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Daphnane diterpenoids isolated from *Trigonostemon thyrsoideum* as HIV-1 antivirals

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ABSTRACT

Four highly oxygenated daphnane diterpenoids, trigonothyrins D–G (**1**–**4**), were isolated from the stems of *Trigonostemon thyrsoideum*, and their structures were elucidated on the basis of extensive spectroscopic studies. Inhibitory activity against HIV-1 was assessed for compounds **1**, **3** and **4**, wherein, **3** showed activity with an EC_{50} value of 0.13 μg/mL and a therapeutic index (TI) of 75.1.

1. Introduction

The genus *Trigonostemon* (Euphorbiaceae) comprising *ca.* 50 species grows mainly in tropical and subtropical regions of Asia (Editorial Committee of Flora Reipublicae Popularis Sinicae, 1997). Previous investigations on *Trigonostemon thyrsoideum*, *T. reidioides*, and *T. chinensis* led to isolation of structurally interesting compounds, including highly functionalized daphnanes (Jayasuriya et al., 2000, 2004; Soonthornchareonnon et al., 2005; Stanoeva et al., 2005; Tempeam et al., 2005; Chen et al., 2009, 2010; Zhang et al., 2010), 3,4-*seco*-cleistanthanes (Yin et al., 2008), tetranorditerpenes (Yin et al., 2008), phenanthrenes (Kokpol et al., 1990; Hu et al., 2009), and a flavonoidal indole alkaloid (Kanchanapoom et al., 2002). Additionally daphnane diterpenoids are known to have various bioactivities, such as anti-HIV-1 (Zhang et al., 2010), antiflea insecticidal (Jayasuriya et al., 2000, 2004), cytotoxic (Soonthornchareonnon et al., 2005), acaricidal (Tempeam et al., 2005), antileukemic and neurotrophic (He et al., 2002a,b; Park et al., 2007; Liao et al., 2009) effects. In this study, four new highly oxygenated daphnanes trigonothyrins D–G (**1**–**4**) were isolated from the stems of *T. thyrsoideum*. Compounds **1**, **3** and **4** were tested for inhibitory activity against HIV-1, and **3** was observed to inhibit HIV-1 induced cytopathic effects with an EC_{50} value of 0.13 μg/mL and a TI value of 75.1.

2. Results and discussion

Air-dried, powdered stems (8.0 kg) of *T. thyrsoideum* were soaked with EtOAc and filtered. The filtrate was concentrated to give a residue (128 g), which was subjected to silica gel column chromatography, MPLC, Sephadex LH-20 and preparative HPLC to afford compounds **1**–**4**.

Compound **1**, amorphous powder, gave a molecular formula of $C_{37}H_{46}O_{14}$ by HRESIMS at *m/z* 737.2785 (calcd for $C_{37}H_{46}O_{14}Na$, 737.2785). The IR absorptions at 3568, 3439, 1746 and 1638 cm^{-1} indicated the presence of hydroxy, ester carbonyl and double bond groups. The ^{13}C NMR (DEPT) (*Fig.* S2 in Supplementary material) spectrum of **1** showed nine methyls, two methylenes, 14 methines, and 12 quaternary carbons, as assigned in Table 1. In addition, an isopropenyl (δ_C 139.1, s, C-15; 119.7, t, C-16; 20.0, q, C-17; δ_H 5.40, 5.45 each 1H, br s, H-16; 1.92, 3H, br s, Me-17), five acetoxy and one benzoyloxy groups were clearly distinguishable in the NMR spectrum (Table 1, *Figs.* S1 and S2). The ^1H NMR spectrum displayed one tertiary methyl (δ_H 1.23, s, Me-20), two secondary methyls (δ_H 1.13, d, J = 6.8 Hz, Me-18; 0.86, d, J = 7.2 Hz, Me-19), and five oxygenated methines (δ_H 6.33, dd, J = 4.0, 1.1 Hz, H-12; 6.05, dd, J = 1.4, 1.1 Hz, H-14; 6.02, s, H-5; 5.61, d, J = 4.2 Hz, H-7; 5.21, d, J = 10.3 Hz, H-3). The above NMR signals indicated that **1** was a daphnane diterpenoid derivative and possessed the same oxygenated pattern as trigonothyrins A–C (Zhang et al., 2010) and trigochinins A–C (Chen et al., 2010) in the diterpenoid skeleton (Fig. 1). Two characteristic carbon resonances at δ_C 91.0 (s, C-4) and 83.7 (s, C-6) in **1** suggested the presence of an oxygen-bridged four-member ring exactly like trigonothyrins A–C and trigochinins

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Table 1
NMR spectroscopic data for trigonothyrins D (**1**) and E (**2**).

