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Synthesis of kaempferide Mannich base derivatives and their antiproliferative activity on three human cancer cell lines

Van-Son Nguyen[1,2], Ling Shi[1], Fang-Qian Luan[1] and Qiu-An Wang[1]✉

[1]College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, P R China; [2]Faculty of Chemical Engineering, Industry University of Hochiminh City, Vietnam

Kaempferide (3,5,7-trihydroxy-4'-methoxyflavone, 1), a naturally occurring flavonoid with potent anticancer activity in a number of human tumour cell lines, was first semisynthesized from naringin. Based on Mannich reaction of kaempferide with various secondary amines and formaldehyde, nine novel kaempferide Mannich base derivatives 2–10 were synthesized. The aminomethylation occurred preferentially in the position at C-6 and C-8 of the A-ring of kaempferide. All the synthetic compounds were tested for antiproliferative activity against three human cancer cell lines (Hela, HCC1954, SK-OV-3) by the standard MTT method. The results showed that compounds 1, 2 and 5–10 were more potent against Hela cells with IC_{50} values of 12.47–28.24 μM than the positive control cis-platin (IC_{50} 41.25 μM), compounds 5, 6, 8 and 10 were more potent against HCC1954 cells with IC_{50} values of 8.82–14.97 μM than the positive control cis-platin (IC_{50} 29.68 μM), and compounds 2, 3, 5, 6 and 10 were more potent against SK-OV-3 cells with IC_{50} values of 7.67-18.50 μM than the positive control cis-platin (IC_{50} 21.27 μM).

Key words: kaempferide, flavonoids, Mannich base derivatives, synthesis, antiproliferative activity

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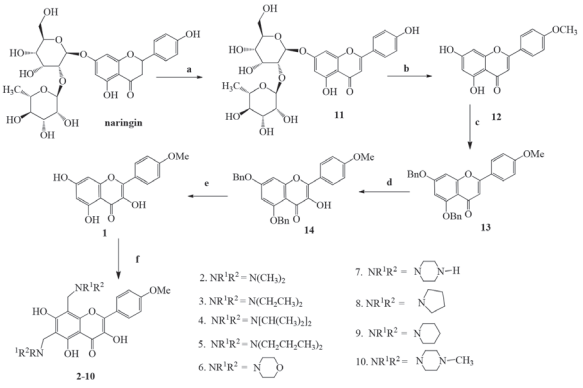
INTRODUCTION

Kaempferide (3,5,7-trihydroxy-4'-methoxyflavone, **1**) is a naturally occurring flavonoid isolated from roots of *Alpinia officinarum* (lesser galangal) (Liu *et al*., 2013), it has been shown to exhibit an interesting spectrum of pharmacological and biological activities, such as antitrypanosomal and antileishmanial (Martineti *et al*., 2010), antioxidant (Gao *et al*., 2010), antiradical and peroxynitrite free radical scavenging (Calgarotto *et al*., 2007). Recently, kaempferide was recognized as a novel anticancer agent by inhibiting growth in *vitro* and arrest in the G_0/G_1 phase of HCT8 line human colon cancer cells (Tasdemir *et al*., 2006), and inhibition of melanogenesis in theophylline-stimulated B16 melanoma 4A5 cells with IC_{50} values of 10–48 μM (Matsuda *et al*., 2009). Kaempferide showed cys-LTs release inhibitory activity from differentiated HL-60 by DMSO with IC_{50} values of 2.0±1.3 μg/mL (Hiroko *et al*., 2010). Kaempferide also showed comparatively strong cytotoxicity toward HT-1080 tumor cells with ED_{50} values of 2.91 μg/mL and colon 26-L5 with ED_{50} values of 5.95 μg/mL (Arjun *et al.,* 1998).

It has recently become apparent that the most of the important classes of drugs, especially those derived from natural products are nitrogen-containing compounds. The Mannich reaction is a versatile reaction that leads to the incorporation of amines into organic molecules. Amine moiety in drugs could enhance physicochemical properties (e.g. water solubility) and improve bioactivity and bioavailability of bioactive molecules (Joshi *et al*., 2013). Kaempferide can undergo regioselective Mannich reactions under certain conditions, and synthesize various Mannich base derivatives.



Scheme 1. Synthesis of kaempferide (1) and its Mannich base derivatives 2–10.

As part of our interest is the extension of systematic investigation of the chemistry and biological activity of flavonoids (Liu *et al.,* 2012; Liu *et al*., 2014; Wu *et al.,* 2013). Herein we report the first semisynthesis of kaempferide (**1**) from commercially low-cost naringin, and synthesis of the series of novel kaempferide Mannich base derivatives **2–10**. Furthermore, all the synthetic compounds were tested for antiproliferative activity against a panel of human cancer cell lines including Hela (cervical carcinoma), HCC1954 (breast cancer) and SK-OV-3 (ovarian cancer) by the standard MTT method.

