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Synthesis and antioxidant evaluation of desmethylxanthohumol analogs and their dimers

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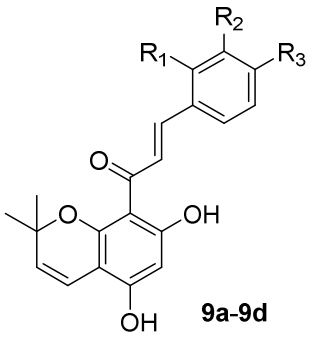
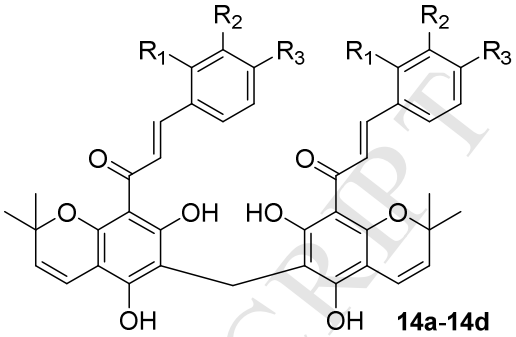
Graphical Abstract

Four ring-closing analogs of natural prenylated chalcone Desmethylxanthohumol (**1**) and their dimers were synthesized. The antioxidant activities of these new chalcone derivatives were evaluated in the PC12 cell model of hydrogen peroxide (H_2O_2)-induced oxidative damage.



9a-9d

R_1 = H, OH, R_2= H, OH, R_3 = H, OH



14a-14d

R_1 = H, OH, R_2= H, OH, R_3 = H, OH

The dimers show better antioxidant activity than the corresponding monomers

The most potent compound increased PC12 cell viability from 25% to 85% under 100 μM

Synthesis and Antioxidant Evaluation of Desmethylxanthohumol Analogs and their Dimers

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Abstract:

Four ring-closed analogs of natural prenylated chalcone desmethylxanthohumol (**1**) and their dimers were synthesized from the commercially available 1-(2,4,6-trihydroxyphenyl)ethan-1-one in five and six linear steps, respectively. The structures of the eight new derivatives were confirmed using ^{1}H NMR, ^{13}C NMR and HRMS. The antioxidant activity of the new chalcone derivatives were evaluated in a PC12 cell model of H_2O_2-induced oxidative damage. The SAR studies suggested that the catechol motif was essential for the antioxidant activity. Moreover, the dimers showed better antioxidant activity than their corresponding monomers did. Among them, compound **14d** was the most potent and increased PC12 cell viability from 25% to 85%. Flow cytometric analysis showed that compound **14d**, the most potent compound, decreased the apoptotic PC12 cell percentage and significantly reduced the LDH release and 8-OHdG generation but increased the GSH levels in H_2O_2-treated PC12 cells. Furthermore, compound **14d** had a higher FRAP value than that of gallic acid. It also reduced the stable $ABTS^{+}$ free radical with a lower EC_{50} than that of gallic acid.

Keywords: Synthesis; Chalcone; Desmethylxanthohumol; Dimer; Antioxidant

1. Introduction

Chalcones with a prenyl group or a benzopyran moiety are widely distributed in fruits, vegetables, tea, and soy-based foodstuff and display a wide spectrum of biological activities [1]. Desmethylxanthohumol (**1**), a prenylated hydroxychalcone, first isolated from hop cones *(Humulus lupulus L.*) by Schulz in 1988 [2] showed antiplasmodial [3], antiproliferative [4,5], and antioxidant bioactivities [6]. Isobavachromene (**2**) isolated from the twigs of *Dorstenia mannii* by Ngadjui et al. [7] in 1998 showed antibacterial [8, 9], anticancer [10], antimalarial [11], and antioxidant activities [12].

(insert Scheme 1)

Oxidative stress can cause cell injury and death, which may be related to numerous diseases and conditions such as liver damage [13], aging [14], cancer [15], stroke [16], myocardial infarction [17], Alzheimer's disease [18], and Parkinson's disease[19]. However, there is still no agreement on whether oxidants trigger these and other diseases, or are produced as a secondary consequence of the diseases. Nevertheless, whether oxidative stress can be considered as a cause or consequence of some diseases, it is important to develop new antioxidant drugs.

As mentioned above, desmethylxanthohumol (**1**) and isobavachromene (**2**) showed antioxidant activity and **2** could be considered as the ring-closed analog of **1**. Some biflavonoids have been reported to exhibit

better bioactivities than their monomers do [20]. Therefore, dimerization of chalcones might increase their activities. Hydrogen peroxide (H_2O_2) is a major reactive oxygen species (ROS) with high cellular membrane permeability. The PC12 cell line was derived from a rat pheochromocytoma of the adrenal medulla, and H_2O_2-induced oxidative damage to these cells constitutes a useful in vitro model system for evaluating the antioxidant properties of compounds [21, 22]. H_2O_2-induced cytotoxicity in PC12 cells results in effects including increased rate of apoptosis, membrane damage with lactate dehydrogenase (LDH) release, nuclear damage with 8-hydroxy-2'-deoxyguanosine (8-OHdG) generation, and decreased cellular glutathione (GSH) levels.

In this study, we synthesized desmethylxanthohumol (**1**) analogs and their respective dimers and subsequently evaluated the antioxidant activity in the H_2O_2-induced PC12 cell oxidative damage model. The antioxidant properties of a selected analog, compound **14d,** and its potential molecular mechanism were also investigated.

2. Chemistry

Desmethylxanthohumol (**1**) is a commercially available compound and, therefore, we synthesized its ring-closed analog, which could also be considered as an analog of Isobavachromene (**2**) (Scheme 1). The

mono-MOM protected 2',4',6'-trihydroxyacetophenone (**5**) was readily synthesized from the commercially available 2',4',6'-trihydroxyacetophenone by treatment with chloromethyl methyl ether and ethyldiisopropylamine followed by regioselective deprotection under acidic conditions. Compound **5** was converted to chalcones (**8a-d**) by cyclization with 3-methyl-2-butenal followed by aldol condensation with four different substituted benzaldehydes (**6a-d**). Full deprotection of **8a-d** by 3N hydrochloric acid (HCl) in methanol (MeOH) and tetrahydrofuran (THF) produced the chalcones **9a-d** at a 16%-38% yield. It is noteworthy that compounds **9a-d** were not stable and partly decomposed in the acid condition.

(insert Scheme 2)

Following the generation of the key intermediate **6**, we attempted to synthesize the corresponding dimers of compound **9a** by condensation with polyformaldehyde followed by aldol and deprotection reactions. However, the desired product was not obtained. To reduce the steric effect of the substrate, the corresponding de-MOM product **11** was evaluated. Fortunately, the desired dimer, **12** was obtained at 71% yield and was subsequently transformed into the bichalcones (**14a-d**) using a similar approach to that used for the monomers.

(insert Scheme 3)

3. Results and discussion

Before the antioxidant activity analysis of our newly synthesized compounds, the toxicity of compound **1** and its ring-closed analog compound **9a** was evaluated using normal human umbilical vein endothelial cells (HUVECs) and rat pheochromocytoma cells (PC12). Firstly, the toxicity of compounds **1** and **9a** against the HUVECs was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The half-minimal inhibitory concentration (IC_{50}) was determined to reflect the toxicity and a lower value indicates a higher cytotoxicity. The result revealed that the IC_{50} of compounds **1** and **9a** in HUVECs was 18.92 and 41.15 μM, respectively

(insert Table 1).

The cytotoxicity of compounds **1** and **9a** against the PC12 cells was then determined, and both compounds showed high toxicity at 200 μM (Figure 1). Furthermore, **9a** only has a slight effect on cell proliferation at 100 μM while **1** showed considerable toxicity against PC12 cells at the same concentration, suggesting that cyclizing the prenyl group of chalcone **1** to benzopyran decreased its toxicity against PC12 cells.

(insert Figure 1)

Based on this information, the ring-closed analogs (compounds **9a-d)** were synthesized, and their antioxidant activity was evaluated in H_2O_2-damaged PC12 cells at a concentration of 100 μM. Gallic acid was used as a positive control, and the results are illustrated in Figure 2. The

viability of PC12 cells decreased to 25.47% after a 2-h exposure to 100 μM H_2O_2. Compounds **9b** and **9d**, which possess 2, 3-dihydroxyl substituted phenyl group and 2, 3, 4-trihydroxyl substituted phenyl groups, respectively, showed antioxidant activity. The viability of the **9b**-pretreated PC12 cells was 54.46%. Moreover, **9d**, which was the most potent compound, protected the PC12 cells against H_2O_2 oxidation by 84.02% at 100 μM, which was comparable to protection shown by gallic acid (88.88%). However, **9a** and **9c**, which possess 4-hydroxyl substituted phenyl group and 2, 4-dihydroxyl substituted phenyl groups, did not exhibit any antioxidant activity.

(insert Figure 2)

As we mentioned above, some dimers show better bioactivities than their monomers do. Therefore, we tested the antioxidant activities of **9a** and its dimer **14a,** which did indeed show superior bioactivity to that of the monomer, **9a** (Figure 3).

(insert Figure 3)

These encouraging results above led us to synthesize the dimers of **14b-d** and subsequently evaluate their antioxidant activity (Figure 4). The bioassay showed that unlike their monomers, all the respective dimers possessed antioxidant activity. Moreover, the results were in agreement with our previous reports that dimers often show better antioxidant activity than their corresponding monomers (**14b-d** versus **9b-d**,

respectively). Among those dimers, **14b** and **14d** protected the PC12 cells against H_2O_2 oxidation by 78% and 85% respectively at 100 μM.

(insert Figure 4)

The effects of the test compounds on cell viability are shown in Table 2. After carefully analyzing the structures of **9a-d** and those of **14a-d**, we found that the catechol motif was essential for the antioxidant activity. We think that compared to the 4-hydroxyl phenyl and 2,4-dihydroxyl phenyl groups, the 2,3-dihydroxyl phenyl and 2,3,4-trihydroxyl phenyl groups can be more easily oxidized to *o*-quinone during the H_2O_2-induced oxidation process.

The structure-activity relationship (SAR) studies of these compounds identified the novel prenylated chalcone derivative, compound **14d**, as a highly potent antioxidant compound. Then, the molecular mechanism by which compound **14d** exerts its antioxidant activity was investigated.

As shown in Table 2, treatment with H_2O_2 (100 μM) for 2 h markedly decreased the cell viability. Hwang *et al*. [23] reported that oxidative stress induces apoptosis rather than necrosis in PC12 cells. The effect of compound **14d** on the morphological alterations in H_2O_2-injured PC12 cells was evaluated to confirm, whether the treatment with H_2O_2 could induce apoptosis in PC12 cells and whether compound **14d** could inhibit this effect. The results (Figure 5A) showed that treatment with H_2O_2 (100 μM) for 2 h markedly increased the typical apoptotic cell morphology

such as cell shrinkage, blebbing, or both. Morphological changes in the compound **14d**-treated PC12 cells suggested that it significantly protected them against H_2O_2-induced cell injury. These results indicate that compound **14d** might protect PC12 cells against oxidative stress injury caused by H_2O_2.

