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•Original article•

Hyparillums A and B: polycyclic polyprenylated acylphloroglucinols from *Hypericum patulum*

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[ABSTRACT] Hyparillums A (**1**) and B (**2**), two previously unidentified polycyclic polyprenylated acylphloroglucinols (PPAPs) with intricate architectures, were isolated from *Hypericum patulum* Thunb. Hyparillum A was the first PPAP with eight-carbon rings based on an unprecedented 6/6/5/6/6/5/6/4 octocyclic system featuring a rare heptacyclo[10.8.1.1^{1,10}.0^{3,8}.0^{8,21}.0^{12,19}.0^{14,17}]docosane core. In contrast, hyparillum B featured a novel heptacyclic architecture (6/6/5/6/6/5/5) based on a hexacyclo[9.6.1.1^{1,9}.0^{3,7}.0^{7,18}.0^{11,16}]nonadecane motif. Furthermore, hyparillums A and B demonstrated promising inhibitory effects on the proliferation of murine splenocytes stimulated by anti-CD3/anti-CD28 monoclonal antibodies and lipopolysaccharide, exhibiting half-maximal inhibitory concentration (IC_{50}) values ranging from 6.13 ± 0.86 to 12.69 ± 1.31 μmol·L^{-1}.

[KEY WORDS] Polycyclic polyprenylated acylphloroglucinols; *Hypericum patulum* Thunb.; Immunosuppressive activities; Structure elucidation.

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Introduction

Polycyclic polyprenylated acylphloroglucinols (PPAPs) are a family of plant secondary metabolites predominantly found in the genus *Hypericum*. Characterized by acylphloroglucinol cores and isoprenyl/geranyl groups [1, 2], more than 1000 PPAPs have been identified, showcasing remarkable structural diversity. These compounds exhibit a broad spectrum of biological activities, including anti-inflammatory, anti-cancer, anti-microbacterial, antidepressant, and hepatoprotective activities [1−7].

Hypericum patulum Thunb. (Clusiaceae), a shrub widely distributed in China and part of the genus *Hypericum*, has traditionally been used in Chinese medicine to treat conditions such as gonorrhea, hepatitis, colds, and bruises [8]. Prior research led to the isolation of several bioactive PPAPs from *H. patulum* [4]. In our continuous pursuit of bioactive metabolites from this plant, two novel PPAPs, hyparillums A (**1**) and B (**2**) (Fig. 1), were isolated. Compound **1** is notably the first PPAP featuring eight-carbon rings with an unprecedented 6/6/5/6/6/5/6/4 octocyclic system based on a heptacyclo [10.8.1.1^{1,10}.0^{3,8}.0^{8,21}.0^{12,19}.0^{14,17}]docosane core. Compound **2** possesses a unique heptacyclic architecture (6/6/5/6/6/5/5) formed around a unique hexacyclo[9.6.1.1^{1,9}.0^{3,7}.0^{7,18}.0^{11,16}]

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These authors have no conflict of interest to declare.

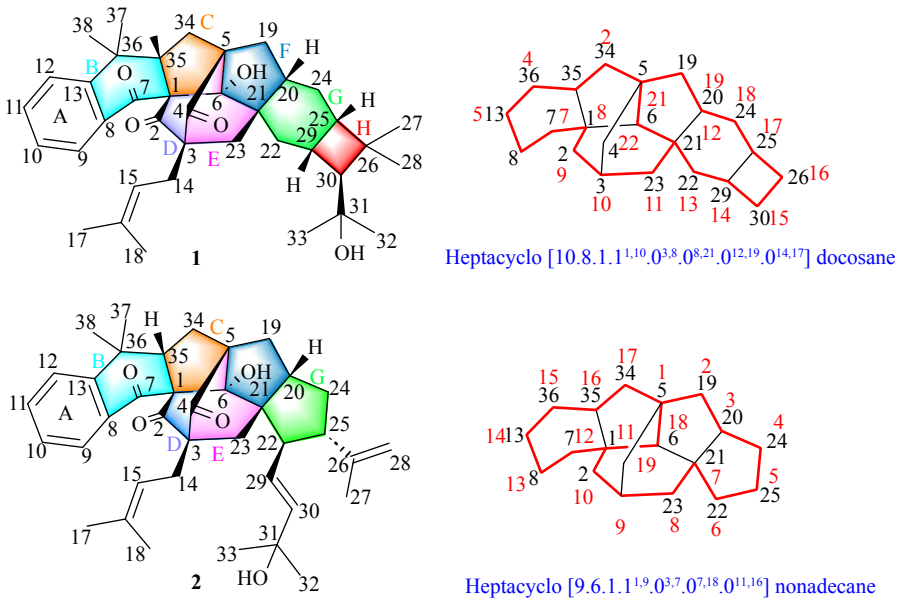

Fig. 1 Structures of 1 and 2 and the nomenclature of the unparalleled bridged systems of 1 and 2 numbered in red.