Reagents and conditions: (a) I_2, Py, reflux, 10 h, 95%; (b) $(CH_3)_2SO_4$, 5% NaOH(aq), 6 h, r.t, then H_2SO_4, reflux, 4 h, 89%; (c) BnBr, K_2CO_3, acetone, reflux, 12 h, 95%; (d) DMDO, acetone, 24 h, r.t, 76%; (e) 5% Pd/C, H_2, 24 h, r.t, 76%; (f) 37% HCHO(aq), CH_3OH, amine, HCl(aq), 80ºC, 3 h, 56–93%.

✉e-mail: wangqa@hnu.edu.cn

Abbreviations: Kaempferide, 3,5,7-trihydroxy-4'-methoxyflavone, **1**

RESULTS AND DISCUSSION

The synthetic pathways adopted for the synthesis of kaempferide **(1)** and its Mannich base derivatives **2–10** are illustrated in Scheme 1, acacetin (**12**) could be obtained by dehydrogenation in I_2/pyridine, regioseletive methylation, hydrolysis of glycosidic bond from the corresponding flavanone glycoside naringin, which is abundant and commercially low-cost. Benzylation of the remaining phenol hydroxyl group of **12** afforded benzyl ether **13.** Oxidation of **13** to the 3-hydroxyflavone benzyl ether **14** proved to be challenging. The method was the oxidation of flavone **13** with dimethyldioxirane (DMDO) generated in *situ* from Oxone and acetone, flowed by acid induced rearrangement that gave the hydroxyflavone benzyl ether **14** in comparable yield. Subsequently, Pd/C catalyzed hydrogenlysis of **14** gave kaempferide (**1**). The sequence from naringin to kaempferide (**1**) was easily conducted on a large scale, and each intermediate could be purified by recrystallization.

Our strategy for the synthesis of the *C*-aminomethylated derivatives relied upon the electrophilic substitution at C-6 and C-8 of the A-ring of kaempferide. This was achieved by the Mannich reaction of the kaempferide (**1**) with formaldehyde and the secondary amines in methanol. The classical conditions of the Mannich reaction for the hydroxyl compounds are based on the substrate, the secondary amine and formaldehyde ratio in alcohol with prolonged heating (Arend *et al*., 1998; Sujith *et al*., 2009; Xu *et al*., 2014). In our case, kaempferide (**1**), formaldehyde and secondary amines in 1: 2: 2 ratio, respectively, were stirred under reflux in methanol for 3 h to afford the *C*-aminomethylated derivatives **2–10**. The structures of the resulting Mannich bases were confirmed by ^{1}H NMR and mass analysis. The ^{1}H NMR spectra of compounds **2–10** clearly indicated the absence of the signal at δ 6.20 of H-6 (C-6) position and δ 6.46 of H-8 (C-8) position of the kaempferide in A-ring system, and a signal at δ 3.61–3.88 indicated the presence of an aminomethyl group at C-6 and C-8 of kaempferide (**1**).

All the synthetic Mannich base derivatives were screened *in vitro* for antiproliferative activity against three human cancer cell lines (Hela, HCC1954 and SK-OV-3) by the standard MTT method (Hattori *et al*., 1952). The antiproliferative activity against these cancer cells was calculated following the formula of the inhibitory ratio and *cis*-platin as positive control. The inhibitory effects of kaempferide (**1**) and its Manich base derivatives **2–10** on three human cancer cells are shown in Table 1. The dose-response curve for MTT assay of compounds **1**, **2** and *cis*-platin on Hela cells, **5**, **6** and *cis*-platin on HCC1954 cells and **5**, **6** on SK-OV-3 cells proliferation as shown in Fig. 1. Overall, most of these derivatives show a broad range of antiproliferative effect against all three cancer cell lines tested. Preliminary bioactive test demonstrated that compounds **1**, **2** and **5**-**10** were more potent (lower IC_{50} values) against Hela cells with IC_{50} values of 12.47–28.24 μM than the positive control *cis*-platin (IC_{50} 41.25 μM), compounds **5**, **6**, **8** and **10** were more potent against HCC1954 cells with IC_{50} values of 8.82–14.97 μM than the positive control *cis*-platin (IC_{50} 29.68 μM), and compounds **2**, **3**, **5**, **6** and **10** were more potent against SK-OV-3 cells with IC_{50} values of 7.67-18.50 μM than the positive control *cis*-platin (IC_{50} 21.27 μM).

