Sophalines E−I, Five Quinolizidine-Based Alkaloids with Antiviral Activities against Hepatitis B Virus from the Seeds of *Sophora alopecuroides*

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List of Supporting Information

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Table S1. NMR data of 1 and 2 (*J* in Hz)

	1[a]		**2**[b]	
no.	δ_H	δ_C	δ_H	δ_C
2α	-	165.4	2.55 (m)	42.0
2β			4.19 (m)	
3a	6.41 (d, 8.8)	116.4	1.57	18.4
3b			1.54	
4a	7.00 (dd, 8.8, 6.9)	140.9	1.66 (m)	25.8
4b			1.57	
5	5.73 (d, 6.9)	107.5	1.83 (m)	32.3
6	-	153.4	3.62 (m)	54.9
7	2.91 (br s)	36.4	2.39 (m)	41.3
8a	1.83 (m)	21.4	6.61 (d, 6.4)	138.3
8b	1.62 (m)			
9	2.25 (m)	33.3	-	130.6
10α	4.36 (d, 15.4)	53.5	-	166.8
10β	3.98 (dd, 15.4, 6.2)			
11	3.83 (d, 12.1)	59.4	3.28	55.5
12a	2.30 (m)	29.2	1.96 (m)	26.3
12b	1.36 (m)		1.58	
13a	4.38 (m)	66.5	1.75 (m)	18.6
13b			1.54	
14a	2.13 (m)	26.6	2.23 (m)	32.5
14b	1.54 (m)		2.23 (m)	
15	4.52 (br d, 12.5)	63.5	-	168.0
17α	3.17 (d, 11.1)	47.7	4.25 (dd, 13.1, 4.3)	40.5
17β	2.24 (m)		2.84 (t, 13.1)	
2′	7.66 (s)	135.3	8.35 (t, 18.8)	133.9
3′	-	115.9	-	116.1
4′	-	127.1	-	125.5
5′	8.09 (d, 7.6)	122.4	8.14 (d, 7.5)	121.3
6′	7.13	123.0	7.16 (td, 7.5, 1.3)	121.6
7′	7.16	123.8	7.20 (td, 7.5, 1.3)	122.7
8′	7.34 (d, 7.6)	112.7	7.47 (d, 7.5)	112.1
9′	-	137.4	-	136.6
10′	-	197.3	-	191.9
11′	-	-	3.79 (s)	41.0

[a]Measured in CD_3OD. [b]Measured at in DMSO. Overlapped signals are reported without designating multiplicity.

Table S2. NMR data of 3 and 4 (*J* in Hz)[a]

	3		**4**	
no.	δ_H	δ_C	δ_H	δ_C
2α	2.87 (td, 12.7, 3.0)	47.4	2.58 (td, 12.1, 2.9)	48.1
2β	3.26 (m)		3.05	
3a	1.82 (m)	25.4	1.64	27.3
3b	1.65 (m)		1.50	
4a	1.97	32.1	1.90	33.3
4b	1.97		1.30	
5	1.91 (m)	33.2	1.73 (m)	34.2
6	2.72	59.3	2.34 (m)	59.1
7	2.20 (m)	45.2	2.09 (m)	46.4
8a	2.79 (dd, 13.4, 3.7)	34.5	2.82 (dd, 13.5, 4.4)	34.7
8b	2.35 (t, 13.4)		2.30 (m)	
11	3.41 (m)	59.9	3.37 (m)	60.0
12a	1.49 (m)	31.3	1.52 (m)	31.1
12b	1.34 (m)		1.30	
13a	1.68 (m)	19.8	1.66	19.8
13b	1.50 (m)		1.50	
14	2.28	32.8	2.25	32.9
15	-	172.7	-	172.6
17α	4.18 (dd, 13.9, 3.7)	45.0	4.02 (dd, 13.8, 5.0)	45.9
17β	3.10 (t, 13.9)		3.06	
2′,10′	3.09	51.3	3.07	51.3
3′,9′	1.96	23.4	1.95 (m)	23.4
4′,8′	2.72	28.6	2.72 (m)	28.6
5′,7′	-	123.5	-	123.5
6′	-	142.8	-	142.6
11′,13′	6.64 (s)	128.7	6.63 (s)	128.6
12′	-	127.0	-	128.4

[a]Measured in CD_3OD. Overlapped signals were reported without designating multiplicity.

Table S3. NMR data of 5 (*J* in Hz)[a]

no.	δ_H	δ_C	no.	δ_H	δ_C
2α	2.04	58.3	2′α	1.94	56.0
2β	2.84		2′β	2.84	
3a	1.88	21.6	3′a	1.63	26.6
3b	1.48		3′b	1.63	
4a	1.65	28.7	4′α	1.12 m	30.4
4b	1.65		4′β	1.72	
5	1.78	36.2	5′	1.98 m	36.6
6	2.23 m	64.7	6′	2.36 m	63.4
7	1.71	42.8	7′	1.84 m	44.0
8a	1.93	27.2	8′α	1.44	19.4
8b	1.48		8′β	1.37 m	
9a	1.75	22.0	9′a	1.75	29.1
9b	1.50		9′b	1.50	
10α	2.04	58.3	10′	3.35	59.8
10β	2.84				
11	3.92 m	52.8	11′	3.62 td (10.6, 4.5)	55.8
12a	2.75 td (18.2, 4.3)	27.8	12′a	2.10 m	28.9
12b	2.30 m		12′b	1.26 m	
13	6.80 t (4.3)	134.5	13′a	1.92	20.3
			13′b	1.66	
14	-	136.5	14′a	2.37 m	32.8
			14′b	2.30 m	
15	-	168.1	15′	-	172.4
17α	4.08 dd (13.1, 4.7)	43.5	17′α	2.93 t (13.5)	47.9
17β	3.15 t (13.1)		17′β	3.58 dd (13.5, 3.8)	

[a]Measured in CD_3OD. Overlapped signals were reported without designating multiplicity.

Isolation Procedures

General. Melting point: X-5 apparatus and was uncorrected; Optical rotation: JASCO P-1020 polarimeter; IR spectra: JASCO FT/IR-480 plus FT-IR spectrometer (KBr pellets); UV spectra: JASCO V-550 UV/VIS spectrophotometer; CD spectra were obtained on a JASCO J-810 spectropolarimeter (JASCO, Tokyo, Japan) at room temperature; NMR spectra: Bruker AV-500 (^{1}H: 500 MHz, ^{13}C: 125 MHz) spectrometer; HRESIMS spectra: Agilent 6210 LC/MSD TOF mass spectrometer. CD spectra: JASCO J-810 spectropolarimeter. X-ray crystallographic analysis: Agilent Gemini S Ultra CCD diffractometer with Cu Kα radiation (λ = 1.54178 Å). Analytical HPLC: Agilent 1200 unit with DAD detector and a RP C18 column (5 μm, 10 mm × 250 mm; Cosmosil, Kyoto, Japan); Preparative HPLC: Varian Prostar system equipped with UV detectors (Varian, California, USA) and a preparative Cosmosil C_{18} column (20 mm × 250 mm; Nacalai Tesque). Thinlayer chromatography (TLC) was performed using precoated silica gel plates (GF_{254}, Yantai Jiangyou Silica Gel Technology Development Co., Ltd., Yantai, China). Open column chromatography (CC) was performed using macroporous resin (Diaion HP-101, Shanghai, China), ODS silica gel (50 mm; YMC, Tokyo, Japan), and Sephadex LH-20 (25–100 mm, Fluka, Buchs, Switzerland). All the reagents were purchased from Tianjin Damao Chemical Company (Damao, Tianjin, China).

Plant Material. The seeds of *Sophora alopecuroides* L. were collected in August 2014 from Wuzhong City, Ningxia Hui Autonomous Region, China, and were authenticated by Prof. Guang-Xiong Zhou, College of Pharmacy, Jinan University. A voucher specimen (No. 20140829) was deposited in the Institute of Traditional Chinese Medicine and Natural Products, Jinan University, Guangzhou, China.

***Extraction and Isolation*.** The air-dried and pulverized seeds (30.0 kg) of *S. alopecuroides* were extracted with 95% ethanol to obtain a crude extract (1.9 kg). The crude extract was dissolved in water and then acidified to pH 3 using 1% HCl. After removal of the neutral components by using $CHCl_3$, the aqueous layer was basified with NH_3 H_2O to pH 8 and re-extracted with $CHCl_3$ to give a total alkaloid fraction (897 g). Then, the alkaloid fraction was subjected repeatedly to column chromatography over macroporous resin (Diaion HP-101) eluting with EtOH/H_2O (10:90, 30:70, 50:50, 70:30, 95:5, *v*:*v*) to afford five major fractions (Fr. 1−5). Fr. 5 (4.9 g) was chromatographed over a ODS column using MeOH/H_2O in a gradient (30:70 to 100:0, *v*:*v*) to yield five subfractions (Fr. 5.1−5.4). Fr. 5.2 (82.2 mg) was purified by preparative HPLC (MeOH/H_2O, 75:25, *v*:*v*) to yield

compound **1** (19.6 mg). Fr. 5.4 (121.8 mg) were chromatographed by Sephadex LH-20 (MeOH), and then purified by preparative HPLC (MeOH/H_2O, 80:20, *v*:*v*) to yield compounds **2** (9.4 mg) and **5** (17.9 mg) . Compounds **3** (8.5 mg) and **4** (27.4 mg) were isolated from Fr.5.5 (58.0 mg) by preparative HPLC (ACN/H_2O, 35:65, *v*:*v*).

Physico-chemical constants of 1–5

Sophaline E (**1**): Colorless crystals in MeOH/H_2O; $[\alpha]_D^{25}$ -19.0 (*c* 0.3, CH_3OH); mp 254-255 ℃; HR-ESI-MS *m/z*: 404.1971 $[M+H]^+$ (calcd for $C_{24}H_{26}N_3O_3$, 404.1969); UV (CH_3OH) λ_{max}: 210, 305 nm; IR (KBr) ν_{max}: 3423, 2934, 2868, 1615, 1457, 1413, 1338, 1117, 658 cm^{-1}.

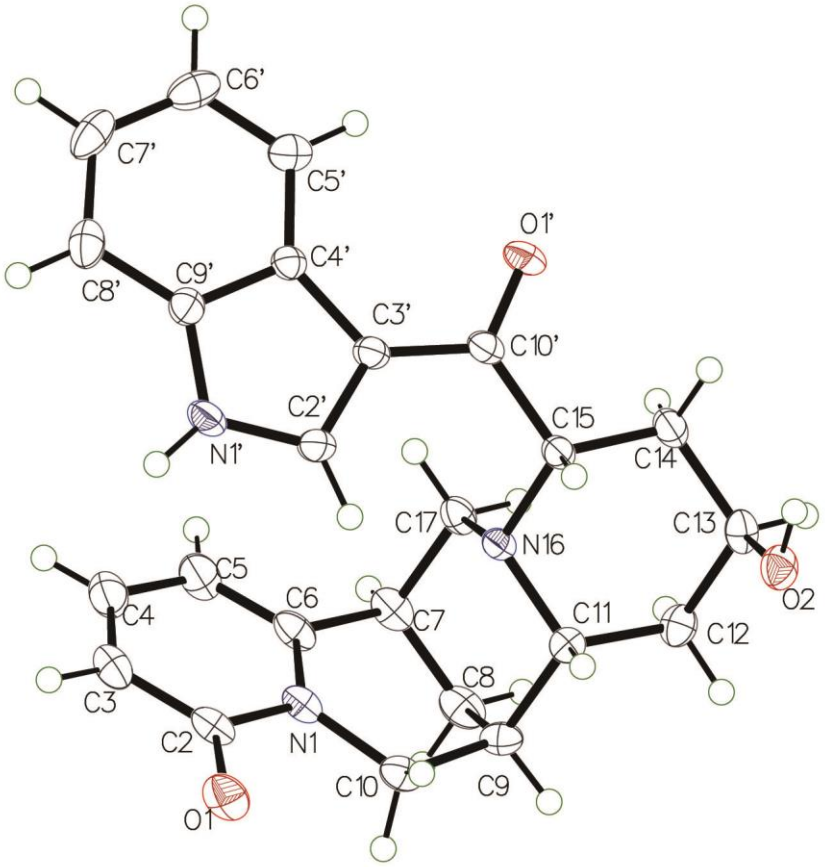
Sophaline F (**2**): Colorless oil; $[\alpha]_D^{25}$ +37.8 (*c* 1.0, CH_3OH); HR-ESI-MS *m/z*: 418.2139 $[M+H]^+$ (calcd for $C_{25}H_{28}N_3O_3$, 418.2125); UV (CH_3OH) λ_{max}: 213, 241, 298 nm; IR (KBr) ν_{max}: 2930, 2866, 1618, 1437, 1343, 1264, 1133, 752 cm^{-1}.

Sophaline G (**3**): Brown oil; $[\alpha]_D^{25}$ -11.0 (*c* 0.2, CH_3OH); HR-ESI-MS *m/z*: 394.2858 $[M+H]^+$ (calcd for $C_{25}H_{36}N_3O$, 394.2853); UV (CH_3OH) λ_{max}: 248 nm; IR (KBr) ν_{max}: 3409, 2947, 1613, 1448, 1384, 1159, 669 cm^{-1}.

Sophaline H (**4**): Brown oil; $[\alpha]_D^{25}$ -14.4 (*c* 0.4, CH_3OH); HR-ESI-MS *m/z*: 394.2856 $[M+H]^+$ (calcd for $C_{25}H_{36}N_3O$, 394.2853); UV (CH_3OH) λ_{max}: 218, 248 nm; IR (KBr) ν_{max}: 3416, 2929, 2854, 1626, 1445, 1338, 1125, 652 cm^{-1}.

Sophaline I (**5**): Colorless crystals in MeOH/H_2O; $[\alpha]_D^{25}$ +15.0 (*c* 0.5, CH_3OH); HR-ESI-MS *m/z*: 493.3539 $[M+H]^+$ (calcd for $C_{30}H_{45}N_4O_2$, 493.3537); UV (CH_3OH) λ_{max}: 211, 250 nm; IR (KBr) ν_{max}: 3429, 2935, 1618, 1415, 1349, 1296, 655 cm^{-1}.

X-Ray Crystallographic analysis of 1



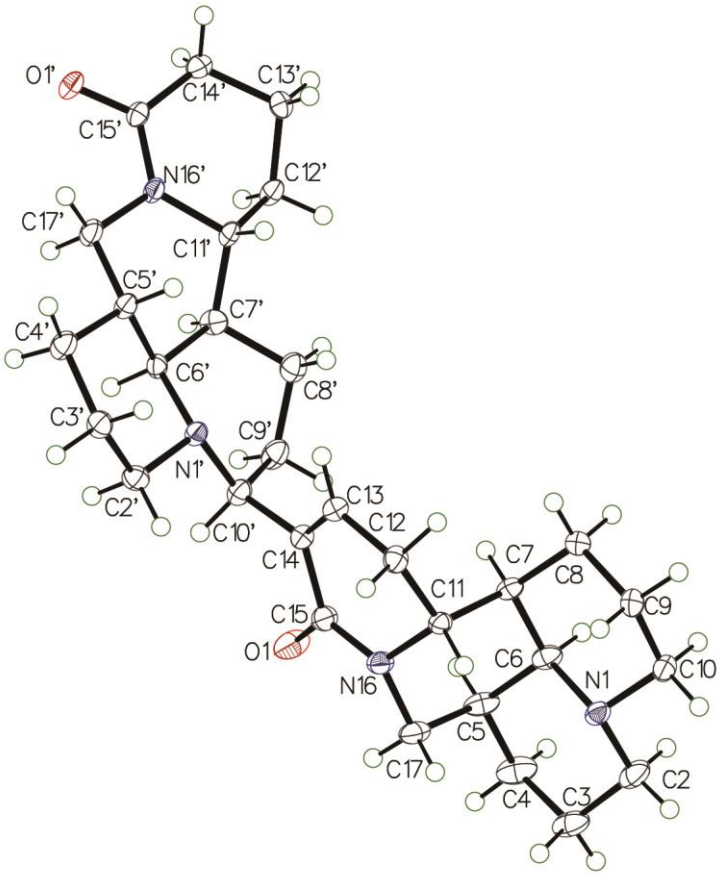
Crytallographic data for **1** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 1813929. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 IEZ, UK (fax: +44-(0)1223-336033 or email: deposit@ccdc.cam.ac.uk).

X-ray crystallographic data of 1

Empirical formula	$C_{24}H_{27}N_3O_4$
Formula weight	421.48
Temperature/K	100.01(10)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	9.68940(10)
b/Å	12.88380(10)
c/Å	16.7991(2)
α/°	90
β/°	90
γ/°	90

Volume/Å^3	2097.14(4)
Z	4
ρ_{calc}g/cm^3	1.335
μ/mm^{-1}	0.746
F(000)	896.0
Crystal size/mm^3	0.14 ×0.12 ×0.11
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	8.65 to 147.156
Index ranges	-12 ≤ h ≤ 11, -15 ≤ k ≤ 15, -20 ≤ l ≤ 20
Reflections collected	30807
Independent reflections	4178 [R_{int} = 0.0632, R_{sigma} = 0.0297]
Data/restraints/parameters	4178/0/284
Goodness-of-fit on F^2	1.032
Final R indexes [I>=2σ (I)]	R_1 = 0.0389, wR_2 = 0.0984
Final R indexes [all data]	R_1 = 0.0395, wR_2 = 0.0989
Largest diff. peak/hole / e Å^{-3}	0.76/-0.25
Hooft parameter	-0.08(6)
Flack parameter	-0.10(6)

X-Ray Crystallographic analysis of 5



Crytallographic data for **5** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 1820013. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 IEZ, UK (fax: +44-(0)1223-336033 or email: deposit@ccdc.cam.ac.uk).

