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Daphnane diterpenoids from the stems of *Trigonostemon lii* and their anti-HIV-1 activity

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ABSTRACT

Thirteen highly oxygenated daphnane diterpenoids, including six known compounds, were isolated from the stems of *Trigonostemon lii*. The structures were elucidated by extensive spectroscopic analyses including 2D NMR spectroscopy (HSQC, ^{1}H–^{1}H COSY, HMBC, and ROESY) and mass spectrometry. The absolute stereochemistries of compounds were established on the basis of CD spectra. Four of the compounds showed modest anti-HIV-1 activity (EC_{50} = 2.04, 9.17, 11.42, and 9.05 μg/ml, TI = 26.49, >21.81, 9.32, and 9.56, respectively) *in vitro*.

1. Introduction

Daphnane diterpenoids are characteristic constituents of the Thymelaeaceae and Euphorbiaceae families (Evans and Taylor, 1983), and these compounds exhibit a wide range of biological activities, such as antileukemic (He et al., 2002a), neurotrophic (He et al., 2000), antihyperglycemic (Carney et al., 1999), and piscicidal activities (Sakata et al., 1971). These constituents, especially the daphnane orthoester derivatives, have been the subject of studies on various aspects of their chemistry, biochemistry and pharmacology due to their complex structure and excellent biological activities (He et al., 2002b; Stanoeva et al., 2005). Recently, a series of new daphnane diterpenoids was reported from the families Thymelaeaceae and Euphorbiaceae by our group and others (Lin et al., 2010; Dong et al., 2011; Li et al., 2011a; Allard et al., 2012; Huang et al., 2012; Vidal et al., 2012).

Trigonostemon lii (Euphorbiaceae) is a tree common in the Yunnan province of China (Editorial Committee of Flora Reipublicae Popularis Sinicae, 1996). The fruits and the leaves of this plant have been used by local residents as herbal medicines to cure some diseases in local residents (Qin et al., 2009). Previous chemical investigations of the leaves of *T. lii* led to the isolation of a series indole alkaloids and phenanthrenone derivatives from the leaves of *T. lii* by our group, and some of these compounds were found to exhibit modest anti-HIV-1 activity (Hu et al., 2009a,b; Tan et al., 2010; Li et al., 2011b). As a continuation of our research work, an in-depth phytochemical investigation was conducted on the stem of *T. lii*. In this work, 13 highly oxygenated daphnane diterpenoids, including seven new ones trigolins A–G (**1**–**7**), were isolated from the stems of this species (Fig. 1). Compounds **1**–**12** were tested for their anti-HIV-1 activity. Among them, compounds **3**, **7**, **8**, and **11** exhibited modest anti-HIV-1 activity (EC_{50} = 2.04, 9.17, 11.42, and 9.05 μg/ml, TI = 26.49, >21.81, 9.32, and 9.56, respectively) *in vitro*. Herein, this paper describes the isolation, structural elucidation, and biological activity of these compounds.

2. Results and discussion

Trigolin A (**1**), obtained as a white powder, had a molecular formula of $C_{38}H_{44}O_{12}$ as determined by the positive HRESIMS ion at *m/z* 715.2716 $[M+Na]^+$ (calcd for $C_{38}H_{44}O_{12}Na$, 715.2730) with 17 degrees of unsaturation. The IR absorptions implied the presence of hydroxyl (3436 cm^{-1}), ester carbonyl (1723 cm^{-1}), and aromatic groups (1603 and 1452 cm^{-1}). In accordance with the molecular formula, all the 38 carbons were well resolved in the ^{13}C NMR spectrum (Table 1) recorded at 276 K (Supplementary data). The

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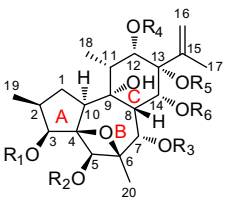

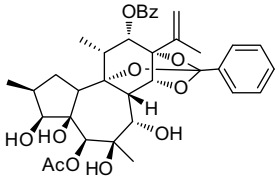

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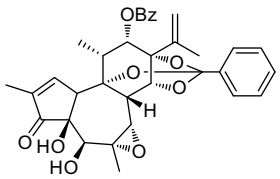

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	R_1	R_2	R_3	R_4	R_5	R_6
1	H	Bz	Ac	Bz	H	Ac
2	Ac	Bz	Ac	Bz	H	H
3	Ac	Bz	Ac	H	H	Bz
4	Ac	Bz	Ac	Bz	H	Ac
5	Ac	Bz	Bz	Ac	H	Ac
6	Ac	Ac	Bz	Bz	H	Ac
7	H	Bz	Bz	Bz	H	Ac
8	H	Bz	Ac	Ac	Bz	Ac
9	Ac	Bz	Ac	Ac	Bz	Ac
10	Ac	Bz	Ac	Bz	Ac	Ac
11	Ac	Ac	Ac	Ac	Bz	Ac

Fig. 1. Compounds from the stems of *Trigonostemon lii*.

