SUPPLEMENTARY MATERIAL

A new indole alkaloid with anti-inflammatory from the branches of *Nauclea officinalis*

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ABSTRACT

A new indole alkaloid, 17-oxo-19-(*Z*)-naucline, and six known alkaloids 2-7 were isolated from the branches of *Nauclea officinalis*. The structure of the new compound 1 was characterised mainly by analysing its physical data including IR, 1D, 2D NMR, and HR-MS. Other compounds were identified by comparisons their data with those reported in the literature. Compound1, 4, 5, 6, 7 showed *in vitro* anti-inflammatory activity decrease the LPS-stimulated production of nitric oxide in RAW264.7 cell, while all compounds exhibited weak cytotoxicity against human tumour cell lines (LOVO, A549 and HepG2).

Keywords: *Nauclea officinalis*; indole alkaloid; anti-inflammatory activity; cytotoxic activity

List of Supplementary Material

Table S1. ^{1}H and ^{13}C NMR spectral data for 17-oxo-19-(Z)-naucline in DMSO-d_{6}. At600/150 MHz.

Atom	1	
	δ_C	δ_H
1		11.83 (1H, s, N-H)
2	127.0	
3	142.0	
4		
5	40.1	4.29 (2H, t, *J* = 7.2Hz)
6	18.9	3.11 (2H, t, *J* = 6.6Hz)
7	116.3	
8	125.1	
9	120.1	7.65 (1H, d, *J* = 7.8 Hz)
10	120.1	7.12 (1H, t, *J* = 7.2 Hz)
11	124.9	7.30 (1H, t, *J* = 7.8 Hz)
12	112.1	7.48 (1H, d, *J* = 8.4Hz)
13	138.8	

14	93.8	7.02 (1H, s)
15	151.2	
16	106.5	
17	160.6	
18	14.0	1.99 (3H, d, *J* = 7.2 Hz)
19	131.2	6.68 (1H, q, *J* = 7.2 Hz)
20	127.4	
21	63.8	4.99 (2H, s)
22	158.4	

Table S2. ^{1}H and ^{13}C NMR spectral data for compounds 2-3 in DMSO-d_6. At400/100 MHz.

Atom	2		3	
	δ_C	δ_H	δ_C	δ_H
1				10.88 (1H, s)
2	135.2		133.9	
3	54.7	4.84 (1H,overlapped)	54.5	4.93 (1H, d, J = 10.7Hz)
4				
5	41.7	5.03 (1H, m)	40.9	2.94 (1H, m)
		2.87 (1H, m)		5.04 (1H, m)
6	22.3	2.72 (2H,overlapped)	22.3	2.71 (1H, m)
				2.77(1H, m)
7	107.9		109.7	
8	128.8		127.8	
9	102.2	6.79 (1H, d, *J* = 2.4 Hz)	117.9	7.42 (1H, d, *J* = 7.8 Hz)
10	150.9		120.2	7.01(1H, td, *J* = 7.6,0.8 Hz)
11	111.9	6.66 (1H,dd, *J* =8.4,2.2Hz)	121.7	7.07 (1H, td, *J* = 7.4,0.8 Hz)

12	112.1	7.13 (1H, d, *J* = 8.2Hz)	111.9	7.29 (1H, d, *J* = 8.0Hz)
13	132.1		138.6	
14	33.2	2.38 (dt, *J* =13.4, 3.6Hz)	32.1	1.45(1H, q, *J* = 13.2Hz)
		1.40 (dd,*J* =13.08, 25.6Hz)		2.46(1H, dt, *J* = 3.7,13Hz)
15	26.9	3.33 (1H, m)	26.8	2.93 (1H, m)
16	108.6		108.7	
17	149.4	7.45 (1H, d, *J* = 2.1 Hz)	148.8	7.46 (1H, d, *J* = 2.4 Hz)
				5.19 (1H, dd, *J* = 10.0,2.6 Hz)
18	120.6	5.17 (1H, dd, *J*=9.6,1.6Hz)	120.5	5.28 (1H, dd, *J* = 12.0,2.6Hz)
		5.27 (1H, dd, *J*=1.6, 16.8Hz)		
19	132.9	5.52 (1H, m)	134.1	5.55 (1H, m)
20	44.7	2.69 (1H, m)	43.7	2.72 (1H, m)
21	96.9	5.49 (1H, d, *J*=1.6)	96.9	5.49 (1H, d, *J* = 1.7Hz)
22	165.6		165.2	
1’	99.6	4.71 (1H, d, *J*=8.1Hz)	99.4	4.70(1H, d, *J* = 7.9Hz)
2’	73.8		73.8	
3’	77.6		78.2	
4’	72.1		71.3	
5’	78.5		77.9	
6’	61.9		62.3	

Table S3. ^{1}H and ^{13}C NMR spectral data for compounds5-6 in DMSO-d_6. At400/100 MHz.