It is interesting to note that compound **2** to Hela cells (IC_{50} 12.47 μM), compound **5,6,8–10** to HCC1954 cells (IC_{50} 8.82–34.69 μM) and compound **2,3,5–8** and **10** to SK-OV-3 cells (IC_{50} 7.67–24.87 μM) were more antiproliferative activities (lower IC_{50} values) than the parent compound kaempferide (**1**) to Hela (IC_{50} 15.18 μM), HCC1954 (IC_{50} 36.27 μM) and SK-OV-3 (IC_{50} 39.80 μM), respectively. Although the other Mannich base derivatives showed no more activity than kaempferide (**1**), they still exhibited some interesting inhibition on these cancer cells. Molecular recognition in the target-binding site in these cancer cells may be the reason for different behavior of these compounds. The presence of amine moiety in flavonoid molecular may increase biological potency due to the greater number of molecular sites for electrophilic attack by cellular constituents, as well as due to the cascade effect of preferential chemosensitization.

Table 1. Half-inhibitory concentration [IC_{50} (μM)][a] of compounds 1–10 on three human cancer cell lines

Compound	Hela	HCC1954	SK-OV-3
1	15.18±2.57	36.27±3.26	39.80±0.04
2	12.47±0.26	63.67±2.41	18.50±0.31
3	70.40±2.77	>100	13.03±0.41
4	70.52±4.16	72.63±10.77	>100
5	20.54±3.12	8.34±0.77	7.67±0.42
6	28.24±5.86	8.82±0.37	11.16±1.08
7	21.01±2.23	36.37±0.38	24.85±0.61
8	20.68±1.38	14.97±0.18	24.87±0.44
9	15.92±0.94	34.69±0.37	63.66±0.39
10	25.66±4.07	10.95±0.53	15.43±0.58
cis-platin[b]	41.25±6.63	29.68±2.08	21.27±2.82

[a]Data are the mean ±S.D. of three independent experiment performed.
[b]*cis*-platin was employed as positive control.

EXPERIMENTAL

General methods

Melting points were determined by an XRC-1 apparatus and were uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker-AV400 spectrometer with internal standards of the different solvents. Mass spectra (MS) and high-resolution mass spectrometry (HRMS) were determined with VG Autospec-3000 or Mat 95 XP spectrometer by the EI or ESI method. The chemical shifts (δ) were measured by ppm, and coupling constant (J) was calculated in hertz (Hz). Column chromatography was carried out using 200–300 mesh silica gel (Qingdao Ocean Chemical Products of China). Commercially available AR or chemical pure reagents, and anhydrous solvent removed water and redistilled were employed.

Synthesis of rhoifolin (11). The solution of naringin (20 g, 34.45 mmol) in pyridine (150 mL) was stirred under reflux for 30 min and then I_2 (12 g, 113.20 mmol) was poured into the solution. After the reaction mixture was refluxed for 15 h, the reaction mixture cooled to room temperature, and the reaction mixture was poured into cold water. Then added 3% HCl(aq) (3×150 mL) and the solution of saturated $Na_2S_2O_3$(aq), filtered and washed with water, recrystallized and dried, gave **11** (18.9 g, yield: 95%) as pale yellow solid, mp 249–251ºC; (lit [(Omayma *et al.,* 2013) 251–253ºC]; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.98 (s, 1H, 5-OH), 10.44 (s, 1H, 4'-OH), 7.95 (d, J=7.7 Hz, 2H, H-2' and H-6'), 6.95 (d, J=7.7 Hz, 2H, H-3' and H-5'), 6.88 (s, 1H, 3-H), 6.80

