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Synthesis and antiproliferative activity of novel aminoalkylated flavones

Lili Yan[1], Haoran Liu[1], Qiuan Wang[1]*, Gangqiang Wang[2]

[1] *College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China; e-mail: WangQA@hnu.edu.cn*

[2] *School of Nuclear Technology and Chemistry & Biology, Hubei University of Science and Technology, Hubei 437100, China; e-mail: wgq20008@163.com*

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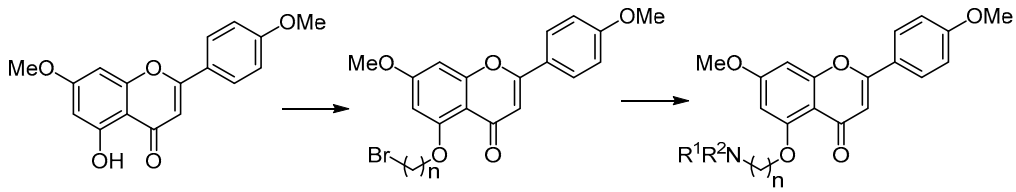


Naturally occurring 5-hydroxy-4',7-dimethoxyflavone (acacetin-7-*O*-methyl ether) was synthesized through dehydrogenation, glycoside hydrolysis, and selective *O*-methylation, using naringin as starting material. Two series of sixteen novel aminoalkylated flavones were synthesized from 5-hydroxy-4',7-dimethoxyflavone. Furthermore, antiproliferative activity of the compounds was evaluated *in vitro* on a panel of three human cancer cell lines (HeLa, HCC1954, and SK-OV-3) using Cell Counting Kit-8 assay. The result showed that most of the synthetic compounds exhibited moderate to potent antiproliferative activities against the three human cancer cell lines with IC_{50} values of 6.95–64.50 μM.

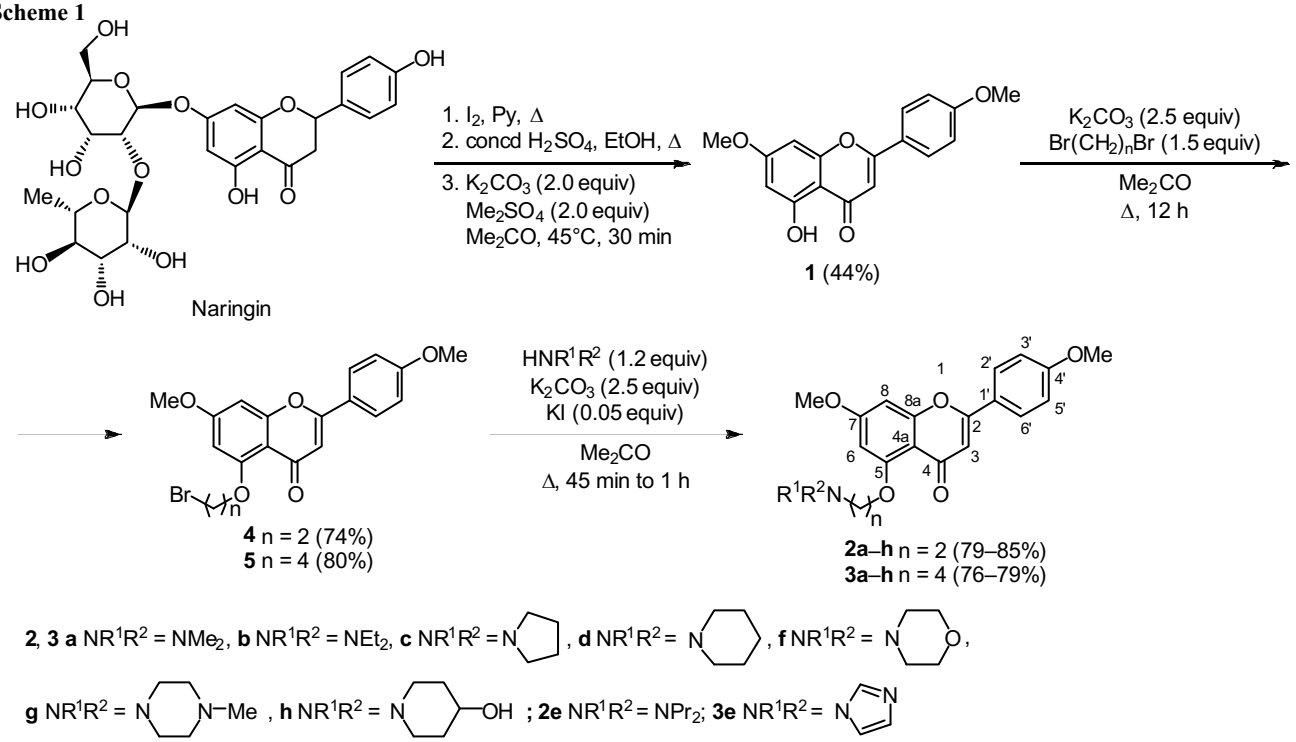
Keywords: 5-hydroxy-4',7-dimethoxyflavone, aminoalkylated flavones, antiproliferative activity, cancer cells, synthesis.