X-ray crystallographic data of 5

Empirical formula	$C_{30}H_{54}N_4O_7$
Formula weight	582.77
Temperature/K	100(2)
Crystal system	monoclinic
Space group	$P2_1$
a/Å	11.62198(16)
b/Å	7.08780(13)
c/Å	19.4487(3)
α/°	90
β/°	106.2877(15)
γ/°	90
Volume/Å^3	1537.77(4)
Z	2
ρ_{calc}g/cm^3	1.259
μ/mm^{-1}	0.722
F(000)	636.0
Crystal size/mm^3	0.28 × 0.26 × 0.21
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	4.734 to 147.44
Index ranges	$-14 \leq h \leq 13$, $-8 \leq k \leq 7$, $-24 \leq l \leq 21$
Reflections collected	18409
Independent reflections	5577 [R_{int} = 0.0307, R_{sigma} = 0.0236]
Data/restraints/parameters	5577/1/405
Goodness-of-fit on F^2	1.029
Final R indexes [I>=2σ (I)]	R_1 = 0.0403, wR_2 = 0.1089
Final R indexes [all data]	R_1 = 0.0410, wR_2 = 0.1096
Largest diff. peak/hole / e Å^{-3}	0.44/-0.24
Hooft parameter	-0.04(6)
Flack parameter	-0.06(8)

Quantum chemical ECD calculation method

The theoretical calculations of compounds **2-4** were performed using Gaussian 09.[1] The systematic random conformational analysis of **2-4** was performed in the SYBYL 8.1 program by using MMFF94s molecular force field, which afforded 42, 54 and 49 conformers for **2-4** respectively, with an energy cutoff of 10 kcal mol^{-1} to the global minima. All the obtained conformers were further optimized using DFT at the B3LYP/6-31+G(d) level in gas phase by using Gaussian09 software.[1] All of the optimized stable conformers were used for TDDFT computation of the excited stats at the same levels, with the consideration of the first 30 excitations. The overall ECD curves of **2-4** were weighted by Boltzmann distribution of each conformer (with a half-bandwidth of 0.33 eV), with a UV correction of 15 nm. The calculated ECD spectra of **2-4** were subsequently compared with the experimental one. The ECD spectra were produced by SpecDis 1.6 software.[2]

Reference

[1] Gaussian 09, Revision A.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.
[2] T. Bruhn, A. Schaumlöffel, Y. Hemberger, G. Bringmann, SpecDis version 1.60, University of Wuerzburg, Germany, 2012.

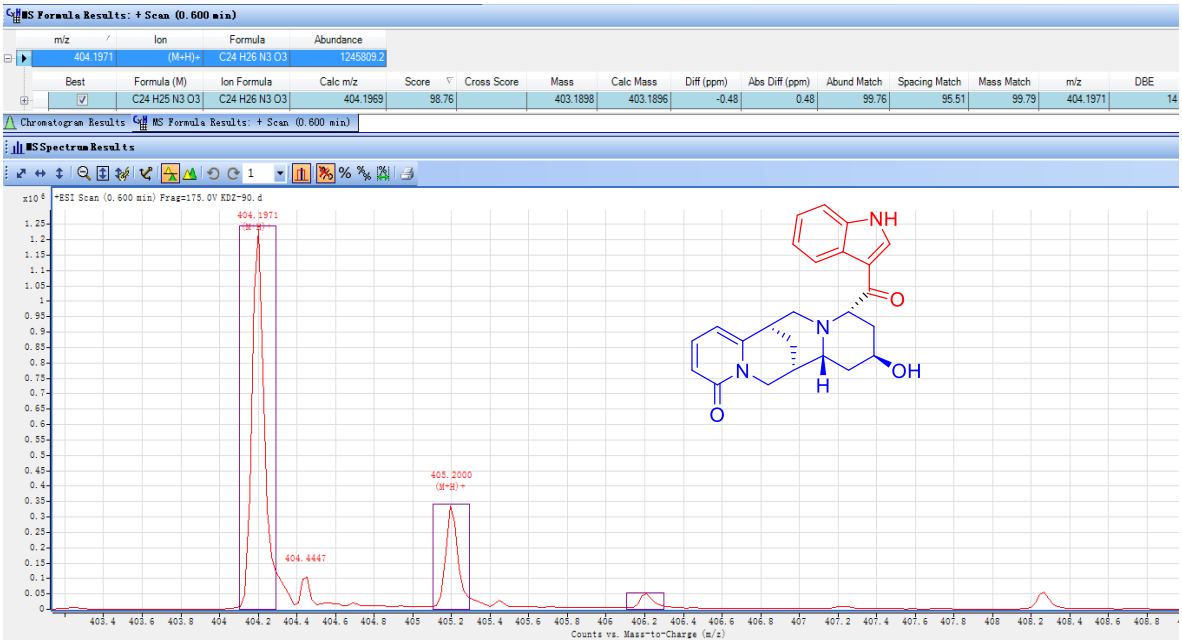


Figure S1. HR-ESI-MS spectrum of 1

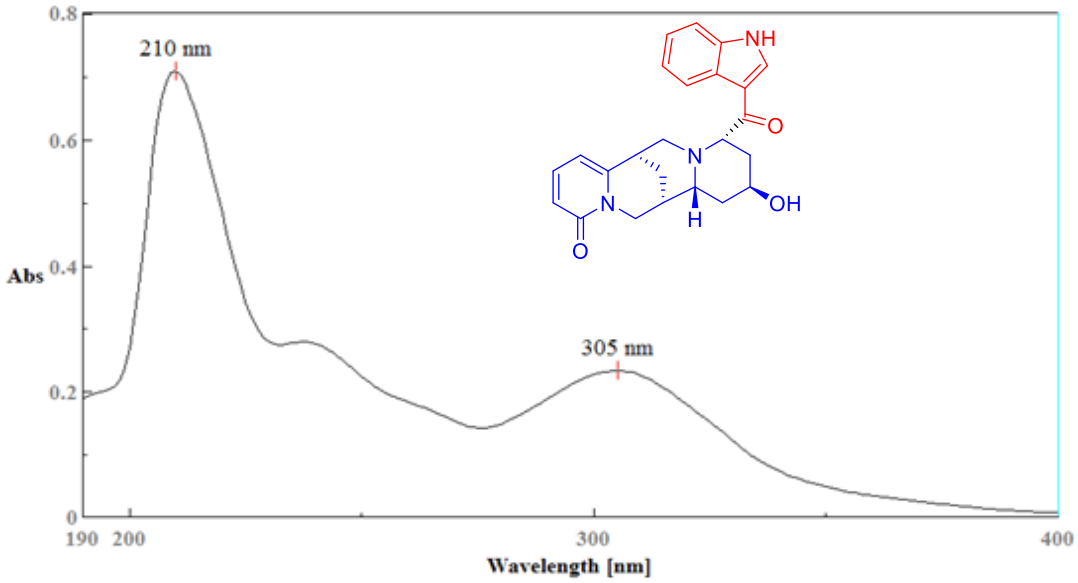


Figure S2. UV spectrum of 1 (CH_3OH)

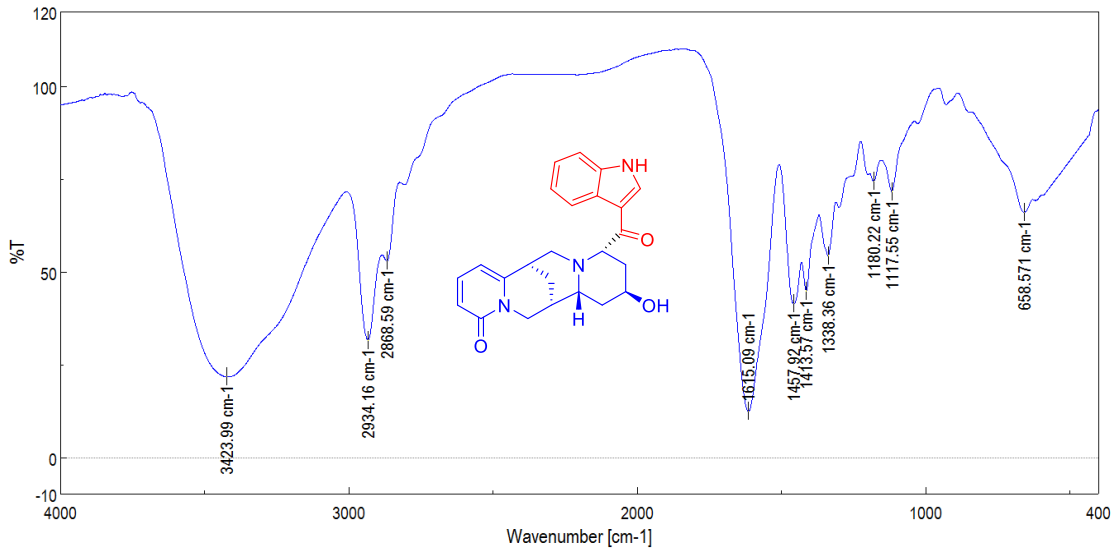


Figure S3. IR spectrum of 1 (KBr disc)

