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Daphnane-type diterpenes with inhibitory activities against human cancer cell lines from *Daphne genkwa*

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ABSTRACT

Four new daphnane-type diterpenes, genkwadanes A–D (**1**–**4**), together with 19 known ones, were isolated from ethanol extract of the flower buds of *Daphne genkwa*. Their structures were determined on the basis of extensive spectroscopic data. Among them, daphnane-type diterpene with a 1,10-double bond (**1**) was isolated from this plant for the first time. The cytotoxicity of all compounds **1**–**23** against the 10 selected human cancer cell lines was assayed. A number of compounds exhibited significant activities against the 10 cancer cell lines (IC_{50} < 9.56 μM). and most interestingly, all the compounds revealed preferred cytotoxicities on the HT-1080 cell line and displayed much stronger inhibitory activities (IC_{50} < 29.94 μM) compared with positive control 5-fluorouracil (IC_{50} = 35.62 μM), particularly, compounds **9**–**11**, **13**, **16** and **19** exhibited the strongest cytotoxicity activities against the HT-1080 cell line (IC_{50} < 0.1 μM).

Daphnane-type diterpenes are the major biologically active constituents existing primarily in Thymelaeaceae and a few in Euphorbiaceae.[1–11] *Daphne genkwa* Sieb. et Zucc. (Thymelaeaceae) is a toxic shrub widespread in China and Korea. Its flower buds are used as a traditional Chinese medicine for diuretic, antitussive, expectorant, and anticancer purposes.[12] Previous studies on the chemical components in the flower buds of this plant have identified diterpenoids,[7–11,13–19] flavonoids,[20–22] and lignans[23] with anticancer, antifertility, pesticidal and irritant activities.

In the course of searching for novel bioactive diterpenes from *Daphne genkwa*, four new daphnane-type diterpenes, genkwadanes A–D (**1–4**), together with 19 known ones pimelea factor P2 (**5**),[24] wikstroelide E (**6**),[25] pimelotide A (**7**),[26] pimelotide C (**8**),[27] yuanhuadine (**9**),[13] yuanhuafine (**10**),[14] genkwadaphnine (**11**),[15] yuanhuacine (**12**),[13] simplexin (**13**),[28] yuanhuahine (**14**),[7] isoyuanhuadine (**15**),[8] yuanhuapine (**16**),[16] yuanhuatine (**17**),[17] orthobenzoate 2 (**18**),[29] genkwanine M (**19**),[9] yuanhuaoate B (**20**),[10] genkwanine F (**21**),[11] yuanhuaoate E (**22**),[18,19] and genkwanine J (**23**),[11] were isolated from the flower buds of *D. genkwa*.[30] Additionally, the cytotoxicity of all the compounds **1–23** was evaluated against 10 human cancer cell lines HeLa (human cervical cancer cell line), HepG2 (human hepatocellular carcinoma cell line), HT-1080 (human sarcoma cell line), HCT116 (human colon cancer cell line), A375-S2 (human melanoma cell line), MCF-7 (human breast adenocarcinoma cell line), A549 (human lung adenocarcinoma cell line), U-937 (human histiocytic lymphoma cell line), K562 (human chronic myelogenous leukemia cell line), and HL60 (human promyelocytic leukemia cell line).

The air-dried flower buds of *D. genkwa* (8 kg) were refluxed with 95% EtOH for 3 × 30 L × 2 h. The solvent was evaporated under vacuum. Then, the extract (867 g) was suspended in H_2O and partitioned with CH_2Cl_2. The CH_2Cl_2 extract (271.5 g)[31] was subjected to the extensive procedures of chromatographic separations to afford 23 daphane-type diterpenoids **1–23** (Fig. 1). Their structures were elucidated on the basis of comprehensive spectroscopic data.

Compound **1**[32] was isolated as a white amorphous powder. Its HRESIMS (positive-ion mode) exhibited a pseudomolecular ion peak at *m/z* 643.2150 $[M+Na]^+$ (calcd for $C_{34}H_{36}O_{11}Na$, 643.2150), corresponding to the molecular formula $C_{34}H_{36}O_{11}$. The ^1H NMR spectrum revealed a singlet methyl at δ_H 1.91, two doublet ones at δ_H 1.30 (3H, d, *J* = 7.2 Hz) and 1.19 (3H, d, *J* = 7.2 Hz), three olefinic protons at δ_H 5.09 (1H, s), 5.04 (1H, s) and 6.28 (1H, d, *J* = 1.2 Hz), together with a oxygenated methylene at δ_H 4.09 (1H, d, *J* = 12.0 Hz) and 3.77 (1H, d, *J* = 12.0 Hz). Accordingly, The ^{13}C NMR of **1** showed carbon resonances due to three methyl moieties (δ_C 18.9, 14.0 and 13.2), a terminal double bond (δ_C 142.5 and 114.2), an endocyclic double bond (δ_C 141.4, 131.5),

