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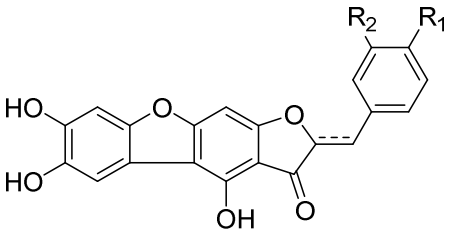
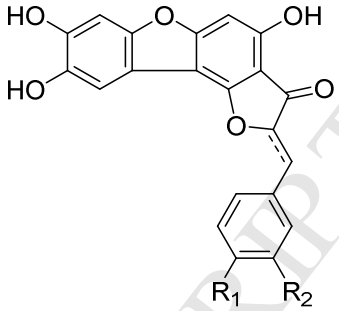
Graphical Abstract

Two novel flavonoids Anastatins A and B as well as their analogs were synthesized. The antioxidant activities of these flavonoids and the key intermediates were evaluated by ferric reducing antioxidant power (FRAP) assay and PC12 cell-based antioxidant assay.



Anastatins A analogs

R_1 = H, OH, R_2= H, OH



Anastatins B analogs

R_1 = H, OH, R_2= H, OH

Aurone derivatives showed better bioactivity than flavone counterparts

The most potent compound protected the PC12 cell against H_2O_2 oxidation by 85.94% at 10 μM

Synthesis and anti-oxidant activity evaluation of (±)-Anastatins A, B and their analogs

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Abstract:

Two novel flavonoids (±)-Anastatins A and B as well as 14 analogs, which containing a benzofuran moiety, were synthesized by using halogenation, Suzuki coupling reaction and an oxidation/Oxa-Michael reaction cascade as the key steps. The structures of the new flavonoids were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS. The antioxidant activities of them as well as the key intermediates were evaluated by ferric reducing antioxidant power (FRAP) assay and the active compounds were evaluated in the PC12 cell model of hydrogen peroxide (H_2O_2)-induced oxidative damage. SAR studies suggested that, for *in vitro* antioxidant activity, aurone derivatives showed better bioactivity than flavone counterparts. However, cyclization to benzofuran and connecting the two conjugated parts as a whole conjugated system by a double bond diminished the *in vitro* antioxidant activity. Among them, the most potent compound **24c** was significantly decreased H_2O_2-caused cell injury. The apoptotic rate (Annexin V^+) of H_2O_2-damaged PC12 cells was 60.7% while that of the compound **24c**-treated cells decreased to 5.9% and 4.1% at 10 μM and 100 μM respectively.

Keywords: Anastatins A, B; Flavone; Aurone; Antioxidant

1. Introduction

Flavonoids are naturally occurring polyphenolic compounds exists in plants such as parsley, onion, orange, chamomile and tea.[1] Many of them are reported to have a variety of pharmacological activities including antioxidant, anti-cancer, anti-viral and anti-inflammatory properties.[2-9] Two novel flavonoids Anastatins A (**1**) and Anastatins B (**2**), which containing a benzofuran moiety and an S-configuration, were isolated from *Anastatica hierochuntica* in Egypt by Yoshikawa et al. in 2003 (Scheme 1). Preliminary biological evaluation revealed that these two compounds showed hepatoprotective effects on D-galactosamine induced cytotoxicity in primary cultured mouse hepatocytes. Moreover, their hepatoprotective activities were stronger than the well-known and commercial hepatoprotective drug silybin.[10]

Liver injury can cause by many factors such as oxidative stress, inflammatory, drugs, excessive intake of ethanol, infection of virus.[11-13] The accumulation of free radicals is a major pathogenetic event occuring in the pathophysiology of various liver injuries. The main sources of the reactive oxygen species (ROS) are represented by the mitochondria and cytochrome P450 enzymes in the hepatocyte, Kupffer cells and neutrophils.[14-16] Overproduction of ROS results in oxidative stress, a deleterious process that can cause membrane lipid peroxidation

(a hallmark of hepatotoxicity), decrease membrane fluidity, suppress enzyme and receptor activity, and damage membrane proteins, which finally triggers cell inactivation and death.[17-19] It is important to develop new antioxidant drugs to prevent or impair the accumulation of free radicals for hepatoprotection.

As mentioned above, Anastatins A and B showed hepatoprotective activities which may be caused by their antioxidant activity. Some aurones, also belong to flavonoids, have been reported to exhibit better antioxidant activity than their flavone counterparts do.[20] Therefore, replace the flavone with aurone might increase their antioxidant activities.

In this study, we synthesized Anastatins A and B as well as their analogs by using halogenation, Suzuki coupling reaction and an oxidation/Oxa-Michael reaction cascade as the key steps and subsequently evaluated the antioxidant activity by ferric reducing antioxidant power (FRAP) assays and H_2O_2-induced PC12 cell oxidative damage model. Based on this data, preliminary SAR was analyzed.

2. Chemistry

Anastatins A and Anastatins B were synthesized according to our previous strategy (Scheme 2 and 3).[21] The 8-iodo substituted apigenin derivative (**5**) and 6-iodo apigenin derivative (**7**) were readily

synthesized from commercially available apigenin (**3**) by protection and iodination sequence according to our previous procedures.[22] With the iodo compounds **5** and **7** in hand, benzo[*d*][1,3] dioxol-5-ylboronic acid (**8)** was coupled with **5** and **7** by using $Pd(PPh_3)_4$ as the catalyst and Cs_2CO_3 as the base in DMF at 100 °C to give compounds **12** and **9** respectively. After dealkyation, oxidation/Oxa-Michael reaction cascade and hydrogenation, (±)-Anastatins A and B were obtained in 54% and 66% overall yield from **12** and **9** respectively (Scheme 3). Then we put our efforts to synthesize the analogs of (±)-Anastatins A and B by replacing a flavone core with aurone. 5-bromo aurone derivatives (**17a**-**17c**) and 7-bromo aurone derivatives (**18a**-**18c**) were prepared from the commercial available aurones (**15a**-**15c**) by isopropyl protection and bromination (Scheme 4). With the bromo compounds **(17a-18c)** in hand, the synthesis of the analogs of (±)-Anastatins A and B (**22a**-**22c** and **26a**-**26c**) was carried out by similar approach (Scheme 5 and 6). Notably, in order to investigate the influence of cyclization (**20c'** versus **22c** and **24c'** versus **26c**), compounds **20c** and **24c** were reduced to compounds **20c'** and **24c'** by hydrogenation.

Results and discussion

The antioxidant activity of our newly synthesized analogs of Anastatins A and B was evaluated using the ferric reducing antioxidant power (FRAP)

assays using Gallic acid as positive control and the results are shown in Table 1. Compound **24c** showed similar activity in the FRAP assay as positive control did. Moreover, compounds **20c, 20c', 22c, 24c'** and **26c** exhibited better activity in the FRAP assay than positive control did. Before the cell-based antioxidant test of these six compounds, the toxicity of them were evaluated using rat pheochromocytoma cells (PC12). The result revealed that compound **22c** showed cytotoxicity and **26c** could promote cell growth (Figure 1 A).

Based on this information, antioxidant activity of other four compounds (**20c**, **20c'**, **24c** and **24c'**) was evaluated in H_2O_2-damaged PC12 cells (Figure 1B) at a concentration of 100 μM and 10 μM. Gallic acid was used as a positive control, and the results are illustrated in Figure 1C and 1D. To our delight, **20c**, **20c'**, **24c** and **24c'** showed better antioxidant activity than Gallic acid. The viability of PC12 cells was nearly 100% after a 2 h exposure to 100 μM H_2O_2 when treated with these four compounds at 100 μM level. Moreover, compounds **20c'** and **24c** showed better antioxidant activity than Gallic acid at 10 μM level. The viability of the **20c'**-pretreated and **24c**-pretreated PC12 cells was 61.46% and 85.94% respectively.

Oxidative stress induces apoptosis rather than necrosis in PC12 cells. The effect of compound **24c** on the morphological alterations was

observed in H_2O_2-injured PC12 cells. As shown in Figure 2A, the typical apoptotic cells such as cell shrinkage, blebbing were clearly observed in PC12 cells after treatment with H_2O_2 (100 μM) for 2 h. Moreover, morphological changes in the gallic acid and compound **24c**-pretreated PC12 cells suggested that both gallic acid and compound **24c** significantly protected them against H_2O_2-induced cell injury. These results indicate that compound **24c** might protect PC12 cells against oxidative stress injury caused by H_2O_2.

To further confirm the effects of compound **24c** 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 2B). The apoptotic rate (Annexin V^+) of the compound -untreated H_2O_2-damaged PC12 cells was 60.7% while that of the compound **24c** -treated cells decreased to 5.9% of the total cells compared to 12.5% observed in the gallic acid-treated control cells (10 μM). Pretreated with compound **24c** protected the PC12 cell against H_2O_2 oxidation and the apoptotic rate decreased to 4.1% at 100 μM, similar to that of gallic acid (2.8%). These results suggest that compound **24c** protected the H_2O_2-injured PC12 cells against apoptosis.

Based on these data, the preliminary structure-activity relationships (SAR) was analyzed. The results are illustrated as following: 1) As we

expected, compound bearing an aurone core showed better *in vitro* antioxidant activity than compound bearing a flavone core (**21b** versus **11** and **25b** versus **14**). 2) In most of the case, the 7-hydroxy group for flavone derivatives and 6-hydroxy group for aurone derivatives could improve the *in vitro* antioxidant activity (**10** versus **11**, **13** versus **14**, **20a** versus **21a**, **20c** versus **21c**, **24a** versus **25a** and **24c** versus **25c**). 3) In most of the case, the α, β-unsaturated double bond, which connected the two conjugated part as a whole conjugated system, could diminish the *in vitro* antioxidant activity (**22a** versus **21a**, **20c'** versus **20c**, **22c** versus **21c**, Anastatins A versus **11**, **26a** versus **25a**, **24c'** versus **24c**, **26c** versus **25c** and Anastatins B versus **14**).

4. Conclusions

In summary, Anastatins A and B as well as their analogs (**22a-c** and **26a-c**) were synthesized from the commercially available apigenin and aurone derivatives respectively. The *in vitro* antioxidant activity as well as PC12 cell-based antioxidant activity evaluation of these compounds and the key intermediates illustrated the following. For *in vitro* antioxidant activity, aurone derivatives showed better bioactivity than flavone counterparts. However, cyclization to benzofuran and connecting the two conjugated part as a whole conjugated system by a double bond diminished the bioactivity. For PC12 cell-based antioxidant activity,

compound **20c**, **20c'**, **24c** and **24c'** showed better activity than Gallic acid at 100 μM level. Moreover, the most potent compound, **24c** protected the PC12 cell against H_2O_2 oxidation by 85.94% at 10 μM. Clarification the mechanism of action of compound **24c** and identify its target molecule is underway in our lab.

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}C NMR 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 l= 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 *(±)-Anastatins B*

Pd/C (10%) 300 mg was added to a solution of **14** (376 mg, 1.0 mmol) in Tetrahydrofuran (THF) (10 mL) and the mixture was stirred at 30□ under hydrogen at balloon pressure for 12 h. Then the suspension was filtrated by Celite and concentrated under reduced pressure to afford (±)-**Anastatins B** (351 mg, 93%) as a yellow solid. ^{1}H NMR (400 MHz, Acetone-d_6) δ 12.18 (s, 1H), 8.64 (s, 1H), 8.20 (s, 2H), 7.54 (d, *J* = 8.0 Hz, 2H), 7.27 (s, 1H), 7.05 (s, 1H), 6.98 (d, *J* = 8.0 Hz, 2H), 6.58 (s, 1H), 5.77 (dd, *J* = 13.2, 2.8 Hz, 1H), 3.41 (dd, *J* = 17.2, 13.2 Hz, 1H), 2.92 (dd, *J* = 17.2, 2.8 Hz, 1H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 198.6, 163.9, 162.6, 159.0, 157.4, 151.0, 146.0, 143.4, 130.7, 129.2, 116.5, 114.9, 107.6, 106.9, 105.0, 99.3, 93.0, 80.9, 43.9; HRMS (ESI) m/z: Calcd for $C_{21}H_{13}O_7$ $[M-H]^-$377.0667; found, 377.0688.

