Supporting Information for

Schincalide A, a Schinortriterpenoid with a Tricyclo[5.2.1.0^{1,6}]decane-Bridged System from the stems and leaves of *Schisandra incarnate*

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Content list:

S1. Experimental Section

S2. HR-ESI-MS interpretation of **1**

S3. Crystal cell diagram for **1**

S4-1. ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **1**

S4-2. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **1**

S5. ^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **1**

S6. DEPT-135 spectrum (101 MHz, $CDCl_3$) of **1**

S7. HSQC spectrum (400 MHz, $CDCl_3$) of **1**

S8. HMBC spectrum (400 MHz, $CDCl_3$) of **1**

S9. ^{1}H-^{1}H COSY spectrum (400 MHz, $CDCl_3$) of **1**

S10. NOESY spectrum (400 MHz, $CDCl_3$) of **1**

S11. IR spectrum of **1**

S12. CD spectrum of **1**

S1. Experimental Section

1.1 General Experimental Procedures.

IR spectrum was recorded with a PerkinElmer Spectrum one FT-IR spectrometer, as KBr pellets. Specific optical rotation was measured on a Perkin-Elmer 341 polarimeter. 1D and 2D NMR spectra were taken on a Bruker-AM-400 spectrometer and a Bruker AVANCE III 600 spectrometer, with TMS as internal standard. The CD spectrum was obtained with a Jasco J-810 spectropolarimeter. HR-ESI-MS was measured on Thermo Scientific LTQ-Orbitrap XL mass spectrometer. X-ray crystallographic data were obtained on a Bruker Kappa *APEX*-Duo CCD diffractometer using Cu Kα radiation. MPLC was performed on EZ Purifier III chromatography with Spherical C18-ODS column (40-60 μm). HPLC was performed on an Agilent 1100 liquid chromatograph with YMC-C18 (5 μm, 250 × 10 mm). Silica gel (SiO_2, 200–300, or 300-400 mesh, Qingdao Marine Chemical Inc., Qingdao, China), Sephadex LH-20 gel (GE Healthcare) and MCI-gel (CHP20P, 75–150 mm, Mitsubishi Chemical Industries Ltd.) were used for column chromatography. Fractions were monitored by TLC (SiO_2, Yan-tai Institute of Chemical Technology). Spots were visualized by heating silica gel plates immersed with 5% H_2SO_4 in EtOH (V/V).

1.2 Plant Material.

The stems and leaves of *Schisandra incarnate* were collected in the Xingshan County of Hubei Province P. R. China, in September 2013, and identified by Han-Li Ruan from Tongji Medical College of Huazhong University of Science and Technology, P. R. China. A voucher specimen (ID 20130901) has been deposited with Herbarium of Materia Medica, Faculty of Pharmacy, Tongji Medical College of Huazhong University of Science and Technology, P. R. China.

1.3 Cellular Proliferation Assay

1.3.1 The test of cytotoxicity against tumor cell lines of 1

The cytotoxicity against tumor cells of **1** were evaluated using HepG2 human hepatocellular carcinoma cell line, A2780 human ovarian cancer cell line, and Panc02 murine pancreatic cell line. All cells were cultured in DMEM medium (Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS, Sijiqing, China) at 37 °C in a humidified atmosphere with 5% CO_2. The

viability of the cells was determined by the MTT colorimetric assay system[1]. Briefly, 180 μL cells (4×10^3) were seeded into each well of a 96-well cell culture plate and allowed to adhere for 20 h before drug addition, then, each tumor cell line was exposed to compound **1** at various concentrations in quadruplicate for 24 h, with 5-Fluorouracil (Sigma, USA) as positive control. After the incubation, 20 μL MTT (Amresco 0793, USA) (5 μg/μL) was added to each well, and the incubation continued for 4 h at 37 °C. 100 μL DMSO (Sigma, USA) was added after the supernatant was doffed off, and the percentage of viable cells was quantified by microplate reader (Bio-Rad650) at 490 nm.

References

(1) Zheng, Y. T.; Zhang, W. F.; Ben, K. L.; Wang, J. H. *Immunopharmacol. Immunotoxicol.* **1995,** *17*, 69.

1.3.2 The test of immunosuppressive activities of 1

Animals: Female BALB/C mice (7–9 weeks old) were obtained from Tongji Experimental Animal Center and were housed in specific conditions (12 h light/12 h dark photoperiod, 22 ± 1 °C, 55% ± 5% relative humidity). All husbandry and experimental contact were made with the mice maintained specific pathogen-free conditions.

Preparation of spleen cells from mice: Female BALB/C mice were sacrificed by cervical dislocation, and the spleens were removed aseptically. Mononuclear cell suspensions were prepared after cell debris, and clumps were removed. Erythrocytes were depleted with ammonium chloride buffer solution. Lymphocytes were washed and suspended in RPMI 1640 medium (Hyclone, Logan, UT, USA) supplemented with 10% FBS, penicillin (100 U/mL, Hyclone, Logan, UT, USA), and streptomycin (100 μg/mL, Hyclone, Logan, UT, USA).

T cell and B cell function assay: Fresh spleen cells were obtained from female BALB/C mice (7–9 weeks old). The 5×10^5 (180 μL per well) spleen cells in 96-well flat plates, were cultured at 37 °C in a humidified and 5% CO_2-containing incubator for 24 h in the presence or absence of various concentrations of compound **1**. The cultures, in the presence or absence of various concentrations of compound **1**, were stimulated with 5 μg/mL of concanavalin A (ConA, Sigma, USA) or 10 μg/mL of lipopolysaccharide (LPS, Sigma, USA) to induce T cells or B cells proliferative responses, respectively. After that, 20 μL MTT (5 μg/μL) was added to each well at the final 4 h of culture.

Then, each 96-well flat plate was pelleted by centrifugation (1500 rpm, 5 min), and 100 μL DMSO was added after the supernatant was doffed off. To the end of the culture, we measured the OD values with microplate reader (Bio-Rad650) at 490 nm. Cyclosporin A (CsA, Sigma, USA) and Mycophenolate mofetil (MMF, Sigma, USA) were used as positive controls.

