Full Paper

Synthesis of Four Natural Prenylflavonoids and Their Estrogen-like Activities

Xiaowu Dong, Yongjian Fan, Lingjun Yu, and Yongzhou Hu

ZJU-ENS Joint Laboratory of Medicinal Chemistry, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, China

Four prenylflavonoids, bavachin **1**, isobavachin **2**, 7,4′-dihydroxy-8-prenylflavone **3**, and 8-prenylapigenin **4** were synthesized and recognized for possessing estrogen-like activity in MCF-7/BOS cells, as evaluated by an estrogen-screening assay. All compounds significantly stimulated the proliferation of MCF-7/BOS cells in a dose-dependent manner. Isobavachin **2** showed the most potent activity, while bavachin **1** was the weakest. The estrogenic potency of these compounds is ranked as follows: **2** > **4** > **3** > **1**.

Keywords: Bavachin / 7,4′-Dihydroxyl-8-prenylflavone / Estrogenic activity / 8-C-Prenylapigenin / Isobavachin

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Introduction

Prenylflavonoids are interesting natural products, isolated from some traditional medicinal plants [1–3]. It was reported that introduction of a prenyl substituent into flavonoids could remarkably improve the various biological activities [4–6]. Among them, bavachin **1**, isolated from *Posralea corylifolia* [7] showed various bioactivities including inhibition of platelet aggregation, antiosteoporosis, antibacterial activity [8–11]. Jain *et al*. have first synthesized bavachin **1** [12], but the poor yield of prenylation and long time of condensation without protection of the hydroxyl are problems, and they are not resolved yet. Recently, we revisited the synthesis of bavachin **1**, and made some improvements, which could increase the total yield from 0.5% [12] to 4.2% and with a more reasonable condensation time [13]. Despite of intensive studies for bavachin **1**, no data have been reported about the estrogen-like effect. In this study, our goal is to evaluate its effect of stimulating MCF-7/BOS cell proliferation. Considering the effect of substituents (C-6 prenyl or C-8 prenyl) and the structural skeleton (flavone or flavonone) in prenylflavonoids, compounds **2**, **3**, and **4**, which are also natural products, were synthesized (Fig. 1) as well. The antioxidant properties, cytotoxicity, and other bioactivities of these compounds had already been reported before [5, 14–18]. However, prenylflavonoids **1**, **2**, **3**, and **4** were also recognized for possessing estrogen-like activities in MCF-7/BOS cells evaluated by an estrogen-screening assay. These effects allow a large variety of therapeutic uses such as for treating osteoporosis and the menopause syndrome, according to the potential value of phytoestrogens [19–21].

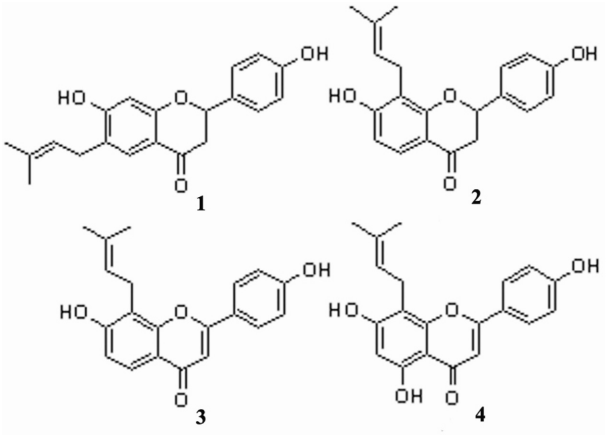
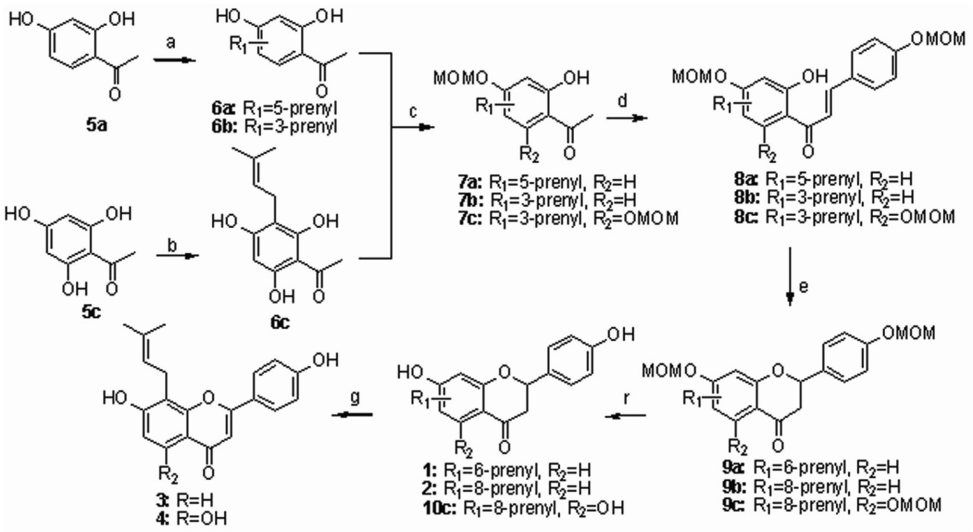


