SUPPLEMENTARY MATERIAL

Aculeaquamide A, cytotoxic paraherquamide from the marine fungus *Aspergillus aculeatinus* WHUF0198

Jun Wu[a]*, Fuqian Wang[b]*, Li-Ming He[a], Si-Yuan Zhou[a], Shou-Bao Wang[c], Jia Jia[d], Kui Hong[a] and You-Sheng Cai[a]

[a] *School of Pharmaceutical Sciences, Wuhan University, Wuhan 430071, P. R. China;* [b] *Department of Pharmacy, Wuhan No. 1 Hospital, Wuhan 430022, P. R. China;* [c] *Beijing Key Laboratory of Drug Targets Identification and Drug Screening, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, 100050, P. R. China;* [d] *Department of Pathogen Biology & Jiangsu Key Laboratory of Pathogen Biology & Helicobacter pylori Research Centre, Nanjing Medical University, Nanjing 211166, P. R. China*

Corresponding author e-mail Kui Hong: kuihong31@whu.edu.cn; You-Sheng Cai: cysh2002@whu.edu.cn

*These authors contributed equally to this work.

Abstract

A new paraherquamide named aculeaquamide A (**1**) was isolated from an EtOAc extract of *Aspergillus aculeatinus* WHF0198 culture media together with five known compounds. The structures of the isolated compounds were elucidated by analysis of NMR and MS data, and the absolute configurations of compound **1** was confirmed by CD spectroscopic methods. All isolated compounds were evaluated for their cytotoxicity against three human cancer cell lines, Bel-7402, A549, and HCT-116. Compounds **1** and **2** showed cytotoxicity against Bel-7402 with IC_{50} values of 3.3 and 1.9 μM, respectively.

Keywords: Aspergillus aculeatinus; paraherquamide; cytotoxicities

List of Supplementary Material

1. Experimental

1.1 Mass Culture of A. aculeatinus WHUF0198

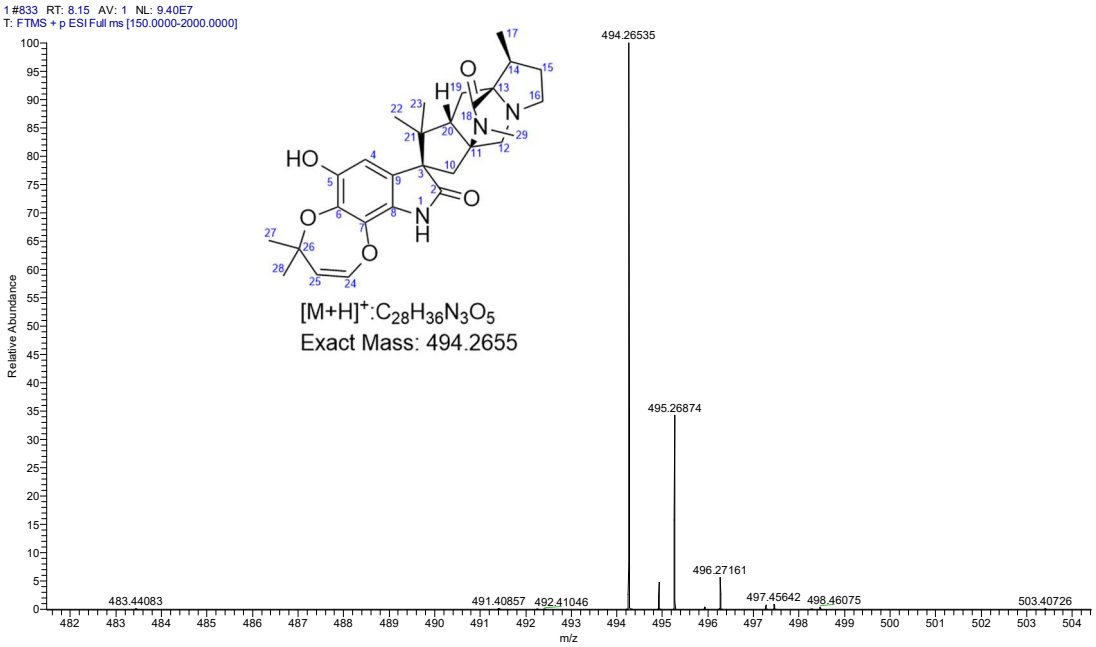
The *A. aculeatinus* WHUF0198 was initially inoculated into number II fungus agar medium (containing 10 g of glucose, 20 g of maltose, 20 g of mannitol, 10 g of MSG, 3 g of yeast extract, 1 g of corn syrup, 0.5 g of KH_2PO_4, 0.3 g of $MgSO_4$, 15 g of sea salt, 20 g of agar per 1 L of sterilized water, adjust to pH 6.5 by a 2 M NaOH solution) and incubated 5 days at 28℃. A portion (10 mL) of the fungal culture was transferred into 300 ml number II fungus liquid medium in a 1 L flask and culture 30 days in a static state at 28℃. A total of 60 flasks of the liquid medium were made for scale-up in this study.

1.2 Cytotoxicity Assay

The cytotoxicity of all the isolated compounds was tested against the A-549 human lung adenocarcinoma, the Bel-7402 human liver adenocarcinoma, and the HCT-116 human colon adenocarcinoma using MTT assay. All cell lines were purchased from China Center for Type Culture Collection (Wuhan, Hubei, People's Republic of China). All human cancer cells were cultured in RPMI-1640 cultural medium. All culture media contained 10% FBS (GIBCO), 100 units/mL penicillin, and 100 μg/mL streptomycin. Three cancer cells were cultured in an incubator under an atmosphere of 5% CO_2 at 37 °C. All compounds were dissolved in dimethyl sulfoxide (DMSO), stored at 4°C and diluted to desired concentrations in the medium. Cells were seeded into 96-well plates at a density of 4×10^3 cells per well. Cells were permitted to adhere for 24 h and

treated with different concentrations of samples for another 72 h. Then, 20 μL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma) in phosphate-buffered saline (PBS) were added to each well for 4 h. The medium was replaced by 100 μL of DMSO and measured at 570 nm by a microplate ELISA reader (Thermo Labsystems). Proliferative inhibition rates (%) were calculated as [1-($A570_{treated}$ /$A570_{control}$)]×100. Three independent repeated trials were conducted for each compound (n = 3). IC_{50} values were determined with the Logit method. 5-Fluorouracil was used as a positive control (purity: 99%, National Institutes for Food and Drug Control).

Figure S1 HR-ESIMS spectra of **1**.