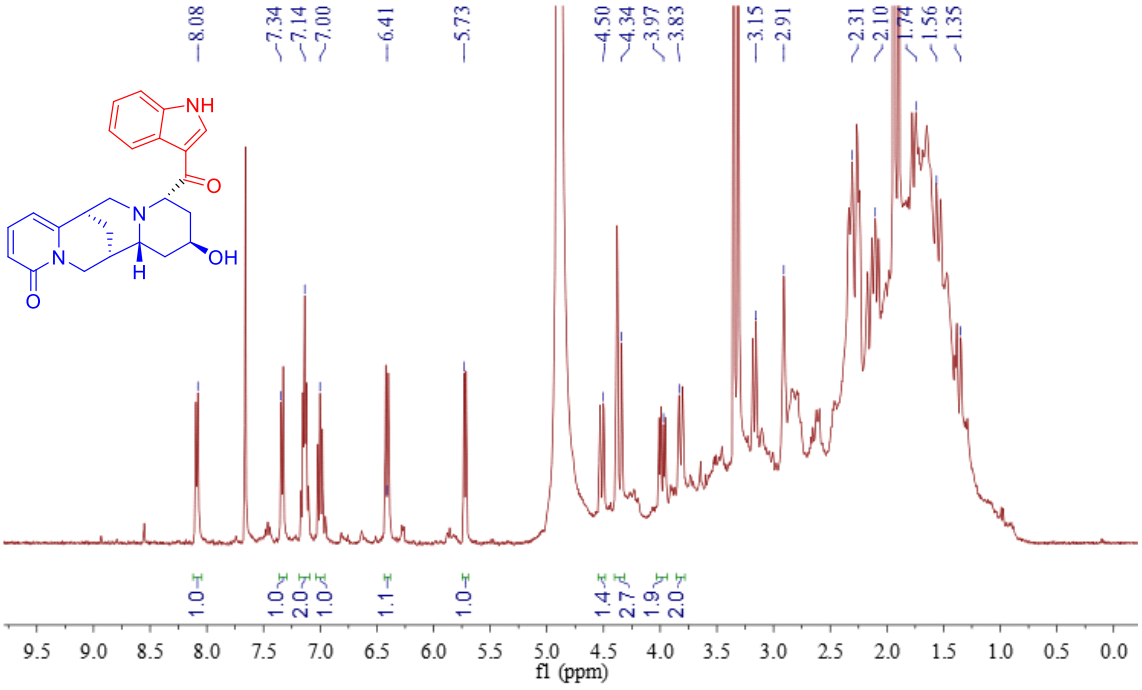


Figure S4. ^{1}H NMR spectrum of 1 in CD_3OD

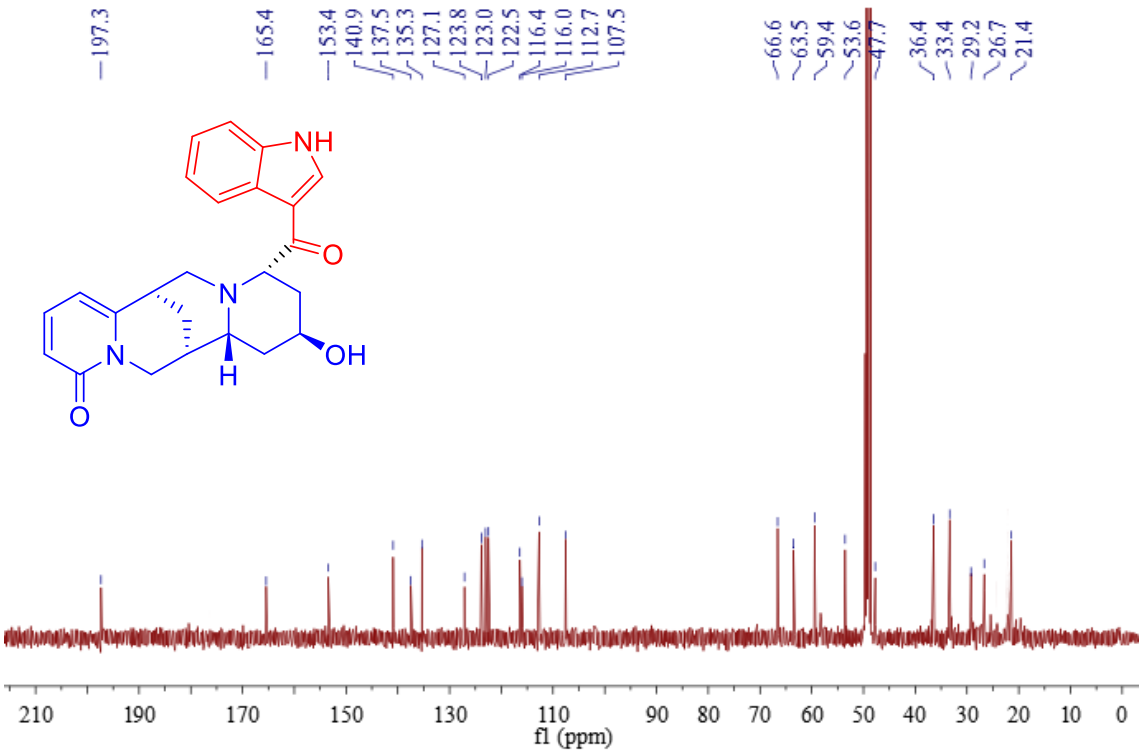


Figure S5. ^{13}C NMR spectrum of 1 in CD_3OD

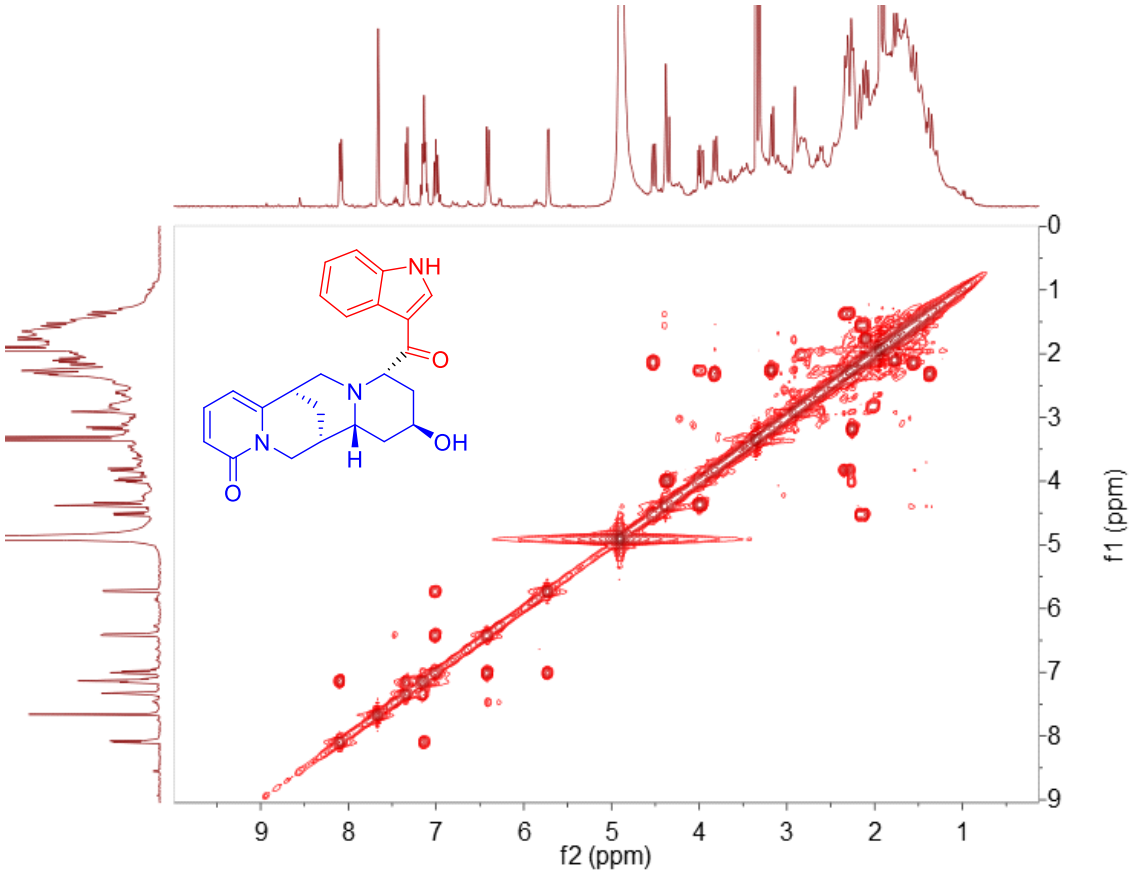


Figure S6. ^{1}H-^{1}H COSY spectrum of 1 in CD_3OD

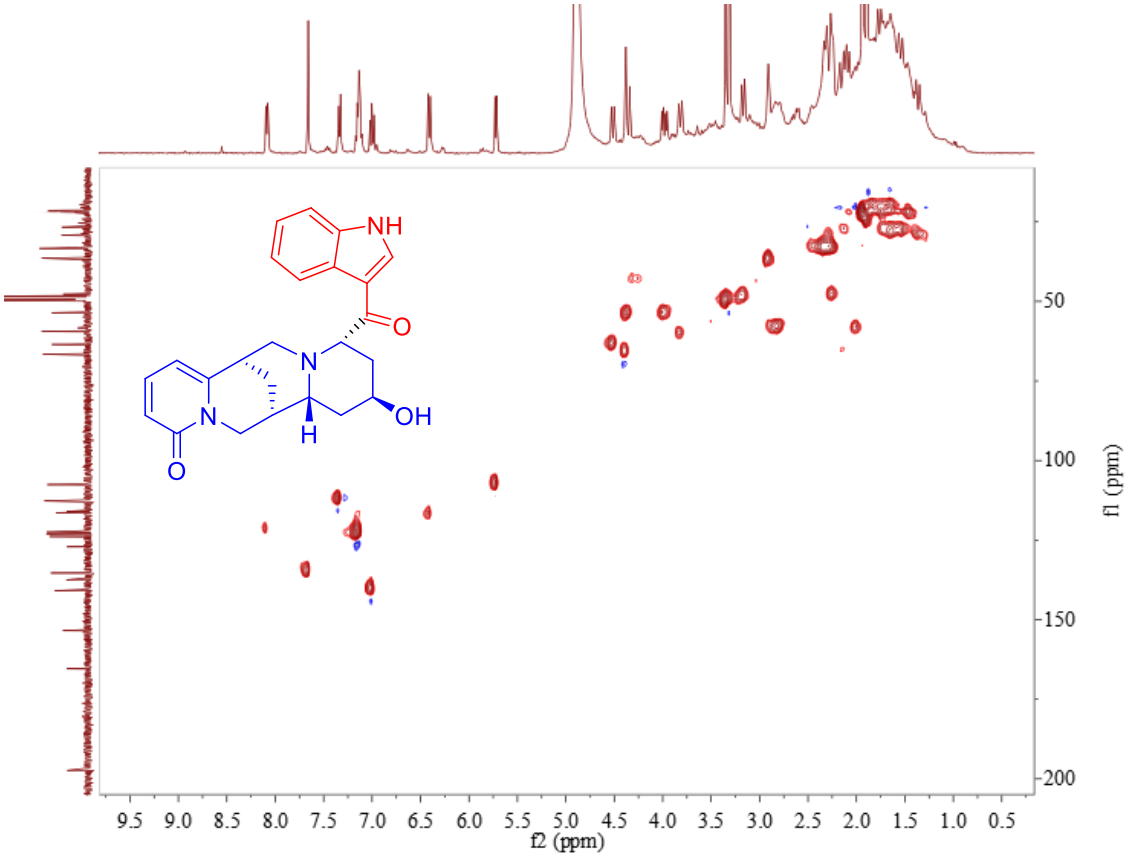


Figure S7. HSQC spectrum of 1 in CD_3OD

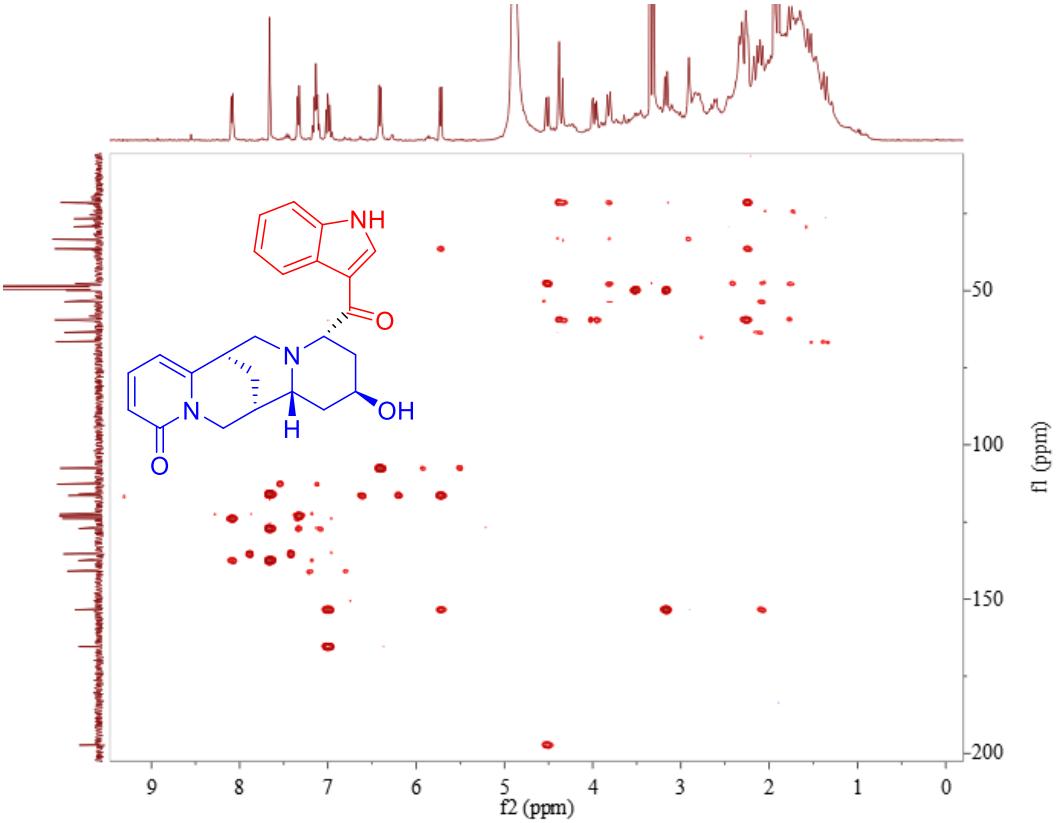


Figure S8. HMBC spectrum of 1 in CD_3OD

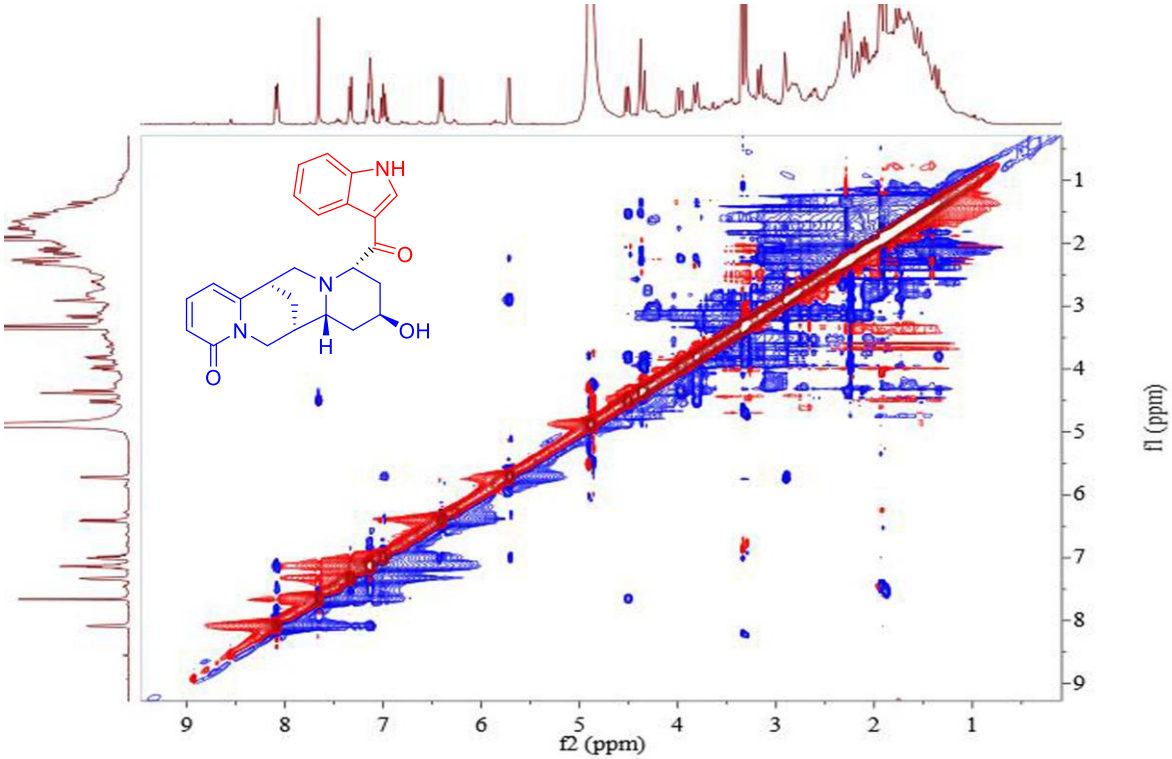


Figure S9. NOESY spectrum of 1 in CD_3OD

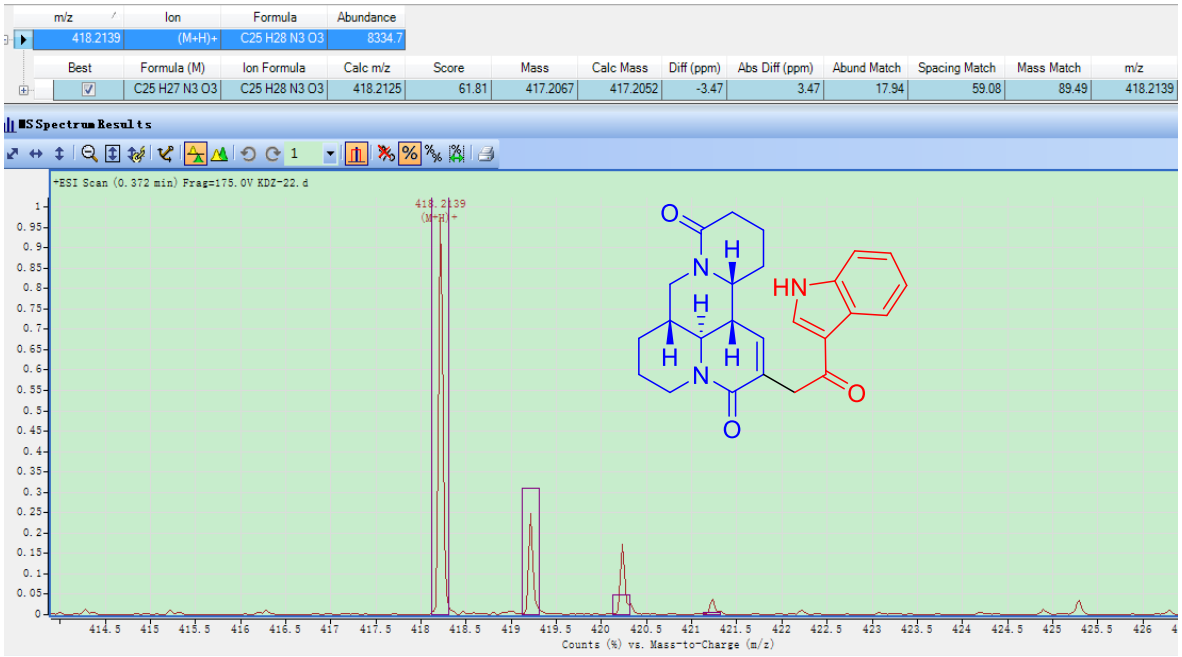


Figure S10. HR-ESI-MS spectrum of 2

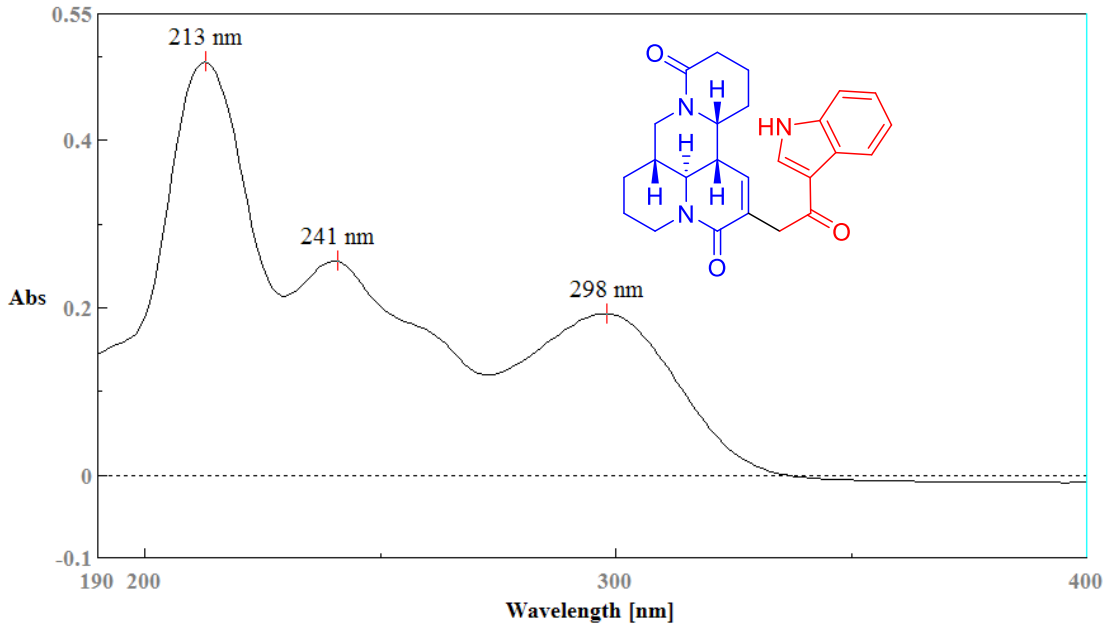


Figure S11. UV spectrum of 2 (CH_3OH)

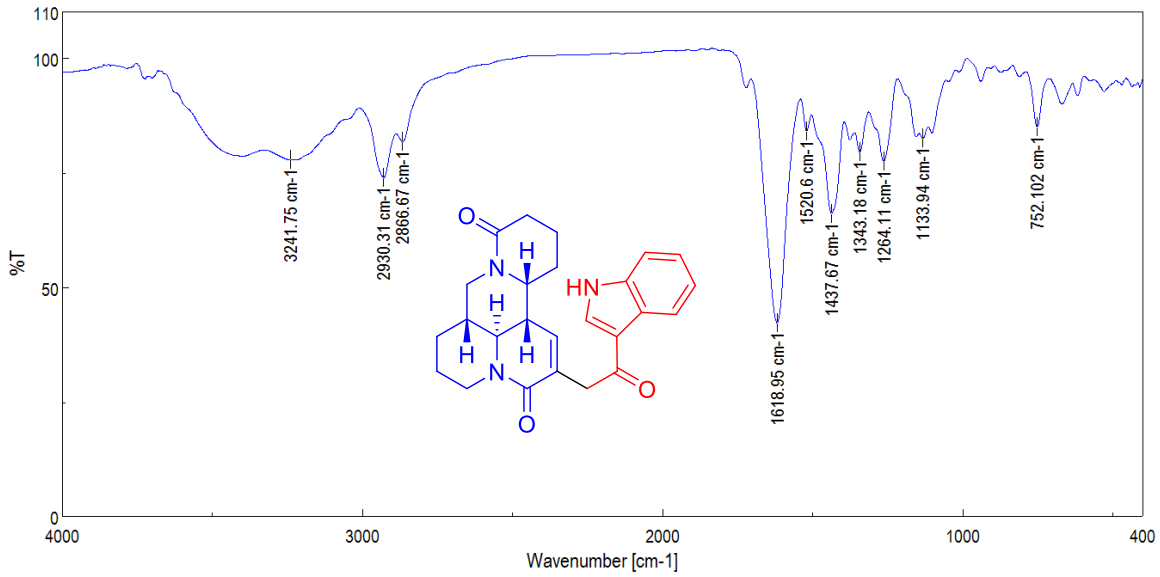


Figure S12. IR spectrum of 2 (KBr disc)

