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Kuguacins F–S, cucurbitane triterpenoids from *Momordica charantia*

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ABSTRACT

Chemical investigation of the vines and leaves of *Momordica charantia* resulted in isolation of fourteen cucurbitane triterpenoids, kuguacins F–S (**1**–**14**), including two pentanorcucurbitacins (**6** and **7**), one octanorcucurbitacin (**8**), and two trinorcucurbitacins (**11** and **12**), along with six known analogues. Their structures were elucidated on the basis of extensive spectroscopic and single-crystal X-ray diffraction analyses. Compounds **1**–**14** exhibited weak anti-HIV-1 activities *in vitro*.

1. Introduction

Momordica charantia L. (Cucurbitaceae) is widely cultivated as a vegetable crop in many tropical and subtropical countries. Its fruit, called kugua in Chinese or bitter melon in English, is a popular vegetable in China. Tissues of this plant have been used as a traditional Chinese medicine for the treatment of toothache, diarrhea, furuncle, and diabetes. In recent decades in Asia, there have been many phytochemical investigations of the fruit of this plant because of its antidiabetic properties. Phytochemical studies on this plant gathered from Japan (Akihisa et al., 2007; Kimura et al., 2005; Miyahara et al., 1981; Murakami et al., 2001; Okabe et al., 1980; Okabe et al., 1982a,b; Yasuda et al., 1984), China (Chang et al., 2006; Chen et al., 2008a; Li et al., 2007; Zhu et al., 1990), Sri Lanka (Matsuda et al., 2007; Nakamura et al., 2006), Pakistan (Begum et al., 1997), India (Harinantenaina et al., 2006), and Nigeria (Fatope et al., 1990), yielded more than fifty cucurbitacins and cucurbitane glycosides. Compared with other cucurbitane triterpenoids, most of the analogues obtained from *M. charantia* are characterized by the presence of oxygenated functionalities at C-7 or C-19.

In the preceding study of this series, we reported the isolation and structure elucidation of five cucurbitacins, kuguacins A–E, from the roots of *M. charantia* collected from Yunnan Province, People's Republic of China (Chen et al., 2008a). In our search for bioactive metabolites, a further study of this species obtained from Yunnan Province led to isolation of twenty cucurbitacins, including fourteen new ones named kuguacins F–S (**1**–**14**). Among the new compounds, **6** and **7** are 23,24,25,26,27-pentanorcucurbitacins, and **8** is a rare 20,21,22,23,24,25,26,27-octanorcucurbitacin. Compounds **4**, **5** and **12** are artefacts formed during the extraction process. In addition, the anti-HIV-1 activities of the new cucurbitacins were tested. Herein, we report the isolation, structure elucidation, and biological activities of compounds **1**–**14** (Fig. 1).

2. Results and discussion

Powdered air-dried vines and leaves of *M. charantia* were extracted with EtOH under conditions of reflux. The combined extracts were concentrated to dryness to afford a crude extract which was suspended in water and partitioned with EtOAc and *n*-BuOH. The EtOAc layer after evaporation of the solvent was repeatedly subjected to silica gel and Sephadex LH-20 column chromatographic purification steps to afford fourteen new cucurbitacins (**1**–**14**), as well as six known ones (**15**–**20**). The structures of the known compounds were identified by spectroscopic data measurement and by comparing the data obtained with the published values, i.e. as momordicine I (**15**) (Yasuda et al., 1984), kuguacin E (**16**) (Chen et al., 2008a), 5β,19-epoxycucurbita-6,23-diene-3β,19,25-triol (**17**) (Mulholland et al., 1997), karavilagenin D (**18**) (Matsuda et al., 2007), 3β,7β,25-trihydroxycucurbita-5,(23*E*)-dien-19-al (**19**) (Fatope et al., 1990), and 3β,7β-dihydroxy-25-methoxycucurbita-5,(23*E*)-dien-19-al (**20**) (Fatope et al., 1990).

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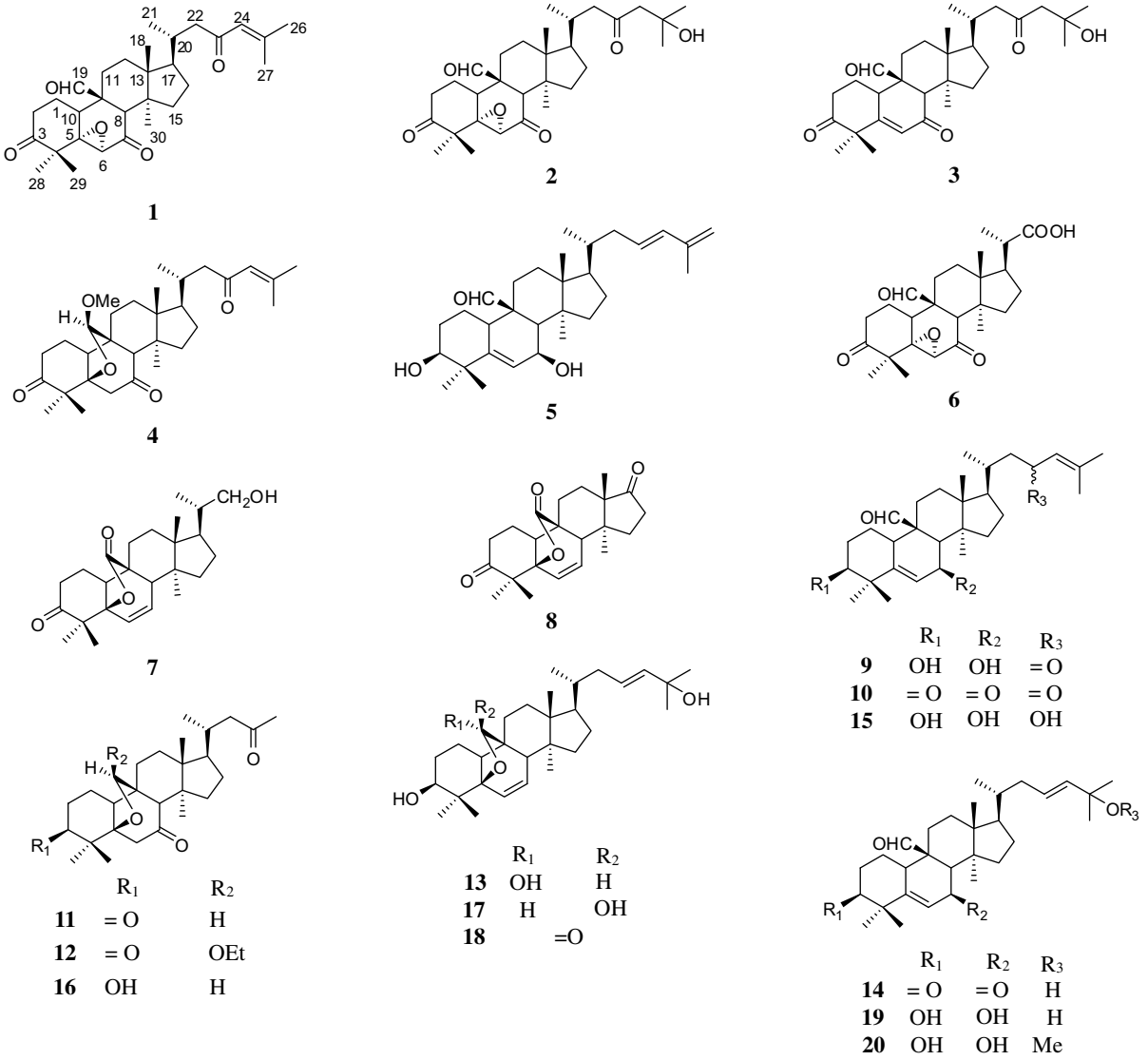

Fig. 1. Chemical structures of **1**–**20**.