Flavones are ubiquitous structures present in a large number of natural compounds comprising a broad range of powerful biological activities.[1] For example, 5-hydroxy-4',7-dimethoxyflavone (acacetin-7-*O*-methyl ether or apigenin-7,4-dimethyl ether) (**1**) (Scheme 1), a natural flavone isolated from plant *Turnera diffusa*, not only possess a "powerful aphrodisiac effects"[2] but also has antiproliferative activity for HeLa cells to some extent.[3] Flavonoids display specific cytotoxic activity toward different cancer cell lines with little or no toxicity toward normal cells at concentrations that are lethal to tumor cells, which has generated large interest in developing flavonoid-based cytostatics for anticancer therapy.[4] The interest, created by biological studies, to develop structural analogs of antitumor agents containing nitrogen heterocycle has often led to enhanced bioactivity.[5] The results obtained in several classes of polyaromatic antitumor agents indicate that the introduction of an aminoalkyloxy side chain can increase significantly biological activity and the potency of the parent compounds.[6]

As part of our screening program dedicated to the search for derivatives of natural flavonoids with anticancer properties,[7] we herein report the synthesis of two series of sixteen novel aminoalkylated flavone derivatives obtained from 5-hydroxy-4',7-dimethoxyflavone (**1**) through extending alkoxy side chain at position 5 and introducing amine–hydrogen bond receptor at the end of the side chain. Furthermore, the *in vitro* antiproliferative activity of the synthesized compounds against three human cancer cell lines HeLa (cervical carcinoma), HCC1954 (breast cancer), and SK-OV-3 (ovarian cancer) was evaluated using Cell Counting Kit-8 (CCK-8) assay.

Synthesis of novel aminoalkylated flavone derivatives **2a**–**h** and **3a**–**h** from flavone **1** is shown in Scheme 1. According to our previous procedure,[8] acacetin-7-*O*-methyl ether (**1**) could be obtained from the corresponding flavonoid glycoside naringin, which is commercially available at a low cost, by a sequence of dehydrogenation, hydrolysis of glycosidic bond, and regioselective methylation. Because the 5-OH group in compound **1** was stabilized by the hydrogen bond, trimethoxy-substituted compounds were not produced under these conditions. Compounds **4** and **5** were the key intermediates, usually prepared from alkylation of the 5-OH group by using dihaloalkanes in the presence of a base. Thus, alkylation of compound **1** with an excess amount of 1,2-dibromoethane or 1,4-dibromobutane in the presence of K_2CO_3 in dry acetone at 60–65°C resulted in intermediates **4**, **5** which were reacted with the corresponding secondary amines in the presence of K_2CO_3 in acetone and catalytic amount of KI to provide the final

Scheme 1



aminoalkylated flavones. This process produced the desired target compounds **2a**–**h** and **3a**–**h** in moderate to good yields. All new compounds were purified by recrystallization or chromatography, and the analytical and spectroscopic data confirmed their structures, as detailed in the Experimental section.

All the synthetic aminoalkylated flavone derivatives **2a**–**h** and **3a**–**h** were tested *in vitro* for their antiproliferative activity against three human cancer cell lines (HeLa, HCC1954, and SK-OV-3) by the CCK-8 method using cisplatin, paclitaxel, and staurosporine as positive controls. Their activities were expressed by the concentration of drug inhibiting 50% cell growth (IC_{50}), and the data presented in the Table 1 is a result of at least three separate experiments. The results showed that most of the synthesized target compounds exhibited moderate to potent antiproliferative activities with IC_{50} values of 6.90–64.50 μM toward these three cancer cell lines. In general, aminoalkylation of flavone **1** causes a significant increase of antiproliferative activity on HCC1954 and SK-OV-3 cells. However, aminoalkylation of flavone **1** causes a marked decrease of activity in HeLa cells. Compounds **1**, **2c**,**d**,**f**, and **3h** were equal or more potent against HeLa cells with IC_{50} values of 7.23–14.88 μM than the positive control cisplatin (IC_{50} 13.30 μM), all compounds except **2f**,**g**, and **3e** were more potent aginst HCC1954 cells with IC_{50} values of 6.95–27.88 μM than the positive control cisplatin (IC_{50} 29.32 μM), and compounds **2d**, **3b**,**d** were more potent against SK-OV-3 cells with IC_{50} values of 15.59–17.59 μM than the positive control cisplatin (IC_{50} 18.66 μM).

It is interesting to note that all compounds except **2f**,**g** and **3a**,**e** had more pronounced antiproliferative activities against HCC1954 cells (IC_{50} 6.95–16.10 μM) than the parent acacetin-7-*O*-methyl ether (**1**) (IC_{50} 23.66 μM).