5.1.2 *(±)-Anastatins A*

The procedure is same as the preparation of (±)-**Anastatins B** by using **11** (100 mg) as substrate to give compound (±)-**Anastatins A** (91 mg, 91%) as a yellow solid. ^{1}H NMR (400 MHz, Acetone-d_6) δ 12.93 (s, 1H), 8.59 (s, 1H), 8.32 (s, 1H), 8.18 (s, 1H), 7.46(s, 1H), 7.44 (d, *J* = 8.4

Hz, 2H), 7.07 (s, 1H), 6.92 (d, *J* = 8.4 Hz, 2H), 6.64 (s, 1H), 5.57 (dd, *J* = 13.2, 2.8 Hz, 1H), 3.34 (dd, *J* = 17.2, 13.2 Hz, 1H), 2.87 (dd, *J* = 17.2, 2.8 Hz, 1H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 199.6, 163.6, 161.6, 158.8, 158.4, 151.0, 145.9, 143.5, 130.7, 129.1, 116.2, 115.0, 108.0, 107.8, 105.0, 99.3, 92.4, 80.4, 43.9; HRMS (ESI) m/z: Calcd for $C_{21}H_{13}O_7$ $[M-H]^-$ 377.0667; found, 377.0676.

5.1.3 *5,7-diisopropoxy-2-(4-isopropoxyphenyl)-4H-chromen-4-one (**4**)*

K_2CO_3 (5.39 g, 39 mmol) and $(CH_3)_2CHI$ (6.63 g, 39 mmol) were added to a stirred solution of Apigenin **3** (2.70 g, 10 mmol) in dry *N*, *N*-Dimethylformamide (DMF) (10 mL). After the addition, the mixture was heated to 45 °C and stirred for 24 h. The reaction was cooled to room temperature, filtered and diluted with ethyl acetate (100 mL) and the resulting solution was poured into aqueous HCl (1 M, 100 mL). The organic layer was separated, washed with saturated NaCl (3×100 mL) and dried over anhydrous Na_2SO_4. After concentrated to dryness under reduced pressure, the crude product was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 40:1) to afford the known compound **4** (3.25 g, 82%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.8 Hz, 2H), 6.96 (d, *J* = 8.8 Hz, 2H), 6.52 (d, *J* = 2.4 Hz, 1H), 6.50 (s, 1H), 6.35 (d, *J* = 2.4 Hz, 1H), 4.69 ~ 4.55 (m, 3H), 1.45 (d, *J* = 6.0 Hz, 6H), 1.40 (d, *J* = 6.0 Hz, 6H), 1.38 (d, *J* = 6.0 Hz,

6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4, 162.0, 160.5, 160.4, 159.9, 159.4, 127.6, 123.7, 115.8, 110.1, 107.6, 100.8, 94.5, 72.5, 70.5, 70.1, 22.0, 21.9; LRMS (ESI) m/z 397 $[M+H]^+$.

5.1.4

*8-iodo-5,7-diisopropoxy-2-(4-isopropoxyphenyl)-4H-chromen-4-one(**5**)*

N-Iodosuccinimide (NIS) (2.47 g, 10.99 mmol) was added to a solution of **4** (3.97 g, 10 mmol) in dry DMF (25 mL) and the resulting solution was stirred at 70□ for 10h. The reaction mixture was then poured into water (200 mL) at 0 °C and extracted with Dichloromethane (DCM) (3×50 mL), the combined organic layers were washed with brine (3×100 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude products was purified by column chromatography on silica (petroleum ether/ethyl acetate 30:1) to afford compound **5** (4.49 g, 86%) as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 8.8 Hz, 2H), 6.99 (d, *J* = 8.8 Hz, 2H), 6.56 (s, 1H), 6.49 (s, 1H), 4.74 ~ 4.53 (m, 3H), 1.46 (d, *J* = 6.0Hz, 12H), 1.38 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 160.8, 160.7, 158.6, 158.4, 155.6, 128.0, 123.2, 115.9, 111.5, 106.8, 100.1, 93.5, 73.7, 72.5, 70.1, 22.04, 21.95, 21.90; HRMS (ESI) m/z: Calcd for $C_{24}H_{27}O_5INa$ $[M+Na]^+$ 545.0795; found, 545.0820.

5.1.5

*5-hydroxy-7-isopropoxy-2-(4-isopropoxyphenyl)-4H-chromen-4-one(**6**)*

K_2CO_3 (3.31 g, 24 mmol) and $(CH_3)_2CHI$ (4.08 g, 24 mmol) were added to a stirred solution of Apigenin **3** (2.70 g, 10 mmol) in dry DMF (10 mL). After the addition, the mixture was stirred at room temperature for 30 h. The reaction was filtered and diluted with ethyl acetate (100 mL) and the resulting solution was poured into aqueous HCl (1 M, 100 mL). The organic layer was separated, washed with saturated NaCl (3×100 mL) and dried over anhydrous Na_2SO_4. After concentrated to dryness under reduced pressure, the crude product was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 10:1) to afford the known compound **6** (3.36 g, 95%) as a yellow solid.

5.1.6

*6-iodo-5,7-diisopropoxy-2-(4-isopropoxyphenyl)-4H-chromen-4-one(**7**)*

NIS (2.47 g, 10.99 mmol) was added to a solution of **6** (3.54 g, 10 mmol) in dry DMF (25 mL) and the resulting solution was stirred at ambient temperature for 10h. The reaction mixture was then poured into water (200 mL) and extracted with DCM (3×50 mL), the combined organic layers were washed with brine (3×100 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude products was used in the next step without further purification. K_2CO_3 (1.04 g, 7.5 mmol) and $(CH_3)_2CHI$ (1.27 g, 7.5 mmol) were added to a stirred solution of rude product (2.40 g, 5 mmol) in dry DMF (10 mL). After the addition, the

mixture was heated to 45 °C and stirred for 24 h. The reaction was cooled to room temperature, filtered and diluted with ethyl acetate (100 mL) and the resulting solution was poured into aqueous HCl (1 M, 100 mL). The organic layer was separated, washed with saturated NaCl (3×100 mL) and dried over anhydrous Na_2SO_4. After concentrated to dryness under reduced pressure, the crude product was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 80:1) to afford the compound **7** (1.12 g, 43%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 9.2 Hz, 2H), 6.98 (d, *J* = 9.2 Hz, 2H), 6.71 (s, 1H), 6.55 (s, 1H), 4.78 ~ 4.62 (m, 3H), 1.49 (d, *J* = 6.0 Hz, 6H), 1.43 (d, *J* = 6.0 Hz, 6H), 1.38 (d, *J* = 6.0Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.1, 161.0, 160.7, 160.5, 159.9, 158.4, 127.7, 123.2, 115.9, 112.7, 107.1, 96.8, 86.6, 79.4, 72.6, 70.2, 22.5, 22.0, 21.9; HRMS (ESI) m/z: Calcd for $C_{24}H_{27}O_5INa$ $[M+Na]^+$ 545.0795; found, 545.0820.

5.1.7

*6-(benzo[d][1,3]dioxol-5-yl)-5,7-diisopropoxy-2-(4-isopropoxyphenyl)-4 H-chromen-4-one (**9**)*

A mixture of compounds **7** (5.22 g, 10 mmol), **8** (2.16 g, 13 mmol), $Pd(PPh_3)_4$ (0.346 g, 0.3 mmol) and Cs_2CO_3 (6.53 g, 20 mmol) in DMF (20 mL) was heated to 100 °C and stirred for 12 h under argon. After cooled to room temperature, the reaction mixture was poured into

ice-water (200 mL) and extracted with DCM (3×50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 30:1) to afford compound **9** (3.57 g, 69%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, *J* = 8.8 Hz, 2H), 6.98 (d, *J* = 8.8 Hz, 2H), 6.91 ~ 6.89 (m, 3H), 6.78 (s, 1H), 6.56 (s, 1H), 6.00 (s, 2H), 4.68 ~ 4.57 (m, 2H), 4.17 ~ 4.11 (m, 1H), 1.38 (d, *J* = 6.0 Hz, 6H), 1.33 (d, *J* = 6.0 Hz, 6H), 1.01 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.3, 160.7, 160.5, 159.6, 158.3, 155.8, 146.6, 146.2, 127.7, 127.3, 125.4, 124.3, 123.6, 115.9, 112.9, 112.2, 107.4, 107.4, 100.8, 97.1, 76.7, 71.2, 70.2, 22.0, 22.0, 21.8; HRMS (ESI) m/z: Calcd for $C_{31}H_{32}O_7Na$ $[M+Na]^+$ 539.2040; found, 539.2063.

5.1.8

*6-(3,4-dihydroxyphenyl)-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one (**10**)*

Boron tri-chloride (70 mL, 70 mmol, 1M solution in hexane) was added to a stirred solution of **9** (5.17 g, 10 mmol) in anhydrous DCM (20 mL) under 0 °C for 30 min. After addition, the reaction mixture was heated to reflux for 4h. Then the reaction was cooled to room temperature, excess ice-water was added and stirred for 10 min. Then the suspension

was filtrated and the residue was dried to afford compound **10** (3.62 g, 96%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.30 (s, 1H), 10.50 (s, 2H), 8.82 (s, 2H), 7.94 (d, *J* = 8.0 Hz, 2H), 6.93 (d, *J* = 8.0 Hz, 2H), 6.79 (s, 1H), 6.75 (s, 1H), 6.74 (d, *J* = 5.2 Hz, 1H), 6.61 (s, 1H), 6.60 (d, *J* = 5.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.0, 163.6, 161.7, 161.2, 158.5, 155.6, 144.4, 144.2, 128.5, 123.1, 122.0, 121.3, 118.5, 116.0, 115.0, 112.8, 103.7, 102.9, 93.6; HRMS (ESI) m/z: Calcd for $C_{21}H_{13}O_7$ $[M-H]^-$ 377.0667; found, 377.0673.

5.1.9

*5,7,8-trihydroxy-2-(4-hydroxyphenyl)-4H-benzofuro[3,2-g]chromen-4-one(**11**)*

Ag_2O (463 mg, 2.0 mmol) was added to a solution of **10** (378 mg, 1.0 mmol) in DMF (10 mL) and the resulting mixture was heated to 50 °C and stirred for 6 h. After cooled to room temperature, EA (50 mL) was added to the reaction mixture and stirred for 30 min. The reaction suspension was filtered through celite and washed with EtOAc, the organic phase was poured into water (100 mL) and extracted with EtOAc (5×100 mL). The combined organic layer was washed with brine (5×50 mL), dried over Na_2SO_4 and concentrated under reduced pressure to afford compound **11** (232 mg, 62%) as a black green solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.88 (s, 1H), 10.41 (s, 1H), 9.31 (s, 2H), 8.02 (d, *J* =

8.4 Hz, 2H), 7.46 (s, 1H), 7.41 (s, 1H), 7.09 (s, 1H), 6.96 (d, *J* = 7.2 Hz, 2H), 6.95 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.8, 165.0, 161.9, 160.1, 154.7, 154.6, 150.1, 146.6, 143.5, 129.2, 121.6, 116.5, 112.9, 109.5, 107.5, 106.0, 102.9, 99.2, 91.7; HRMS (ESI) m/z: Calcd for $C_{21}H_{11}O_7$ $[M-H]^-$ 375.0510; found, 375.0526.

5.1.10

*8-(benzo[d][1,3]dioxol-5-yl)-5,7-diisopropoxy-2-(4-isopropoxyphenyl)-4 H-chromen-4-one (**12**)*

The procedure is same as the preparation of **9** by using **5** (5.22 g, 10 mmol) as substrate to give compound **12** (3.82 g, 74%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 8.8 Hz, 2H), 6.95 ~ 6.89 (m, 3H), 6.84 (d, *J* = 8.8 Hz, 2H), 6.52 (s, 1H), 6.51 (s, 1H), 6.05 (s, 2H), 4.65 ~ 4.53 (m, 3H), 1.49 (d, *J* = 6.0 Hz, 6H), 1.34 (d, *J* = 6.0 Hz, 6H), 1.29 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8, 160.6, 160.4, 159.0, 158.2, 156.3, 147.1, 146.5, 127.6, 126.2, 124.7, 123.5, 115.8, 113.3, 111.8, 110.6, 107.8, 106.5, 101.0, 100.1, 73.5, 71.4, 70.0, 22.02, 22.01, 21.95; HRMS (ESI) m/z: Calcd for $C_{31}H_{32}O_7Na$ $[M+Na]^+$ 539.2040; found, 539.2065.

