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Synthesis and antiproliferative activity of flavonoid triazolyl glycosides

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Abstract: Sixteen flavonoid triazolyl glycosides **4–19** were synthesized in good yields *via* Cu(I)-catalyzed azide-alkyne cycloadditions of terminal alkynes of flavonoids **1–3** with acetylated sugar azides followed by deacetylation with sodium methoxide in anhydrous methanol. The antiproliferative activity of the synthesized compounds against three human cancer cell lines (Hela, HCC1954 and SK-OV-3) *in vitro* was evaluated. Flavonoids **1**, **2** and flavonoid triazolyl glycosides **7**, **12**, **17** exhibit potent antiproliferative activity against these cancer cell lines.

Keywords: alkyne; antiproliferative activity; azide; cycloaddition; flavonoids; glycosides; 1,2,3-triazole.

Introduction

Flavonoid glycosides exhibit a wide range of biological activities, such as anti-inflammatory, cardioprotective, antitumor, antiviral and enzymatic inhibition properties [1–8]. In order to study the function of glycosylated flavonoids at the molecular level, numerous glycosylation methods have been developed for the assembly of complex flavonoid glycosides [9–12]. However, these traditional approaches often involve laborious synthetic transformations and tremendous protecting group manipulations, which complicate the overall synthesis and decrease the synthetic efficiency. Hence, development of new strategies and tactics in glycosylation reactions is of growing importance.

Towards our goal of designing new flavonoid compounds, we attempted to develop a practical general strategy for the synthesis of triazole-linked flavonoid glycoconjugates by a 'click chemistry' approach. The Cu(I) catalyzed azide-alkyne cycloaddition (CuAAc) can be considered as a typical 'click chemistry', in which a 1,2,3-triazole is formed in high yield and good regioselectivity [13–15]. On the basis of our previous experience in flavonol glycoconjugates as antitumor agents [16–19], in this work we synthesized two series of glucose and maltose glycosyl derivatives. Overall, 16 new flavonoid triazole glycosides were synthesized and tested *in vitro* for their antiproliferative activity against three human cancer cell lines Hela (cervical cancer), HCC1954 (breast cancer) and SK-OV-3 (ovarian cancer).

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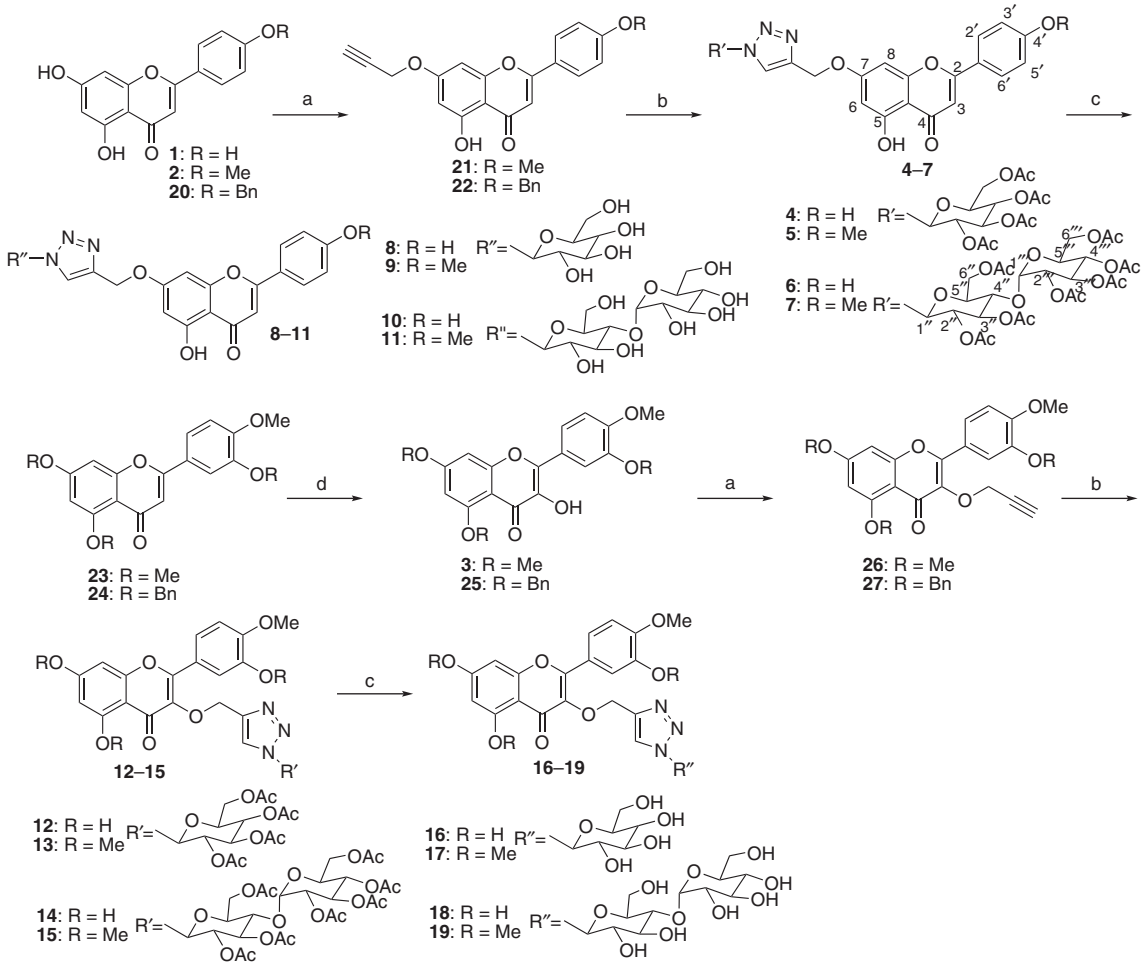
Results and discussion