nonadecane moiety. Both compounds **1** and **2** exhibited moderate inhibitory activities against the proliferation of murine splenocytes induced by anti-CD3/anti-CD28 monoclonal antibodies (mAbs) and lipopolysaccharide (LPS), with half-maximal inhibitory concentration (IC_{50}) values ranging from 6.13 ± 0.86 to 12.69 ± 1.31 μmol·L^{-1}. Herein, the isolation, structural elucidation, and biosynthetic pathways of **1** and **2** are reported.

Results and Discussion

Hyparillum A (**1**) was obtained as a colorless oil. The molecular formula of this metabolite, $C_{38}H_{48}O_5$, was established by HR-ESI-MS data, indicating 15 degrees of unsaturation. The ^{1}H nuclear magnetic resonance (NMR) data exhibited signals for eight methyls [δ_H 1.06 (3H, s), 1.08 (3H, s), 1.20 (3H, s), 1.26 (3H, s), 1.27 (3H, s), 1.36 (3H, s), 1.58 (3H, s), 1.66 (3H, s)], one olefinic proton [δ_H 5.01 (1H, t, J = 7.2 Hz)], and one *ortho*-disubstituted phenyl group [δ_H 7.35 (1H, m), 7.36 (1H, m), 7.54 (1H, td, J = 7.6, 1.4 Hz), 7.68 (1H, m)]. The ^{13}C NMR data suggested the existence of eight methyl, six methylene, and ten methine groups (including one olefinic and four aromatics), along with fourteen nonprotonated carbon atoms (including two oxygenated and three carbonyls).

Comparative analysis with NMR data of known PPAPs from the genus *Hypericum* [9–11] suggested that **1** belongs to the PPAP class. The presence of six aromatic carbons, two olefinic carbons, and three carbonyls, contributing to seven degrees of unsaturation, confirmed that compound **1** is an octocyclic natural product.

The unique nature of compound **1**, a PPAP with an atypical carbon skeleton, was further elucidated through detailed 2D NMR analysis (Fig. 2). The Heteronuclear Multiple Bond Correlations (HMBCs) from H_2-34 to C-1, C-4, C-5, C-6, and C-19, from H_2-23 to C-6, C-20, and C-21, from H_2-14 to C-2, C-3, C-4, and C-23, from H-35 to C-1 and C-2, as well as the proton spin systems of H_2-34/H-35 and H_2-19/H-20, established the complex cage-like moiety (rings C/D/E/F). Furthermore, the clear HMBCs from H-9 to C-7 and C-13, from H-12 to C-8, from H_3-38 to C-13, C-36, and C-37, and from H_3-37 to C-35, from H-35 to C-1 and C-7, coupled with the ^{1}H–^{1}H COSY correlations of H-9/H-10/H-11/H-12, definitively established the presence of benzene (ring A) attached at ring C *via* the C-13–C-36–C-35 and C-8–C-7–C-1 bonds, forming the six-membered ring B. Additionally, the HMBCs from H-20 to C-24, from H_2-22 to C-23, C-25, and C-30, from H_2-24 to C-21 and C-25, from H_3-27 to C-25, C-26, and C-28, from H_3-28 to C-30, from H_3-32 to C-30 and C-31, from H_3-33 to C-31, in conjunction with ^{1}H–^{1}H COSY correlations of H_2-19/H-20/H_2-24/H-25/H-29/H-30 and H_2-22/H-29, illustrated that the four-membered ring H was connected to ring F *via* C-20–C-24–C-25 and C-21–C-22–C-29 bonds, forming ring G. Therefore, the planar structure of **1**, the first PPAP with an octocyclic carbon system based on a heptacyclo[10.8.1.1^{1,10}.0^{3,8}.0^{8,21}.0^{12,19}.0^{14,17}]docosane core, was defined (Fig. 1).

