SUPPLEMENTARY MATERIAL

Cytotoxic activities of flavonoids from a Traditional Mongolian medicinal herb *Clematis aethusifolia* Turcz.

Yanxi Chang,^{a,†} Ping Zhang,^{a,†} Xuan Zhang,^b Jianping Chen,^a Wolf-Dieter Rausch,^c Gula A,^d Baoquan Bao^{a,*}

^aCollege of Pharmacy, Inner Mongolia Medical University, Hohhot 010000, China;

^bCollege of Basic Medical Sciences, Inner Mongolia Medical University, Hohhot 010000, China; ^cDepartment of Biomedical Sciences, University of Veterinary Medicine, Vienna 1210, Austria; ^dCollege of Traditional Mongolian Medicine, Inner Mongolia Medical University, Hohhot 010000, China.

Abstract: In the course of our search for anti-tumor constituents from the traditional Mongolian medicinal herb *Clematis aethusifolia* Turcz., 11 flavonoids were isolated for the first time from the dried aerial parts of the plant by flash C₁₈ column chromatography, Sephadex LH-20, and reversed-phase preparative HPLC. The planar structures of these flavonoids were established based on 1D and 2D NMR and high-resolution mass spectrometry. Compounds 1, 2, 4, and 5 showed moderate cytotoxicity against a panel of five human solid tumor cell lines, including A-375, a human melanoma cell line; SK-OV-3, a human ovarian cancer cell line; A549, a human lung cancer cell line; HCT-15, a human colorectal adenocarcinoma cell line; and SH-SY5Y, a human neuroblastoma cell line (with IC₅₀ values of 20-70 μM). The obtained cytotoxic apigenin and its derivatives may be useful as standard compounds for the quality control of the crude drug and its preparations.

Keywords: *Clematis aethusifolia*; chemical constituents; cytotoxicity; flavonoids; traditional Mongolian medicine

Table S1. Cytotoxicity of flavonoids from *Clematis aethusifolia* against five human tumor cell lines^a

Compound	A375	SK-OV-3	A549	HCT-15	SH-SY5Y
1	57.4	100.0	100.0	>200.0	20.0
2	>200.0	>200.0	>200.0	200.0	46.9
3	>200.0	>200.0	>200.0	>200.0	100.0
4	100.0	28.5	40.7	100	>200.0
5	>200.0	70.2	>200.0	>200.0	200.0
6	>200.0	>200.0	>200.0	>200.0	>200.0
7	100.0	>200.0	>200.0	>200.0	>200.0
8	>200.0	>200.0	>200.0	>200.0	>200.0
9	>200.0	>200.0	>200.0	>200.0	>200.0
10	>200.0	>200.0	>200.0	>200.0	100.0
11	>200.0	>200.0	>200.0	>200.0	>200.0
Paclitaxel	4.21	4.68	4.89	827.51	26.39

^a Data expressed in IC₅₀ values (compound **1-11**: μ M, Paclitaxel: nM). A-375, human melanoma cell line; SK-OV-3, human ovarian cancer cell line; A549, human lung cancer cell line; HCT-15, human colorectal adenocarcinoma cell line; SH-SY5Y, human neuroblastoma cell line.

Experimental

General Experimental Procedures

1D and 2D NMR spectra were recorded on a Bruker AVANCE III 600 MHz and Bruker AV 500MHz instruments (Billerica, MA, USA). Chemical shifts were reported with reference to the respective residual solvent or deuterated solvent peaks (δ_{H} 2.5 and δ_{C} 39.5 for DMSO-*d*₆; δ_{H} 3.3 and δ_{C} 49.0 for CD₃OD). High-resolution mass spectrometry (HRMS) data were obtained on a Thermo Fisher Scientific Q-Exactive

high-resolution mass instrument with a heated electrospray ionization source, a Quadropole-Orbitrap mass analyzer, and an X-caliber data processing system. Pre-HPLC was performed on a Shimadzu LC-20AT series with RID-10A detector (Kyoto, Japan), and a Shim pack PRC-ODS column (250 × 20 mm i.d., 15 μm, 80 Å).