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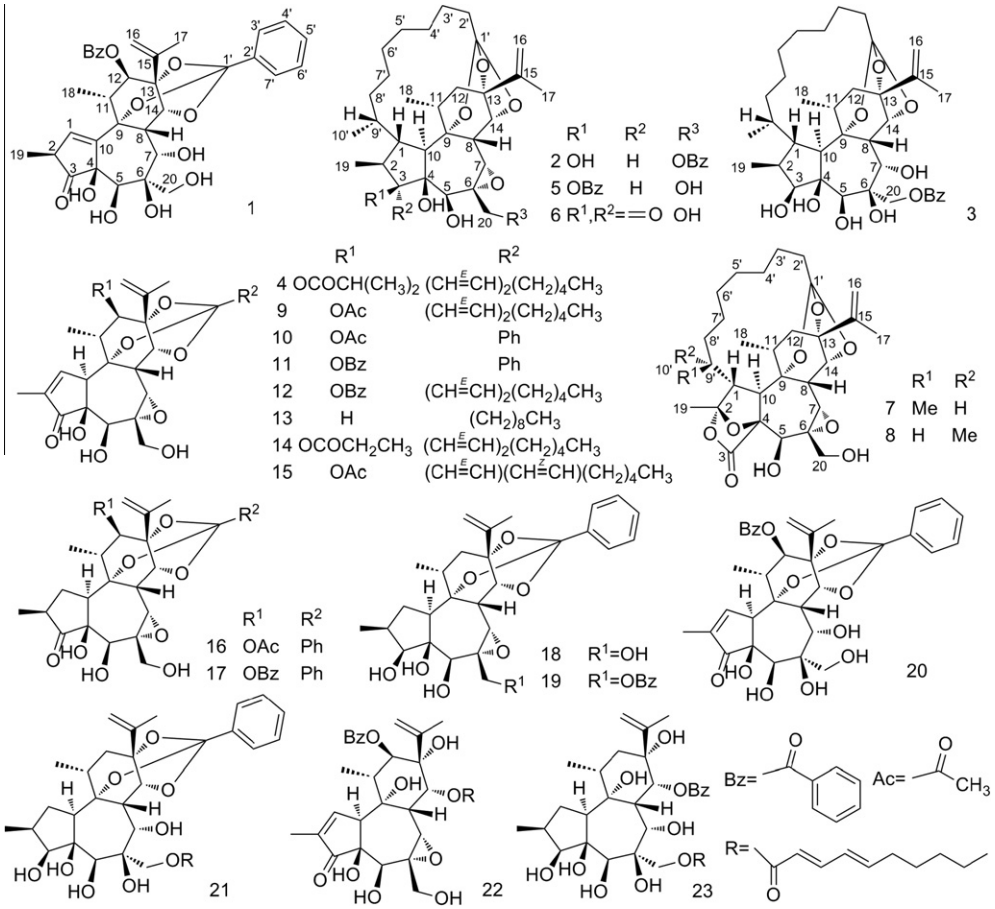

Figure 1. Structures of compounds **1**–**23**.

Table 1
^{1}H and ^{13}C NMR data for compound **1** (in $CDCl_3$, 600/150 MHz)

Position	^{1}H	^{13}C	Position	^{1}H	^{13}C
1	6.28, d (1.2)	131.5	16	5.04, s 5.09, s	114.2
2	3.31, m	42.1	17	1.91, s	18.9
3		213.3	18	1.30, d (7.2)	14.0
4		84.6	19	1.19, d (7.2)	13.2
5	4.16, s	72.0	20	4.09, d (12.0)	70.1
				3.77, d (12.0)	
6		76.5	1′		117.9
7	4.39, s	78.8	2′		134.6
8	3.48, d (2.4)	35.5	3′, 7′	7.68, m	125.9
9		77.6	4′, 6′	7.43, m	128.6
10		141.4	5′	7.45, m	130.1
11	2.39, q (7.8)	46.4	1″		165.1
12	5.51, s	75.6	2″		129.6
13		85.3	3″, 7″	7.96, m	129.5
14	5.13, d (2.4)	82.5	4″, 6″	7.44, m	128.3
15		142.5	5″	7.57, m	133.4

