Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Significant Anti-inflammatory Aziridne-containing Indole Alkaloids from the Chinese Medicinal Plant *Alstonia scholaris*

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1. Experimental Section

General experimental procedure

Optical rotations were recorded on an Autopol VI, Serial #91058. UV spectra were obtained on a Shimadzu UV-2700 spectrometer. CD spectra were determined on a chiral scan instrument. IR spectra were obtained on a NICOLET iS10 infrared spectrophotometer using KBr pellets. NMR spectra were measured on a Bruker AVANCE NEO 400MHz spectrometer, with TMS as an internal standard. HRESIMS analyses were measured on Agilent 1290 UPLC/6545 Q-TOF mass spectrometer. Silica gel (200−300 mesh; Qingdao Marine Chemical Inc., PR China), C-18 silica gel (40−60 μm; Daiso Co., Japan), and Sephadex LH-20 (Amersham Pharmacia, Sweden) were used for column chromatography. Fractions were monitored by TLC on silica gel plates (GF254, Qing-dao Haiyang Chemical Co., Ltd.).

Extraction and isolation

The leaves of *Alstonia scholaris* were collected in June 2013 from Pu'er city, Yunnan Province, P. R. China. The dried and powdered leaves of *A. scholaris* (10.0 kg) were extracted with EtOH (300 L×3) at room temperature for three times (48 h×3), and the solvent was evaporated under vacuo. The residue was dissolved in 0.3 % HCl and then adjusted to pH 2−3. Then the solution was adjusted to pH 9−10 with 10% ammonia. The basic solution was subsequently partitioned with EtOAc to afford the alkaloidal extract. Then the extract (300 g) was obtained and subjected to column chromatography (CC) over a silica gel eluting with $CHCl_3$-MeOH (from 50:1 to 1:1, v/v) to afford eight fractions (Fr. A−H). Fr. B (21.0 g) and Fr. C (8.2 g) was merged and subjected to a C-18 column eluted with aqueous MeOH (from 30:70 to 100:0, v/v) to yield ten fractions (Fr. BC.1−10). Fr.BC.4 (3.0 g) was submitted to Sephadex LH-20 (MeOH) to produce three fractions Fr.BC.4.1−Fr.BC.4.3. Fr.BC.4.2 (847.6 mg) was purified by a silica gel eluting with $CHCl_3$-MeOH (from 1:0 to 0:1, v/v) and following re-crystal method to

afforded compound **2** (9.8 mg). Fr.BC.5 (3.1 g) was submitted to Sephadex LH-20 (MeOH) to produce three fractions Fr.BC.5.1−Fr.BC.5.3. Compound **1** (15.8 mg) was purified from Fr.BC.5.2 (2.5 g) by a silica gel eluting with $CHCl_3$-acetone (from 25:1 to 1:1, v/v).

Alstolactine D (**1**): colorless block; $[\alpha]_D^{23}$ −95.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 300 (3.48), 240 (3.89), 200 (4.46); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 307 (− 0.84), 275 (+ 0.28), 225 (− 12.57), 205 (+ 11.34); IR (KBr) ν_{max} 3235, 2934, 1723, 1608, 1484, 1213, 1041 cm^{-1}; HRESIMS *m/z*: 377.1471 $[M + Na]^+$ (calcd for $C_{20}H_{22}N_2O_4Na$, 377.1472); ^1H and ^{13}C NMR data, see Table 1.

Alstolactine E (**2**): colorless block; $[\alpha]_D^{23}$ −197.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 300 (3.64), 240 (4.03), 200 (4.61); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 290 (+ 1.06), 261 (+ 0.18), 220 (− 14.72), 204 (+ 12.26); IR (KBr) ν_{max} 3439, 2952, 1725, 1634, 1485, 1214, 1039 cm^{-1}; HRESIMS *m/z*: 377.1464 $[M + Na]^+$ (calcd for $C_{20}H_{22}N_2O_4Na$, 377.1472); ^1H and ^{13}C NMR data, see Table 1.

2. Table of the 2D NMR correlations data for compounds 1 and 2

Table S1. 2D NMR correlations data for compound **1**

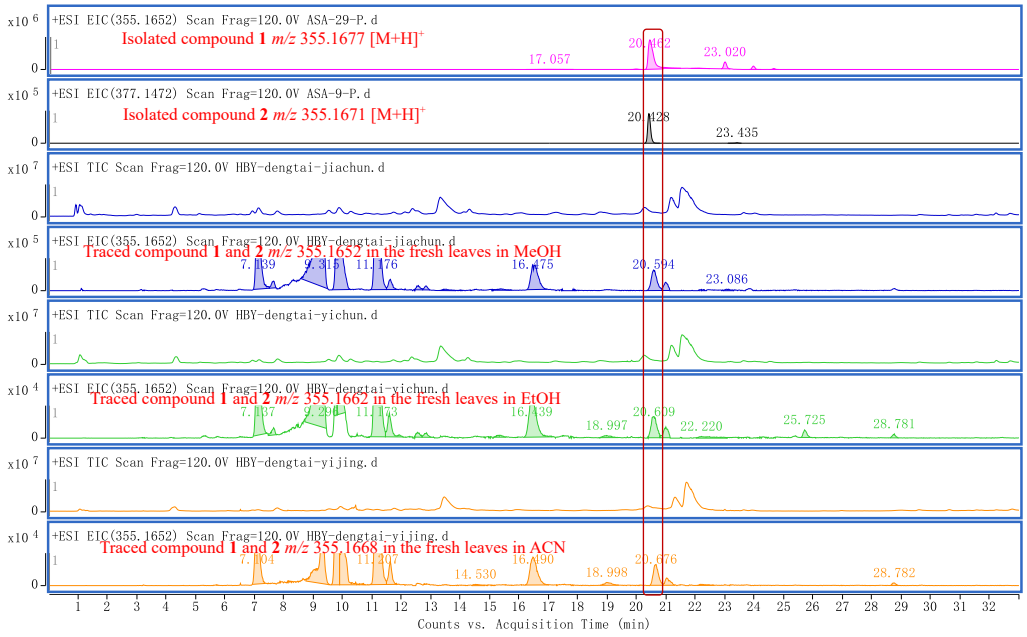
δ_H	HSQC	^1H-^1H COSY	HMBC	ROESY
δ_H 4.43 (N-H)			δ_C 51.7 (C-7), 135.2 (C-8), 109.4 (C-12), 146.5 (C-13)	
δ_H 3.47 (H-3)	δ_C 63.7 (C-3)	δ_H 1.70 (H_2-14)	δ_C 102.4 (C-2), 51.7 (C-7), 29.2 (C-15), 47.1 (C-20)	δ_H 2.00 (Ha-21)
δ_H 4.75 (H-5)	δ_C 105.6 (C-5)	δ_H 2.43 (Ha-6)	δ_C 102.4 (C-2)	δ_H 2.43 (Ha-6)
δ_H 2.43 (Ha-6)	δ_C 43.3 (C-6)	δ_H 4.75 (H-5)	δ_C 102.4 (C-2), 51.7 (C-7), 135.2 (C-8)	δ_H 7.50 (H-9), 4.75 (H-5)
δ_H 2.22 (Hb-6)	δ_C 43.3 (C-6)	δ_H 4.75 (H-5)		δ_H 3.44 (H_3-1'), 4.62 (H-19)
δ_H 7.50 (H-9)	δ_C 124.4 (C-9)	δ_H 6.86 (H-10)	δ_C 51.7 (C-7), 146.5 (C-13)	δ_H 2.43 (Ha-6)
δ_H 6.86 (H-10)	δ_C 120.6 (C-10)	δ_H 7.50 (H-9), 7.12 (H-11)		
δ_H 7.12 (H-11)	δ_C 128.8 (C-11)	δ_H 6.86 (H-10), 6.60 (H-12)		
δ_H 6.60 (H-12)	δ_C 109.4 (C-12)	δ_H 7.12 (H-11)	δ_C 135.2 (C-8)	
δ_H 1.70 (H_2-14)	δ_C 24.1 (C-14)	δ_H 3.47 (H-3), 2.59 (H-15)	δ_C 51.2 (C-16)	δ_H 2.00 (Ha-21)
δ_H 2.59 (H-15)	δ_C 29.2 (C-15)	δ_H 1.70 (H_2-14), 2.99 (H-16)		δ_H 1.48 (H_3-18), 2.00 (Ha-21)
δ_H 2.99 (H-16)	δ_C 51.2 (C-16)	δ_H 2.59 (H-15)	δ_C 102.4 (C-2), 51.7 (C-7), 135.2 (C-8), 170.9 (C-17)	
δ_H 1.48 (H_3-18)	δ_C 21.6 (C-18)	δ_H 4.62 (H-19)	δ_C 47.1 (C-20)	δ_H 2.59 (H-15)
δ_H 4.62 (H-19)	δ_C 79.4 (C-19)	δ_H 1.48 (H_3-18)	δ_C 170.9 (C-17), 47.1 (C-20), 29.4 (C-21)	δ_H 2.22 (Hb-6), 1.78 (Hb-21)
δ_H 2.00 (Ha-21)	δ_C 29.4 (C-21)		δ_C 63.7 (C-3), 29.2 (C-15), 47.1 (C-20)	δ_H 3.47 (H-3), 2.59 (H-15), 1.70 (H_2-14)

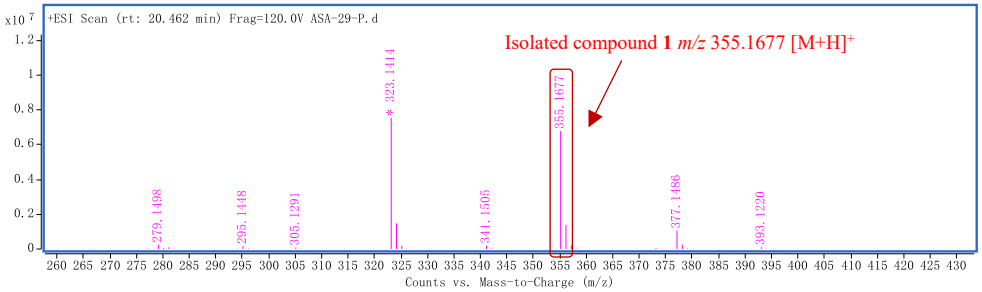
δ_H 1.78 (Hb-21)	δ_C 29.4 (C-21)		δ_C 63.7 (C-3), 29.2 (C-15), 47.1 (C-20)	δ_H 4.62 (H-19)
δ_H 3.44 (H_3-1')	δ_C 58.1 (C-1')		δ_C 105.6 (C-5)	δ_H 2.22 (Hb-6)

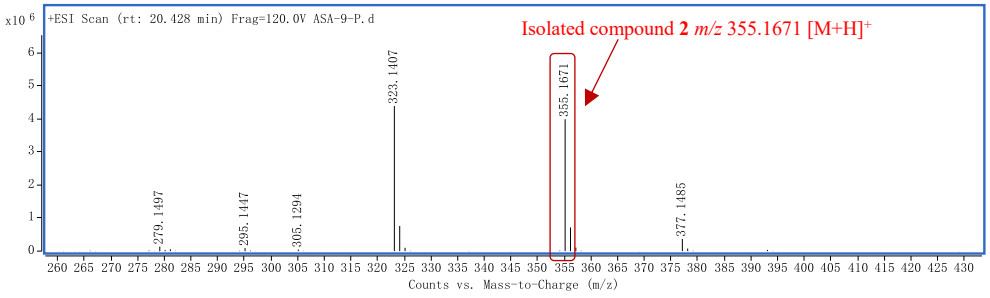
Table S2. 2D NMR correlations data for compound **2**

