd, 2H, $J_{7,8/7',8'}$ = 8.7, $J_{7,5/7',5'}$ = 2.4 Hz, H-7/7′),7.31 (d, 2H, $J_{8,7/8',7'}$ = 8.7 Hz, H-8/8′), 6.59 (d, 1H, $J_{6'',5''}$ = 6.0 Hz, H-6″), 6.54 (s, 1H, H-2″),6.49 (d, 1H, $J_{5'',6''}$ = 8.1 Hz, H-5″), 6.12 (s, 1H, Ar$_3$CH), 5.61 (s, 1H, OH), 3.53 (s, 3H, OCH$_3$); Anal. Calcd for $C_{26}H_{16}Cl_2O_8$, C = 59.22, H = 3.06, Cl = 13.45, Found C = 59.23, H = 3.04; EI-MS *m/z* (rel. int. %): 527 (M$^+$, 42), 329 (100), 313 (56), 299 (73), 196 (51).

*4.1.7. 3,3′-((3-Hydroxyphenyl) methylene)bis(6-chloro-4-hydroxy-2H-chromen-2-one) (**7**)*

Yield: 0.22 g (82%); ^{1}H NMR (DMSO-d_6, 300 MHz); δ 7.74 (d, 2H, $J_{5,7/5',7'} = 2.7$ Hz, H-5/5′), 7.55 (dd, 2H, $J_{7,8/7',8'} = 8.7$, $J_{7,5/7',5'} = 2.7$ Hz, H-7/7′), 7.32 (d, 2H, $J_{8,7/8',7'} = 8.7$ Hz, H-8/8′), 6.92 (t, 1H, $J_{5''(4'',6'')} = 7.8$ Hz, H-5″), 6.51 (m, 3H, H-2″/4″/6″), 6.14 (s, 1H, Ar_3CH); Anal. Calcd for $C_{25}H_{14}Cl_2O_7$, C = 60.38, H = 2.84, Found C = 60.36, H = 2.82; EI-MS *m/z* (rel. int. %): 497 (M^+, 42), 299 (95), 283 (74), 196 (41), 154 (100).

*4.1.8. 3,3′-((3-Hydroxy-4-methoxyphenyl)methylene)bis(6-chloro-4-hydroxy-2H-chromen-2-one) (**8**)*

Yield: 0.23 g (84%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.73 (d, 2H, $J_{5,7/5',7'} = 2.7$ Hz, H-5/5′), 7.54 (dd, 2H, $J_{7,8/7',8'} = 8.7$, $J_{7,5/7',5'} = 2.7$ Hz, H-7/7′),7.32 (d, 2H, $J_{8,7/8',7'} = 9.0$ Hz, H-8/8′), 6.69 (d, 1H, $J_{5'',6''} = 8.4$ Hz, H-5″),6.52 (s, 1H, H-2″), 6.43 (d, 1H, $J_{6'',5''} = 8.4$ Hz H-6″), 6.09 (d, 1H, Ar_3CH); Anal. Calcd for $C_{26}H_{16}Cl_2O_8$, C = 59.22, H = 3.06, Found C = 59.24, H = 3.04; EI-MS *m/z* (rel. int. %): 527 (M^+, 42), 329 (89), 313 (100), 299 (53), 196 (48).

*4.1.9. 3,3′-((3-Ethoxy-4-hydroxyphenyl)methylene)bis(6-chloro-4-hydroxy-2H-chromen-2-one) (**9**)*

Yield: 0.22 g (82%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.73 (d, 2H, $J_{5,7/5'7'} = 2.4$ Hz, 2 × H-5), 7.54 (dd, 2H, $J_{7,8/7',8'} = 8.7$, $J_{7,5/7',5'} = 2.7$ Hz, H-7/7′),7.31 (d, 2H, $J_{8,7/8',7'} = 8.7$ Hz, H-8/8′), 6.58 (d, 2H, $J_{5'',6''/2'',6''} = 8.1$ Hz, H-5″/2″),6.48 (d, 1H, $J_{6'',5''} = 8.1$ Hz, H-6″), 6.10 (s, 1H, Ar_3CH), 3.80 (q, 2H, O**CH$_2$**CH_3), 1.17 (t, 3H, OCH_2**CH$_3$**); Anal. Calcd for $C_{27}H_{18}Cl_2O_8$, C = 59.91, H = 3.35, Found C = 59.92, H = 3.34; EI-MS *m/z* (rel. int. %):541 (M^+, 42), 299 (100), 287 (25), 154 (74), 126 (38).