Coupling constants (*J*) in Hz are given in parentheses. Chemical shift values are expressed in ppm.

a hydroxymethyl (δ_C 70.1) and a ketone carbonyl (δ_C 213.3). In addition, the presence of a characteristic quaternary carbon resonance at δ_C 117.9 suggested that **1** is a daphnane-type diterpene orthoester.[3] Comparison of its NMR (Table 1) and MS spectroscopic data with those of yuanhuaoate B (**20**),[10] showed that **1** was the geometric isomer of **20**. However, the distinctive differences in the NMR spectra were observed between the A ring resonances of **1** and those of yuanhuaoate B (**20**). Namely, in the ^{13}C NMR spectrum, **1** showed resonance for an upfield shifted carbon at δ_C 42.1 (C-2) and a downfield shifted carbon at δ_C 141.4 (C-10) instead of δ_C 137.2 and δ_C 50.5 in **20**, respectively, which suggested a 1,10 double bond in A ring instead of a 1, 2 one in **20**. In the ^{1}H NMR spectrum, a multiplet proton at δ_H 3.31 (H-2) assignable to H-19 was observed, and the proton signal of H-10 was absent, meanwhile, the HMBC correlations from H-11 (δ_H 2.39) and H-2 (δ_H 3.31) to C-10 (δ_C 141.4) (Fig. 2), which further indicated the presence of a 1,10 double bond in **1**. The relative configuration of the daphnane-type diterpene scaffold in compound **1** was established by NOESY correlations and molecular modeling and determined to be similar to those of yuanhuaoate B (**20**) with β-orientations of H-7, H-8, H-14 and H_3-19, and α-orientations of H-12 and H_3-18. It is important to note that the presence of correlation of δ_H 1.19 (H_3-19) with δ_H 1.30 (H_3-18) in the NOESY spectrum, and in the molecular modeling of **1** (Fig. 4), only when the H_3-19 was β-oriented, the NOESY correlations of H_3-19/H_3-18 could be observed. Thus, a β-orientation of the 19-methyl moiety was confirmed, which is also based on the fact that all naturally occurring daphnane esters isolated to date are β-oriented methyl group (C-19) at C-2.[3] Consequently, the structure and relative configuration of compound **1** was elucidated, and named genkwadane A.

Compound **2**[33] was also obtained as a white amorphous powder, with a molecular formula $C_{37}H_{50}O_9$ established from HRESIMS (*m*/*z* 639.3528, $[M+H]^+$, calcd for $C_{37}H_{51}O_9$, 639.3528) and NMR data. The ^{1}H and ^{13}C NMR spectra of **2** indicated the presence of four methyl groups [δ_C 19.0, δ_H 1.77 (3H, s); δ_C 21.2, δ_H 1.27 (3H, d, J = 7.2 Hz); δ_C 14.7, δ_H 1.07 (3H, d, J = 6.6 Hz); δ_C 19.0, δ_H 0.91 (3H, d, J = 7.2 Hz)], a terminal double bond [δ_C 146.9, 111.1; δ_H 5.00 (1H, s), 4.88 (1H, s)], a hydroxymethyl [δ_C 68.5; δ_H 5.03 (1H, d, J = 12.0 Hz), 4.05 (1H, d, J = 12.0 Hz)] and a benzoyl moiety

Table 2
^{1}H and ^{13}C NMR data for compounds **2–3** (in $CDCl_3$, 400/100 MHz)

