The Antigluconeogenic Activity of Cucurbitacins from *Momordica charantia*

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- antigluconeogenic activity
- PEPCK activity

Abstract

Five new cucurbitacins, kuguacins II–VI (**1**–**5**), along with five known analogues (**6**–**10**), were obtained from the fruit of *Momordica charantia*. Structures of the new compounds were elucidated as 5*β*,19-epoxycucurbit-23-en-7-on-3*β*,25-diol (**1**), 5*β*,19-epoxycucurbit-7,23-dion-3*β*,25-diol (**2**), 5*β*,19-epoxycucurbit-6-en-19,23-dion-3*β*,25-diol (**3**), 5*β*,19-epoxy-23,24,25,26,27-pentanorcucurbit-6-en-7,19-dion-3*β*,22-diol (**4**), and cucurbit-5-en-7,23-dion-3*β*,19,25-triol (**5**) by extensive spectroscopic and single-crystal X-ray diffraction analyses. Some cucurbitane compounds from this species were screened for their potential antidiabetic properties in terms of antigluconeogenic activity. As a result, compounds **1, 10, 11**, and **12** (at 25–100 µM) showed concentration-dependent inhibition on glucose production from liver cells. In addition, compounds **11** and **12** (at 100 µM) showed around 20–30% inhibition on PEPCK activity.

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Introduction

The fruit of *Momordica charantia* L. (Cucurbitaceae), known as kugua in Chinese and bitter melon in English, is a popular vegetable in the southern part of China. Tissues of this species are used as traditional Chinese medicine to treat a variety of ailments. The stem and root are applied to treat toothaches, diarrhea, furuncle, and diabetes. The fruit is used to cure diarrhea, furuncle, heat stroke, and diabetes and the seeds are used to remedy asynodia [1].

Besides antidiabetic properties, *M. charantia* compounds showed many other bioactivities, such as anticancer [2–7], agonist/antagonist [8], antimalarial [9], and antioxidant activities [10, 11]. The two major compounds, 5*β*,19-epoxy-3*β*,25-dihydroxycucurbita-6,23(*E*)-diene and 3*β*,7*β*,25-trihydroxycucurbita-5,23(*E*)-dien-19-al, showed hypoglycemic effects in diabetes-induced male ddY mice at 400 mg/kg [12]. Moreover, in studies using both L6 myotubes and 3 T3-L1 adipocytes, momordicoside S and karaviloside XI could significantly stimulate GLUT4 translocation from cytosol to the cell membrane [13]. Furthermore, at concentrations of 10 and 25 µg/mL, momordicin II and kuguaglycoside G significantly stimulated insulin secretion compared to the vehicle control, $p \le 0.007$ and $p = 0.002$, respectively [14], and the compound 7*β*,25-dihydroxycucurbita-5,23(*E*)-dien-19-al 3-*O*-*β*-D-allopyranoside showed potent hypoglycemic activities by the glucose uptake assay [15]. Although there are many reports on the antidiabetic activities of the constituents from *M. charantia* plant, we noticed that the majority of the reported compounds exhibited weak or no activities, while the active compounds are available in minute quantities. Therefore, further research is still required to find antidiabetic active components from this plant by using a new bioassay mode.

Our previous phytochemical investigations demonstrated a series of new cucurbitane triterpenoids as well as the anti-HIV activities of some cucurbitacins isolated from the fruit, root, and stem of *M. charantia* [16–20]. In our search for bioactive components, a further study of the fruit led to the isolation of five new cucurbitacins (**1**–**5**) and five known ones (**6**–**10**) (**Fig. 1**), momordicin I (**6**) [21], 5*β*,19-epoxycucurbita-6,23-dien-3*β*,19(*R*),25-triol (**7**) [22], balsaminol E (**8**) [23], kuguacin A (**9**) [16], and 3*β*,7*β*,25-trihydroxycucurbita-5,(23*E*)-dien-19-al (**10**) [24]. Furthermore, compounds relatively abundant in different

