Supporting Information

Hyperprins A and B, Two Complex Meroterpenoids from *Hypericum przewalskii*

Jian-Fa Zong,[†] Zhu Hu,[†] Ying-Ying Shao,[‡] Qing Shi,[†] Meng-Meng Zhang,[‡] Yu-Bo Zhou,[‡] Jia Li,[‡] and Ai-Jun Hou*[,†]

[†]*School of Pharmacy, State Key Laboratory of Medical Neurobiology, Fudan University, Shanghai 201203, People's Republic of China*

[‡]*National Center for Drug Screening, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China*

*Corresponding Author

E-mail: ajhou@shmu.edu.cn.

Contents

Experimental section

General experimental procedures

Melting points were measured on an SGM X-4 apparatus. Optical rotations were measured on a Rudolph Autopol IV-T polarimeter. IR spectra were obtained on a ThermoFisher Nicolet iS5 FT-IR spectrometer. UV spectra were recorded on a Hitachi U-2900 UV-Vis spectrophotometer. ESIMS were measured on an Agilent 1100 LC-MS spectrometer; HRESIMS were obtained on an AB SCIEX TripleTOF 5600+ LC-MS spectrometer. NMR spectra were acquired on a Bruker Avance III HD 600 MHz spectrometer or a Varian Mercucy Plus-400 MHz spectrometer using $CDCl_3$ as solvent. Chemical shifts were reported with respect to $CDCl_3$ (δ_H 7.26 and δ_C 77.16). ECD spectra were recorded on a JASCO J-810 spectrometer. X-ray crystallographic data were acquired on a Bruker D8 Venture diffractometer (Ga Kα radiation: λ = 1.34139 Å) or a Bruker APEX-II CCD diffractometer (Mo Kα radiation: λ = 0.71073 Å). Semi-preparative HPLC was performed on a Shimadzu Essentia LC-16 (UV detector: 210 and 254 nm) and a XTerra RP_{18} column (250 × 10 mm, 10 μm, Waters Co., USA). Silica gel (100–200 mesh, Qingdao Haiyang Chemical Co., Ltd., China), MCI gel CHP20/P120 (75–150 μm, Mitsubishi Chemical Co., Japan), and ODS gel (50 μm, YMC Co., Ltd., Japan) were used for column chromatography. Precoated silica gel GF254 plates (Qingdao Haiyang Chemical Co., Ltd., China) were used for TLC analysis.

Plant material

The aerial parts of *Hypericum przewalskii* were collected in August 2016 in Mei County from Shanxi Province, People's Republic of China. The plant material was identified by Dr. Yun Kang, School of Pharmacy, Fudan University, and a voucher specimen (TCM 2016-08-02 Hou) has been deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Fudan University.

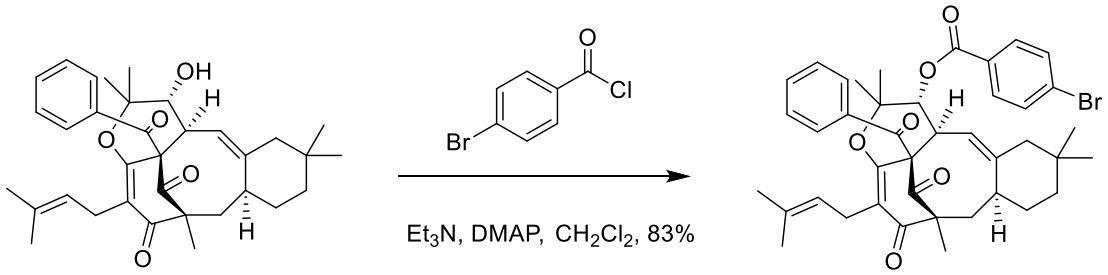
Extraction and isolation

The air-dried aerial parts of *Hypericum przewalskii* (10 kg) were powdered and extracted with 95% EtOH (100 L) at room temperature. The filtrate was evaporated under reduced pressure to produce a crude extract (1500 g), which was subjected to silica gel column chromatography (CC) eluted with $CHCl_3$. The $CHCl_3$ fraction (106 g)

was separated over a MCI gel column (MeOH–H_2O, 7:3–10:0) to afford three fractions (Frs. A–C). Fr. A (10 g) was separated on an ODS gel column (MeOH–H_2O, 6:4–10:0) to give six fractions (Frs. A1–A6). Fr. A3 (170 mg) was purified by semi-preparative HPLC (MeOH–H_2O, 7:3) to afford **1** (5.5 mg). Fr. A4 (230 mg) was isolated by semi-preparative HPLC (MeOH–H_2O, 8:2) to give **2** (21.2 mg).

Preparation of the *p*-bromobenzoate ester 3

Triethylamine (3.0 equiv, 6.3 μL, 0.045 mmol) was added to a stirred solution of **2** (8.0 mg, 0.015 mmol), 4-bromobenzoyl chloride (3.0 equiv, 9.8 mg, 0.045 mmol), and DMAP (2.0 equiv, 3.6 mg, 0.03 mmol) in CH_2Cl_2 (0.40 mL). After heating at 40 °C for 1 h, the reaction mixture was quenched with saturated NH_4Cl solution and extracted by EtOAc three times. The combined EtOAc layers were concentrated under reduced pressure. Purification of the residue by semi-preparative HPLC (MeOH–H_2O, 85:15) yielded **3** (8.9 mg, 83%) as a white solid.



2 **3**

Physical and spectroscopic data of 1–3

Hyperprin A (**1**): colorless crystals (MeOH); mp 177–178 °C; $[\alpha]_D^{25}$ –4.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.10), 250 (4.04), 270 (3.99) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 214 (+4.64), 275 (–3.80), 330 (+0.74) nm; IR (KBr) ν_{max} 3499, 2958, 2933, 2873, 1734, 1679, 1644, 1584, 1470, 1450, 1360, 1260, 1128, 998 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; positive ESIMS *m/z* 547.2 $[M + H]^+$; positive HRESIMS *m/z* 547.3038 $[M + H]^+$ (calcd for $C_{34}H_{43}O_6$, 547.3054).

Hyperprin B (**2**): colorless oil (MeOH); $[\alpha]_D^{25}$ –314.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.36), 246 (4.32) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 205 (+1.91), 220 (–0.66), 234 (+0.80), 259 (–5.26), 283 (+0.13), 319 (–4.51) nm; IR (KBr) ν_{max} 3484, 2978, 2928,

2868, 1719, 1688, 1654, 1622, 1450, 1375, 1327, 1228, 1078, 1046, 1028 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; positive ESIMS *m/z* 531.2 $[M + H]^+$; positive HRESIMS *m/z* 531.3102 $[M + H]^+$ (calcd for $C_{34}H_{43}O_5$, 531.3105).

p-Bromobenzoate ester of hyperprin B (**3**): colorless crystals (MeOH); mp 130–131 °C. positive ESIMS *m/z* 713.2 $[M + H]^+$, 715.2 $[M + 2 + H]^+$; negative ESIMS *m/z* 711. 0 $[M - H]^-$, 713.0 $[M + 2 - H]^-$. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 2.42 (1H, br d, *J* = 13.6 Hz, H-6a), 1.85 (1H, overlapped, H-6b), 2.63 (1H, m, H-7), 7.76 (2H, br d, *J* = 7.6 Hz, H-12, 16), 7.33 (2H, t, *J* = 7.6 Hz, H-13, 15), 7.49 (1H, br t, *J* = 7.6 Hz, H-14), 3.24 (1H, dd, *J* = 13.6, 7.6 Hz, H-17a), 3.16 (1H, dd, *J* = 13.6, 7.6 Hz, H-17b), 5.11 (1H, br t, *J* = 6.8 Hz, H-18), 1.70 (3H, br s, H_3-20), 1.60 (3H, br s, H_3-21), 1.40 (3H, s, H_3-22), 0.81 (3H, s, H_3-26), 0.51 (3H, s, H_3-27), 6.14 (1H, br d, *J* = 11.2 Hz, H-29), 4.01 (1H, t, *J* = 10.4 Hz, H-30), 5.69 (1H, d, *J* = 9.2 Hz, H-31), 1.37 (3H, s, H_3-33), 1.43 (3H, s, H_3-34), 7.80 (2H, d, *J* = 8.0 Hz, H-37, 41), 7.56 (2H, d, *J* = 8.0 Hz, H-38, 40); ^{13}C NMR (150 MHz, $CDCl_3$) δ_C: 73.1 (C-1), 166.1 (C-2), 123.9 (C-3), 198.9 (C-4), 58.6 (C-5), 43.7 (C-6), 36.7 (C-7), 145.7 (C-8), 205.4 (C-9), 193.5 (C-10), 136.4 (C-11), 128.6 (C-12, 16), 128.5 (C-13, 15), 133.0 (C-14), 22.6 (C-17), 120.1 (C-18), 134.0 (C-19), 25.8 (C-20), 18.0 (C-21), 24.8 (C-22), 30.0 (C-23), 36.2 (C-24), 31.7 (C-25), 30.0 (C-26), 26.6 (C-27), 46.3 (C-28), 122.6 (C-29), 49.0 (C-30), 74.4 (C-31), 89.1 (C-32), 21.7 (C-33), 28.8 (C-34), 164.7 (C-35), 128.7 (C-36), 132.0 (C-37, 41), 131.3 (C-38, 40), 128.7 (C-39).

Bioassay

PTP1B activity assay

The PTP1B bioassay procedure was the same as the described previously.[1,2] The IC_{50} values were calculated with Prism 4 software (Graphpad, San Diego, CA).

Antiproliferation activity assay

The antiproliferation activity was evaluated by the MTS assay, as reported in the literature.[3] Briefly, MV-4-11 cells were incubated for 72 h at 37 °C in 5% CO_2 in the presence of serial dilutions of the tested compounds, and the final concentration of DMSO was 0.2%. Each sample was treated in triplicate. Then, 20 μL of MTS solution was added to each well and incubated for 3 h. The optical density of each well was measured at 490 nm on a SpectraMAX 340 microplate reader (Molecular Devices, CA) with a reference wavelength at 690 nm. The IC_{50} value was calculated with Prism 4 software (Graphpad, San Diego, CA).

References

1. Shi, L.; Yu, H. P.; Zhou, Y. Y.; Du, J. Q.; Shen, Q.; Li, J. Y.; Li, J. *Acta Pharmacol. Sin.* **2008**, *29*, 278–284.

2. Wang, M.; Yu, B. W.; Yu, M. H.; Gao, L. X.; Li, J. Y.; Wang, H. Y.; Li, J.; Hou, A. J. *Chem. Biodiversity* **2015**, *12*, 937–945.

3. Guo, Y.; Wang, Y.; Li, H; Wang, K.; Wan, Q.; Li, J.; Zhou, Y.; Y. Chen. *ACS Med. Chem. Lett.* **2018**, *9*, 502–506.

X-ray crystallographic data

Table S1. X-ray crystallographic data for **1**

Empirical formula	$C_{34}H_{42}O_6 \cdot \frac{1}{2}CH_3OH$	
Formula weight	562.69	
Temperature	169.98 K	
Wavelength	1.34139 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 12.8538(4) Å	α = 90°.
	b = 13.9193(4) Å	β = 90°.
	c = 33.8404(9) Å	γ = 90°.
Volume	6054.6(3) $Å^3$	
Z	8	
Density (calculated)	1.235 Mg/m^3	
Absorption coefficient	0.434 mm^{-1}	
F(000)	2424	
Crystal size	0.12 x 0.08 x 0.05 mm^3	
Theta range for data collection	4.229 to 54.953°.	
Index ranges	-15<=h<=15, -16<=k<=16, -38<=l<=41	
Reflections collected	68032	
Independent reflections	11481 [R(int) = 0.0382]	
Completeness to theta = 53.594°	99.5 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7508 and 0.6612	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	11481 / 1 / 757	
Goodness-of-fit on F^2	1.081	
Final R indices [I>2sigma(I)]	R1 = 0.0383, wR2 = 0.1016	
R indices (all data)	R1 = 0.0417, wR2 = 0.1048	
Absolute structure parameter	0.06(4)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.342 and -0.440 $e.Å^{-3}$	

Table S2. X-ray crystallographic data for **3**

Empirical formula	C_{41} H_{45} Br O_6	
Formula weight	713.68	
Temperature	293(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 21	
Unit cell dimensions	a = 10.5014(4) Å	α = 90°.
	b = 18.7473(8) Å	β = 117.9370(10)°.
	c = 10.6528(4) Å	γ = 90°.
Volume	1852.84(13) $Å^3$	
Z	2	
Density (calculated)	1.279 Mg/m^3	
Absorption coefficient	1.154 mm^{-1}	
F(000)	748	
Crystal size	0.190 x 0.170 x 0.120 mm^3	
Theta range for data collection	2.421 to 25.496°.	
Index ranges	-12<=h<=12, -22<=k<=22, -12<=l<=12	
Reflections collected	27144	
Independent reflections	6847 [R(int) = 0.0374]	
Completeness to theta = 25.242°	99.7 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7456 and 0.6290	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	6847 / 13 / 450	
Goodness-of-fit on F^2	1.020	
Final R indices [I>2sigma(I)]	R1 = 0.0408, wR2 = 0.0907	
R indices (all data)	R1 = 0.0621, wR2 = 0.1019	
Absolute structure parameter	0.020(4)	
Extinction coefficient	0.016(3)	
Largest diff. peak and hole	0.264 and -0.274 $e.Å^{-3}$	

