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Research paper

Total synthesis of 8-(6″-umbelliferyl)-apigenin and its analogs as anti-diabetic reagents

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ABSTRACT

The naturally occurring flavone 8-(6″-umbelliferyl)apigenin, a hybrid structure of apigenin and coumarin, as well as seven of its analogues were synthesized for the first time by using iodination and Suzuki coupling reactions as key steps. The synthesis of 8-(6″-umbelliferyl)-apigenin was achieved in seven linear steps from the commercially available 1-(2,4,6-trihydroxyphenyl)ethan-1-one and 7-hydroxyl coumarine with 31% overall yield. Effects of these compounds on glucose disposal were investigated in adipocytes. All of the flavonoid and coumarin hydrids were found to have better bioactivities than their corresponding flavonoid cores. The most potent compound **15** (10 μM) could promote glucose consumption by 57% which exhibited similar effect as the positive control metformin at 1 mM. Moreover, fluorescence microscopy showed that four 8-(6″-umbelliferyl)apigenin analogues **2**, **15**, **30** and **31** could promote the 2-NBDG uptake into 3T3-L1 cells, which consist with those observed in the regulation of glucose.

1. Introduction

Apigenin, a naturally occurring flavonoid and abundantly present in common fruits, vegetables and herbs such as parsley, onions, oranges, chamomile, tea and wheat sprouts, has been shown to process many bioactivities including anti-cancer [1], anti-inflammatory [2], antioxidant [3] and anti-diabetic properties [4]. Coumarins are a prominent class of benzopyrones of either natural or synthetic origins with diverse biological activities including antimicrobial [5], monoamine oxidase (MAO) inhibition [6], anti-tumor [7], antioxidant [8] and anti-diabetic properties [9]. In 2002, two novel flavonoids, 6-(8″-umbelliferyl)apigenin (**1**) and 8-(6″-umbelliferyl)apigenin (**2**), both belonging to the hybrids of apigenin and coumarin, were isolated from *Gnidia socotrana* by Frank et al. (Fig. 1) [10].

Due to their structural uniqueness and good bioactivities of apigenin and coumarin, along with our on-going interest in the synthesis of natural flavonoids and chalcones, we embarked on a synthetic effort towards these types of molecules [11]. In this paper, we would like to report a facile synthesis of compound **2** and its analogs by using iodination and Suzuki coupling reactions as key steps, as well as anti-diabetic activity evaluation of these new flavonoids.

2. Chemistry

Our retro-synthetic analysis for the synthesis of 8-(6″-umbelliferyl)apigenin was depicted in Scheme 1. The target molecule could be synthesized from iodo apigenin derivative **3** and coumarin boronic ester **4** by Suzuki coupling followed by deprotection. The required iodo compound **3** can be readily prepared from a

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commercially available starting material 1-(2,4,6-trihydroxyphenyl)ethan-1-one (**5**) and the boronic ester fragment **4** can be derived from 7-hydroxyl coumarin (**6**).

Compound **3** was synthesized in four steps from **5** via a regio-selective iodination of the apigenin derivative **9** according to our previously reported procedures (Scheme 2) [11a]. The coumarin boronic ester intermediates **4** was prepared from iodide **13** by coupling with bis(pinacolato)diboron in the presence of PdCl2(dppf). The key intermediate **13** was synthesized from 7-hydroxyl coumarin **6** through methylation, transesterification, regio-selective iodination and relactonization in 62% overall yield according to literature procedures (Scheme 3) [12].

With the key intermediates **3** and **4** in hand, the Suzuki coupling reaction was initially attempted using Pd(PPh3)4 as the catalyst and Cs_2CO_3 as the base in DMF at 100 °C. The desired coupling product **14** was obtained in 14% yield. To improve the yield, the reaction was optimized by screening a variety of solvents and palladium catalysts. (Entries 1–4, Table 1). This led us to the identification of $Pd(PPh_3)_4$ along with Cs_2CO_3 in dioxane at 100 °C as the optimal reaction conditions for the formation of **14**, which was isolated in 70% yield.

The final deprotection was tested by using the common reagent BCl_3. Unfortunately, no formation of the desired product was detected and the reaction gave the partially deprotected intermediate **15** resulting from selective cleavage of the isopropyl groups was obtained. However, when a stronger dealkylation reagent BBr3 was employed, the final target, 8-(6″-umbelliferyl)-apigenin (**2**) was formed in nearly quantitative yield (Scheme 4).

3. Results and discussion

With the natural product **2** and its methyl ether **15** in hand, the anti-diabetic activities of these two compounds were evaluated by using glucose disposal bioassay in adipocytes [13]. To our delight, the 8-(6″-umbelliferyl)-apigenin (**2**) (10 μM) and compound **15** (10 μM) could promote glucose consumption by 48% and 57% respectively.

Encouraged by the results of compounds **2** and **15**, we further designed and synthesized three series of analogous compounds by replacing apigenin with other flavonoids and changing the protection pattern of the 7-hydroxyl on the coumarin moiety. These compounds were synthesized in a similar way as shown in Scheme 5, that *N*-iodosuccinimide (NIS) was used as a highly efficient reagent for the regio-selective iodination when chrysin, luteolin and kaempferol isopropyl ethers were used as substrates according to our previous research [11a].

To gain SAR information of this type of compounds, the activities of the natural product and the analogues were evaluated and compared to apigenin (**16**), chrysin (**17**), quercetin (**18**), luteolin (**19**), 7-hydroxyl coumarin (**6**) (Fig. 2) as well as Metformin which was used as positive control.

The bioactivities of this compounds were summarized in Fig. 3. Generally, the flavonoids including apigenin (**16**), chrysin (**32**), quercetin (**18**) and luteolin (**19**) could promote glucose consumption in adipocytes to some extent, but 7-hydroxyl coumarin (**6**) did not showed this kind of activity. Most of the hybrid compounds including 7-methoxyl coumarin substituted flavonoids and 7-hydroxyl coumarin substituted flavonoids show stronger glucose disposal activity than their corresponding flavonoids cores (**15** and **2** versus apigenin, **29** and **32** versus chrysin, **30** versus quercetin, **31** and **34** versus luteolin), which suggests that introducing a coumarin substitution at 8-position of flavonoids could increase the glucose disposal activity.

Moreover, 7-methoxyl coumarin substituted flavonoids showed stronger glucose disposal activity than the corresponding 7-hydroxyl coumarin substituted flavonoids (**15** versus **2**, **29** versus **32**, **30** versus **33**, **31** versus **34**), which indicated that 7-phenol group on the coumarin diminished the bioactivity (Fig. 3). Although the electronic effect and steric effect of the methoxyl group did not have much difference with the phenol group, an intramolecular hydrogen bond between 7-phenol group on the coumarin and 7-phenol group on the flavonoid may undermine the intermolecular hydrogen bond between target proteins with the latter.

However, the analogues bearing alkyl protection groups including **14**, **26**, **27** and **28** have no glucose disposal activity, which suggested that the phenol groups on the flavonoids of the hybrids were required for the activity (Fig. 4).

In order to further confirm the beneficial effects of four compounds **2**, **15**, **30** and **31** on glucose homeostasis, we then detected their influence on 2-*N*-7-(nitrobenz-2-oxa-1,3-diazol-4-yl)-amino-2-deoxy-D-glucose (2-NBDG) uptake under basal condition by using Metformin as control. As shown in Fig. 5, these four compounds promoted basal glucose uptake in adipocytes evidenced by stronger fluorescence in cytoplasma. These findings were in agreement with those observed in the regulation of glucose consumption.