Atom	6		5	
	δ_C	δ_H	δ_C	δ_H
1		11.80 (1H, s, N-H)		11.93 (1H, s)
2	128.4		127.0	

3	136.0		136.3	
5	40.3	4.37 (2H, t, *J* = 6.4Hz)	41.7	4.39 (2H, t, *J*=6.7Hz)
6	19.2	3.11 (2H, t, *J* = 6.4Hz)	19.6	3.11 (2H, t, *J*=6.7Hz)
7	114.5		113.9	
8	126.2		124.9	
9	119.5	7.66 (1H, d, *J* = 7.8Hz)	119.4	7.64 (1H, d, *J*=7.6Hz)
10	119.8	7.11(1H, t, *J* = 7.5 Hz)	119.6	7.11 (1H, t, *J* =7.3Hz)
11	125.2	7.28 (1H, t, *J* = 7.5 Hz)	123.9	7.25 (1H, td, *J* =14.1, 7.0Hz)
12	111.3	7.45 (1H, d, *J* = 7.8Hz)	111.3	7.49 (1H, d, *J*=7.6Hz)
13	139.4		138.6	
14	95.7	7.02 (1H, s)	92.9	7.34 (1H, s)
15	142.3		139.0	
16	119.3		126.6	
17	161.0		149.7	9.28 (1H, s)
18	118.9	7.50 (1H, d, *J* = 5.4Hz)	118.9	6.07 (1H,d, *J*=17.5Hz)
				5.65 (1H,d, *J*=10.4Hz)
19	150.5	8.68 (1H, d, *J* = 5.3 Hz)	130.0	7.29 (1H, dd, *J*=17.5 10.8Hz)
20			118.8	
21	150.9	9.36(1H, s)	147.8	8.88 (1H, s)
22			161.0	

Table S4. ^{1}H and ^{13}C NMR spectral data for compounds4,7 in DMSO-d_6. At400/100 MHz.

Atom	4		Atom	7	
	δ_C	δ_H		δ_C	δ_H
1		11.87 (1H, s)	1	162.7	
2	127.7		2	41.0	7.05 (1H, brs)
3	136.4		3	18.7	4.38 (2H, t, *J* =7.2Hz)

4			4	115.8	3.13 (2H, t, *J* =7.2Hz)
5	40.2	4.40 (2H, m)	4a	124.8	
6	19.1	3.11 (2H, t, *J*=6Hz)	4b	120.0	
			5	119.8	7.63 (1H, d, *J* =8.4Hz)
7	114.5		6	124.9	7.09 (1H, t, *J* =7.2Hz)
8	125.3		7	112.2	7.26 (1H, t, *J* =7.2Hz)
9	119.6	7.62 (1H, d, *J*=7.8Hz)	8	138.9	7.45 (1H, d, *J* =8.4Hz)
10	119.8	7.09 (1H, t, *J* =7.2Hz)	8a		
11	124.3	7.26 (1H, t, *J* =7.2Hz)	9		12.18 (1H, brs)
12	111.8	7.47 (1H, d, *J*=7.8Hz)	9a	126.7	
13	138.3				
14	93.8	7.24 (1H, s)			
15	138.4				
16	118.8				
17	149.1	9.24 (1H, s)			
18	25.2	1.51 (3H, d, *J*=6.6Hz)			
19	63.8	5.32(1H, m)			
20	134.8				
21	147.5	8.77 (1H, s)			
22	161.1				

Table S5 *In vitro* cytotoxicities of compounds1-7.

compound	IC_{50}[a] (μM)		
	A549	HepG2	LOVO
1	54.8±1.14	30.8±1.87	40.5±2.22
2	>100	>100	>100
3	>100	>100	>100

4	>100	36.50±2.62	31.22±1.52
5	94.99±1.53	73.77±2.45	91.80±1.52
6	>100	74.48±1.53	67.33±1.78
7	30.76±2.2	35.16±2.51	44.01±1.85
cisplatin[b]	0.34±0.17	0.99±0.28	0.38±0.21

[a] Means ± S.D. From three independent experiments (n=3)

[b] Positive control

Table S6 *In vitro* inflammatory activity of compounds1-7.

compound	IC_{50}[a] (μM)
	RAW264.7
1	4.61±1.12 μM
2	25.55±1.13 μM
3	32.84±1.18 μM
4	3.76±0.94 μM
5	2.85±0.67 μM
6	1.87±0.58 μM
7	4.66±0.85 μM
Hydrocortisone[b]	3.76 ±0.49 μM.