Table 1. Cytotoxicity of Compound 1 against HepG2, A2780, and Panc02 Cell Lines

compound	concentration (μg/mL)	concentration (μM)	Cell Viability (%)		
			HepG2	A2780	Panc02
Compound 1	50	95.38	49.58	56.88	82.28
	25	47.69	57.42	59.41	82.59
	12.5	23.85	66.39	67.21	83.97
	6.25	11.92	68.95	73.03	92.94
	3.125	5.96	71.51	78.76	98.16
5-Fu		100	22.05	52.06	56.19
		40	32.5	60.04	65.91
		20	52.85	77.37	68.24
		10	63.16	90.62	72.78
		5	87.59	95.29	79.49

Table 2. Immunosuppressive Effects of Compound 1 on Murine Lymphocyte Proliferation Induced by ConA (5 μg/mL) or LPS (10 μg/mL)

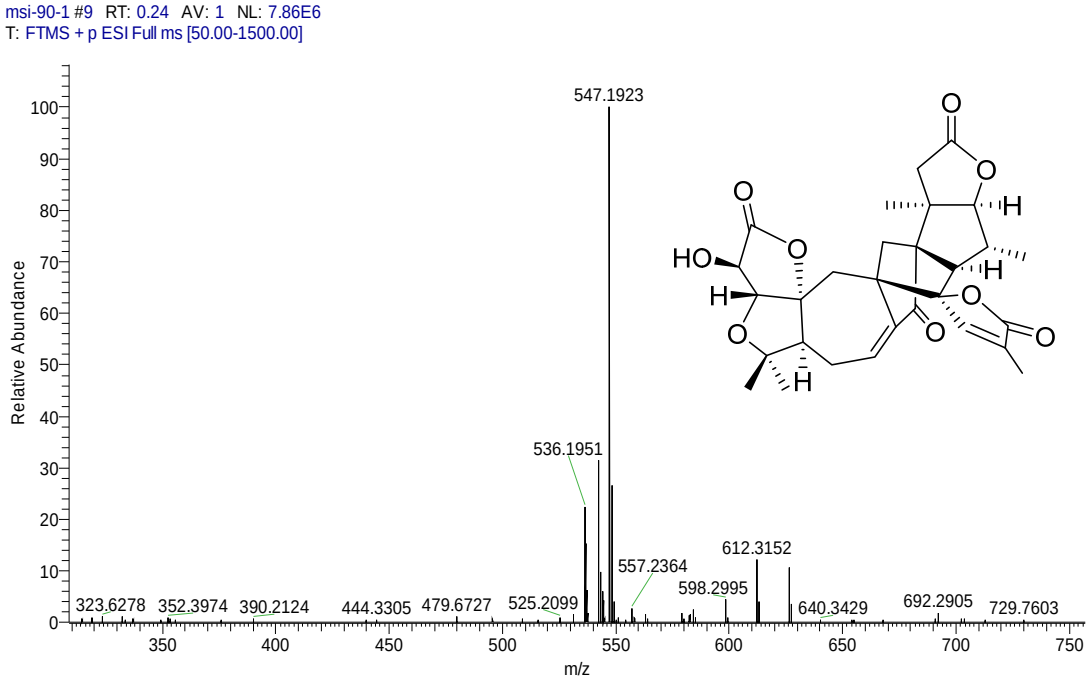
compound	concentration (μg/mL)	Inhibitory Rate of Proliferation (%)	
		T cells	B cells
Compound 1	50	36.76	11.89
	25	29.46	9.97
	12.5	17.71	8.2
	6.25	24.55	2.46
	3.125	10.12	-4.37
CsA	0.5	91.67	
	0.2	78.37	
	0.1	62.95	
	0.05	45.39	
	0.025	35.27	
MMF	10		84.42
	5		77.36
	3		47.42
	1.5		41.98
	0.75		33.02

1.4 Physical-chemical properties of compound 1

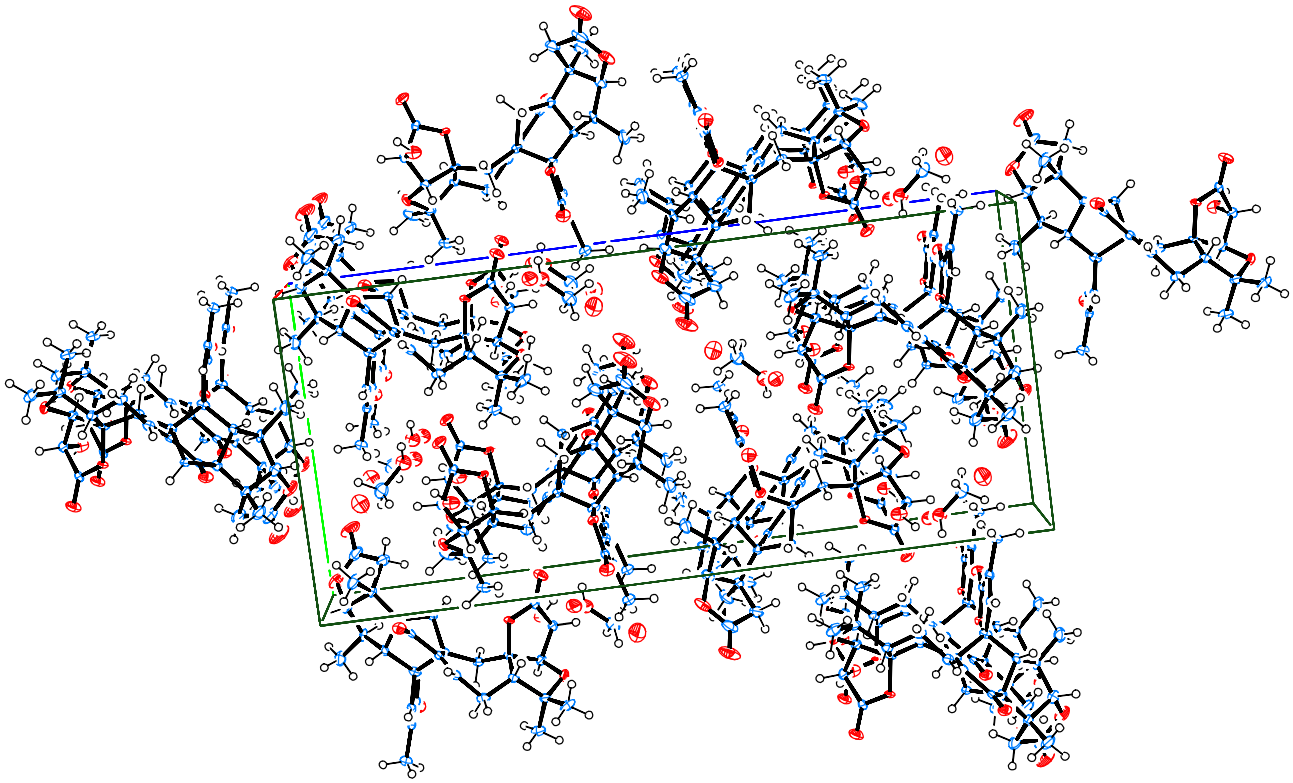
Schincalide A (1): white needles ($CHCl_3$); mp 218-220 °C; $[\alpha]_D^{26.4}$: +58.21 ($CHCl_3$, *c* 0.39); UV: λ_{max}^{MeOH}: 220nm; IR (KBr) ν_{max}: 3460, 1788, 1765, 1735, 1720, 1632, 1485, 1478, 1462, 1453, 1074 cm^{-1}; HRESIMS *m/z* 547.1923 $[M + Na]^+$, calculated 547.1944.

