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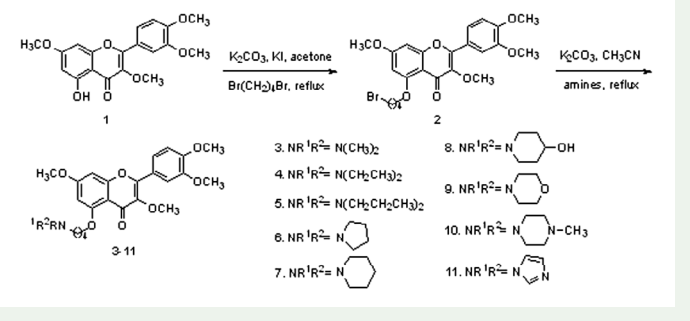
Synthesis and antiproliferative activities of aminoalkylated polymethoxyflavonoid derivatives

Xue-Li Li, Yan-Hua Zhang, Cai-Fang Wang and Qiu-An Wang

College of Chemistry and Chemical Engineering, Hunan University, Changsha, China

ABSTRACT

A series of novel aminoalkylated polymethoxyflavonoid derivatives **3–11** was synthesised from 5-hydroxy-3,7,3′,4′-tetramethoxyflavonoid (**1**) through extending alkoxy chain at the 5-position, and introducing amine hydrogen bond receptor at the end of the side chain. Their antiproliferative activities were evaluated *in vitro* on a panel of three human cancer cell lines (Hela, HCC1954 and SK-OV-3). The results showed that all the target compounds exhibited antiproliferative activities against investigated cancer cells with IC_{50} values of 9.51–53.33 μM. Compounds **5**, **7**, **8**, **11** on Hela cells and compounds **4–9**, **11** on HCC1954 exhibited more potency as compared to positive control *cis*-Platin.



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Polymethoxyflavonoids; aminoalkylated derivatives; synthesis; antiproliferative activity

1. Introduction

Flavonoids are secondary metabolites widely distributed in higher plants that show various biological effects including antioxidative, anti-inflammatory, antiviral, antifungal anticarcinogenic, cardioprotective and neurite outgrowth stimulatory activities (Singh et al. 2014; Chang et al. 2017; Phan et al. 2017). Nevertheless, the most promising bioactive flavonoids such as quercetin, chrisin, kaempferol and apigenin have very low bioavailability, making them largely ineffective *in vivo* (Chen and Chen 2013). In fact, such compounds are

CONTACT Qiu-An Wang Wangqa@hnu.edu.cn
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polyhydroxylated flavonoids (PHFs), and the free hydroxyl groups limit the intestinal absorption and are quickly conjugated by glucuronidation and sulfation (Walle 2007).