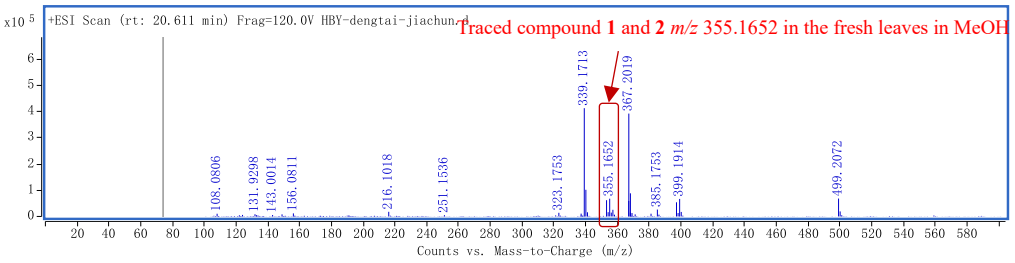
δ_H	HSQC	^{1}H-^{1}H COSY	HMBC	ROESY
δ_H 4.39 (N-H)			δ_C 52.2 (C-7), 137.5 (C-8), 109.0 (C-12), 145.8 (C-13)	
δ_H 3.39 (H-3)	δ_C 64.0 (C-3)	δ_C 23.5 (C-14)	δ_C 105.3 (C-2), 52.2 (C-7), 28.9 (C-15), 46.9 (C-20)	δ_H 1.93 (Ha-21)
δ_H 5.09 (H-5)	δ_C 104.4 (C-5)	δ_C 45.0 (C-6)	δ_C 105.3 (C-2)	δ_H 2.27 (Hb-6), 4.42 (H-19)
δ_H 2.32 (Ha-6)	δ_C 45.0 (C-6)	δ_C 104.4 (C-5)	δ_C 105.3 (C-2), 52.2 (C-7), 137.5 (C-8)	δ_H 7.42 (H-9), 2.97 (H_3-1')
δ_H 2.27 (Hb-6)	δ_C 45.0 (C-6)	δ_C 104.4 (C-5)		δ_H 5.09 (H-5), 4.42 (H-19)
δ_H 7.42 (H-9)	δ_C 123.8 (C-9)	δ_C 120.5 (C-10)	δ_C 52.2 (C-7), 145.8 (C-13)	δ_H 2.32 (Ha-6)
δ_H 6.78 (H-10)	δ_C 120.5 (C-10)	δ_C 123.8 (C-9), 128.2 (C-11)		
δ_H 6.99 (H-11)	δ_C 128.2 (C-11)	δ_C 120.5 (C-10), 109.0 (C-		
δ_H 6.49 (H-12)	δ_C 109.0 (C-12)	δ_C 128.2 (C-11)	δ_C 137.5 (C-8)	
δ_H 1.61 (Ha-14)	δ_C 23.5 (C-14)	δ_C 64.0 (C-3), 28.9 (C-15)	δ_C 51.9 (C-16)	δ_H 1.93 (Ha-21)
δ_H 1.53 (Hb-14)				
δ_H 2.53 (H-15)	δ_C 28.9 (C-15)	δ_C 23.5 (C-14), 51.9 (C-16)		δ_H 1.93 (Ha-21), 1.41 (H_3-18)
δ_H 3.07 (H-16)	δ_C 51.9 (C-16)	δ_C 28.9 (C-15)	δ_C 105.3 (C-2), 52.2 (C-7), 137.5 (C-8), 170.8 (C-17)	
δ_H 1.41 (H_3-18)	δ_C 21.7 (C-18)	δ_C 79.4 (C-19)	δ_C 46.9 (C-20)	δ_H 2.53 (H-15)
δ_H 4.42 (H-19)	δ_C 79.4 (C-19)	δ_C 21.7 (C-18)	δ_C 170.8 (C-17), 46.9 (C-20), 29.1 (C-21)	δ_H 5.09 (H-5), 2.27 (Hb-6), 1.63 (Hb-21)
δ_H 1.93 (Ha-21)	δ_C 29.1 (C-21)		δ_C 64.0 (C-3), 28.9 (C-15), 46.9 (C-20)	δ_H 3.39 (H-3), 2.53 (H-15), 1.61 (Ha-14)
δ_H 1.63 (Hb-21)	δ_C 29.1 (C-21)			δ_H 4.42 (H-19)
δ_H 2.97 (H_3-1')	δ_C 54.5 (C-1')		δ_C 104.4 (C-5)	δ_H 2.32 (Ha-6)

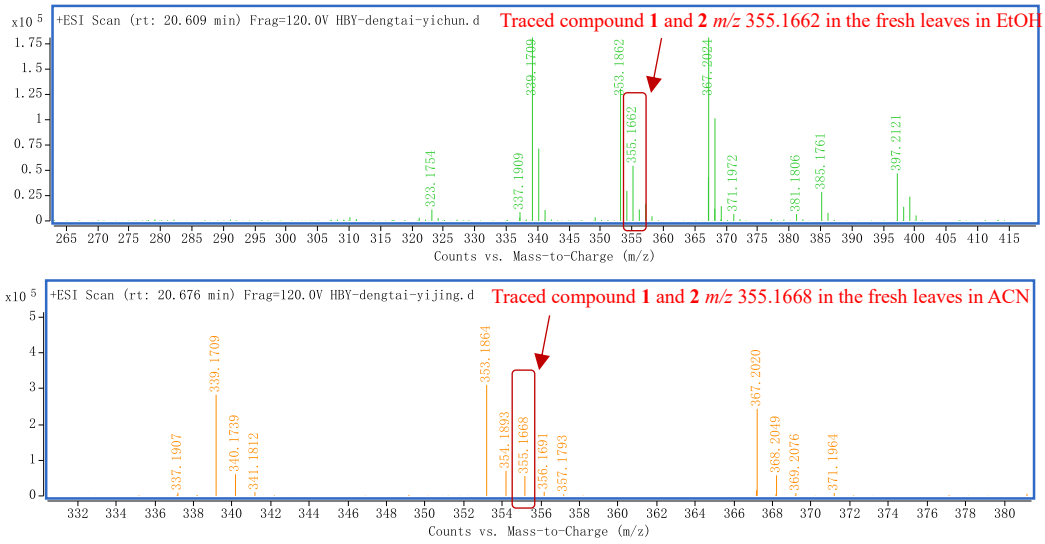
3. Detection of 1 and 2 in the fresh leaves of *Alstonia scholaris* by UPLC-ESI-Q-TOF-MS











Solvent Composition

	Channel	Ch. 1 Solv.	Name 1	Ch2 Solv.	Name 2	Selected	Used	Percent
1	A	100.0 % Water V.03		100.0 % Water V.03		Ch. 1	Yes	96.00 %
2	B	100.0 % Acetonitrile V.03		100.0 % Methanol V.03		Ch. 1	Yes	4.00 %

Timetable

	Time	A	B	Flow	Pressure
1	7.00 min	88.00 %	12.00 %	--- mL/min	--- bar
2	12.00 min	83.00 %	17.00 %	--- mL/min	--- bar
3	16.00 min	83.00 %	17.00 %	--- mL/min	--- bar
4	23.00 min	70.00 %	30.00 %	--- mL/min	--- bar
5	28.00 min	55.00 %	45.00 %	--- mL/min	--- bar
6	35.00 min	35.00 %	65.00 %	--- mL/min	--- bar
7	40.00 min	2.00 %	98.00 %	--- mL/min	--- bar
8	45.00 min	2.00 %	98.00 %	--- mL/min	--- bar

Figure S1. Detection of **1** and **2** in the fresh leaves of *Alstonia scholaris*

4. Computational data of 2

ECD calculation for compounds **2**

To establish the absolute configurations for the isolated compounds, the theoretical ECD calculations were carried out using Gaussian 09[1]. The selected conformers **2-1** (98.54%), **2-2** (0.03%), **2-3** (1.43%) were conducted at B3LYP/6-31(d, p) level for the ECD calculations. The solvent effects were taken into account by the polarizable-conductor calculation model (PCM, methanol as the solvent). The final calculated ECD spectra was simulated using the ECD/UV analysis tool according to the Boltzmann-calculated contribution of each conformer. As a result, the overall pattern of calculated ECD spectra of (2*R*,3*S*,5*R*,7*S*,15*R*,16*R*,19*S*,20*S*)-**2** well match the experimental data of

2. Therefore, qualitative analysis of the predicted and experimental ECD spectra allow the assignments of the absolute configurations of compounds **2**.

(1) Gaussian 09, Revision C.01,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, T. Keith, 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, 2010.

Table S3. Extracted heats and weighting factors of the optimized conformers of **2-1**, **2-2**, and **2-3** at B3LYP/6-31(d, p) level

		B3LYP/6-31(d, p)	
compound	Conformer	Extracted heats	Boltzmann-calculated contribution (%)
2	**2-1**	-1185.692251	98.54%
	2-2	-1185.684668	0.03%
	2-3	-1185.688256	1.43%

Table S4. The Cartesian coordinates of the lowest energy conformers for **2-1**, **2-2**, **2-3**

2-1	X axis(Å)	Y axis(Å)	Z axis(Å)	**2-2**	X axis(Å)	Y axis(Å)	Z axis(Å)
C	3.4392	2.4414	-0.1391	C	3.6027	2.2398	-0.0194
C	4.1051	1.6858	-1.1156	C	4.2364	1.5047	-1.032
C	3.5373	0.5104	-1.615	C	3.6107	0.3923	-1.6012
C	2.3169	0.1007	-1.114	C	2.3668	0.0205	-1.1302
C	1.642	0.868	-0.1641	C	1.7266	0.765	-0.1396
C	2.1938	2.0366	0.3425	C	2.3332	1.8761	0.4308
N	1.5891	-1.0144	-1.5235	N	1.5811	-1.0242	-1.6069
C	0.4523	-1.1343	-0.6094	C	0.4495	-1.1522	-0.6888
C	0.2998	0.231	0.1515	C	0.3468	0.1851	0.1289
O	0.7854	-2.1653	0.3273	O	0.7862	-2.2217	0.1929

