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Novel diterpenoids with potent inhibitory activity against endothelium cell HMEC and cytotoxic activities from a well-known TCM plant *Daphne genkwa*

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Abstract—Twelve highly oxygenated novel daphnane-type diterpenoids genkwanines A–L (**1**–**12**), together with four known diterpenes (**13**–**16**), were isolated from the bud of *Daphne genkwa*, a well-known traditional Chinese medicine (TCM). The new structures were elucidated on the basis of spectral methods, especially 2D NMR spectra (HMQC, HMBC, NOESY). Inhibitory activity against endothelium cell HMEC proliferation and cytotoxic activities against two tumor cell lines were assessed for all the compounds **1**–**16**, and found to be significant and structure related. A number of compounds showed very potent cytotoxic activities against two tumor cell lines at the IC_{50} levels of 0.15–8.40 μM, and most interestingly, five of the compounds **4**, **8**, **10**, **13**, and **14** exhibited strong activity to inhibit the endothelium cell HMEC at the IC_{50} levels of 2.90–15.0 μM.

1. Introduction

Daphnane-type diterpenes mostly occur in the families Euphorbiaceae and Thymelaeaceae,[1] which showed interesting biological activity, such as antileukemic,[2–6] skin irritant,[7–9] neurotrophic,[10] antihyperglycemic,[11] antifertility,[12–16] pesticide activities,[17] and curing bladder hyper-reflexia.[18] The flower bud of *Daphne genkwa* Sieb. *et* Zucc. (Thymelaeaceae), a well-known traditional Chinese medicine, is mainly used for diuretic, antitussive, expectorant, and anticancer purposes.[19] Previous studies on the chemical constituents of this plant resulted in the isolation of a few daphnane-type diterpenes, which showed antifertility and antileukemic activity,[12–15,20,21] and some phenolic compounds.[22–24] In the current project for biologically active compounds from traditional Chinese medicinal plants, 12 highly oxygenated novel daphnane-type diterpenoids, namely genkwanines A–L (**1**–**12**), together with four known diterpenoids (**13**–**16**), were isolated from this medicinal plant. Compounds **1**–**16** have been tested for inhibitory activity against endothelium cell (HMEC) proliferation and cytotoxic activities against two tumor cell lines P-388 and A-549. The tests of inhibitory activity against HMEC proliferation and cytotoxic activities against two tumor cell lines for compounds **1**–**16** found to be significant and structure related. A number of compounds showed very potent cytotoxic activities against two tumor cell lines at the IC_{50} levels of 0.15–8.40 μM. Most interestingly, five of the compounds **4**, **8**, **10**, **13**, and **14** exhibited strong activity to inhibit HMEC cell line at the IC_{50} levels of 2.90–15.0 μM. The endothelial cells, such as HMEC cell line, present good targets for new anticancer drug discovery, and the inhibitors against endothelium cells abnormal proliferation and migration offer hope for a new way of cancer chemotherapy.[25–27] We reported herein the isolation and structural elucidation of these new diterpenoids, their inhibitory activity against HMEC proliferation and cytotoxic activities, and a brief discussion on their structure–activity relationship.

2. Results and discussion

2.1. Structural chemistry

The $CHCl_3$-soluble fraction of the bud of *D. genkwa* was subjected to the extensive procedures of column

Keywords: Novel daphnane-type diterpenoids; *Daphne genkwa*; Inhibitory activity against endothelium cell HMEC; Cytotoxic activities.
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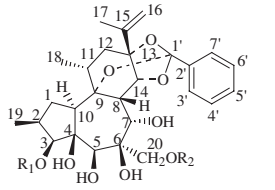
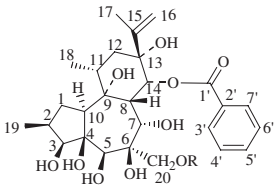
chromatography to afford 16 daphnane-type diterpenoids **1**–**16** (Fig. 1). Among them, 12 are new highly oxygenated daphnane-type diterpenoids, namely genkwanines A–L (**1**–**12**), and the four known ones, yuanhuacine (**13**), yuanhuadine (**14**), yuanhuafine (**15**), and yuanhuapin (**16**), were previously isolated from this plant.

Genkwanine A (**1**) was obtained as a white amorphous powder, $[\alpha]_D^{20}$ +42.7 (*c* 0.73, $CHCl_3$). It showed pseudomolecular ion at *m*/*z* 505 $[M+H]^+$ in positive ESIMS, and at *m*/*z* 503 $[M-H]^-$ in negative ESIMS. The molecular formula of **1** was determined as $C_{27}H_{36}O_9$ by HREIMS, indicating the presence of 10 degrees unsaturation. The strong IR absorption at 3415 cm^{-1} was assignable to the hydroxyls. Twenty seven carbon signals comprising three methyls, four methylenes, thirteen methines, and seven quaternary carbons were evident from its ^{13}C NMR and DEPT spectra, in which, the following functionalities of one terminal double bond (δ_C 146.1, C-15; 111.5, C-16), an oxygenated methylene (δ_C 69.7, C-20), four oxygenated methine carbons (δ_C 76.1, C-3; 74.5, C-5; 80.2, C-7; 84.1, C-14), four oxygenated quaternary carbons (δ_C 81.2, C-4; 76.5, C-6; 85.0, C-9; 85.3, C-13) and one phenyl group were distinguishable. A quaternary carbon signal at δ 117.2 assignable to an orthoester group is the most notably structural feature of daphnane-type diterpenoids. The ^1H NMR spectrum of **1** further defined some above proton bearing functionalities and their connectivity patterns judging from the chemical shifts and coupling constants. In the ^1H NMR, three methyl groups were displayed as one singlet at δ 1.82 (3H, s, CH_3-17), two doublets at δ 1.31, (3H, d, J = 6.8 Hz, CH_3-18) and 1.02 (3H, d, J = 6.2 Hz, CH_3-19); the only oxygenated methylene resolved at δ 3.95 (1H, d, J = 11.2 Hz, H-20) and 3.79 (1H, d, J = 11.2 Hz, H-20), is indicative that it was attached to a quaternary carbon; the four oxygenated methine protons were appeared at δ 4.57 (1H, d, J = 1.4 Hz, H-14), 4.28 (1H, s, H-7), 4.22 (1H, d, J = 5.1 Hz, H-3) and 3.71 (1H, s, H-5); the olefinic protons observed as a typical AB system at δ 5.04 (1H, s, H-16) and 4.92 (1H, s, H-16) were a typical feature of terminal double bond.

Analysis of the ^1H NMR, ^{13}C NMR, and HMQC spectra enabled us to allot all the protons to their bonded carbons, and further demonstrated that **1** was a daphnane-type diterpenoid derivative. Compared with the known compounds **13**–**15**, the upfield shifted methyl protons of H-19 (δ 1.02) and the H-3 at δ_H 4.22 (d, J = 5.1 Hz; δ_C 76.1) indicated that there was no enone





1 R_1 = H; R_2 = H

2 R_1 = CO(CH=CH)$_2$(CH$_2$)$_4$CH$_3$ (1″, 10″); R_2 = H

3 R_1 = CO(CH=CH)$_3$(CH$_2$)$_2$CH$_3$; R_2 = H

4 R_1 = COPh; R_2 = H

5 R_1 = H; R_2 = CO(CH=CH)$_3$(CH$_2$)$_2$CH$_3$

6 R_1 = H; R_2 = CO(CH=CH)$_2$(CH$_2$)$_4$CH$_3$

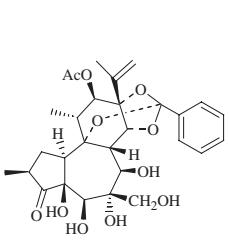
7 R_1 = H; R_2 = CO(CH=CH)(CH$_2$)$_6$CH$_3$