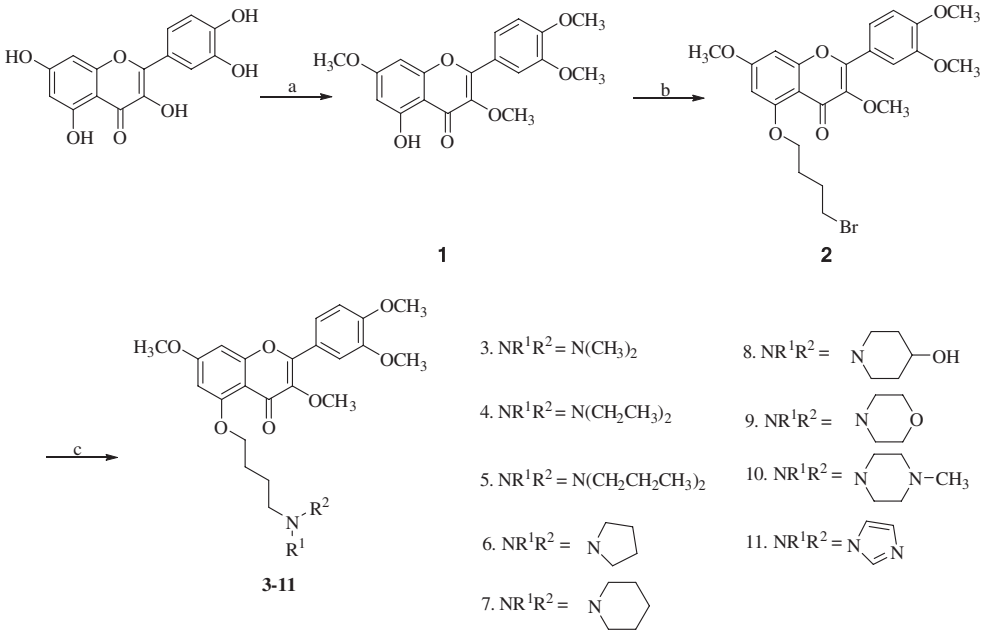
Polymethoxylated flavonoids (PMFs) are readily absorbed in the intestine and show wide tissue distribution and metabolic stability (Ruiu et al. 2015). PMFs may be more biologically active flavonoids compared to their hydroxylated analogues. Some results suggest that methylation of some natural phenolic compounds could improve P-gp modulating activity, and polymethoxy-substituted phenyl ring may be an important pharmacophore for anticancer effect (Yuan et al. 2015). 5-Hydroxy-3,7,3′,4′-tetramethoxyflavonoid (**1**), a polymethoxyflavonoid, occurred in sweet orange (*Citrus sinensis*) peel, can be employed as safe and effective modulators of BCRP-mediated drug resistance in cancer (Li et al. 2006). Our previous studies also suggested that the polymethoxy flavonoids possess significantly enhanced cytotoxic activity (Nguyen et al. 2017).

The introduction of the aminoalkyl group to the structure of compounds is a general strategy for improving bioavailability and water solubility (Bonesi et al. 2008). The results obtained in several classes of polyaromatic antitumour agent indicate that the introduction of an aminoalkyl side chain can increase significantly the biological activity and the potency of the parent compounds (Fu et al. 2012). These findings encourage us to investigate whether the flavonoid skeletons which possess aminoalkyl side chain can be improved antiproliferative activity. In the present work, we aimed to synthesise a novel series of methylated quercetin derivatives through extending alkoxy chain at the 5-position, and introducing amine hydrogen bond receptor at the end of the side chain. Furthermore, their antiproliferative activities were evaluated *in vitro* on a panel of three human cancer cell lines Hela (cervical carcinoma), HCC1954 (breast cancer) and SK-OV-3 (ovarian cancer) using CCK-8 assay.

2. Results and discussion

The novel aminoalkylated polymethoxyflavonoid derivatives **3–11** were synthesised according to the synthetic route shown in Scheme 1. Regiospecific methoxylation of quercetin with dimethylsulfate and K_2CO_3 provides the key intermediate 5-hydroxy-3,7,3′,4′-tetramethoxy flavonoid (**1**), because of the strong intramolecular H-bond at hydroxyl of C-5 with the adjacent carbonyl at C-4, quercetin was readily prepared from the commercial material rutin by acid hydrolysis of rutinose unit as previously described (Nguyen et al. 2015). The bromoderivative **2,** which was obtained in simple one-step alkylation of **1** with 1,4-dibromobutane refluxed in K_2CO_3 and acetone, was further reacted with corresponding amines in the presence of K_2CO_3 in CH_3 CN to provide the final aminoalkylated polymethoxyflavonoid derivatives **3–11**. All new compounds were purified by recrystallisation or chromatography, and their structures were confirmed by the analytical and spectroscopic data.