The flavonoid triazolyl glycosides were synthesized as shown in Scheme 1. According to previous procedures reported by us, the flavonoids apigenin (**1**), acacetin (**2**), 5,7,3′,4′-tetramethoxyflavonol (**3**) and *O*-benzyl or *O*-methylflavonoids **20**, **23**, **24**, **25** were prepared starting with naringin or hesperidin [20]. The synthesis of the target triazolyl glycosides involved the initial preparation of acetylenic flavonoids and acetylated sugar azides. Sugar azides were synthesized from *D*-glycose by adopting the literature procedure [21, 22]. In the next step, an *O*-alkylation reaction was used to obtain the acetylenic flavonoids **21**, **22**, **26** and **27**. Then, the copper-catalyzed CuAAc reaction of sugar azides and flavonoids followed by deacetylation furnished the desired products. The azide-alkyne cycloaddition reactions are regioselective and produce triazolyl glycosides in the β-configuration exclusively. Existence of the β-anomeric form in these sugar derivatives was confirmed by the large axial-axial coupling constant of 8.0–9.2 Hz between the anomeric atoms H(1) and H(2) in the proton nuclear magnetic resonance (^{1}H NMR) spectra of glycosyl derivatives.

Flavonoids **1–3** and 16 novel synthesized flavonoid triazolyl glycosides **4–19** were investigated for their antiproliferative activity employing the Cell Counting Kit-8 (CCK-8) assay using *cis*-platin as a positive control against three human cancer cell lines (Hela, HCC1954 and SK-OV-3). Antiproliferative activities of the compounds indicated by half maximal inhibitory concentration (IC_{50})values were calculated by linear regression analysis of the concentration-response curves obtained for each compound.



Scheme 1 Synthetic routes to flavonoids **1–3** and their triazolyl glycosides **4–19**.
Reagents and conditions: (a) 3-bromo-1-propyne, K_2CO_3, acetone, reflux; (b) acetyl glycosyl azide, $CuSO_4$, ascorbic acid, CH_2Cl_2, $NaHCO_3$, room temperature, then H_2, Pd/C for **4**, **6**, **12** and **14**; (c) CH_3ONa, CH_3OH; (d) oxone, acetone, CH_2Cl_2, Na_2CO_3, $NaHCO_3$.

Table 1 The antiproliferative activity [IC_{50} (μM)][a] of flavonoids and their triazolyl glycosides on human cancer cell lines.

Compounds	Hela	HCC1954	SK-OV-3
1	8.40 ± 0.04	13.40 ± 0.30	42.54 ± 0.92
2	44.51 ± 0.79	39.37 ± 0.46	>100
3–6	>100	>100	>100
7	14.67 ± 0.67	>100	>100
8–11	>100	>100	>100
12	36.67 ± 0.48	30.56 ± 0.46	43.42 ± 1.02
13–16	>100	>100	>100
17	53.33 ± 1.99	39.79 ± 1.21	>100
18, 19	>100	>100	>100
cis-Platin[b]	21.30 ± 0.21	33.57 ± 0.20	12.07 ± 0.77

[a]Data are mean results ± S.D. of three independent experiments.
[b]*cis*-Platin was employed as a positive control.

The results are summarized in Table 1. As can be seen, parent flavonoid **1** is active against all three cancer cells, and flavonoid **2** exhibits antiproliferative activity against Hela and HCC1954 cells. The flavonoid triazolyl glycosides **7**, **12** and **17** exhibit moderate to potent antiproliferative activities. In particular, compound **7** is active against Hela cells and the activity of compound **12** against HCC1954 cells is greater than that of *cis*-platin.

Conclusion

A synthetic route to flavonoid triazolyl glycosides using a 'click chemistry' approach was developed. The flavonoids and flavonoid triazolyl glycosides were evaluated *in vitro* for antiproliferative activity against three human cancer cells (Hela, HCC1954 and SK-OV-3). Most of the compounds have low activity or are inactive compared with the positive control *cis*-platin. However, the flavonoid **1** against Hela (IC_{50} 8.40 μM) and HCC1954 (IC_{50} 13.40 μM) cells, new flavonoid triazolyl glycosides **7** against Hela cells (IC_{50}

14.67 μM) and **12** against HCC1954 cells (IC_{50} 30.56 μM) possess higher activity than *cis*-platin.

Experimental

^{1}H NMR spectra were recorded at 400 MHz on a Bruker AM-400 instrument, using tetramethylsilane as an internal standard. Mass spectra were determined with a ZAB-HS spectrometer operating in the electrospray ionization (ESI) mode. Infrared (IR) spectra were measured on a Bruker Tensor-27 spectrometer. Melting points were measured on a XRC-l apparatus and are uncorrected. Column chromatography was carried out using 200–300 mesh silica gel (Qingdao Ocean Chemical Products of China). Apigenin (**1**), acacetin (**2**), 5,7,3′,4′-tetramethoxyflavonol (**3**), 4′-*O*-benzylapigenin (**20**), 5,7,3′,4′-tetramethoxyluteolin (**23**), 5,7,4′-tribenzyloxydiosmetin (**24**) and 3-hydroxy-4′-methoxy-5,7,3′-tribenzyloxyflavonol (**25**) were prepared from naringin and hesperidin as reported previously [17, 18]. β-*D*-acetyl glucosyl azide and β-*O*-acetyl maltosyl azide were prepared by a minor modification of the procedure published previously [21].

