Med Chem Res
DOI 10.1007/s00044-017-1966-y

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antiproliferative properties of novel naringenin derivatives

Su-You Liu[1] · Jiang Zeng[1] · Kun-Jian Peng[2] · Li-Chao Zhang[1] · Li-Jun Liu[2] · Zhi-Yong Luo[2] · Da-You Ma[1]

Received: 1 March 2016 / Accepted: 19 June 2017

Abstract In order to improve the antiproliferative activity of naringenin, a naturally occurred flavonoid in citrus fruits, a series of naringenin derivatives with a tertiary amino side chain were prepared. The antiproliferative activities of these naringenin derivatives were evaluated on four human cancer cell lines, namely, MCF-7, HCT116, Hela, and A549. Compounds **4a**, **9a**, and **10a** exhibited remarkably enhanced growth inhibition activity. Based on the observed results, the structure–activity relationship of these derivatives was discussed.

Keywords Naringenin · Antiproliferative activity · Synthesis · Derivatives

Introduction

Naringenin (NG) is one of the most abundant flavonoids in citrus fruits (Kawaii et al. 1999). It has been shown to exert a variety of biological effects, such as antioxidant, anti-inflammatory, cholesterol-lowing, antitumor, etc (Lee et al. 1999; Chen et al. 2002; Fuhr et al. 1993; Benavente-Garcia et al. 1997; Heo et al. 2004). Owing to its low-toxicity, (Moon et al. 2006) the anticancer activity of NG is especially attractive. Both in vitro and in vivo studies had revealed that NG can effectively inhibit the growth of several types of human tumor cell lines (Kanno et al. 2005; Arul and Subramanian 2013; Sabarinathan et al. 2010). Mechanistic studies indicated that the proapoptotic activity of NG on cancer cell lines may involve the inhibition of PI3K/Akt signaling pathways and caspase-3 activation (Sabarinathan and Vanisree 2013; Bak et al. 2011; Park et al. 2008). However, the antineoplastic activity of NG is not strong enough for clinical application. Furthermore, the clinical potential of NG is seriously restricted by its low oral bioavailability (Semalty et al. 2010; Felgines et al. 2000). Several NG derivatives had been designed in order to improve the anticancer potential (Yoon et al. 2013; Lee et al. 2007; Liu et al. 2013; Kim et al. 2007). For instance, NG derivatives modified at position 7 with bulky substituents exhibited enhanced inhibitory effects on HCT116 human colon cancer cells and Human Lung epithelial carcinoma A549 cells (Yoon et al. 2013; Lee et al. 2007).

In this study, eleven NG derivatives with an aliphatic tertiary amino side chain were synthesized. Their antiproliferative effects were evaluated on four human cancer cell lines, namely MCF-7, HCT116, Hela, and A549. Based on the observed results, the structure–activity relationship of these derivatives was discussed.

Material and methods

Chemistry

All chemicals were of analytical grade and used as received unless otherwise stated. ^{1}H and ^{13}C NMR spectra were

✉ Zhi-Yong Luo
luozhiyong@csu.edu.cn

✉ Da-You Ma
madayou@csu.edu.cn

[1] School of Pharmaceutical Sciences, Central South University, Changsha 410013, China

[2] School of Life Sciences, Central South University, Changsha 410013, China

Published online: 11 July 2017

recorded on a Bruker DRX 400 NMR instrument. MS were recorded on a Qstar mass spectrometer.

*Synthesis of 7-(2-Bromoethoxy)-5-hydroxy-2-(4-hydroxyphenyl)chroman-4-one (**1**)*

A mixture of NG (2.72 g, 10 mmol), potassium carbonate (0.69 g, 5 mmol) and 1,2-dibromoethane (21.6 mL, 250 mmol) in 100 mL DMF was stirred at 50 °C for 3 days. The resultant mixture was cooled to room temperature and filtrated. The filtrate was mixed with 1 L H_2O, and then extracted with ethyl acetate (150 mL × 3). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with Petroleum ether/EtOAc (2:1) to afford **1** (0.95 g, 25%): mp 180.0–181.0 °C. ^{1}H nuclear magnetic resonance (NMR) (400 MHz, dimethylsulfoxide (DMSO)-d_6): δ 2.73 (dd, J = 2.4, 17.2 Hz, 1H, H-3*cis*), 3.33 (m, 1H, H-3*trans*), 3.78 (t, J = 5.2 Hz, 2H, H-2″), 4.38 (t, J = 5.2 Hz, 2H, H-1″), 5.49 (dd, J = 2.4, 14.4 Hz, 1H, H-2), 6.10 (d, J = 1.6 Hz, 1H, H-8), 6.12 (d, J = 1.6 Hz, 1H, H-6), 6.79 (d, J = 8.4 Hz, 2H, H-3′,5′), 7.33 (d, J = 8.4 Hz, 2H, H-2′,6′), 9.61 (s, 1H, 4′-OH), 12.10 (s, 1H, 5-OH).

*Synthesis of 7-(3-chloropropoxy)-5-hydroxy-2-(4-hydroxyphenyl)chroman-4-one (**2**)*

According to same procedure as preparation of **1**, compound **2** was obtained as a pale yellowish solid, yield 58%, mp 96.0–98.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.19–2.25 (m, 2H, H-2″), 2.79 (dd, J = 3.2, 17.2 Hz, 1H, H-3*cis*), 3.09 (dd, J = 10.2, 17.2 Hz, 1H, H-3*trans*), 3.71 (t, J = 6.4 Hz, 2H, H-3″), 4.13 (t, J = 6.0 Hz, 2H, H-1″), 5.36 (dd, J = 2.8, 12.8 Hz, 1H, H-2), 6.04 (d, J = 2.4 Hz, 1H, H-8), 6.07 (d, J = 2.4 Hz, 1H, H-6), 6.87–6.91 (m, 2H, H-3′, 5′), 7.32–7.35 (m, 2H, H-2′, 6′).