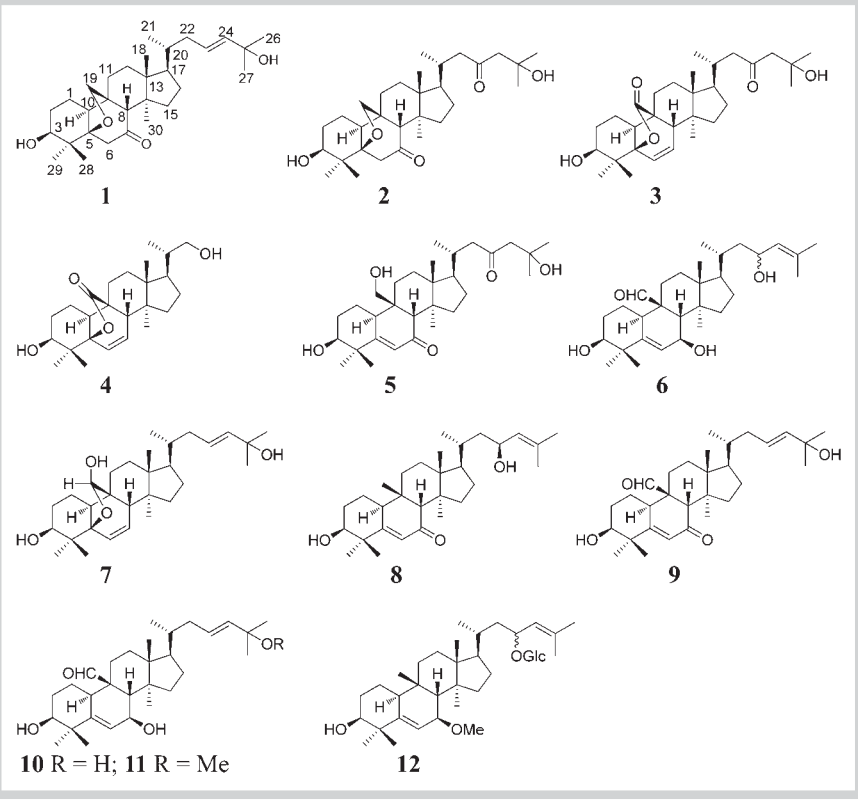

Fig. 1 Chemical structures of compounds **1**–**12**.

tissues of this species were subjected to the screening for their potential antidiabetic properties with a special focus on antigluconeogenic activities. Herein, we describe the isolation and structural elucidation of the new compounds as well as the antigluconeogenic activities of tested compounds.

Results and Discussion

Kuguacin II (**1**) was isolated as colorless prismatic crystals and was assigned the molecular formula of $C_{30}H_{48}O_4$ on the basis of a molecular ion peak at 472.3554 $[M]^+$. The IR spectrum showed the absorption bands of hydroxyl (3441 cm^{-1}) and isolated carbonyl (1723 cm^{-1}) functionalities. The UV maximum absorption at 202 nm indicated the absence of a conjugated carbonyl group. Obvious signals observed in the ^1H NMR spectrum were six tertiary methyl groups [δ_H 1.56 (6H), 1.28 (3H), 0.86 (6H), and 0.75 (3H)], one secondary methyl group [δ_H 0.94 (d, J = 5.5 Hz)], a pair of methylene protons [δ_H 2.96 (d, J = 17.9 Hz) and 2.48 (d, J = 17.9 Hz)], and a pair of oxygenated methylene protons [δ_H 3.85 (d, J = 8.5 Hz) and 3.55 (d, J = 8.5 Hz)]. The typical methine proton signal at δ_H 2.66 (dd, J = 11.2, 6.6 Hz), which was tentatively ascribed to H-10, suggested that compound **1** had a cucurbitane skeleton [18]. The ^{13}C and DEPT studies of compound **1** demonstrated the presence of 29 resonances, including seven methyl signals (δ_C 30.9, 26.2, 21.5, 21.2, and 15.6), one carbonyl carbon (δ_C 212.8), two olefinic carbons (δ_C 141.8 and 124.0), one oxymethine carbon (δ_C 76.4), one oxymethylene carbon (δ_C 79.2), and two oxygenated quaternary carbons (δ_C 89.1 and 69.7). From the ^1H and ^{13}C NMR data, compound **1** was proposed to be a cucurbitacin, namely, a 19-norlanostane compound. The oxymethylene signal (δ_C 79.2) was assigned to C-19 by comparison with those of (23*E*)-5*β*,19-epoxycucurbita-6,23-diene-3*β*,25-diol as well as signals for C-3 (δ_C 76.4), C-5 (δ_C 89.1), C-23 (δ_C 124.0), C-24 (δ_C 141.8), and C-25 (δ_C 69.7) [25]. The differences between compound **1** and (23*E*)-5*β*,19-epoxycucurbita-6,23-diene-3*β*,25-diol are that the olefinic signals at δ_C 131.9 (C-6) and 131.5 (C-7) in the known compound were replaced by a carbonyl carbon at δ_C 212.8 and a methylene signal at δ_C 50.9 in compound **1**. In the HSQC spectrum, correlations were observed between the methylene protons at δ_H 2.96 (1H, d, J = 17.9 Hz) and 2.48 (1H, d, J = 17.9 Hz) and a carbon signal at δ_C 50.9, by which the two methylene protons were ascribed to the carbon (δ_C 50.9). Furthermore, the methylene protons at δ_H 2.96 (1H, d, J = 17.9 Hz) and 2.48 (1H, d, J = 17.9 Hz) also showed HMBC correlations with the carbon signals at δ_C 89.1 (C-5), δ_C 63.1 (C-8), δ_C 41.0 (C-10), and the carbonyl carbon at δ_C 212.8. All the above 2D NMR spectra indicated that the methylene was assigned at C-6 and the carbonyl carbon at C-7. The side chain and rings A, C, and D were identical to those of (23*E*)-5*β*,19-epoxycucurbita-6,23-diene-3*β*,25-diol, as shown by detailed analysis of the ^{13}C, ^1H, HMBC, HSQC, and ^1H-^1H COSY spectra of compound **1**. The single-crystal X-ray crystallographic results (**Fig. 2**) of compound **1** also confirmed the proposed structure.