No.	**1**[a]		**2**[b]	
	δ_H	δ_C	δ_H	δ_C
1	1.25 (1H, m, H_β) 1.96 (1H, m, H_α)	34.6 (t)	1.08 (1H, m, H_β) 1.98 (1H, m, H_α)	36.0 (t)
2	2.34 (1H, m)	30.9 (d)	2.18 (1H, m)	33.6 (d)
3	5.21 (1H, d, 10.3)	72.5 (d)	4.01 (1H, d, 10.3)	73.7 (d)
4		91.0 (s)		93.4 (s)
5	6.02 (1H, s)	73.1 (d)	6.04 (1H, s)	75.1 (d)
6		83.7 (s)		85.3 (s)
7	5.61 (1H, d, 4.2)	79.2 (d)	5.60 (1H, d, 3.9)	80.9 (d)
8	2.89 (1H, dd, 4.2, 1.4)	39.1 (d)	2.92 (1H, dd, 3.9, 2.0)	40.5 (d)
9		76.7 (s)		77.9 (s)
10	2.08 (1H, m)	49.5 (d)	2.07 (1H, m)	50.4 (d)
11	2.17 (1H, m)	40.2 (d)	2.23 (1H, m)	41.2 (d)
12	6.33 (1H, dd, 4.0, 1.1)	72.5 (d)	6.25 (1H, dd, 4.4, 1.2)	74.3 (d)
13		81.7 (s)		83.4 (s)
14	6.05 (1H, dd, 1.4, 1.1)	75.2 (d)	6.08 (1H, dd, 2.0, 1.2)	76.4 (d)
15		139.1 (s)		141.2 (s)
16	5.40 (1H, br s) 5.45 (1H, br s)	119.7 (t)	5.45 (1H, br s) 5.48 (1H, br s)	120.2 (t)
17	1.92 (3H, br s)	20.0 (q)	1.94 (3H, br s)	20.0 (q)
18	1.13 (3H, d, 6.8)	11.5 (q)	1.12 (3H, d, 6.8)	11.9 (q)
19	0.86 (3H, d, 7.2)	15.8 (q)	0.91 (3H, d, 6.8)	16.3 (q)
20	1.23 (3H, s)	19.5 (q)	1.24 (3H, s)	20.0 (q)
1′		163.9 (s)		165.4 (s)
2′		129.8 (s)		131.4 (s)
3′, 7′	7.86 (2H, d, 7.9)	129.4 (2 × d)	7.83 (2H, d, 7.8)	130.4 (2 × d)
4′, 6′	7.38 (2H, dd, 7.9, 7.3)	128.5 (2 × d)	7.45 (2H, dd, 7.8, 7.3)	129.7 (2 × d)
5′	7.52 (1H, t, 7.3)	133.1 (d)	7.59 (1H, t, 7.3)	134.5 (d)
3-CH_3CO		170.1 (s)		
3-CH_3CO	2.16 (3H, s)	20.8 (q)		
5-CH_3CO		170.1 (s)		172.7 (s)
5-CH_3CO	2.03 (3H, s)	20.5 (q)	2.12 (3H, s)	20.6 (q)
7-CH_3CO		170.0 (s)		172.1 (s)
7-CH_3CO	2.07 (3H, s)	21.2 (q)	2.08 (3H, s)	21.5 (q)
12-CH_3CO		169.3 (s)		171.6 (s)
12-CH_3CO	1.91 (3H, s)	20.8 (q)	1.95 (3H, s)	20.9 (q)
14-CH_3CO		168.9 (s)		171.3 (s)
14-CH_3CO	2.10 (3H, s)	21.4 (q)	2.14 (3H, s)	21.9 (q)
9-OH	3.42 (1H, s)			

The *J* values are in parentheses and reported in Hz.
[a] Determined in $CDCl_3$.
[b] Determined in CD_3OD.

