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Trinorcucurbitane and cucurbitane triterpenoids from the roots of *Momordica charantia*

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Abstract

Five cucurbitacins, kuguacins A–E (**1**–**5**), together with three known analogues, 3β,7β,25-trihydroxycucurbita-5,(23*E*)-diene-19-al (**6**), 3β,25-dihydroxy-5β,19-epoxycucurbita-6,(23*E*)-diene (**7**), and momordicine I (**8**), were isolated from roots of *Momordica charantia*. Structures of **1**–**5** were elucidated by NMR and MS spectroscopic analysis. Among them, compounds **3**–**5** possess an unprecedented 25,26,27-trinorcucurbitane backbone. Compounds **3** and **5** showed moderate anti-HIV-1 activity with EC_{50} values of 8.45 and 25.62 μg/ml, and exerted minimal cytotoxicity against C8166 cells ($IC_{50} > 200$ μg/ml), with a selectivity index more than 23.68 and 7.81, respectively.

Keywords: *Momordica charantia*; Cucurbitaceae; Roots; Kuguacins A–E; Trinorcucurbitane triterpenoids; Anti-HIV-1

1. Introduction

Momordica charantia L. (Cucurbitaceae) is distributed in Asian countries and widely cultivated as a vegetable crop. Its fruit, called kugua, is a favorable vegetable in China. The fruits, vines, leaves, and roots of *M. charantia* have been used in China to treat toothache, diarrhea, furuncle, and diabetes. Many phytochemical studies on these tissues have been reported, which resulted in isolation of nearly 50 new cucurbitins and cucurbitane glycosides (Chang et al., 2006; Fatope et al., 1990; Harinantenaina et al., 2006; Kimura et al., 2005; Miyahara et al., 1981; Murakami et al., 2001; Nakamura et al., 2006; Okabe et al., 1980, 1982a,b; Yasuda et al., 1984). However, no work has been reported on the constituent of the roots of this plant.

Cucurbitane compounds isolated from *M. charantia* are noted for antidiabetic and anticancer activities (Harinantenaina et al., 2006; Zhu et al., 1990). In the course of our search for potential bioactive cucurbitacins from cucurbitaceous plants, five new ones named kuguacins A–E (**1**–**5**), together with three known compounds (**6**–**8**) (Fig. 1), were isolated from the roots of *M. charantia* collected in Yunnan, China. This paper reports the isolation and structure elucidation of the new compounds and their anti-HIV activity.

2. Results and discussion

The methanol extract of the roots of *M. charantia* was subjected to silica gel and Sephadex LH-20 column chromatography to afford five new cucurbitacins (**1**–**5**), in addition to three known ones (**6**–**8**).

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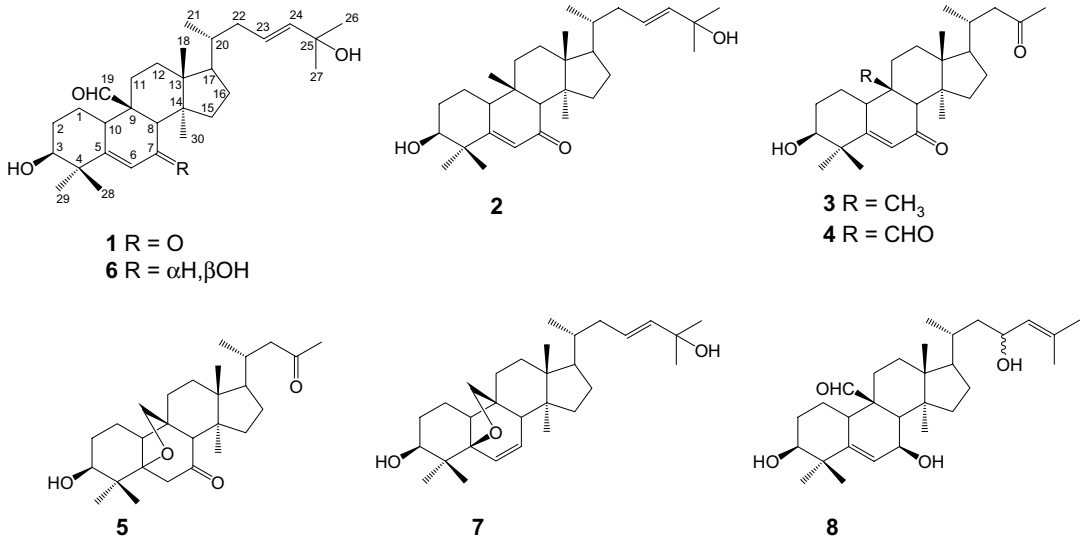

Fig. 1. Structures of **1**–**8**.