Plant Materials

Clematis aethusifolia was collected in Zhenglan Banner of the Inner Mongolian autonomous region in August 2014, and the original plant was identified by Prof. Baoquan Bao. The voucher specimen (No. 20140817) was stored in the College of Pharmacy, Inner Mongolia Medical University, Hohhot, China.

Cell lines

The human melanoma cell line A-375, human ovarian cancer cell line SK-OV-3, human lung cancer cell line A549, and human colorectal adenocarcinoma cell line HCT-15 were obtained from Shanghai Chinese Academy of Sciences (Shanghai, China). The human neuroblastoma cell line SH-SY5Y was purchased from Beijing Chinese Academy of Medical Sciences (Beijing, China). All the cells were maintained in suitable medium supplemented with 10% fetal bovine serum (DMEM for A-375, McCoy's 5A for SK-OV-3, F12K for A549, RPMI-1640 for HCT-15 and SH-SY5Y) and incubated at 37°C with 5% CO₂.

Extraction and Isolation

Dried and powdered aerial parts of *C. aethusifolia* (4.10 kg) were extracted with 95 % ethanol 3 times. The extract was concentrated by reducing the pressure and then dried by vacuum, then suspended in water and partitioned with EtOAc and *n*-BuOH in turn. The EtOAc fraction (23.25 g) was subjected to reversed-phase C₁₈ flash column chromatography with a stepped gradient solvent system of 30 %, 50 %, 80 %, and 95 % MeOH in water to yield 4 fractions. The 80 % MeOH/H₂O fraction (1.68 g) was applied to a reversed-phase preparative HPLC C₁₈ and eluted with 80 % MeOH/H₂O, and compounds **1**, **2**, **3**, and **5** (**1**: 4.31 mg, **2**: 33.4 mg, **3**: 13.99 mg, **5**: 31.80 mg) were obtained. Next, the BuOH fraction (23.05 g) was subjected to reversed-phase C₁₈ flash column chromatography with a stepped gradient solvent system of 10-100 % MeOH in water to collect 10 fractions. The 30 % MeOH/H₂O fraction (1.45 g) was applied to Sephadex LH-20 column chromatography (solvent: 40 % MeOH/H₂O) for purification

and then pre-HPLC C₁₈ elution with 40 % MeOH/H₂O to give compounds **6**, **7**, and **10** (**6**: 1.0 mg, **7**: 2.5 mg, **10**: 0.6 mg). The 40 % MeOH/H₂O fraction (0.99 g) was applied for Sephadex LH-20 column chromatography (solvent: 50 % MeOH/H₂O) for purification, and then use pre-HPLC C₁₈ elution with 55 % MeOH/H₂O to give compounds **4**, **8**, **9**, and **11** (**4**: 1.3 mg, **8**: 10.3 mg, **9**: 1.6 mg, **11**: 5.2 mg).

Compound 1

Yellow amorphous powder. The ¹H-NMR spectrum (CD₃OD, 600 MHz): δ 7.83 (2H, d, J=8.4 Hz, H-2', 6'), 6.92 (2H, d, J=8.4 Hz, H-3', 5'), 6.58 (1H, s, H-3), 6.44 (1H, d, J=1.8 Hz, H-8), 6.20 (1H, d, J=1.8 Hz, H-6). The ¹³C-NMR spectrum (CD₃OD, 150 MHz): δ 166.0 (C-2), 105.3 (C-3), 183.9 (C-4), 159.4 (C-5), 100.1 (C-6), 166.3 (C-7), 95.0 (C-8), 162.8 (C-9), 103.9 (C-10), 123.3 (C-1'), 129.5 (C-2', 6'), 117.0 (C-3', C-5'), 163.2 (C-4').

