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Compounds from *Kadsura heteroclita* and related anti-HIV activity

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Abstract

Phytochemical investigation of the stems of *Kadsura heteroclita* led to isolation of 16 compounds, including the triterpenoid named longipedlactone J (**2**), and two dibenzocyclooctadiene type lignans named heteroclitin I and J (**3**, **4**). Compounds **8**–**10**, **14**, and **15** were weakly active as anti-HIV agents, whereas compounds **6** and **12** exhibited moderate anti-HIV activity with EC_{50} values of 1.6 μg/mL, and 1.4 μg/mL, therapeutic index (TI) values of 52.9, and 65.9, respectively. Their structures were established by spectroscopic methods, including application of 2D NMR techniques and CD spectra.
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Keywords: *Kadsura heteroclita*; Schisandraceae; Triterpenoid; Dibenzocyclooctadiene type lignans; Anti-HIV-1 activity

1. Introduction

The rapid worldwide spread of acquired immunodeficiency syndrome (AIDS) has prompted an intense research effort to discover compounds that can effectively inhibit the human immunodeficiency virus (HIV), the etiological agent of AIDS (Yu et al., 2007). Natural products are a rich source of biologically active compounds, and are important sources of new drugs and leads besides tailored synthesis. Medicinal herbs may have practical value as an alternative medical therapy in inhibition of HIV infection (Kaleab et al., 2005). The genus *Kadsura* belongs to the family Schisandraceae. Some species of this genus have been reported to contain dibenzocyclooctadiene lignans, lanostane and cycloartane triterpenoids (Wang et al., 2006, 2007; Chen et al., 2006; Kuo et al., 2005). Lignans, especially of the dibenzocyclooctadiene lignans, are the principal bioactive constituents of *Kadsura* medicinal plant. Pharmacological studies have indicated various beneficial activities; including antitumor, anti-hepatitis and anti-lipid peroxidative activities; some dibenzocyclooctadiene lignans also exhibit potent anti-HIV activities (Yang et al., 1992; Kuo et al., 2001; Liu and Li, 1993, 1995; Shen et al., 2005; Chen et al., 1992, 1996, 2002). Therefore, in the research field of phytochemistry, this genus has aroused scientific interest to identify new natural compounds with anti-HIV activities, and to investigate the occurrence of natural compounds that could be used as natural sources of intermediates for synthesis of high-added-value compounds. The stems of *Kadsura heteroclita* (Roxb.) Craib, a plant indigenous to southern China, are known as the one of major source of "Ji-Xue-Teng" in Chinese traditional medicine for treatment of menstrual irregularities, blood deficiencies, and other feminine disorders (Lu and

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Chen, 2006). This paper deals with isolation and structural elucidation of three new compounds, named as longipedlactone J (**2**), heteroclitin I (**3**) and J (**4**), respectively, together with 13 known compounds, kadsulignan K (**5**) (Liu et al., 1992), interiorin (**6**), heteroclitin D (**7**), kadsurin (**8**) (Chen et al., 1992), heteroclitin F (**9**) (Yang et al., 1992), acetoxyl oxokadsurane (**10**), benzoyl oxokadsurane (**11**) (Li and Xue, 1990), interiorin B (**12**) (Ding and Luo, 1990), (−)-epicatechin (**13**) (Markham, 1976), quercetin (**14**) (Shen et al., 1993), taxifolin (**15**) (Markham, 1976), β-sitosterol (**16**); daucosterol (**17**) (Tan et al., 1996). Seven of these compounds (**6**, **8–10, 12, 14,** and **15**) showed activity in an HIV growth inhibition assay with TI values >5. In particular, interiorin (**6**) and interiorin B (**12**) exhibited moderate anti-HIV activity with EC_{50} values of 1.6 μg/mL, and 1.4 μg/mL, TI values of 52.9, and 65.9, respectively.

2. Results and discussion

2.1. Isolation, structure determinations

Longipedlactone J (**2**, 10.1 mg), heteroclitin I (**3**, 2.1 mg) and J (**4**, 6.5 mg) were obtained from the Me_2CO extract of the stems of *K. heteroclita* by column chromatography using normal phase and semipreparative HPLC. Their structural assignments were made by analysis of spectroscopic data including application of extensive 2D NMR techniques and CD spectra.