Table 1
^{13}C NMR Spectroscopic Data for Compounds **1**–**7** in $CDCl_3$.

Position	**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5**[b]	**6**[a]	**7**[a]
1	34.9	34.5	35.1	34.6	34.4	34.9	34.8
2	31.1	30.9	31.2	31.0	31.0	31.1	32.4
3	72.8	74.5	74.1	74.0	73.8	72.8	72.2
4	91.7	91.8	91.3	91.3	91.7	91.7	93.3
5	73.4	74.7	74.9	74.2	73.6	73.4	73.7
6	84.0	83.3	84.3	84.0	84.0	84.0	84.6
7	79.3	79.0	79.7	79.2	78.7	79.3	78.8
8	40.1	41.0	40.5	39.8	40.3	40.1	40.0
9	77.0	77.1	77.6	76.8	76.3	77.0	76.7
10	49.4	49.4	50.3	49.4	49.1	49.4	48.8
11	39.2	39.9	38.4	39.5	39.0	39.2	38.9
12	76.0	76.9	75.8	76.2	74.4	76.0	75.9
13	74.7	74.7	75.0	74.7	73.5	74.7	74.6
14	75.6	75.0	76.6	75.6	75.4	75.6	75.3
15	144.3	144.1	144.6	144.2	144.7	144.3	144.1
16	115.1	115.4	114.9	115.2	114.7	115.1	115.0
17	19.7	20.0	19.8	19.8	19.7	19.7	19.6
18	12.3	12.2	12.4	12.4	11.3	12.3	12.0
19	16.0	16.2	15.8	16.0	16.0	16.0	15.7
20	19.4	19.4	19.5	19.9	19.3	19.4	19.3
1′	165.7	166.1	165.9	166.1	165.6	165.7	165.8
2′	129.7	129.5	129.6	129.4	128.9	129.7	129.2
3′/7′	130.3	129.7	129.5	129.6	129.1	130.3	129.8
4′/6′	128.8	128.7	128.4	128.7	128.2	128.8	128.4
5′	133.8	133.5	133.2	133.7	133.5	133.8	133.3
1″	167.2	168.6	166.9	167.7	165.7	167.2	165.6
2″	129.4	129.9	130.1	129.8	128.7	129.4	129.5
3″/7″	129.8	130.2	130.0	129.9	129.8	129.8	130.2
4″/6″	128.6	128.9	128.7	128.8	128.5	128.6	128.6
5″	133.6	133.8	133.3	133.8	133.5	133.6	133.6
1‴							167.0
2‴							129.3
3‴/7‴							129.6
4‴/6‴							128.4
5‴							133.4
3-OAc	21.1, 170.5	20.7, 170.5	20.7, 170.0	20.9, 170.6	20.2, 170.4	21.1, 170.5	
5-OAc	20.8, 170.3					20.8, 170.3	
7-OAc		22.0, 171.7	20.5, 170.2	21.6, 170.4			
12-OAc					20.7, 171.2		
13-OAc							
14-OAc	20.7, 171.0			21.5, 170.4	20.1, 170.7	20.7, 171.0	20.5, 170.8

[a] Recorded at 150 MHz.
[b] Recorded at 125 MHz.

carbons were further classified by DEPT experiments as six methyls, two methylenes (one olefinic), 19 methines (five oxygenated and 10 olefinic carbons), and 11 quaternary carbons (four ester carbonyls, four oxygenated carbons, and three olefinics carbons). In addition, two tertiary methyls at δ_H 2.00 (s, 3H) and 1.34 (s, 3H), two secondary methyls at δ_H 1.23 (d, J = 5.9 Hz, 3H) and 0.94 (d,