Compound 2

Yellow amorphous powder. HRMS (m/z): 431.09866 [M-H]⁻, (cal.431.09837, Δ +0.6727ppm) established the molecular formula as C₂₁H₂₀O₁₀. The ¹H-NMR spectrum (DMSO-*d*₆, 600 MHz): δ 7.97 (1H, d, J=8.4 Hz, H-2', 6'), 6.95 (1H, d, J=8.4 Hz, H-3', 5'), 6.89 (1H, s, H-3), 6.84 (1H, d, J=1.8 Hz, H-8), 6.46 (1H, d, J=1.8 Hz, H-6), 5.09 (1H, d, J=7.2 Hz, Glc-H-1''), 3.73 (1H, d, J=10.8 Hz, Glc-H-6''a), 3.50 (1H, dd, J=10.6, 5.4 Hz, Glc-H-6''b), 3.47 (1H, t, J=9.0 Hz, Glc-H-5''), 3.33 (1H, t, J=9.0 Hz, Glc-H-3''), 3.29 (1H, t, J=9.0 Hz, Glc-H-2''), 3.21 (1H, t, J=9.0 Hz, Glc-H-4''). The ¹³C-NMR spectrum (DMSO-*d*₆, 150 MHz): δ 164.4 (C-2), 103.2 (C-3), 182.2 (C-4), 161.3 (C-5), 99.6 (C-6), 163.1 (C-7), 95.0 (C-8), 157.1 (C-9), 105.5 (C-10), 121.2 (C-1'), 128.8 (C-2', 6'), 116.2 (C-3', 5'), 161.5 (C-4'), 100.0 (Glc-C-1''), 73.2 (Glc-C-2''), 76.6 (Glc-C-3''), 69.7 (Glc-C-4''), 77.3 (Glc-C-5''), 60.7 (Glc-C-6'').

Compound 3

Yellow amorphous powder. HRMS (m/z): 473.10967 [M-H]⁻ (cal. 473.10894, Δ +1.543ppm), and 431.09899 [M-C₂H₃O]⁻ (Cal. 431.09837, Δ +1.438ppm) established the molecular formula as C₂₃H₂₂O₁₁. The ¹H-NMR spectrum (DMSO-*d*₆, 600 MHz): δ 7.96 (2H, d, J=8.4 Hz, H-2', 6'), 6.96 (2H, d, J=8.4 Hz, H-3', 5'), 6.88 (1H, s, H-3), 6.81 (1H, s, H-8), 6.44 (1H, s, H-6), 5.11 (1H, d, J=7.2 Hz, Glc-H-1''), 4.34 (1H, d, J=10.8 Hz, Glc-H-6''a), 4.06 (1H, dd, J=12.0, 7.2 Hz, Glc-H-6''b), 3.75 (1H, t, J=7.8 Hz, Glc-H-2''),

3.27-3.43 (3H, m, Glc-H-3'', 4'', 5''), 2.01 (3H, s, H-2'''). The ^{13}C -NMR spectrum (DMSO- d_6 , 150 MHz): δ 164.3 (C-2), 103.2 (C-3), 182.1 (C-4), 161.7 (C-5), 99.6 (C-6, Glc-C-1''), 162.8 (C-7), 94.9 (C-8), 157.0 (C-9), 105.5 (C-10), 120.9 (C-1'), 128.6 (C-2', 6'), 116.1 (C-3', 5'), 164.4 (C-4'), 74.0 (Glc-C-2''), 76.3 (Glc-C-3''), 69.9 (Glc-C-4''), 73.1 (Glc-C-5''), 63.2 (Glc-C-6''), 170.3 (C-1'''), 21.1 (C-2'''). The HMBC correlations from H-6''a, b (δ_{H} 4.34, 4.06) to C-1''' (δ_{C} 170.3) and H-1'' (δ_{H} 5.11) to C-7 (δ_{C} 162.8) indicated the correlations of acetyl moiety to glucopyranosyl and the glucopyranosyl moiety to apigenin.

