Hypatulone A, a Homoadamantane-type Acylphloroglucinol with Intricately Caged Core from *Hypericum patulum*

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Supporting Information

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2. HRESIMS, UV, IR, CD, and NMR Spectra

1. EXPERIMENTAL SECTION

1.1 General Experimental Procedures. Optical rotations were measured on a JASCOP-1020 polarimeter in MeOH at room temperature. Nuclear magnetic resonance (NMR) spectra were on a Bruker AVIII-600 NMR instrument (^{1}H: 600 MHz, ^{13}C: 150 MHz) equipped with CryoProbe (Bruker, Karlsruhe, Germany), with tetramethylsilane (TMS) as an internal standard. Chemical shift values (δ) are given in parts per million (ppm) and coupling constants in Hertz (Hz). The following abbreviations are used to designate multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Electrospray ionization (ESI) and high-resolution electrospray ionization (HRESIMS) were carried out an Agilent 1100 series LC/MSD ion trap mass spectrometer and an Agilent 6529B Q-TOF instrument (Agilent Technologies, Santa Clara, CA, USA), respectively. Preparative high-performance liquid chromatography (*Pre*-HPLC) was performed on a Shimadzu LC-6A system (Shimadzu, Tokyo, Japan) equipped with a Shim-pack RP-C_{18} column (200 mm × 20 mm i.d., 10 μm, Shimadzu, Tokyo, Japan) with flow rate at 10.0 ml/min and column temperature at 25 ^{o}C, detected by a binary channel UV detector at 210 and 270 nm. All solvents used were of analytical grade (Jiangsu Hanbang Science and Technology. Co., Ltd.). Silica gel (200-300 mesh, Qingdao Haiyang Chemical Co., Ltd, Qingdao China) and RP-C_{18} silica (40-63 μm, FuJi, Japan) were used for column chromatography. Fractions obtained from column chromatography (CC) were monitored by thin-layer chromatography (TLC) with precoated silica gel GF_{254} (Qingdao Haiyang Chemical Co., Ltd, China) plates.

1.2 Plant Material. Air-dried flowers of *Hypericum patulum* were collected from Yunnan

Province, People's Republic of China, in August 2017, and were authenticated by Professor Mian Zhang of the Research Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University (accession number 2017-HPF).

1.3 Extraction and isolation.

The dried powder of flowers of *H. patulum* (2.0 kg) was extracted three times (3 × 5L) with 95% aqueous EtOH under ultrasonic agitation, and the crude (468 g) was suspended in H_2O and extracted with petroleum ether (PE) (3 × 1L). The petroleum ether extract (90.0 g) was subjected to a silica gel column, eluted with a gradient of PE-EtOAc (1:0, 9:1, 8:2, *v*/*v*) to give five fractions (A-E), which were combined based on TLC. Fraction B (13.6 g) was chromatographed over a C_{18} silica gel column eluted with a gradient system of MeOH-H_2O (7:3, 8:2, 9:1, 1:0, *v*/*v*) to give four subfractions (B1-B4). B2 (4.8 g) was sequentially purified by columns of C_{18} silica gel (MeOH-H_2O, 70% to 85%, *v*/*v*) and then further separated over *Pre*-HPLC (MeOH−H_2O, 80:20, *v*/*v*) to obtain compound **1** (14.2 mg). B4 (1.8 g) was sequentially purified by columns of C_{18} silica gel (MeOH-H_2O, 80% to 90%, *v*/*v*) and then further separated over *Pre*-HPLC (MeOH−H_2O, 85:15, *v*/*v*) to obtain compound **2** (141 mg).

1.4 NO production bioassay

The RAW264.7 cell line was purchased from the Chinese Academic of Sciences. The cells were cultured in DMEM containing 10% FBS with penicillin (100 U/mL) and streptomycin (100 U/mL) at 37 ºC in a humidified atmosphere with 5% CO_2. The cells were allowed to grow in 96-well plates with 1×10^5 cells/well to treat test compounds. After

being incubated for 2 h, the cells were treated with 100 ng/mL of LPS for 18 h. Nitrite in culture media was measured to assess NO production using Griess reagent. The absorbance at 540 nm was measured on a microplate reader. *N*-monomethyl-L-arginine was used as the positive control. Cytotoxicity was determined by the MTT method, after 48 h incubation with test compounds. All the experiments were performed in three independent replicates.