Figure 1. Structure of presented compounds **1**–**4**.

Correspondence: Yongzhou Hu, ZJU-ENS Joint Laboratory of Medicinal Chemistry, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, Zhejiang, 310031, China.
E-mail: huyz@zju.edu.cn
Fax: +86 571 8820-8460



(*a*) 1.3N NaOH, acetone; 3,3-dimethylallyl bromide, benzene, reflux; (*b*) 3,3-dimethylallyl bromide, 10% KOH; (c) MOMCl, K_2CO_3, acetone, 0°C to r.t.; (*d*) *p*-methoxymethoxylbenzaldehyde, 10% KOH (H_2O/EtOH); (*e*) NaOAc, EtOH, reflux; (*f*) 3N HCl, MeOH, reflux; (*g*) I_2, pyridine, 90°C.

Scheme 1. Synthesis route of compounds **1 – 10.**

Result and discussion

The synthetic pathway is outlined in Scheme 1. Bavachin **1** and isobavachin **2** were synthesized using 2,4-dihydroxyacetophenone **5a** as the starting material, which was prenylated with 3,3-dimethylallyl bromide, to furnish **6a** and **6b,** according to the reported method [13]. Compound **6c** was achieved by treating 2,4,6-trihydroxyacetophenone **5c** with 3,3-dimethylallyl bromide, according to the approach in the literature [22]. Selective methoxymethylation of **6a**, **6b**, and **6c** with chloromethoxylmethyl ether and anhydrous K_2CO_3 in dry acetone gave compounds **7a**, **7b**, and **7c**, respectively. Condensation of **7a**, (**7b** or **7c**) with *p*-methoxymethoxylbenzaldehyde, proceeded in aqueous alcoholic alkali, to obtain chalcone **8a, 8b**, or **8c**. Compound **8a**, (**8b** or **8c**) was cyclized by refluxing in a solution of NaOAc in EtOH, to afford the flavonone **9a**, **9b**, or **9c**. Demethoxymethylation of **9a**, **9b**, and **9c** was carried out in 3 N HCl/MeOH to obtain the products **1**, **2**, and **10c**, respectively. Finally, **3** and **4** were obtained by dehydrogenation of the corresponding prenylflavanones **2** and **10c** with iodine and pyridine in good yields (76% and 80%, respectively).

The estrogenic effects of the selected compounds were examined by an E-SCREEN assays in MCF-7/BOS cells. The maximum cell proliferative effect was observed with 1 nM 17β-estradiol. The proliferative effect of these compounds is expressed as relative proliferative effect (RPE) (Fig. 2), relative to 17β-estradiol's one (1 nM, 100%).

Previous studies of the structure-activity relationship of flavone and isoflavone indicated that their potent

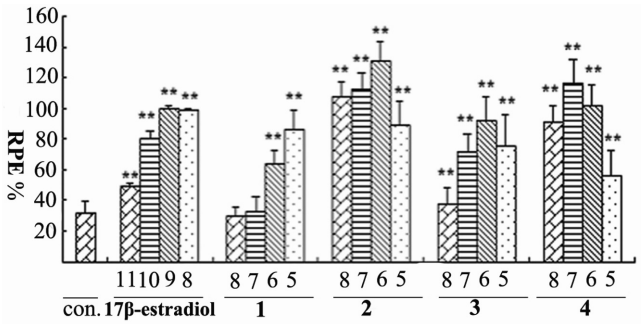


Figure 2. Relative Proliferative Effect (RPE) of compounds **1 – 4** relative to that of 17ß-estradiol (1 nM, 100%).