5.1.11

*8-(3,4-dihydroxyphenyl)-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen -4-one (**13**)*

The procedure is same as the preparation of **10** by using **12** (5.17 g, 10 mmol) as substrate to give compound **13** (3.66 g, 97%) as a black-red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.12 (s, 1H), 10.65 (s, 1H), 10.37 (s, 1H), 8.98 (s, 2H), 7.67 (d, *J* = 8.8 Hz, 2H), 6.85 ~ 6.80 (m, 5H), 6.72 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.38 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.3, 163.7, 161.3, 161.1, 159.7, 154.0, 144.7, 144.4, 128.3, 122.5, 122.1, 121.3, 118.5, 115.8, 115.1, 108.3, 103.7, 102.4, 98.7; HRMS (ESI) m/z: Calcd for $C_{21}H_{13}O_7$ $[M-H]^-$ 377.0667; found, 377.0688.

5.1.12

*5,9,10-trihydroxy-2-(4-hydroxyphenyl)-4H-benzofuro[2,3-h]chromen-4-one (**14**)*

The procedure is same as the preparation of **11** by using **13** (378 mg) as substrate to give compound **14** (274 mg, 73%) as a red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.20 (s, 1H), 10.56 (s, 1H), 9.52 (s, 2H), 8.10 (d, *J* = 8.8 Hz, 2H), 7.56 (s, 1H), 7.12 (s, 1H), 7.06 (d, *J* = 8.8 Hz, 2H), 7.05 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.4, 163.9, 161.4, 159.7, 158.5, 149.5, 149.1, 145.6, 143.2, 128.4, 120.9, 116.1, 111.8, 106.1, 105.9, 104.8, 103.8, 98.8, 95.0; HRMS (ESI) m/z: Calcd for $C_{21}H_{11}O_7$ $[M-H]^-$ 375.0510; found, 375.0525.

5.1.13 *(Z)-2-benzylidene-4,6-diisopropoxybenzofuran-3(2H)-one(**16a**)*

K_2CO_3 (3.59 g, 26 mmol) and $(CH_3)_2CHI$ (4.42 g, 26 mmol) were added to a stirred solution of **15a** (2.54 g, 10 mmol) in dry DMF (10 mL). After the addition, the mixture was heated to 45 °C and stirred for 24 h. The reaction was cooled to room temperature, filtered and diluted with ethyl acetate (100 mL) and the resulting solution was poured into aqueous HCl (1 M, 100 mL). The organic layer was separated, washed with saturated NaCl (3×100 mL) and dried over anhydrous Na_2SO_4. After concentrated to dryness under reduced pressure, the crude product was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 40:1) to afford the known compound **16a** (2.98 g, 88%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 ~ 7.85 (m, 2H), 7.50 ~ 7.48 (m, 3H), 6.61 (s, 1H), 6.54 (d, *J* = 2.0 Hz, 1H), 6.36 (d, *J* = 2.0 Hz, 1H), 4.69 ~ 4.55 (m, 2H), 1.46 (d, *J* = 6.0 Hz, 6H), 1.41 (d, *J* = 6.0 Hz, 6H).

5.1.14

(Z)-4,6-diisopropoxy-2-(4-isopropoxybenzylidene)benzofuran-3(2H)-one (16b)

The procedure is same as the preparation of **16a** by using **15b** (2.70 g, 10 mmol) as substrate to give known compound **16b** (3.09 g, 78%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.8 Hz, 2H), 6.93 (d, *J* = 8.8 Hz, 2H), 6.68 (s, 1H), 6.32 (d, *J* = 1.6 Hz, 1H), 6.09 (d, *J* = 1.6

Hz, 1H), 4.75 ~ 4.59 (m, 3H), 1.43 (d, *J* = 6.0 Hz, 6H), 1.40 (d, *J* = 6.0 Hz, 6H), 1.36 (d, *J* = 6.0 Hz, 6H).

5.1.15

*(Z)-2-(3,4-diisopropoxybenzylidene)-4,6-diisopropoxybenzofuran-3(2H)-one(**16c**)*

The procedure is same as the preparation of **16a** by using **15c** (2.86 g, 10 mmol) as substrate to give known compound **16c** (3.77 g, 83%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 ~ 7.36 (m, 2H), 7.46 ~ 7.38 (m, 3H), 6.87 (d, *J* = 8.4 Hz, 1H), 6.57 (s, 1H), 6.23 (d, *J* = 1.6 Hz, 1H), 6.02 (d, *J* = 1.6 Hz, 1H), 4.69 ~ 4.39 (m, 4H), 1.36 (d, *J* = 6.0 Hz, 6H), 1.33 (d, *J* = 6.0 Hz, 6H), 1.31 (d, *J* = 6.0 Hz, 6H), 1.30 (d, *J* = 6.4 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.3, 167.9, 165.9, 157.2, 149.6, 147.7, 146.1, 125.1, 124.8, 120.1, 115.8, 109.4, 104.8, 96.4, 89.3, 71.7, 71.1, 70.8, 70.0, 21.2, 21.1, 20.9, 20.9.

5.1.16

*(Z)-5-bromo-2-benzylidene-4,6-diisopropoxy-benzofuran-3(2H)-one (**17a**) and (Z)-7-bromo-2-benzylidene-4,6-diisopropoxy-benzofuran-3(2H)-one (**18a**)*

N-Bromosuccinimide (NBS) (1.1 mmol) was added to a solution of **16a** (3.38 g, 10 mmol) in dry DCM (25 mL) and the resulting solution was stirred at room temperature for 30 min. The reaction mixture was

then poured into water (200 mL) at 0 °C and extracted with DCM (3×50 mL), the combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude products was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 40:1) to afford compound **18a** (2.13 g, 51%) and compound **17a** (1.83 g, 44%) as yellow solids respectively. Data for **17a:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 8.4 Hz, 2H), 7.46 ~ 7.36 (m, 3H), 6.77 (s, 1H), 6.55 (s, 1H), 5.25 ~ 5.14 (m, 1H), 4.76 ~4.66 (m, 1H), 1.48 (d, *J* = 6.0 Hz, 6H), 1.42 (d, *J* = 6.0 Hz, 6H). Data for **18a:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 8.4 Hz, 2H), 7.48 ~ 7.36 (m, 3H), 6.77 (s, 1H), 6.21 (s, 1H), 4.89 ~ 4.81 (m, 1H), 4.76 ~ 4.64 (m, 1H), 1.46 (d, *J* = 6.0 Hz, 6H), 1.44 (d, *J* = 6.0 Hz, 6H).

5.1.17

*(Z)-5-bromo-4,6-diisopropoxy-2-(4-isopropoxybenzylidene)benzofuran-3(2H)-one (**17b**)*

*(Z)-7-bromo-4,6-diisopropoxy-2-(4-isopropoxybenzylidene)benzofuran-3(2H)-one (**18b**)*

The procedure is same as the preparation of **18a** by using **16b** (3.97 g, 10 mmol) as substrate to give known compound **18b** (2.52 g, 53%) and compound **17b** (2.14 g, 45%) as yellow solids respectively. Data for **17b:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.8 Hz, 2H), 6.94 (d, *J* = 8.8

Hz, 2H), 6.74 (s, 1H), 6.54 (s, 1H), 5.30 - 5.12 (m, 1H), 4.77 - 4.55 (m, 2H), 1.48 (d, *J* = 6.1 Hz, 6H), 1.41 (d, *J* = 6.1 Hz, 6H), 1.37 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.7, 167.3, 162.4, 159.3, 154.9, 146.3, 133.1, 124.7, 116.0, 111.9, 108.2, 102.7, 92.2, 79.1, 72.9, 70.0, 22.7, 22.0, 21.9. LRMS (ESI) *m/z* 475 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd for $C_{24}H_{28}O_5Br$ $[M+H]^+$ 475.1115; found, 475.1119. Data for **18b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 8.8 Hz, 2H), 6.96 (d, *J* = 8.8 Hz, 2H), 6.75 (s, 1H), 6.20 (s, 1H), 4.91 – 4.79 (m, 1H), 4.72 – 4.58 (m, 2H), 1.45 (d, *J* = 6.1 Hz, 6H), 1.43 (d, *J* = 6.1 Hz, 6H), 1.37 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.2, 164.7, 162.5, 159.3, 157.3, 146.4, 133.3, 124.8, 116.1, 111.9, 107.5, 96.5, 86.9, 73.4, 72.9, 70.0, 22.0. LRMS (ESI) *m/z* 475 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd for $C_{24}H_{28}O_5Br$ $[M+H]^+$ 475.1115; found, 475.1111.

5.1.18

*(Z)-5-bromo-4,6-diisopropoxy-2-(3,4-diisopropoxybenzylidene)benzofuran-3(2H)-one (**17c**)*

*(Z)-7-bromo-4,6-diisopropoxy-2-(3,4-diisopropoxybenzylidene)benzofuran-3(2H)-one (**18c**)*

The procedure is same as the preparation of **18a** by using **16c** (4.54 g, 10 mmol) as substrate to give known compound **18c** (2.61 g, 49%) and compound **17c** (2.13 g, 40%) as yellow solids respectively. Data for **17c**:

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 2.0 Hz, 1H), 7.45 (d, *J* = 2.0 Hz, 1H), 6.95 (d, *J* = 9.0 Hz, 1H), 6.70 (s, 1H), 6.52 (s, 1H), 5.25-5.18 (m, 1H), 4.74-4.67 (m, 1H), 4.62-4.56 (m, 1H), 4.54-4.48 (m, 1H), 1.48 (d, *J* = 6.0 Hz, 6H), 1.41 (d, *J* = 6.0 Hz, 6H), 1.38 (d, *J* = 6.0 Hz, 6H), 1.37 (d, *J* = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.65, 167.35, 162.45, 154.89, 151.24, 148.63, 146.46, 126.33, 125.58, 121.71, 116.44, 112.04, 108.16, 102.78, 92.25, 79.07, 72.96, 72.88, 71.72, 22.63, 22.27, 22.15, 21.90; LRMS m/z Calcd for $C_{27}H_{33}BrO_6$ $[M+H]^+$ 533.1; found:533.1. Data for **18c**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 2.0 Hz, 1H), 7.37 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.95 (d, *J* = 8.4 Hz, 1H), 6.72 (s, 1H), 6.20 (s, 1H), 4.88-4.82 (m, 1H), 4.69-4.54 (m, 3H), 1.44 (t, *J* = 6.0 Hz, 12H), 1.41 (d, *J* = 6.0 Hz, 6H), 1.37 (d, *J* = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.21, 164.67, 162.48, 157.32, 150.71, 149.07, 146.54, 126.09, 125.79, 119.47, 116.93, 112.12, 107.44, 96.50, 86.82, 73.43, 72.90, 72.11, 72.06, 22.37, 22.20, 22.00; LRMS m/z Calcd for $C_{27}H_{33}BrO_6$ $[M+H]^+$ 533.1; found:533.1.

5.1.19

*(Z)-5-(benzo[d][1,3]dioxol-5-yl)-2-benzylidene-4,6-diisopropoxybenzofuran-3(2H)-one (**19a**)*

The procedure is same as the preparation of **9** by using **17a** (4.17 g, 10 mmol) as substrate to give compound **19a** (3.16 g, 69%) as a yellow

solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 8.4 Hz, 2H), 7.46 ~ 7.35 (m, 3H), 6.84 ~ 6.74 (m, 4H), 6.57 (s, 1H), 5.99 (s, 2H), 4.77 (hept, *J* = 6.0 Hz, 1H), 4.62 (hept, *J* = 6.0 Hz, 1H), 1.32 (d, *J* = 6.0 Hz, 6H), 1.11 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.7, 168.0, 164.5, 155.5, 147.9, 146.7, 146.1, 132.7, 131.1, 129.3, 128.8, 127.1, 124.8, 120.7, 111.8, 110.7, 107.8, 107.5, 100.8, 91.8, 77.6, 71.7, 22.4, 21.8. HRMS (ESI) m/z: Calcd for $C_{28}H_{26}NaO_6$ $[M+Na]^+$ 481.1622; found, 481.1618.

5.1.20

*(Z)-5-(benzo[d][1,3]dioxol-5-yl)-4,6-diisopropoxy-2-(4-isopropoxybenzyl idene)benzofuran-3(2H)-one (**19b**)*

The procedure is same as the preparation of **9** by using **17b** (4.76 g, 10 mmol) as substrate to give compound **19b** (3.26 g, 63%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 8.4 Hz, 2H), 6.94 (d, *J* = 8.4 Hz, 2H), 6.84 ~ 6.74 (m, 4H), 6.56 (s, 1H), 5.98 (s, 2H), 4.80 ~ 4.73 (m, 1H), 4.65 ~ 4.58 (m, 2H), 1.37 (d, *J* = 6.0 Hz, 6H), 1.31 (d, *J* = 6.0 Hz, 6H), 1.10 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.6, 167.7, 164.2, 159.1, 155.3, 146.7, 146.1, 132.9, 127.3, 125.0, 124.8, 120.5, 116.0, 111.8, 111.1, 108.1, 107.4, 100.8, 91.8, 77.5, 71.7, 70.0, 22.4, 22.0, 21.8; HRMS (ESI) m/z: Calcd for $C_{31}H_{32}NaO_7$ $[M+Na]^+$ 539.2040; found, 539.2065.