Table 1. Half-maximal inhibitory concentrations (IC_{50} in μM) of compounds **1**, **2a**–**h**, and **3a**–**h** on the human cancer cell lines

Compound	HeLa	HCC1954	SK-OV-3
1	7.23	23.66	>100
2a	34.98	12.54	28.42
2b	23.06	12.80	24.66
2c	12.86	16.10	24.32
2d	14.40	8.26	17.59
2e	27.83	8.68	20.92
2f	14.88	>100	>100
2g	50.98	34.62	64.50
2h	18.32	12.69	19.83
3a	17.21	27.88	43.81
3b	16.90	12.60	17.33
3c	17.23	14.23	23.39
3d	16.27	6.95	15.59
3e	36.27	37.14	48.47
3f	19.51	13.13	24.70
3g	17.82	12.48	24.29
3h	14.22	11.27	23.80
Cisplatin*	13.30	29.32	18.66
Paclitaxel*	0.0055	0.009	0.0028
Staurosporine*	0.0112	0.037	0.0031

* Cisplatin, paclitaxel, and staurosporine were employed as positive control.

Although parent flavone **1** is inactive against SK-OV-3 (IC_{50} >100 μM), all of aminoalkyl-substituted flavones except **2f** presented moderate antiproliferative activity against SK-OV-3 cells (IC_{50} 15.59–64.50 μM). In particular, compound **1** on HeLa cells, compounds **2d**,**e** and **3d** on HCC1954 cells showed the gratest potency with IC_{50}

value below 10 μM. Compound **2f** showed selective antiproliferative activity against HeLa cells. Molecular recognition in the target binding site in these cancer cells may be the reason for different behavior of these compounds.

In summary, two series of sixteen novel aminoalkylated flavones derivatives were synthesized from 5-hydroxy-4',7-dimethoxyflavone. The biological activity study results showed that most of synthesized target compounds exhibit moderate antiproliferative activities toward three cancer cell lines. While aminoalkylation of the parent flavone causes a significant increase of antiproliferative activity toward HCC1954 and SK-OV-3 cells it appears to decrease the activity toward HeLa cells. Synthesized compounds are potential and selective anticancer agent and worthy of further investigation.

Experimental

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker-AV400 spectrometer (400 and 100 MHz, respectively) with TMS as internal standard. Mass spectra were recorded on a Mat 95 XP spectrometer by the EI (70 eV) method or paper spray tandem mass spectrometry (PS-MS/MS) by the ESI method. High-resolution mass spectra were recorded on a Mat 95 XP spectrometer using the EI (70 eV) method. Melting points were determined by an XRC-1 apparatus and are uncorrected. Column chromatography was carried out on silica gel 200–300 mesh (Qingdao Ocean Chemical Products of China).

Starting compounds and analytical reagents were obtained from commercial sources and utilized without further purification. Solvents were dehydrated and distilled using standard experimental procedures. Acacetin-7-*O*-methyl ether (**1**) was prepared from the corresponding flavonoid glycoside naringin according to previously described procedure.[8]

Synthesis of 5-(2-bromoethoxy)-7-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (4) and 5-(4-bromobutoxy)-7-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (5) (General method). Compound **1** (1.00 g, 3.4 mmol) was dissolved in dry acetone (25 ml); anhydrous K_2CO_3 (1.17 g, 8.5 mmol) was added and the reaction was refluxed for about 45 min. Then dibromoethane or dibromobutane (5.1 mmol) was added dropwise and refluxed for about 12 h, the reaction mixture was cooled to room temperature, filtered, and evaporated to dryness, the residue was purified by column chromatography, eluent petroleum ether–EtOAc, 1:1.

5-(2-Bromoethoxy)-7-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (4). Yield 1.02 g (74%), yellow solid, mp 187–189°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.75 (2H, d, *J* = 8.2, H-2',6'); 6.93 (2H, d, *J* = 8.3, H-3',5'); 6.54 (1H, s, H-8); 6.48 (1H, s, H-3); 6.34 (1H, s, H-6); 4.31 (2H, t, *J* = 6.9, 5-OCH_2); 3.83 (3H, s, 4'-OCH_3); 3.81 (3H, s, 7-OCH_3); 3.69 (2H, t, *J* = 6.9, CH_2Br). ^{13}C NMR spectrum, δ, ppm: 176.2; 162.7; 161.1; 160.9; 159.9; 158.7; 158.2; 126.6; 122.8; 113.4; 106.6; 97.9; 93.2; 68.9; 54.6; 54.2; 27.2.