8 R_1 = H; R_2 = COPh

9 R = H

10 R = CO(CH=CH)$_2$(CH$_2$)$_4$CH$_3$

11 R = COPh



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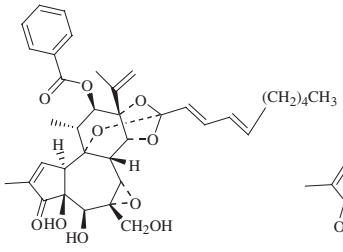

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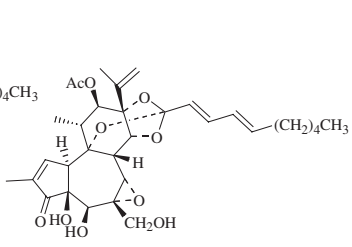

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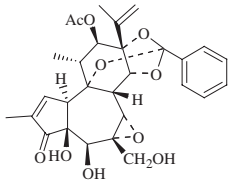

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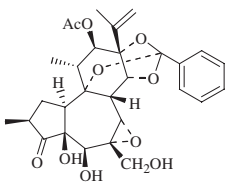

16

Figure 1. Structures of **1**–**16**.

group in **1**; the proton signal of H-7 (δ 4.28 s) and the carbon signals C-7 (δ 80.2) and C-6 (δ 76.5) were all downfield shifted, suggesting the presence of 6,7-dihydroxyls in **1** instead of an 6,7-expoxy. In the HMBC spectrum (see Fig. 2), the carbon signal at δ 117.2 (C-1′) correlated with the proton signals at δ 4.57 (H-14), 7.67 (H-3′ and H-7′) and 7.37 (H-4′ and H-6′) verified the presence of an orthoester group in **1**; the H-7 at δ 4.28 showed correlations with C-5 (δ_C 74.5), C-6 (δ_C 76.5), C-8 (δ_C 36.5) and C-20 (δ_C 69.7), and H-3 at δ 4.22 correlated with C-1 (δ_C 35.5), C-2 (δ_C 36.4), C-4 (δ_C 81.2), C-5 (δ_C 74.5), and C-10 (δ_C 51.7), were indicative of the linkages and oxygenated patterns of these carbons. The planar structure of **1** was thus figured out.

The relative stereochemistry of **1** was completely established by the coupling constants of ^{1}H NMR and the correlations of NOESY spectrum (Fig. 3). In the ^{1}H NMR spectrum, the H-7 at δ 4.28 appeared as singlet indicated that the 7-OH was α-oriented, and if the 7-OH was β-oriented, the coupling constant was normally appeared at ca. $J_{7,8}$ = 10 Hz.[28] In the NOESY spectrum, the H-7 was strongly correlated with H-8 and H-14, indicating that the H-7, H-8, and H-14 were all in the β-configuration. The significant NOESY correlation pairs of H-2/H-3, H-2/H-5, H-3/H-5, H-5/H-10, and H-10/H-18 permitted the assignment of H-2, H-3, H-5, H-10, and H_3-18 in the α-orientation. The H-5 showed correlation with one of H_2-20 proton at δ 3.95 suggested that they were at the same side and the hydroxyl at C-6 was β-orientation. The structure and relative stereochemistry of **1** was therefore elucidated (Fig. 1), namely genkwanine A. The complete assignments of ^{1}H and ^{13}C NMR data were made by the interpretation of its HMQC, HMBC, and NOESY spectra (Tables 1 and 3).

Genkwanine B (**2**), a white amorphous powder, showed pseudo-molecular ions at *m/z* 655 $[M+H]^+$ and 653 $[M-H]^-$ in the positive and negative ESIMS, respectively. The HREIMS of **2** provided a molecular formula $C_{37}H_{50}O_{10}$, inferring the occurrence of 13 degrees of unsaturation. The IR absorption bands at 3430 and 1689 cm^{-1} were attributed to the hydroxyls and ester carbonyl, respectively. The presence of an ester carbonyl was confirmed by a quaternary carbon signal at δ 169.2 in the ^{13}C NMR. The ^{1}H and ^{13}C NMR spectral data of **2** were very similar to those of **1**, except for the presence of a decadienoyl which was determined by ^{1}H and ^{13}C NMR data, and especially the fragment ion at *m/z* 151 $[C_9H_{15}CO]^+$ in the EIMS spectrum. Furthermore, the H-3″ at δ 7.31 showed correlations with the carbon signals at δ 169.2 (C-1″), 146.2 (C-4″), and 128.3 (C-5″), indicating the occurrence of a $\Delta^{2,4}$ double bonds in the acyl. The acyl group was assigned as (2*E*,4*E*)-decadienoyl on the basis of coupling constants ($J_{2'',3''}$ = 15.2 Hz) of ^{1}H NMR and compared with the ^{13}C NMR data reported in the literature.[5] The H-3 at δ 5.13 (1H, d, *J* = 5.3 Hz) was severely shifted downfield compared with that of **1**, suggesting the acyloxy group was allocated to C-3, and confirmed by HMBC spectrum, in which, the carbonyl at δ 169.2 (C-1″) correlated with the proton signals at δ 5.13 (H-3), 5.89 (H-2″) and 7.31 (H-3″). The structure of **2** was unambiguously assigned herein, namely genkwanine B.

Genkwanine C (**3**), a white amorphous powder, showed pseudo-molecular ions at *m/z* 653 $[M+H]^+$ and 651 $[M-H]^-$ in the positive and negative ESIMS mode,

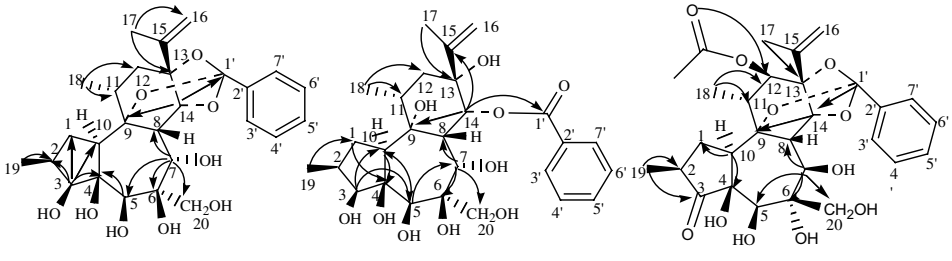

Figure 2. The Selected HMBC of **1**, **9**, and **12**.

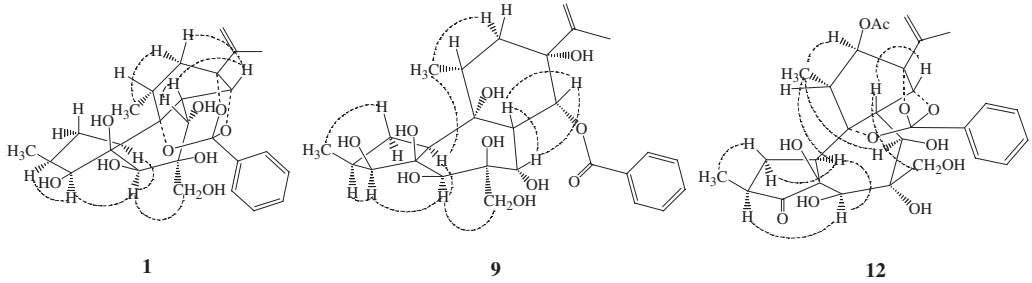

Figure 3. The key NOESY of **1**, **9**, and **12**.