5.1.21

*(Z)-5-(benzo[d][1,3]dioxol-5-yl)-2-(3,4-diisopropoxybenzylidene)-4,6-dii sopropoxybenzofuran-3(2H)-one (**19c**)*

The procedure is same as the preparation of **9** by using **17c** (5.33 g, 10 mmol) as substrate to give compound **19c** (3.9 g, 68%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 ~ 7.47 (m, 2H), 6.96 (d, *J* = 8.8 Hz, 1H), 6.84 ~ 6.74 (m, 3H), 6.70 (s, 1H), 6.54 (s, 1H), 5.99 (s, 2H), 4.76 (hept, *J* = 6.0 Hz, 1H), 4.66 ~ 4.48 (m, 3H), 1.38 (d, *J* = 6.0 Hz, 12H), 1.32 (d, *J* = 6.0 Hz, 6H), 1.10 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.5, 167.7, 164.2, 155.3, 151.0, 148.7, 146.8, 146.7, 146.1, 127.2, 126.1, 125.9, 124.8, 121.6, 120.6, 116.6, 111.8, 111.2, 108.0, 107.4, 100.8, 91.9, 77.5, 72.9, 71.8, 71.7, 22.4, 22.3, 22.2, 21.7; HRMS (ESI) m/z: Calcd for $C_{34}H_{38}NaO_8$ $[M+Na]^+$ 597.2459; found, 597.2449.

5.1.22

*(Z)-2-benzylidene-5-(3,4-dihydroxyphenyl)-4,6-dihydroxybenzofuran-3(2 H)-one (**20a**)*

The procedure is same as the preparation of **10** by using **19a** (4.58 g, 10 mmol) as substrate to give compound **20a** (3.51 g, 97%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.87 (s, 1H), 9.51 (s, 1H), 8.81 (s, 1H), 7.93 (d, *J* = 8.0 Hz, 2H), 7.52 ~ 7.40 (m, 3H), 6.75 (d, *J* = 8.0 Hz,

1H), 6.68 (s, 1H), 6.64 (d, *J* = 2.0 Hz, 1H), 6.50 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.44 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.7, 165.8, 155.1, 148.1, 145.1, 144.8, 132.8, 131.3, 129.8, 129.4, 123.5, 122.6, 119.1, 115.7, 113.3, 109.1, 103.1, 91.4; HRMS (ESI) m/z: Calcd for C21H13O6 $[M-H]^-$ 361.0718; found, 361.0705.

5.1.23

*(Z)-5-(3,4-dihydroxyphenyl)-4,6-dihydroxy-2-(4-hydroxybenzylidene)benzofuran-3(2H)-one (**20b**)*

The procedure is same as the preparation of **10** by using **19b** (5.17 g, 10 mmol) as substrate to give compound **20b** (3.74 g, 99%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.74 (s, 1H), 10.07 (s, 1H), 9.36 (s, 1H), 8.79 (s, 2H), 7.80 (d, *J* = 8.4 Hz, 2H), 6.89 (d, *J* = 8.4 Hz, 2H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.64 (s, 1H), 6.62 (s, 1H), 6.50 (d, *J* = 8.0 Hz, 1H), 6.41 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.8, 165.4, 165.3, 159.4, 154.8, 146.3, 145.0, 144.7, 133.4, 123.7, 123.6, 122.6, 119.1, 116.5, 115.6, 113.0, 110.2, 103.4, 91.2; HRMS (ESI) m/z: Calcd for $C_{21}H_{14}NaO_7$ $[M+Na]^+$ 401.0632; found, 401.0627.

5.1.24

*(Z)-2-(3,4-dihydroxybenzylidene)-5-(3,4-dihydroxyphenyl)-4,6-dihydroxybenzofuran-3(2H)-one (**20c**)*

The procedure is same as the preparation of **10** by using **19c** (5.74 g, 10 mmol) as substrate to give compound **20c** (3.82 g, 97%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s, 1H), 9.29 (bs, 3H), 7.46 (d, *J* = 2.0 Hz, 1H), 7.21 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.85 (d, *J* = 8.0 Hz, 1H), 6.75 (d, *J* = 8.0 Hz, 1H), 6.65 (d, *J* = 2.0 Hz, 1H), 6.53 (s, 1H), 6.51 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.42 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ180.8, 165.3, 154.8, 148.1, 146.2, 145.9, 145.0, 144.7, 124.7, 124.0, 123.6, 122.6, 119.1, 118.0, 116.5, 115.6, 112.9, 110.7, 103.4, 91.1; HRMS (ESI) m/z: Calcd for $C_{21}H_{13}O_8$ $[M-H]^-$ 393.0616; found, 393.0638.

5.1.25

*2-(3,4-dihydroxybenzyl)-5-(3,4-dihydroxyphenyl)-4,6-dihydroxybenzofuran-3(2H)-one(**20c'**)*

The procedure is same as the preparation of **(±)-Anastatins A** by using **20c** (100 mg) as substrate to give compound **20c'** (95 mg, 95%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86 (m, 6H), 6.72 (d, *J* = 8.4 Hz, 1H), 6.67 (d, *J* = 2.4 Hz, 1H), 6.64 (d, *J* = 8.0 Hz, 1H), 6.59 (d, *J* = 2.0 Hz, 1H), 6.53 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.46 (dd, *J* = 8.0, 2.0 Hz, 1H), 4.81 (dd, *J* = 8.4, 4.0 Hz, 1H), 3.04 (dd, *J* = 14.8, 4.0 Hz, 1H), 2.72 (q, *J* = 14.8, 8.4 Hz, 1H).^{13}C NMR (101 MHz, DMSO-d_6) δ 197.0, 172.0, 166.3, 154.2, 145.3, 144.9, 144.5, 144.3, 127.7, 123.7,

122.6, 120.6, 119.0, 117.2, 115.8, 115.6, 111.3, 102.8, 90.7, 86.3, 36.6; HRMS (ESI) m/z: Calcd for $C_{21}H_{15}O_8$ $[M-H]^-$ 395.0772; found, 395.0770.

5.1.26 *(Z)-4,6,7-trihydroxy-2-benzylidene-2H-benzofuro [3,2-f] benzofuro-3-one(**21a**)*

The procedure is same as the preparation of **11** by using **20a** (100 mg) as substrate to give compound **21a** (61 mg, 61%) as a black solid. ^{1}H NMR (400 MHz, DMSO) δ 9.42 (s, 1H), 9.18 (s, 1H), 7.97 (d, J = 7.6 Hz, 2H), 7.55 ~ 7.42 (m, 3H), 7.40 (s, 1H), 7.16 (s, 1H), 6.82 (s, 1H), 6.75 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.6, 166.9, 162.3, 151.8, 149.8, 148.0, 145.7, 143.7, 132.6, 131.5, 130.1, 129.5, 113.4, 110.5, 110.2, 107.0, 100.6, 99.2, 93.6; HRMS (ESI) m/z: Calcd for $C_{21}H_{11}O_6$ $[M-H]^-$ 359.0561; found, 359.0550.

5.1.27 *(Z)-4,6,7-trihydroxy-2-(4-hydroxybenzylidene)-2H-benzofuro [3,2-f] benzofuro-3-one (**21b**)*

The procedure is same as the preparation of **11** by using **20b** (100 mg) as substrate to give compound **21b** (63 mg, 63%) as a red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1H), 10.26 (s, 1H), 9.44 (s, 1H), 9.23 (s, 1H), 7.84 (d, *J* = 8.8 Hz, 2H), 7.40 (s, 1H), 7.16 (s, 1H), 6.93 (d, *J* = 8.8 Hz, 2H), 6.86 (s, 1H), 6.76 (s, 1H).^{13}C NMR (100 MHz, DMSO-d_6) δ 178.5, 166.5, 162.0, 159.8, 151.7, 149.7, 146.2, 145.6, 143.6,

133.7, 123.5, 116.6, 113.5, 111.6, 109.9, 107.0, 100.8, 99.2, 93.5; HRMS (ESI) m/z: Calcd for $C_{21}H_{12}NaO_7$ $[M+Na]^+$ 399.0475; found, 399.0470.

5.1.28 *(Z)-4,6,7-trihydroxy-2-(3,4-dihydroxybenzylidene)-2H-benzofuro [3,2-f] benzofuro-3-one*(**21c**)

The procedure is same as the preparation of **11** by using **20c** (100 mg) as substrate to give compound **21c** (51 mg, 51%) as a red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.68 (s, 1H), 9.38 (s, 1H), 9.32 (s, 1H), 9.14 (s, 1H), 7.48 (d, *J* = 2.0 Hz, 1H), 7.39 (s, 1H), 7.26 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.13 (s, 1H), 6.85 (d, *J* = 8.0 Hz, 1H), 6.66 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.3, 166.5, 151.8, 149.7, 148.4, 146.2, 146.0, 145.5, 143.6, 127.1, 126.0, 125.0, 123.9, 118.3, 116.5, 113.6, 111.9, 110.0, 107.0, 99.2, 93.4; HRMS (ESI) m/z: Calcd for $C_{21}H_{11}O_8$ $[M-H]^-$ 391.0459; found, 391.0461.

5.1.29 *4,6,7-trihydroxy-2-benzyl-2H-benzofuro [3,2-f] benzofuro-3-one(**22a**)*

The procedure is same as the preparation of **(±)-Anastatins A** by using **21a** (100 mg) as substrate to give compound **22a** (88 mg, 88%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.83 (s, 1H), 9.32 (s, 1H), 9.09 (s, 1H), 7.31 ~ 7.17 (m, 6H), 7.10 (s, 1H), 6.37 (s, 1H), 5.08 (dd, *J* = 8.0, 3.6 Hz, 1H), 3.28 (dd, *J* = 14.8, 3.6 Hz, 1H), 2.98 (dd, *J* = 14.8, 8.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.4, 173.5, 162.4,

151.3, 149.5, 145.3, 143.5, 136.7, 129.8, 128.7, 127.1, 113.5, 108.6, 106.8, 100.6, 99.2, 93.1, 86.4, 37.1; HRMS (ESI) m/z: Calcd for $C_{21}H_{13}O_6$ $[M-H]^-$ 361.0718; found, 361.0703.

5.1.30 *4,6,7-trihydroxy-2-(4-hydroxybenzyl)-2H-benzofuro [3,2-f] benzofuro-3-one(**22b**)*

The procedure is same as the preparation of **(±)-Anastatins A** by using **21b** (376 mg) as substrate to give compound **22b** (362 mg, 96%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.81 (s, 1H), 9.29 (s, 1H), 9.19 (s, 1H), 9.08 (s, 1H), 7.30 (s, 1H), 7.08 ~ 7.05 (m, 3H), 6.92 (d, *J* = 8.0 Hz, 2H), 6.36 (s, 1H), 4.98 (m, 1H), 3.15 (d, *J* = 14.0 Hz, 1H), 2.87 (dd, *J* = 14.0, 7.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.5, 173.6, 162.4, 156.4, 151.3, 149.5, 145.2, 143.5, 130.8, 126.6, 115.4, 113.5, 108.6, 106.8, 100.7, 99.1, 93.0, 86.7, 36.4; HRMS (ESI) m/z: Calcd for $C_{21}H_{14}NaO_7$ $[M+Na]^+$ 401.0632; found, 401.0625.

5.1.31 *4,6,7-trihydroxy-2-(3,4-dihydroxybenzyl)-2H-benzofuro [3,2-f] benzofuro-3-one(**22c**)*

The procedure is same as the preparation of **(±)-Anastatins A** by using **21c** (100 mg) as substrate to give compound **22c** (86 mg, 86%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 1H), 9.30 (s, 1H), 9.09 (s, 1H), 8.76 (s, 1H), 8.69 (s, 1H), 7.30 (s, 1H), 7.09 (s, 1H), 6.65 (s, 1H), 6.59 (d, *J* = 7.6 Hz, 1H), 6.51 (d, *J* = 7.6 Hz, 1H), 6.37 (s, 1H), 4.94

(m, 1H), 3.08 (d, *J* = 14.4 Hz, 1H), 2.80 (dd, *J* = 14.4, 7.6 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 194.1, 173.0, 161.7, 150.7, 148.9, 144.7, 143.8, 142.9, 126.7, 120.0, 116.7, 115.2, 112.9, 108.0, 106.2, 100.1, 98.6, 92.5, 86.2 ; HRMS (ESI) m/z: Calcd for $C_{21}H_{13}O_8$ $[M-H]^-$ 393.0616; found, 393.0600.