(insert Table 2)

To further study the effects of compound **14d** on apoptosis of pretreated H_2O_2-damaged PC12 cells, they were double-labeled with Annexin V-FITC and propidium iodide (PI) and then analyzed using flow cytometry (Figure 5B). The apoptotic rate (Annexin V^+/PI^- and Annexin V^+/PI^+) of the untreated H_2O_2-damaged PC12 cells was 77.6% while that of the compound **14d**-treated cells decreased to 3.8% of the total cells compared to 13.9% observed in the gallic acid-treated control cells. These results suggest that compound **14d** protected the H_2O_2-injured PC12 cells against apoptosis.

(insert Figure 5)

The effects of compound **14d** for the release of LDH, generation of 8-OHdG, and GSH activity were assessed. The cells were pretreated with 10 μM compound **14d** or gallic acid for 30 min and then incubated with H_2O_2 (100 μM, 2 h). LDH is a stable cytoplasmic enzyme present in all cells, and is rapidly released into the culture medium when the plasma membrane is damaged by an oxidant [24]. An increased LDH activity in

culture supernatant indicates an increase in the number of dead or plasma membrane-damaged cells. As shown in Figure 6A, treatment with H_2O_2 markedly increased the LDH level ($P < 0.01$) from 238.73 to 479.77 U/L. However, pre-incubation with gallic acid and compound **14d** significantly decreased the LDH levels to 326.59 and 251.45 U/L ($P < 0.05$ and $P < 0.01$), respectively.

DNA damage is one of the earliest recognized and most extensively studied manifestations of oxygen toxicity in biology. In nuclear and mitochondrial DNA, 8-hydroxy-2'-deoxyguanosine (8-OHdG) is one of the predominant forms of free radical-induced oxidative lesions and, therefore, has been widely used as a biomarker for oxidative stress and carcinogenesis [25]. As shown in Figure 6B, the 8-OHdG level in PC12 cells exposed to H_2O_2 significantly increased (123.97 ng/L, $P < 0.01$) compared to that in the control group (47.12 ng/L). However, pretreatment of PC12 cells with compound **14d** decreased 8-OHdG levels ($P < 0.01$) to 62.80 ng/L, which was better than the effect of gallic acid treatment (85.70 ng/L).

Antioxidant nutrients such as GSH act as the primary defense mechanisms that protect living systems from oxidative damage [26]. Exposure of PC12 cells to H_2O_2 significantly ($P < 0.01$) reduced the GSH level (Figure 6C) from 127.85 to 59.93 μmol/gprot, whereas pretreatment

with gallic acid and compound **14d** attenuated this effect (99.73 or 105.71 μmol/gprot, respectively.

(insert Figure 6)

The antioxidant activity of compound **14d** was evaluated using the ferric reducing antioxidant power (FRAP) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assays and the results are shown in Table 3. Compound **14d** exhibited better activity in the FRAP assay than the positive control, gallic acid did (648.44 and 531.02 mg/mM, equivalent amounts of vitamin C [Vc], respectively). The scavenging activity of compound **14d** against the ABTS radical was also evaluated and expressed as the half-maximal effective concentration (EC_{50}). Compound **14d** exhibited a high ABTS radical scavenging activity with an EC_{50} of 0.54 mM, which was slightly less than that of gallic acid (0.35 mM). This result suggests that compound **14d** possesses potent *in vitro* antioxidant properties.

4. Conclusions

In summary, ring-closed analogs (**9a-d**) of the natural prenylated chalcone desmethylxanthohumol (**1**) and their dimers (**14a-14d**) were synthesized from the commercially available 1-(2,4,6-trihydroxyphenyl)ethan-1-one (**3**) in five and six linear steps

respectively. The biological activity evaluation illustrated the following. 1) The ring-closed analog, **9a** showed a lower toxicity than compound **1** did on HUVECs and PC12 cells. 2) Compounds bearing 2,3-dihydroxyl phenyl and 2,3,4-trihydroxyl phenyl groups showed better antioxidant activity than their counterparts with 4-hydroxyl phenyl and 2,4-dihydroxyl phenyl groups did. 3) The dimers showed better antioxidant activity than their corresponding monomers did. 4) The most potent compound, **14d** protected the PC12 cell against H_2O_2 oxidation by 85% at 100 μM, which was comparable to the activity shown by gallic acid. 5) Pretreatment with compound **14d** significantly prevented the H_2O_2-induced apoptosis. 6) Compound **14d** exhibited protective effects on the release of LDH (a plasma membrane damage marker), production of 8-OHdG (a DNA damage marker), and a decrease in GSH (an antioxidant enzyme).

Studies are currently underway to clarify the mechanism of action of compound **14d** and identify its target molecule. Although compound **14d** showed a superior antioxidant activity *in vitro* to that of its parent structure, the chalcones, the molecular mechanism of this action is still unclear. Therefore, it is necessary to further study the *in vivo* antioxidant action and associated mediating pathways of compound **14d** in animal models [27-29].

5. Experimental section

5.1. Chemistry

All commercial materials and reagents were used without further purification, unless otherwise stated. All solvents were distilled prior to use. The solvents for reaction were distilled to remove water over Na or CaH_2. All reactions were carried out in oven-dried glassware under an inert atmosphere (nitrogen or argon). For chromatography, 200-300 mesh silica gel (Qingdao, China) was employed. ^{1}H and ^{13}CNMR spectra were recorded at 400 MHz and 100 MHz with a Brucker ARX 400 spectrometer. The chemical shifts (δ) for ^{1}H NMR spectra were given in parts per million (ppm) referenced to the residual proton signal of the duterated solvent ($CDCl_3$ at δ = 7.26 ppm, DMSO-d_6 at δ = 2.50 ppm and Acetone-d_6 at δ = 2.05 ppm); coupling constants were expressed in hertz (Hz). ^{13}C NMR spectra were referenced to the carbon signal of $CDCl_3$ (δ = 77.0 ppm), DMSO-d_6 (δ = 40.0 ppm) and Acetone-d_6 (δ = 30.0 ppm and 206.0 ppm). The following abbreviations are used to describe NMR signals: s = singlet, d = doublet, t = triplet, m = multiple, and dd = doublet of doublets. HRMS were recorded on Bruker Daltonics, Inc. APEXIII 7.0 TESLA FTMS (ESI). Known products were characterized by comparing to their corresponding ^{1}H-NMR reported in the literatures.

5.1.1. 1-(2-hydroxy-4,6-bis(methoxymethoxy)phenyl)ethan-1-one (4)

1-(2,4,6-trihydroxyphenyl)ethan-1-one (16.8 g, 100 mmol) in dry CH_2Cl_2 (500 mL) were added DMF (20 mL). The mixture was cooled to 0°C and N,N-diethylpropan-2-amine (39.3 g, 250 mmol) was added slowly. After addtion chloro(methoxy)methane (17.7 g, 220 mmol) in dry CH_2Cl_2 (100 mL) was added dropwised. The reaction mixture was warmed to room temperature and stirred for 6 h. The reaction was quenched with NH_4Cl (aq) and extracted with CH_2Cl_2 (3x500 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (petroleum ether/ethyl acetate 20:1) to afford a compound **4** (21.0 g, 82%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.66 (s, 1H), 6.17 (s, 2H), 5.19 (s, 2H), 5.10 (s, 2H), 3.46 (s, 3H), 3.41 (s, 3H), 2.58 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 203.1, 166.8, 163.4, 160.3, 106.8, 97.0, 94.4, 93.9, 93.9, 56.6, 56.3, 32.9.

5.1.2 1-(2,6-dihydroxy-4-(methoxymethoxy)phenyl)ethan-1-one (5)

Compound **4** (2.56 g, 10.0 mmol) in MeOH (50 mL) was cooled to 0 °C. Then 2N HCl (10 mL) was added dropwised. After addition, the reaction mixture was warmed to 40 °C and stirred for 3h. The reaction was quenched with $NaHCO_3$ (aq) and extracted with EtOAc (3x100 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (petroleum ether/ethyl acetate 10:1)

to afford a compound **5** (1.30 g, 61%) as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 12.26 (s, 2H), 6.02 (s, 2H), 5.17 (s, 2H), 3.36 (s, 3H), 2.58 (s, 3H).^{13}C NMR (100 MHz, DMSO) δ 203.8, 164.4, 163.6, 105.8, 95.1, 93.9, 56.3, 33.0.

5.1.3

1-(7-hydroxy-5-(methoxymethoxy)-2,2-dimethyl-2H-chromen-8-yl)ethan-1-one (6)

Compound **5** (2.12 g, 10.0 mmol) in dry pyridine (20 mL) was added 3-Methyl-2-butenal (1.26 g, 15.0 mmol). The reaction was heated to 120 °C for 16h. The mixture was cooled and pyridine was removed by distillation under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 mL) and poured into 1N HCl (20 mL). The organic phase was washed with $NaHCO_3$ (aq) (10 mL) and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (petroleum ether/ethyl acetate 50:1) to afford a compound **6** (2.09 g, 75%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.64 (s, 1 H), 6.58 (d, *J* = 10.0 Hz, 1 H), 6.20 (s, 1 H), 5.43 (d, *J* = 10.0 Hz, 1 H), 5.20 (s, 2 H), 3.47 (s, 3 H), 2.67 (s, 3 H), 1.50 (s, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.4, 164.9, 157.4, 155.4, 123.8, 115.5, 105.5, 102.2, 93.9, 93.2, 77.0, 55.5, 32.2, 26.9.

5.1.4 4-(methoxymethoxy)benzaldehyde (7a)

4-hydroxybenzaldehyde (1.22 g, 10 mmol) in dry CH_2Cl_2 (10 mL) was cooled to 0 °C. Then NaH (900 mg, 15 mmol) was added. After addition, chloro(methoxy)methane (965 mg, 12 mmol) in dry CH_2Cl_2 (10 mL) was added dropwised. The reaction was warmed to room temperature and stirred for 24 h. The reaction was quenched with NH_4Cl (aq) and extracted with CH_2Cl_2 (3x10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (petroleum ether/ethyl acetate 20:1) to afford a compound **7a** (1.50 g, 90%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 1H), 7.83(d, *J* = 8.8 Hz, 2H), 7.14(d, *J* = 8.8 Hz, 2H), 5.25(s, 2H), 3.49(s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 190.8 , 162.2 , 131.8 , 130.7 , 116.3 , 94.1 , 56.3 .

5.1.5 2,3-bis(methoxymethoxy)benzaldehyde (7b)

2,3-dihydroxybenzaldehyde (1.38 g, 10 mmol) in dry CH_2Cl_2 (10 mL) was cooled to 0 °C. Then NaH (1.80 g, 30 mmol) was added. After addition, chloro(methoxy)methane (1.93 g, 24 mmol) in dry CH_2Cl_2 (10 mL) was added dropwised. The reaction was warmed to room temperature and stirred for 24 h. The reaction was quenched with NH_4Cl (aq) and extracted with CH_2Cl_2 (3x10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column

chromatography on silica (petroleum ether/ethyl acetate 15:1) to afford a compound **7b** (1.90 g, 84%) as a colorless oil. ^{1}H NMR (400 MHz, CDCl3) δ 10.47 (s, 1H), 7.50 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.41 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.17-7.11 (m, 1H), 5.26 (s, 2H), 5.23 (s, 2H), 3.58 (s, 3H), 3.52 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 190.2, 150.1, 149.9, 130.6, 124.6, 122.3, 120.9, 99.7, 95.3, 57.9, 56.4.