Compound 4

Yellow amorphous powder. HRMS (m/z): 461.11049 [$\text{M}-\text{H}$] $^-$ (cal. 461.10894, Δ +3.361ppm) established the molecular formula as $\text{C}_{22}\text{H}_{20}\text{O}_{11}$. The ^1H -NMR spectrum (CD_3OD , 500MHz): δ 7.53 (1H, dd, $J=8.5, 2.0$ Hz, H-6'), 7.47 (1H, brs, H-2'), 6.89 (1H, d, $J=8.5$ Hz, H-5'), 6.83 (1H, d, $J=2.0$ Hz, H-8), 6.66 (1H, brs, H-3), 6.45 (1H, d, $J=2.0$ Hz, H-6), 5.05 (1H, d, $J=7.0$ Hz, Glc-H-1''), 3.93 (3H, s, 3'- OCH_3), 3.92 (1H, dd, $J=15.0, 4.5$ Hz, Glc-H-6''b), 3.70 (1H, dd, $J=15.0, 4.5$ Hz, Glc-H-6''a), 3.55 (1H, m, Glc-H-5''), 3.50 (1H, m, Glc-H-3''), 3.49 (1H, m, Glc-H-2''), 3.40 (1H, m, Glc-H-4''). The ^{13}C -NMR spectrum (CD_3OD , 500MHz): δ 167.1 (C-2), 104.0 (C-3), 187.1 (C-4), 162.0 (C-5), 101.2 (C-6), 165.0 (C-7), 96.1 (C-8), 159.0 (C-9), 108.0 (C-10), 122.2 (C-1', 6'), 110.7 (C-2'), 148.4 (C-3'), 150.9 (C-4'), 117.2 (C-5'), 101.7 (Glc-C-1''), 74.8 (Glc-C-2''), 77.9 (Glc-C-3''), 71.3 (Glc-C-4''), 78.5 (Glc-C-5''), 62.5 (Glc-C-6''), 56.7 (3'- OCH_3). The HMBC correlations from - OCH_3 (δ_{H} 3.93) to C-3' (δ_{C} 148.4) indicated that the methoxy moiety was connected to carbon C-3'.

Compound 5

Yellow amorphous powder. HRMS (m/z): 577.13617 [$\text{M}-\text{H}$] $^-$ (cal. 577.13515, Δ +1.780ppm) established the molecular formula as $\text{C}_{30}\text{H}_{26}\text{O}_{12}$. The ^1H -NMR spectrum (DMSO- d_6 , 600 MHz): δ 7.96 (2H, d, $J=9.0$ Hz, H-2', 6', 2'', 6''), 7.50 (1H, d, $J=15.6$ Hz, H-7''), 6.90 (2H, d, $J=9.0$ Hz, H-3', 5'), 6.89 (1H, s, H-3), 6.83 (1H, d, $J=1.2$ Hz, H-8), 6.68 (2H, d, $J=7.2$ Hz, H-3'', 5''), 6.46 (1H, d, $J=1.2$ Hz, H-6), 6.35 (1H, d, $J=15.6$ Hz, H-8''), 5.18 (1H, d, $J=7.2$ Hz, Glc-H-1'''), 4.47 (1H, d, $J=10.8$ Hz, Glc-H-6'''a), 4.19 (1H, m, Glc-H-6'''b), 3.85 (1H, m, Glc-H-2'''), 3.27~3.36 (3H, m, Glc-H-3''', 4''', 5'''). The ^{13}C -NMR spectrum (DMSO- d_6 , 150 MHz): δ 161.5 (C-2), 103.1 (C-3), 182.1 (C-4),

161.3 (C-5), 99.6 (C-6, Glc-C-1'''), 162.8 (C-7), 94.8 (C-8), 157.0 (C-9), 105.4 (C-10), 121.2 (C-1'), 128.7 (C-2', 6'), 116.1 (C-3', 5'), 164.4 (C-4'), 124.9 (C-1''), 130.2 (C-2'', 6''), 115.7 (C-3'', 5''), 159.9 (C-4''), 145.1 (C-7''), 113.8 (C-8''), 166.6 (C-9''), 73.0 (Glc-C-2'''), 76.4 (Glc-C-3'''), 70.0 (Glc-C-4'''), 73.9 (Glc-C-5'''), 63.5 (Glc-C-6'''). The HMBC correlations from Glc-H-6'''b (δ_{H} 4.19) to C-9'' (δ_{C} 166.6) and Glc-H-1''' (δ_{H} 5.18) to C-7 (δ_{C} 162.8) indicated that the E-*p*-coumaroyl moiety was connected to glucopyranosyl and the glucopyranosyl moiety was connected to apigenin.