Antiproliferative activities *in vitro* of all synthesised compounds against three cancer cell lines (Hela, HCC1954 and SK-OV-3) were evaluated based on a CCK-8 assay using *cis*-Platin and paclitaxel as positive controls. Antiproliferative activities of the compounds indicated by IC_{50} values were calculated by linear regression analysis of the concentration–response curves obtained for each compound. The results were summarised in Table 1. Table 1 shows that parent compound 5-hydroxy-3,7,3′,4′-tetramethoxyflavonoid(**1**) did not exhibit any inherent cytotoxicity to investigated cancer cell lines (IC_{50} > 100 μM), while all aminoalkylated



Reagents and conditions : a. K_2CO_3, acetone, NaH, $(CH_3)_2SO_4$, Δ ; b. K_2CO_3, KI, $Br(CH_2)_4Br$, acetone, reflux;

c. K_2CO_3, CH_3CN, amines, reflux

Scheme 1. Synthesis of aminoalkylated polymethoxyflavonoid derivatives **3**–**11.**

Table 1. The antiproliferative activity (IC_{50} in μM) of compounds **1**, **3**–**11** on the human cancer cell lines.

compound	Hela	HCC1954	SK-OV-3
1	>100	>100	>100
3	53.33	43.82	40.90
4	23.05	20.75	28.29
5	9.51	14.63	20.93
6	21.74	18.73	29.43
7	16.56	23.27	32.02
8	12.02	19.84	26.71
9	33.33	24.75	38.37
10	34.43	37.99	43.42
11	14.66	20.61	29.01
cis-Platin[a]	21.30	33.57	12.07
paclitaxel[a]	0.0021	0.0011	0.0017

[a]*cis*-Platin and paclitaxel were employed as positive controls.

polymethoxyflavonoid derivatives showed moderate to potent anticancer activity against tested cell lines with IC_{50} values ranging from 9.51 to 53.33 μM. Therefore, the presence of an aminobutyl group in the flavonoid moiety (compounds **3–11**) resulted to be a key substitution for the activity of compounds against these three cancer cells. Compounds **5**, **7**, **8**, **11** towards Hela Cells and compounds **4–9**, **11** on HCC1954 displayed more potency as compared to positive control *cis*-Platin. Compound **4** revealed to be the most active with IC_{50} values ranging from 9.51 to 20.93 μM against all cancer cell lines.

3. Experimental

3.1. General information

Melting points were measured on a XRC-I apparatus, uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Varian INOVA-400 and Bruker AM-400 instrument, using tetramethylsilane as internal standard, chemical shifts (δ) in ppm, coupling constants (J) in Hz. Mass spectra were determined with ZAB-HS spectrometer. Column chromatography was carried out using 200–300 mesh silica gel (Qingdao Ocean Chemical Products of China). AR or chemical pure reagents and solvents were purchased from commercial sources, and anhydrous solvents were freshly distilled or purified according to standard procedures.

5-Hydroxy-3,7,3′,4′-tetramethoxyflavone (**1**) was prepared from quercetin according to the previous methods (Yuan et al. 2015).

3.2. 5-Bromobutoxy-3,7,3′,4′-tetramethoxyflavone (2)

Anhydrous potassium carbonate (250 mg, 1.81 mmol) and a catalytic amount of KI were added in turn to a solution of 5-hydroxy-3,7,3′,4′-tetramethoxyflavone (0.25 g, 0.70 mmol) in acetonitrile (20 mL). The reaction mixture refluxed for 1 h. Then 1,4-dibromobutane (0.12 mL, 1.44 mmol) was added dropwise. After addition completed, the reaction mixture was refluxed for 22 h. The reaction was monitored by TLC. After cooling to room temperature, the solvent was removed *in vacuo*. The residue was extracted with CH_2Cl_2 (3 × 50 mL). The organic phase was combined and dried over anhydrous sodium sulphate. Then, the solvent was removed *in vacuo* and crude product was purified by silica gel column chromatography (ethyl acetate/petroleum ether, *v/v*, 1:3 as the eluent) to afford white solid **2** in 84% yield. mp 131–133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, J = 1.9 Hz, 1H, 6′-H), 7.63 (s, 1H, 2′-H), 6.91 (d, J = 9.1 Hz, 1H, 5′-H), 6.43 (s, 1H, 8-H), 6.26 (s, 1H, 6-H), 4.03 (dd, J = 11.4, 5.5 Hz, 2H, 5-OCH_2), 3.90 (s, 6H, 3′-OCH_3 and 4′-OCH_3), 3.83 and 3.77 (2s, 6H, 3-OCH_3 and 7-OCH_3), 3.28 (t, J = 6.6 Hz, 2H, CH_2), 2.19–2.08 (m, 2H, CH_2), 2.05–1.95 (m, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 160.7,157.2,155.7,149.7,147.8, 145.7, 138.1, 120.4, 118.6, 108.1, 107.8, 93.6, 89.5, 65.2, 65.0, 56.9,53.0, 52.9, 52.7, 26.8, 26.6, 24.4. MS(ESI): *m/z* 492.1 $[M + H]^+$.