5.1.6 2,4-bis(methoxymethoxy)benzaldehyde (7c)

2,4-dihydroxybenzaldehyde (1.38 g, 10 mmol) in dry CH_2Cl_2 (10 mL) was cooled to 0 °C. Then NaH (1.80 g, 30 mmol) was added. After addition, chloro(methoxy)methane (1.93 g, 24 mmol) in dry CH_2Cl_2 (10 mL) was added dropwised. The reaction was warmed to room temperature and stirred for 24 h. The reaction was quenched with NH_4Cl (aq) and extracted with CH_2Cl_2 (3x10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (petroleum ether/ethyl acetate 15:1) to afford a compound **7c** (1.94 g, 86%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.27 (s, 1H), 7.73 (d, *J* = 8.8 Hz, 1H), 6.76 (d, *J* = 2.0 Hz, 1H), 6.66-6.69 (m, 1H), 5.21 (s, 2H), 5.15 (s, 2H), 3.45 (s, 3H), 3.41 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 188.4, 163.5, 161.3, 130.2, 120.2, 109.5, 102.6, 94.7, 94.2, 56.6, 56.4.

5.1.7 2,3,4-tris(methoxymethoxy)benzaldehyde (7d)

2,3,4-trihydroxybenzaldehyde (1.54 g, 10 mmol) in dry CH_2Cl_2 (10 mL) was cooled to 0 °C. Then NaH (2.10 g, 35 mmol) was added. After addition, chloro(methoxy)methane (2.57 g, 32 mmol) in dry CH_2Cl_2 (10 mL) was added dropwised. The reaction was warmed to room temperature and stirred for 24 h. The reaction was quenched with NH_4Cl (aq) and extracted with CH_2Cl_2 (3x10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (petroleum ether/ethyl acetate 15:1) to afford a compound **7d** (2.70 g, 94%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.29 (s, 1H), 7.61 (d, *J* = 8.8 Hz, 1H), 7.04 (d, *J* = 8.8 Hz, 1H), 5.27 (s, 2H), 5.27 (s, 2H), 5.15 (s, 2H), 3.62 (s, 3H), 3.58 (s, 3H), 3.51 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.9 , 156.6 , 154.2 , 138.9 , 124.9 , 124.4 , 111.3 , 100.3 , 98.8 , 94.8 , 57.9 , 57.4 , 56.5 .

5.1.8

(E)-1-(7-hydroxy-5-(methoxymethoxy)-2,2-dimethyl-2H-chromen-8-yl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one (8a)

Compound **6** (0.10 g, 0.36 mmol) in EtOH (3 mL) was cooled to 0 °C. Then KOH (30% aq) (1 mL) was added slowly. After addtion, compound **7a** (72 mg, 0.43 mmol) was added. The reaction was heated to 50 °C for 6h. After cooling down to 0 °C, 2N HCl was added to the reaction mixture until the pH = 5-6. The percipitate was filtered and dried

to give compound **8a** (0.13 g, 83%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.0 (s, 1H), 8.02 (d, *J* = 15.6 Hz, 1H), 7.76 (d, *J* = 15.6 Hz, 1H), 7.55 (d, *J* = 8.8 Hz, 2H), 7.07 (d, *J* = 8.8 Hz, 2H), 6.62 (d, *J* = 10.0 Hz, 1H), 6.24 (s, 1H), 5.47 (d, *J* = 10.0 Hz, 1H), 5.22 (s, 4H), 3.493 (s, 3H), 3.489 (s, 3H), 1.54 (s, 6H).^{13}C NMR (100 MHz, $CDCl_3$) δ 193.0, 166.9, 159.0, 158.5, 155.8, 142.3, 129.9, 129.4, 125.6, 124.8, 116.8, 116.6, 107.0, 103.6, 95.3, 94.3, 94.2, 78.0, 56.5, 56.2, 28.0.

5.1.9

(E)-3-(2,3-bis(methoxymethoxy)phenyl)-1-(7-hydroxy-5-(methoxymethoxy)-2,2-dimethyl-2H-chromen-8-yl)prop-2-en-1-one (8b)

Compound **6** (0.10 g, 0.36 mmol) in EtOH (3 mL) was cooled to 0 °C. Then KOH (30% aq) (1 mL) was added slowly. After addtion, compound **7b** (98 mg, 0.43 mmol) was added. The reaction was heated to 50 °C for 6h. After cooling down to 0 °C, 2 NHCl was added to the reaction mixture until the pH = 5-6. The percipitate was filtered and dried to give compound **8b** (0.15 g, 86%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.05 (s, 1H), 8.23 (d, *J* = 16.0 Hz, 1H), 8.08 (d, *J* = 16.0 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.19 (d, *J* = 8.8 Hz, 1H), 7.08 (t, *J* = 8.0 Hz, 1H), 6.61 (d, *J* = 10.0 Hz, 1H), 6.25 (s, 1H), 5.47 (d, *J* = 10.0 Hz, 1H), 5.22 (s, 4H), 5.19 (s, 2H), 3.64 (s, 3H), 3.51 (s, 3H), 3.49 (s, 3H), 1.54 (s, 6H).^{13}C NMR (100 MHz, $CDCl_3$) δ 192.0, 165.9, 157.6, 154.9, 149.5,

145.4, 136.3, 129.8, 127.4, 123.9, 123.5, 119.0, 117.0, 115.7, 106.0, 102.6, 98.4, 94.3, 94.2, 93.3, 77.0, 56.9, 55.5, 55.3, 27.0 .

5.1.10

(E)-3-(2,4-bis(methoxymethoxy)phenyl)-1-(7-hydroxy-5-(methoxymethoxy)-2,2-dimethyl-2H-chromen-8-yl)prop-2-en-1-one (8c)

Compound **6** (0.10 g, 0.36 mmol) in EtOH (3 mL) was cooled to 0 °C. Then KOH (30% aq) (1 mL) was added slowly. After addtion, compound **7c** (98 mg, 0.43 mmol) was added. The reaction was heated to 50 °C for 6h. After cooling down to 0 °C, 2 NHCl was added to the reaction mixture until the pH = 5-6. The percipitate was filtered and dried to give compound **8c** (146 mg, 84%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.16 (s, 1H), 8.21 (d, *J* = 15.6 Hz, 1H), 8.02 (d, *J* = 15.6 Hz, 1H), 7.60 (d, *J* = 8.8 Hz, 1H), 6.87 (d, *J* = 2.4 Hz, 1H), 6.76 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H), 6.62 (d, *J* = 10.0 Hz, 1H), 6.24 (s, 1H), 5.47 (d, *J* = 10.0 Hz, 1H), 5.26 (s, 2H), 5.22 (s, 2H), 5.20 (s, 2H), 3.51 (s, 3H), 3.50 (s, 3H), 3.49 (s, 3H), 1.54 (s, 6H).^{13}C NMR (100 MHz, $CDCl_3$) δ 193.0, 166.9, 158.6, 155.9, 150.5, 146.4, 137.4, 130.8, 128.4, 125.0, 124.5, 120.0, 118.0, 116.7, 107.0, 103.6, 99.5, 95.3, 95.2, 94.3, 78.0, 58.0, 56.5, 56.3, 28.0.

5.1.11

(E)-1-(7-hydroxy-5-(methoxymethoxy)-2,2-dimethyl-2H-chromen-8-yl)-3-(2,3,4-tris(methoxymethoxy)phenyl)prop-2-en-1-one (8d)

Compound **6** (0.10 g, 0.36 mmol) in EtOH (3 mL) was cooled to 0 °C. Then KOH (30% aq) (1 mL) was added slowly. After addtion, compound **7d** (123 mg, 0.43 mmol) was added. The reaction was heated to 50 °C for 6h. After cooling down to 0 °C, 2 NHCl was added to the reaction mixture until the pH = 5-6. The percipitate was filtered and dried to give compound **8d** (180 mg, 91%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.2 (s, 1H), 8.16 (d, *J* = 15.6 Hz, 1H), 8.04 (d, *J* = 15.6 Hz, 1H), 7.39 (d, *J* = 8.8 Hz, 1H), 7.00 (d, *J* =8.8 Hz, 1H), 6.61 (d, *J* = 10.0 Hz, 1H), 6.24 (s, 1H), 5.47 (d, *J* = 10.0 Hz, 1H), 5.25 (s, 2H), 5.21 (s, 2H), 5.20 (s, 2H), 5.16 (s, 2H), 3.64 (s, 3H), 3.62 (s, 3H), 3.52 (s, 3H), 3.49 (s, 3H), 1.54 (s, 6H).^{13}C NMR (100 MHz, $CDCl_3$) δ192.9, 166.9, 158.5, 155.8, 153.0, 150.9, 139.7, 137.5, 126.7, 124.9, 124.7, 122.5, 116.8, 111.9, 107.0, 103.5, 100.0, 98.8, 95.3, 95.0, 94.3, 78.0, 58.1, 57.4, 56.5, 56.4, 28.0.

5.1.12

(E)-1-(5,7-dihydroxy-2,2-dimethyl-2H-chromen-8-yl)-3-(4-hydroxyphenyl)prop-2-en-1-one (9a)

Compound **8a** (50 mg, 0.12 mmol) in MeOH (4 mL) and THF (1 mL) was added. The reaction mixture was cooled to 0 °C, then 2N HCl (1 mL) was added. After addtion, the mixture was heated to 60 °C for 30 min. The mixture was poured in cold $NaHCO_3$ (aq) and extracted with EtOAc (3x10 mL). The combined organic layers were washed with brine,

dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (petroleum ether/ethyl acetate 4:1) to afford a compound **9a** (15 mg, 38%) as a red solid. ^{1}H NMR (400 MHz, DMSO) δ 13.84 (s, 1H), 10.83 (s, 1H), 10.17 (s, 1H), 7.84 (d, *J* = 16.0 Hz, 1H), 7.64 (d, *J* = 16.0 Hz, 1H), 7.55 (d, *J* = 8.6 Hz, 2H), 6.87 (d, *J* = 8.6 Hz, 2H), 6.52 (d, *J* = 10.0 Hz, 1H), 5.96 (s, 1H), 5.57 (d, *J* = 10.0 Hz, 1H), 1.49 (s, 6H).^{13}C NMR (100 MHz, DMSO) δ 192.4, 165.3, 160.5, 160.1, 155.9, 143.3, 130.8, 126.4, 125.1, 124.0, 116.9, 116.5, 105.6, 102.5, 96.2, 78.1, 28.0; HRMS (ESI) m/z: Calcd for $C_{20}H_{17}O_5$ $[M-H]^-$ 337.1081; found, 337.1086.