Compound 6

Yellow amorphous powder. HRMS (m/z): 649.13904 $[\text{M}+\text{Na}]^+$ (cal. 649.13752, $\Delta+2.342\text{ppm}$) established the molecular formula as $\text{C}_{27}\text{H}_{30}\text{O}_{17}$. The ^1H -NMR spectrum (CD_3OD , 500 MHz): δ 7.69 (1H, d, $J=2.0$ Hz, H-2'), 7.55 (1H, dd, $J=7.5, 2.0$ Hz, H-6'), 6.90 (1H, d, $J=7.5$ Hz, H-5'), 6.37 (1H, d, $J=2.0$ Hz, H-8), 6.19 (1H, d, $J=2.0$ Hz, H-6), 5.35 (1H, d, $J=7.5$ Hz, Glc-H-1''), 4.78 (1H, d, $J=7.5$ Hz, Glc-H-1'''), 3.81 (1H, dd, $J=12.0, 2.0$ Hz, Glc-H-6''a), 3.74 (1H, dd, $J=12.0, 4.0$ Hz, Glc-H-6''b), 3.72 (1H, dd, $J=12.0, 2.0$ Hz, Glc-H-6'''a), 3.55 (1H, dd, $J=12.0, 4.0$ Hz, Glc-H-6'''b), 3.45-3.20 (8H, m, Glc-H-2'', 2''', 3'', 3''', 4'', 4''', 5'', 5'''). The ^{13}C -NMR spectrum (CD_3OD , 125 MHz): δ 156.3 (C-2), 133.3 (C-3), 177.3 (C-4), 161.2 (C-5), 98.6 (C-6), 164.1 (C-7), 93.5 (C-8), 156.3 (C-9), 104.0 (C-10), 121.1 (C-1'), 115.2 (C-2'), 144.7 (C-3'), 148.4 (C-4'), 116.2 (C-5'), 121.6 (C-6'), 100.9 (Glc-C-1''), 81.3 (Glc-C-2''), 76.5 (Glc-C-3''), 70.1 (Glc-C-4''), 76.3 (Glc-C-5''), 60.7 (Glc-C-6'', 6'''), 103.1 (Glc-C-1'''), 74.0 (Glc-C-2'''), 76.5 (Glc-C-3'''), 69.5 (Glc-C-4'''), 76.4 (Glc-C-5''').

Compound 7

Yellow amorphous powder. HRMS (m/z): 487.08595 $[\text{M}(\text{C}_{21}\text{H}_{20}\text{O}_{12})+\text{Na}]^+$ (cal. 487.08470, $\Delta+2.566\text{ppm}$). The ^1H -NMR spectrum (CD_3OD , 500 MHz): δ 7.73 (1H, d, $J=2.0$ Hz, H-6'), 7.61 (1H, dd, $J=6.5, 2.0$ Hz, H-2'), 6.89 (1H, dd, $J=6.5, 2.0$ Hz, H-5'), 6.40 (1H, d, $J=2.0$ Hz, H-8), 6.21 (1H, d, $J=2.0$ Hz, H-6), 5.27 (1H, d, $J=7.5$ Hz, Glc-H-1''), 3.74 (1H, dd, $J=12.0, 2.5$ Hz, Glc-H-6''a), 3.60 (1H, dd, $J=12.0, 6.0$ Hz, Glc-H-6''b), 3.50 (1H, t, $J=7.5$ Hz, Glc-H-2''), 3.38 (1H, m, Glc-H-4''), 3.37 (1H, m, Glc-H-3''), 3.25 (1H, m, Glc-H-5''). The ^{13}C -NMR spectrum (CD_3OD , 125 MHz): δ 157.1 (C-2), 135.6 (C-3), 179.9 (C-4), 163.0 (C-5), 98.6 (C-6), 166.0 (C-7), 93.4 (C-8), 158.5 (C-9), 104.1 (C-10), 121.8 (C-1'), 114.7 (C-2'), 144.5 (C-3'), 148.5 (C-4'), 116.3 (C-5'),