General procedure for the synthesis of flavonoids substituted with a terminal alkyne 21, 22, 26 and 27

A mixture of flavonoid compounds **2**, **3**, **20** or **25** (1.8 mmol) and anhydrous K_2CO_3 (1 g, 7.2 mmol) in acetone (50 mL) was treated dropwise with propargyl bromide (1.5 mL, 2 mmol) and then heated under reflux for 2 h. The progress of the reaction was monitored by thin-layer chromatography (TLC). After cooling to room temperature, the resultant precipitate was filtered, washed with acetone and crystallized from ethyl acetate.

7-*O*-Propargylacacetin (21) Yellow solid; yield 85%; mp 184–186°C; ^{1}H NMR ($CDCl_3$): δ 12.84 (s, 1H, 5-OH), 7.84 (d, 2H, *J* = 9 Hz, 2′-H, 6′-H), 7.03 (d, 2H, *J* = 9 Hz, 3′-H, 5′-H), 6.59 (s, 1H, 3-H), 6.57 (d, 1H, *J* = 2 Hz, 8-H), 6.44 (d, 1H, *J* = 2 Hz, 6-H), 4.78 (d, 2H, *J* = 2 Hz, OC*H*$_2$), 3.9 (s, 3H, 4′-OC*H*$_3$), 2.59 (t, 1H, *J* = 2 Hz, C*H*); ESI-MS: *m/z* 323 $[M+Na]^+$. Anal. Calcd for $C_{19}H_{14}O_5$: C, 70.80; H, 4.38. Found: C, 70.68; H, 4.22.

4′-*O*-Benzyl-7-*O*-propargylapigenin (22) Yellow solid; yield 83%, mp 192–194°C; ^{1}H NMR ($CDCl_3$): δ 12.83 (s, 1H, 5-OH), 7.83 (d, 2H, *J* = 8 Hz, 2′-H, 6′-H), 7.62–7.25 (m, 5H, C$_6$*H*$_5$), 7.07 (d, 2H, *J* = 12 Hz, 3′-H, 5′-H), 6.58 (s, 1H, 3-H), 6.56 (d, 1H, *J* = 2 Hz, 8-H), 6.43 (d, 1H, *J* = 2 Hz, 6-H), 5.15 (s, 2H, C*H*$_2$), 4.76 (d, 2H, *J* = 2 Hz, OC*H*$_2$Ph), 2.59 (t, 1H, *J* = 2 Hz, C*H*); ESI-MS: *m/z* 399 $[M+Na]^+$. Anal. Calcd for $C_{25}H_{18}O_5$: C, 75.37; H, 4.55. Found: C, 75.25; H, 4.49.

3-*O*-Propargyl-5,7,3′,4′-tetramethoxyflavonoid (26) White solid; yield 80%; mp 148–150°C; ^{1}H NMR ($CDCl_3$): δ 7.83 (d, 1H, *J* = 2 Hz, 2′-H), 7.73 (m, 1H, 6′-H), 2.35 (t, 1H, *J* = 2 Hz, C*H*), 3.91, 3.97, 3.96, 3.99 (4s, 12H, 4OC*H*$_3$), 6.98 (d, 1H, *J* = 9 Hz, 5′-H), 6.52 (d, 1H, *J* = 2 Hz, 8-H), 6.36 (d, 1H, *J* = 2 Hz, 6-H), 4.98 (d, 2H, *J* = 2 Hz, C*H*$_2$); ESI-MS: *m/z* 397 $[M+Na]^+$. Anal. Calcd for $C_{22}H_{20}O_7$: C, 66.66; H, 5.09. Found: C, 66.59; H, 4.98.

3-Hydroxy-4′-methoxy-5,7,3′-tribenzyloxyflavonoid (27) White solid; yield 85%; mp 148–150°C; ^{1}H NMR ($CDCl_3$): δ 7.82 (d, 1H, *J* = 2 Hz, 2′-H), 7.73 (dd, 1H, *J* = 2 Hz and 8 Hz, 6′-H), 7.30–7.64 (m, 15H, Ar-H), 7.00 (d, 1H, *J* = 8 Hz, 5′-H), 6.55 (d, 1H, *J* = 2 Hz, 8-H), 6.47 (d, 1H, *J* = 2 Hz, 6-H), 5.27 (s, 2H, 3′-OC*H*$_2$Ph), 5.24 (s, 2H, 7-OC*H*$_2$Ph), 5.11 (s, 2H, 5-OC*H*$_2$Ph), 4.86 (s, 2H, C*H*$_2$), 3.97 (s, 3H, OC*H*$_3$), 2.37 (t, 1H, *J* = 2 Hz, C*H*); ESI-MS: *m/z* 625 $[M+Na]^+$. Anal. Calcd for $C_{40}H_{32}O_7$: C, 76.91; H, 5.16. Found: C, 76.82; H, 5.23.