The highly rigid skeleton of hyparillum A (**1**), encompassing rings B/C/D/E/F/G [9–11], along with the observed Nuclear Overhauser effect spectroscopy (NOESY) cross-peaks of OH-6/H-22a, OH-6/H_3-38, and H-35/H_3-37, facilitated the determination of its relative configurations. These configurations at C-1, C-3, C-5, C-6, C-21, and C-35 of **1** were the same as those of hyperuralone B [9] (Fig. 2). Furthermore, the NOESY correlations of OH-6/H-22a and H-20/H-23b unquestionably indicated that OH-6 and H-20 were oriented in opposite directions, with OH-6 being α-oriented and H-20 β-oriented. The distinct NOESY correlations from H-23b to H-20 and H-29, from H_3-27 to H-25 and H-29, and from H_3-28 to H_2-24 and H-30 elucidated that H-25, H-29, and the side chain (C-32–C-31–C-33) were β-oriented and on

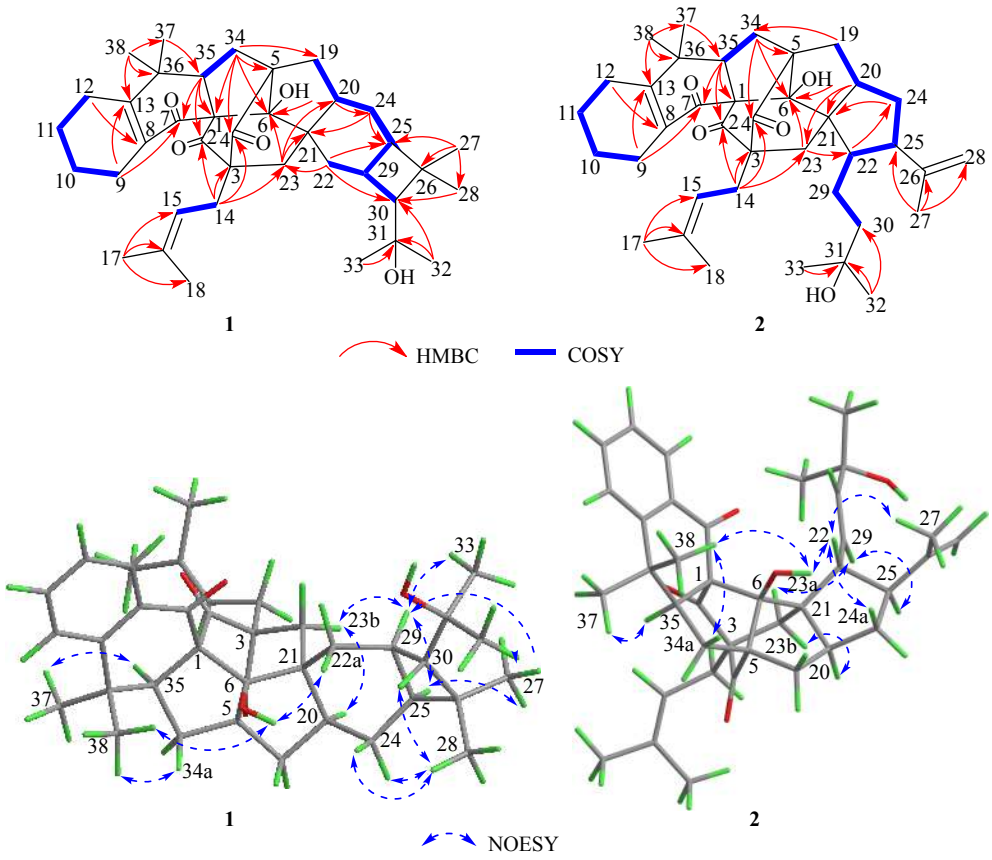

Fig. 2 Key 2D NMR correlations of 1 and 2.

the same side, while H-30 was *α*-oriented (Fig. 2). Therefore, the relative configuration of **1** was identified.

Additionally, considering that **1** possessed a highly complex undescribed carbon skeleton, which was derived from a complex biosynthetic pathway, the calculations of ^{13}C NMR chemical shifts of this compound at the B972/pcSseg-2 level in PCM chloroform were performed, and the results agreed well with the experimental data with the correlation coefficient (R^2) value of 0.9991 (Fig. 3). This strong agreement supported our conclusion on the planar construction and relative configuration of this novel PPAP. Furthermore, the calculated electronic circular dichroism (ECD) spectra of **1** at CAM-B3LYP/def2-TZVP level were in harmony with the experimental ECD data (Fig. 4), allowing for a definitive assignment of its absolute structure as 1*S*,3*S*,5*R*,6*S*,20*S*,21*R*,25*S*,29*R*,30*S*,35*S*.