5.1.13

(E)-1-(5,7-dihydroxy-2,2-dimethyl-2H-chromen-8-yl)-3-(2,3-dihydroxyphenyl)prop-2-en-1-one (9b)

Compound **8b** (50 mg, 0.102 mmol) in MeOH (4 mL) and THF (1 mL) was added. The reaction mixture was cooled to 0 °C, then 3N HCl (1 mL) was added. After addtion, the mixture was heated to 60 °C for 30 min. The mixture was poured in cold $NaHCO_3$ (aq) and extracted with EtOAc (3x10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (petroleum ether/ethyl acetate 2:1) to afford a compound **9b** (6 mg, 16%) as a red solid. ^{1}H NMR (400 MHz, DMSO) δ 13.83 (s, 1H), 10.83 (s, 1H), 9.70 (s,

1H), 9.19 (s, 1H), 8.04 (d, *J* = 16.0 Hz, 1H), 7.64 (d, *J* =16.0 Hz, 1H), 7.01 (d, *J* = 8.0 Hz, 1H), 6.85 (d, *J* = 8.0 Hz, 1H), 6.70-6.74 (m, 1H), 6.50 (d, *J* = 10.0 Hz, 1H)， 5.9,5 (s, 1H), 5.54 (d, *J* = 10.0 Hz, 1H), 1.48 (s, 6H).^{13}C NMR (100 MHz, DMSO) δ 192.8, 165.8, 160.3, 156.1, 146.4, 146.3, 139.2, 126.9, 125.3, 122.8, 119.8, 119.3, 117.4, 116.8, 105.8, 102.6, 96.2, 78.3, 27.8; HRMS (ESI) m/z: Calcd for $C_{20}H_{17}O_6$ $[M-H]^-$ 353.1031; found, 353.1041.

5.1.14

(E)-1-(5,7-dihydroxy-2,2-dimethyl-2H-chromen-8-yl)-3-(2,4-dihydroxyphenyl)prop-2-en-1-one (9c)

Compound **8c** (50 mg, 0.102 mmol) in MeOH (4 mL) and THF (1 mL) was added. The reaction mixture was cooled to 0 °C, then 3N HCl (1 mL) was added. After addtion, the mixture was heated to 60 °C for 30 min. The mixture was poured in cold $NaHCO_3$ (aq) and extracted with EtOAc (3x10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (petroleum ether/ethyl acetate 2:1) to afford a compound **9c** (10 mg, 27%) as a red solid. ^{1}H NMR (400 MHz, DMSO) δ 14.21 (s, 1H), 10.74 (s, 1H), 10.31 (s, 1H), 9.98 (s, 1H), 8.05 (d, *J* = 15.6 Hz, 1H), 7.85 (d, *J* = 15.6 Hz, 1H), 7.34 (d, *J* = 8.4 Hz, 1H), 6.50 (d, *J* = 10.0 Hz, 1H), 6.41 (d, *J* = 2.4 Hz, 1H), 6.35-6.30 (m, 1H), 5.92 (s, 1H), 5.53 (d, *J* = 10.0 Hz, 1H), 1.49 (s,

6H).^{13}C NMR (100 MHz, DMSO) δ 192.8 , 166.0 , 161.7, 159.9, 159.8, 156.1, 140.6, 132.2, 125.1, 123.3, 117.0, 114.2, 108.5, 105.6, 103.2, 102.5, 96.2, 78.1, 27.7; HRMS (ESI) m/z: Calcd for $C_{20}H_{17}O_6$ $[M-H]^-$ 353.1031; found, 353.1034.

5.1.15

(E)-1-(5,7-dihydroxy-2,2-dimethyl-2H-chromen-8-yl)-3-(2,3,4-trihydroxyphenyl)prop-2-en-1-one (9d)

Compound **8d** (50 mg, 0.09 mmol) in MeOH (4 mL) and THF (1 mL) was added. The reaction mixture was cooled to 0 °C, then 3N HCl (1 mL) was added. After addtion, the mixture was heated to 60 °C for 30 min. The mixture was poured in cold $NaHCO_3$ (aq) and extracted with EtOAc (3x10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (petroleum ether/ethyl acetate 2:1) to afford a compound **9d** (6 mg, 18%) as a red solid. ^{1}H NMR (400 MHz, DMSO) δ 14.12 (s, 1H), 10.73 (s, 1H), 9.84 (s, 1H), 9.24 (s, 1H), 8.61 (s, 1H), 7.99 (d, *J* = 16.0 Hz, 1H), 7.90 (d, *J* = 16.0 Hz, 1H), 6.92 (d, *J* = 8.8 Hz, 1H), 6.49 (d, *J* = 10.0 Hz, 1H), 6.41 (d, *J* = 8.8 Hz, 1H), 5.93 (s, 1H), 5.53 (d, *J* = 10.0 Hz, 1H), 1.48 (s, 6H).^{13}C NMR (100 MHz, DMSO) δ 192.7, 165.9, 160.0, 156.0, 149.6, 148.1, 140.8, 133.5, 125.2, 123.4, 120.6, 116.9, 115.2, 108.6, 105.7, 102.5, 96.2,

78.1, 27.8; HRMS (ESI) m/z: Calcd for $C_{20}H_{17}O_7$ $[M-H]^-$ 369.0980; found, 369.0981.

5.1.16 1-(5,7-dihydroxy-2,2-dimethyl-2H-chromen-8-yl)ethan-1-one (11)

Compound **6** (557 mg, 2.0 mmol) in MeOH (10 mL) was cooled to 0 °C, then 3N HCl (2.5 mL) was added. After addtion, the mixture was heated to 60 °C for 6h. The mixture was poured in cold $NaHCO_3$ (aq) and extracted with EtOAc (3x20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (petroleum ether/ethyl acetate 20:1) to afford a compound **11** (250 mg, 53%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 13.55 (s, 1H), 10.83 (s, 1H), 6.47 (d, *J* = 10.0 Hz, 1H), 5.90 (s, 1H), 5.52 (d, *J* = 10.0 Hz, 1H), 2.58 (s, 3H), 1.45 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ 203.0, 165.3, 160.4, 157.0, 125.2, 116.7, 105.1, 102.3, 95.8, 78.3, 33.3, 27.8.

5.1.17 1,1'-(methylenebis(5,7-dihydroxy-2,2-dimethyl-2H-chromene-6,8-diyl))bis(ethan-1-one) (12)

Compound **11** (234 mg, 1.0 mmol) in CH_2Cl_2 (10 mL) was added polyformaldehyde (15 mg, 0.5 mmol). The reaction mixture was heated to 60 °C for 8h. Solvent was removed under reduced pressure and the residue was purified by by column chromatography on silica (petroleum

ether/ethyl acetate 50:1) to afford a compound **12** (170 mg, 71%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.81 (s, 2H), 9.43 (s, 2H), 6.63 (d, *J* = 10.0 Hz, 2H), 5.43 (d, *J* = 10.0 Hz, 2H), 3.75 (s, 2H), 2.67 (s, 6H), 1.47 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.8, 160.2, 157.3, 154.9, 124.0, 116.0, 104.9, 104.0, 102.4, 77.1, 31.7, 26.9, 14.4.

5.1.18

(2E,2'E)-1,1'-(methylenebis(5,7-dihydroxy-2,2-dimethyl-2H-chromene-6,8-diyl))bis(3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one) (13a)

Compound **12** (0.24 g, 0.5 mmol) in EtOH (3 mL) was cooled to 0 °C. Then KOH (30% aq) (1 mL) was added slowly. After addtion, compound **7a** (200 mg, 1.2 mmol) was added. The reaction was heated to 50 °C for 6h. After cooling down to 0 °C, 2N HCl was added to the reaction mixture until the pH = 5-6. The percipitate was filtered and dried to give compound **13a** (343 mg, 88%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.69 (s, 2H), 8.11 (d, *J* = 15.6 Hz, 2H), 7.82 (d, *J* = 15.6 Hz, 2H), 7.56 (d, *J* = 8.8 Hz, 4H), 7.08 (d, *J* = 8.8 Hz, 4H), 6.68 (d, *J* = 10.0 Hz, 2H), 5.48 (d, *J* = 10.0 Hz, 2H), 5.22 (s, 4H), 3.82 (s, 2H), 3.49 (s, 6H), 1.53 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.7, 162.9, 159.1, 158.7, 155.3, 143.2, 130.1, 129.3, 125.0, 124.9, 117.4, 116.6, 106.6, 105.3, 103.8, 94.2, 78.1, 56.2, 28.0, 15.8.

5.1.19

(2E,2'E)-1,1'-(methylenebis(5,7-dihydroxy-2,2-dimethyl-2H-chromen

e-6,8-diyl))bis(3-(2,3-bis(methoxymethoxy)phenyl)prop-2-en-1-one) (13b)

Compound **12** (0.24 g, 0.5 mmol) in EtOH (3 mL) was cooled to 0 °C. Then KOH (30% aq) (1 mL) was added slowly. After addtion, compound **7b** (270 mg, 1.2 mmol) was added. The reaction was heated to 50 °C for 6h. After cooling down to 0 °C, 2N HCl was added to the reaction mixture until the pH = 5-6. The percipitate was filtered and dried to give compound **13b** (367 mg, 82%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.70 (s, 2H), 8.30 (d, *J* = 16.0 Hz, 2H), 8.17 (d, *J* = 16.0 Hz, 2H), 7.34 (dd, *J* = 8.0, 1.2 Hz, 2H), 7.20 (dd, *J* = 8.0, 1.2 Hz, 2H), 7.09 (t, *J* = 8.0 Hz, 2H), 6.67 (d, *J* = 10.0 Hz, 2H), 5.48 (d, *J* = 10.0 Hz, 2H), 5.22 (s, 4H), 5.20 (s, 4H), 3.82 (s, 2H), 3.66 (s, 6H), 3.52 (s, 6H), 1.52 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.9, 163.0, 158.8, 155.4, 150.5, 146.5, 138.4, 130.7, 127.7, 125.1, 124.6, 120.1, 118.2, 117.2, 106.6, 105.3, 103.8, 99.5, 95.2, 78.2, 58.0, 56.3, 28.0, 15.8.

5.1.20 (2E,2'E)-1,1'-(methylenebis(5,7-dihydroxy-2,2-dimethyl-2H-chromen e-6,8-diyl))bis(3-(2,4-bis(methoxymethoxy)phenyl)prop-2-en-1-one) (13c)

Compound **12** (0.24 g, 0.5 mmol) in EtOH (3 mL) was cooled to 0 °C. Then KOH (30% aq) (1 mL) was added slowly. After addtion, compound **7c** (270 mg, 1.2 mmol) was added. The reaction was heated to

50 °C for 6h. After cooling down to 0 °C, 2N HCl was added to the reaction mixture until the pH = 5-6. The percipitate was filtered and dried to give compound **13c** (250 mg, 56%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.71 (s, 2H), 8.26 (d, *J* = 16.0 Hz, 2H), 8.09 (d, *J* = 16.0 Hz, 2H), 7.60 (d, *J* = 8.8 Hz, 2H), 6.87 (d, *J* = 2.4 Hz, 2H), 6.76 (dd, *J* = 8.8, 2.4 Hz, 2H), 6.68 (d, *J* = 10.0Hz, 2H), 5.47 (d, *J* = 10.0 Hz, 2H), 5.27 (s, 4H), 5.20 (s, 4H), 3.82 (s, 2H), 3.52 (s, 6H), 3.50 (s, 6H), 1.52 (s, 12H).^{13}C NMR (100 MHz, $CDCl_3$) δ 192.9, 163.0, 160.5, 158.6, 157.8, 155.3, 138.2, 128.4, 125.0, 124.7, 119.3, 117.4, 109.6, 106.6, 105.3, 103.7, 103.6, 94.8, 94.3, 78.0, 56.4, 56.3, 28.0, 15.8.