J = 7.1 Hz, 3H), a terminal double bond at δ_H 5.24 (br s, 1H) and 5.20 (br s, 1H), two acetyl groups, and two benzoyl groups could be distinguished in the NMR spectra (Tables 1 and 2). Those functionalities accounted for 13 out of the 17 degrees of unsaturation, and the remaining four degrees of unsaturation required that **1** was tetracyclic. The aforementioned evidence indicated that compound **1** had the typical A, B, and C rings of a daphnane diterpenoid. The remaining one degree of unsaturation was accounted for by an oxetane ring. The downfield-shifted C-4 (δ_C 91.7) and C-6 (δ_C 84.0) signals suggested formation of this ring between C-4 and C-6. This conclusion was also supported by comparing the NMR spectra of this compound with those of trigochinins A and B (Chen et al., 2010a). The gross structure of **1** was finally determined by 2D NMR spectroscopic analyses (Fig. 2a). The HMBC correlations of H-7 and H-14 to the corresponding acetyl carbonyls confirmed that the two acetyl groups were located at C-7 and C-14, respectively. Likewise, the two benzoyloxy groups were placed at C-5 and C-12, respectively, which were assigned based on the HMBC correlations of H-5 and H-12 to the corresponding benzoyloxy carbonyls. Two hydroxyl proton resonances at δ_H (2.85, s, and 3.55, s) without any corresponding carbons in the HSQC spectrum, were attributable to C-3 and C-9, respectively, which were confirmed by the HMBC correlation of the relevant protons to C-3 and C-9. In addition to the above substituted groups, the presence of another hydroxyl group was detected at C-13 due to its chemical shift (δ_C 74.7) even there was no direct HMBC correlation evidence.

The relative configuration of **1** was established by a combination of the ROESY spectrum and the analogous correlation of the NMR spectroscopic data for **1** with of the data for **8** (Chen et al., 2010a). The ROESY correlations (Fig. 2b) of H-1β/H-8, H-8/H-7, H-11 and H-14, and H-11/H-12 indicated that these protons were co-facial, and they were arbitrarily assigned to the β-orientation. Consequently, the ROESY correlations of H-5/H-10, H-3, H_3-20, and OH-9, and of H-10/H-2, H-3, H_3-18 and OH-9 suggested they were α-oriented. The strong ROESY correlations of H_3-17/H-14 and H_2-16/H-12 also indicated that C-15 occupied the axial orientation at C-13, which was in a β-direction. Thus, the structure of compound **1** was established.

Trigolins B (**2**) and C (**3**) were found to have the same molecular formula of $C_{38}H_{44}O_{12}$ as **1**, as determined by their HRESIMS. Detailed comparison of the NMR data of **2** and **3** with those of **1** suggested that these compounds were structural analogs. The main differences between them were the location of the acetoxy and benzoyl groups. The acetoxy group was located at C-3 in **2**, which was confirmed by the HMBC correlation of H-3 to its corresponding acetyl carbonyl. Likewise, the benzoyl group was located at C-14 in **3**, which was confirmed by the HMBC correlation of H-14 to its corresponding benzoyl carbonyl. The structures of **2** and **3** were confirmed by 2D NMR experiments, including HSQC, HMBC, and ROESY experiments.

Trigolin D (**4**) possessed a molecular formula of $C_{40}H_{46}O_{13}$ as determined by HRESIMS. The analysis of the ^1H and ^{13}C NMR spectroscopic data of **4** showed that it is likely an acetylated derivative of **2**, a conclusion that was supported by the presence of 42

Table 2
^1H NMR [δ_H (mult, J (Hz))] Spectroscopic Data for Compounds **1**–**7** in $CDCl_3$.