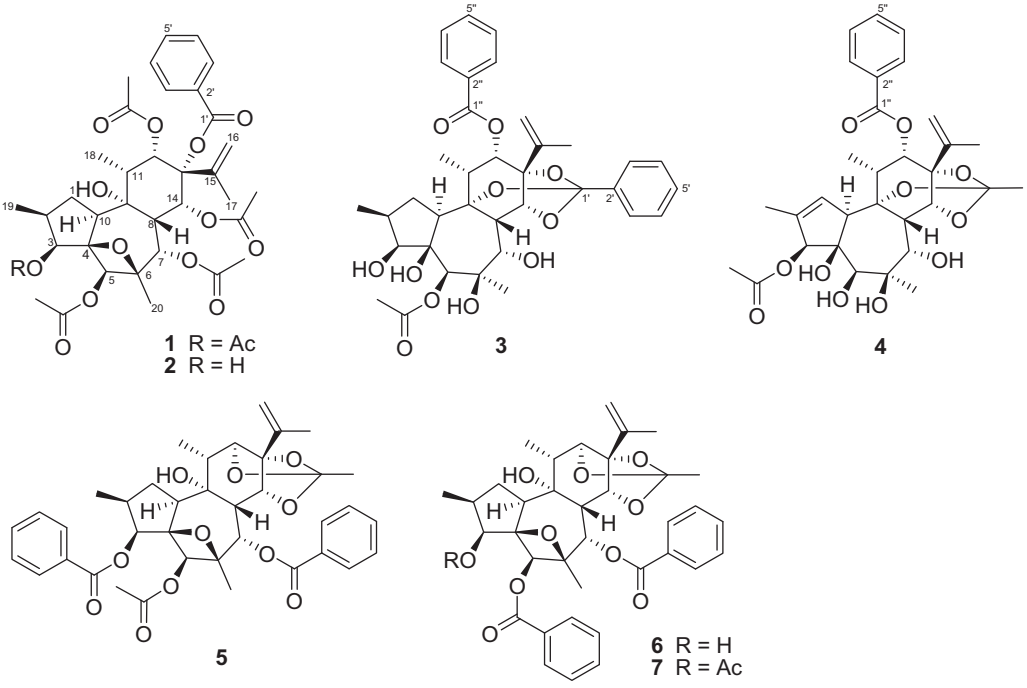

Fig. 1. Structures of compounds **1**–**7**.

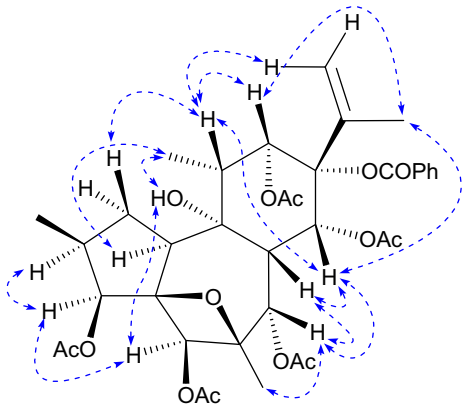

Fig. 2. Important ROESY correlations of **1**.

A–C, which was also supported by the fact that the ^{13}C NMR signals of C-4 and C-6 were shifted downfield by comparison with those of the 4,6-dihydroxy analogues **3** and **4**. HMBC correlations (*Fig.* S4) from δ_H 5.21 (1H, H-3) to δ_C 170.1 (s), from δ_H 6.02 (1H, H-5) to δ_C 170.1 (s), from δ_H 5.61 (1H, H-7) to δ_C 170.0 (s), from δ_H 6.33 (1H, H-12) to δ_C 169.3 (s), and from δ_H 6.05 (1H, H-14) to δ_C 168.9 (s) were observed, indicating that the five acetoxy groups were located at C-3, C-5, C-7, C-12 and C-14, respectively. The correlations from δ_H 3.42 (1H, s, 9-OH) to δ_C 39.1 (d, C-8), 76.7 (s, C-9) and 49.5 (d, C-10) demonstrated that an hydroxyl group was located at C-9. Consequently, the benzoyloxy group was unambiguously assigned to C-13. Quite recently, the absolute configuration of trigochinin A, bearing the same 4,6-oxetane ring as **1**, has been determined by X-ray crystallography and CD analysis (Chen et al., 2010). The NMR spectroscopic data including ROESY information (Fig. 2, *Fig.* S5) of **1** were in very close accord with those of trigochinin A, which established that the two compounds had the same configuration. On the basis of the above, the structure of **1** was assigned as shown in Fig. 1, named trigonothyrin D.