3.3. General procedure for synthesis of aminoalkylated polymethoxy flavonoid derivatives (3–11)

Anhydrous potassium carbonate (0.28 g, 2.05 mmol) was added to a solution of 5-bromobutoxy-3,7,3′,4′-tetramethoxyflavone (0.20 g, 0.41 mmol) in acetonitrile (15 mL). The reaction mixture refluxed for 1 h. Then secondary amines (0.5 mmol) were added dropwise to the reaction mixture and refluxing was continued. Completion of the reaction was monitored by TLC. After cooling to room temperature, the solvent was removed *in vacuo*. The residue was diluted with water and extracted with CH_2Cl_2 (3 × 10 mL). The organic phase was combined and washed with brine (3 × 10 mL), dried over anhydrous Na_2SO_4 and concentrated. The residue was subjected to silica gel column chromatography purification (ethyl acetate/petroleum ether, *v/v*, 2:3 with 3–5 drops of triethylamine as the eluent), to afford white solids **3–11** in 71–94% yield.

3.3.1. 5-(4′-Dimethylamino)butoxy-3,7,3′,4′-tetramethoxyflavone (3)

3 was afforded as a white solid in 77% yield; mp 67–69 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, J = 3.1 Hz, 1H, 6′-H), 7.71 (s, 1H, 2′-H), 6.50 (d, J = 4.5 Hz, 1H, 5′-H), 6.50 (s, 1H, 8-H), 6.34 (s, 1H, 6-H), 4.10 (t, J = 6.5 Hz, 2H, 5-OCH_2), 3.97 (s, 6H, 3′-OCH_3 and 4′-OCH_3), 3.90 and 3.85 (2s, 6H, 3-OCH_3 and 7-OCH_3), 2.43–2.35 (m, 2H, CH_2), 2.26 (s, 6H, CH_3NCH_3), 2.00–1.95 (m, 2H, CH_2), 1.75–1.78 (m, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.0, 160, 159.0, 150.9, 148.9, 141.4, 123.7, 121.8, 111.2, 96.9, 92.6, 69.5, 60.3, 59.5, 56.3, 56.2, 56.0, 45.7, 27.0, 24.3. MS(EI): m/z 457.1 $[M]^+$.

3.3.2. 5-(4′-Diethylamino)butoxy-3,7,3′,4′-tetramethoxyflavone (4)

4 was afforded as a white solid in 71% yield; mp 212–214 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (s, 2H, 6′-H and 2′-H), 6.91 (d, J = 9.0 Hz, 1H, 5′-H), 6.42 (s, 1H, 8-H), 6.26 (s, 1H, 6-H), 4.02 (t, J = 6.5 Hz, 2H, 5-OCH_2), 3.89 (s, 6H, 3′-OCH_3 and 4′-OCH_3), 3.82 and 3.77 (2s, 6H, 3-OCH_3 and 7-OCH_3), 2.52 (dd, J = 13.1, 6.1 Hz, 6H, $N(CH_2)_3$), 1.89 (dd, J = 13.6, 6.7 Hz, 2H, CH_2), 1.70 (d, J = 6.7 Hz, 2H, CH_2), 0.98 (td, J = 7.0, 3.2 Hz, 6H, $2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 163.7, 160.3, 158.7, 152.4, 150.7, 148.6, 141.1, 123.4, 121.5, 111.1, 110.7, 96.6, 92.3, 69.2, 59.9, 55.9, 55.7, 52.3, 46.7, 26.9, 23.1, 11.3. MS(ESI): m/z 485.2 $[M + H]^+$.