[a] Means ± S.D. From three independent experiments (n=3)

[b] Positive control

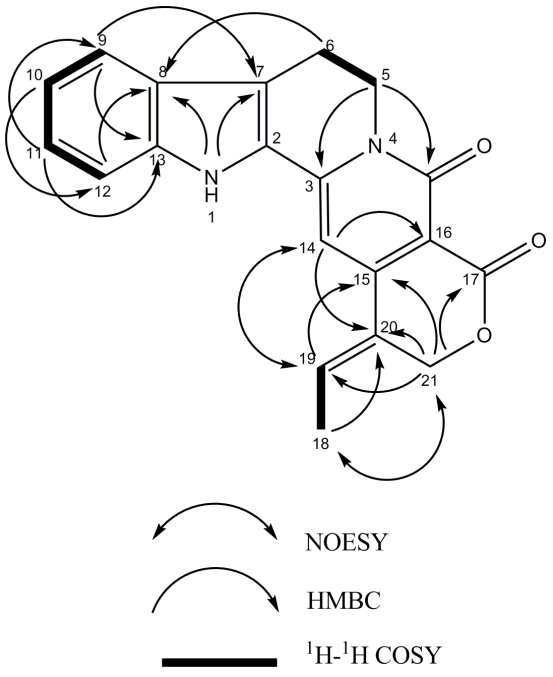


Figure S1. Selected 2D NMR correlations for17-oxo-19-(Z)-naucline.

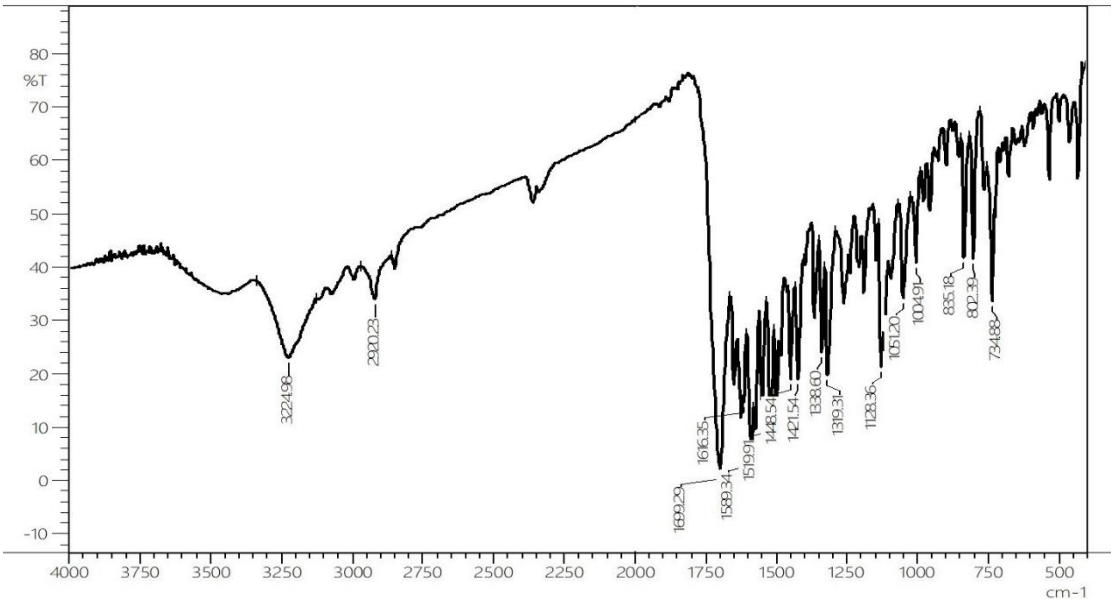


Figure S2. IR spectrum of 17-oxo-19-(*Z*)-naucline.

