Petchiennes A–E, Meroterpenoids from *Ganoderma petchii*

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Petchiennes A–E (1-5), five new meroterpenoids, were isolated from the fruiting bodies of *Ganoderma petchii*. Their structures, including absolute configurations, were elucidated by means of spectroscopic and computational methods. Compound 4 was isolated as a racemic mixture, which was finally purified by chiral HPLC to yield individual (+)- and (-)-antipodes. Biological evaluation showed that compounds 2 and (+)-4 could increase intracellular free calcium concentration at 10 μM in HEK-293 cells.

**Keywords:** Ganoderma petchii, Ganodermae, Meroterpenoids, Spiro-compound.

Meroterpenoids from the genus *Ganoderma* are characterized by a 1,4-diarylxybenzene residue conjugated with a terpenoidal moiety. Since the discovery from *G. pfeifferi* of ganomycins A and B in this class of meroterpenoids [1], few other investigations associated with this compound class from *Ganoderma* species have been reported. We isolated a novel meroterpenoid termed lingzhioil from *G. lucidum* [2a], and since then, several structurally diverse meroterpenoids with renal or neural protective effects were characterized by us [2b–2e]. In our continuing efforts focused on the search of bioactive meroterpenoids from *Ganoderma, G. petchii* became our research target. This fungus is distributed in China, Sri Lanka, Malaysia, Singapore and Indonesia. In China, it is used as a healthcare mushroom and seen in several markets of Chinese medical materials. Our efforts on this fungus led to the isolation of five new meroterpenoids, and here we describe their isolation, structure characterization and biological evaluation.

![Figure 1: The chemical structures of compounds 1–5.](image)

Petchiene A (1) has the molecular formula C_{16}H_{19}O_{4} derived from its HRESIMS, ^{13}C NMR and DEPT spectra. The ^{1}H NMR spectrum (Table 1) of 1 contains a typical ABX spin system (δH: 7.32 (1H, d, J = 2.7 Hz, H-3), 7.07 (1H, dd, J = 8.9, 2.7 Hz, H-5), 6.79 (1H, d, J = 8.9 Hz, H-6)), suggesting the presence of a 1,2,4-trisubstituted benzene ring, along with a resonance for an olefinic proton. The ^{13}C NMR and DEPT spectra contain resonances for 16 carbons including five aliphatic methylenes (one oxygenated), four methines

![Figure 2: COSY and key HMBC correlations of 1.](image)

![Figure 3: Calculated and experimental ECD spectra of (+)-1 (red, at the B3LYP-SCRF (PCM)/6-311+G(2d,p)//B3LYP/6-311+G(2d,p) level in MeOH; black, experimentally observed in MeOH).](image)

Figure 3: Calculated and experimental ECD spectra of (+)-1 (red, at the B3LYP-SCRF (PCM)/6-311+G(2d,p)//B3LYP/6-311+G(2d,p) level in MeOH; black, experimentally observed in MeOH).

(all sp³), and seven quaternary carbons (one ketone, one carboxylic acid, four olefinic including two oxygenated). The ^{1}H–^{1}H COSY spectrum (Figure 2) showed correlations between H-5/H-6 and H-6'/H-7'/H-8'. In the HMBC spectrum, the correlation from H-3 to C-1' (δC: 204.9) indicates that the phenyl unit is linked to C-1', and from H-2' (δH: 3.49, 3.46) to C-2 (δC: 120.1), C-1', C-3' (δC: 44.8) indicate that C-2' is linked to C-1'. In addition, HMBC correlations between H-7'/C-3', C-5' and H-4'/C-6', C-8', in combination with COSY correlations of H-6'/H-7'/H-8' and degrees of unsaturation of 1, indicate the presence of a six-membered ring. The positions of a carboxylic acid and a hydroxymethyl were assigned by the HMBC correlations between H-2', H-4'/C-10 and H-4', H-6'/C-9'. Hence, the planar structure of 1 was identified as shown. 1 was isolated as a non-racemic compound indicated by its optical rotation. The absolute configuration at the single chiral center of 1 was clarified by electronic circular dichroism (ECD) calculations. For this
purpose, DFT and TD-DFT calculations were carried out at 298 K in the gas phase with Gaussian 09 [3]. The ECD spectrum of (3’5)-1, correlates well with the experimental ECD spectrum of 1, leading to the unambiguous assignment of the absolute configurations at the stereogenic centers in 1 as 3’S (Figure 3).