Position	2		3	
	^{1}H	^{13}C	^{1}H	^{13}C
1	2.04, m	49.2	2.27, m	50.1
2	1.60, o	35.7	1.83, o	36.0
3	3.79, d (4.8)	79.4	4.14, m	78.6
4		78.8		83.9
5	3.76, s	71.2	3.63, s	75.3
6		60.1		77.2
7	3.34, s	64.3	4.45, s	76.2
8	3.03, d (2.4)	37.0	2.42, d (2.4)	36.1
9		81.3		82.5
10	2.79, d (12.6)	48.8	2.74, d (11.2)	50.9
11	2.38, m	35.7	2.60, m	35.6
12	2.19, m 1.67, m	36.6	2.18, m 1.68, m	36.8
13		84.0		84.8
14	4.24, d (3.0)	82.0	4.32, o	84.6
15		146.9		146.3
16	5.00, s 4.88, s	111.1	4.98, s 4.88, s	111.2
17	1.77, s	19.0	1.75, s	18.9
18	1.27, d (7.2)	21.2	1.31, d (6.8)	21.6
19	1.07, d (6.6)	14.7	1.04, d (7.2)	14.7
20	5.03, d (12.0)	68.5	4.91, d (12.0)	67.3
	4.05, d (12.0)		4.59, d (12.0)	
1′		119.9		119.7
2′	1.91, o 1.86, m	33.9	1.93, m 1.84, o	33.7
3′	1.55, o 1.26, o	27.4	1.55, o 1.28, o	27.6
4′	1.56, o 1.24, o	24.7*	1.32, o	25.6
5′	1.34, o 1.28, o	24.2*	1.45, o 1.23, o	24.2
6′	1.56, o 1.24, o	24.5*	1.54, o 1.31, o	24.7
7′	1.12, m 0.99, m	19.6	1.11, m 0.98, m	19.8
8′	1.34, o 1.28, o	24.0*	1.43, m 1.26, o	22.5
9′	1.60, o	37.9	1.64, o	38.0
10′	0.91, d (7.2)	19.0	0.90, d (7.2)	18.5
1″		166.8		167.2
2″		130.0		129.9
3″, 7″	8.08, m	129.9	8.07, m	129.8
4″, 6″	7.46, m	128.5	7.45, m	128.5
5″	7.57, m	133.3	7.57, m	133.2

o: The abbreviation for overlapped. * Interchangeable.

Table 3
^{1}H and ^{13}C NMR data for compound **4** (in $CDCl_3$, 300/75 MHz)

Position	^{1}H	^{13}C	Position	^{1}H	^{13}C
1	7.57, s	160.8	19	1.79, d (1.5)	10.2
2		137.2	20	3.92, d (12.3)	65.2
3		209.8		3.83, d (12.3)	
4		72.5	1′		117.3
5	4.24, s	72.2	2′	5.65, d (15.3)	122.5
6		60.8	3′	6.66, dd	135.4
7	3.56, s	64.4		(10.5, 15.3)	
8	3.47, d (2.7)	35.9	4′	6.04, dd	128.9
9		78.4		(10.5, 15.3)	
10	3.83, o	47.8	5′	5.85, m	139.6
11	2.33, m	44.4	6′	2.10, m	32.9
12	4.96, s	78.2	7′	1.39, m	29.0
13		84.0	8′	1.26, m	31.6
14	4.77, d (2.4)	80.7	9′	1.27, m	22.8
15		143.3	10′	0.88, s	14.3
16	4.94, s 5.01, s	113.7	1″		176.0
17	1.83, s	19.2	2″	2.45, m	34.2
18	1.30, d (7.2)	18.5	3″, 4″	1.10, d (6.9)	19.0

(δ_C 166.8, 130.0, 129.9 × 2, 128.8 × 2, 133.3), especially a typical quaternary carbon resonance at δ_C 119.9 (C-1′), deducing compound **2** is a 1-alkyldaphnane derivatives.[3] The NMR spectra of **2** resembled those of pimelea factor P2 (**5**),[24] suggested that they had the same molecular skeleton and substituent group. Detailed comparison of its ^{1}H NMR spectrum with that of **5** showed that the two proton signals of H-20 in **5** were shifted downfield to δ_H 5.03 (H-20β) and 4.05 (H-20α) in **2**, indicating that the benzoyl group at C-20 instead of C-3 in **2**. The HMBC cross-peak from

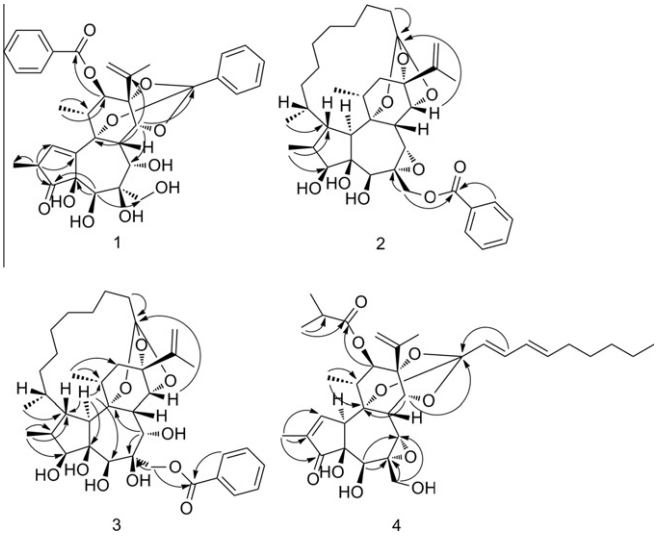

Figure 2. Key HMBC correlations of compounds **1–4**.