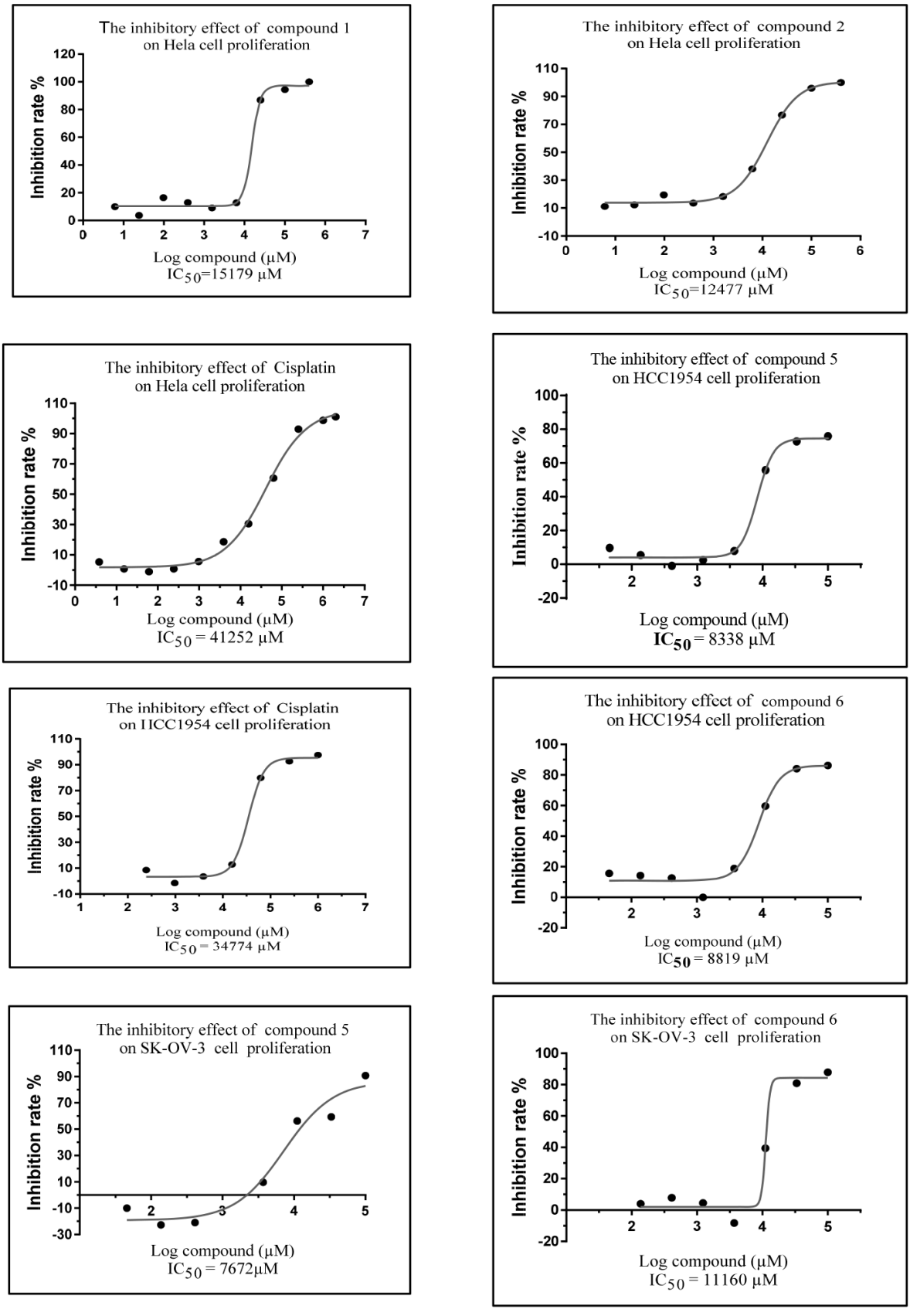


Figure 1. The dose-response curve for MTT assay of compounds 1, 2 and *cis*-platin on Hela cell, 5, 6 and cis-platin on HCC1954 cell and 5, 6 on SK-OV-3 cell proliferation.
Data are mean value ± S.D. of three similar experiments

(s, 1H, 8-H), 6.38 (s, 1H, 6-H), 5.38 (s, 1H, sugar-H), 5.24 (d, *J*=5.7 Hz, 1H, sugar-H), 5.20 (s, 1H, sugar-H), 5.14 (s, 1H, sugar-H), 4.76 (s, 1H, sugar-H), 4.71 (s, 1H, sugar-H), 4.52 (s, 1H, sugar-H), 3.78–3.70 (m, 3H, sugar-H), 3.52–3.49 (m, 6H, sugar-OH), 3.23 (d, *J*=5.5 Hz, 2H, sugar-CH_2), 1.21 (d, *J*=5.5 Hz, 3H, sugar-CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.4, 164.7, 163.0, 161.9, 161.6, 157.4, 136.7, 129.1, 121.4, 116.5, 105.9, 103.6, 100.9, 99.8, 98.2, 95.0, 77.6, 76.7, 72.3, 70.9, 70.1, 68.8, 60.9, 18.5; ESIMS: *m/z* 601 $[M+Na]^+$.

Synthesis of acacetin (12). $(CH_3)_2SO_4$ (17 mL, 63.05 mmol) was slowly added to the solution of compound **11** (10 g, 17.3 mmol) in 150 mL of 5% NaOH(aq). The reaction mixture was stirred at room temperature for 6 h. Then 15 mL of concentrated sulfuric acid was added dropwise, the mixture was stirred and refluxed for 4 h, the reaction mixture cooled to room temperature, and the reaction mixture was poured into cold water, the solids was filtered and washed with water, dried, and the residue was recrystallized from petroleum ether and ethyl acetate mixture (*v/v*, 8:2) to afford **3** (4.6 g, yield: 89%) as yellow solid, mp 258–259ºC; [lit (Sharma *et al*., 1963). 261–263ºC]; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.98 (s, 1H, 5-OH), 10.92 (s, 1H, 7-OH), 8.08 (d, *J*=8.7 Hz, 2H, H-2' and H-6'), 7.15 (d, *J*=8.7 Hz, 2H, H-3' and H-5'), 6.91 (s, 1H, H-3), 6.55 (d, *J*=2.1 Hz, 1H, H-8), 6.25 (d, *J*=2.1 Hz, 1H, H-6), 3.91 (s, 3H, 4'-OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.2, 164.7, 163.7, 162.8, 161.9, 157.8, 128.8, 123.3, 115.0, 104.2, 103.9, 99.4, 94.5, 56.0; EIMS: *m/z* 284 $[M]^+$.