General procedure for the synthesis of compounds 4–7 and 12–15

A mixture of acetylated sugar azide (1.05 mmol), alkyne (1 mmol), $CuSO_4 \cdot 5H_2O$ (1 N, 0.05 mmol), sodium ascorbate (0.1 mmol), tetrabutylammonium bromide (TBAB, 0.1 mmol), dichloromethane (15 mL) and aqueous $NaHCO_3$ (1 M, 15 mL) was stirred at room temperature until TLC showed no trace of the alkyne. The aqueous layer was washed with dichloromethane, and the combined extract was dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was subjected to silica gel chromatography eluting with EtOAc/petroleum ether, 1:2, to afford the benzyl-protected products **4**, **6**, **12**, **14**. Removal of the benzyl groups by hydrogenation over 10% Pd/C at room temperature for 16 h afforded compounds **5**, **7**, **13**, **15**.

1-[*N*-(2,3,4,6-Tetra-*O*-acetyl-β-*D*-glucosyl)]-4-(7-*O*-methyleneapigenin)-[1,2,3]-triazole (4) Yellow solid; yield 86%; mp 201–202°C; ^{1}H NMR ($CDCl_3$): δ 12.98 (s, 1H, 5-OH), 10.40 (s, 1H, 4′-OH), 7.49 (s, 1H, triazole-H), 7.9 (d, 2H, *J* = 9 Hz, 2′-H, 6′-H), 7.15 (d, 2H, *J* = 9 Hz, 3′-H, 5′-H), 6.54 (s, 2H, 6-H, 8-H), 6.42 (s, 1H, 3-H), 5.76 (d, 1H, *J* = 9 Hz, 1″-H), 5.6 (s, 2H, 6″-C*H*$_2$OAc), 5.36 (s, 2H, OC*H*$_2$), 5.18 (d, 1H, *J* = 3 Hz, 5″-H), 4.20–4.11 (m, 3H, 2″, 3″, 4″-H), 2.32–1.75 (4s, 12H, 4COC*H*$_3$); ESI-MS: *m/z* 704 $[M+Na]^+$. Anal. Calcd for $C_{32}H_{31}N_3O_{14}$: C, 56.39; H, 4.58; N, 6.16. Found: C, 56.43; H, 4.46; N, 6.11.

1-[*N*-(2,3,4,6-Tetra-*O*-acetyl-β-*D*-glucosyl)]-4-(7-*O*-methyleneacacetin)-1,2,3]-triazole (5) Yellow solid; yield 76%; mp 198–200°C; ^{1}H NMR ($CDCl_3$): δ 12.82 (s, 1H, 5-OH), 7.8 (d, 2H, *J* = 8.8 Hz, 2′-H, 6′-H), 7.59 (s, 1H, triazole-H), 7.03 (d, 2H, *J* = 9.2 Hz, 3′-H, 5′-H), 6.58 (s, 2H, 6-H, 8-H), 6.40 (s, 1H, 3-H), 5.80 (d, 1H, *J* = 9 Hz, 1″-H), 5.5 (s, 2H, 6″-C*H*$_2$OAc), 5.29 (s, 2H, OC*H*$_2$), 5.20 (d, 1H, *J* = 3 Hz, 5′-H), 4.20–4.10 (m, 3H, 2″, 3″, 4″-H), 3.89 (s, 3H, 4′-OC*H*$_3$), 2.20–1.80 (4s, 12H, 4C*H*$_3$CO); ESI-MS: *m/z* 718 $[M+Na]^+$. Anal. Calcd for $C_{33}H_{33}N_3O_{14}$: C, 56.98; H, 4.78; N, 6.04. Found: C, 56.83; H, 4.65; N, 6.19.

1-[*N*-(2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-β-*D*-maltosyl)]-4-(7-*O*-methyleneapigenin)-[1,2,3]-triazole (6) Yellow solid; yield 81%; mp 226–228°C; ^{1}H NMR ($CDCl_3$): δ 12.78 (s, 1H, 5-OH), 7.85 (s, 1H, triazole-H), 7.75 (d, 2H, *J* = 8 Hz, 2′-H, 6′-H), 6.95 (d, 2H, *J* = 8.0 Hz, 3′-H, 5′-H), 6.76 (s, 1H, 4′-OH), 6.54–6.52 (m, 2H, 2-H, 6-H), 6.39 (d, 1H, *J* = 2 Hz, 3-H), 5.92 (d, 1H, *J* = 8 Hz, 1‴-H), 5.50–5.45 (m, 2H, 6‴-C*H*$_2$OAc), 5.41–5.30 (m, 2H, 6″-C*H*$_2$OAc), 5.26 (s, 2H, OC*H*$_2$-triazole), 5.08 (t, 1H, *J* = 8 Hz, 1″-H), 4.88 (dd, 1H, *J* = 4 Hz and 10 Hz, 4‴-H), 4.52 (dd, 1H, *J* = 2 Hz and 12 Hz, 4″-H), 4.30–3.97 (m, 6H, sugar-H), 2.13, 2.11, 2.07, 2.04, 2.03, 2.02, 1.85 (s, 3H/each, COC*H*$_3$); ESI-MS: *m/z* 992 $[M+Na]^+$. Anal. Calcd for $C_{44}H_{47}N_3O_{22}$: C, 54.49; H, 4.88; N, 4.33. Found: C, 54.36; H, 4.78; N, 4.25.