Position	1[a]	2[a]	3[b]	4[a]	5[b]	6[a]	7[a]
1	1.94 (m) 1.13 (m)	1.98 (m) 1.26 (m)	2.00 (m) 1.21 (m)	2.01 (m) 1.28 (m)	1.99 (m) 1.23 (m)	1.97 (m) 1.26 (m)	1.98 (m) 1.17 (m)
2	2.22 (m)	2.42 (m)	2.41 (m)	2.45 (m)	2.43 (m)	2.37 (m)	2.24 (m)
3	4.19 (t, 9.3)	5.14 (d 10.2)	5.23 (d, 10.2)	5.23 (d, 7.2)	5.17 (overlapped)	5.23 (d, 10.4)	4.20 (t, 10.2)
5	6.36 (s)	6.46 (s)	6.05 (s)	6.34 (s)	6.44 (s)	6.33 (s)	6.61 (br s)
7	5.72 (s)	5.68 (d, 4.0)	5.73 (br s)	5.71 (br s)	5.94 (d, 3.4)	5.89 (s)	6.00 (br s)
8	2.94 (br s)	2.62 (br d 4.0)	3.22 (br s)	3.01 (br s)	3.03 (br s)	3.13 (br s)	3.13 (br s)
10	2.19 (overlapped)	2.23 (dd 13.7, 5.8)	2.27 (dd 13.4, 4.7)	2.27 (dd, 13.1 4.9)	2.27 (dd, 10.4, 6.5)	2.17 (dd, 13.0, 5.3)	2.28 (dd, 13.7,5.4)
11	2.05 (m)	2.02 (m)	1.64 (m)	2.04 (m)	1.87 (m)	1.96 (m)	2.03 (m)
12	5.67 (br s)	5.66 (br d 2.8)	3.96 (br s)	5.67 (br s)	5.43 (s)	5.63 (br s)	5.68 (br s)
14	5.91 (br s)	4.47 (br s)	6.23 (br s)	5.90 (br s)	5.90 (s)	5.94 (s)	6.01 (br s)
16	5.24 (br s) 5.20 (br s)	5.20 (br s) 5.16 (br s)	5.17 (br d 6.8)	5.22 (br s) 5.19 (br s)	5.19 (br s) 5.15 (br s)	5.19 (br s) 5.17 (br s)	5.28 (br s) 5.24 (br s)
17	2.00 (s)	1.93 (s)	1.82 (s)	2.00 (s)	1.92 (s)	1.96 (s)	2.02 (s)
18	1.23 (d, 5.9)	1.20 (d, 6.8)	1.34 (d, 6.3)	1.24 (d, 6.3)	1.12 (d, 6.6)	1.18 (d, 6.6)	1.23 (d, 6.6)
19	0.94 (d, 7.1)	0.83 (d, 7.1)	0.83 (d, 6.7)	0.86 (d, 7.1)	0.81 (d, 6.9)	0.87 (d, 7.1)	0.96 (d, 7.2)
20	1.34 (s)	1.49 (s)	1.30 (s)	1.41 (s)	1.48 (s)	1.31 (s)	1.46 (s)
3′/7′	8.15 (dd, 8.2 1.1)	8.04 (d, 7.5)	7.90 (d, 7.5)	8.00 (d, 7.4)	7.93 (d, 7.5)	8.15 (d, 7.5)	8.11 (dd 8.2,1.2)
4′/6′	7.48 (t, 8.2)	7.47 (t, 7.5)	7.44 (t, 7.5)	7.48 (t, 7.4)	7.42 (t, 7.5)	7.48 (t, 7.5)	7.44 (t, 8.2)
5′	7.60 (t, 8.2)	7.60 (t, 7.5)	7.59 (m)	7.61 (t, 7.4)	7.56 (t, 7.5)	7.59 (t, 7.5)	7.56 (t, 8.2)
3″/7″	8.10 (br d, 7.6)	8.09 (d, 7.6)	8.03 (d, 7.5)	8.10 (dd, 8.0 1.2)	8.13 (d, 7.6)	7.94 (d, 7.6)	8.21 (d, 7.6)
4″/6″	7.51 (t, 7.6)	7.50 (t, 7.6)	7.47 (t, 7.5)	7.51 (t, 8.0)	7.47 (t, 7.6)	7.30 (t, 7.6)	7.52 (t, 7.6)
5″	7.63 (t, 7.6)	7.62 (t, 7.6)	7.63 (m)	7.63 (t, 8.0)	7.59 (t, 7.6)	7.50 (t, 7.6)	7.63 (t, 7.6)
3‴/7‴							8.00 (d, 7.5)
4‴/6‴							7.35 (t, 7.5)
5‴							7.54 (overlapped)
3-OH	2.85 (s)						2.87 (d, 10.2)
9-OH	3.55 (s)		4.03 (s)	3.56 (s)		3.19 (s)	3.59 (s)
13-OH							1.68 (s)
14-OH		3.14 (s)					
3-OAc		1.94 (s)	2.06 (s)	2.05 (s)	1.94 (s)	2.17 (s)	
5- OAc						2.00 (s)	
7-OAc	2.16 (s)	2.12 (s)	1.41 (s)	2.15 (s)			
12-OAc					2.05 (s)		
13-OAc							
14-OAc	1.74 (s)			1.74 (s)	1.29 (s)	0.95 (s)	1.00 (s)

[a] Recorded at 600 MHz.
[b] Recorded at 500 MHz.

