Full Paper

Synthesis and Antiproliferative *In-Vitro* Activity of Natural Flavans and Related Compounds

Liang Zhang[1,2], Wei-Ge Zhang[1], En-Long Ma[3], Lan Wu[4], Kai Bao[1], Xiao-Long Wang[3], Yu-Ling Wang[2], and Hong-Rui Song[2]

[1] Department of Medicinal Chemistry, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang, P. R. China
[2] Department of Organic Chemistry, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang, P. R. China
[3] Department of Pharmacology, School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, P. R. China
[4] The First Affiliated College, Chinese Medical University, Shenyang, P. R. China

The total synthesis of natural flavan racemates (±) **1**, (±) **2** and natural flavones **3**, **4** had thus been achieved. A straightforward synthetic procedure of flavans *via* the Pd-C catalyzed hydrogenation/hydrogenolysis of corresponding flavones was developed. Furthermore, the antiproliferative activities of racemic flavans (±) **1**, (±) **2**, flavones **3**, **4**, and five synthetic intermediates toward human SGC-7901, BEL-7402, HeLa, and HL-60 cell lines *in vitro* were evaluated by MTT assay, and the racemic flavans (±) **1** were found to have significant antiproliferative activity against all four cell lines.

Keywords: Antiproliferative activity / Flavan / Flavone / Hydrogenation – Hydrogenolysis

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Introduction

Flavans are a set of naturally occurring flavonoids possessing a 2-phenylchroman nucleus; they are widely distributed in the plant kingdom and many exhibit interesting and useful biological activities [1, 2, 3, 4]. Because of their widespread occurrence and valuable properties, numerous syntheses for the construction of flavans have been developed, including inter- and intramolecular Mitsunobu reaction [5], acid-catalyzed cyclization [6], reduction of flavanones [7, 8] or flav-2-enes [9], etc. However, to the best of our knowledge, the straightforward approach to flavans from flavones, a large family of flavonoids, has never been documented.

Two flavans, 2(*S*)-7,8,3′,4′,5′-pentamethoxyflavan **1** and 2(*S*)-5′-hydroxy-7,8,3′,4′- tertramethoxyflavan **2**, along with flavones 7,8,3′,4′,5′-pentamethoxyflavone **3** and 5′-hydroxy-7,8,3′,4′-tertramethoxyflavone **4**, had been isolated from the roots of *Muntingia calabura* L. by N. Kaneda and co-workers (Fig. 1) [10]. These natural flavonoids demonstrate significant cytotoxic activity against human colon carcinoma Co12 and murine lymphocytic leukemia P-388 cell lines, but the total synthesis of these natural flavonoids has not been reported yet.

Our continued interest in searching for new antitumor agents and understanding their structure-activity relationships prompted us to develop a general synthetic route to racemic flavans (±) **1**, (±) **2** and flavones **3**, **4**. The key step for the synthesis of flavans (±) **1** and (±) **2** is the Pd-C-catalyzed hydrogenation/hydrogenolysis of corresponding flavones. Furthermore, the antiproliferative activities against human SGC-7901, BEL-7402, HeLa, and HL-60 cell lines *in vitro* of racemic flavans (±) **1**, (±) **2**, fla-

Correspondence: Wei-Ge Zhang, Department of Medicinal Chemistry, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, P. R. China.
E-mail: zhangweige2000@sina.com
Fax: +86 24 2398 6393

Hong-Rui Song, Department of Organic Chemistry, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, P. R. China.
E-mail: hongruisong@163.com
Fax: +86 24 2398 6393

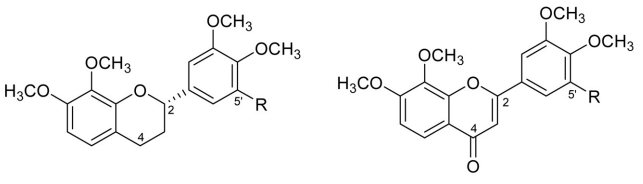


Figure 1. Molecular structures of natural flavans **1, 2** and flavones **3, 4**.