Hyparillum B (**2**) was also obtained as a colorless oil. Its molecular formula, $C_{38}H_{46}O_5$, was identified by analyzing the HR-ESI-MS data, suggesting 16 indices of hydrogen deficiency. Its ^{1}H NMR data contained the signals of four aromatic protons [δ_H 7.35 (1H, m), 7.36 (1H, m), 7.54 (1H, m), 7.62 (1H, m)], five olefinic protons [δ_H 4.76 (2H, s), 5.03 (1H, m), 5.50 (1H, dd, J = 15.7, 8.6), 5.96 (1H, d, J = 15.7)], and seven methyl group protons [δ_H 1.10 (3H, s), 1.31 (3H, s), 1.32 (3H, s), 1.35 (3H, s), 1.57 (3H, s), 1.65 (3H, s), and 1.72 (3H, s)]. The ^{13}C and DEPT NMR data showed 38 carbon resonances corresponding to seven methyl, six methylene (including one olefinic), and eleven methine groups, and fourteen nonprotonated carbon atoms (including two oxygenated and three carbonyls). The NMR data manifested that **2** was a PPAP with a heptacyclic carbon skeleton.

A detailed comparison of the NMR data of **1** and **2** suggested that they shared a common arrangement of rings A–F (Table 1), which was supported by the HMBCs from H-9 to C-7 and C-13, from H-12 to C-8, from H_3-37 to C-35, from H_3-38 to C-13, C-36, and C-37, from H-35 to C-1, C-2, and C-7, from H_2-34 to C-4, C-5, and C-6, from H_2-19 to C-34, from H_2-14 to C-2, C-3, C-4, and C-23, from H_2-23 to C-6 and C-22, and from H-20 to C-21 and C-23, along with the ^{1}H–^{1}H COSY correlations of H-9/H-10/H-11/H-12, H_2-19/H-20 and H_2-34/H-35. Furthermore, the clear HMBCs from H-22 to C-24, from H_2-24 to C-21, from H_3-27 to C-25, C-26, and C-28, from H_3-32 to C-30 and C-31, from H_3-33 to C-31, as well as the ^{1}H–^{1}H COSY correlations of H-20/H_2-24/H-25/H-22/H-29/H-30, undisputedly confirmed that a five-membered ring G was attached at ring F in **2**. Thus, the planar structure of this heptacyclic PPAP, bearing a unique hexacyclo[9.6.1.1^{1,9}.0^{3,7}.0^{7,18}.0^{11,16}]nonadecane moiety, was established (Fig. 1).

The relative configurations at C-1, C-3, C-5, C-6, C-20, C-21, and C-35 of **2** were determined to be identical to those of **1**. This conclusion was drawn based on the configuration-

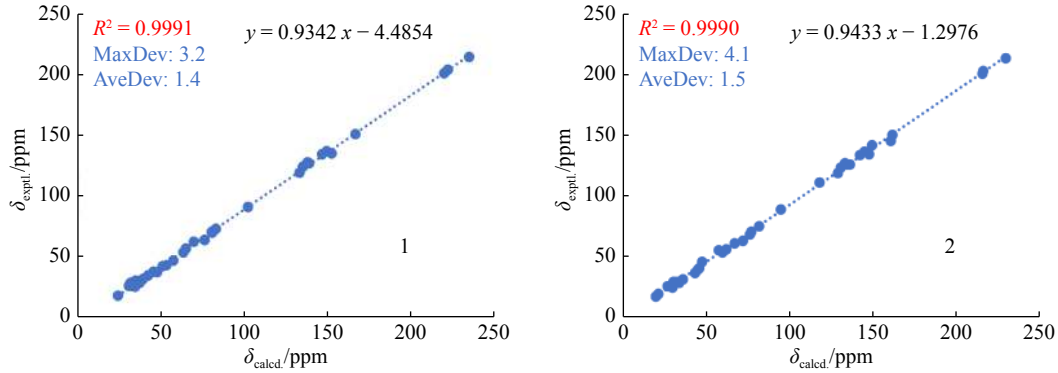

Fig. 3 Linear correlations between the experimental and calculated ^{13}C NMR chemical shifts for 1 and 2.