4. Conclusions

In summary, the apigenin and coumarin hybrid natural product 8-(6″-umbelliferyl)-apigenin (**2**) were synthesized for the first time in seven liner steps from the commercially available 1-(2,4,6-trihydroxyphenyl)ethan-1-one (**5**) and 7-hydroxyl coumarin in 31% overall yield by employing iodination and the Suzuki coupling

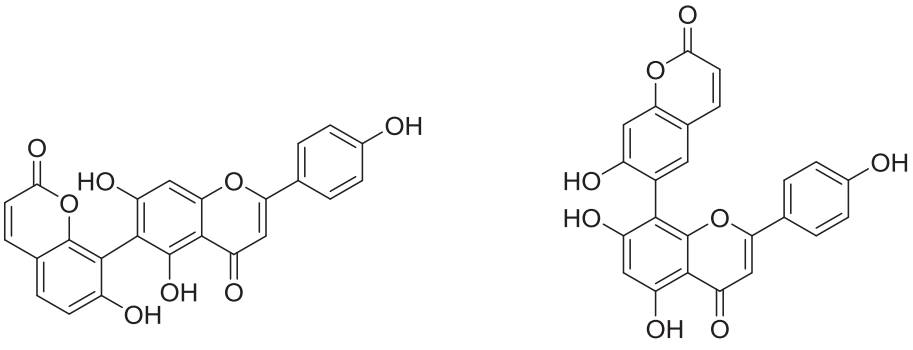
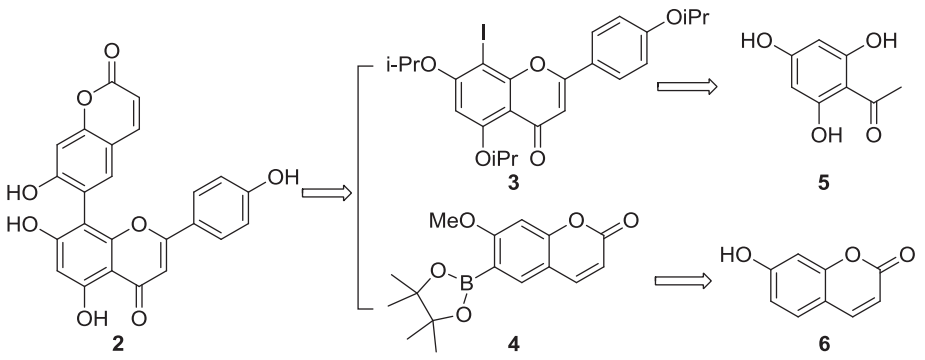
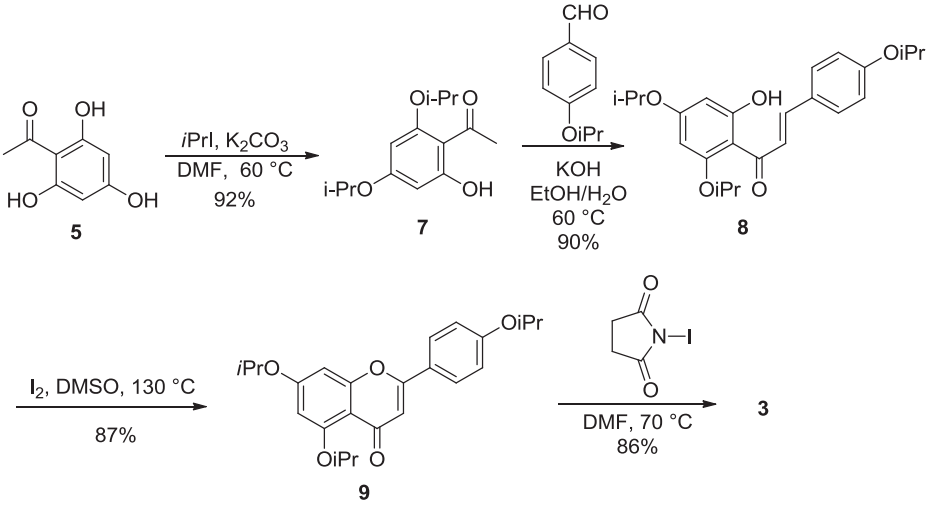


Fig. 1. 6-(8″-umbelliferyl)-apigenin and 8-(6″-umbelliferyl)-apigenin.

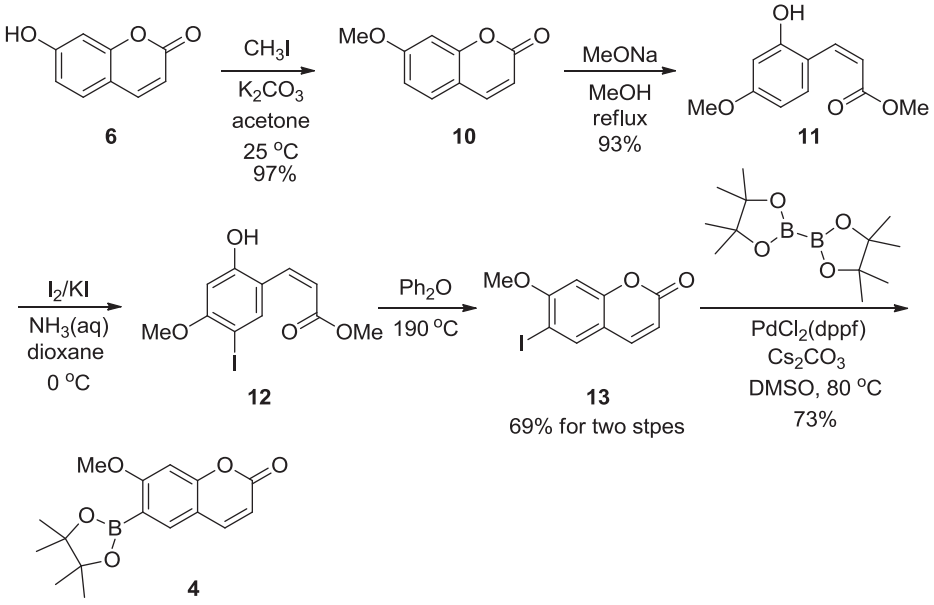


Scheme 1. Retro-synthetic analysis of 8-(6″-umbelliferyl)apigenin (**2**).

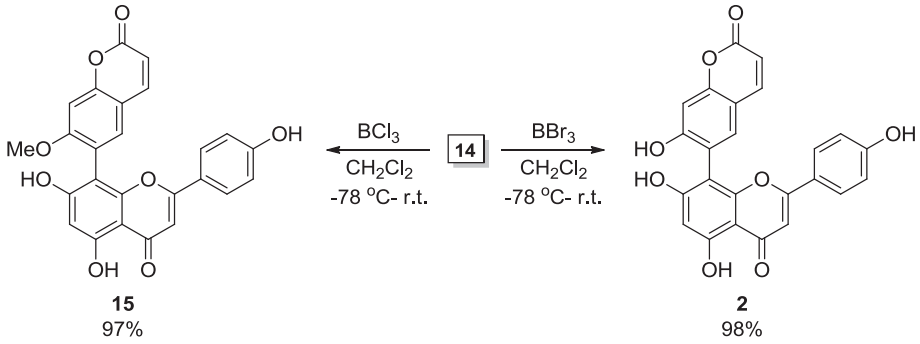


Scheme 2. Synthesis of key intermediate **3**.

reaction as key steps. Moreover seven analogues were synthesized by similar procedures. The biological evaluations of glucose disposal activities in adipocytes for these compounds were performed. The results reveal that attaching 7-methoxyl coumarin to the C-8 position of apigenin, chrysin, quercetin and luteolin would increase the bioactivities compared to those with a flavonoid core. The bioactivity of four compounds **2**, **15**, **30** and **31** was confirmed by detection of 2-NBDG uptake in 3T3-L1 cells with fluorescence



Scheme 3. Synthesis of key intermediate **4**.



Scheme 4. Synthesis of 8-(6″-umbelliferyl)-apigenin **2** and its methyl ether **15**.

microscopy. The syntheses of other 8-(6″-umbelliferyl)-apigenin derivatives and analogues and other biological evaluations for these compounds are ongoing in our laboratory.

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 deuterated solvent ($CDCl_3$ at δ = 7.26 ppm and DMSO-d_6 at δ = 2.50 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) and DMSO-d_6 (δ = 40.0 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 with their corresponding ^{1}H NMR and ^{13}C NMR reported in the literature.

5.1.1. 7-Methoxycoumarin (**10**)

K_2CO_3 (2.0 g, 14.5 mmol) and MeI (3.42 g, 24.1 mmol) were added to a solution of 7-hydroxyl coumarin (**6**) (2.0 g, 12.3 mmol) in acetone (100 mL) and the mixture was reacted for 5 h. After filtration and dilution with EtOAc, the resulting solution was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed to afford compound **10** (2.1 g, 97%) as a crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 16.1 Hz, 1H), 7.40 (d, J = 8.7 Hz, 1H), 6.53–6.48 (m, 2H), 6.38 (s, 1H), 3.81 (s, 3H).