121.6 (C-6'), 102.9 (Glc-C-1''), 74.3 (Glc-C-2''), 76.7 (Glc-C-3''), 69.8 (Glc-C-4''), 76.9 (Glc-C-5''), 61.2 (Glc-C-6'').

Compound 8

Yellow amorphous powder. HRMS (m/z): 609.14677 [M-H]⁻ (cal.609.14611, Δ +1.083ppm) established the molecular formula as C₂₇H₃₀O₁₆. The ¹H-NMR spectrum (CD₃OD, 500 MHz): δ 7.64 (1H, s, H-2'), 7.62 (1H, d, J=8.5 Hz, H-6'), 6.87 (1H, d, J=8.5 Hz, H-5'), 6.38 (1H, s, H-8), 6.20 (1H, s, H-6), 5.10 (1H, d, J=7.5 Hz, Glc-H-1''), 4.52 (1H, s, Rha-H-1'''), 3.81 (1H, d, J=12.0 Hz, Glc-H-6''a), 3.64 (1H, brs, Rha-H-2'''), 3.54 (1H, dd, J=9.5, 3.0 Hz, Rha-H-3'''), 3.47 (1H, t, J=7.5 Hz, Glc-H-2''), 3.45 (1H, m, Rha-H-5'''), 3.45 (1H, m, Glc-H-3''), 3.40 (1H, m, Glc-H-6''b), 3.30 (1H, m, Glc-H-5''), 3.28 (1H, m, Rha-H-4'''), 3.26 (1H, m, Glc-H-4''), 1.12 (1H, d, J=6.0 Hz, Rha-H-6'''). The ¹³C-NMR spectrum (CD₃OD, 125 MHz): δ 158.5 (C-2), 135.6 (C-3), 179.3 (C-4), 162.9 (C-5), 100.0 (C-6), 166.2 (C-7), 94.9 (C-8), 159.3 (C-9), 105.5 (C-10), 123.1 (C-1'), 116.0 (C-2'), 145.8 (C-3'), 149.8 (C-4'), 117.6 (C-5'), 123.5 (C-6'), 104.7 (Glc-C-1''), 75.7 (Glc-C-2''), 78.2 (Glc-C-3''), 71.4 (Glc-C-4''), 77.2 (Glc-C-5''), 68.5 (Glc-C-6''), 102.4 (Rha-C-1'''), 72.1 (Rha-C-2'''), 72.2 (Rha-C-3'''), 73.9 (Rha-C-4'''), 69.7 (Rha-C-5'''), 17.9 (Rha-C-6''').