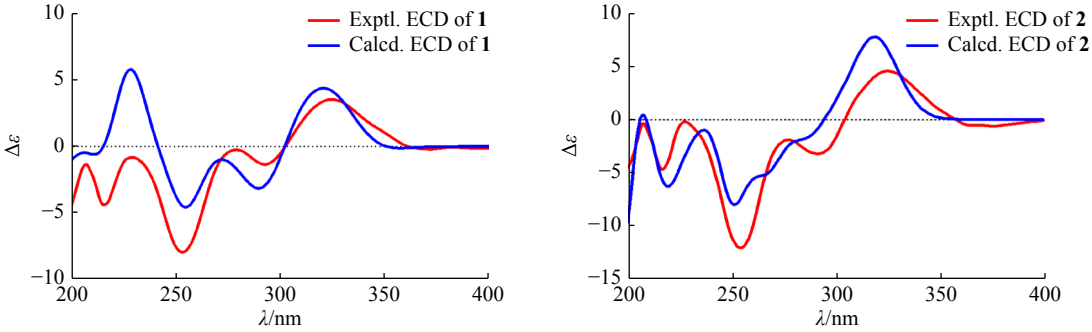

Fig. 4 Calculated and experimental ECD spectra for 1 and 2 (in MeOH).

al characteristics of the cage-liked moiety (rings B/C/D/E/F) and a detailed analysis of its NOESY data, specifically the correlations of OH-6/H-22, OH-6/H_3-38, H-35/H_3-37, and H-20/H-23b (Fig. 2). Additionally, the NOESY correlation of OH-6/H-22 further illustrated that they were *α*-oriented. Furthermore, the coupling constant of H-22/H-25 (J = 11.2 Hz), along with the distinct NOESY cross-peaks of H-22/H-24a, H-22/H_3-27, and H-25/H-29, clarified that H-25 was *β*-oriented. Thus, the relative structure of **2** was defined.

To corroborate the relative configuration of **2**, the calculations of ^{13}C NMR data were applied. The calculated data closely aligned with the experimental findings, as evidenced by an R^2 value of 0.9990 (Fig. 3). Additionally, ECD calculations were carried out to determine the absolute configuration of **2**. The high similarity between the calculated ECD curves of (1*S*,3*S*,5*R*,6*S*,20*S*,21*R*,22*R*,25*S*,35*S*)-**2** and its experimental curves unambiguously confirmed the absolute configuration of this metabolite (Fig. 4).

From a structural perspective, compound **1** was the first PPAP with highly complex eight-carbon rings based on a unique heptacyclo[10.8.1.1^{1,10}.0^{3,8}.0^{8,21}.0^{12,19}.0^{14,17}]docosane core. **2** possessed a unique heptacyclic architecture decorated by an unparalleled hexacyclo[9.6.1.1^{1,9}.0^{3,7}.0^{7,18}.0^{11,16}]nonadecane moiety. Therefore, a hypothetical biogenetic pathway for **1** and **2** was proposed (Fig. 5).

The biosynthesis of hyparillums A (**1**) and B (**2**) was hypothesized to commence with an intramolecular Diels-Alder cyclization between the bonds C-6–C-1–C-2–C-3 and C-21–C-23 in monocyclic polyprenylated acylphloroglucinols (MPAPs), forming intermediate **i** [9, 12]. Subsequent Diels-Alder cyclization involving bonds C-1–C-7–C-8–C-13 and C-35–C-36, followed by oxidation, could yield intermediate **ii** [9, 13]. From the intermediate **ii**, a key [2 + 2] cyclization process imparted **1** with its unique 6/6/5/6/6/5/6/4 carbon skeleton [14, 15]. Conversely, hyparillum B (**2**), with its unique 6/6/5/6/6/5/5 heptacyclic architecture, was proposed to originate from **ii** *via* oxidation and cyclization reactions involving the attack from C-25 to C-22 [16]. Significantly, both Diels-Alder and [2 + 2] cyclization played crucial roles in the formation of **1** and **2**, facilitating the generation of complex polycyclic systems with multiple chiral centers [2, 17].

In biological assays, compounds **1** and **2** displayed potential inhibitory activities against the proliferation of murine splenocytes stimulated by anti-CD3/anti-CD28 mAbs, with IC_{50} values of 6.13 ± 0.86 and 7.13 ± 0.89 μmol·L^{-1}, respectively. Additionally, **1** and **2** also showed potential activities against LPS-induced murine splenocyte proliferation, with IC_{50} values of 8.26 ± 0.85 and 12.69 ± 1.31 μmol·L^{-1}, respectively.

Experimental

General experimental procedures

The 1D and 2D NMR data were measured on Bruker AV-600 spectrometers with TMS as internal standard. HR-MS (ESI-TOF) data were obtained using a Bruker micOTOF II and SolariX 7.0 spectrometer (Bruker, Karlsruhe, Germany).