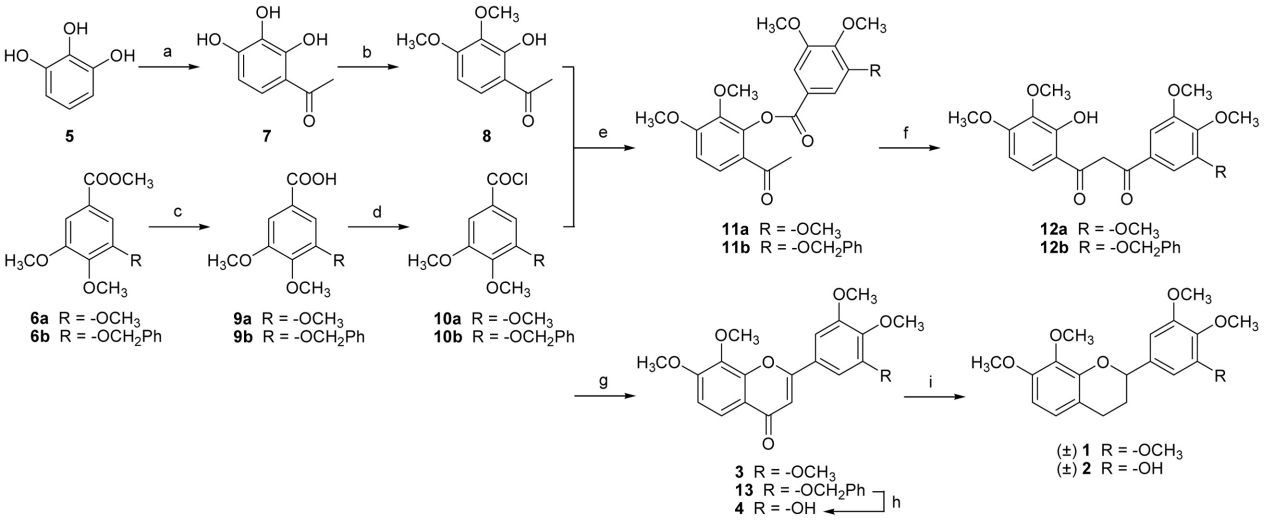
vones **3**, **4**, and some synthetic intermediates were preliminarily evaluated by MTT assay.

Results and discussion

Retro-synthetic analysis of (±) **1** and (±) **2** led to benzene-1,2,3-triol **5** and methyl 3,4,5-trimethoxybenzoate **6a** or methyl 3-benzyloxy-4,5-dimethoxybenzoate **6b**, while the general approach to the synthesis of (±) **1**, (±) **2** and **3**, **4** was described in Scheme 1.

Our synthesis commenced with the commercially available benzene-1,2,3-triol **5** which was acetylated with Ac_2O in the presence of traces of concentrated H_2SO_4 to afford ketone **7** [11]. Selective dimethylation of **7** with $(CH_3O)_2SO_2$ under basic condition gave intermediate **8** in 79.8% yield. After hydrolysation of benzoates **6a** and **6b**, being prepared from methyl 3,4,5-trihydroxybenzoate as described in the literature [12, 13], benzoic acids **9a** and **9b** were converted to corresponding benzoic chlorides **10a** and **10b** by treating with $SOCl_2$. These benzoic chlorides could be used directly in the next step without further purification. The esterfication of **8** with **10a** or **10b** was carried out in dry pyridine at 110°C for one hour to give **11a** or **11b** in 91.7% and 83.0% yield, respectively. Baker-Venkataraman rearrangement of **11a** and **11b** in the presence of NaOH in pyridine could be completed at room temperature in four hours to form 1,3-diketones **12a** and **12b** in 75.4% and 73.1% yield, respectively [14]. Treatment of **12a** with acetic acid by refluxing for six hours gave natural flavone **3** in 72.4% yield. In a similar way, compound **13** was obtained in 72.6% yield. Carrying out the deprotection of **13** via Pd-C-catalyzed hydrogenolysis in EtOAc at atmospheric pressure and room temperature for 12 hours, we obtained the desired natural flavone **4** in 81.5% yield, meanwhile separated a hydrophobic by-product in about 5% yield. The ESI-MS and ^{1}H-NMR spectrum in $CDCl_3$ solution of the by-product indicated that this compound was the racemic flavan (±) **2**. This result demonstrated that flavones could be converted to corresponding flavans directly *via* the Pd-C-catalyzed hydrogenation / hydrogenolysis.