Kuguacin F (**1**) was isolated as colorless needles. Its molecular formula, $C_{30}H_{42}O_5$, was deduced by HRESIMS (calc. *m*/*z* 505.2929; found 505.2938, $[M+Na]^+$). The IR spectrum displayed absorptions at 1690, 1710, and 1712 cm^{-1}, suggestive of isolated and conjugated carbonyl groups. The ^{13}C NMR spectroscopic data in combination with analysis of DEPT and HSQC spectra showed the presence of 30 carbon signals due to seven methyls, seven methylenes, six methines, nine quaternary carbons (including three carbonyl carbons), and one aldehyde group, which were assigned to a triterpene skeleton. Careful analysis of the NMR data indicated that it was a highly oxygenated cucurbitane derivative. A comparison between the ^{13}C NMR spectroscopic data of **1** with those of momordicine I (**15**) (Yasuda et al., 1984) indicated that the three oxymethines at C-3, C-7, and C-23 in **15** were oxidized to three ketones (δ_C 210.6, 206.1, and 200.5) in **1**, and the double bond between C-5 and C-6 in **15** was replaced by an oxygenated quaternary carbon (δ_C 70.8) and an oxymethine (δ_C 57.8) in **1**. HMBC correlations between H-8 (δ_H 3.30) and C-6 (δ_C 57.8), C-7 (δ_C 206.1), C-19 (δ_C 202.9), and between H-6 (δ_H 3.64) and C-4 (δ_C 48.8), C-5 (δ_C 70.8), C-7 (δ_C 206.1), and C-10 (δ_C 38.1), enabled the carbonyl to be placed at C-7, the oxymethine at C-6 and the oxygenated quaternary carbon at C-5. A conjugated ketone located at C-23 was confirmed by HMBC correlations of H-24 (δ_H 6.17) with C-22 (δ_C 51.6), C-23 (δ_C 200.5), C-25 (δ_C 154.3), C-26 (δ_C 27.3), and C-27 (δ_C 20.6). Further HMBC correlations of H-2/C-1, C-3, and C-10 also proved the presence of a carbonyl group at C-3.

An epoxide group between C-5 and C-6 instead of two hydroxyl groups at C-5 and C-6 was deduced from the molecular formula, which was substantiated by the absence of a hydroxyl absorption in the IR spectrum. The NOESY correlations of H-8 to H-18 and H-19, of H-10 to H-28 and H-30, and of H-17 to H-30 established the stereochemistry of H-8β, H-10α, and H-17α. The β-orientation of H-6 was suggested by the correlations between H-6 and H-29, and between H-10 and H-28 in its NOESY spectrum (Fig. 2).

The molecular formula of kuguacin G (**2**) was determined to be $C_{30}H_{44}O_6$ by the positive ion at *m*/*z* 523.3048 $[M+Na]^+$ in the HRESIMS. Its IR spectrum displayed absorptions attributable to hydroxyl (3428 cm^{-1}), aldehyde (2881 cm^{-1}), and isolated carbonyl (1712 cm^{-1}) groups. The ^1H and ^{13}C NMR spectroscopic data of **2** were similar to those of **1** with the exception of a conjugated double bond between C-24 and C-25 in **1**, instead of a methylene (δ_C 56.0; δ_H 2.65 (*d*, *J* = 14.2 Hz) and 2.60 (*d*, *J* = 14.2 Hz)) and an

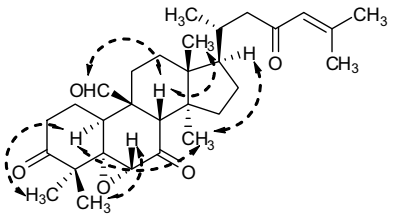

Fig. 2. Key ROESY correlations for **1**.

Table 1
Anti-HIV-1 activities of compounds **1**–**14**.

No.	Anti-HIV-1 activity EC_{50} (μg/ml)	Cytotoxicity IC_{50} (μg/ml)	Selectivity index SI (IC_{50}/EC_{50})
1	40.5	>200	4.9
2	13.7	62.8	4.6
3	11.4	68.2	6.0
4	23.7	>200	8.4
5	11.2	16.2	1.4
6	59.1	145.0	2.5
7	17.5	>200	11.4
8	61.3	>200	3.3
9	12.5	76.3	6.1
10	10.1	92.1	9.1
11	21.6	165.2	7.6
12	7.2	54.8	7.6
13	11.1	60.0	5.4
14	3.7	49.3	13.3
AZT	0.003	>200	>50000

oxygenated quaternary carbon (δ_C 69.5) in **2**. On the basis of these observations, it was reasonable to assume that **2** is a hydrated derivative of **1** with a hydroxyl group at C-25. HMBC and ^1H–^1H COSY spectra confirmed the above deduction. The relative stereochemistry at C-6 was determined to be the same as that of **1** by analysis of the ROESY spectrum.

Kuguacin H (**3**) was obtained as colorless needles with the empirical molecular formula of $C_{30}H_{44}O_5$, in agreement with the HRESIMS (*m*/*z* 507.3078 $[M+Na]^+$, calc. for $C_{30}H_{44}O_5Na$, 507.3086) and ^{13}C NMR spectroscopic data. The IR spectrum showed absorptions for hydroxyl (3436 cm^{-1}), aldehyde (2883 cm^{-1}), isolated carbonyl (1713 cm^{-1}), and conjugated carbonyl (1651 cm^{-1}) functionalities. Careful analysis of the ^{13}C NMR spectrum of **3** and **2** made it clear that these two compounds were similar except for the presence of two olefinic signals at δ_C 126.1 and δ_C 166.8 and the absence of the epoxide group between C-5 and C-6 in **3**. HMBC correlations observed from the olefinic proton signal at δ_H 6.39 (*d*, *J* = 1.8 Hz, H-6) to C-5 (δ_C 166.8), C-7 (δ_C 199.1), C-8 (δ_C 50.9), and C-10 (δ_C 39.7) corroborated the proposed structure of **3**.

Kuguacin I (**4**) was isolated as colorless needles and showed a $[M+Na]^+$ ion at 521.3250 ($C_{31}H_{46}O_5Na$) in the HRESIMS. The 1D NMR spectroscopic data (Tables 2 and 4) were in accordance with a methoxycucurbitane derivative. Detailed comparison of the ^{13}C NMR and DEPT spectra of **4** with those of **3** showed similarities with the exception of the signals in ring B. The signals due to the C-5, C-6 double bond and the aldehyde at C-19 in **3** were absent, but a methylene, an oxygenated quaternary carbon, and a hemiacetal group were indicated by resonances at δ_C 49.5, 90.4, 110.5, and 57.5, respectively. The corresponding ^1H NMR signals at δ_H 2.92 (*d*,

Table 2
^1H NMR spectroscopic data of compounds **1**–**8**.