5.1.2. 6-Iodo-7-methoxycoumarin (**13**)

A solution of freshly prepared MeONa [made from Na (1.0 g) and dried methanol (25 mL)] were added to a suspension of **10** (1.0 g, 5.7 mmol) in dry methanol (15 mL) under N_2, and the mixture was refluxed for 4.5 h. After cooling and neutralization with 2 N HCl, the mixture was filtered and dried to give **11** (1.1 g, 93%) as a white solid. NH_4OH (12.5 mL 25% aq) was added to a solution of **11** (0.6 g, 2.9 mmol) in dioxane (5 mL). Then a solution of iodine (0.79 g, 3.1 mmol) in 25 mL of aqueous KI (5% w/v) was added dropwised with stirring at 0 °C. After stirring for 1 h, the mixture was slightly acidified with 2.5 N H_2SO_4. The mixture was filtered and dried to give the mixture of **12** and **13.** Then diphenyl ether (8 mL) was added and the mixture was heated under N_2 at 190 °C for 4 h. After cooling, the mixture purified by column chromatography on silica gel (Hexane: EA = 9: 1) to afford the desired product **13** (600 mg, 69% for two steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.58 (d, J = 12.0 Hz, 1H), 6.77 (s, 1H), 6.27 (d, J = 12.0 Hz, 1H), 3.95 (s, 3H).

5.1.3. 7-methoxy-6-(4, 4, 5, 5-tetramethyl-1, 3, 2-dioxaborolan-2-yl)-coumarin (**4**)

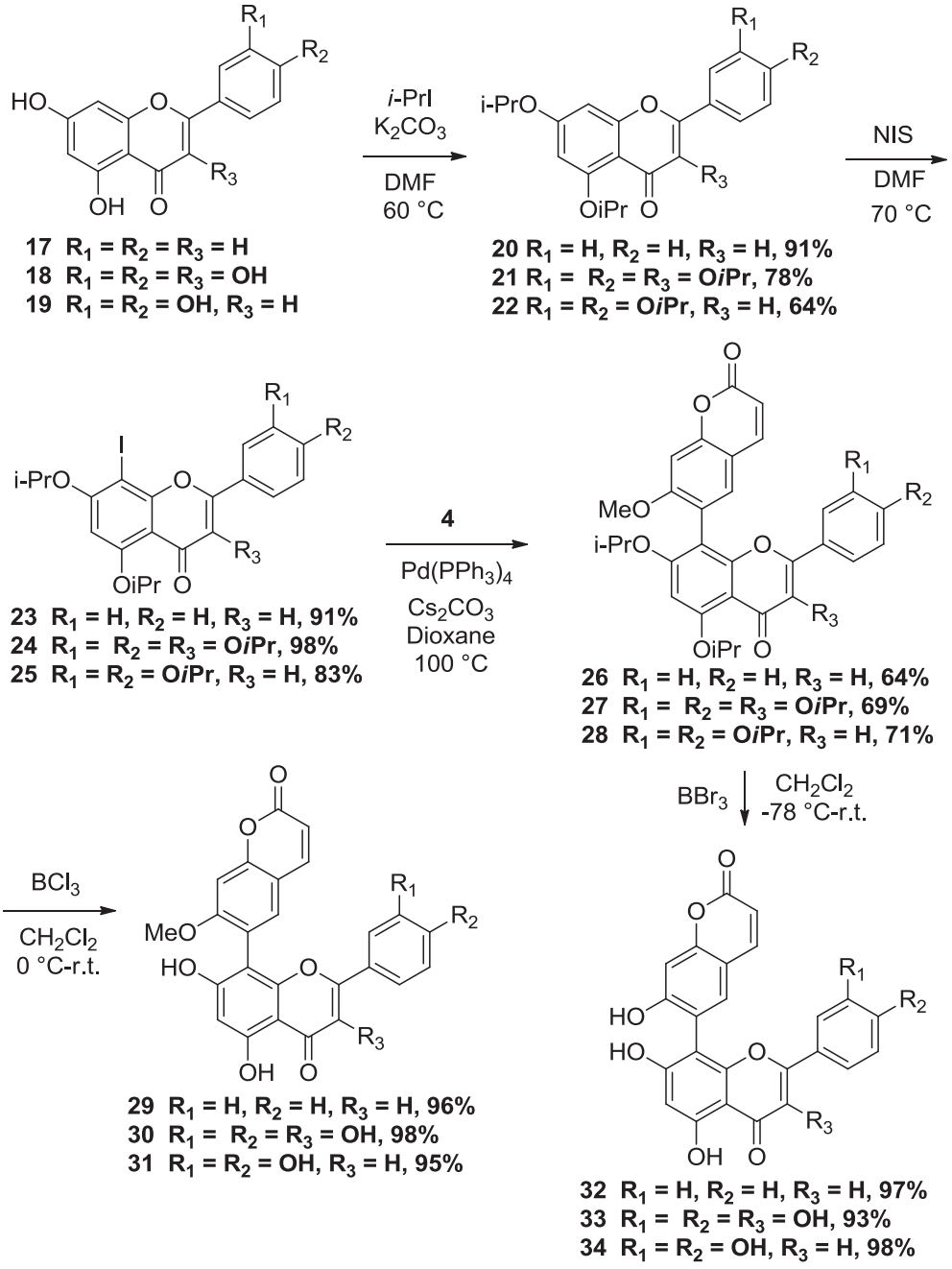
Compound **13** (4.0 g, 13.24 mmol, 1.0 equiv), bis(pinacolato) diboron (6.73 g, 26.48 mmol, 2.0 equiv), $PdCl_2$(dppf) (969 mg, 1.32 mmol, 0.1 equiv) and Cs_2CO_3 (8.63 g, 26.48 mmol, 2.0 equiv) were dissolved in DMSO (60 mL) under argon and the resulting suspension was heated to 80 °C with stirring for 24 h. After cooled to room temperature, the reaction mixture was poured into ice-water (200 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (3 × 200 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 10:1) to afford compound **4** (2.92 g, 9.67 mmol, 73%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.64 (d, J = 8.0 Hz, 1H), 6.76 (s, 1H), 6.23 (d, J = 8.0 Hz, 1H), 3.90 (s, 3H), 1.37 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 161.1, 158.1, 143.5, 137.0, 112.9, 112.1, 98.6, 83.9, 56.2, 24.8; LRMS (ESI) m/z 302 $[M+H]^+$; HRMS (ESI)m/z: Calcd for $C_{16}H_{20}BO_5$ $[M+H]^+$ 302.1434; found, 302.1435.

5.1.4. 1-(2-hydroxy-4,6-diisopropoxyphenyl)ethanone (**7**)

K_2CO_3 (9.86 g, 71.37 mmol) and $(CH_3)_2CHI$ (12.13 g, 71.37 mmol) were added to a stirred solution of **5** (5.00 g, 29.74 mmol) in dry DMF (10 mL). After the addition, the mixture was heated to 60 °C and stirred for 10 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 brine (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 100:1) to afford the known compound **7** (6.90 g, 92%) as a yellow oil.

5.1.5. 5,7-diisopropoxy-2-(4-isopropoxyphenyl)-4H-chromen-4-one (**9**)

4-isopropoxybenzaldehyde (1.65 g, 9.99 mmol) and compound **7** (2.52 g, 9.99 mmol) were added to ethanol (3 mL) with stirring for 5 min. Then a solution of 50% KOH (aq.) (2.80 g, 49.94 mmol) was added to the reaction mixture and heated to 60 °C with stirring for 6 h. After cooled to room temperature, the mixture was poured into



Scheme 5. Synthesis of 8-(6″-umbelliferyl)-apigenin analogues **29**–**34**.

ice water and acidized with concentrated hydrochloric acid to pH = 7.0. Then the suspension was 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 100:1) to afford intermediate **8** (3.58 g, 90%) as a yellow oil.

8 (3.98 g, 9.99 mmol) and I2 (254 mg, 1.0 mmol) were added to a flask with DMSO (5 mL). The reaction mixture was stirred at 130 °C for 7 h. After completion of the reaction, the mixture was 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 (petroleum ether/ethyl acetate 40:1) to afford compound **9** (3.45 g, 87%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 8.8 Hz, 2H), 6.97 (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 = 6.1 Hz, 6H), 1.40 (d, J = 6.1 Hz, 6H), 1.38 (d, J = 6.1 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 $[M+H]^+$.