estrogen-like activities were ascribed to their structural similarity to mammalian estrogens [23]. Insight into the observed effects revealed that the 8-prenyl substituent could markedly improve the estrogenic activity because the maximal proliferative effect of compounds **2 – 4** (8-prenyl) was more potent than compound **1** (6-prenyl). Le Bail *et al*. reported that structural spatial conformation could play an important role in estrogenic activity. For example, naringenin (flavonone) and apigenin (flavone) have a 4′,5,7-trihydroxyl substituent, however, the former conformation is much more estrogen-like [24]. The result could be well validated by the maximal proliferative effect of compound **2** (flavonone), which was more efficient than compounds **3** (flavone). Furthermore, a 5-hydroxyl substituent in compound **4** appeared to slightly increase the estrogenic activity compared to that of **3**.

In conclusion, the synthesized compounds **1 – 4** exhibited moderate or great estrogenic activity, which could

be further studied as a potent non-steroidal phytoestrogen used for the treatment of certain hormone-dependent diseases, such as osteoporosis and the menopause syndrome.

Experiment

Chemistry

Melting points were determined on a Büchi B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. All ^{1}H-NMR spectra were recorded on Bruker400M spectrometer (Bruker Bioscience, Billerica, MA, USA) with $CDCl_3$ or DMSO-d_6 as solvent. Chemical shifts were reported in d values (ppm), relative to internal TMS, and *J* values were reported in Hertz. Mass spectral data were obtained by Finnigan LCQ DECA spectrometer (Thermo Electron Corporation, Bremen, Germany). IR spectra were measured with Bruker vector 200 (Bruker). Reagents and solvents were purchased from common commercial suppliers and were used without further purification.

*General method for synthesis of compounds **7a–c***

To a cool solution of **6a,** (**6b** or **6c**), dry potassium carbonate in anhydrous acetone, chloromethoxylmethyl ether was added. After stirring for 2 h at room temperature, the potassium carbonate was filtered off. The filtrate was concentrated to give a residue, which was purified by silica gel column chromatography to afford **7a**, **7b**, or **7c**.

*4-Methoxymethyl-5-(3, 3-dimethylallyl)-2-hydroxylacetonphenone **7a***

Reagent: compound **6a** (660 mg, 3.0 mmol), chloromethoxylmethyl ether (300 mg, 3.73 mmol), dry potassium carbonate (550 mg, 4.0 mmol), anhydrous acetone (50 mL); Purification: silica gel column chromatography using petroleum ether : ethyl acetate (20 : 1). Yellow oil (580 mg, 73%); IR (KBr) ν (cm^{-1}): 3242, 2920, 1636, 1492, 1368 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ): 1.74 (s, 6H), 2.54 (s, 3H), 3.28 (d, 2H, *J* = 6.8 Hz), 3.47 (s, 3H), 5.24 (s, 2H), 5.27 (m, 1H), 6.60 (s, 1H), 7.44 (s, 1H), 12.51 (s, 1H). ESI-MS: *m/z* 265 $[M+H]^+$.

*4-Methoxymethyl-3-(3, 3-dimethylallyl)-2-hydroxylacetonphenone **7b***

Reagent: compound **6b** (660 mg, 3.0 mmol), chloromethoxylmethyl ether (300 mg, 3.73 mmol), dry potassium carbonate (550 mg, 4.0 mmol), anhydrous acetone (50 mL); Purification: silica gel column chromatography using petroleum ether : ethyl acetate (20 : 1). Yellow oil (580 mg, 70%); IR (KBr) ν (cm^{-1}): 3230, 2915, 1628, 1488, 1360 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ): 1.69 (s, 3H), 1.81 (s, 3H), 2.58 (s, 3H), 3.37 (d, 2H, *J* = 7.2 Hz), 5.21 (m, 1H), 6.66 (d, 1H, *J* = 8.8 Hz), 7.60 (d, 1H, *J* = 8.8 Hz), 12.80 (s, 1H). ESI-MS: *m/z* 265 $[M+H]^+$.