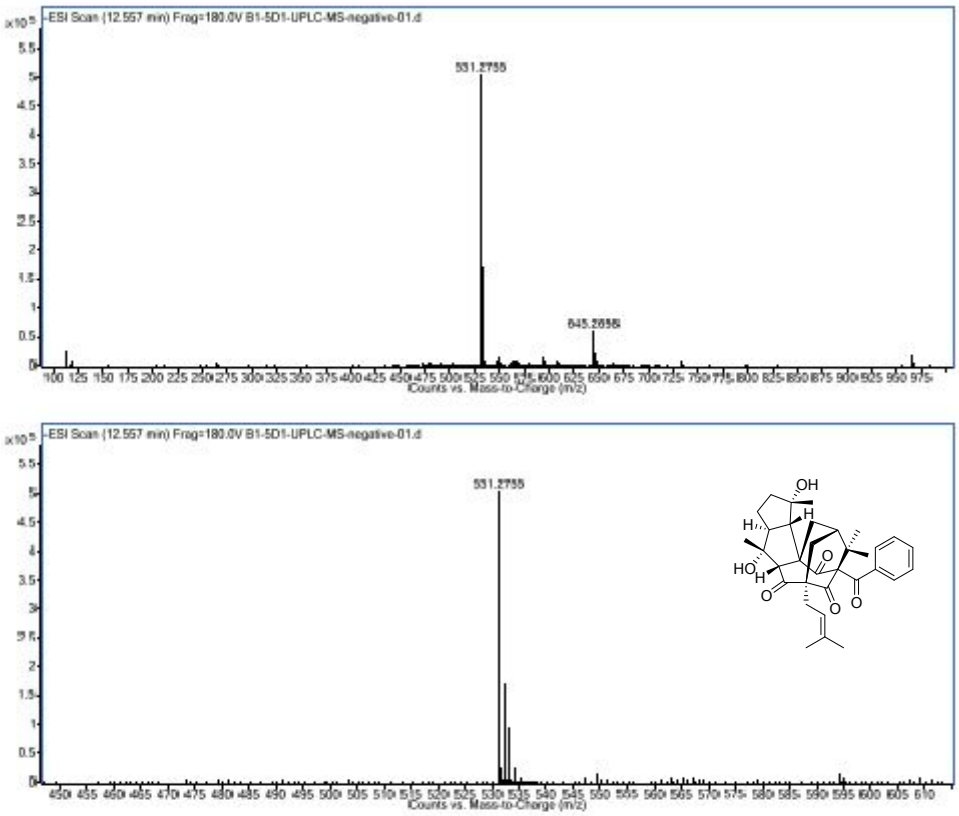
1.5 Physical and chemical data

Hypatulone A (1): light yellow oil, $[\alpha]^{25}_{D}$= +11.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) = 204 (3.96), 247 (3.71); IR (KBr) ν_{max} 3461, 2963, 2926, 2852, 1743, 1713, 1684, 1637, 1452, 1384, 1261, 1096, 1024, 803 cm^{-1}; ECD (MeOH) λ_{max} (Δε) 205 (+18.34), 238 (-11.60), 264 (+3.36), 295 (+3.66), 346 (-4.85); ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1; positive ESIMS *m*/*z* 533.2 $[M + H]^+$; HRESIMS *m*/*z* 531.2755 $[M – H]^-$ (calcd for $C_{33}H_{39}O_6$, 531.2752).

Hyperbeanol B (2): yellow gum. HRESIMS *m*/*z* 557.2869 $[M + Na]^+$ (calcd for $C_{33}H_{42}O_6Na$, 557.2874). ^{1}H NMR ($CDCl_3$) δ_H 7.53 (1H, t, *J*=7.5 Hz, H-31), 7.47 (2H, d, *J*=7.7 Hz, H-29 and H-33), 7.39 (2H, t, *J*=7.7 Hz, H-30 and H-32), 4.98 (1H, t, *J*=7.5 Hz, H-18), 4.73 (1H, t, *J*=7.7 Hz, H-23), 2.90 (1H, dd, *J*=13.5, 9.4 Hz, H-17), 2.73 (1H, m, H-17), 2.71 (1H, m, H-22), 2.59 (1H, dd, *J*=13.4, 7.3 Hz, H-22), 7.39 (2H, t, *J*=7.7 Hz, H-30, 32), 2.16 (1H, m, H-14*β*), 2.13 (1H, m, H-7*α*), 1.81 (1H, m, H-10*β*), 1.75 (1H, m, H-12), 1.73 (1H, m, H-11*β*), 1.71 (1H, m, H-10*α*), 1.69 (1H, m, H-11*α*), 1.66 (3H, s, H_3-26), 1.61 (3H, s, H_3-25), 1.60 (1H, m, H-14*β*), 1.55 (3H, s, H_3-21), 1.49 (3H, s, H_3-20), 1.33 (3H, s, H_3-16), 1.20 (3H, s, H_3-15). ^{13}C NMR ($CDCl_3$) δ_C 210.5 (C-5), 199.9 (C-3), 199.1 (C-27), 193.5 (C-1), 138.4 (C-24), 137.7 (C-28), 136.6 (C-19), 132.5 (C-31), 128.3 (C-30 and C-

32), 127.8 (C-29 and C-33), 118.8 (C-18), 116.7 (C-23), 113.4 (C-2), 79.7 (C-9), 68.8 (C-13), 64.3 (C-4), 60.8 (C-6), 51.5 (C-12), 44.0 (C-8), 41.0 (C-10), 40.7 (C-14), 40.2 (C-14), 36.0 (C-17), 34.5 (C-7), 29.3 (C-15), 26.4 (C-16), 26.1 (C-21 and C-26), 21.0 (C-11), 18.0 (C-20 and C-25).

2. NMR, HRESIMS, and UV Spectra



Elemental Composition Calculator

Target m/z:	531.2755	**Result type:**	Negative ions	**Species:**	$[M-H]^-$
Elements:		C (0-80); H (0-120); O (0-30); N(0-10); Cl (0-5)			
Ion Formula		**Calcalated m/z**		**PPM Error**	
C33H39O6		531.2752		-0.53	

Figure S1. HRESIMS spectrum of **1**.