Original spectroscopic data

Figure S1. Positive ESIMS spectrum of **1**

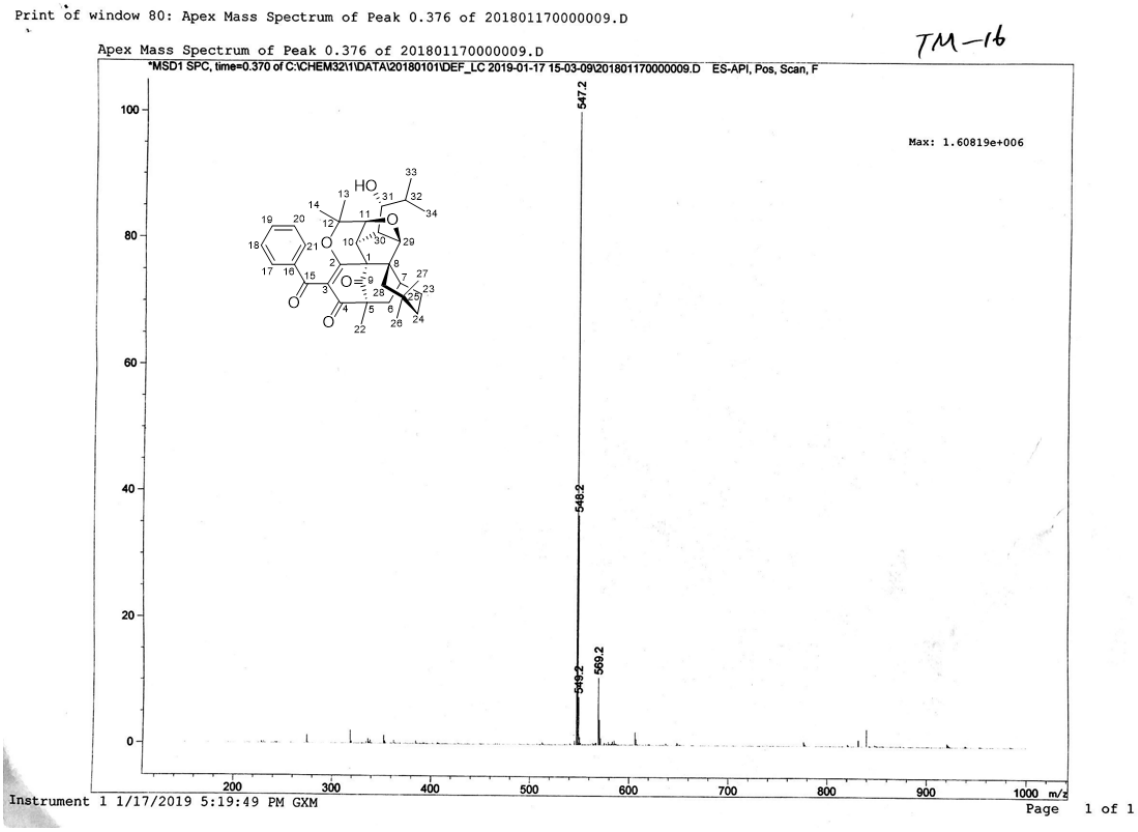
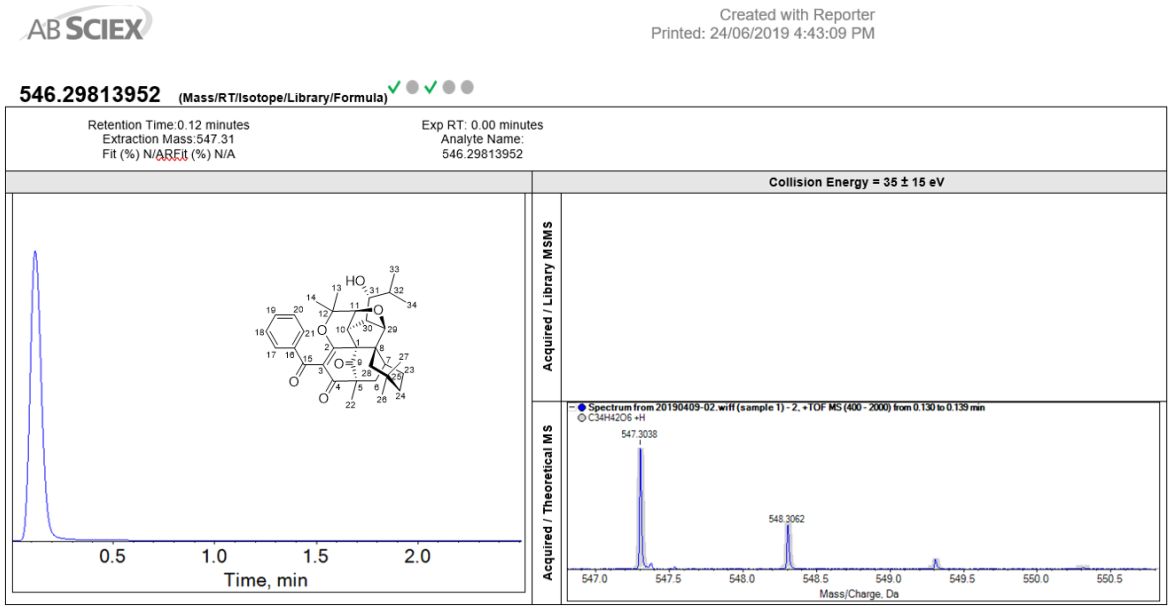


Figure S2. Positive HR-ESIMS spectrum of **1**



Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score(%)
546.29813952(TM-16)	90%	C34H42O6	4192062	5	547.3054	547.3038	-2.9	0.00	0.12	0.12	2.2%	N/A