C	0.9349	-1.5806	1.6188	C	0.5396	-1.8062	1.5292
C	0.2066	-0.2436	1.6026	C	0.2201	-0.3133	1.5694
C	-0.8409	1.1772	-0.3717	C	-0.7308	1.2064	-0.3921
C	-0.8343	-1.5333	-1.3908	C	-0.8623	-1.4772	-1.4627
C	-1.8898	0.5429	-1.3008	C	-1.8161	0.6455	-1.3291
C	-1.2039	-0.36	-2.3102	C	-1.1841	-0.2725	-2.3592
C	-3.2945	-1.7073	-1.0445	C	-3.3231	-1.5432	-1.0944
N	-1.982	-1.6924	-0.4233	N	-2.0069	-1.5937	-0.4853
C	-2.7142	-0.4063	-0.4828	C	-2.6829	-0.2745	-0.5219
O	-2.6472	1.332	1.2752	O	-2.5639	1.4935	1.2044
C	-1.5269	1.884	0.7674	C	-1.3821	1.9503	0.7431
O	-1.0536	2.9006	1.2676	O	-0.838	2.9159	1.2712
O	2.3008	-1.3637	1.9566	O	1.5639	-2.1966	2.4381
C	-3.3589	0.1844	0.7382	C	-3.2861	0.3291	0.7154
C	-4.7744	0.6686	0.4353	C	-4.714	0.8046	0.46
C	3.0346	-2.5766	2.0243	C	2.8706	-1.7853	2.0674
H	-0.3945	1.9778	-0.9813	H	-0.2321	1.9804	-0.9958
H	-3.4051	-0.555	1.5463	H	-3.2977	-0.3969	1.5367
H	3.894	3.3528	0.2413	H	4.0992	3.1074	0.409
H	5.0706	2.0188	-1.4876	H	5.2199	1.8084	-1.3824
H	4.0499	-0.0672	-2.3758	H	4.0966	-0.1653	-2.3943
H	1.674	2.6299	1.0892	H	1.8359	2.4607	1.1999
H	2.0836	-1.8529	-1.7921	H	2.0236	-1.8663	-1.948
H	0.4891	-2.2455	2.3677	H	-0.3508	-2.3527	1.8609
H	-0.8333	-0.4423	1.8762	H	-0.8034	-0.2266	1.9436
H	0.6168	0.4689	2.3259	H	0.84	0.235	2.2865
H	-0.6809	-2.464	-1.9487	H	-0.7588	-2.405	-2.0364
H	-2.5123	1.3026	-1.786	H	-2.4015	1.4452	-1.7959
H	-1.8969	-0.6735	-3.099	H	-1.8989	-0.5397	-3.1456
H	-0.3559	0.1219	-2.806	H	-0.3194	0.1771	-2.8562
H	-3.3766	-1.8711	-2.1115	H	-3.4224	-1.6883	-2.1627
H	-4.064	-2.2241	-0.4791	H	-4.1082	-2.0366	-0.5292
H	-5.2255	1.1171	1.3271	H	-5.1287	1.2745	1.3586
H	-5.418	-0.1473	0.0931	H	-5.371	-0.0189	0.1653
H	-4.765	1.4445	-0.3387	H	-4.7372	1.5612	-0.3325
H	4.0545	-2.342	2.3411	H	3.5723	-2.1955	2.7995
H	2.5915	-3.2572	2.7578	H	3.1456	-2.1747	1.0833
H	3.0828	-3.0576	1.0428	H	2.9634	-0.6977	2.0917
2-3							
C	3.5603	2.1784	-0.516				
C	4.1612	1.2935	-1.4234				
C	3.5226	0.1042	-1.786				
C	2.2971	-0.1881	-1.2196				

C	1.6884	0.7046	-0.3376				
C	2.3106	1.8891	0.0331				
N	1.5058	-1.3027	-1.4955				
C	0.3862	-1.2685	-0.5525				
C	0.3284	0.1699	0.0749				
O	0.6793	-2.2228	0.474				
C	0.928	-1.524	1.6941				
C	0.266	-0.1565	1.5682				
C	-0.7802	1.1234	-0.5021				
C	-0.9391	-1.6651	-1.2672				
C	-1.8825	0.4651	-1.3485				
C	-1.269	-0.5618	-2.2834				
C	-3.3954	-1.6734	-0.8583				
N	-2.0695	-1.6707	-0.2656				
C	-2.7354	-0.3594	-0.431				
O	-2.53	1.5155	1.1674				
C	-1.3959	1.9588	0.5891				
O	-0.8501	2.9804	0.9973				
O	2.331	-1.4219	1.9039				
C	-3.3209	0.3738	0.7409				
C	-4.7092	0.9173	0.4144				
C	2.6778	-1.2248	3.2653				
H	-0.3135	1.8459	-1.1893				
H	-3.3977	-0.2888	1.6109				
H	4.0694	3.0998	-0.2439				
H	5.1315	1.5353	-1.8493				
H	3.9855	-0.5754	-2.4926				
H	1.8409	2.5827	0.7243				
H	1.9664	-2.1905	-1.6444				
H	0.479	-2.1106	2.5039		-		
H	-0.7727	-0.2763	1.89				
H	0.7274	0.6093	2.1984				
H	-0.8502	-2.65	-1.7393				
H	-2.4759	1.2102	-1.8896				
H	-1.9964	-0.9078	-3.0265				
H	-0.4093	-0.1738	-2.8378				
H	-3.5102	-1.931	-1.9035				
H	-4.1767	-2.0945	-0.2328				
H	-5.1171	1.4665	1.27				
H	-5.4066	0.1165	0.1509				
H	-4.6662	1.6229	-0.423				
H	3.7658	-1.14	3.3327				
H	2.2351	-0.3053	3.6578				

H	2.3605	-2.0809	3.8685				

5. Biological evaluation

Chemicals

Lipopolysaccharide (LPS; Escherichia coli) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Human bronchial epithelial (16HBE) cells were supplied from American Type Culture Collection (Manassas, VA, USA). Antibodies against p-p65, caspase-1 and IL-18 were obtained by Cell Signalling Technology (Danvers, MA, USA). Anti-β-tubulin was purchased from Proteintech (Rosemont, IL, USA). Enzyme-linked immune-sorbent assay (ELISA) reagents sets for IL-6 and TNF-α were purchased from R&D Systems (Minneapolis, MN, USA). All the other chemicals and solvents were of the highest purity grade available.

Animals

Pathogen-free ICR male mice (22–24 g) were purchased from Kunming Medical University (License SCXK, 2020–0004). All mice were kept in room temperature (20–25 ◦C) and constant humidity (40–70%) SPF grade laboratory, under a 12 h light–dark cycle. Standard diet and water were free access. According to the international rules considering animal experiments and the internationally accepted ethical principles for laboratory animal use and care, the animal study was performed. This study was reviewed and approved (No. Kib202107007) by the Institutional Animal Care and Use Committee of Kunming Institute of Botany, Chinese Academy of Sciences (SYXK, 2018–0005).

Anti-inflammatory activity against LPS-induced 16HBE Cells

LPS-induced 16HBE cells inflammation model was set up as previously reported with slight modifications[2]. 16HBE cells in the logarithmic growth phase were harvested and diluted in Dulbecco's modified Eagle's medium (DMEM) and seeded on 6-well plates at 4×10^5 cells/well overnight. Then the supernatant was discarded and the stimulation of LPS (final concentration 1 μg/ml) was added in each well accompanied by the addition of compounds **1** and **2** (5 μg/ml) and positive control dexamethasone (DEX, 5 μg/ml). Subsequently, ATP (final concentration 1 mmol) was supplemented

into wells after 24 h of incubation. After 1.5 h, the PCT and CRP levels from the cell-free supernatant were determined using commercial ELISA kits (Shanghai Jining Industrial Co., Ltd., Chengdu, China) according to the protocols of the manufacturer, and cellular protein was extracted for western blot analysis.

Western blot analysis

Total protein was extracted from the 16 HBE cells using lysis buffer. The protein concentration of the supernatant was measured and quantified using the BCA kit. P-p65, caspase-1, IL-18 and *β*-tubulin were detected. The transferred proteins were visualized with an enhanced chemiluminescence detection kit (Tiangen Biotech, Beijing, China) on a FluorChem E System (Protein Simple, Santa Clara, CA, USA).

LPS-induced Acute lung injury (ALI) model in mice

The LPS-induced ALI mice model was set up as described in literature[3]. Specifically, the male mice aged 6-8 weeks were randomly divided into 7 groups (10 ones each group). (1) Control group: sham sensitization with saline via intranasal (i.n.); (2) LPS: sensitization with LPS via i.n.; (3) DXM: dexamethasone via intragastrical (i.g.) 5 mg/kg plus challenge with LPS via i.n.; (4−7) Testing groups: Compound **1** and **2** at dose of 2 mg/kg and 5 mg/kg plus challenge with LPS via i.n.. Mice were pre-administrated with DEX and/or different tested articles daily for 2 days by intraperitoneal injection, while those in the other groups were treat with 0.1% dimethylsulfoxide (10 ml/kg) every day. On the 3rd day, mice were administrated with DXM and/or tested articles after LPS stimulation (60 μg/mice, i.n.) for 1 h, except for mice in the sham group, which were administered with normal saline. After 6 h, the animals were euthanized, closely followed by lungs harvest, which were used for detection of cytokines and histopathological analysis.

Detection of cytokines in lung homogenate

The lung tissue supernatants were prepared as described previously[4]. 50 mg lungs were homogenized in 0.45 ml of ice-cold phosphate-buffered saline using tissue homogenizer (SurgiVet, Madison, WI, USA) to obtain the lung homogenate, and was cooled on ice. Then the lung homogenate was centrifuged at 1000 × g for 10 minutes at 4 °C to obtain the supernatant, and stored at −80 °C. IL-6 and TNF-*α* levels in the

lung homogenate were measured using commercially ELISA kits.

Histological examination of lungs

To histologically analyze the mice lungs, the histological examination was investigated as previously reported[5]. The right upper lobes of lung were removed when the mice under terminal anesthesia. Then the tissue was washed in phosphate buffer, and fixed in 4% formaldehyde buffered with a phosphate solution (0.1 M, pH 7.4) at room temperature. The lung fragments were dehydrated in graded concentrations of ethanol, embedded in paraffin, and cut into 5-μm-thick sections. Then tissue sections were prepared and stained with hematoxylin and eosin to evaluate the general morphology. The lung index was also detected.

Statistical Analysis.

Results were expressed as the mean ± standard error of the mean (SD). Statistical significance was determined using one-way ANOVA, with ($^{\#\#}$) $p < 0.01$, (**) $p < 0.01$ or (*) $p < 0.05$ accepted as the significance value.

(2) G. M. Liu, Q. F. Wan, J. W. Li, X. Y. Hu, X. L. Gu, S. C. Xu, *Thorac. Cancer.* **2020**, *11*, 1297−1308.

(3) K. Zhao, X. T. Li, J. R. Yang, Z. B. Huang, C. L. Li, H. R. Huang, K. Zhang, D. L. Li, L. Y. Zhang, X. Zheng, *J. Ethnopharmacol.* **2022**, *290*, 115119.

(4) Y. L. Zhao, Z. F. Yang, B. F. Wu, J. H. Shang, X. D. Luo, *J. Ethnopharmacol.* **2022**, *259*, 112949.

(5) Y. L. Zhao, S. B. Pu, Y. Qi, B. F. Wu, X. D. Luo, *J. Ethnopharmacol.* **2020**, *267*, 113506.

6. NMR, HRESIMS, IR, UV ORD and CD of 1

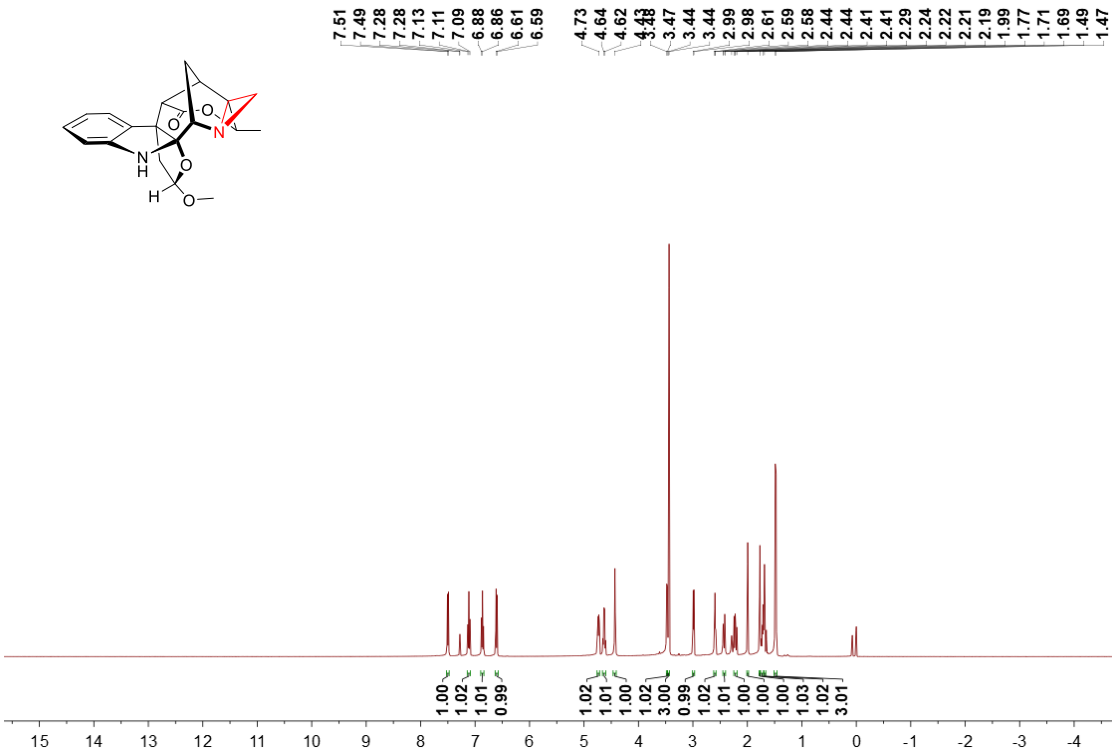


Figure S2. ^{1}H NMR (400 MHz) spectrum of compound **1** in $CDCl_3$.