5.1.6. 5,7-diisopropoxy-2-phenyl-4H-chromen-4-one (**20**)

K_2CO_3 (3.45 g, 24.98 mmol) and $(CH_3)_2CHI$ (4.25 g, 24.98 mmol) were added to a stirred solution of **17** (2.54 g, 9.99 mmol) in dry DMF (10 mL). After the addition, the mixture was heated to 45 °C and stirred for 30 h. The reaction was cooled to room temperature, filtered and diluted with ethyl acetate (100 mL) and the resulting

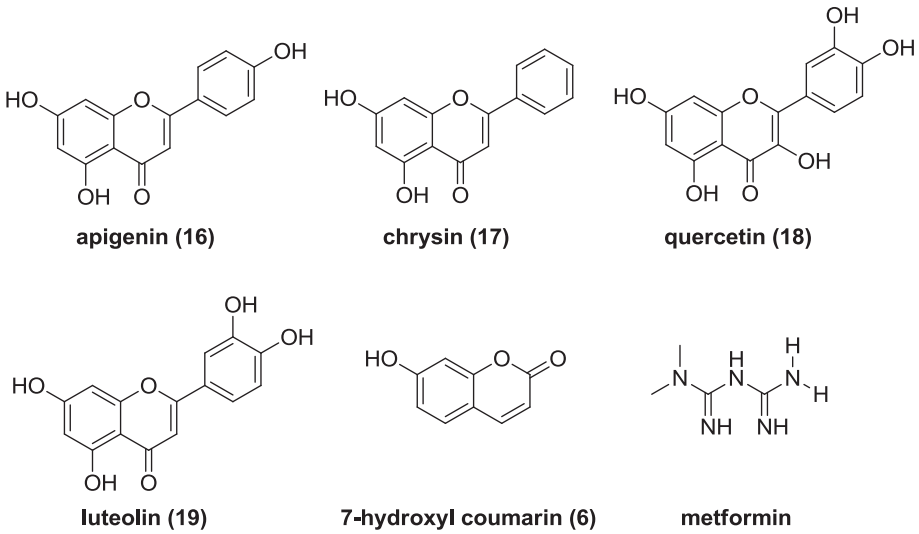


Fig. 2. The structures of apigenin, chrysin, quercetin, luteolin, metformin and 7-hydroxyl coumarin.

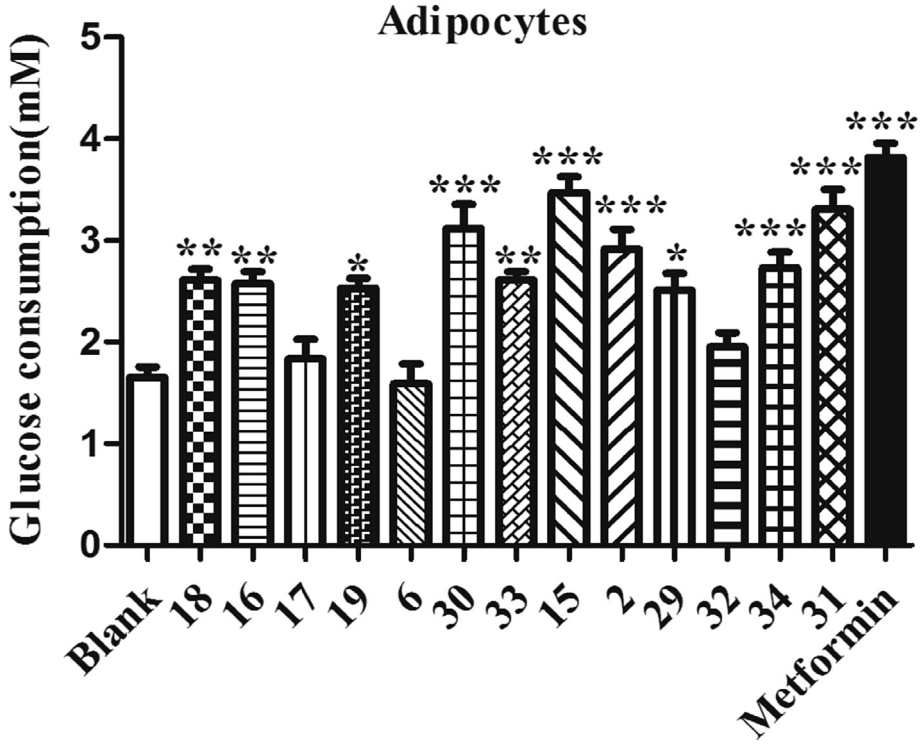


Fig. 3. Apigenin (**16**), chrysin (**17**), quercetin (**18**), luteolin (**19**) metformin, 7-hydroxyl coumarin (**6**), natural product (**2**) and its analogues promoted glucose consumption in adipocytes under basal condition. Data are mean ± SEM ($n = 3$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs*. Blank.

solution was poured into aqueous HCl (1 M, 100 mL). The organic layer was separated, washed with brine (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 100:1) to afford the known compound **20** (3.08 g, 91%) as a pale yellow solid.

*5.1.7. 2-(3,4-diisopropoxyphenyl)-3,5,7-triisopropoxy-4H-chromen-4-one (**21**)*

The procedure is the same as the preparation of **20** by using compound **18** (3.02 g, 9.99 mmol) as substrate to give known compound **21** (4.0 g, 78%) as a pale yellow solid.

*5.1.8. 2-(3,4-diisopropoxyphenyl)-5,7-diisopropoxy-4H-chromen-4-one (**22**)*

The procedure is the same as the preparation of **20** by using compound **19** (2.86 g, 9.99 mmol) as substrate to give compound **22** (2.91 g, 64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (dd, $J = 8.4$, 2.4 Hz, 1H), 7.41 (d, $J = 2.0$ Hz, 1H), 6.98 (d, $J = 8.4$ Hz, 1H), 6.53 (d, $J = 2.4$ Hz, 1H), 6.50 (s, 1H), 6.36 (d, $J = 2.4$ Hz, 1H), 4.68–4.51 (m, 4H), 1.46 (d, $J = 6.4$ Hz, 6H), 1.41 (d, $J = 6.0$ Hz, 6H), 1.38 (d, $J = 6.0$ Hz,

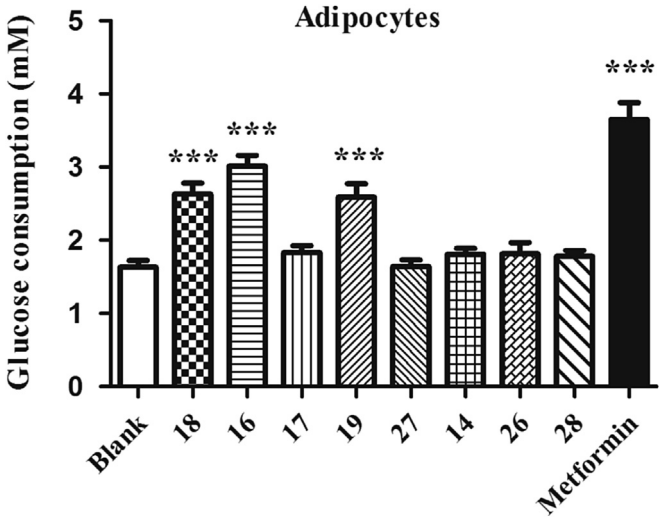


Fig. 4. Apigenin (**16**), chrysin (**17**), quercetin (**18**), luteolin (**19**), metformin and analogues bearing alkyl protection groups promoted glucose consumption in adipocytes under basal conditions.

6H), 1.38 (d, $J = 6.0$ Hz, 6H).

*5.1.9. 8-iodo-5,7-diisopropoxy-2-(4-isopropoxyphenyl)-4H-chromen-4-one(**3**)*

NIS (2.47 g, 10.99 mmol) was added to a solution of **9** (3.97 g, 10 mmol) in dry DMF (25 mL) and the resulting solution was stirred at 70 °C for 10 h. 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 (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 **3** (4.49 g, 86%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, $J = 8.4$ Hz, 2H), 6.97 (d, $J = 8.4$ Hz, 2H), 6.54 (s, 1H), 6.43 (S, 1H), 4.71–4.55 (m, 3H), 1.44 (d, $J = 6.0$ Hz, 12H), 1.36 (d, $J = 6.0$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 161.1, 161.0, 160.7, 159.9, 157.9, 128.2, 123.2, 115.9, 111.5, 106.7, 99.3, 73.6, 72.6, 70.1, 22.1, 22.0, 21.9; LRMS (ESI) *m/z* 523 $[M+H]^+$; HRMS (ESI)*m/z*: Calcd for $C_{24}H_{27}O_5I$ $[M+Na]^+$ 545.0795; found, 545.0820.