Figure S3. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of **1**

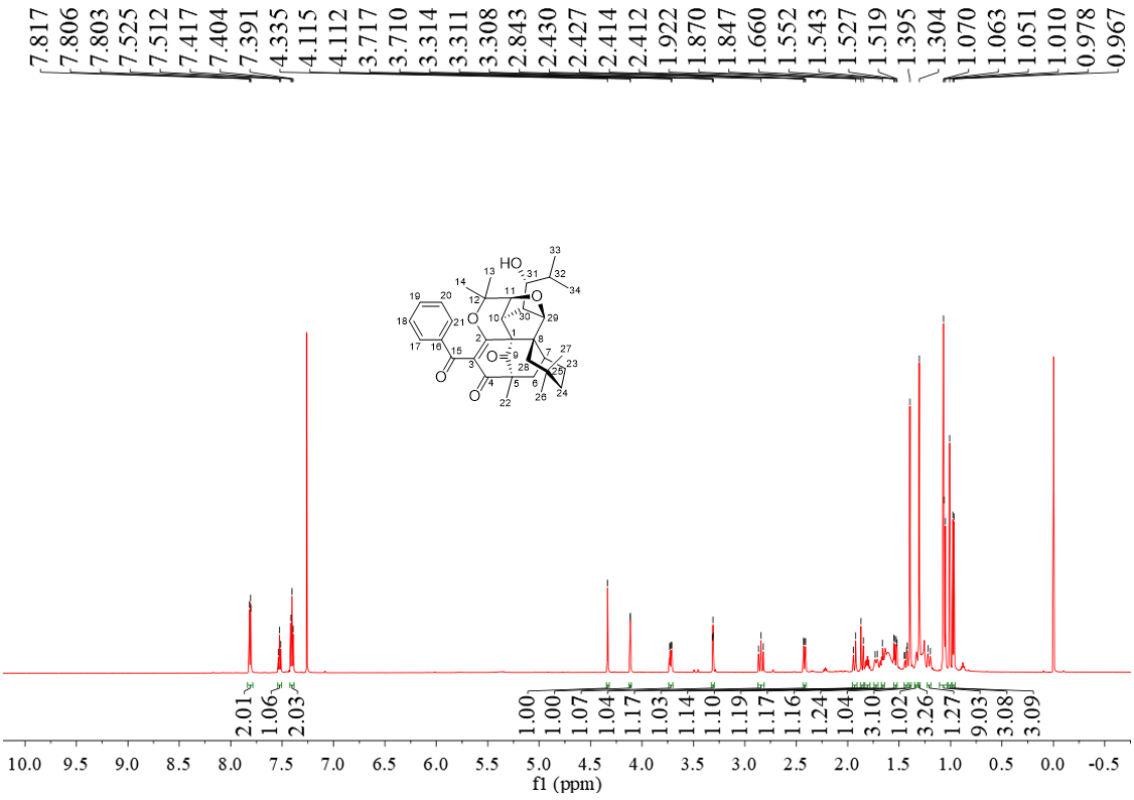


Figure S4. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of **1**

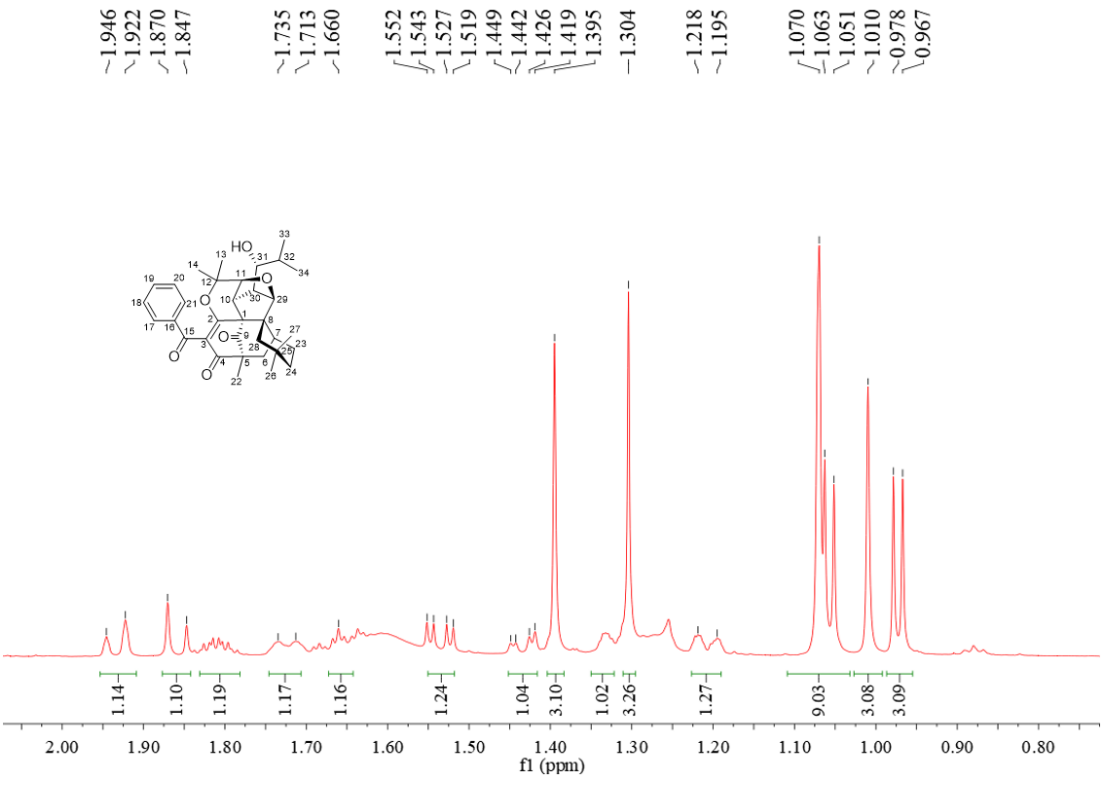


Figure S5. ^{13}C NMR and DEPT (150 MHz, $CDCl_3$) spectra of **1**

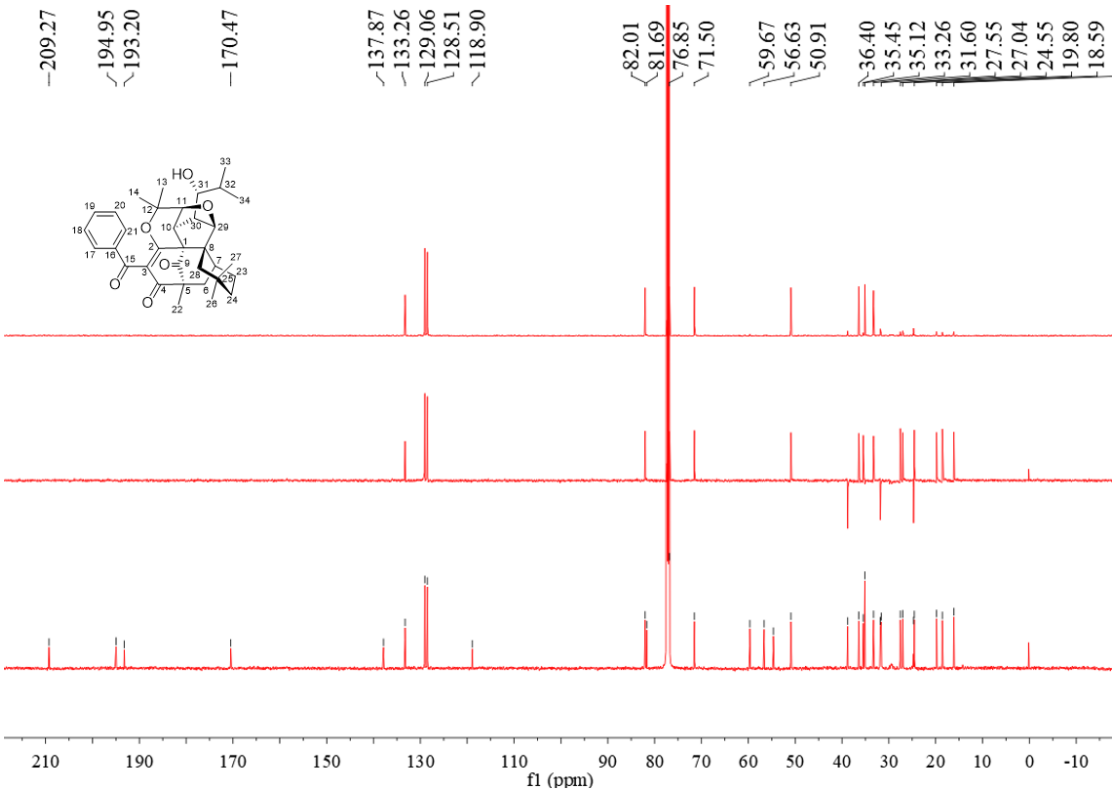


Figure S6. HSQC (600 MHz, $CDCl_3$) spectrum of **1**

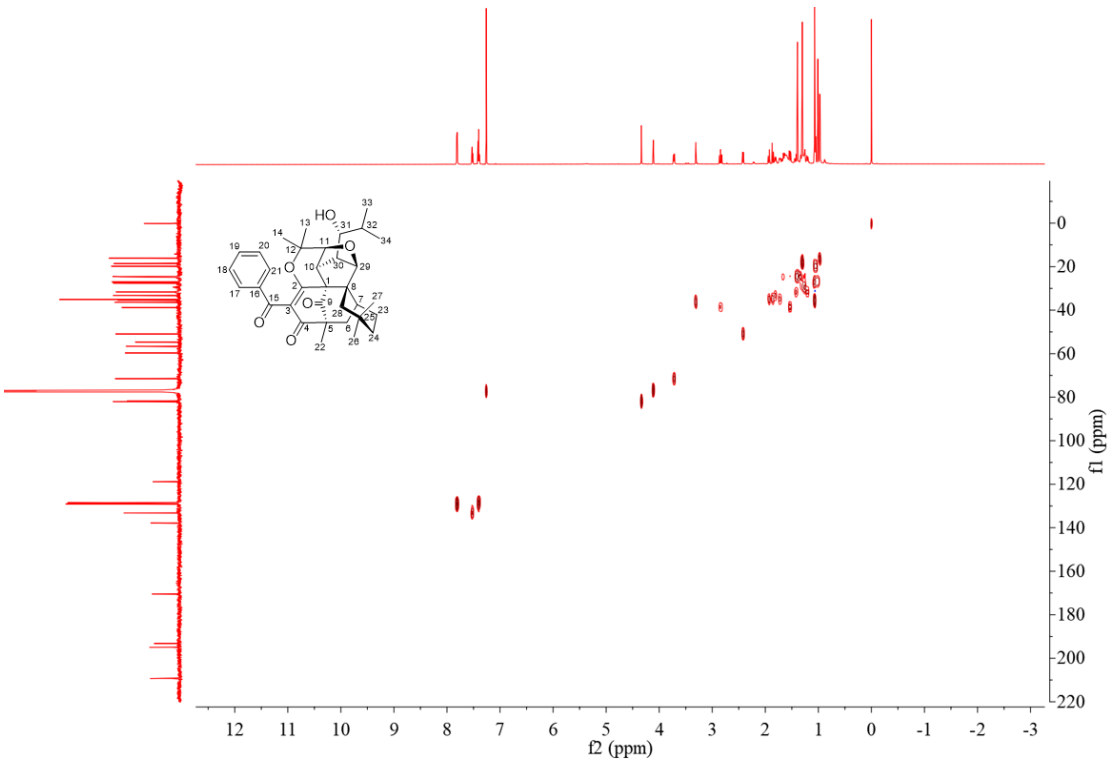


Figure S7. HSQC (600 MHz, $CDCl_3$) spectrum of **1**

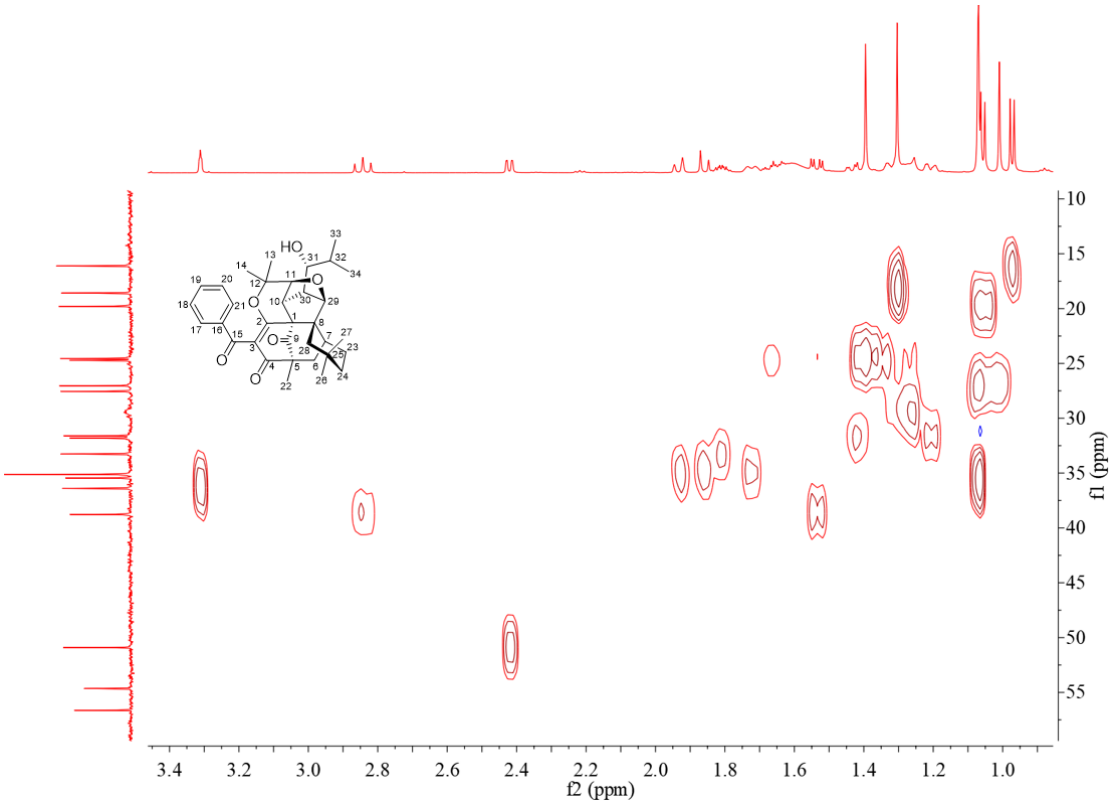


Figure S8. HMBC (600 MHz, $CDCl_3$) spectrum of **1**

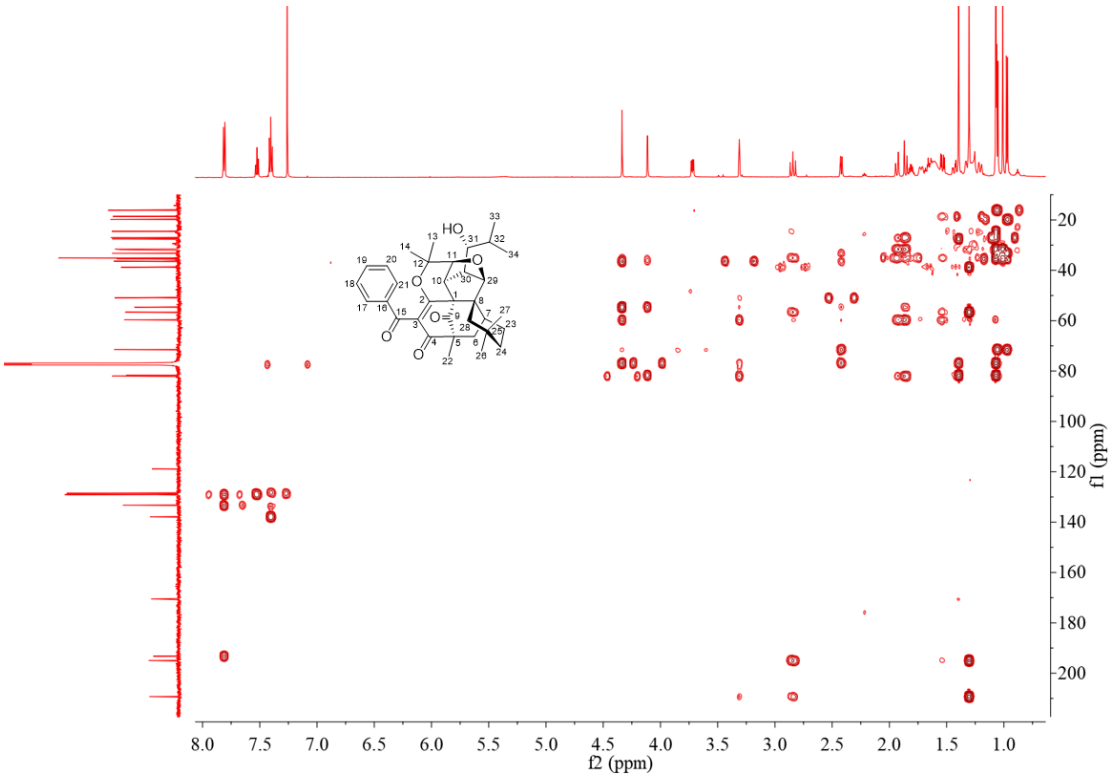


