DOI 10.1007/s10600-019-02605-x
Chemistry of Natural Compounds, Vol. 55, No. 1, January, 2019

SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF NATURAL AND NON-NATURAL POLYMETHOXYCHALCONES AND POLYMETHOXYFLAVONES

Kingsadingthongkham Vongdeth,[1,2] Peipei Han,[1] Wei Li,[1] and Qiu-An Wang[1*]

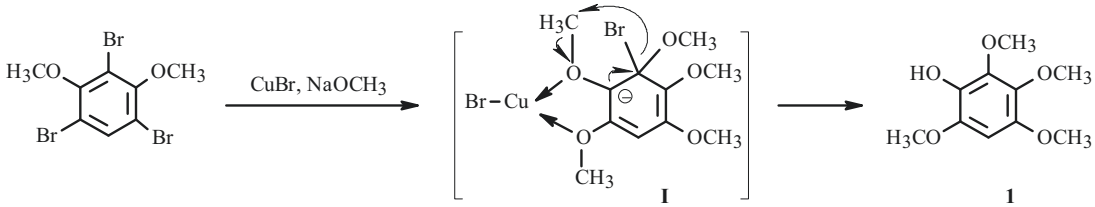
*Two series of polymethoxychalcones and polymethoxyflavones, including the natural products 2′-hydroxy-3,4,5,4′,6′-pentamethoxychalcone (**8c**), 5,6,7,8,3′,4′,5′-heptamethoxyflavone (**6**), 5,7,3′,4′,5′-pentamethoxyflavone (**9c**), 3-hydroxy-5,6,7,8,3′,4′,5′-heptamethoxyflavone (**7**), and 3-hydroxy-5,7,3′,4′,5′-pentamethoxyflavone (**10**), were synthesized. The antiproliferative activity* in vitro *was evaluated against a panel of three human cancer cell lines (HeLa, HCC1954, and SK-OV-3) by the cell counting kit-8 (CCK-8) assay. The results showed that most of the synthetic compounds exhibited moderate to potent antiproliferative activities. Some compounds displayed equal or higher potential than the positive control drug cisplatin. In particular, compounds **4c**, **4e**, **8a**, and **9a** possess IC_{50} values equal to or below 10 μM and are worthy of further investigation.*

Keywords: polymethoxychalcone, polymethoxyflavone, synthesis, antiproliferative activity.

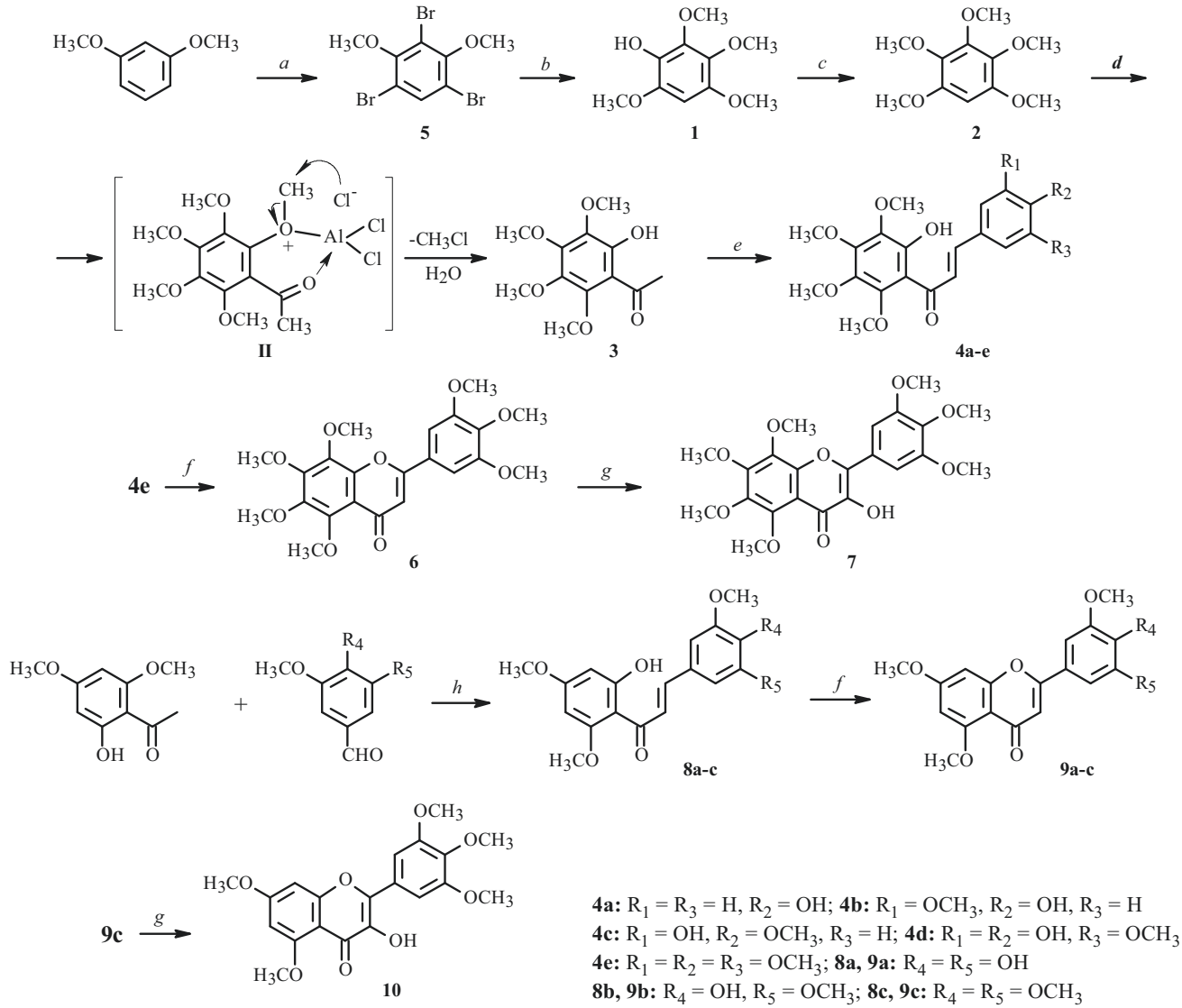
Flavones are a large and well-known family of compounds of natural and synthetic origin with great structural variety responsible for a wide range of biological activities and pharmacological effects [1–3]. Chalcones are prominent secondary metabolite precursors of flavonoids and isoflavonoids in plants. Chemically, they consist of two aromatic rings linked by a three-carbon α,β-unsaturated system and are known to exhibit an impressive array of biological properties [4, 5].