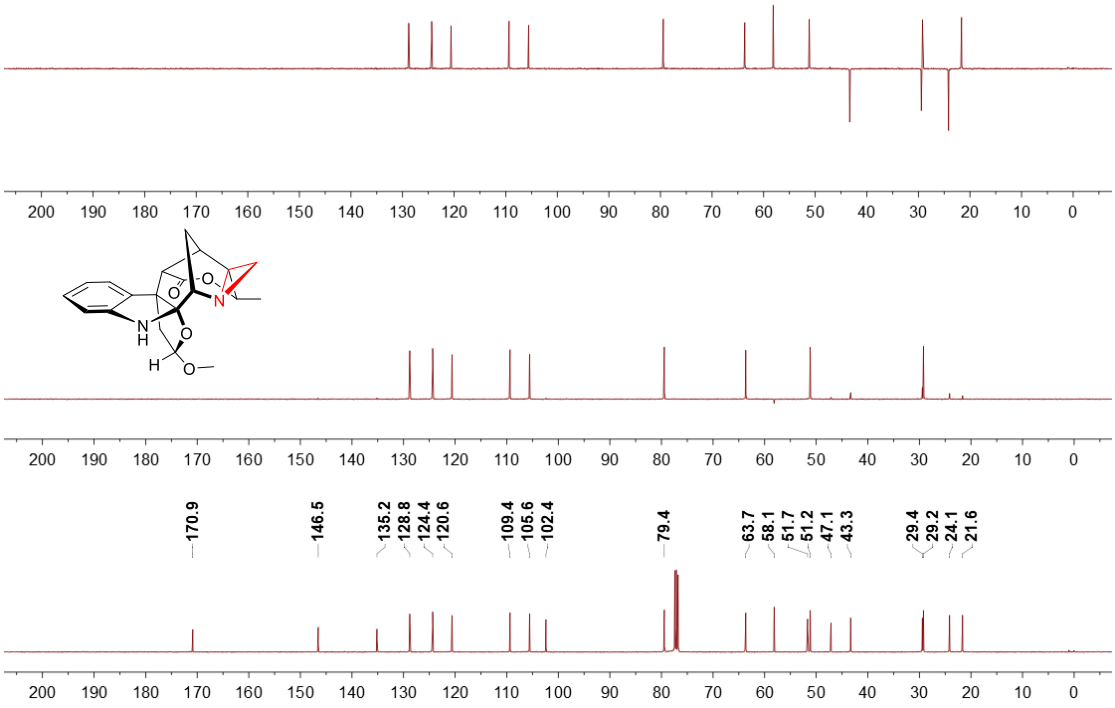


Figure S3. ^{13}C NMR (100 MHz) and DEPT spectra of compound **1** in $CDCl_3$.

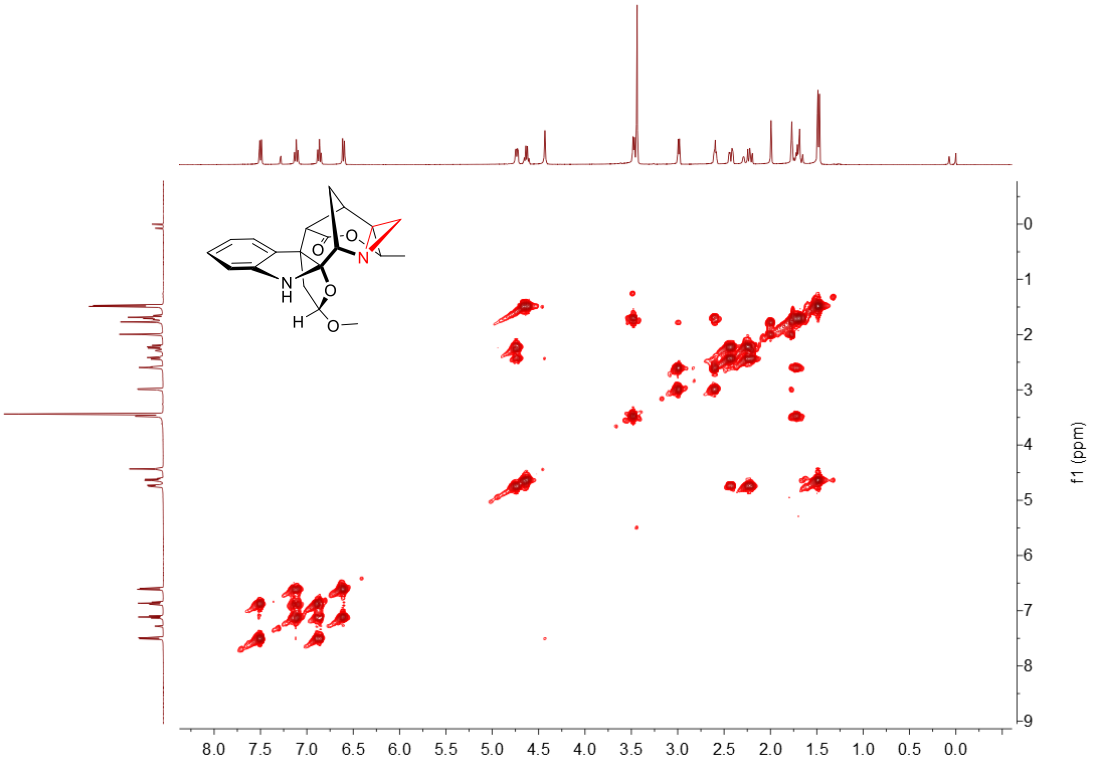


Figure S4. ^{1}H-^{1}H COSY spectrum of compound **1** in $CDCl_3$.

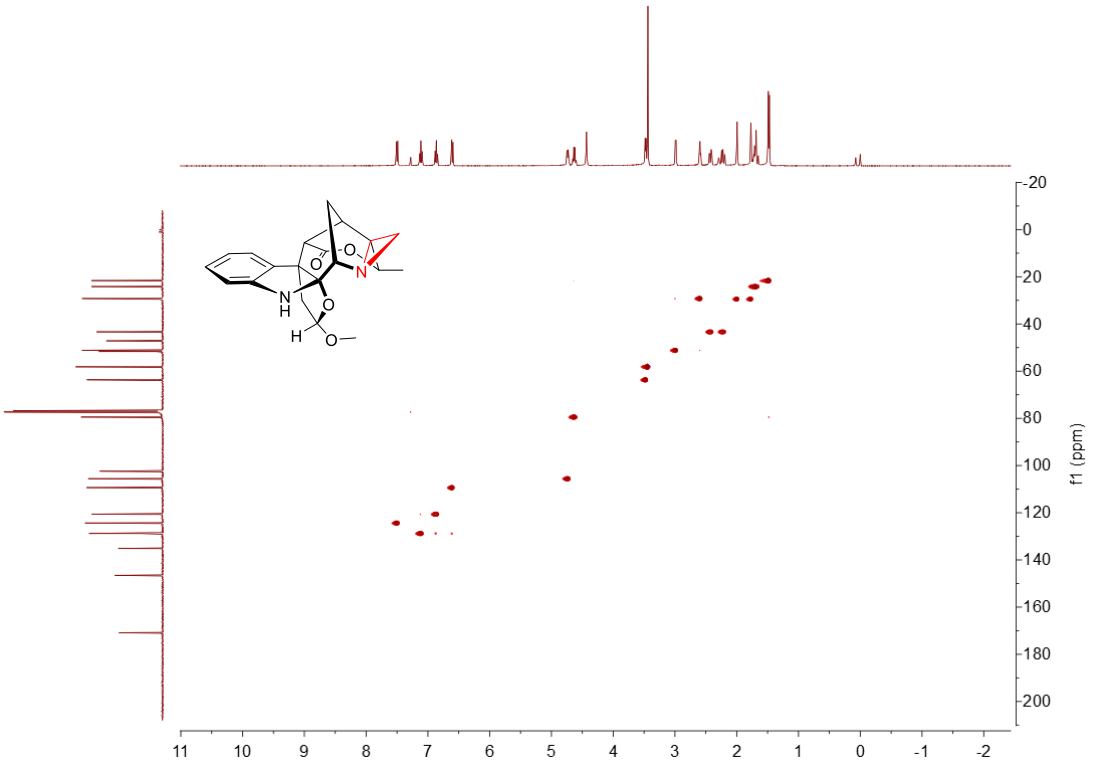


Figure S5. HSQC spectrum of compound **1** in $CDCl_3$.

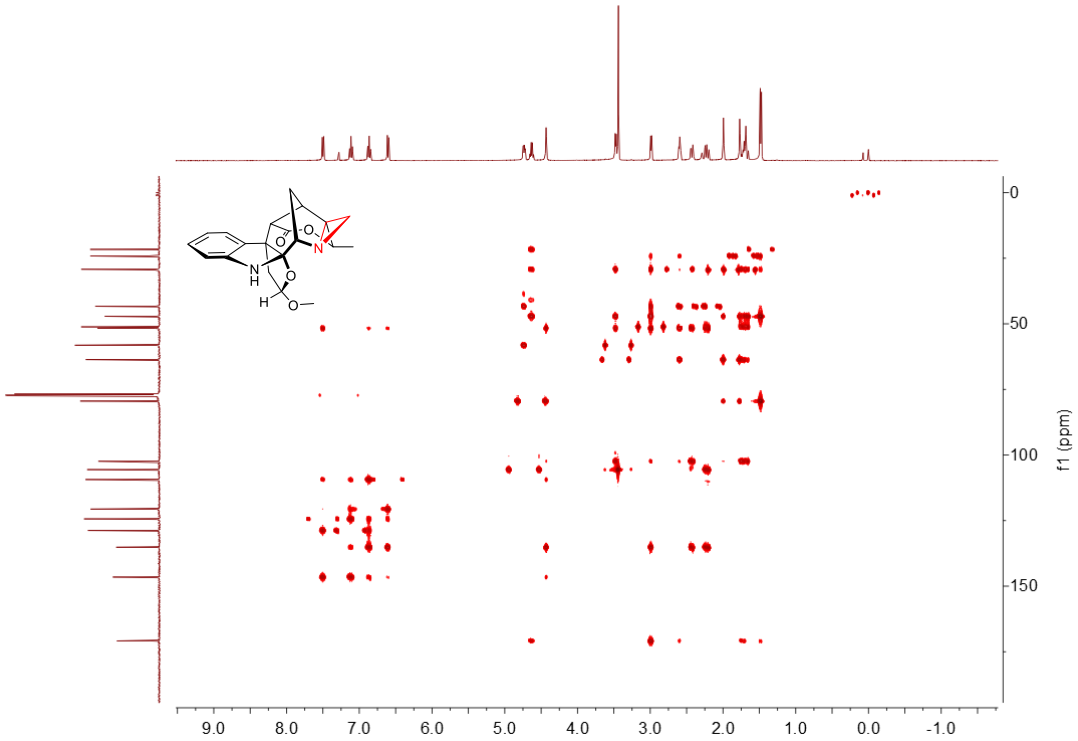


Figure S6. HMBC spectrum of compound **1** in $CDCl_3$.