5-(4-Bromobutoxy)-7-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (5). Yield 1.18 g (80%), yellow solid, mp 160–162°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.72 (2H, d, *J* = 8.8, H-2',6'); 6.91 (2H, d, *J* = 8.8, H-3',5'); 6.46 (1H, s, H-8); 6.44 (1H, s, H-3); 6.25 (1H, s, H-6); 4.01 (2H, t, *J* = 5.9, 5-OCH_2); 3.82 (3H, s, 4'-OCH_3); 3.79 (3H, s, 7-OCH_3); 3.51 (2H, t, *J* = 6.4, CH_2Br); 2.19–2.11 (2H, m, CH_2); 2.03–1.96 (2H, m, CH_2). ^{13}C NMR spectrum, δ, ppm: 177.4; 163.8; 162.0; 160.6; 160.1; 159.8; 127.6; 123.9; 114.3; 109.4; 107.7; 97.0; 92.9; 68.2; 55.7; 55.5; 34.1; 29.4; 27.6. Mass spectrum (ESI), *m*/*z* (I_{rel}, %): 433 $[M+H]^+$ (100). Found, *m*/*z*: 432.0567 $[M]^+$. $C_{21}H_{21}BrO_5$. Calculated, *m*/*z*: 432.0572.

Synthesis of 5-*O*-aminoalkyled flavones 2a–h and 3a–h (General method). The bromoalkyled flavone intermediate **4** or **5** (0.5 mmol) and anhydrous K_2CO_3 (172.5 mg, 1.25 mmol) were added to anhydrous acetone (15 ml), the mixture refluxed for 1 h. Then corresponding secondary amine (0.6 mmol) and KI (4.2 mg, 0.025 mmol) were added and mixture refluxed for 45 min to 1 h. The mixture was cooled to room temperature, precipitate filtered off and filtrate evaporated to dryness. Then water (30 ml) was slowly added, the aqueous phase was extracted with dichloromethane (3×25 ml). The organic phases were combined, dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography, eluent petroleum ether–EtOAc, 4:1 gradient to 1:1, with several drops of Et_3N added.

5-[2-(Dimethylamino)ethoxy]-7-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (2a). Yield 153 mg (82%), white solid, mp 165–167°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.75 (2H, d, *J* = 8.3, H-2',6'); 6.93 (2H, d, *J* = 8.3, H-3',5'); 6.52 (1H, s, H-8); 6.46 (1H, s, H-3); 6.33 (1H, s, H-6); 4.23 (2H, t, *J* = 5.2, 5-OCH_2); 3.83 (3H, s, 4'-OCH_3); 3.81 (3H, s, 7-OCH_3); 3.15 (2H, t, *J* = 5.2, CH_2); 2.60 (6H, s, 2NCH_3). ^{13}C NMR spectrum, δ, ppm: 176.6; 163.1; 161.3; 160.2; 158.6; 158.3; 126.7; 124.6; 122.7; 113.5; 106.4; 96.8; 92.8; 65.6; 56.0; 54.6; 54.0; 44.0. Mass spectrum (ESI), *m*/*z* (I_{rel}, %): 370 $[M+H]^+$ (100).

5-[2-(Diethylamino)ethoxy]-7-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (2b). Yield 159 mg (80%), white solid, mp 86–88°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.69 (2H, d, *J* = 8.6, H-2',6'); 6.88 (2H, d, *J* = 8.6, H-3',5'); 6.44 (2H, s, H-3,8); 6.28 (1H, s, H-6); 4.04 (2H, t, *J* = 6.4, 5-OCH_2); 3.79 (3H, s, 4'-OCH_3); 3.76 (3H, s, 7-OCH_3); 2.94 (2H, t, *J* = 6.4, CH_2); 2.61 (4H, q, *J* = 7.1, 2CH_2); 1.01 (6H, t, *J* = 7.1, 2CH_3). ^{13}C NMR spectrum, δ, ppm: 176.4; 162.8; 160.9; 159.5; 159.0; 158.7; 126.5; 122.7; 113.3; 108.2; 106.5; 95.9; 92.0; 67.2; 54.7; 54.4; 50.4; 46.9. Mass spectrum (ESI), *m*/*z* (I_{rel}, %): 398 $[M+H]^+$ (100).

7-Methoxy-2-(4-methoxyphenyl)-5-[2-(pyrrolidin-1-yl)-ethoxy]-4*H*-chromen-4-one (2c). Yield 156 mg (79%), white solid, mp 139–141°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.74 (2H, d, *J* = 7.8, H-2',6'); 6.92 (2H, d, *J* = 7.8, H-3',5'); 6.48 (1H, s, H-8); 6.46 (1H, s, H-3); 6.31 (1H, s, H-6); 4.15 (2H, t, *J* = 5.9, 5-OCH_2); 3.82 (3H, s, 4'-OCH_3); 3.80 (3H, s, 7-OCH_3); 3.02 (2H, t, *J* = 5.9, CH_2); 2.71–2.65 (4H, m, 2NCH_2); 1.77–1.73 (4H, m, 2CH_2). ^{13}C NMR spectrum, δ, ppm: 176.4; 162.8; 161.0; 159.6; 159.0; 158.8; 126.6; 122.9; 113.3; 110.4; 106.7; 96.1; 92.1; 67.6; 54.7; 54.5; 53.8; 53.4; 22.6. Mass spectrum (ESI), *m*/*z* (I_{rel}, %): 396 $[M+H]^+$ (100). Found, *m*/*z*: 395.1727 $[M]^+$. $C_{23}H_{25}NO_5$. Calculated, *m*/*z*: 395.1733.