Over the past two decades, increasing attention has been given to polymethoxyflavones (PMFs) [6, 7]. It was reported that flavones and chalcones containing a high number of methoxy groups are effective as colorectal cancer chemopreventive agents, and polymethoxylated flavonoids and chalcones in general appear to have great potential as cancer chemopreventive/chemotherapeutic agents in contrast with their nonmethylated analogues, even though they occur in much lower concentrations [8–10].

However, systematic studies on the synthesis and structure–activity relationships of PMFs have not been widely reported. Due to our continuous interest in discovering and developing flavones and chalcones as potential anticancer compounds [11–13], in the present study, we developed a versatile method for the synthesis of natural and non-natural polymethoxychalcones and polymethoxyflavones, and evaluated *in vitro* their antiproliferative activities on a panel of three human cancer cell lines, including HeLa (cervical carcinoma), HCC1954 (breast cancer), and SK-OV-3 (ovarian cancer) by the CCK-8 assay.



1) College of Chemistry and Chemical Engineering, Hunan University, 410082, Changsha, P. R. China, e-mail: wangqa@hnu.edu.cn; 2) Department of Chemistry, Faculty of Natural Sciences, National University of Laos, Dong Dok Campus, North 13 rd, Vientiane Capital, Lao PDR, P. O. Box 7322. Published in *Khimiya Prirodnykh Soedinenii*, No. 1, January–February, 2019, pp. 12–17. Original article submitted July 13, 2017.



a. Br_2, CH_2Cl_2, r.t; *b*. CuBr, $NaOCH_3$, DMF, microwave, 120°C; *c*. $(CH_3)_2SO_4$, K_2CO_3, acetone, reflux; *d*. CH_3COCl, $AlCl_3$, ether, r.t; *e*. substituted benzaldehydes, 50% NaOH (aq), C_2H_5OH, r.t; *f*. I_2, DMSO, H_2SO_4, reflux; *g*. oxone, acetone–CH_2Cl_2, Na_2CO_3–$NaHCO_3$, 5°C; *h*. 50% NaOH (aq), C_2H_5OH, r.t.

Scheme 1.

Base on the results, we proposed a reaction mechanism that includes the intermediate **I**, in which CuBr coordinates with the two *ortho*-position methoxy groups. The complex is the subsequent thermal elimination of volatile CH_3Br to form the salt of **1**, which is converted under acidic conditions into **1**. Although we did not find in the literature the detailed mechanism of this reaction, it seems that nucleophilic attack of the bromide anion on the methyl group is involved in the demethylation reaction. Such a mechanism has been proposed for the catalytic demethoxylation of aryl ethers at the position *ortho* to the carbonyl group by magnesium iodide [14].

Methoxylation of **1** was achieved using dimethylsulfate as methylating agent in the presence of anhydrous potassium carbonate and acetone to yield pentamethoxybenzene (**2**) (Scheme 1). 2-Hydroxy-3,4,5,6-tetramethoxyacetophenone (**3**), prepared from **2** with Friedel-Crafts acetylation and demethylation with aluminium trichloride, proceeded in a highly regioselective manner including the intermediate **II**. Claisen-Schmidt aldol condensation of the acetophenone **3** or 2-hydroxy-4,6-dimethoxyacetophenone with various hydroxyl or methoxyl-substituted benzaldehyde yielded a series of polymethoxychalcones **4a–e** and **8a–c**. All synthesized chalcones were purified by recrystallization or chromatography, and the analytical and spectroscopic data confirmed their structures, as detailed in the experimental section. The coupling constant of J = 15–16 Hz observed in olefinic protons Hα and Hβ indicates the formation of only the expected *E* isomers. Compound **8c** is a natural product isolated from *Merrillia caloxylon* (Rutaceae) [15].