Table 1 The ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of 1 and 2 in $CDCl_3$ (δ in ppm, J in Hz)

No.	1		2	
	δ_H	δ_C	δ_H	δ_C
1		69.6		69.0
2		203.1		202.6
3		63.6		63.5
4		213.8		213.3
5		70.5		75.5
6		90.6		89.3
7		200.4		200.6
8		136.3		136.5
9	7.68 m	126.5	7.62 m	126.3
10	7.35 m	127.1	7.36 m	127.2
11	7.54 td (7.6, 1.4)	133.9	7.54 m	133.8
12	7.36 m	123.8	7.35 m	123.7
13		150.6		150.2
14	2.27 d (7.1)	25.4	2.28 m	25.4
15	5.01 t (7.2)	118.9	5.03 m	119.0
16		134.5		134.6
17	1.58 s	18.1	1.57 s	18.1
18	1.66 s	26.1	1.65 s	26.0
19	*a* 1.97 dd (11.2, 7.0), *b* 1.67 overlap	31.6	*a* 2.05 m, *b* 1.70 overlap	32.0
20	1.51 overlap	53.6	2.31 m	61.5
21		42.8		54.3
22	*a* 1.90 dd (13.3, 11.6), *b* 2.22 m	34.5	2.98 dd (11.2, 8.6)	46.3
23	*a* 1.76 overlap, *b* 1.70 overlap	46.7	*a* 2.11 d (13.8), *b* 1.50 d (13.8)	41.0
24	1.57 overlap	27.9	*a* 1.63 overlap, *b* 2.00 m	40.2
25	1.59 overlap	42.2	2.58 m	55.8
26		37.8		145.6
27	1.26 s	26.6	1.72 s	19.9
28	1.06 s	27.5	4.76 s	111.4
29	2.43 m	26.3	5.50 dd (15.7, 8.6)	126.3
30	1.77 overlap	62.2	5.96 d (15.7)	141.9
31		72.9		71.1
32	1.27 s	28.4	1.31 s	30.0
33	1.20 s	30.4	1.32 s	30.0
34	*a* 2.20 m, *b* 2.05 dd (11.6, 7.9)	28.6	*a* 2.27 m, *b* 2.04 m	29.3
35	2.68 dd (9.6, 7.9)	56.7	2.66 dd (9.9, 7.8)	56.5
36		37.4		37.4
37	1.36 s	26.2	1.35 s	26.3
38	1.08 s	30.0	1.10 s	29.8
6-OH	2.84 s		3.09 s	

Optical rotations were detected on an AUTOPOL IV-T Automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). ECD spectra were collected on a JASCO-810 spectrometer (JASCO, Tokyo, Japan). IR spectra were acquired by using a Bruker Vertex 70 FT-IR spectrophotometer (Bruker, Karlsruhe, Germany). UV spectra were obtained on a Lambda 35 instrument (PerkinElmer Inc., Fremont, California, USA). Semi-preparative HPLC was conducted on an Agilent 1200 system or a Dionex HPLC system with a reversed-phase (RP) C_{18} column (5 μm, 10 mm × 250 mm, Welch Ultimate XB-C_{18}) to separate and purify the samples. Column chromatography (CC) including silica gel (80–120 and 100–200 mesh; Qingdao Marine Chemical Inc., China), Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden), MCI gel (75–150 μm, Merck, Germany), and ODS (50 μm, YMC Co. Ltd., Japan) were used to separate and purify the samples.

Plant material

The dried leaves of *H. patulum* were collected from the Enshi Autonomous Prefecture, Hubei Province (GPS coordinates: 29°50′33″–30°39′30″ N, 109°4′48″–109°58′42″ E), China, in August 2018. The plants were identified by Prof. ZHANG C. G. of Huazhong University of Science and Technology (HUST). A voucher sample (No. HP20180824) was deposited in the herbarium of Tongji Medical College of HUST.

