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Macathiohydantoins B–K, novel thiohydantoin derivatives from *Lepidium meyenii*

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ABSTRACT

Macathiohydantoins B–K (**1**–**10**), two new group of naturally occurring thiohydantoin derivatives, together with one known analogues (**11**), were isolated from the rhizomes of *Lepidium meyenii* (Maca). Compounds **1**–**11** were all initially obtained as racemic mixtures and further separated by chiral HPLC chromatography to afford the eleven pairs of enantiomers. The structures of **1**–**10** including their absolute configurations were fully established by the comprehensive spectroscopic analysis and electronic circular dichroism (ECD) calculations. All isolates were evaluated for their cytotoxic and antimicrobial activities.

1. Introduction

Lepidium meyenii, a species of family Brassicaceae and well-known as maca, has been usually used as a folk medicine to improve sexual behavior, fertility and reduce stress and menopausal symptoms.[1–5] Previous investigations on maca led to reports of sterol,[6,7] glucosinolate,[8–11] macamides, macaenes,[12–14] flavones.[15] In addition, maca has been proved to exhibit various bioactivities, such as enhancement of sexual drive in humans, increasing overall vigour and energy levels and increasing sexual fertility in humans and domestic livestock,[16] antioxidant,[17,18] immunostimulation and memory improvement.[19] Considering the fact that the plant secondary metabolites can be influenced significantly by the ecological environment, phytochemical investigation on the rhizomes of title species cultivated in Lijiang, Yunnan province of China, was conducted. As a result, ten novel thiohydantoin derivatives (Fig. 1), macathiohydantoins B–K (**1**–**10**), were obtained and characterized. Herein, we report the isolation, structural elucidation, and biological activities of these compounds.

2. Results and discussion

Macathiohydantoin B (**1**) was isolated as colorless oil. The molecular formula of **1** was assigned as $C_{13}H_{14}O_2N_2S$ by HRESIMS at *m/z* 263.0847 $[M+H]^+$ and ^{13}C NMR spectrum, requiring eight degrees of unsaturation. The IR spectrum showed the absorption bands for carbonyl (1743 cm^{-1}) and aromatic ring (1632 and 1432 cm^{-1}). The ^1H NMR spectrum of **1** displayed signals for four aromatic protons signals at δ_H 6.93 (br s, H-3a), 6.75 (dd, $J = 8.0$, 1.6 Hz, H-5a), 7.18 (t, $J = 8.0$ Hz, H-6a), and 7.00 (d, $J = 8.0$ Hz, H-7a). With the aid of DEPT and HSQC experiments, the ^{13}C NMR of **1** exhibited 14 carbon signals categorized into four methylenes, five methines (including four aromatic ones), four quaternary carbons, and two carbonyl groups. The above-mentioned NMR data accounted for six degrees of unsaturation and the remaining two ones suggested the presence of a dicyclic ring system. The HMBC correlations (Fig. 2) from H-3a to C-5a/C-7a and from H-6a to C-2a/C-4a verified the presence of a 1,3-disubstituted benzene ring (A) and one hydroxy group was located at C-4a. Likewise, the observed HMBC correlations from H-4 to C-7 and the substructure (H-4–H_2-5–H_2-6–H_2-7) revealed by

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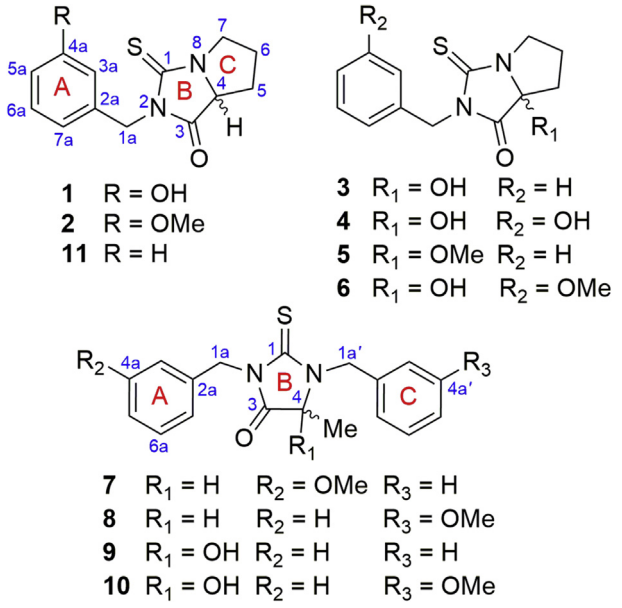

Fig. 1. Structures of compounds **1**–**11** isolated from *L. meyenii*.

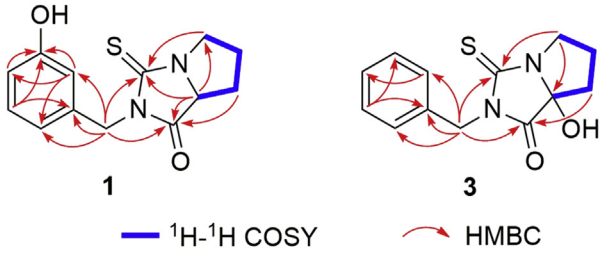

Fig. 2. Key 2D correlations of **1** and **3**.