5.1.21 (2E,2'E)-1,1'-(methylenebis(5,7-dihydroxy-2,2-dimethyl-2H-chromene-6,8-diyl))bis(3-(2,3,4-tris(methoxymethoxy)phenyl)prop-2-en-1-one) (13d)

Compound **12** (0.24 g, 0.5 mmol) in EtOH (3 mL) was cooled to 0 °C. Then KOH (30% aq) (1 mL) was added slowly. After addtion, compound **7d** (340 mg, 1.2 mmol) was added. The reaction was heated to 50 °C for 6h. After cooling down to 0 °C, 2N HCl was added to the reaction mixture until the pH = 5-6. The percipitate was filtered and dried to give compound **13d** (387 mg, 76%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.72 (s, 2H), 8.24 (d, *J* = 15.6 Hz, 2H), 8.13 (d, *J* = 15.6 Hz, 2H), 7.40 (d, *J* = 8.8 Hz, 2H), 7.01 (d, *J* = 8.8 Hz, 2H), 6.66 (d, *J* = 10.0

Hz, 2H), 5.47 (d, *J* = 10.0 Hz, 2H), 5.25 (s, 4H), 5.21 (s, 4H), 5.16 (s, 4H), 3.81 (s, 2H), 3.65 (s, 6H), 3.62 (s, 6H), 3.52 (s, 6H), 1.52 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.7, 163.1, 158.7, 155.3, 153.2, 151.1, 139.7, 138.5, 125.9, 125.0, 124.6, 122.6, 117.3, 112.0, 106.6, 105.2, 103.7, 100.0, 98.8, 95.0, 78.1, 58.2, 57.4, 56.4, 28.0, 15.8.

5.1.22

(2E,2'E)-1,1'-(methylenebis(5,7-dihydroxy-2,2-dimethyl-2H-chromene-6,8-diyl))bis(3-(4-hydroxyphenyl)prop-2-en-1-one) (14a)

Compound **13a** (78 mg, 0.10 mmol) in MeOH (3 mL) and THF (3 mL) was added. The reaction mixture was cooled to 0 °C, then 2N HCl (1 mL) was added. After addtion, the mixture was heated to 40 °C for 10 h. The mixture was poured in cold $NaHCO_3$ (aq) and extracted with EtOAc (3x10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (petroleum ether/ethyl acetate 4:1) to afford a compound **14a** (28 mg, 40%) as a red solid. ^{1}H NMR (400 MHz, DMSO) δ 15.36 (s, 2H), 10.17 (s, 2H), 7.92 (d, *J* = 15.6 Hz, 2H), 7.72 (d, *J* = 15.6 Hz, 2H), 7.56 (d, *J* = 8.8 Hz, 4H), 6.87 (d, *J* = 8.8 Hz, 4H), 6.63 (d, *J* = 10.0 Hz, 2H), 5.60 (d, *J* = 10.0 Hz, 2H), 3.78 (s, 2H), 1.50 (s, 12H). ^{13}C NMR (100 MHz, DMSO) δ 192.5, 163.7, 160.7, 154.5, 143.9, 130.9, 126.4, 125.7, 123.6, 117.2, 116.6, 107.3, 105.2,

103.0, 78.0, 27.9, 16.3; HRMS (ESI) m/z: Calcd for $C_{41}H_{35}O_{10}$ $[M-H]^-$ 687.2236; found, 687.2219.

5.1.23

(2E,2'E)-1,1'-(methylenebis(5,7-dihydroxy-2,2-dimethyl-2H-chromene-6,8-diyl))bis(3-(2,3-dihydroxyphenyl)prop-2-en-1-one) (14b)

Compound **13b** (90 mg, 0.10 mmol) in MeOH (3 mL) and THF (3 mL) was added. The reaction mixture was cooled to 0 °C, then 2N HCl (1 mL) was added. After addtion, the mixture was heated to 40 °C for 10 h. The mixture was poured in cold $NaHCO_3$ (aq) and extracted with EtOAc (3x10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (petroleum ether/ethyl acetate 2:1) to afford a compound **14b** (26 mg, 37%) as a red solid. ^{1}H NMR (400 MHz, DMSO) δ 15.30 (s, 2H), 9.71 (s, 2H), 9.22 (s, 2H), 8.15 (d, *J* = 15.6 Hz, 2H), 7.99 (d, *J* = 15.6 Hz, 2H), 7.02 (d, *J* = 7.2 Hz, 2H), 6.86 (d, *J* = 7.6 Hz, 2H), 6.72 (t, *J* = 7.8 Hz, 2H), 6.61 (d, *J* =10.0 Hz, 2H), 5.55 (d, *J* = 9.6 Hz, 2H), 3.76 (s, 2H), 1.48 (s, 12H). ^{13}C NMR (100 MHz, DMSO) δ 193.0, 163.7, 154.6, 146.6, 146.3, 139.8, 126.5, 125.9, 122.7, 119.8, 119.4, 117.6, 117.2, 107.2, 105.3, 103.0, 79.6, 78.2, 27.8, 16.3; HRMS (ESI) m/z: Calcd for $C_{41}H_{35}O_{12}$ $[M-H]^-$ 719.2134; found, 719.2112.

5.1.24

(2E,2'E)-1,1'-(methylenebis(5,7-dihydroxy-2,2-dimethyl-2H-chromene-6,8-diyl))bis(3-(2,4-dihydroxyphenyl)prop-2-en-1-one) (14c)

Compound **13c** (90 mg, 0.10 mmol) in MeOH (3 mL) and THF (3 mL) was added. The reaction mixture was cooled to 0 °C, then 2N HCl (1 mL) was added. After addtion, the mixture was heated to 40 °C for 10 h. The mixture was poured in cold $NaHCO_3$ (aq) and extracted with EtOAc (3x10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (petroleum ether/ethyl acetate 2:1) to afford a compound **14c** (25 mg, 35%) as a red solid. ^{1}H NMR (400 MHz, DMSO) δ 16.20 (s, 2H), 10.41 (s, 2H), 10.06 (s, 2H), 8.16 (d, *J* = 15.6 Hz, 2H), 7.94 (d, *J* = 15.6 Hz, 2H), 7.36 (d, *J* = 8.8 Hz, 2H), 6.60 (d, *J* = 10.0 Hz, 2H), 6.43 (d, *J* = 2.4 Hz, 2H), 6.34 (dd, *J* = 8.8, 2.4 Hz, 2H), 5.57 (d, *J* = 10.0 Hz, 2H), 3.75 (s, 2H), 1.49 (s, 12H). ^{13}C NMR (100 MHz, DMSO) δ 192.8, 163.8, 162.0, 160.2, 154.7, 141.7, 132.5, 125.7, 122.7, 117.2, 114.3, 108.7, 107.2, 105.1, 103.2, 103.1, 78.1, 66.8, 27.7, 16.0; HRMS (ESI) m/z: Calcd for $C_{41}H_{35}O_{12}$ $[M-H]^-$ 719.2134; found, 719.2106.

5.1.25

(2E,2'E)-1,1'-(methylenebis(5,7-dihydroxy-2,2-dimethyl-2H-chromene-6,8-diyl))bis(3-(2,3,4-trihydroxyphenyl)prop-2-en-1-one) (14d)

Compound **13d** (102 mg, 0.10 mmol) in MeOH (3 mL) and THF (3 mL) was added. The reaction mixture was cooled to 0 °C, then 2N HCl (1 mL) was added. After addtion, the mixture was heated to 40 °C for 10 h. The mixture was poured in cold $NaHCO_3$ (aq) and extracted with EtOAc (3x10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (petroleum ether/ethyl acetate 1:1) to afford a compound **14d** (20 mg, 26%) as a red solid. ^{1}H NMR (400 MHz, DMSO) δ 16.07 (s, 2H), 9.89 (s, 2H), 9.32 (s, 2H), 8.66 (s, 2H), 8.09 (d, *J* = 15.6 Hz, 2H), 7.98 (d, *J* = 15.6 Hz, 2H), 6.93 (d, *J* = 8.8 Hz, 2H), 6.60 (d, *J* = 10.0 Hz, 2H), 6.42 (d, *J* = 8.8 Hz, 2H), 5.56 (d, *J* = 10.0 Hz, 2H), 3.74 (s, 2H), 1.48 (s, 12H).^{13}C NMR (100 MHz, DMSO) δ 192.8, 163.7, 154.7, 149.9, 148.5, 133.5, 126.0, 122.7, 121.2, 117.1, 115.2, 108.5, 107.1, 105.2, 103.0, 79.6, 78.2, 27.7, 16.2; HRMS (ESI) m/z: Calcd for $C_{41}H_{35}O_{14}$ [M-H]- 751.2032; found, 751.2030

5.2 Biology evaluation

5.2.1 Cell lines and culture conditions

PC12 cell line was obtained from the Shanghai Institutes of Biological Sciences (Shanghai, China). Cells were grown at 37 °C in RPMI-1640 supplemented with 10% fetal bovine serum, 2.05 mM glutamine, and 1%

penicillin/streptomycin in a humidified atmosphere containing 5% CO_2. The medium was replaced once every third day.

5.2.2 Cytotoxic analysis in human normal cells line HUVEC

HUVEC cells (100 μL) were cultured in 96-well plates at a density of 5 ×10^3 cells per well for 24 h. 0.5 μL of compounds (solved in DMSO) were added to each well to culture for additional 48 h. Then 20 μL of MTT solution (5 mg/ml) was added to each well and incubated for another 4 h. The cells in each well were then solubilized with DMSO (100 μL for each well) and the optical density (OD) was recorded at 490 nm. DMSO was used as positive control and the IC_{50} values were derived from the mean OD values of the triplicate tests versus using Graph Pad Prism 5.0.

5.2.3 MTT assay in H_2O_2-damaged PC12 cells

H_2O_2-induced PC12 cells oxidative-damage model was established and the cell viability was evaluated by MTT assay as a measure of the antioxidant activity of test compounds. Gallic acid, a well-known potent antioxidant, was used as a positive control. Briefly, PC12 cells were plated in 96-well plates at a density of 5×10^5 cells per well in 90 μL medium. After 24 h incubation, DMSO, test compound, or gallic acid (0.01, 0.1, 1, 10 and 100 μM) were added to each well and incubated for 0.5 h. Subsequently, H_2O_2 (100 μM) was added and cells were incubated for 2 h to induce cell injury. MTT assay was performed as mentioned

above. For each treatment, the mean cell viability was calculated from three independent experiments. The DMSO-treated controls were assigned a cell viability value of 100%.