Crystal data for schincalide A (**1**): $C_{29}H_{32}O_9 \cdot (CH_4O)_{0.64} \cdot O_{0.72}$, M= 556.72, orthorhombic, a = 6.9086 (4) Å, b = 13.0711 (8) Å, c = 29.2663 (18) Å, α = 90.00°, β = 90.00°, γ = 90.00°, V = 2642.8 (3) $Å^3$, T= 150 K, space group $P212121$, Z = 4, μ(CuKα) = 0.884 mm^{-1}, 13672 reflections measured, 4377 independent reflections (R_{int} = 0.0618). The final R_1 values were 0.0636 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.1807 ($I > 2\sigma(I)$). The final R_1 values were 0.0662 (all data). The final $wR(F^2)$ values were 0.1826 (all data). The goodness of fit on F^2 was 1.116. The Flack parameter was 0.08 (10). The Hooft parameter was 0.09 (12) for 1784 Bijvoet pairs.

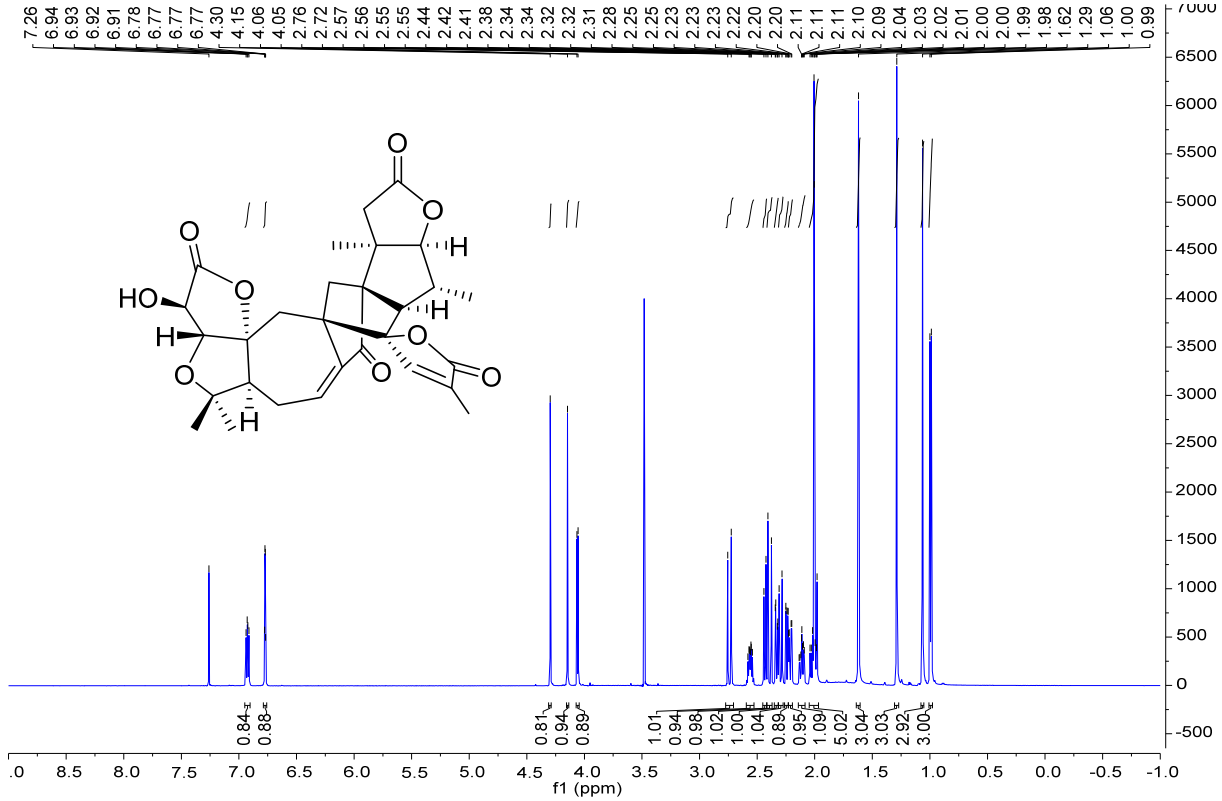
S2. HR-ESI-MS interpretation of 1

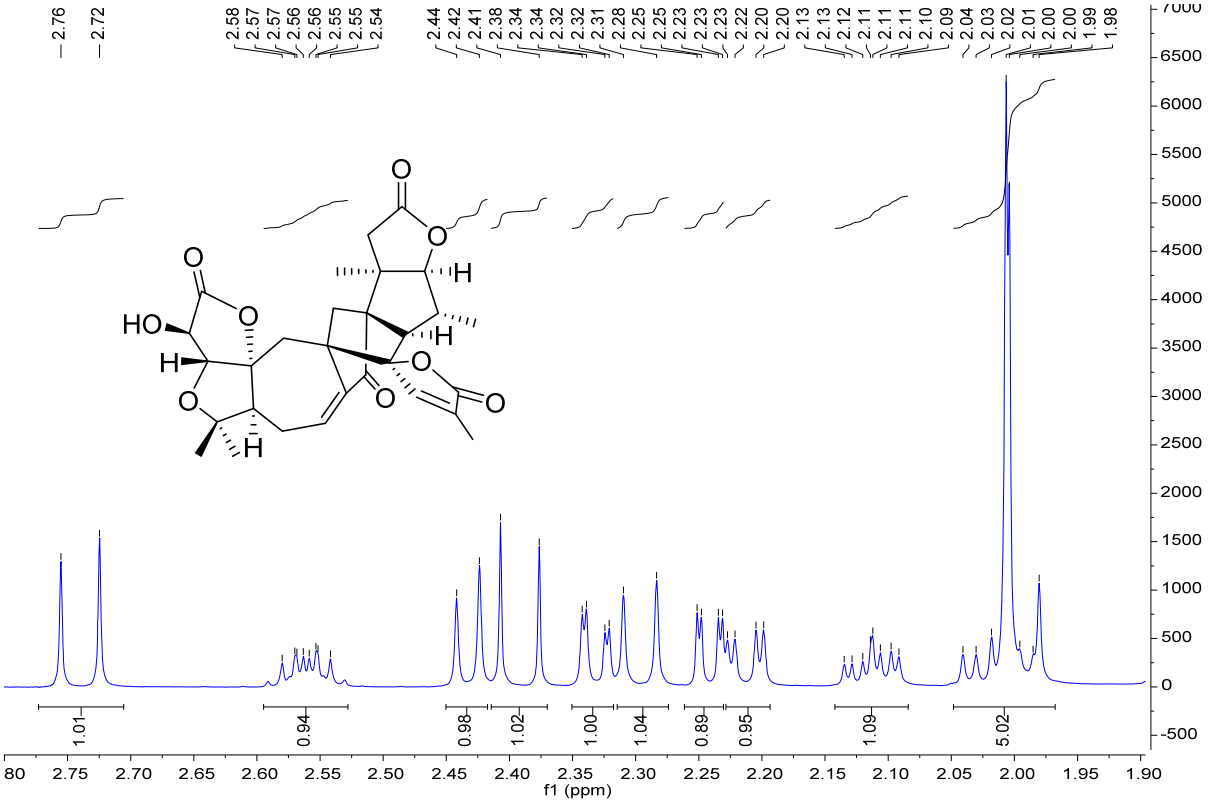


S3. Crystal cell diagram for 1

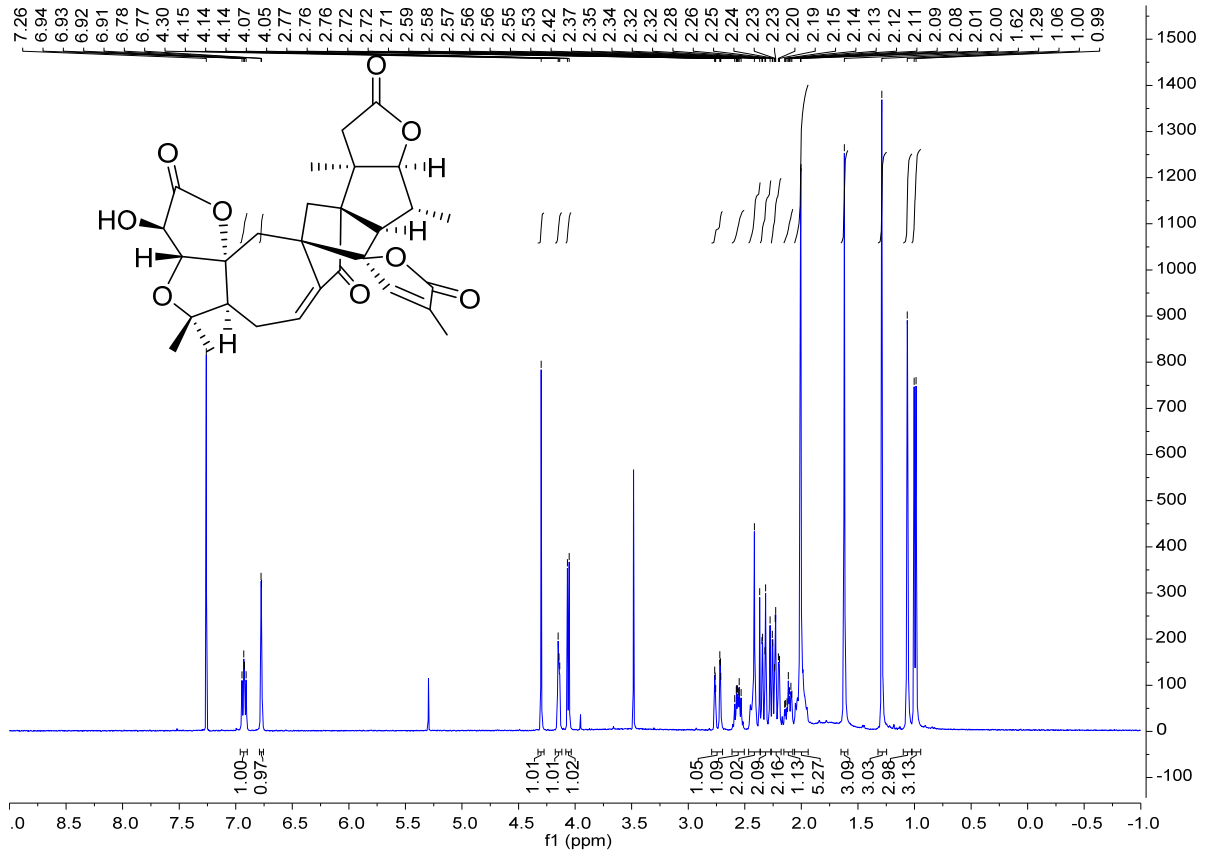


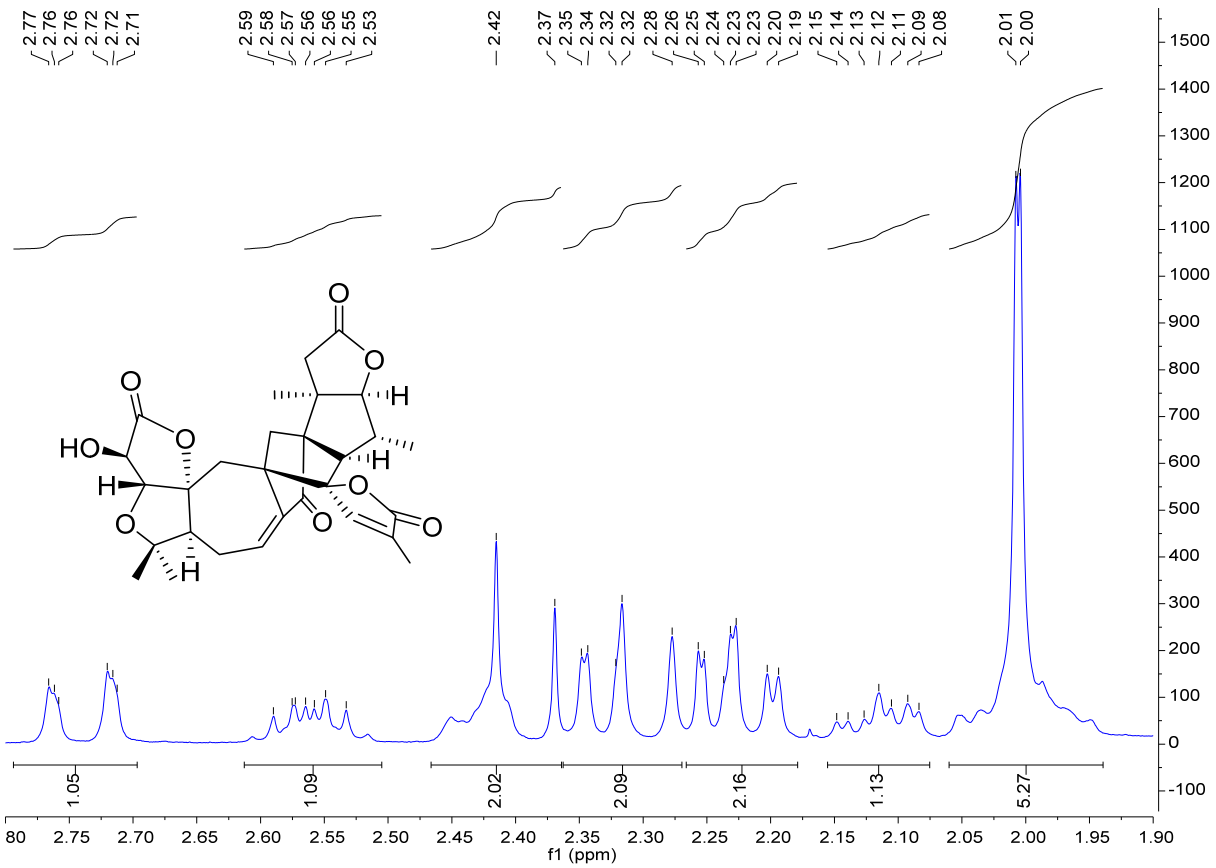
S4-1. ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of 1