The known compounds were identified by using spectroscopic methods including EIMS, ^{1}H and ^{13}C NMR spectroscopic analysis and also by comparing experimental data with those described in the literature as 3β,7β,25-trihydroxycucurbita-5,(23*E*)-dien-19-al (**6**) (Fatope et al., 1990), 3β,25-dihydroxy-5β,19-epoxycucurbita-6,(23*E*)-diene (**7**) (Chang et al., 2006), and momordicine I (**8**) (Yasuda et al., 1984), respectively.

Kuguacin A (**1**) was isolated as colorless needles. Its molecular formula was determined as $C_{30}H_{46}O_4$ by HRESIMS ($[M+Na]^+$ at *m/z* 493.3292, cacld 493.3293). Its IR spectrum showed absorptions for a hydroxyl group (3429 cm^{-1}), an aldehyde group (2873 cm^{-1}), a conjugated carbonyl group (1722 cm^{-1}) and a double bond (1649 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **1** (Tables 2 and 3) showed the presence of six tertiary methyl groups [δ_H 0.83, 0.87, 1.15, 1.28 (3H each, *s*) and 1.29 (3H ×2, *s*); δ_C 18.3 (*q*), 14.9 (*q*), 27.2 (*q*), 24.9 (*q*), 29.9 (*q*) and 30.0 (*q*)], a secondary methyl group [δ_H 0.90 (3H, *d*, $J = 6.0$ Hz); δ_C 18.8 (*q*)], two oxygenated carbons [δ_H 3.67 (1H, *s*); δ_C 76.6 (*d*) and 70.0 (*s*)], an aldehyde group [δ_H 9.56 (1H, *s*); δ_C 203.4 (*d*)], a disubstituted double bond [δ_H 5.56 (2H, *m*); δ_C 124.9 (*d*) and 139.8 (*d*)], and an α, β-unsaturated carbonyl system [δ_H 6.19 (1H, *br s*); δ_C 127.1 (*d*), 168.1 (*s*) and 199.4 (*s*)]. In addition, the upfield region of the ^{13}C NMR and DEPT spectra displayed seven methylenes, four methines, and four quaternary carbons. Comparison of the ^{1}H and ^{13}C NMR spectroscopic data between **1** and **6** (Fatope et al., 1990) showed similarities except that an oxymethine at C-7 in **6** was replaced by a carbonyl group (δ_C 199.4, *s*) in **1**. This was confirmed by the observation of HMBC correlations from H-6 (δ_H 6.19, 1H, *br s*) to C-4 (δ_C 43.6, *s*), C-7 (δ_C 199.4, *s*), C-8 (δ_C 51.2, *d*) and C-10 (δ_C 37.9, *d*). Thus, **1** was concluded to be 3β,25-dihydroxycucurbita-5,(23*E*)-dien-7-on-19-al.

Kuguacin B (**2**) was assigned the molecular formula $C_{30}H_{48}O_3$, as deduced from its positive HRESIMS (*m/z* 479.3511 $[M+Na]^+$) and NMR data. The ^{13}C NMR spectrum of **2** (Table 3) was similar to that of **1**, except for the presence of a signal at δ_C 27.8 (*q*) and the absence of an aldehyde signal at δ_C 203.4 (*d*), indicating that the aldehyde group at C-19 of **1**was replaced by a methyl group in **2**. The suggestion was in accord with the observation of the downfield shift of C-8 signal from δ_C 51.2 in **1** to δ_C 59.8 in **2**. This was further established by the HMBC correlations from both H-8 (δ_H 2.38, 1H, *s*) and H-10 (δ_H 2.67, 1H, *t-like*, $J = 8.3$ Hz) to C-19 (δ_C 27.8). Therefore, compound **2** was elucidated as 3β,25-dihydroxycucurbita-5,(23*E*)-dien-7-one.