*Synthesis of 7-methoxy-5-hydroxy-2-[4-(2-bromoethoxy) phenyl]chroman-4-one (**7**)*

To a solution of 7-methoxy-5-hydroxy-2-(4-hydroxyphenyl) chroman-4-one (4.29 g, 15 mmol) in 150 mL acetone, potassium carbonate (2.07 g, 15 mmol) and 1,2-dibromoethane (56.4 g, 0.3 mol) was added successively. The resulting mixture was stirred at 80 °C for 60 h. Acetone was removed by rotavaper. 100 mL ethyl acetate was added to the residue, and washed by H_2O (20 mL × 3). The organic solution was concentrated. The residue was purified with a silica gel column and was eluted with Petroleum ether/EtOAc 5:1 to afford **7** (1.14 g, 19%): mp 125.5–126.7 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.79 (dd, J = 2.4, 16.4 Hz, 1H, H-3*cis*), 3.09 (dd, J = 13.6, 16.4 Hz, 1H, H-3*trans*), 3.66 (t, J = 6.0 Hz, 2H, H-2″), 3.81(s, 3H, OCH_3), 4.32 (t, J = 6.0 Hz, 2H, H-1″), 5.37 (dd, J = 12.4, 14.4 Hz, 1H, H-2), 6.05 (d, J = 2.4 Hz, 1H, H-8), 6.08 (d, J = 2.4 Hz, 1H, H-6), 6.97 (d, J = 8.0 Hz, 2H, H-3′, 5′), 7.39 (d, J = 8.0 Hz, 2H, H-2′, 6′).

*Synthesis of 7-methoxy-5-hydroxy-2-[4-(2-choloropropoxy) phenyl]chroman-4-one (**8**)*

According to same procedure as preparation of **7**, Compound **8** was obtained as a white solid, yield 24%: mp 88.0–90.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$):δ 2.22–2.28 (m, 2H, H-2″), 2.79 (dd, J = 3.2, 17.2 Hz, 1H, H-3*cis*), 3.10 (dd, J = 13.2, 17.2 Hz, 1H, H-3*trans*), 3.78 (t, J = 6.4 Hz, 2H, H-3″), 3.81 (s, 3H, OCH_3), 4.14 (t, J = 6 Hz, 2H, H-1″), 5.37 (dd, J = 3.2, 13.2 Hz, 1H, H-2), 6.04 (d, J = 2.4 Hz, 1H, H-8), 6.07 (d, J = 2.4 Hz, 1H, H-6), 6.95–6.97 (m, 2H, H-3′, 5′), 7.37–7.39 (m, 2H, H-2′, 6′).

*General procedure for the preparation of **3a–3d**, **4a–4c**, **9a**, **9b**, **10a**, **10b***

To a solution of the halide compound (**1**, **2**, **7** or **8**, 1 mmol) in 5 mL DMF, a secondary amine (5 mmol) was added. The resulting solution was stirred at 80 °C for 70 min, then cooled to room temperature. The solution was diluted with 30 mL H_2O and extracted with ethyl acetate (10 mL × 3). The combined organic phase was concentrated and the resulting residue was purified on a silica gel column eluted with Petroleum ether/EtOAc to afford the substituted product.

*7-(2-Diethylaminoethoxy)-5-hydroxy-2-(4-hydroxyphenyl) chroman-4-one (**3a**)*

White solid, yield 62%, R_f = 0.35 (MeOH/CH_2Cl_2 = 1:10, v/v), mp 121.0–123.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.95 (t, J = 6.8 Hz, 6H, H-2‴), 2.49–2.54 (tetra, J = 6.8 Hz, 4H, H-1‴), 2.70–2.75 (m, 3H, H-3*cis*), 3.33–3.35 (m, 1H, H-3*trans*), 4.06 (t, J = 6.4 Hz, 2H, H-1″), 5.48 (dd, J = 2.8, 12.8 Hz, 1H, H-2), 6.06 (d, J = 2.0 Hz, 1H, H-8), 6.08 (d, J = 2.0 Hz, 1H, H-6), 6.79 (d, J = 8.8 Hz, 2H, H-3′, 5′), 7.32 (d, J = 8.8 Hz, 2H, H-2′, 6′), 9.60 (s, 1H, 4′-OH), 12.10 (s, 1H, 5-OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 12.1 (C-2‴), 42.3 (C-3), 47.1 (C-1‴), 51.2 (C-2″), 67.3 (C-1″), 78.8 (C-2), 94.4 (C-8), 95.2 (C-6), 102.8 (C-4a), 115.4 (C-3′, 5′), 128.6 (C-2′,6′), 128.9 (C-1′), 158.0 (C-4′), 163.1 (C-8b), 163.4 (C-5), 166.9 (C-7), 197.1 (C=O). IR (KBr, cm^{-1}) V_{max}: 3421.4, 3122.3, 2970.9, 1634.9, 1568.9, 1514.6, 1398.1, 1161.2, 1087.4, 838.8. HRMS (ESI) m/z $[M+H]^+$ calcd. for $C_{21}H_{26}O_5N$, 372.1811; found 372.1808.