TABLE 1. Half-inhibitory Concentrations of Compounds **4a–e**, **8a–c**, **6**, **9a–c**, **7**, and **10** on the Human Cancer Cell Lines (IC_{50}, μM)

Compound	Hela	HCC1954	SK-OV-3
4a	34.09	33.85	56.41
4b	29.23	25.25	32.53
4c	1.44	9.28	1.60
4d	32.95	19.47	48.40
4e	4.99	15.98	10.53
8a	5.14	8.26	12.97
8b	> 100	> 100	> 100
8c	> 100	15.05	23.76
6	35.80	> 100	63.04
9a	4.83	8.58	10.64
9b	> 100	> 100	> 100
9c	40.82	53.84	30.17
7	28.56	62.33	31.19
10	> 100	> 100	> 100
Cisplatin[a]	13.30	29.32	18.66
Paclitaxel[a]	0.0055	0.009	0.0028
Staurosporin[a]	0.0112	0.037	0.0031

[a] Cisplatin, paclitaxel, and staurosporin were employed as positive controls.

The synthesis of polymethoxychalcones and polymethoxyflavones was accomplished according to the general pathway in Scheme 1. The approaches rely on polymethoxyacetophenone **3** as a key compound. However, efficient synthesis of such a highly oxygenated acetophenone derivative remains to be resolved. With this mind, we commenced with the development of an alternative access to key compound **4**. We chose commercially available 1,3-dimethoxybenzene as a starting material. Our efforts concentrated on methoxylation of the 2,4,6-positions of 1,3-dimethoxybenzene using an Ulmann-type reaction. 1,3-Dimethoxybenzene was brominated with bromide in methylene chloride to give 1,3,5-tribromo-2,4-dimethoxybenzene (**5**). Compound **5** was subjected to a CuBr-mediated Ulmann-type reaction with a large excess amount of sodium methoxide, which gave rise to 2-hydroxy-1,3,4,5-tetramethoxybenzene instead of the anticipated pentamethoxybenzene (**2**).

The treatment of chalcone **4e** and **8a–c** with concentrated H_2SO_4 and a catalytic amount of iodine in DMSO under reflux gave the desired flavones **6** and **9a–c** in good yield. Compound **6** is a natural product isolated from *Eupatorium coelestinum* [16]. Introduction of a 3-OH group to flavones **6** and **9c** was achieved using dimethyldioxirane (DMDO), followed by opening of the formed epoxide with catalytic *p*-toluenesulfonic acid to afford the desired flavonols **7** and **10** in 63–65% yield. Compound **7** is another natural product isolated from *Athrixia phylicoides* (Asteraceae) [17]. Compounds **9c** and **10** are also natural products isolated from *Merrillia caloxylon* (Rutaceae) [18].

The synthesized target chalcones and flavones were investigated for their antiproliferative activity *in vitro* employing the CCK-8 assay with cisplatin, paclitaxel, and staurosporin as positive control against three human cancer cell lines (HeLa, HCC1954, and SK-OV-3). The antiproliferative activities of compounds, as indicated by IC_{50} values, were calculated by linear regression analysis of the concentration-response curves obtained for each compound. The results from the antiproliferation assay are summarized in Table 1.

The result indicated that most of these polymethoxychalcones and flavonoids exhibited moderate to potent antiproliferative activities, except compounds **8b**, **9b**, and **10**, toward all three cells. The activity of compound **8c** to HeLa cells and compound **6** to HCC1954 was negative at the highest tested concentration (> 100 μM). Some compounds displayed equal or higher (lower IC_{50} values) potential than the positive control drug cisplatin. Compounds **4c**, **4e**, **8a**, and **9a** were more potent against HeLa cells, with IC_{50} values of 1.44–5.13 μM, than the positive control cisplatin (IC_{50} 13.30 μM). Compounds **4b–e**, **8a**, **8c**, and **9a** were more potent against HCC1954 cells, with IC_{50} values of 8.26–25.25 μM, than the positive control cisplatin (IC_{50} 29.32 μM), and compounds **4c**, **4e**, **8a**, and **9a** were more potent against SK-OV-3 cells, with IC_{50} values of 1.60–12.97 μM, than the positive control cisplatin (IC_{50} 18.66 μM).

It is interesting to note that several compounds showed promising cytotoxicity, such as **4c**, **4e**, **8a**, and **9a**, which give IC_{50} values equal to or below 10 μM. Compound **4c** is the most potent among all the tested compounds, with the highest

potency toward HeLa cells (IC_{50} 1.44 μM) and SK-OV-3 cells (IC_{50} 1.60 μM), which are 9-fold and 12-fold greater compared to the positive control cisplatin, respectively. The antiproliferative activity of compound **8c** to HCC1954 and SK-OV-3 and compound **6** to HeLa and SK-OV-3 was selective.