Petchiene B (2) was found to have the molecular formula C_{15}H_{16}O_{4} (8 degrees of unsaturation) deduced from its HRESIMS, 13C NMR and DEPT spectra. The 1H and 13C NMR spectra of 2 are similar to those of 1. The only difference is that the 3’ double bond of 2 was formed by deacetylation of 1 (Figure 4), which could be supported by HMBC correlations between H-2’ (δH 6.77)/C-1’, C-2’, C-3’ and H-7’, H-8’/C-3’ (δC 157.7). In addition, a ROESY correlation between H-2’ (δH 6.77)/H-4’ (δH 6.39) was observed, indicating a trans double bond between C-2’ and C-3’.

![Figure 4: COSY and key HMBC correlations of 2 and 3.](image1)

Table 1: 1H NMR data of 1-3 (δH in ppm, J in Hz).

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<td>7.26 overlap, 7.25 d (2.7)</td>
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<td>5</td>
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<td>6.77 overlap</td>
<td>6.86 d (8.8)</td>
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<td></td>
<td>b: 3.46 d (18.1)</td>
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<td>6.56 s</td>
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<td>4’</td>
<td>5.82 s</td>
<td>6.39 s</td>
<td>6.06 s</td>
</tr>
<tr>
<td>6’</td>
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<tr>
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<td></td>
<td>b: 1.72 m</td>
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<td>8’</td>
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<td>3.02 t (6.0)</td>
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<td>9’</td>
<td>3.96 s</td>
<td>4.14 s</td>
<td>1.91 s</td>
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*600 MHz in acetone-δ, 500 MHz in methanol-δ, 300 MHz in CDCl3.

Petchiene C (3), isolated as a yellow solid, has the molecular formula C_{16}H_{16}O_{4} (8 degrees of unsaturation), based on its HRESIMS, 13C NMR and DEPT spectra. The NMR data of 3 closely resemble those of 2, indicating that they are analogues. The only difference is that a hydroxymethyl group in 2 was replaced by a methyl in 3 evidenced from HMBC correlations between H-1’/9’ (δH 1.91)/C-4’, C-5’, C-6’ (Figure 4). A ROESY correlation between H-2’ (δH 6.56 in methanol-δ) /H-8’ (δH 2.48) was observed, indicating the configuration of the double bond.

The molecular formula of racemic petchiene D (4) was determined to be C_{16}H_{16}O_{4} (8 degrees of unsaturation) by analysis of its HRESIMS, 13C NMR and DEPT spectra. Compound 4 could be generated from 2 via Michael addition reaction to construct a spiro compound. This conclusion is supported by the HMBC correlations between H-2’/C-1’, C-2’, C-3’ (δC 79.1), C-4’ and H-8’/C-2’, C-3’, C-4’. In addition, ROESY correlations between 4-OH (δH 9.43 in DMSO-δ D-3, H-5 were observed, indicating the position of the free hydroxyl group in the benzene ring. Thus far, the planar structure of 4 was identified as shown. Chiral HPLC analysis indicated that 4 is racemic. Subsequent separation by chiral HPLC yielded (+)- and (-)-4 in a ratio of 1.3:1; the absolute configuration at the stereogenic center was assigned to be 3’S for (-)-4 by the same methods as those of 1 (Figure 6).

![Figure 5: COSY and key HMBC correlations of 4 and 5.](image2)

Table 2: 1H NMR data of 4 and 5 (δH in ppm, J in Hz).

<table>
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<tr>
<td></td>
<td>b: 1.99 m</td>
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<tr>
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<td>a: 1.89 m</td>
<td>7.43 s (7.6)</td>
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<tr>
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<td>b: 1.67 overlap</td>
<td></td>
</tr>
<tr>
<td>9’</td>
<td>3.95 s</td>
<td></td>
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</table>

*600 MHz in methanol-δ, 500 MHz in acetone-δ, 300 MHz in CDCl3.

Petchiene E (5) was determined to have the molecular formula C_{16}H_{16}O_{4} (10 degrees of unsaturation) on the basis of HRESIMS analysis. The 1H NMR spectrum of 5 (Table 2) contains seven aromatic protons. The 13C NMR and DEPT spectra (Table 3) contain 15 signals ascribed to one sp3 methylene, seven methines (seven sp3), and seven quaternary carbon signals (one ketone, one carboxylic acid, and five sp3). The 1H-1H COSY spectrum shows (Figure 5) the existence of fragments H-5/H-6, and H-6’/H-7’/H-8’. In the HMBC spectrum, correlations between H-3/C-1’ and H-2/C-2, HMBC spectrum, correlations between H-3/C-1’ and H-2/C-2, HMBC spectrum, correlations between H-3/C-1’ and H-2/C-2, HMBC spectrum, correlations between H-3/C-1’ and H-2/C-2.