5.2.4 Flow cytometric analysis of apoptosis

Cells were assayed by the Annexin-V-FITC Apoptosis Detection Kit (BD Biosciences, USA) according to the manufacturer's instructions [30]. Briefly, PC12 cells were treated using the same method as for MTT assay. The concentrations of compound **14d** or gallic acid were 10 μM. Damaged PC12 cells were harvested, washed twice with ice-cold PBS, and resuspended in 1× binding buffer at a concentration of 1×10^6 cells/mL. Subsequently, the cells were stained with 5 μL Annexin-V-FITC and 5 μL PI (50 μg/mL) for 15 min in the dark at 25 °C, and analyzed by flow cytometry.

5.2.5 LDH assay

The plasma membrane damage of PC12 cells was determined by the release of LDH into the medium [24] . PC12 cells (3×10^5 cells/mL in 6 cm dish) were treated as described in MTT assay. The concentration of compound **14d** or gallic acid was 10 μM. The supernatant was harvested for the spectrophotometric determination of the LDH release using an assay kit (Nanjing Jiancheng Co., China) according to the manufacturer's protocol; the absorbance of samples was read at 490 nm.

5.2.6 8-OHdG assay

8-hydroxy-2' -deoxyguanosine (8-OHdG) is one of the predominant forms of free radical-induced oxidative lesions, and has therefore been widely used as a biomarker for oxidative stress [25]. PC12 cells (3×10^5 cells/mL in 6 cm dish) were treated using the same method as described for MTT assay. The concentration of compound **14d** or gallic acidwas 10 μM. Damaged PC12 cells were harvested by centrifugation, washed twice with PBS (pH 7.0), and resuspended with CHAPS lysis buffer (110 μL) for 30 min on ice. After repeated freezing and thawing for 5 times, the lysate was centrifuged (15000 rpm, 15 min) to obtain supernatant. Oxidative DNA damage (8-hydroxy-2'-deoxyguanosine/deoxyguanosine ratio) was assayed by the assay kit (Nanjing Jiancheng Co., China) according to the manufacturer's instructions.

5.2.7 GSH assay

Glutathione (GSH) is an important antioxidant enzyme. Reduced GSH levels indicate oxidative stress and decreased efficiency of antioxidant system [26] . GSH peroxidase activity was measured at 405 nm based on the rate of oxidation of reduced glutathione to oxidized glutathione by H_2O_2 under the catalysis of glutathione peroxidase. PC12 cells (3×10^5 cells/mL in 6 cm dish) were treated using the same method as described in MTT assay. The concentration of compound **14d** or gallic acidwas 10 μM. Damaged PC12 cells were harvested by centrifugation, washed twice with PBS (pH 7.0), and resuspended in CHAPS lysis buffer

(110 μL) for 30 min on ice. The lysate was centrifuged (15000 rpm, 15 min) to obtain supernatant. GSH assay was performed using an assay kit (Nanjing Jiancheng Co., China) according to the manufacturer's instructions.

5.2.8 Antioxidant activity by FRAP assay

The ferric reducing antioxidant power (FRAP) measures the antioxidant capacity to reduce the Fe^{3+}/tripyridyl-s-triazine (TPTZ) complex to the ferrous form [31]. A mixture containing phosphate buffer (0.2 M, pH 6.6, 25 μL) and potassium ferricyanide (1.0% w/v, 25 μL) was freshly prepared and warmed to 37 °C. Different concentrations of the compound **14d** or DMSO (10 μL) were added to the mixture and incubated at 50 °C for 20 min. Subsequently, trichloroacetic acid (25 μL 10%), H_2O (25 μL) and ferric chloride (25 μL) were added to the mixture and incubated for 10 min. The decrease in absorption of the complex was measured at 660 nm. Each analysis was performed in triplicate. The vitamin C (Vc) standard was diluted to different concentrations (100-600 mg/mL) for the experiment. The ferric-reducing antioxidant power in the reaction medium was calculated from the calibration curve derived from a serial dilution of the Vc standard. The equivalent amounts of Vc for test compound were calculated from three independent experiments.

5.2.9 Antioxidant activity by ABTS assay

Radical scavenging activity of compound **14d** was measured by a modified ABTS assay [32]. The ABTS radical cation assay is one of the most commonly used methods for screening and evaluating antioxidants currently. To prepare ABTS stock solution, ABTS powder was dissolved in sodium acetate buffer to a final concentration of 10 mM. H_2O_2 (35%, 172.7 μL) was added to the ABTS solution to produce ABTS radical cation ($ABTS^+$). The $ABTS^+$ mixture was kept in dark for 12 h before use. The ABTS working solution was freshly prepared by diluting $ABTS^+$ mixture with sodium acetate to an absorbance of 0.70 at 650 nm. The ABTS assay was performed in 96-well plates containing test compounds (5.5 μL) and ABTS working solution (130 μL). The mixture was gently shaken by a microplate reader for 30 seconds. The absorption of the complex was measured after 10 min at 650 nm. The EC_{50} values for the test compound were calculated from three independent experiments by nonlinear regression using Graph Pad Prism 5.0.

5.3 Statistical analysis

All data were expressed as mean ± S.D. Results were analyzed by one-way analysis of variance (ANOVA), and significant differences were determined by post-hoc Tukey test using SPSS 21.0 software. ##$P < 0.01$ compared to control cells; **$P < 0.01$ compared to H_2O_2-treated cells.

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Figure captions:

Fig. 1 Toxicity of compound **1** and **9a** on PC12 cell. Cell viability of test compound-treated PC12 cells was evaluated by MTT assay. IC_{50} was calculated to reflect the cytotoxicity of test compounds.

Fig. 2 Antioxidant activity of compound 9a, 9b, 9c, 9d and Gallic acid. Antioxidant activity of test compounds was evaluated using the H_2O_2-induced PC12 cells oxidative-damage model. Cell viability in test compound (9a, 9b, 9c, 9d, 100 μM) pre-treated PC12 cells was evaluated by MTT assay. The DMSO-treated controls were assigned a cell viability value of 100%. Gallic acid was used as a positive control. ##P < 0.01 compared to control cells; **P < 0.01 compared to H_2O_2-treated cells.

Fig. 3 Comparsion of antioxidant activity between compound 9a and 14a.

Antioxidant activity of test compounds was evaluated using the H_2O_2-induced PC12 cells oxidative-damage model. Cell viability in test compound (9, 14a, 100 μM) pre-treated PC12 cells was evaluated by MTT assay. The DMSO-treated controls were assigned a cell viability value of 100%. Gallic acid was used as appositive control. ##P < 0.01 compared to control cells; **P < 0.01 compared to H_2O_2-treated cells.

Fig. 4 Comparsion of antioxidant activity between monomer 9b, 9c, 9d and 14b, 14c, 14d

Antioxidant activity of test compounds was evaluated using the H_2O_2-induced PC12 cells oxidative-damage model. Cell viability in test compound (9b, 9c, 9d, 14b, 14c, 14d, 100 μM) pre-treated PC12 cells was evaluated by MTT assay. The DMSO-treated controls were assigned a cell viability value of 100%. Gallic acid was used as appositive control. ##P < 0.01 compared to control cells; **P < 0.01 compared to H_2O_2-treated cells.

Fig. 5 Compound **14d** attenuated H_2O_2-induced apoptosis of PC12 cells. (A) Morphological changes (at 200 ×) in PC12 cells treated with H_2O_2 (100 μM, 2 h) and compound **14d** (10 μM, 0.5 h). Control cells were only treated with medium containing 0.1% dimethyl sulfoxide (DMSO). Cells treated with gallic acid (10 μM) were used as positive control. (B) The protective effect of compound **14d** (10 μM) on apoptosis in H_2O_2-injured PC12 cells using double staining with Annexin V/PI. Gallic acid (10 μM) was used as positive control.

Fig. 6 Effects of compound **14d** pretreatment on the release of LDH (A); the generation of 8-OHdG (B); and the level of GSH (C), in H_2O_2-injured PC12 cells. Cells were pretreated with compound **14d** (10 μM) for 0.5 h and then incubated with H_2O_2 (100 μM, 2 h). All data are presented as means ± SD, n = 3. ##P < 0.01 compared to control cells; *P < 0.05, **P < 0.01 compared to H_2O_2-treated cells.

Table 1 Toxicity of compound **1** and **9a** on HUVEC cell.

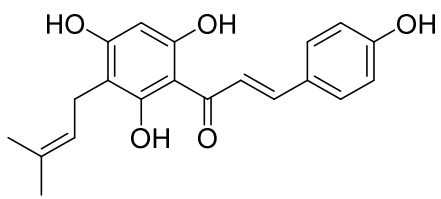
(μM)	Compound 1	Compound 9a
Mean IC_{50}	18.92	41.15
S.D.	1.02	7.56

Table 2 The cell viability after pre-treated with test compound (100 μM) in H_2O_2-damaged PC12 cells.

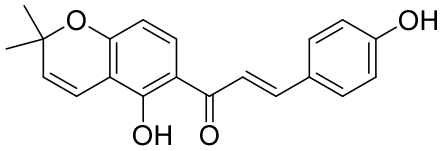
Compound	9a	9b	9c	9d	14a	14b	14c	14d
Cell Viability (%)	20.59	54.46	24.08	84.02	44.86	77.54	43.40	85.25
S.D.	4.46	2.97	2.04	2.53	1.79	3.90	1.80	3.39

Table 3 Equivalent amounts of Vc and EC_{50} values representing $ABTS^{+}$ radicals scavenging activity of compound **14d** and gallic acid. Each value represents percent means ± SD of three independent experiments conducted in triplicate. EC_{50} indicates concentration of drug causing 50% scavenging of $ABTS^{+}$ radicals.

Compound	Equivalent amount of Vc (mg/mmol)	Scavenging activity of $ABTS^{+}$ (EC_{50}, μM)
Gallic acid	531.02 ± 26.47	0.35 ± 0.01
Compound **14d**	648.44 ± 9.50	0.54 ± 0.09