Kuguacin C (**3**) was obtained as colorless needles. Its molecular formula, $C_{27}H_{42}O_3$, was established from the quasimolecular ion peak at *m/z* 437.3038 $[M+Na]^+$ in the HRESIMS data. Its IR spectrum showed absorptions for hydroxyl group (3521 cm^{-1}) and a conjugated carbonyl group (1698 cm^{-1}). The UV spectrum exhibited a conjugated group based on the absorption at 248 nm. In the ^{1}H NMR spectrum, the signals of six tertiary methyl groups [δ_H 0.82, 0.90, 0.96, 1.13, 1.24 and 2.11 (3H each, s)] and one secondary methyl group (δ_H 0.90, 3H, *d*, $J = 6.4$ Hz) were detected. The ^{13}C NMR spectrum of **3** showed signals for 27 carbons due to seven methyl groups, two carbonyl groups, one olefinic group, seven methylenes, five methines (including an oxygenated one), and four quaternary carbons. Considering the fact that the tetracyclic triterpenoids isolated thus from *M. charantia* were generally cucurbitane compounds, compound **3** was tentatively presumed to have the basic skeleton of trinorcucurbitacin, and a fragmentation at *m/z* 166 $[C_{10}H_{42}O_2]^+$ (base peak) due to the loss of rings-C, D by cleavage of C-7/C-8 and C-9/C-10 implied that **3** had a Δ^5-7-one structure (Akihisa et al., 1994).

Comparison of the ^{13}C NMR spectrum of **3** with that of **2** displayed similarities in rings A–D, except for the absence of the signals for C-25, 26, 27, and the presence of a carbonyl group at δ_C 209.1 and a methyl group at δ_C 30.5 in side chain of **3**. A further analysis of the methyl group at δ_H 2.11 (3H, *s*) in the ^1H NMR spectroscopic data led to the conclusion that the carbonyl group was positioned at C-23. This conclusion was substantiated by the HMBC correlations from H-21 (δ_H 0.90, 3H, *d*, $J = 6.4$ Hz) to C-20 (δ_C 32.8, *d*) and C-22 (δ_C 51.1, *t*), and from H-22 (δ_H 2.10, 1H, *m*; δ_H 2.44, 1H, *br d*, $J = 15.7$ Hz) to C-23 (δ_C 209.1, 9is) and C-24 (δ_C 30.5, *q*). Thus, compound **3**was assigned as 3β-hydroxy-25,26,27-trinorcucurbita-5-en-7,23-dione.

Kuguacin D (**4**) was assigned as $C_{27}H_{40}O_4$ by HRESIMS ($[M+Na]^+$, *m/z* 451.2826) and ^{13}C NMR data. Its IR peaks at 1716 and 1643 cm^{-1} suggested the presence of an α,β-conjugated carbonyl group, which was confirmed by the absorption at 246 nm in the UV spectrum. Comparison of the 1D NMR spectroscopic data of **4** with those of **3** showed similarities except that the methyl group at C-19 in **3** was replaced by an aldehyde group [δ_H 9.91 (1H, *s*); δ_C 204.7 (*d*)] in **4**. The upfield chemical shift of C-8 from δ_C 59.7 in **3** to δ_C 52.0 in **4** also supported the above deduction. HMBC correlations observed from H-19 (δ_H 9.91, 1H, *s*) to C-8 (δ_C 52.0, *d*) and C-10 (δ_C 39.2, *d*) also corroborated the proposed structure. Kuguacin C (**4**) was therefore determined as 3β-hydroxy-25,26,27-trinorcucurbita-5-en-7,23-dion-19-al.