7-Methoxy-2-(4-methoxyphenyl)-5-[2-(piperidin-1-yl)-ethoxy]-4*H*-chromen-4-one (2d). Yield 162 mg (79%),

white solid, mp 138–140°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.74 (2H, d, *J* = 8.3, H-2',6'); 6.93 (2H, d, *J* = 8.3, H-3',5'); 6.49 (1H, s, H-8); 6.47 (1H, s, H-3); 6.31 (1H, s, H-6); 4.17 (2H, t, *J* = 5.9, 5-OCH$_2$); 3.82 (3H, s, 4'-OCH$_3$); 3.80 (3H, s, 7-OCH$_3$); 2.94 (2H, t, *J* = 5.8, CH$_2$); 2.67–2.53 (4H, m, 2NCH$_2$); 1.61–1.55 (4H, m, 2CH$_2$); 1.41 (2H, d, *J* = 4.6, CH$_2$). ^{13}C NMR spectrum, δ, ppm: 123.7; 122.8; 122.1; 113.3; 111.5; 108.3; 107.4; 106.6; 98.5; 96.1; 95.9; 92.2; 66.2; 56.2; 54.7; 54.5; 54.1; 24.6; 22.9. Mass spectrum (EI), *m*/*z* (*I*$_{rel}$, %): 111 (100), 298 (31).

5-[2-(Dipropylamino)ethoxy]-7-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (2e). Yield 168 mg (79%), yellow solid, mp 91–93°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.74 (2H, d, *J* = 8.7, H-2',6'); 6.92 (2H, d, *J* = 8.7, H-3',5'); 6.47 (2H, s, H-3,8); 6.32 (1H, s, H-6); 4.06 (2H, t, *J* = 6.6, 5-OCH$_2$); 3.82 (3H, s, 4'-OCH$_3$); 3.80 (3H, s, 7-OCH$_3$); 2.98 (2H, t, *J* = 6.6, CH$_2$); 2.53–2.43 (4H, m, 2NCH$_2$); 1.45 (4H, dd, *J* = 15.0, *J* = 7.4, 2CH$_2$); 0.82 (6H, t, *J* = 7.3, 2CH$_3$). ^{13}C NMR spectrum, δ, ppm: 176.4; 162.8; 161.0; 159.6; 159.2; 158.8; 126.6; 122.9; 113.3; 108.4; 106.7; 96.1; 92.1; 67.2; 56.1; 54.7; 54.5; 51.6; 19.3; 10.8. Mass spectrum (ESI), *m*/*z* (*I*$_{rel}$, %): 426 [M+H]$^+$ (100).

7-Methoxy-2-(4-methoxyphenyl)-5-(2-morpholinoethoxy)-4*H*-chromen-4-one (2f). Yield 175 mg (85%), white solid, mp 174–176°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.74 (2H, d, *J* = 8.6, H-2',6'); 6.92 (2H, d, *J* = 8.6, H-3',5'); 6.46 (1H, s, H-8); 6.48 (1H, s, H-3); 6.29 (1H, s, H-6); 4.13 (2H, t, *J* = 5.6, 5-OCH$_2$); 3.82 (3H, s, 4'-OCH$_3$); 3.80 (3H, s, 7-OCH$_3$); 3.73–3.65 (4H, m, O(CH$_2$)$_2$); 2.89 (2H, t, *J* = 5.6, CH$_2$); 2.73–2.58 (4H, m, N(CH$_2$)$_2$). ^{13}C NMR spectrum, δ, ppm: 176.3; 162.8; 161.0; 159.7; 158.8; 158.8; 126.6; 122.8; 113.3; 108.4; 106.6; 96.2; 92.1; 66.8; 66.0; 56.2; 54.7; 54.5; 53.3. Mass spectrum (ESI), *m*/*z* (*I*$_{rel}$, %): 412 [M+H]$^+$ (100). Found, *m*/*z*: 411.1676 [M]$^+$. $C_{23}H_{25}NO_6$. Calculated, *m*/*z*: 411.1682.