*4.1.10. 3,3′-((3,4,5-Trimethoxyphenyl)methylene)bis(6-chloro-4-hydroxy-2H-chromen-2-one) (**10**)*

Yield: 0.23 g (84%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.75 (d, 2H, $J_{5,7/5',7'} = 2.7$ Hz, 2 × H-5), 7.54 (dd, 2H, $J_{7,8/7',8'} = 8.7$, $J_{7,5/7',5'} = 2.4$ Hz, H-7/7′),7.32 (d, 2H, $J_{8,7/8',7'} = 8.7$ Hz, H-8/8′), 6.37 (s, 2H, H-2″/6″), 6.16 (s, 1H, Ar_3CH), 3.60 (s, 3H, OCH_3), 3.54 (s, 6H, 2 × OCH_3); Anal. Calcd for $C_{28}H_{20}Cl_2O_9$, C = 58.86, H = 3.53; Found C = 58.84, H = 3.54; EI-MS *m/z* (rel. int. %): 571 (M^+, 42), 374 (89), 343 (100), 196 (37), 154 (61).

*4.1.11. 3,3′-((3,4-Dimethoxyphenyl)methylene)bis(6-chloro-4-hydroxy-2H-chromen-2-one) (**11**)*

Yield: 0.23 g (84%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.74 (d, 2H, $J_{5,7/5',7'} = 1.5$ Hz, H-5/5′), 7.54 (dd, 2H, $J_{7,8/7',8'} = 5.1$, $J_{7,5/7',5'} = 1.5$ Hz, H-7/7′), 7.32 (d, 2H, $J_{8,7/8',7'} = 5.4$ Hz, 2 × H-8), 6.75 (d, 1H, $J_{5'',6''} = 5.1$ Hz, H-5″), 6.63 (s, 1H, H-2″), 6.61 (d, 1H, $J_{6'',5''} = 5.1$ H-6″), 6.16 (s, 1H, Ar_3CH), 3.67 (s, 3H, 3-OCH_3), 3.52 (s, 3H, 4-OCH_3); Anal. Calcd for $C_{27}H_{18}Cl_2O_8$, C = 59.91, H = 3.35; Found C = 59.93, H = 3.33; EI-MS *m/z* (rel. int. %): 541 (M^+, 42), 343 (34), 313 (100), 196 (36), 154 (65).

*4.1.12. 3,3′-((2-4-Dichlorophenyl)methylene)bis(6-chloro-4-hydroxy-2H-chromen-2-one) (**12**)*

Yield: 0.23 g (84%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.76 (s, 1H, H-3″), 7.73 (d, 2H, $J_{5,7/5',7'} = 2.4$ Hz, H-5/5′), 7.54 (dd, 2H, $J_{7,8/7',8'} = 8.7$, $J_{7,5/7',5'} = 2.4$ Hz, H-7/7′), 7.36 (d, 2H, $J_{8,7/8',7'} = 8.7$ Hz, 2 × H-8/8′), 7.40 (m, 2H, H-5″/6″), 6.07 (s, 1H, Ar_3CH); Anal. Calcd for $C_{25}H_{12}Cl_4O_6$, C = 54.58, H = 2.20, Found C = 54.56, H = 2.19; EI-MS *m/z* (rel. int. %): 550 (M^+, 42), 317 (100), 196 (37), 154 (72), 126 (37).

*4.1.13. 3,3′-((3-Nitrophenyl) methylene)bis(6-chloro-4-hydroxy-2H-chromen-2-one) (**13**)*

Yield: 0.22 g (82%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.73 (d, 2H, $J_{5,7/5',7'} = 2.4$ Hz, H-5/5′), 7.57 (dd, 2H, $J_{7,8/7',8'} = 8.7$, $J_{7,5/7',5'} = 2.7$ Hz, H-7/7′), 7.48 (m, 4H, H-2″/4″/5″/6″), 7.32 (d, 2H, $J_{8,7/8',7'} = 8.7$ Hz, H-8/8′), 6.31 (s, 1H, Ar_3CH); Anal. Calcd for $C_{25}H_{13}Cl_2NO_8$, C = 57.05, H = 2.49, N = 2.66, Found C = 57.07, H = 2.48, N = 2.67; EI-MS *m/z* (rel. int. %): 511 (M^+, 42), 313 (100), 283 (69), 196 (23), 154 (44).

*4.1.14. 3,3′-((3-Nitrophenyl)methylene)bis(4-hydroxy-6-methyl-2H-chromen-2-one) (**14**)*

Yield: 0.24 g (86%); ^{1}H NMR: (DMSO-d_6, 300 MHz): δ 8.00 (d, 1H, $J_{4'',5''} = 8.1$ Hz, H-4″), 7.86 (br s, 1H, H-2″), 7.61 (s, 2H, H-5/5′), 7.56 (d, 1H, $J_{6'',5''} = 7.2$ Hz, H-6″), 7.48 (t, 1H, $J_{5''(4'',6'')} = 7.2$ Hz, H-5″), 7.36 (dd, 2H, $J_{7,8/7',8'} = 8.7$, $J_{7,5/7',5'} = 2.4$ Hz, H-7/7′), 7.19 (d, 2H, $J_{8,7/8',7'} = 8.4$ Hz, 2H-8/8′), 6.33 (s, 1H, Ar_3CH), 2.33 (s, 6H, CH_3); Anal. Calcd for $C_{27}H_{19}NO_8$, C = 66.80, H = 3.95, N = 2.89, Found C = 66.82, H = 3.97, N = 2.87; EI-MS *m/z* (rel. int. %): 485 (M^+, 42), 309 (24), 176 (31), 134 (100), 106 (24).