Kuguacin E (**5**) was established as $C_{27}H_{42}O_4$ by HRESIMS ($[M+Na]^+$, *m/z* 453.2986) and ^{13}C NMR data. Its IR spectrum showed absorption at 3536 cm^{-1}, indicating the presence of hydroxyl group. The UV spectrum displayed no conjugated group based on the absence of an absorption from 230 nm to 350 nm. Obvious signals in the ^1H NMR spectrum were five methyl singlets at δ_H 0.76 (3H, *s*), 0.86 (3H, *s*), 0.93 (3H, *s*), 1.27 (3H, *s*), and 2.10 (3H, *s*), an oxymethylene at δ_H 3.55 (1H, *d*, $J = 8.5$ Hz) and 3.85 (1H, *d*, $J = 8.5$ Hz), as well as a methylene at δ_H 2.49 (1H, *d*, $J = 17.9$ Hz) and 2.96 (1H, *d*, $J = 17.9$ Hz). Analysis of the 1D NMR spectra of **5** also showed a 25,26,27-trinorcucurbitane skeleton. Comparison of ^1H and ^{13}C NMR data spectroscopic of **5** with those of **4** showed that the differences can be rationalized by the replacement of an aldehyde group present at C-19 and two olefin carbon signals at C-5 and C-6 in **4** by an oxymethylene group at C-19, an oxygenated quaternary carbon at C-5, and a methylene group at C-6 in **5**. The molecular formula, indicating seven degrees of unsaturation, still required the additional ring, thus C-19 (δ_C 79.2, *t*) was presumed to be linked to C-5 (δ_C 89.1, *s*) via an oxygen atom. The obvious long-range HMBC correlations (Fig. 2) from H-19 [δ_H 3.55 (1H, *d*, $J = 8.5$ Hz) and 3.85 (1H, *d*, $J = 8.5$ Hz)] to C-5 undoubtedly demonstrated that there was an oxygen bridge between C-5 and C-19. Following the stereochemistry of cucurbitane compounds, O-5 was established to be β-oriented (Mulholland et al., 1997). Kuguacin E (**5**) was thus determined as 3β-hydroxy-5β,19-epoxy-25,26,27-trinorcucurbita-7,23-dione. Though compound **5** was obtained as white prism crystals, an attempt of X-ray diffracting did not succeed for the small amount.

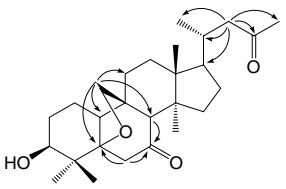

Fig. 2. Key HMBC correlations of **5**.

The cucurbitane-type triterpenoid skeleton is very stable. It has been reported that among more than two hundred cucurbitanes, only several lacked C-28 (or 29) in ring A, or C-22, 23, 24, 25, 26, and 27 in their side-chain. The novel cleavage between C-24 and 25 has never been described thus far. To the best of our knowledge, compounds **3**–**5** also represent the first occurrence of tetracyclic triterpenoids with a methyl group at C-24 and a carbonyl group at C-23. When treated with hot alkali, ecballic acid, a tetranorcucurbitacin, was formed from cucurbitacins J and K (Enslin and Norton, 1964). It was also reported that the structure of 5,19-epoxy-6-en-cucurbitane was obtained from momordicoside K on treatment of $NaBH_4$ and then acetic acid in MeOH (Okabe et al., 1982b). The possibility of the three compounds being artifacts produced during the separation could be excluded since the extraction and isolation processes did not involve the use of either alkali or acid.

Cucurbitane compounds are generally bitter, including the three trinorcucurbitacins. However, compound **6**, from *M. charantia*, is nearly tasteless.

The compounds were tested for in vitro inhibitory effects against HIV replication in C8166 cells. As shown in Table 1, compounds **3** and **5** displayed moderate anti-HIV-1 activity with EC_{50} values of 8.45 and 25.62 μg/ml, and exerted minimal cytotoxicity against C8166 cells ($IC_{50} > 200$ μg/ml), with a selectivity index more than 23.68 and 7.81, respectively. The other compounds were inactive with a selectivity index of 7.01 for **1**, 3.09 for **2**, 1.54 for **4**, 11.87 for **6**, 1.92 for **7**, and 3.76 for **8**. This is the first report of (weak) anti-HIV activity data of cucurbitacins.

Table 1
Anti-HIV activity of compounds**1**–**8**

No.	Cytotoxicity IC_{50} (μg/ml)	Anti-HIV-1 activity EC_{50} (μg/ml)	Selectivity index SI (IC_{50}/EC_{50})
1	75.56	10.78	7.01
2	37.35	12.08	3.09
3	>200	8.45	23.68
4	83.87	54.14	1.54
5	>200	25.62	>7.81
6	67.33	5.67	11.87
7	92.57	48.56	1.91
8	20.18	5.37	3.76
AZT	>200	0.0034	58823

3. Experimental

3.1. General experimental procedures

Melting points were obtained on an XRC-1 apparatus and are uncorrected. Optical rotations were carried out on a Perkin–Elmer model 241 polarimeter. UV spectra were measured in a UV 210A spectrometer. IR spectra were measured in a Bio-Rad FTS-135 spectrometer with KBr pellets. MS were recorded on a Finnigan MAT 90 instrument. 1D and 2D NMR spectra were measured on either a Bruker AM-400 or a Bruker DRX-500 instrument with TMS as internal standard. Column chromatography was performed either on silica gel (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), or Lichroprep RP-18 gel (40–63 μm; Merck, Darmstadt, Germany). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 15% H_2SO_4 in H_2O.