*5.1.10. 8-iodo-5,7-diisopropoxy-2-phenyl-4H-chromen-4-one (**23**)*

The procedure is the same as the preparation of **3** by using compound **20** (3.38 g, 1.0 mmol) as substrate to give compound **23** (4.22 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02–7.99 (m, 2H), 7.53–7.51 (m, 3H), 6.66 (s, 1H), 6.50 (s, 1H), 4.75–4.66 (m, 1H), 4.64–4.55 (m, 1H), 1.46 (dd, $J = 6.0$, 1.4 Hz, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.1, 160.7, 158.8, 158.4, 155.6, 131.4, 131.3, 129.0, 126.2, 111. 4, 108.3, 99.9, 93.4, 73.6, 72.6, 22.0, 21. 9; LRMS (ESI) *m/z* 465 $[M+H]^+$; HRMS (ESI)*m/z*: Calcd for $C_{21}H_{21}O_4I$ $[M+H]^+$ 465.0557; found, 465.0568.

*5.1.11. 8-iodo-2-(3,4-diisopropoxyphenyl)-3,5,7-triisopropoxy-4H-chromen-4-one (**24**)*

The procedure is the same as the preparation of **3** by using compound **21** (5.13 g, 10.0 mmol) as substrate to give compound **24** (6.25 g, 98%).

*5.1.12. 8-iodo-2-(3,4-diisopropoxyphenyl)-5,7-diisopropoxy-4H-chromen-4-one (**25**)*

The procedure is same as the preparation of **3** by using compound **22** (4.54 g, 10.0 mmol) as substrate to give compound **25** (4.81 g, 83%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, $J = 2.0$ Hz, 1H), 7.62 (dd, $J = 8.5$, 2.1 Hz, 1H), 7.01 (d, $J = 8.5$ Hz, 1H), 6.58 (s, 1H), 6.45 (s, 1H), 4.71 (m, 1H), 4.66–4.54 (m, 3H), 1.47 (d, $J = 6.0$ Hz, 12H), 1.41 (d, $J = 6.0$ Hz, 6H), 1.39 (d, $J = 6.$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 161.0, 160.0, 157.9, 152.1, 149.0, 124.1, 120.5, 116.7, 115.7, 111.4, 107.0, 99.2, 73.6, 72.6, 72.5, 72.0, 22.3, 22.1, 22.1, 21.9; LRMS (ESI) *m/z* 581 $[M+H]^+$; HRMS (ESI)*m/z*: Calcd for $C_{27}H_{33}O_6I$ $[M+Na]^+$ 603.1214; found, 603.1247.

*5.1.13. 5′,7′-diisopropoxy-7-methoxy-2′-phenyl-2H,4′H-[6,8′-bichromene]-2,4′-dione (**26**)*

Compound **23** (464 mg, 1.0 mmol), **4** (393 mg, 1.3 mmol), tetrakis(triphenylphosphine)palladium (116 mg, 0.1 mmol) and Cs_2CO_3 (652 mg, 2.0 mmol) were dissolved in dioxane (5 mL) under argon and the resulting suspension was heated to 100 °C with stirring for 12 h. After cooled to room temperature, the reaction mixture was poured into ice-water (50 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (3 × 50 mL), dried over anhydrous 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 10:1) to get crude product, then washed with PE:EA = 1:1 to afford a new compound **26** (328 mg, 0.64 mmol, 64%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, $J = 9.5$ Hz, 1H), 7.44–7.37 (m, 4H), 7.32 (m, 2H), 6.97 (s, 1H), 6.62 (s, 1H), 6.53 (s, 1H), 6.32 (d, $J = 9.4$ Hz, 1H), 4.63 (m, 2H), 3.77 (s, 3H), 1.51 (d, $J = 6.1$ Hz, 6H), 1.28 (d, $J = 6.0$ Hz, 3H), 1.24 (d, $J = 6.0$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$)

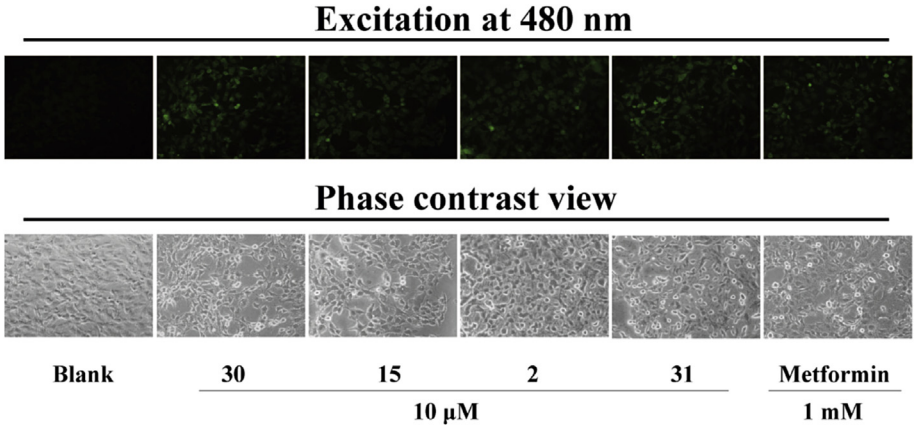
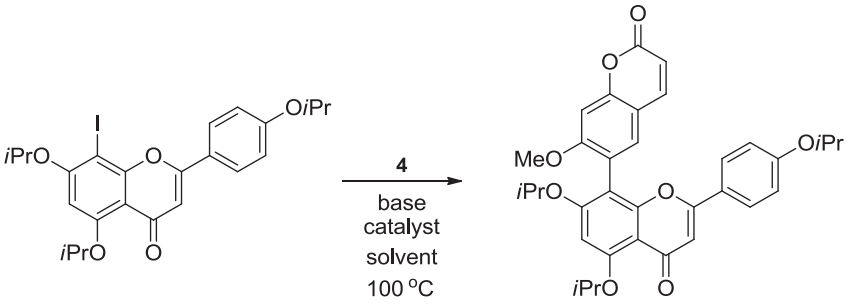


Fig. 5. Metformin as well as the best four compounds **2**, **15**, **30** and **31** regulated 2-NBDG uptake in 3T3-L1 adipocytes under basal condition.

Table 1
Suzuki coupling reaction of compound **3** under different conditions.[a]



3 **14**

Entry	Solvent	Catalyst	Base	Yield[b] (%)
1	DMF	$Pd(PPh_3)_4$	Cs_2CO_3	14
2	DMSO	$Pd(PPh_3)_4$	Cs_2CO_3	16
3	Dioxane	$Pd(PPh_3)_4$	Cs_2CO_3	70
4	Dioxane	$PdCl_2$(dppf)	Cs_2CO_3	11

[a] Reaction conditions: **3** (1.0 mmol), **4** (1.2 mmol), catalyst (0.1 mmol) and base (2.0 mmol) in solvent (3 mL) heated to 100 °C for 12 h.
[b] Isolated yield after purification by silica gel chromatography.

δ 177.6, 161.3, 161.0, 160.0, 159.6, 159.2, 156.6, 155.7, 143.5, 131.5, 131.5, 131.1, 128.9, 125.5, 119.5, 113.2, 112.1, 110.4, 108.2, 107.9, 99.0, 98.9, 73.3, 71.4, 56.0, 22.1, 22.1, 22.0, 21.9; LRMS (ESI) *m/z* 513 $[M+H]^+$; HRMS (ESI)*m/z*: Calcd for $C_{31}H_{28}O_7$ $[M+Na]^+$ 535.1727; found, 535.1742.