1-[*N*-(2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-β-*D*-maltosyl)]-4-(7-*O*-methyleneacacetin)-[1,2,3]-triazole (7) Yellow solids, yield 68%, mp 215–217°C. ^{1}H NMR ($CDCl_3$) δ 12.83 (s, 1H, 5-OH), 7.90 (s, 1H, triazole-H), 7.84 (2H, d, *J* = 8 Hz, 2′-H, 6′-H), 7.02 (d, 2H, *J* = 8 Hz, 3′-H, 5′-H), 6.95–6.58

(m, 2H, 6-H, 8-H), 6.41 (d, 1H, *J*=2 Hz, 3-H), 5.92 (d, 1H, *J*=8 Hz, 1‴-H), 5.48–5.45 (m, 2H, 6‴-C*H*$_2$OAc), 5.41–5.35 (m, 2H, 6″-C*H*$_2$OAc), 5.28 (s, 2H, OC*H*$_2$-triazole), 5.08 (t, 1H, *J*=8 Hz, 1″-H), 4.90–4.87 (dd, 1H, *J*=4 Hz and 10 Hz, 4‴-H), 4.52–4.49 (dd, 1H, *J*=2 Hz and 12 Hz, 4″-H), 4.29–3.98 (m, 8H, sugar-H), 3.90 (s, 3H, 4′-OC*H*$_3$), 2.13, 2.11, 2.07, 2.04, 2.03, 2.02, 1.84 (s, 3H/each, COC*H*$_3$); ESI-MS: *m/z* 1006 [M+Na]$^+$. Anal. Calcd for $C_{45}H_{49}N_3O_{22}$: C, 54.93; H, 5.02; N, 4.27. Found: C, 54.82; H, 5.24; N, 4.25.

1-[*N*-(2,3,4,6-Tetra-*O*-acetyl-β-*D*-glucosyl)]-4-[3-*O*-methylene-(5,7,3′-trihydroxy-4′-methoxyflavonol)]-[1,2,3]-triazole (12) Yellow solid; yield 77%; mp 185–187°C; ^{1}H NMR ($CDCl_3$): δ 12.52 (s, 1H, OH), 8.67 (s, 1H, OH), 7.88 (s, 1H, triazole-H), 7.63 (dd, 2H, *J*=2 x 2 Hz, 6′-H, 2′-H), 7.61–7.57 (m, 1H, 5′-H), 7.54 (s, 1H, 8-H), 6.86 (s, 1H, 6-H), 5.82 (d, 1H, *J*=9 Hz, 1″-H), 5.44–5.34 (m, 2H, 6″-C*H*$_2$OAc), 5.32–5.29 (m, 2H, OC*H*$_2$), 5.27–5.24 (m, 1H, 2″-H), 4.33 (dd, 1H, *J*=2 x 5 Hz, 3″-H), 4.23–4.20 (m, 1H, 4″-H), 4.12–4.09 (m, 1H, 5″-H), 3.95 (s, 3H, 4′-OC*H*$_3$), 2.11, 2.08, 2.03, 1.87 (4s, 12H, 4COC*H*$_3$); ESI-MS: *m/z* 750 [M+Na]$^+$. Anal. Calcd for $C_{33}H_{33}N_3O_{16}$: C, 54.47; H, 4.57; N, 5.77. Found: C, 54.56; H, 4.59; N, 5.88.

1-[*N*-(2,3,4,6-Tetra-*O*-acetyl-β-*D*-glucosyl)]-4-[3-*O*-methylene-(5,7,3′,4′-tetramethoxyflavonol)]-[1,2,3]-triazole (13) Yellow solid; yield 82%; mp 118–120°C; ^{1}H NMR ($CDCl_3$): δ 8.12 (s, 1H, triazole-H) 7.74 (d, 1H, *J*=2 Hz, 6′-H), 7.72 (d, 1H, *J*=2 Hz, 2′-H), 6.98 (d, 1H, *J*=9 Hz, 5′-H), 6.53 (d, 1H, *J*=2 Hz, 8-H), 6.38 (d, 1H, *J*=2 Hz, 6-H), 5.83 (d, 1H, *J*=9 Hz, 1″-H), 5.48–5.38 (m, 2H, 3″-H, 4″-H), 5.28–5.20 (m, 3H, 2″-H, C*H*$_2$), 4.31–4.27 (m, 2H, 6″-C*H*$_2$OAc), 4.16–4.12 (m, 1H, 5″-H), 4.00, 3.95, 3.93, 3.92 (4s, 12H, 4OC*H*$_3$), 2.10, 2.07, 2.03, 1.83, (4s, 12H, 4COC*H*$_3$); ESI-MS: *m/z* 770 [M+Na]$^+$. Anal. Calcd for $C_{36}H_{39}N_3O_{16}$: C, 56.18; H, 5.11; N, 5.46. Found: C, 56.29; H, 5.24; N, 5.56.

1-[*N*-(2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-β-*D*-maltosyl)]-4-[3-*O*-methylene-(5,7,3′-trihydroxy-4′-methoxyflavonol)]-[1,2,3]-triazole (14) Yellow solid; yield 74%; mp 143–144°C; ^{1}H NMR ($CDCl_3$): δ 12.50, 8.90 (s, 2H, 2OH), 7.78 (d, 2H, *J*=10 Hz, 2′, 6′-H), 7.75 (s, 1H, triazole-H), 6.82 (d, 1H, *J*=9 Hz, 5′-H), 6.20 (s, 1H, 8-H), 6.18 (s, 1H, 6-H), 5.74 (d, 1H, *J*=9 Hz, 1″-H), 5.40–5.28 (m, 3H, 2‴-H, 3‴-H, 1‴-H), 5.20 (s, 2H, OC*H*$_2$), 5.15–5.12 (m, 1H, 3″-H), 5.03–5.02 (m, 1H, 2″-H), 4.59–4.57 (m, 2H, 4‴-H, 4″-H), 4.20–4.03 (m, 4H, 6‴-C*H*$_2$OAc, 6″-C*H*$_2$OAc), 4.93–3.94 (m, 2H, 5″-H, 5‴-H), 3.95 (s, 3H, OC*H*$_3$),2.18, 2.17, 2.08, 2.07, 1.99, 1.84, 1.73 (s, 3H/each, COC*H*$_3$); ESI-MS: *m/z* 1038 [M+Na]$^+$. Anal. Calcd for $C_{45}H_{49}N_3O_{24}$: C, 53.20; H, 4.86; N, 4.14. Found: C, 53.35; H, 4.98; N, 4.23.