H	1	2	3	4	5	6	7	8
1	2.55 *m*, 2.14 *m*	2.49 *m*, 2.16 *m*	2.40 *m*, 1.62 *m*	2.04 *m*, 1.70 *m*	2.25 *m*, 1.68 *m*	2.49 *m*, 2.13 *m*	2.06 *m*, 1.33 *m*	2.03 *m*, 1.35 *m*
2	3.01 *ddd* (15.3, 15.3, 5.4), 2.70 *ddd* (15.3, 3.8, 3.8)	2.82 *m*, 2.52 *m*	2.82 *m*, 2.58 *m*	2.85 *ddd* (13.7, 13.7, 5.6), 2.38 *ddd* (3.8, 3.8, 13.7)	2.04 *m*, 1.89 *m*	3.02 *m*, 2.68 *m*	2.82 *ddd* (14.2, 14.2, 5.9), 2.28 *m*	2.83 *ddd* (14.2, 14.2, 5.9), 2.28 *m*
3					3.55 *br s*			
6	3.64 *s*	3.62 *s*	6.39 *d* (1.8)	2.92 *d* (18.3), 2.74 *d* (18.3)	5.88 *d* (5.4)	3.62 *s*	6.31 *dd* (9.8, 2.0)	6.42 *dd* (9.8, 2.2)
7					3.97 *br d* (5.4)		5.74 *dd* (9.8, 3.3)	5.54 *dd* (9.8, 3.2)
8	3.30 *s*	3.28 *s*	2.80 *s*	3.36 *s*	2.03 *br s*	3.31 *s*	2.58 *br s*	2.64 *br s*
10	3.37 *dd* (12.1, 3.5)	3.36 *dd* (12.1, 3.6)	3.30 *m*	3.10 *dd* (11.0, 6.4)	2.49 *dd* (12.8, 4.2)	3.36 *dd* (12.1, 3.5)	3.05 *dd* (12.1, 5.7)	3.01 *dd* (12.1, 5.8)
11	2.10 *m*, 1.58 *m*	2.10 *m*, 1.57 *m*	2.09 *m*, 1.82 *m*	1.56 *m* (2H)	2.27 *m*, 1.54 *m*	1.94 *m*, 1.56 *m*	2.41 *m*, 1.69 *m*	2.30 *m*, 1.76 *m*
12	1.59 *m* (2H)	1.66 *m* (2H)	1.65 *m* (2H)	1.58 *m* (2H)	1.66 *m* (2H)	1.76 *m*, 1.56 *m*	1.64 *m*, 1.27 *m*	1.70 *m*, 1.63 *m*
15	1.75 *m*, 1.42 *m*	1.75 *m*, 1.41 *m*	1.34 *m* (2H)	1.87 *m*, 1.68 *m*	1.36 *m* (2H)	1.82 *m*, 1.44 *m*	1.24 *m* (2H)	1.68 *m*, 1.30 *m*
16	1.70 *m*, 1.24 *m*	1.75 *m*, 1.23 *m*	1.79 *m*, 1.23 *m*	1.86 *m*, 1.31 *m*	1.90 *m*, 1.37 *m*	2.13 *m*, 1.67 *m*	1.97 *m*, 1.40 *m*	2.47 *m*, 2.20 *m*
17	1.49 *m*	1.48 *m*	1.52 *m*	1.48 *m*	1.51 *m*	2.17 *m*	1.76 *m*	
18	0.75 *s*	0.71 *s*	0.76 *s*	0.87 *s*	0.87 *s*	0.75 *s*	0.87 *s*	1.05 *s*
19	9.86 *s*	9.84 *s*	9.71 *s*	4.68 *s*	9.78 *s*	9.86 *s*		
20	2.14 *m*	2.14 *m*	2.15 *m*	2.17 *m*	1.51 *m*	2.67 *m*	1.70 *m*	
21	0.98 *d* (6.3)	0.96 *d* (6.4)	1.00 *d* (6.3)	1.00 *d* (5.8)	0.89 *d* (6.3)	1.31*d* (6.8)	1.23 *d* (6.3)	
22	2.57 *m*, 2.18 *m*	2.60 *m*, 2.45 *m*	2.68 *dd* (16.8, 2.4), 2.42 *dd* (16.8, 9.9)	2.54 *br d* (12.3), 2.13 *m*	2.22 *m*, 1.78 *m*		3.88 *dd* (10.3, 2.9), 3.59 *dd* (10.3, 6.0)	
23					5.61 m			
24	6.17 *s*	2.65 *d* (14.2), 2.60 *d* (14.2)	2.82 *d* (14.3), 2.77 *d* (14.3)	6.17 *s*	6.10 *d* (15.6)			
26	1.77 *s*	1.49 *s*	1.50 *s*	1.76 *s*	4.84 *br s*			
27	2.20 *s*	1.49 *s*	1.50 *s*	2.21 *s*	1.82 *s*			
28	1.07 *s*	1.05 *s*	1.37 *s*	1.11 *s*	1.03 *s*	1.06 *s*	1.14 *s*	1.19 *s*
29	1.44 *s*	1.45 *s*	1.35 *s*	1.28 *s*	1.22 *s*	1.40 *s*	1.36 *s*	1.39 *s*
30	0.96 *s*	0.94 *s*	0.90 *s*	0.99 *s*	0.73 *s*	0.97 *s*	0.86 *s*	0.86 *s*
CH_3O				3.30 *s*				

Table 3
^{1}H NMR spectroscopic data of compounds **9–14**.

H	9	10	11	12	13	14
1	2.09 *m*, 1.75 *m*	2.30 *m*, 1.70 *m*	2.00 *m*, 1.59 *m*	2.10 *m*, 1.69 *m*	1.52 *m* (2H)	2.30 *m*, 1.70 *m*
2	2.09 *m*, 1.92 *m*	2.86 *m*, 2.61 *m*	2.87 *ddd* (13.5, 13.5, 5.9), 2.35 *ddd* (13.5, 4.3, 4.3)	2.85 *ddd* (13.7, 13.7, 6.0), 2.38 *ddd* (3.8, 3.8, 13.7)	1.90 *m* (2H)	2.83 *m*, 2.51 *m*
3	3.82 *br s*				3.60 *br s*	
6	6.28 *br d* (4.7)	6.43 *s*	2.44 *d* (13.6), 2.13 *d* (13.6)	2.93 *d* (18.2), 2.74 *d* (18.2)	6.25 *br d* (9.8)	6.43 *s*
7	4.36 *br d* (5.1)				5.56 *dd* (9.8, 3.2)	
8	2.37 *s*	2.84 *s*	2.73 *br s*	3.40 *s*	2.31 *br s*	2.82 *s*
10	2.70 *m*	3.31 *m*	3.00 *dd* (10.0, 6.5)	3.10 *dd* (11.1, 6.8)	2.43 *m*	3.31 *m*
11	2.66 *m*, 1.57 *m*	2.10 *m*, 1.72 *m*	1.49 *m*, 1.33 *m*	1.57 *m*, 1.35 *m*	1.89 *m*, 1.31 *m*	2.12 *m*, 1.78 *m*
12	1.60 *m* (2H)	1.65 *m* (2H)	1.59 *m*, 1.50 *m*	1.59 *m* (2H)	1.57 *m* (2H)	1.61 *m* (2H)
15	1.37 *m* (2H)	1.78 *m*, 1.36 *m*	1.78 *m*, 1.42 *m*	1.85 *m*, 1.56 *m*	1.33 *m* (2H)	1.78 *m*, 1.36 *m*
16	1.94 *m*, 1.37 *m*	1.84 *m*, 1.26 *m*	1.76 *m*, 1.23 *m*	1.81 *m*, 1.22 *m*	1.92 *m*, 1.34 *m*	1.86 *m*, 1.23 *m*
17	1.57 *m*	1.53 *m*	1.47 *m*	1.45 *m*	1.53 *m*	1.49 *m*
18	0.89 *s*	0.79 *s*	0.77 *s*	0.82 *s*	0.81 *s*	0.74 *s*
19	10.65 *s*	9.75 *s*	3.63 *d* (8.7), 3.54 *d* (8.7)	4.96 *s*	5.33 *d* (4.0)	9.76 *s*
20	2.17 *m*	2.14 *m*	2.13 *m*	2.11 *m*	1.51 *m*	1.48 *m*
21	1.03 *d* (5.5)	1.01 *d* (5.3)	0.92 *d* (5.9)	0.86 *d* (6.4)	0.88 *d* (5.8)	0.95 *d* (5.4)
22	2.54 *d* (12.2), 2.17 *m*	2.58 *m*, 2.18 *m*	2.91 *br d* (18.1), 2.73 *br d* (17.9)	2.47 *m*, 2.14 *m*	2.26 *m*, 1.85 *m*	2.24 *m*, 1.83 *m*
23					5.94 *m*	5.94 *br s*
24	6.17 *s*	6.19 *s*	2.11 *s*	2.10 *s*	5.94 *m*	5.94 *br s*
26	1.75 *s*	1.79 *s*			1.55 *s*	1.56 *s*
27	2.21 *s*	2.24 *s*			1.55 *s*	1.56 *s*
28	1.19 *s*	1.39 *s*	1.12 *s*	1.11 *s*	0.94 *s*	1.39 *s*
29	1.48 *s*	1.41 *s*	1.28 *s*	1.29 *s*	1.48 *s*	1.40 *s*
30	0.86 *s*	0.95 *s*	0.98 *s*	1.00 *s*	0.96 *s*	0.91 *s*
CH_3CH_2O				3.72 *m*, 3.43 *m*		
CH_3CH_2O				1.03 *t* (7.1)		

Table 4
^{13}C NMR spectroscopic data of compounds **1–14**.