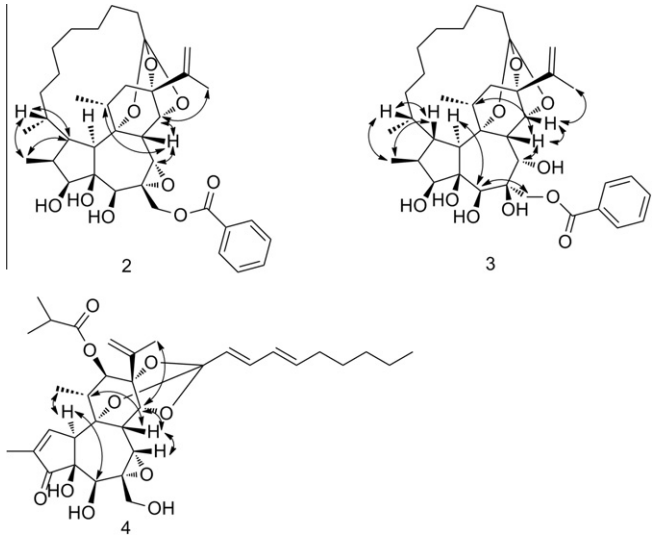

Figure 3. Key NOESY correlations of compounds **2–4**.

H-20β (δ_H 5.03) to C-6 (δ_C 60.1) and C-1″ (δ_C 166.8) confirmed this deduction. The configuration of the 1-alkyldaphnane scaffold in **2** was elucidated by NOESY correlations to be the same as those of pimelea factor P2 (**5**). Thus, the structure of **2** was unambiguously established, and named genkwadane B.

Compound **3**[34] was assigned to be $C_{37}H_{52}O_{10}$, according to the HRESIMS (*m/z* 657.3637 $[M+H]^+$, calcd for $C_{37}H_{53}O_{10}$, 657.3633) and NMR data. The ^{1}H and ^{13}C NMR spectra of **3** and **2** were similar, suggesting that **3** is a 1-alkyldaphnane derivatives as well. Detailed comparison of the ^{13}C NMR spectrum showed that the carbon resonances at δ_C 60.1 (C-6) and 64.3 (C-7) were shifted downfield to δ_C 77.2 and 76.2, indicating that a 6,7-epoxide moiety was oxygenated into two hydroxyl groups in compound **3**. This deduction was further confirmed by HSQC and HMBC spectra. The HMBC correlations from H-7 (δ_H 4.45) to C-6 (δ_C 77.2) and from H-20β (δ_H 4.91) to C-6 (δ_C 77.2) were observed. In the NOESY spectrum of **3**, the H-7 (δ_H 4.45) was significant correlated with H-8 (δ_H 2.42) and H-14 (δ_H 4.32), suggesting that the H-7 was in the β-orientation. The H-5 (δ_H 3.63) showed correlations with H_2-20 protons at δ_H 4.91 and 4.59 indicated that they were at the same side

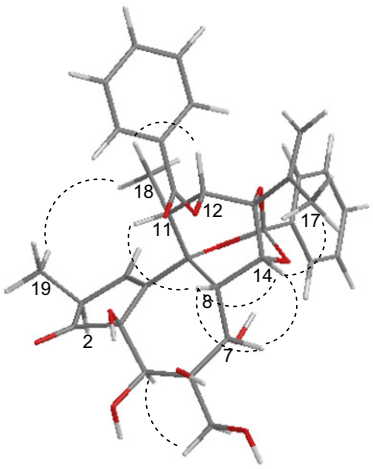

Figure 4. Significant NOESY correlations of genkwadane A (**1**).

and the hydroxyl at C-6 was β-configuration. Thus compound **3** was elucidated as shown in Figure 1, and named genkwadane C.

Compound **4**[35] was obtained as a white amorphous powder, and its molecular formula was formulated as $C_{34}H_{46}O_{10}$ according to its HRESIMS (*m/z* 615.3164 $[M+H]^+$, calcd for $C_{34}H_{47}O_{10}$, 615.3164). The closely similar ^1H and ^{13}C NMR spectra of **4** to those of yuanhuahine (**14**)[7] suggesting that it had a similar structure to compound **14**. The main difference among them was that the presence of an *iso*-propyl group at C-2″ in **4** instead of an ethyl group in **14**. This deduction was confirmed by the key HMBC correlations from H_3-3″ and H_3-4″ to C-2″ (34.2) and C-1″ (176.0). The daphnane-type diterpene skeleton configuration of **4** was established by NOESY correlations and determined to be the same as the skeleton of compounds **9–15** (Fig. 3). Therefore, the structure of **4** was assigned as shown in Figure 1, and named genkwadane D.