Kuguacin III (**2**), a white amorphous solid, had a molecular formula of $C_{30}H_{48}O_5$ on the basis of its HREIMS, ^{13}C NMR, and DEPT data. Its IR spectrum showed the presence of hydroxyl (3420 cm^{-1}) and isolated carbonyl (1718 cm^{-1}) groups. In the ^1H NMR spectrum of compound **2**, six tertiary methyl groups [δ_H 1.23 (6H), 1.14 (3H), 0.90 (3H), and 0.89 (6H)], and two pairs of methylene protons [δ_H 3.83 (d, J = 8.7 Hz) and 3.51 (d, J = 8.7 Hz); δ_H 2.77 (d, J = 18.2 Hz) and 2.28 (d, J = 18.2 Hz)] were distinguishable from other signals. The ^{13}C and DEPT spectra of compound **2** displayed the presence of 30 resonances, including seven methyl signals (δ_C 29.3, 29.2, 26.2, 21.2, 21.0, and 15.6), two carbonyl carbons (δ_C 213.4 and 213.2), one oxymethine carbon (δ_C 76.5), one

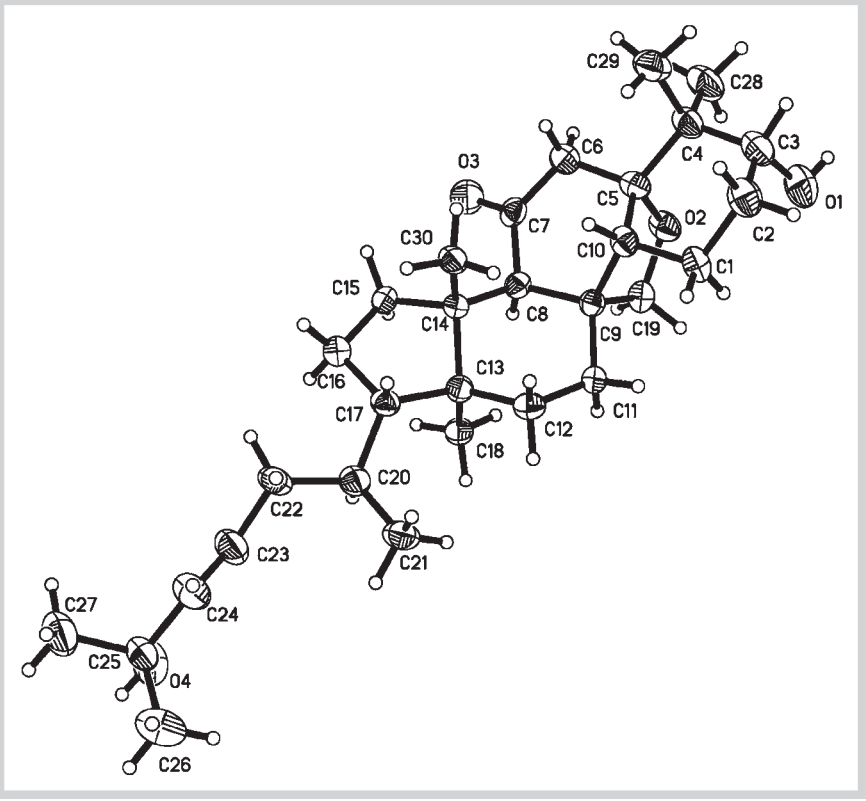

Fig. 2 X-ray structure of compound **1** showing the relative configuration.