7-Methoxy-2-(4-methoxyphenyl)-5-[2-(4-methylpiperazin-1-yl)ethoxy]-4*H*-chromen-4-one (2g). Yield 176 mg (83%), white solid, mp 129–131°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.74 (2H, d, *J* = 8.5, H-2',6'); 6.93 (2H, d, *J* = 8.5, H-3',5'); 6.49 (1H, s, H-8); 6.46 (1H, s, H-3); 6.30 (1H, s, H-6); 4.14 (2H, t, *J* = 5.4, 5-OCH$_2$); 3.83 (3H, s, 4'-OCH$_3$); 3.81 (3H, s, 7-OCH$_3$); 2.95 (2H, t, *J* = 5.3, CH$_2$); 2.83–2.75 (4H, m, 2CH$_2$); 2.63–2.53 (4H, m, 2CH$_2$); 2.33 (3H, s, CH$_3$). ^{13}C NMR spectrum, δ, ppm: 176.4; 162.8; 161.1; 159.8; 158.8; 126.6; 122.8; 113.4; 108.3; 107.9; 106.6; 96.4; 92.3; 66.6; 55.4; 54.7; 54.5; 53.6; 52.0; 44.4. Mass spectrum (ESI), *m*/*z* (*I*$_{rel}$, %): 425 [M+H]$^+$ (100).

5-[2-(4-Hydroxypiperidin-1-yl)ethoxy]-7-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (2h). Yield 174 mg (82%), yellow solid, mp 189–191°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.75 (2H, d, *J* = 7.8, H-2',6'); 6.93 (2H, d, *J* = 7.9, H-3',5'); 6.50 (1H, s, H-8); 6.47 (1H, s, H-3); 6.31 (1H, s, H-6); 4.16 (2H, t, *J* = 5.1, 5-OCH$_2$); 3.83 (3H, s, 4'-OCH$_3$); 3.81 (3H, s, 7-OCH$_3$); 3.70–3.64 (1H, m, CH); 2.97–2.92 (4H, m, 2NCH$_2$); 2.45–2.43 (2H, m, NCH$_2$); 1.97–1.84 (4H, m, 2CH$_2$). ^{13}C NMR spectrum, δ, ppm: 176.5; 162.9; 161.1; 159.8; 158.8; 126.6; 122.9; 113.4; 108.4; 106.6; 96.3; 92.3; 85.4; 66.5; 55.5; 54.7; 54.5; 50.5; 33.1. Mass spectrum (ESI), *m*/*z* (*I*$_{rel}$, %): 426 [M+H]$^+$ (100).

5-[4-(Dimethylamino)butoxy]-7-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (3a). Yield 153 mg (77%), white solid, mp 119–121°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.74 (2H, d, *J* = 8.3, H-2',6'); 6.92 (2H, d, *J* = 8.3, H-3',5'); 6.47 (1H, s, H-8); 6.45 (1H, s, H-3); 6.28 (1H, s, H-6); 3.99 (2H, t, *J* = 6.5, 5-OCH$_2$); 3.82 (3H, s, 4'-OCH$_3$); 3.80 (3H, s, 7-OCH$_3$); 2.62 (2H, t, *J* = 5.6, CH$_2$); 2.35 (6H, s, 2NCH$_3$); 1.94–1.86 (2H, m, CH$_2$); 1.85–1.76 (2H, m, CH$_2$). ^{13}C NMR spectrum, δ, ppm: 176.5; 162.9; 161.0; 159.7; 159.1; 158.8; 126.6; 122.8; 113.3; 108.3; 106.6; 96.0; 91.9; 68.1; 57.8; 54.7; 54.5; 43.7; 25.5; 22.5. Mass spectrum (ESI), *m*/*z* (*I*$_{rel}$, %): 398 [M+H]$^+$ (100).

5-[4-(Diethylamino)butoxy]-7-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (3b). Yield 161 mg (76%), yellow solid, mp 77–80°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.78 (2H, d, *J* = 8.8, H-2',6'); 6.96 (2H, d, *J* = 8.9, H-3',5'); 6.54 (1H, s, H-8); 6.48 (1H, s, H-3); 6.32 (1H, d, *J* = 2.2, H-6); 4.05 (2H, t, *J* = 6.5, 5-OCH$_2$); 3.87 (3H, s, 4'-OCH$_3$); 3.85 (3H, s, 7-OCH$_3$); 2.57 (6H, q, *J* = 7.3, 3NCH$_2$); 1.95–1.90 (2H, m, CH$_2$); 1.73 (2H, dt, *J* = 15.1, *J* = 7.5, CH$_2$); 1.03 (6H, t, *J* = 7.2, 2CH$_3$). ^{13}C NMR spectrum, δ, ppm: 177.4; 163.7; 161.9; 160.4; 160.2; 159.7; 127.5; 123.8; 114.2; 109.3; 107.5; 96.9; 92.7; 69.1; 55.6; 55.4; 52.3; 46.6; 26.9; 23.0; 11.3. Found, *m*/*z*: 425.2197 [M]$^+$. $C_{25}H_{31}NO_5$. Calculated, *m*/*z*: 425.2202.