Phytochemical study of the flower buds of *D. genkwa* has resulted in the isolation of four new daphnane-type diterpenes, genkwadanes A–D (**1–4**), and 19 known ones (**5–23**). The new compounds (**2–3**) and pimelea factor P2 (**5**), wikstroelide (**6**) belong to 1-alkyldaphnane type diterpenes, the known compounds pimelotide A (**7**) and C (**8**) are daphnane ketal-lactone type diterpenes, the new compounds genkwadane A (**1**) and D (**4**) and the known ones (**9–21**) belong to daphnane-type diterpene orthoesters, and the two known compounds yuanhuaoate E (**22**) and genkwanine J (**23**) are daphnane diterpenes without 9,13,14-orthoester. It is worthwhile to mention that a daphnane-type diterpene with a 1,10 double bond (**1**) was isolated from this plant for the first time.

Previous studies on this plant had revealed that daphnane-type diterpenes exhibited proliferating inhibitory activity against human cancer line,[7,36] all compounds (**1–23**) were evaluated for their in vitro cytotoxicity against HeLa, HepG2, HT-1080, HCT116, A375-S2, MCF-7, A549, U-937, K562 and HL60 cell lines by the MTT method.[37] The results showed that the majority of compounds exhibited cytotoxicity in all 10 cell lines (see Table 4). Specially, compounds **4** and **12** possessed pronounced cytotoxicities against the cell lines K562 and HepG2 at the IC_{50} levels of 5.11 and 8.04 μM, respectively. It is interesting that both compound **12** and **15** revealed significant activities with IC_{50} of 5.56 and 0.64 μM against the human cervical HeLa cells, respectively. Compounds **8–9**, **11–12**, **17** and **20** exhibited potent cytotoxicities to the MCF-7 cell line with IC_{50} values in the range of 0.37–7.36 μM. Three compounds **12**, **17** and **19** showed obvious inhibitory effect against the A375-S2 cell line at the IC_{50} levels of 8.72, 9.31 and 3.62 μM, respectively. In addition, all the compounds revealed preferred cytotoxicities on the HT-1080 cell line as shown in Table 4, compared with positive control 5-fluorouracil (IC_{50} = 35.62 μM), they displayed much stronger inhibitory activities (IC_{50} < 29.94 μM), particularly compounds **9–11**, **13**, **16** and **19** exhibited the strongest activities to the HT-1080 cell line (IC_{50} < 0.1 μM), and compounds **2–4**, **7**, **12** and **20** showed very

Table 4
Inhibition effects of compounds **1–23** on the growth of tumor cells in vitro (IC_{50}, μM)

Compound	IC_{50} (μM) Cell line									
	HeLa	MCF-7	HepG2	HCT116	A549	A375-S2	HT1080	HL60	U937	K562
1	24.76	33.70	27.85	>50	26.95	14.29	16.10	27.70	29.87	46.88
2	>50	19.91	11.35	28.92	18.78	29.36	9.56	18.08	16.49	18.69
3	25.15	>50	28.55	>50	44.99	28.64	7.71	>50	>50	>50
4	>50	20.80	17.75	25.15	23.05	24.87	4.55	29.99	27.61	5.11
5	18.30	>50	19.12	19.59	17.03	25.67	13.22	17.86	16.68	25.18
6	17.06	>50	11.37	29.93	19.26	29.03	15.14	22.11	19.46	24.04
7	28.14	20.29	28.11	35.98	35.66	>50	0.83	>50	>50	>50
8	23.25	7.36	25.16	26.49	>50	22.98	20.79	34.08	22.92	26.28
9	17.06	4.11	26.27	32.65	>50	15.75	<0.1	30.05	14.66	22.16
10	42.37	>50	>50	37.53	>50	>50	<0.1	>50	>50	>50
11	19.09	2.36	24.10	29.28	19.09	16.88	<0.1	24.35	13.22	22.53
12	5.56	2.39	8.04	22.96	13.11	8.72	2.02	26.81	11.79	16.08
13	21.72	25.41	38.82	>50	26.96	10.65	<0.1	>50	>50	>50
14	25.52	>50	13.50	46.79	18.78	>50	28.80	22.70	27.36	29.35
15	0.64	23.45	30.00	32.31	24.12	21.52	14.89	17.72	17.43	17.54
16	50.00	23.62	>50	>50	>50	14.24	<0.1	>50	>50	>50
17	20.31	0.37	24.05	24.87	12.46	9.31	14.35	26.02	12.35	24.64
18	31.71	12.36	48.91	49.86	>50	47.23	24.49	>50	>50	42.31
19	20.17	25.28	29.90	28.14	23.38	3.62	<0.1	30.85	18.11	20.16
20	20.97	0.37	25.09	26.47	15.91	23.34	0.59	27.03	14.43	21.31
21	26.17	>50	22.66	27.43	22.29	25.41	29.94	20.50	23.84	24.19
22	16.67	>50	11.05	16.44	13.34	26.51	18.40	18.51	11.62	29.03
23	21.05	>50	17.85	35.77	20.91	23.69	22.91	10.06	15.63	24.54
5-Fu[a]	7.34	11.50	40.34	27.76	44.05	6.67	35.62	10.05	23.93	23.55