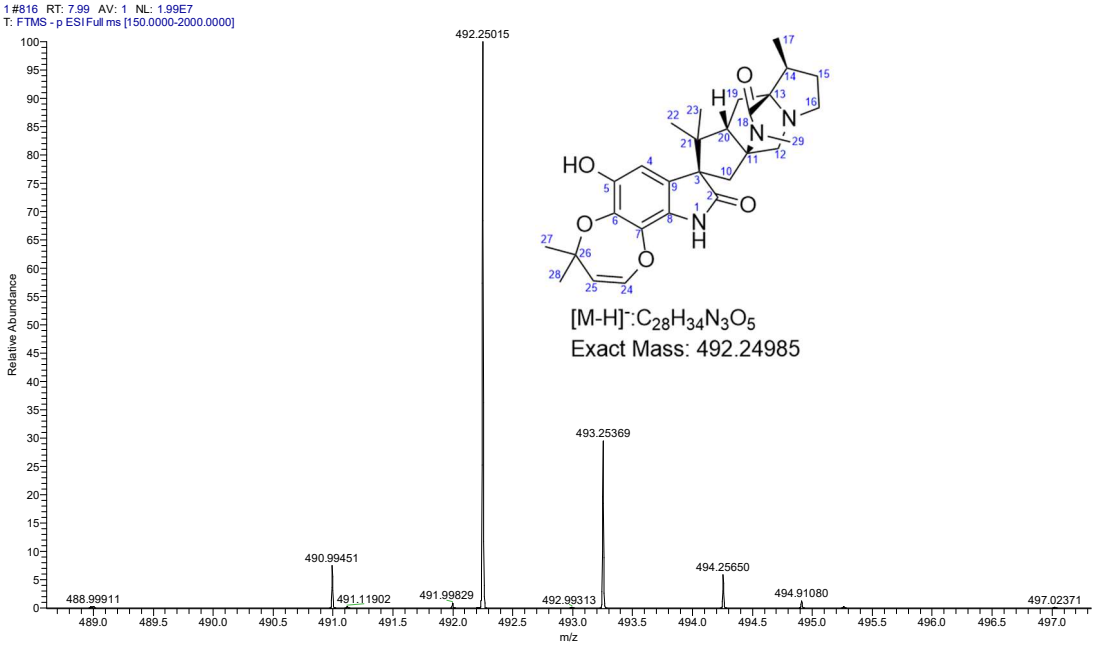


Figure S2 ^{1}H NMR spectrum of **1** (600 MHz, pyridine-d_5).

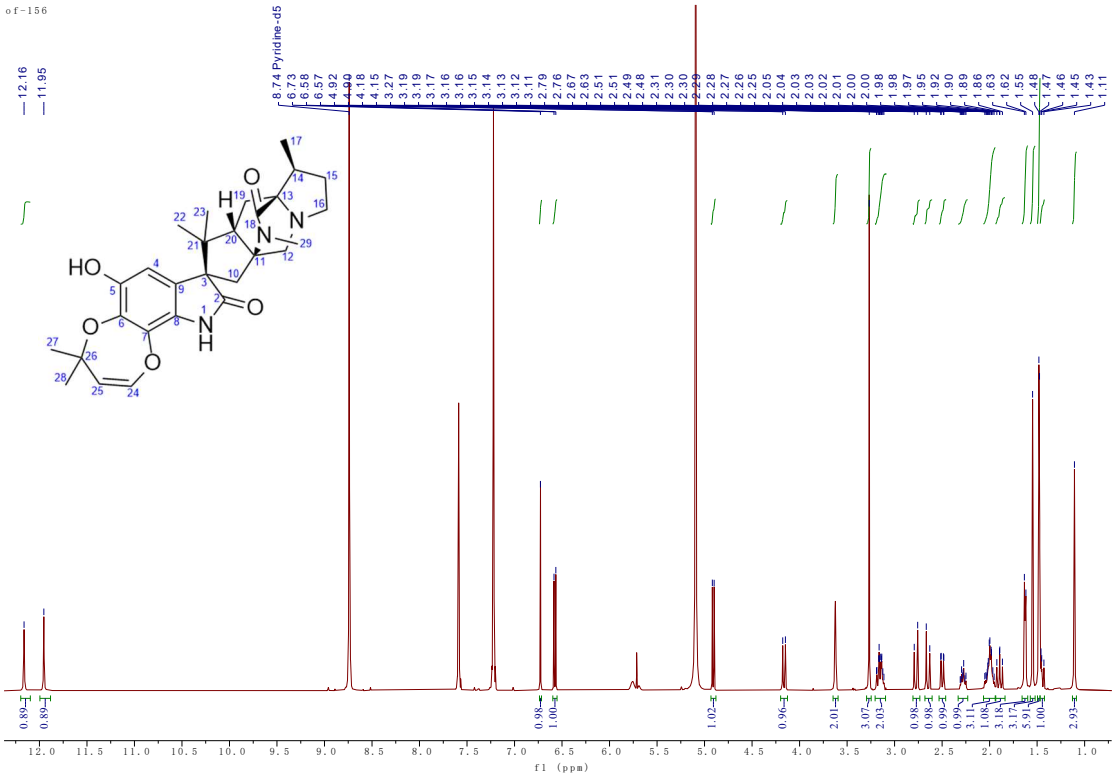


Figure S3 ^{13}C NMR spectrum of **1** (150 MHz, pyridine-d_5).

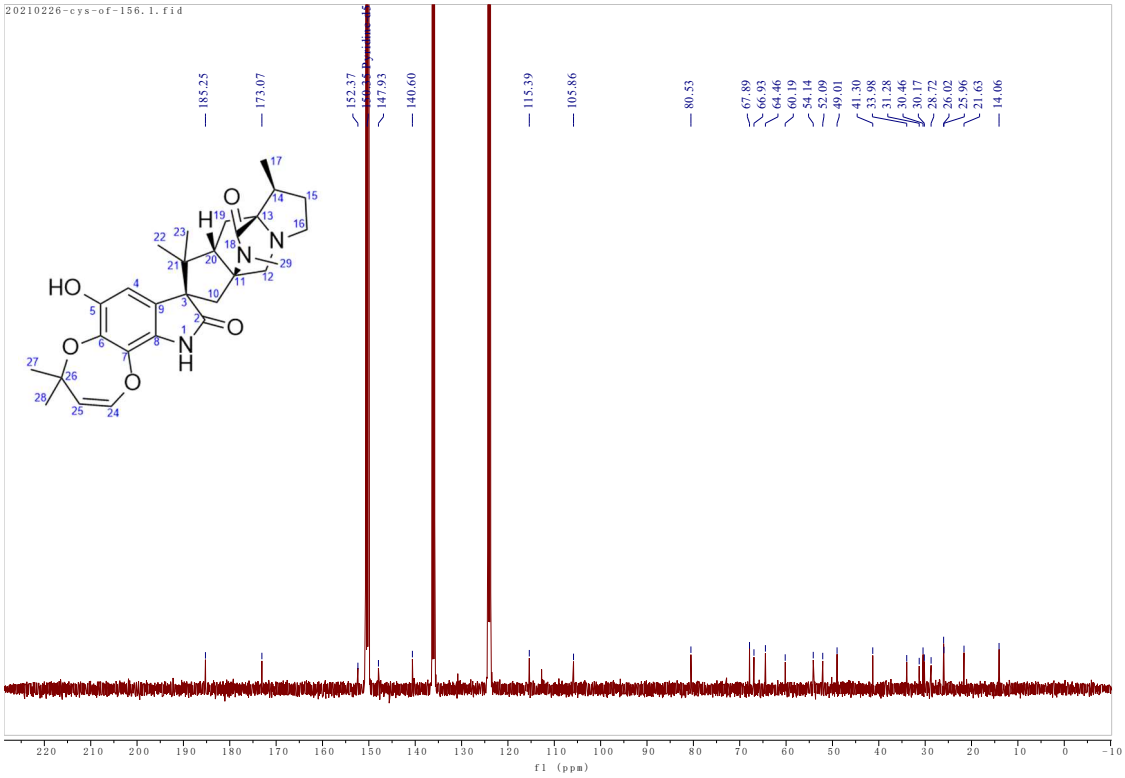


Figure S4 ^{1}H-^{1}H COSY spectrum of **1** (600 MHz, pyridine-d_5).