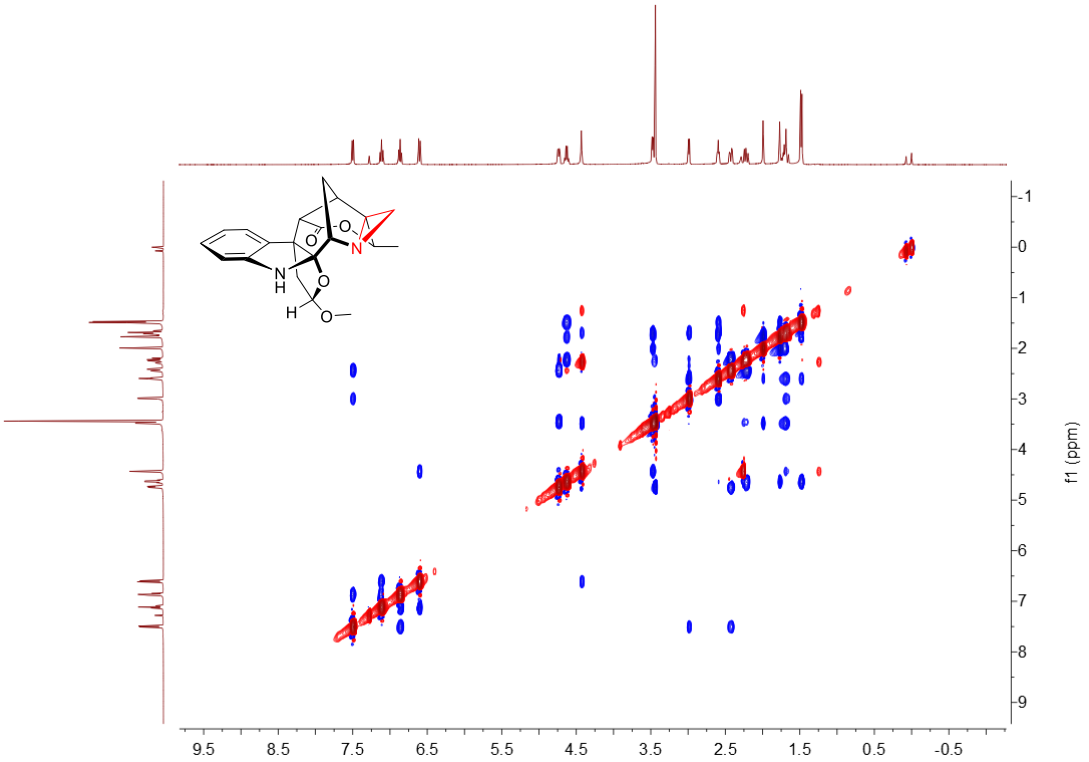


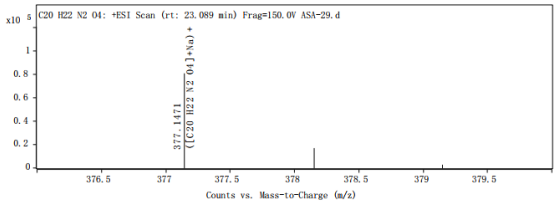
Figure S7. ROESY spectrum of compound **1** in $CDCl_3$.

Qualitative Analysis Report

Data File	ASA-29.d	**Sample Name**	ASA-29
Sample Type	Sample	**Position**	P2-E2
Instrument Name	Instrument 1	**User Name**	
Acq Method	20210527-DTY-MSMS.m	**Acquired Time**	2021/5/31 11:03:22 (UTC+08:00)
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			
Sample Group		**Info.**	
Stream Name	LC 1	**Acquisition Time (Local)**	2021/5/31 11:03:22 (UTC+08:00)
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.09.00 (B9044.0)	**QTOF Driver Version**	8.00.00
QTOF Firmware Version	25.723	**DDE Mode**	1
Tune Mass Range Max.	3200		

Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
150	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
108.0809		100431.83		
130.0651		79209.08		
216.0652		58281.01		
279.1491		102446.55		
323.14	1	2230475.75		
323.2055		143245.63		
324.1425	1	401843.91		
377.1471	1	80428.47	C20 H22 N2 O4	(M+Na)+
731.3058	1	144189.16		
732.3087	1	64922.21		

Element	Min	Max
C	3	60
H	0	120
O	0	30
N	0	30
S	0	5
Cl	0	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C20 H22 N2 O4	True	354.1579	354.158	0.2	C20 H22 N2 Na O4	99.26

--- End Of Report ---

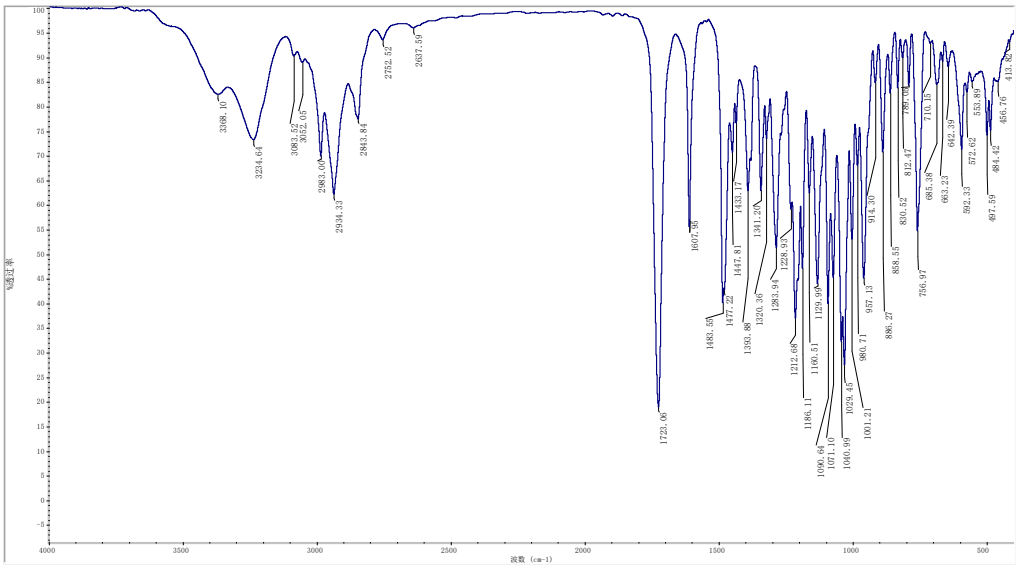
Spectrum Identification Results: + Scan (rt: 23.089 min) (ASA-29.d)

Best	ID Sourc	Nam	Formula	Specie	m/z	Score	Diff (ppm	Score (MFG
☑	MFG		C20 H22 N2 O4	(M+Na)+	377.147	99.26	0.2	99.26

Species	m/z	Score (iso. abund	Score (mass	Score (MFG, MS/M	Score (MS	Score (MFG)	Score (iso. spacing	Heigh	Ion Formula
(M+Na)+	377.147	97.72	99.97		99.26	99.26	99.69	80428.5	C20 H22 N2 Na O4

Height (Calc)	Height Sum% (Cal	Height % (Calc	m/z (Calc	Diff (mDa	Heigh	Height	Height Sum	m/z	Diff (ppm
79149.4	**79.1**	**100**	**377.1472**	**0.1**	**80428.5**	**100**	**80.4**	**377.147**	**0.3**

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



Sample Name: WASA-29
KBr压片
采集时间: 星期五 4月 16 15:48:44 2021 (GMT+08:00)
仪器型号：NICOLET iS10
Software version: OMNIC 9.8.372

样品扫描次数: 16
背景扫描次数: 16
分辨率: 4.000
采样增益: 1.0
动镜速度: 0.4747
光阑: 80.00

Figure S9. IR spectrum of compound **1**.

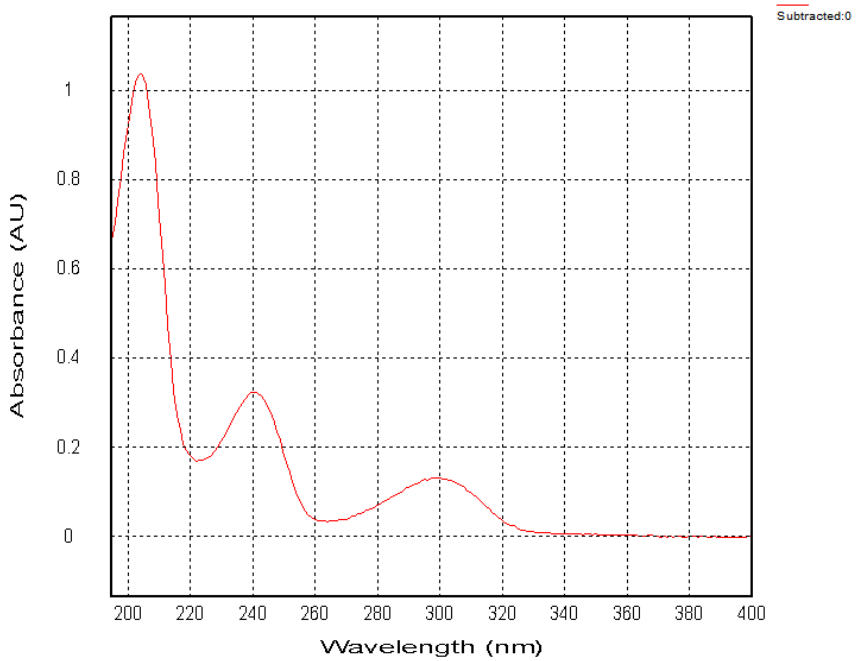


Figure S10. UV spectrum of compound **1** (0.1372mg/mL MeOH).

Rudolph Research Analytical

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

Measurement Date : Thursday, 11-MAR-2021

Set Temperature : OFF

Time Delay : Disabled

Delay between Measurement : Disabled

n	Average	Std.Dev.	% RSD	Maximum	Minimum
5	-95.00	0.20	-0.21	-94.80	-95.31

S.No	Sample ID	Time	Result	Scale	OR °Arc	WLG.nm	Lg.mm	Conc.g/100ml	Temp.
1	WASA29	04:43:32 PM	-95.31	SR	-0.0934	589	100.00	0.098	23.3
2	WASA29	04:43:41 PM	-94.80	SR	-0.0929	589	100.00	0.098	23.3
3	WASA29	04:43:49 PM	-94.90	SR	-0.0930	589	100.00	0.098	23.3
4	WASA29	04:43:57 PM	-95.10	SR	-0.0932	589	100.00	0.098	23.3
5	WASA29	04:44:05 PM	-94.90	SR	-0.0930	589	100.00	0.098	23.3

Figure S11. Experimental ORD compound **1**.