^{1}H–^{1}H COSY spectrum established the partial structure of a five-membered moiety (ring C) via C–4-N-8–C-7. Moreover, the HMBC correlations from H_2-7 to C-1, from H_2-5 to C-3, and from H_2-1a to C-1/C-3/C-3a/C-7a substantiated the construction of ring C and the benzene ring was connected to ring B via C-2a–C-1a–N-2. Based on the aforementioned evidence and the X-ray diffraction analysis of **11** (Fig. 4), the planar structure of **1** was thus established as shown in Fig. 1.

The specific rotation value [−4.2 (*c* 0.028, MeOH)] of **1** suggested that it should a racemate mixture, which was further substantiated by a chiral analysis (Fig. S1). The subsequent chiral HPLC resolution of **1** afforded the anticipated enantiomers (+)-**1** and (−)-**1**, whose electronic circular dichroism (ECD) curves were definitely opposite to each other. As depicted in Fig. S10 (see Supplementary data), the well matched ECD curves of (+)-**1** and (−)-**1** with the calculated ECD curves of 4*R*-1 and 4*S*-1 allowed the establishment of the absolute configurations of 4*R* for (+)-**1** and 4*S* for (−)-**1**, respectively.

Macathiohydantoin C (**2**) gave a molecular formula of $C_{14}H_{16}O_2N_2S$ by HRESIMS at *m*/*z* 277.1003 $[M+H]^+$ (calcd for $C_{14}H_{17}O_2N_2S$, 277.1005) and ^{13}C NMR spectrum. The ^{1}H and ^{13}C NMR spectral data (Table 1) of **2** were very similar to those of **1**, except for presence of one methoxy group (δ_H 3.76, δ_C 55.2) in **2** instead of hydroxy group in **1**, which was assigned by HMBC correlation from H_3-OMe to C-4a. The planar structure of unit B and C in **2** was further verified to be the same as that of **1** by its ^{1}H–^{1}H COSY and HMBC experiments. A chiral HPLC analysis of **2** afforded anticipated enantiomers (+)-**2** and (−)-**2** (Fig. S2). Their specific rotation values suggested the absolute configurations of 4*R* and 4*S* for (+)-**2** and (−)-**2**, respectively.

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of **1** and **2** in $CDCl_3$.

No.	1		2	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		186.6, s		186.7, s
3		173.6, s		173.4, s
4	4.20, dd (7.0, 10.4)	65.1, d	4.19, dd (7.0, 10.2)	65.0, d
5	1.68, m	26.8, t	1.67, m	26.8, t
	2.28, overlapped		2.26, overlapped	
6	2.16, m	26.8, t	2.15, m	26.8, t
	2.28, overlapped		2.26, overlapped	
7	3.57, ddd (3.2, 9.3, 11.9)	48.4, t	3.56, ddd (3.0, 9.2, 11.9)	48.4, t
	3.96, dt (8.3, 11.5)		3.96, dt (8.5, 11.6)	
1a	4.87, d (14.5)	44.8, t	4.89, d (14.5)	44.9, t
	4.97, d (14.5)		4.99, d (14.5)	
2a		137.4, s		137.2, s
3a	6.93, brs	115.4, d	7.0, overlapped	114.1, d
4a		155.7, s		159.6, s
5a	6.75, dd (1.6, 8.0)	114.9, d	6.80, dd (2.0, 8.0)	113.4, d
6a	7.18, t (7.9)	129.8, d	7.21, t (8.1)	129.5, d
7a	7.00, d (8.0)	121.1, d	7.0, overlapped	120.8, d
OMe			3.76, s	55.2, q

Macathiohydantoin D (**3**) had the same molecular formula ($C_{13}H_{14}O_2N_2S$) as **1** on the basis of the quasimolecular ion at *m*/*z* 263.0850 $[M+H]^+$ (calcd for $C_{13}H_{14}O_2N_2S$, 263.0849) in the HRESIMS and ^{13}C NMR spectrum. The similar ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) of **3** and **1** implied that **3** was an analogue of **1**. The observed ^{13}C NMR signals [δ_C 135.0 (s, C-2a), 128.6 × 2 (d, C-3a, 7a), 128.5 × 2 (d, C-4a, 6a), and 127.9 (d, C-5a)] for the ring A moiety indicated the presence of a monosubstituted benzene ring. Besides, further examination of the ^{1}H and ^{13}C NMR spectra of **3** with those of **1** indicated that the remanent hydroxy group was placed at C-4 in **3**, which was supported by HMBC correlations (Fig. 2) from H-5a/H-7a to C-4. The absolute configuration at C-4 in **3** was assigned by comparison of the experimental and calculated ECD spectra. As shown in Fig. S10, the experimental ECD spectra of (+)-**3** and (−)-**3** well matched with the calculated ECD of 4*S*-**3** and 4*R*-**3**, which unequivocally determined the absolute configurations of 4*S* for (+)-**3** and 4*R* for (−)-**3**, respectively.