Previous assignments of configuration at C-6 in trigonothyrins A–C (**5**–**7**) (Zhang et al., 2010) were based solely on the ROESY correlation between H-7 and Me-20 and placed Me-20 in the β position. However ring B in daphnanes is seven-membered and in fact, H-7 were spatially adjacent with Me-20, according to the crystal structure of trigochinin A (Chen et al., 2010). Therefore, the previously reported configuration at C-6 in trigonothyrins A–C (**5**–**7**) (Zhang et al., 2010) should be revised as current form (Fig. 1), in accord with trigonothyrin D (**1**).

Compound **2** had a molecular formula of $C_{35}H_{44}O_{13}$ by HRESIMS (pos.) *m/z* 695.2668 (calcd for $C_{35}H_{44}O_{13}Na$, 695.2679). By comparison of the NMR spectroscopic data with those of **1** (Table 1), **2** was identical to **1**, except for the lack of an acetoxy group at C-3. This

Table 2
NMR spectroscopic data for trigonothyrins F (**3**) and G (**4**) in $CDCl_3$.

No.	**3**		**4**	
	δ_H	δ_C	δ_H	δ_C
1	1.64 (1H, m, H_β) 1.96 (1H, m, H_α)	34.6 (t)	5.74 (1H, br s)	127.5 (d)
2	1.77 (1H, m)	36.6 (d)		134.1 (s)
3	3.65 (1H, br d, 4.4)	76.9 (d)	5.73 (1H, br s)	81.9 (d)
4		83.8 (s)		84.8 (s)
5	5.06 (1H, s)	76.9 (d)	3.61 (1H, s)	76.9 (d)
6		76.0 (s)		76.5 (s)
7	4.16 (1H, br s)	81.8 (d)	4.14 (1H, s)	81.6 (d)
8	2.98 (1H, br d, 2.6)	34.7 (d)	2.60 (1H, d, 2.2)	34.5 (d)
9		81.7 (s)		80.8 (s)
10	2.90 (1H, dd, 13.3, 5.3)	51.9 (d)	3.64 (1H, br s)	53.9 (d)
11	3.26 (1H, m)	39.1 (d)	3.32 (1H, m)	39.0 (d)
12	5.49 (1H, d, 7.7)	72.5 (d)	5.38 (1H, d, 7.8)	72.2 (d)
13		87.3 (s)		87.0 (s)
14	4.72 (1H, d, 2.6)	84.4 (d)	4.50 (1H, d, 7.2)	83.9 (d)
15		142.0 (s)		142.1 (s)
16	5.06 (1H, br s) 5.28 (1H, br s)	113.2 (t)	5.01 (1H, br s) 5.20 (1H, br s)	112.8 (t)
17	1.81 (3H, br s)	19.5 (q)	1.73 (3H, br s)	19.3 (q)
18	1.20 (3H, d, 7.0)	11.6 (q)	1.09 (3H, d, 7.1)	11.2 (q)
19	1.01 (3H, d, 6.6)	13.0 (q)	1.61 (3H, br s)	13.2 (q)
20	1.34 (3H, s)	26.2 (q)	1.48 (3H, s)	25.4 (q)
1′		118.0 (s)		119.2 (s)
2′		135.1 (s)	1.74 (3H, s)	21.5 (q)
3′, 7′	7.72 (2H, m)	126.0 (2 × d)		
4′, 6′	7.42 (2H, m)	128.2 (2 × d)		
5′	7.42 (1H, m)	129.8 (d)		
1″		166.1 (s)		166.0 (s)
2″		129.4 (s)		129.4 (s)
3″, 7″	8.04 (2H, d, 8.1)	129.8 (2 × d)	8.05 (2H, d, 8.1)	129.8 (2 × d)
4″, 6″	7.44 (2H, dd, 8.1, 7.3)	128.5 (2 × d)	7.44 (2H, dd, 8.1, 7.3)	128.5 (2 × d)
5″	7.58 (1H, t, 7.3)	133.4 (d)	7.58 (1H, t, 7.3)	133.4 (d)
3-CH_3CO				169.8 (s)
3-CH_3CO			2.18 (3H, s)	21.0 (q)
5-CH_3CO		170.9 (s)		
5-CH_3CO	2.21 (3H, s)	21.0 (q)		
3-OH	2.96 (1H, br s)			
4-OH	4.60 (1H, s)			
6-OH	4.64 (1H, br s)			
7-OH	3.42 (1H, s)			

The *J* values are in parentheses and reported in Hz.

was supported by analysis of the HMBC experiment. The detectable ROESY correlations were also similar to those of **1**. Therefore, the structure of **2** was assigned, named trigonothyrin E.