Synthesis of 2-(4-methoxyphenyl)-5,7-bis(benzyloxy)-4H-chromen-4-one (**13**). The solution of **12** (4 g, 14.08 mmol) and anhydrous K_2CO_3 (15 g, 108.6 mmol) in dry acetone (120 mL) was stirred and refluxed for 1 h. Then BnBr (4.5 mL, 37.83 mmol) was added dropwise. After the reaction mixture was refluxed for 10 h, the organic phase was separated. The solvent was removed, and the residue was recrystallized from petroleum ether and ethyl acetate (*v/v*, 8:2) to give **13** (6.2 g, yield: 95%) as white solid, mp 150–152ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 (d, *J*=8.6 Hz, 2H, H-2' and H-6'), 7.62 (d, *J*=8.6 Hz, 2H, H-3' and H-5'), 7.02–7.44 (m, 10H, Ar-H), 6.65 (d, *J*=8.6 Hz, 2H, H-6), 6.59 (d, *J*=8.6 Hz, 1H, H-3), 6.50 (s, 1H, H-8), 5.24 (d, *J*=5.5 Hz, 2H, 5-OCH_2), 5.12 (s, 7-OCH_2), 3.88 (s, 3H, 4'-OMe); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.4, 163.6, 162.8, 162.0, 160.7, 159.7, 136.4, 135.8, 128.8, 128.6, 127.7, 126.6, 123.8, 114.4, 107.7, 104.6, 98.4, 94.4, 70.8, 70.5, 55.5; EIMS: *m/z* 464 $[M]^+$.

Synthesis of 3-hydroxy-2-(4-methoxyphenyl)-5,7-bis(benzyloxy)-4*H*-chromen-4-one (**14**). The mixture of compound **13** (3 g, 6.46 mmol) in 100 mL solvent (acetone and CH_2Cl_2, *v/v*, 3:4) and the solution [Na_2CO_3 (16 g) and $NaHCO_3$ (7 g) in water (140 mL)], was stirred at 0ºC, then Oxone (23 g) in water (300 mL) was added dropwise for 5 h. The solution was adjusted to pH 9. After stirring for 24 h, the organic phase was separated. The aqueous phase was extracted with dichloromethane (3×30 ml). The organic phase was combined and washed with NaCl(aq) and $Na_2S_2O_3$(aq), dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, the residue was added 10 mg *p*-toluensulfonic acid in dry acetone. The solution was stirred at room temperature for 1 h. The crude solid was recrystallized from CH_3OH to give **14** (2.35 g, yield: 76%) as yellow powder, mp 254–256ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (d, *J*=8.6 Hz, 2H, H-2' and H-6'), 7.61 (d, *J*=7.3 Hz, 2H, Ar-H), 7.45–7.35 (m, 8H, Ar-H), 7.04 (d, *J*=8.6 Hz, 2H, H-3' and H-5'), 6.66 (d, *J*=2. 1 Hz, 1H, H-8), 6.50 (d, *J*=2.1 Hz, 1H, H-6), 5.31 (s, 1H, 3-OH), 5.25 (s, 2H, 5-OCH_2), 5.16–5.12 (m, 2H, 7-OCH_2), 3.89 (s, 3H, 4'-OMe); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.8, 166.4, 160.6, 159.2, 158.5, 142.5, 137.6, 136.4, 135.7, 130.9, 128.9, 128.5, 127.8, 126.9, 126.7, 122.2, 114.0, 106.7, 97.5, 95.3, 71.3, 70.4, 58.0; EIMS: *m/z* 480 $[M]^+$.