![Figure 6: Calculated and experimental ECD spectra of (-)-4 (red, at the B3LYP SFF (PCM)/6-311+G(2d,p)//B3LYP/6-311+G(2d,p) level in MeOH, black, experimentally observed in MeOH).](image3)

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![Figure 7: Calculated and experimental ECD spectra of (-)-4 (red, at the B3LYP SFF (PCM)/6-311+G(2d,p)//B3LYP/6-311+G(2d,p) level in MeOH, black, experimentally observed in MeOH).](image4)
Mentheroids from Ganoderma petchii

Figure 7: Effect of the isolates on intracellular Ca²⁺ concentration in HEK-293 cells.

Correlations between H-4', H-6'/C-9' (δC 170.0) suggest that the carboxylic acid group is connected to C-5'. As a result, the structure of 5 was determined.

Biological evaluation: Calcium (Ca²⁺) is a fundamental second messenger that is involved in a wide range of cellular processes and, therefore, is of potential interest in drug discovery [4]. The intracellular free Ca²⁺ concentration is regulated through multiple mechanisms in neurons, and abnormalities in Ca²⁺ signaling have been implicated in many neurological disorders [5]. Considering that many species of Ganoderma are used for the treatment of central nervous system associated diseases, the isolates were evaluated for their potential on the changes in Ca²⁺ in HEK-293 cells. The results showed that compounds 2 and (−)-4 could significantly elevate intracellular Ca²⁺ concentration at 10 μM (Figure 7). Whether these two compounds are beneficial for neurological disorders needs further investigation.

Experimental

General: Column chromatography (CC) was performed on silica gel (200–300 mesh; Qingdao Marine Chemical Inc., People’s Republic of China), C-18 silica gel (40–60 μm; Daiso Co., Japan), MCI gel CHP 20P (75–150 μm, Mitsubishi Chemical Industries, Tokyo, Japan) and Sephadex LH-20 (Amersham Pharmacia, Sweden). Optical rotations were recorded on a Horiba SEPA-300 polarimeter. UV spectra were recorded on a Shimadzu UV-2401PC spectrophotometer, IR spectra on a Brucker Tensor-27 spectrophotometer, and CD spectra on a Chirascan instrument. Semi-preparative HPLC was carried out using an Agilent 1200 liquid chromatography; the column used was a 250 mm × 9.4 mm, i.d., 5 μm, Zorbax SB-C18 and a 250 mm × 10 mm, i.d., 5 μm, Daicel Chiralpak IC, flow rate: 3 mL/min. NMR spectra were recorded on a Bruker AV-600 spectrometer, with TMS as an internal standard. EIMS and HRESIMS were determined on an AutoSpec Premier P776 spectrometer. ESI-MS and HRESIMS were measured on an API QSTAR Pulsar 1 spectrometer.

Fungal material: The fruiting bodies of G. petchii (Lloyd) Steyaert were purchased from a market of Chinese medical materials located at Zhonghao-Luoshi-Wan of Kunming, People’s Republic of China, in July 2014. A voucher specimen (CHXY-0588) was authorized by Prof. Zhu-Liang Yang at Kunming Institute of Botany and deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, People’s Republic of China.

Extraction and isolation: The powdered fruiting bodies of G. petchii (60 kg) were extracted under reflux with 70% EtOH (150 L × 2 h × 3) to give a crude extract (3.39 kg), which was suspended in H₂O followed by extraction with EtOAc to afford an EtOAc soluble extract. Separation of this extract (2.09 kg) using a MCI gel CHP 20P column eluted with a gradient of aqueous MeOH (10%–100%) produced 8 portions (Fr.1–Fr.8). Among them, fr.5 (410 g) was further separated via MCI gel CHP 20P washed with a gradient of aqueous MeOH (20%–100%) to yield 7 fractions (Fr.5.1–Fr.5.7). Fr.5.4 (3.48 g) was submitted to a RP-18 column with a gradient of aqueous MeOH (30%–80%) to afford 8 sub-fractions (Fr.5.4.1–Fr.5.4.8). Fr.5.4.4 (233 mg) was purified by preparative TLC (2 drops of formic acid in CHCl₃/MeOH, 9:1) followed by semi-preparative HPLC (MeOH/H₂O, 43%) to yield compounds 1 (6.8 mg, tᵣ = 23 min), 2 (9.7 mg, tᵣ = 18 min) and 5 (1.0 mg, tᵣ = 25 min). Fr. 6 (50 g) was separated using MCI gel CHP 20P (MeOH/H₂O, 10:90, 30:70, 50:50, 70:30, 80:20) to yield 4 fractions (Fr.6.1–Fr.6.4). Fr. 6.3 (12 g) was subjected to gel filtration on Sephadex LH-20 (MeOH) followed by chromatography on a RP-18 column (MeOH/H₂O, 50%–70%) to yield 3 (5.3 mg, tᵣ = 28 min) and 4 (6.0 mg, tᵣ = 26 min). Racemic compound 4 was subjected to chiral HPLC to afford (+)-4 (2.2 mg) and (−)-4 (2.1 mg) (n-hexane/ethanol, 90:10).