Longipedlactone J (**2**) was isolated as an optically active ($[\alpha]_D^{15.0}$ −241.6) white powder. The HRESIMS ($[M-H]^-$, *m/z* 467.3156, calcd. 467.3161), in combination with ^{1}H- and ^{13}C NMR spectroscopic data (Table 1), indicated that its molecular formula was $C_{32}H_{40}O_7$, necessitating a total of 13 degrees of unsaturation. The UV spectrum of **1** showed absorption maxima at 224, 256, 262, and 276 nm, suggesting the presence of several conjugated systems. The IR spectrum showed the presence of a hydroxyl group (3437 cm^{-1}), and two α, β-unsaturated lactone groups (1694, and 1707 cm^{-1}). The ^{1}H NMR spectrum (Table 1) exhibited signals for one secondary methyl (δ_H 1.08, d, $J = 7.2$ Hz), four tertiary methyls (δ_H 1.21, 1.46, 1.50, and 1.85), five olefinic proton resonances (δ_H 5.71, 6.00, 6.45, 6.86, and 6.71), a characteristic exocyclic methylene (δ_H 4.93, 5.09, each br s) group, as well as an acetyl singlet (δ_H 1.98, s). Analysis of the ^{13}C NMR, DEPT, and HSQC data established that **2** contains three carbonyl carbons (δ_C 166.0, 166.5, and 170.5), ten quaternary carbons (including four olefinic and two oxygenated carbons), eleven methines (including five olefinic and two oxygenated ones), five methylenes (including an exocyclic methylene), five methyls (including one secondary methyl) and an acetyl group. Apart from five double bonds and three carbonyl groups, the remaining elements of unsaturation in **2** were assumed to be those of a pentacyclic skeleton. These data were consistent with the HRMS empirical formula and suggested that **2** was probably a pentacyclic triterpene. Comparison of the ^{1}H- and ^{13}C NMR spectroscopic data with those of the known longipedlactone A (**1**) (Pu et al., 2006) showed the presence of the same pentacyclic triterpenoid skeleton, except for the presence of an oxygenated methine (δ_C 68.9, d) and an acetyl group (δ_C 21.1, 170.5) in **2**. They also differed by the absence of a methylene assigned to C-6 (δ_C 29.5, t) of **1**, indicating that the CH_2-6 of **1** was replaced by an acetyl group in **2**. This assignment was in accordance with the observation of significant downfield shifts of the C-4, C-5, C-6, C-7, and C-8 signals from δ_C 82.5 (s), 49.5 (d), 29.5 (d), 28.2 (t), and 57.4 (d) in **1** to δ_C 79.8 (s), 50.4 (d), 68.9 (d), 32.0 (t), and 50.4 (d) in **2**, respectively. This was further established by HMBC correlations observed from H-6 (δ_H 6.07, br s) to the acetyl carbonyl, and the ^{1}H, ^{1}H-COSY correlations of H-5/H-6/H-7/H-8 (Fig. 2a). The β-configuration of AcO–C(6) was deduced on the basis of the ROESY correlations (H-6/H-5α, H-6/CH_3-30α, H-6/H-7α, and H-6/H-7β) (Fig. 2b), which was

Table 1
^{1}H and ^{13}C NMR spectroscopic assignments of compounds **1** and **2**