7-Methoxy-2-(4-methoxyphenyl)-5-[4-(pyrrolidin-1-yl)-butoxy]-4*H*-chromen-4-one (3c). Yield 167 mg (79%), white solid, mp 136–138°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.73 (2H, d, *J* = 8.5, H-2',6'); 6.91 (2H, d, *J* = 8.5, H-3',5'); 6.45 (2H, s, H-8 and H-3); 6.27 (1H, s, H-6); 4.00 (2H, t, *J* = 6.4, 5-OCH$_2$); 3.81 (3H, s, 4'-OCH$_3$); 3.79 (3H, s, 7-OCH$_3$); 2.61–2.49 (6H, m, 3NCH$_2$); 1.94–1.86 (2H, m, CH$_2$); 1.81–1.70 (6H, m, 3CH$_2$). ^{13}C NMR spectrum, δ, ppm: 176.5; 162.8; 160.1; 159.5; 159.2; 158.8; 126.6; 122.9; 113.3; 108.4; 106.6; 95.9; 91.8; 68.2; 54.9; 54.7; 54.5; 53.0; 25.9; 24.1; 22.4. Mass spectrum (EI), *m*/*z* (*I*$_{rel}$, %): 298 (100). Found, *m*/*z*: 423.2040 [M]$^+$. $C_{25}H_{29}NO_5$. Calculated, *m*/*z*: 423.2046.

7-Methoxy-2-(4-methoxyphenyl)-5-[4-(piperidin-1-yl)-butoxy]-4*H*-chromen-4-one (3d). Yield 170 mg (78%), white solid, mp 116–118°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.80 (2H, d, *J* = 8.8, H-2',6'); 6.98 (2H, d, *J* = 8.8, H-3',5'); 6.53 (1H, d, *J* = 2.1, H-8); 6.51 (1H, s, H-3); 6.33 (1H, d, *J* = 2.1, H-6); 4.06 (2H, t, *J* = 5.6, 5-OCH$_2$); 3.88 (3H, s, 4'-OCH$_3$); 3.86 (3H, s, 7-OCH$_3$); 2.75 (2H, t, *J* = 7.2, CH$_2$); 2.72–2.62 (4H, m, 2NCH$_2$); 1.96–1.92 (4H, m, 2CH$_2$); 1.75–1.70 (4H, m, 2CH$_2$); 1.54–1.47 (2H, m, CH$_2$). ^{13}C NMR spectrum, δ, ppm: 177.4; 163.8; 162.0; 160.6; 160.0 (2C); 127.5; 123.7; 114.3; 109.2; 107.4; 96.9; 92.9; 69.1; 58.3, 55.7; 55.4; 53.9; 26.6; 24.7; 23.6; 22.6. Found, *m*/*z*: 437.2197 [M]$^+$. $C_{26}H_{31}NO_5$. Calculated, *m*/*z*: 437.2202.

5-[4-(1*H*-Imidazol-1-yl)butoxy]-7-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (3e). Yield 166 mg (79%), white solid, mp 98–100°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.74 (2H, d, *J* = 8.6, H-2',6'); 7.50 (1H, s, H-2 imidazole); 6.96 (2H, d, *J* = 9.4, H-4,5 imidazole); 6.92 (2H, d, *J* = 8.6, H-3',5'); 6.48 (1H, s, H-8); 6.46 (1H, s, H-3); 6.23 (1H, s, H-6); 4.10 (2H, t, *J* = 7.0, 5-OCH$_2$); 3.97 (2H, t, *J* = 5.7, CH$_2$); 3.82 (3H, s, 4'-OCH$_3$); 3.80 (3H, s, 7-OCH$_3$); 2.10–2.04 (2H, m, CH$_2$); 1.84–1.76 (2H, m, CH$_2$). ^{13}C NMR spectrum, δ, ppm: 177.4; 163.8; 162.1; 160.8; 160.0; 159.8; 137.3; 129.2, 127.6; 123.8; 119; 114.4;

109.3; 107.6; 97.0; 93.0; 68.7; 55.8; 55.5; 46.7; 28.1; 25.8. Mass spectrum (EI), *m*/*z* (I_{rel}, %): 327 (6), 355 (16), 385 (100), 403 (9), 418 (73). Found, *m*/*z*: 420.1680 $[M]^+$. $C_{24}H_{24}N_2O_5$. Calculated, *m*/*z*: 420.1685.