*5.1.14. 5′,7′-diisopropoxy-2′-(4-isopropoxyphenyl)-7-methoxy-2H,4′H-[6,8′-bichromene]-2,4′-dione (**14**)*

The procedure is same as the preparation of **26** by using compound **3** (522 mg, 1.0 mmol) as substrate to give compound **14** (410 mg, 0.72 mmol, 72%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 9.6 Hz, 1H), 7.41 (s, 1H), 7.33 (d, *J* = 8.8 Hz, 2H), 6.97 (s, 1H), 6.79 (d, *J* = 8.8 Hz, 2H), 6.52 (d, *J* = 7.2 Hz, 2H), 6.32 (d, *J* = 9.6 Hz, 1H), 4.68–4.54 (m, 3H), 3.77 (s, 3H), 1.50 (d, *J* = 6.0 Hz, 6H), 1.32 (dd, *J* = 5.9, 2.5 Hz, 6H), 1.28 (d, *J* = 6.0 Hz, 3H), 1.24 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.6, 161.4, 161.0, 160.4, 160.2, 159.4, 159.1, 156.5, 155.6, 143.5, 131.5, 127.2, 123.2, 119.6, 115.8, 113.1, 112.1, 110.3, 107.8, 106.6, 99.0, 98.9, 73.3, 71.3, 70.1, 56.0, 22.1, 22.1, 22.0, 21.9; LRMS (ESI) *m/z* 571 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd for $C_{34}H_{34}O_8$ $[M+ Na]^+$ 593.2146; found, 593.2177.

*5.1.15. 2′-(3,4-diisopropoxyphenyl)-3′,5′,7′-triisopropoxy-7-methoxy-2H,4′H-[6,8′-bichromene]-2,4′-dione (**27**)*

The procedure is same as the preparation of **26** by using compound **24** (638 mg, 1.0 mmol) as substrate to afford a new compound **27** (473 mg, 0.69 mmol, 69%) as a light brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 12.0 Hz, 1H), 7.48 (d, *J* = 8.0, 1H), 7.39 (s, 1H), 7.30 (s, 1H), 6.93 (s, 1H), 6.75 (d, *J* = 8.0 Hz, 1H), 6.49 (s, 1H), 6.28 (d, *J* = 8.0 Hz, 1H), 4.80–4.63 (m, 1H), 4.58–4.49 (m, 3H), 4.13–4.09 (m, 1H), 3.75 (s, 3H), 1.49 (d, *J* = 8.0 Hz, 6H), 1.28 (dd, *J* = 8.0 Hz, *J* = 28.0 Hz, 6H), 1.21–1.15 (m, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5, 161.3, 161.1, 159.3, 159.2, 155.6, 155.5, 152.7, 151.0, 147.8, 143.6, 138.4, 131.5, 124.2, 123.0, 119.7, 118.4, 115.2, 113.0, 112.1, 110.3, 107.3, 99.0, 98.2, 73.9, 73.1, 72.5, 71.4, 71.3, 56.0, 22.4, 22.4, 22.2, 22.1, 22.1, 22.0, 21.9; LRMS (ESI) *m/z* 687 $[M+H]^+$; HRMS (ESI) *m/z*: Calcd for $C_{40}H_{47}O_{10}$ $[M+H]^+$687.3161; found, 687.3164.

*5.1.16. 2′-(3,4-diisopropoxyphenyl)-5′,7′-diisopropoxy-7-methoxy-2H,4′H-[6,8′-bichromene]-2,4′-dione (**28**)*

The procedure is same as the preparation of **26** by using compound **25** (580 mg, 1.0 mmol) as substrate to afford a new compound **28** (446 mg, 0.71 mmol, 71%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 9.5 Hz, 1H), 7.40 (s, 1H), 7.08 (dd, *J* = 8.5, 1.8 Hz, 1H), 6.95 (s, 1H), 6.86 (d, *J* = 1.7 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 6.50 (d, *J* = 4.0 Hz, 2H), 6.30 (d, *J* = 9.6 Hz, 1H), 4.66–4.46 (m, 3H), 4.11–4.02 (m, 1H), 3.77 (s, 3H), 1.49 (d, *J* = 6.0 Hz, 6H), 1.30 (d, *J* = 6.0 Hz, 6H), 1.24 (dd, *J* = 13.6, 6.0 Hz, 6H), 1.16 (d, *J* = 6.0 Hz, 3H), 1.13 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.6, 161.2, 161.1, 160.1, 159.4, 159.1, 156.5, 155.6, 152.0, 148.6, 143.6, 131.4, 124.1, 119.8, 119.7, 116.3, 115.1, 113.1, 112.0, 110.3, 107.7, 106.8, 98.9, 98.8, 73.2, 72.3, 71.8, 71.3, 56.1, 22.1, 22.1, 22.0, 21.9, 21.9; LRMS (ESI) *m/z* 629 $[M+H]^+$; HRMS (ESI)*m/z*: Calcd for $C_{37}H_{40}O_9$ $[M+Na]^+$ 651.2565; found, 651.2592.

*5.1.17. 5′,7′-dihydroxy-7-methoxy-2′-phenyl-2H,4′H-[6,8′-bichromene]-2,4′-dione (**29**)*

Boron tri-chloride (0.7 mL, 0.7 mmol, 1 M solution in hexane) was added to a stirred solution of **26** (51 mg, 0.1 mmol) in anhydrous DCM (5 mL) under 0 °C for 30 min. After addition, the reaction mixture was heated to room temperature for 4 h. Then excess ice-water was added to the reaction mixture with stirring for 10 min. Then the suspension was filtrated and the residue was recrystallized in methanol to afford compound **29** (41 mg, 96%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.97 (s, 1H), 10.93 (s, 1H), 8.06 (d, *J* = 9.6 Hz, 1H), 7.70 (s, 1H), 7.66 (d, *J* = 7.3 Hz, 2H), 7.53 (d, *J* = 6.6 Hz, 1H), 7.47 (d, *J* = 7.0 Hz, 2H), 7.27 (s, 1H), 7.04 (s, 1H), 6.45 (s, 1H), 6.37 (d, *J* = 9.1 Hz, 1H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.64, 163.4, 162.5, 161.1, 161.1, 160.8, 155.8, 155.0, 144.7, 132.5, 131.1, 129.6, 126.4, 118.3, 113.2, 112.4, 105.4, 104.3, 103.7, 99.8, 99.2, 56.8; LRMS (ESI) *m/z* 429 $[M+H]^+$; HRMS (ESI)*m/z*: Calcd for $C_{25}H_{16}O_7$ $[M-H]^-$ 427.0823; found, 427.0836.

*5.1.18. 5′,7′-dihydroxy-2′-(4-hydroxyphenyl)-7-methoxy-2H,4′H-[6,8′-bichromene]-2,4′-dione (**15**)*

The procedure is same as the preparation of **29** by using **14** (57 mg, 0.1 mmol) as substrate, Boron tri-chloride (0.8 mL, 0.8 mmol, 1 M solution in hexane) to afford compound **15** (43 mg, 97%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.10 (s, 1H), 10.84 (s, 1H), 10.34 (s, 1H), 8.05 (d, *J* = 9.6 Hz, 1H), 7.67 (s, 1H), 7.49 (d, *J* = 8.8 Hz, 2H), 7.27 (s, 1H), 6.82–6.78 (m, 3H), 6.41 (s, 1H), 6.37 (d, *J* = 9.2 Hz, 1H), 3.79 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.5, 164.0, 162.2, 161.6, 161.1, 161.1, 160.9, 155.7, 154.9, 144.7, 132.5, 128.5, 121.6, 118.4, 116.4, 113.1, 112.4, 104.0, 103.5, 103.0, 99.7, 99.0, 56.8; LRMS (ESI) *m/z* 445 $[M+H]^+$; HRMS (ESI)*m/z*: Calcd for

$C_{25}H_{17}O_8$ $[M-H]^-$ 443.0772; found, 448.0810.

*5.1.19. 2′-(3,4-dihydroxyphenyl)-3′,5′,7′-trihydroxy-7-methoxy-2H,4′H-[6,8′-bichromene]-2,4′-dione (**30**)*

The procedure is same as the preparation of **29** by using **27** (69 mg, 0.1 mmol) as substrate, Boron tri-chloride (1.0 mL, 1.0 mmol, 1 M solution in hexane) to afford compound **30** (47 mg, 98%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.61 (s, 1H), 10.76 (s, 1H), 9.62 (s, 1H), 9.42 (s, 1H), 9.06 (s, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.64 (s, 1H), 7.39 (s, 1H), 7.23 (s, 1H), 7.02 (d, J = 8.0 Hz, 1H), 6.70 (d, J = 8.0 Hz, 1H), 6.39 (s, 1H), 6.35 (d, J = 12.0 Hz, 1H), 3.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ184.5, 176.5, 161.8, 161.2, 160.9, 160.3, 155.7, 153.8, 148.1, 147.3, 145.4, 144.8, 136.1, 132.5, 122.5, 119.7, 118.5, 115.8, 113.0, 112.4, 103.4, 103.1, 99.7, 98.4, 56.7; LRMS (ESI) *m/z* 477.0 $[M+H]^+$; HRMS (ESI)*m/z*: Calcd for $C_{25}H_{16}O_{10}$ $[M+H]^+$ 477.0816; found, 477.0808.