Position	1	2	
	δ_C (mult.)	δ_H (mult., *J*, Hz)	δ_C (mult.)
1	146.8 (d)	6.86 (d, 12.3)	145.7 (d)
2	118.5 (d)	6.00 (d, 12.3)	119.2 (d)
3	169.5 (s)		166.5 (s)
4	82.5 (s)		79.8 (s)
5	49.5 (d)	4.46 (br s)	50.4 (d)
6	29.5 (t)	6.07 (br s)	68.9 (d)
7α	28.2 (t)	2.00 (dd, 2.0, 15.1)	32.0 (t)
7β		1.74 (m)	
8	57.4 (d)	2.00 (overlapped)	50.4 (d)
9	80.8 (s)		79.2 (s)
10	146.5 (s)		140.4 (s)
11α	51.7 (t)	2.58 (dd, 8.2, 13.1)	51.2 (t)
11β		1.65 (dd, 10.9, 13.1)	
12	54.1 (d)	2.78 (dd, 8.2, 10.9)	53.1 (d)
13	149.7 (s)		148.2 (s)
14	44.9 (s)		43.6 (s)
15α	38.1 (t)	1.60 (dd, 3.0, 15.9)	37.6 (t)
15β		2.01 (overlapped)	
16	126.7 (d)	5.71 (d, 3.0)	125.8 (d)
17	141.7 (s)		140.2 (s)
18a	108.2 (t)	5.09 (br s)	108.5 (t)
18b		4.93 (br s)	
19	149.1 (d)	6.71 (s)	148.2 (d)
20	41.1 (d)	3.00 (m)	40.0 (d)
21	15.8 (q)	1.08 (d, 7.2)	15.0 (q)
22	82.5 (d)	4.44 (overlapped)	80.7 (d)
23α	27.5 (t)	2.05 (m)	26.4 (t)
23β		2.36 (m)	
24	141.6 (d)	6.45 (br d, 6.0)	139.5 (d)
25	128.9 (s)		128.2 (s)
26	168.2 (s)		166.0 (s)
27	16.9 (q)	1.85 (s)	17.1 (q)
28	27.4 (q)	1.21 (s)	27.0 (q)
29	29.3 (q)	1.50 (s)	29.8 (q)
30	26.0 (q)	1.46 (s)	26.1 (q)
9-OH		6.83 (s)	
6-OAc		1.98	21.1 (q)
			170.5 (s)

All spectra were recorded in pyridine-d_5 at 500 MHz. δ in ppm, *J* in Hz.