7-Methoxy-2-(4-methoxyphenyl)-5-[4-morpholinobutoxy]-4*H*-chromen-4-one (3f). Yield 169 mg (77%), yellow solid, mp 166–168°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.71 (2H, d, *J* = 7.3, H-2',6'); 6.89 (2H, d, *J* = 7.5, H-3',5'); 6.44 (2H, s, H-3,8); 6.25 (1H, s, H-6); 3.99 (2H, t, *J* = 6.4, 5-OCH_2); 3.77 (3H, s, 4'-OCH_3); 3.80 (3H, s, 7-OCH_3); 3.62 (4H, t, 2OCH_2); 2.36 (6H, t, *J* = 16.3, 3NCH_2); 1.90–1.85 (2H, m, CH_2); 1.74–1.66 (2H, m, CH_2). ^{13}C NMR spectrum, δ, ppm: 176.2; 162.8; 160.8; 159.5; 159.1; 158.5; 126.6; 122.9; 113.0; 108.3; 106.6; 95.9; 91.7; 68.0; 66.0; 57.7; 54.7; 54.2; 52.2; 25.4; 21.0. Found, *m*/*z*: 439.1989 $[M]^+$. $C_{25}H_{29}NO_6$. Calculated, *m*/*z*: 439.1995.

7-Methoxy-2-(4-methoxyphenyl)-5-[4-(4-methylpiperazin-1-yl)butoxy]-4*H*-chromen-4-one (3g). Yield 176 mg (78%), white solid, mp 124–126°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.73 (2H, d, *J* = 8.5, H-2',6'); 6.91 (2H, d, *J* = 8.5, H-3',5'); 6.46 (2H, s, H-3,8); 6.27 (1H, s, H-6); 4.00 (2H, t, *J* = 6.4, 5-OCH_2); 3.82 (3H, s, 4'-OCH_3); 3.80 (3H, s, 7-OCH_3); 2.38 (10H, m, 5NCH_2); 2.20 (3H, s, NCH_3); 1.93–1.83 (2H, m, CH_2); 1.75–1.65 (2H, m, CH_2). ^{13}C NMR spectrum, δ, ppm: 176.4; 162.8; 161.0; 159.5; 159.3; 158.8; 126.6; 122.9; 113.3; 108.4; 106.7; 95.9; 91.7; 68.1; 57.1; 54.7; 54.4; 54.1; 52.1; 45.0; 25.9; 22.3. Mass spectrum (ESI), *m*/*z* (I_{rel}, %): 453 $[M+H]^+$ (100). Found, *m*/*z*: 452.2306 $[M]^+$. $C_{26}H_{32}N_2O_5$. Calculated, *m*/*z*: 452.2311.

5-[4-(4-Hydroxypiperidin-1-yl)butoxy]-7-methoxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one (3h). Yield 174 mg (77%), white solid, mp 182–184°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.80 (2H, d, *J* = 8.9, H-2',6'); 6.98 (2H, d, *J* = 9.0, H-3',5'); 6.53 (1H, s, H-8); 6.52 (1H, d, *J* = 2.3, H-3); 6.34 (1H, d, *J* = 2.3, H-6); 4.07 (2H, t, *J* = 6.5, 5-OCH_2); 3.89 (3H, s, 4'-OCH_3); 3.87 (3H, s, 7-OCH_3); 3.68 (1H, m, *J* = 11.1, *J* = 7.0, C<u>H</u>OH); 2.88–2.74 (2H, m, NCH_2); 2.50–2.37 (2H, m, NCH_2); 2.14 (2H, t, *J* = 10.0, CH_2); 1.97–1.87 (4H, m, 2CH_2); 1.76 (2H, dt, *J* = 14.8, *J* = 7.4, CH_2); 1.58 (2H, qd, *J* = 9.8, *J* = 3.7, CH_2). ^{13}C NMR spectrum, δ, ppm: 177.4; 163.7; 161.9; 160.5; 160.2; 159.7; 127.5; 123.8; 114.2; 109.3; 107.6; 96.9; 92.6; 69.1; 58.0; 55.6; 55.4; 51.0; 34.4; 26.9; 23.4. Mass spectrum (ESI), *m*/*z* (I_{rel}, %): 454 $[M+H]^+$ (100).

Assay for antiproliferative activity. The antiproliferative activity was evaluated by CCK-8 assay against three human cancer cell lines (HeLa, HCC1954, and SK-OV-3).[9] Briefly, cells (5×10^3 per well in a 96-well plate) were treated with different concentrations of compounds **1**, **2a**–**h**, and **3a**–**h** (100, 25, 6.25, 1.56, 0.39, 0.0976, 0.0244, and 0.0061 μM) for 48 h. Then 5% CCK-8 solution was added to each well and incubated under conditions of 90% humidity and 5% CO_2 for another 1–3 h. Color development was quantified photometrically at 450 nm.). An absorbance microplate reader (EL×808, Bio-Rad 680) was used to determine the concentration that killed 50% of cells (IC_{50}). 10 μl of 1% sodium dodecyl sulfate (SDS) (dissolving 0.1 g of SDS in phosphate buffer saline (PBS) to prepare 10 ml of solution) or 10 μl of 0.1 mol/l acid, such as hydrochloric acid was added to stop the color reaction. The data represent the means of at least three separate experiments.

Supplementary information file containing analytical data characterizing the synthesized compounds and dose-response curves is available at the journal website http://link.springer.com/journal/10593.

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