*4.1.15. 3,3′-((3-Ethoxy-4-hydroxyphenyl)methylene)bis(6-methyl-4-hydroxy-2H-chromen-2-one) (**15**)*

Yield: 0.22 g (82%); ^{1}H NMR: (DMSO-d_6, 300 MHz): δ 7.63 (br s, 2H, H-5/5′), 7.36 (dd, 2H, $J_{7,8/7',8'} = 8.4$, $J_{7,5/7',5'} = 1.5$ Hz, H-7/7′), 7.20 (d, 2H, $J_{8,7/8',7'} = 8.4$ Hz, H-8/8′), 6.61 (d, 2H, $J_{5'',6''/6'',5''} = 8.4$ Hz, H-5″/6″), 6.50 (d, 1H, $J_{2'',6''} = 7.8$ Hz, H-3″/5″), 6.16 (s, 1H, Ar_3CH_3), 2.35 (s, 6H, CH_3), 2.31 (q, 2H, O**CH$_2$**CH_3), 1.18 (t, 3H, OCH_2**CH$_3$**); Anal. Calcd for $C_{29}H_{24}O_8$, C = 69.59, H = 4.83, Found C = 69.61, H = 4.84; EI-MS: *m/z* (rel. int. %):500 (M^+, 42), 324 (95), 295 (25), 279 (60), 267 (100).

*4.1.16. 3,3′-((2-Nitrophenyl)methylene)bis(6-methyl-4-hydroxy-2H-chromen-2-one) (**16**)*

Yield: 0.22 g (82%); ^{1}H NMR: (DMSO-d_6, 300 MHz): δ 7.56 (m, 4H,H-5/5′/3″/6″), 7.37 (m, 4H, H-7/7′/3″/6″), 7.15 (d, 2H, $J_{8,7/8',7'} = 8.4$ Hz, H-8/8′), 6.46 (s, 1H, Ar_3CH), 2.31 (s, 6H, CH_3); Anal. Calcd for $C_{27}H_{19}NO_8$, C = 66.80, H = 3.95, N = 2.89, Found C = 66.82, H = 4.83, N = 2.91; EI-MS: *m/z* (rel. int. %): 485 (M^+, 42), 263 (100), 176 (29), 134 (81), 106 (17).

*4.1.17. 3,3′-((4-Methoxyphenyl)methylene)bis(4-hydroxy-6-methyl-2H-chromen-2-one) (**17**)*

Yield: 0.23 g (84%); ^{1}H NMR: (DMSO-d_6, 300 MHz): 7.65 (br s, 2H, H-5/5′), 7.38 (dd, 2H, $J_{7,8/7',8'} = 8.4$, $J_{7,5/7',5'} = 2.4$ Hz, H-7/7′), 7.22 (d, 2H, $J_{8,7/8',7'} = 8.4$ Hz, H-8/8′), 7.01 (d, 2H, $J_{2'',3''/6'',5''} = 8.4$ Hz, H-2/6), 6.75 (d, 2H, $J_{3'',2''/5'',6''} = 8.7$ Hz, H-3″/5″), 6.22 (s, 1H, Ar_3CH), 3.68 (s, 3H, OCH_3) 2.34 (s, 6H, $2CH_3$); Anal. Calcd for $C_{28}H_{22}O_7$, C = 71.48, H = 4.71, Found C = 71.46, H = 4.73; EI-MS *m/z* (rel. int. %): 470 (M^+, 42), 293 (100), 263 (52), 176 (16), 134 (47).

*4.1.18. 3,3′-((4-Methoxyphenyl)methylene)bis(6-chloro-4-hydroxy-2H-chromen-2-one) (**18**)*

Yield: 0.23 g (84%); ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.73 (d, 2H, $J_{5,7/5',7'} = 2.4$ Hz, H-5/5′), 7.54 (dd, 2H, $J_{7,8/7',8'} = 8.7$, $J_{7,5/7',5'} = 2.4$ Hz, H-7/7′),7.32 (d, 2H, $J_{8,7/8',7'} = 8.7$ Hz, H-8/8′), 6.98 (d, 2H, $J_{2'',3''/6'',5''} = 8.1$ Hz, H-2″/6″),6.73 (d, 2H, $J_{3'',2''/5'',6''} = 8.7$ Hz, H-3″/5″), 6.15 (s, 1H, Ar_3CH), 3.66 (s, 3H, OCH_3); Anal. Calcd for $C_{26}H_{16}Cl_2O_7$, C = 61.07, H = 3.15, Found C = 61.06, H = 3.16; EI-MS *m/z* (rel. int. %): 511 (M^+, 42), 313 (100), 283 (69), 196 (23), 154 (44).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.05.010.

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