The HRESIMS of **3** gave an ion at *m/z* 673.2648 (calcd for $C_{36}H_{42}O_{11}Na$, 673.2624), corresponding to the molecular formula of $C_{36}H_{42}O_{11}$. The IR absorptions at 3449, 1721, 1639, and 1630 cm^{-1} suggested the presence of hydroxy, carbonyl and double bond groups. By analysis of the NMR spectroscopic data (Table 2, *Figs.* S11 and S12), **3** was determined to be a daphnane. In the HMBC spectrum (*Fig.* S14), significant correlations from δ_H 5.06 (1H, s, H-5) to δ_C 170.9 (s) and from δ_H 5.49 (1H, d, J = 7.7 Hz, H-12) to δ_C 166.1 (s), indicated that the acetoxy and benzoyloxy groups were connected at C-5 and C-12, respectively. Additionally, correlations from δ_H 4.60 (1H, s, 4-OH) to δ_C 51.9 (d, C-10), 76.9 (d, C-3/C-5) and 83.8 (s, C-4), from δ_H 4.64 (1H, br s, 6-OH) to δ_C 26.2 (q, Me-20) and 76.0 (s, C-6), and from δ_H 3.42 (1H, s, 7-OH) to δ_C 34.7 (d, C-8), 76.0 (s, C-6) and 81.8 (d, C-7), established the presence of hydroxy groups at C-4, C-6 and C-7. Observation of a quaternary carbon at δ 118.0, which is characteristic of an orthoester group, in combination with signals of a monosubstituted benzene group, allowed the remaining oxygenated carbons in ring C to be assigned to a 9,13,14-orthobenzoate, a familiar linkage in daphnanes (Liao et al., 2009). This conclusion was supported by the HMBC correlations from δ_H 4.72 (1H, d, J = 2.6 Hz, H-14) and 7.72 (2H, m, H-3′/H-7′) to δ_C 118.0 (s, C-1′). Therefore, the gross structure of **3** was established on the basis of the above spectral interpretation.

A diagnostic coupling constant of $^3J_{11,12}$ is 7.7 Hz, suggesting an α-oxygenated group at C-12 in **3** (Kasai et al., 1981; Adolf and Hecker, 1984; Tyler and Howden, 1985; Carney et al., 1999; He et al., 2000), which was verified by the ROESY correlations (*Fig.* S15) of H-12/H-8, H-12/H-11, H-12/H-14 and H-12/Me-17. Meanwhile, the correlations of 4-OH/H-8 and 4-OH/H-11 indicated a β-orientation of the hydroxy group at C-4. The configuration at other positions was in accord with that of **1** and **2** by the ROESY experiment. The structure of **3** was determined as shown in Fig. 1, named trigonothyrin F.

Compound **4** produced a quasi-molecular ion at *m/z* 609.2325 in HRESIMS, suggesting a molecular formula of $C_{31}H_{38}O_{11}$ (calcd for $C_{31}H_{38}O_{11}Na$, 609.2311). The NMR data of **4** were very similar to those of **3**, with obvious differences was as follows: instead of a monosubstituted benzene group, a singlet methyl (δ_H 1.74, 3H, s, Me-2′; δ_C 21.5, q, C-2′) appeared, suggesting the presence of a orthoacetate in **4**, which was supported by the HMBC correlation from δ_H 1.74 (3H, s, Me-2′) to δ_C 119.2 (s, C-1′); signals for a trisubstituted olefin were newly observed, and the correlations from δ_H 5.74 (1H, br s, H-1) to δ_C 13.2 (q, Me-19), 53.9 (d, C-10), 84.8 (s, C-4) and 134.1 (s, C-2) assigned the double bond at C-1. Furthermore, the position of an acetoxy group was determined at C-3 by the HMBC correlations from δ_H 5.73 (1H, br s, H-3) to δ_C 169.8 (s), 76.9 (d, C-5) and 127.5 (d, C-1). The similarity of the ROESY correlations of **4** and **3** indicated their identical stereochemistry. The structure of **4** was thus established, named trigonothyrin G (Fig. 1).