Macathiohydantoin E (**4**) was shown to have the molecular formula of $C_{13}H_{14}O_3N_2S$ by an ion peak at *m*/*z* 279.0802 $[M+H]^+$ (calcd for $C_{13}H_{15}O_3N_2S$, 279.0798) in the HRESIMS and ^{13}C NMR spectrum, differing from **3** by 16 mass units, indicating replacement of H by OH. Inspection of the NMR (Tables 1 and 2) data indicated a high similarity between **4** and **2** except for the difference in the aromatic moiety. In its HMBC spectrum, correlations from H-3a and H-5a to 4a revealed that the hydroxy group was placed at C-4a. Furthermore, the other parts of **4** were the same as that of **3**, which was supported by its ^{1}H–^{1}H COSY and HMBC spectra.

Macathiohydantoins F (**5**) and G (**6**) showed pseudo-molecular ion peaks at *m*/*z* 277.1007 $[M+H]^+$ and 293.0953 $[M+H]^+$ in their HRESIMS spectra, corresponding to the molecular formulas of $C_{14}H_{17}O_2N_2S$ and $C_{14}H_{17}O_3N_2S$, respectively. As shown in Table 2, the ^{1}H and ^{13}C NMR spectra suggested that the structure of compounds **5** and **6** were similar to **3** and **4**, respectively, except for the chemical shift values of C-4 in **5** and C-4a in **6**, as well as the presence of the signals (δ_H 3.12 and δ_C 51.9 in **5**; δ_H 3.77 and δ_C 55.2 in **6**) due to methoxy groups. On the basis of HMBC correlations from the methoxy protons to C-4 and C-4a, the methoxy groups were unambiguously located at C-4 in **5** and C-4a in **6**, respectively. The structures of **5** and **6** were in good agreement with the molecular formulas deduced from HRESIMS and NMR spectra, and this further confirmed by ^{1}H–^{1}H COSY, HMQC, and HMBC experiments.

Table 2
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of compounds **3**–**6**.

No.	3[a]		4[b]		5[a]		6[a]	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		185.7, s		187.2, s		186.3, s		185.5, s
3		172.3, s		174.0, s		170.2, s		171.9, s
4		92.5, s		93.8, s		97.2, s		92.5, s
5	1.66, m 2.12, m	32.8, t	1.74, m 2.05, m	33.6, t	1.75, m 2.19, m	32.4, t	1.73, m 2.18, m	33.0, t
6	2.17, m 2.41, m	24.8, t	2.20, m 2.41, m	25.9, t	2.19, overlapped 2.37, m	24.9, t	2.23, m 2.47, m	24.9, t
7	3.64, ddd (2.6, 9.1, 11.7) 3.90, dt (8.6, 11.5)	48.0, t	3.62, ddd (2.8, 9.3, 11.7) 3.95, dt (8.6, 11.3)	49.0, t	3.58, ddd (3.2, 9.3, 12.1) 4.05, dt (8.2, 12.0)	48.0, t	3.69 ddd (2.8, 9.3, 11.5) 3.96 dt (8.6, 11.5)	48.2, t
1a	4.91, d (12.0) 4.87, d (12.0)	45.0, t	4.88, br s	45.7, t	4.97, d (14.4) 5.04, d (14.4)	45.1, t	4.89, d (14.5) 4.95, d (14.5)	45.0, t
2a		135.3, s		138.8, d		135.6, s		136.9, s
3a	7.37, d (6.0)	128.6, d	6.82, br s	116.1, d	7.31, m	128.6, d	6.99, br s	114.3, d
4a	7.26, t (6.0)	128.5, d		158.6, s	7.43, d (7.0)	128.6, d		159.6, s
5a	7.23, m	127.9, d	6.66, dd (1.5, 8.0)	115.6, d	7.27, d (8.7)	128.0, d	6.80, dd (2.2, 8.2)	113.4, d
6a	7.26, t (6.0)	128.5, d	7.09, t (7.8)	130.4, d	7.43, d (7.0)	128.0, d	7.21, t	129.6, d
7a	7.37, d (6.0)	128.6, d	6.84, d (7.7)	120.4, d	7.31, m	128.6, d	7.00, d (7.7)	120.9, d
OMe					3.11, s	51.9, q	3.77, s	55.21, q

[a] Measured in $CDCl_3$.
[b] Measured in CD_3OD.

According to the fact that the hydroxy and methoxy groups substituted at benzene ring could not change the absolute configurations of **4**–**6** as those of (+)-**3** and (−)-**3**, the assignment of absolute configurations of 4*S* for (+)-**4**–(+)-**6** and 4*R* for (−)-**4**–(−)-**6** were determined by comparison of their specific rotation values of each enantiomer with those of **3**.