3.3.3. 5-(4′-Dipropylamino)butoxy-3,7,3′,4′-tetramethoxyflavone (5)

5 was afforded as a white solid in 80% yield; mp 60–62 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (dd, J = 4.2, 2.5 Hz, 2H, 6′-H and 2′-H), 6.92 (d, J = 4.6 Hz, 5′-H), 6.42 (s, 1H, 8-H), 6.28 (s, 1H, 6-H), 4.02 (t, J = 6.6 Hz, 2H, 5-OCH_2), 3.90 (s, 6H, 3′-OCH_3 and 4′-OCH_3), 3.83 and 3.79 (2s, 6H, 3-OCH_3 and 7-OCH_3), 2.48–2.45 (m, 2H, CH_2), 2.37–2.33 (m, 4H, CH_2NCH_2), 1.89 (dd, J = 14.5, 7.0 Hz, 2H, CH_2), 1.68–1.64 (m, 2H, CH_2), 1.40 (d, J = 7.0 Hz, 4H, $2CH_2$), 0.80 (t, J = 7.3 Hz, 6H, $2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3, 164.2, 160.9, 159.2, 151.2, 149.1, 123.9, 122.0, 111.6, 111.2, 110.1, 102.8, 97.1, 92.8, 69.8, 60.4, 56.5, 56.4, 56.2, 54.1, 32.1, 27.4, 23.1, 20.5, 12.4; MS (m/z, EI): 513.2$[M]^+$. HRMS (EI): m/z $[M^+]$ calcd for $C_{29}H_{39}O_7$ N: 513.2721, found: 513.2736.

3.3.4. 5-(4′-(Pyrrolidin-1-yl)butoxy)-3,7,3′,4′-tetramethoxyflavone (6)

6 was afforded as a white solid in 84% yield; mp 160–162 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (dd, J = 8.5, 2.5 Hz, 2H, 6′-H and 2′-H), 6.92 (d, J = 8.5 Hz, 1H, 5′-H), 6.46 (s, 1H, 8-H), 6.26 (s, 1H, 6-H), 4.04 (t, J = 5.3 Hz, 2H, 5-OCH_2), 3.90 (s, 6H, 3′-OCH_3 and 4′-OCH_3), 3.84 and 3.73 (2s, 6H, 3-OCH_3 and 7-OCH_3), 3.55–3.46 (m, 2H, CH_2), 3.02–2.92 (m, 2H, CH_2), 2.23–2.10 (m, 4H, CH_2NCH_2), 1.97 (dd, J = 11.7, 5.7 Hz, 2H, CH_2), 1.79–1.76 (m, 4H, $2CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 163.9, 159.7, 158.7, 152.9, 150.9, 148.7, 140.9, 123.1, 121.7, 111.1, 110.8, 109.3, 96.6, 92.8, 68.9, 59.8, 56.0, 55.9, 55.8, 55.2, 53.4, 25.8, 23.4. MS(ESI): m/z 488.2 $[M + H]^+$.

3.3.5. 5-(4′-(Piperidin-1-yl)butoxy)-3,7,3′,4′-tetramethoxyflavone (7)

7 was afforded as a white solid in 91% yield (recrystallisation from ethyl acetate instead of flash column chromatography purification); mp 111–113 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66–7.60 (m, 2H, 6′-H and 2′-H), 6.90 (d, J = 9.0 Hz, 1H, 5′-H), 6.40 (s, 1H, 8-H), 6.25 (s, 1H, 6-H), 4.01 (t, J = 4.9 Hz, 2H, 5-OCH_2), 3.89 (s, 6H, 3′-OCH_3 and 4′-OCH_3), 3.82 and 3.78 (2s, 6H, 3-OCH_3 and 7-OCH_3), 2.51–2.23 (m, 6H, $N(CH_2)_3$), 1.88 (dd, J = 14.4, 6.8 Hz, 2H, CH_2), 1.71 (dd, J = 14.8, 7.7 Hz, 2H, CH_2), 1.51 (dd, J = 11.0, 5.4 Hz, 4H, $2CH_2$), 1.40–1.32 (m, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 163.7, 160.3, 158.7, 152.4, 150.7, 148.6, 141.0, 123.4, 121.5, 110.9, 109.6, 96.5,

92.3, 69.2, 59.9, 58.8, 55.9, 55.7, 54.4, 26.9, 25.7, 24.3, 23.1. MS (*m/z*, EI): 497.2 $[M]^+$. HRMS (EI): *m/z* $[M^+]$ calcd for $C_{28}H_{35}O_7$ N: 497.2408, found: 497.2390.