Compounds **1**, **3** and **4** were tested for inhibitory activity against HIV-1, and the results are summarized in Table 3. Compound **2** was obtained in a limited amount, and not tested for its activity. Compound **3** showed significant activity to prevent the cytopathic effects of HIV-1 in C8166 cells with an EC_{50} of 0.13 μg/mL, and a TI of 75.1.

Table 3
Anti-HIV-1 activities of compounds **1**, **3** and **4**.

Compound	Anti-HIV-1 activity EC_{50} (μg/mL)	Cytotoxicity CC_{50} (μg/mL)	Therapy index (TI) CC_{50}/EC_{50}
1	78.8	117	1.48
3	0.13	9.76	75.1
4	9.93	56.5	5.69
AZT	0.004	1390	347,500

3. Concluding remarks

Trigonothyrins D (**1**) and E (**2**) are a type of rare oxetane-containing daphnanes, and the configuration at C-6 in trigonothyrins A–C (**5**–**7**) has been revised. Compounds **1**, **3** and **4** were tested for inhibitory activity against HIV-1 (Table 3). Among them, **3** can markedly prevent the cytopathic effects of HIV-1 in C8166 cells with an EC_{50} of 0.13 μg/mL and a TI of 75.1.

4. Experimental

4.1. General experimental procedures

Optical rotations were obtained on a Horiba SEPA-300 or Jasco P-1020 polarimeter. IR spectra were taken on a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were recorded with a Bruker DRX-500 or Bruker AV-400 instrument at room temperature. ESIMS (including HRESIMS) were measured on an API QSTAR Pulsar i mass spectrometer. Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. TLC spots were visualized by heating silica gel plates immersed in vanillin–H_2SO_4 in ethanol. MPLC was performed on a Büchi Sepacore System (Büchi Labortechnik AG, Switzerland), and columns packed with Chromatorex C-18 (40–75 μm, Fuji Silysia Chemical Ltd., Japan). Preparative HPLC was performed by using an Agilent 1100 series system equipped with a Zorbax SB-C_{18}, 9.4 mm × 150 mm column.

4.2. Plant material

The stems of *Trigonostemon thyrsoideum* Stapf were collected in Xishuangbanna of Yunnan Province, China, in May 2008, and identified by Mr. Yu Chen of Kunming Institute of Botany. A voucher specimen (HFG2009001TT) was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

4.3. Extraction and isolation

Air-dried, powdered stems (8.0 kg) of *T. thyrsoideum* were soaked with EtOAc (35 L × 3, with each soaking for 7 days) at room temperature and filtered. The filtrate was concentrated in vacuo to give a residue (128 g), which was subjected to silica gel column chromatography (CC) eluted with a gradient of petroleum ether (b.p. 60–90 °C)/acetone (1:0–1:2) and then MeOH to obtain 10 fractions. Fraction 5 (600 mg) eluted with petroleum ether (b.p. 60–90 °C)/acetone (60:40) was further isolated and purified by MPLC (MeOH–H_2O, 85:15), Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) and preparative HPLC (CH_3CN–H_2O, eluting from 45:55 to 65:35 for 40 min with a flow rate of 10 ml/min) to afford compounds **1** (45 mg), **3** (14.5 mg) and **4** (9.9 mg). Fraction 6 (300 mg) eluted with petroleum ether (b.p. 60–90 °C)/acetone (50:50) was subjected to Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) and then preparative HPLC (CH_3CN–H_2O, eluting from 35:65 to 75:25 for 25 min with a flow rate of 10 ml/min) to yield compound **2** (3.3 mg).

4.4. Trigonothyrin D (*1*)

Amorphous powder, $[\alpha]_D^{15}$ −4.6 (*c* 0.46, $CHCl_3$); IR (KBr) ν_{max} 3568, 3439, 2980 2934, 1746, 1638, 1603, 1452, 1375, 1251, 1105, 1081, 1035, 714 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic

data, see Table 1; ESIMS *m/z* 737 $[M+Na]^+$, HRESIMS (pos.) *m/z* 737.2785 (calcd for $C_{37}H_{46}O_{14}Na$, 737.2785).