3.2. Plant material

The plants were cultivated at Dahanying Village, Anning County, Yunnan Province, People's Republic of China, in August 2005. The sample was identified by Prof. Shukun Chen, and a voucher specimen has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy Sciences.

3.3. Extraction and isolation

Air-dried roots of *M. charantia* (1.9 kg) were extracted with MeOH (3 × 5 l, 6 h each) at 60 °C. After removal of the solvent under reduced pressure, a residue (160 g) was obtained. This residue was dissolved in H_2O (2 L) and then extracted successively with EtOAc (1 L ×3) and *n*-BuOH (1 L ×4). The EtOAc extract (40 g) was subjected to silica gel CC, eluted with a gradient system of $CHCl_3$/MeOH (1:0, 30:1, 20:1, 10:1) to yield fractions I–V monitored by TLC. Fraction I (5 g) was repeatedly applied to silica gel CC using $CHCl_3$/$(Me)_2CO$ (50:1, 20:1, 15:1) as eluent and then purified further over Sephadex LH-20 using MeOH to give compounds **3** (17 mg), **4** (14 mg), and **5** (9 mg). In the same way, compounds **1** (21 mg) and **2** (11 mg) were successively obtained from Fraction II (1.2 g). Compound **6** (310 mg) was purified from fraction III (1.5 g) by recrystallization. Compounds **7** (23 mg) and **8** (40 mg) were isolated and purified from fraction IV (3 g) by silica gel CC with $CHCl_3$/MeOH (20:1) as eluent

Table 2
^{1}H NMR (400 MHz) spectroscopic data of compounds **1**–**5**

	1	**2**	**3**[a]	**4**[b]	**5**[a]
1	1.74 *m*	1.75 *m*	1.76 *m*	1.93 *m*	1.51 *m*
	1.84 *m*			2.06 *m*	1.76 *m*
2	1.85 *m*	1.78 *m*	1.81 *m*	1.93 *m*	1.78 *m*
	2.01 *m*	1.97 *m*	2.00 *m*	2.09 *m*	1.80 *m*
3	3.67 *s*	3.64 *s*	3.65 *br s*	3.82 *br s*	3.56 *s*
6	6.19 *br s*	6.08 *br s*	6.08 s	6.48 *s*	2.49 *d* (17.9); 2.96 *d* (17.9)
8	2.47 s	2.38 *s*	2.40 *s*	2.71 *s*	2.72 *s*
10	2.89 *m*	2.67 *t-like* (8.3)	2.70 *m*	3.06 *br s*	2.70 *dd* (6.7, 11.6)
11	1.77 *m*	1.45 *m*	1.49 *m*	1.78 *m*	1.27 *m*
	2.16 *m*	1.74 *m*	1.77 *m*	2.20 *m*	1.61 *m*
12	1.66 *m*	1.57 *m* 1.73 *m*	1.56 *m* 1.72 *m*	1.63 *m*	1.50 *m* 1.61 *m*
15	1.26 *m*	1.09 *m*	1.08 *m*	1.26 *m*	1.41 *m*
	1.65 *m*	1.57 *m*	1.55 *m*	1.74 *m*	1.77 *m*
16	1.29 *m*	1.27 *m*	1.28 *m*	1.20 *m*	1.26 *m*
	1.77 *m*	1.81 *m*	1.82 *m*	1.78 *m*	1.78 *m*
17	1.53 *m*	1.45 *m*	1.46 *m*	1.49 *m*	1.49 *m*
18	0.87 *s*	0.86 *s*	0.90 *s*	0.74 *s*	0.76 *s*
19	9.56 *s*	0.95 *s*	0.96 *s*	9.91 *s*	3.55 *d* (8.5) 3.85 *d* (8.5)
20	1.56 *m*	1.47 *m*	2.03 *m*	2.05 *m*	2.12 *m*
21	0.90 *d* (6.0)	0.87 *d* (6.5)	0.90 *d* (6.4)	0.94 *d* (6.1)	0.92 overlapped
22	1.78 *m*	1.76 *m*	2.10 *m*	2.07 overlapped	2.10 *m*
	2.14 *m*	2.13 *m*	2.44 *br d* (15.7)	2.43 *d* (14.2)	2.43 *d* (13.5)
23	5.56 *m*	5.56 *m*			
24	5.56 *m*	5.56 *m*	2.11 *s*	2.09 *s*	2.10 *s*
26	1.29 *s*	1.28 *s*			
27	1.29 *s*	1.28 *s*			
28	1.28 *s*	1.23 *s*	1.24 *s*	1.41 *s*	1.27 *s*
29	1.15 s	1.12 *s*	1.13 *s*	1.19 *s*	0.86 *s*
30	0.83 *s*	0.81 *s*	0.82 s	0.92 *s*	0.92 *s*