Figure S9. HMBC (600 MHz, $CDCl_3$) spectrum of **1**

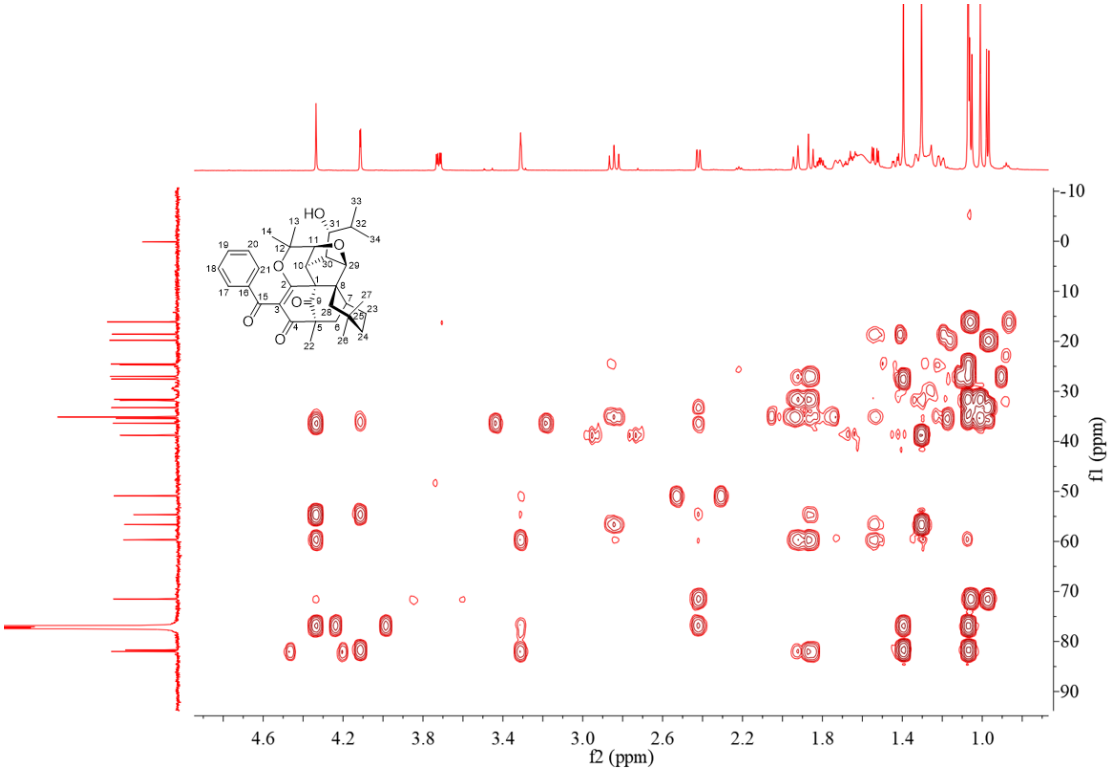


Figure S10. ^{1}H-^{1}H COSY (600 MHz, $CDCl_3$) spectrum of **1**

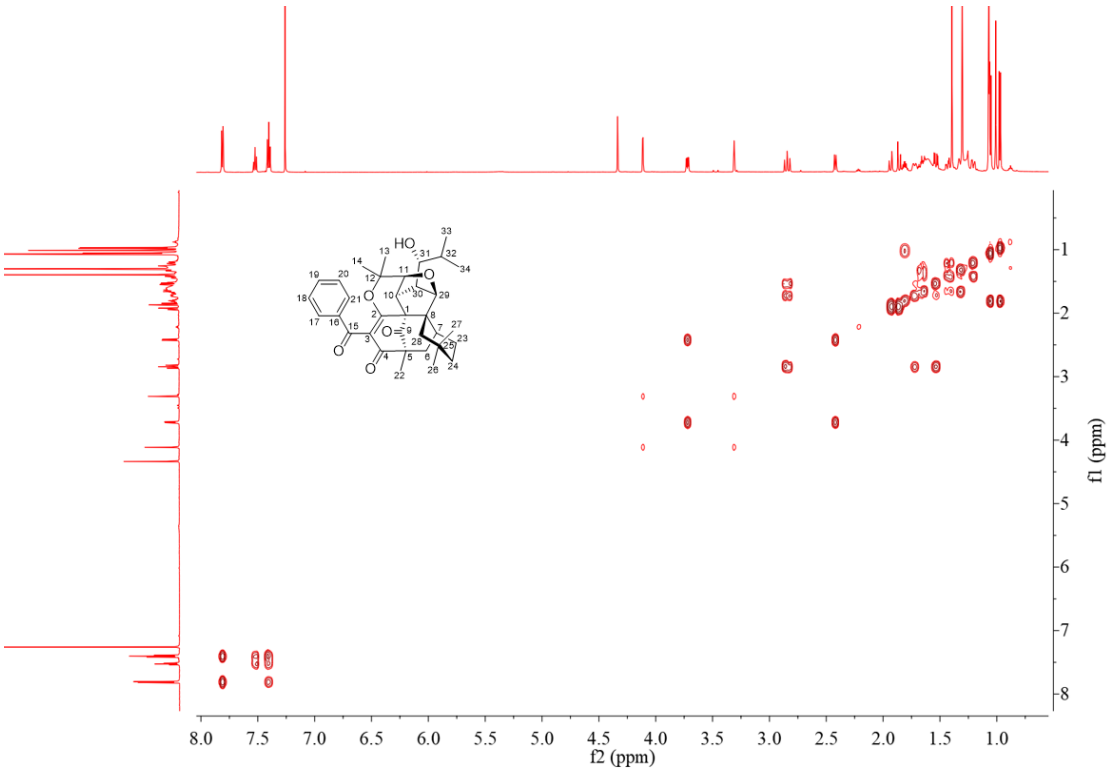


Figure S11. ^{1}H-^{1}H COSY (600 MHz, $CDCl_3$) spectrum of **1**

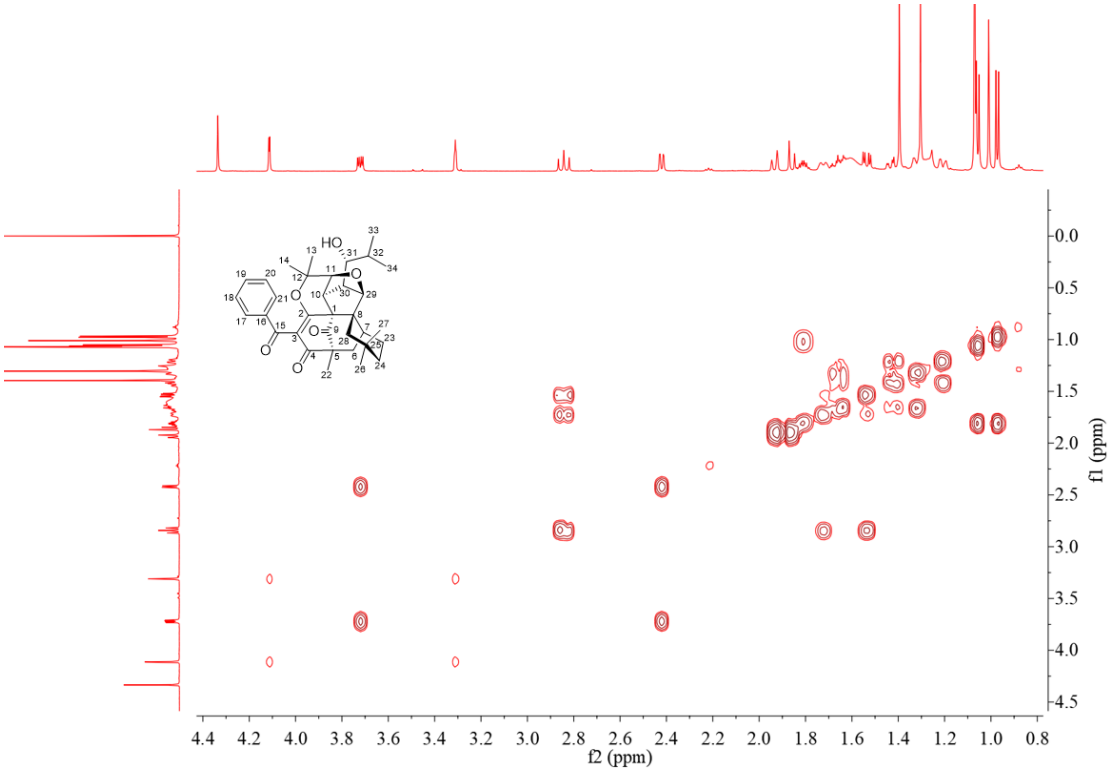


Figure S12. ROESY (600 MHz, $CDCl_3$) spectrum of **1**

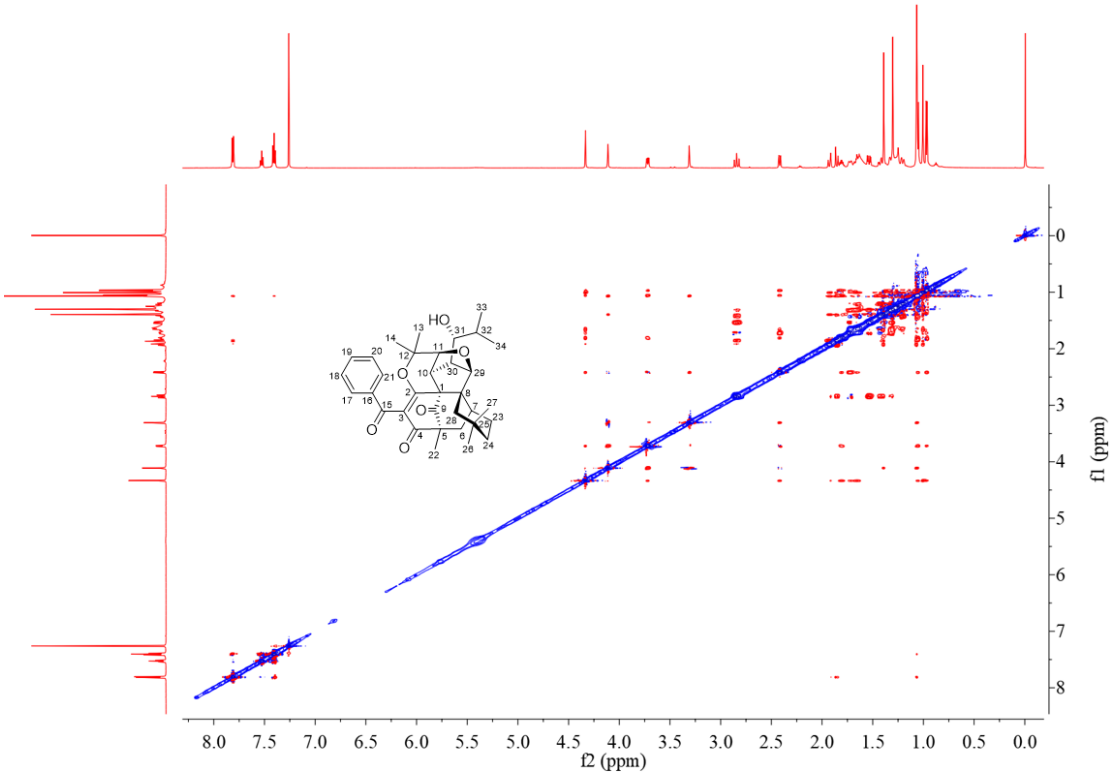


Figure S13. ROESY (600 MHz, $CDCl_3$) spectrum of **1**

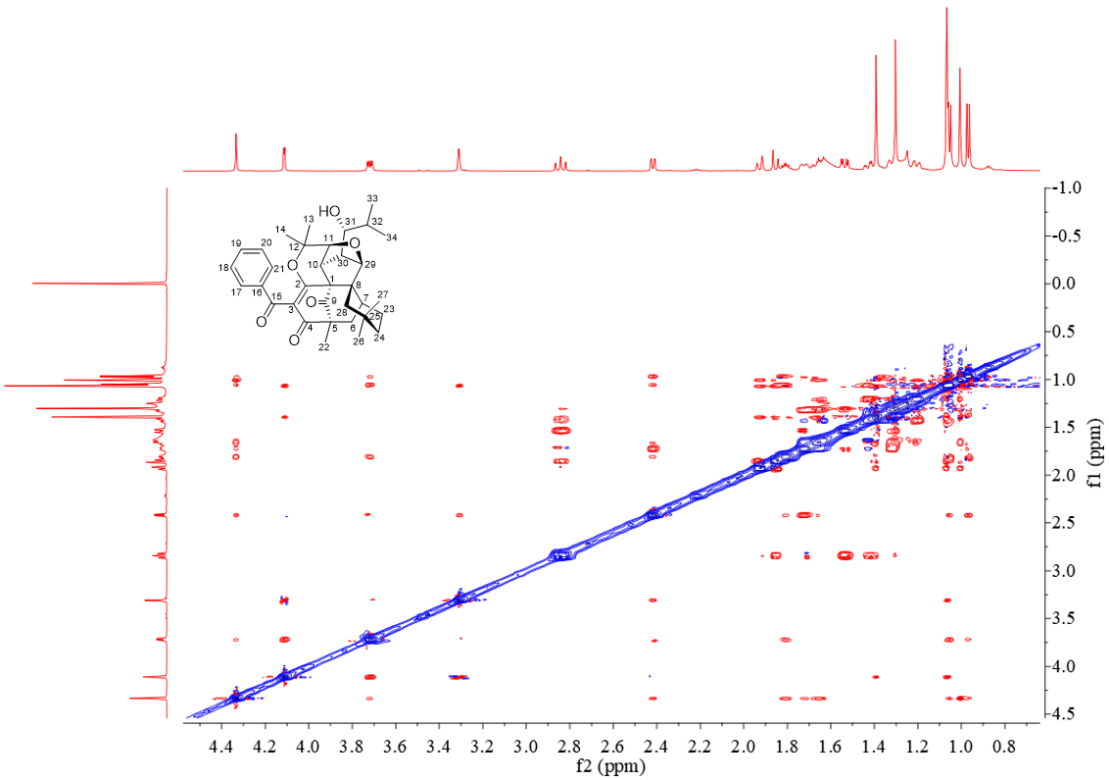


Figure S14. UV spectrum of **1**

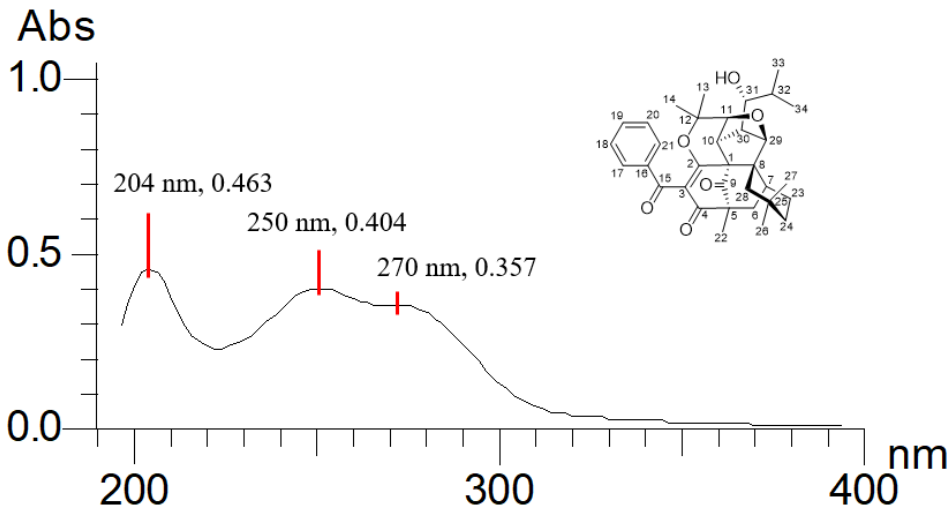