[a] 5-Fu (5-fluorouracil) was used as positive control.

potent cytotoxicities to it at the IC_{50} levels of 9.56, 7.71, 4.55, 0.83, 2.02 and 0.59 μM, respectively.

According to the structural features of **1–23**, genkwadane A (**1**), was less active than yuanhuaoate B (**20**) against all 10 cell lines (except the human melanoma A375-S2 cells), indicating that the presence of a 1,10 double bond will reduce the anticancer activity. The reduced activity of genkwadane B (**2**) against the seven cell lines (including HepG2, HCT116, MCF-7, A549, U-937, K562 and HL60 cells) as compared to genkwadane C (**3**), suggested that opening of the 6,7-epoxide group is detrimental to the inhibitory activity. Compounds **9** and **12** with aliphatic (2*E*,4*E*-tetradecadienoate) group at C-1′ exhibited stronger activity against all 10 cell lines than compounds **10** and **11** with phenyl group, indicating that the replacement of an aromatic group with an unsaturated alkyl group in the orthoester chain will enhance the anticancer activity. Additionally, compounds with a benzoyl moiety at C-12 generally displayed more potent activity than analogs of the same structural type, suggesting that the presence of a benzoyl moiety may be an important pharmacophore of inhibitory activity.

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30. Plant material: The flower buds of *D. genkwa* were collected from Mianyang, Sichuan province, PR China, in June 2010, and were identified by Professor J.C. Lu (Department of Natural Products Chemistry, Shenyang Pharmaceutical University, PR China). A voucher specimen (No. 20100701) has been deposited in the Herbarium of Shenyang Pharmaceutical University, Liaoning, PR China.
31. The CH_2Cl_2 extract (271.5 g) was subjected to vacuum liquid chromatography (VLC) with a CH_2Cl_2–MeOH gradient system (100:0, 99:1, 94:6, 88:12, 0:100, v/v). The main part (195.0 g) was further chromatographed on silica gel column with a petroleum ether–acetone gradient system (100:0, 98:2, 90:10, 65:35, 0:100, v/v) to obtain 6 fractions (A–F). Fraction B was chromatographed on Sephadex LH-20 eluted with MeOH, and then subjected to a C-18 reversed-phase open column to give 5 subfractions (B1–B5). Subfraction B2 was subjected to silica gel column chromatography with a petroleum ether–acetone gradient system, and further purified by Prep-HPLC eluted with MeOH–H_2O (85:15) to afford compounds **13** (t_R 37.9 min, 6.8 mg) and **14** (t_R 28.6 min, 10.0 mg). In the same way, compounds **3** (t_R 79.4 min, 9.4 mg) and **21** (t_R 51.6 min, 16.0 mg) were isolated from subfraction B3 by silica gel column and Prep-HPLC eluted with petroleum ether–acetone and MeOH–H_2O (88:12), respectively. Subfraction B4 was purified by Prep-HPLC eluted with MeOH–H_2O (85:15) to obtain compounds **2** (t_R 77.6 min, 6.1 mg), **5** (t_R 91.2 min, 56.9 mg) and **6** (t_R 53.2 min, 7.9 mg). Fraction C was chromatographed on a MCI-GEL CHP-20P column and purified by Prep-HPLC eluted with MeOH–H_2O (84:16) to give compounds **4** (t_R 68.6 min, 7 mg) and **12** (t_R 64.6 min, 894 mg). Fraction D was chromatographed on a MCI-GEL CHP-20P column, and then subjected to a C-18 reversed-phase open column to obtain 5 subfractions (D1–D5). Subfraction D2 was isolated and purified by silica gel column and Prep-HPLC eluted with petroleum ether–acetone and MeOH–H_2O (72:28) to afford