[a] Data were recorded in C_5D_5N.
[b] Data were recorded in $CDCl_3$.

Table 3
^{13}C NMR (100 MHz) spectroscopic data of compounds **1**–**5**

	1[a]	**2**[b]	**3**[a]	**4**[a]	**5**[a]
1	21.6 *t*	20.8 *t*	20.8 *t*	22.5 *t*	18.5 *t*
2	28.7 *t*	29.7 *t*	28.6 *t*	29.6 *t*	27.3 *t*
3	76.1 *d*	76.6 *d*	76.7 *d*	75.6 *d*	76.4 *d*
4	43.6 *s*	42.8 *s*	42.8 *s*	43.4 *s*	38.5 *s*
5	168.1 *s*	169.0 *s*	169.0 *s*	169.3 *s*	89.1 *s*
6	127.1 *d*	125.9 *d*	125.9 *d*	126.9 *d*	50.9 *t*
7	199.4 *s*	202.8 *s*	202.7 *s*	199.0 *s*	212.8 *s*
8	51.2 *d*	59.8 *d*	59.7 *d*	52.0 *d*	63.0 *d*
9	51.2 *s*	35.8 *s*	35.8 *s*	51.1 *s*	47.0 *s*
10	37.9 *d*	40.3 *d*	40.2 *d*	39.2 *d*	41.0 *d*
11	22.3 *t*	31.3 *t*	31.2 *t*	23.0 *t*	22.3 *t*
12	28.4 *t*	28.6 *t*	29.8 *t*	29.1 *t*	30.7 *t*
13	45.3 *s*	45.7 *s*	45.8 *s*	45.4 *s*	46.1 *s*
14	48.2 *s*	48.5 *s*	48.5 *s*	48.8 *s*	49.0 *s*
15	34.5 *t*	34.5 *t*	34.5 *t*	35.1 *t*	34.7 *t*
16	27.4 *t*	27.8 *t*	28.0 *t*	27.8 *t*	28.1 *t*
17	49.5 *d*	49.5 *d*	49.8 *d*	50.0 *d*	49.7 *d*
18	14.9 *q*	15.4 *q*	15.4 *q*	14.8 *q*	15.5 *q*
19	203.4 *d*	27.8 *q*	27.8 *q*	204.7 *d*	79.2 *t*
20	36.2 *d*	36.2 *d*	32.8 *d*	32.9 *d*	32.8 *d*
21	18.8 *q*	18.7 *q*	19.8 *q*	20.0 *q*	19.9 *q*
22	39.0 *t*	39.0 *t*	51.1 *t*	50.8 *t*	50.8 *t*
23	124.9 *d*	125.1 *d*	209.1 *s*	208.1 *s*	208.2 *s*
24	139.8 *d*	139.6 *d*	30.5 *q*	30.4 *q*	30.5 *q*
25	70.7 *s*	70.7 *s*			
26	29.9 *q*	29.9 *q*			
27	30.0 *q*	29.9 *q*			
28	24.9 *q*	24.8 *q*	24.8 *q*	25.6 *q*	21.2 *q*
29	27.2 *q*	27.8 *q*	27.8 *q*	27.1 *q*	26.2 *q*
30	18.3 *q*	18.0 *q*	18.0 *q*	18.5 *q*	21.5 *q*

[a] Data were recorded in C_5D_5N.
[b] Data were recorded in $CDCl_3$.

followed by a reversed-phase column (RP-18) developing with MeOH/H_2O (60:40 → 70:30, v/v) and then Sephadex LH-20 (MeOH).