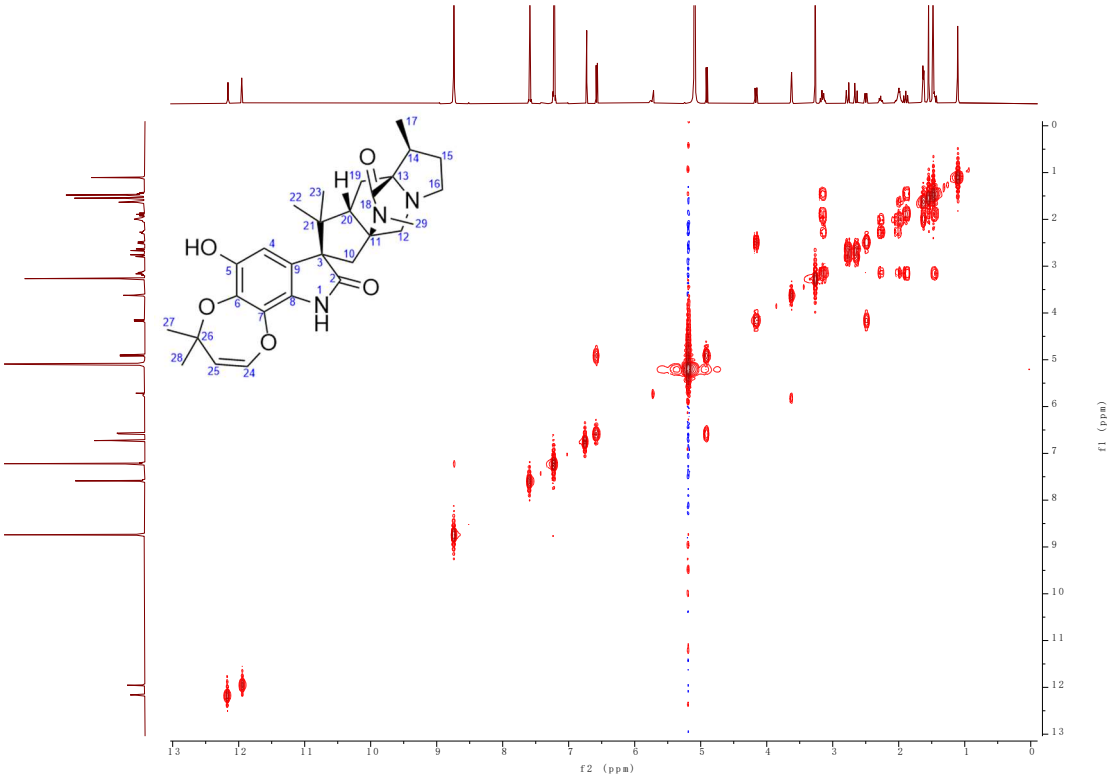


Figure S5 HSQC spectrum of **1** (600 MHz, pyridine-d_5).

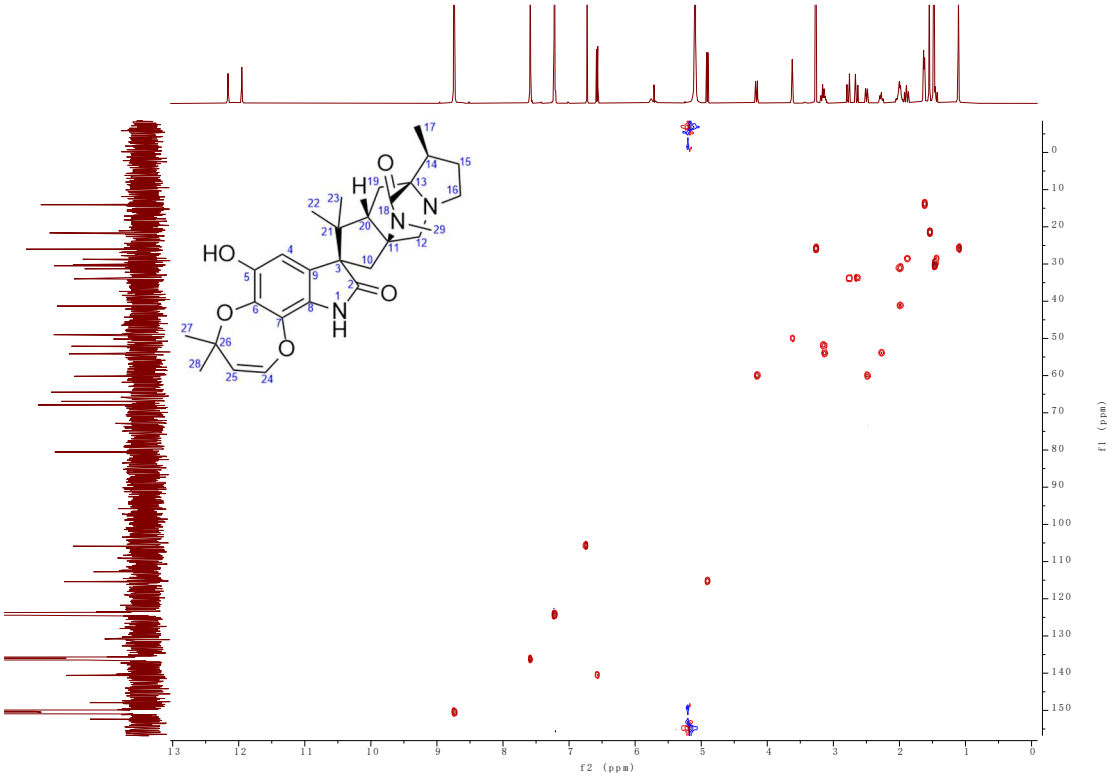


Figure S6 HMBC spectrum of **1** (600 MHz, pyridine-d_5).