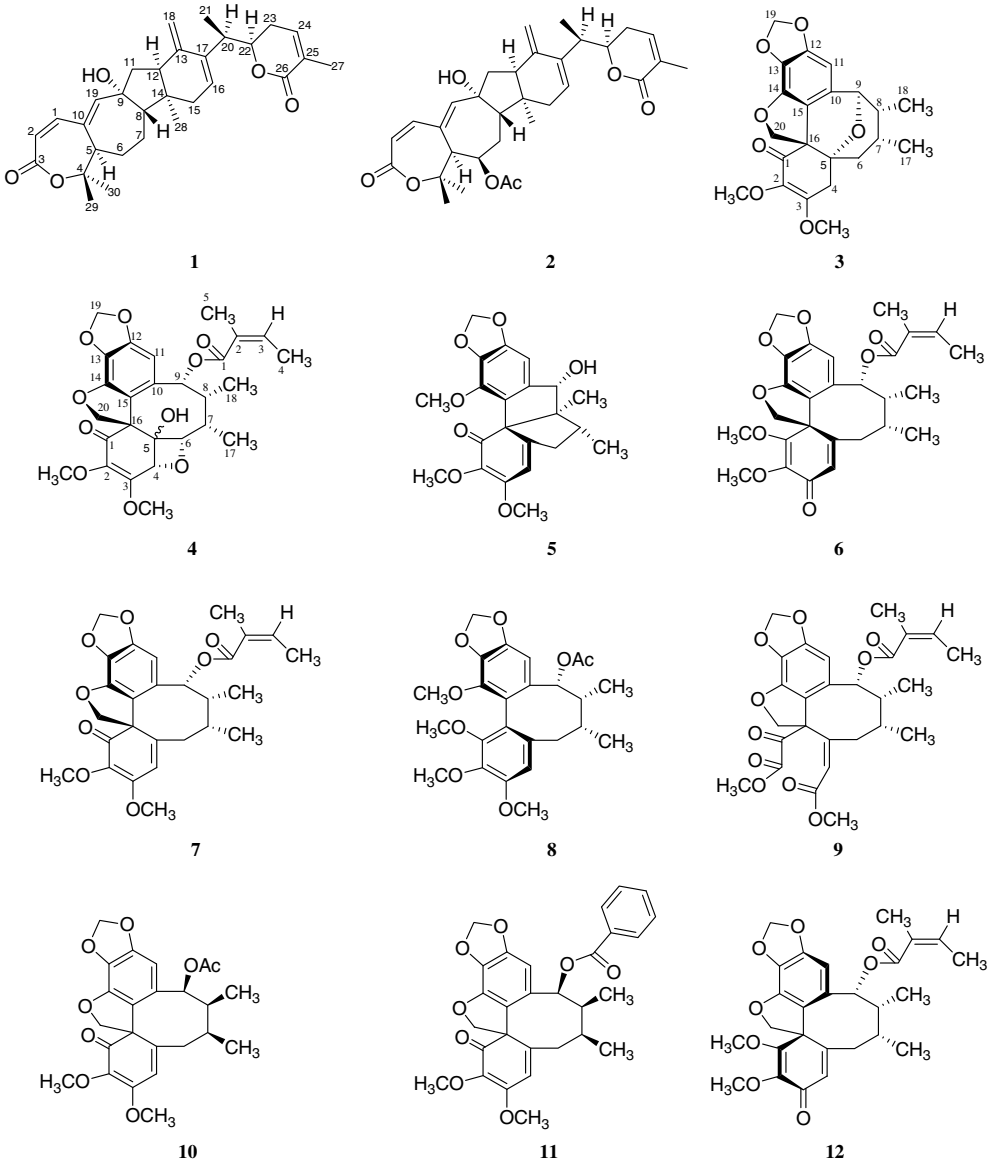

Fig. 1. Structures of compounds **1**–12.

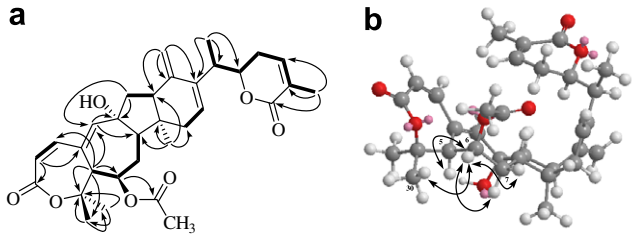

Fig. 2. (a) ^{1}H, ^{1}H COSY (−) and key HMBC (H ⟶ C) correlations of **2**; (b) key ROESY (↔) correlations of **2**.

also supported by the upfield shift of C-8 ($\Delta\delta_C$ −7.0) caused by the syn-γ effect between AcO–C(6) and H_β-8 (Han et al., 2003). Thus, the structure of **1** was determined as 6β-acetyl-longipedlactone A, and named as longipedlactone J.

Heteroclitin I (**3**), obtained as white powder, had the molecular formula $C_{22}H_{24}O_7$ as determined by positive HRESIMS (*m/z* 423.1428 $[M+Na]^+$, calcd. 423.1419). The UV bands (218, 271 nm) and IR absorptions at 1666, 1627 cm^{-1} indicated the presence of benzyl and carbonyl groups. Characteristic *AB* quartet signals at δ_H 4.41 and 4.50 in the ^{1}H NMR spectrum (Table 2), and a quaternary C-atom at δ_C 58.0 in the ^{13}C NMR spectrum (Table 2), indicated that **3** was a dibenzocyclooctane-type lignan with a spirobenzofuranoid skeleton (Li and Xue, 1990). The ^{1}H NMR spectrum of **3** showed the presence of two secondary methyl groups (δ_H 0.70, and 1.06, 3H each, d, $J = 6.8$, 7.2, respectively), assignable to CH_3-17 and CH_3-18 groups, respectively; one methylenedioxy moiety (δ_H 5.90, and 5.93, *AB*, $J = 0.8$ Hz, 1H each), and two methoxy groups (δ_H 3.56, and 4.01, 3H each, s) on an aromatic ring, and one aromatic proton (δ_H 6.16 for H-11).