Compound 9

Yellow amorphous powder. HRMS (m/z): 623.16398 [M-H]⁻ (cal.623.16176, Δ +3.562ppm) established the molecular formula as C₂₈H₃₂O₁₆. The ¹H-NMR spectrum (CD₃OD, 500 MHz): δ 7.95 (1H, d, J=2.0 Hz, H-2'), 7.62 (1H, dd, J=8.5, 2.0 Hz, H-6'), 6.91 (1H, d, J=8.5 Hz, H-5'), 6.39 (1H, s, H-8), 6.19 (1H, s, H-6), 5.22 (1H, d, J=7.5 Hz, Glc-H-1''), 4.53 (1H, brs, Rha-H-1'''), 3.95 (3H, s, 3'-OCH₃), 3.82 (1H, d, J=10.0 Hz, Glc-H-6''a), 3.62 (1H, m, Rha-H-2'''), 3.50 (1H, m, Rha-H-3''), 3.49 (1H, m, Rha-H-3'''), 3.48 (1H, m, Glc-H-2''), 3.45 (1H, m, Glc-H-5''), 3.42 (1H, m, Glc-H-6b''), 3.41 (1H, m, Rha-H-5'''), 3.27 (1H, m, Glc-H-4''), 3.24 (1H, m, Rha-H-4'''), 1.11 (3H, d, J=6.5 Hz, Rha-H-6'''). The ¹³C-NMR spectrum (CD₃OD, 125 MHz): δ 158.6 (C-2), 135.4 (C-3), 179.2 (C-4), 162.8 (C-5), 100.0 (C-6), 167.1 (C-7), 95.1 (C-8), 158.7 (C-9), 105.0 (C-10), 123.0 (C-1'), 114.6 (C-2'), 148.4 (C-3'), 150.9 (C-4'), 116.1 (C-5'), 124.0 (C-6'), 104.5 (Glc-C-1''), 75.9 (Glc-C-2''), 77.4 (Glc-C-3''), 73.9 (Glc-C-4''), 78.2 (Glc-C-5''), 68.5 (Glc-C-6''), 102.5 (Rha-C-1'''), 72.1 (Rha-C-2'''), 72.3 (Rha-C-3'''), 71.6 (Rha-C-4'''), 69.8

(Rha-C-5'''), 17.9 (Rha-C-6'''), 56.8 (3'-OCH₃). The HMBC correlations from -OCH₃ (δ_H 3.95) to C-3' (δ_C 148.4) indicate that the methoxy moiety was connected to carbon C-3'.

Compound 10

Yellow amorphous powder. HRMS (m/z): 633.14392 [M+Na]⁺ (cal.633.14261, Δ +2.069ppm) established the molecular formula as C₂₇H₃₀O₁₆. The ¹H-NMR spectrum (CD₃OD, 500 MHz): δ 8.03 (2H, d, J=8.5 Hz, H-2', 6'), 6.89 (2H, d, J=8.5 Hz, H-3', 5'), 6.40 (1H, d, J=2.0 Hz, H-8), 6.20 (1H, d, J=2.0 Hz, H-6), 5.44 (1H, d, J=7.5 Hz, Glc-H-1''), 4.61 (1H, s, J=7.5 Hz, Glc-H-1'''), 3.78-3.61 (4H, m, Glc-H-6'', 6'''), 3.49-3.20 (8H, m, Glc-H-2'', 2''', 3'', 3''', 4'', 4''', 5'', 5'''). The ¹³C-NMR spectrum (CD₃OD, 125 MHz): δ 156.2 (C-2), 133.3 (C-3), 177.5 (C-4), 161.3 (C-5), 98.8 (C-6), 160.4 (C-7), 93.8 (C-8), 156.6 (C-9), 103.2 (C-10), 120.9 (C-1'), 132.3 (C-2', 6'), 116.2 (C-3', 5'), 156.8 (C-4'), 99.1 (Glc-C-1''), 81.5 (Glc-C-2''), 77.9 (Glc-C-3''), 69.8 (Glc-C-4''), 77.9 (Glc-C-5''), 62.7 (Glc-C-6''), 103.9 (Glc-C-1'''), 74.3 (Glc-C-2'''), 77.9 (Glc-C-3'''), 69.8 (Glc-C-4'''), 77.9 (Glc-C-5'''), 62.7 (Glc-C-6''').