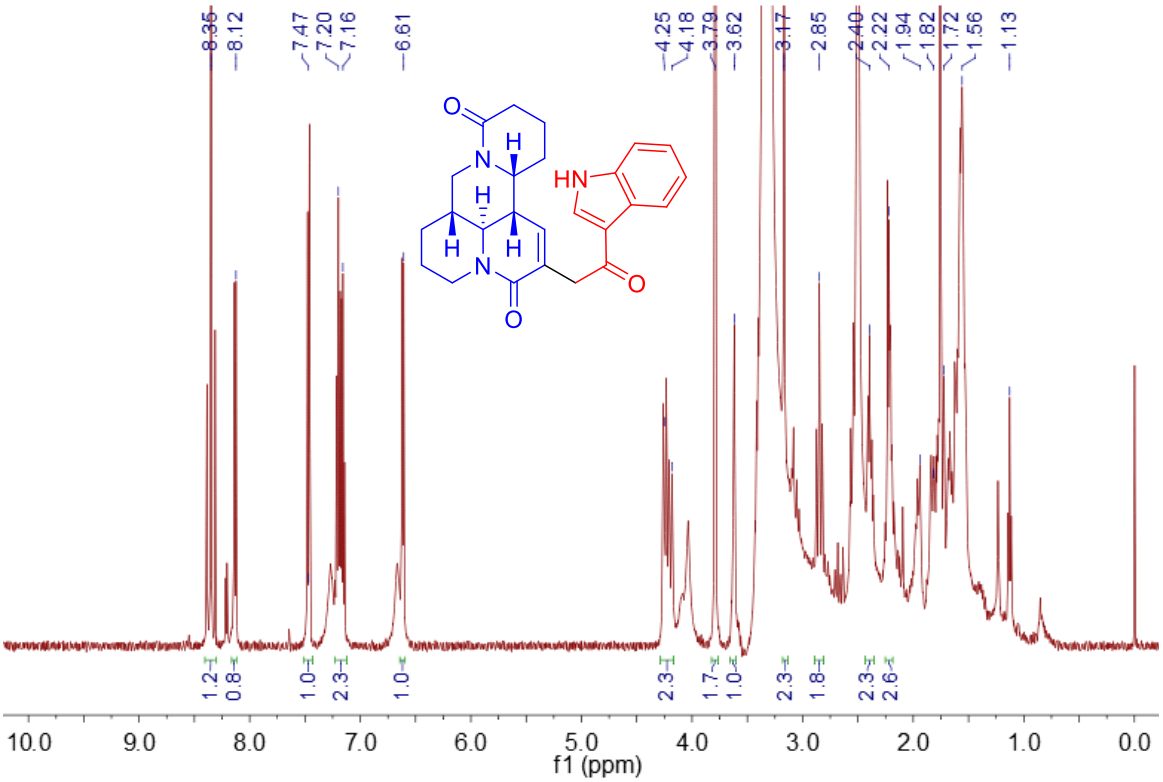


Figure S13. ^{1}H NMR spectrum of 2 in DMSO

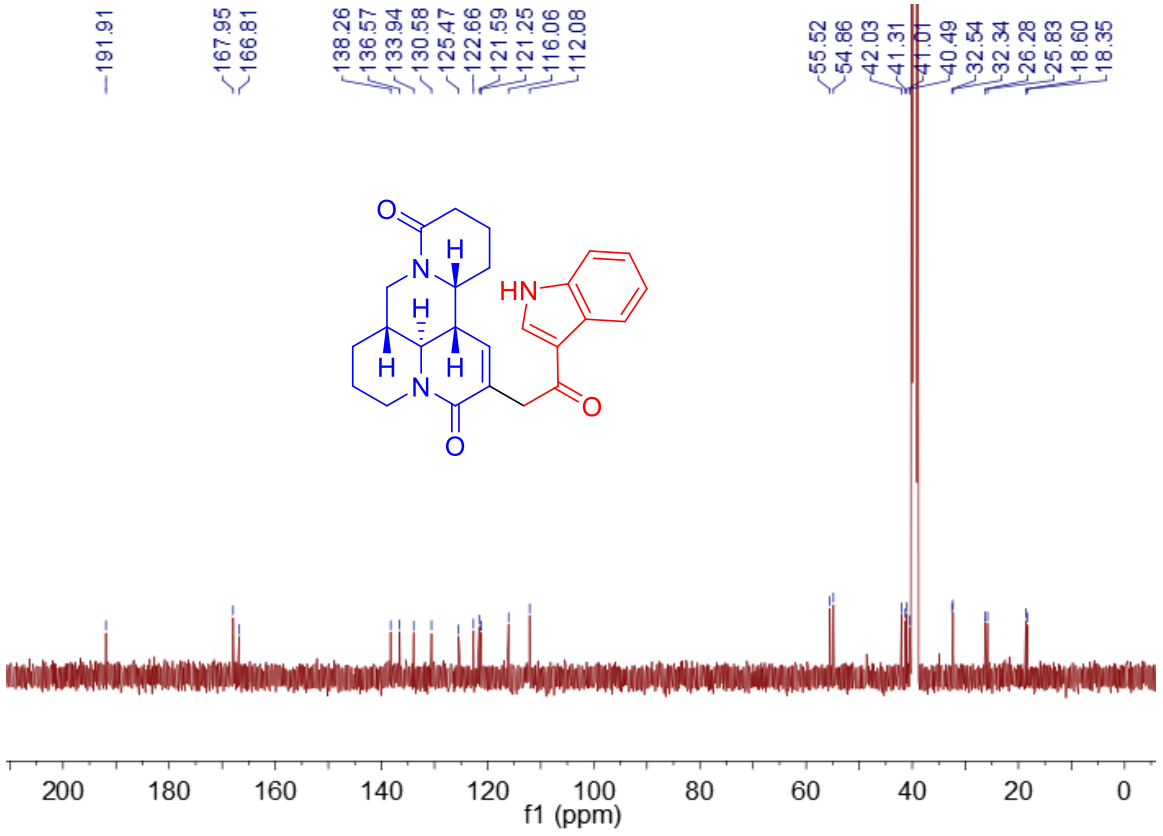


Figure S14. ^{13}C NMR spectrum of 2 in DMSO

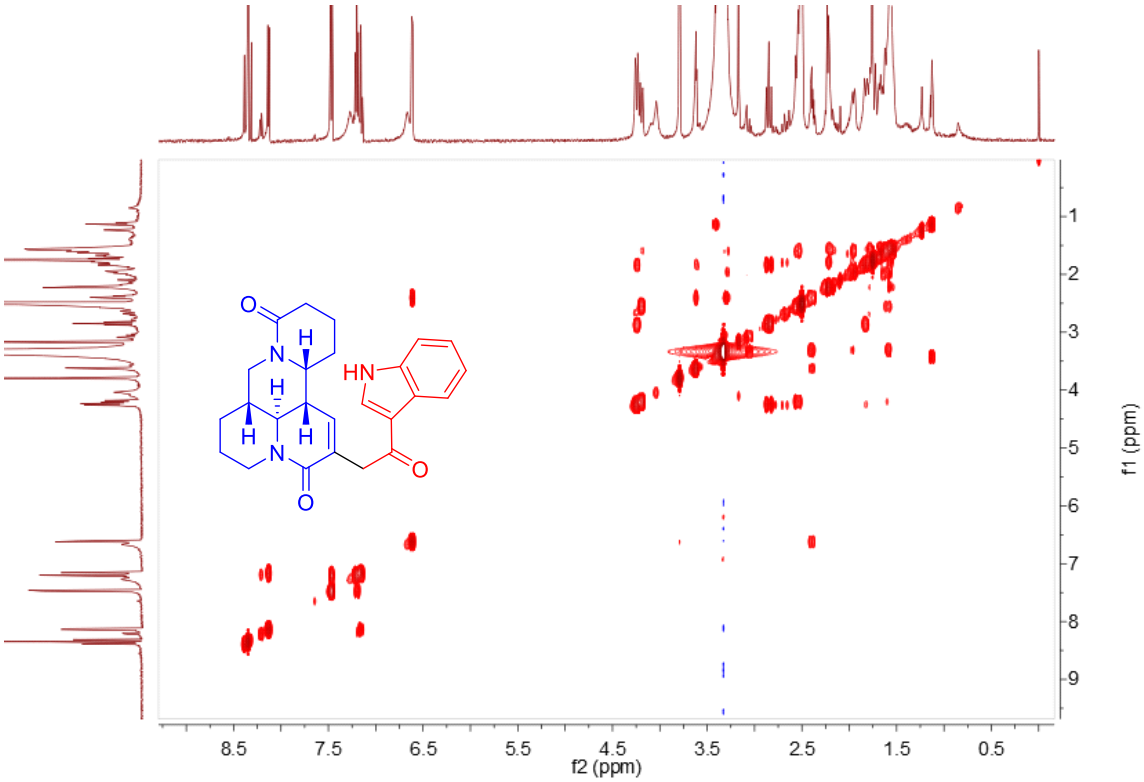


Figure S15. ^{1}H-^{1}H COSY spectrum of 2 in DMSO

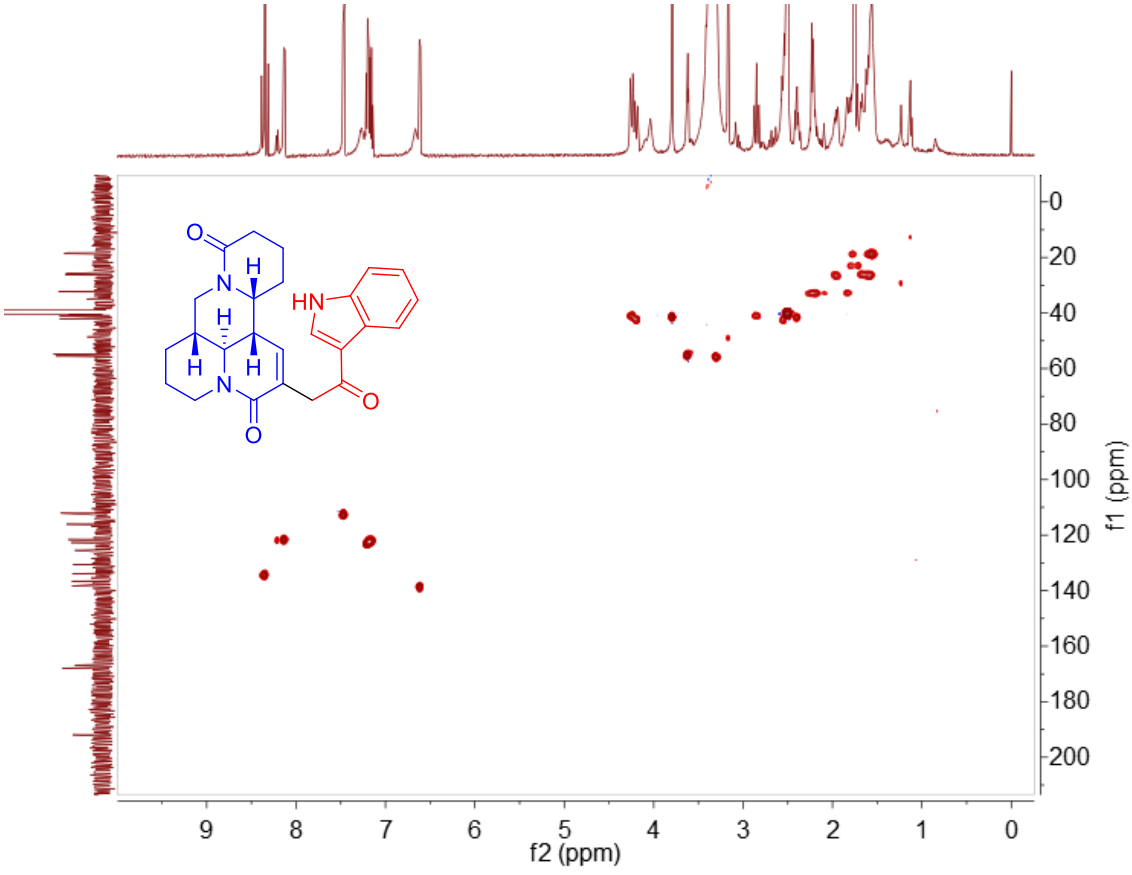


Figure S16. HSQC spectrum of 2 in DMSO

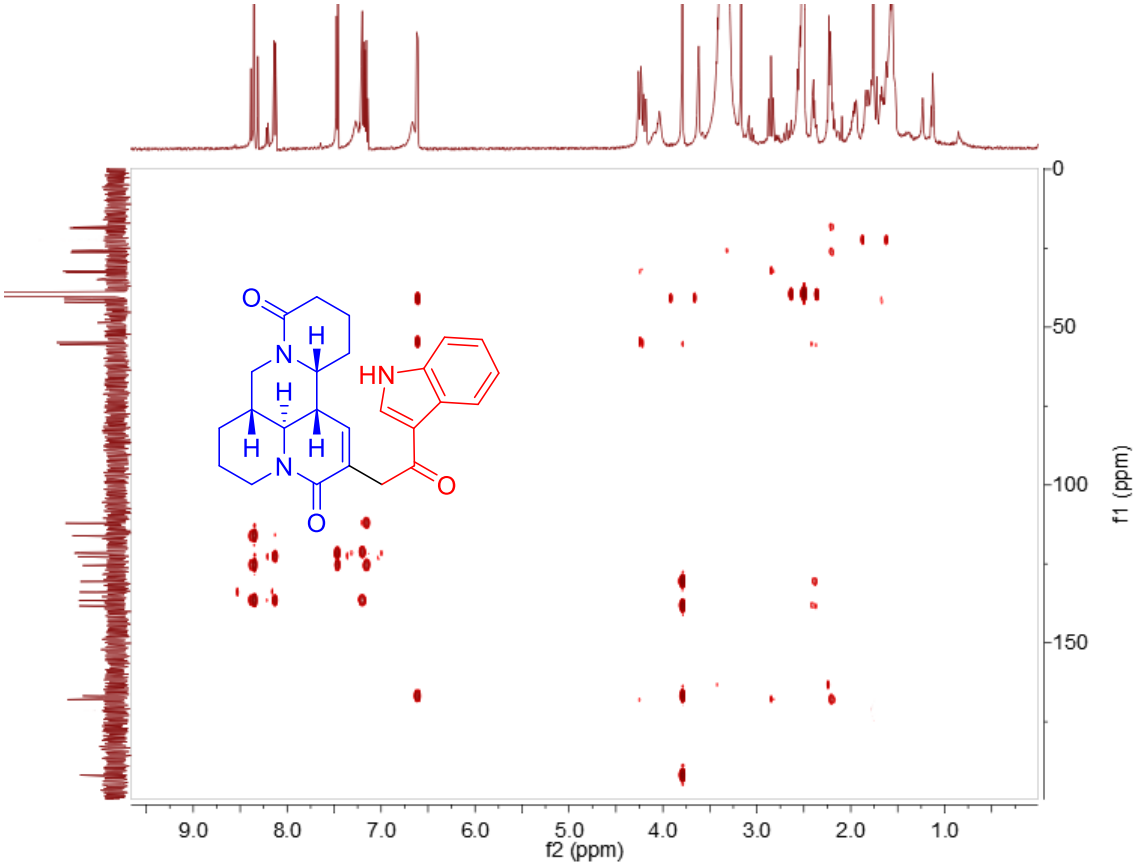


Figure S17. HMBC spectrum of 2 in DMSO

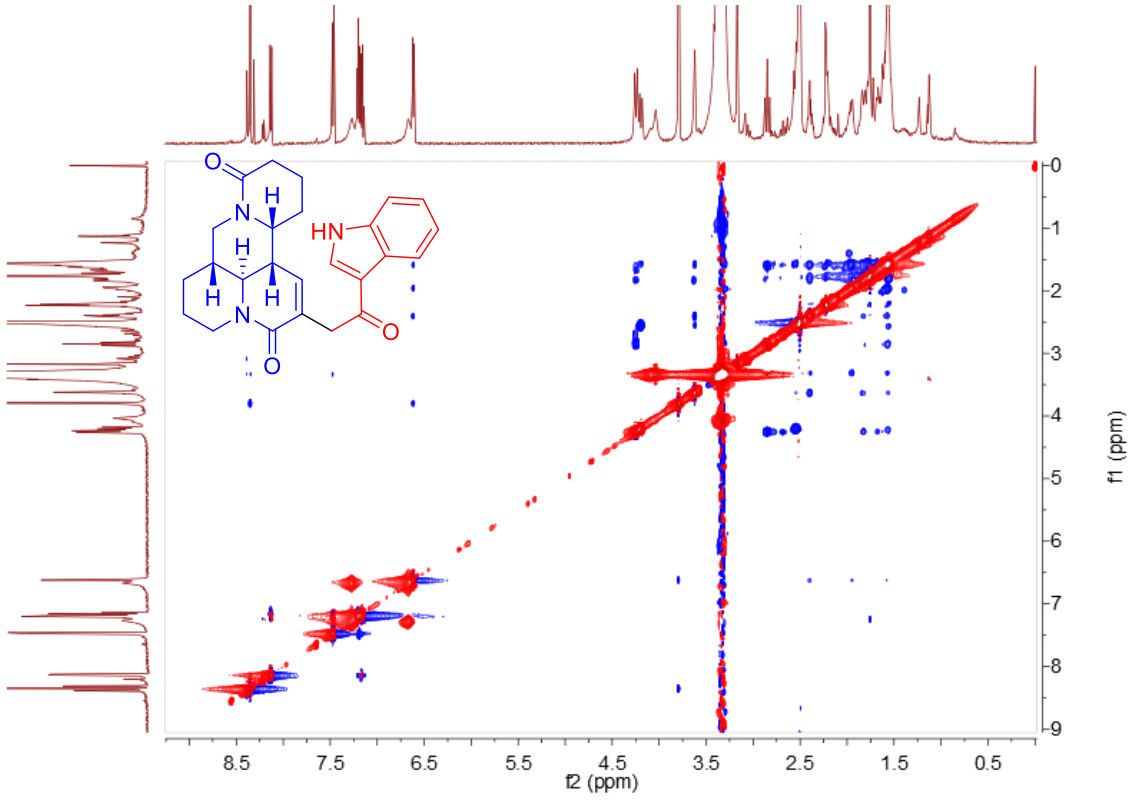


Figure S18. NOESY spectrum of 2 in DMSO

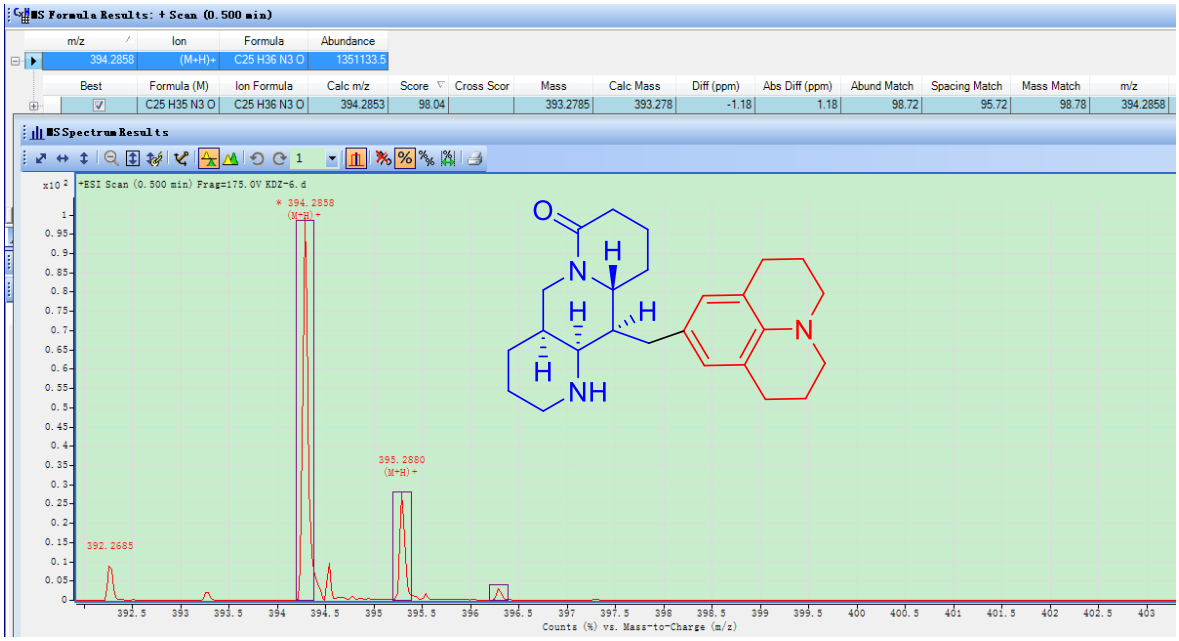


Figure S19. HR-ESI-MS spectrum of 3

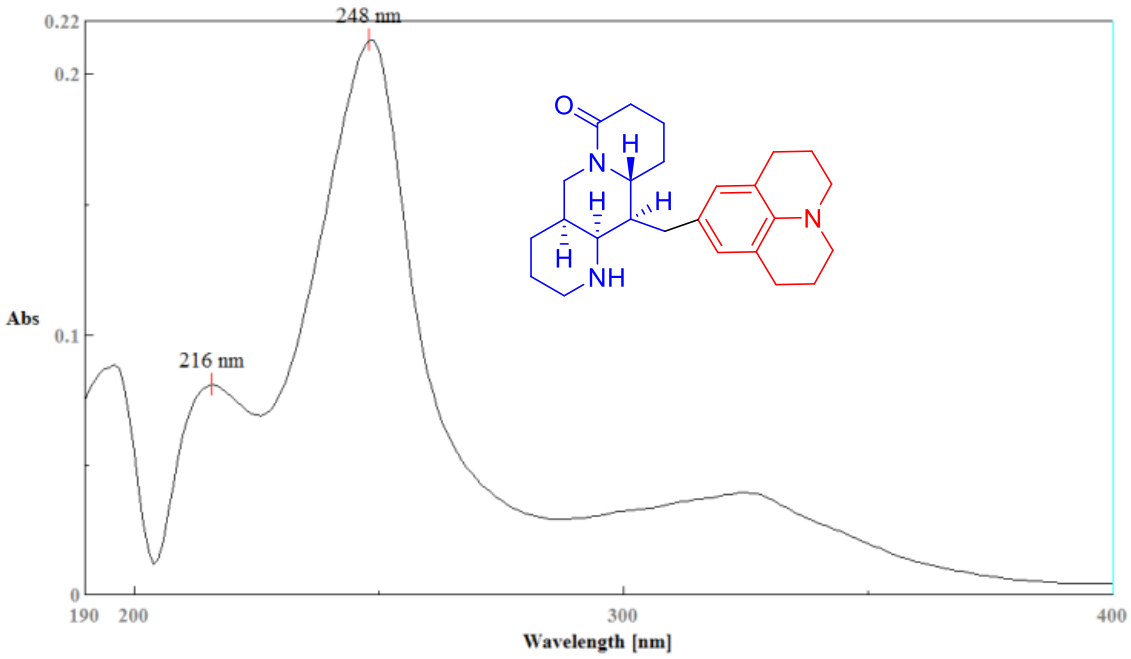


Figure S20. UV spectrum of 3 (CH_3OH)