Table 1. ^{1}H NMR spectral data for genkwanines A–D (**1**–**4**)

No	1	2	3	4
1β	2.00 (1H, m)	2.00 (1H, m)	2.06 (1H, m)	2.10 (1H, m)
1a	1.90 (1H, m)	1.90 (1H, m)	1.92 (1H, m)	1.90 (1H, m)
2	1.82 (1H, m)	1.82 (1H, m)	1.85 (1H, m)	1.80 (1H, m)
3	4.22 (1H, d, 5.1)	5.13 (1H, d, 5.2)	5.18 (1H, d, 5.0)	5.31 (1H, d, 5.2)
4	—	—	—	—
5	3.71 (1H, s)	3.88 (1H, s)	3.94 (1H, s)	3.92 (1H, s)
6	—	—	—	—
7	4.28 (1H, s)	4.28 (1H, s)	4.24 (1H, s)	4.28 (1H, s)
8	2.49 (1H, d, 2.0)	2.49 (1H, d, 2.0)	2.48 (1H, 2.3)	2.48 (1H, d, 2.5)
9	—	—	—	—
10	2.65 (1H, dd, 13.1, 5.3)	2.79 (1H, dd, 12.6, 5.4)	2.90 (1H, dd, 12.6, 5.4)	2.85 (1H, dd, 13.3, 5.2)
11	2.73 (1H, m)	2.73 (1H, m)	2.74 (1H, m)	2.75 (1H, m)
12β	2.20 (1H, dd, 14.3, 7.0)	2.21 (1H, dd, 14.3, 7.2)	2.21 (1H, dd, 14.0, 7.3)	2.19 (1H, dd, 14.3, 7.0)
12a	1.78 (1H, d, 14.3)	1.75 (1H, d, 14.3)	1.78 (1H, d, 14.0)	1.75, d, 14.3
13	—	—	—	—
14	4.57 (1H, d, 2.0)	4.56 (1H, d, 2.0)	4.58 (1H, d, 2.3)	4.54 (1H, d, 2.5)
15	—	—	—	—
16	5.04 (1H, s)	5.04 (1H, s)	5.04 (1H, s)	5.02 (1H, s)
	4.92 (1H, s)	4.92 (1H, s)	4.95 (1H, s)	4.91 (1H, s)
17	1.82 (3H, s)	1.82 (3H, s)	1.78 (3H, s)	1.78 (3H, s)
18	1.31 (3H, d, 6.8)	1.35 (3H, d, 6.7)	1.35 (3H, d, 6.5)	1.29 (3H, d, 7.1)
19	1.02 (3H, d, 6.2)	1.07 (3H, d, 6.0)	1.02 (3H, d, 6.0)	1.08 (3H, d, 7.1)
20	3.95 (1H, d, 11.2)	4.07 (1H, d, 11.3)	4.08 (1H, d, 11,3)	4.06 (1H, d, 11.3)
	3.79 (1H, 11.2)	3.65 (1H, d, 11.3)	3.64 (1H, d, 11.3)	3.66 (1H, d, 11.3)
1′	—	—	—	—
2′	—	—	—	—
3′, 7′	7.67 (2H, m)	7.67 (2H, m)	7.70 (2H, m)	7.71 (2H, m)
4′, 6′	7.37 (2H, m)	7.31 (2H, m)	7.37 (2H, m)	7.37 (2H, m)
5′	7.35 (1H, m)	7.32 (1H, m)	7.38 (1H, m)	7.38 (1H, m)
1″		—	—	—
2″		5.89 (1H, d, 15.2)	5.90 (1H, d, 15.4)	—
3″		7.31 (1H, dd, 15.2, 9.8)	7.35 (1H, dd, 15.4, 10.6)	8.05 (1H, d, 7.1)
4″		6.20 (1H, m)	6.60 (1H, dd, 14.8 10.6)	7.45 (1H, t, 7.5)
5″		6.20 (1H, m)	6.24 (1H, dd, 14.8, 11.4)	7.60 (1H, d, 7.4)
6″		2.20 (2H, m)	6.15 (1H, 15.4, 11.4)	7.45 (1H, t, 7.5)
7″		1.44 (2H, m)	5.95 (1H, m)	8.05 (1H, d, 7.1)
8″		1.29 (2H, m)	1.72 (2H, m)	
9″		1.31 (2H, m)	1.40 (2H, m)	
10″		0.90 (3H, t, 6.8)	0.86 (3H, 7.0)	

Data were measured in $CDCl_3$ at 400 MHz. *J* values were given in Hz.

respectively. The compound **3** with molecular formula $C_{37}H_{48}O_{10}$ as determined by HREIMS has 14 degrees of unsaturation, one more degree of unsaturation than that of **2**. The ^{1}H and ^{13}C NMR spectral data of the diterpenoid moiety were almost identical with those of **2**, and the only difference was the presence of one more double bond in the acyl part judging from the existence of two more olefinic protons in **3**. A significant fragment ion at *m*/*z* 149 $[C_9H_{13}CO]^+$ in the EIMS suggested the presence of a decatrienoyloxy at C-3 of **3**. In the ^{1}H NMR, the coupling constants of the olefinic protons of acyl part in **3** ($J_{2'',3''}$ = 15.4 Hz, $J_{4'',5''}$ = 14.8 Hz, and $J_{6'',7''}$ =15.4 Hz) showed that the acyl moiety was 2*E*,4*E*,6*E*-decatrienoyl. The structure of **3** thus characterized to be 3-(2*E*,4*E*,6*E*)-decatrienoyl of **1**, namely genkwanine C.

Genkwanine D (**4**) has a molecular formula of $C_{34}H_{40}O_{10}$ as determined by HREIMS at *m*/*z* 608.2619 (calcd for $C_{34}H_{40}O_{10}$, 608.2622). Comparison of the spectral data of **4** with those of compounds **1**–**3** (Tables 1 and 3) showed that the presence of a benzoyloxy at C-3 in compound **4**. The structure of genkwanine D was definitely assigned.

Genkwanine E (**5**) showed molecular formula of $C_{37}H_{48}O_{10}$ as assigned by the HREIMS. The ^{1}H and ^{13}C NMR spectra of **5** (Tables 2 and 3) were similar to those of **1**, except for the presence of an acyl group, which has led to a severely downfield shifted two proton signals H-20 (δ 4.74, 4.41) in the ^{1}H NMR spectrum, suggesting that the acyloxy group was allocated to the C-20. The ^{1}H, ^{13}C NMR, and EIMS spectra of **5** revealed that the acyl was decatrienoyl, and the large coupling constants ($J_{2'',3''}$ = 15.4 Hz, $J_{4'',5''}$ = 14.8 Hz, $J_{6'',7''}$ = 15.4 Hz) further defined a 2*E*,4*E*,6*E*-decatrienoyl. The linkage of 2*E*,4*E*,6*E*-decatrienoyloxy to C-20 was confirmed by the HMBC correlation between C-1″ (δ 168.1) and H_2-20 (4.74, 4.41).

Genkwanines F–H (**6**–**8**) were elucidated to be the most closely related homologues of genkwanine E (**5**) in very similar ways. Genkwanines F–H were thus assigned as 20-*O*-(2*E*,4*E*)-decadienoyl-genkwanine A (**6**),

Table 2. ^{1}H NMR spectral data for genkwanines E–H (**5**–**8**)