C	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	25.0	25.0	24.7	24.8	21.8	25.0	25.9	25.9	21.8	24.7	24.7	24.8	17.4	24.7
2	38.5	38.5	38.1	36.0	29.6	38.5	35.6	35.4	29.9	38.0	36.1	36.1	22.6	38.2
3	210.6	210.6	211.1	212.6	76.8	210.6	211.4	211.0	75.7	210.3	213.1	212.7	76.6	210.4
4	48.8	48.7	51.9	49.8	42.0	48.8	48.1	48.1	41.8	51.9	49.9	49.8	45.4	51.4
5	70.8	70.8	166.8	90.4	146.0	70.8	88.6	88.5	145.8	166.8	91.9	90.3	84.6	166.9
6	57.8	57.8	126.1	49.5	124.4	57.8	131.0	132.0	124.3	126.1	50.9	49.7	134.0	126.1
7	206.1	206.1	199.1	211.4	66.7	206.0	134.6	132.2	65.7	199.1	208.3	211.6	130.6	199.2
8	50.3	50.2	50.9	53.8	48.2	50.3	44.9	43.9	50.7	50.9	63.0	53.9	50.1	50.9
9	49.4	49.4	51.3	49.5	50.5	49.4	51.4	51.9	50.6	51.3	47.0	49.6	50.0	51.3
10	38.1	38.1	39.7	42.1	37.2	38.2	40.1	41.0	36.9	39.7	41.5	42.5	38.7	39.7
11	24.4	24.4	23.7	23.4	24.0	24.4	22.2	21.3	22.7	23.7	22.8	23.3	28.2	23.7
12	29.3	29.2	29.0	30.6	29.6	29.4	30.0	30.1	29.5	29.0	30.7	30.6	28.0	29.0
13	45.2	45.7	45.5	46.2	46.0	45.6	45.4	43.2	45.9	45.5	46.2	46.2	37.5	45.4
14	48.9	48.6	48.4	48.6	48.6	48.8	47.7	52.1	48.4	48.5	49.0	48.6	49.0	48.4
15	35.3	35.2	35.1	35.4	35.3	35.5	33.5	30.1	34.9	35.1	34.8	35.3	33.7	35.2
16	27.7	27.6	27.8	28.3	28.2	27.0	27.3	33.2	28.0	27.9	28.2	28.2	28.1	27.7
17	49.8	50.2	49.9	49.5	50.7	47.3	47.4	216.3	50.7	50.2	49.7	49.7	50.3	49.7
18	15.2	15.2	14.8	15.6	15.5	15.6	14.7	16.5	14.9	14.8	15.6	15.4	15.1	15.0
19	202.9	202.9	204.0	110.5	208.7	202.8	181.0	180.4	207.8	204.0	79.6	109.3	107.8	204.1
20	33.4	32.6	32.7	33.4	37.2	43.7	39.7		33.5	33.5	32.9	32.9	36.7	36.7
21	20.0	20.1	20.2	20.0	19.4	17.9	17.5		20.0	20.0	20.0	21.4	18.9	19.0
22	51.6	52.1	52.2	51.8	40.3	179.0	67.1		51.9	51.7	50.2	50.9	39.7	39.4
23	200.5	210.6	210.3	200.6	129.8				200.7	200.6	212.5	208.2	124.3	126.1
24	124.9	56.0	56.0	124.9	134.9				124.9	124.9	30.5	30.5	141.7	141.9
25	154.3	69.5	69.5	154.2	142.8				154.1	154.3			69.7	69.8
26	27.3	30.5	30.5	27.3	114.8				27.3	27.3			30.9	30.9
27	20.6	30.2	30.2	20.6	19.4				20.6	20.5			30.9	30.9
28	16.2	16.2	22.4	25.0	27.6	16.2	23.0	22.9	27.4	22.4	25.5	24.9	24.5	22.5
29	25.0	25.1	28.3	17.6	26.0	25.1	17.3	17.3	26.2	28.3	17.9	17.7	21.5	28.4
30	20.5	20.5	18.5	21.5	18.6	20.5	19.4	19.1	18.3	20.6	21.4	21.4	19.0	18.5
CH_3CH_2O												65.9		
CH_3CH_2O												15.5		
CH_3O				57.5										

J = 18.3 Hz) and 2.38 (*d*, *J* = 18.3 Hz) were assigned to H-6, and the resonance at δ_H 4.68 (*s*) was ascribed to H-19, on the basis of HMBC correlations between H-6/C-5, C-7, C-8, and between H-19/C-8, C-9, C-10. In the mass spectrum, the loss of a HCO_2CH_3 fragment confirmed the placement of the additional methoxy group at C-19 (Mulholland et al., 1997). Further HMBC correlations observed from H-19 to C-5 also demonstrated that there was an oxygen bridge between C-5 and C-19. The (*R*)-configuration of C-19 was supported by the absence of ROESY cross-peak of H-19/H-8 (Mulholland et al., 1997).

The molecular formula of kuguacin J (**5**) gave a quasi-molecular ion at *m*/*z* 477.3335 $[M+Na]^+$, corresponding to the molecular formula of $C_{30}H_{46}O_3$, indicating 14 mass units less than the known compound (23*E*)-3β-hydroxy-7β-methoxycucurbita-5,23,25-trien-19-al (Kimura et al., 2005). The only difference between the ^{13}C NMR spectra of the two compounds was that **5** lacked a methoxy group at C-7 and the signal for C-7 was shifted upfield to δ_C 66.7 in **5**. The HMBC correlations from δ_H 3.97 (*br d*, *J* = 5.4 Hz, H-7) to C-5, C-6, and C-8 also substantiated the above proposal.

Kuguacin K (**6**) was isolated as amorphous powder and exhibited a quasi-molecular ion peak at *m*/*z* 453.2261 $[M+Na]^+$ in the HRESIMS, appropriate for a molecular formula of $C_{25}H_{34}O_6$. Detailed analysis of the NMR spectra of **6** and **1** made it clear that the two compounds were similar, except for the presence of a carboxylic group at δ_C 179.0 for C-22 and the absence of the signals for C-23, C-24, C-25, C-26, and C-27, in **6**. These differences could be explained to be the result of an oxidative cleavage between C-22 and C-23 of **1**, which was confirmed by the HMBC correlations of H-21 to C-17, C-20, and C-22. The β-orientation of H-6 was assigned in the same way as for **1**. Pentanorcucurbitane derivatives have previously been obtained by synthesis, but this is the first report of their occurrence in nature (Miyahara et al., 1981; Okabe et al., 1980).

Kuguacin L (**7**) had a molecular formula of $C_{25}H_{36}O_4$ by HRESIMS (*m*/*z* 423.2507 $[M+Na]^+$, calc. 423.2511). Detailed comparison of the NMR spectroscopic data of **7** with those of **18** (Matsuda et al., 2007) showed similarities with the exception of the presence of a carbonyl functionality (δ_C 211.4) at C-3 and an oxygenated methylene (δ_C 67.2; δ_H 3.88 (*dd*, *J* = 10.3, 2.9 Hz) and 3.59 (*dd*, *J* = 10.3, 6.0 Hz)) at C-22, and the absence of the hydroxyl group for C-3 and signals for C-23, C-24, C-25, C-26, and C-27, in **7**. HMBC correlations observed from H-28 and H-29 to C-3, C-4, and C-5, and from H-22 to C-20, C-21, and C-17, proved the above presumption.