1-[*N*-(2,3,6,2′,3′,4′,6′-Hepta-*O*-acetyl-β-*D*-maltosyl)]-4-[3-*O*-methylene-(5,7,3′,4′-tetramethoxy flavonol)]-[1,2,3]-triazole (15) Yellow solid; yield 87%; mp 129–132°C; ^{1}H NMR ($CDCl_3$): δ 8.17 (s, 1H, triazole-H), 7.76 (d, 2H, *J*=9 Hz, 2′, 6′-H), 6.99 (d, 1H, *J*=9 Hz, 5′-H), 6.55 (d, 1H, *J*=2.4 Hz, 8-H), 6.40 (d, 1H, *J*=2 Hz, 6-H), 5.89 (d, 1H, *J*=9 Hz, 1″-H), 5.51–5.38 (m, 3H, 2‴-H, 3‴-H, 1‴-H), 5.19 (s, 2H, OC*H*$_2$), 5.13–5.08 (m, 1H, 3″-H), 4.94–4.90 (dd, 1H, *J*=3 Hz and 9 Hz, 2″-H), 4.51–4.47 (m, 1H, 4‴-H), 4.25–4.32 (m, 4H, 6‴-C*H*$_2$OAc, 6″-C*H*$_2$OAc), 4.19–4.07 (m, 3H, 4″-H, 5″-H, 5‴-H), 4.02, 3.98, 3.96, 3.95 (4s, 12H, 4OC*H*$_3$), 2.17, 2.06, 2.04, 1.84 (s, 3H/each, C*H*$_3$CO); ESI-MS: *m/z* 1058 [M+Na]$^+$. Anal. Calcd for $C_{48}H_{55}N_3O_{24}$: C, 54.49; H, 5.24; N, 3.97. Found: C, 54.53; H, 5.36; N, 5.37.

General procedure for the synthesis of compounds 8–11 and 16–19

A solution of compounds **4–7** or **12–15** in $NaOCH_3$ in anhydrous CH_3OH (0.3 M, 3 mL) was stirred for 4–6 h at room temperature, during which time the color of the solution changed to light yellow from dark orange. Then the solution was concentrated *in vacuo* and the residue was purified by silica gel chromatography eluting with EtOAc MeOH, 3:1.

1-(*N*-β-*D*-Glucosyl)-4-(7-*O*-methylene apigenin)-[1,2,3]-triazole (8) Yellow solid; yield 76%; mp 230–232°C; ^{1}H NMR (DMSO-d_6): δ 12.98 (s, 1H, 5-OH), 10.40 (s, 1H, 4′-OH), 8.51 (s, 1H, triazole-H), 7.9 (d, 2H, *J*=9 Hz, 2′-H, 6′-H), 6.95 (s, 1H, 6-H), 6.93 (d, 2H, *J*=2 Hz, 3′-H, 5′-H), 6.86 (s, 1H, 3-H), 6.50 (d, 1H, *J*=2 Hz, 8-H), 5.58 (d, 1H, *J*=5 Hz, 2″-OH), 5.44 (d, 1H, *J*=3 Hz, 3″-OH), 5.30 (d, 1H, *J*=6 Hz, 4″-OH), 5.29 (s, 2H, OC*H*$_2$), 5.17 (d, 1H, *J*=6 Hz, 6″-OH), 4.63 (t, 1H, *J*=6 Hz, 1″-H), 3.7–3.1 (m, 5H, sugar-H); ESI-MS: *m/z* 536 [M+Na]$^+$. Anal. Calcd for $C_{24}H_{23}N_3O_{10}$: C, 56.14; H, 4.52; N, 8.18. Found: C, 56.36; H, 4.68; N, 8.06.

1-(*N*-β-*D*-Glucosyl)-4-(7-*O*-methylene acacetin)-[1,2,3]-triazole (9) Yellow solid; yield 82%; mp 229–230°C, ^{1}H NMR (DMSO-d_6): δ 12.95 (s, 1H, 5-OH), 8.53 (s, 1H, triazole-H), 8.07 (d, 2H, *J*=9 Hz, 2′-H, 6′-H), 7.13 (d, 2H, *J*=9 Hz, 3′-H, 5′-H), 6.98 (s, 1H, 3-H), 5.57 (d, 1H, *J*=9 Hz, 2″-OH), 5.45 (d, 1H, *J*=6 Hz, 3″-OH), 5.33 (d, 1H, *J*=5 Hz, 4″-OH), 5.19 (d, 1H, *J*=5 Hz, 6″-OH), 5.29 (s, 2H, OC*H*$_2$), 3.87 (s, 3H, 4′-OC*H*$_3$), 3.22–3.21 (m, 6H, sugar-H); IR: 3413, 2042, 1637, 1617, 1503, 1400, 1301, 1114, 828, 626 cm^{-1}; ESI-MS: *m/z*) 550 [M+Na]$^+$. Anal. Calcd for $C_{25}H_{25}N_3O_{10}$: C, 56.92; H, 4.78; N, 7.97. Found: C, 56.79; H, 4.83; N, 8.06.