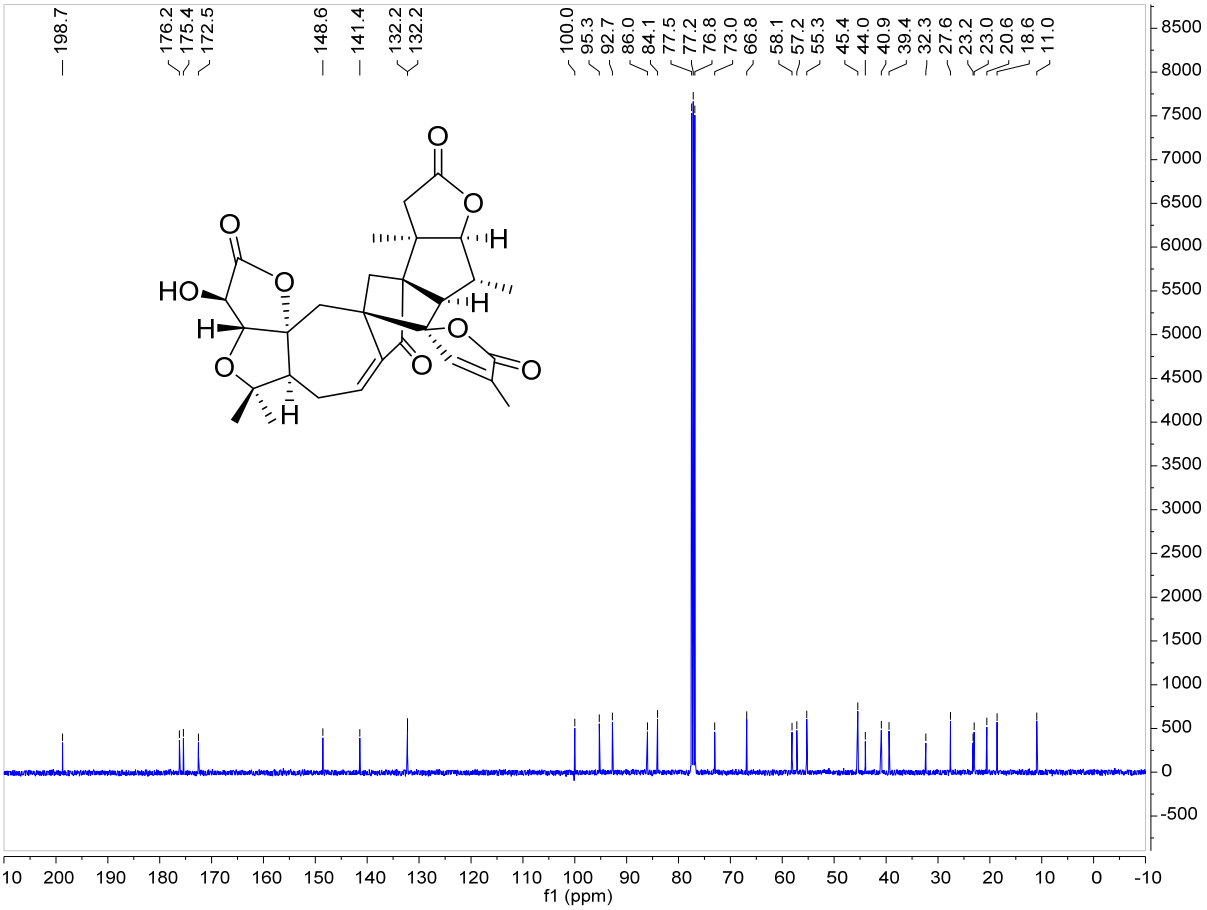


S4-2. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of 1