oxymethylene carbon (δ_C 79.0), and two oxygenated quaternary carbons (δ_C 89.2 and 69.6). Comparison of ^{1}H and ^{13}C NMR data of compound **2** with those of compound **1** showed that both compounds possessed the same structure in rings A–D, except for the differences in the side chain, which could be presumed by the replacement of two olefin carbon signals at C-23 and C-24 in compound **1** by a carbonyl carbon at δ_C 213.2 and a methylene signal at δ_C 53.6 in compound **2**. Obvious HMBC correlations from the methyl group signals at δ_H 1.23 (6H, H-26 and H-27) to δ_C 69.6 (C-25) and 53.6, and from the other methylene signals at δ_H 2.47 (m, H_a – 22) and 2.16 (m, H_b – 22) to δ_C 32.5 (C-20), 213.2, and 53.6, proved that the position of the methylene carbon was ascribed to C-24, while the carbonyl carbon was assigned as C-23. The positive HREIMS of kuguacin IV (**3**) showed a molecular ion peak at 486.3345 $[M]^+$, according to the molecular formula of $C_{30}H_{46}O_5$. Its IR spectrum showed absorptions attributable to hydroxyl (3506 cm^{-1}) and isolated carbonyl (1715 cm^{-1}) groups. The ^{1}H NMR spectrum of compound **3** showed the distinct presence of six tertiary methyl groups [δ_H 1.28 (3H), 1.25 (6H), 0.97 (3H), 0.95 (3H), 0.87 (3H)], one secondary methyl group [δ_H 0.94 (d, J = 6.4 Hz)], a pair of methylene protons [δ_H 2.61 (d, J = 17.4 Hz) and 2.55 (d, J = 17.4 Hz)], and two olefinic protons [δ_H 6.21 (dd, J = 9.8, 1.9 Hz) and 5.72 (J = 9.8, 3.3 Hz)]. The ^{13}C and DEPT studies of compound **3** displayed the presence of 30 resonances, including seven methyl signals, two carbonyl carbons (δ_C 213.3 and 181.4), two olefinic carbons (δ_C 133.2 and 131.3), one oxymethine carbon (δ_C 75.3), and one oxygenated quaternary carbon (δ_C 85.4). Accordingly, compound **3** was suggested to be a cucurbitacin. The carbonyl signal (δ_C 181.4) was assigned to C-19 by analysis of the HMBC correlations [δ_H 2.54 (H-8)/δ_C 131.3 (C-6), 133.2 (C-7), 51.0 (C-9), 181.4; and δ_H 2.64 (H-10)/δ_C 85.4 (C-5), 131.3 (C-6), 51.0 (C-9), 181.4]. Comparison of ^{1}H and ^{13}C NMR data of compound **3** with those of karavilagenin D [26] showed that both compounds possessed the same structure in rings A–D. The differences lied in that the two olefinic carbon signals at C-23 and C-24 in karavilagenin D were replaced by a carbonyl carbon at δ_C 213.3 and a methylene signal at δ_C 53.5 in compound **3**. The observed HMBC correlations from the methyl group signals at δ_H 1.25 (6H, H-26 and H-27) to δ_C 69.7 (C-25) and 53.5 manifested that the position of the methylene carbon was ascribed to C-24. Furthermore, HMBC correlations from H-24 [δ_H 2.61 (d, J = 17.4 Hz) and 2.55 (d, J = 17.4 Hz)] to δ_C 213.3, 51.7 (C-22) and 69.7 (C-25) also proved the position of the carbonyl carbon at C-23.