*7-(2-Morpholinoethoxy)-5-hydroxy-2-(4-hydroxyphenyl) chroman-4-one (**3b**)*

White solid, yield 90%, $R_f = 0.45$ (ethyl acetate/petroleum ether = 2:1, v/v), mp 192.5–193.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.44 (t, $J = 4.4$ Hz, 4H, H-1‴), 2.66 (t, $J = 6.4$ Hz, 2H, H-2″), 2.74 (dd, $J = 2.8$, 17.2 Hz, 1H, H-3*cis*), 3.34 (dd, $J = 12.8$, 17.2 Hz, 1H, H-3*trans*), 3.56 (t, $J = 4.4$ Hz, 4H, H-2‴), 4.13 (t, $J = 5.6$ Hz, 2H, H-1″), 5.48 (dd, $J = 2.8$, 12.8 Hz, 1H, H-2), 6.08 (d, $J = 2.0$ Hz, 1H, H-8), 6.10 (d, $J = 2.0$ Hz, 1H, H-6), 6.79 (d, $J = 8.8$ Hz, 2H, H-3′, 5′), 7.32 (d, $J = 8.8$ Hz, 2H, H-2′, 6′), 9.60 (s, 1H, 4′-OH), 12.10 (s, 1H, 5-OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 42.3 (C-3), 53.8 (C-1‴), 56.9 (C-2″), 66.3 (H-2‴), 66.5 (C-1″), 78.9 (C-2), 94.5 (C-8), 95.4 (C-6), 102.9 (C-4a), 115.5 (C-3′, 5′), 128.7 (C-2′, 6′), 129.0 (C-1′), 158.1 (C-4′), 163.2 (C-8b), 163.5 (C-5), 166.9 (C-7), 197.3 (C=O). IR (KBr, cm^{-1}) V_{max}: 3440.8, 2959.2, 2924.3, 2862.14, 1643.9, 1522.3, 1440.8, 1306.8, 1172.5, 1115.1, 831.7, 590.3. HRMS (ESI) *m/z* $[M + H]^+$ calcd. for $C_{21}H_{24}O_6N$, 386.1604; found 386.1603.

*7-(2-(Piperidin-1-yl)ethoxy)-5-hydroxy-2-(4-hydroxyphenyl)chroman-4-one (**3c**)*

White solid, yield 71%, $R_f = 0.35$ (MeOH/CH_2Cl_2 = 1:10, v/v), mp 168.0–170.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.35–1.39 (m, 2H, H-3‴), 1.41–1.49 (m, 4H, H-2‴, 4‴), 2.39 (t, $J = 2.0$ Hz, 4H, H-1‴, 5‴), 2.61 (t, $J = 6.0$ Hz, 2H, H-2″), 2.73 (dd, $J = 3.2$, 17.2 Hz, 1H, H-3*cis*), 3.33 (dd, $J = 12.8$, 17.2 Hz, 1H, H-3*trans*), 4.11 (t, $J = 5.6$ Hz, 2H, H-1″), 5.47 (dd, $J = 2.8$, 12.8 Hz, 1H, H-2), 6.07 (d, $J = 2.4$ Hz, 1H, H-8), 6.09 (d, $J = 2.4$ Hz, 1H, H-6), 6.79 (d, $J = 8.8$ Hz, 2H, H-3′, 5′), 7.32 (d, $J = 8.8$ Hz, 2H, H-2′, 6′), 9.61 (s, 1H, 4′-OH), 12.10 (s, 1H, 5-OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 23.9 (C-3‴), 25.5 (C-2‴, 4‴), 42.0 (C-3‴, 5‴), 54.3 (C-1‴), 57.0 (C-2″), 66.3 (C-1″), 78.6 (C-2), 94.2 (C-8), 95.1 (C-6), 102.5 (C-4a), 115.1 (C-3′, 5′), 128.3 (C-2′, 6′), 128.7 (C-1′), 157.7 (C-4′), 162.8 (C-8b), 163.2 (C-5), 166.6 (C-7), 196.8 (C=O). IR (KBr, cm^{-1}) V_{max}: 3433.0, 3118.5, 2935.9, 1638.8, 1576.7, 1522.3, 1440.8, 1196.1, 1168.9, 1087.4, 834.9. HRMS (ESI) *m/z* $[M + H]^+$ calcd. for $C_{22}H_{26}O_5N$, 384.1811; found 384.1810.