*4,6-Dimethoxymethyl-3-(3, 3-dimethylallyl)-2-hydroxylacetonphenone **7c***

Reagent: compound **6c** (708 mg, 3.0 mmol), methoxymethylchloride (600 mg, 7.45 mmol), dry potassium carbonate (1.1 g, 8.0 mmol), anhydrous acetone (50 mL); Purification: silica gel column chromatography using petroleum ether : ethyl acetate (18 : 1). Yellow oil (680 mg, 70%); IR (KBr) ν (cm^{-1}): 3190, 2932, 1632, 1478, 1355 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ): 1.67 (s, 3H), 1.77 (s, 3H), 2.65 (s, 3H), 3.30 (d, 1H, *J* = 7 Hz), 3.47 (s, 3H), 3.50 (s, 3H), 5.20 (m, 1H), 5.23(s, 2H), 5.24 (s, 2H), 6.39 (s, 1H), 13.81 (s, 1H). ESI-MS: *m/z* 325 $[M+H]^+$.

*General method for synthesis of compounds **8a**, **8b**, and **8c***

To a cold solution of the acetophenone **7a**, (**7b** or **7c)** and *p*-methoxymethoxylbenzaldehyde in 3 mL H_2O-EtOH (1/4), 20% KOH in 3 mL H_2O-EtOH (1/4) was added with stirring. The resulting mixture was stirred under argon at room temperature for 36 h. The whole mixture was poured into ice water, acidified to pH~2 with 1 N HCl, and extracted with ethyl acetate. The organic phase was washed with saturated NaCl solution, dried (Na_2SO_4), and concentrated *in vacuo*. The residue was purified to give chalcone **8a**, **8b**, or **8c**.

*7,4′-Dimethoxymethyl-5-(3,3-dimethylallyl)-chalcone **8a***

Reagent: compound **7a** (470 mg, 1.78 mmol), *p*-methoxymethoxylbenzaldehyde (296 mg, 1.78 mmol); Purification: silica gel column chromatography using petroleum ether : ethyl acetate (15 : 1). Yellow oil (550 mg, 75%); IR (KBr) ν (cm^{-1}): 3447, 2923, 1635, 1570, 1364 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ): 1.78 (s, 6H), 3.31 (d, 2H, *J* = 6.8 Hz), 3.50 (s, 6H), 5.25 (s, 4H,), 5.31 (m, 1H), 6.67 (s, 1H), 7.11 (d, 2H, *J* = 8.8 Hz), 7.62 (d, 2H, *J* = 8.8 Hz), 7.65 (s, 1H), 7.47 (d, 1H, *J* = 15.6 Hz), 7.86 (d, 1H, *J* = 15.6 Hz), 13.32(s, 1H, OH). ESI-MS: *m/z* 413 $[M+H]^+$.

*7,4′-Dimethoxymethyl-3-(3,3-dimethylallyl)-chalcone **8b***

Reagent: compound **7b** (470 mg, 1.78 mmol), *p*-methoxymethoxylbenzaldehyde (296 mg, 1.78 mmol); Purification: silica gel column chromatography using petroleum ether : ethyl acetate (15 : 1). Yellow oil (572 mg, 72%); IR (KBr) ν (cm^{-1}): 3433, 2955, 1640, 1574, 1360 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ): 1.72 (s, 3H), 1.82 (s, 3H), 3.44 (d, 2H, *J* = 6.8 Hz), 3.51 (s, 6H), 5.25 (s, 2H), 5.27 (m, 1H), 5.31 (s, 2H), 6.70 (d, 1H, *J* = 8.4 Hz), 7.08 (d, 2H, *J* = 8.8 Hz), 7.51 (d, 1H, *J* = 15.6 Hz), 7.64 (d, 2H, *J* = 8.8 Hz), 7.80 (d, 1H, *J* = 8.4 Hz), 7.88 (d, 1H, *J* = 15.6 Hz), 12.8 (s, 1H, OH). ESI-MS: *m/z* 413 $[M+H]^+$.