No	5	6	7	8
1β	2.12 (1H, m)	2.10 (1H, m)	2.12 (1H, m)	2.12 (1H, m)
1a	1.95 (1H, m)	1.84 (1H, m)	1.93 (1H, m)	1.93 (1H, m)
2	1.78 (1H, m)	1.70 (1H, m)	1.70 (1H, m)	1.73 (1H, m)
3	4.26 (1H, d, 5.2)	4.25 (1H, d, 5.0)	4.24 (1H, 5.0)	4.26 (1H, d, 5.0)
4	—	—	—	—
5	3.44 (1H, s)	3.38 (1H, s)	3.40 (1H, s)	3.45 (1H, s)
6	—	—	—	—
7	4.47 (1H, s)	4.42 (1H, s)	4.45 (1H, s)	4.46 (1H, s)
8	2.48 (1H, d, 2.0)	2.45 (1H, d, 2.1)	2.42 (1H, d, 2.0)	2.50 (1H, d, 2.0)
9	—	—	—	—
10	2.71 (1H, dd, 13.0, 5.4)	2.68 (1H, dd, 13.1, 5.3)	2.70 (1H, dd, 12.8, 5.5)	2.85 (1H, dd, 13.3, 5.2)
11	2.62 (1H, m)	2.60 (1H, m)	2.60 (1H, m)	2.75 (1H, m)
12β	2.23 (1H, dd, 14.2, 8.0)	2.18 (1H, dd, 14.0, 8.0)	2.23 (1H, dd, 13.9, 8.0)	2.12 (1H, dd, 14.0, 7.5)
12a	1.76 (1H, d, 14.2)	1.70 (1H, d, 14.0)	1.78 (1H, d, 13.9)	1.75 (1H, d, 14.0)
13	—	—	—	—
14	4.59 (1H, d, 2.0)	4.48 (1H, d, 2.1)	4.60 (1H, d, 2.0)	4.58 (1H, d, 2.0)
15	—	—	—	—
16	5.05 (1H, s)	5.05 (1H, s)	5.05 (1H, s)	5.02 (1H, s)
	4.92 (1H, s)	4.90 (1H, s)	4.92 (1H, s)	4.91 (1H, s)
17	1.83 (3H, s)	1.82 (3H, s)	1.80 (3H, s)	1.78 (3H, s)
18	1.33 (3H, d, 7.0)	1.28 (3H, d, 6.8)	1.30 (3H, d, 7.0)	1.25 (3H, d, 6.8)
19	1.05 (3H, d, 6.3)	1.00 (3H, d, 6.3)	1.02 (3H, d, 6.5)	1.01 (3H, d, 7.0)
20	4.41 (1H, d, 11.5)	4.35 (1H, d, 11.5)	4.41 (1H, d, 11.6)	4.60 (1H, d, 11.0)
	4.74 (1H, d, 11.5)	4.70 (1H, d, 11.5)	4.70 (1H, d, 11.6)	4.70 (1H, d, 11.0)
1′	—	—	—	—
2′	—	—	—	—
3′, 7′	7.65 (2H, m)	7.69 (2H, m)	7.65 (2H, m)	7.71 (2H, m)
4′, 6′	7.37 (2H, m)	7.37 (2H, m)	7.37 (2H, m)	7.37 (2H, m)
5′	7.38 (1H, m)	7.38 (1H, m)	7.38 (1H, m)	7.38 (1H, m)
1″	—	—	—	—
2″	5.90 (1H, d, 15.4)	5.82 (1H, d, 15.3)	5.75 (1H, d, 13.2)	—
3″	7.35 (1H, dd, 15.4, 10.6)	7.31 (1H, dd, 15.3, 9.8)	7.24 (1H, m)	8.05 (1H, d, 7.1)
4″	6.60 (1H, dd, 14.8, 10.6)	6.18 (1H, m)	1.64 (2H, m)	7.45 (1H, t, 7.5)
5″	6.26 (1H, dd, 14.8, 11.4)	6.18 (1H, m)	1.25–1.40	7.60 (1H, d, 7.4)
6″	6.20 (1H, 15.4, 11.4)	2.20 (2H, m)	1.25–1.40	7.45 (1H, t, 7.5)
7″	5.95 (1H, m)	1.44 (2H, m)	1.25–1.40	8.05 (1H, d, 7.1)
8″	1.70 (2H, m)	1.29 (2H, m)	1.25–1.40	
9″	1.40 (2H, m)	1.31 (2H, m)	1.25–1.40	
10″	0.86 (3H, 7.0)	0.90 (3H, t, 6.8)	0.90 (3H, t, 7.5)	

Data were measured in $CDCl_3$ at 400 MHz. *J* values were given in Hz.

20-*O*-(2*E*)-decaenoyl-genkwanine A (**7**) and 20-*O*-benzoyl-genkwanine A (**8**), respectively. The complete assignments of their spectral data were also achieved (Tables 2 and 3, experimental).

Genkwanine I (**9**), a white amorphous powder, showed pseudo-molecular ion at *m*/*z* 523 $[M+H]^+$ in the positive mode of ESIMS, and ion at *m*/*z* 521 $[M-H]^-$ in the negative mode of ESIMS. The HREIMS of **9** at *m*/*z* 504.2358 $[M-H_2O]^+$ (calcd for $C_{27}H_{36}O_9$, 504.2359) revealed a molecular of $C_{27}H_{38}O_{10}$, which implied the presence of 9 degrees of unsaturation. IR absorptions at 3407 and 1706 cm^{-1} implied the presence of hydroxyls and ester carbonyl, respectively. The ^{1}H NMR and ^{13}C NMR indicated that **9** had similar oxygenated pattern as that of **1**. In the ^{13}C NMR of **9**, the following functionalities of one terminal double bond, an oxygenated methylene, four oxygenated methines, four oxygenated quaternary carbons, and one phenyl group were distinguishable. Compared with compound **1**, the absence of the typical quaternary carbon for an orthoester group, and in stead of the presence of one ester carbonyl at δ_C 167.1 (C-1′) in **9** suggested that a benzoyloxy was likely located at C-14 and two hydroxyls were linked to the C-9 and C-13. The significant downfield shifted H-14 proton signal at δ 5.70 (d, J = 5.3 Hz) of **9** proved the presence of an benzoyl group at C-14.[5] The planar structure of 9 was further confirmed by a outstanding performance of HMBC experiment (see Fig. 2), in which, the carbon signal at δ 167.1 (C-1′) correlated with the proton signals at δ 5.70 (H-14), 8.02 (H-3′ and H-7′) and 7.41 (H-4′ and H-6′) indicated the attachment of benzoyloxy at C-14; and the locations of all the hydroxyls were also assignable.

The relative stereochemistry of **9** was demonstrated by the coupling constants and NOESY correlations (Fig. 3). The singlet proton signal of H-7 at δ 4.05 and the doublet proton signal of H-8 at δ 3.20 ($J_{8,14}$ = 5.3 Hz) in the ^{1}H NMR indicated that the hydroxyl at C-7 was α-oriented.[26] In the NOESY spectrum, the H-14 and H-8 strongly correlated with H-7, indicating that H-7, H-8, and H-14 are all β-oriented; the NOESY correlation pairs of H-2/H-3, H-2/H-5, H-3/H-5, H-5/H-10,

Table 3. ^{13}C NMR spectral data for genkwanines A–H (**1**–**8**)