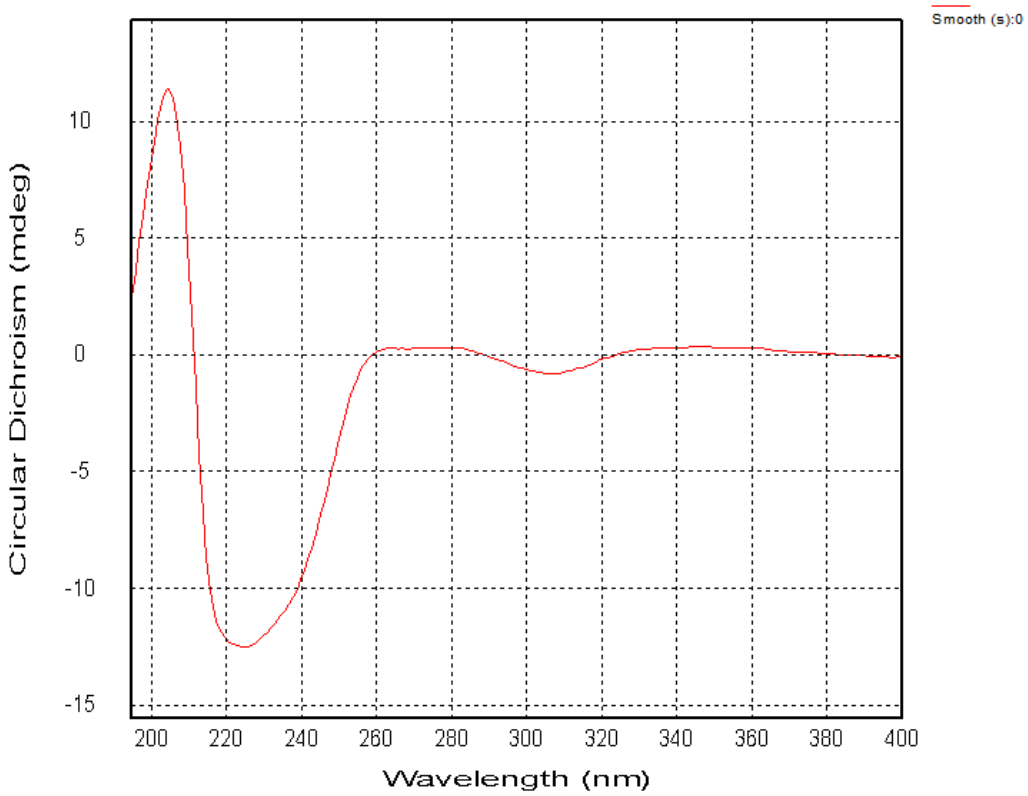


Figure S12. Experimental CD compound **1**.

7. NMR, HRESIMS, IR, UV, ORD and CD of 2

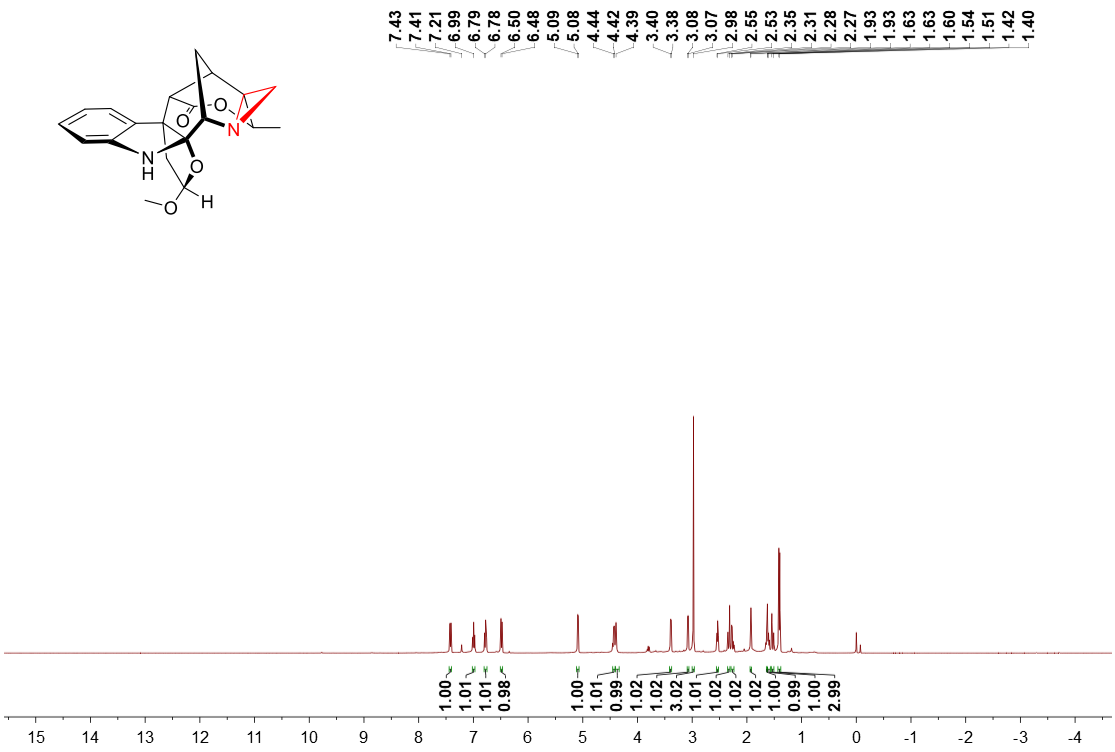


Figure S13. ^{1}H NMR (400 MHz) spectrum of compound **2** in $CDCl_3$.

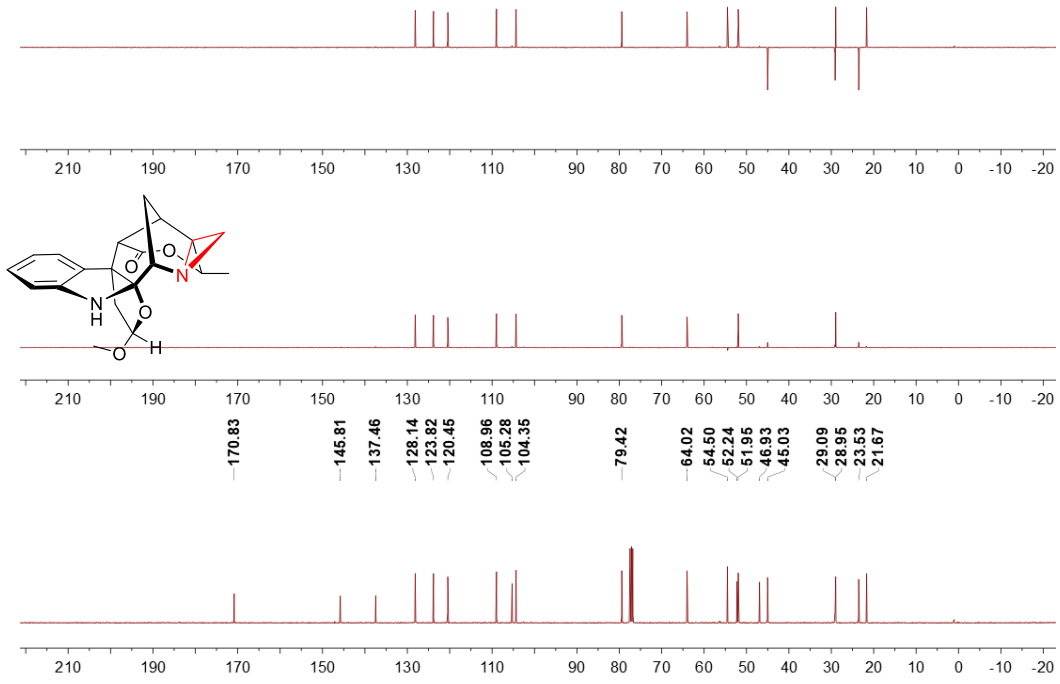


Figure S14. ^{13}C NMR (100 MHz) and DEPT spectra of compound **2** in $CDCl_3$.

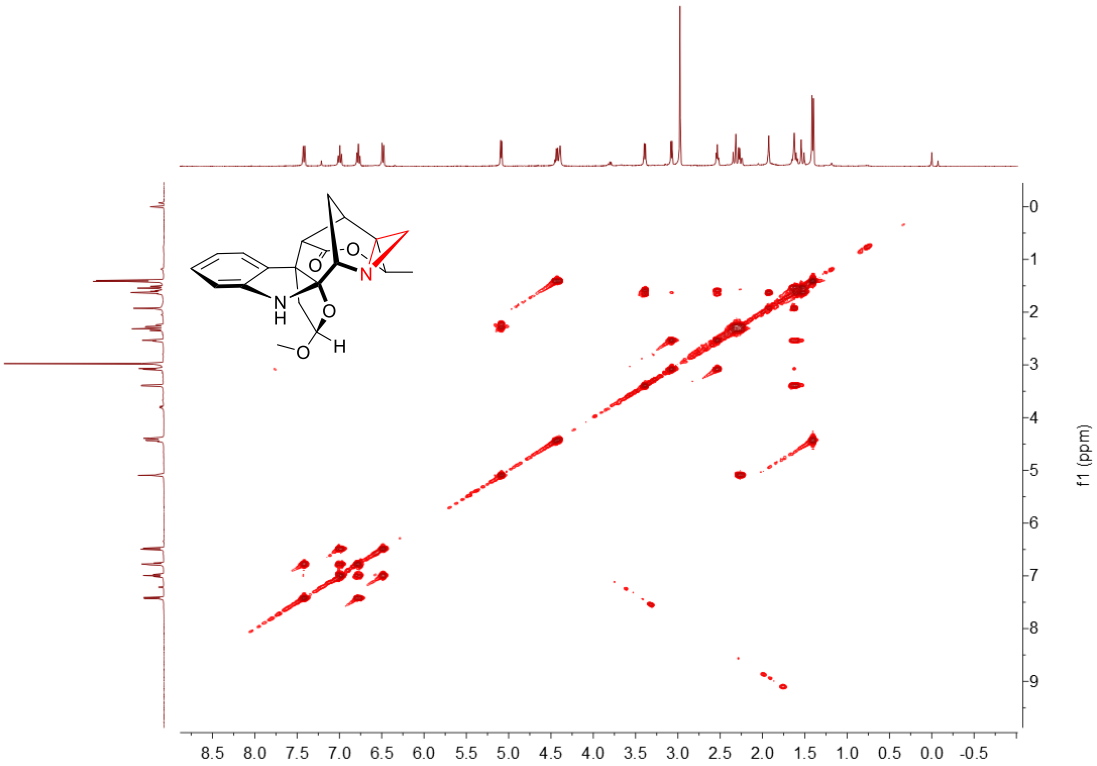


Figure S15. ^{1}H-^{1}H COSY spectrum of compound **2** in $CDCl_3$.

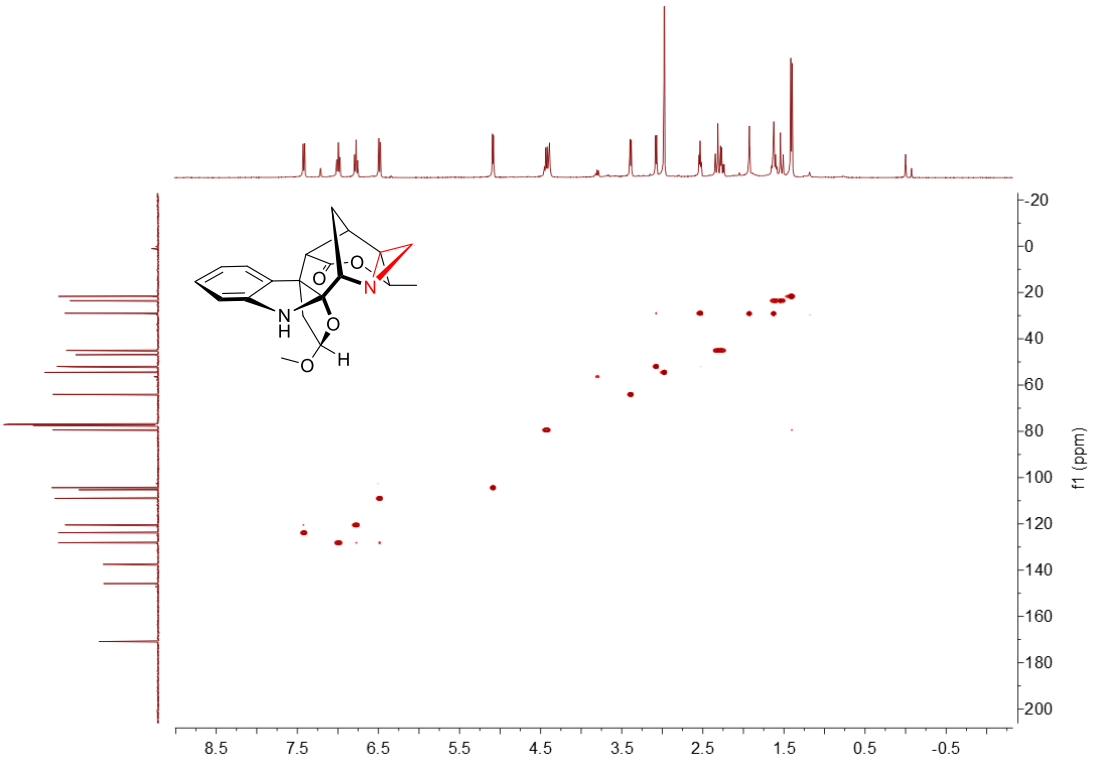


Figure S16. HSQC spectrum of compound **2** in $CDCl_3$.