5.1.32

*(Z)-7-(benzo[d][1,3]dioxol-5-yl)-2-benzylidene-4,6-diisopropoxybenzofuran-3(2H)-one (**23a**)*

The procedure is same as the preparation of **12** by using **18a** (4.17 g, 10 mmol) as substrate to give compound **23a** (3.71 g, 81%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 6.8 Hz, 2H), 7.37 ~ 7.30 (m, 3H), 7.13 ~ 7.11 (m, 2H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.69 (s, 1H), 6.25 (s, 1H), 6.04 (s, 2H), 4.90 ~ 4.84 (m, 1H), 4.66 ~ 4.60 (m, 1H), 1.47 (d, *J* = 6.0 Hz, 6H), 1.35 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.9, 165.2, 163.4, 157.3, 147.8, 147.3, 146.6, 132.7, 131.0, 129.1, 128.7, 124.6, 124.3, 111.2, 110.0, 108.4, 107.8, 105.9, 101.0, 95.7, 73.0, 71.9, 22.1, 22.0; HRMS (ESI) m/z: Calcd for $C_{28}H_{26}NaO_6$ $[M+Na]^+$ 481.1622; found, 481.1621.

5.1.33

*(Z)-7-(benzo[d][1,3]dioxol-5-yl)-4,6-diisopropoxy-2-(4-isopropoxybenzylidene)benzofuran-3(2H)-one (**23b**)*

A mixture of compounds **18b** (4.76 g, 10 mmol), **8** (1.83 g, 11 mmol), $Pd(PPh_3)_4$ (1.16 g, 1.0 mmol) and Cs_2CO_3 (6.53 g, 20 mmol) in DMF (20 mL) was heated to 100 °C and stirred for 12 h under argon. After cooled to room temperature, the reaction mixture was poured into ice-water (200 mL) and extracted with DCM (3×50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (petroleum ether/ethyl acetate 30:1) to afford compound **23b** (3.88 g, 75%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 7.6 Hz, 2H), 7.12 (m, 2H), 6.93 (d, *J* = 7.6 Hz, 1H), 6.85 (d, *J* = 7.6 Hz, 2H), 6.68 (s, 1H), 6.24 (s, 1H), 6.04 (s, 2H), 4. 90 ~ 4. 84 (m, 1H), 4. 64 ~ 4.57 (m, 2H), 1.46 (d, *J* = 6.0 Hz, 6H), 1.35 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.8, 165.0, 163.0, 158.9, 157.1, 147.2, 146.6, 146.5, 132.9, 125.1, 124.8, 124.3, 115.9, 111.2, 110.5, 108.4, 107.7, 106.2, 101.0, 95.9, 73.0, 71.8, 69.9, 22.1, 22.0, 21.98; HRMS (ESI) m/z: Calcd for $C_{31}H_{32}NaO_7$ $[M+Na]^+$539.2040; found, 539.2034.

5.1.34

(Z)-7-(benzo[d][1,3]dioxol-5-yl)-4,6-diisopropoxy-2-(3,4-diisopropoxybenzylidene)benzofuran-3(2H)-one (23c)

The procedure is same as the preparation of **23b** by using 18c (5.33 g, 10 mmol) as substrate to give compound **23c** (4.07 g, 71%) as a yellow

solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, *J* = 2.0 Hz, 1H), 7.21 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.06 ~ 7.03 (m, 2H), 6.92 (d, *J* = 8.0 Hz, 1H), 6.86 (d, *J* = 8.0 Hz, 1H), 6.65 (s, 1H), 6.24 (s, 1H), 6.02 (s, 2H), 4.91 ~ 4.82 (m, 1H), 4.65 ~ 4.48 (m, 2H), 4.30 ~ 4.21 (m, 1H), 1.47 (d, *J* = 6.0 Hz, 6H), 1.34 (d, *J* = 6.0 Hz, 6H), 1.33 (d, *J* = 6.0 Hz, 6H), 1.22 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.8, 165.0, 163.2, 157.2, 150.5, 148.8, 147.2, 146.7, 146.5, 126.2, 125.9, 125.0, 124.2, 119.3, 117.0, 111.3, 110.7, 108.6, 108.0, 106.2, 101.0, 95.8, 73.0, 72.0, 71.8, 71.8, 22.2, 22.1, 22.0, 21.9; HRMS (ESI) m/z: Calcd for $C_{34}H_{38}NaO_8$ $[M+Na]^+$597.2459; found, 597.2458.

5.1.35

*(Z)-2-benzylidene-7-(3,4-dihydroxyphenyl)-4,6-dihydroxybenzofuran-3(2H)-one (**24a**)*

The procedure is same as the preparation of **13** by using **23a** (4.58 g, 10 mmol) as substrate to give compound **24a** (3.51 g, 97%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 10.93 (s, 1H), 10.87 (s, 1H), 8.93 (s, 2H), 7.83 (d, *J* = 7.6 Hz, 2H), 7.38 ~ 7.36 (m, 3H), 7.03 (s, 1H), 6.88 ~ 6.82 (m , 2H), 6.60 (s, 1H), 6.29 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.0, 164.8, 164.6, 157.0, 148.3, 145.2, 144.8, 133.0, 131.1, 129.5, 129.3, 122.4, 122.0, 118.4, 115.5, 108.3, 105.5, 103.1, 98.5; HRMS (ESI) m/z: Calcd for $C_{21}H_{13}O_6$ $[M-H]^-$ 361.0718; found, 361.0698.

5.1.36

*(Z)-7-(3,4-dihydroxyphenyl)-4,6-dihydroxy-2-(4-hydroxybenzylidene)benzofuran-3(2H)-one (**24b**)*

Boron tri-chloride (70 mL, 70 mmol, 1M solution in hexane) was added to a stirred solution of **23b** (5.17 g, 10 mmol) in anhydrous DCM (20 mL) under 0 °C for 30 min. After addition, the reaction mixture was heated to reflux for 4h. Then the reaction was cooled to room temperature, excess ice-water was added and stirred for 10 min. Then the suspension was filtrated and the residue was dried to afford compound **24b** (3.74 g, 99%) as a black-red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 10.82 (s, 1H), 10.03 (s, 1H), 8.94 (s, 1H), 8.93 (s, 1H), 7.69 (d, J = 8.4 Hz, 2H), 7.03 (s, 1H), 6.85 ~ 6.83 (m, 2H), 6.77 (d, J = 8.4 Hz, 2H), 6.53 (s, 1H), 6.32 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.0, 164.6, 164.1, 159.1, 156.7, 146.5, 145.1, 144.6, 133.1, 123.9, 122.6, 122.0, 118.3, 116.3, 115.4, 109.3, 105.2, 103.4, 98.3; HRMS (ESI) m/z: Calcd for $C_{21}H_{14}NaO_7$ $[M+Na]^+$ 401.0632; found, 401.0628.

5.1.37

*(Z)-2-(3,4-dihydroxybenzylidene)-7-(3,4-dihydroxyphenyl)-4,6-dihydroxybenzofuran-3(2H)-one (**24c**)*

The procedure is same as the preparation of **13** by using **23c** (5.74 g, 10 mmol) as substrate to give compound **24c** (3.82 g, 97%) as a yellow

solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.78 (s, 2H), 9.66 (s, 1H), 8.92 (s, 3H), 7.26 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.13 (d, *J* = 1.6 Hz, 1H), 7.01 (d, *J* = 1.6 Hz, 1H), 6.88 ~ 6.81 (m, 2H), 6.74 (d, *J* = 8.4 Hz, 1H), 6.43 (s, 1H), 6.26 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.0, 164.6, 164.1, 156.7, 147.7, 146.5, 145.7, 145.1, 144.6, 124.3, 123.6, 122.6, 122.0, 119.1, 118.3, 116.3, 115.5, 109.8, 105.3, 103.4, 98.3; HRMS (ESI) m/z: Calcd for $C_{21}H_{13}O_8$ $[M-H]^-$ 393.0616; found, 393.0606.

5.1.38

*2-(3,4-dihydroxybenzyl)-7-(3,4-dihydroxyphenyl)-4,6-dihydroxybenzofuran-3(2H)-one(**24c'**)*

The procedure is same as the preparation of **(±)-Anastatins B** by using **24c** (100 mg) as substrate to give compound **24c'** (96 mg, 96%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.81 (bs, 6H), 6.80 (d, *J* = 2.0 Hz, 1H), 6.59 (d, *J* = 8.4 Hz, 1H), 6.54 (dd, *J* = 8.4, 1.6 Hz, 1H), 6.60 (s, 1H), 6.59 (d, *J* = 8.4 Hz, 1H), 6.48 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.03 (s, 1H), 4.64 (dd, *J* = 6.4, 4.0 Hz, 1H), 2.98 (dd, *J* = 14.8, 4.0 Hz, 1H), 2.76 (dd, *J* = 14.8, 6.4 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 196.2, 171.6, 164.7, 156.0, 145.1, 144.7, 144.3, 144.1, 127.3, 123.2, 122.3, 121.0, 118.5, 117.5, 115.6, 115.4, 105.1, 103.2, 96.6, 85.9, 36.6. HRMS (ESI) m/z: Calcd for $C_{21}H_{15}O_8$ $[M-H]^-$ 395.0772; found, 395.0766.

5.1.39 *(Z)-4,8,9-trihydroxy-2-benzylidene-2H-benzofuro [2,3-g] benzofuro-3-one(**25a**)*

The procedure is same as the preparation of **14** by using **24a** (100 mg) as substrate to give compound **25a** (82 mg, 82%) as a black solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.56 (s, 1H), 9.44 (s, 1H), 8.06 (d, J = 7.6 Hz, 2H), 7.62 ~ 7.50 (m, 3H), 7.38 (s, 1H), 7.08 (s, 1H), 6.87 (s, 1H), 6.75 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.4, 163.8, 159.6, 156.3, 150.2, 147.9, 146.1, 143.8, 132.7, 131.5, 130.2, 129.6, 111.8, 110.7, 106.4, 105.9, 102.6, 99.5, 94.2; HRMS (ESI) m/z: Calcd for $C_{21}H_{11}O_6$ $[M-H]^-$ 359.0561; found, 359.0592.

5.1.40 *(Z)-4,8,9-trihydroxy-2-(4-hydroxybenzylidene)-2H-benzofuro [2,3-g] benzofuro-3-one (**25b**)*

Ag_2O (115 mg) was added to a solution of **24b** (100 mg) in DMF (10 mL) and the resulting mixture was heated to 50 °C and stirred for 5 h. After cooled to room temperature, MeOH (5 mL) was added to the reaction mixture and stirred for 30 min. The reaction suspension was filtered through celite and washed with EtOAc, the organic phase was poured into water (100 mL) and extracted with EtOAc (4×100 mL). The combined organic layer was washed with brine (4×30 mL), dried over Na_2SO_4 and concentrated under reduced pressure to afford compound **25b** (75 mg, 75%) as a red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.18 (s,

1H), 10.25 (s, 1H), 9.48 (s, 1H), 9.44 (s, 1H), 7.92 (d, *J* = 8.4 Hz, 2H), 7.40 (s, 1H), 7.08 (s, 1H), 7.00 (d, *J* = 8.0 Hz, 2H), 6.79 (s, 1H), 6.76 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.2, 163.4, 159.8, 159.2, 156.0, 150.1, 146.2, 146.0, 143.7, 133.7, 123.6, 116.7, 111.9, 111.8, 106.3, 106.2, 102.6, 99.5, 93.9; HRMS (ESI) m/z: Calcd for $C_{21}H_{12}NaO_7$ $[M+Na]^+$ 399.0475; found, 399.0471.

5.1.41 *(Z)-4,8,9-trihydroxy-2-(3,4-dihydroxybenzylidene)-2H-benzofuro [2,3-g] benzofuro-3-one(**25c**)*

The procedure is same as the preparation of **25b** by using **24c** (100 mg) as substrate to give compound **25c** (57 mg, 57%) as a red solid. ^{1}H NMR (400 MHz, DMSO) δ 11.16 (s, 1H), 9.78 (s, 1H), 9.59 (s, 1H), 9.38 (s, 1H), 9.24 (s, 1H), 7.47 (s, 1H), 7.45 (m, 2H), 7.08 (s, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 6.75 (s, 1H), 6.70 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.2, 163.4, 159.3, 156.0, 150.1, 148.6, 146.1, 146.0, 143.7, 124.6, 123.9, 118.8, 116.8, 112.3, 112.0, 106.9, 106.3, 102.6, 99.4, 93.9; HRMS (ESI) m/z: Calcd for $C_{21}H_{11}O_8$ $[M-H]^-$ 391.0459; found, 391.0431.