*5,7,4′-Trimethoxymethyl-3-(3,3-dimethylallyl)-chalcone **8c***

Reagent: compound **7c** (500 mg, 1.54 mmol), *p*-methoxymethoxylbenzaldehyde (256 mg, 1.54 mmol); Purification: silica gel column chromatography using petroleum ether : ethyl acetate (12 : 1). Yellow oil (552 mg, 71%); IR (KBr) ν (cm^{-1}): 3420, 2940, 1644, 1568, 1359 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ): 1.67(s, 3H,), 1.79(s, 3H), 3.34 (d, 2H, *J* = 6.8 Hz), 3.49 (s, 3H), 3.50 (s, 3H), 3.52 (s, 3H), 5.21 (m, 1H), 5.22 (s, 2H), 5.25 (s, 2H), 5.27 (s, 2H), 6.40 (s, 1H,), 7.06 (d, 2H, *J* = 8.8 Hz), 7.55 (d, 2H, *J* = 8.8 Hz), 7.76 (d, 1H, *J* = 15.6 Hz), 7.83 (d, 1H, *J* = 15.6 Hz), 13.83 (s, 1H). ESI-MS: *m/z* 473 $[M+H]^+$.

*General method for synthesis of compounds **9a**, **9b**, and **9c***

A stirred solution of **8a**, (**8b** or **8c**) and sodium acetate in 5 mL ethanol containing three drops of water was refluxed for 24 h. The mixture was poured into cold water and extracted with

ethyl acetate. The organic phase was washed with brine, dried, and filtered. After removing the solvent, the residue was purified to afford flavanone **9a**, **9b**, or **9c**.

*7,4′-Dimethoxymethyl-6-(3,3-dimethylallyl)-flavanone **9a***

Reagent: compound **8a** (500 mg, 1.21 mmol), sodium acetate (500 mg); Purification: silica gel column chromatography using petroleum ether : ethyl acetate (10 : 1). Pale yellow syrup (360 mg, 72%); Purification: silica gel column chromatography using petroleum ether: ethyl acetate (10 : 1). IR (KBr) ν (cm^{-1}): 2960, 1682, 1574, 1238 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ): 1.73, 1.76 (3H, each s), 2.81 (dd, 1H, *J* = 16.8 Hz, 0.8 Hz), 3.03 (d, 1H, *J* = 12.0 Hz, 16.8 Hz), 3.29 (d, 2H, *J* = 7.2 Hz), 3.49 (s, 6H), 5.20-5.29 (m, 5H), 5.41 (dd, 1H, *J* = 12.0 Hz, 0.8 Hz), 6.70 (s, 1H), 7.10 (d, 2H, *J* = 8.4 Hz), 7.41(d,2H, *J* = 8.4 Hz), 7.72 (s, 1H). ESI-MS: *m/z* 413 $[M+H]^+$.

*7,4′-Dimethoxymethyl-8-(3,3-dimethylallyl)-flavanone **9b***

Reagent: compound **8b** (500 mg, 1.21 mmol), sodium acetate (500 mg); Purification: silica gel column chromatography using petroleum ether : ethyl acetate (10 : 1). Pale yellow syrup (373 mg, 70%); IR (KBr) ν (cm^{-1}): 2965, 1675, 1568, 1250 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ): 1.67 (s, 3H), 1.78 (s, 3H), 2.70 (d, 1H, *J* = 16.8 Hz), 3.07 (d, 1H, *J* = 12.0 Hz, 16.8 Hz), 3.21 (d, 2H, *J* = 7.2 Hz), 3.40(s, 3H), 3.40 (s, 3H), 5.15 (t, 1H, *J* = 7.2 Hz), 5.28 (s, 2H), 5.35 (s, 2H), 5.47 (d, 1H, *J* = 12.0 Hz), 6.60 (d, 1H, *J* = 8.8 Hz), 6.83 (m, 2H, *J* = 8.4 Hz), 7.37 (m, 2H, *J* = 8.4 Hz), 7.55 (d, 1H, *J* = 8.8 Hz). ESI-MS: *m/z* 413 $[M+H]^+$.