Macathiohydantoins H (**7**) and I (**8**) were assigned the same molecular formula of $C_{19}H_{20}O_2N_2S$ with 11 degrees of unsaturation based on their HRESIMS and ^{13}C NMR data. The ^{13}C NMR data (Table 3) of **7** and **8** displayed characterized signals responsible for 10 degrees of unsaturation, consisting of an amide thiocarbonyl, an amide carbonyl, and one monosubstituted and one 1,3-disubstituted aromatic rings. Furthermore, combined with the molecular information and the remaining one degree of unsaturation, HMBC correlations from H-4 to C-1/C-3 certified the formation of a 2-thioxoimidazolidin-4-one moiety (ring B, Fig. 3). Interestingly, the observed HMBC correlations from H_2-1a to C-1/C-3/C-3a/C-7a, from H-3a/H_3-OMe to C-4a, and from H-4/H-2aa to C-1aa for **7** suggested that the 1,3-disubstituted aromatic ring was connected to N-2 via a C-2a–C-1a–N-2 and the other monosubstituted one was attached to the N-5 via a C-2aa–C-1aa–N-5 bond in **7**. Whereas, the key HMBC correlations from H_2-1a to C-1/C-3/C-3a/C-7a, from H_2-1aa to C-1/C-4/C-3aa/C-7aa, and from H-

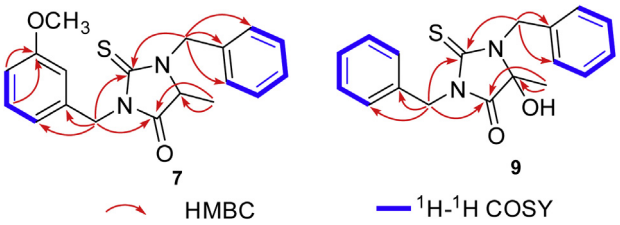

Fig. 3. Key 2D correlations of **7** and **9**.

Table 3
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data of compounds **7**–**10** in $CDCl_3$.

No.	7		8		9		10	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		182.5, s		182.0, s		182.2, s		182.1, s
3		174.0, s		173.7, s		173.2, s		173.4, s
4	3.88, q (7.0)	56.8, d	3.90, q (6.8)	56.8, d		85.5, s		85.4, s
1a	5.06, d (14.6) 5.02, d (14.6)	45.3, t	5.08, d (14.6) 5.04, d (14.6)	45.2, t	5.06, 2H br s	45.3, t	5.02, 2H d (1.8)	45.0, t
2a		137.4, s		136.0, s		135.7, s		135.8, s
3a	7.04, d (2.0)	114.0, d	7.32, t (7.5)	128.5, d	7.04, d (7.8)	127.8, d	7.43, dd (1.5, 7.8)	128.4, d
4a		159.6, s	7.47, d (7.3)	128.6, d	7.32, overlapped	128.6, d	7.25, overlapped	128.7, d
5a	6.82, dd (2.2, 8.3)	113.5, d	7.28, m	127.8, d	7.28, overlapped	127.7, d	7.25, overlapped	127.8, d
6a	7.23, t (8.0)	129.5, d	7.47, d (7.3)	128.6, d	7.32, overlapped	128.6, d	7.25, overlapped	128.7, d
7a	7.05, d (2.0)	120.8, d	7.32, t (7.5)	128.5, d	7.04, d (7.8)	127.8, d	7.43, dd (1.5, 7.8)	128.4, d
1a′	4.43, d (15.0) 5.70, d (15.0)	48.4, t	4.38, d (15.0) 5.68, d (15.0)	48.3, t	4.85, d (15.0) 5.22, d (15.0)	46.6, t	4.85, d (15.6) 5.22, d (15.6)	46.0, t
2a′		135.0, s		136.3, s		136.8, s		138.6, s
3a′	7.31, m	128.1, d	6.85, overlapped	113.7, d	7.32, overlapped	128.6, d	6.99, d (2.4)	113.5, d
4a′	7.34, m	129.0, d		159.7, s	7.46, d (7.4)	128.7, d		159.7, s
5a′	7.32, m	128.3, d	6.85, overlapped	113.6, d	7.28, overlapped	128.0, d	6.80, dd (2.2, 8.7)	113.0, d
6a′	7.34, m	129.0, d	7.25, overlapped	130.1, d	7.46, d (7.4)	128.7, d	7.22, t (7.8)	129.6, d
7a′	7.31, m	128.1, d	6.86, d (8.3)	120.2, d	7.32, overlapped	128.6, d	6.98, brs	120.1, d
OMe	3.80, s	55.2, q	3.77, s	55.3, q			3.74, s	55.2, q
4-Me	1.41, d (7.0)	15.1, q	1.41, d (6.8)	15.1, q	1.42, s	22.8, q	1.46, s	22.6, q

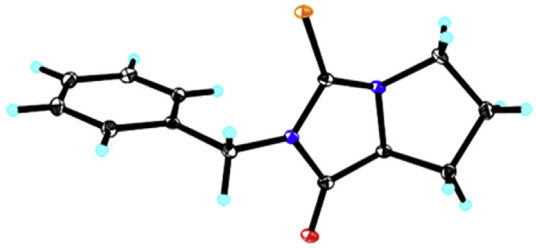

Fig. 4. X-Ray crystallographic structure of **11**.