*7-(2-Dimethylanimoethoxy)-5-hydroxy-2-(4-hydroxyphenyl) chroman-4-one (**3d**)*

White solid, yield 30%, $R_f = 0.35$ (MeOH/CH_2Cl_2 = 1:10, v/v), mp 165.0–167.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.16 (s, 6H, NCH_3), 2.73 (dd, $J = 2.4$, 17.2 Hz, 1H, H-3*cis*), 3.30 (dd, $J = 12.8$, 17.2 Hz, 1H, H-3*trans*), 3.32 (t, $J = 5.6$ Hz, 2H, H-2″) 4.08 (t, $J = 5.6$ Hz, 2H), 5.47 (dd, $J = 2.4$, 12.8 Hz, 1H, H-2), 6.07 (d, $J = 2.4$ Hz, 1H, H-8), 6.09 (d, $J = 2.4$ Hz, 1H, H-6), 6.78 (d, $J = 8..4$ Hz, 2H, H-3′,5′), 7.32 (d, $J = 8.4$ Hz, 2H, H-2′,6′), 12.02 (br, 1H, 5-OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 41.9 (C-3), 45.3 (N-CH_3), 57.2 (C-2″), 66.4 (C-1″), 78.5 (C-2), 94.1 (C-8), 94.9 (C-6), 102.5 (C-4a), 115.0 (C-3′, 5′), 128.3 (C-2′, 6′), 128.6 (C-1′), 157.6 (C-4′), 162.8 (C-8b), 163.1 (C-5), 166.6 (C-8), 196.7 (C=O). IR (KBr, cm^{-1}) V_{max}: 34.25.3, 3137.9, 2951.5, 1634.9, 1572.8, 1518.4, 1401.9, 1277.7, 1165.0, 1087.4, 838.8. HRMS (ESI) *m/z* $[M + H]^+$ calcd. for $C_{19}H_{22}O_5N$, 344.1498; found 344.1496.

*7-(3-Diethylaminopropoxy)-5-hydroxy-2-(4-hydroxyphenyl) chroman-4-one (**4a**)*

Pale yellowish oil, 39.0%, $R_f = 0.35$ (MeOH/CH_2Cl_2 = 1:10, v/v),. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.05 (t, $J = 7.2$ Hz, 6H, H-2‴), 1.90-1.96 (m, 2H, H-2″), 2.56–2.65 (m, 6H, H-1‴, H-3″), 2.77 (dd, $J = 2.8$, 17.2 Hz, 1H, H-3*cis*), 3.08 (dd, $J = 12.8$, 17.2 Hz, 1H, H-3*trans*), 4.00 (t, $J = 6.0$ Hz, 2H, H-1″), 5.33 (dd, $J = 2.8$, 12.8 Hz, 1H, H-2), 6.02 (d, $J = 2.4$ Hz, 1H, H-8), 6.05 (d, $J = 2.4$ Hz, 1H, H-6), 6.83–6.86 (m, 2H, H-3′, 5′), 7.29–7.32 (m, 2H, H-2′, 6′), 12.00 (br, 1H, 5-OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.7 (C-2‴), 25.7 (C-2″), 43.0 (C-3), 46.4 (C-1‴), 48.8 (C-3″), 66.6 (C-1″), 79.0 (C-2), 94.4 (C-8), 95.5 (C-6), 103.0 (C-4a), 116.0 (C-3′,5′), 127.8 (C-2′,6′), 129.3 (C-1′), 157.3 (C-4′), 162.9 (8b), 164.0 (C-5), 167.2 (C-7), 196.2 (C=O). IR (KBr, cm^{-1}) V_{max}: 3163.6, 2969.6, 2918.6, 1649.1, 1564.0, 1513.0, 1455.1, 1390.4, 1298.5, 1267.9, 1159.0, 1090.9, 832.2, 736.9, 594.0. HRMS (ESI) *m/z* $[M + H]^+$ calcd. for $C_{22}H_{28}O_5N$, 386.1967; found 386.1962.

*7-(3-Morpholinopropoxy)-5-hydroxy-2-(4-hydroxyphenyl) chroman-4-one (**4b**)*

Pale yellowish oil, yield 76%, $R_f = 0.36$ (ethyl acetate/petroleum ether = 2:1, v/v), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.97 (m, 2H, H-2″), 2.52–2.55 (m, 6H, H-1‴, H-3″), 2.75 (dd, $J = 2.8$, 17.2 Hz, 1H, H-3*cis*), 3.07 (dd, $J = 13.2$, 17.2 Hz, 1H, H-3*trans*), 3.75 (t, $J = 4.4$ Hz, 4H, H-2‴), 4.01 (t, $J = 6.4$ Hz, 2H, H-1″), 5.30 (dd, $J = 2.8$, 13.2 Hz, 1H, H-2), 6.00 (d, $J = 2.0$ Hz, 1H, H-8), 6.04 (d, $J = 2.0$ Hz, 1H, H-6), 6.83 (d, $J = 8.4$ Hz, 2H, H-3′, 5′), 7.27 (d, $J = 8.4$ Hz, 2H, H-2′, 6′), 12.00 (br, 1H, 5-OH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 25.7 (C-2″), 43.0 (C-3), 53.5 (C-1‴), 55.2 (C-3″), 66.4 (C-2‴), 66.6 (C-1″), 78.9 (C-2), 94.5 (C-8), 95.4 (C-6), 103.0 (C-4a), 115.7 (C-3′, 5′), 127.9 (C-2′, 6′), 129.7 (C-1′), 156.7 (C-4′), 162.9 (C-8b), 163.9 (C-5), 167.2 (C-7), 196.1 (C=O). IR (KBr, cm^{-1}) V_{max}: 3425.2, 3188.3, 2955.3, 2850.5, 2807.8, 1646.6, 1572.8, 1518.4, 1440.8, 1301.0, 1165.0, 1095.2, 834.9. HRMS (ESI) *m/z* $[M + H]^+$ calcd. for $C_{22}H_{26}O_6N$, 400.1760; found 400.1757.