No	1	2	3	4	5	6	7	8
1	35.5	35.5	35.5	35.7	34.9	35.0	34.9	34.9
2	36.4	36.0	36.0	36.0	36.0	36.0	35.9	35.9
3	76.1	80.6	80.5	81.0	77.4	77.4	77.5	77.2
4	81.2	81.1	81.0	81.2	81.2	81.2	81.2	81.2
5	74.5	76.1	76.0	75.8	73.9	73.8	73.9	73.7
6	76.5	75.3	75.3	75.4	76.9	76.7	76.7	76.7
7	80.2	80.2	80.1	80.0	76.2	75.9	76.0	76.2
8	36.5	36.5	36.4	36.5	36.5	36.5	36.4	36.5
9	85.0	85.2	85.2	85.1	85.0	85.0	85.2	85.0
10	51.7	52.2	52.2	52.2	51.5	51.5	51.4	51.4
11	35.3	35.0	34.9	34.9	35.3	35.3	35.2	35.2
12	36.3	36.3	36.2	36.3	36.3	36.3	36.3	36.1
13	85.3	85.3	85.3	83.2	85.3	85.2	85.3	85.2
14	84.1	84.7	84.7	84.6	84.3	84.3	84.3	84.4
15	146.1	146.1	146.0	145.7	146.1	146.2	146.1	146.1
16	111.5	111.5	111.5	111.4	111.4	111.3	111.4	111.5
17	19.1	19.1	19.1	19.2	19.1	19.1	19.1	19.2
18	20.9	20.9	20.8	21.0	20.8	20.8	20.9	20.8
19	13.2	13.2	13.2	13.6	13.2	13.1	13.2	13.1
20	69.7	70.8	70.7	70.5	66.9	66.8	66.8	67.2
1′	117.2	117.2	117.2	117.0	117.2	117.1	117.2	117.2
2′	135.8	135.7	135.7	135.5	135.8	135.8	135.7	135.7
3′, 7′	125.9	125.9	125.9	125.7	125.9	125.9	125.9	125.8
4′, 6′	128.1	128.1	128.1	128.4	128.1	128.0	128.1	128.1
5′	129.6	129.6	129.5	129.3	129.5	129.4	129.5	129.5
1″		169.2	169.0	167.9	168.0	168.1	168.1	167.2
2″		118.0	118.7	129.5	119.2	118.4	130.4	129.9
3″		146.8	146.6	129.6	146.2	146.3	146.1	129.9
4″		146.2	142.4	128.4	142.0	145.7	35.0	128.4
5″		128.3	141.3	133.3	141.0	128.2	32.9	133.2
6″		33.0	130.0	128.4	130.0	33.0	29.6	128.4
7″		28.3	127.5	129.6	127.6	28.3	29.3	129.9
8″		31.4	35.1		35.1	31.1	31.3	
9″		22.5	22.1		22.2	22.6	22.5	
10″		14.0	13.7		13.7	13.9	13.7	

Measured in $CDCl_3$ at 100 MHz. Chemical shift values are expressed in ppm.

and H-10/H-18 permitted the assignment of H-2, H-3, H-5, H-10, and H_3-18 to be in α-orientation. The NOESY correlation between H-5 and H_2-20 showed that the C-6–OH took an β-orientation. The relative structure of **9** was therefore determined and designated as genkwanine I. The complete assignments of ^1H and ^{13}C NMR data were resolved by the interpretation of its HMQC, HMBC, and NOESY spectra (Tables 4 and 5).

Genkwanine J (**10**), a white amorphous powder, showed pseudo-molecular ion at *m/z* 673 $[M+H]^+$ and *m/z* 671 $[M-H]^-$ in the positive and negative modes of ESIMS. The HREIMS revealed the molecular formula $C_{37}H_{52}O_{11}$ at *m/z* 672.3505 (calcd for $C_{37}H_{52}O_{11}$, 672.3510). The spectral feature of the diterpenoid core of **10** was very similar to that of **9**, except for the proton signals of H_2-20 at δ 4.56 (1H, d, $J = 11.9$ Hz) and 4.38 (1H, d, $J = 11.9$ Hz) were downfield shifted in the ^1H NMR, suggesting the existence of an acyloxy group at C-20. The spectral data of **10**, especially the strong ion at *m/z* 151 $[C_9H_{15}CO]^+$ in the EIMS and the large coupling constants of the olefinic proton signals of the acyl ($J_{2'',3''} = 15.1$ Hz, $J_{4'',5''} = 15.2$ Hz) in the ^1H NMR, indicated the acyl group was (2*E*,4*E*)-decadienoyl. The structure of genkwanine J (**10**) was thereby assigned.

Genkwanine K (**11**) was obtained as a white amorphous powder. The structure of compound **11** was determined in a very similar way as in **10**. The only difference of two compounds was the acyloxy at C-20, a benzoyloxy at C-20 in **11** was assignable by its spectral data.

Genkwanine L (**12**), a white amorphous powder, showed the molecular formula $C_{29}H_{36}O_{11}$ as determined by HREIMS, indicating the presence of 12 degrees of unsaturation. Both the positive and negative modes of ESIMS gave pseudo-molecular ions at *m/z* 561 $[M+H]^+$ and 559 $[M-H]^-$, respectively. All the 29 carbons were resolved in the ^{13}C NMR including four methyls, three secondary carbons, thirteen tertiary carbons, and nine quaternary carbons. A most distinguishable quaternary carbon at δ 117.2 in the ^{13}C NMR was attributable to an orthoester group, inferring a typically structural feature of daphnane-type diterpenoids. The quaternary carbon signals at δ 219.6 and 169.4 in the ^{13}C NMR were assignable to a cyclopentanone,[21] and an ester groups, respectively, corresponding to the IR absorption bands at 1739 cm^{-1} (cyclopentanone)

Table 4. ^{1}H NMR spectral data for genkwanines I–L (**9**–**12**)

No	**9**	**10**	**11**	**12**
1β	1.88 (1H, m)	1.93 (1H, m)	1.83 (1H, m)	2.12 (1H, m)
1a	1.53 (1H, m)	1.52 (1H, m)	1.57 (1H, m)	1.61 (1H, m)
2	1.64 (1H, m)	1.65 (1H, m)	1.70 (1H, m)	2.31 (1H,m)
3	4.06 (1H, d, 4.8)	4.26 (1H, d, 5.3)	4.21(1H, d, 5.9)	—
4	—	—	—	—
5	3.38 (1H, s)	3.21 (1H, s)	3.30 (1H, s)	3.72 (1H, s)
6	—	—	—	—
7	4.05 (1H, s)	4.09 (1H, s)	4.19 (1H, s)	4.28 (1H, d, 9.6)
8	3.20 (1H, d, 5.3)	3.09 (1H, d, 5.9)	3.15 (1H, d, 6.3)	2.78 (1H, dd, 9.6, 2.6)
9	—	—	—	—
10	2.17 (1H, dd, 13.0, 5.4)	2.23 (1H, dd, 13.0, 5.4)	2.29 (1H, dd, 13.0, 5.4)	2.29 (1H, m)
11	2.30 (1H, m)	2.31 (1H, m)	2.30 (1H, m)	2.55 (1H, q, 7.1)
12β	2.01 (1H, dd, 14.0, 8.0)	2.00 (1H, dd, 14.1, 8.0)	2.03 (1H, dd, 14.3, 8.1)	
12a	1.63 (1H, d, 14.0)	1.63 (1H, d, 14.1)	1.63 (1H, d, 14.3)	5.06 (1H, s)
13	—	—	—	—
14	5.70 (1H, d, 5.3)	5.63 (1H, d, 5.9)	5.71 (1H, d, 6.3)	5.17 (1H, d, 2.6)
15	—	—	—	—
16	5.06 (1H, s)	5.06 (1H, s)	5.05 (1H, s)	4.99 (1H, s)
	4.88 (1H, s)	4.83 (1H, s)	4.83 (1H, s)	4.97 (1H, s)
17	1.82 (3H, s)	1.79 (3H, s)	1.80 (3H, s)	1.83 (3H, s)
18	1.20 (3H, d, 6.6)	1.23 (3H, d, 7.0)	1.22 (3H, d, 7.0)	1.39 (3H, d, 6.9)
19	1.00 (3H, d, 6.7)	1.04 (3H, d, 6.7)	1.05 (3H, d, 6.7)	1.05 (3H, d, 6.0)
20	3.66 (1H, d, 11.7)	4.56 (1H, d, 11.9)	4.64 (1H, d, 11.7)	4.25 (1H, d, 11.3)
	3.61 (1H, d, 11.7)	4.38 (1H, d, 11.9)	4.54 (1H, d, 11.7)	4.00 (1H, d, 11.3)
1′	—	—	—	—
2′	—	—	—	—
3′, 7′	8.02 (2H, d, 7.6)	8.01 (2H, d, 7.7)	7.96 (2H, d, 7.9)	7.68 (2H, m)
4′, 6′	7.41 (2H, t, 7.5)	7.40 (2H, t, 7.5)	7.40 (2H, m)	7.37 (2H, m)
5′	7.54 (1H, t, 7.3)	7.56 (1H, t, 7.9)	7.55 (1H, m)	7.38 (1H, m)
1″		—	—	—
2″		5.86 (1H, d, 15.1)	—	2.00 (3H, s, OAc)
3″		7.46 (1H, dd, 15.1, 10.0)	7.90 (1H, d, 8.1)	
4″		6.10 (1H, dd, 15.2, 10.0)	7.40 (1H, m)	
5″		6.12 (1H, m)	7.55 (1H, m)	
6″		2.15 (2H, m)	7.40 (1H, m)	
7″		1.44 (2H, m)	7.90 (1H, d, 8.1)	
8″		1.30 (2H, m)		
9″		1.31 (2H, m)		
10″		0.90 (3H, t, 6.8)		

Data were measured in $CDCl_3$ at 400 MHz. *J* values were given in Hz.