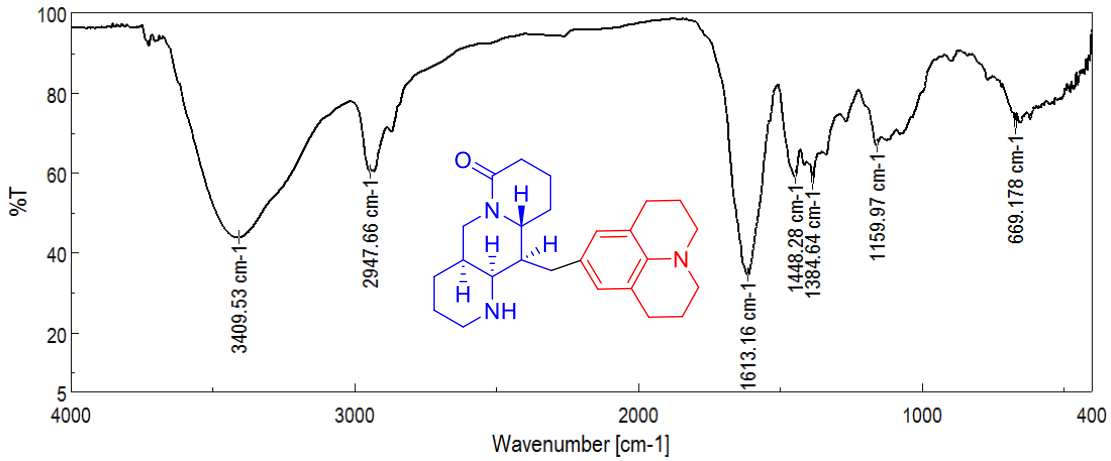


Figure S21. IR spectrum of 3 (KBr disc)