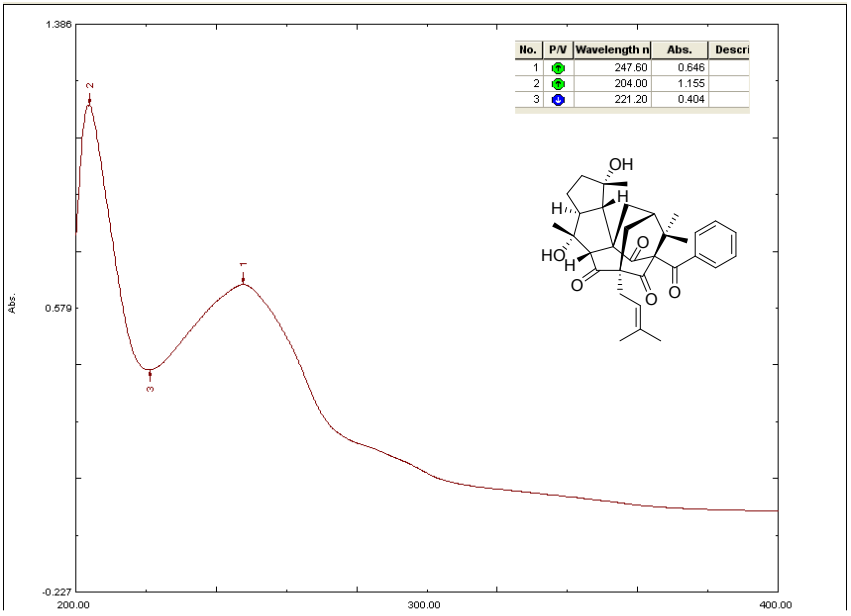


Figure S2. UV (MeOH) spectrum of **1**.

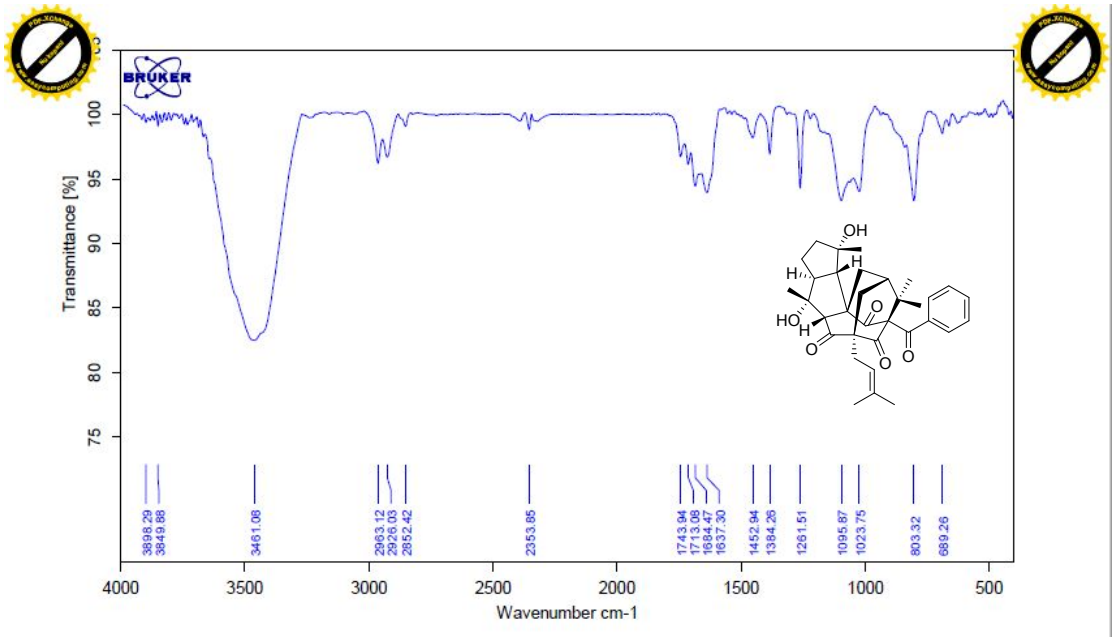


Figure S3. IR (MeOH) spectrum of **1**.