Extraction and isolation

The dried leaves of *H. patulum* (35.0 kg) were ground and extracted with 95% EtOH (25 L) five times at room temperature to obtain the crude extract. The crude extract was then suspended in water and partitioned with CH_2Cl_2, yielding a CH_2Cl_2 fraction (2.5 kg). The CH_2Cl_2 fraction was chromatographed on silica gel CC (80–120 mesh) and eluted with a stepwise gradient of petroleum ether–ethyl acetate (50 ∶ 1–0 ∶ 1), resulting in seven fractions (A–G). Fr. D (105.0 g) was further separated into six fractions (D1–D6) loaded on a silica gel CC with an eluent of petroleum ether–ethyl acetate (40 ∶ 1 to 1 ∶ 1, *V*/*V*). Fr. D4 (10 g) was loaded on an MCI column (MeOH–H_2O, 90 ∶ 10 to 100 ∶ 0, *V*/*V*) to remove pigment and then on an ODS column (MeOH–H_2O, 40 ∶ 60 to 100 ∶ 0, *V*/*V*) to afford nine subfractions, D4a–D4i. Fr. D4c (1.0 g) was subjected to Sephadex LH-20 CC (MeOH–CH_2Cl_2, 1 ∶ 1, *V*/*V*), resulting in three subfractions, D4c1–D4c3. Fr. D4c2 (280 mg) was purified by semi-preparative HPLC with an RP-C_{18} column repeatedly to obtain **1** (2.9 mg, 2 mL·min^{-1}, t_R 29.5 min, MeOH–H_2O, 92%). Fr. D4g (900 mg) was divided into three subfractions, D4g1–D4g3, by using Sephadex LH-20 CC (MeOH–CH_2Cl_2, 1 ∶ 1, *V*/*V*). Then, Fr. D4g2 (300 mg) was further purified by semi-preparative HPLC with an RP-C_{18} column to yield **2** (1.5 mg, 2 mL/min^{-1}, t_R 30.5 min, MeOH–H_2O, 90%).

Hyparillum A (***1***): colorless oil; $[\alpha]_D^{28}$ –26.1 (*c* 0.2, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 252 (3.95) nm; IR (KBr) ν_{max}: 3470, 2965, 2925, 2871, 1739, 1708, 1675, 1599, 1452, 1384, 1368, 1309, 1259, 1160, 1117, 826 and 763 cm^{-1}; ECD