Figure S15. ECD spectrum of **1**

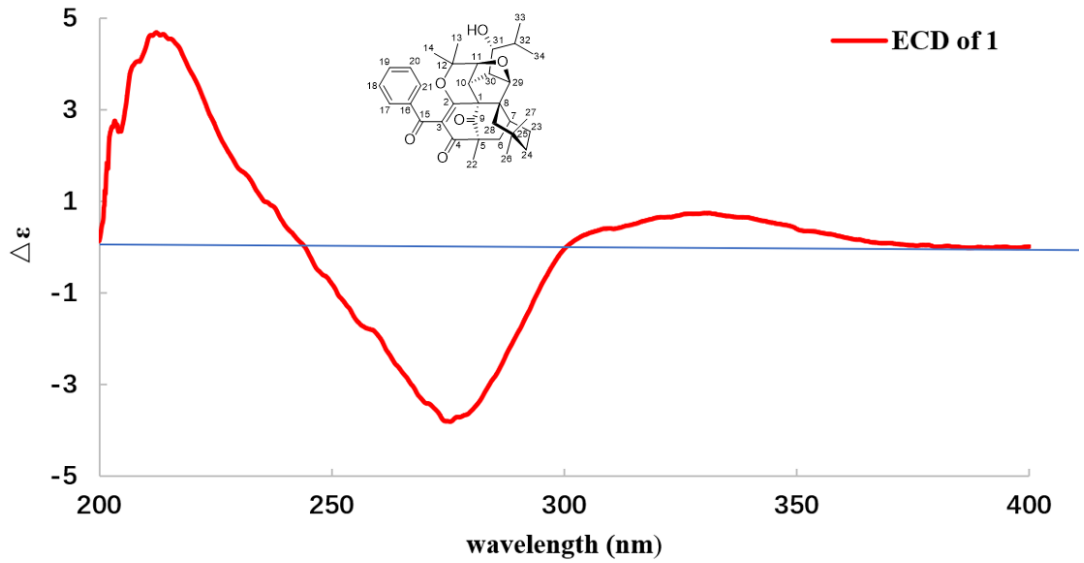


Figure S16. IR spectrum of **1**

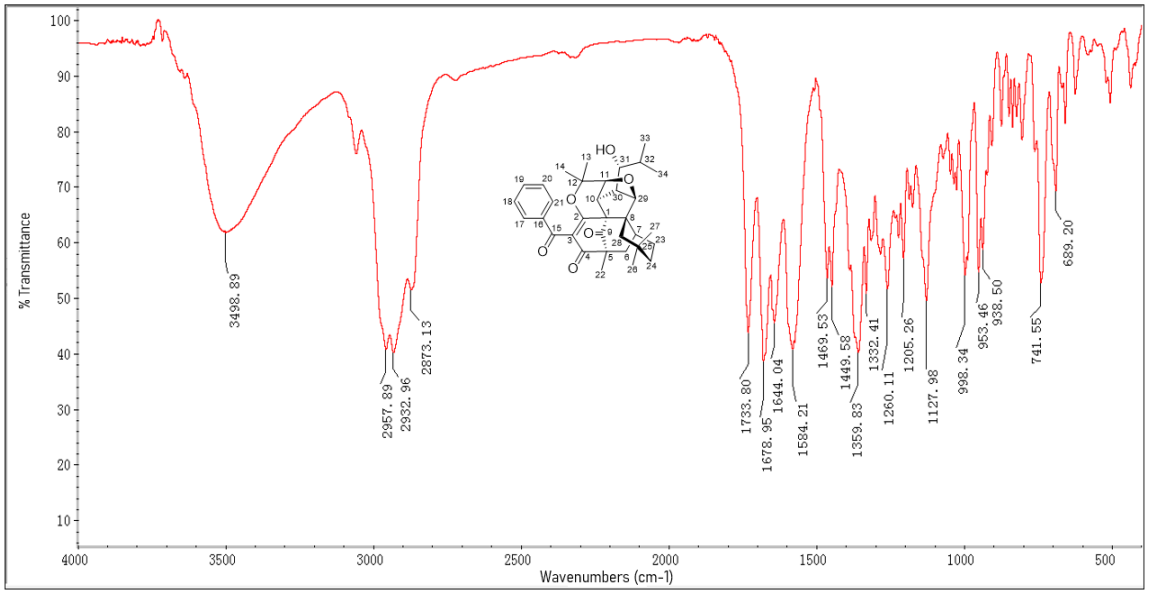


Figure S17. Positive ESIMS spectrum of **2**

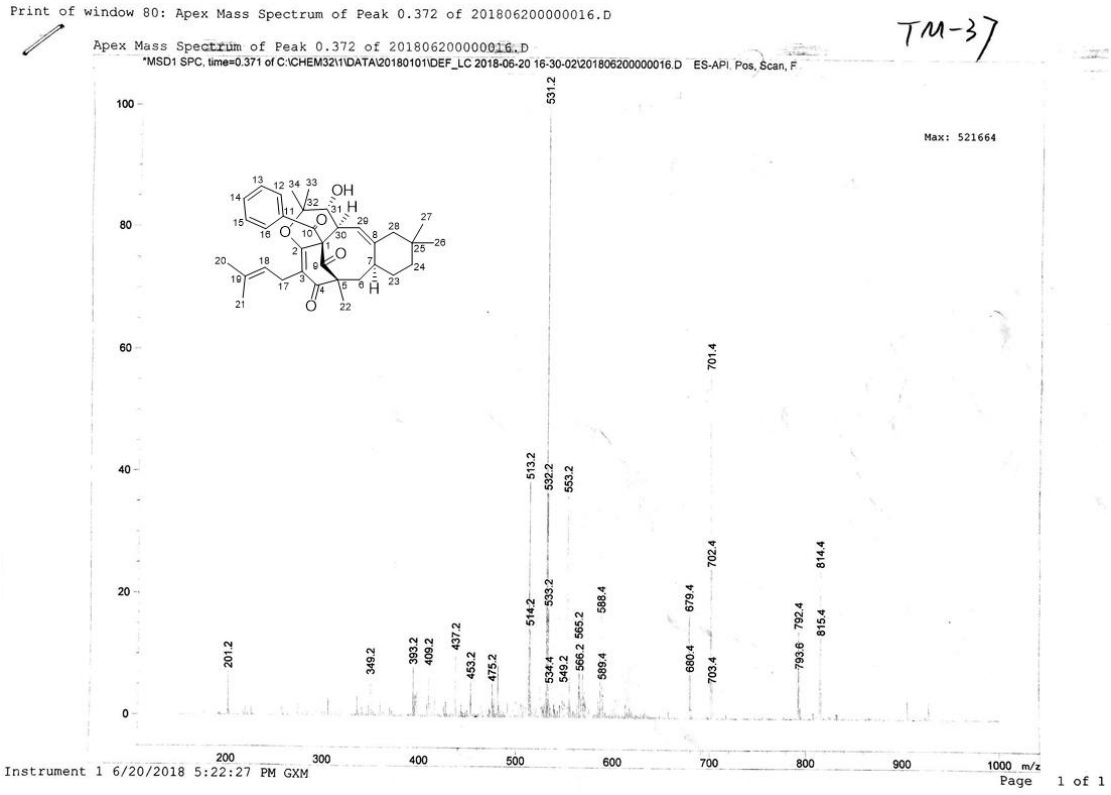
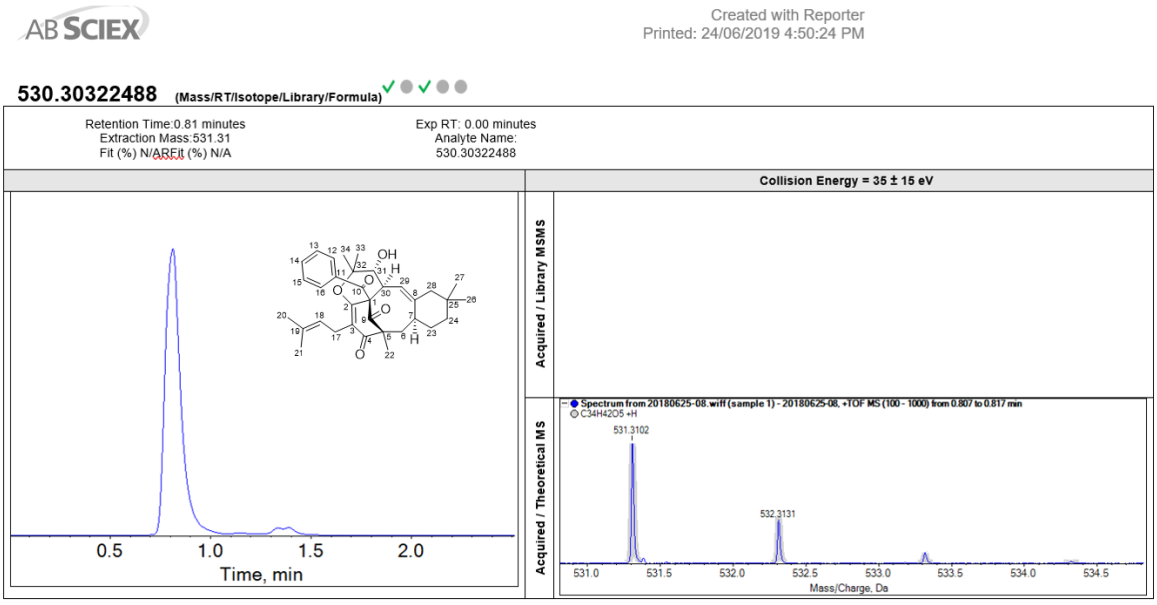


Figure S18. Positive HR-ESIMS spectrum of **2**



	Compound Name (Library Hit)	Score	Formula	Intensity	Threshold	Expected m/z	Found at m/z	Error (ppm)	Expected RT (min)	Found RT (min)	RT Delta (min)	Isotope Diff (%)	Library Score(%)
✓●✓●●	530.30322488 (TM-37)	90%	C34H42O5	2190925	50	531.3105	531.3102	-0.7	0.00	0.81	0.81	2.5%	N/A

