Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Obscurinin A, a unique *Lycopodium* alkaloid possessing an 8/6/6/6/5 pentacyclic system isolated from *Lycopodium obscurum* L.

Bei-Bei Gao,§[a,b] Wen-Yan Li ,§[a,b] Shuai Jiang,[a,b] and Qin-Shi Zhao ➲,[a]

[a]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China. Email: qinshizhao@mail.kib.ac.cn

[b]University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China.

§ These authors contributed equally to this work.

Supporting Information

Table of Contents

1. General experimental procedures

1D and 2D NMR were measured on the Bruker Avance III 600 spectrometer (Bruker, Fällanden, Switzerland) with TMS as an internal standard. X-ray crystal diffraction data was recorded on the Bruker D8 Quest diffractometer (Bruker, Karlsruhe, Germany) using a copper target (Cu Kα) as a light source. HRESIMS spectrum was run on an Agilent 1290 UPLC/6540 Q-TOF spectrometer (Agilent, California, America). IR was carried out on a Bruker PMA-50 Vibrational Circular Dichroism Spectrometer (Bruker Optics, Ettlingen, Germany) with the KBr pellet. The optical rotation data was run on a JASCO P-1020 digital polarimeter (JASCO, Tokyo, Japan). The Shimadzu UV-2401A spectrophotometer (Shimadzu, Tokyo, Japan) was used to obtain the UV spectrum data. C_{18}-CE column (40 μm, 50 × 310 mm, Acchrom, Taizhou, China) and a Lisure EZ Purifier apparatus (Lisure Technology, Suzhou, China) were utilized. Melting points were measured using a WRX-4 micro melting point apparatus. Column chromatography was performed on Silica gel (200–300 mesh, Marine Chem. Co., Ltd., Qingdao, China). Semi-preparative HPLC was performed on an Agilent 1260 instrument with an X-Bridge C18 column (5 μm, 10 × 250 mm, Waters, Massachusetts, America).

2. Cell cultivation and expression

Human embryonic kidney (HEK) 293 cells (purchased from ATCC) were grown in DMEM (VivaCell, Shanghai, China) plus 10% newborn calf serum (VivaCell, Shanghai, China) and penicillin (100 U/ml)/streptomycin (0.1 mg/mL) (VivaCell, Shanghai, China) at 37 ℃ with 5% CO_2. HEK 293 cells were transfected using Lipofectamine 3000 (Invitrogen) with pCDNA3.1-human $Ca_v3.1$ and pCDNA3.1-eGFP and used in 48 hours.

3. Whole-Cell Voltage-Clamp Recordings

All the recordings were performed at room temperature (24°C). Cell membrane potential was held at -80 mV. The peak currents of $Ca_v3.1$ were elicited by 150 ms depolarization from a holding potential of -100 mV to -40 mV at 4 s intervals. the voltage step recording was elicited from -80 mV to +60 mV in 150 ms depolarization at 1.5 s intervals. Borosilicate glass micropipettes were pulled to produce a resistance of 2-6 MΩ (P-1000, Sutter Instrument) and filled with intracellular recording solution containing 130 mM CsCl, 2 mM $MgCl_2$, 10 mM EGTA, 5 mM Na-ATP, 10 mM HEPES (pH 7.2 with CsOH). The extracellular recording solution was composed of 145 mM CsCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM Glucose, 10 mM HEPES (pH 7.4 with CsOH). The current signals are amplified by the amplifier (SUTTER IPA-2). The currents are passed through a low energy filter at 2 kHz and then sampled at 10 kHz.

4. Data analysis and statistics

Data fitting and statistical analyses were performed using Graphpad Prism 8.0. IC_{50} values were determined by fitting the data points to a Hill equation with the form of $Y = {}_{Min} + ({}_{Max} - {}_{Min})/ [1+10(\text{Log } IC_{50} - C) \times \text{Hillslope}]$. Where IC_{50} is the concentration at which half-maximal currents were inhibited at the testing concentration range, C is the concentration of compounds, I_{Min} is the minimum stimulation ratio, I_{Max} is the maximum stimulation ratio, and

Hillslope is the Hill coefficient. All the data were presented as mean ± SD.