Kuguacin M (**8**) was obtained as colorless needles, and was analyzed for molecular formula $C_{22}H_{28}O_4$ based on its HRESIMS (*m*/*z* 379.1881 $[M+Na]^+$, calc. 379.1885). The ^{13}C NMR spectrum of **8** displayed resonances for four tertiary methyl groups, six methylenes, four methines, and eight quaternary carbons. A careful comparison of the ^1H and ^{13}C NMR spectra of **8** with those of **7** indicated that both compounds had identical A, B and C rings. HMBC correlations established the structure of ring D in **8**: one methyl singlet resonance at δ_H 1.05 corresponding to Me-18 showed cross-peaks with a ketone (C-17, δ_C 216.3), a methylene (C-12, δ_C 30.1), and two quaternary carbons (C-13, δ_C 43.2, and C-14, δ_C 52.1); another methyl singlet resonance at δ_H 0.86 (Me-30) correlated with a methine (C-8, δ_C 43.9), a methylene (C-15, δ_C 30.1), and the two quaternary carbons (C-13, C-14). The above observations suggested that compound **8** is an octanorcucurbitane with a ketone at C-17. The position of the carbonyl group at C-17 was also supported by the downfield chemical shift of C-16 from δ_C 27.3 in **7** to δ_C 33.2 in **8**. Kuguacin M (**8**) is the first octanorcucurbitane triterpenoid isolated from the genus *Momordica*, while the closest analogues would appear to be khekadaengoside L (Kanchanapoom et al., 2002), and endecaphyllacins A and B (Chen et al., 2008b), previously isolated from *Trichosanthes tricuspidata* and *Hemsleya endecaphylla*, respectively.

Kuguacin N (**9**) was obtained as amorphous powder, and its molecular formula was determined as $C_{30}H_{46}O_4$ by HRESIMS (*m*/*z* 493.3297 $[M+Na]^+$). Its NMR spectra were similar to those of **15** (Yasuda et al., 1984) except that the hydroxyl group at C-23 in **15** was replaced by a carbonyl group (δ_C 200.7) to form an α,β-unsaturated functionality in **9**, which was confirmed by the downfield shift of C-24 by 6.8 ppm and the abnormal upfield shift of C-25 by 23.4 ppm. The olefinic proton at δ_H 6.17 (s, H-24) correlated in the HSQC spectrum with CH at δ_C 124.9, and in the HMBC spectrum with C-23, C-25, C-26, and C-27, proved the proposed structure of **9**.

The positive HRESIMS of kuguacin O (**10**) showed a molecular ion peak at 489.3162 $[M+Na]^+$, in according with an empirical molecular formula of $C_{30}H_{42}O_4$, which possessed ten degrees of unsaturation. Careful comparison of the NMR spectroscopic data between **9** and **10** led to the conclusion that the oxymethines at C-3 and C-7 were oxidized to two carbonyl groups, on the basis of HMBC correlations between H-28, H-29 and C-3 (δ_C 210.3), and between H-6 and C-7 (δ_C 199.1), C-8, and C-10.

Kuguacin P (**11**) was obtained as colorless prisms, and was determined to have the molecular formula $C_{27}H_{40}O_4$ by HRESIMS (found $[M+Na]^+$ 451.2813, calc. 451.2824). Analysis of the NMR spectra indicated that it was a trinorcucurbitane derivative. Comparison of the ^1H and ^{13}C NMR spectroscopic data of **11** with those of kuguacin E (**16**) (Chen et al., 2008a) displayed similarities except for the hydroxyl group at C-3 in **16** being replaced by a carbonyl carbon (δ_C 213.1) in **11**, which was validated by HMBC correlations from H-28 and H-29 to C-3, C-4, C-5. The single-crystal X-ray crystallographic results of **11** confirmed the proposed structure (Fig. 3).

Kuguacin Q (**12**) was obtained as colorless needles. Its molecular formula $C_{29}H_{44}O_5$ was established by the $[M+Na]^+$ ion peak at *m*/*z* 495.3083 (calc. for $C_{29}H_{44}O_5Na$, 495.3086) in the HRESIMS. Comparison of the ^1H and ^{13}C NMR spectroscopic data of **12** with those of **11**, suggested that both compounds were closely similar and shared the same trinorcucurbitane skeleton. The only difference was in the signals due to the functionality at C-19, including the absence of an oxygenated methylene and the appearance of an oxygenated methine (δ_C 109.3 and δ_H 4.96 (s)). The signals of δ_C 65.9, δ_C 15.5, δ_H 1.03 (3H, *t*, *J* = 7.1 Hz), δ_H 3.43 (1H, *m*), and δ_H 3.72 (1H, *m*) were diagnostic of an ethoxyl group, which was connected to C-19 based on HMBC correlations between H-19/C-1′, H-1′/C-19, C-2′. No NOE was observed between H-19 and H-8, suggesting that C-19 has the (*R*)-configuration.

Kuguacin R (**13**) and **17** were isolated as nearly 1:2 mixtures of inseparable isomers. Their HRESIMS spectra gave a pseudomolecular ion peak at 495.3460 $[M+Na]^+$, consistent with the molecular formula $C_{30}H_{48}O_4$. Careful analysis of the NMR spectroscopic data of the mixture showed that the two compounds were C-19 epimers. In the NMR data of **13**, the oxymethine at δ_H 5.33 (*d*, *J* = 4.0 Hz, H-19) correlated in the HSQC spectrum with the CH at δ_C 107.8, and in the HMBC with C-8, C-9, and C-10. Since compound **17**, the major epimer, has the (*R*)-configuration at C-19 (Mulholland et al., 1997), compound **13** must have the (*S*)-configuration at the same position. This was confirmed by the NOESY correlation between H-19 and H-8. The assignment was in accordance with the observation of the C-8 signal being shifted downfield by 8.7 ppm and the C-10 signal being shifted upfield by 1.9 ppm in **13**. Fortunately, the mixture crystallized as prisms from methanol. X-ray crystallographic analysis confirmed the proposed structure (Fig. 4).

Kuguacin S (**14**) had the molecular formula of $C_{30}H_{44}O_4$ established by HRESIMS at *m*/*z* 491.3126 $[M+Na]^+$ (calc. for $C_{30}H_{44}O_4Na$, 491.3137), corresponding to nine degrees of unsaturation. The NMR spectroscopic data are in good agreement with those of (23*E*)-25-hydroxycucurbita-5,23-diene-3,7-dione (Chang et al., 2006), except that the methyl group was replaced by an aldehyde

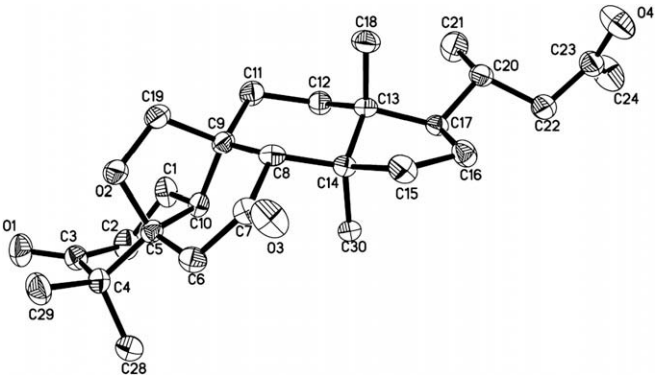

Fig. 3. X-ray crystal structure of **11**.

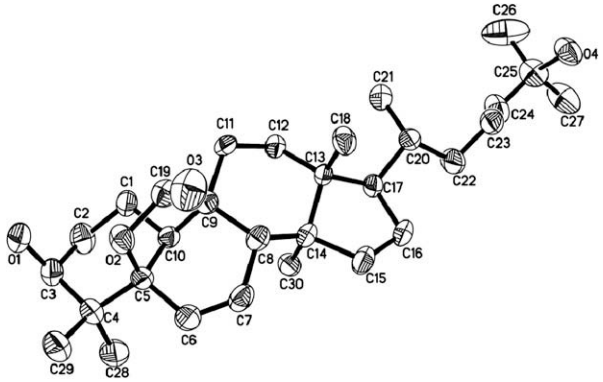

Fig. 4. X-ray crystal structure of **13** and **17**.

group (δ_C 204.1; δ_H 9.76 (s)) at C-19. Two groups of HMBC correlations supported the above deduction: an aldehyde singlet resonance at δ_H 9.76 corresponding to H-19 showed cross-peaks with two methines (C-8, δ_C 50.9, and C-10, δ_C 39.7), and a quaternary carbon (C-9, δ_C 51.3); another methine singlet resonance at δ_H 2.82 (H-8) correlated with a quaternary carbon (C-9), a methine (C-10), an aldehyde group (C-19, δ_C 204.1), a ketone carbon (C-7, δ_C 199.2), and an olefinic methine (C-6, δ_C 126.1). The proposed structural feature was also substantiated by the fragment ion peak in the EIMS at *m/z* 420 $[M–H_2O–CHO]^+$.