*5,7,4′-Trimethoxymethyl-8-(3,3-dimethylallyl)-flavanone **9c***

Reagent: compound **8c** (500 mg, 1.06 mmol), sodium acetate (500 mg); Purification: silica gel column chromatography using petroleum ether: ethyl acetate (8 : 1). Pale yellow syrup (350 mg, 70%); IR (KBr) ν (cm^{-1}): 2980, 1655, 1565, 1245 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz, δ): 1.64 (s, 3H), 1.65 (s, 3H), 2.80 (dd, 1H, *J* = 16.4 Hz, 2.8 Hz), 2.98 (dd, 1H, *J* = 13.4 Hz, 16.4 Hz), 3.31 (d, 2H, *J* = 6.8 Hz), 3.48 (s, 3H), 3.49 (s, 3H), 3.54 (s, 3H), 5.17 (m, 1H), 5.20 (s, 2H), 5.24 (s, 2H), 5.26 (s, 2H), 5.36 (dd, 1H, *J* = 2.8 Hz, 13.4 Hz), 6.57 (s, 1H), 7.08 (d, 2H, *J* = 8.4 Hz), 7.38 (d, 1H, *J* = 8.4 Hz). ESI-MS: *m/z* 473 $[M+H]^+$.

*General method for synthesis of compounds **1**, **2**, and **10c***

To a solution of **9a**, (**9b** or **9c**) in methanol (10 mL), 3 N HCl (2 mL) was added. The resulting mixture was refluxed for 45 min, then poured into cold water and extracted with ethyl acetate. The organic phase was washed with saturated NaCl solution and then dried (Na_2SO_4). After removal of the solvent, the residue was chromatographed over silicon gel. Elution with petroleum ether-ethyl acetate gave **1**, **2**, or **10c**.

(±)-Bavachin 1

Reagent: compound **9a** (300 mg, 0.73 mmol); Purification: silica gel column chromatography using petroleum ether: ethyl acetate (4 : 1). White amorphous powder (180 mg, 76%), mp.: 184-1850C (lit. [25] mp. 1890C); IR (KBr) ν (cm^{-1}): 3126, 2961, 1654, 1518, 1461 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz, δ): 1.66(s, 3H), 1.71 (s, 3H), 2.63 (dd, 1H, *J* = 16.8 Hz, 0.8 Hz), 3.03 (d, 2H, *J* = 7.2 Hz), 3.17 (d, 1H, *J* =12.0, 16.8 Hz), 5.26 (m,1H), 5.40 (dd, 1H, *J* = 12.0, 0.8 Hz), 6.38 (s, 1H), 6.78 (d, 2H, *J* = 8.4 Hz), 7.31 (d, 2H, *J* = 8.4 Hz), 7.45 (s, 1H), 9.55 (s, 1H, OH), 10.60 (s, 1H, OH). ESI-MS: *m/z* 325 $[M+H]^+$.

(±)-Isobavachin 2

Reagent: compound **9b** (300 mg, 0.73 mmol); Purification: silica gel column chromatography using petroleum ether : ethyl acetate (4 : 1). White amorphous powder (165 mg, 70%), mp.: 188-1890C (lit. [26] mp. 187-1880C); IR (KBr) ν (cm^{-1}): 3266, 2966, 1646, 1520, 1441 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz, δ): 1.64 (s, 3H), 1.67 (s, 3H), 2.75 (dd, 1H, *J* = 16.8 Hz, 0.8 Hz), 3.12 (d, 1H, *J* =12.0 Hz, 16.8 Hz), 3.26 (d, 2H, *J* = 7.0 Hz), 5.20 (t, 1H, *J* = 7.0 Hz), 5.50 (dd, 1H, *J* = 12.0 Hz, 0.8 Hz), 6.64 (d, 1H, *J* = 8.8 Hz), 6.86 (d, 2H, *J* = 8.4 Hz), 7.39 (d, 2H, *J* = 8.4 Hz), 7.58 (d, 1H, *J* = 8.8 Hz), 9.63 (s, 1H), 10.53 (s,1H). ESI-MS: *m/z* 325 $[M+H]^+$.