Figure S19. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of **2**

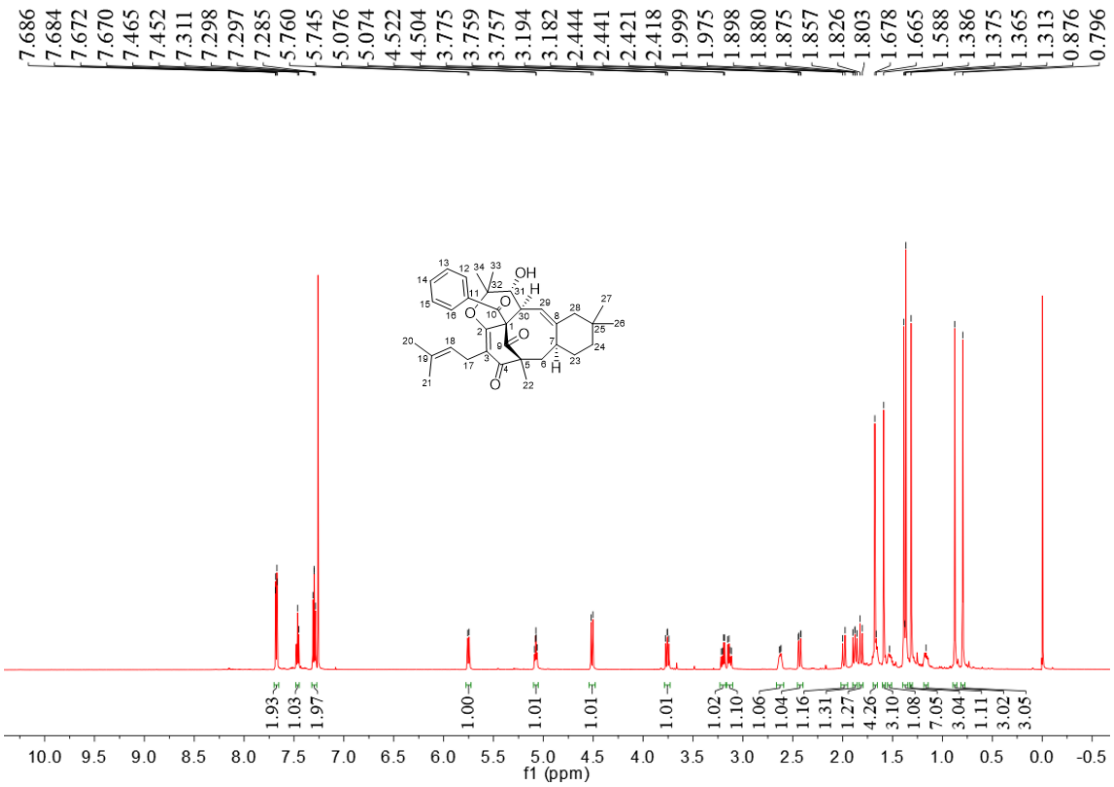


Figure S20. ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of **2**

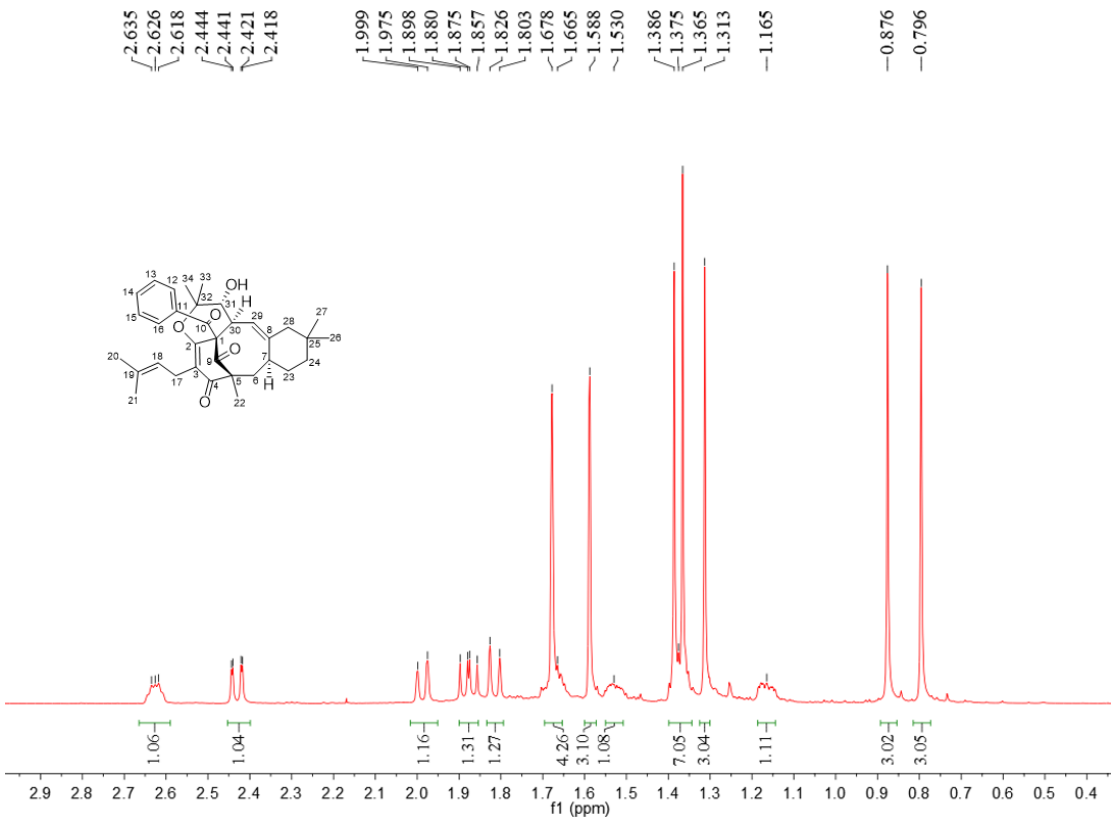


Figure S21. ^{13}C NMR and DEPT (150 MHz, $CDCl_3$) spectra of **2**

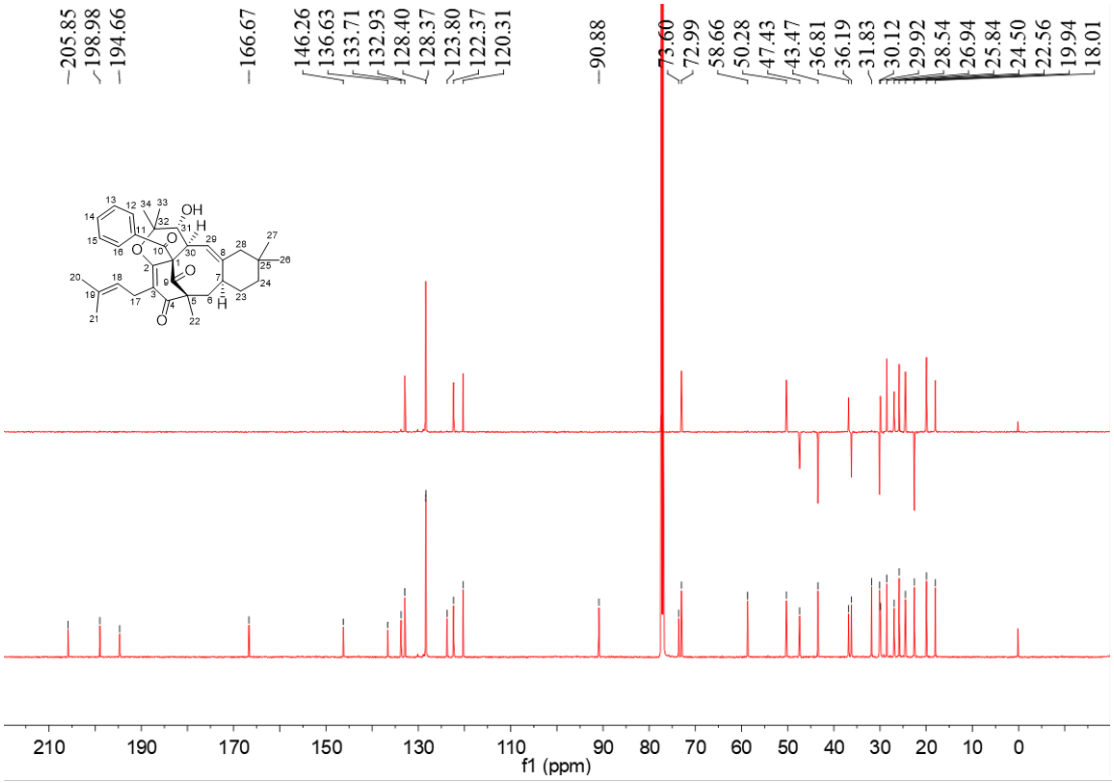


Figure S22. HSQC (600 MHz, $CDCl_3$) spectrum of **2**

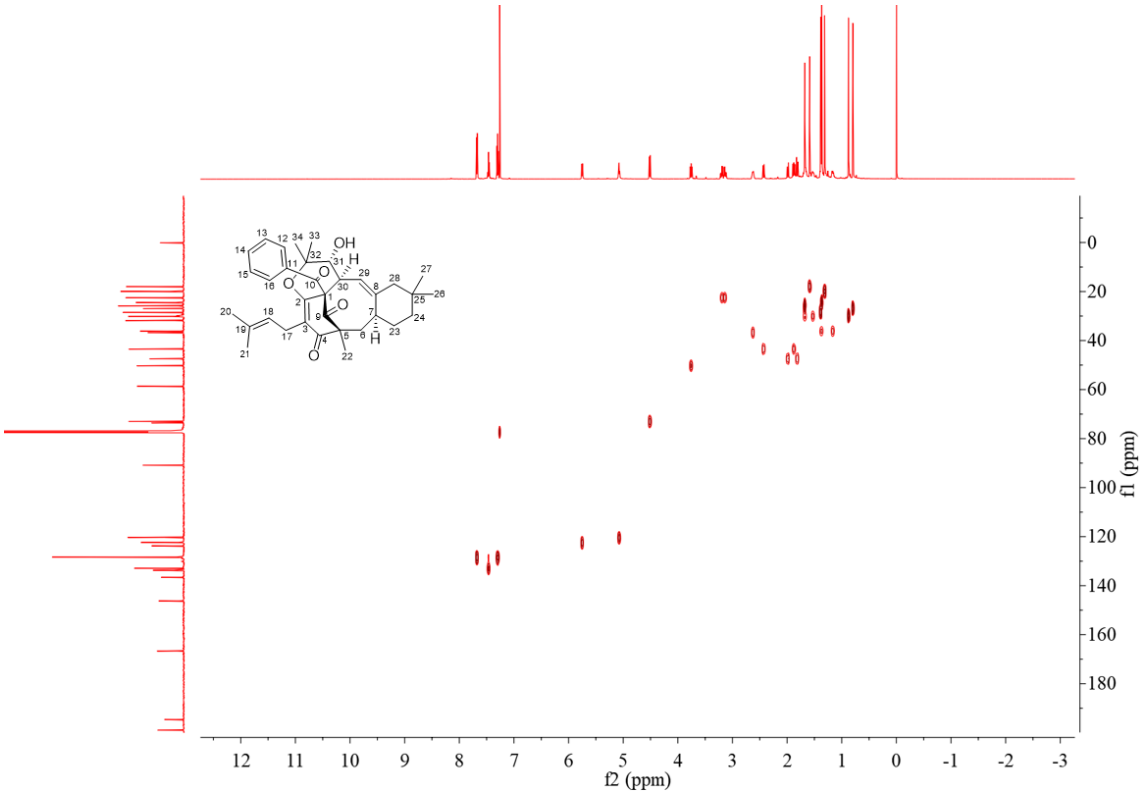


Figure S23. HSQC (600 MHz, $CDCl_3$) spectrum of **2**

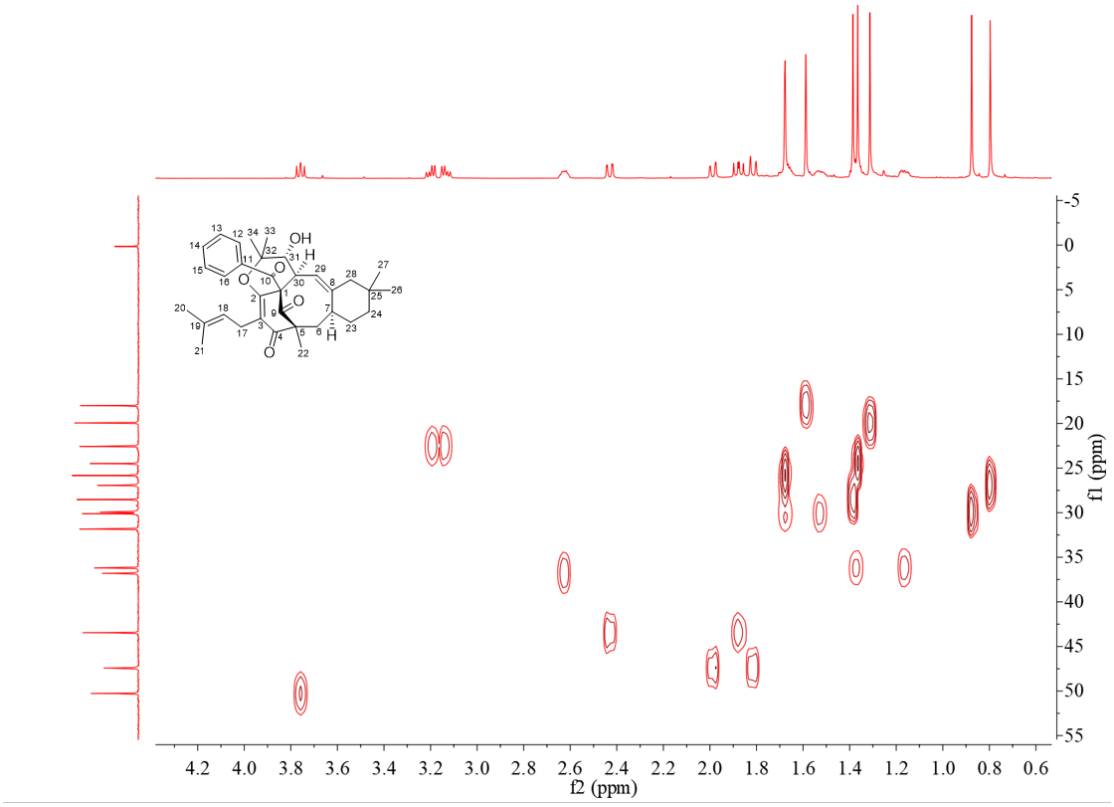


Figure S24. HMBC (600 MHz, $CDCl_3$) spectrum of **2**