5.1.42 *4,8,9-trihydroxy-2-benzyl-2H-benzofuro [2,3-g] benzofuro-3-one(**26a**)*

The procedure is same as the preparation of **(±)-Anastatins B** by using **25a** (100 mg) as substrate to give compound **26a** (92 mg, 91%) as a red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (s, 1H), 9.36 (s, 1H),

9.22 (s, 1H), 7.36 ~ 7.21 (m, 5H), 7.11 (s, 1H), 7.02 (s, 1H), 6.56 (s, 1H), 5.14 (dd, *J* = 8.0, 3.6 Hz, 1H), 3.31 (dd, *J* = 14.8, 3.6 Hz, 1H), 3.03 (dd, *J* = 14.8, 8.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.1, 166.8, 163.9, 155.6, 149.6, 145.5, 143.5, 136.9, 129.8, 128.7, 127.1, 112.5, 106.2, 105.7, 102.6, 99.3, 92.3, 86.8, 37.2; HRMS (ESI) m/z: Calcd for $C_{21}H_{13}O_6$ $[M-H]^-$ 361.0718; found, 361.0703.

5.1.43 *4,8,9-trihydroxy-2-(4-hydroxybenzyl)-2H-benzofuro [2,3-g] benzofuro-3-one(**26b**)*

Pd/C (10%) 40 mg was added to a solution of **25b** (376 mg, 1.0 mmol) in EtOH (10 mL) and the mixture was stirred at room temperature under hydrogen at balloon pressure for 12 h. Then the suspension was filtrated by Celite and concentrated under reduced pressure to afford **26b** (370 mg, 98%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H), 9.32 (s, 1H), 9.23 (s, 1H), 9.20 (s, 1H), 7.12 (s, 1H), 7.11 (d, *J* = 8.4 Hz, 2H), 7.01 (s, 1H), 6.66 (d, *J* = 8.4 Hz, 2H), 6.54 (s, 1H), 5.04 (dd, *J* = 8.0, 3.6 Hz, 1H), 3.18 (dd, *J* = 14.8, 3.6 Hz, 1H), 2.91 (dd, *J* = 14.8, 8.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 195.7, 166.3, 163.3, 155.9, 154.9, 149.1, 144.9, 142.9, 130.2, 126.2, 114.9, 112.0, 105.7, 105.2, 102.0, 98.7, 91.6, 86.6, 35.9; HRMS (ESI) m/z: Calcd for $C_{21}H_{14}NaO_7$ $[M+Na]^+$401.0632; found, 401.0623.

5.1.44 *4,8,9-trihydroxy-2-(3,4-dihydroxybenzyl)-2H-benzofuro [2,3-g] benzofuro-3-one(**26c**)*

The procedure is same as the preparation of **(±)-Anastatins A** by using **25c** (100 mg) as substrate to give compound **26c** (92 mg, 92%) as a red solid100. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.93 (s, 1H), 9.37 (s, 1H), 9.21 (s, 1H), 8.81 (s, 1H), 8.75 (s, 1H), 7.14 (s, 1H), 7.02 (s, 1H), 6.70 (s, 1H), 6.63 ~ 6.55 (m, 3H), 5.03 (dd, *J* = 7.6, 3.6 Hz, 1H), 3.13 (dd, *J* = 14.8, 3.6 Hz, 1H), 2.85 (dd, *J* = 14.8, 7.6 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.3, 166.9, 163.9, 155.5, 149.6, 145.43, 145.35, 144.3, 143.5, 127.4, 120.5, 117.2, 115.8, 112.6, 106.3, 105.8, 102.6, 99.3, 92.2, 87.2, 36.6; HRMS (ESI) m/z: Calcd for $C_{21}H_{13}O_8$ $[M-H]^-$ 393.0616; found, 393.0601.

5.2 Biology evaluation

5.2.1 Antioxidant activity by FRAP assay

FRAP assay measures the antioxidant capacity to reduce ferric 2,4,6-tripyridyl-s-triazine (TPTZ) complex to its ferrous form. Antioxidant activity by FRAP assay of compound 1 and 2 were performed as described previously.[23] 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. Equivalent amounts of Vc for the test compounds were calculated from three independent experiments.

5.2.2 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.

5.2.3 Cytotoxic analysis in PC12 cells

PC12 cells (100 μL) were cultured in 96-well plates at a density of 5 $\times 10^5$ 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.

5.2.4 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 (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%.

5.2.5 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. [24, 25] Briefly, PC12 cells were treated using the same method as for MTT assay. The concentrations of compound **24c** or gallic acid were 10 μM and 100 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.

5.3 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

and #P < 0.05 compared to control cells (DMSO); **P < 0.01 compared to H_2O_2-treated cells.

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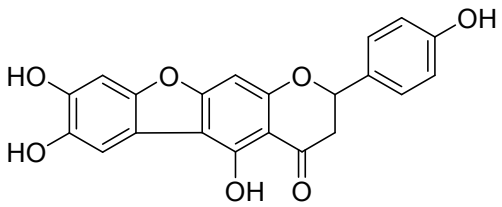
Figure captions:

Fig. 1 The cytotoxicity of target compounds against PC-12 cells and their cytoprotective activities in H_2O_2-induced oxidative-damage PC12 cell model. (A) The cell viabilities of PC-12 treated by 100 μM target compounds for 48 h were detected by MTT assay. The DMSO-treated controls were assigned a cell viability value of 100%. Gallic acid was used as appositive control. $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ compared to control cells (DMSO); (B) H_2O_2-induced oxidative-damage PC12 cell model was established by treated with 100 μM H_2O_2 for 2 h. $^{\#\#}P < 0.01$ compared to control cells (H_2O_2-). The cytoprotective activity of 100 μM (C) or 10 μM (D) target compounds were evaluated by MTT assay in H_2O_2-induced oxidative-damage PC12 cell model. Gallic acid (GA) was used as a positive control. $^{**}P < 0.01$ compared to H_2O_2-treated cells.

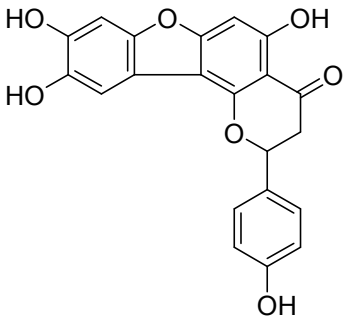
Fig. 2 Compound **24c** attenuated H_2O_2-induced apoptosis of PC12 cells. Cells treated with gallic acid (10 μM and 100 μM) were used as positive control. (A) Morphological changes (at 200 x) in PC12 cells treated with H_2O_2 (100 μM, 2 h) and compound **24c** (10 μM and 100 μM, 0.5 h). Control cells were only treated with medium containing 0.1% dimethyl sulfoxide (DMSO). (B) The protective effect of compound **24c** (10 μM and 100 μM) on apoptosis in H_2O_2-injured PC12 cells using double staining with Annexin V/PI.

Table 1 The results of total reducing power testing ($\bar{x} \pm S$)

Compound	FRAP (Equivalent amount of Vc , mg/mmol)	Compound	FRAP (Equivalent amount of V mg/mmol)
20a	237.24±36.46	24a	269.02±16.34
21a	134.24±3.30	25a	116.47±6.13
22a	227.47±32.37	26a	262.24±2.67
20b	240.80±26.08	24b	263.47±1.26
21b	264.36±20.74	25b	270.06±10.66
22b	253.47±28.91	26b	248.36±52.83
20c	372.21±46.97	24c	318.13±8.28
20c'	394.36±0.01	24c'	375.91±0.01
21c	277.10±30.92	25c	259.61±21.34
22c	395.80±79.04	26c	372.69±51.70
10	275.99±34.56	13	271.47±34.46
11	116.24±8.01^{g}	14	137.02±15.09^{g}
Anastatins A	259.76±23.61^{cdef}	Anastatins B	223.58±20.58^{f}
Vc	147.99±6.13^{g}	Gallic acid	332.52±39.17^{b}