*7-(3-(Piperidin-1-yl)propoxy)-5-hydroxy-2-(4-hydroxyphenyl)chroman-4-one (**4c**)*

Pale yellowish oil, yield 81%, $R_f = 0.35$ (MeOH/CH_2Cl_2 = 1:10, v/v), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.47 (br, 2H, H-3‴), 1.62–1.68 (m, 4H, H-2‴), 1.96–2.03 (m, 2H, H-2″), 2.52–2.55 (m, 6H, H-1‴, 3″), 2.75 (dd, $J = 2.8$, 17.2 Hz, 1H, H-3*cis*), 3.07 (dd, $J = 13.2$, 17.2 Hz, 1H, H-3*trans*), 3.98 (t, $J = 6.0$ Hz, 2H, H-1″), 5.31 (dd, $J = 2.8$, 13.2 Hz, 1H, H-2), 5.98 (d, $J = 2.0$ Hz, 1H, H-8), 6.03 (d, $J = 2.0$ Hz, 1H, H-6), 6.82 (d, $J = 7.2$ Hz, 2H, H-3′, 5′), 7.27 (d, $J = 7.2$ Hz, 2H, H-2′, 6′), 12.01 (br, 1H, 5-OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.9 (C-3‴), 25.1 (C-2‴), 25.8 (C-2″), 43.0 (C-3), 54.3 (C-1‴), 55.6 (C-3″), 66.6 (C-1″), 79.0 (C-2), 94.4 (C-8), 95.5 (C-6), 103.0 (C-4a), 115.9 (C-3′,5′), 127.8 (C-2′,6′), 129.3 (C-1′), 157.3 (C4′), 162.9 (C-8b), 163.9 (C-5), 167.1 (C-7), 196.2 (C=O). IR (KBr, cm^{-1}) V_{max}: 3440.1, 3123.3, 2927.5, 2856.3, 2799.4, 1642.4, 1571.2, 1514.2, 1439.5, 1297.1, 1158.2, 1087.1, 837.9. HRMS (ESI) *m/z* $[M + H]^+$ calcd. for $C_{23}H_{28}O_5N$, 398.1967; found 398.1964.

*5-Hydroxy-7-methoxy-2-(4-(2-diethylaminoethoxy)phenyl) chroman-4-one (**9a**)*

Pale yellowish oil, 72%, $R_f = 0.45$ (MeOH/CH_2Cl_2 = 1:10, v/v), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.08 (t, $J = 7.2$ Hz, 6H, H-2‴), 2.63-2.69 (m, 4H, H-1‴), 2.78 (dd, $J = 2.8$, 17.2 Hz, 1H, H-3*cis*), 2.90 (t, $J = 6.4$ Hz, 2H, H-2″), 3.10 (dd, $J = 12.8$, 17.2 Hz, 1H, H-3*trans*), 3.80 (s, 3H, OCH_3), 4.08 (t, $J = 6.4$ Hz, 2H, H-1″), 5.36 (dd, $J = 2.8$, 12.8 Hz, 1H, H-2), 6.04 (d, $J = 2.0$ Hz, 1H, H-8), 6.06 (d, $J = 2.0$ Hz, 1H, H-6), 6.95 (d, $J = 8.8$ Hz, 2H, H-3′,5′), 7.36 (d, $J = 8.8$ Hz, 2H, H-2′,6′), 12.03 (s, 1H, 5-OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.8 (C-2‴), 43.1 (C-3), 47.8 (C-1‴), 51.7 (C-2″), 55.6 (OCH_3), 66.7 (C-1′), 79.0 (C-2), 94.1 (C-8), 95.0 (C-6), 103.1 (C-4a), 114.8 (C-3′, 5′), 127.6 (C-2′, 6′), 130.3 (C-1′), 159.3 (C-4′), 162.9 (C-8b), 164.1 (C-5), 167.9 (C-7), 196.0 (C=O). IR (KBr, cm^{-1}) V_{max}: 2970.9, 1642.7, 1572.8, 1518.4, 1444.7, 1374.8, 1293.2, 1250.5, 1153.4, 1091.3, 1025.2, 827.2, 737.9. HRMS (ESI) *m/z* $[M + H]^+$ calcd. for $C_{22}H_{28}O_5N$, 386.1967; found 386.1961.

*5-Hydroxy-7-methoxy-2-(4-(2-morpholinoethoxy)phenyl) chroman-4-one (**9b**)*

White solid, yield 82%, $R_f = 0.40$ (ethyl acetate/petroleum ether = 2:1, v/v), mp 101.6–102.7 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.60 (br, 4H, H-1‴), 2.78 (dd, $J = 3.2$, 17.2 Hz, 1H, H-3*cis*), 2.83 (t, $J = 5.6$ Hz, 2H, H-2″), 3.08 (dd, $J = 13.2$, 17.2 Hz, 1H, H-3*trans*), 3.75 (t, $J = 4.4$ Hz, 4H, H-2‴), 3.80 (s, 3H, OCH_3), 4.15 (t, $J = 5.6$ Hz, 2H, H-1″), 5.36 (dd, $J = 3.2$, 13.2 Hz, 1H, H-2), 6.04 (d, $J = 2.0$ Hz, 1H, H-8), 6.07 (d, $J = 2.0$ Hz, 1H, H-6), 6.94–6.98 (m, 2H, H-3′, 5′), 7.36–7.39 (m, 2H, H-2′, 6′), 12.03 (s, 1H, 5-OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 43.1 (C-3), 54.0 (C-1‴), 55.6 (OCH_3), 57.5 (C-2″), 65.8 (C-2‴), 66.8 (C-1″), 78.9 (C-2), 94.1 (C-8), 95.0 (C-6), 103.0 (C-4a), 114.8 (C-3′,5′), 127.7 (C-2′,6′), 130.5 (C-1′), 159.1 (C-4′), 162.8 (8b), 164.0 (C-5), 167.8 (C-7), 195.9 (C=O). IR (KBr, cm^{-1}) V_{max}: 3436.9, 3126.2, 2951.5, 2869.9, 2800.0, 1627.2, 1572.8, 1514.6, 1301.0, 1207.8, 1153.4, 1114.6, 1087.4. HRMS (ESI) *m/z* $[M + H]^+$ calcd. for $C_{22}H_{26}O_6N$, 400.1760; found 400.1758.