Table 2
^{1}H and ^{13}C NMR spectroscopic assignments of compounds **3** and **4**

Position	3		4	
	δ_H (mult., *J*, Hz)	δ_C (mult.)	δ_H (mult., *J*, Hz)	δ_C (mult.)
1		196.1 (s)		194.2 (s)
2		162.6 (s)		161.3 (s)
3		133.8 (s)		137.0 (s)
4α	2.68 (d, 18.7)	40.8 (t)	4.02 (d, 3.9)	72.4 (d)
4β	2.87 (d, 18.7)			
5		73.8 (s)		67.3 (s)
6α	1.35 (t, 13.2)	36.4 (t)	3.27 (d, 9.4)	74.7 (d)
6β	1.79 (dd, 5.2, 15.1)			
7	1.11 (m)	25.2 (d)	1.70 (m)	35.3 (d)
8	1.46 (m)	40.5 (d)	1.90 (m)	43.9 (d)
9	4.70 (s)	78.6 (d)	5.79 (d, 5.0)	81.4 (d)
10		128.7 (s)		130.7 (s)
11	6.16 (s)	97.9 (d)	6.35 (s)	102.1 (d)
12		130.1 (s)		131.5 (s)
13		152.8 (s)		151.5 (s)
14		142.0 (s)		145.7 (s)
15		123.2 (s)		121.4 (s)
16		58.0 (s)		61.0 (s)
17	0.70 (d, 6.8)	19.6 (q)	1.08 (d, 7.2)	18.2 (q)
18	1.06 (d, 7.2)	12.0 (q)	1.06 (d, 7.2)	11.5 (q)
19a	5.93 (d, 0.8)	102.6 (t)	5.96 (d, 1.1)	103.3 (t)
19b	5.90 (d, 0.8)		5.94 (d, 1.1)	
20α	4.50 (d, 9.5)	81.0 (t)	4.87 (d, 9.4)	81.9 (t)
20β	4.41 (d, 9.5)		4.56 (d, 9.4)	
1′				169.4 (s)
2′				128.4 (s)
3′				139.9 (s)
4′			1.78 (overlapped)	16.2 (q)
5′			1.79 (s)	21.7 (q)
2-OCH_3	4.01 (s)	58.3 (q)	4.06 (s)	58.2 (q)
3-OCH_3	3.56 (s)	60.7 (q)	3.54 (s)	60.3 (q)

All spectra were recorded in CD_3OD at 500 MHz. δ in ppm, *J* in Hz.

Moreover, in the downfield region, an oxymethine was observed at δ_H 4.70 (H-9). The ^{13}C NMR spectrum and DEPT established that **3** possessed corresponding signals including two methyl (δ_C 12.0, and 19.6), an α, β-unsaturated carbonyl group (δ_C 196.1), one pentasubstituted aromatic ring, two olefinic carbons (C-2, δ_C 162.6 and C-3, δ_C 133.8), one methylenedioxy carbon (δ_C 102.6), three methine carbons (including a oxygenated carbon, δ_C 78.6), two quaternary carbons (including an oxygenated carbon, δ_C 73.8), three methylene carbons (including an oxygenated carbon, δ_C 81.0) and two methoxyl (δ_C 58.3, 60.7) groups. The functional groups were assigned on the basis of HMBC and ^{1}H, ^{1}H COSY studies (Fig. 3a). Thus, the correlations of a methylenedioxy proton (–OCH_2O–) to C-12 and C-13, the oxygenated methylene protons CH_2-20 to C-1, C-5, C-16, and the two methoxy groups to C-2 and C-3, respectively, confirmed the substituent group positions. The HMBC correlation of H-9 to C-5 confirmed there was an oxo-bridge between C-9 (δ_C 78.6, d) and C-5 (δ_C 73.8, s), which was further confirmed by MS analysis. The relative configuration of **3** was shown to be as depicted in Fig. 3b by the correlations observed in a ROESY experiment. ROESY correlations were also