3.3.6. 5-(4′-(4-Hydroxypiperidin-1-yl)butoxy)-3,7,3′,4′-tetramethoxyflavone (8)

8 was afforded as a white solid in 89% yield; mp 134–136 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65–7.43 (m, 2H, 6′-H and 2′-H), 7.41 (d, J = 8.7 Hz, 1H, 5′-H), 6.46 (s, 1H, 8-H), 6.28 (s, 1H, 6-H), 6.05 (s, 1H, -CH-OH), 4.04 (t, J = 5.8 Hz, 2H, 5-OCH_2), 3.90 (s, 6H, 3′-OCH_3 and 4′-OCH_3), 3.84 and 3.76 (2s, 6H, 3-OCH_3 and 7-OCH_3), 3.21 (t, J = 6.5 Hz, 2H, CH_2), 2.35–2.22 (m, 4H, CH_2NCH_2), 2.06–2.02 (m, 4H, $2CH_2$), 1.69–1.61 (m, 1H, CH), 1.23–1.15 (m, 2H, CH_2), 0.87–0.56 (m, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.3, 168.3, 164.5, 163.2, 155.3, 145.6, 141.8, 133.8, 126.1, 123.4, 115.6, 115.2, 101.1, 97.1, 73.2, 64.5, 60.5, 60.4, 60.3, 51.2, 32.5, 30.2. MS(ESI): *m/z* 513.2 $[M + H]^+$.

3.3.7. 5-(4′-Morpholinobutoxy)-3,7,3′,4′-tetramethoxyflavone (9)

9 was afforded as a white solid in 87% yield; mp 56–58 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64–7.62 (m, 2H, 6′-H and 2′-H), 6.91 (d, J = 9.0 Hz, 1H, 5′-H), 6.42 (s, 1H, 8-H), 6.26 (s, 1H, 6-H), 4.03 (t, J = 5.2 Hz, 2H, 5-OCH_2), 3.90 (s, 6H, 3′-OCH_3 and 4′-OCH_3), 3.83 and 3.78 (2s, 6H, 3-OCH_3 and 7-OCH_3), 3.64–3.62 (m, 4H, CH_2NCH_2), 2.41–2.38 (m, 4H, CH_2CH_2), 1.92–1.89 (m, 4H, $2CH_2$), 1.76–1.70 (m, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.1, 161.6, 151.9, 149.9, 142.4, 124.6, 122.9, 112.4, 111.9, 97.9, 93.6, 70.3, 68.2, 61.3, 59.8, 57.3, 57.0, 54.9, 28.1, 24.1. MS (*m/z*, EI): 499.2 $[M]^+$. HRMS (EI): *m/z* $[M^+]$ calcd for $C_{27}H_{33}O_8$ N: 499.2201, found: 499.2188.

3.3.8. 5-(4′-(4-methylpiperazin-1-yl)butoxy)-3,7,3′,4′-tetramethoxyflavone (10)