Compound 11

Yellow amorphous powder. HRMS (m/z): 593.15010 [M(C₂₇H₃₀O₁₅)-H]⁻ (cal.593.15119, Δ -1.838ppm). The ¹H-NMR spectrum (CD₃OD, 500 MHz): δ 8.05 (2H, d, J=9.0 Hz, H-2', 6'), 6.89 (2H, d, J=9.0 Hz, H-3', 5'), 6.38 (1H, d, J=2.0 Hz, H-8), 6.19 (1H, d, J=2.0 Hz, H-6), 5.11 (1H, d, J=7.5 Hz, Glc-H-1''), 4.51 (1H, brs, Rha-H-1'''), 3.81 (1H, d, J=10.0 Hz, Glc-H-6''a), 3.63 (1H, m, Rha-H-2'''), 3.53 (1H, m, Rha-H-3'''), 3.47 (1H, m, Rha-H-5'''), 3.46 (1H, m, Glc-H-2''), 3.45 (1H, m, Glc-H-3''), 3.39 (1H, m, Glc-H-6''b), 3.32 (1H, m, Glc-H-5''), 3.28 (1H, m, Rha-H-4'''), 3.25 (1H, m, Glc-H-4''), 1.12 (1H, d, J=6.0 Hz, Rha-H-6'''). The ¹³C-NMR spectrum (CD₃OD, 125 MHz): δ 158.6 (C-2), 135.3 (C-3), 179.3 (C-4), 163.0 (C-5), 100.0 (C-6), 166.7 (C-7), 95.0 (C-8), 159.3 (C-9), 105.4 (C-10), 122.7 (C-1'), 132.3 (C-2', 6'), 116.1 (C-3', C-5'), 161.5 (C-4'), 104.6 (Glc-C-1''), 75.7 (Glc-C-2''), 78.1 (Glc-C-3''), 71.4 (Glc-C-4''), 77.2 (Glc-C-5''), 68.5 (Glc-C-6''), 102.4 (Rha-C-1'''), 72.1 (Rha-C-2'''), 72.3 (Rha-C-3'''), 73.9 (Rha-C-4'''), 69.7 (Rha-C-5'''), 17.9 (Rha-C-6''').

Cell Counting Kit-8 (CCK-8) assay

The CCK-8 assay was performed to evaluate the effect of all 11 compounds on cell proliferation of the various tumor cell lines. Cells were seeded into 96-well plates and

incubated overnight. The 11 compounds were serially diluted to the following concentrations: 200, 100, 50, 25, 5, and 1 μ M (paclitaxel served as reference drug: 1000, 100, 10, 1, 0.1, 0.01 nM) and were added to the cells. The assay plates were further cultured for 72 hours at 37°C. Next, 100 μ l of CCK-8 reagents (DOJINDO, Kumamoto, Japan) was added to each well and incubated for an additional 30 min to 180 min. Subsequently, the optical density (OD) values were measured at 450 nm in a with anmicroplate reader (Thermo Fisher, Waltham, MA, USA). The effects of each compound were given as 50% inhibitory concentrations (IC_{50}), which were calculated based on sigmoidal curve fittings. The experiments were repeated 3 times.

Spectrums of compound 1

Spectrums of compound 2

Spectrums of compound 3

AV-600-HMBC
Sample: JCE9

JCE9-F125 RT: 3.31-4.27 AU: 28.16.181E9
 1.7766-p (ES+data) (110.05-1300.0)

芹菜素-7-O-(6-2酯)-葡萄糖

$[M-H]^-$

$C_{22}H_{22}O_{11}$ 474

Cal 48.10894

$\Delta = 1.58916$

JCE9

Spectrums of compound 4

ZDC-40-LH-20-19-2: #1-29 RT: 0.01-0.27 AV: 29 NL: 1.12E7
T: FTMS - p ESI Full ms [110.00-1000.00]

Spectrums of compound 5

Spectrums of compound 6

Spectrums of compound 7

NL: 7.73E5
 C₂₁H₂₀O₁₂+Na₁
 C₂₁H₂₀O₁₂Na₁
 pos Chrg 1

Spectrums of compound 8

Spectrums of compound 9

Spectrums of compound 10

Spectrums of compound 11

NL:
4.05E7
ZDC-40-3-2#1-29
RT: 0.01-0.27 AV:
29 T: FTMS - p ESI
Full ms
[110.00-1000.00]

NL:
7.19E5
C₂₇H₂₉O₁₅
C₂₇H₂₉O₁₅
pa Chg 1