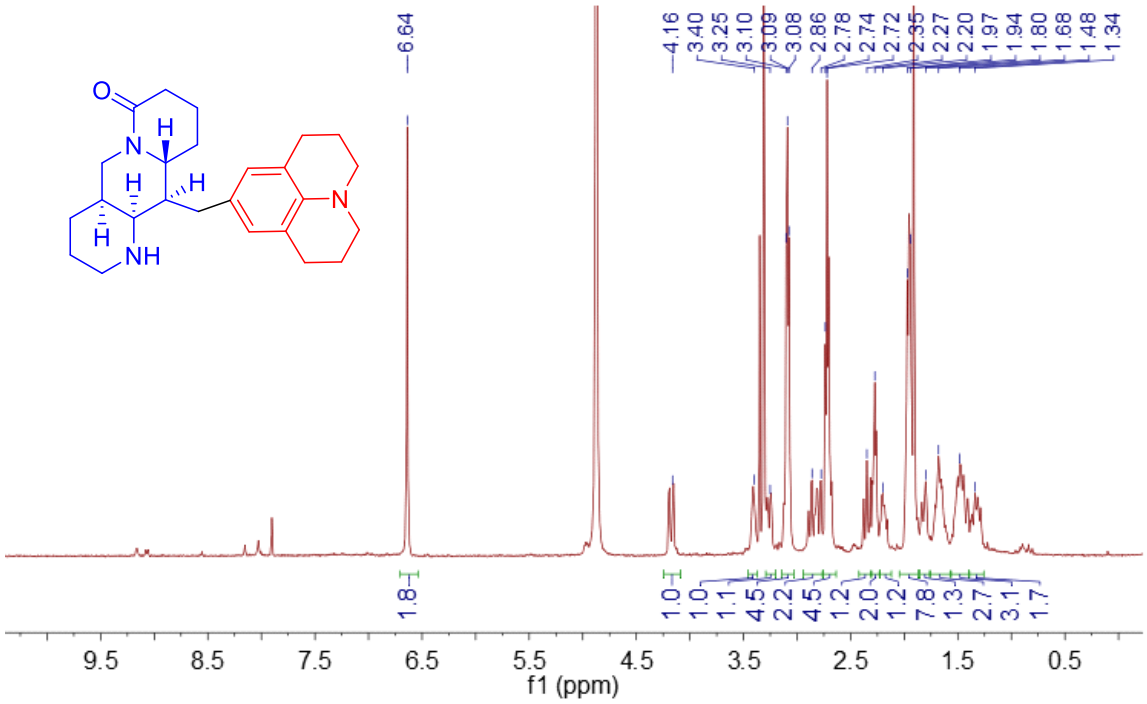


Figure S22. ^{1}H NMR spectrum of 3 in CD_3OD

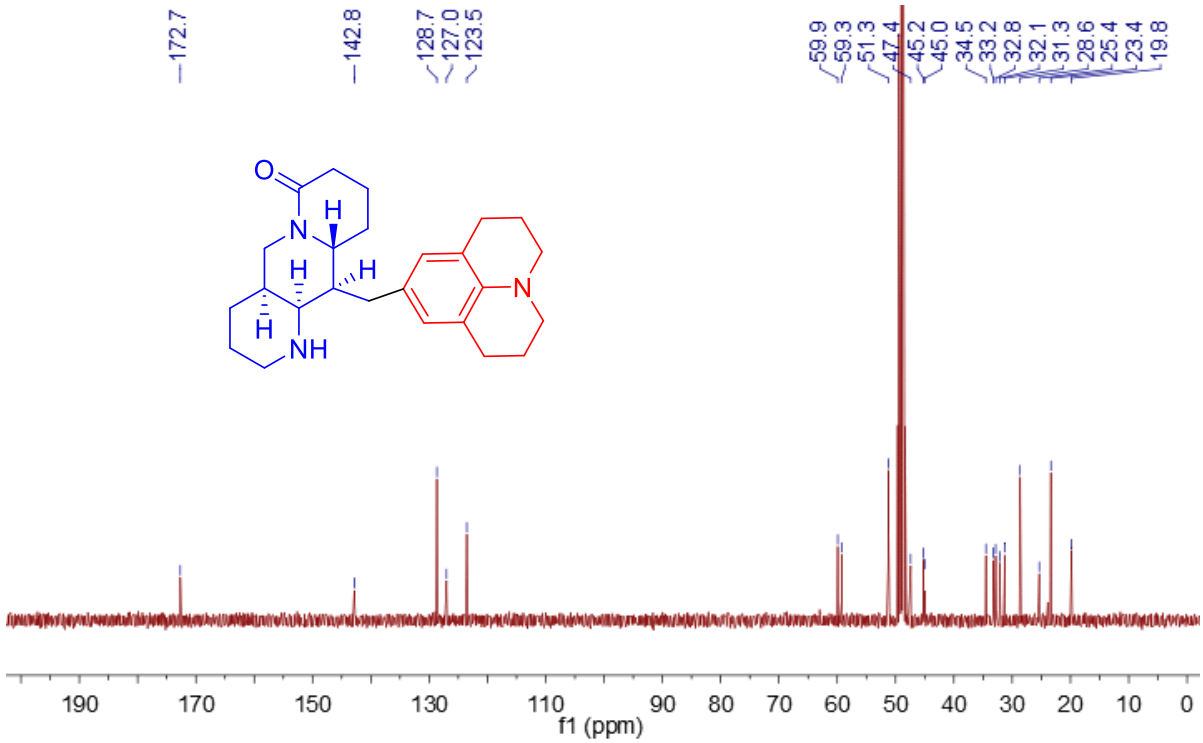


Figure S23. ^{13}C NMR spectrum of 3 in CD_3OD

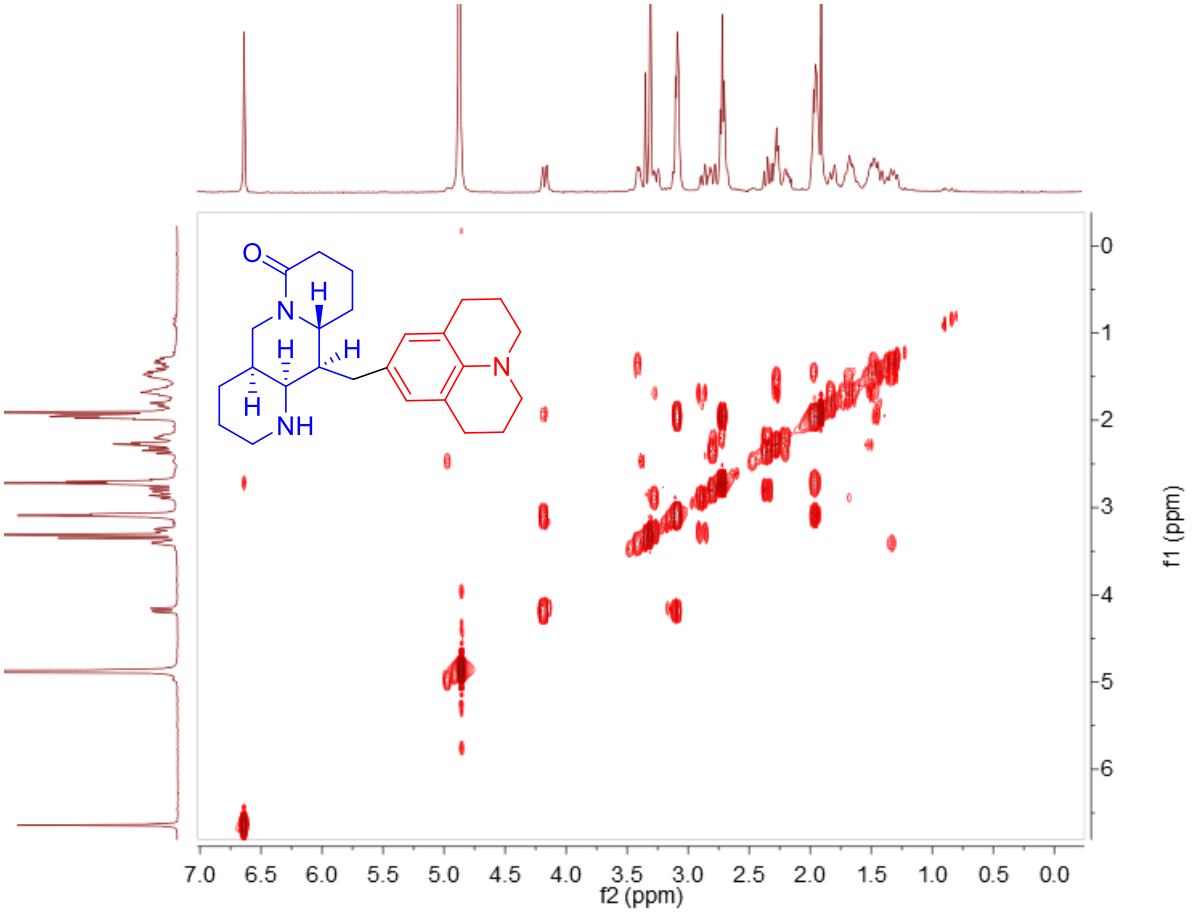


Figure S24. ^{1}H-^{1}H COSY spectrum of 3 in CD_3OD

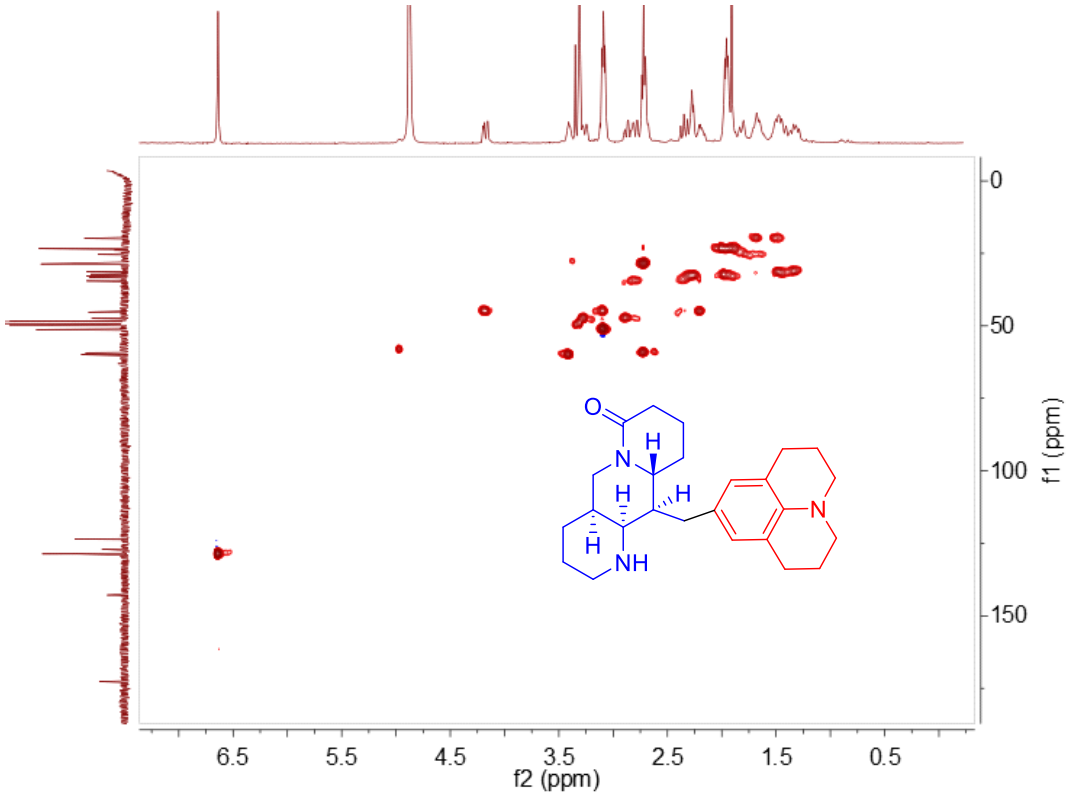


Figure S25. HSQC spectrum of 3 in CD_3OD

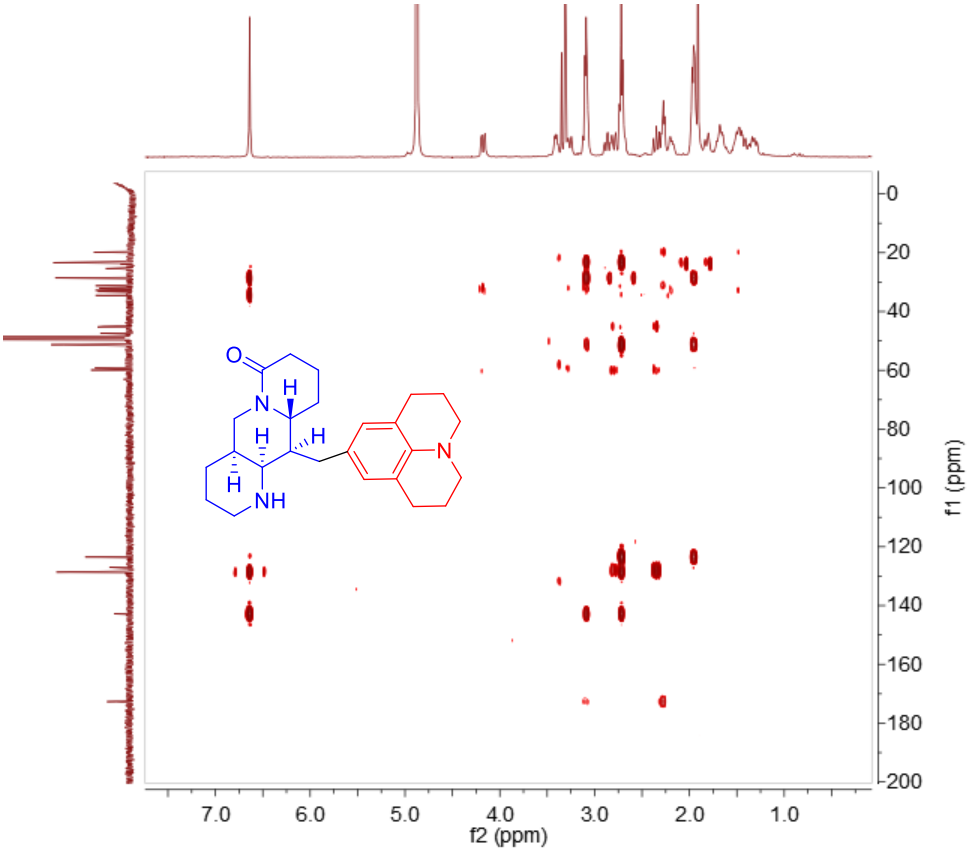


Figure S26. HMBC spectrum of 3 in CD_3OD

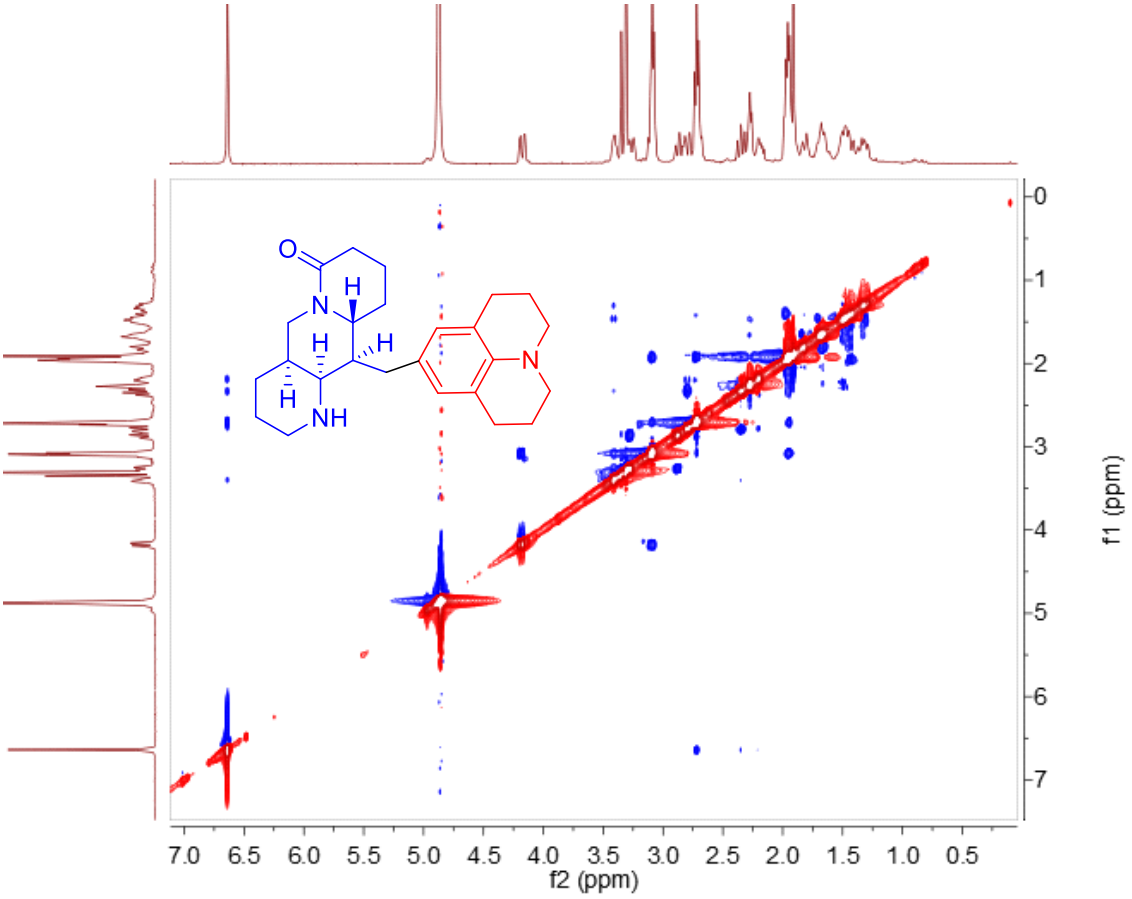


Figure S27. NOESY spectrum of 3 in CD_3OD

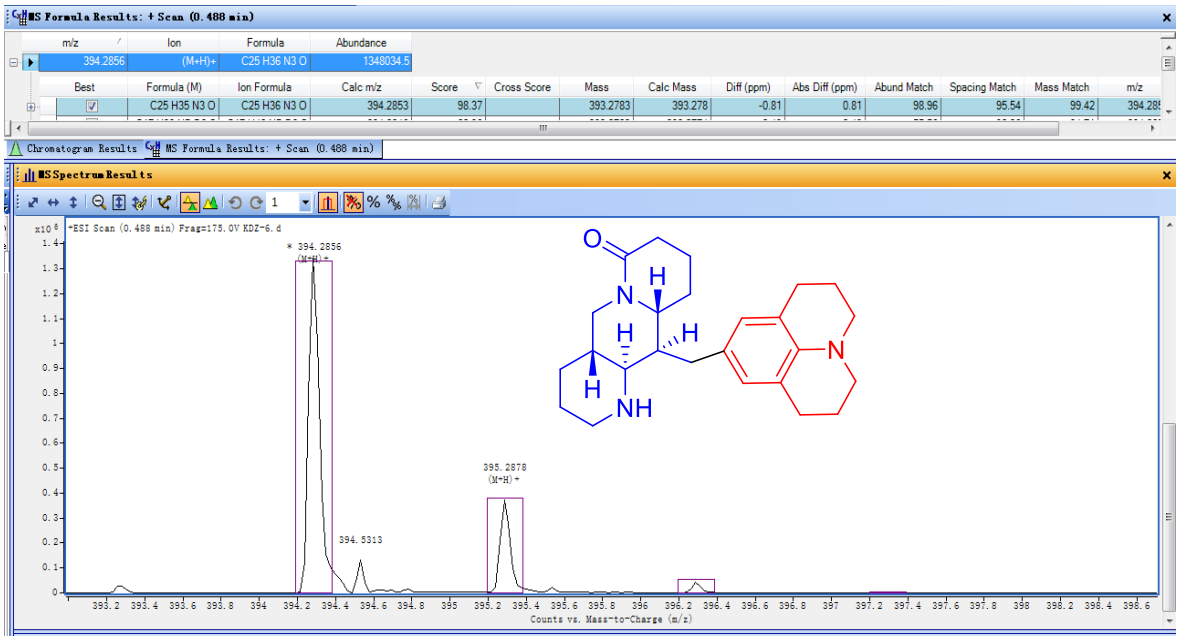


Figure S28. HR-ESI-MS spectrum of 4

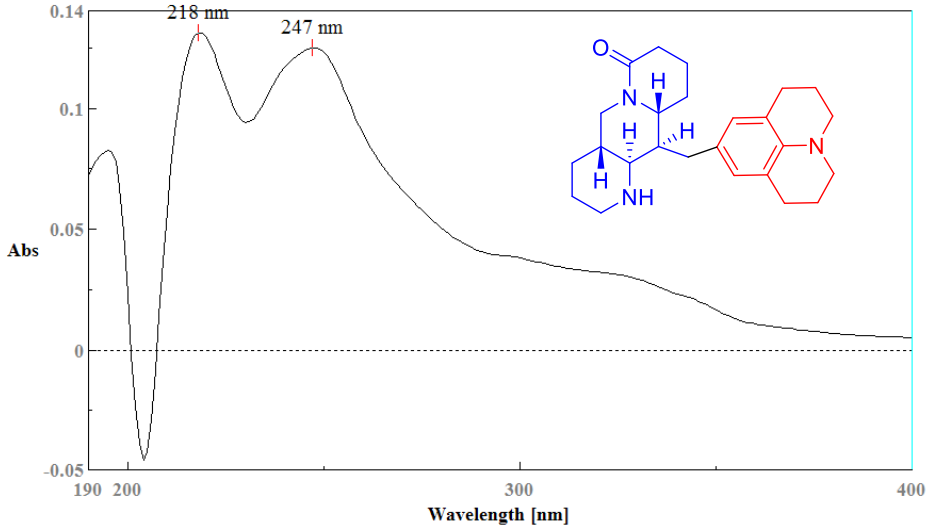


Figure S29. UV spectrum of 4 (CH_3OH)

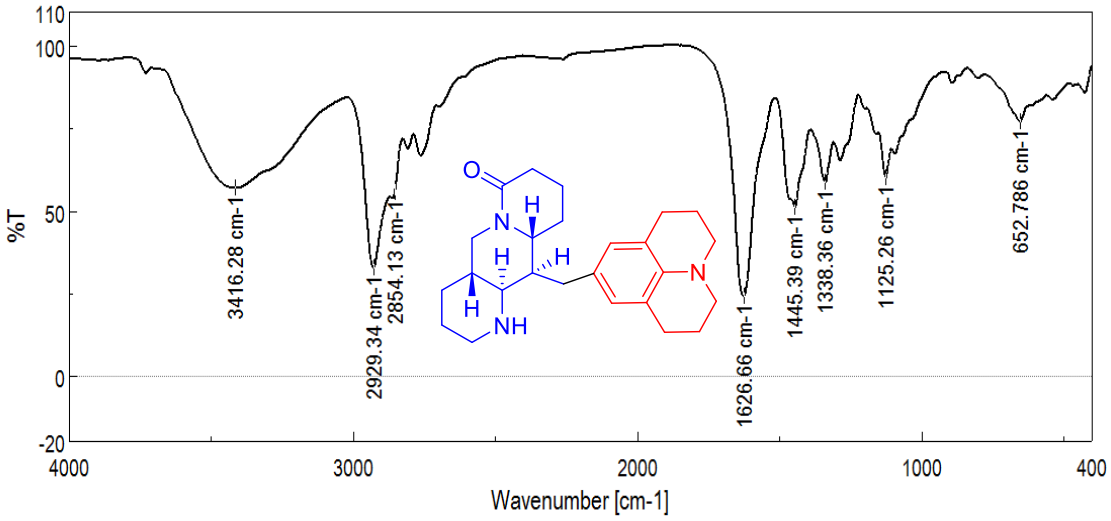


Figure S30. IR spectrum of 4 (KBr disc)