*5-Hydroxy-7-methoxy-2-(4-(3-diethylaminopropoxy)phenyl) chroman-4-one (**10a**)*

Pale yellowish oil, yield 45%, $R_f = 0.45$ (MeOH/CH_2Cl_2 = 1:10, v/v), ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.05 (t, $J = 7.2$ Hz, 6H, H-2‴), 1.98 (m, 2H, H-2″), 2.56–2.58 (m, 4H, H-1‴), 2.63 (t, $J = 7.4$ Hz, 2H, H-3″), 2.78 (dd, $J = 3.2$, 17.2 Hz, 1H, H-3*cis*), 3.09 (dd, $J = 13.6$, 17.2 Hz, 1H, H-3*trans*), 3.80 (s, 3H, OCH_3), 4.03 (t, $J = 6.0$ Hz, 2H, H-1″), 5.36 (dd, $J = 2.8$, 12.8 Hz, 1H, H-2), 6.04 (d, $J = 2.4$ Hz, 1H, H-8), 6.07 (d, $J = 2.4$ Hz, 1H, H-6), 6.94 (d, $J = 8.8$ Hz, 2H, H-3′,5′), 7.36 (d, $J = 8.8$ Hz, 2H, H-2′,6′), 12.04 (s, 1H, 5-OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.6 (C-2‴), 26.8 (C-2″), 43.1 (C-3), 46.9 (C-1‴), 49.2 (C-3″), 55.6 (OCH_3), 66.3 (C-1′), 79.0 (C-2), 94.1 (C-8), 95.0 (C-6), 103.0 (C-4a), 114.7 (C-3′,5′), 127.6 (C-2′,6′), 130.1 (C-1′), 159.4 (C-4′), 162.8 (C-8b), 164.0 (C-5), 167.8 (C-7), 196.1 (C=O). IR (KBr, cm^{-1}) V_{max}: 3444.7, 3130.5, 2964.8, 2923.4, 1642.7, 1570.2, 1515.0, 1397.6, 1297.5, 1204.3, 1156.0, 1090.4, 834.9. HRMS (ESI) *m/z* $[M + H]^+$ calcd. for $C_{21}H_{30}O_5N$, 400.2124; found 400.2121.

*5-Hydroxy-7-methoxy-2-(4-(3-morpholinopropoxy)phenyl) chroman-4-one (**10b**)*

White solid, yield 85%, $R_f = 0.40$ (ethyl acetate/petroleum ether = 2:1, v/v), mp 126.9–127.9 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.00 (m, 2H, H-2″), 2.52 (m, 6H, H-1‴, 3″), 2.78 (dd, $J = 2.4$, 17.2 Hz, 1H, H-3*cis*), 3.09 (dd, $J = 13.2$, 17.2 Hz, 1H, H-3*trans*), 3.74 (t, 4H, H-2‴), 3.80 (s, 3H, OCH_3), 4.05 (t, $J = 6.0$ Hz, 2H, H-1″), 5.36 (dd, $J = 2.4$, 12.4 Hz, 1H, H-2), 6.04 (d, $J = 2.0$ Hz, 1H, H-8), 6.06 (d, $J = 2.0$ Hz, 1H, H-6), 6.94 (d, $J = 8.4$ Hz, 2H, H-3′, 5′), 7.36 (d, $J = 8.4$ Hz, 2H, H-2′, 6′), 12.03 (s, 1H, 5-OH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 26.3 (C-2″), 43.1 (C-3), 53.7 (C-1‴), 55.4 (C-3″), 55.6 (OCH_3), 66.2 (C-1″), 66.9 (C-2‴), 78.9 (C-2), 94.2 (C-8), 95.0 (C-6), 103.1 (C-4a), 114.7 (C-3′, 5′), 127.6 (C-2′, 6′), 130.3 (C-1′), 159.4 (C-4′), 162.8 (C-8b), 164.1 (C-5), 167.9 (C-7), 195.9 (C=O). IR (KBr, cm^{-1}) V_{max}: 3433.0, 3126.2, 2955.3, 2846.6, 1631.1, 1580.6, 1522.3, 1382.5, 1273.8, 1207.8, 1157.3, 1087.4, 819.4. HRMS

(ESI) *m/z* $[M + H]^+$ calcd. for $C_{23}H_{28}O_6N$, 414.1917; found 414.1913.