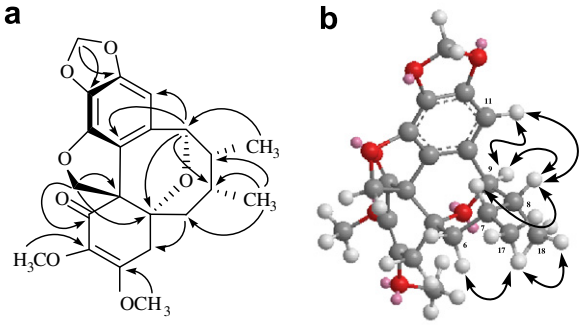

Fig. 3. (a) Key HMBC (H ⟶ C) correlations of **3**; (b) key ROESY (↔) correlations of **3**.

observed between H-7 and H-8, CH_3-17 and CH_3-18; CH_3-17 also gave a ROESY correlation to H-6α, which established that CH_3-17 and CH_3-18 were in an α-orientation, and the ROESY correlations of H-9 with H-8, and H-11 indicated the oxo-bridging was in α-orientation, too. The similar CD spectrum of **3** with that of kadsutherin C (Lu and Chen, 2006) (negative Cotton effects at 216 and 246 nm, and positive ones at 228 and 277 nm) indicated an axially chiral (aS)-1,1′-biphenyl unit, with the cyclooctane ring deduced to be in a boat-like conformation. Thus, the structure of **3** was determined, and named as heteroclitin I. Note that compound **3** was the first example of a dibenzocyclooctane type lignan connecting between C-9 and C-5 with an oxygen atom in *Kadsura* species.

Heteroclitin J (**4**), obtained as white powder, had the molecular formula $C_{27}H_{30}O_{10}$, as derived from HRESIMS at m/z 537.1731 ($[M+Na]^+$, calcd. 537.1736). The UV, IR and NMR spectra established that **4** possessed a C_{18} lignan skeleton with an additional oxygenated methylene group, which was similar to those of known C_{19} homolignans (Lu and Chen, 2006). The presence of signals at $\delta_H = 6.09$ (brq, 1H, $J = 7.6$, H-3′), 1.78 (overlapped, 3H, H-4′), 1.79 (s, 3H, H-5′), and δ_C 169.4 (C-1′), 128.4 (C-2′), 139.9 (C-3′), 16.2 (C-4′), 21.7 (C-5′) (Table 2) indicated an angeloxy group in **4**. The positive FABMS fragments at *m/z* 415 $[M-C_4H_7COOH+H]^+$ also confirmed this deduction. The structure of **4** was elucidated from its COSY, HSQC, and HMBC studies. The HMBC experiment (Fig. 4a) of **4** also indicated correlations of H-9 to C-1′, and that an angeloxy group was located at C-9. The HMBC correlations of H-4 to C-5 (δ_C 67.3, s), C-6 (δ_C

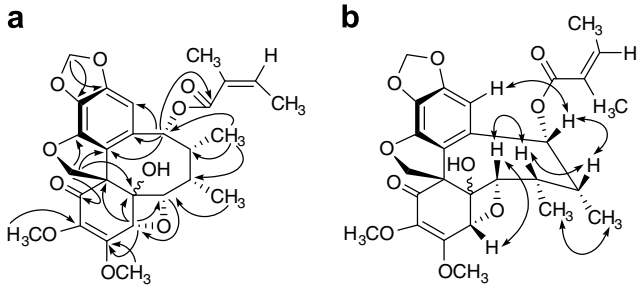

Fig. 4. (a) Key HMBC (H ⟶ C) correlations of **4**; (b) key ROESY (↔) correlations of **4**.

74.7, d), and C-16 (δ_C 61.0, s), and H-6 to C-4 (δ_C 72.4, d), together with the 13 degrees of unsaturation of **4**, establishing that one ether ring was required between C-4 and C-6. The ROESY spectrum (Fig. 4b) further determined the stereochemistry of **4**: correlations of H-7 with H-8, and CH_3-17 with CH_3-18 suggested that CH_3-17 and CH_3-18 were in a *cis*-configuration; correlations of H-6 with H-7, and H-4 showed that the quaternary ring system should be positioned on the α-orientation, and correlations of H-9 with H-11, and H-8 indicated the angeloxy group was in the α-orientation too. Because there would be no positive ROESY correlations of OH-5, the relative configuration could not be determined. Since compound **4** had a similar CD Cotton effect curve to that of the known skeletally similar compound kadsutherin C, the conformation of **4** might be assigned as 6S, 7S, 8S, 9R, 16S. Therefore, heteroclitin J was assigned as showed in Fig. 1.