5. The NMR, HRESIMS, UV and IR Spectra of Compound 1

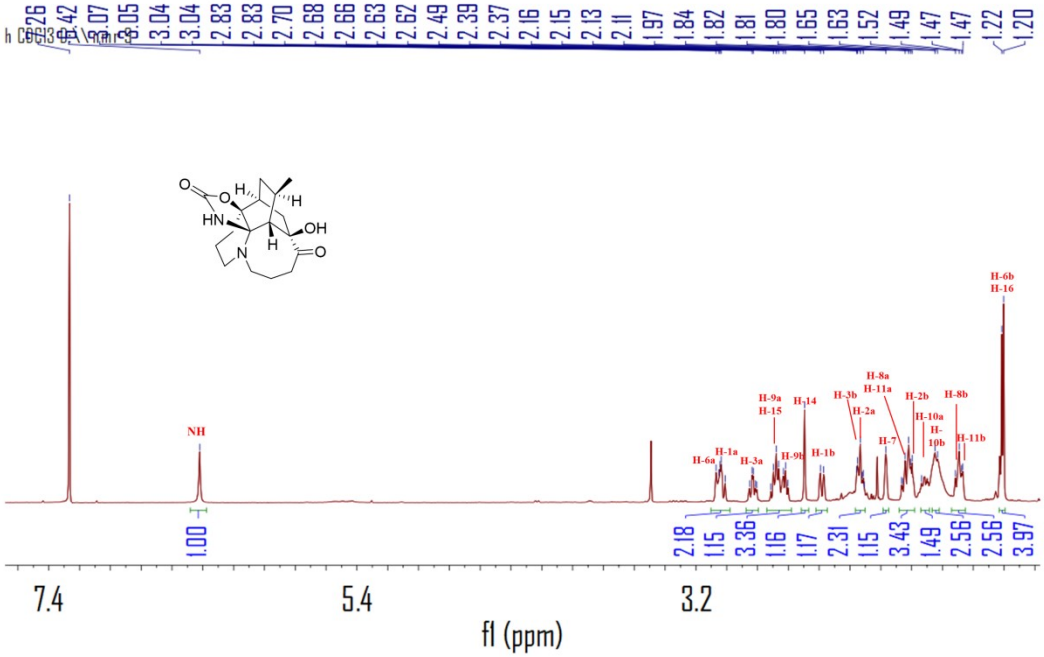


Figure S1. ^{1}H NMR spectrum of compound **1** (in $CDCl_3$, 600 MHz)

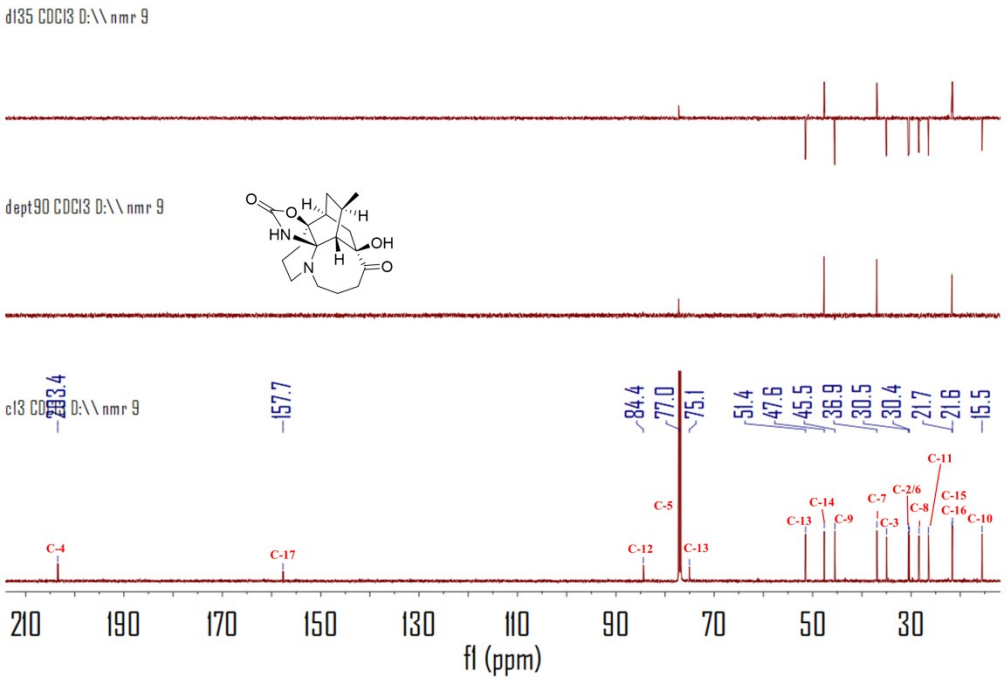


Figure S2. ^{13}C NMR and DEPT spectrum of compound **1** (in $CDCl_3$, 150 MHz)