*7-Diethylaminocarbonylmethoxy-5-hydroxy-2-(4-hydroxyphenyl)chroman-4-one (**5**)*

To a solution of NG (272 mg, 1 mmol) in 20 mL acetone, was added *N,N*-diethyl-2-chloro-acetoamide (150 mg, 1 mmol), potassium carbonate (69 mg, 0.5 mmol) and potassium iodide (16 mg, 0.1 mmol). The resulting mixture was stirred at 45 °C for 2 days. 40 mL H_2O was added to the mixture. The precipitates collected by filtration was washed with ethyl acetated to afford 5 (240 mg, 62%) as white solid: $R_f = 0.55$ (ethyl acetate/petroleum ether = 2:1, v/v), mp 229.5–230.7 °C, ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.02 (t, $J = 7.2$ Hz, 3H, CH_{3a}), 1.13 (t, $J = 7.2$ Hz, 3H, CH_{3b}), 2.71 (dd, $J = 2.8$, 17.2 Hz, 1H, H-3*cis*), 3.28 (m, 4H, NCH_2), 3.31 (dd, $J = 12.8$, 17.2 Hz, 1H, H-3*trans*), 4.86 (s, 2H, H-1″), 5.48 (dd, $J = 2.8$, 12.8 Hz, 1H, H-2), 6.04 (d, $J = 2.0$ Hz, 1H, H-8), 6.07 (d, $J = 2.0$ Hz, 1H, H-6), 6.79 (d, $J = 8.0$ Hz, 2H, H-3′,5′), 7.32 (d, $J = 8.0$ Hz, 2H, H-2′,6′), 9.57 (s, 1H, 4′-OH), 12.07 (s, 1H, 5-OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.2 (CH_{3a}), 14.3 (CH_{3b}), 42.3 (NCH_2), 66.2 (C-1″), 78.9 (C-2), 94.6 (C-8), 95.5 (C-6), 102.9 (C-4a), 115.4 (C-3′, 5′), 128.7 (C-2′, 6′), 129.0 (C-1′), 157.9 (C-4′), 163.0 (C-8b), 163.1 (C-5), 165.6 (C-7), 166.6 (2″-C=O), 197.2 (4-C = O). IR (KBr, cm^{-1}) V_{max}: 3231.0, 2974.8, 2932.0, 1634.9, 1576.7, 1518.4, 1448.5, 1161.2, 1141.7, 1087.4, 842.7. HRMS (ESI) *m/z* $[M + H]^+$ calcd. for $C_{21}H_{24}O_6N$, 386.1604; found 386.1607.

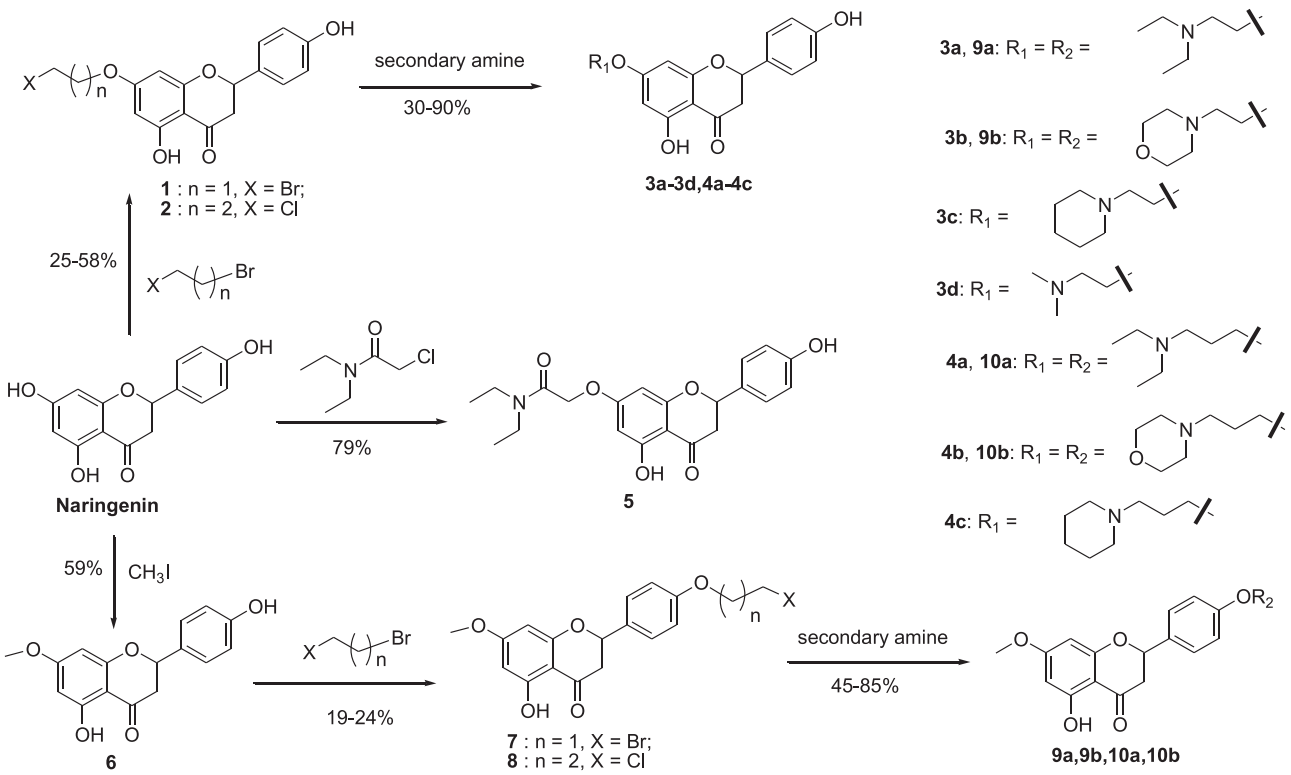
Antiproliferative assay

Cell growth inhibition was analyzed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, cells cultured overnight on 96-well plates were washed twice with PBS, administered media containing different concentrations of NG derivatives and incubated for 48 h. Twenty microliter of MTT stock solution (5 mg/mL) were added to each well, followed by incubation for an additional 4 h. Blue formazans were eluted from cells by the addition of 100 μL of DMSO with gentle shaking for 10 min at room temperature. Absorbencies were measured at 570 nm using an enzyme-linked immunosorbent assay reader. Data were collected from three separate experiments and the percentage of NG derivatives induced cell growth inhibition was determined by comparison to DMSO-treated control cells. Growth inhibition rate was calculated in the following way: GI (%) = $(A_0 - A_1)/A_0$*100, where A_0 is the absorbance of the control, A_1 is the absorbance of tested samples. The GI_{50} values were calculated using SPSS software.