4.5. Trigonothyrin E (**2**)

Amorphous powder, $[\alpha]_D^{20}$ 0.0 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3570, 3445, 2975 2931, 1751, 1630, 1602, 1452, 1376, 1275, 1236, 1105, 1027, 713 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 1; ESIMS *m/z* 695 $[M+Na]^+$, HRESIMS (pos.) *m/z* 695.2668 (calcd for $C_{35}H_{44}O_{13}Na$, 695.2679).

4.6. Trigonothyrin F (**3**)

Amorphous powder, $[\alpha]_D^{21}$ +9.7 (*c* 0.58, $CHCl_3$); IR (KBr) ν_{max} 3449, 2958, 2932, 1721, 1639, 1630, 1452, 1375, 1351, 1318, 1282, 1237, 1176, 1121, 1079, 1029, 990, 712 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 2; ESIMS *m/z* 673 $[M+Na]^+$, HRESIMS (pos.) *m/z* 673.2648 (calcd for $C_{36}H_{42}O_{11}Na$, 673.2624).

4.7. Trigonothyrin G (**4**)

Amorphous powder, $[\alpha]_D^{22}$ −28.6 (*c* 1.35, MeOH); IR (KBr) ν_{max} 3450, 2973, 2952, 1721, 1648, 1604, 1452, 1401, 1376, 1280, 1258, 1177, 1107, 1027, 933, 712 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 2; ESIMS *m/z* 609 $[M + Na]^+$, HRESIMS (pos.) *m/z* 609.2325 (calcd for $C_{31}H_{38}O_{11}Na$, 609.2311).

4.8. Assays for anti-HIV-1 activity

Cytotoxicity was measured by the MTT method as described previously (Zheng et al., 1995, 1999).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2010.08.008.

References

Adolf, W., Hecker, E., 1984. On the active principles of the spurge family X. Skin irritants, cocarcinogens, and cryptic carcinogens from the latex of the manchineel tree. J. Nat. Prod. 47, 482–496.

Carney, J.R., Krenisky, J.M.R., Williamson, T., Luo, J., Carlson, T.J., Hsu, V.L., Moswa, J.L., 1999. A new daphnane diterpenoid with potent antihyperglycemic activity from *Maprounea africana*. J. Nat. Prod. 62, 345–347.

Chen, H.D., He, X.F., Ai, J., Geng, M.Y., Yue, J.M., 2009. Trigochilides A and B, two highly modified daphnane-type diterpenoids from *Trigonostemon chinensis*. Org. Lett. 11, 4080–4083.

Chen, H.D., Yang, S.P., He, X.F., Ai, J., Liu, Z.K., Liu, H.B., Geng, M.Y., Yue, J.M., 2010. Trigochinins A–C: three new daphnane-type diterpenes from *Trigonostemon chinensis*. Org. Lett. 12, 1168–1171.

Editorial Committee of Flora Reipublicae Popularis Sinicae, Flora Reipublicae Popularis Sinicae, vol. 44, Academic Press, Beijing, 1997, pp. 162–163.

He, W.D., Cik, M., Lesage, A., Linden, I.V.D., Kimpe, N.D., Appendino, G., Bracke, J., Mathenge, S.G., Mudida, F.P., Leysen, J.E., Puyvelde, L.V., 2000. Kirkinine, a new daphnane orthoester with potent neurotrophic activity from *Synaptolepis kirkii*. J. Nat. Prod. 63, 1185–1187.

He, W., Cik, M., Appendino, G., Puyvelde, L.V., Leysen, J.E., Kimpe, N.D., 2002a. Daphnane-type diterpene orthoesters and their biological activities. Mini-Rev. Med. Chem. 2, 185–200.

He, W., Cik, M., Puyvelde, L.V., Dun, J.V., Appendino, G., Lesage, A., Lindin, I.V., Leysen, J.E., Wouters, W., Mathenge, S.G., Mudida, F.P., Kimpe, N.D., 2002b. Neurotrophic and antileukemic daphnane diterpenoids from *Synaptolepis kirkii*. Bioorg. Med. Chem. 10, 3245–3255.

Hu, X.J., Wang, Y.H., Kong, L.Y., He, H.P., Gao, S., Liu, H.Y., Ding, J., Xie, H., Di, Y.T., Hao, X.J., 2009. New phenanthrenes from *Trigonostemon lii* Y.T. Chang. Tetrahedron Lett. 50, 2917–2919.