3aa/H_3-OMe to C-4aa for **8** proved that the monosubstituted aromatic ring and the 1,3-disubstituted one were placed at N-2 and N-5 in **8** via C-2a–C-1a–N-2 and C-2aa–C-1aa–N-5 bonds, respectively. Finally, good agreement was obtained between the calculated and experimental ECD spectra (Fig. S11) established the assignment of absolute configuration of 4*R* (+)-**7**/(+)-**8** and 4*S* for (−)-**7**/(−)-**8**, respectively.

Macathiohydantoin J (**9**) was showed a $[M+H]^+$ ion at *m*/*z* 327.1160 (calcd for $C_{18}H_{19}O_2N_2S$, 327.1162) in HRESIMS, corresponding to a molecular formula of $C_{18}H_{18}N_2O_2S$. The ^1H and ^{13}C NMR spectra of **9** exhibited signals attributable to two benzyl units. HMBC correlations from H_2-1a to C-1/C-3 and from H-1aa to C-1 confirmed these two rings were placed at N-2 and N-5, respectively. However, the presence of an oxyquternary carbon at δ_C 85.5 indicated that the H-4 was oxygenated to hydroxy group, which was further corroborated by HMBC correlations from Me-5 to C-1/C-3/C-4. The absolute configurations of (+)-**9** and (−)-**9** were thus deduced to be 4*S* and 4*R*, respectively, as supported by the experimental and calculated ECD spectra (Fig. S10).

The molecular formula of macathiohydantoins K (**10**) was determined as $C_{19}H_{20}N_2O_3S$ by HREIMS and ^{13}C NMR data with 30 mass units higher than that of **9**, indicating a OCH_2 moiety difference with the latter. Comparison of ^1H and ^{13}C NMR spectra (Table 3) of **10** with those of **9** indicated that a methoxy group (δ_H 3.72, δ_C 55.2) appeared in **10**, which was linked to C-4a′ by HMBC correlations from H_3-OMe and H-3a′ to C-4a′. The similar specific rotation values of (+)-**10** and (−)-**10** with those of (+)-**9** and (−)-**9** suggested the absolute configurations of C-4 to be *S* and *R*, respectively.

In addition, the structure of macathiohydantoin A (**11**)[20] was further confirmed by an X-ray diffraction experiment using Cu Ka radiation (Fig. 4). **11** was also found to be in a racemic form, and its absolute configuration were assigned by ECD calculation evidence. Compounds **1**–**11** were evaluated for their cytotoxic and antimicrobial activities. However, all the isolates were found be devoid of significant cytotoxic and antimicrobial effects.

All the isolated compounds were evaluated for their cytotoxicities against five human cancer cells (HL-60, SMMC-7721, A-549, MCF-7, SW480), as well as antimicrobial activities against three bacterial strains (*S. aureus*, *E. coli*, and *P. aeruginosa*) and three fungal strains (*A. fumigatus*, *C. parapsilosis*, and *C. albicans*).[21] Unfortunately, none of them was active.

3. Conclusion

In summary, ten novel thiohydantoin derivatives, named macathihydantoins B–K, along with one known analogue, were isolated from *L. meyenii*. Unlike well-known macamides and macaenes from title species, these thiohydantoin derivatives obtained in this study could be the characteristic components of maca. Macathihydantoins B–K were all isolated as racemic mixtures and their absolute configurations were further established further chiral separation and ECD calculation or specific rotation values. Although these interesting molecules did not show notable biological activities, more attention would be attracted from synthetic and pharmacological communities.

4. Experimental section

4.1. General experimental procedures

Optical rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded using a Shimadzu UV2401PC spectrophotometres. IR spectra were measuring on a Bruker Tensor-27 infrared spectrophotometer using KBr disks. 1D and 2D NMR spectra were acquired on DRX-500 and AVANCE III-600 spectrometers (Bruker BioSpin GmBH, Rheinstetten, Germany). The Waters Xevo TQ-S and Waters AutoSpec Premier P776 mass spectrometers gave the ESIMS and HREIMS data, respectively. ECD spectra were taken on a JASCO J-815 spectropolarimeter in MeOH. Column Chromatography (CC) was carried out by silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China) and RP-18 (40–63 μm, merk). Fractions were monitored by TLC (GF254, Qingdao Marine Chemical Ltd., Qingdao, China), and by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol. An agilent 1100 series instrument equipped with chiral chromatography columns (CHIRALCEL OD-H, 5 μm, 4.6 mm × 150 mm; CHIRALCEL OJ-H, 5 μm, 4.6 mm × 150 mm) was used to analyze and separate the enantiomers.