In order to optimize the synthetic procedure, we examined the influence of solvent, reaction time, and amount of catalyst Pd^0 on the yield of flavan (±) **2**. The results were summarized in Table 1. The influence of solvent could be seen from entries 1–6 of Table 1, and MeOH was confirmed to be the preferred solvent. From entries 6-9, with the amount of catalyst Pd^0 increasing from 0.10 to 0.67 equiv, reaction time was extended from 12 to 24 hours while the isolation yields rose from 15.6% to 82.6%. Comparing entries 10 with 9, it could be found that increas-



Reagents and conditions: (a) $(CH_3CO)_2O$, H_2SO_4, reflux, 1 h; (b) $(CH_3O)_2SO_2$, K_2CO_3, CH_3COCH_3, reflux, 12 h; (c) 10% NaOH, 8 h; (d) $SOCl_2$, 4 h; (e) dry pyridine, 110°C, 1 h; (f) pyridine, NaOH, rt, 4 h; (g) CH_3COOH, 6 h; (h) H_2, Pd-C, $CH_3COOC_2H_5$, rt, 12 h; (i) H_2, Pd-C, CH_3OH, rt, 24 h.

Scheme 1. Synthesis of presented compounds.

Table 1. The synthesis of flavan (±) **2** under different conditions.

Entry	Solvent	Reaction time (h)	Amount of catalyst Pd^0 (equiv)	Isolated yield (%)
1	EtOAc	12	0.03	5.2[a)]
2	EtOAc	12	0.10	7.7[a)]
3	THF	12	0.10	Trace[b)]
4	HOAc	12	0.10	Trace[b)]
5	EtOH	12	0.10	9.3[a)]
6	MeOH	12	0.10	15.6[a)]
7	MeOH	24	0.10	25.8[a)]
8	MeOH	24	0.33	46.7[a)]
9	MeOH	24	0.67	82.6[a)]
10	MeOH	48	0.67	80.3[a)]

[a)] The desired product flavan (±) **2** was isolated by column chromatography on silica gel (EtOAc/hexane = 1 : 10, v/v) as the first fraction.

[b)] Only a small quantity of flavan (±) **2** could be found on TLC under this condition.

Table 2. *In-vitro* antiproliferative activity of racemic flavans (±) **1** and (±) **2**, flavone **3** and **4**, and synthetic intermediates **11a–13** against four human cancer cell lines.

Compounds	IC_{50} (μM)			
	SGC-7901	BEL-7402	HeLa	HL-60
11a	>100	>100	>100	>100
11b	>100	>100	>100	>100
12a	72.7	>100	63.7	28.9
12b	>100	>100	>100	>100
13	>100	>100	>100	>100
3	>100	>100	>100	>100
4	>100	>100	>100	>100
(±) **1**	4.1	12.0	5.3	1.8
(±) **2**	50.5	38.1	58.8	29.7
Cisplatin	9.6	20.1	8.7	10.2

Data are expressed as means ± SE from the dose–response curves of at least three independent experiments.

ing the time from 24 to 48 hours did not affect the yields of flavan (±) **2**. Hence, the best result was obtained when the substrate **13** was reacted in MeOH under H_2 at atmospheric pressure and room temperature in the presence of Pd-C (0.67 equiv) for 24 hours.

Following the procedure described above, racemic flavans (±) **1** was prepared in 81.9% yield by using flavone **3** as the substrate.

The antiproliferative activity of racemic flavans (±) **1** and (±) **2**, flavone **3** and **4**, and synthetic intermediates **11a–13** was assessed *in vitro* against four human cancer cell lines, SGC-7901 gastric carcinoma, BEL-7402 hepatic carcinoma, HeLa cervical carcinoma, and HL-60 acute promyelocytic leukemia, by colorimetric MTT assay using cisplatin as positive control and expressed as IC_{50} values. IC_{50} is the concentration (μM) required to inhibit tumor cell proliferation by 50% after 72 hours of exposure of the cells to a tested compound. The measured IC_{50} values for all compounds are summarized in Table 2.