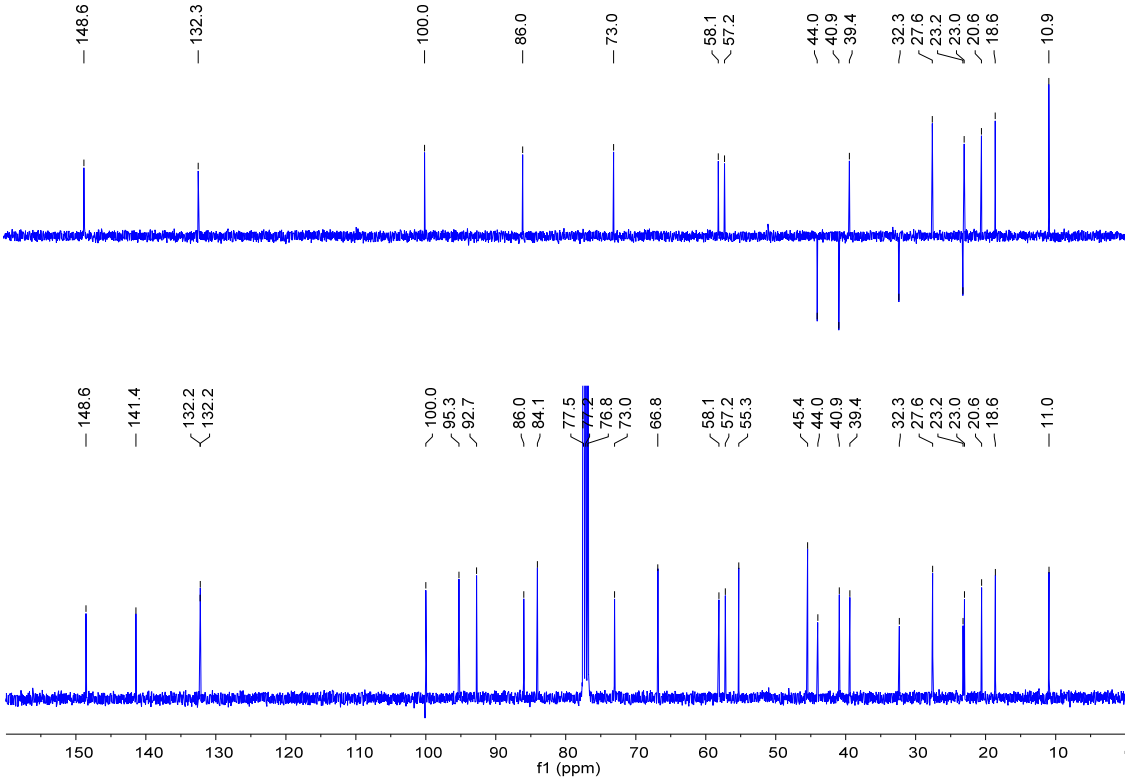




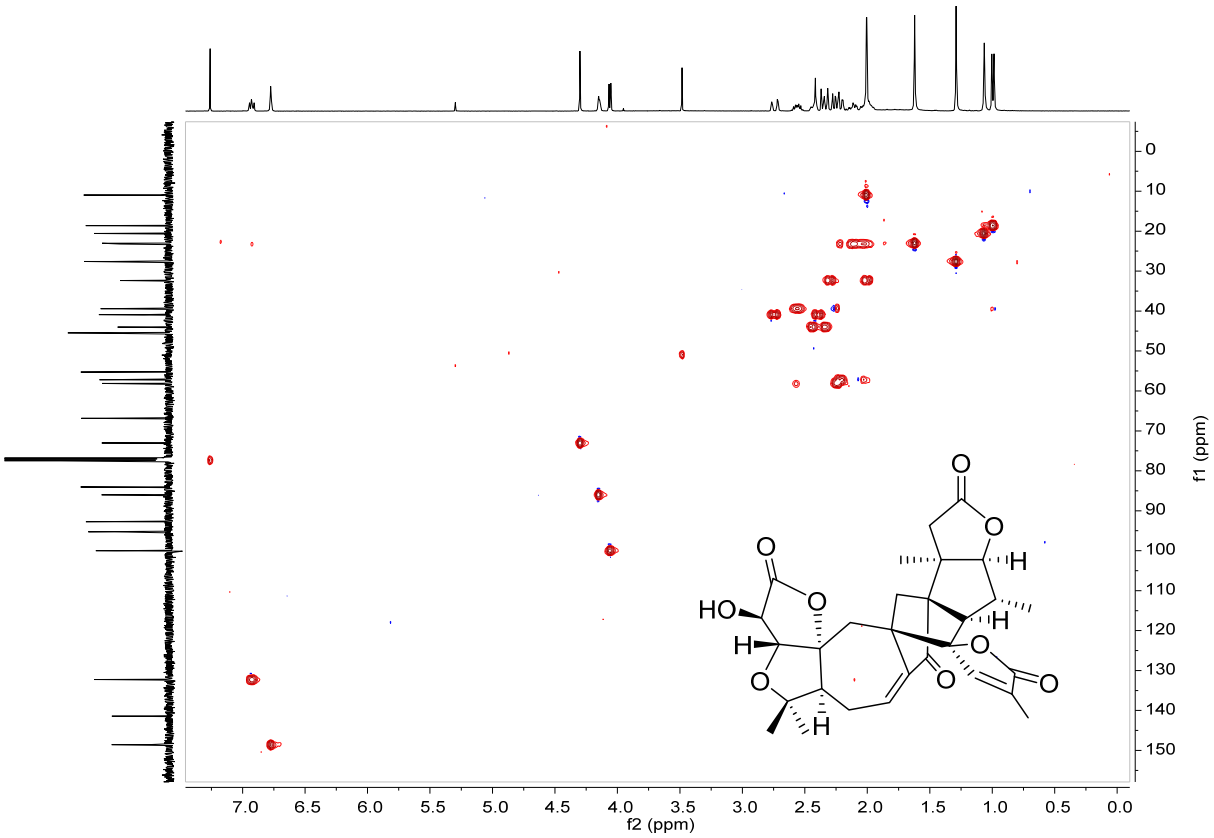
S5. ^{13}C NMR spectrum (101 MHz, $CDCl_3$) of 1