The anti-HIV-1 activities were evaluated in preventing the cytopathic effects of HIV-1_{IIIB} in C8166, and cytotoxicity was measured in parallel with the determination of antiviral activity using AZT as a positive control. Compounds **12** and **14** showed anti-HIV-1 activity with EC_{50} values of 7.2 and 3.7 μg/mL, respectively, and compounds **1**, **4**, **7**, and **8** exerted minimal cytotoxicity against C8166 cells (IC_{50} > 200 μg/mL) (Table 1).

3. Conclusion

Fourteen new cucurbitacins possessing several skeletons were obtained from the vines and leaves of *M. charantia*. However, the methyl and ethyl acetal products, such as **4** and **12**, and compound **5**, the one bearing diene functionality on side-chain, are clearly artefacts formed during the extraction process. The trinorcucurbitane skeleton may arise from a retro-aldolisation reaction on some of its co-metabolites. For example, compound **12** may be transformed from kuguacin O (**10**). All the new compounds showed weak anti-HIV-1 activity *in vitro*.

4. Experimental

4.1. General experimental procedures

^1H, ^{13}C, and 2D NMR spectra were recorded on a Bruker DRX-500 instrument, with TMS as internal standard. MS data were obtained on a VG AutoSpec-3000 spectrometer. UV spectra were measured on a Shimadzu double-beam 210A spectrophotometer. The IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. Melting points were obtained on an X-4 digital display micromelting point apparatus and are uncorrected. Optical rotations were carried out on a Perkin–Elmer model 241 polarimeter. Column chromatography (CC) 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.

4.2. Plant material

The vines and leaves were cultivated at Dahanying Village, Anning County, Yunnan Province, People's Republic of China, in August 2005. The sample was identified by Prof. Shu-Kun Chen, and a voucher specimen (No. KIB 2005-8-10) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy Sciences.

4.3. Extraction and isolation

Powdered air-dried vines and leaves of *M. charantia* (30 kg) were extracted with EtOH under conditions of reflux (3 × 100 L) to give a crude extract after concentrating under vacuum. This extract was dissolved in H_2O (4 L) and then extracted with EtOAc (5 L × 3) to furnish a residue, which was then subjected to silica gel CC and eluted with a gradient system of $CHCl_3$/MeOH (1:0, 20:1, 0:1) to yield fractions I–III. Fraction II (127 g) was applied to a DIAION HP-20 column, eluted with H_2O/MeOH (100:0, 10:90, 0:100) to afford fractions A–C. Fraction B (70 g) was subjected to silica gel CC and eluted with a gradient system of $CHCl_3$/MeOH (1:0, 50:1, 30:1, 20:1) to yield fractions 1–4 monitored by TLC. Fraction 2 (20 g) was submitted to repeated chromatography over silica gel ($CHCl_3$/MeOH, from 60:1 to 35:1) and RP-18 (MeOH/H_2O, from 60:40 to 75:25), followed by Sephadex HL-20 (MeOH), to yield compounds **1** (14 mg, 0.000047%), **2** (7 mg, 0.000023%), **3** (8 mg, 0.000027%), **4** (6 mg, 0.000020%), **6** (39 mg, 0.000130%), **7** (9 mg, 0.000030%), **8** (2 mg, 0.000007%), **10** (6 mg, 0.000020%), **11** (88 mg, 0.000293%), **12** (4 mg, 0.000011%), and **14** (5 mg, 0.000017%). Compounds **13** and **17** (407 mg, 0.001357%), **16** (9 mg, 0.000030%), and **18** (137 mg, 0.000457%) were obtained from fraction 3 by over silica gel CC ($CHCl_3$/MeOH, from 40:1 to 20:1) and RP-18 (MeOH/H_2O, from 60:40 to 75:25), then Sephadex LH-20 (MeOH). Fraction 4 (14 g) was separated by RP-18 (MeOH/H_2O, from 50:50 to 90:10) to yield compounds **5** (73 mg, 0.000243%), **9** (74 mg, 0.000247%), **15** (150 mg, 0.000500%), **19** (2 g, 0.006667%), and **20** (90 mg, 0.000300%).

4.3.1. Kuguacin F (*1*)

Colorless needles (MeOH); mp 275–276 °C; α_D^{20} −47.3 (*c* 0.09, MeOH); UV λ_{max}^{MeOH} nm (log ε): 220 (4.3), 390 (1.4); IR ν_{max}^{KBr} cm^{-1}: 2969, 2881, 1712, 1710, 1690, 1617, 1458, 1386, 1036; For ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data, see Tables 2 and 4; EIMS, *m/z* (rel. int.): 357 [M – side-chain ($C_9H_{15}O$)]$^+$ (4), 356 (5), 339 (9), 323 (28), 135 (18),

125 (81), 98 (65), 83 (100), 55 (23); HRESIMS, *m/z*: 505.2938 $[M+Na]^+$ (calc. for $C_{30}H_{42}O_5Na$, 505.2929).

*4.3.2. Kuguacin G (**2**)*

Colorless needles (MeOH); mp 250–252 °C; α_D^{20} −12.0 (*c* 0.08, MeOH); UV λ_{max}^{MeOH} nm (log ε): 216 (3.7), 251 (2.6); IR ν_{max}^{KBr} cm^{-1}: 3428, 2960, 2881, 1712, 1470, 1391; For ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data, see Tables 2 and 4; EIMS, *m/z* (rel. int.): 426 $[M–CH_3–CH_3–CHO]^+$ (12), 398 (15), 369 (73), 339 (77), 243 (52), 203 (71), 175 (56), 136 (98), 121 (100), 55 (34); HRESIMS, *m/z*: 523.3048 $[M+Na]^+$ (calc. for $C_{30}H_{44}O_6Na$, 523.3035).

*4.3.3. Kuguacin H (**3**)*

Colorless needles (MeOH); mp 226–228 °C; α_D^{20} −4.8 (*c* 0.15, MeOH); UV λ_{max}^{MeOH} nm (log ε): 218 (3.7), 248 (3.6); IR ν_{max}^{KBr} cm^{-1}: 3436, 2961, 2932, 2883, 1713, 1651, 1620, 1467, 1381; For ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data, see Tables 2 and 4; EIMS, *m/z* (rel. int.): 442 $[M–CH_3–CH_3–CHO]^+$ (3), 414 (7), 396 (17), 323 (100), 175 (56), 135 (77), 121 (86), 55 (35); HRESIMS, *m/z*: 507.3078 $[M+Na]^+$ (calc. for $C_{30}H_{44}O_5Na$, 507.3086).

*4.3.4. Kuguacin I (**4**)*

Colorless needles (MeOH); mp 235–237 °C; α_D^{20} −8.5 (*c* 0.16, MeOH); UV λ_{max}^{MeOH} nm (log ε): 213 (3.2), 239 (2.8); IR ν_{max}^{KBr} cm^{-1}: 2978, 2944, 2885, 1716, 1680, 1609, 1468, 1443, 1384, 1114; For ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data, see Tables 2 and 4; EIMS, *m/z* (rel. int.): 467 $[M–OCH_3]^+$ (1), 438 $[M–HCO_2CH_3]^+$ (1), 369 (30), 340 (20), 325 (13), 125 (95), 98 (80), 83 (100); HRESIMS, *m/z*: 521.3250 $[M+Na]^+$ (calc. for $C_{31}H_{46}O_5Na$, 521.3242).