Table 5. ^{13}C NMR spectral data for genkwanines I–L (**9**–**12**)

No	**9**	**10**	**11**	**12**	**No**	**9**	**10**	**11**	**12**
1	34.9	34.6	34.6	32.2	19	13.2	13.2	13.2	12.7
2	36.5	36.4	36.4	43.7	20	67.4	66.7	67.2	61.7
3	76.2	75.7	75.7	218.4	1′	167.1	166.7	166.5	117.2
4	83.2	83.3	83.5	78.4	2′	129.7	129.5	129.6	135.2
5	74.5	74.7	74.2	80.7	3′, 7′	129.9	129.8	129.9	125.8
6	77.0	76.8	77.2	75.8	4′, 6′	128.6	128.3	128.3	127.9
7	76.5	77.4	76.7	69.0	5′	133.4	133.0	133.2	129.5
8	40.2	40.1	40.3	36.8	1″		169.1	168.0	169.4 (OAc)
9	77.4	77.5	77.6	78.0	2″		117.4	129.1	21.2 (OAc)
10	54.4	54.7	54.4	47.0	3″		147.2	130.0	
11	34.5	34.1	34.1	42.7	4″		146.3	128.4	
12	37.8	37.8	37.6	78.3	5″		128.2	133.4	
13	75.2	74.8	75.3	83.6	6″		33.0	128.4	
14	76.7	77.0	77.0	76.7	7″		28.3	130.0	
15	147.4	147.7	147.8	143.0	8″		31.3		
16	111.8	111.5	111.4	113.2	9″		22.5		
17	19.1	19.2	19.2	18.9	10″		13.7		
18	18.7	18.7	18.8	18.9					

Measured in $CDCl_3$ at 100 MHz. Chemical shift values are expressed in ppm.

and 1702 cm^{-1}. The ^1H NMR spectrum displayed two olefinic proton signals at δ 4.99 (1H, s, H-16) and 4.97 (1H, s, H-16) assignable to a terminal double bond; two proton signals at δ 4.25 (1H, d, J = 11.3 Hz, H-20) and 4.00 (1H, d, J = 11.3 Hz, H-20) attributable to an oxygenated methylene; five protons at δ 7.68 (2H, m, H-3′ and H-7′), 7.37 (2H, m, H-4′ and H-6′), and 7.38 (1H, m, H-5′) ascribable to a monosubstituted phenyl.

The ^1H NMR, ^{13}C NMR, ^1H–^1H COSY and HMQC made it possible to assign the protons to their bonding carbons and figure out the structural fragments, and also confirmed a feature of daphnane-type diterpenoid for **12**. In the HMBC spectrum (Fig. 2), the correlation between the carbon signal at δ 169.5 and the proton signal at δ 5.06 (H-12) indicated that the acetoxyl was attached to the C-12; the quaternary carbon signal at δ 117.2 (C-1′) gave correlation with the signal at δ 5.17 (H-14), which in turn correlated with the carbon signals at δ 83.6 (C-13) and 78.0 (C-9), confirming the occurrence of the orthoester group; the correlations of H_3-19 with C-2 and C-3 (δ 219.6) assigned the ketone group to the C-3. The planar structure of **12** could be established by the above spectral interpretation.

The relative stereochemistry was determined by the analysis of ^1H NMR and NOESY spectra (Fig. 3). The virtual zero coupling between the H-11 and H-12 was highly diagnostic of orthoester-type daphnanes with an β-oxygenated functional group at C-12,[6,10,20,28] which was confirmed by the correlation between H-12 and H_3-18 in the NOESY spectrum. If the oxygenated functional group at C-12 is α-oriented, the coupling constant of $J_{11,12}$ will appear at about 8–11 Hz.[11] A β-hydroxyl at C-7 was deduced from the large coupling constant of $J_{7,8}$ = 9.6 Hz,[28] and was confirmed by the strong correlation between H-7 and H_3-18 in the NOESY spectrum. The strong NOESY correlation pairs of H-8/H-14 and H-14/H-11 indicated H-8, H-14, and H-11 were β-oriented. As a consequence, the NOESY correlation between H-8 and H_2-20 indicated that the C-6–OH was α-oriented. The correlation pairs of H-10/H-1α, H-10/H-2, H-10/H-5, and H-10/H-18 indicated that the H-1, H-2, H-5, H-10, and H-18 were in the α-orientation. The structure of **12** was unambiguously assigned, namely genkwanine L.

The four known diterpenoids were identified on the basis of spectral data (^1H and ^{13}C NMR, ESI) as yuanhuacine (**13**),[12] yuanhuadine (**14**),[13] yuanhuafine (**15**),[14] and yuanhuapine (**16**),[21] which were isolated previously from this plant.

2.2. Biological activity and structure–activity relationship

Compounds **1**–**16** have been tested for inhibitory activity against endothelium cell (HMEC) proliferation and cytotoxic activities against two tumor cell lines P-388 and A-549, and found to be significant and structure related. A number of the compounds showed very potent cytotoxic activities against two tumor cell lines at the IC_{50} levels of 0.15–8.40 μM, and most interestingly, five of the compounds **4**, **8**, **10**, **13**, and **14** exhibited strong activity to inhibit the endothelium cell HMEC at the IC_{50} levels of 2.90–15.0 μM (Table 6). The HMEC cell is one of the endothelial cells presenting good targets for new anticancer drug discovery, and the inhibitors against endothelium cells abnormal proliferation and migration offer hope for a new way of cancer chemotherapy.

For the compounds **1**–**8** having same basic backbone with an orthoester group, their inhibitory activity against endothelium cells proliferation and cytotoxic activities were obviously related with the acyloxyl groups at C-3 and C-20, which are: (1) acyloxyl groups at C-3/C-20 are necessary for the inhibitory activity against endothelium cells proliferation and cytotoxic activities, compounds **2**–**8** having an acyloxyl group at C-3/C-20 are active, while the compound **1** bearing two hydroxyl groups at C-3 and C-20 is inactive (defined as IC_{50} > 100 μM). (2) It seems that benzoyloxy group at C-3/C-20 is better than fatty acyloxy group, for example, compounds **4** and **8** showed more stronger activities than **2**, **3**, and **5**–**7**, and it is also observed that the fatty acyloxy group with less unsaturation will remarkable attenuate the activity, for example, in the case of compound **7**. The absence of orthoester group is detrimental to the inhibitory activity against endothelium cells proliferation and cytotoxic activities, for example, compound **11** showed less active than compound **8**. For compounds **13**–**16** with a 6,7-epoxyl group showed more potent activities, especially compounds **13** and **14** with a

Table 6. Inhibitory activity against HMEC cell and cytotoxic activities of **1**–**16**

Compounds	IC_{50} (μM)			Compounds	IC_{50} (μM)		
	P388	A-549	HMEC		P388	A-549	HMEC
1	>100	>100	>100	**9**	61.0	>100	>100
2	8.40	6.80	52.0	**10**	25.0	4.20	8.10
3	18.0	8.00	>100	**11**	>100	42.0	>100
4	8.00	0.79	2.90	**12**	>100	56.0	>100
5	75.0	8.70	>100	**13**	30.0	0.15	14.0
6	39.0	24.0	50.0	**14**	1.50	4.10	9.00
7	>100	57.0	>100	**15**	>100	1.06	81.0
8	13.0	1.60	15.0	**16**	73.0	0.42	>100
PAB[a]	0.32	0.86	1.03				

[a] Pseudo-laric acid B (PAB) was used as positive control.

long-chain fatty orthoester group showed more promising inhibitory activity against endothelium cell HMEC and cytotoxic activities, and these four compounds seem more sensitive to A-549 cell line.