*(±)-8-Prenylnaringenin **10c***

Reagent: compound **9c** (300 mg, 0.64 mmol); Purification: silica gel column chromatography using petroleum ether : ethyl acetate (5 : 1). White amorphous powder (98 mg, 72%), mp.: 183 – 184°C (lit. [27], mp. 183 – 184°C); IR (KBr) ν (cm^{-1}): 3334, 2964, 1654, 1524, 1441 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz, d): 1.70 (s, 3H), 1.77 (s, 3H), 2.68 (dd, 1H, *J* = 16.4 Hz, 0.8 Hz), 2.92 (d, 1H, *J* = 12.0 Hz, 16.4 Hz), 3.30 (d, 2H, *J* = 7.0 Hz), 5.08 (m, 1H, *J* = 7.0 Hz), 5.41 (dd, 1H, *J* = 12.0 Hz, 0.8 Hz), 5.93 (s, 1H), 7.05 (d, 2H, *J* = 8.0 Hz), 7.35 (d, 1H, *J* = 8.0 Hz), 10.38 (s, 1H), 10.65 (s, 1H), 12.31 (s, 1H). ESI-MS: *m/z* 341 $[M+H]^+$.

*General method for synthesis of compounds **3** and **4***

A stirred solution of corresponding prenylflavonone and iodine in dry pyridine (4 mL) was heated to 90°C for 6 h. The mixture was cooled and poured into cold water. The precipitate was separated and the mixture was extracted with ethyl acetate. The combined organic phases were washed with saturated sodium thiosulfate and water, successively. Then, the organic layer was dried with sodium sulfate and concentrated. The residue was purified by silica gel column chromatography using eluent mixtures of solvents in the proportions indicated for each case.

*7,4′-Dihydroxy-8-prenylflavone **3***

Reagents: isobavachin **2** (100 mg, 0.31 mmol), iodine (78 mg, 0.31 mmol). Purification: silica gel column chromatography using petroleum ether : ethyl acetate (1 : 1). Light yellow solid (76 mg, 76%), mp.: 240 – 241°C (lit. [27], mp. 240 – 241°C); IR (KBr) ν (cm^{-1}): 3197, 2923, 1656, 1507, 1422 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz, δ): 1.65 (s, 3H), 1.79(s, 3H), 3.58 (d, 2H, *J* = 5.6 Hz), 5.24 (m, 1H), 6.72 (s, 1H), 6.93 (d, 2H, *J* = 8.4 Hz), 6.97(d, 1H, *J* = 8.4 Hz), 7.74 (d, 1H, *J* = 8.4 Hz), 7.89 (d, 2H, *J* = 8.4 Hz), 10.28(s, 1H), 10.65(s, 1H). ESI-MS: *m/z* 323 $[M+H]^+$.

*8-C-Prenylapigenin **4***

Reagents: 8-C-prenylnaringenin **10c** (100 mg, 0.29 mmol), iodine (75 mg, 0.29 mmol). Purification: silica gel column chromatography using petroleum ether : ethyl acetate (1 : 1). Light yellow solid (78 mg, 80%), mp. 239 – 240°C (lit. [28], mp. 173 – 174°C, decomposed); IR (KBr) ν (cm^{-1}): 3385, 3197, 2925, 1622, 1508, 1435 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz, d): 1.62 (s, 3H), 1.75 (s, 3H), 3.43 (d, 2H, *J* = 6.4 Hz), 5.19 (m,1H), 6.28 (s, 1H), 6.77 (s, 1H), 6.93 (d, 2H, *J* = 8.0 Hz), 7.89 (d, 2H, *J* = 8.0 Hz), 10.38 (s, 1H), 10.79 (s, 1H), 12.90 (s, 1H). ESI-MS: *m/z* 339 $[M+H]^+$.

Biological assay

Cell culture

Estrogen receptor-positive human breast adenocarcinoma MCF-7/BOS cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing antibiotics (100 IU/mL^{-1} penicillin and 100 Ag/mL^{-1} streptomycin), supplemented with 10% fetal bovine serum (FBS). Cells were grown at 37°C in a humidified atmosphere of 5% CO_2 in air. Medium was renewed 2–3 times per week.

Proliferation assay of cell

Confluent MCF-7/BOS cells were washed twice with *D*-Hanks solution before the addition of 0.25% trypsin–EDTA and seeded into 24-well plates at a density of 1×10^4 cells/well in normal growth medium. After 48 h, the cells were washed with *D*-Hanks solution and the estrogen-free medium (phenol red-free DMEM with 5% charcoal-dextran stripped human serum) was added. Following another 48 h pretreatment, the cells were exposed to increasing concentrations of test compounds. Cell proliferation was assessed by MTT method after seven days, during which the medium was changed every three days. The results are expressed as proliferation compared, with the treatment by 1 nM 17β-estradiol as reference.

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