As could be seen from the presented data, the natural flavan racemate (±) **1** was found to have significant antiproliferative activity, which was 1.6- to 5.7-fold more potent than cisplatin. The other natural flavan racemate, (±) **2**, showed moderate activity against all four cell lines. By comparing the result obtained above, it could be confirmed that conversion of the 5-OMe to a hydrophilic 5′-OH group in these racemic flavans caused a 3.1- to 16.5-fold decrease in inhibition of the growth of these cell lines. In our hands, the natural flavone **3** and **4** were found to have no activity to all against the four cell lines. Within the synthetic intermediates, the 1,3-dione **12a** exhibited weak to moderate activity against SGC-7901, HeLa, and HL-60. Removal of the *m*-OMe group on 3,4,5-trimethoxy phenyl moiety of **12a** and replacement with a bulky benzyloxy group, to afford its analogue **12b**, resulted in a marked decrease in the activity against GC-7901, HeLa, and HL-60. Moreover, it was noted that activity of the natural flavan racemates (±) **1** and (±) **2** against the leukemia HL-60 cell line are generally higher than that against the solid tumour SGC-7901, BEL-7402, and HeLa cell lines.

In summary, the first total synthesis of natural flavan racemates (±) **1**, (±) **2**, and natural flavones **3**, **4** had thus been achieved in 26.1%, 23.1%, 31.9%, and 24.0% overall yield, respectively. The ^{1}H-NMR, ^{13}C-NMR, MS data of all synthetic compounds were identical with that of the natural products. In addition, a straightforward synthetic procedure of flavans *via* the Pd-C-catalyzed hydrogenation / hydrogenolysis of corresponding flavones, which was simple and economical compared with the multistep methods, was developed and applied in the synthesis of natural flavan racemates. Furthermore, the antiproliferative activities of racemic flavans (±) **1**, (±) **2**, flavones **3**, **4**, and five synthetic intermediates toward human SGC-7901, BEL-7402, HeLa, and HL-60 cell lines *in vitro* were evaluated by MTT assay, and the racemic flavans (±) **1** was found to have significant antiproliferative activity against all four cell lines.

The authors have declared no conflict of interest.

Experimental

Melting points for the compounds were determined using a hot-stage microscope and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were taken in $CDCl_3$ solution on Bruker ARX-300 spectrometers with TMS as the internal reference (Bruker BioSciences, USA). MS spectra were obtained using Micromass Quattro micro™ API

mass spectrometer (Waters Corp, Milford, MA, USA). Elemental analyses (C and H) were performed by Jilin University (Changchun, China). Column chromatography was run on silica gel (200 – 300 mesh) from Qingdao Ocean Chemicals (Qingdao, China). Unless otherwise noted, all the materials were obtained from commercially available sources and were used without further purification.

Chemistry

1-(2,3,4-Trihydroxyphenyl)ethanone ***7***

Pyrogallol (50.10 g, 400 mmol) and Ac_2O (38.0 mL, 410 mmol) were refluxed with 1 mL of concentrated H_2SO_4 for 1 h. The contents were cooled and poured into 200 mL of H_2O containing 40 mL EtOH. Then, 2 mL concentrated HCl was added and the solution was refluxed for 45 min to decompose excess Ac_2O. The reaction mixture, on concentration under reduced pressure, yielded a brown solid, which on crystallization from saturated $NaHSO_3$ aqueous solution gave compound **7** (53.25 g, 79.7%) as a yellow solid: mp. 171 – 173°C (lit [15] mp 173°C).

1-(2-Hydroxy-3,4-dimethoxyphenyl)ethanone ***8***

A mixture of **7** (10.0 g, 59.6 mmol) and anhydrous K_2CO_3 (18.0 g, 130.7 mmol) in dry acetone was stirred well at room temperature for 1 h, then $(MeO)_2SO_2$ (5.5 mL, 57.6 mmol) was added. The mixture was refluxed for 12 h. After filtration, the filtrate on evaporation afforded a solid, which after column chromatography over silica gel (*n*-hexane : EtOAc = 10 : 1) gave compound **8** (9.31 g, 79.8%) as a light-yellow solid: mp. 62 – 64°C (lit [11] mp. 62 – 64°C). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.58 (3H, s), 3.92 (3H, s), 3.98 (3H, s), 6.50 (1H, d, *J* = 9.0 Hz), 7.50 (1H, d, *J* = 9.0 Hz), 12.98 (1H, s). MS (ESI) *m/z*: 197 $[M+H]^+$.

3,4,5-Trimethoxybenzoic acid ***9a***

The suspension of methyl 3,4,5-trimethoxybenzoate (10.1 g, 44.6 mmol, 99.8%) in 10% NaOH (50 mL) was stirred at room temperature for about 8 h until the solid disappeared then the mixture was adjusted to pH 1 – 2 with concentrated HCl. The solid obtained was filtered, washed with water to give **9a** (9.20 g, 97.3%) as a white solid: mp. 167 – 169°C (lit [16] 168 – 171°C), which was used in the next step without further purification.