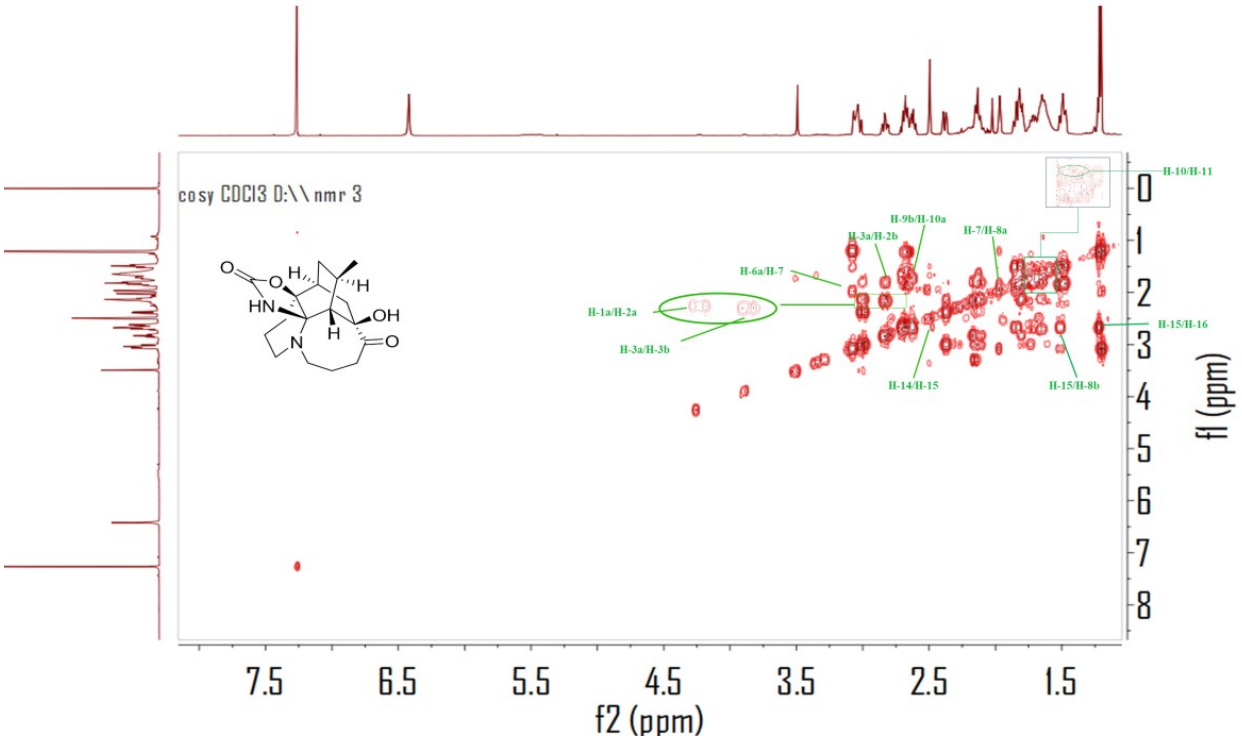


Figure S3. ^{1}H-^{1}H COSY spectrum of compound **1** (in $CDCl_3$, 600 MHz)

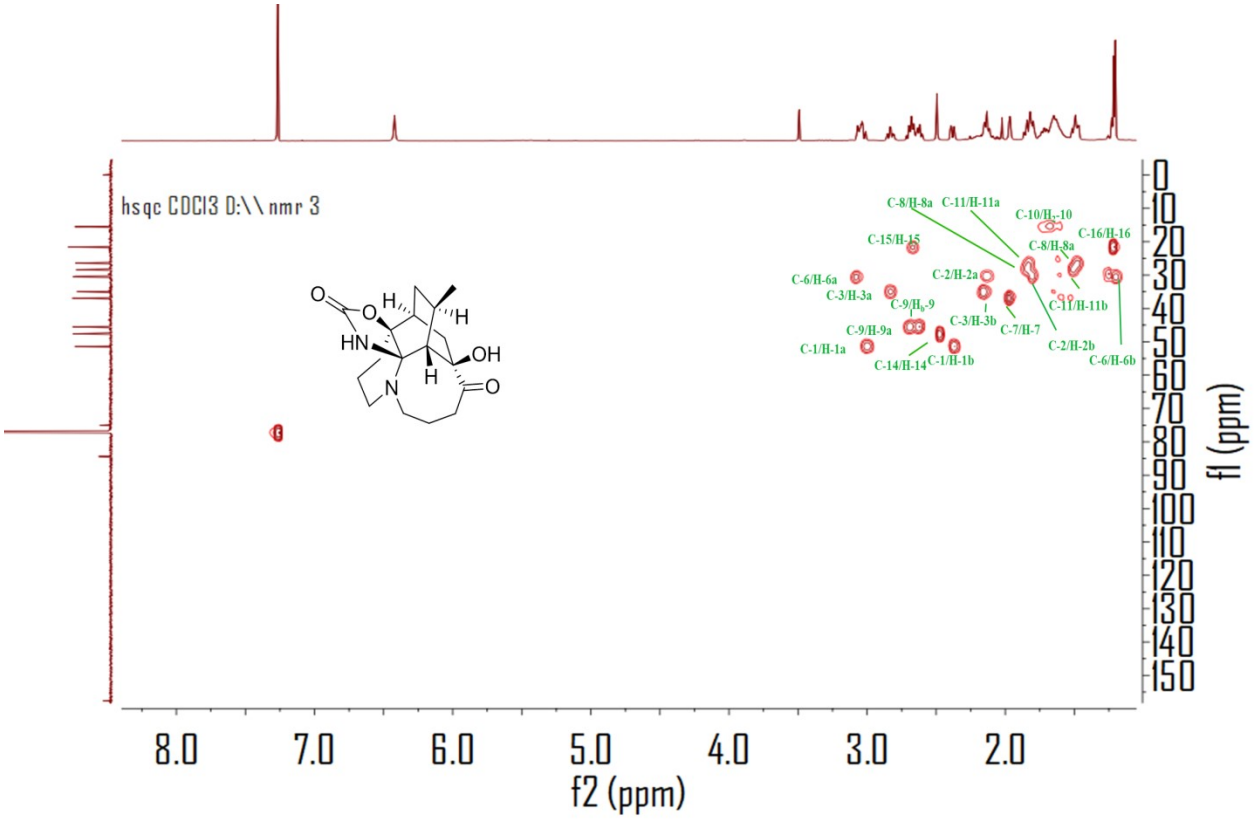


Figure S4. ^{1}H-^{13}C HSQC spectrum of compound **1** (in $CDCl_3$, 600 MHz)