3. Conclusion

Sixteen highly oxygenated novel daphnane-type diterpenoids **1**–**16** were isolated from a well-known traditional Chinese medicine, the bud of *D. genkwa*. Among them, genkwanines A–L (**1**–**12**) are new compounds. The most pronounced biological activities were seen for compounds **4**, **8**, **10**, **13**, and **14** in the inhibitory activity against endothelium cell (HMEC) proliferation and cytotoxic tests, especially compounds **4**, **10**, and **14** with remarkable inhibitory activity (IC_{50}: 2.90, 8.10, and 9.00 μM, respectively) against the endothelial cell line HMEC. Clearly, the potent inhibitory activity against endothelium cells proliferation and cytotoxic activities of these diterpenoids isolated and tested in this study make them suitable as a rich resource/and lead structures for the development of potential anticancer agents.

4. Experimental

4.1. General experimental procedures

IR spectra were recorded on a Perkin–Elmer 577 spectrometer with KBr disc. Optical rotations were determined on a Perkin–Elmer 341 polarimeter at room temperature. NMR spectra were measured on a Bruker AM-400 spectrometer with TMS as internal standard. EIMS (70 eV) was carried out on a Finnigan MAT 95 mass spectrometer, and ESIMS was recorded on a Finnigan LCQ^{DECA} Mass spectrometer. All solvents used were of analytical grade (Shanghai Chemical Plant, Shanghai, People's Republic of China). Silica gel (230–400 mesh) was used for column chromatography, and pre-coated silica gel GF_{254} plates (Qingdao Marine Chemical Plant, Qingdao, People's Republic of China) were used for TLC. C_{18} reverse-phased silica gel (150–200 mesh, Merck) and MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd.) were also used for column chromatography.

4.2. Plant material

The bud of *D. genkwa* was collected from Shanghai area and identified by Dr. Ying Xiang of Shanghai Institute of Materia Medica. A voucher specimen (Accession number: Dgenk-2002-1Y) was deposited at Shanghai Institute of Materia Medica, CAS, People's Republic of China.

4.3. Extraction and isolation

The powder of the flower bud of *D. genkwa* (5.0 kg) was extracted with 95% ethanol extensively at room temperature to give a dark crude extract (534 g), which was then dissolved in water (3 L) to form a suspension, and partitioned with $CHCl_3$ to afford $CHCl_3$ soluble fraction C (157 g). The fraction C was subjected to column chromatography eluted with petroleum ether containing increasing amount of acetone to offer fractions 1–5. The fraction 1 (6.7 g) was purified by silica gel column chromatography eluted with petroleum ether–acetone (6:1) to yield compound **13** (55 mg) and **14** (22 mg). The fraction 2 (7.3 g) was subjected to a column of MCI gel CHP20P eluted with MeOH–H_2O (5:5–10:0) to afford fractions 2a and 2b; the fraction 2a (680 mg) was further chromatographied over a silica gel column and eluted with $CHCl_3$–MeOH (60:1) to give compound **4** (12 mg) and **8** (14 mg); the fraction 2b (750 mg) was purified by C_{18} silica gel column eluted with 85% MeOH in water to afford compound **2** (20 mg), **3** (11 mg), **5** (13 mg), **6** (15 mg) and **7** (6.5 mg). The fraction 3 (11.3 g) was applied to a column of MCI gel CHP20P eluted with MeOH–H_2O (5:5–8:2) to collect the major fraction, which was further purified by a reversed phase C_{18} silica gel column eluted with 70% MeOH in water to afford compound **15** (45 mg) and **16** (60 mg). The fraction 4 (7.3 g) was subjected to a column of MCI gel CHP20P eluted with MeOH–H_2O (5:5–10:0) to afford fractions 4a and 4b; compound **10** (45 mg) was obtained from fraction 4a (1.1 g) by silica gel column chromatography eluted with $CHCl_3$–MeOH (20:1); fraction 4b (870 mg) was purified over silica gel column using $CHCl_3$–MeOH (25:1) for elution to give **11** (11 mg). The fraction 5 (5.3 g) was subjected to a column containing MCI gel CHP20P eluted with MeOH–H_2O (5:5–10:0) to afford fraction 5a (1.1 g), which was then applied to silica gel column chromatography eluted by $CHCl_3$–MeOH (15:1) to give **1**(130 mg), **9** (235 mg), and **12** (50 mg).

4.3.1. Genkwanine A (1). White amorphous powder; $[\alpha]_D^{20}$ +42.7 (*c* 0.73, $CHCl_3$); IR (KBr) ν_{max} 3415 (OH), 2958, 1647, 1452, 1350, 1078, 1029, 980, 916, 696 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; positive ESIMS *m/z* 505 $[M+H]^+$, negative ESIMS *m/z* 503 $[M-H]^-$; EIMS *m/z* (%): 504 $[M]^+$ (2), 473 $[M-CH_2OH]^+$ (8), 455 (2), 353 (28), 291 (20), 273 (8), 151 (12), 105 (100), 77 (22). HREIMS *m/z*: 504.2336 (calcd for $C_{27}H_{36}O_9$, 504.2359).

4.3.2. Genkwanine B (2). White amorphous powder; $[\alpha]_D^{20}$ −11.0 (*c* 0.61, $CHCl_3$); IR (KBr) ν_{max} 3430 (OH), 2929, 1689 (C=O) 1641 (C=C), 1452, 1350, 1313, 1122, 1078, 1030, 999, 696 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; positive ESIMS *m/z* 655 $[M+H]^+$, negative ESIMS *m/z* 653 $[M-H]^-$; EIMS *m/z* (%): 654 $[M]^+$ (2), 636 $[M-H_2O]^+$ (10), 623 $[M-CH_2OH]$ (11), 503 $[M-C_9H_{15}CO]^+$ (12), 151 (45), 105 (100), 77 (42). HREIMS *m/z*: 654.3393 (calcd for $C_{37}H_{50}O_{10}$, 654.3404).

4.3.3. Genkwanine C (3). White amorphous powder; $[\alpha]_D^{20}$ −15.3 (*c* 0.69, $CHCl_3$); IR (KBr) ν_{max} 3434 (OH), 2927, 1680 (C=O), 1616 (C=C), 1452, 1352, 1272, 1122, 1078, 1030, 916, 696 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; positive ESIMS *m/z* 653 $[M+H]^+$, negative ESIMS *m/z* 651 $[M-H]^-$; EIMS *m/z* (%): 652 $[M]^+$ (2), 634 $[M-H_2O]^+$ (8) 621 $[M-CH_2OH]^+$ (12), 503 $[M-C_9H_{13}CO]^+$ (10), 429 (26), 149 (49), 105 (100), 77

(42), 57 (65). HREIMS *m/z*: 652.3276 (calcd for $C_{37}H_{48}O_{10}$, 652.3247).