3-Benzyloxy-4,5-dimethoxybenzoic acid ***9b***

The suspension of methyl 3-benzyloxy-4,5-dimethoxybenzoate (4.21 g, 13.9 mmol, 99.8%) in 10% NaOH (20 mL) was stirred at room temperature for about 8 h until the solid disappeared then the mixture was adjusted to pH 1 – 2 with concentrated HCl. The solid obtained was filtered, washed with water to give **9b** (3.47 g, 86.4%) as a yellow solid: mp. 171 – 172°C (lit [17] 170 – 172°C), which was used in the next step without further purification.

6-Acetyl-2,3-dimethoxyphenyl-3,4,5-trimethoxybenzoate ***11a***

The solution of 3,4,5-trimethoxybenzoic acid (2.38 g, 11.2 mmol) in $SOCl_2$ (8 mL) was refluxed for 4 h then the solvent was removed by evaporation to give compound **10a** as a yellow solid. Then, 1-(2-hydroxy-3,4-dimethoxyphenyl)ethanone **7** (2.00 g, 10.2 mmol) and dry pyridine (5 mL) were added. The resulting mixture was heated for 1 h at 105°C and poured with vigorous stirring onto 10 mL of a concentrated HCl / ice mixture. The product was collected by filtration after standing overnight. The crude product was purified by column chromatography over silica gel (*n*-hexane : EtOAc = 10 : 1) to give **11a** (3.65 g, 91.7%) as a white solid: mp. 141 – 143°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.51 ($3H_2$ s), 3.83 (3H, s), 3.95 (9H, s), 3.97 (3H, s), 6.91 (1H, d, *J* = 9.0 Hz), 7.49 (2H, s), 7.69 (1H, d, *J* = 9.0 Hz). MS (ESI) *m/z*: 413 $[M+Na]^+$. Anal. Calcd. for $C_{20}H_{22}O_8$: C, 61.53; H, 5.68. Found: C, 61.46; H, 5.61.

6-Acetyl-2,3-dimethoxyphenyl 3-(benzyloxy)-4,5-dimethoxybenzoate ***11b***

The solution of 3-benzyloxy-4,5-dimethoxybenzoic acid (3.23 g, 11.2 mmol) in $SOCl_2$ (10 mL) was refluxed for 4 h, then the solvent was removed by evaporation to give compound **10b** as a yellow solid. Then, 1-(2-hydroxy-3,4-dimethoxyphenyl)ethanone (2.00 g, 10.2 mmol) and dry pyridine (10 mL) were added. The resulting mixture was heated for 1 h at 105°C and poured onto 50 mL of a concentrated HCl / ice mixture with vigorous stirring. The product was collected by filtration after standing overnight. The crude product was purified by column chromatography over silica gel (*n*-hexane : EtOAc = 10 : 1) to give **11b** (3.95 g, 83.0%) as a light yellow solid: mp. 108 – 110°C. ^{1}H-NMR (300 MHz, $CDCl_3$) d: 2.46 (3H, s), 3.79 (3H, s), 3.94 (3H, s), 3.96 (3H, s), 3.97 (3H, s), 5.21 (2H, s), 6.90 (1H, d, *J* = 9.0 Hz), 7.33 – 7.47 (5H, m), 7.48 (1H, d, *J* = 1.8 Hz), 7.55 (1H, d, *J* = 1.8 Hz), 7.69 (1H, d, *J* = 9.0 Hz). MS (ESI) *m/z*: 467 $[M+H]^+$. Anal. Calcd. for $C_{26}H_{26}O_8$: C, 66.94; H, 5.62. Found: C, 66.86; H, 5.59.