1-(*N*-β-*D*-Maltosyl)-4-(7-*O*-methyleneapigenin)-[1,2,3]-triazole (10) Yellow solid; yield 73%; mp 194–196°C; ^{1}H NMR (DMSO-d_6): δ 12.99 (s, 1H, 5-OH), 10.41 (s, 1H, 4′-OH), 8.53 (s, 1H, triazole-H), 7.96 (d, 2H, *J*=8 Hz, 2′-H, 6′-H), 6.95–6.93 (m, 3H, 3′-H, 5′-H and 8-H), 6.87 (s, 1H, 6-H), 6.51 (d, 1H, *J*=2 Hz, 3-H), 5.77 (d, 1H, *J*=4 Hz, 6‴-OH), 5.66 (d, 2H, *J*=8 Hz, 6″-OH, 6‴-OH), 5.60 (d, 1H, *J*=4 Hz), 5.55 (d, 1H, *J*=4 Hz, 3″-OH), 5.30 (s, 2H, OC*H*$_2$-triazole), 5.08 (d, 1H, *J*=4 Hz, 4‴-OH), 4.93 (d, 2H, *J*=4 Hz, 2″-OH, 2‴-OH), 4.62 (t, 1H, *J*=6 Hz, 1‴-H), 4.54 (t, 1H, *J*=4 Hz, 1″-H), 3.87 (dd, 1H, *J*=8 Hz and 15 Hz, 4‴-H), 3.75–3.39 (m, 11H, sugar-H), 3.30–3.25 (dd, 1H, *J*=6 Hz and 10 Hz, 3‴-OH), 3.12–3.06 (dd, 1H, *J*=9 Hz and 14 Hz, 3″-OH); IR: 486, 592, 615, 704, 773, 883, 907, 1038, 1056, 1099, 1127, 1175, 1209, 1254, 1275, 1304, 1400, 1447, 1505, 1603, 1637, 1662, 2039, 3231, 3412 cm^{-1}; ESI-MS: *m/z* 698 [M+Na]$^+$. Anal. Calcd for $C_{30}H_{33}N_3O_{15}$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.47; H, 5.11; N, 6.03.

1-(*N*-β-*D*-Maltosyl)-4-(7-*O*-methyleneacacetin)-[1,2,3]-triazole (11) Yellow solid; yield 77%; mp 198–200°C; ^{1}H NMR (DMSO-d_6): δ 12.93 (s, 1H, 5-OH), 8.53 (s, 1H, triazole-H), 8.07 (d, 2H, *J*=8 Hz, 2′-H, 6′-H), 7.13 (d, 2H, *J*=8 Hz, 3′-H, 5′-H), 6.96 (s, 2H, 6-H, 8-H), 6.51 (s, 1H, 3-H), 5.78 (d, 1H, *J*=4 Hz, 6‴-OH), 5.67–5.61 (m, 3H, 3″-OH, 3‴-OH and 6″-OH), 5.30 (s, 2H, OC*H*$_2$-triazole), 5.08 (d, 1H, *J*=6 Hz, 4‴-OH), 4.95 (m, 2H, 2″-OH, 2‴-OH), 4.63–4.55 (m, 2H, 4″-H, 4‴-H), 3.86 (s, 3H, 4′-OC*H*$_3$), 3.61–3.09 (m, 12H, sugar-H); IR: 481, 616, 768, 830, 907, 1035, 1073, 1119, 1178, 1251, 267, 1301, 1400, 1505, 1617, 1637, 2054, 3412; ESI-MS: *m/z* 712 cm^{-1}; [M+Na]$^+$. Anal. Calcd for $C_{31}H_{35}N_3O_{15}$: C, 53.99; H, 5.12; N, 6.09. Found: C, 54.09; H, 5.23; N, 6.20.

1-(*N*-β-*D*-Glucosyl)-4-[3-*O*-methylene-(5,7,3′-trihydroxy-4′-methoxyflavonol)]-[1,2,3]-triazole (16) Yellow solid; yield 92%; mp 210–212°C; ^{1}H NMR (DMSO-d_6): δ 12.68 (s, 1H, OH), 8.30 (s, 1H, triazole-H), 7.59 (d, 2H, *J*=9 Hz, 2′ -H, 6′-H), 7.04 (d, 1H, *J*=9 Hz, 5′-H), 6.39 (s, 1H, 8-H), 6.18 (s, 1H, 6-H), 5.48 (d, 1H, *J*=9 Hz, 1″-H), 5.46 (s, 1H, 6″-OH), 5.36 (s, 1H, 4″-OH), 5.25 (s, 1H, 3″-OH), 5.12 (s, 2H, OC*H*$_2$), 4.67 (s, 1H, 2″-OH), 4.02 (t, 1H, *J*=6 Hz, 2″-H), 3.84 (s, 3H, OC*H*$_3$), 3.72 (d, 1H, *J*=6 Hz, 5″-H), 3.51–3.47 (m, 3H, 6″-H, 3″-H), 3.46–3.45 (1H, m,

4″-H); ESI-MS: *m/z* 582 $[M+Na]^+$. Anal. Calcd for $C_{25}H_{25}N_3O_{12}$: C, 53.67; H, 4.50; N, 7.51. Found: C, 53.84; H, 4.63; N, 7.42.