*4.3.5. Kuguacin J (**5**)*

Amorphous powder; mp 166–169 °C; α_D^{20} 0.86 (*c* 0.12, MeOH); UV λ_{max}^{MeOH} nm (log ε): 224 (3.8), 256 (3.1), 262 (3.0); IR ν_{max}^{KBr} cm^{-1}: 3432, 2950, 2878, 1709, 1460, 1380, 967; For ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data, see Tables 2 and 4; EIMS, *m/z* (rel. int.): 436 $[M–H_2O]^+$ (7), 309 (21), 203 (34), 187 (60), 173 (81), 172 (77), 123 (82), 109 (100), 107 (75), 95 (74), 55 (54); HRESIMS, *m/z*: 477.3335 $[M+Na]^+$ (calc. for $C_{30}H_{46}O_3Na$, 477.3344).

*4.3.6. Kuguacin K (**6**)*

Amorphous powder; mp 275–277 °C; α_D^{20} −12.7 (*c* 0.13, MeOH); UV λ_{max}^{MeOH} nm (log ε): 215 (4.8), 262 (4.2); IR ν_{max}^{KBr} cm^{-1}: 3426, 2960, 2853, 1706, 1465, 1390; For ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data, see Tables 2 and 4; EIMS, *m/z* (rel. int.): 430 $[M]^+$ (2), 402 (8), 384 (12), 369 (11), 341 (26), 203 (47), 177 (53), 149 (71), 137 (100), 121 (95), 107 (97), 55 (68); HRESIMS, *m/z*: 453.2261 $[M+Na]^+$ (calc. for $C_{25}H_{34}O_6Na$, 453.2253).

*4.3.7. Kuguacin L (**7**)*

White needles (MeOH); mp 320–321 °C; α_D^{20} −41.0 (*c* 0.08, MeOH); UV λ_{max}^{MeOH} nm (log ε): 215 (4.2), 264 (3.5); IR ν_{max}^{KBr} cm^{-1}: 3461, 2969, 2942, 2878, 1761, 1714, 1468, 1382, 1166, 915; For ^1H NMR (C_5D_5 N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data, see Tables 2 and 4; EIMS, *m/z* (rel. int.): 356 $[M–CO_2]^+$ (26), 227 (41), 201 (42), 188 (95), 173 (63), 137 (91), 109 (100), 55 (29); HRESIMS, *m/z*: 423.2507 $[M+Na]^+$ (calc. for $C_{25}H_{36}O_4Na$, 423.2511).

*4.3.8. Kuguacin M (**8**)*

Colorless needles (MeOH); mp 332–333 °C; α_D^{20} −36.7 (*c* 0.08, MeOH); UV λ_{max}^{MeOH} nm (log ε): 215 (3.9), 262 (2.8); IR ν_{max}^{KBr} cm^{-1}: 2963, 2868, 1753, 1720, 1466, 1169, 1027, 910; For ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data, see Tables 2 and 4; EIMS, *m/z* (rel. int.): 356 $[M]^+$ (2), 341 (7), 312 (80), 297 (61), 279 (38), 255 (100), 227 (52), 91 (29); HRESIMS, *m/z*: 379.1881 $[M+Na]^+$ (calc. for $C_{22}H_{28}O_4Na$, 379.1885).

*4.3.9. Kuguacin N (**9**)*

Amorphous powder; mp 140–143 °C; α_D^{20} −5.9 (*c* 0.13, MeOH); UV λ_{max}^{MeOH} nm (log ε): 228 (3.6); IR ν_{max}^{KBr} cm^{-1}: 3412, 2950, 2876, 1712, 1619, 1465, 1445, 1249, 1040, 944; For ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data, see Tables 3 and 4; EIMS, *m/z* (rel. int.): 470 $[M]^+$ (1), 452 (3), 405 (14), 293 (11), 187 (24), 136 (44), 125 (43), 83 (100), 55 (23); HRESIMS, *m/z*: 493.3297 $[M+Na]^+$ (calc. for $C_{30}H_{46}O_4Na$, 493.3293).

*4.3.10. Kuguacin O (**10**)*

Colorless needles (MeOH); mp 267–269 °C; α_D^{20} −12.8 (*c* 0.13, MeOH); UV λ_{max}^{MeOH} nm (log ε): 215 (4.2), 244 (3.9); IR ν_{max}^{KBr} cm^{-1}: 2977, 2925, 2867, 1710, 1681, 1618, 1459; For ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data, see Tables 3 and 4; EIMS, *m/z* (rel. int.): 369 $[M–(C_{22}–C_{27})]^+$ (73), 341 (16), 340 (14), 313 (13), 203 (16), 125 (58), 98 (42), 83 (100), 55 (24); HRESIMS, *m/z*: 489.3162 $[M+Na]^+$ (calc. for $C_{30}H_{42}O_4Na$, 489.3161).

*4.3.11. Kuguacin P (**11**)*

Colorless prisms (MeOH); mp 229–231 °C; α_D^{20} −3.7 (*c* 0.16, MeOH); UV λ_{max}^{MeOH} nm (log ε): 215 (4.6), 264 (3.9); IR ν_{max}^{KBr} cm^{-1}: 2971, 2845, 1707, 1703; For ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data, see Tables 3 and 4; EIMS, *m/z* (rel. int.): 428 $[M]^+$ (25), 370 (12), 191 (100), 178 (53), 121 (27); HRESIMS, *m/z*: 451.2813 $[M+Na]^+$ (calc. for $C_{27}H_{40}O_4Na$, 451.2824).

*4.3.12. Kuguacin Q (**12**)*

Colorless needles (MeOH); mp 219–221 °C; α_D^{20} −25.0 (*c* 0.08, MeOH); UV λ_{max}^{MeOH} nm (log ε): 215 (4.4), 264 (3.2); IR ν_{max}^{KBr} cm^{-1}: 2976, 1717, 1116, 968; For ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data, see Tables 3 and 4; EIMS, *m/z* (rel. int.): 427 $[M–OEt]^+$ (1), 398 (25), 340 (46), 313 (76), 204 (79), 121 (100), 107 (47); HRESIMS, *m/z*: 495.3083 $[M+Na]^+$ (calc. for $C_{29}H_{44}O_5Na$, 495.3086).

*4.3.13. Kuguacin R (**13**) and **17***

Colorless prisms (MeOH); UV λ_{max}^{MeOH} nm (log ε): 215 (4.7), 264 (4.0); IR ν_{max}^{KBr} cm^{-1}: 3426, 2968, 2949, 2875, 1378, 1119, 1082, 973, 916; For ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data, see Tables 3 and 4; EIMS, *m/z* (rel. int.): 426 $[M–CH_2O_2]^+$ (8), 408 (12), 389 (26), 309 (20), 203 (31), 172 (100), 157 (60), 109 (88), 81 (46), 55 (34); HRESIMS, *m/z*: 495.3460 $[M+Na]^+$ (calc. for $C_{30}H_{48}O_4Na$, 495.3450).

*4.3.14. Kuguacin S (**14**)*

Amorphous powder; mp: 174–177 °C; α_D^{20} −7.7 (*c* 0.13, MeOH); UV λ_{max}^{MeOH} nm (log ε): 214 (4.2), 251 (3.7), 256 (3.7); IR ν_{max}^{KBr} cm^{-1}: 3372, 2969, 2928, 2874, 1714, 1651, 1621, 1464, 1180, 1293, 972; For ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectroscopic data, see Tables 3 and 4; EIMS, *m/z* (rel. int.): 468 $[M]^+$ (4), 450 (10), 421 (20), 369 (34), 341 (34), 311 (26), 203 (61), 121 (65), 109 (100), 55 (50); HRESIMS, *m/z*: 491.3126 $[M+Na]^+$ (calc. for $C_{30}H_{44}O_4Na$, 491.3137).

4.4. Anti-HIV-1 and cytotoxicity assay

The anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}), and cytotoxicity assay

against C8166 cells (IC_{50}) was assessed using the MTT method as described in the literature (Zhang et al., 2005).