S6. DEPT-135 spectrum (101 MHz, $CDCl_3$) of 1



S7. HSQC spectrum (400 MHz, $CDCl_3$) of 1



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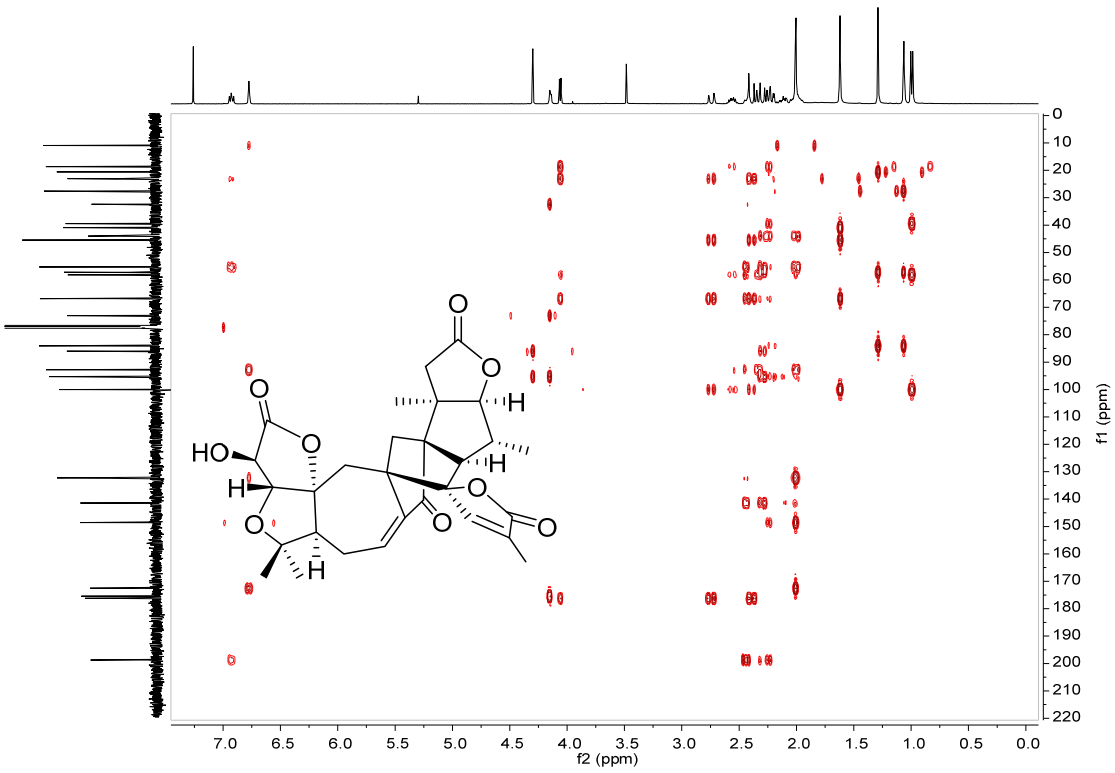
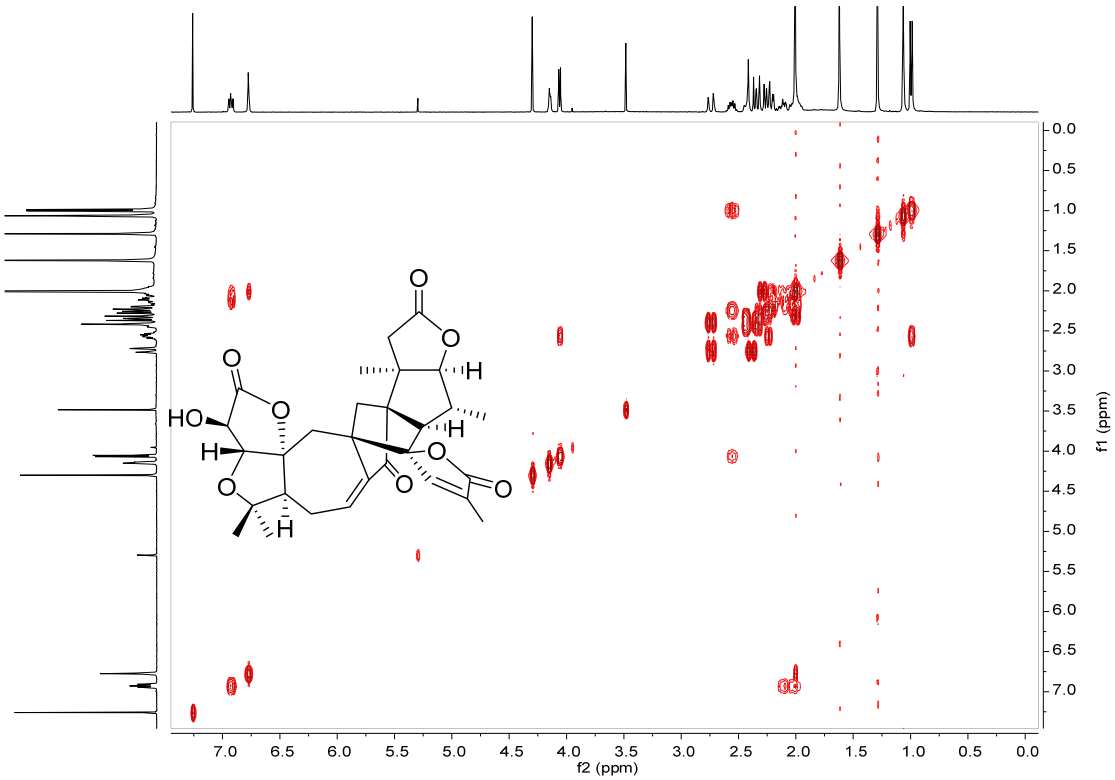
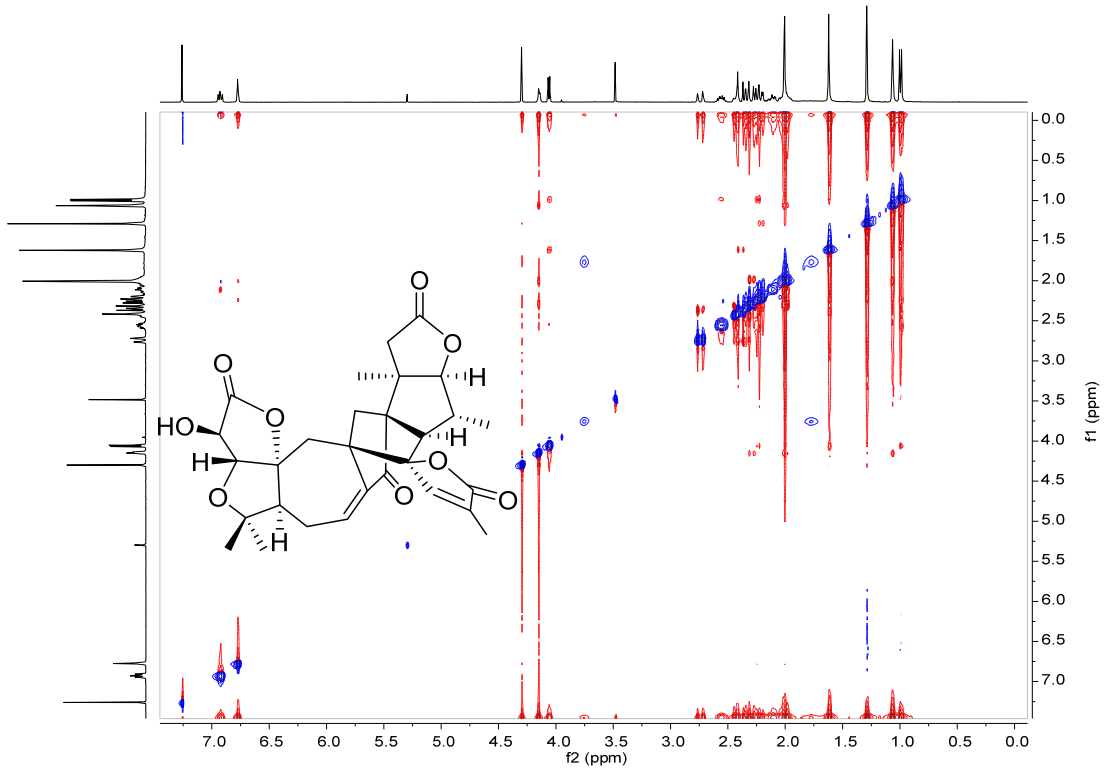