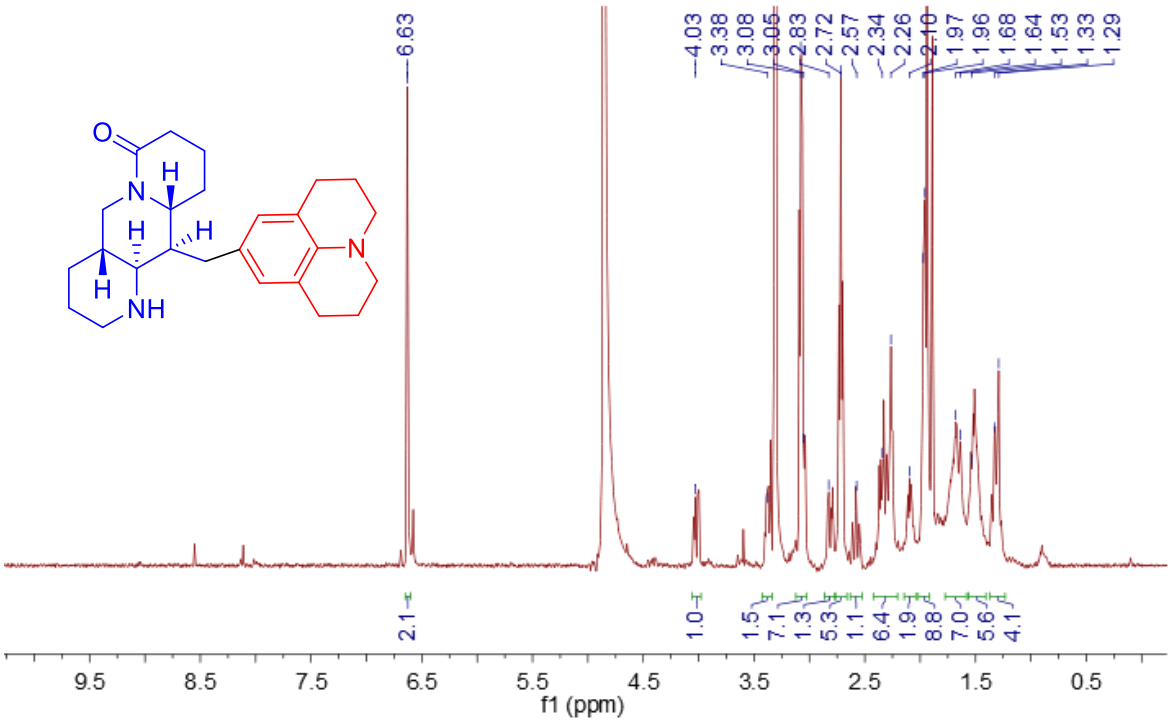


Figure S31. ^{1}H NMR spectrum of 4 in CD_3OD

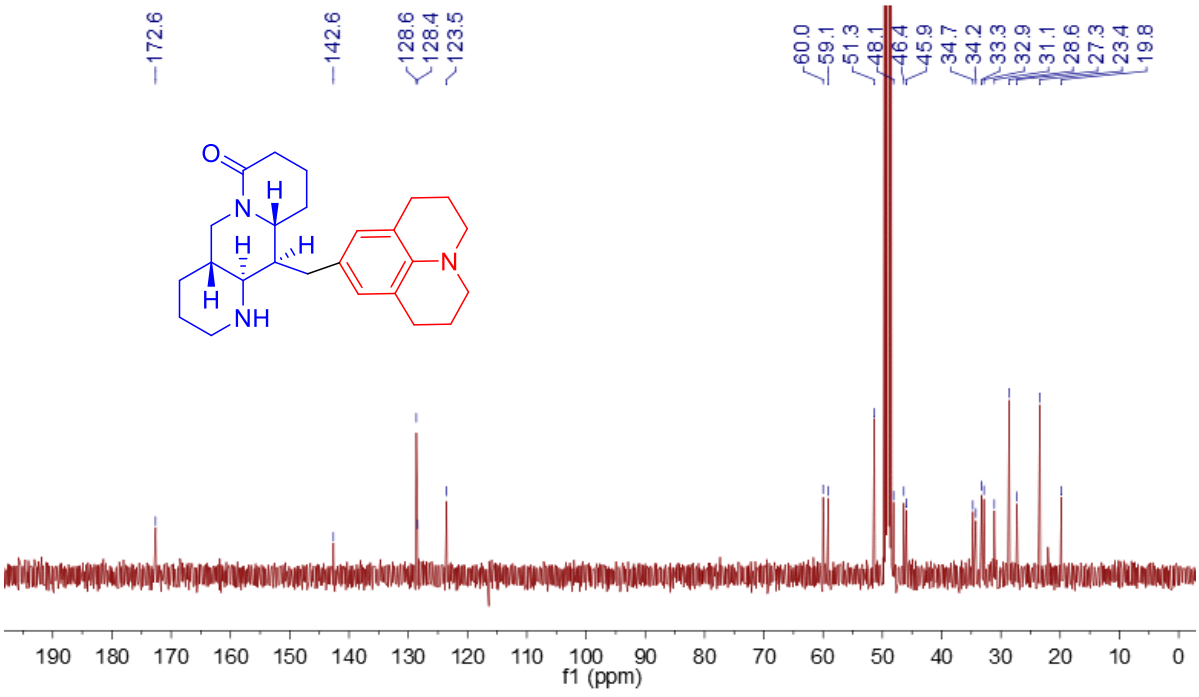


Figure S32. ^{13}C NMR spectrum of 4 in CD_3OD

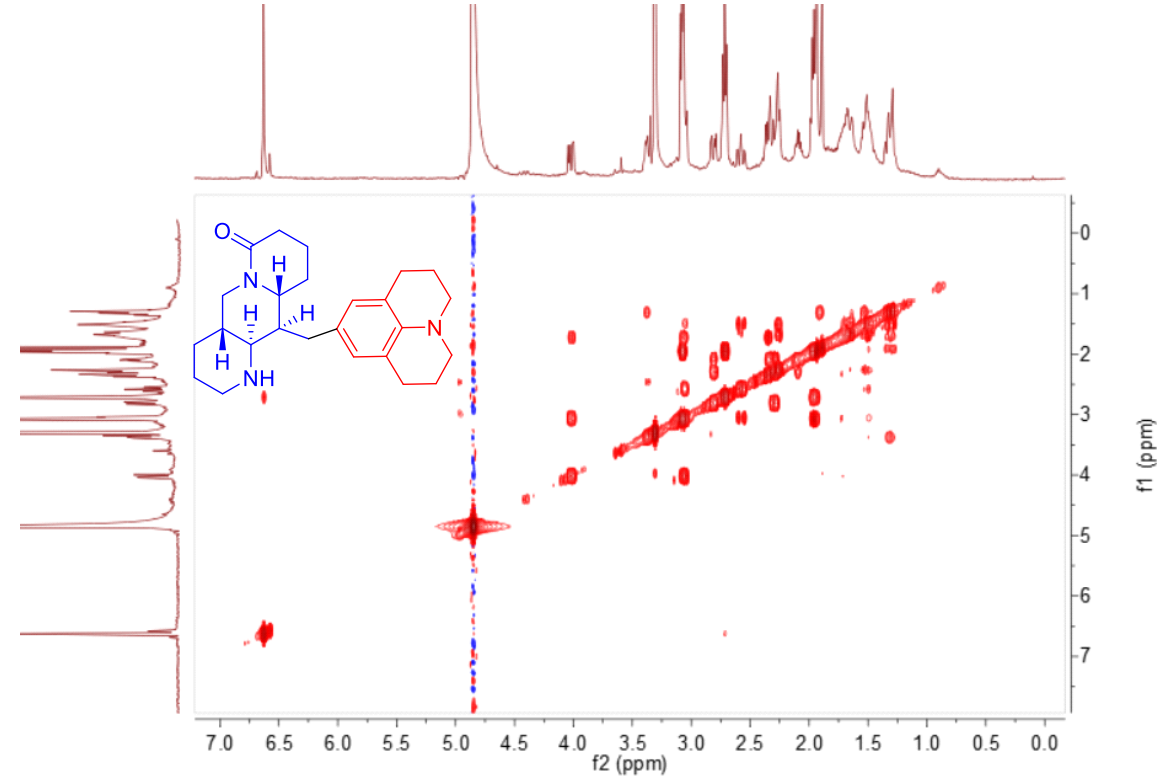


Figure S33. ^{1}H-^{1}H COSY spectrum of 4 in CD_3OD

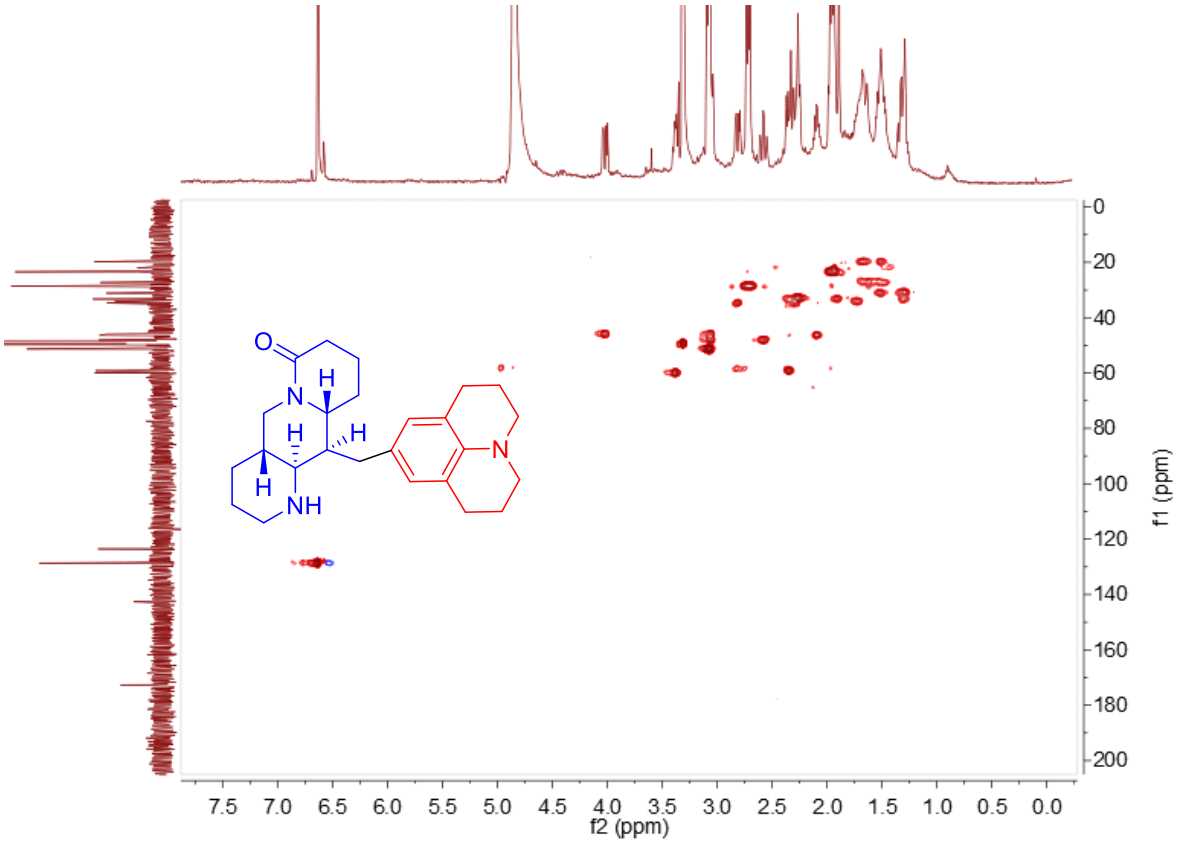


Figure S34. HSQC spectrum of 4 in CD_3OD

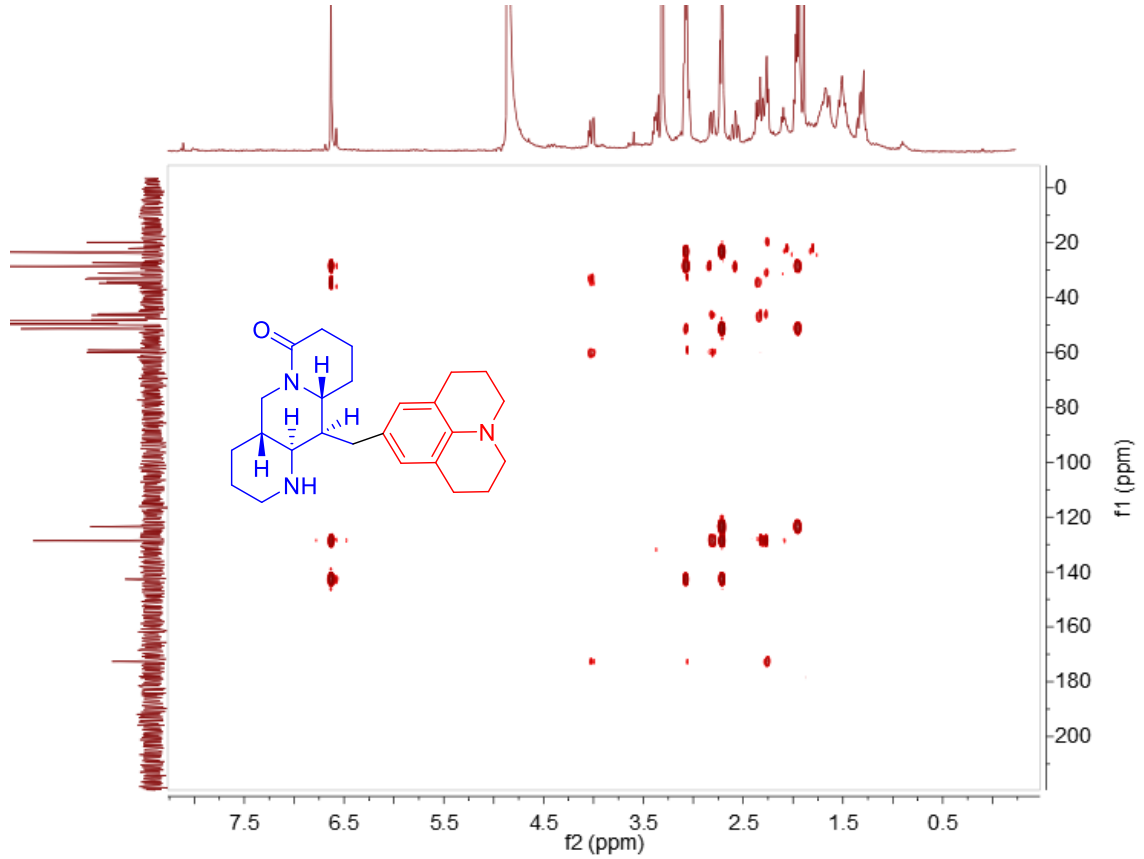


Figure S35. HMBC spectrum of 4 in CD_3OD

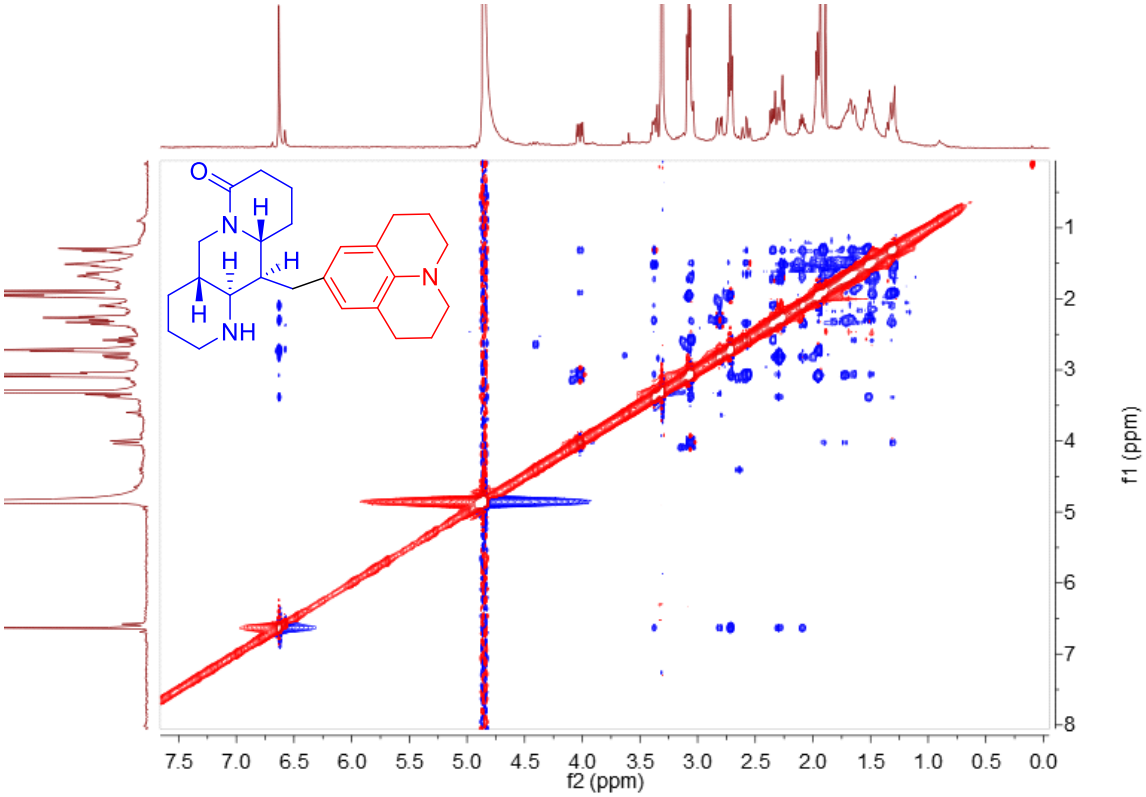


Figure S36. ROESY spectrum of 4 in CD_3OD

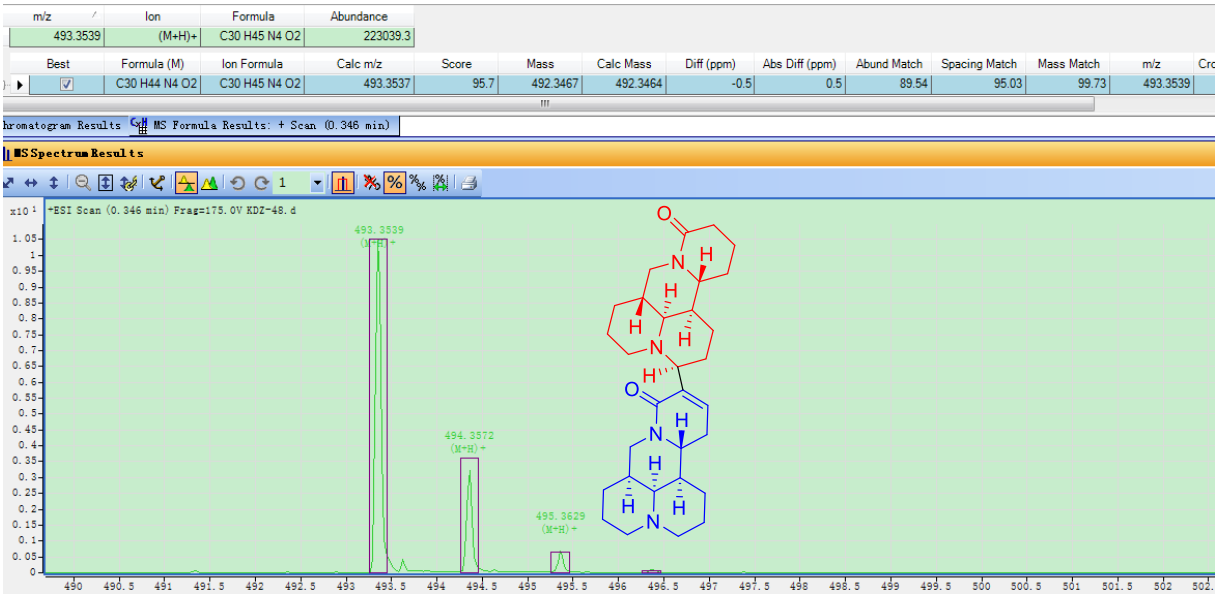


Figure S37. HR-ESI-MS spectrum of 5

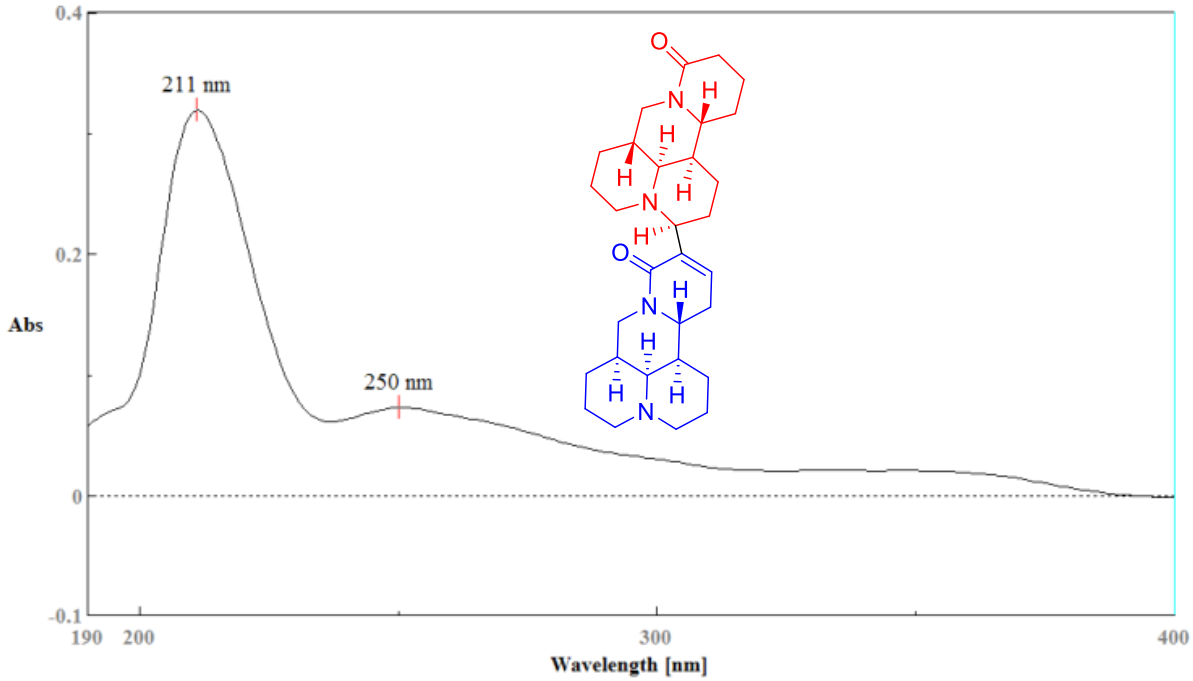


Figure S38. UV spectrum of 5 (CH_3OH)

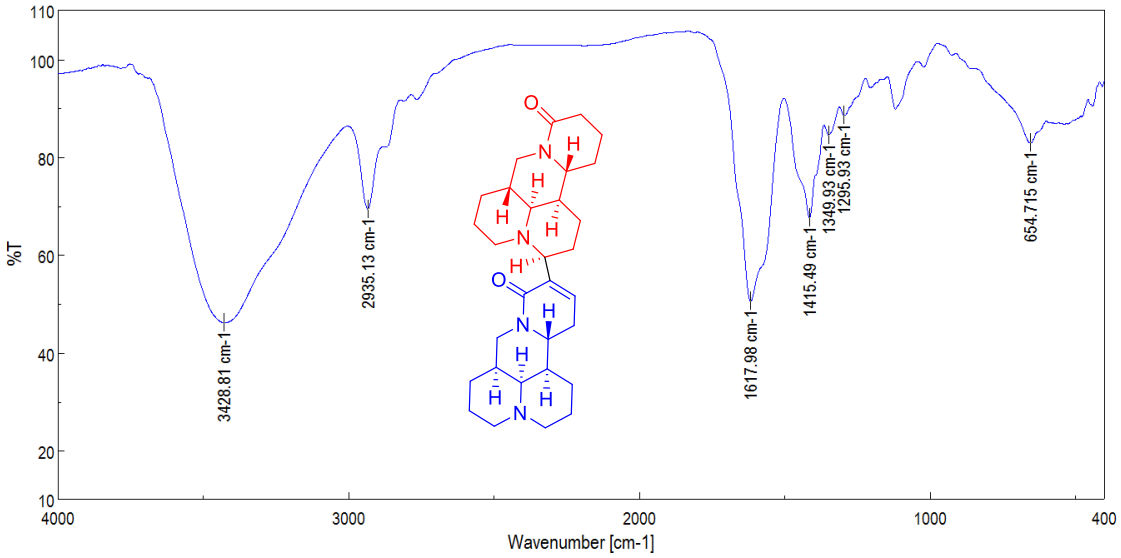


Figure S39. IR spectrum of 5 (KBr disc)