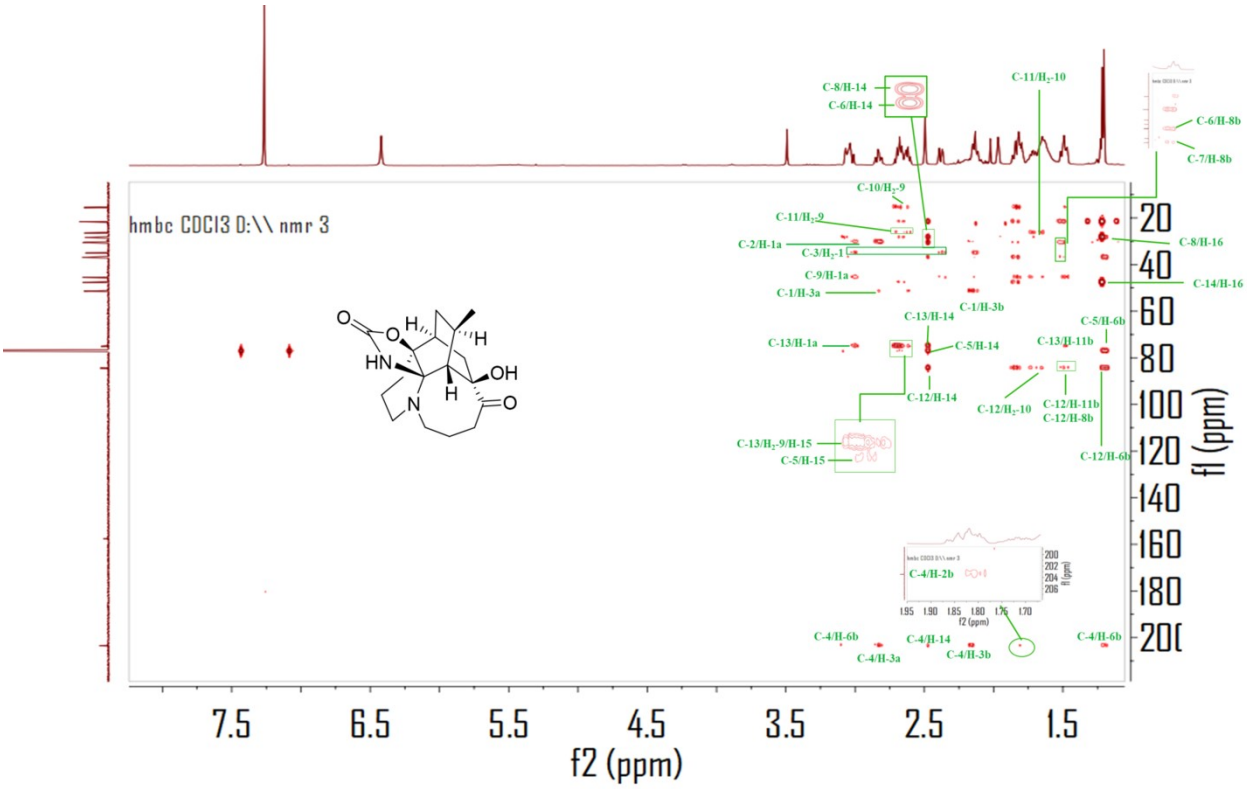


Figure S5. ^{1}H-^{13}C HMBC spectrum of compound **1** (in $CDCl_3$, 600 MHz)