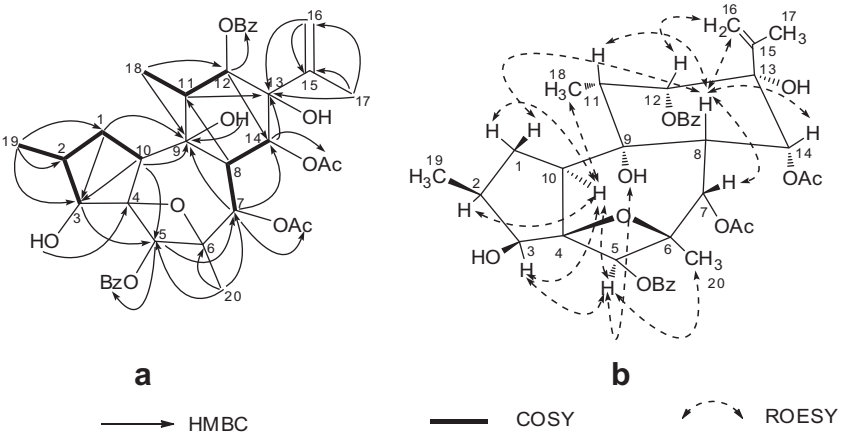

Fig. 2. Selected 2D NMR correlations of **1**.

more mass units in the molecular formula relative to the mass of **2**. The direct comparison the ^{1}H NMR data with those of compound **2** showed that H-14 of **4** at δ_H (5.90) was shifted downfield by δ 1.43 due to the acetylating effect at C-14. This means that an acetoxy group was located at C-14, which was further supported by the HMBC correlation between H-14 and the corresponding acetyl carbonyl. The relative configuration of **4** was assigned to be the same as that of **2** based on a comparison of their NMR data and ROESY data. Therefore, the structure of **4** was established as shown.

Trigolins E (**5**) and F (**6**), obtained as white amorphous powders, were determined to have the same molecular formulas of $C_{40}H_{46}O_{13}$ as **4** based on HRESIMS. Detailed comparison of the UV, IR, NMR, and MS data of compounds **5** and **6** with those of **4** showed that they were structural congeners with the same number of benzoyloxy groups and acetoxy groups. The primary differences between them were due to the different location of these benzoyloxy groups and acetoxy groups. The HMBC correlations of H-7 and H-3″ to benzoyloxy carbonyl along with the correlations of H-12 and a methyl proton to an acetoxy carbonyl indicated that the benzoyloxy group was located at C-7 and the acetoxy group was located at C-12 in **5**. In **6**, the locations of these benzoyloxy and acetoxy groups were assigned to C-7 and C-5, respectively. These positions were confirmed by the HMBC correlations from the protons to the corresponding carbonyl groups.

Trigolin G (**7**) had 62 mass units more than **1**. Comparison of the ^{1}H and ^{13}C NMR data of **7** (Tables 1 and 2) with those of **1** indicated that the only difference was that the acetoxy group at C-12 in **1** was replaced by a benzoyloxy group in **7**. This assignment was further confirmed by the HMBC correlation of H-12 to the benzoyloxy carbonyl.

The known compounds were identified as trigochinins A (**8**) (Chen et al., 2010a), B (**9**) (Chen et al., 2010a), E (**10**) (Chen et al., 2010b), and F (**11**) (Chen et al., 2010b); trigonothyrin F (**12**) (Zhang et al., 2010); and trigoxyphin A (**13**) (Lin et al., 2010) by comparison of their spectroscopic data with the reported data. Compounds **1**–**11** are a group daphnane-type diterpenoids bearing an oxetane ring that formed between two oxygenated quaternary carbons, C-4 and C-6. This oxetane ring is rare in the family of daphnane diterpenes. The absolute configuration of compound **8** was previously elucidated by CD analysis (Chen et al., 2010a). To determine the absolute configurations of all of the new compounds, the CD spectra of compounds **1**–**8** were determined as shown in the Supplementary data. Compounds **1**–**7** showed a similar positive Cotton effect at λ_{max} 233 nm and a negative Cotton effect at λ_{max} 211 nm, indicating that the chiral centers of compounds **1**–**7** have an absolute configuration identical to that of **8.**