2.2. Bioassay experiments

The anti-HIV activity was indicated as potencies of compounds **2**, **4–15** in preventing the cytopathic effects of HIV-1 in C8166 cells, with cytotoxicity measured in parallel with the determination of antiviral activity using AZT as a positive control ($EC_{50} = 2.03$ ng/mL and $CC_{50} =$ 1146.08 μg/mL). Compounds **6** and **12** exhibited moderate anti-HIV-1 activity with EC_{50} values of 1.6 μg/mL, and 1.4 μg/mL, and therapeutic index (TI) values of 52.9, and 65.9, respectively (Table 3). Compounds **8–10**, **14**, and **15** showed weak activity with TI values >5. Comparison of the dibenzocyclooctadiene lignans (**4–12**) with the same skeleton, compounds **6** and **12** demonstrated higher activity than the others in anti-HIV-1 activity, indicating that αα′ββ′-dienone mignt be an important functional group. In addition, because of a small quantity of compounds **3**, its anti-HIV activity was not tested.

Table 3
Anti-HIV activities of the compounds from *K. heteroclita*

Compound	CC_{50} (μg/mL)[a]	EC_{50} (μg/mL)[b]	TI (CC_{50}/EC_{50})[c]
2	7.3	3.8	1.9
4	112.4	64.4	1.7
5	>200	79.5	>2.5
6	84.6	1.6	52.9
7	82.1	17.8	4.6
8	97.1	17.4	5.6
9	>200	19.9	>10.1
10	99.7	7.5	13.3
11	69.3	22.1	3.1
12	92.2	1.4	65.9
13	>200	45.0	>4.4
14	87.8	5.3	16.6
15	118.9	13.8	8.6
Positive control AZT	1146.1	2.03 ng/ml	564571.4

[a] CC_{50} = concentration causing 50% cellular cytotoxicity.
[b] EC_{50} = 50% effective concentration.
[c] TI = therapeutic index.

2.3. Conclusion

In this study, the stems of *K. heteroclita* were investigated chemically, and a new triterpenoid (five ring systems), and two new lignans together with 13 known compounds were isolated. The new lignans were the first examples of the dibenzocyclooctadiene type lignan connecting between C-9 and C-5 in **3**, C-4 and C-6 in **4**, respectively, with an oxygen atom in *Kadsura* species. In the anti-HIV-1 activity assays, five compounds (**8–10**, **14**, and **15**) showed weak activity with TI values >5, and two compounds (**6** and **12**) showed moderate activity with TI values >50.

3. Experimental part

3.1. General experimental procedures

Optical rotations were measured on a JASCO DIP-370 digital polarimeter, whereas CD spectra were measured on a JASCO J-810 spectropolarimeter. IR spectra were obtained on a Bio-Rad FtS-135 spectrophotometer with KBr pellets, whereas UV spectral data were obtained using a UV-210A spectrometer. MS were recorded on a VG Auto Spec-3000 spectrometer. 1D and 2D NMR spectra were obtained on the Bruker DRX-500 instruments with TMS as an internal standard. CC and TLC: silica gel (200–300 mesh) from Qingdao Marine Chemical Factory, Quingdao, People's Republic of China.