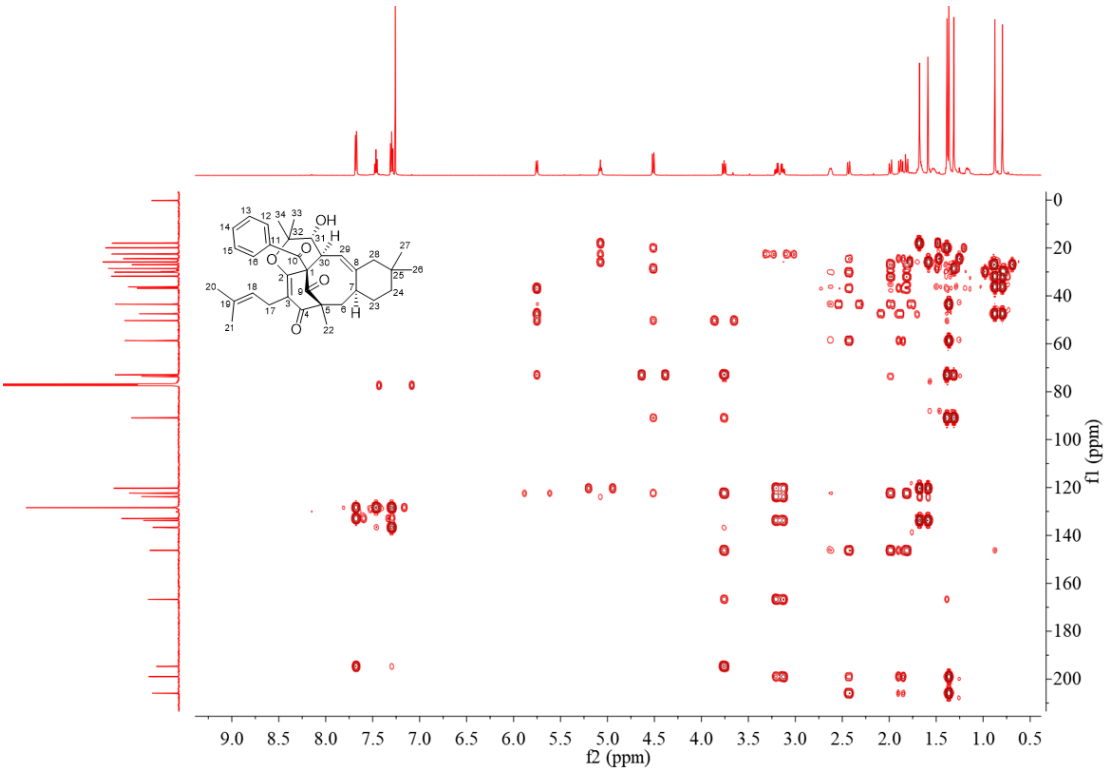


Figure S25. HMBC (600 MHz, $CDCl_3$) spectrum of **2**

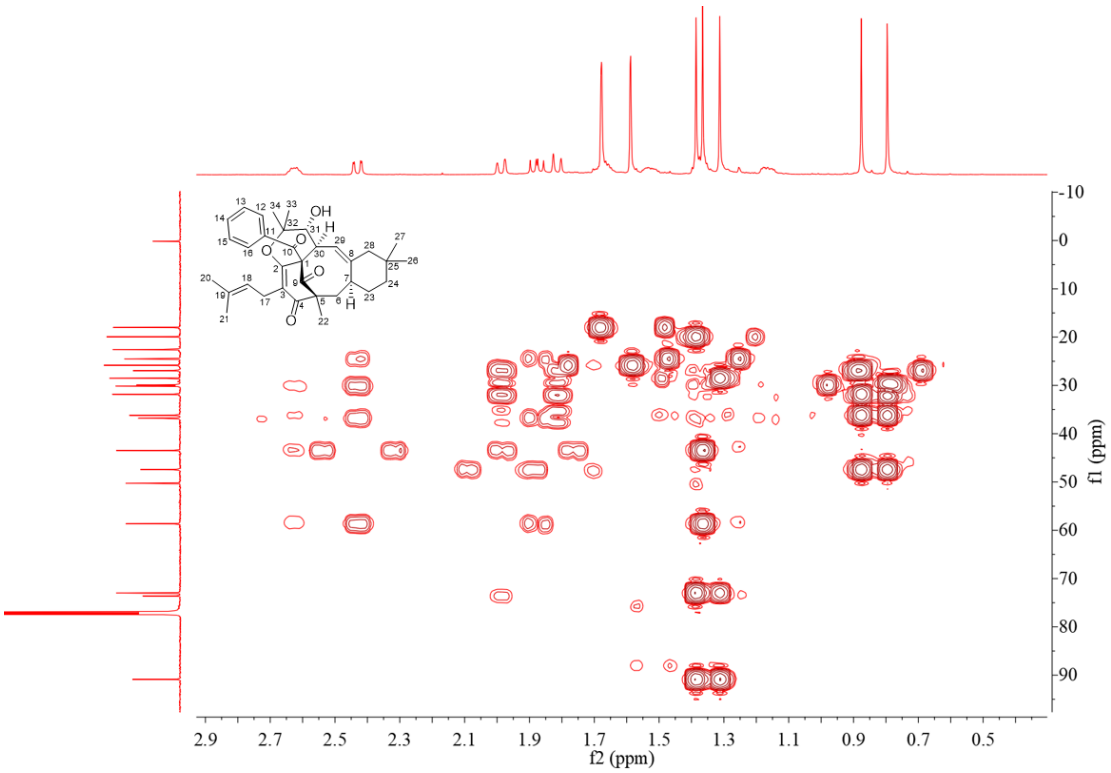


Figure S26. ^{1}H-^{1}H COSY (600 MHz, $CDCl_3$) spectrum of **2**

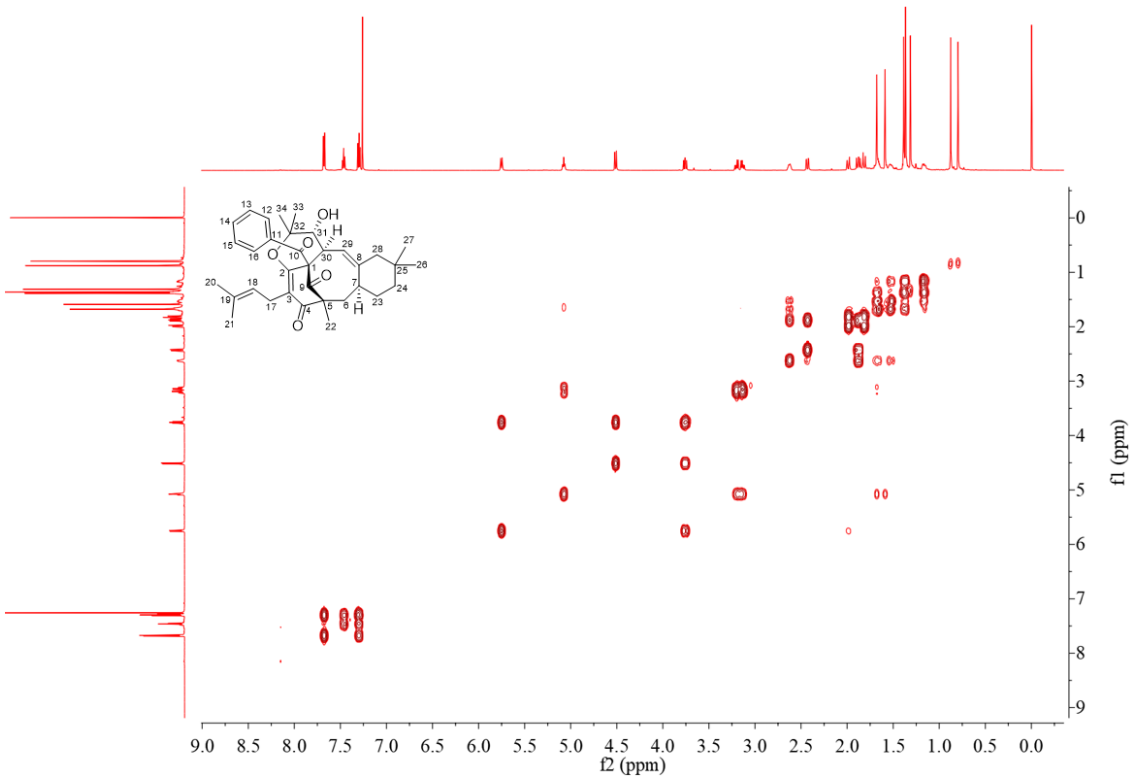


Figure S27. ^{1}H-^{1}H COSY (600 MHz, $CDCl_3$) spectrum of **2**

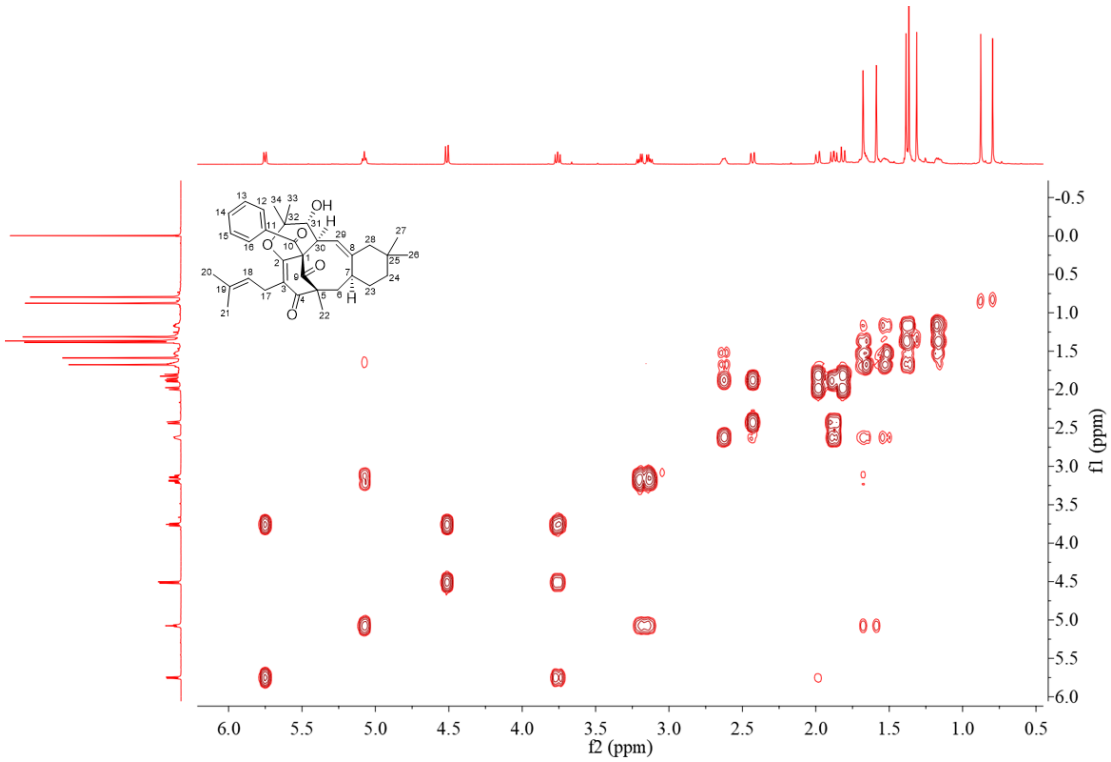


Figure S28. ROESY (600 MHz, $CDCl_3$) spectrum of **2**

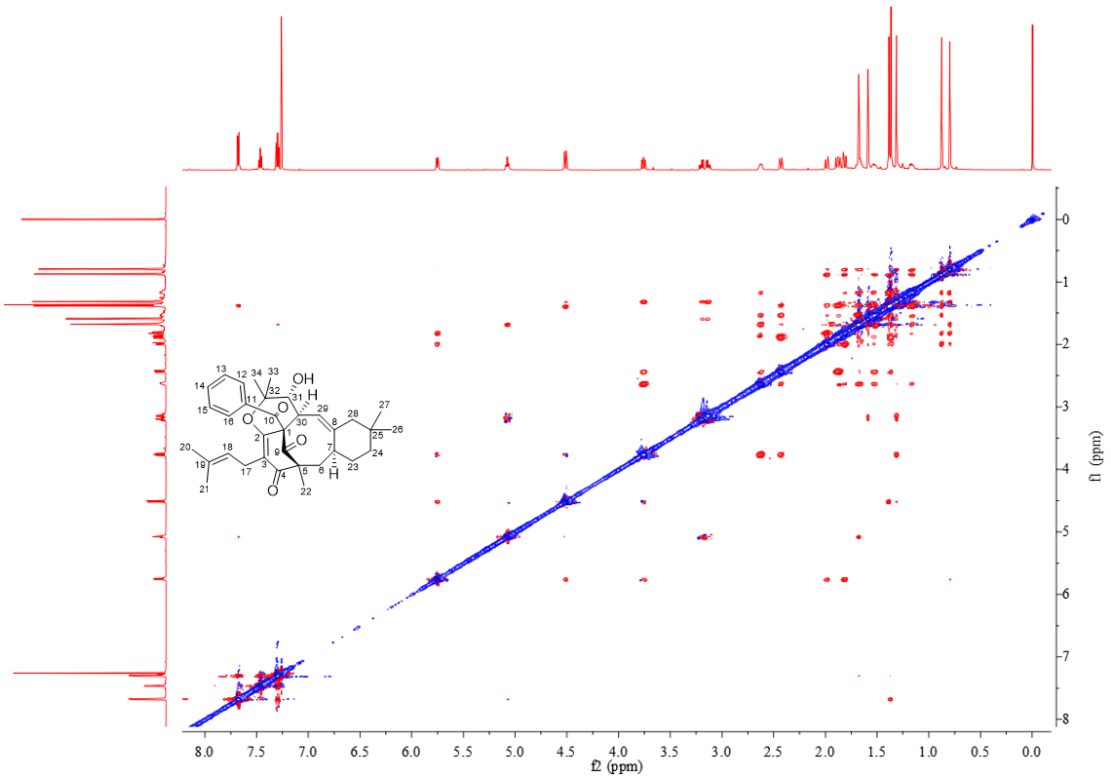


Figure S29. ROESY (600 MHz, $CDCl_3$) spectrum of **2**

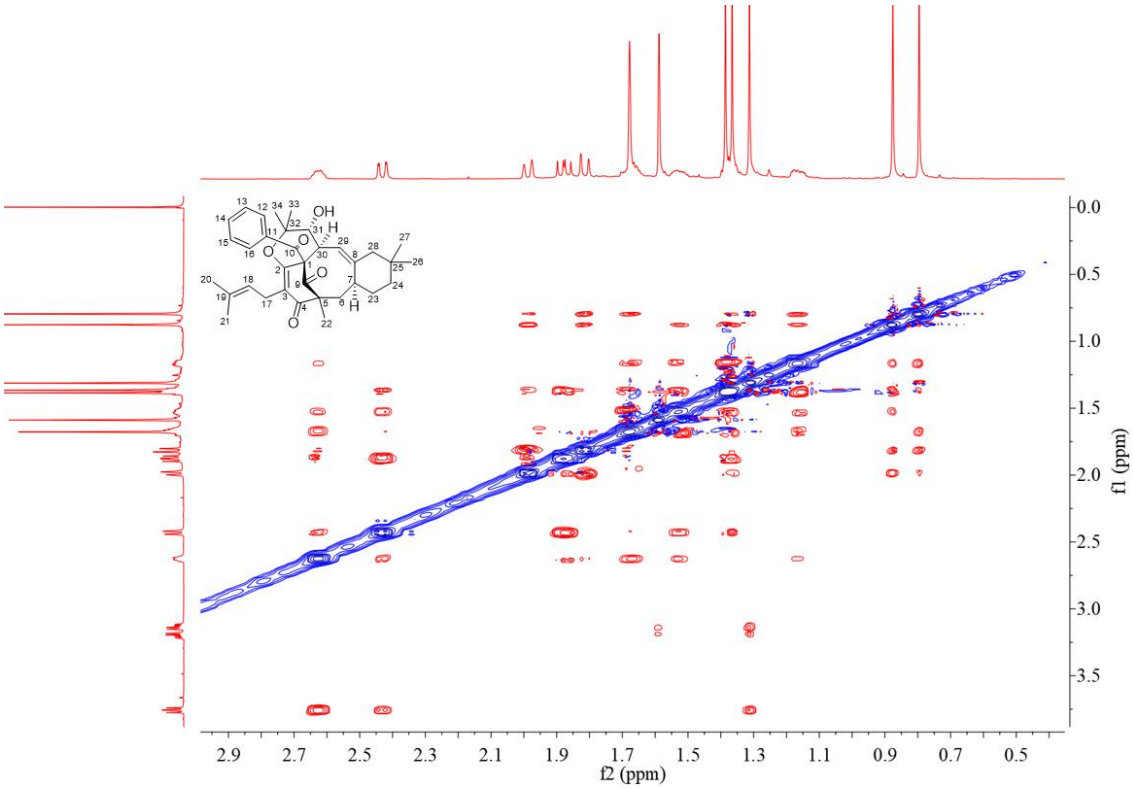