It was interesting that the NMR resonances of all the new compounds except **2** showed unresolved signals in the 1D NMR spectra at room temperature. However, the NMR resonances returned to normal when the temperature was changed from 276 K to 293 K or to 253 K (Supplementary data). This change implied the presence of an unstable conformation in those compounds in solvent at room temperature. Compounds **2** and **8**–**11** exhibited clear signals at room temperature, and the H-12 resonances of these compounds were observed as doublet peaks with a coupling constant of approximately 3.0 Hz. These data implied that the C ring of these compounds have a chair conformation, which was further confirmed by X-ray analysis (Chen et al., 2010a). The H-12 signals of compounds **1** and **3**–**7** were observed as a singlet peaks when the temperature for NMR was changed from 276 K to 293 K or to 253 K, which indicated that the C ring of these compounds also have a chair conformation after the temperature change. Detailed comparison of their structures with those of **8**–**11** indicated the presence of an ester carbonyl at C-13 in compounds **8**–**11** but a hydroxyl group in **1**–**7**. This ester carbonyl could stabilize the C ring through hydrogen bonding and steric hindrance. Thus, all compounds **1**–**7** except **2** showed unresolved signals due to the loss of these effects. Compound **2** was stable, perhaps because the hydroxyl group at C-14 formed a hydrogen bond with the acetyl carbonyls at C-12, as in the structure of 9,12,14-orthoester daphnane diterpenoid (Jayasuriya et al., 2000, 2004; Allard et al., 2012).

Compounds **1**–**12** were tested for cytotoxicity against C8166 cells (CC_{50}), and anti-HIV-1 activity was evaluated using an inhibition assay for the cytopathic effects of HIV-1 (EC_{50}). AZT was used

Table 3
Cytotoxicity and Anti-HIV-1 Activity of Compounds **1**–**12**.

Compounds	Cytotoxicity	Anti-HIV-1	Selectivity index
	CC_{50} (μg/ml)	EC_{50} (μg/ml)	CC_{50}/EC_{50}
1	183.40	71.05	2.58
2	>200	62.49	>3.20
3	54.04	2.04	26.49
4	>200	67.68	>2.96
5	41.95	11.89	3.52
6	>200	51.04	>3.92
7	>200	9.17	>21.81
8	106.45	11.42	9.32
9	97.92	61.21	1.60
10	19.20	12.55	1.53
11	86.54	9.05	9.56
12	>200	57.95	>3.45
AZT (positive control)	1139.47	0.0032	351688.27

as a positive control. Compounds **3**, **7**, **8**, and **11** showed modest anti-HIV-1 activity with EC_{50} values of 2.04, 9.17, 11.42, and 9.05 μg/ml, respectively, along with TI values of 26.49, >21.81, 9.32, and 9.56, respectively (Table 3).

3. Concluding remarks

Chemical investigation of the stems of *T. lii* resulted in the isolation of 13 daphnane-type diterpenoids including seven new compounds. Compounds **1**–**11** belong to the 4,6-oxetane type of daphnane diterpenoids. The NMR resonances of compounds **1**–**7** exhibited unresolved signals in the 1D NMR spectra at room temperature, and the reason for this behavior was discussed in this paper. The absolute stereochemistries of compounds **1**–**7** were elucidated based on the comparison of the CD Cotton effect with the Cotton effect of the known compound **8**. Compounds **1**–**12** were evaluated for inhibitory activity against HIV-1 using AZT as the positive control. Compounds **3**, **7**, **8**, and **11** showed modest anti-HIV-1 activity (EC_{50} = 2.04, 9.17, 11.42, and 9.05 μg/ml, TI = 26.49, >21.81, 9.32, and 9.56, respectively) *in vitro*.

4. Experimental

4.1. General experimental procedures

Optical rotations were obtained on a JASCO DIP-370 digital polarimeter, whereas IR spectra were measured in a Bio-Rad FTS-135 spectrometer with KBr pellets, and UV data were obtained using a UV-210A spectrometer. CD spectra were recorded with an Applied Photophysics Chirascan spectrometer. 1D and 2D NMR spectra were acquired on Bruker AM-400, DRX-500, and AV-600 NMR spectrometers using a TMS as an internal standard. ESIMS were recorded using a Finnigan MAT 90 instrument and a VG Auto Spec-3000 spectrometer. Column chromatography (CC) was performed on Si gel H (10–40 μm; Qingdao Marine Chemical Factory) and Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden). MPLC was performed on 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 1200 series system equipped with a Zorbax XDB-C18, 9.4 mm × 150 mm column.