3.2. Plant material

The stems of *K. heteroclita* were collected in fengqing county of yunnan province, China, in August 2005, and identified by Prof. Xi-Wen Li, Kunming Institute of Botany. A voucher specimen (No. 0043643) has been deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and isolation

The air-dried and powdered stems (4.1 kg) of *K. heteroclita* were extracted three times with H_2O:acetone (7:3, v/v, 15 L) at room temperature to yield an extract, which was successively extracted with petroleum ether and EtOAc. The EtOAc extract was evaporated to dryness under reduced pressure to give an extract (120 g) that was separated by silica gel CC (1 kg, 200–300 mesh) and eluted with a $CHCl_3/Me_2CO$ gradient system (9:1, 8:2, 7:3, 6:4, 5:5) to give fractions 1–5. Fraction 1 (30.1 g) and 2 (12 g) was subjected to CC with $CHCl_3/(CH_3)_2CHOH$ (20:1) to afford 6 fractions, and 4 fractions, respectively, which were further purified by semipreparative HPLC separation (Agilent 1100 HPLC system, USA; Zorbax SB-C-18, Agilent, 9.4mm × 25 cm, USA, MeOH–H_2O) to give compound **2** (10.1 mg), **3** (2.1 mg), **4** (6.5 mg), **5** (8.3 mg), **6** (73.4 mg), **7** (15.2 mg), **8** (3.0 mg), **9** (41.7 mg), **10** (86.0 mg), **11**

(11.1 mg), **12** (10.0 mg), and **13** (9.2 mg). Fraction 3 was subjected to CC with $CHCl_3/CH_3OH$ (10:1) to afford 3 fractions, which were further purified by Sephadex LH-20 (CH_3OH) to afford compounds **14** (63.0 mg), **15** (23.2 mg), **16** (80.4 mg), and **17** (26.1 mg).

3.3.1. Longipedlactone J (2)

White powder, $[\alpha]_D^{15.0}$ −241.6 (*c* 0.69, C_5H_5N); UV (MeOH) λ_{max} (log ε): 361 (2.70), 276 (4.42), 262 (4.40), 256 (4.38), 224 (4.42) nm; IR (KBr) v_{max} 3437, 2976, 2930, 2886, 2838, 1718, 1694, 1373, 1237, 1132, 1034, 989 cm^{-1}; For NMR spectroscopic analysis, see Table 1. Negative FABMS *m/z* 720 $[M+2Gly]^-$ (33%), 627 $[M+Gly-H]^-$ (100%), 536 $[M]^-$ (70%); negative HR-ESIMS found 535.2699, calcd. 535.2695 for $C_{32}H_{39}O_7$ $[M-H]^-$.

3.3.2. Heteroclitin I (3)

White powder, $[\alpha]_D^{15.1}$ +88.6 (*c* 0.43, C_5H_5N); UV (MeOH) λ_{max} (log ε): 271 (4.40), 218 (4.80) nm; IR (KBr) v_{max} 3438, 2957, 2926, 2880, 1666, 1627, 1477, 1465, 1372, 1271, 1247, 1198, 1114, 1053, 998 cm^{-1}; For NMR spectroscopic analysis, see Table 2. Negative FABMS *m/z* 491 $[M+Gly-H]^-$ (1%), 399$[M-H]^-$ (100%); positive HR-ESIMS found 423.1428, calcd. 423.1419 for $C_{22}H_{24}O_7Na$ $[M+Na]^+$. CD (CH_3OH): Δε (nm) = 31.26 (277), −5.75 (247), 2.72 (228), −41.91 (216).

3.3.3. Heteroclitin J (4)

White powder, $[\alpha]_D^{16.6}$ +51.3 (*c* 0.80, C_5H_5N); UV (MeOH) λ_{max} (log ε): 268 (4.44), 255 (4.35), 217 (4.91) nm; IR (KBr) v_{max} 3489, 2969, 2944, 2878, 2585, 1715, 1668, 1630, 1505, 1456, 1382, 1229, 1198, 1148, 1115, 1070, 1033 cm^{-1}; For NMR spectroscopic analysis, see Table 2. Positive FABMS *m/z* 1029 $[2M+H]^+$ (1%), 513$[M-H]^+$ (8%), 415$[M-C_5H_8O_2+H]^+$ (100%); positive HR-ESIMS found 537.1731, calcd. 537.1736 for $C_{27}H_{30}O_{10}Na$ $[M+Na]^+$. CD (CH_3OH): Δε (nm) = 14.41 (276), −21.34 (246), 30.30 (224), −9.43 (209).

3.4. Anti-HIV-1 assay

The cytotoxicity assay 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}) (Wang et al., 2004).

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