Synthesis of kaempferide (**1**). The mixture of compound **14** (2 g, 4.16 mmol) and 1.6 g of 5% Pd/C in 25 mL of (CH_3OH: EtOAc: 1:1) was stirred under H_2 atmosphere (balloon) at room temperature for 24 h. The organic phase was separated. The solvent was removed under reduced pressure, the residue was recrystallized from petroleum ether and ethyl acetate mixture (*v/v*, 6:4) to give **1** (900 mg, yield: 76%) as yellow powder, mp 224–226ºC [lit (Hattori *et al*., 2011). 225–227ºC]; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.44 (s, 1H, 5-OH), 10.97 (s, 1H, 7-OH), 9.54 (s, 1H, 3-OH), 8.13 (s, 2H, H-2' and H-6'), 7.11 (s, 2H, H-3' and H-5'), 6.46 (s, 1H, 8-H), 6.20 (s, 1H, 6-H), 3.84 (s, 3H, 4'-OMe); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.5, 164.5, 161.2, 160.9, 156.7, 146.7, 136.5, 129.8, 123.7, 114.5, 103.6, 98.70, 93.9, 55.8; HRMS (EI): *m/z* calcd for $C_{16}H_{12}O_6$, 300.0622 $[M]^+$, found 300.0628.

General experimental procedure for synthesis of Mannich base derivatives. The solution of **kaempferide** (80 mg, 0.16 mmol) with 0.02 mL of 15% HCl(aq) in 10 mL of MeOH and formaldehyde (0.32 mmol), was stirred at room temperature, then secondary amine (0.32 mmol) was added dropwise and the reaction mixture was stirred and refluxed at 80ºC for 3 h. The solvent was removed under reduced pressure and the residue diluting with H_2O, the solution was extracted by EtOAc (3×30 mL), the extracts were combined and the solvent was removed under reduced pressure, dried over anhydrous Na_2SO_4, the crude solid was recrystallized with EtOAc/petroleum ether to afford **2–10** in 56–93% yields.

3,5,7-Trihydroxy-2-(4-methoxyphenyl)-6,8-bis[(dimethylamino)methyl]-4*H*-chromen -4-one (2): Yellow crystals, 72% yield, mp 305-306ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.96 (s, 1H, 5-OH), 7.74 (d, *J*=8.7 Hz, 2H, H-2' and H-6'), 6.72 (d, *J*=8.7 Hz, 2H, H-3' and H-5'), 3.84 (s, 3H, 4'-OCH_3), 3.71 (s, 4H, 6-CH_2N and 8-CH_2N), 2.38 (s, 12H, 2CH_3NCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.1, 162.0, 161.7, 160.2, 158.2, 154.3, 146.4, 135.9, 129.2, 124.1, 114.0, 105.0, 101.0, 55.9, 55.6, 43.8; EIMS: m/z 414 $[M]^+$; HRMS (EI): *m/z* calcd for $C_{22}H_{26}N_2O_6$, 414.1782 $[M]^+$, found 414.1791.

3,5,7-Trihydroxy-2-(4-methoxyphenyl)-6,8-bis[(diethylamino)methyl]-4*H*-chromen-4-one (3): Yellow crystals, 76% yield, mp 298–299ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.23 (s, 1H, 5-OH), 7.61 (d, *J*=8.6 Hz, 2H, H-2' and H-6'), 6.88 (d, *J*=8.6 Hz, 2H, H-3' and H-5'), 3.86 (s, 3H, 4'-OCH_3), 3.79 (s, 2H, 8-CH_2N), 3.77 (s, 2H, 6-CH_2N), 2.75 (q, *J*=5.2 Hz, 4H, CH_2NCH_2), 1.21 (t, *J*=5.2 Hz, 12H, 4CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.7, 163.8, 162.3, 159.2, 158.1, 146.6, 132.8, 127.5, 123.1, 113.5, 104.3, 103.9, 55.5, 55.3, 46.33, 13.3; EIMS: m/z 470$[M]^+$; HRMS (EI): *m/z* calcd for $C_{26}H_{34}N_2O_6$ 470.2417 $[M]^+$, found 470.2413.

3,5,7-Trihydroxy-2-(4-methoxyphenyl)-6,8-bis[(diisobutylamino)methyl]-4*H*-chromen-4-one (4): Yellow crystals, 56% yield, mp 296–297ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.95 (s, 1H, 5-OH), 9.50 (s, 1H, 3-OH), 8.07 (d, *J*=8.5 Hz, 2H, H-2' and H-6'), 7.16 (d, *J*=8.5 Hz, 2H, H-3' and H-5'), 3.87 (s, 3H, 4'-OCH_3), 3.71 (s, 2H, 8-NCH_2), 3.67 (s, 2H, 6-NCH_2), 2.82 (s, 4H, 4CH), 1.35 (t, *J*=5.1 Hz, 24H, 6CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.0, 163.7, 161.4, 160.9, 158.4, 146.3, 136.4, 129.2, 121.5, 114.0, 105.9, 103.9, 103.0, 55.8, 50.1, 46.8, 19.3; EIMS: *m/z* 526 $[M]^+$; HRMS

(EI): *m/z* calcd for $C_{30}H_{42}N_2O_6$, 526.3035 [M]$^+$, found 526.3031.