4.2. Plant material

Stems of *T. lii* were collected in Xishuangbanna in Yunnan Province, People's Republic of China, in July 2008, and identified by Prof. Shun-Cheng Zhang of Xishuangbanna Institute of Botany, Chinese Academy of Sciences (CAS). A voucher specimen (KIB08070615) was deposited in the Herbarium of Kunming Institute of Botany.

4.3. Extraction and isolation

Air-dried, powdered stems (50.0 kg) of *T. lii* were extracted with acetone (3 × 300 L) at 50 °C. After removal of the solvent by evaporation, the residue (1.1 kg) was suspended in H_2O (4 L) and then partitioned with petroleum ether (4 × 3 L). The petroleum ether (500.0 g) fraction was subjected to silica gel CC with a gradient elution system of petroleum ether/acetone (100:0–30:70) to obtain eight fractions (A–H). Fraction C (40.0 g) was separated and purified by MPLC (MeOH–H_2O, 85:15) to yield six fractions (C1–C6). Fraction C3 was subjected to Sephadex LH-20 CC (MeOH–H_2O, 10:1) to yield compounds **11** (20.0 mg), **12** (30.0 mg) and **13** (10.0 mg). Fraction C4 was purified using Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) and then by the semi-preparative HPLC (CH_3OH–H_2O, eluting from 60:40 for 45 min with a flow rate of 8 ml/min) to afford compounds **1** (5.0 mg), **2** (7.0 mg), **3** (6.0 mg), **4** (7.0 mg), **5** (9.0 mg), **6** (11.0 mg), **7** (5.0 mg), **8** (9.0 mg), **9** (25.0 mg), and **10** (30.0 mg) (Supplementary data).

4.3.1. Trigolin A (*1*)

White powder; $[\alpha]^{26}_D$ + 12.3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.22), 230 (4.44), 271 (3.45) nm; CD (0.00040 M, MeOH) λ_{max} ($\Delta\varepsilon$) 199 (+10.3), 229 (+2.6) nm; IR (KBr) ν_{max} 3436, 2970, 2929, 1723, 1603, 1452, 1374, 1278, 1239, 1120, 1071, 1025, 714 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS *m/z* 715.0 $[M+Na]^+$; HRESIMS *m/z* 715.2716 $[M+Na]^+$ (calcd for $C_{38}H_{44}O_{12}Na$, 715.2730).

4.3.2. Trigolin B (*2*)

White powder; $[\alpha]^{26}_D$ + 45.1 (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.19), 230 (4.40), 271 (3.42) nm; CD (0.00016 M, MeOH) λ_{max} ($\Delta\varepsilon$) 198 (+4.3), 233 (+0.2) nm; IR (KBr) ν_{max} 3459, 2974, 2931, 1726, 1602, 1452, 1374, 1315, 1278, 1177, 1121, 1071, 1026, 714 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS *m/z* 715.0 $[M+Na]^+$; HRESIMS *m/z* 715.2728 $[M+Na]^+$ (calcd for $C_{38}H_{44}O_{12}Na$, 715.2730).

4.3.3. Trigolin C (*3*)

White powder; $[\alpha]^{26}_D$ − 16.0 (*c* 0.41, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.14), 230 (4.32), 271 (3.32) nm; CD (0.00041 M, MeOH) λ_{max} ($\Delta\varepsilon$) 202 (−4.1), 222 (−6.2), 240 (+6.7) nm; IR (KBr) ν_{max} 3449, 2974, 2931, 1736, 1603, 1452, 1374, 1315, 1278, 1177, 1115, 1072, 1026, 714 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS *m/z* 693.0 $[M+H]^+$, 715.0 $[M+Na]^+$; HRESIMS *m/z* 715.2725 $[M+Na]^+$ (calcd for $C_{38}H_{44}O_{12}Na$, 715.2730).