Kuguacin V (**4**), obtained as an amorphous solid, displayed the molecular formula of $C_{25}H_{38}O_4$ by HREIMS data, in accordance with its ^{13}C NMR and DEPT spectra. The IR spectrum showed the absorption bands of hydroxyl (3481 cm^{-1}) and isolated carbonyl (1720 cm^{-1}) groups. The ^{1}H NMR spectrum of compound **4** displayed four tertiary methyl groups (δ_H 1.28, 0.95, 0.94, and 0.87), one secondary methyl group [δ_H 1.04 (d, J = 5.8 Hz)], two olefinic protons [δ_H 6.21 (dd, J = 9.8, 2.0 Hz) and 5.73 (J = 9.8, 3.3 Hz)], and a methine proton signal at δ_H 2.66 (dd, J = 12.3, 5.8 Hz). The ^{13}C NMR and DEPT studies of compound **4** demonstrated the presence of 25 resonances, including five methyl signals (δ_C 23.5, 20.3, 19.3, 18.4, and 14.7), one carbonyl carbon (δ_C 181.5), two olefinic carbons (δ_C 133.4 and 131.2), one oxymethine carbon (δ_C 75.3), one oxymethylene carbon (δ_C 68.1), and one oxygenated quaternary carbon (δ_C 85.4). From these data, compound **4** was deduced to be a pentanorcucurbitacin. A detailed comparison of the 1D NMR data with those of compound **3** showed that both compounds possessed the same structure in rings A–D. The differences lied in the absence of structural moieties for C-23, C-24, C-25, C-26, and C-27, and the presence of an oxymethylene for C-22 in compound **4**. This deduction was further proved by the HMBC correlations from the oxymethylene

protons at δ_H 3.67 (br d, J = 11.0 Hz) and 3.41 (dd, J = 10.4, 5.8 Hz) to C-17 (δ_C 46.8), C-20 (δ_C 38.8), and C-21 (δ_C 18.4).

Kuguacin VI (**5**) was obtained as a white amorphous solid with the empirical molecular formula of $C_{30}H_{48}O_5$, in agreement with the HREIMS (*m/z* 488.3493 $[M]^+$) and ^{13}C NMR spectroscopic data. Its IR spectrum showed the presence of hydroxyl (3305 cm^{-1}), isolated carbonyl (1715 cm^{-1}), and conjugated carbonyl (1655 cm^{-1}) groups. The UV maximum absorption at 275 also indicated the presence of a conjugated carbonyl group. The ^1H NMR spectrum of **5** clearly displayed six tertiary methyl groups [δ_H 1.26 (3H), 1.25 (6H), 1.14 (3H), 0.93 (3H), and 0.86 (3H)], one secondary methyl group [δ_H 0.92 (d, J = 6.3 Hz)], a single olefinic proton [δ_H 6.11], a pair of methylene protons [δ_H 2.60 (d, J = 17.3 Hz) and 2.54 (d, J = 17.3 Hz)], and a pair of oxygenated methylene protons [δ_H 3.66 (d, J = 11.0 Hz) and 3.32 (d, J = 11.0 Hz)]. The ^{13}C NMR and DEPT spectra of compound **5** showed the presence of 30 resonances, including six methyl signals (δ_C 29.4, 29.1, 27.2, 25.0, 18.3, and 14.9), two carbonyl carbons (δ_C 213.5 and 202.3), two olefinic carbons (δ_C 168.3 and 127.3), one oxymethine carbon (δ_C 76.5), one oxymethylene carbon (δ_C 70.6), and one oxygenated quaternary carbon (δ_C 69.6). The detailed comparison of the 1D NMR data of compound **5** with those of compound **1** displayed that both compounds possessed the same structure in rings A, C, D, and the side chain except for a double bond in compound **5** instead of an oxygenated quaternary carbon and a methylene carbon in compound **1**. The oxymethylene carbon signal (δ_C 70.6) was assigned to C-19 by analysis of the HMBC correlations [δ_H 2.76 (H-8)/δ_C 40.3 (C-9), 70.6; and δ_H 2.73 (H-10)/δ_C 40.3 (C-9), 70.6]. The carbon chemical shift signal of C-19 in compound **1** at δ_C 79.2 was shifted upfield to δ_C 70.6 in **5**, suggesting the absence of the oxygen bridge between C-5 and C-19 in compound **5**. HMBC correlations [δ_H 2.76 (H-8)/δ_C 202.3 (C-7) and 127.3; and δ_H 2.73 (H-10)) /δ_C 168.3 and 127.3] further elucidated that the double bond located between C-5 and C-6, and the carbon signals at δ_C 168.3 and 127.3 were assigned to C-5 and C-6, respectively.