Preliminary structure–activity relationship analysis revealed that the position of the hydroxyl or methoxyl group and the number of methoxyl groups may be closely linked with their antiproliferative activity against these cancer cells. These compounds contain a high number of methoxyl groups and thus exhibit higher antiproliferative activity. Compound **8a** and **9a**, which contain two hydroxyl groups (at the *ortho* position) in the B-ring, significantly increase the antiproliferative activity, with IC_{50} values in the range of 4.83–12.97 μM. Chalcone **8b** and flavone **9b**, having 3,5-dimethoxy-4-hydroxy group in the B-ring, did not demonstrate any antiproliferative activity below 100 μM concentration. Compound **10**, which has a hydroxyl group at the 3-position, also showed inactivity, while the presence of 3,4-dihydroxyl-5-methoxyl phenyl or 3,4,5-trimethoxyl aryl ring moieties was very important with respect to the inhibitory activity against these three cancer cell lines. Clarification of the structural determinants of potency will guide the design of novel potent molecules for future development.

To the best of our knowledge, such observations on the antiproliferative activity against these three cells have not been reported previously. Although we have limited information on the structure–activity relationship (SAR), the findings of this study provide important information for the exploitation and utilization of PMFs as anticancer agents.

In summary, eight polymethoxychalcones **4a–e** and **8a–c** and six polymethoxyflavones **6**, **9a–c**, **7**, and **10** were synthesized. Among the synthesized target compounds, **8c**, **6**, **9c**, **7**, and **10** are natural products. The majority of the target compounds exhibited moderate to potent antiproliferative activities against human cancer cell lines (HeLa, HCC1954, and SK-OV-3). Among them, compounds **4c**, **4e**, **8a**, and **9a** were the most active with IC_{50} values ranging from 1.44 to 15.98 μM against all three cancer cell lines and are worthy of further development.

EXPERIMENTAL

General Experimental Procedures. Melting points were measured on an XRC-I apparatus and were uncorrected. Microwave reaction was performed with an XH-MC-1 microwave reactor (Beijing Xianghua Science and Technology Development Co., China). ^1H and ^{13}C NMR spectra were recorded on a Bruker AM-400 instrument using tetramethylsilane as an internal standard. Chemical shifts (δ) are in ppm, and coupling constants (J) are in Hz. Mass spectra (MS) or high-resolution mass spectrometry (HR-MS) was determined with a VG Autospec-3000 or Mat 95 XP spectrometer by the ESI or FAB method. Column chromatography was carried out on 200–300 mesh silica gel (Qingdao Ocean Chemical Products of China). Commercially available AR or chemically pure reagents and anhydrous solvents with the water removed and redistilled were employed.

3,4-Dihydroxy-5-methoxybenzaldehyde and 3,4,5-trimethoxybenzaldehyde were prepared according to our previous methods [19]. 2-Hydroxy-4,6-dimethoxyacetophenone was prepared from resorcinol *via* Friedel-Crafts acetylation and selective *O*-methylation [20].

Synthesis of 1,3,5-Tribromo-2,4-dimethoxybenzene (5). To a solution of 1,3-dimethoxybenzene (5 g, 36 mmol) in CH_2Cl_2 (30 mL) at room temperature was added bromine (5 mL), and the reaction mixture was stirred for 1 h at room temperature. The reaction mixture was taken up in water and extracted with CH_2Cl_2. Standard workup of the organic layer provided a white crystallized residue 13.13 g, yield 98%; mp 70–71°C [lit. [21] 70–71°C]. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.63 (1H, s, Ar-H), 3.88 (6H, s, OCH_3).

Synthesis of 2,3,4,6-Tetramethoxyphenol (1). To a solution of **5** (1 g, 2.7 mol) in DMF (20 mL) at room temperature were added CuBr (1.79 g, 12.62 mmol) and $NaOCH_3$ (0.44 g, 8.1 mmol) in CH_3OH (15 mL), and the reaction mixture was exposed to microwave irradiation (700 W) at 120°C for 1 h and then cooled to room temperature. The cooled reaction mixture was taken up in water (50 mL) and extracted at pH 6 with ethyl acetate (3 × 50 mL), and the organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated to yield a residue that was chromatographed on silica gel using petroleum ether–ethyl acetate (7:3) as eluent to afford a white solid 430 mg, yield 75%; mp 85–86°C [lit. [22] 84–85°C] ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 6.84 (1H, s, Ar-H), 5.25 (1H, s, OH), 3.89, 3.86, 3.83, 3.65 (each 3H, s, OCH_3). MS (FAB^+) *m/z* 215 $[M + 1]^+$.

Synthesis of 1,2,3,4,5-Pentamethoxybenzene (2). A solution of **1** (1 g, 4.6 mmol) and anhydride K_2CO_3 (2 g, 13.53 mmol) in dry acetone (20 mL) was refluxed for 30 min. Then dimethyl sulfate (0.5 mL, 5.19 mmol) was added dropwise. The reaction mixture was stirred at 30°C for 4 h, filtered, and the filtrate evaporated to afford a crude solid, which was

crystallized from petroleum ether–ethyl acetate (3:1) to afford a white solid (950 mg, yield 91%, mp 57–58°C [lit. [21] 57–58°C]). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 3.83 (6H, s, OCH_3), 3.85 (6H, s, OCH_3), 3.96 (3H, s, OCH_3), 6.30 (1H, s, Ar-H). MS (FAB$^+$) *m/z* 229 $[M + 1]^+$.