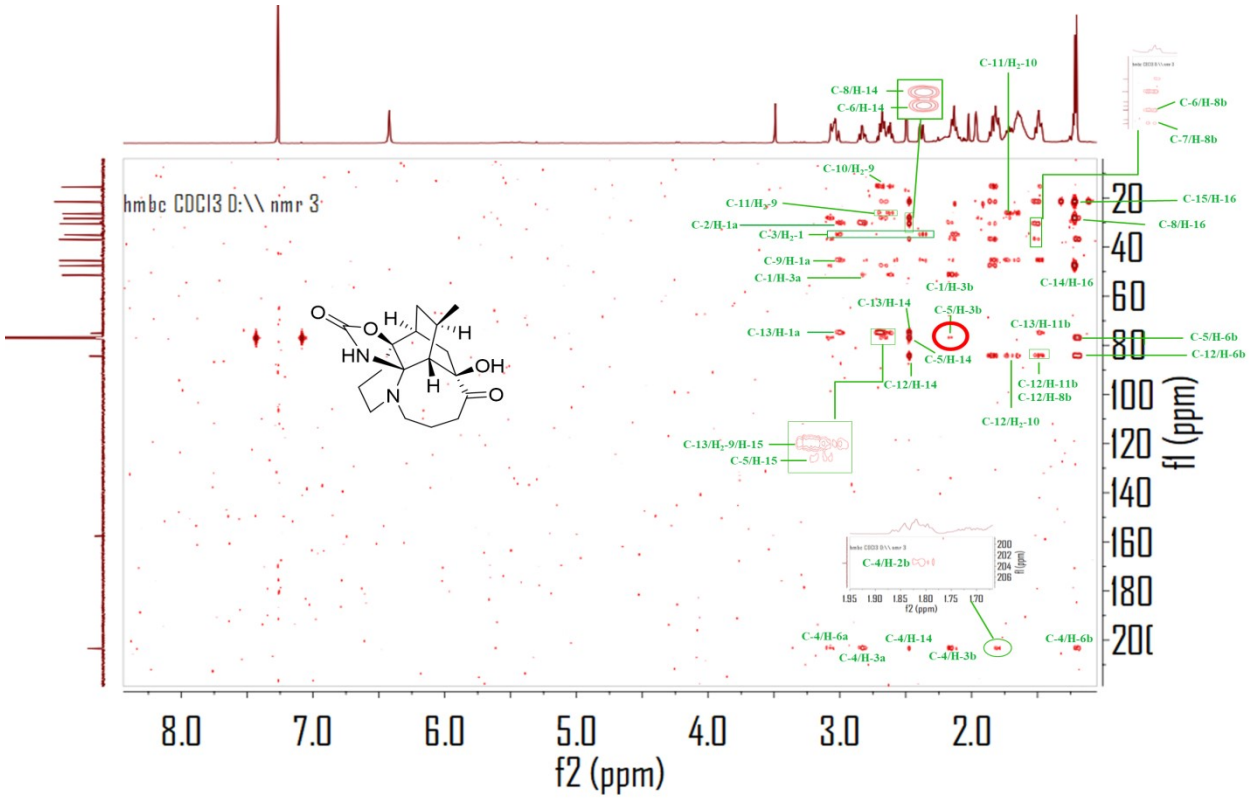


Figure S6. Amplified ^{1}H-^{13}C HMBC spectrum of compound **1** (in $CDCl_3$, 600 MHz)

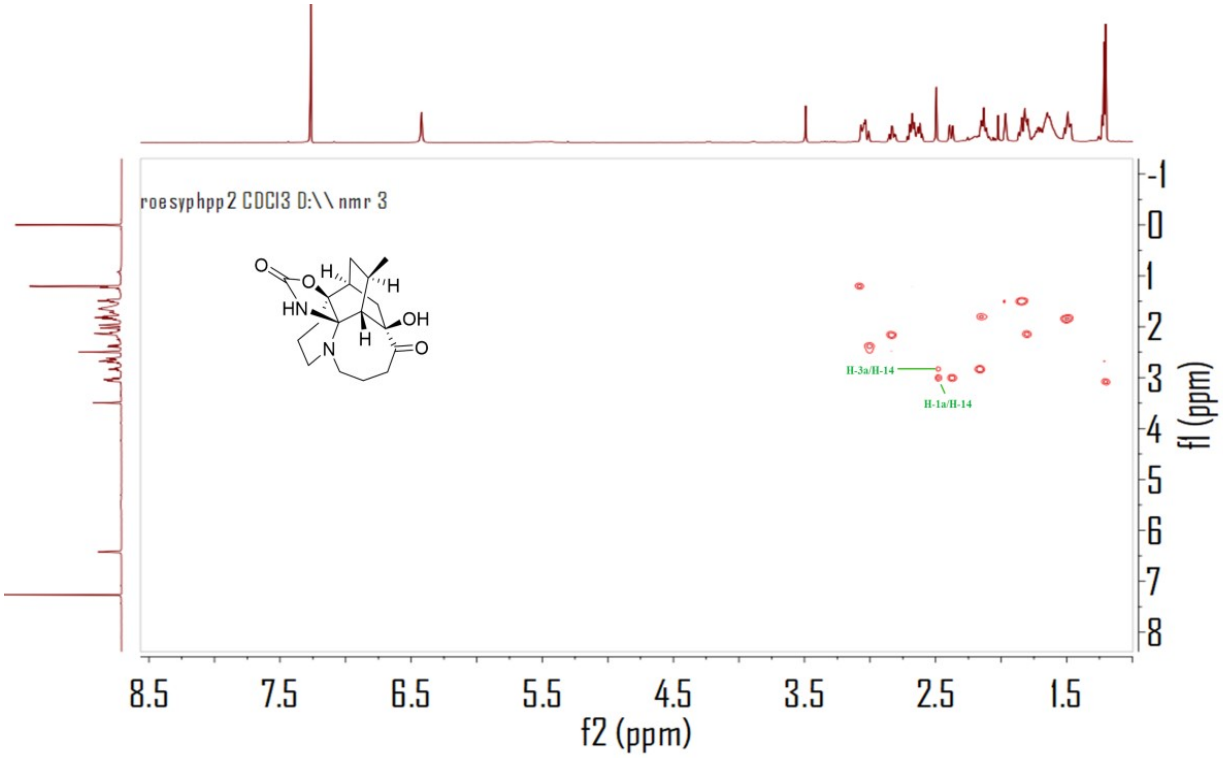


Figure S7. ^{1}H-^{1}H ROESY spectrum of compound **1** (in $CDCl_3$, 600 MHz)