1-(2-Hydroxy-3,4-dimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl)propane-1,3-dione ***12a***

Compound **11a** (3.41 g, 8.74 mmol) and powdered sodium hydroxide (2.62 g, 65.6 mmol) were shaken for 4 h in pyridine (10 mL) at room temperature. The reaction mixture was poured into an excess of concentrated HCl / ice to yield a light-yellow precipitate. Pure **12a** was obtained by column chromatography over silica gel (*n*-hexane : EtOAc = 7 : 1) as a yellow solid (2.57 g, 75.4%): mp. 122 – 124°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 3.91 – 3.95 (15H, m), 4.54 (2H, s), 6.54 (1H, d, *J* = 9.0 Hz), 6.65 (2H, s), 7.56 (1H, d, *J* = 9.0 Hz), 12.21 (1H, s). The characteristic signals for the enol isomer were found: 6.65 (1H, s), 12.28 (1H, s) and 15.84 (1H, s). The ratio of the two forms is about 1 : 3 in $CDCl_3$. MS (ESI) *m/z*: 391 $[M+H]^+$. Anal. Calcd. for $C_{20}H_{22}O_8$: C, 61.53; H, 5.68. Found: C, 61.48; H, 5.69.

1-(3-(Benzyloxy)-4,5-dimethoxyphenyl)-3-(2-hydroxy-3,4-dimethoxyphenyl)propane-1,3-dione ***12b***

Compound **11b** (3.05 g, 6.50 mmol) and powdered sodium hydroxide (1.96 g, 49.0 mmol) were shaken for 4 h in pyridine (10 mL) at room temperature. The reaction mixture was poured into an excess of concentrated HCl / ice to yield a brown precipitate. Purification by column chromatography over silica gel (*n*-hexane : EtOAc = 7 : 1) yielded **12b** (2.23 g, 73.1%) as a yellow solid: mp. 158 – 161°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 3.90 – 3.95 (12H, m), 4.47 (2H, s), 5.21 (2H, s), 6.54 (1H, d, *J* = 7.2 Hz), 7.15 (1H, d, *J* = 1.8 Hz), 7.34 (6H, m), 7.52 (1H, d, *J* = 7.2 Hz), 15.49 (1H, s). Also the characteristic signals for the enol isomer were found: 6.54 (1H, s), 12.66 (1H, s) and 15.48 (1H, s). The ratio of the two forms is about 1 : 2 in $CDCl_3$. MS (ESI) *m/z*: 489 $[M+Na]^+$. Anal. Calcd. for $C_{26}H_{26}O_8$: C, 66.94; H, 5.62. Found: C, 66.87; H, 5.61.

7,8,3′,4′,5′-Pentamethoxyflavone ***3***

Compound **12a** (2.20 g, 5.64 mmol) was refluxed for 6 h in 10 mL of acetic acid. The mixture then was poured onto 120 mL of ice water to precipitate the flavone. After purification by column chromatography over silica gel (*n*-hexane : EtOAc = 5 : 1), the natural flavone **3** was obtained (1.52 g, 72.4%) as a white solid: mp. 205–206°C (lit [10] mp 206°C). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 3.94 (3H, s), 3.97 (6H, s), 4.02 (3H, s), 4.05 (3H, s), 6.80 (1H, s), 7.07 (1H, d, *J* = 9.0 Hz), 7.21 (2H, s), 7.97 (1H, d, *J* = 9.0 Hz). ^{13}C-NMR ($CDCl_3$) δ: 56.1, 56.2, 56.3, 60.7, 61.5, 103.5, 106.1, 109.9, 118.1, 120.5, 126.7, 136.7, 141.3, 150.5, 153.5, 156.7, 162.6, 177.5. MS (ESI) *m/z*: 395 $[M+Na]^+$. Anal. Calcd. for $C_{20}H_{20}O_7$: C, 64.51; H, 5.41. Found: C, 64.49; H, 5.38.

5′- Benzyloxy-7,8,3′,4′-tertramethoxyflavone ***13***

Compound **12b** (1.20 g, 2.58 mmol) was refluxed for 6 h in acetic acid (50 mL). The mixture then was poured onto 120 mL of ice water and 10% NaOH was used to adjusted the pH 6–7. Then, the mixture was extracted with EtOAc (3 × 50 mL). The organic layer was washed by brine (2 × 30 mL) and dried over anhydrous Na_2SO_4. After filtration, the liquid was concentrated and the residue could be purified by column chromatography over silica gel (*n*-hexane : EtOAc = 5 : 1) to give **13** (0.84 g, 72.6%) as a yellow solid: mp. 155–157 C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 3.96 (6H, s), 3.99 (3H, s), 4.01 (3H, s), 5.24 (2H, s), 6.69 (1H, s), 7.06 (1H, d, *J* = 9.0 Hz), 7.19 (1H, d, *J* = 1.8 Hz), 7.24 (1H, d, *J* = 1.8 Hz), 7.33–7.49 (5H, m), 7.95 (1H, d, *J* = 9.0 Hz). MS (ESI) *m/z*: 471 $[M+Na]^+$. Anal. Calcd. for $C_{26}H_{24}O_7$: C, 69.63; H, 5.39. Found: C, 69.51; H, 5.34.