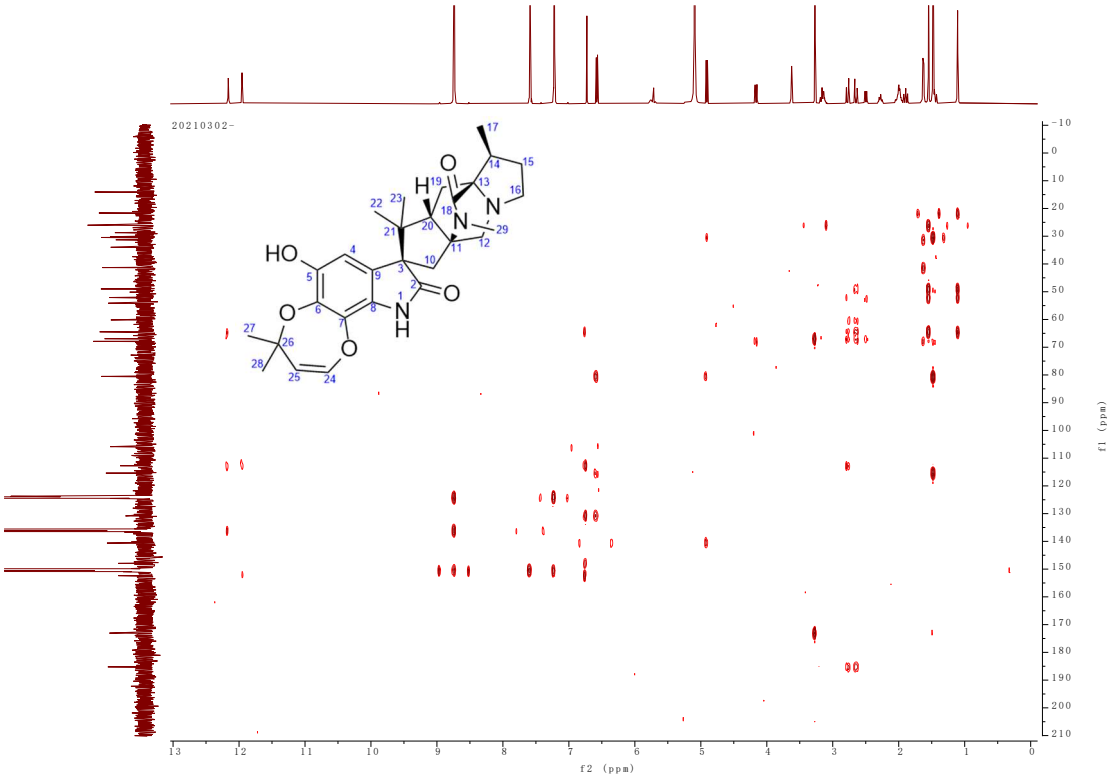


Figure S7 NOESY spectrum of **1** (600 MHz, pyridine-d_5).

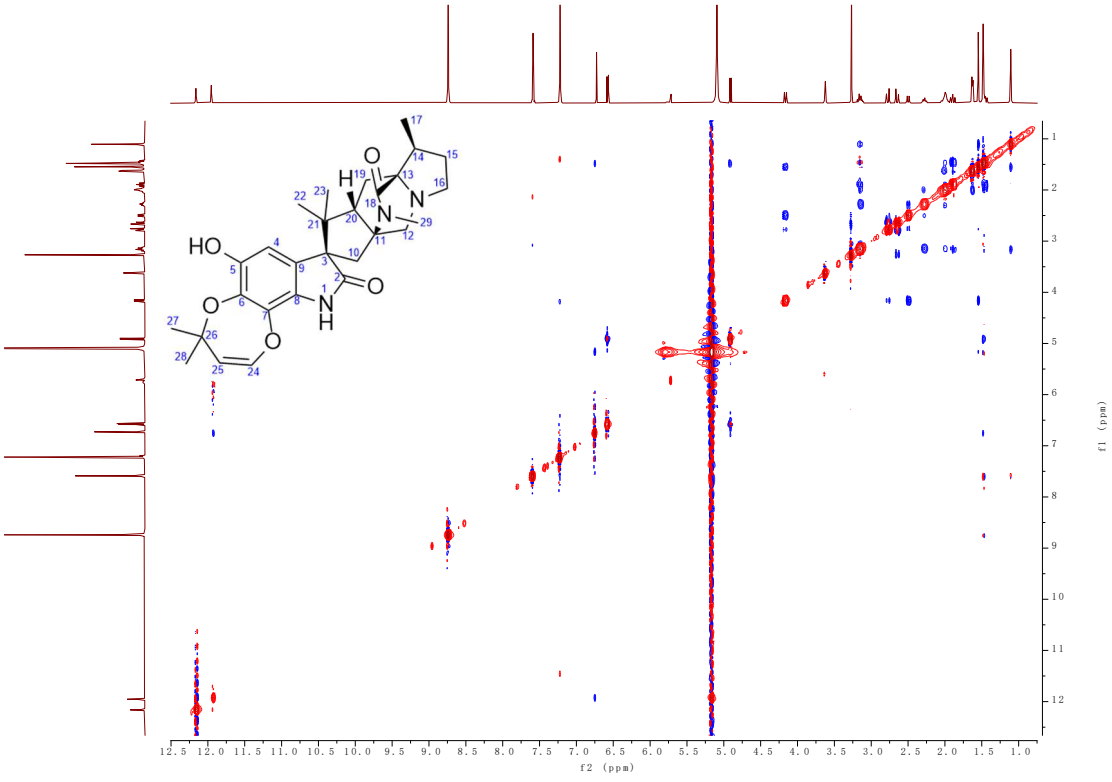


Figure S8 Experimental ECD spectrum of **1**.

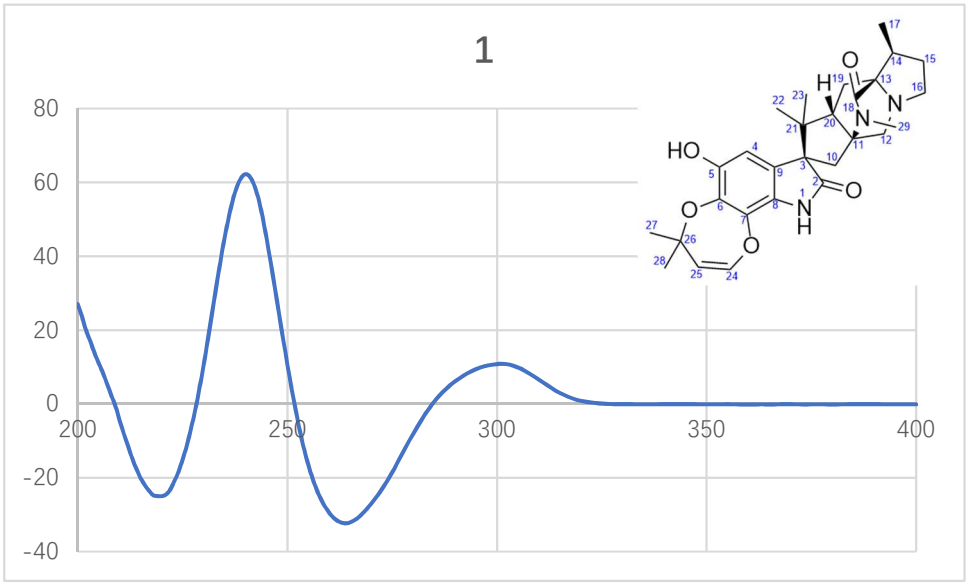


Figure S9 Experimental ECD spectrum of **2**.