10 was afforded as a white solid in 84% yield; mp 157–159 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71–7.69 (m, 2H, 6′-H and 2′-H), 6.99 (d, J = 4.5 Hz, 1H, 5′-H), 6.49 (s, 1H, 8-H), 6.34 (s, 1H, 6-H), 4.09 (t, J = 6.6 Hz, 2H), 3.97 (s, 6H, 3′-OCH_3 and 4′-OCH_3), 3.90 and 3.85 (2s, 6H, 3-OCH_3 and 7-OCH_3), 2.72–2.36 (m, CH_3 $N(CH_2)_2$), 2.36–2.10 (m, 6H, $N(CH_2)_3$), 2.00–1.93 (m, 2H, CH_2), 1.79–1.72 (m, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 163.0, 159.7, 158.0, 151.8, 149.9, 147.9, 140.4, 122.7, 120.9, 110.4, 109.9, 108.9, 95.9, 91.6, 68.5, 59.3, 57.4, 55.3, 55.0, 54.4, 52.5, 45.4, 26.2, 22.5. MS (*m/z*, EI): 512.2 $[M]^+$. HRMS (EI): *m/z* $[M^+]$ calcd for $C_{28}H_{36}O_7N_2$: 512.2517, found: 512.2525.

3.3.9. 5-(4′-(1H-imidazol-1-yl)butoxy)-3,7,3′,4′-tetramethoxyflavone (11)

11 was afforded as a white solid in 94% yield (recrystallisation from ethyl acetate instead of flash column chromatography purification); mp 132–134 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, J = 7.6 Hz, 2H, 6′-H and 2′-H), 7.54 (s, 1H, NHN), 6.95 (d, J = 9.4 Hz, 2H, NHHN), 6.91 (d, J = 8.7 Hz, 1H, 5′-H), 6.44 (s, 1H, 8-H), 6.22 (s, 1H, 6-H), 4.11 (t, J = 6.8 Hz, 2H, 5-OCH_2), 3.98 (t, J = 5.6 Hz, 2H, CH_2), 3.89 (s, 6H, 3′-OCH_3 and 4′-OCH_3), 3.82 and 3.77 (2s, 6H, 3-OCH_3 and 7-OCH_3), 2.15–2.04 (m, 2H, CH_2), 1.89–1.78 (m, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.7, 163.7, 159.9, 158.7, 150.8, 148.6, 141.1, 137.2, 129.0, 123.2, 121.6, 118.8, 110.9, 109.5, 96.6, 92.6, 68.6, 59.9, 56.0, 55.9, 55.7, 46.7, 27.9, 25.7; MS (*m/z*, EI): 480.2 $[M]^+$. HRMS (EI): *m/z* $[M^+]$ calcd for $C_{26}H_{28}O_7N_2$: 480.1891, found: 480.1897.

3.4. Assay for antiproliferative activity

The antiproliferative activity *in vitro* of compounds **1**, **3–11** was measured using the CCK-8 assay (Song et al. 2015). Hela, HCC1954 and SK-OV-3 cell lines were obtained from the Tumor

Cell Resoures Bank, Chinese Academy of Medical Sciences. Cell counting kit-8 was obtained from Dojindo (Japan).

Hela, HCC1954 and SK-OV-3 (5×10^3 per well in a 96-well plate) were treated with different concentrations of compounds **1**, **3–11** (100, 25, 6.25, 1.56, 0.39, 0.0976, 0.0244, 0.0061 μM) for 48 h. Then, 10% CCK-8 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 450–490 nm by automated Fluorimeter (Novostar, BMG LABTECH, Germany) to determine the concentration that killed 50% of cells (IC_{50}). Data represent the means of at least three separate experiments. The IC_{50} value was defined as the concentration that caused 50% inhibition of cell proliferation.

4. Conclusions

In summary, a series of novel aminoalkylated polymethoxyflavonoid derivatives were synthesised. The antiproliferative activity results showed that all the target compounds exhibited antiproliferative activities against investigated cancer cells with IC_{50} values of 9.51–53.33 μM. The introduction of aminoalkyl group in the flavonoid moiety (compounds **3–11**) resulted to be a key substitution for the activity of polymethoxyflavonoid **1** against these three cancer cells. Compounds **5**, **7**, **8**, **11** on Hela cells and compounds **4–9**, **11** on HCC1954 cells displayed more potency as compared to positive control *cis*-Platin. Compound **5** revealed to be the most active with IC_{50} values ranging from 9.51 to 20.93 μM against all cancer cell lines, and worthy of further development.

Disclosure statement

No potential conflict of interest was reported by the authors.

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