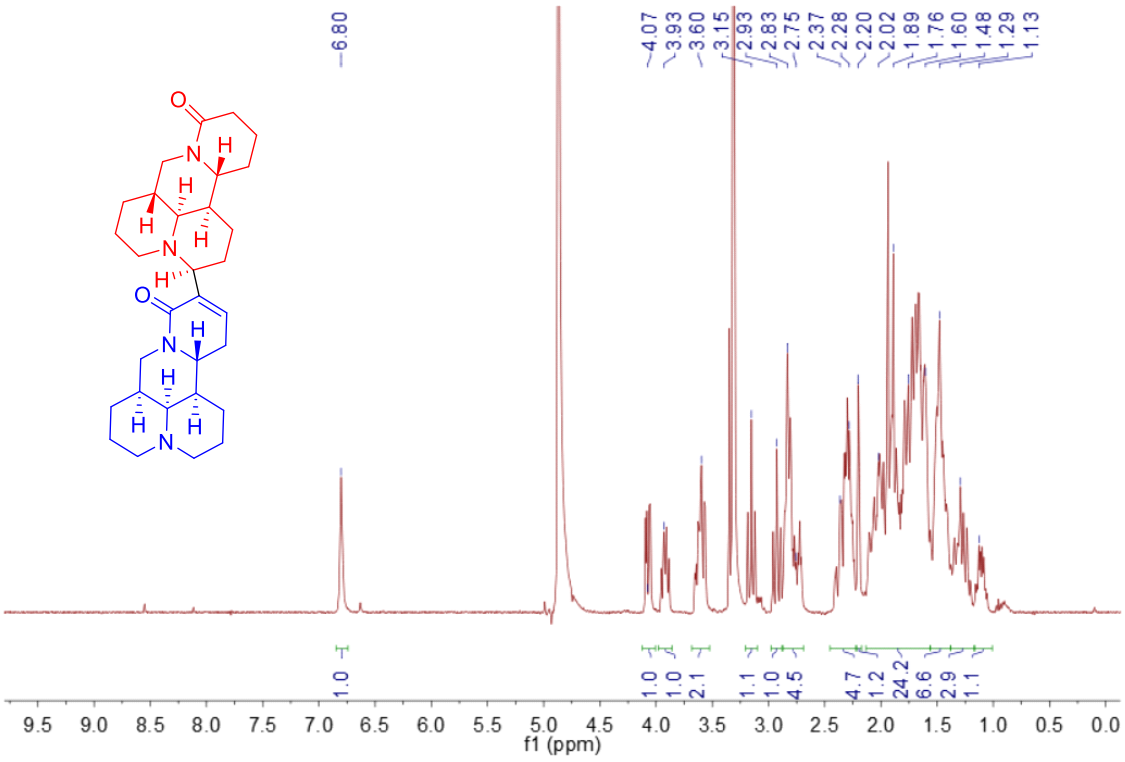


Figure S40. ^{1}H NMR spectrum of 5 in CD_3OD

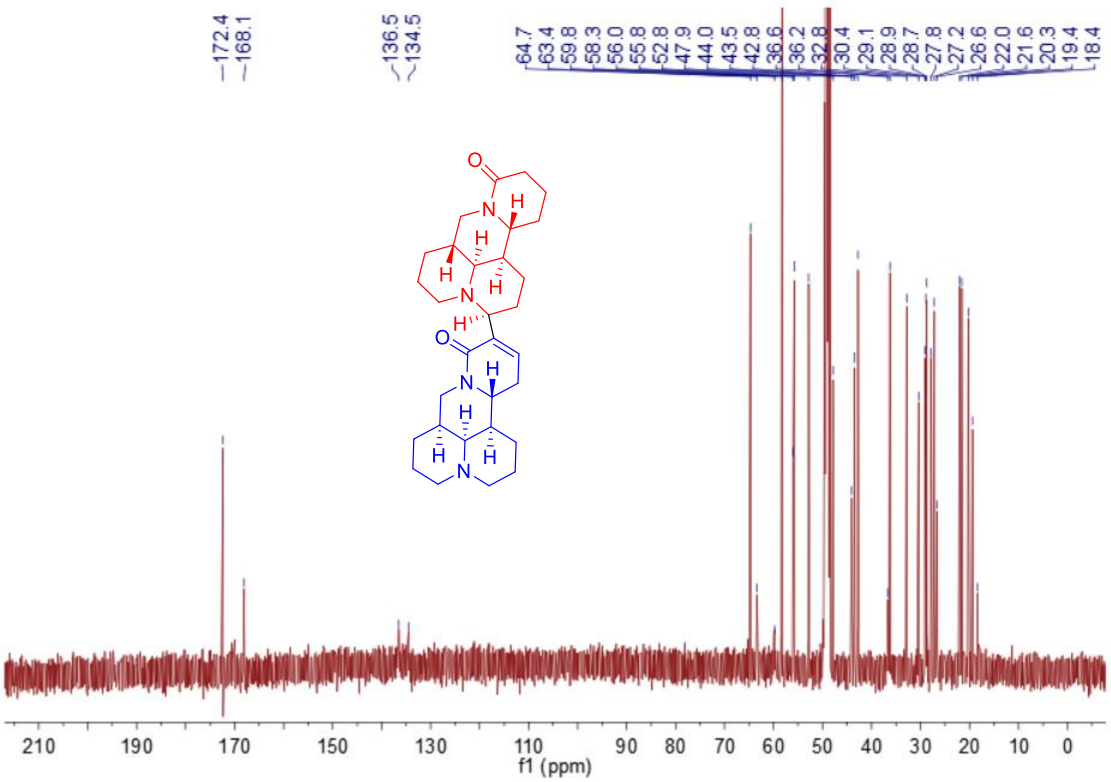


Figure S41. ^{13}C NMR spectrum of 5 in CD_3OD

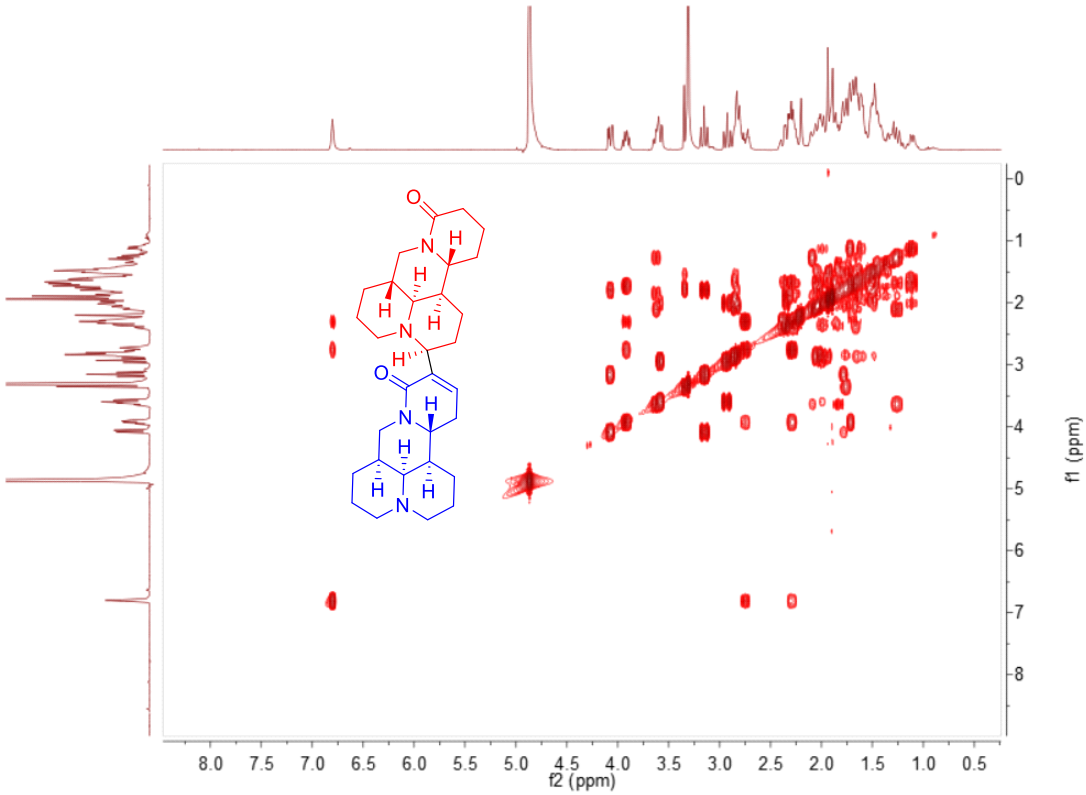


Figure S42. ^{1}H-^{1}H COSY spectrum of 5 in CD_3OD

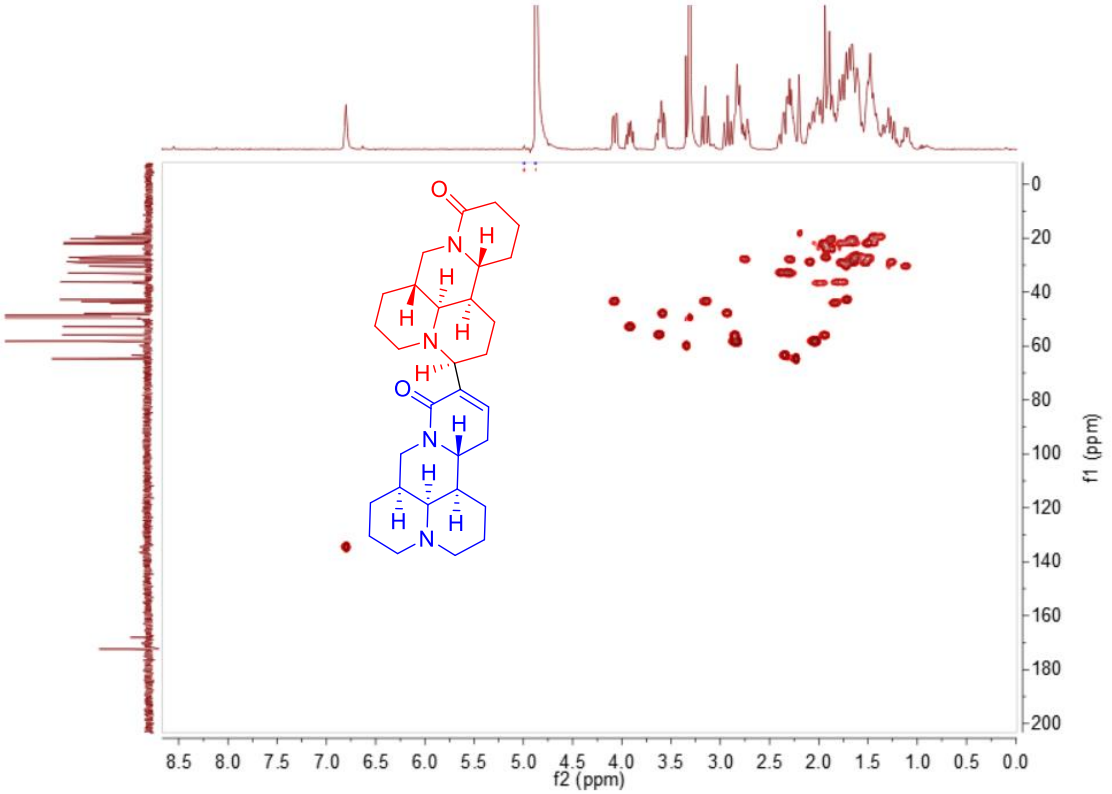


Figure S43. HSQC spectrum of 5 in CD_3OD

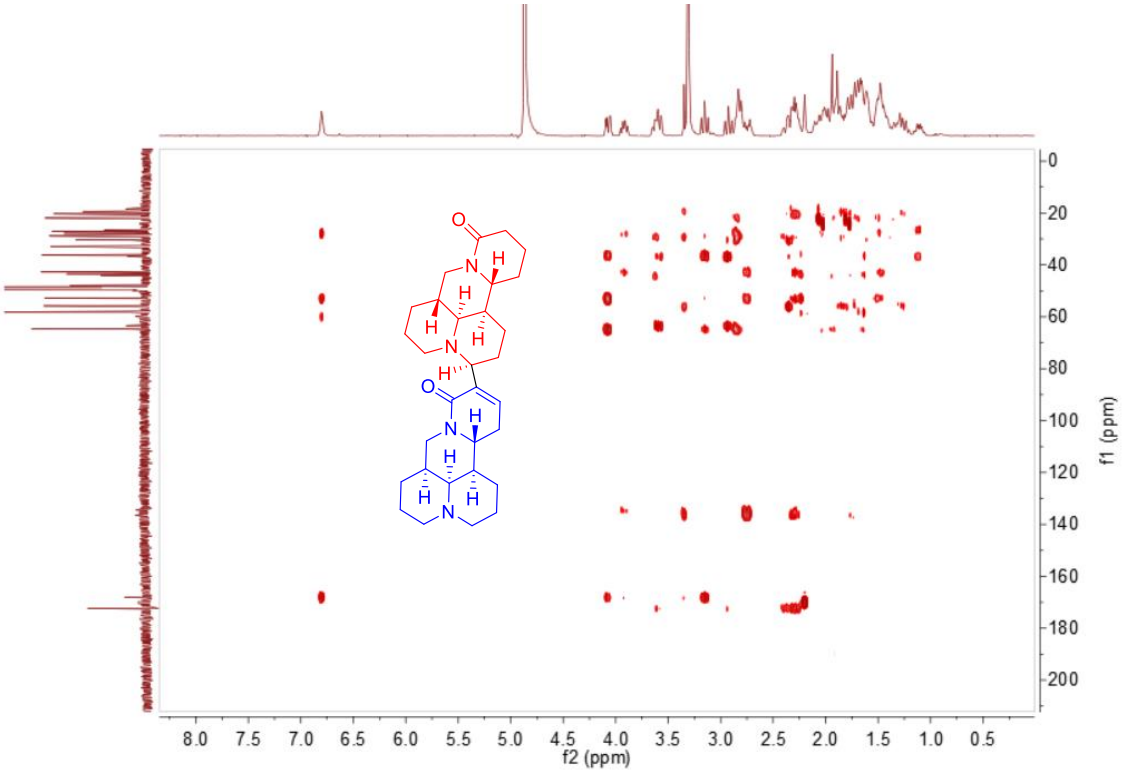


Figure S44. HMBC spectrum of 5 in CD_3OD

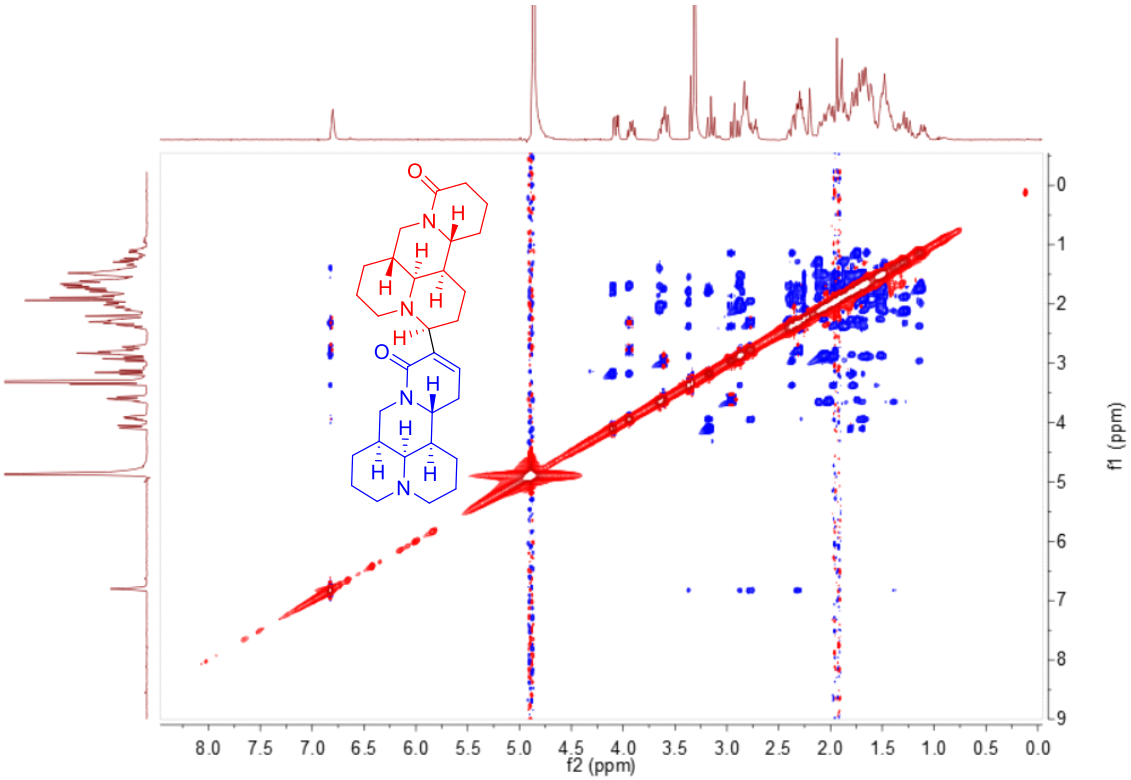


Figure S45. NOESY spectrum of 5 in CD_3OD

Anti-HBV Effect *in Vitro*

According to previous studies,[1-2] the anti-HBV assay *in vitro* was performed. HepG2.2.15 cells (2×10^4 cells/well) were seeded in 24-well plates. After 48 h incubation, cells were incubated in the presence of various concentrations of samples (1.0, 0.8, 0.4 and 0.2 mM, respectively; maximal noncytotoxic concentration as the highest concentration) at 37 ℃ for 9 days, and the medium was refreshed by fresh drug-containing medium every 3 days. Finally, culture medium was harvested to determine the HBsAg and HBeAg secretion by a diagnostic kit for HBsAg and HBeAg (ELISA) (Shanghai SIIC KEHUA Biotech Co., Ltd). Positive control was performed using lamivudine (3TC) (Sigma), and the medium without sample was used as control group. Each test was performed in triplicate, and the SEM (standard error of the mean) of inhibition values varied no more than 5%. MTT assay was used to evaluate cell damage.

Table S4. Inhibitory activity of compounds 1–5 against HBsAg and HBeAg secretion

Compound	Concentration (mM)	HBsAg (inhibition %)	HBeAg (inhibition %)
1[a]	0.4	39.5 ± 2.3	13.8 ± 0.2
2[b]	0.035	53.8 ± 5.7	39.8 ± 2.5
3[b]	0.035	39.8 ± 6.1	21.5 ± 3.6
4[b]	0.035	32.4 ± 2.8	20.8 ± 1.9
5[a]	0.4	15.6 ± 0.7	11.3 ± 0.2
Matrine[a]	0.4	34.9 ± 3.5	21.8 ± 0.8
3TC[c]	1.0	35.7 ± 1.6	34.4 ± 2.7

[a]This compound showed cytotoxicity against HepG2.2.15 cell line at the concentration of 0.8 mM. Cell damage was assessed using MTT assay, and cell growth inhibition against HepG2.2.15 cell line ≥25% was considered as cytotoxic.

[b] These compounds showed cytotoxicity against HepG2.2.15 cell line at the concentration of 0.07 mM.

[c] Positive control.

(1) Ding, P. L.; Liao, Z. X.; Huang, H.; Zhou, P.; Chen, D. F. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1231–1235.

(2) Lupberger, J.; Mund, A.; Kock, J.; Hildt, E. *J. Hepatol.* **2006**, *45*, 547–552.