The 21 cucurbitane compounds, compounds **1** (98%), **10** (97%), **11** (97%), and **12** (98%, kuguaglycoside A), momordicoside A (99%), momordicaside B (98%), momordicacoside G (96%), momordicaside L (97%), momordicaside P (95%), momordicoside S (95%), karaviloside XI (97%), karaviloside V (98%), kuguaglycoside D (96%), kuguaglycoside E (97%), kuguaglycoside H (96%), kuguaglycoside G (98%), kuguacin G (98%), kuguacin E (99%), goyaglycoside-d (97%), momordicine II (98%), and momordicaside F2 (97%), isolated from *M. charantia* in our present and previous researches, were evaluated for their antigluconeogenic activities (**Fig. 31S**, Supporting Information). Among all compounds tested, compounds **1, 10, 11**, and **12** (at 25–100 µM) showed concentration-dependent inhibition on glucose production from liver cells (**Fig. 32S**, Supporting Information). In addition, compounds **11** and **12** (at 100 µM) showed around 20–30% inhibition on PEPCK activity (**Fig. 33S**, Supporting Information). However, except for compound **10**, compounds **1, 11**, and **12** are present in a low content in dried *M. charantia* tissues. The percentage content of **10** was 0.016% in the root [16], 0.0067% in the vine and leaf [18], and 0.0032% in the fruit, while the percentage content of **1** was 0.0018% from the fruit, **11** was 0.0003% in the vine and leaf [18], and **12** was 0.0008% in the root [17].

Materials and Methods

The dried fruit of *M. charantia* (1.5 kg) was collected at Huanian Farm, Yuxi City, Yunnan Province, People's Republic of China in August 2011. The sample was identified by Prof. Shu-Kun Chen, and a voucher specimen (No. KIB 2011–08–14) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Air-dried fruit of *M. charantia* (1.5 kg) was extracted with methanol at 60 °C three times (5 L each). After removal of the solvent under reduced pressure, a residue (140 g) was obtained. This residue was dissolved in water (2 L) and then extracted with EtOAc (1 L × 3). The EtOAc extract (20 g) was subjected to silica gel column chromatography (300 g, 200–300 mesh, 6 × 60 cm), eluted with a gradient system of $CHCl_3$-MeOH (1 : 0, 30 : 1, 20 : 1, 10 : 1, each 4 L) to yield fractions I–V monitored by TLC. Fraction II (2 g) was repeatedly chromatographed over silica gel (50 g, 200–300 mesh, 3.5 × 35 cm), using $CHCl_3$-$(Me)_2CO$ (50 : 1, 20 : 1, 15 : 1, each 800 mL) as the eluent followed by a reversed-phase column (RP-18) developing with aqueous MeOH (60% → 70%) to give compounds **8** (21 mg) and **9** (17 mg). Fraction III (1.2 g) was chromatographed over silica gel (40 g, 200–300 mesh, 3 × 35 cm), using $CHCl_3$-MeOH (50 : 1, 30 : 1, 20 : 1, each 500 mL) as the eluent to afford fractions III a–III c. Compound **1** (27 mg) was crystallized from fraction III a (260 mg). Fraction III b (92 mg) was repeatedly chromatographed over silica gel (6 g, 200–300 mesh, 2 × 20 cm), using $CHCl_3$-MeOH (50 : 1, 30 : 1, each 100 mL) as the eluent followed by semipreparative HPLC (60 : 40 → 70 : 30, MeOH-H_2O) to yield **2** (3 mg) and **3** (2 mg). Compounds **4** (2 mg) and **5** (4 mg) were purified by semipreparative HPLC (55 : 45 → 65 : 35, MeOH-H_2O) from fraction III c (19 mg). Compounds **6** (150 mg), **7** (23 mg), and **10** (48 mg) were isolated and purified from fraction V (390 mg) by column chromatography over silica gel (20 g, 200–300 mesh, 3 × 50 cm) with $CHCl_3$-MeOH (20 : 1, 50 mL) as the eluent followed by a reversed-phase column (RP-18) developing with aqueous MeOH (60% → 70%) and then Sephadex LH-20 (MeOH, 3 × 150 cm, 1 L).