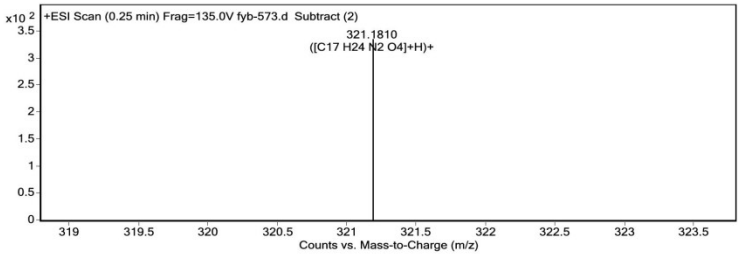
Qualitative Analysis Report

Data Filename	fyb-573.d	**Sample Name**	fyb-573
Sample Type	Sample	**Position**	P1-F1
Instrument Name	Instrument 1	**User Name**	
Acq Method	s.m	**Acquired Time**	1/3/2023 5:22:31 PM
IRM Calibration Status	Success	**DA Method**	PCDL.m
Comment			

Sample Group	**Info.**
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.05.01 (B5125.2)

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	ESI



Peak List

m/z	z	Abund
274.2738	1	1936.58
280.1916	1	1076.64
290.2112	1	642.72
302.3054	1	985.01
318.3003	1	606.73
330.3374	1	1837.9
353.2657	1	701.47
374.3623	1	677.23
437.1936	1	1272.86
453.1681	1	1360.95

Formula Calculator Element Limits

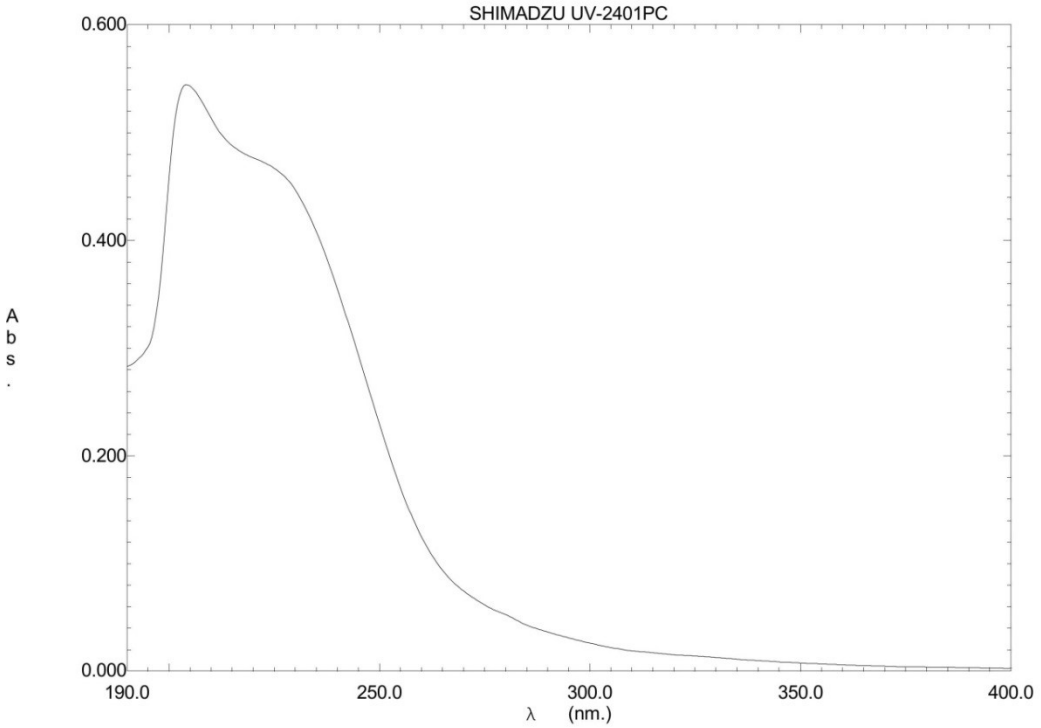
Element	Min	Max
C	3	100
H	0	200
O	0	30
N	0	10

Formula Calculator Results

Formula	CalculatedMass	CalculatedMz	Mz	Diff. (mDa)	Diff. (ppm)	DBE
C17 H24 N2 O4	320.1736	321.1809	321.1810	-0.10	-0.31	7.0000