*5.1.20. 2′-(3,4-dihydroxyphenyl)-5′,7′-dihydroxy-7-methoxy-2H,4′H-[6,8′-bichromene]-2,4′-dione (**31**)*

The procedure is same as the preparation of **29** by using **28** (63 mg, 0.1 mmol) as substrate, Boron tri-chloride (0.9 mL, 0.9 mmol, 1 M solution in hexane) to afford compound **31** (44 mg, 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.13 (s, 1H), 10.83 (s, 1H), 9.99 (s, 1H), 9.20 (s, 1H), 8.05 (d, J = 8.8 Hz, 1H), 7.66 (s, 1H), 7.25 (s, 1H), 7.02 (s, 1H), 6.96 (s, 1H), 6.77 (d, J = 8.0 Hz, 1H), 6.70 (s, 1H), 6.41 (s, 1H), 6.36 (d, J = 8.8 Hz, 1H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.4, 164.3, 162.1, 161.1, 161.1, 160.9, 155.8, 154.9, 150.2, 146.1, 144.8, 132.4, 122.0, 119.0, 118.4, 116.3, 113.9, 113.1, 112.4, 104.1, 103.5, 103.1, 99.8, 99.0, 56.7; LRMS (ESI) *m/z* 461 $[M+H]^+$; HRMS (ESI)*m/z*: Calcd for $C_{25}H_{16}O_9$ $[M-H]^-$ 459.0722; found, 459.0751.

*5.1.21. 5′,7,7′-trihydroxy-2′-phenyl-2H,4′H-[6,8′-bichromene]-2,4′-dione (**32**)*

Tribromoborane (175 mg, 0.7 mmol, 7.0 equiv) was added to a stirred solution of **26** (51 mg, 0.1 mmol) in anhydrous DCM (5 mL) under −78 °C for 30 min. After addition, the reaction mixture was slowly heated to room temperature for 12 h. Then the reaction was cooled to 0 °C, excess ice-water was added with stirring for 10 min. DCM was removed under reduced pressure, then the suspension was filtrated and the residue was dried to afford the product **32** as a yellow solid (40 mg, 97%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.97 (s, 1H), 10.89 (s, 1H), 10.63 (s, 1H), 8.00 (d, J = 9.5 Hz, 1H), 7.73 (d, J = 7.4 Hz, 2H), 7.62 (s, 1H), 7.54 (d, J = 7.2 Hz, 1H) 7.52–7.44 (m, 3H), 7.00 (d, J = 16.0 Hz, 2H), 6.45 (s, 1H), 6.27 (d, J = 9.4 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.2, 163.1, 162.1, 160.5, 160.5, 159.5, 154.8, 154.7, 144.5, 132.5, 132.0, 130.8, 129.1, 126.1, 116.8, 111.6, 111.2, 104.9, 103.9, 103.5, 102.0, 98.7; LRMS (ESI) *m/z* 415 $[M+H]^+$; HRMS (ESI)*m/z*: Calcd for $C_{24}H_{14}O_7$ $[M-H]^-$ 413.0667; found, 413.0677.

*5.1.22. 5′,7,7′-trihydroxy-2′-(4-hydroxyphenyl)-2H,4′H-[6,8′-bichromene]-2,4′-dione (**2**)*

The procedure is same as the preparation of **32** by using **14** (57 mg, 0.1 mmol) as substrate, Tribromoborane (200 mg, 0.8 mmol, 8.0 equiv) to afford compound **2** (42 mg, 98%). ^{1}H NMR (400 MHz, acetone-d_6) δ 13.18 (s, 1H), 9.47 (s, 3H), 7.94 (d, J = 9.5 Hz, 1H), 7.64 (S, 1H), 7.64 (d, J = 8.8 Hz, 2H), 6.99 (s, 1H), 6.88 (d, J = 8.8 Hz, 2H), 6.67 (s, 1H), 6.43 (s, 1H), 6.24 (d, J = 9.5 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.1, 163.7, 161.8, 161.1, 160.6, 159.6, 154.9, 154.6, 144.5, 132.5, 128.2, 121.3, 117.0, 115.9, 111.7, 111.3, 103.7, 103.4, 102.7, 102.1, 98.6; LRMS (ESI) *m/z* 431 $[M+H]^+$; HRMS (ESI)*m/z*: Calcd for $C_{24}H_{14}O_8$ $[M-H]^-$ 429.0616; found, 429.0646.

*5.1.23. 2′-(3,4-dihydroxyphenyl)-3′,5′,7,7′-tetrahydroxy-2H,4′H-[6,8′-bichromene]-2,4′-dione (**33**)*

The procedure is same as the preparation of **32** by using **27** (69 mg, 0.1 mmol) as substrate, Tribromoborane (250 mg, 1.0 mmol) to afford compound **33** (43 mg, 93%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.63 (s, 1H), 11.46 (s, 1H), 9.52 (s, 1H), 9.30 (s, 1H), 9.04 (s, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.65 (s, 1H), 7.52 (s, 1H), 7.16 (d, J = 8.0 Hz, 1H), 6.82 (s, 1H), 6.69 (d, J = 8.0 Hz, 1H), 6.26 (s, 1H), 6.19 (d, J = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.3, 162.3, 161.2, 160.1, 155.3, 154.0, 147.9, 146.8, 145.3, 145.1, 135.9, 132.7, 122.8, 119.9, 118.9, 116.0, 115.8, 111.1, 110.8, 104.5, 103.3, 102.8, 99.6; LRMS (ESI) *m/z* 463 $[M+H]^+$; HRMS (ESI)*m/z*: Calcd for $C_{24}H_{15}O_{10}$ $[M+H]^+$ 463.0660; found, 463.0668.

*5.1.24. 2′-(3,4-dihydroxyphenyl)-5′,7,7′-trihydroxy-2H,4′H-[6,8′-bichromene]-2,4′-dione (**34**)*

The procedure is same as the preparation of **32** by using **28** (63 mg, 0.1 mmol) as substrate, Tribromoborane (225 mg, 0.9 mmol) to afford compound **34** (44 mg, 98%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (d, J = 9.6 Hz, 1H), 7.58 (s, 1H), 7.09 (d, J = 8.0 Hz, 1H), 7.04 (s, 1H), 6.98 (s, 1H), 6.78 (d, J = 8.0 Hz, 1H), 6.69 (s, 1H), 6.42 (s, 1H), 6.26 (d, J = 9.2 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.5, 164.4, 162.2, 161.0, 160.0, 155.3, 155.1, 150.1, 146.1, 145.0, 132.9, 122.2, 119.2, 117.4, 116.2, 114.1, 112.0, 111.7, 104.1, 103.8, 103.0, 102.6, 99.0; LRMS (ESI) *m/z* 447 $[M+H]^+$; HRMS (ESI)*m/z*: Calcd for $C_{24}H_{14}O_9$ $[M-H]^-$ 445.0565; found, 445.0596.

5.2. Biology evaluation

5.2.1. Cell culture and differentiation

3T3-L1 cells (a cell line of preadipocyte from ATCC) were cultured in Dulbecco's Minimum Essential Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS), 100 μg/mL of streptomycin and 100 U/mL of penicillin at 37 °C in a 5% CO_2 atmosphere. Two days after full confluence, cells were differentiated via incubation in DMEM containing 0.5 mM isobutylmethylxanthine (IBMX), 1 μM dexamethasone (Dex), 10 μg/mL insulin for 48 h, and then for 2 days in DMEM (10% FBS) containing 10 μg/mL insulin alone [13]. Adipocytes were used 8–10 days after the initiation of differentiation.

5.2.2. Glucose consumption

Serum-starved adipocytes (2×10^5 cells per well) were cultured in 48-well plates for 4 h in KRH (Krebs–Ringer phosphate–HEPES buffer, containing 118 mmol/L NaCl, 5 mmol/L KCl, 1.3 mmol/L $CaCl_2$, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4, and 30 mmol/L HEPES, containing 0.5% BSA, pH 7.4) containing 11 mmol/L glucose and pretreated with 10 μmol/L compounds or 1 mmol/L metformin respectively. The glucose concentration in KRH at 0 h and 4 h after incubation were determined with a commercial kit based on the glucose oxidase peroxidase (GOD-POD) method and the difference between the two concentrations was regarded as the amount of consumed glucose.