5′-Hydroxy-7,8,3′,4′-tertramethoxyflavone ***4***

A solution of **13** (0.26 g, 0.58 mmol) in EtOAc (10.0 mL) was stirred in the presence of 10% Pd-C (0.02 g) under a hydrogen atmosphere at room temperature for 10 h. The catalyst was filtrated and washed with EtOAc. The filtrate was concentrated *in vacuo* and purified by column chromatography silica gel (*n*-hexane : EtOAc = 5 : 1) to give the natural flavone **4** (0.19 g, 85.5%) as a light yellow solid: mp. 211–213°C (lit [10] mp. 212–213°C). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 3.94 (3H, s), 3.97 (3H, s), 4.01 (3H, s), 4.05 (3H, s), 4.72 (1H, s), 6.70 (1H, s), 7.09 (1H, d, *J* = 9.0 Hz), 7.12 (1H, d, *J* = 1.8 Hz), 7.24 (1H, d, *J* = 1.8 Hz), 7.94 (1H, d, *J* = 9.0 Hz). ^{13}C-NMR ($CDCl_3$) δ: 55.7, 56.2, 60.5, 61.3, 101.6, 105.6, 107.1, 109.8, 117.5, 120.6, 126.5, 136.7, 139.4, 150.3, 150.5, 153.1, 156.7, 163.2, 178.8. MS (ESI) *m/z*: 359 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{18}O_7$: C, 63.68; H, 5.06. Found: C, 63.61; H, 5.04.

2(R,S)-7,8,3′,4′,5′-Pentamethoxyflavan (±) ***1***

Compound **3** (0.81 g, 2.17 mmol) was stirred in MeOH (5 mL) in the presence of 10% Pd-C (1.55 g, 1.45 mmol Pd^0) under a hydrogen atmosphere at room temperature for 12 h. Pd/C was filtrated and washed with EtOAc. After removing the solvent, the oil was purified by column chromatography silica gel (*n*-hexane : EtOAc = 10 : 1). The natural flavan racemate (±) **1**, was obtained (0.64 g, 81.9%) as a oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.05 (1H, m), 2.19 (1H, m), 2.71 (1H, m), 2.89 (1H, m), 3.85 (3H, s), 3.86 (9H, s), 3.89 (3H, s), 5.07 (1H, dd, J_1 = 2.4 Hz, J_2 = 9.9 Hz), 6.51 (1H, d, *J* = 8.5 Hz), 6.78 (1H, d, *J* = 8.5 Hz), 6.67 (2H, s). ^{13}C-NMR ($CDCl_3$) δ: 24.3, 30.0, 55.9, 56.1, 60.5, 60.7, 77.5, 102.8,104.1, 115.8, 123.4, 137.2, 137.3, 148.5, 151.7, 153.1. MS (ESI) *m/z*: 383 $[M+Na]^+$. Anal. Calcd. for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71. Found: C, 66.61; H, 6.69.

2(R,S)-5′-Hydroxy-7,8,3′,4′-tertramethoxyflavan (±) ***2***

Compound **13** (0.50 g, 1.12 mmol) was stirred in MeOH (5 mL) in the presence of 10% Pd-C (0.80 g, 0.75 mmol Pd^0) under a hydrogen atmosphere at room temperature for 12 h. The catalyst was filtrated and washed with EtOAc. The filtrate was concentrated *in vacuo* and purified by column chromatography silica gel to give The natural flavan racemate (±) **2** (0.32 g, 82.6%) as a oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.01 (1H, m), 2.18 (1H, m), 2.74 (1H, m), 2.88 (1H, m), 3.85 (6H, m), 3.88 (6H, s), 5.02 (1H, dd, J_1 = 2.5 Hz, J_2 = 9.6 Hz), 5.80 (1H, s), 6.49 (1H, d, *J* = 8.5 Hz), 6.76 (1H, d, *J* = 8.5 Hz), 6.59 (1H, d, *J* = 1.7 Hz), 6.65 (1H, d, *J* = 1.8 Hz). ^{13}C-NMR ($CDCl_3$) δ: 24.1, 29.4, 55.4, 55.8, 60.3, 60.5, 77.1, 101.4, 104.2, 105.3, 115.1, 123.0, 134.6, 136.8, 137.5, 148.3, 148.7, 151.2, 152.2. MS (ESI) *m/z*: 347 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{22}O_6$: C, 65.88; H, 6.40. Found: C, 65.81; H, 6.37.