4.3.4. Trigolin D (*4*)

White powder; $[\alpha]^{26}_D$ + 27.9 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε) 200 (4.21), 230 (4.42), 272 (3.15) nm; CD (0.00039 M, MeOH) λ_{max} ($\Delta\varepsilon$) 199 (+17.0), 239 (+4.0) nm; IR (KBr) ν_{max} 3445, 2977, 2933, 1727, 1602, 1452, 1373, 1315, 1278, 1240, 1176, 1119, 1071, 1026, 714 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS *m/z* 757.0 $[M+Na]^+$, 1491 $[2M+Na]^+$; HRESIMS *m/z* 757.2846 $[M+Na]^+$ (calcd for $C_{40}H_{46}O_{13}Na$, 757.2836).

4.3.5. Trigolin E (*5*)

White powder; $[\alpha]^{26}_D$ − 5.3 (*c* 0.42, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.15), 231 (4.36), 272 (3.35) nm; CD (0.00039 M, MeOH) λ_{max} ($\Delta\varepsilon$) 202 (−5.2), 223 (−7.4), 238 (+16.1) nm; IR (KBr) ν_{max} 3566, 3438, 2976, 2933, 1731, 1602, 1452, 1375, 1315, 1276, 1245, 1177, 1120, 1071, 1026, 714 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS *m/z* 735.0 $[M+H]^+$, 757.0 $[M+Na]^+$, 773.0 $[M+K]^+$, 1491 $[2M+Na]^+$; HRESIMS *m/z* 757.2848 $[M+Na]^+$ (calcd for $C_{40}H_{46}O_{13}Na$, 757.2836).

4.3.6. Trigolin F (*6*)

White powder; $[\alpha]^{26}_D$ + 24.0 (*c* 0.34, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.17), 229 (4.33), 272 (3.37) nm; CD (0.00040 M, MeOH) λ_{max} ($\Delta\varepsilon$) 198 (+10.5), 234 (+4.1) nm; IR (KBr) ν_{max} 3444, 2975, 2931, 1740, 1602, 1452, 1371, 1315, 1277, 1249, 1177, 1119, 1071, 1026, 712 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS 757.0 $[M+Na]^+$; HRESIMS *m/z* 757.2833 $[M+Na]^+$ (calcd for $C_{40}H_{46}O_{13}Na$, 757.2836).

4.3.7. Trigolin G (*7*)

White powder; $[\alpha]^{26}_D$ + 40.3 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.33), 230 (4.55), 272 (3.58) nm; CD (0.00038 M,

MeOH) λ_{max} ($\Delta\varepsilon$) 199 (+13.4), 238 (+11.4) nm; IR (KBr) ν_{max} 3437, 2972, 2929, 1725, 1602, 1452, 1379, 1315, 1278, 1177, 1121, 1070, 1025, 712 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS 777.0 $[M+Na]^+$; HRESIMS *m/z* 777.2893 $[M+Na]^+$ (calcd for $C_{43}H_{46}O_{12}Na$, 777.2886).

4.4. Anti-HIV-1 assay

Cytotoxicity against C8166 cells (CC_{50}) was assessed using the MTT method, and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}) (Zheng et al., 2000; Wang et al., 2004). Briefly, cells were seeded on a microtiter plate in the absence or presence of various concentrations of compounds in triplicate and incubated at 37 °C in a humid atmosphere of 5% CO_2 for 3 days. 20 μL MTT reagent (5 mg/mL in PBS) was added to each well, then incubated at 37 °C for 4 h, 50% DMF-20% SDS (100 μL) was added. After the formazan was dissolved completely, the plates were read on a Bio-Tek ELx 800 ELISA reader at 595 nm/630 nm (A595/630). The cytotoxic concentration that caused the reduction of viable cells by 50% (CC_{50}) was calculated from dose–response curve. In 100 μL various concentrations of compounds, C8166 cells (4×10^5/mL) were infected with virus ($HIV\text{-}1_{IIIB}$) at a multiplicity of infection (M.O.I) of 0.06. The final volume per well was 200 μL. Control assays were performed without the testing compounds in $HIV\text{-}1_{IIIB}$ infected and uninfected cultures. AZT was included as positive control. After 3 days of culture, the cytopathic effect (CPE) was measured by counting the number of syncytia (multinucleated giant cell). Percentage inhibition of syncytia formation was calculated and 50% effective concentration (EC_{50}) was calculated. The therapeutic index (SI) was calculated from the ratio of CC_{50}/EC_{50}.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2013.03.003.

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