3,5,7-Trihydroxy-2-(4-methoxyphenyl)-6,8-bis[(dipropylamino)methyl]-4*H*-chromen-4-one (5): Yellow crystals, 75% yield, mp 301–303ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.94 (s, 1H,5-OH), 9.21 (s, 1H, 3-OH), 8.01 (d, *J*=8.4 Hz, 2H, H-2' and H-6'), 6.93 (d, *J*=8.5 Hz, 2H, H-3' and H-5'), 3.83 (s, 3H, 4'-OCH_3), 3.78 (s, 2H, 8-NCH_2), 3.72 (s, 2H, 6-NCH_2), 2.47 (d, *J*=8.5 Hz, 8H, $2CH_2NCH_2$), 1.38–1.35 (m, 8H, $4CH_2$), 0.89 (m, 12H, $4CH_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.4, 161.5, 160.6, 159.4, 158.4, 146.4, 136.0, 129.6, 124.2, 114.2, 105.1, 103.4, 103.3, 55.8, 55.7, 54.9, 19.0, 11.7; EIMS: *m/z* 526 [M]$^+$; HRMS (EI): *m/z* calcd for $C_{30}H_{42}N_2O_6$, 526.3035 [M]$^+$, found 526.3030.

3,5,7-Trihydroxy-2-(4-methoxyphenyl)-6,8-bis(morpholinomethyl)-4*H*-chromen-4-one (6): Yellow crystals, 93% yield, mp 269–270ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.04 (s, 1H, 5-OH), 10.04 (s, 1H, 7-OH), 8.19 (d, *J*=8.4 Hz, 2H, H-2' and H-6'), 7.12 (d, *J*=8.4 Hz, 2H, H-3' and H-5'), 3.85 (s, 3H, 4'-OCH_3), 3.76 (s, 4H, 6-CH_2N and 8-CH_2N), 3.61 (d, *J*=5.5 Hz, 8H, $2CH_2OCH_2$), 2.53 (d, *J*=5.6 Hz, 8H, $2CH_2NCH_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.7, 163.5, 160.9, 160.1, 158.7, 146.2, 136.7, 129.7, 126.4, 114.6, 104.8, 103.8, 103.4, 66.6, 66.4, 55.8, 53.4, 52.8, 50.9; ESIMS: m/z 498 [M]$^+$; HRMS (EI): *m/z* calcd for $C_{26}H_{30}N_2O_8$, 498.2002 [M]$^+$, found 498.2006.

3,5,7-Trihydroxy-2-(4-methoxyphenyl)-6,8-bis(piperazin-1-ylmethyl)-4*H*-chromen-4-one (7): Yellow crystals, 87% yield, mp 294–295ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.98 (s, 1H, 5-OH), 8.16 (d, *J*=8.6 Hz, 2H, H-2' and H-6'), 7.14 (d, *J*=8.6 Hz, 2H, H-3' and H-5'), 3.85 (s, 3H, 4'-OCH_3), 3.78 (s, 2H, 8-CH_2N), 3.74 (s, 2H, 6-CH_2N), 2.81-2.47 (m, 8H, $2CH_2NCH_2$), 2.37-2.27 (m, 8H, $2CH_2NCH_2$), 1.99 (s, 2H, 2HN) ^{13}C NMR (100 MHz, DMSO-d_6): δ 177.0, 164.3, 161.0, 160.3, 159.4, 145.6, 136.6, 129.7, 123.9, 114.7, 104.0, 103.3, 57.6, 55.8, 52.0, 46.7; EIMS: *m/z* 496 [M]$^+$; HRMS (EI): *m/z* calcd for $C_{26}H_{32}N_4O_6$, 496.2316 [M]$^+$, found 496.2322.

3,5,7-Trihydroxy-2-(4-methoxyphenyl)-6,8-bis(pyrrolidin-1-ylmethyl)-4*H*-chromen-4-one (8): Orange crystals, 89% yield, mp 280–281ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.93 (s. 1H, 5-OH) 8.16 (d, *J*=8.9 Hz, 2H, H-2' and H-6'), 7.12 (d, *J*=8.9 Hz, 2H, H-3' and H-5'), 3.84 (s, 3H, 4'-OCH_3), 3.79 (s, 2H, 8-CH_2N), 3.70 (s, 2H, 6-CH_2N), 2.94 (d, *J*=5.6 Hz, 8H 2CH-$_2NCH_2$), 1.83 (d, *J*=5.6 Hz, 8H, $4CH_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.8, 161.8, 161.6, 159.8, 158.7, 146.7, 135.6, 129.5, 123.3, 114.5, 107.8, 104.9, 61.4, 55.8, 50.6, 23.5; EIMS: *m/z* 466 [M]$^+$; HRMS (EI): *m/z* calcd for $C_{26}H_{30}N_2O_6$, 466.2100 [M]$^+$, found 466.2106.