3.3.1. Kuguacin A (*1*)

Colorless needles; m.p. 196–198 °C; $[\alpha]_D^{20}$ +14.3 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε): 206 (10.0), 210 (10.0), 247 (1.1) nm; IR (KBr) ν_{max}: 3429, 2959, 2934, 2873, 1722, 1649 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; EIMS *m*/*z*: 470 $[M]^+$ (25), 452 (5), 423 (20), 405 (24), 371 (24), 343 (21), 187 (43), 175 (48), 133 (48), 121 (71), 109 (100), 95 (57), 81 (51); HRESIMS *m*/*z*: 493.3292 (calcd 493.3293 for $C_{30}H_{46}O_4Na$).

3.3.2. Kuguacin B (*2*)

Colorless needles; m.p. 124–126 °C; $[\alpha]_D^{20}$ +7.1 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε): 206 (10.0), 210 (10.0), 247 (1.6) nm; IR (KBr) ν_{max}: 3431, 2957, 2932, 1644, 1465, 1380, 1299 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; EIMS *m*/*z*: 456 $[M]^+$ (41), 438 (36), 423 (35), 357 (100), 329 (23), 189 (64), 166 (94), 148 (64), 133 (69), 121 (75), 95 (74), 69 (61), 55 (72); HRESIMS *m*/*z*: 479.3511 (calcd 479.3501 for $C_{30}H_{48}O_3Na$).

3.3.3. Kuguacin A (*3*)

Colorless needles; m.p. 220–222 °C; $[\alpha]_D^{20}$ +12.0 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 248 (1.14) nm; IR (KBr) ν_{max}: 3521, 2951, 2870, 1698, 1640, 1613, 1465, 1375, 1355, 1306, 1237, 980 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; EIMS *m*/*z*: 414 $[M]^+$ (16), 381 (31), 357 (42), 356 (19), 189 (65), 166 (100), 148 (69), 121 (88); HRESIMS *m*/*z*: 437.3038 (calcd 437.3031 for $C_{27}H_{42}O_3Na$).

3.3.4. Kuguacin B (*4*)

Colorless needles; m.p. 212–214 °C; $[\alpha]_D^{20}$ +5.0 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε): 246 (0.52), 201 (0.53) nm; IR (KBr) ν_{max}:3498, 2955, 2927, 2874, 1716, 1699, 1643, 1466, 1378, 1295 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; EIMS *m*/*z*: 428 $[M]^+$ (22), 381 (24), 323 (30), 187 (48), 175 (60), 135 (65), 121 (100); HRESIMS *m*/*z*: 451.2826 (calcd 451.2824 for $C_{27}H_{40}O_4Na$).

3.3.5. Kuguacin C (*5*)

White prism crystals; m.p. 216–218 °C; $[\alpha]_D^{20}$ +6.4 (*c* 0.14, MeOH); UV (MeOH): no absorption from 230 nm to 350 nm; IR (KBr) ν_{max}: 3536, 2955, 2933, 1706, 1691, 1468, 1440, 1378, 1355, 1035 cm^{-1}; for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 2 and 3; EIMS *m*/*z*: 430 $[M]^+$ (4), 194 (16), 193 (100), 180 (60), 167 (13); HRESIMS *m*/*z*: 453.2986 (calcd 453.2980 for $C_{27}H_{42}O_4Na$).

3.4. Anti-HIV assays

Cytotoxicity was measured by MTT method as described previously (Zheng et al., 1995). Briefly, cells were seeded in the absence or presence of various concentrations of compounds in triplicate for 3–7 days. The percentage of viable cells was quantified at 595/630 nm ($A_{595/630}$) in an ELISA reader. The cytotoxic concentration that caused the reduction of viable cells by 50% (IC_{50}) was determined from dose–response curve.

The cytopathic effect was measured by counting the number of syncytia (multinucleated giant cell) in each well under an inverted microscope (Zheng et al., 1999). The percentage inhibition of syncytial cell formation was calculated by percentage of syncytial cell numbers in compound treated cultures to that of infected control culture. AZT was used for drug control. The concentration of the antiviral sample reducing HIV-1 replication by 50% (EC_{50}) was determined from the dose response curve. The selectivity index (SI) was calculated from the ratio of IC_{50}/EC_{50}.

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