--- End Of Report ---

Figure S8. HRESIMS spectrum of compound **1**



Figur

e S9. UV spectrum of compound **1** in MeOH

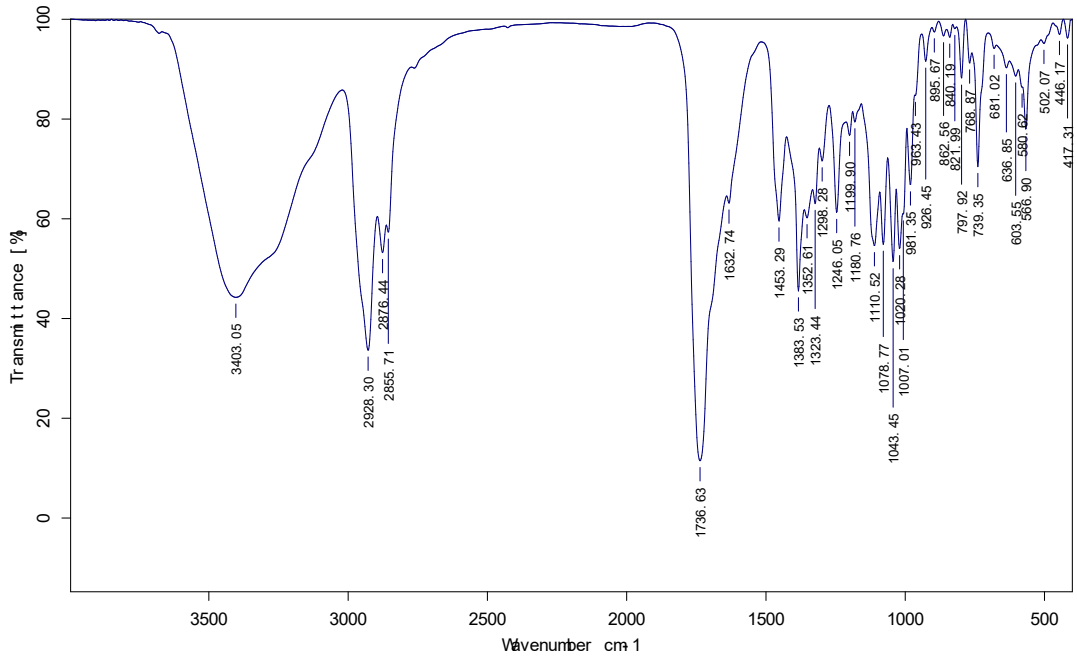


Figure S10. IR spectrum of compound **1**

Rudolph Research Analytical

This sample was measured on an Autopol VI, Serial #91058
Manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA.

Measurement Date : Friday, 10-MAR-2023

Set Temperature : OFF

Time Delay : Disabled

Delay between Measurement : Disabled

n	Average	Std.Dev.	% RSD	Maximum	Minimum
5	-106.72	0.50	-0.46	-106.36	-107.27

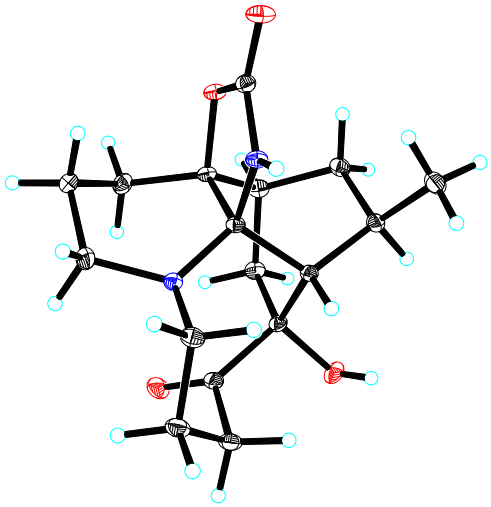
S.No	Sample ID	Time	Result	Scale	OR °Arc	WLG.nm	Lg.mm	Conc.g/100ml	Temp.
1	FYB-57	04:56:04 PM	-107.27	SR	-0.118	589	100.00	0.110	22.6
2	FYB-57	04:56:10 PM	-107.27	SR	-0.118	589	100.00	0.110	22.6
3	FYB-57	04:56:17 PM	-106.36	SR	-0.117	589	100.00	0.110	22.6
4	FYB-57	04:56:23 PM	-106.36	SR	-0.117	589	100.00	0.110	22.6
5	FYB-57	04:56:29 PM	-106.36	SR	-0.117	589	100.00	0.110	22.6

Page 1 of 1

Figure S11. $[\alpha]_D$ spectrum of compound **1** in MeOH

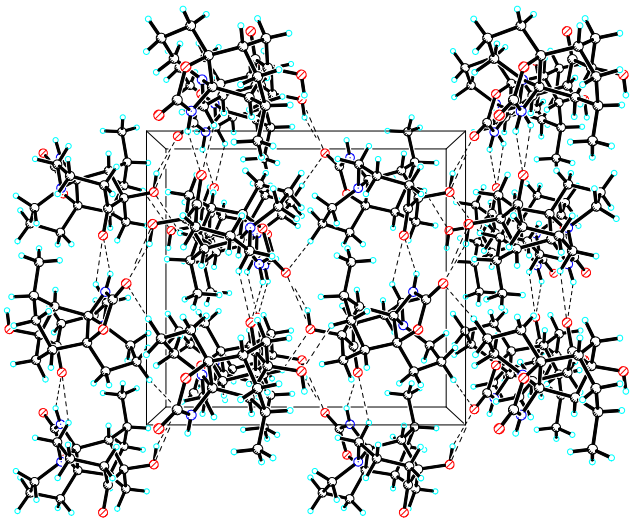
6. Crystallographic Data of compound 1

Crystal data for fyb57: $C_{17}H_{24}N_2O_4$, M = 320.38, a = 9.2974(3) Å, b = 12.2423(4) Å, c = 13.2392(5) Å, α = 90°, β = 90°, γ = 90°, V = 1506.91(9) Å^3, T = 150.(2) K, space group $P212121$, Z = 4, μ(Cu Kα) = 0.824 mm^{-1}, 14835 reflections measured, 2934 independent reflections (R_{int} = 0.0464). The final R_1 values were 0.0280 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0722 ($I > 2\sigma(I)$). The final R_1 values were 0.0283 (all data). The final $wR(F^2)$ values were 0.0724 (all data). The goodness of fit on F^2 was 1.072. Flack parameter = 0.06(6).