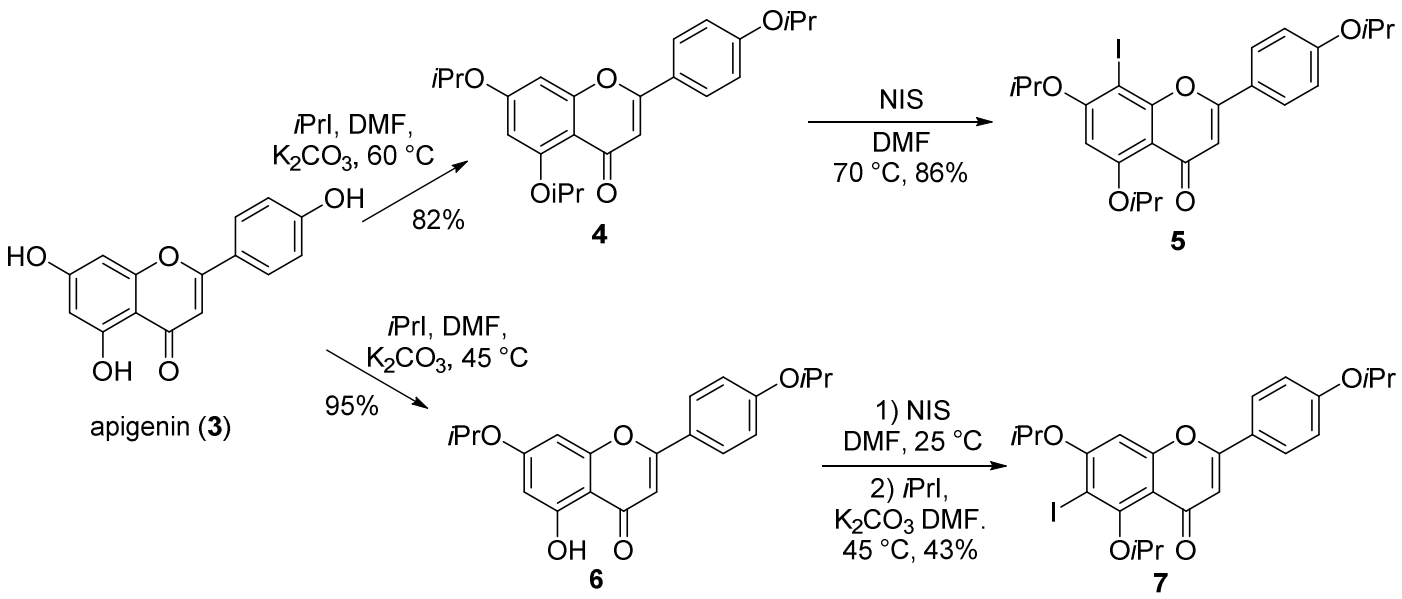


(±)-**Anastatins A**

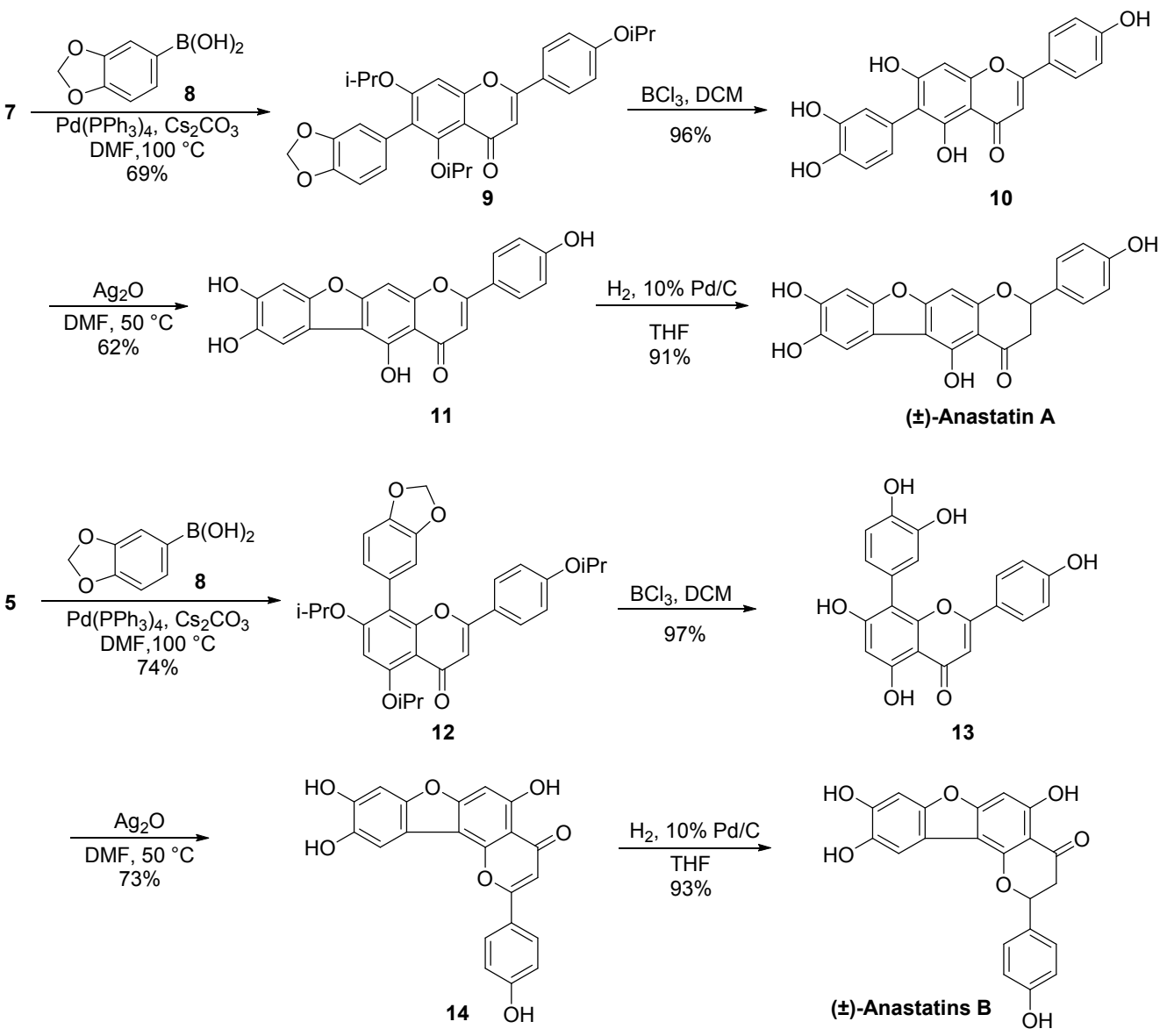


(±)-**Anastatins B**

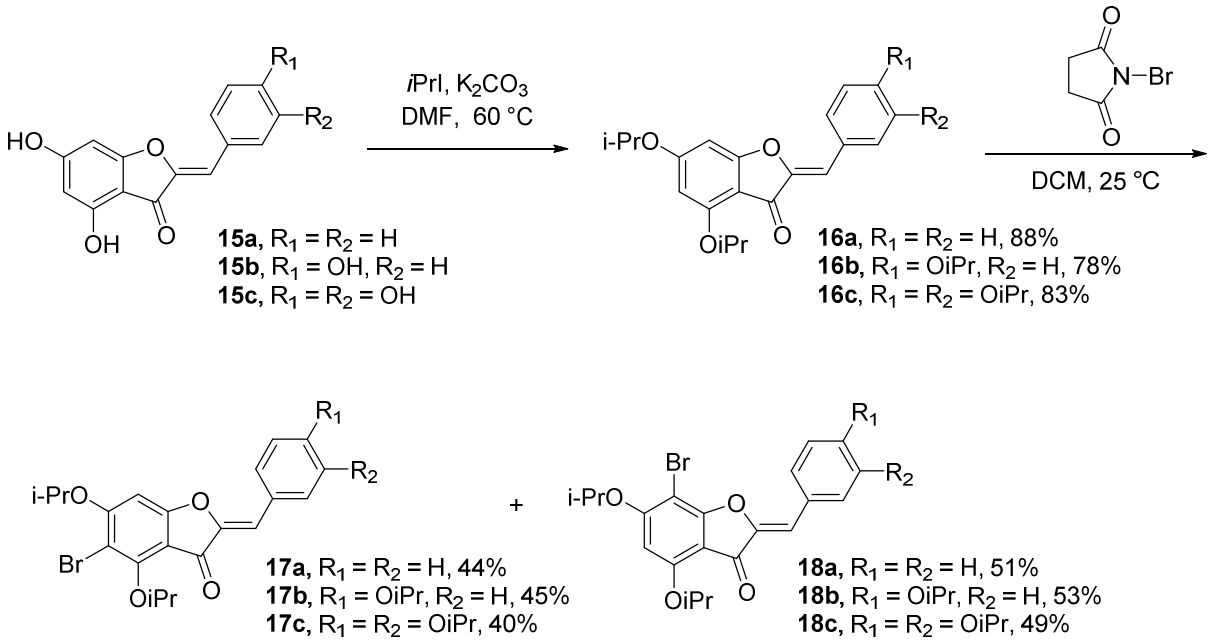
Scheme 1 The structure of Anastatins A and B



Scheme 2 Synthesis of iodo-intermediate **5** and **7**



Scheme 3 Synthesis of Anastatins A and Anastatins B



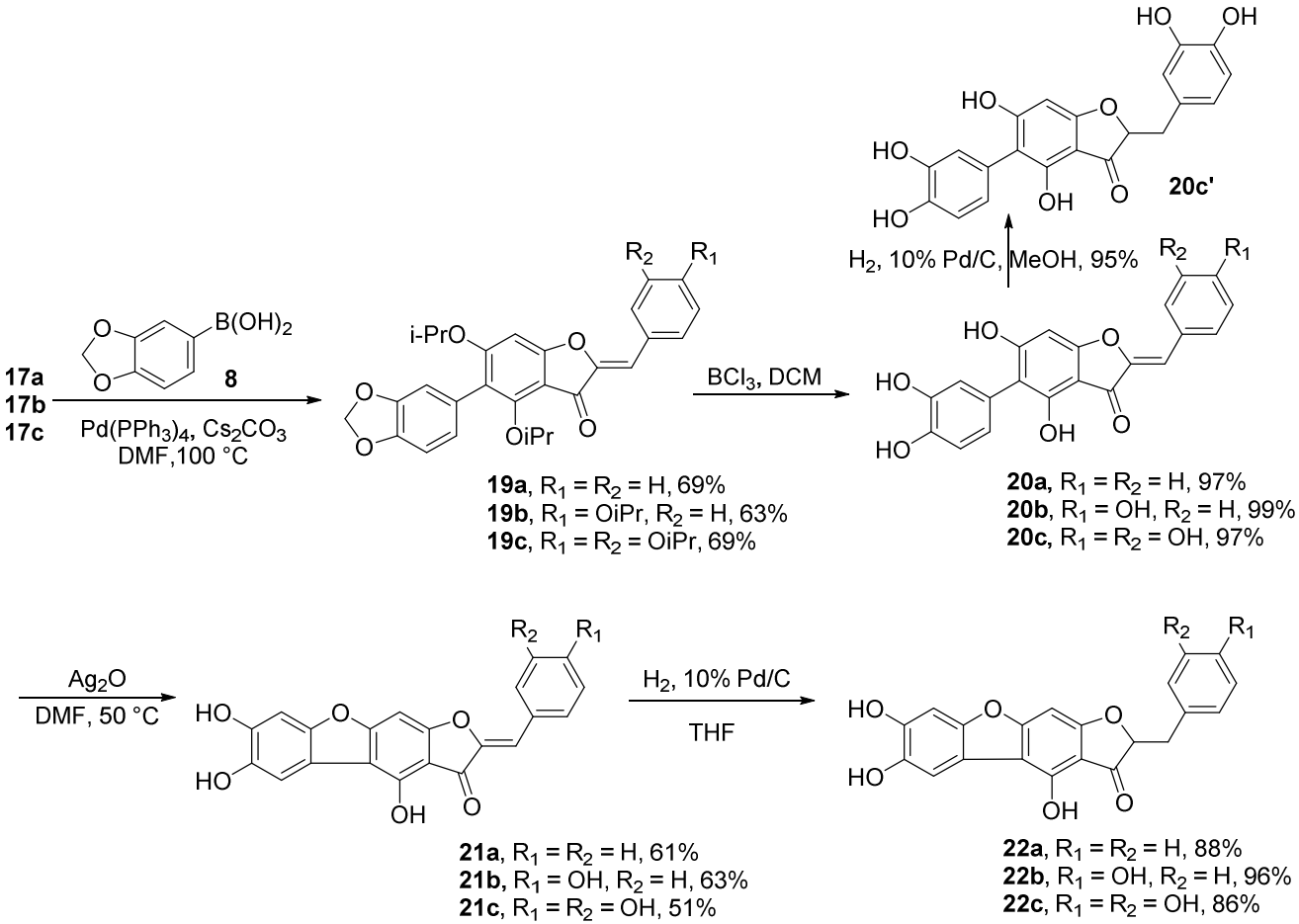
15a, $R_1 = R_2 = H$
15b, $R_1 = OH$, $R_2 = H$
15c, $R_1 = R_2 = OH$

16a, $R_1 = R_2 = H$, 88%
16b, $R_1 = OiPr$, $R_2 = H$, 78%
16c, $R_1 = R_2 = OiPr$, 83%

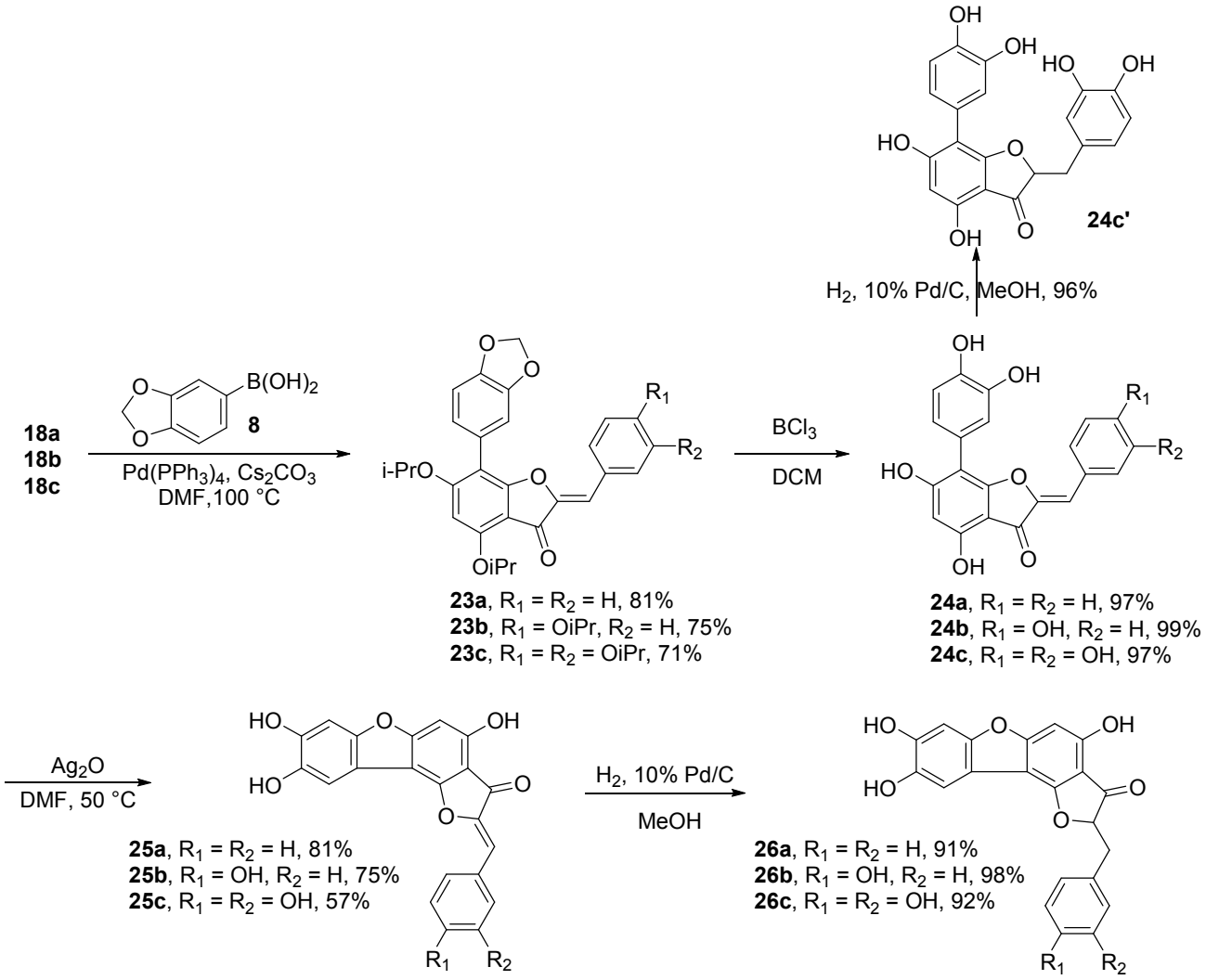
17a, $R_1 = R_2 = H$, 44%
17b, $R_1 = OiPr$, $R_2 = H$, 45%
17c, $R_1 = R_2 = OiPr$, 40%