Figure S30. UV spectrum of **2**

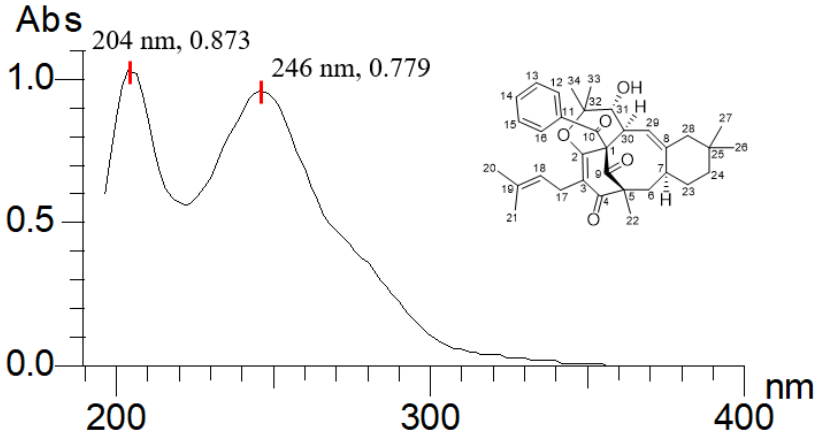


Figure S31. ECD spectrum of **2**

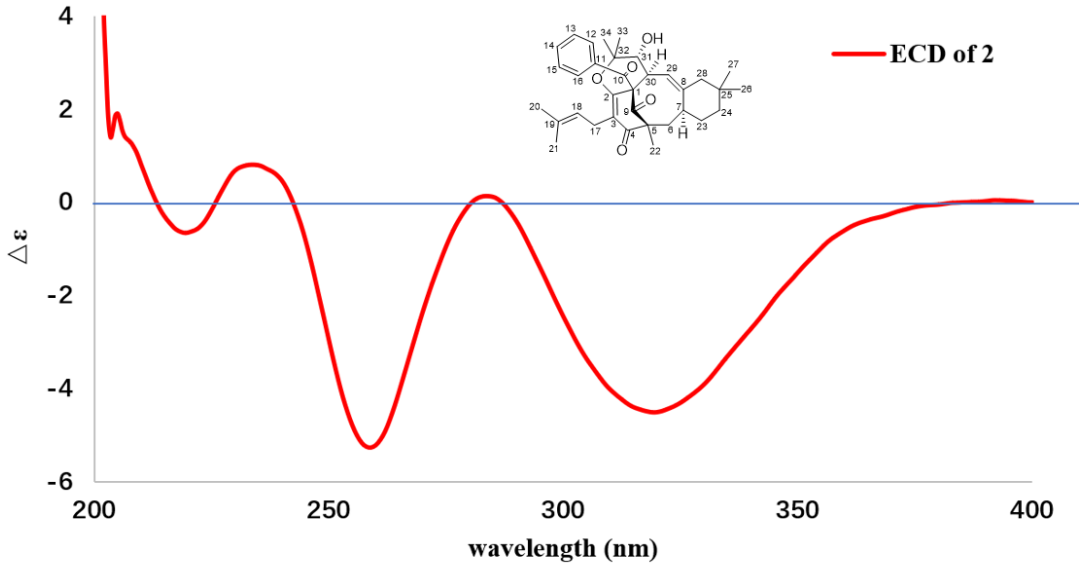


Figure S32. IR spectrum of **2**

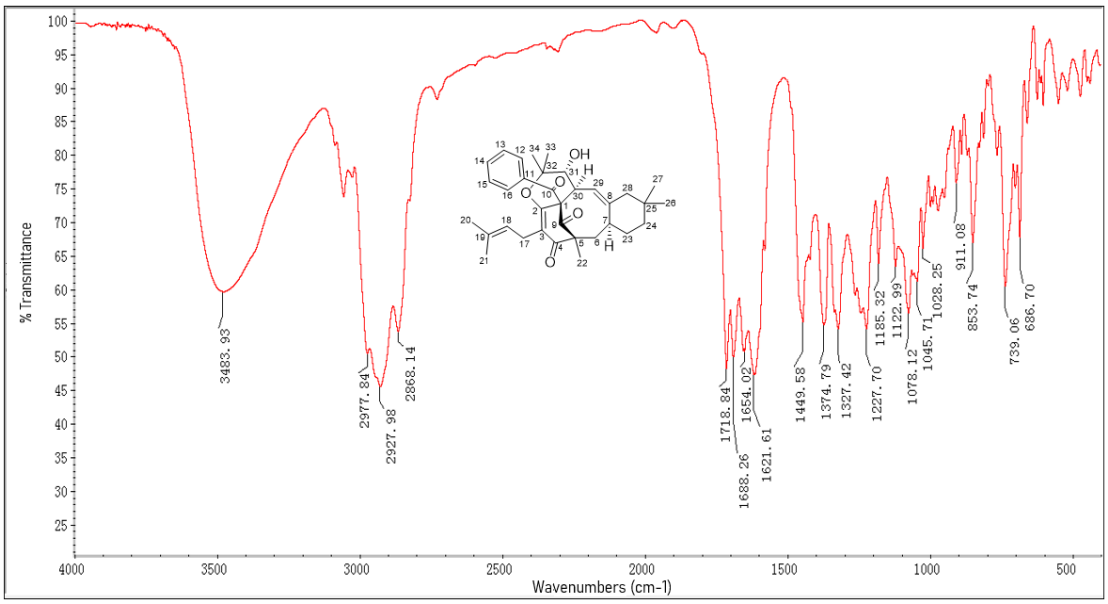


Figure S33. Positive ESIMS spectrum of **3**

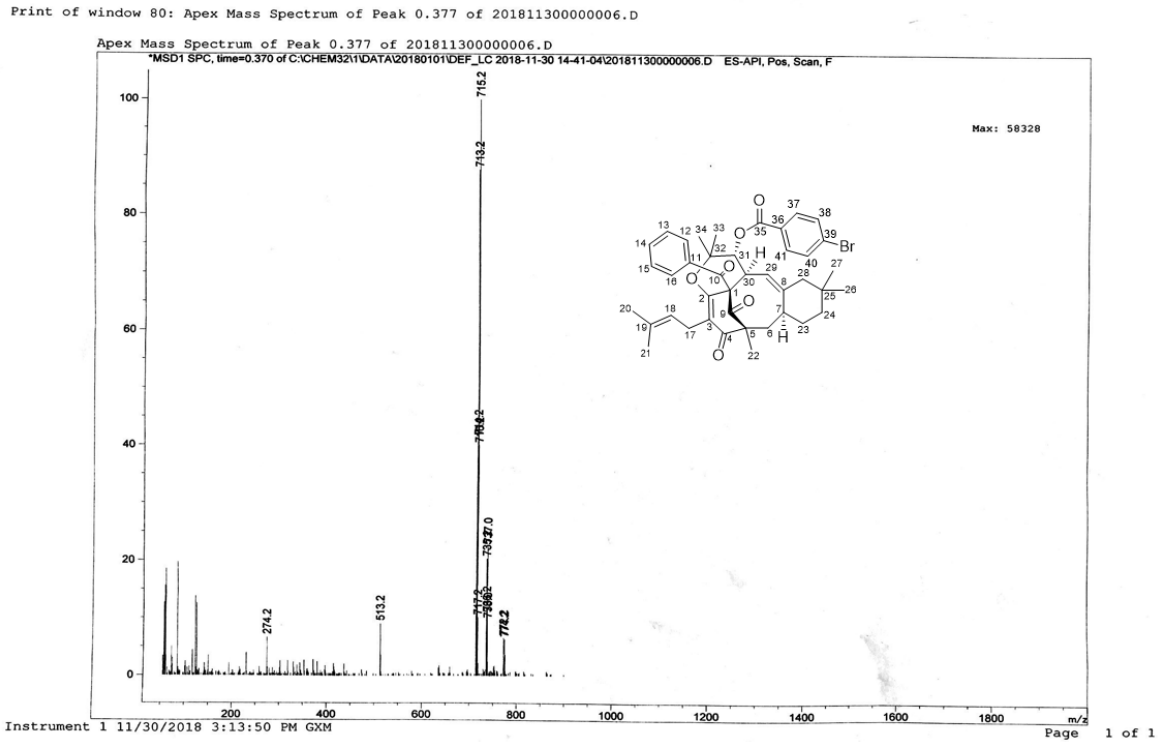


Figure S34. Negative ESIMS spectrum of **3**

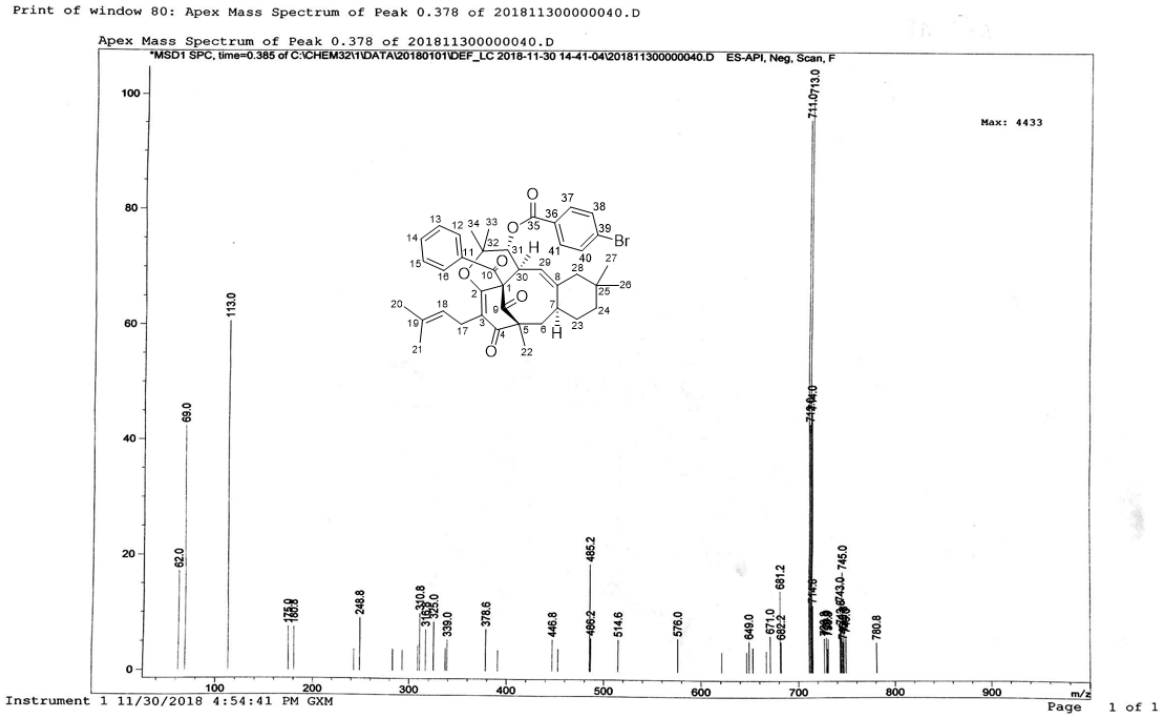


Figure S35. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **3**

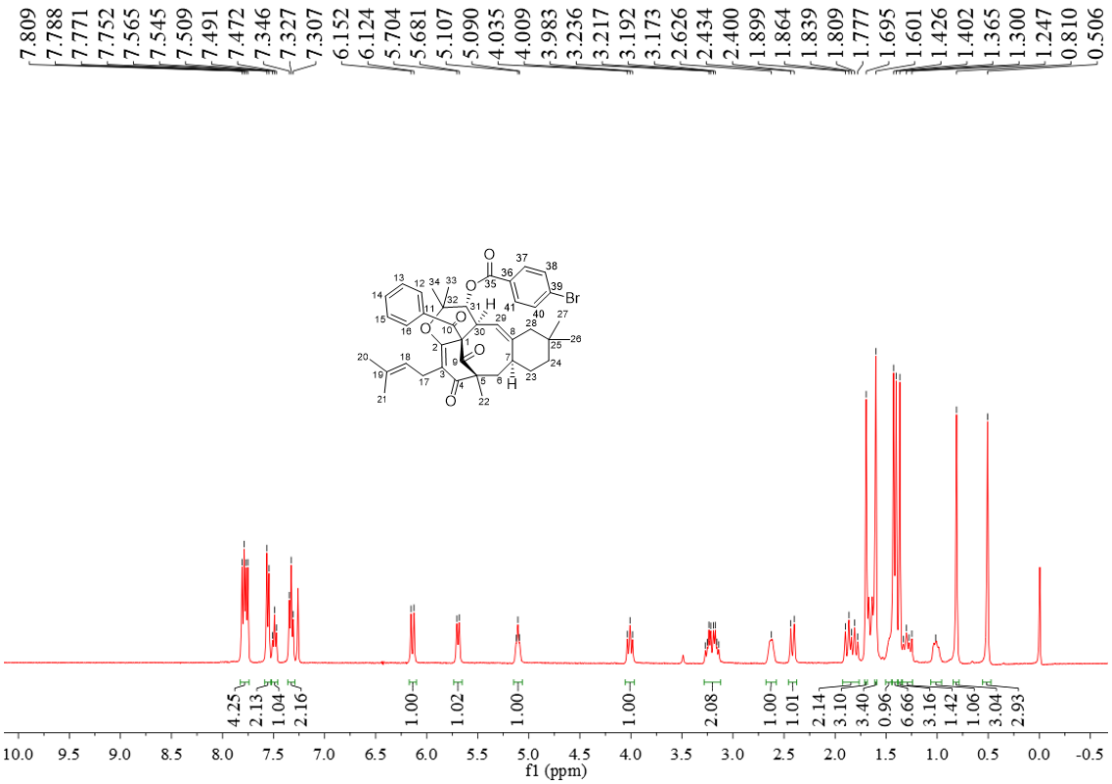


Figure S36. ^{13}C NMR and DEPT (150 MHz, $CDCl_3$) spectra of **3**