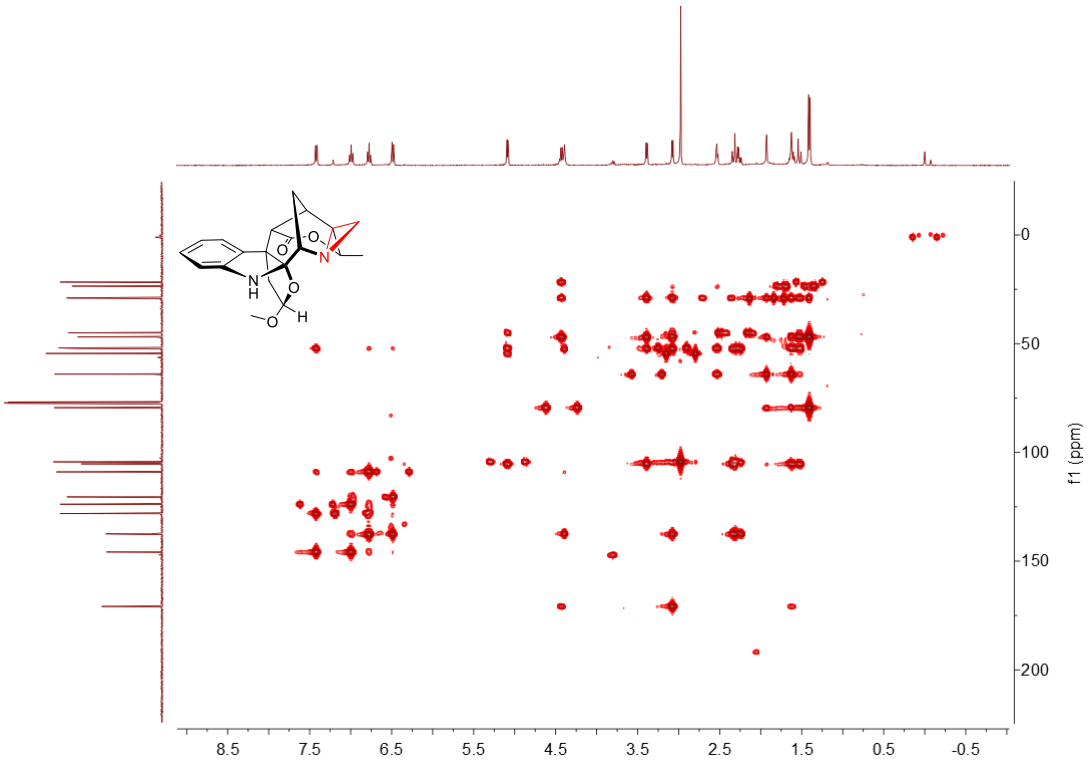


Figure S17. HMBC spectrum of compound **2** in $CDCl_3$.

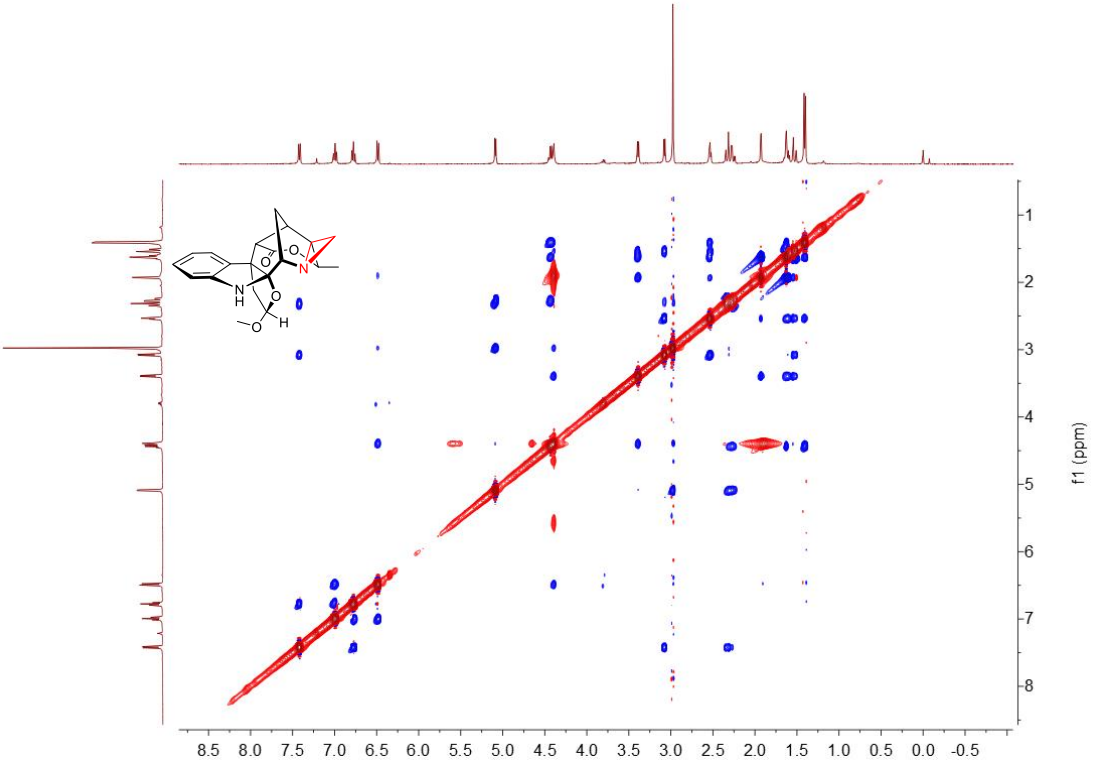


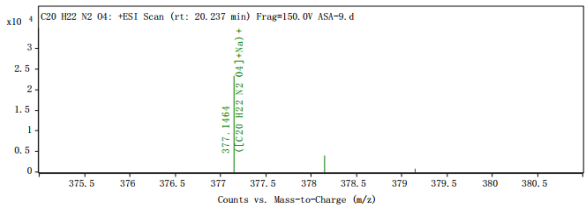
Figure S18. ROESY spectrum of compound **2** in $CDCl_3$.

Qualitative Analysis Report

Data File	ASA-9.d	**Sample Name**	ASA-9
Sample Type	Sample	**Position**	P2-D5
Instrument Name	Instrument 1	**User Name**	
Acq Method	20210527-DTY-MSMS.m	**Acquired Time**	2021/5/31 6:01:16 (UTC+08:00)
IRM Calibration Status	Success	**DA Method**	Default.m
Comment			
Sample Group		**Info.**	
Stream Name	LC 1	**Acquisition Time (Local)**	2021/5/31 6:01:16 (UTC+08:00)
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.09.00 (B9044.0)	**QTOF Driver Version**	8.00.00
QTOF Firmware Version	25.723	**DDE Mode**	1
Tune Mass Range Max.	3200		

Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
150	0	ESI



Peak List

m/z	z	Abund	Formula	Ion
108.0804		33047.37		
130.065		17537.05		
216.065		17842.22		
279.1485		31859.3		
295.1438		21459.55		
323.1392	1	749140.5		
324.1425	1	143036.09		
355.1658	1	416659.63		
356.1686	1	95170.41		
377.1464	1	23307.05	C20 H22 N2 O4	(M+Na)+

Element	Min	Max
C	3	60
H	0	120
O	0	30
N	0	30
S	0	5
Cl	0	3

Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	Score
C20 H22 N2 O4	True	354.1573	354.158	1.8	C20 H22 N2 Na O4	91.76

--- End Of Report ---

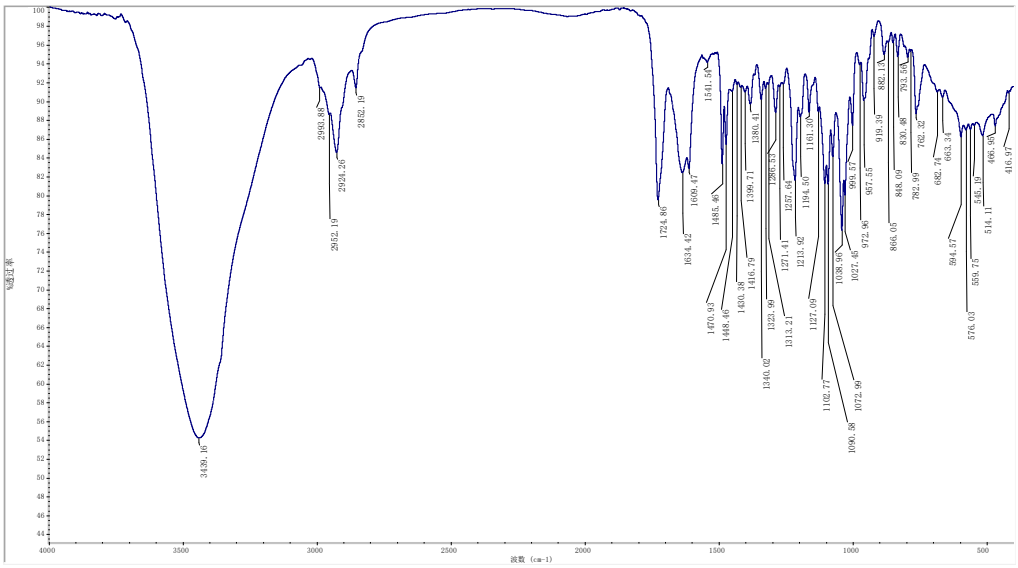
Spectrum Identification Results: + Scan (rt: 20.237 min) (ASA-9.d)

Best	ID Sourc	Nam	Formula	Specie	m/z	Scor	Diff (ppm	Score (MFG
☑	MFG		C20 H22 N2 O4	(M+Na)+	377.146	91.76	1.8	91.76

Species	m/z	Score (iso. abund	Score (mass	Score (MFG, MS/M	Score (MS	Score (MFG	Score (iso. spacing	Heigh	Ion Formula
(M+Na)+	377.146	76.59	97.62		91.76	91.76	98.26	23307	C20 H22 N2 Na O4

Height (Calc)	Height Sum% (Cal	Height % (Calc	m/z (Calc	Diff (mDa	Heigh	Height	Height Sum	m/z	Diff (ppm
22156.9	79.3	100	377.1472	0.7	23307	100	83.4	377.146	1.98

Figure S19. HRESIMS of compound **2**.