Petchiene A (1)
Pale yellow solid.

[pδ]D₂⁴ = +12.3 (c 0.64, MeOH).

IR (KBr, νmax): 3423, 2935, 2872, 1707, 1644, 1624, 1590, 1486, 1449, 1369, 1280, 1222, 1179, 998, 869, 831, 785 cm⁻¹.

UV (MeOH) λmax (log ε): 365 (3.57), 256 (3.87), 224 (4.17) nm.

CD (acetone): ΔS₉₀ = -4.89, ΔS₅₄ = -0.80.

¹H and ¹³C NMR: Tables 1 and 3.

ESIMS: m/z 305 [M-H].

HRESIMS: m/z 305.1043 [M-H] (calcd for C₁₈H₃₁O₇, 305.1031).

Petchiene B (2)
Yellow solid.

UV (MeOH) λmax (log ε): 380 (3.59), 320 (4.07), 220 (3.99), 202 (4.12) nm.

¹H and ¹³C NMR: Tables 1 and 3.

ESIMS: m/z 259 [M-H].


Petchiene C (3)
Yellow solid.

UV (MeOH) λmax (log ε): 384 (1.50), 327 (3.66), 226 (3.99) nm.

¹H and ¹³C NMR: Tables 1 and 3.

ESIMS: m/z 243 [M-H].


(±)-Petchiene D (4)
Pale yellow solid.

[pδ]D₂⁴ = +102.2 (c 0.27 MeOH); (−)-4: [pδ]D₂⁴ = -123.3 (c 0.17, MeOH); (−)-4.

UV (MeOH) λmax (log ε): 362 (0.81), 255 (1.47), 228 (4.15) nm;


¹H and ¹³C NMR: Tables 2 and 3.

ESIMS: m/z 259 [M-H].


Petchiene E (5)
Pale yellow solid.
UV (MeOH) λ_max (log ε): 368 (3.51), 256 (3.76), 223 (4.20), 202 (4.34) nm.

IR (KBr) ν_max: 3395, 3076, 2927, 1701, 1634, 1612, 1487, 1446, 1380, 1351, 1324, 1267, 1205, 1182, 784 cm⁻¹.

1H and 13C NMR: Tables 2 and 3.

ESIMS: m/z 271 [M-H]⁻.

HRESIMS: m/z 271.0616 [M-H]⁻ (calcld for C₁₃H₁₄O₅, 271.0612).

Calcium imaging assay: The calcium imaging assay was performed by an automated, cell-based fluorescence-imaging system (Array scan), as previously reported, with slight modification [6,7]. The HEK293 cells were seeded at a density of 2×10⁵ cells/well in 96-well plate coated with poly-L-lysine and incubated for 12 h. Then, the HEK-293 cells were washed 3 times with HBSS (1.26 mM CaCl₂, 0.493 mM MgCl₂, 0.407 mM MgSO₄, 5.33 mM KCl, 0.441 mM KH₂PO₄, 4.17 mM NaHCO₃, 137.93 mM NaCl, 0.338 mM NaHPO₄, 5.56 mM D-Glucose, pH 7.2-7.4) and loaded finally with 2 μM Fluo-4 AM (DOJINDO) for 80 min at 37°C in HBSS. After incubation, the cells were washed mildly 3 times by HBSS. After the loading, calcium imaging was analyzed using Array scan VTI HCS Reader (Cellomics, The Thermo Scientific, Pittsburgh, PA). Fluorescence of Fluo-4 AM was excited at 488 nm, and emitted at 543 nm and recorded as times-series mode per 5 sec. The value of the changed fluorescence was expressed as the relative intensity compared with the initial value.

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References