4.2. Plant material

The rhizomes of *Lepidium meyenii* were collected from Li Jiang of Yunnan Province of China in 2014 and authenticated by Xiwen Li of Kunming Institute of Botany, Chinese Academy of Science.

4.3. Extraction and isolation

The air-dried and powdered maca stem (40 kg), were extracted three times with 95% MeOH and evaporated to remove solutions solutions to yield the crude extract (10 kg). The aqueous residue was extracted with petroleum (PE) and ethyl acetate (EtOAc), respectively. The PE part (900.8 g) was separated by a silica gel column with PE/acetone (50:1 → 1:1, v/v) to yield seven fractions (Fr. I–VII). Fr. V (90 g) was separated by an RP-18 column with MeOH/H_2O (50:50 → 100:0, v/v) to afford five subfractions (Fr. V_1–V_5). Fr. V_3 (9.0 g) was further separated by a silica gel column eluted with $CHCl_3$/acetone 20:1 to afford compounds **1** (17 mg), **2** (21 mg), **3** (15 mg), and **11** (14 mg). Similarly, the EtOAc moiety (200 g) was chromatographed on a silica gel column eluted with $CHCl_3$/acetone (30:1 → 1:1, v/v) to give five major fractions (Fr. A–E). Fr. B (57 g) was separated by RP-18 column with MeOH/H_2O (50:50 → 100:0, v/v) to give five fractions (Fr. B_1–B_5). Compound **4** (17 mg) was obtained from Fr. B_3 (6 g) by a Sephadex LH-20 column (MeOH) and followed by a silica gel column eluted with $CHCl_3$-MeOH (10:1, v/v). Fr. B_4 (5.0 g) was chromatographed on an RP-18 column eluted with MeOH/H_2O (1:1 → 1:0, v/v) to afford **5** (7 mg). Likewise, Fr. B_5 (4.3 g) was separated by an RP-18 column eluted with MeOH-H_2O (50:50 → 100:0, v/v) and further purified by TCL with $CHCl_3$/acetone (3:1, v/v) as developing agent to afford **6** (11 mg). Fr. B_1 was subjected to a silica gel column eluted with $CHCl_3$/acetone (35:1, v/v) and then purified by HPLC chiral separation eluted with *n*-hexane/isopropanol (90:10, v/v) to give compounds (±)-**7** (1.5 mg), (±)-**8** (1.6 mg), (±)-**9** (2.1 mg), and (±)-**10** (1.8 mg). Compounds (±)-**1**, (±)-**2**, (±)-**3**, (±)-**4**, (±)-**5**, (±)-**6**, and (±)-**11** were then successively separated from each other by chiral semi-preparative HPLC with *n*-hexane/2-propanol (98:2 v/v,

3.0 mL/min).

*4.3.1. Macathiohydantoin B (**1**)*

Light yellow oil; $[\alpha]_D^{19}$ −4.24 (*c* 0.028, MeOH); $[\alpha]_D^{19}$ +64.3(c 0.009, MeOH) for (+)-**1**; $[\alpha]_D^{19}$ −60.2 (c 0.010, MeOH) for (−)-**1**; UV (MeOH) λ_{max} (log ε) 217 (3.85), 245 (3.79), 272 (3.97) nm; ECD (MeOH) 226 ($\Delta\varepsilon$ +1.13), 247 ($\Delta\varepsilon$ −1.36), 273 ($\Delta\varepsilon$ +0.93) nm for (+)-**1**; ECD (MeOH) 226 ($\Delta\varepsilon$ −1.13), 247 ($\Delta\varepsilon$ +1.36), 273 ($\Delta\varepsilon$ −0.93) nm for (−)-**1**; IR (KBr) ν_{max} 3430, 2954, 2736, 1743, 1619, 1432, 1429, 1343, 1228, cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS *m/z* 263.0847 $[M + H]^+$ (calcd for $C_{13}H_{15}O_2N_2S$, 263.0849).

*4.3.2. Macathiohydantoin C (**2**)*

Light yellow oil; $[\alpha]_D^{19}$ −0.45 (*c* 0.011, MeOH); $[\alpha]_D^{19}$ +70.1(c 0.005, MeOH) for (+)-**2**; $[\alpha]_D^{19}$ −66.7 (c 0.007, MeOH) for (−)-**2**; UV (MeOH) λ_{max} (log ε) 217 (3.92), 244 (2.81), 271 (2.98) nm; IR (KBr) ν_{max} 3446, 2924, 1747, 1639, 1432, 1043 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS *m/z* 277.1003 $[M + H]^+$ (calcd for $C_{14}H_{17}O_2N_2S$, 277.1005).