Isolates

Kuguacin II (**1**): colorless prismatic crystals (MeOH); m. p. 215–216 °C; $[\alpha]_D^{23}$+41.2 (*c* 0.090, MeOH); UV (MeOH) λ_{max} nm (log ε) 202 (4.39); IR (KBr) ν_{max} 3441, 2966, 2928, 2871, 1714, 1467, 1378, 1093 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz): **Table 1**; ^{13}C NMR (C_5D_5N, 125 MHz): **Table 2**. ESI-MS (positive): *m/z* 495 $[M + Na]^+$, 967 $[2M + Na]^+$; HREIMS *m/z* 472.3554 $[M]^+$ (calcd. for $C_{30}H_{48}O_4$, 472.3553).

Kuguacin III (**2**): white amorphous solid ($CHCl_3$); $[\alpha]_D^{23}$ +39.7 (*c* 0.110, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε) 210 (3.91); IR (KBr) ν_{max} 3420, 2982, 2870, 1718, 1464, 1381 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): **Table 1**; ^{13}C NMR ($CDCl_3$, 150 MHz): **Table 2**. HREIMS *m/z* 488.3491 $[M]^+$ (calcd. for $C_{30}H_{48}O_5$, 488.3502).

Kuguacin IV (**3**): white amorphous solid ($CHCl_3$); $[\alpha]_D^{23}$ −35.5 (*c* 0.014, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε) 211 (4.68); IR (KBr) ν_{max} 3506, 2950, 2874, 1758, 1715, 1623, 1444, 1036 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): **Table 1**; ^{13}C NMR ($CDCl_3$, 150 MHz): **Table 2**. HREIMS *m/z* 486.3345 $[M]^+$ (calcd. for $C_{30}H_{46}O_5$, 486.3345).

Kuguacin V (**4**): white amorphous solid ($CHCl_3$); $[\alpha]_D^{23}$ −85.3 (*c* 0.010, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε) 220 (4.76); IR (KBr) ν_{max} 3481, 2978, 2893, 1753, 1720, 1621, 1160, 1030 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): **Table 1**; ^{13}C NMR ($CDCl_3$, 150 MHz):

Table 1 ^{1}H NMR data for compounds **1**–**5** (δ in ppm, J in Hz).

Pos.	1[a]	2[b]	3[b]	4[b]	5[b]
1	1.76 m, 1.58 m	1.62 m, 1.57 m	1.65 m, 1.31 m	1.65 m, 1.30 m	1.81 m, 1.75 m
2	1.84 m, 1.81 m	1.82 m, 1.78 m	1.84 m, 1.79 m	1.84 m, 1.80 m	2.00 m, 1.86 m
3	3.58 s	3.47 s	3.45 br s	3.47 br s	3.66 s
6	2.96 d (17.9), 2.48 d (17.9)	2.77 d (18.2), 2.28 d (18.2)	6.21 dd (9.8, 1.9)	6.21 dd (9.8, 2.0)	6.11 s
7			5.72 dd (9.8, 3.3)	5.73 dd (9.8, 3.3)	
8	2.72 s	2.62 overlap	2.54 br s	2.53 br s	2.76 s
10	2.66 dd (11.2, 6.6)	2.61 overlap	2.64 m	2.66 dd (12.3, 5.8)	2.73 m
11	1.62 m, 1.28 m	1.77 m, 1.43 m	2.26 m, 1.78 m	2.26 m, 1.79 m	1.53 m, 0.94 m
12	1.56 m, 1.49 m	1.68 m, 1.64 m	1.72 m, 1.63 m	1.72 m, 1.64 m	1.74 m, 1.63 m
15	1.73 m, 1.36 m	1.63 m, 1.26 m	2.08 m, 1.31 m	2.05 m, 1.37 m	1.64 m, 1.14 m
16	1.82 m, 1.29 m	1.85 m, 1.32 m	1.90 m, 1.35 m	1.93 m, 1.44 m	1.85 m, 1.33 m
17	1.49 m	1.43 m	1.53 m	1.61 m	1.51 m
18	0.75 s	0.89 s	0.97 s	0.94 s	0.93 s
19	3.85 d (8.5), 3.55 d (8.5)	3.83 d (8.7), 3.51 d (8.7)			3.66 d (11.0), 3.32 d (11.0)
20	1.49 m	2.05 m	2.08 m	1.63 m	2.06 m
21	0.94 d (5.5)	0.91 overlap	0.94 d (6.4)	1.04 d (5.8)	0.92 d (6.3)
22	2.21 m, 1.87 m	2.47 m, 2.16 m	2.49 m, 2.19 m	3.67 br d (11.0), 3.41 dd (10.4, 5.8)	2.47 m, 2.14 m
23	5.91 m				
24	6.00 br s	2.55 overlap	2.61 d (17.4), 2.55 d (17.4)		2.60 d (17.3), 2.54 d (17.3)
26	1.56 s	1.23 s	1.25 s		1.25 s
27	1.56 s	1.23 s	1.25 s		1.25 s
28	1.28 s	1.14 s	1.28 s	1.28 s	1.26 s
29	0.86 s	0.90 s	0.95 s	0.95 s	1.14 s
30	0.86 s	0.89 s	0.87 s	0.87 s	0.86 s