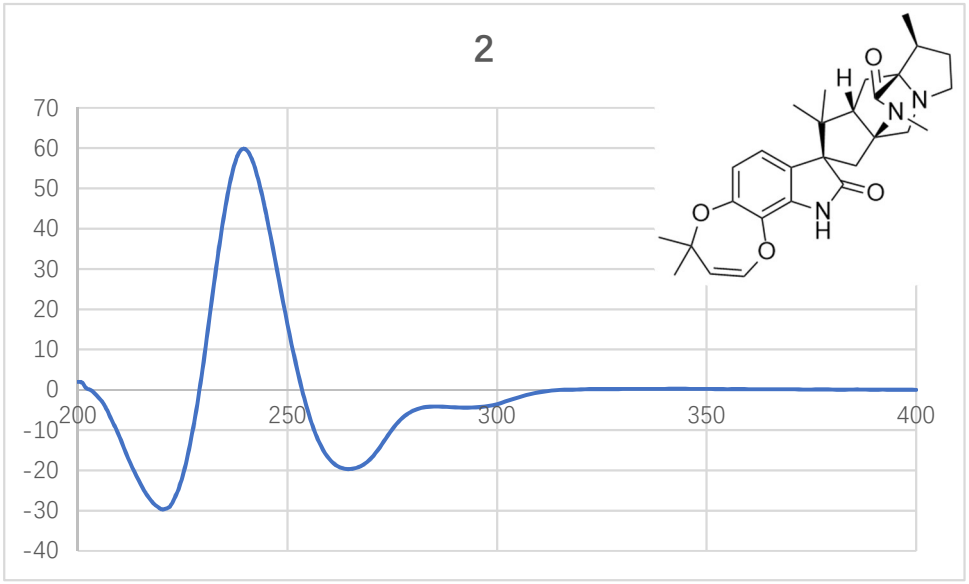


Figure S10 ^{1}H NMR spectrum of **2** (400 MHz, CD_3OD).

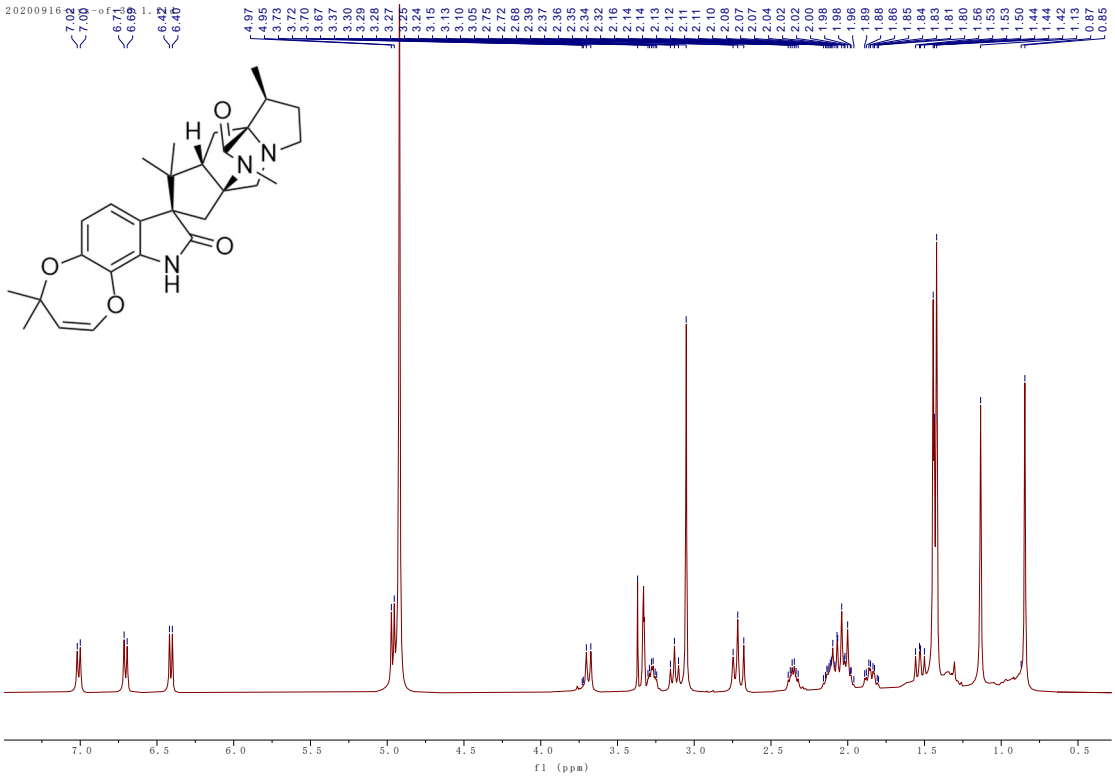


Figure S11 ^{13}C NMR spectrum of **2** (100 MHz, CD_3OD).