*4.3.3. Macathiohydantoin D (**3**)*

Light colorless oil, $[\alpha]_D^{19}$ −1.04 (*c* 0.017, MeOH); $[\alpha]_D^{19}$ +47.0 (c 0.005, MeOH) for (+)-**3**; $[\alpha]_D^{19}$ −124.5 (c 0.009, MeOH) for (−)-**3**; UV (MeOH) λ_{max} (log ε) 253 (3.16), 270 (3.25) nm; ECD (MeOH) 233 ($\Delta\varepsilon$ −1.45), 251 ($\Delta\varepsilon$ +1.71), 279 ($\Delta\varepsilon$ −1.06) nm for (+)-**3**; ECD (MeOH) 233 ($\Delta\varepsilon$ +1.45), 251 ($\Delta\varepsilon$ −1.71), 279 ($\Delta\varepsilon$ +1.06) nm for (−)-**3**; IR (KBr) ν_{max} 3426, 2954, 2925, 2853, 1740, 1630, 1429, 1345, 1230 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table 2; HRESIMS *m/z* 263.0850 $[M + H]^+$ (calcd for $C_{14}H_{17}O_2N_2S$, 263.0849).

*4.3.4. Macathiohydantoin E (**4**)*

Light yellow oil; $[\alpha]_D^{19}$ −1.71 (*c* 0.040, MeOH); $[\alpha]_D^{19}$ +49.0 (c 0.007, MeOH) for (+)-**4**; $[\alpha]_D^{19}$ −47.6 (c 0.009, MeOH) for (−)-**4**; UV (MeOH) λ_{max} (log ε) 216 (3.54), 252 (3.57), 271 (4.01) nm; IR (KBr) ν_{max} 3427, 2961, 2923, 2735, 2618, 1747, 1602, 1430, 1341, 1232, cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table 2; HRESIMS *m/z* 279.0802 $[M + H]^+$ (calcd for $C_{13}H_{15}O_3N_2S$, 279.0798).

*4.3.5. Macathiohydantoin F (**5**)*

Light yellow oil; $[\alpha]_D^{20}$ +0.89 (*c* 0.012, MeOH); $[\alpha]_D^{20}$ +17.9 (c 0.010, MeOH) for (+)-**5**; $[\alpha]_D^{20}$−19.6 (c 0.009, MeOH) for (−)-**5**; UV (MeOH) λ_{max} (log ε) 203 (3.75), 270 (3.74) nm; IR (KBr) ν_{max} 3423, 2926, 1655, 1550, 1420, 1196 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table 2; HRESIMS *m/z* 277.1007 $[M + H]^+$ (calcd for $C_{14}H_{17}O_2N_2S$, 277.1005).

*4.3.6. Macathiohydantoin G (**6**)*

Light yellow oil; $[\alpha]_D^{25}$ −1.47 (*c* 0.040, MeOH); $[\alpha]_D^{25}$ +40.0 (c 0.010, MeOH) for (+)-**6**; $[\alpha]_D^{25}$ −55.7 (c 0.009, MeOH) for (−)-**6**; UV (MeOH) λ_{max} (log ε) 215 (3.54), 251 (3.52), 271 (3.67) nm; IR (KBr) ν_{max} 3442, 3424, 2926, 1754, 1628, 1429, 1341, 1232 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table 2; HRESIMS *m/z* 293.0953 $[M + H]^+$ (calcd for $C_{14}H_{17}O_3N_2S$, 293.0954).

*4.3.7. Macathiohydantoin H (**7**)*

Light yellow oil, $[\alpha]_D^{20}$ +4.13 (*c* 0.005, MeOH); $[\alpha]_D^{20}$ +18.5 (c 0.004, MeOH) for (+)-**7**; $[\alpha]_D^{20}$ −16.4 (c 0.003, MeOH) for (−)-**7**; UV (MeOH) λ_{max} (log ε) 252 (4.23), 271 (4.21) nm; ECD (MeOH) 256 ($\Delta\varepsilon$ −1.79) 275 ($\Delta\varepsilon$ +2.16) nm for (+)-**7**; ECD (MeOH) 252 ($\Delta\varepsilon$ +1.79), 277 ($\Delta\varepsilon$−2.16) nm for (−)-**7**; IR (KBr) ν_{max} 3426, 1956, 1753, 1567, 1410 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data ($CDCl_3$), see Table 3; HRESIMS *m/z* 341.1317 $[M + H]^+$ (calcd for $C_{19}H_{21}O_2N_2S$, 341.1318).

*4.3.8. Macathiohydantoin I (**8**)*

Light yellow oil, $[\alpha]_D^{20}$ +4.77 (*c* 0.007, MeOH); $[\alpha]_D^{20}$ +19.7 (c 0.003, MeOH) for (+)-**8**; $[\alpha]_D^{20}$ −24.8 (c 0.003, MeOH) for (−)-**8**; UV (MeOH) λ_{max} (log ε) 252 (4.25), 271 (4.23) nm; IR (KBr) ν_{max} 3426, 1956, 1751, 1560, 1413, 734 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table 3; HRESIMS *m/z* 341.1317 $[M + H]^+$ (calcd for $C_{19}H_{21}O_2N_2S$, 341.1318).