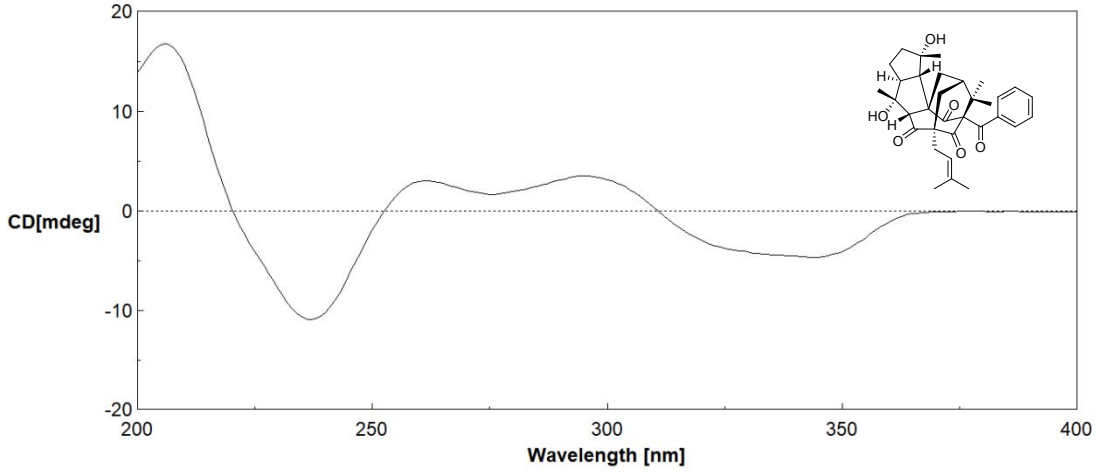


Figure S4. Experimental ECD (MeOH) spectrum of **1**.

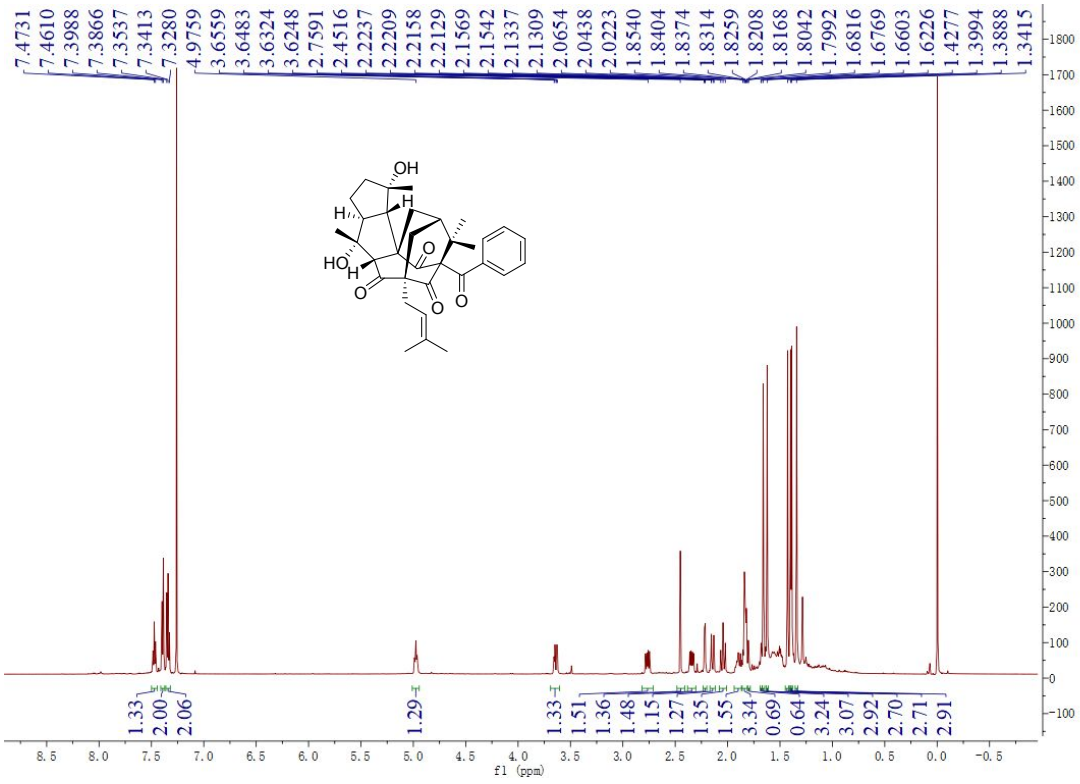


Figure S5. ^{1}H NMR (600 MHz; $CDCl_3$) spectrum of **1**.