18a, $R_1 = R_2 = H$, 51%
18b, $R_1 = OiPr$, $R_2 = H$, 53%
18c, $R_1 = R_2 = OiPr$, 49%

Scheme 4 Synthesis of intermediates **17a**-**18c**



Scheme 5 Synthesis of (±)-Anastatins A derivatives **22a-22c**



Scheme 6 Synthesis of (±)-Anastatins B derivatives **26a-26c**

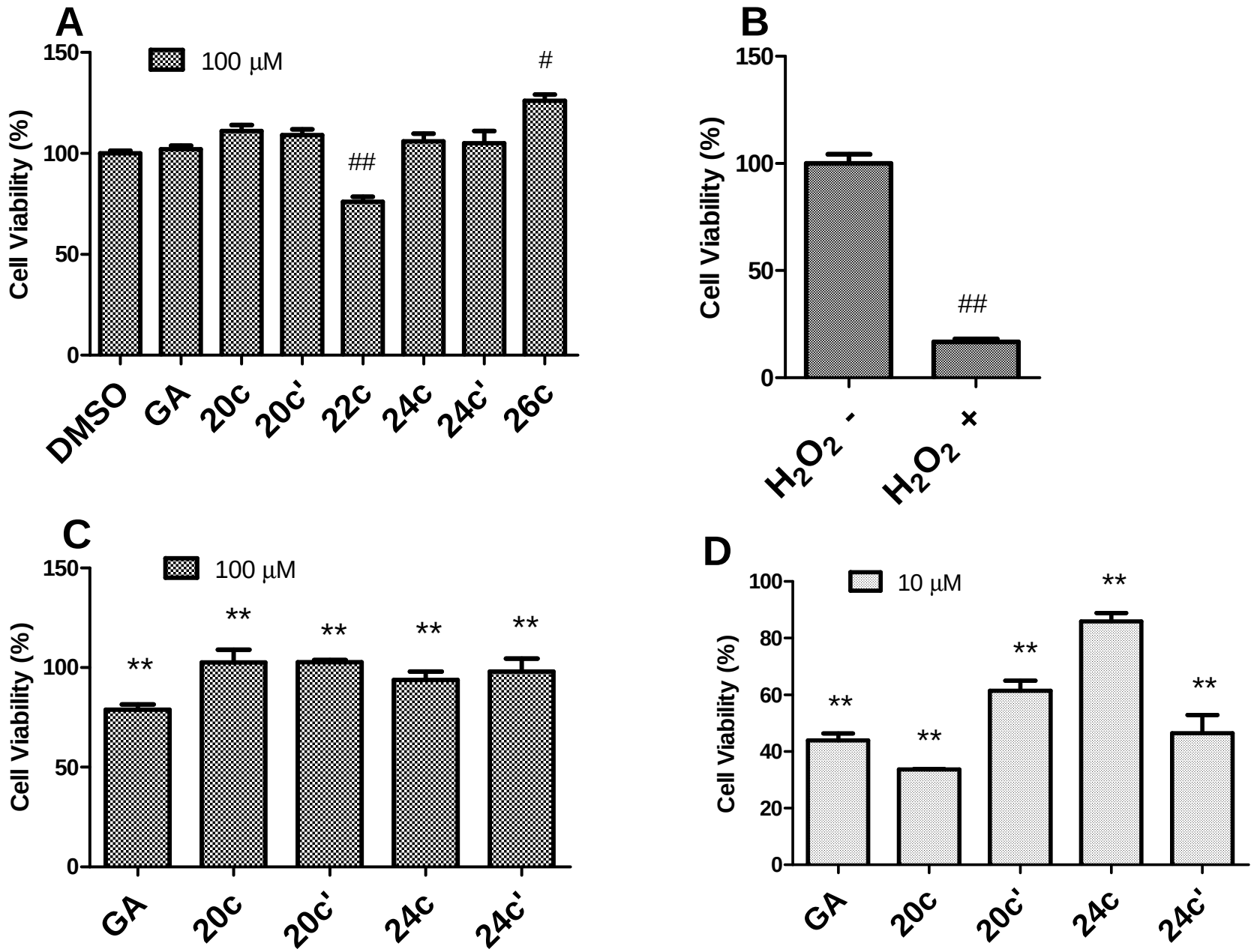
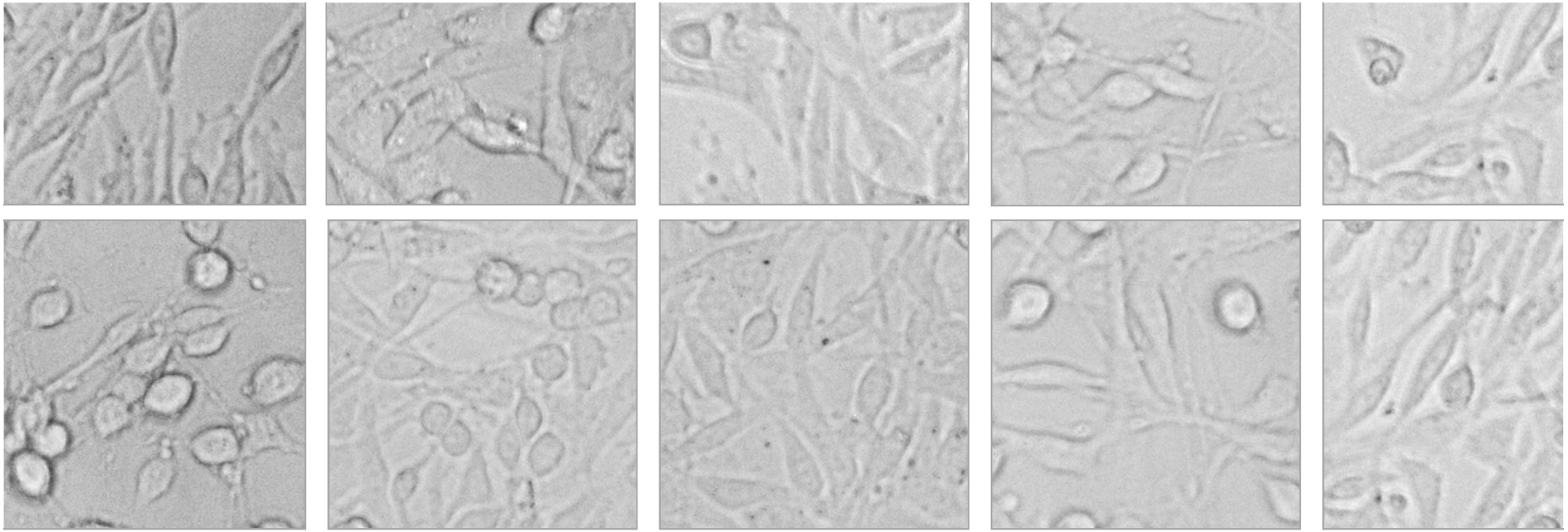
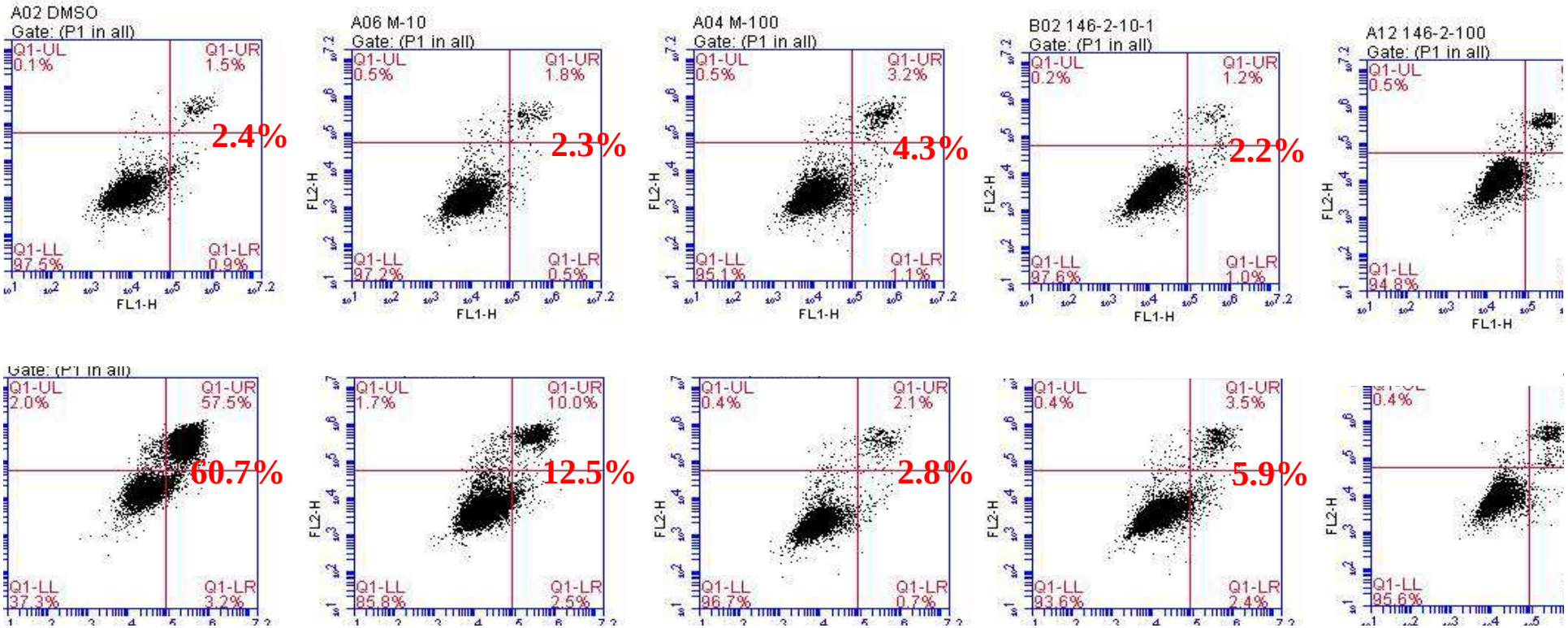


Figure 1 The cytotoxicity of target compounds against PC-12 cells and their cytoprotective activities in H_2O_2-induced oxidative-damage PC12 cell model




A02 DMSO
Gate: (P1 in all)
Q1-UL 0.1%
Q1-UR 1.5%
2.4%
Q1-LL 97.5%
Q1-LR 0.9%
A06 M-10
Gate: (P1 in all)
Q1-UL 0.5%
Q1-UR 1.8%
2.3%
Q1-LL 97.2%
Q1-LR 0.5%
A04 M-100
Gate: (P1 in all)
Q1-UL 0.5%
Q1-UR 3.2%
4.3%
Q1-LL 95.1%
Q1-LR 1.1%
B02 146-2-10-1
Gate: (P1 in all)
Q1-UL 0.2%
Q1-UR 1.2%
2.2%
Q1-LL 97.6%
Q1-LR 1.0%
A12 146-2-100
Gate: (P1 in all)
Q1-UL 0.5%
Q1-LL 94.8%
Gate: (P1 in all)
Q1-UL 2.0%
Q1-UR 57.5%
60.7%
Q1-LL 37.3%
Q1-LR 3.2%
Q1-UL 1.7%
Q1-UR 10.0%
12.5%
Q1-LL 85.8%
Q1-LR 2.5%
Q1-UL 0.4%
Q1-UR 2.1%
2.8%
Q1-LL 96.7%
Q1-LR 0.7%
Q1-UL 0.4%
Q1-UR 3.5%
5.9%
Q1-LL 93.6%
Q1-LR 2.4%
Q1-UL 0.4%
Q1-LL 95.6%
FL1-H
FL2-H

Highlights

1. Anastatins A and B as well as their analogs were synthesized
2. Aurone derivatives showed better antioxidant activity than flavone counterparts
3. Compound **24c** was identified as the most potent antioxidant activity.
4. Compound **24c** decreased apoptosis in H_2O_2-treated PC12 cells.