Sample Name: WASA-9
KBr压片
采集时间: 星期五 4月 16 15:28:05 2021 (GMT+08:00)
仪器型号：NICOLET iS10
Software version: OMNIC 9.8.372

样品扫描次数: 16
背景扫描次数: 16
分辨率: 4.000
采样增益: 1.0
动镜速度: 0.4747
光阑: 80.00

Figure S20. IR spectrum of compound **2**.

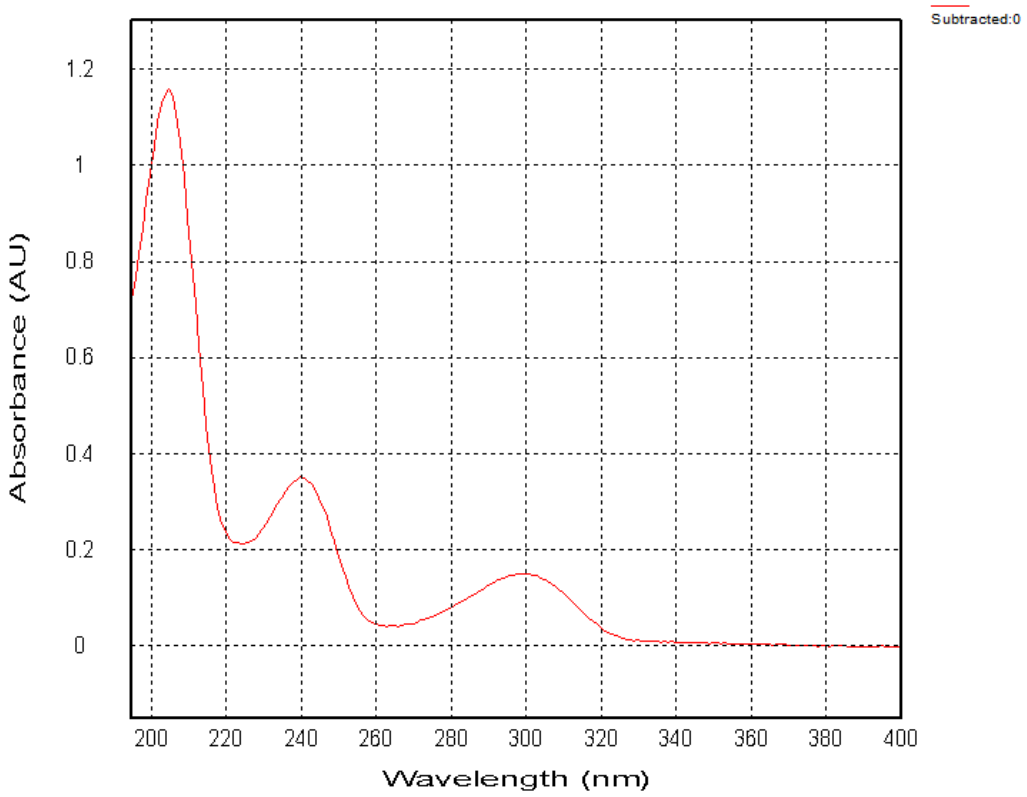


Figure S21. UV spectrum of compound **2** (0.1428mg/mL MeOH).

Rudolph Research Analytical

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

Measurement Date : Thursday, 11-MAR-2021

Set Temperature : OFF

Time Delay : Disabled

Delay between Measurement : Disabled

n	Average	Std.Dev.	% RSD	Maximum	Minimum
5	-197.20	0.44	-0.22	-196.67	-197.75

S.No	Sample ID	Time	Result	Scale	OR °Arc	WLG.nm	Lg.mm	Conc.g/100ml	Temp.
1	WASA9	04:55:31 PM	-197.75	SR	-0.2017	589	100.00	0.102	23.4
2	WASA9	04:55:39 PM	-197.55	SR	-0.2015	589	100.00	0.102	23.4
3	WASA9	04:55:47 PM	-196.96	SR	-0.2009	589	100.00	0.102	23.4
4	WASA9	04:55:56 PM	-197.06	SR	-0.2010	589	100.00	0.102	23.4
5	WASA9	04:56:04 PM	-196.67	SR	-0.2006	589	100.00	0.102	23.4

Figure S22. Experimental ORD of compound **2**.

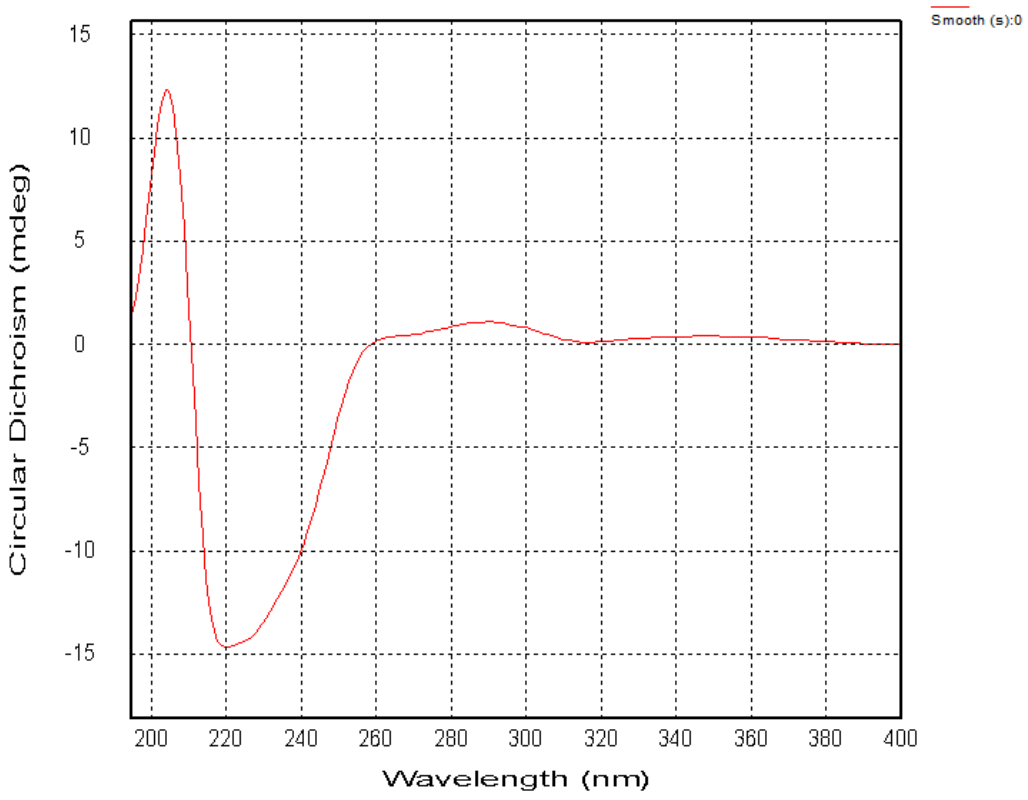
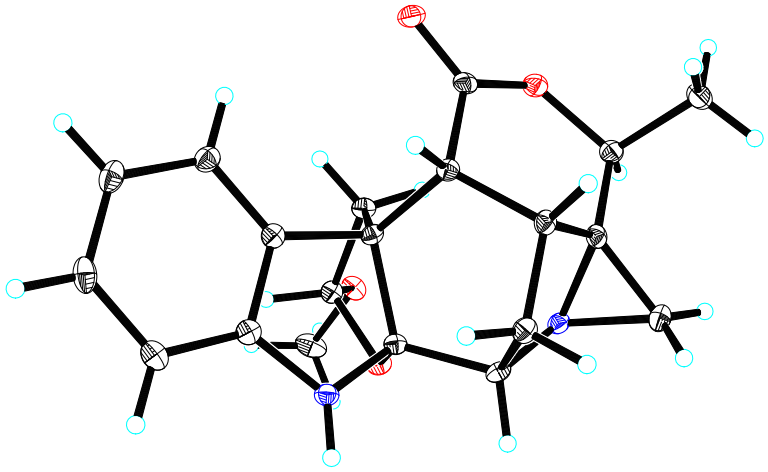


Figure S23. CD spectrum of compound **2** (0.1428mg/mL MeOH).

8. Crystal data and structure refinement for 1

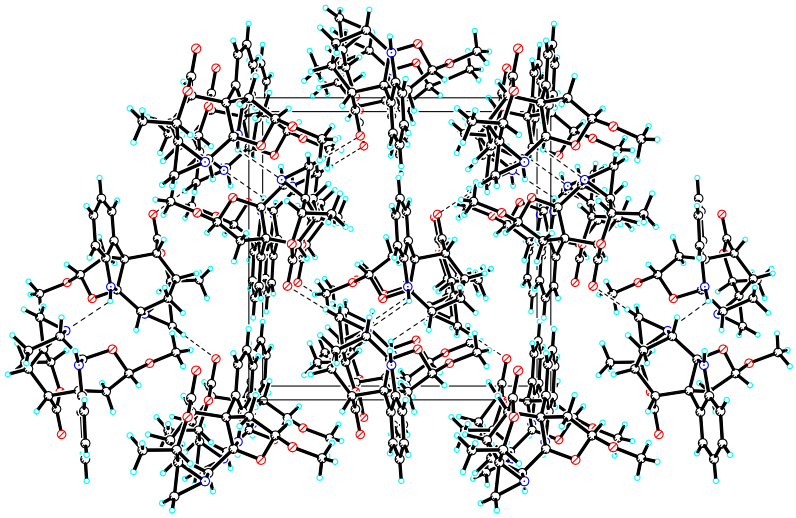
Crystal data for wasa29: $C_{20}H_{22}N_2O_4$, M = 354.39, a = 8.9449(2) Å, b = 13.8365(4)

Å, c = 13.8542(4) Å, α = 90°, β = 90°, γ = 90°, V = 1714.68(8) Å^3, T = 100.(2) K, space group $P212121$, Z = 4, μ(Cu Kα) = 0.787 mm^{-1}, 30048 reflections measured, 3373 independent reflections (R_{int} = 0.0388). The final R_1 values were 0.0308 ($I > 2\sigma(I)$). The final $wR(F^2)$ values were 0.0814 ($I > 2\sigma(I)$). The final R_1 values were 0.0309 (all data). The final $wR(F^2)$ values were 0.0815 (all data). The goodness of fit on F^2 was 1.054. Flack parameter = 0.02(4).



View of a molecule of wasa29 with the atom-labelling scheme.

Displacement ellipsoids are drawn at the 30% probability level.



View of the pack drawing of wasa29.

Hydrogen-bonds are shown as dashed lines.

Table 1. Crystal data and structure refinement for wasa29_0m.

Identification code	global	
Empirical formula	C20 H22 N2 O4	
Formula weight	354.39	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 8.9449(2) Å	α= 90°.
	b = 13.8365(4) Å	β= 90°.
	c = 13.8542(4) Å	γ = 90°.
Volume	1714.68(8) $Å^3$	
Z	4	
Density (calculated)	1.373 Mg/m^3	
Absorption coefficient	0.787 mm^{-1}	
F(000)	752	
Crystal size	0.520 x 0.480 x 0.380 mm^3	
Theta range for data collection	5.89 to 72.45°.	
Index ranges	-11<=h<=11, -17<=k<=17, -15<=l<=17	
Reflections collected	30048	
Independent reflections	3373 [R(int) = 0.0388]	
Completeness to theta = 72.45°	99.2 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.75 and 0.56	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3373 / 0 / 237	
Goodness-of-fit on F^2	1.054	
Final R indices [I>2sigma(I)]	R1 = 0.0308, wR2 = 0.0814	
R indices (all data)	R1 = 0.0309, wR2 = 0.0815	
Absolute structure parameter	0.02(4)	
Largest diff. peak and hole	0.340 and -0.399 $e.Å^{-3}$	