Results and discussion

Tertiary amino group is a common moiety in drugs. Introduction of a tertiary amino group could enhance the hydrophilicity of a chemical entity through the corresponding salt form and in the meanwhile may improve its bioactivity. Here, we attempted to introduce a tertiary amino moiety to NG. As we know, the 7-hydroxy group of NG can be selectively alkylated. As shown in Scheme 1, nucleophilic substitution with 1,2-dibromoethane or 1-bromo-3-chloropropane gave alkylated halo-intermediate **1** and **2** respectively, which were further substituted with a secondary amine to afford tertiary amino group bearing NG derivatives **3a–3d**, **4a–4c**. 7-methylated NG **6** was obtained using methyl iodide as electrophile. Further alkylation with 1,2-dibromoethane or 1-bromo-3-chloropropane give the 4′ substituted halo-intermidiates **7** and **8**, respectively. In a similar manner, tertiary amino group bearing NG derivatives **9a**, **9b**, **10a**, and **10b** were also obtained using a secondary amine. Meanwhile, compound **5**, a NG derivative bearing an amide side chain at 7-hydroxy group was also prepared (Scheme 1).

The antiproliferative activities of the synthesized NG derivatives were evaluated by MTT method on four human cancer cell lines, namely, MCF-7 human breast cancer cells, HCT116 human colon cancer cells, Hela human cervical cancer cell and A549 human Lung epithelial carcinoma cells. The growth inhibitory rate at 100 μM ($GI_{100\mu M}$) and GI_{50} value for each compound on each cell line were collected. As shown in Table 1, NG showed very weak antiproleferative activity to these four cancer cell lines even at 100 μM concentration (entry 13, Table 1). As we expected, the tertiary amino side chain bearing NG dereivatives generally exihibited much stronger growth inhibitory effect on these cell lines than NG did while the amide side chain bearing compound **5** did not show any enhanced activity (entry 1–11 and 12, Table 1). Among these compounds, **10a** exhibited the most potent growth inhibitory activity to the tested cancer cell lines with GI_{50} value of 15.0, 6.0, 8.7 and 11.4 μM to MCF7, HCT116, Hela and A549, respectively (entry 10, Table 1). Further SAR analysis indicated that: (1) the distance between N and O on the side chain has an notable influence on the antiproliferative activity, namely, the compounds with three methlenes between N and O (compounds **4a**, **10a**, entry 5 and 10, Table 1) showed stronger activity than the compounds with two methlenes between N and O (compound **3a**, **9a**, entry 1 and 8, Table 1); (2) a morphlino group is not a good tertiary amino moiety for the side chain, since the morpholino group bearing compounds **3b**, **4b**, **9b**, and **10b** (entry 2, 6, 9, 11, Table 1) showed much higher GI_{50}s than corresponding diethyl amino group bearing compounds **3a**, **4a**, **9a**, and **10a** did (entry 1, 5,8, 10, Table 1).



Scheme 1 Synthesis of naringenin derivatives

Table 1 Antiproliferative activity of NG derivatives on human cancer cell lines, $GI_{100\mu M}$ (%), GI_{50} (μM)

Entry	Compound	MCF-7		HCT116		Hela		A549	
		$GI_{100\ \mu M}$	GI_{50}	$GI_{100\ \mu M}$	GI_{50}	$GI_{100\ \mu M}$	GI_{50}	$GI_{100\ \mu M}$	GI_{50}
1	**3a**	44	>100	93	22.3	94	55.2	92	52.0
2	**3b**	0	>100	55	107.0	0	>100	16	>100
3	**3c**	48	>100	91	35.1	93	63.9	93	48.6
4	**3d**	35	>100	93	43.7	88	66.3	86	87.3
5	**4a**	99	26.5	92	11.0	92	25.1	92	20.6
6	**4b**	19	>100	82	75.7	0	>100	28	>100
7	**4c**	88	44.4	92	12.9	93	28.7	92	25.1
8	**9a**	99	19.7	89	10.5	92	10.0	94	17.7
9	**9b**	11	>100	85	68.8	8	>100	22	>100
10	**10a**	99	15.0	90	6.0	94	8.7	91	11.4
11	**10b**	30	>100	29	>100	11	>100	16	>100
12	**5**	0	>100	19	>100	6	>100	1	>100
13	Naringenin	4	>100	20	>100	0	>100	0	>100

$GI_{100\mu M}$ (%) = Growth inhibition rate at 100 μM; GI_{50} (μM) = Median growth inhibition concentration (μM)

Conclusion

In summary, by attaching a tertiary amino side chain on 7-hydroxy group or 4′-hydroxy group of naringenin, several novel NG derivatives were prepared. Some of these naringenin derivatives exhibited remarkably enhanced antiproliferative activity to four tested human cancer cell lines. The mechanism behind the enhanced sensitivity of these NG derivatives towards cancer cells will be further explored.

Acknowledgements This work was partially supported by the grants from the National Natural Science Foundation of China (No. 20672147, No. 21472247).

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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