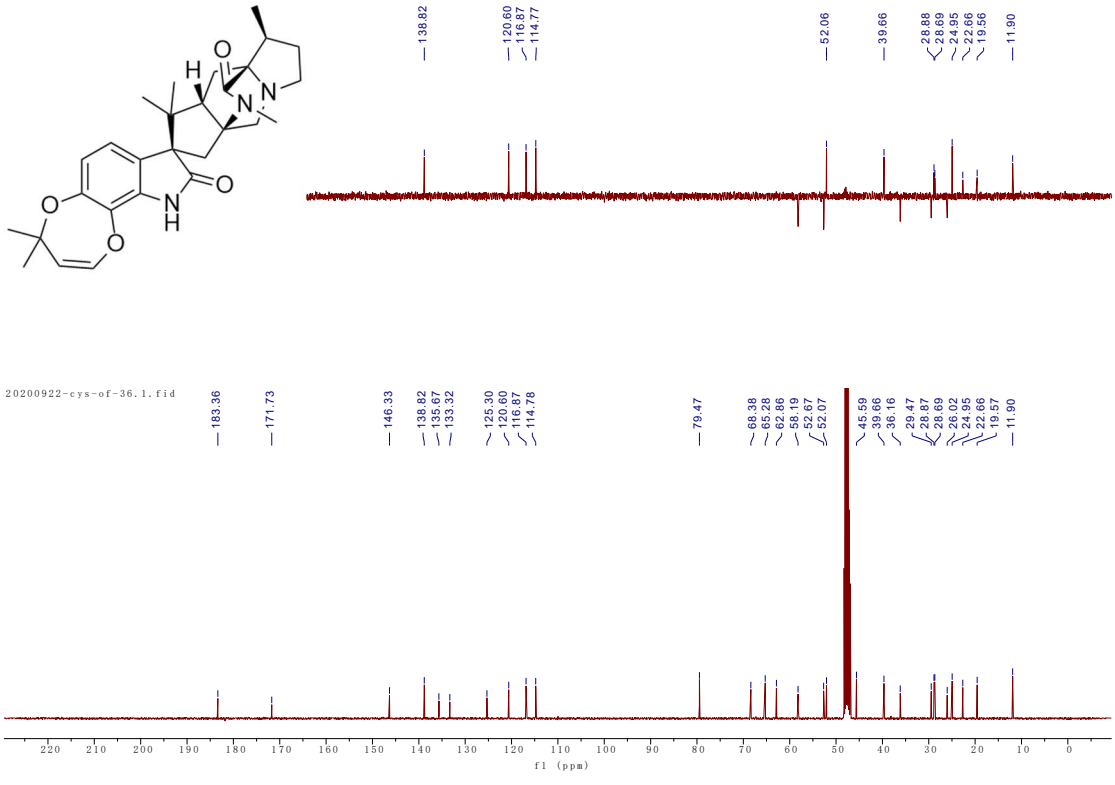


Figure S12 ^{1}H NMR spectrum of **3** (400 MHz, $CDCl_3$).

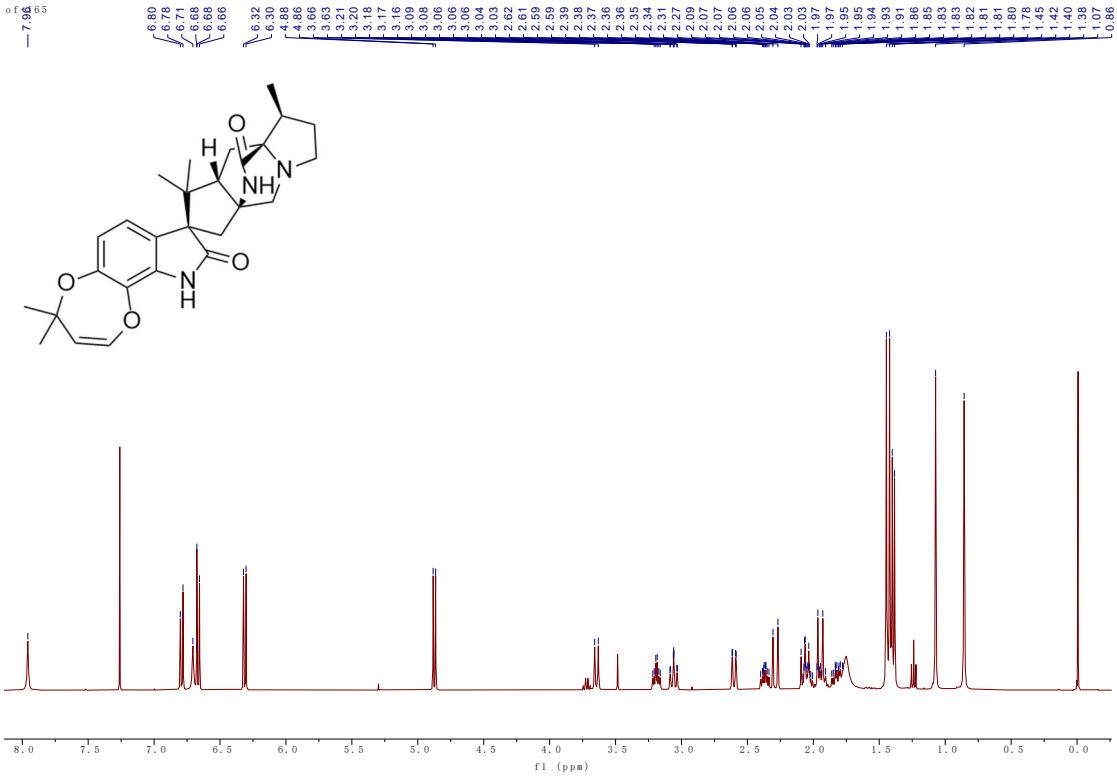


Figure S13 ^{1}H NMR spectrum of **4** (400 MHz, $CDCl_3$).

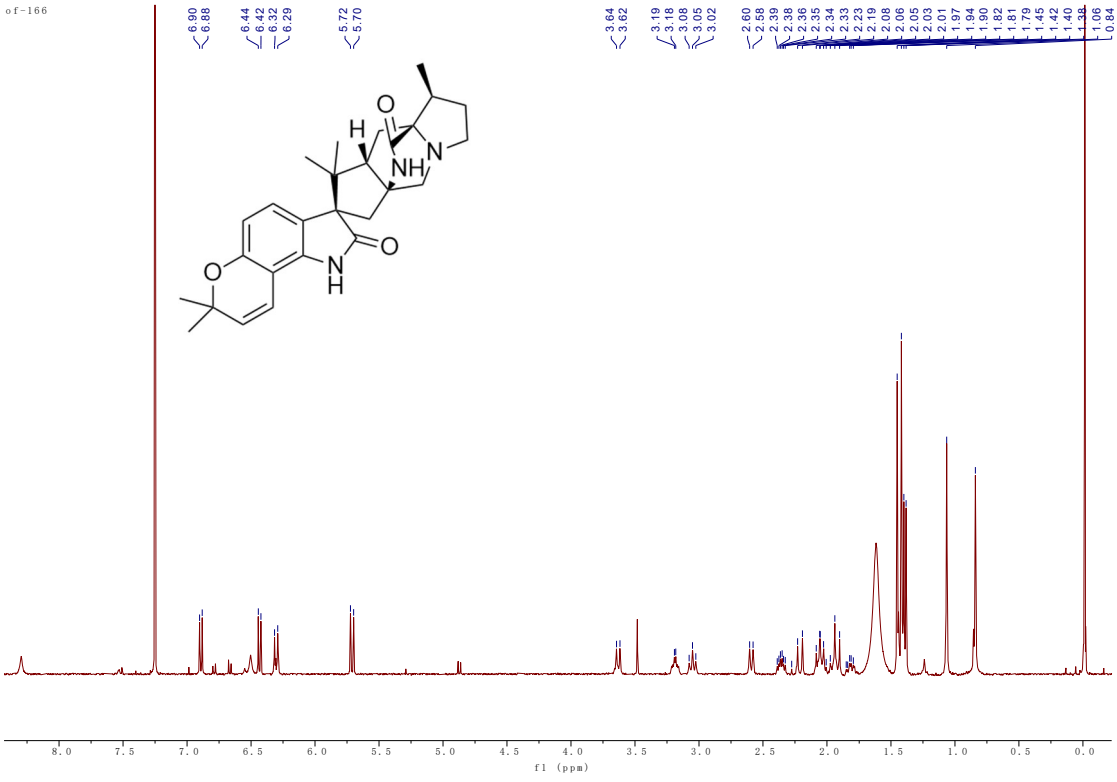


Figure S14 ^{1}H NMR spectrum of **5** (400 MHz, $CDCl_3$).