Cytotoxicity assays

SGC-7901, BEL-7402, HeLa, and HL-60 cell lines were obtained from Chinese Academy of Medical Sciences (Beijing, China). Cells were cultured in RPMI-1640 medium (Sigma, USA) supplemented with 10% heat-inactivated fetal calf serum (HyClone, USA), 2 mmol/L glutamin (GIBCO, USA), 100 KU/L penicillin and 100 g/L streptomycin (GIBCO, USA) at 37°C in 5% CO_2. SGC-7901, BEL-7402, HeLa, and HL-60 cells were plated at 5×10^3 cells per 96-well cell culture plate, and incubated for 24 h at 37°C. The various concentrations of series of the test compounds were then added to growth medium. As a control group, DMSO (end-concentration of 0.1%) was added to growth medium. After 72 h treatment, cell viability was determined by MTT assay [18]. The percentage of cell growth inhibition was calculated as follows:

$$\text{Inhibition (\%)} = [A_{570}(\text{control}) - A_{570}(\text{drug})]/A_{570}(\text{control}) \times 100$$

References

[1] D. D. Majo, M. Giammanco, M. L. Guardia, E. Tripoli, *et al., Food Res. Intern.* **2005**, *38*, 1161–1166.

[2] H. Y. Sohn, K. H. Son, C. S. Kwon, G. S. Kwon, S. S. Kang, *Phytomedicine* **2004**, *11*, 666–672.

[3] T. Morikawa, F. M. Xu, H. Matsuda, M. Yoshikawa, *Chem. Pharm. Bull.* **2006**, *54*, 1530–1534.

[4] A. F. Abdel-Razik, M. I. Nassar, E.-D. A. El-Khrisy, A.-A. M. Dawidar, T. J. Mabry, *Fitoterapia* **2005**, *76*, 762–764.

[5] K. J. Hodgetts, *Tetrahedron* **2005**, *61*, 6860–6870.

[6] J. Xue, X. Zhang, X. Chen, Y. Zhang, Y. Li, *Synthetic Commun.* **2003**, *33*, 3527–3536.

[7] K. Oyama, T. Kondo, *J. Org. Chem.* **2004**, *69*, 5240–5246.

[8] M. C. Jimenez, M. A. Miranda, R. Tormos, *Tetrahedron* **1997**, *53*, 14729–14736.

[9] T. G. C. Bird, B. R. Brown, I. A. Stuart, A. W. R. Tyrrell, *J. Chem. Soc. Perkin Trans. I* **1983**, *8*, 1831–1846.

[10] N. Kaneda, J. M. Pezzuto, D. D. Soejarto, A. D. Kinghorn, *et al., J. Nat. Prod.* **1991**, *54*, 196–205.

[11] Y. K. Rao, C. V. Rao, P. H. Kishore, D. Gunasekar, *J. Nat. Prod.* **2001**, *64*, 368–367.

[12] A. S. Chida, P. V. S. N. Vani, M. Chandrasekharam, R. Srinivasan, A. K. Singh, *Synthetic Commun*. **2001**, *31*, 657 – 660.

[13] M. Tanaka, Y. Ikeya, H. Mitsuhashi, M. Maruno, T. Wakamatsu, *Tetrahedron* **1995**, *51*, 11703 – 11724.

[14] P. V. Tekade, K. N. Patil, P. S. Bodkhe, *Asian J. Chem.* **2005**, *17*, 1340 – 1342.

[15] A. J. Buckingham *Dictionary of Organic Compounds*, 5th ed., Chapman & Hall, London, **1982**, *5*, p. 5484.

[16] X. Li, Y. Li, W. Xu, *Bioorg. Med. Chem*. **2006**, *14*, 1287 – 1293.

[17] E. Späth, H. Röder, *Monatsh. Chem.* **1922**, *43*, 93 – 97.

[18] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55 – 63.