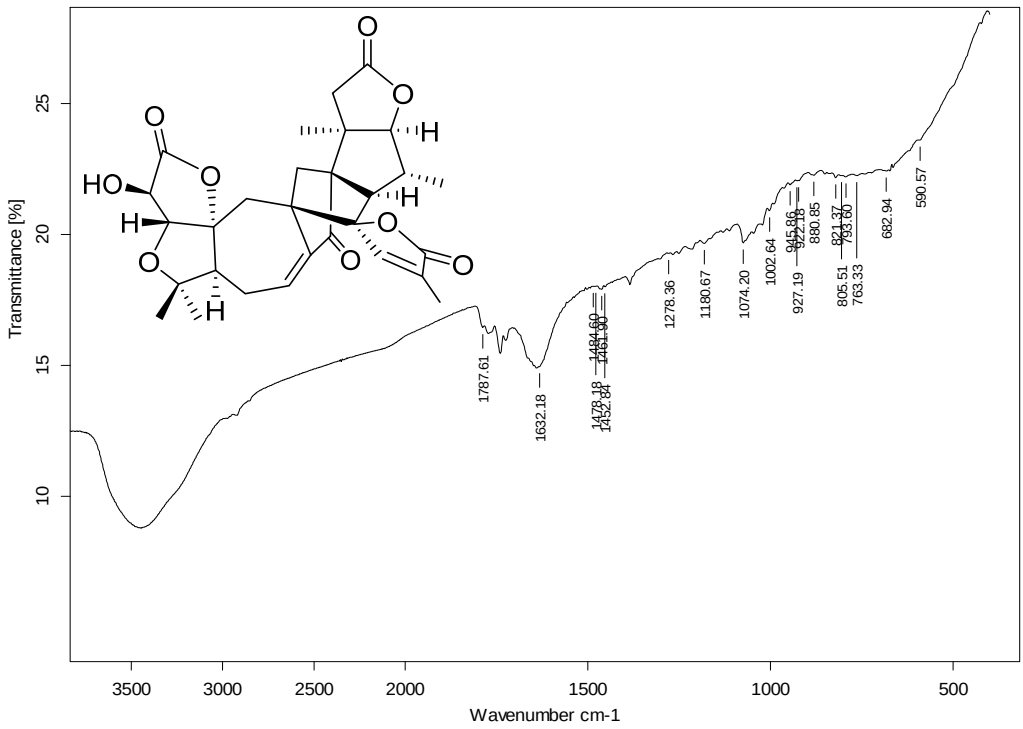
S9. ^{1}H-^{1}H COSY spectrum (400 MHz, $CDCl_3$) of 1



S10. NOESY spectrum (400 MHz, $CDCl_3$) of 1



S11. IR spectrum of 1



S12. CD spectrum of 1