3,5,7-Trihydroxy-2-(4-methoxyphenyl)-6,8-bis(piperidin-1-ylmethyl)-4*H*-chromen-4-one (9): Yellow crystals, 89% yield, mp 230–231ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.25 (s, 1H, 5-OH), 8.17 (d, *J*=8.5 Hz, 2H, 2'-H, and 6'-H), 7.00 (d, *J*=8.5 Hz, 2H, H-3' and H-5'), 3.88 (s, 3H, 4'-OCH_3), 3.83 (s, 2H, 8-CH_2N), 3.80 (s, 2H, 6-CH_2N), 2.59 (s, 8H, $2CH_2NCH_2$), 1.79 – 1.55 (m, 8H, $2CH_2CH_2$), 1.48 (d, *J*=5.3 Hz, 4H, $2CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.6, 165.8, 160.8, 157.6, 154.3, 144.8, 136.1, 129.3, 123.9, 114.0, 103.9, 102.1, 55.4, 54.2, 53.7, 53.6, 25.7, 25.5, 24.1, 23.8; EIMS: *m/z* 494 [M]$^+$; HRMS (EI): *m/z* calcd for $C_{28}H_{34}N_2O_6$, 494.2411 [M]$^+$, found 494.2417.

3,5,7-Trihydroxy-2-(4-methoxyphenyl)-6,8-bis[(4-methylpiperazin-1-yl)methyl]-4*H*-chromen-4-one (10): Yellow crystals, 86% yield, mp 200–201ºC; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.99 (s, 1H, 5-OH), 8.16 (d, *J*=8.7 Hz, 2H, H-2' and H-6'), 7.14 (d, *J*=8.7 Hz, 2H, H-3' and H-5'), 3.88 (s, 2H, 8-CH_2N), 3.85 (s, 3H, 4'-OCH_3), 3.77 (s, 2H, 6-CH_2N), 2.64 (s, 8H, $2CH_2NCH_2$), 2.40 (s, 8H, $4CH_2$), 2.20 (s, 6H, $2NCH_3$), ^{13}C NMR (100 MHz, DMSO-d_6): δ 176.5, 165.7, 160.9, 157.7, 154.5, 146.3, 136.5, 129.6, 124.0, 114.6, 102.9, 102.3, 57.6, 55.8, 54.5, 51.9, 45.9; EIMS: *m/z* 524 [M] $^+$; HRMS (EI): *m/z* calcd for $C_{28}H_{36}N_4O_6$, 524.2631 [M]$^+$, found 524.2625.

Assay for antiproliferative activity

Cell culture and determination of IC_{50}. The antiproliferative activity of compounds **1–10** were measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay (Cao *et al*., 2009; Elbaz *et al*., 2012) Human cancer cells (Hela, HCC1954 and SK-OV-3) (5×10^3 per well in a 96-well plate) were treated with different concentrations of compounds **1–10** (100, 25, 6.25, 1.56, 0.39, 0.0976, 0.0244, 0.0061 μM) for 48 h. Then 5% MTT was added into each well and incubated with 90% humidity and 5% CO_2 for another 1–3 h. The supernatant was discarded, and 0.1 ml of DMSO was added to dissolve precipitation. The mixture was shaken on a microvibrator for 5 min, and the absorbance was measured at 570 nm by Automated Microplated EL × 800 (Bio-Rad 680) spectrophotometer to determine the concentration that killed 50% of cells (IC_{50}). The IC_{50} value was defined as the concentration that caused 50% inhibition of cell proliferation.

(1 — Average absorbance of treated group/Average absorbance of control group) ×100%

Statistical analysis. Data represent the means of at least three separate experiments. Statistical analysis was performed using SAS statistical software. A value of $p<0.05$ was considered significant.

CONCLUSION

In this paper, kaempferide (**1**) was first semisynthesized from naringin. Based on Mannich reaction of kaempferide with various secondary amines and formaldehyde, nine new flaovonoid Mannich base derivatives **2–10** were synthesized. The antiproliferative activities test demonstrated that compounds **1**, **2** and **5–10** were more potent (lower IC_{50} values) against Hela cells with IC_{50} values of 12.47–28.24 μM than the positive control *cis*-platin (IC_{50} 41.25 μM), compounds **5**, **6**, **8** and **10** were more potent against HCC1954 cells with IC_{50} values of 8.82–14.97 μM than the positive control *cis*-platin (IC_{50} 29.68 μM), and compounds **2**, **3**, **5**, **6** and **10** were more potent against SK-OV-3 cells with IC_{50} values of 7.67–18.50 μM than the positive control *cis*-platin (IC_{50} 21.27 μM). The results indicated that these compounds are potential anticancer agents and are promising for further development.

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