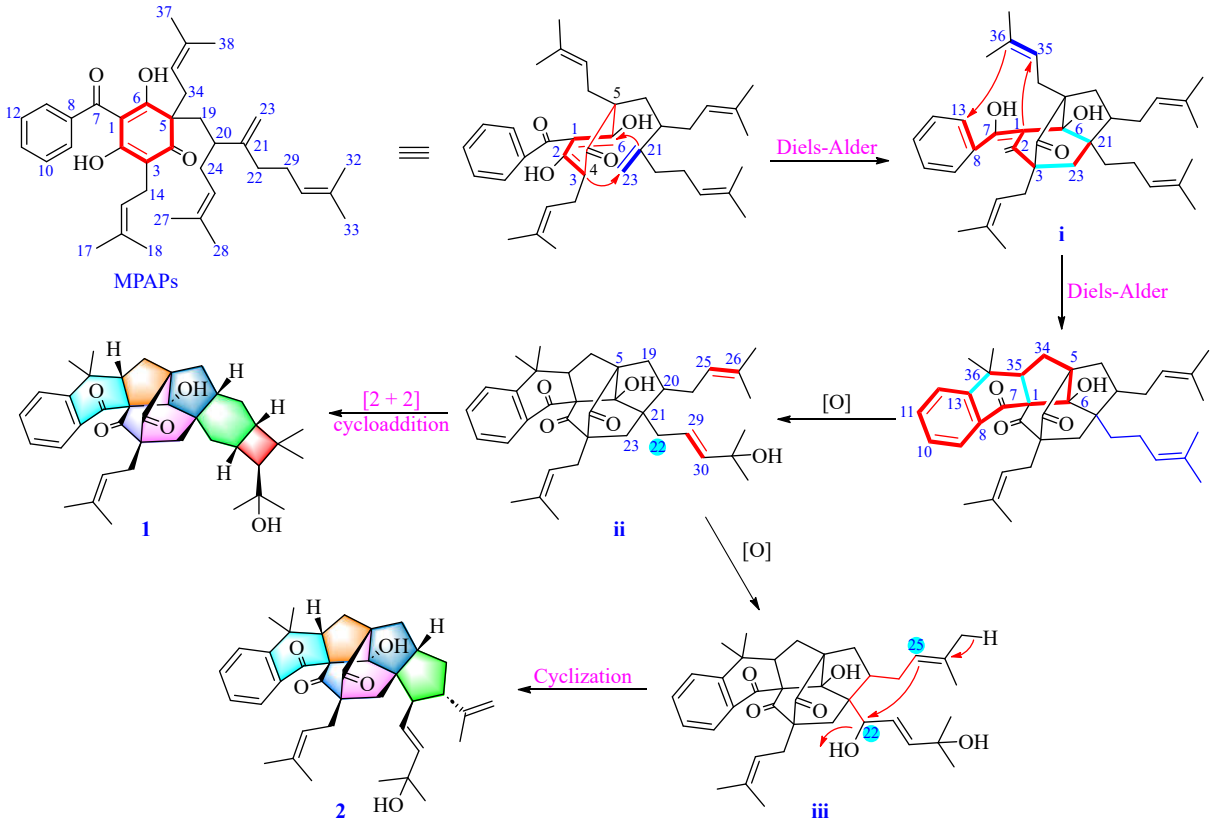

Fig. 5 Plausible biosynthetic pathways of 1 and 2.

(CH_3OH) λ_{max} ($\Delta\varepsilon$): 216 (−4.73), 230 (−0.71), 255 (−7.86), 280 (−0.24), 293 (−1.38), 326 (+3.51) nm. ^1H and ^{13}C NMR data (Table 1); positive HR-ESI-MS *m/z* 607.3373 $[M + Na]^+$ (Calcd. for $C_{38}H_{48}O_5Na^+$, 607.3394).

Hyparillum B (2): colorless oil; $[\alpha]_D^{28}$ −51.5 (*c* 0.1, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 252 (4.03) nm; IR (KBr) ν_{max}: 3433, 2968, 2926, 2871, 1739, 1709, 1676, 1600, 1454, 1383, 1311, 1255, 1161, 1118, 886 and 762 cm^{-1}; ECD (CH_3OH) λ_{max} ($\Delta\varepsilon$): 217 (−5.26), 227 (−0.11), 254 (−12.00), 277 (−1.84), 292 (−3.19), 326 (+4.52) nm.^1H and ^{13}C NMR data (Table 1); positive HR-ESI-MS *m/z* 605.3213 $[M + Na]^+$ (Calcd. for $C_{38}H_{46}O_5Na^+$, 605.3237).

Computational methods

The details of NMR and ECD calculations for compounds **1** and **2** were included in the Supporting Information.

Cell culture and treatment

Male C57BL/6 J mice (20–25 g, 6–8 weeks old), purchased from Beijing HFK Bio-Technology Co., Ltd., were used as a source of the spleen cells. Firstly, the mice were euthanized humanely using cervical dislocation under anesthesia. After removal of the spleen under rigorous sterile operation, splenocytes were purified by homogenization with syringe plungers, passing through a 0.1-mm sterile nylon mesh, and erythrocytes were removed to purify the splenocytes. Then, the purified splenocytes were suspended in PBS supplemented with 0.1% BSA and further labeled with 5-carboxyfluorescein diacetate succinimide ester (CFSE) at a density of 1×10^7 cells/mL. Furthermore, LPS (10 μg·mL^{-1}, Sigma, USA) or anti-CD3/anti-CD28 mAbs (0.5 μg·mL^{-1}) were further used to stimulate the murine splenocytes. The splenocytes were then cultured for 72 h at 2.5×10^5 cells/well in 96-well round-bottom plates (Costar, Cambridge, MA) containing RPMI-1640 medium (200 μL) supplemented with 10% heat-inactivated FBS in a humidified atmosphere with 5% CO_2 at 37 °C. Finally, the proliferation of the cells, indicated by the CFSE signal in gated cells, was detected using a FACSCelesta flow cytometer (BD Biosciences).

Conclusion

In this study, we successfully isolated two novel and structurally complex PPAPs, hyparillums A (**1**) and B (**2**), from *H. patulum*. Hyparillum A (**1**) represented the first PPAP with eight-carbon rings based on an unprecedented 6/6/5/6/6/5/6/4 octocyclic system decorated by a heptacyclo [10.8.1.1^{1,10}.0^{3,8}.0^{8,21}.0^{12,19}.0^{14,17}]docosane core. Hyparillum B (**2**) possessed a unique heptacyclic architecture (6/6/5/6/6/5/5) constituted by an unparalleled hexacyclo[9.6.1.1^{1,9}.0^{3,7}.0^{7,18}.0^{11,16}]nonadecane fragment. More significantly, both compounds exhibited inhibitory activities against the proliferation of murine splenocytes stimulated by anti-CD3/anti-CD28 mAbs and LPS. In this paper, our findings not only enrich the structural diversity of PPAPs but also lay the foundation for further study on immunosuppressive activity.

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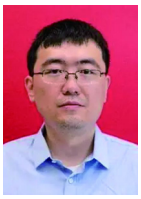

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