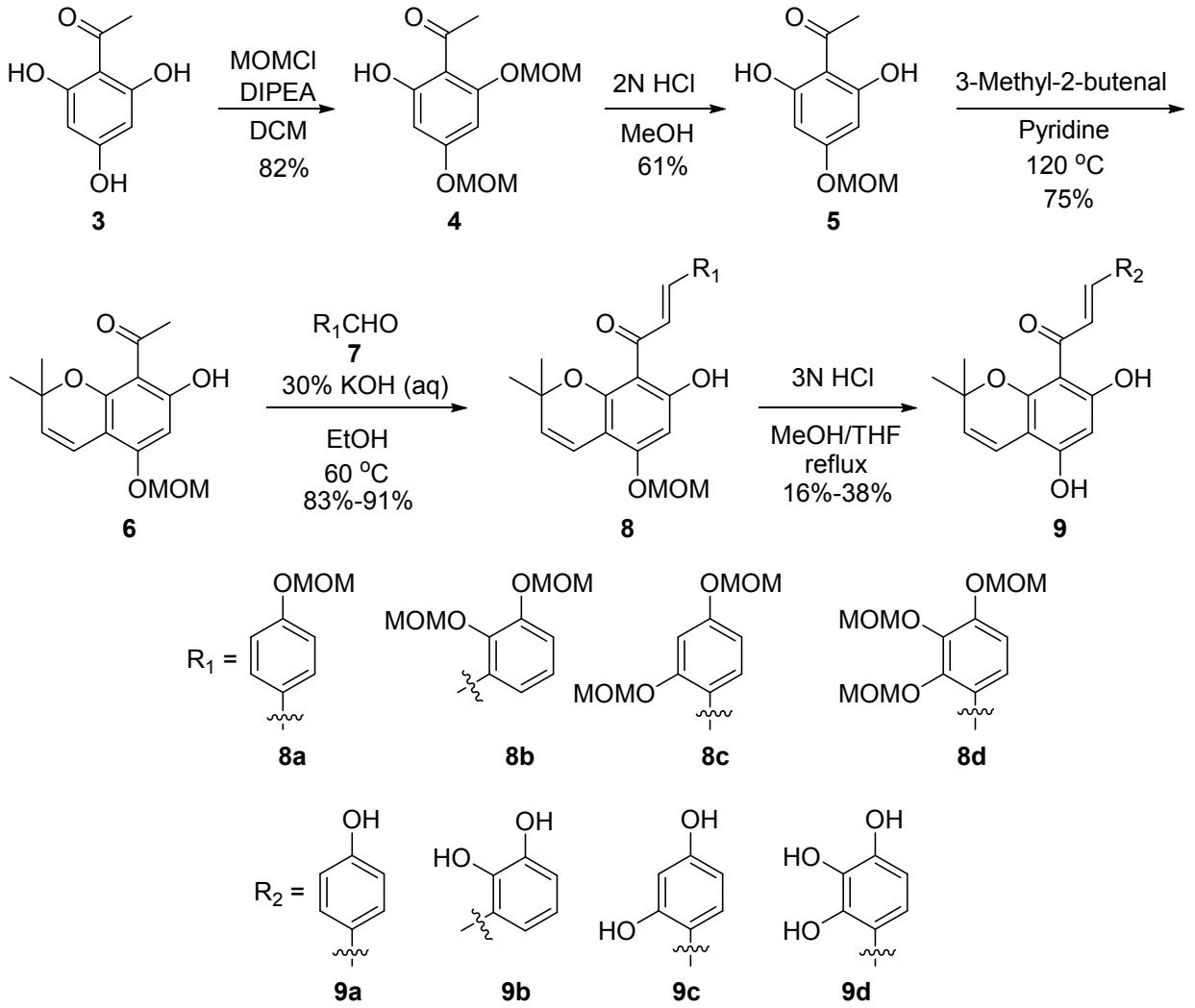


Desmethylxanthohumol (**1**)

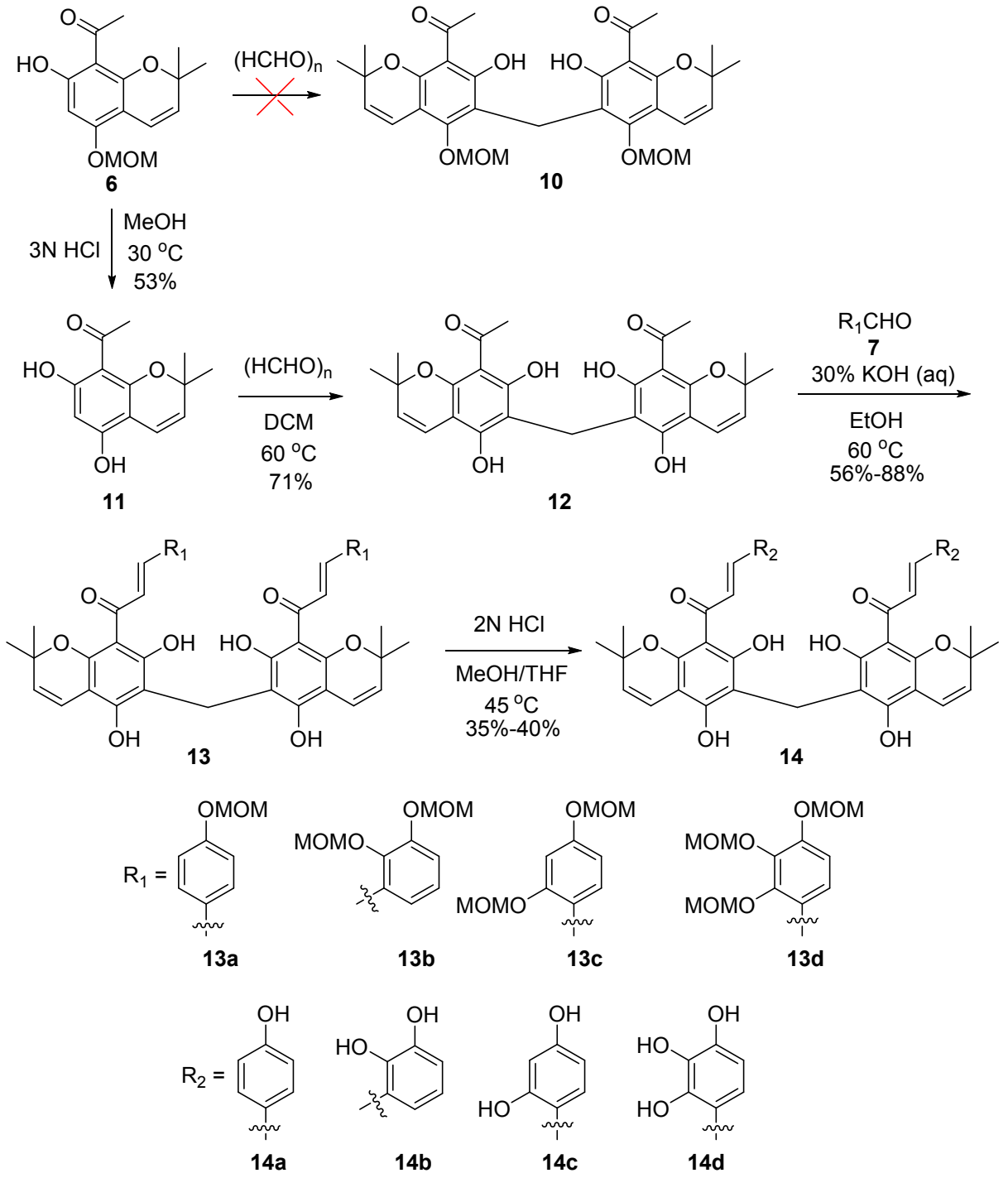


Isobavachromene (**2**)

Scheme 1 The structure of Desmethylxanthohumol (**1**) and Isobavachromene (**2**)



Scheme 2 Synthesis of ring-closing analogs of Desmethylxanthohumol **(1)**


(HCHO)n
6
10
3N HCl
MeOH
30 °C
53%
11
(HCHO)n
DCM
60 °C
71%
12
R1CHO
7
30% KOH (aq)
EtOH
60 °C
56%-88%
13
2N HCl
MeOH/THF
45 °C
35%-40%
14
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13b
13c
13d
R2 =
14a
14b
14c
14d

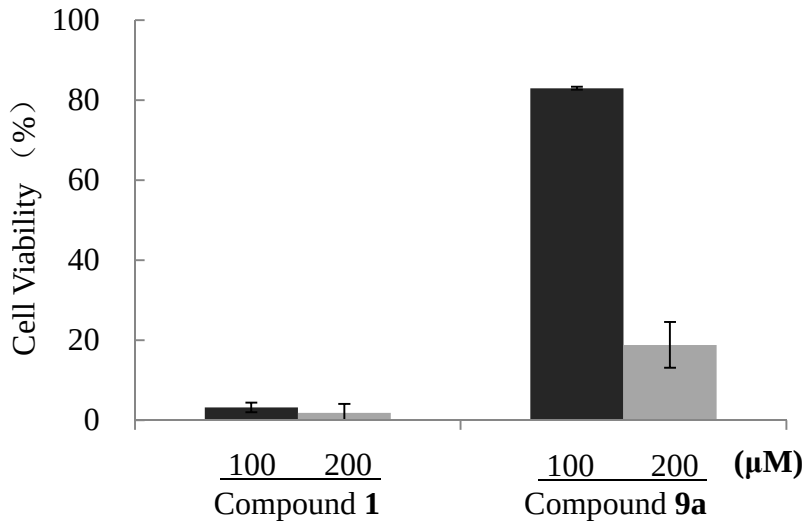

Figure 1 Toxicity of compound **1** and **9a** on PC12 cell.

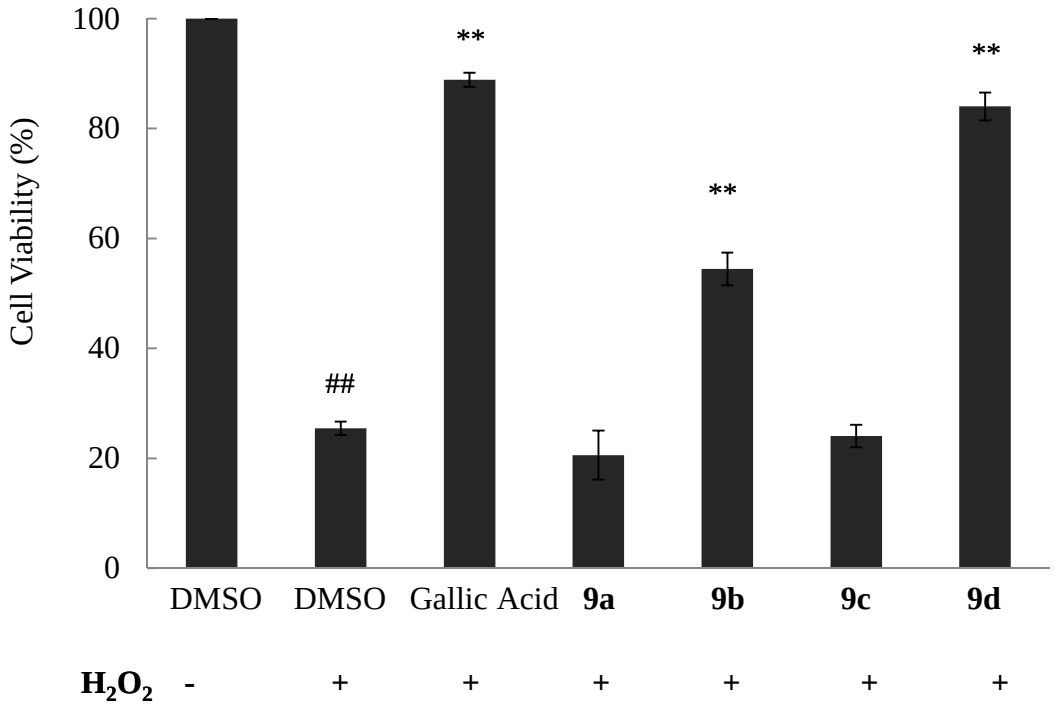

Figure 2 Antioxidant activity of compound **9a**, **9b**, **9c**, **9d** and Gallic acid