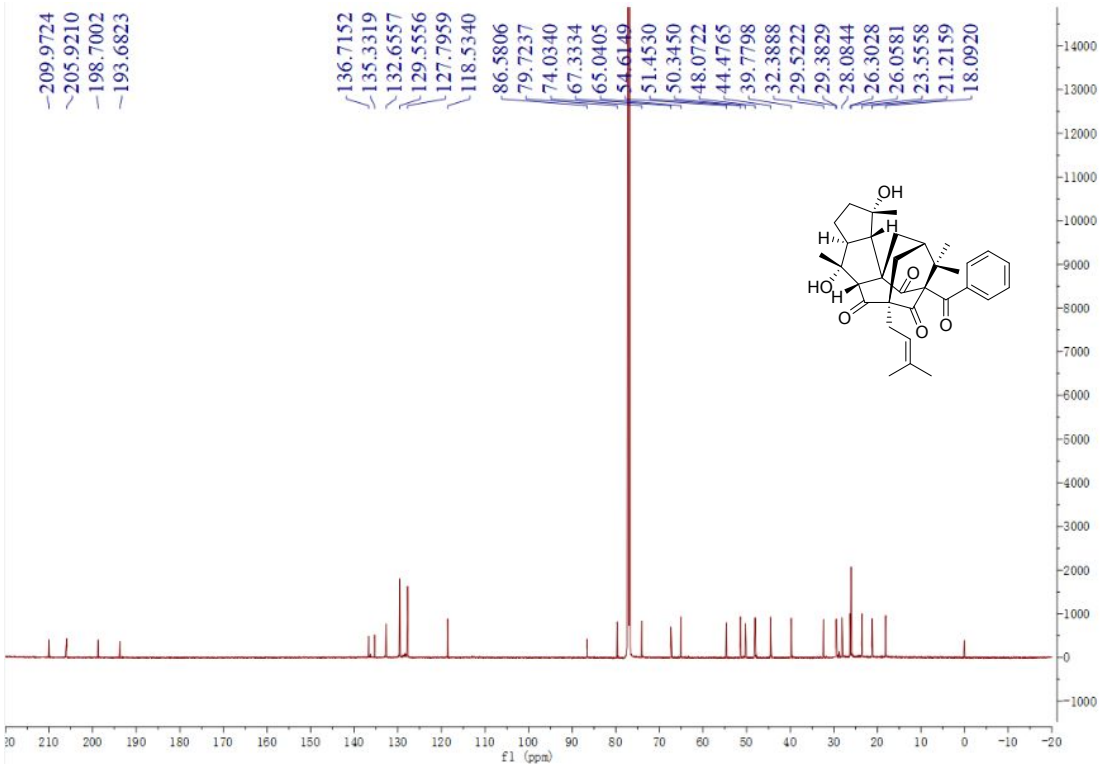


Figure S6. ^{13}C NMR (150 MHz; $CDCl_3$) spectrum of **1**.

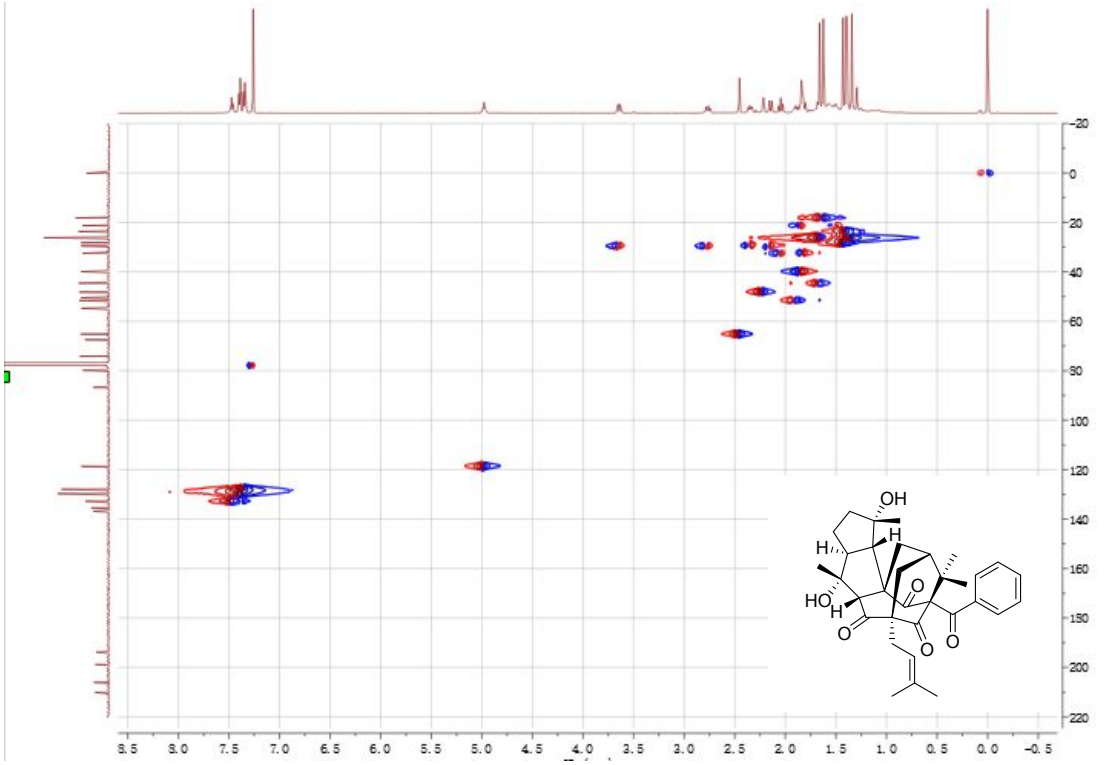


Figure S7. HSQC ($CDCl_3$) spectrum of **1**.