compounds **1** (t_R 77.6 min, 6.0 mg), **10** (t_R 32.0 min, 93 mg), **11** (t_R 80.0 min, 120 mg), **16** (t_R 36.7 min, 142 mg), **17** (t_R 91.3 min, 16 mg), **20** (t_R 83.7 min, 19 mg) and **22** (t_R 75.8 min, 5.2 mg). Subfraction D3 was fractionated by chromatography on Sephadex LH-20 eluted with MeOH, and then purified by Prep-HPLC using MeOH–H_2O (79:21) as eluent to obtain compounds **7** (t_R 75.5 min, 214 mg), **9** (t_R 68.0 min, 648 mg) and **15** (t_R 54.0 min, 3.1 mg). Subfraction D4 was subjected to silica gel column chromatography with petroleum ether–acetone, and then purified by Prep-HPLC eluted with MeOH–H_2O (84:16) to give compounds **8** (t_R 39.4 min, 6.6 mg), **18** (t_R 17.6 min, 7.4 mg), **19** (t_R 28.6 min, 6.2 mg), and **23** (t_R 21.2 min, 11.6 mg).
32. Genkwadane A (**1**): white, amorphous powder; $[\alpha]_D^{25}$ +65.5 (c = 0.058, MeOH); UV (MeOH) λ_{max} (log ε): 230 (4.3) nm, 218 (4.1) nm; IR (KBr) ν_{max}: 3378, 2944, 2833, 1720, 1645, 1450, 1384, 1116, 1030, 620 cm^{-1}; HRESIMS m/z 643.2150 $[M+Na]^+$ (calcd for $C_{34}H_{36}O_{11}Na$, 643.2150); ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 1.
33. Genkwadane B (**2**): white, amorphous powder; $[\alpha]_D^{25}$ +30.4 (c = 0.092, MeOH); UV (MeOH) λ_{max} (log ε): 228 (4.5) nm, 212 (4.3) nm; IR (KBr) ν_{max}: 3385, 2926, 2853, 1722, 1645, 1450, 1384, 1276, 1117, 1030, 778, 713, 619 cm^{-1}; HRESIMS m/z 639.3528 $[M+H]^+$ (calcd for $C_{37}H_{51}O_9$, 639.3528); ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2.
34. Genkwadane C (**3**): white, amorphous powder; $[\alpha]_D^{25}$ +33.3 (c = 0.096, MeOH); UV (MeOH) λ_{max} (log ε): 229 (4.5) nm, 211 (4.2) nm; IR (KBr) ν_{max}: 3417, 2927, 2833, 1725, 1692, 1660, 1645, 1551, 1531, 1449, 1384, 1275, 1117, 1030, 712, 619 cm^{-1}; HRESIMS m/z 657.3637 $[M+H]^+$ (calcd for $C_{37}H_{53}O_{10}$, 657.3633); ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2.
35. Genkwadane D (**4**): white, amorphous powder; $[\alpha]_D^{25}$ +24.2 (c = 0.066, MeOH); UV (MeOH) λ_{max} (log ε): 232 (4.4) nm, 207 (3.9) nm; IR (KBr) ν_{max}: 3419, 2925, 2852, 1736, 1708, 1630, 1450, 1384, 1120, 1030, 621 cm^{-1}; HRESIMS m/z 615.3164 $[M+H]^+$ (calcd for $C_{34}H_{47}O_{10}$, 615.3164); ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3.
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37. *Cell growth inhibition assay*: Cell growth inhibition was assessed by MTT assay, as previously reported.[38] Each tested compound and 5-fluorouracil (positive control) were dissolved in DMSO and diluted with the medium to the test concentrations, and the final concentration of DMSO in the culture medium was controlled at less than 0.5% (v/v). Briefly, cells were cultured at 37 °C and dispersed in replicates in 96-well plates with HeLa, HepG2, HT-1080, HCT116, A375-S2, MCF-7, A549, U-937, K562 and HL60 for 24 h. Fresh medium with compounds (**1**–**23**) at different concentrations (0.1 μM, 1 μM, 10 μM, 50 μM) was then added to individual wells and incubated for 48 h, with 5-fluorouracil as the positive control. After 48 h, the cell was incubated with MTT solution (0.5 mg/ml) for an additional 4 h at 37 °C. The produced formazan crystals were solubilized with DMSO and the optical density of solution was measured at 492 nm using a Spectra Shell reader (Tecan, Austria).
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