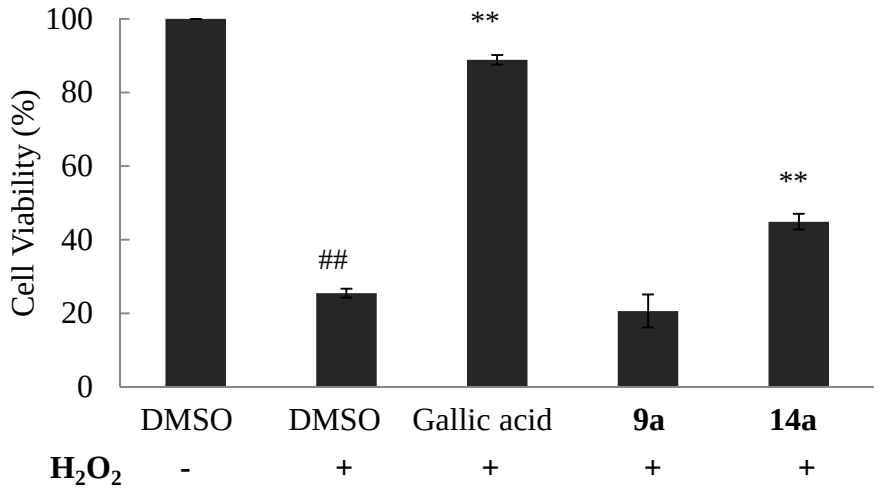

Figure 3 Comparsion of antioxidant activity between compound **9a** and **14a.**

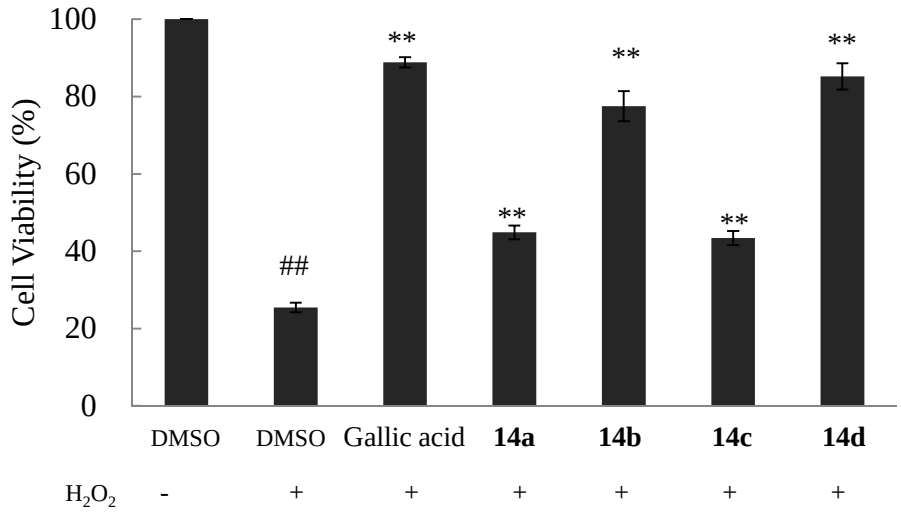

Figure 4 Comparsion of antioxidant activity between monomer **9b, 9c, 9d** and **14b, 14c, 14d**

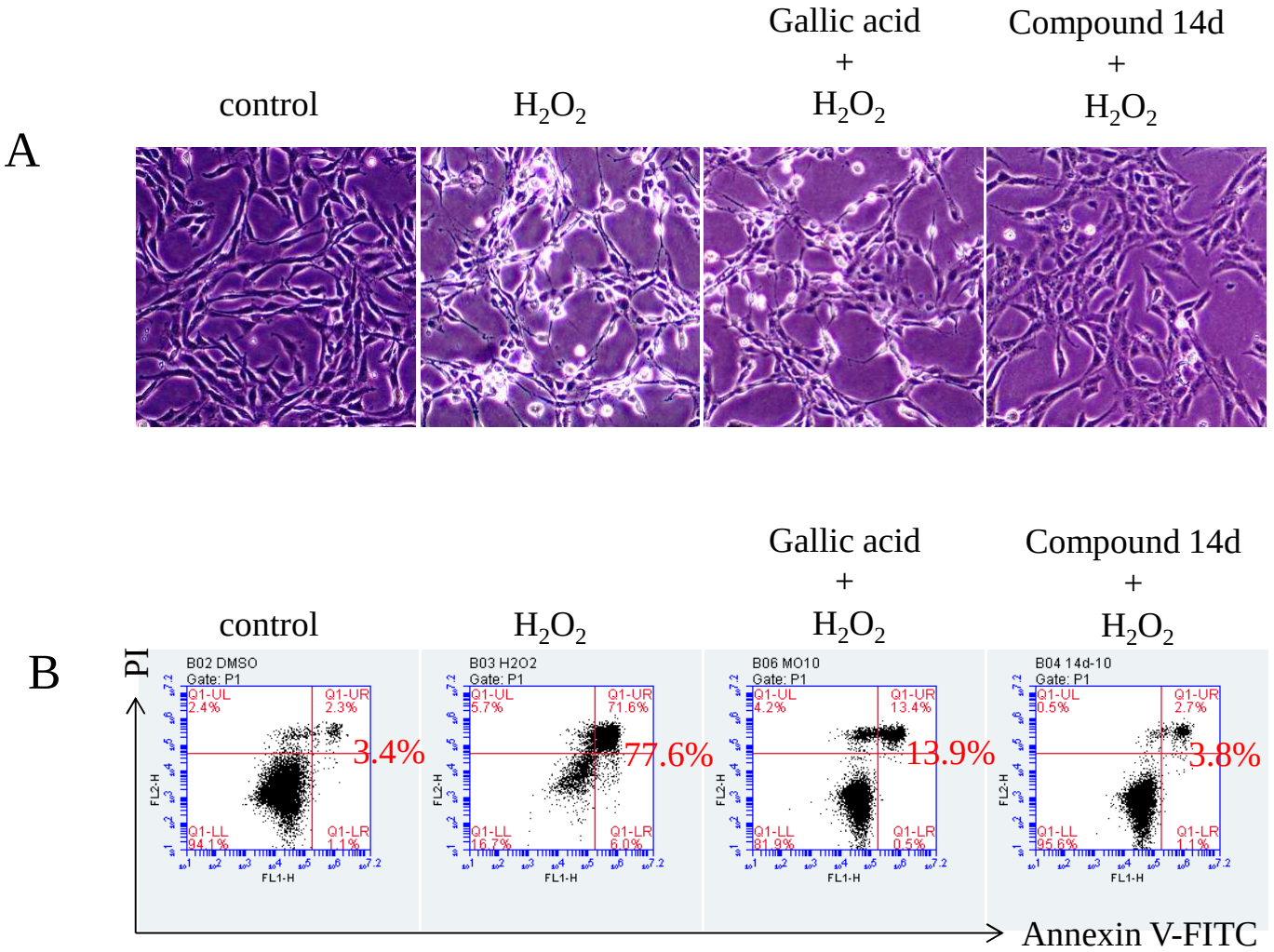

Figure 5 Compound **14d** attenuated H_2O_2-induced apoptosis of PC12 cells.

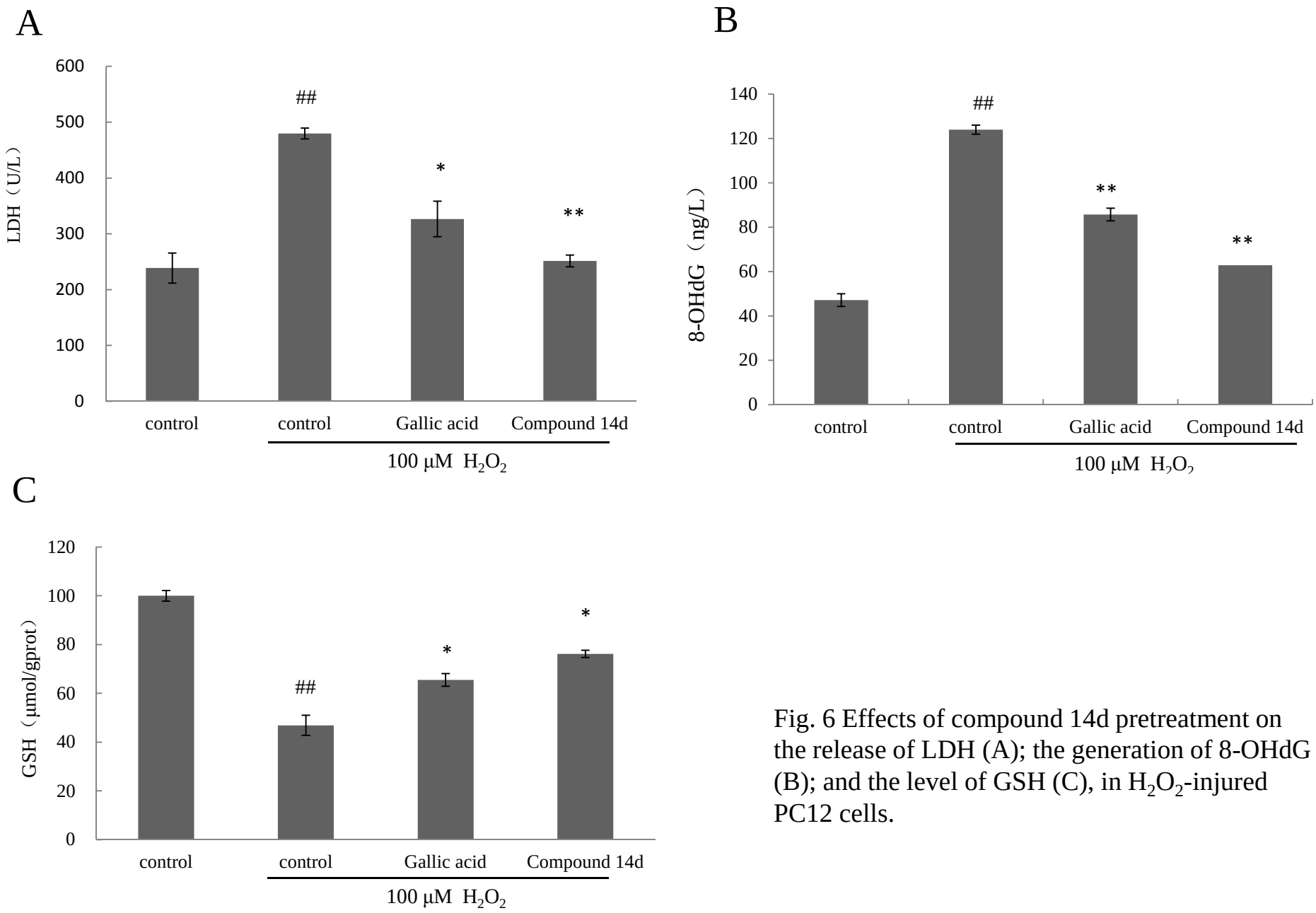

Fig. 6 Effects of compound 14d pretreatment on the release of LDH (A); the generation of 8-OHdG (B); and the level of GSH (C), in H_2O_2-injured PC12 cells.

Highlights

1. Four ring-closing analogs of Desmethylxanthohumol were synthesized.
2. The catechol motif is critical for the antioxidant activity.
3. The dimers show better antioxidant activity than the corresponding monomers.
4. Compound **14d** was identified as the most potent antioxidant activity.
5. Compound **14d** decreased apoptosis in H_2O_2-treated PC12 cells.