4.5. X-ray crystallographic studies of **11**, **13** and **17**

4.5.1. Kuguacin P (**11**)

$C_{27}H_{40}O_4$, $MW = 428.59$, orthorhombic system, space group $P2_12_12_1$, $a = 8.741$ (8) Å, $b = 11.552$ (10) Å, $c = 23.232$ (2) Å, $V = 2346.0$ (4) Å^3, $Z = 4$, $D_{calc} = 1.213$ g/cm^3, crystal dimensions $0.31 \times 0.27 \times 0.23$ mm was used for measurements on a Bruker APEX II diffractometer with a graphite monochromator (ω scans, $2\theta_{max} = 56.78^o$), Mo Kα radiation. The total number of independent reflections measured was 20190, of which 15301 were observed ($|F|^2 \geqslant 2\sigma|F|^2$). Final indices: $R_1 = 0.0476$, $wR_2 = 0.0959$, $S = 1.017$.

4.5.2. Kuguacin R (**13**) and **17**

$C_{30}H_{48}O_6$, $MW = 504.18$, orthorhombic system, space group $P2_12_12_1$, $a = 19.768$ (3) Å, $b = 19.986$ (3) Å, $c = 7.743$ (10) Å, $V = 3059.0$ (4) Å^3, $Z = 4$, $D_{calc} = 1.095$ g/cm^3, crystal dimensions $0.46 \times 0.38 \times 0.32$ mm was used for measurements on a Bruker APEX II diffractometer with a graphite monochromator (ω scans, $2\theta_{max} = 56.96^o$), Mo Kα radiation. The total number of independent reflections measured was 3984, of which 1986 were observed ($|F|^2 \geqslant 2\sigma|F|^2$). Final indices: $R_1 = 0.1059$, $wR_2 = 0.2857$, $S = 1.083$. Both the crystal structures of **11**, **13** and **17** were solved by the direct method SHELXS-97 (Sheldrich, G.M., University of Gottingen, Gottingen, Germany, 1997) and expanded using difference Fourier techniques, refined by the program SHELXL-97 (Sheldrich, G.M., University of Gottingen, Gottingen, Germany, 1997) and the full-matrix least-squares calculations. Crystallographic data for the structures have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 668007 of **11**, and 668008 of **13** and **17**). Copies of these data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0) 1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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References

Akihisa, T., Higo, N., Tokuda, H., Ukiya, M., Akazawa, H., Tochigi, Y., Kimura, Y., Suzuki, T., Nishino, H., 2007. Cucurbitane-type triterpenoids from the fruits of *Momordica charantia* and their cancer chemopreventive effects. J. Nat. Prod. 70, 1233–1239.

Begum, S., Ahmed, M., Siddiqui, B.S., Khan, A., Saify, Z.S., Arif, M., 1997. Triterpenes, a sterol and a monocyclic alcohol from *Momordica charantia*. Phytochemistry 44, 1313–1320.

Chang, C.I., Chen, C.R., Liao, Y.W., Cheng, H.L., Chen, Y.C., Chou, C.H., 2006. Cucurbitane-type triterpenoids from *Momordica charantia*. J. Nat. Prod. 69, 1168–1171.

Chen, J.C., Tian, R.R., Qiu, M.H., Lu, L., Zheng, Y.T., Zhang, Z.Q., 2008a. Trinorcucurbitane and cucurbitane triterpenoids from the roots of *Momordica charantia*. Phytochemistry 69, 1043–1048.

Chen, J.C., Zhang, G.H., Zhang, Z.Q., Qiu, M.H., Zheng, Y.T., Yang, L.M., Yu, K.B., 2008b. Octanorcucurbitane and cucurbitane triterpenoids from the tubers of *Hemsleya endecaphylla* with HIV-1 inhibitory activity. J. Nat. Prod. 71, 153–155.

Fatope, M.O., Takeda, Y., Yamashita, H., Okabe, H., Yamauchi, T., 1990. New cucurbitane triterpenoids from *Momordica charantia*. J. Nat. Prod. 53, 1491–1497.

Harinantenaina, L., Tanaka, M., Takaoka, S., Oda, M., Mogami, O., Uchida, M., Asakawa, Y., 2006. *Momordica charantia* constituents and antidiabetic screening of the isolated major compounds. Chem. Pharm. Bull. 54, 1017–1021.

Kanchanapoom, T., Kasai, R., Yamasaki, K., 2002. Cucurbitane and octanorcucurbitane glycosides from fruits of *Trichosanthes tricuspidata*. Phytochemistry 59, 215–228.

Kimura, Y., Akihisa, T., Yuasa, N., Ukiya, M., Suzuki, T., Toriyama, M., Motohashi, S., Tokuda, H., 2005. Cucurbitane-type triterpenoids from the fruit of *Momordica charantia*. J. Nat. Prod. 68, 807–809.

Li, Q.Y., Chen, H.B., Liu, Z.M., Wang, B., Zhao, Y.Y., 2007. Cucurbitane triterpenoids from *Momordica charantia*. Magn. Reson. Chem. 45, 451–456.

Matsuda, H., Nakamura, S., Murakami, T., Yoshikawa, M., 2007. Structures of new cucurbitane-type triterpenes and glycosides, karavilagenins D and E, and karavilosides VI, VII, VIII, IX, X, and XI, from the fruit of *Momordica charantia*. Heterocycles 71, 331–341.

Miyahara, Y., Okabe, H., Yamauchi, T., 1981. Studies on the constituents of *Momordica charantia* L. II. Isolation and characterization of minor seed glycosides, momordicosides C, D and E.. Chem. Pharm. Bull. 29, 1561–1566.

Mulholland, D.A., Sewram, V., Osborne, R., Pegel, K.H., Connolly, J.D., 1997. Cucurbitane triterpenoids from the leaves of *Momordica foetida*. Phytochemistry 45, 391–395.

Murakami, T., Emoto, A., Matsuda, H., Yoshikawa, M., 2001. Medicinal foodstuffs. XXI. Structures of new cucurbitane-type triterpene glycosides, goyaglycosides-a, -b, -c, -d, -e, -f, -g, and -h, and new oleanane-type triterpene saponins, goyasaponins I, II, and III, from the fresh fruit of Japanese *Momordica charantia* L. Chem. Pharm. Bull. 49, 54–63.

Nakamura, S., Murakami, T., Nakamura, J., Kobayashi, H., Matsuda, H., Yoshikawa, M., 2006. Structures of new cucurbitane-type triterpenes and glycosides, karavilagenins and karavilosides, from the dried fruit of *Momordica charantia* L. in Sri Lanka. Chem. Pharm. Bull. 54, 1545–1550.

Okabe, H., Miyahara, Y., Yamauchi, T., Miyahara, K., Kawasaki, T., 1980. Studies on the constituents of *Momordica charantia* L. I. Isolation and characterization of momordicosides A and B, glycosides of a pentahydroxy-cucurbitane triterpene. Chem. Pharm. Bull. 28, 2753–2762.

Okabe, H., Miyahara, Y., Yamauchi, T., 1982a. Studies on the constituents of *Momordica charantia* L. III. Characterization of new cucurbitacin glycosides of the immature fruits. (1) Structures of momordicosides G, F_1, F_2 and I. Chem. Pharm. Bull. 30, 3977–3986.

Okabe, H., Miyahara, Y., Yamauchi, T., 1982b. Studies on the constituents of *Momordica charantia* L. IV. Characterization of the new cucurbitacin glycosides of the immature fruits. (2) Structures of the bitter glycosides, momordicosides K and L. Chem. Pharm. Bull. 30, 4334–4340.

Yasuda, M., Iwamoto, M., Okabe, H., Yamauchi, T., 1984. Structures of momordicines I, II and III, the bitter principles in the leaves and vines of *Momordica charantia* L. Chem. Pharm. Bull. 32, 2044–2047.

Zhang, G.H., Wang, Q., Chen, J.J., Zhang, X.M., Tam, S.C., Zheng, Y.T., 2005. Anti-HIV-1 effect of scutellarin. Biochem. Biophys. Res. Commun. 334, 812–816.

Zhu, Z.J., Zhong, Z.C., Luo, Z.Y., Xiao, Z.Y., 1990. Studies on the active constituents of *Momordica charantia* L. Acta Pharm. Sin. 25, 898–903.