Synthesis of 2-Hydroxy-3,4,5,6-tetramethoxyacetobenzene (3). To a solution of **2** (1 g, 4.4 mmol) and $AlCl_3$ anhydride (1.15 g, 9 mmol) in dry ether (20 mL) was added dropwise acetyl chloride (1.05 g, 15 mmol). The reaction mixture was stirred at room temperature for 5 h and acidified with 2 N aqueous HCl. The mixture was stirred for a further 2 h and extracted with EtOAc (3 × 50 mL). The organic phase was washed with 5% NaOH (aq), then neutralized with 2N HCl (aq) and dried over anhydrous Na_2SO_4. The solvent was evaporated, and the crude residue was chromatographed on silica gel using petroleum ether–ethyl acetate (8:2) to afford a yellow oil 0.35 g, yield 31%. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 13.08 (1H, s, OH), 4.00, 3.88, 3.78, 3.73 (each 3H, s, OCH_3), 2.60 (3H, s, $C(O)CH_3$). ESI-MS *m/z* 255 $[M - H]^-$.

General Procedure for the Synthesis of Chalcones 4a–e and 8a–c. To a stirred solution of substituted benzaldehyde (2.0 g, 15.62 mmol) and 2-hydroxy-3,4,5,6-tetramethoxyacetophenone or 2-hydroxy-4,6-dimethoxyacetophenone (2.5 g, 15.62 mmol) in ethanol (30 mL) was added 20 mL of 20% aqueous KOH, and the reaction mixture was stirred at room temperature for 48 h. The reaction mixture was cooled to 0°C (ice-water bath) and acidified with 10% aqueous HCl. A yellow precipitate was formed, which was filtered and washed with 10% aqueous HCl. The yellow solid was recrystallized from petroleum ether–EtOAc to give chalcone **4a–e** and **8a–c**.

4,2′-Dihydroxy-3′,4′,5′,6′-tetramethoxychalcone (4a). Yellow solid, yield 84%, mp 156–158°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 13.22 (1H, s, 3-OH), 7.77 (1H, d, J = 15.5, H-β), 7.71 (1H, d, J = 15.6, H-α), 7.46 (2H, d, J = 7.6, H-2, 6), 6.82 (2H, d, J = 7.7, H-3, 5), 6.22 (1H, s, 2′-OH), 4.02 (3H, s, 3′-OCH_3), 3.82 (3H, s, 4′-OCH_3), 3.81 (3H, s, 5′-OCH_3), 3.79 (3H, s, 6′-OCH_3). ESI-MS *m/z* 359 $[M - H]^-$.

4,2′-Dihydroxy-3,3′,4′,5′,6′-pentamethoxychalcone (4b). Yellow solid, yield 87%, mp 94–96°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 13.18 (1H, s, 4-OH), 7.74 (1H, d, J = 15.5, H-β), 7.69 (1H, d, J = 15.5, H-α), 7.14 (1H, dd, J = 8.2, 1.5, H-6), 7.03 (1H, d, J = 1.4, H-2), 6.86 (1H, d, J = 8.2, H-5), 6.11 (1H, s, 2′-OH), 4.01 (3H, s, 3′-OCH_3), 3.85 (3H, s, 4′-OCH_3), 3.80 (6H, s, 5′, 6′-OCH_3), 3.78 (3H, s, 3-OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 192.50, 153.86, 152.27, 149.84, 147.42, 145.89, 143.49, 137.42, 136.26, 126.74, 122.85, 122.34, 114.01, 110.13, 109.33, 61.21, 60.60, 60.33, 60.03, 54.95. ESI-MS *m/z* 391 $[M + H]^+$.

3,2′-Dihydroxy-4,3′,4′,5′,6′-pentamethoxychalcone (4c). Yellow solid, yield 92%, mp 94–96°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 13.23 (1H, s, 3-OH), 7.68 (1H, d, J = 15.5, H-β), 7.65 (1H, d, J = 15.5, H-α), 7.14 (1H, d, J = 1.5, H-2), 6.98 (1H, dd, J = 8.3, 1.5, H-6), 6.72 (1H, d, J = 8.3, H-5), 6.26 (1H, s, 2′-OH), 3.96 (3H, s, 3′-OCH_3), 3.76 (6H, s, 5′, 6′-OCH_3), 3.75 (3H, s, 4′-OCH_3), 3.74 (3H, s, 4-OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 193.55, 154.82, 153.28, 0.97, 149.25, 146.06, 144.30, 138.40, 137.11, 128.59, 124.26, 122.87, 113.30, 111.07, 110.74, 62.03, 61.50, 61.29, 60.96, 55.90. ESI-MS *m/z* 391 $[M + H]^+$.

3,4,2′-Trihydroxy-5,3′,4′,5′,6′-pentamethoxychalcone (4d). Yellow solid, yield 90%, mp 76–78°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 13.20 (1H, s, 4-OH), 7.73 (1H, d, J = 15.5, H-β), 7.68 (1H, d, J = 15.5, H-α), 7.12 (1H, d, J = 8.2, 1.4, H-2), 7.02 (1H, d, J = 1.1, H-5), 6.85 (1H, d, J = 8.2, 3-OH), 6.23 (1H, s, 2′-OH), 4.00 (3H, s, 3′-OCH_3), 3.83 (3H, s, 4′-OCH_3), 3.80 (6H, s, 5, 5′-OCH_3), 3.78 (3H, s, 6′-OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 193.51, 154.87, 153.29, 150.86, 148.50, 146.96, 144.56, 138.43, 137.26, 127.72, 123.81, 123.36, 115.07, 111.13, 110.36, 62.22, 61.61, 61.34, 61.04, 55.93. MS (FAB$^+$) *m/z* 407 $[M + 1]^+$.