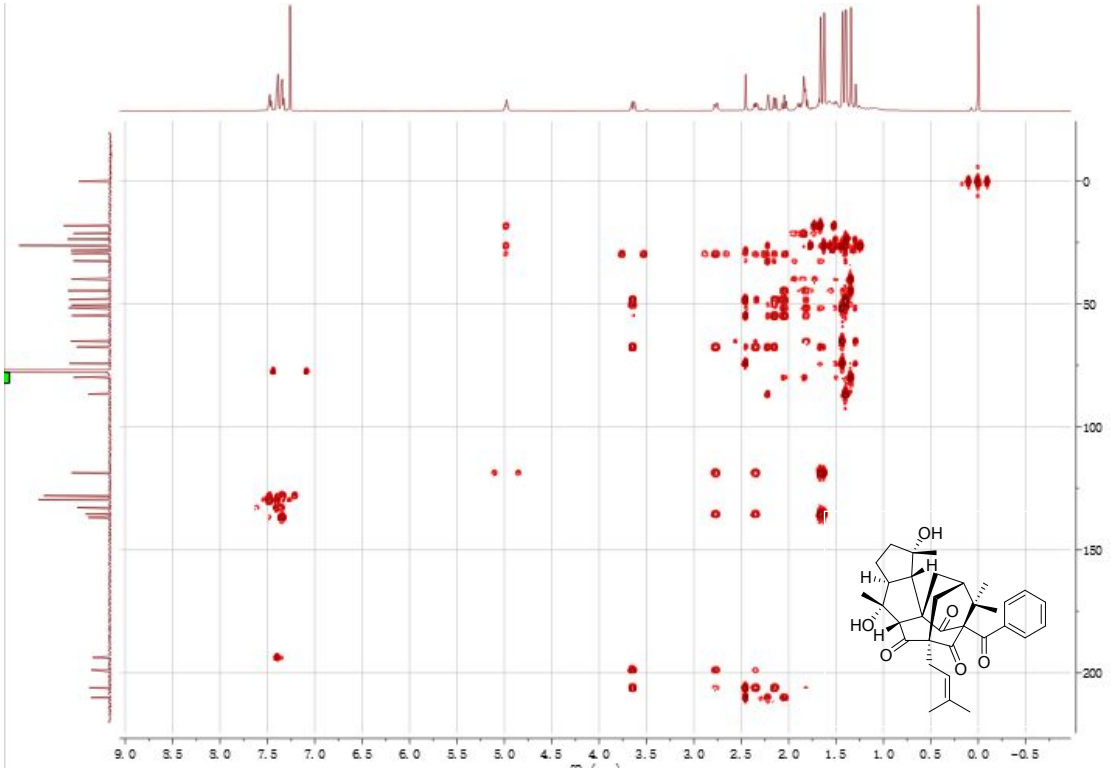


Figure S8. HMBC ($CDCl_3$) spectrum of **1**.

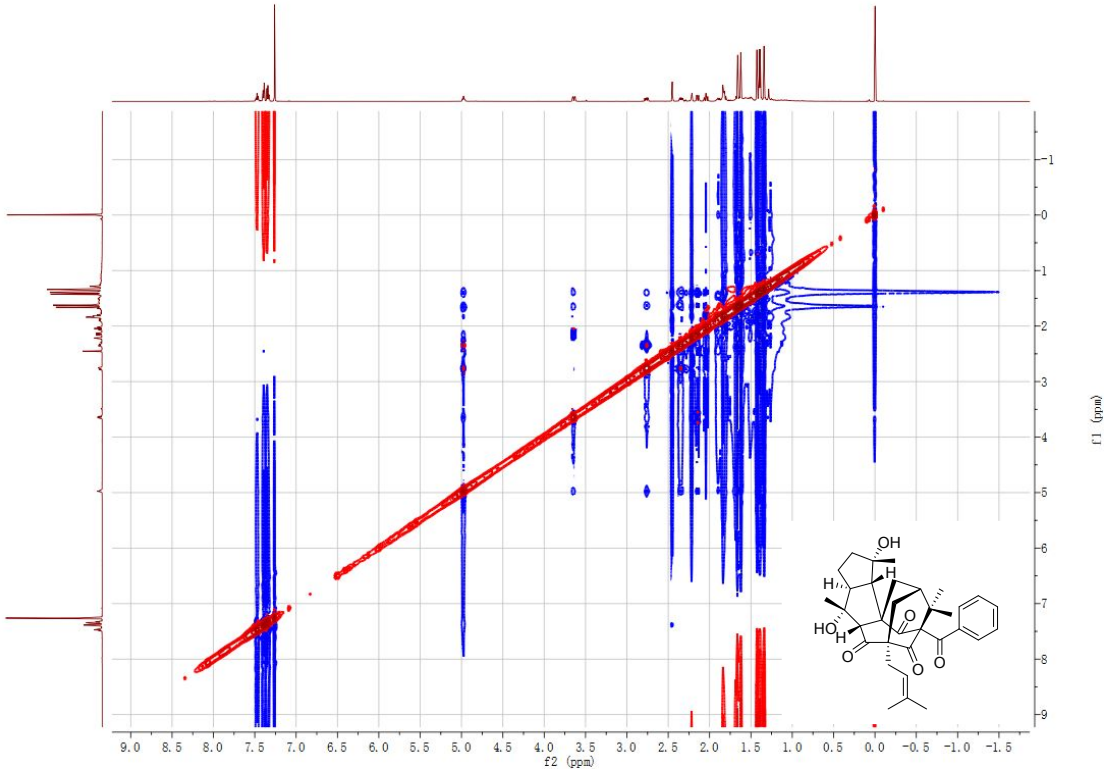


Figure S9. NOESY ($CDCl_3$) spectrum of **1**.