5.2.3. Detection of 2-N-7-(nitrobenz-2-oxa-1,3-diazol-4-yl)-amino-2-deoxy-D-glucose uptake by 3T3-L1 cells with fluorescence microscopy

After 1-h serum starvation, 3T3-L1 cells were incubated with agents in KRH for 0.5 h, and cultured with 500 μmol/L 2-*N*-7-(nitrobenz-2-oxa-1,3-diazol-4-yl)-amino-2-deoxy-D-glucose (2-NBDG) for another 1 h. After washed with KRH for 3 times, cells were observed by inverted fluorescence microscope (Olympus IX81).

5.2.4. Statistics

All data were expressed as mean ± SEM (standard error of the mean), with n representing the number of independent experiments. GraphPad Prism6.0 was used for statistical analysis and

graphs. Mean values were compared by the one-way ANOVA with Dunnett's post-hoc test. Values of $p < 0.05$ were regarded as being statistically significant.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2016.07.015. These data include MOL files and InChiKeys of the most important compounds described in this article.

References

[1] (a) H.F. Lu, Y.J. Chie, M.S. Yang, C.S. Lee, J.J. Fu, J.S. Yang, T.W. Tan, S.H. Wu, Y.S. Ma, S.W. Ip, J.G. Chung, Apigenin induces caspase-dependent apoptosis in human lung cancer A549 cells through Bax- and Bcl-2-triggered mitochondrial pathway, Int. J. Oncol. 36 (2010) 1477–1484;
(b) D. Patel, S. Shukla, S. Gupta, Apigenin and cancer chemoprevention: progress, potential and promise, Int. J. Oncol. 30 (2007) 233–245;
(c) S. Shukla, S. Gupta, Apigenin suppresses insulin-like growth factor I receptor signaling in human prostate cancer: an in vitro and in vivo study, Mol. Carcinog. 48 (2009) 243–252.

[2] (a) J. Wang, Y.T. Liu, L. Xiao, L. Zhu, Q. Wang, T. Yan, Anti-inflammatory effects of apigenin in lipopolysaccharide-induced inflammatory in acute lung injury by suppressing COX-2 and NF-kB pathway, Inflammation 37 (2014) 2085–2090;
(b) G.S. Jeong, S.H. Lee, S.N. Jeong, Y.C. Kim, E.C. Kim, Anti-inflammatory effects of apigenin on nicotine- and lipopolysaccharide-stimulated human periodontal ligament cells via heme oxygenase-1, Int. Immunopharmacol. 9 (2009) 1374–1380.

[3] (a) W.W. Jung, Protective effect of apigenin against oxidative stress-induced damage in osteoblastic cells, Int. J. Mol. Med. 33 (2014) 1327–1334;
(b) E. Wang, F. Chen, X. Hu, Y. Yuan, Protective effects of apigenin against furan-induced toxicity in mice, Food Funct. 5 (2014) 1804–1812.

[4] (a) J.S. Choi, I.M. Nurul, A.M. Yousof, E.J. Kim, Y.M. Kim, H.A. Jung, Effects of C-glycosylation on anti-diabetic, anti-Alzheimer's disease and anti-inflammatory potential of apigenin, Food. Chem. Toxicol. 64 (2014) 27–33.

[5] M.J. Matos, S. Vazquez-Rodriguez, L. Santana, E. Uriarte, C. Fuentes-Edfuf, Y. Santos, A. Muñoz-Crego, Looking for new targets: simple coumarins as antibacterial agents, Med. Chem. 8 (2012) 1140–1145.

[6] (a) M.J. Matos, G. Ferino, E. Cadoni, R. Laguna, F. Borges, E. Uriarte, L. Santana, 8-Substituted 3-arylcoumarins as potent and selective MAO-B inhibitors: synthesis, pharmacological evaluation, and docking studies, Chem. Med. Chem. 7 (2012) 464–470;
(b) M.J. Matos, S. Vazquez-Rodriguez, E. Uriarte, L. Santana, D. Viña, MAO inhibitory activity modulation: 3-Phenylcoumarins versus 3-benzoylcoumarins, Bioorg. Med. Chem. Lett. 21 (2011) 4224–4227;
(c) D. Secci, S. Carradori, A. Bolasco, P. Chimenti, M. Yañez, F. Ortuso, S. Alcaro, Synthesis and selective human monoamine oxidase inhibition of 3-carbonyl, 3-acyl, and 3-carboxyhydrazido coumarin derivatives, Eur. J. Med. Chem. 46 (2011) 4846–4852.

[7] S. Serra, A. Chicca, G. Delogu, S. Vazquez-Rodriguez, L. Santana, E. Uriarte, L. Casu, J. Gertsch, Synthesis and cytotoxic activity of non-naturally substituted 4-oxycoumarin derivatives, Bioorg. Med. Chem. Lett. 22 (2012) 5791–5794.

[8] I. Kostova, S. Bhatia, P. Grigorov, S. Balkansky, V.S. Parmar, A.K. Prasad, L. Saso, Coumarins as antioxidants, Curr. Med. Chem. 18 (2011) 3929–3951.

[9] (a) H. Cao, Y. Ji, W. Li, Y. Liu, R. Fu, M. Xiang, Protective effects of the total Coumarin fraction of urtica dentata on experimental diabetic nephropathy in vitro and in vivo, Planta Med. 81 (2015) 1353–1360;
(b) A. Pangal, M. Gazge, V. Mane, J.A. Shaikh, Various pharmacological aspects of coumarin derivatives: a review, Int. J. Pharm. Res. Bio-Sci. 2 (2013) 168–194;
(c) K. Raj, N. Misra, G. Pachauri, M. Sharma, A.K. Tamrakar, A.B. Singh, A.K. Srivastava, K. Phani Kiran, C.V. Narasimha Rao, S.R. Prubhu, Novel class of hybrid natural products as antidiabetic agents, Nat. Prod. Res. 23 (2009) 60–69.

[10] K. Franke, A. Porzel, J. Schmidt, Flavone-coumarin hybrids from Gnidia socotrana, Phytochemistry 61 (2002) 873–878.

[11] (a) K. Lu, J. Chu, H. Wang, X. Fu, D. Quan, H. Ding, Q. Yao, P. Yu, Regioselective iodination of flavonoids by N-iodosuccinimide under neutral conditions, Tetrahedron Lett. 54 (2013) 6345–6348;
(b) H. Wang, Z. Yan, Y. Lei, K. Sheng, Q. Yao, K. Lu, P. Yu, Concise synthesis of prenylated and geranylated chalcone natural products by regiospecific iodination and Suzuki coupling reactions, Tetrahedron Lett. 55 (2014) 897–899;
(c) H. Wang, L. Zhang, J. Liu, Z. Yang, H. Zhao, Y. Yang, D. Shen, K. Lu, Z. Fan, Q. Yao, Y. Zhang, Y. Teng, P. Yu, Synthesis and anti-cancer activity evaluation of novel prenylated and geranylated chalcone natural products and their analogs, Eur. J. Med. Chem. 92 (2015) 435–448;
(d) G. Pan, K. Yang, Y. Ma, X. Zhao, K. Lu, P. Yu, Synthesis of 6- or 8-bromo flavonoids by regioselective mono-bromination and deprotection protocol from flavonoid alkyl ethers, Bull. Korean Chem. Soc. 36 (2015) 1460–1466;
(e) G. Pan, Y. Ma, K. Yang, X. Zhao, H. Yang, Q. Yao, K. Lu, T. Zhu, P. Yu, Total synthesis of (±)-Anastatins A and B, Tetrahedron Lett. 56 (2015) 4472–4475.

[12] E. Zubia, F.R. Luis, G.M. Massanet, I.G. Collado, An efficient synthesis of furanocoumarins, Tetrahedron 48 (1992) 4239–4246.

[13] M. Wang, X.J. Gao, W.W. Zhao, W.J. Zhao, C. Jiang, F. Huang, J.P. Kou, B.L. Liu, K. Liu, Opposite effects of genistein on the regulation of insulin-mediated glucose homeostasis in adipose tissue, Br. J. Pharmacol. 170 (2013) 328–340.