2′-Hydroxy-3,4,5,3′,4′,5′,6′-heptamethoxychalcone (4e). Yellow solid, yield 75%, mp 89–91°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.82 (1H, d, J = 15.5, H-β), 7.77 (1H, d, J = 15.6, H-α), 6.87 (2H, s, H-2, 6), 4.10 (3H, s, OCH_3), 3.92 (6H, s, OCH_3), 3.89 (9H, s, OCH_3), 3.87 (3H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 190.1, 157.7, 151.2, 150.1, 147.5, 140.7, 137.1, 135.1, 133.9, 127.4, 122.4, 107.7, 102.4, 59.0, 58.4, 58.1, 57.8, 57.7, 52.9. ESI-MS *m/z* 433 $[M - H]^-$.

3,4,2′-Trihydroxy-5,4′,6′-trimethoxychalcone (8a). Yellow solid, yield 74%, mp 162–164°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 14.23 (1H, s, 2′-OH), 7.67 (1H, d, J = 15.5, H-β), 7.56 (1H, d, J = 15.5, H-α), 7.32 (1H, s, H-2), 6.93 (1H, s, H-6), 6.15 (1H, s, H-3′), 6.03 (1H, s, H-5′), 5.89 (1H, s, 2′-OH), 3.88, 3.84, 3.76 (each 3H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 191.14, 167.37, 165.21, 161.38, 146.20, 143.84, 140.10, 127.85, 125.48, 124.13, 108.80, 107.69, 105.23, 92.80, 90.29, 55.34, 54.89, 54.59. ESI-MS *m/z* 347 $[M + H]^+$.

4,2′-Dihydroxy-3,5,4′,6′-tetramethoxychalcone (8b). Yellow solid, yield 76%, mp, 89–91°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 13.51 (1H, s, 2′-OH), 9.09 (1H, s, 4-OH), 7.66 (1H, d, J = 15.6, H-β), 7.63 (1H, d, J = 15.5, H-α), 7.02 (2H, s, H-2, 6), 6.13 (2H, d, J = 7.9, H-3′, 5′), 3.90, 3.85, 3.82 (12H, each s, 4 × OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 192.71, 165.71, 165.60, 162.15, 148.59, 144.30, 139.12, 125.64, 125.10, 106.94, 106.76, 94.34, 91.41, 56.53, 56.48, 56.04. ESI-MS *m/z* 359 $[M - H]^-$.

2′-Hydroxy-3,4,5,4′,6′-pentamethoxychalcone (8c). Yellow solid, yield 85%, mp 188–190°C [lit. [18] 186–187°C]. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 14.33 (1H, s, OH), 7.82 (1H, d, J = 15.5, H-*β*), 7.72 (1H, d, J = 15.5, H-*α*), 6.86 (2H, s, H-2, 6), 6.13 (1H, d, J = 2.3, H-5′), 5.98 (1H, d, J = 2.3, H-3′), 3.93 (9H, s, 3, 4, 5-OCH_3), 3.91 (6H, s, 4′, 6′-OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 192.2, 168.3, 166.1, 162.3, 153.3, 142.3, 139.0, 131.0, 126.8 106.2, 105.5, 93.8, 91.2, 61.0, 56.0, 55.7. ESI-MS *m/z* 373 $[M - H]^-$.

General Procedure for Synthesis of Polymethoxyflavones 6, 9a–c. Polymethoxychalcones **4e**, **8a–c** (0.46 mmol) and iodine (18.5 mg, 0.074 mmol) were added to a solution of conc. H_2SO_4 (0.07 mL) in DMSO (5 mL). The mixture was warmed to 80–85°C and stirred for 24 h. When the reaction was over (TLC analysis), saturated aqueous $Na_2S_2O_3$ was added. Then a mixture of 1N HCl (aq)/ethyl acetate (30 mL:30 mL) was added at room temperature. The organic layer was discarded, and concentrated ammonium hydroxide was added the aqueous solution until it was clearly basic (pH > 9). The product was extracted with ethyl acetate (3 × 30 mL). The extract was washed with brine and dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (eluent, petroleum ether–EtOAc, 8:2–6:4) to afford the desired products.

5,6,7,8,3′,4′,5′-Heptamethoxyflavone (6). Yellow solid, yield 62%, mp 105–106°C [lit. [16]: 101.5–102.5°C]. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.12 (2H, s, H-2′, 6′), 6.67 (1H, s, H-3), 4.12, 4.03, 3.99 (each 3H, s, OCH_3), 3.87 (6H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.79 (3H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 189.8, 176.0, 160.3, 158.9, 152.5, 147.7, 144.1, 125.4, 112.7, 111.0, 108.7, 103.3, 91.6, 59.9, 59.8, 58.1, 58.1, 57.9, 57.7. ESI-MS *m/z* 433 $[M + H]^+$.