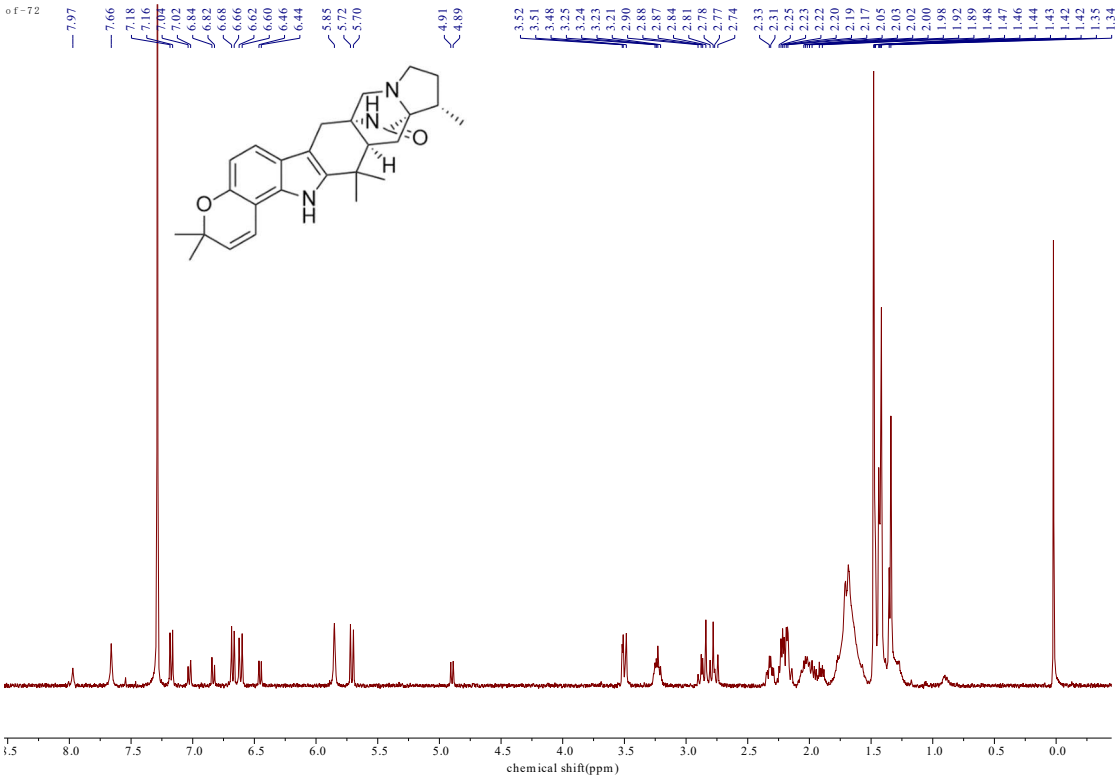


Figure S15 HR-ESIMS spectrum of **6**.

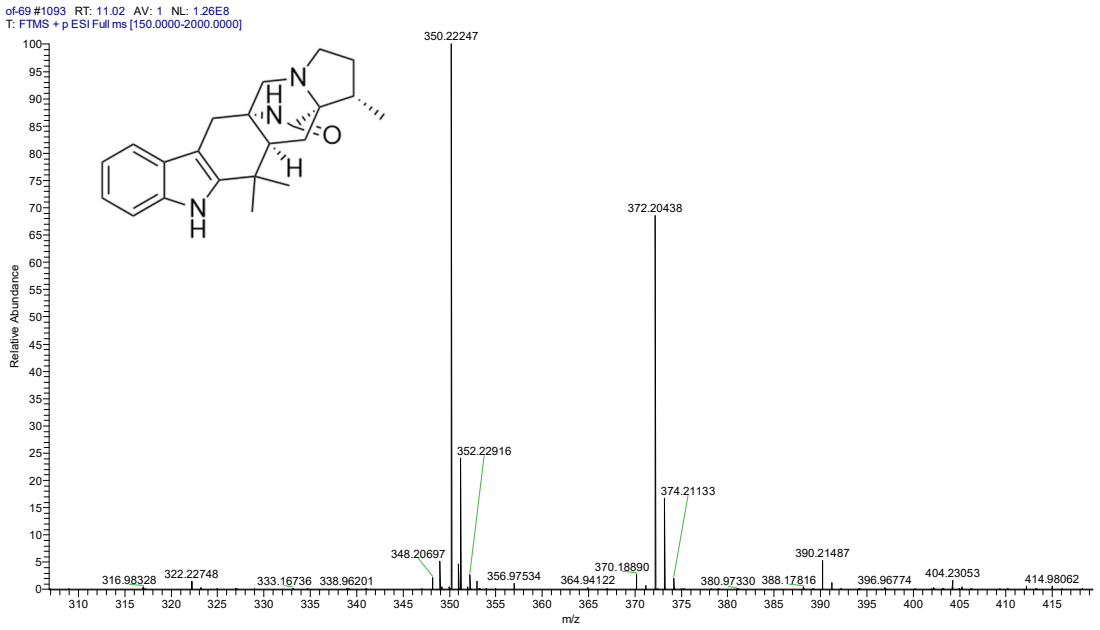


Figure S16 ^{1}H NMR spectrum of **6** (400 MHz, CD_3OD).