View of a molecule of fyb57 with the atom-labelling scheme.

Displacement ellipsoids are drawn at the 30% probability level.



View of the pack drawing of fyb57.

Hydrogen-bonds are shown as dashed lines.

Table 1. Crystal data and structure refinement for fyb57_0m.

Identification code	global	
Empirical formula	C17 H24 N2 O4	
Formula weight	320.38	
Temperature	150(2) K	
Wavelength	1.54178 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 9.2974(3) Å	α= 90°.
	b = 12.2423(4) Å	β= 90°.
	c = 13.2392(5) Å	γ = 90°.
Volume	1506.91(9) $Å^3$	
Z	4	
Density (calculated)	1.412 Mg/m^3	
Absorption coefficient	0.824 mm^{-1}	
F(000)	688	
Crystal size	0.900 x 0.670 x 0.580 mm^3	
Theta range for data collection	4.92 to 72.12°.	

Index ranges	-11<=h<=11, -15<=k<=15, -16<=l<=12
Reflections collected	14835
Independent reflections	2934 [R(int) = 0.0464]
Completeness to theta = 72.12°	99.2 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.65 and 0.47
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2934 / 0 / 210
Goodness-of-fit on F^2	1.072
Final R indices [I>2sigma(I)]	R1 = 0.0280, wR2 = 0.0722
R indices (all data)	R1 = 0.0283, wR2 = 0.0724
Absolute structure parameter	0.06(6)
Largest diff. peak and hole	0.235 and -0.154 e.$Å^{-3}$

7. HPLC-MS analyses of the alkaloidal extract and compound 1

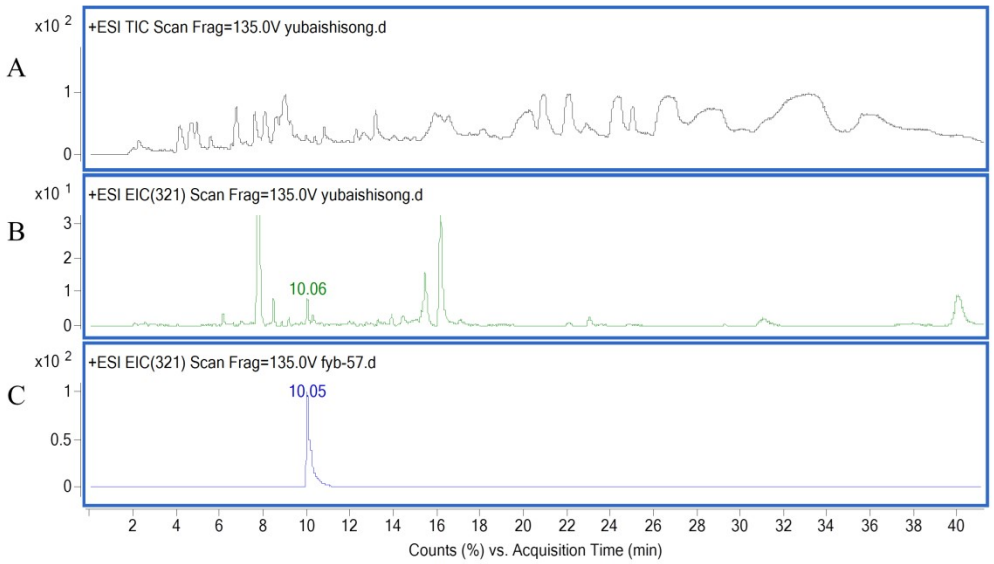


Figure S12. HPLC-MS analyses of the alkaloidal extract and compound **1**. (A) The alkaloidal extract from *L. obscurum*. (B) Traced compound **1** m/z 321 in alkaloidal extract. (C) Isolated compound **1** m/z 321.

HPLC-MS condition: HPLC-MS analyses were performed on an Agilent 1290 UHPLC-ESI-Q-TOF/MS system with an X-Bridge C18 column (5 μm, 4.6 × 250 mm, Waters). The alkaloidal extract and compound **1** were analyzed with a gradient elution of MeCN/H_2O: 0.0 min, MeCN/H_2O (10:90); 40.0 min, MeCN/H_2O (100:0). The Q-TOF/MS data were acquired in positive mode and conditions of MS analysis were as follows: drying gas (N_2) flow-rate, 9 L/min; drying gas temperature, 350 ºC; nebulizing gas (N_2) pressure, 40 psi; capillary voltage, 3500 V; fragmentor, 135 V.