4′,5′-Dihydroxy-5,7,3′,-trimethoxyflavone (9a). Yellow solid, yield 75%, mp 241–243°C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 9.20 (1H, s, OH), 9.04 (1H, s, OH), 7.15 (1H, s, H-2′), 7.07 (1H, s, H-6′), 6.65, 6.57 (1H, d, J = 2.3, H-8), 6.43 (1H, d, J = 2.3, H-6), 6.34 (1H, s, H-3), 3.93, 3.89, 3.82 (each 3H, s, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 179.26, 169.01, 168.34, 159.25, 148.76, 146.35, 146.20, 137.38, 122.76, 112.68, 111.72, 108.05, 104.78, 94.76, 90.14, 56.92, 56.56, 56.47. ESI-MS *m/z* 345 $[M + H]^+$.

4′-Hydroxy-5,7,3′,5′-tetramethoxyflavone (9b). Yellow solid, yield 75%, mp 240–242°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.07 (2H, s, H-2′, 6′), 6.62 (1H, d, J = 2.4, H-8), 6.26 (1H, d, J = 2.4, H-6), 6.06 (1H, s, H-3), 5.77 (1H, s, OH), 3.89, 3.88, 3.85 (12H, each s, 4 × OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 179.47, 167.75, 167.69, 158.39, 146.11, 145.79, 135.63, 122.98, 110.63, 107.35, 104.40, 92.98, 88.18, 55.38, 55.19, 55.12. ESI-MS *m/z* 359 $[M + H]^+$.

5,7,3′,4′,5′-Pentamethoxyflavone (9c). Yellow solid, yield 65%, mp 200–202°C [lit. [18]: 195–196°C]. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.01 (2H, s, H-2′, 6′), 6.57 (1H, s, H-3), 6.51 (1H, d, J = 2.3, H-8), 6.33 (1H, d, J = 2.3, H-6), 3.90 (3H, s, OCH_3), 3.89 (6H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.85 (3H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 177.5, 164.2, 160.9, 160.6, 159.8, 153.5, 140.9, 130.9, 128.8, 126.6, 103.4, 96.2, 92.9, 61.0, 56.3, 55.8. MS (FAB^+) *m/z* 373 $[M + 1]^+$.

Synthesis of Polymethoxyflavones 7 and 10. A solution of flavones **6** or **8c** (1.34 mmol) in acetone–CH_2Cl_2 (6:5, 70 mL) was mixed with a buffer of Na_2CO_3 (8 g) and $NaHCO_3$ (4 g) in water (100 mL), and the mixture was vigorously stirred at 5°C. A solution of oxone (6.0 g, 9.8 mmol) in 70 mL water was added dropwise over 2 h after the addition was complete, and the pH of the reaction mixture was adjusted to pH 8. Then the mixture was kept again at 5°C for another 12 h. The water layer was extracted with dichloromethane (3 × 50 mL). The combined organic extracts were washed with saturated aqueous $Na_2S_2O_3$ and saturated aqueous NaCl and dried over anhydrous Na_2SO_4. The solution was filtered, and *p*-tuluenesulfonic acid (5 mg, 0.03 mmol) was added. After stirring for 1 h, the solvent was removed under reduced pressure, and the solid residue was recrystallized from methanol to afford flavonol **7** or **10** as yellow crystals.

3-Hydroxy-5,6,7,8,3′,4′,5′-heptamethoxyflavone (7). Yellow solid, yield 63%, mp 110–112°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 13.96 (1H, s, OH), 5.98 (1H, d, J = 2.3, H-2′), 5.85 (1H, d, J = 2.3, H-6′), 3.78 (9H, s, OCH_3), 3.75 (6H, s, OCH_3), 2.54 (6H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 194.2, 162.3, 158.7, 158.0, 153.3, 144.3, 140.0, 130.4, 128.5, 111.9, 105.4, 91.4, 91.0, 61.0, 56.7, 56.1, 55.9, 55.4, 52.6. MS (FAB^+) *m/z* 449 $[M + 1]^+$.

3-Hydroxy-5,7,3′,4′,5′-pentamethoxyflavone (10). Yellow solid, yield 65%, mp 218–220°C [lit. [17]: 232–234°C]. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 12.66 (1H, s, OH), 7.02 (1H, s, H-2′), 6.54 (1H, s, H-6′), 6.44 (1H, d, J = 2.2, H-8), 6.32 (1H, d, J = 2.2, H-6), 3.89 (9H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.83 (3H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 180.5, 168.9, 168.8, 159.4, 153.2, 147.3, 139.5, 128.0, 111.0, 108.5, 105.2, 94.0, 89.2, 61.0, 56.2, 55.5. ESI-MS *m/z* 389 $[M + H]^+$.

For *in vitro* antiproliferative activity screening, HeLa, HCC1954, and SK-OV-3 cell lines were maintained in DMEM + 5% fetal bovine serum (FBS), RPMI 1640 + 5% FBS, and Mccoy′s 5 A + 10% FBS medium in a humidified atmosphere of 5% CO_2 at 37°C, respectively. Stock solutions of the tested compounds (20 mL) including controls, were

prepared with DMSO. The highest DMSO concentration of medium (0.5%) did not have a significant effect on the determined cellular functions.