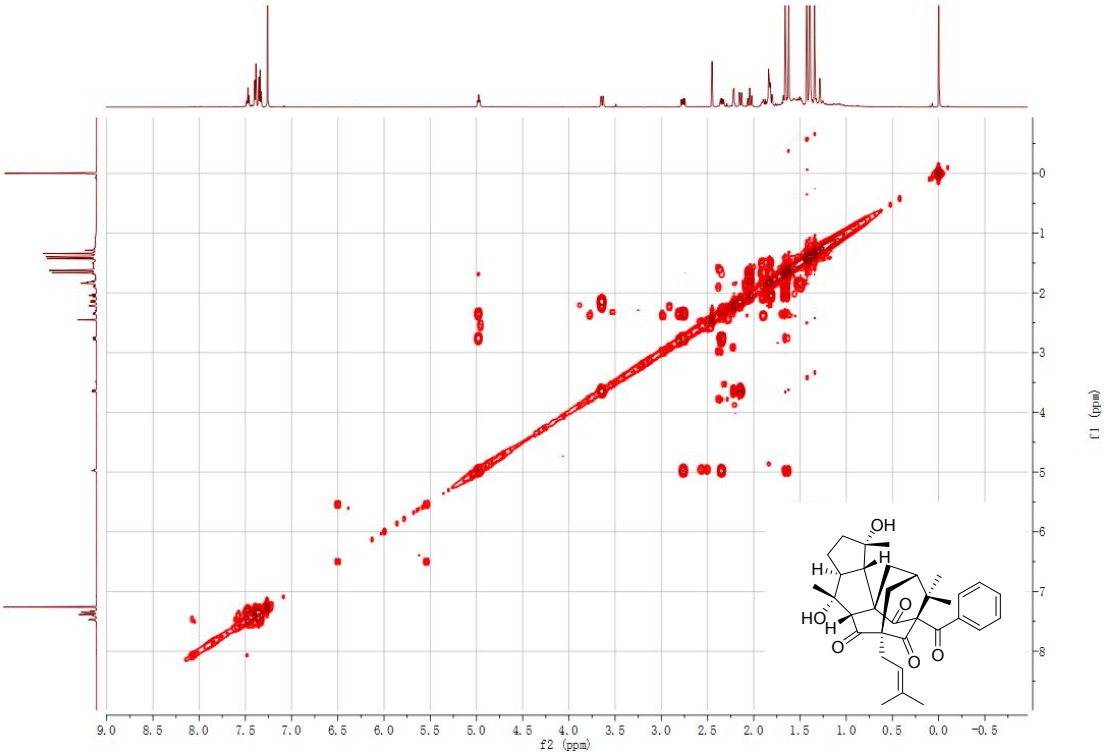
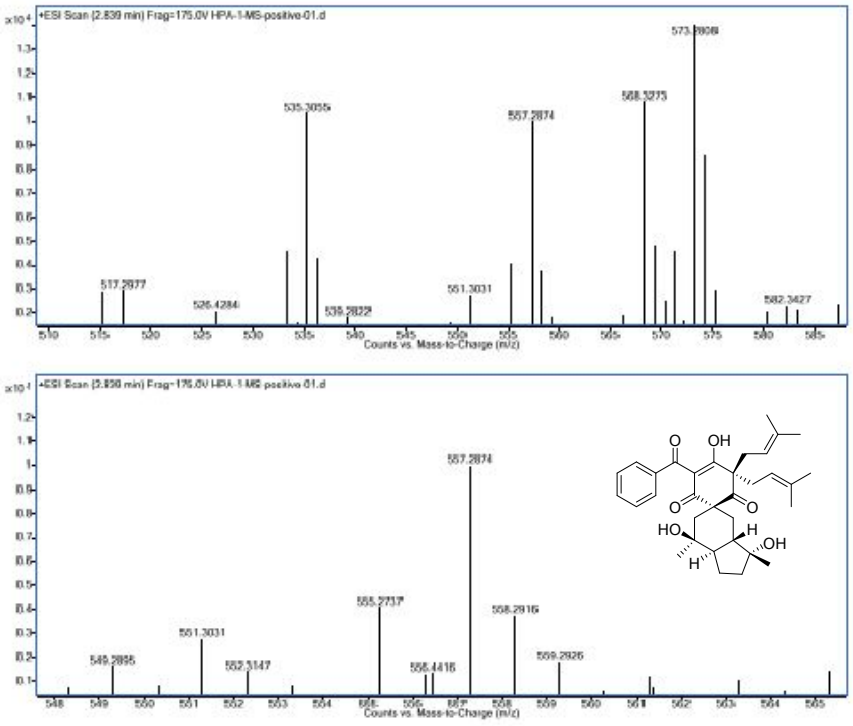


Figure S10. $^{1}H–^{1}H$ COSY ($CDCl_3$) spectrum of **1**.



Elemental Composition Calculator

Target m/z:	557.2869	**Result type:**	Positive ions	**Species:**	$[M+Na]^+$
Elements:		C (0-80); H (0-120); O (0-30); Na (0-5)			
Ion Formula		**Calculated m/z**		**PPM Error**	
C33H42NaO6		557.2874		0.82	

Figure S11. HRESIMS spectrum of **2**.