Jayasuriya, H., Zink, D.L., Singh, S.B., Borris, R.P., Nanakorn, W., Beck, H.T., Balick, M.J., Goetz, M.A., Slayton, L., Gregory, L., Zakson-Aiken, M., Shoop, W., Singh, S.B., 2000. Structure and stereochemistry of rediocide A, a highly modified daphnane from *Trigonostemon reidioides* exhibiting potent insecticidal activity. J. Am. Chem. Soc. 122, 4998–4999.

Jayasuriya, H., Zink, D.L., Borris, R.P., Nanakorn, W., Beck, H.T., Balick, M.J., Goetz, M.A., Gregory, L., Shoop, W.L., Singh, S.B., 2004. Rediocides B–E, potent insecticides from *Trigonostemon reidioides*. J. Nat. Prod. 67, 228–231.

Kanchanapoom, T., Kasai, R., Chumsri, P., Kraisintud, K., Yamasakia, K., 2002. Lotthanongine, an unprecedented flavonoidal indole alkaloid from the roots of Thai medicinal plant, *Trigonostemon reidioides*. Tetrahedron Lett. 43, 2941–2943.

Kasai, R., Lee, K.H., Huang, H.C., 1981. Genkwadaphnin, a potent antileukemic diterpene from *Daphne genkwa*. Phytochemistry 20, 2592–2594.

Kokpol, U., Thebpatiphat, S., Boonyaratavej, S., Chedchuskulcai, V., Ni, C.Z., Clardy, J., Chaichantipyuth, C., Chittawong, V., Miles, D.H., 1990. Structure of trigonostemone, a new phenanthrenone from the Thai plant *Trigonostemon reidioides*. J. Nat. Prod. 53, 1148–1151.

Liao, S.G., Chen, H.D., Yue, J.M., 2009. Plant orthoesters. Chem. Rev. 109, 1092–1140.

Park, B.Y., Min, B.S., Ahn, K.S., Kwon, O.K., Joung, H., Bae, K.H., Lee, H.K., Oh, S.R., 2007. Daphnane diterpene esters isolated from flower buds of *Daphne genkwa* induce apoptosis in human myelocytic HL-60 cells and suppress tumor growth in Lewis lung carcinoma (LLC)-inoculated mouse model. J. Ethnopharmacol. 111, 496–503.

Soonthornchareonnon, N., Sakayarojkul, M., Isaka, M., Mahakittikun, V., Chuakul, W., Wongsinkongman, P., 2005. Acaricidal daphnane diterpenoids from *Trigonostemon reidioides* (Kurz) Craib roots. Chem. Pharm. Bull. 53, 241–243.

Stanoeva, E., He, W., De Kimpe, N., 2005. Natural and synthetic cage compounds incorporating the 2,9,10-trioxatricyclo-[4.3.1.$0^{3,8}$]decane type moiety. Bioorg. Med. Chem. 13, 17–28.

Tempeam, A., Thasana, N., Pavaro, C., Chuakul, W., Siripong, P., Ruchirawat, S., 2005. A new cytotoxic daphnane diterpenoid, rediocide G, from *Trigonostemon reidioides*. Chem. Pharm. Bull. 53, 1321–1323.

Tyler, M.I., Howden, M.E.H., 1985. Antineoplastic and piscicidal 1-alkyldaphnane orthoesters from *Pimelea* species. J. Nat. Prod. 48, 440–445.

Yin, S., Su, Z.S., Zhou, Z.W., Dong, L., Yue, J.M., 2008. Antimicrobial diterpenes from *Trigonostemon chinensis*. J. Nat. Prod. 71, 1414–1417.

Zhang, L., Luo, R.H., Wang, F., Jiang, M.Y., Dong, Z.J., Yang, L.M., Zheng, Y.T., Liu, J.K., 2010. Highly functionalized daphnane diterpenoids from *Trigonostemon thyrsoideum*. Org. Lett. 12, 152–155.

Zheng, Y.T., Ben, K.L., Zhang, F., Wang, J.H., 1995. In vitro immunotoxicity and cytotoxicity of trichosanthin against human normal immunocytes and leukemia–lymphoma cells. Immunopharm. Immunotoxicol. 17, 69–79.

Zheng, Y.T., Ben, K.L., Jin, S.W., 1999. Alpha-momorcharin inhibits HIV-1 replication in acutely but not chronically infected T-lymphocytes. Acta Pharmacol. Sin. 20, 239–243.