*4.3.9. Macathiohydantoin J (**9**)*

Light yellow oil, $[\alpha]_D^{26}$ −2.33 (*c* 0.004, MeOH); $[\alpha]_D^{26}$ +11.0 (c 0.004, MeOH) for (+)-**9**; $[\alpha]_D^{26}$ −9.8 (c 0.005, MeOH) for (−)-**9**; UV (MeOH) λ_{max} (log ε) 246 (3.38), 273 (3.40) nm; ECD (MeOH) 252 ($\Delta\varepsilon$ +2.57), 277 ($\Delta\varepsilon$ −2.43) nm for (+)-**9**; ECD (MeOH): 252 ($\Delta\varepsilon$ −2.57), 277 ($\Delta\varepsilon$ +2.43) nm for (−)-**9**; IR (KBr) ν_{max} 3420, 2925, 1956, 1751, 1642, 1560, 1417, 1341, 1238, 1190, 737 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table 3; HRESIMS *m/z* 327.1160 $[M + H]^+$ (calcd for $C_{18}H_{19}O_2N_2S$, 327.1162).

*4.3.10. Macathiohydantoin K (**10**)*

Light yellow oil, $[\alpha]_D^{20}$ +0.97 (*c* 0.007, MeOH); $[\alpha]_D^{20}$+17.7 (c 0.004, MeOH) for (+)-**10**; $[\alpha]_D^{20}$ −19.9 (c 0.003, MeOH) for (−)-**10**; UV (MeOH) λ_{max} (log ε) 245 (3.62), 270 (3.61) nm; IR (KBr) ν_{max} 3433, 2926, 1895, 1750, 1416, 1119 cm^{-1}; ^1H NMR and ^{13}C NMR spectroscopic data, see Table 3; HRESIMS *m/z* 379.1083 $[M + Na]^+$ (calcd for $C_{19}H_{20}O_3N_2SNa$, 379.1087).

*4.3.11. Macathiohydantoin A (**11**)*

Light yellow oil; $[\alpha]_D^{25}$ +60.3 (*c* 0.013, MeOH); $[\alpha]_D^{25}$ +110.5 (c 0.012, MeOH) for (+)-**11**; $[\alpha]_D^{25}$ −67.9 (c 0.01, MeOH) for (−)-**11**; ECD (MeOH) 232 ($\Delta\varepsilon$ +1.36), 252 ($\Delta\varepsilon$ −1.67), 275 ($\Delta\varepsilon$ +1.54) nm for (+)-**11**; ECD (MeOH) 232 ($\Delta\varepsilon$ −1.36), 252 ($\Delta\varepsilon$ +1.67), 275 ($\Delta\varepsilon$ −1.54) nm for (−)-**11**; Crystal data for **11**: $C_{13}H_{14}N_2OS$, $M = 246.32$, $a = 11.8325(2)$ Å, $b = 5.70770(10)$ Å, $c = 18.2337(3)$ Å, $\alpha = 90°$, $\beta = 99.97°$, $\gamma = 90°$, $V = 1212.83(4)$ $Å^3$, $T = 100(2)$ K, space group *P*21/*n*, $Z = 4$, $\mu(CuK\alpha) = 2.242$ mm^{-1}, 11991 reflections measured, 2199 independent reflections ($R_{int} = 0.0398$). The final R_1 values were 0.0405 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1221 ($I > 2\sigma(I)$). The final R_1 values were 0.0408 (all data). The final $wR(F^2)$ values were 0.1225 (all data). The goodness of fit on F^2 was 1.122.

4.4. Quantum chemical ECD calculations

The theoretical calculations were achieved using Gaussian 09.[22] The conformations generated by the MM2 force field in Chem-Bio3D software were optimized via the Density Functional Theory (DFT) at the B3LYP/6-31+G(d) level in gas phase. The optimized conformations were subjected to ECD calculations by means of Time Dependent DFT (TDDFT) at the B3LYP/6-311++G(2d,p) level in MeOH using the CPCM model. The calculated ECD curves were generated by SpecDis version 1.63 software.[23]

4.5. Cytotoxic assay

All the compounds were evaluated for their cytotoxicities against five human cancer cells (HL-60, SMMC-7721, A-549, MCF-7, SW480) by MTT assay as described in the literature.[21]

4.6. Antibacterial assay

All the isolated compounds were evaluated for their antimicrobial activities against three bacterial strains (*S. aureus*, *E. coli*, and *P. aeruginosa*) and three fungal strains (*A. fumigatus*, *C. parapsilosis*, and *C. albicans*). The MICs were determined using a dilution

antimicrobial susceptibility test.[21] The experiments were conducted for three independent replicates.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2017.05.096.

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