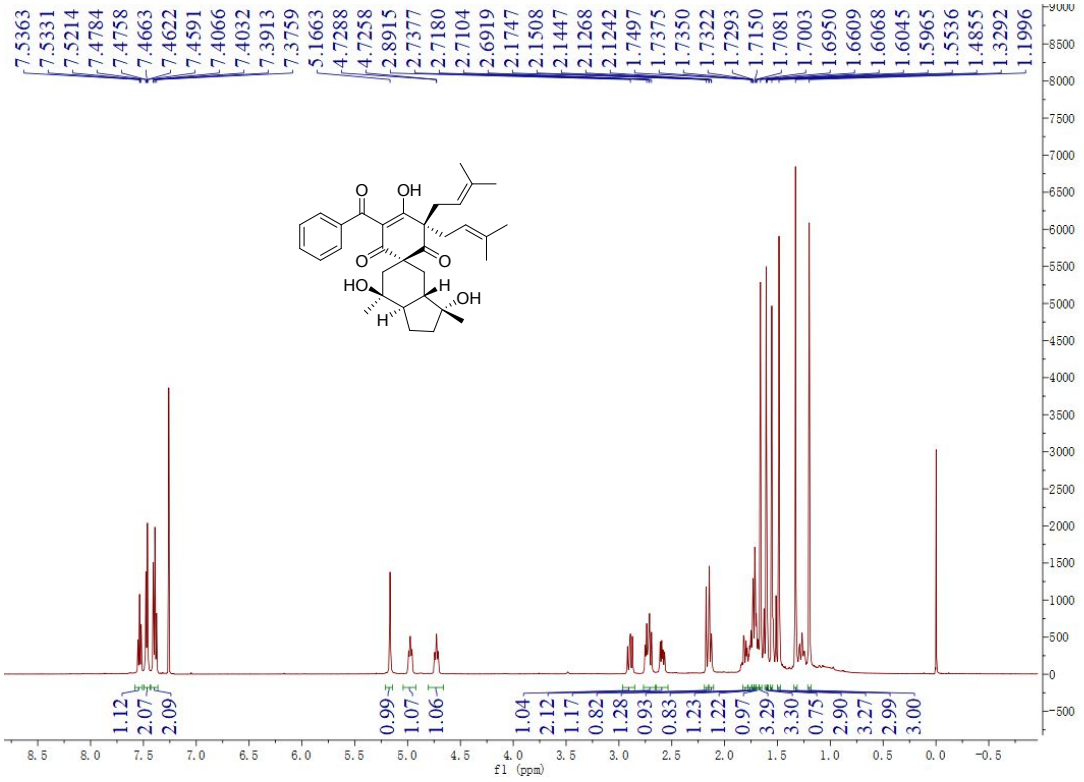


Figure S12. ^{1}H NMR (600 MHz; $CDCl_3$) spectrum of **2**.

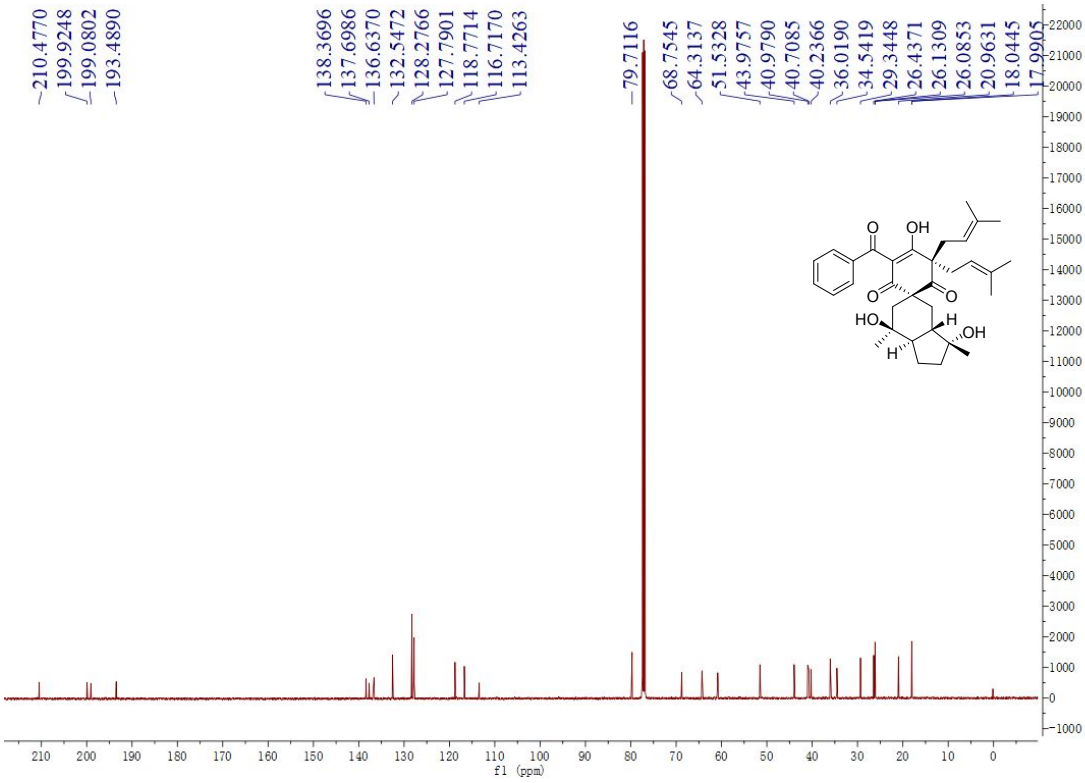


Figure S13. ^{13}C NMR (150 MHz; $CDCl_3$) spectrum of **2**.