4.3.4. Genkwanine D (4). White amorphous powder; $[\alpha]_D^{20}$ +13.4 (*c* 1.3, $CHCl_3$); IR (KBr) ν_{max} 3438 (OH), 2929, 1697 (C=O), 1616 (C=C), 1452, 1350, 1288, 1124, 1078, 1028, 977, 711 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; positive ESIMS *m/z* 609 $[M+H]^+$, negative ESIMS *m/z* 607 $[M-H]^-$; EIMS *m/z* (%): 608 $[M]^+$ (1), 577 $[M-CH_2OH]$ (8), 503 (28), 105 (100), 77 (22). HREIMS *m/z*: 608.2619 (calcd for $C_{34}H_{40}O_{10}$, 608.2622).

4.3.5. Genkwanine E (5). White amorphous powder; $[\alpha]_D^{20}$ +50.3 (*c* 1.2, $CHCl_3$); IR (KBr) ν_{max} 3431 (OH), 2929, 1716 (C=O), 1616 (C=C), 1452, 1350, 1120, 1078, 1029, 980, 916, 696 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; Positive ESIMS *m/z* 653 $[M+H]^+$, negative ESIMS *m/z* 651 $[M-H]^-$; EIMS *m/z* (%): 652 $[M]^+$ (5), 634 $[M-H_2O]^+$ (12) 621 $[M-CH_2OH]^+$ (15), 503 $[M-C_9H_{13}CO]^+$ (14), 429 (20), 149 (40), 105 (100), 77 (40), 57 (60). HREIMS *m/z*: 652.3259 (calcd for $C_{37}H_{48}O_{10}$, 652.3247).

4.3.6. Genkwanine F (6). White amorphous powder; $[\alpha]_D^{20}$ +67.1 (*c* 1.9, $CHCl_3$); IR (KBr) ν_{max} 3427 (OH), 2929, 1712 (C=O), 1643 (C=C), 1452, 1348, 1265, 1137, 1078, 980, 916, 696 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; Positive ESIMS *m/z* 655 $[M+H]^+$, negative ESIMS *m/z* 653 $[M-H]^-$; EIMS *m/z* (%): 654 $[M]^+$ (4), 636 $[M-H_2O]^+$ (14), 621 $[M-CH_2OH]$ (17), 503 $[M-C_9H_{15}CO]^+$ (9), 151 (40), 105 (100), 77 (40). HREIMS *m/z*: 654.3379 (calcd for $C_{37}H_{50}O_{10}$, 654.3404).

4.3.7. Genkwanine G (7). White amorphous powder; $[\alpha]_D^{20}$ +56.9 (*c* 0.41, $CHCl_3$); IR (KBr) ν_{max} 3429 (OH), 2927, 1709 (C=O), 1614 (C=C), 1452, 1350, 1269, 1120, 1078, 1030, 979, 916, 696 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; Positive ESIMS *m/z* 657 $[M+H]^+$, negative ESIMS *m/z* 655 $[M-H]^-$; EIMS *m/z* (%): 656 $[M]^+$ (2), 625 $[M-CH_2OH]^+$ (12), 503 $[M-C_9H_{17}CO]^+$ (15), 153 (23), 105 (100), 77 (42), 57 (65). HREIMS *m/z*: 608.2620 (calcd for $C_{34}H_{40}O_{10}$, 608.2622).

4.3.8. Genkwanine H (8). White amorphous powder; $[\alpha]_D^{20}$ +42.5 (*c* 1.6, $CHCl_3$); IR (KBr) ν_{max} 3427 (OH), 2929, 1716 (C=O), 1616 (C=C), 1452, 1350, 1276, 1118, 1078, 1028, 980, 916, 713 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; Positive ESIMS *m/z* 609 $[M+H]^+$, negative ESIMS *m/z* 607 $[M-H]^-$; EIMS *m/z* (%): 608 $[M]^+$ (4), 577 $[M-CH_2OH]$ (10), 503 (28), 105 (100), 77 (22). HREIMS *m/z*: 608.2625 (calcd for $C_{34}H_{40}O_{10}$, 608.2622).

4.3.9. Genkwanine I (9). White amorphous powder; $[\alpha]_D^{20}$ +17.5 (*c* 0.77, $CHCl_3$); IR (KBr) ν_{max} 3407 (OH), 2928, 1706 (C=O), 1616 (C=C), 1452, 1280, 1116, 1078, 1028, 980, 916, 758, 712 cm^{-1}; ^1H and ^{13}C NMR, see Tables 4 and 5; Positive ESIMS *m/z* 523 $[M+H]^+$, negative ESIMS *m/z* 521 $[M-H]^-$; EIMS *m/z*: M^+ (no see), 504 $[M-H_2O]^+$ (4), 473 $[M-H_2O-CH_2OH]^+$ (28) 455 (12), 400 (12), 122 (20), 105 (100), 77 (30). HREIMS *m/z*: 504.2358 $[M-H_2O]^+$ (calcd for $C_{27}H_{36}O_9$, 504.2359).

4.3.10. Genkwanine J (10). White amorphous powder; $[\alpha]_D^{20}$ +21.7 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3419 (OH), 2929, 1701 (C=O), 1637 (C=C), 1452, 1278, 1112, 1026, 711 cm^{-1}; ^1H and ^{13}C NMR, see Tables 4 and 5; Positive ESIMS *m/z* 673 $[M+H]^+$, negative ESIMS *m/z* 671 $[M-H]^-$; EIMS *m/z* (%): 672 $[M]^+$ (2), 654 $[M-H_2O]^+$ (8) 641 $[M-CH_2OH]^+$ (12), 503 $[M-H_2O-C_9H_{15}CO]^+$ (15), 429 (26), 151 (33), 105 (100), 77 (52), 57 (65). HREIMS *m/z*: 672.3505 (calcd for $C_{37}H_{52}O_{11}$, 672.3510).

4.3.11. Genkwanine K (11). White amorphous powder; $[\alpha]_D^{20}$ +45.2 (*c* 0.99, $CHCl_3$); IR (KBr) ν_{max} 3423 (OH), 2931, 1716, 1616 (C=C), 1450, 1317, 1280, 1116, 1078, 1026, 711 cm^{-1}; ^1H and ^{13}C NMR, see Tables 4 and 5; Positive ESIMS *m/z* 626 $[M+H]^+$, negative ESIMS *m/z* 625 $[M-H]^-$; EIMS *m/z* (%): 626 $[M]^+$ (5), 608 $[M-H_2O]^+$ (8) 595 $[M-CH_2OH]^+$ (12), 503 $[M-H_2O-C_6H_5CO]^+$ (13), 105 (100), 77 (40), 57 (45). HREIMS *m/z*: 626.2730 (calcd for $C_{34}H_{42}O_{11}$, 626.2727).

4.3.12. Genkwanine L (12). White amorphous powder; $[\alpha]_D^{20}$ +103.5 (*c* 0.46, $CHCl_3$); IR (KBr) ν_{max} 3442 (OH), 2971, 1743 (C=O), 1645 (C=C), 1452, 1351, 1234, 1083, 1028, 916, 698 cm^{-1}; ^1H and ^{13}C NMR, see Tables 4 and 5; Positive ESIMS *m/z* 561 $[M+H]^+$, negative ESIMS *m/z* 559 $[M-H]^-$; EIMS *m/z* (%): 560 $[M]^+$ (4), 542 (10), 517 (18) 455 (23), 105 (100), 77 (42), 57 (65), 43 (51). HREIMS *m/z*: 560.2267 (calcd for $C_{29}H_{36}O_{11}$, 560.2258).

4.4. Biological assays of inhibiting HMEC cell proliferation and cytotoxicity

Compounds **1**–**16** were tested for the inhibitory activity against endothelium cell HMEC and cytotoxic activities against P-388 and A-549 tumor cell lines according to standard protocols,[29,30] and the natural anticancer agent pseudo-laric acid B[31] was used as positive control. Purities of all the tested compounds **1**–**16** checked by HPLC analyses are higher than 95%.

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