1-(*N*-β-*D*-Glucosyl)-4-[3-*O*-methylene-(5,7,3′,4′-tetramethoxyflavonol)]-[1,2,3]-triazole (17) Yellow solid; yield 91%; mp 154–156°C; ^{1}H NMR (DMSO-d_6): δ 8.46 (s, 1H, triazole-H), 7.80 (d, 2H, *J* = 9 Hz, 2′-H, 6′-H), 7.20 (d, 1H, *J* = 9 Hz, 5′-H), 6.94 (d, 1H, *J* = 2 Hz, 8-H), 6.61 (d, 1H, *J* = 2 Hz, 6-H), 5.64 (d, 1H, *J* = 9 Hz, 1″-H), 5.47 (d, 1H, *J* = 6 Hz, 6″-OH), 5.38 (d, 1H, *J* = 4 Hz, 4″-OH), 5.23 (s, 2H, OCH_2), 5.26 (d, 1H, *J* = 5 Hz, 3″-OH), 4.73 (t, 1H, *J* = 5 Hz, 2″-OH), 4.00 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.80–3.78 (m, 2H, 6″-H), 3.56–3.51 (m, 3H, 2″-H, 3″-H, 5″-H), 3.36–3.32 (m, 1H, 4″-H); ESI-MS (*m/z*): 624 $[M+Na]^+$. Anal. Calcd for $C_{28}H_{31}N_3O_{12}$: C, 55.90; H, 5.19; N, 6.99. Found: C, 55.83; H, 5.05; N, 7.11.

1-(*N*-β-*D*-Maltosyl)-4-[3-*O*-methylene-(5,7,3′-trihydroxy-4′-methoxyflavonol)]-[1,2,3]-triazole (18) Yellow solid; yield 87%; mp 202–205°C; ^{1}H NMR (DMSO-d_6): δ 12.47 (s, 1H, OH), 9.49 (s, 1H, OH), 8.32 (s, 1H, triazole-H), 7.62 (m, 1H, 6′-H), 7.47 (d, 1H, *J* = 2 Hz, 2′-H), 7.05 (d, 1H, *J* = 9 Hz, 5′-H), 6.42 (s, 1H, 8-H), 6.21 (s, 1H, 6-H), 5.71 (d, 1H, *J* = 9 Hz, 1″-H), 5.61 (d, 1H, *J* = 6 Hz, 1‴-H), 5.23 (s, 1H, OH), 5.12 (s, 2H, OCH_2), 4.91 (s, 1H, OH), 4.83 (d, 1H, *J* = 4 Hz, OH), 4.71 (s, 2H, 2OH), 4.52 (d, 1H, *J* = 7 Hz, OH), 4.22 (d, 1H, *J* = 7 Hz, OH), 3.87–3.64 (m, 4H, 3″-H, OCH_3), 3.61–3.53 (m, 2H, 6‴-H), 3.51–3.29 (m, 9H, sugar-H); ESI-MS: *m/z* 744 $[M+Na]^+$. Anal. Calcd for $C_{31}H_{35}N_3O_{17}$: C, 51.60; H, 4.89; N, 5.82. Found: C, 51.52; H, 4.78; N, 5.96.

1-(*N*-β-*D*-Maltosyl)-4-[3-*O*-methylene-(5,7,3′,4′-tetramethoxyflavonol)]-[1,2,3]-triazole (19) Yellow solid; yield 84%; mp 186–189°C; ^{1}H NMR (DMSO-d_6): δ 8.39 (s, 1H, triazole-H), 7.71 (m, 2H, 2′-H, 6′-H), 7.10 (d, 1H, *J* = 8 Hz, 5′-H), 6.84 (s, 1H, 8-H), 6.51 (s, 1H, 6-H), 5.78 (s, 1H, OH), 5.55 (d, 2H, *J* = 4 Hz, sugar-H), 5.51 (s, 1H, OH), 5.17–5.08 (m, 3H, 3OH), 4.95 (s, 2H, OCH_2), 4.63 (d, 1H, *J* = 6 Hz, OH), 4.56 (d, 1H, *J* = 2 Hz, OH), 3.90, 3.87, 3.84, 3.75 (4s, 12H, 4OCH_3), 3.73–3.41 (m, 10H, sugar-H), 3.29–3.06 (m, 3H, sugar-H); ESI-MS: *m/z* 786 $[M+Na]^+$. Anal. Calcd for $C_{34}H_{41}N_3O_{17}$: C, 53.47; H, 5.41; N, 5.50. Found: C, 53.33; H, 5.50; N, 5.67.

Assay for antiproliferative activity

Proliferation of Hela, HCC1954 and SK-OV-3 cells was evaluated by the CCK-8 (Dojingdo, Kumamoto, Japan) assay according to the manufacturer's instructions [22]. This assay is based on the cleavage of the tetrazolium salt WST-8 by mitochondrial dehydrogenase in viable cells. Briefly, 1×10^3 cells/well were incubated with 45 μL culture medium in 384-well plates. After being adhered to the well, the cells were treated with 5 μL of tested compounds at different concentrations, and then cultured for 72 h before addition of 5 μL CCK-8 to the culture medium in each well. After a 2 h incubation at 37°C, absorbance at 450 nm of each well was measured (Novostar, BMG LABTECH, Germany). Each experiment was repeated 3 times, and the data represent the mean values of all measurements. The IC_{50} values were calculated using the Graphpad prism 5 software.

Statistical analysis

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

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