Proliferation of HeLa, HCC1954, and SK-OV-3 cells was evaluated by the CCK-8 (Cell Counting Kit-8, Dojindo Laboratories, Kumamoto, Japan) assay [23]. This assay was based on the cleavage of the tetrazolium salt WST-8 by mitochondrial dehydrogenase in viable cells. Briefly, 5×10^3 cells/well were incubated with 40 μL culture medium in 96-well plates. After adherence to the well, the cells were treated with 5 μL of the tested compounds at different concentrations at 37°C; absorbance at 450 nm of each well was measured with a Bio-Rad 680 microplate read. Each experiment was repeated three times.

Mean absorbance for each drug dose was expressed as the percentage of the control and plotted versus drug concentration. Dose-response curves were fitted, and IC_{50} values were calculated by SPSS statistical software. IC_{50} values represent drug concentrations that reduce the mean absorbance at 450 nm to 50%.

ACKNOWLEDGMENT

We thank the National Natural Science Foundation of China (No. J1210040, 21173074) for financial support.

REFERENCES

1. M. Singh, M. Kaur, and O. Silakari, *Eur. J. Med. Chem.*, **84**, 206 (2014).
2. Y. Yanqing, D. Wei, L. Chunbo, L. Ping, S. Qinpeng, Y. Juanxia, W. Yuede, Z. Kun, J. Bingkun, G. Xuemei, Z. Min, and H. Qiufen, *Chem. Nat. Compd.*, **52**, 359 (2016).
3. T. K.-D. Hoang, T. K.-C. Huynh, and T. -D. Nguyen, *Bioorg. Chem.*, **63**, 45 (2015).
4. D. Mahapatra, S. K. Bharti, and V. Asati, *Eur. J. Med. Chem.*, **98**, 69 (2015).
5. N. Bathelemy, W. F. Ghislain, A. Pantaleon, K. Justin, D. Arif, and T. N. Bonaventure, *Chem. Nat. Compd.*, **53**, 207 (2017).
6. Y. Miyata, T. Sshitari, Y. Okuyama, A. Schimada, H. Takahashi, H. Natsugari, and H. Kosano, *Bioorg. Med. Chem. Lett.*, **23**, 183 (2013).
7. B. P. Bandgar, S. S. Gawande, R. G. Bodade, and J. V. Totre, *Bioorg. Med. Chem.*, **18**, 1364 (2010).
8. T. Walle, N. Ta, T. Kawamori, X. Wen, P. A. Tsuji, and V. Walle, *Pharmacology*, **73**, 1288 (2007).
9. S. Kawaii, T. Ikuina, T. Hikima, T. Tokiwano, and Y. Yoshizawa, *Anticancer Res.*, **32**, 5239 (2012).
10. S. Li, M.-H. Pan, C.-S. Lai, C.-Y. Lo, S. Dushenkov, and C.-T. Ho, *Bioorg. Med. Chem.*, **15**, 3381 (2007).
11. V.-S. Nguyen, L. Shi, F.-Q. Luan, and Q.-A. Wang, *Acta Biochim. Pol.*, **62**, 547 (2015).
12. V.-S. Nguyen, W. Li, Y. Li, and Q.-A. Wang, *Med. Chem. Res.*, **26**, 1585 (2017).
13. V.-S. Nguyen, L. Shi, S.-C. Wang, and Q.-A. Wang, *Anti-cancer Agents Med. Chem.*, **17**, 134 (2016).
14. A. Miroslaw, S. Katarzyna, and Z. Anna, *Tetrahedron*, **64**, 9544 (2008).
15. P. N. Marta, T. L. Raquel, C. Kanthima, P. Panee, S. J. N. Maria, V. M. Helena, P. Madalena, M. S. S. Artur, and C. Honorina, *Chem. Biodivers.*, **9**, 1133 (2012).
16. L.-V. Ngo and T. V. C. Pham, *Phytochemistry*, **18**, 1859 (1979).
17. M. J. Mashimbye, P. Soundy, and R. T. Van, *J. Chem.*, **59**, 1 (2006).
18. K. Takeshi and F. Kurnia, *Phytochemistry*, **45**, 179 (1997).
19. S. L. Cai, S. Liu, L. Liu, and Q. A.Wang, *Chem. Res. Chin. Univ.*, **28**, 631 (2012).
20. A. Detsi, M. Majdalani, C. A. Kontogiorgis, H. L. Dimitra, and P. Kefalas, *Bioorg. Med. Chem.*, **17**, 8073 (2009).
21. Q. A. Wang, Z. Wu, L. Liu, L. H. Zou, and M. Luo, *Chin. J. Org. Chem.*, **30**, 1682 (2010).
22. M. Tsukayama, E. Kusunoki, and M. M. Hossain, *Heterocycles*, **71**, 1589 (2007).
23. Y. P. Song, Z. Y. Xin, Y. M. Wan, J. B. Li, B. P. Ye, and X. W. Xue, *Eur. J. Med. Chem.*, **90**, 695 (2015).