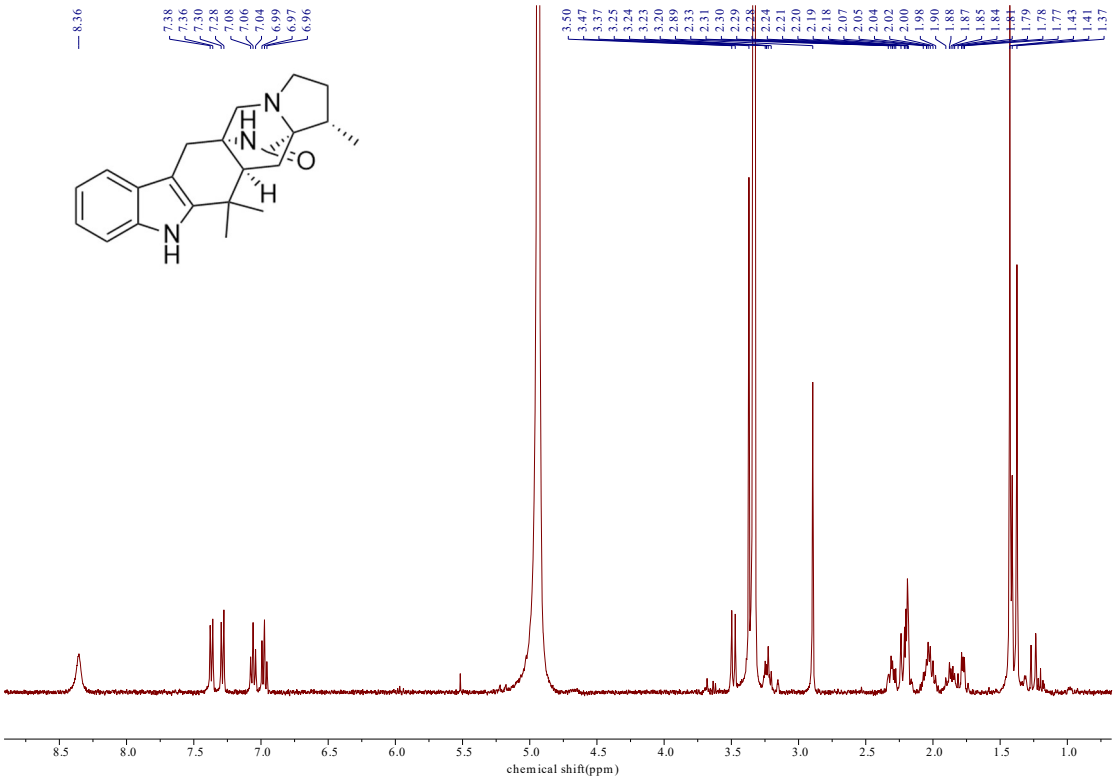


Figure S17 ^{13}C NMR spectrum of **6** (100 MHz, CD_3OD).

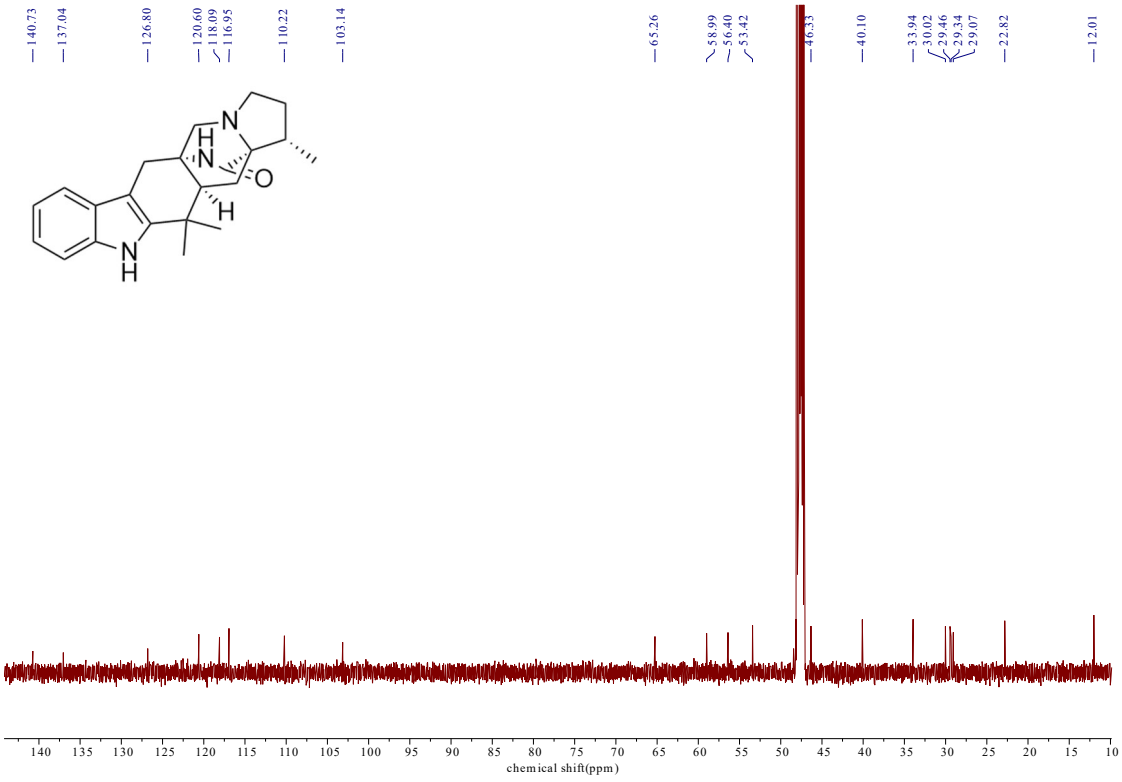


Figure S18. ^{1}H-^{1}H COSY, HMBC, and NOESY correlations of compound **1**.

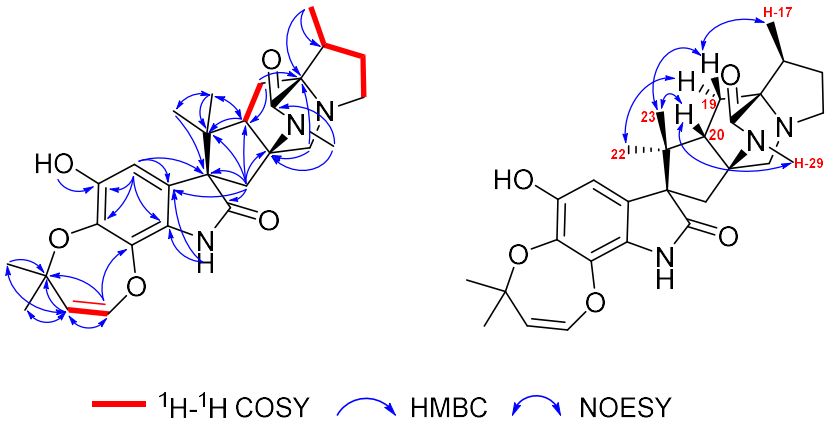


Table S1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Data for **1** in pyridine-d_5.

No.	δ_C	δ_H, J (Hz)	No.	δ_C	δ_H, J (Hz)
2	185.3		17	14.1	1.63 d (6.3)
3	64.5		18	173.1	
4	105.9	6.73 s	19	28.7	1.89 dd (12.4, 11.1, H-19$_\beta$); 1.46 m (H-19$_\alpha$)
5	152.4		20	52.1	3.16 m
6	147.9		21	49.0	
7	130.8		22	21.6	1.55 s
8	136.6		23	26.0	1.11 s
9	112.7		24	140.6	6.58 d (7.6)
10	34.0	2.78 d (14.5, H-10$_\alpha$); 2.65 d (14.5, H-10$_\beta$)	25	115.4	4.91 d (7.6)
11	66.9		26	80.5	
12	60.2	4.16 d (11.0); 2.50 dd (11.0, 1.6)	27	30.5	1.48 s
13	67.9		28	30.2	1.47 s
14	41.3	2.00 m	29	26.0	3.27 s
15	31.3	2.00 m	5-OH		11.95 s
16	54.2	3.14 m; 2.28 m	1-NH		12.16 s