[a] Measured in C_5D_5N at 500 MHz; [b] measured in $CDCl_3$ at 600 MHz

Table 2 ^{13}C NMR and DEPT data for compounds **1**–**5**.

Pos.	1[a]	2[b]	3[b]	4[b]	5[b]
1	18.6 t	18.0 t	18.4 t	18.4 t	20.6 t
2	27.3 t	26.5 t	26.5 t	26.5 t	28.0 t
3	76.4 d	76.5 d	75.3 d	75.3 d	76.5 d
4	38.5 s	37.9 s	37.0 s	37.0 s	42.9 s
5	89.1 s	89.2 s	85.4 s	85.4 s	168.3 s
6	50.9 t	49.8 t	131.3 d	131.2 d	127.3 d
7	212.8 s	213.4 s	133.2 d	133.4 d	202.3 s
8	63.1 d	62.8 d	44.4 d	44.4 d	54.6 d
9	47.1 s	46.8 s	51.0 s	51.1 s	40.3 s
10	41.0 d	40.4 d	39.8 d	39.7 d	39.5 d
11	22.3 t	22.3 t	21.6 t	21.6 t	25.2 t
12	30.7 t	30.4 t	29.8 t	29.7 t	29.3 t
13	46.0 s	45.9 s	45.1 s	45.1 s	45.6 s
14	49.0 s	48.8 s	47.8 s	47.5 s	48.2 s
15	34.8 t	34.1 t	33.1 t	33.3 t	34.5 t
16	28.0 t	28.0 t	27.7 t	26.9 t	28.6 t
17	49.4 d	49.4 d	50.4 d	46.8 d	49.8 d
18	15.6 q	15.6 q	14.6 q	14.7 q	14.9 q
19	79.2 t	79.0 t	181.4 s	181.5 s	70.6 t
20	36.6 d	32.5 d	32.6 d	38.8 d	32.8 d
21	18.9 q	19.7 q	18.4 q	18.4 q	19.9 q
22	39.4 t	51.6 t	51.7 t	68.1 t	51.7 t
23	124.0 d	213.2 s	213.3 s		213.5 s
24	141.8 d	53.6 t	53.5 t		53.4 t
25	69.7 s	69.6 s	69.7 s		69.6 s
26	30.9 q	29.3[c] q	29.4[d] q		29.4[e] q
27	30.9 q	29.2[c] q	29.2[d] q		29.1[e] q
28	21.2 q	21.0 q	19.3 q	19.3 q	25.0 q
29	26.2 q	26.2 q	23.5 q	23.5 q	27.2 q
30	21.5 q	21.2 q	20.3 q	20.3 q	18.3 q

[a] Measured in C_5D_5N at 125 MHz; [b] measured in $CDCl_3$ at 150 MHz; [c–e] Data may be interchangeable

Table 2. HREIMS *m/z* 402.2757 $[M]^+$ (calcd. for $C_{25}H_{38}O_4$, 402.2770).

Kuguacin V (**5**): white amorphous solid ($CHCl_3$); $[\alpha]_D^{23}$ +4.1 (*c* 0.009, $CHCl_3$); UV (MeOH) λ_{max} nm (log ε) 275 (4.62); IR (KBr) ν_{max} 3305, 2950, 2862, 1715, 1655, 1164, 1070 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): **Table 1**; ^{13}C NMR ($CDCl_3$, 150 MHz): **Table 2**. HREIMS *m/z* 488.3493 $[M]^+$ (calcd. for $C_{30}H_{48}O_5$, 488.3502).

Supporting information

The NMR and HREIMS spectra of compounds **1**–**5**, general experimental procedures, glucose production assay, PEPCK activity assay, X-ray crystallographic analysis, and the effects for selected compounds on antigluconeogenic activities, on glucose production from liver cells, and on inhibition of PEPCK activity are available as Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

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