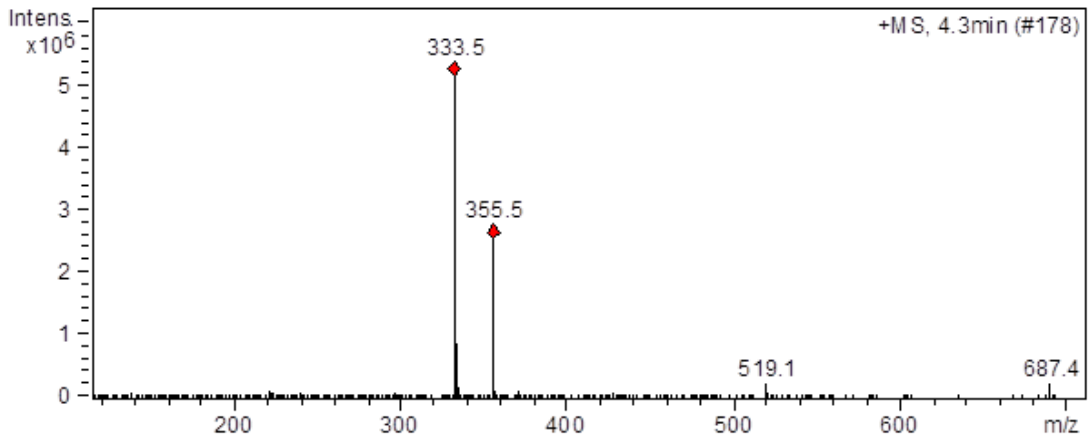
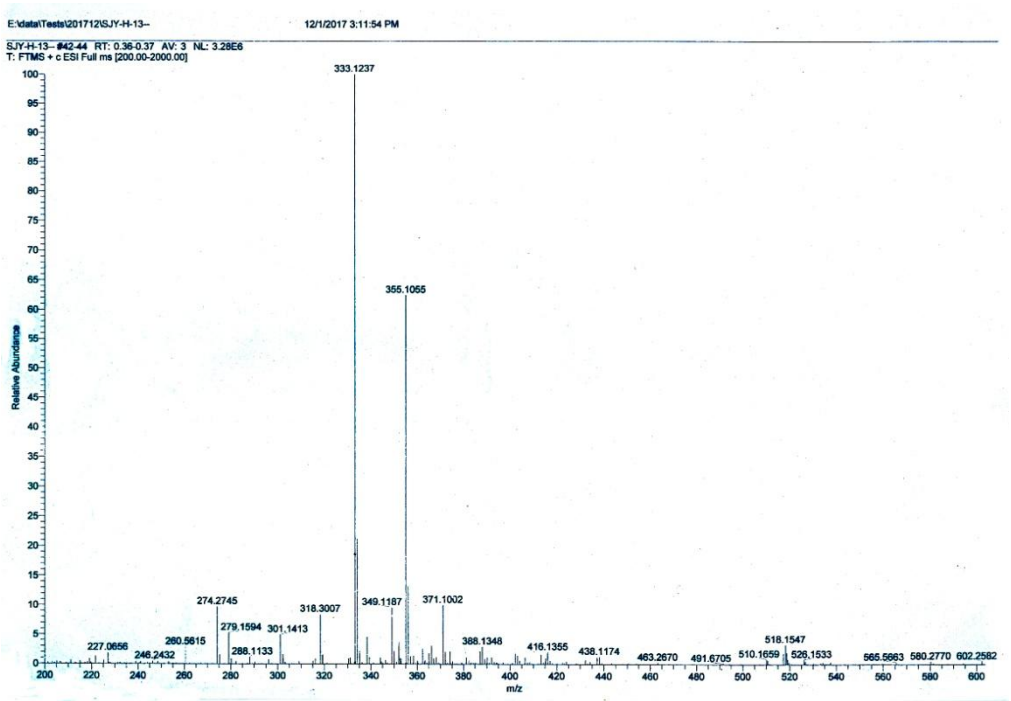


Figure S3. ESI-MS spectrum of 17-oxo-19-(*Z*)-naucline



H-13 m/z	Theo. Mass	Delta (ppm)	RDB	Composition
333.1237	333.1234	1.08	13.5	[12]C20 H17 O3 N2

Figure S4. HR-ESI-MS spectrum of 17-oxo-19-(Z)-naucline .

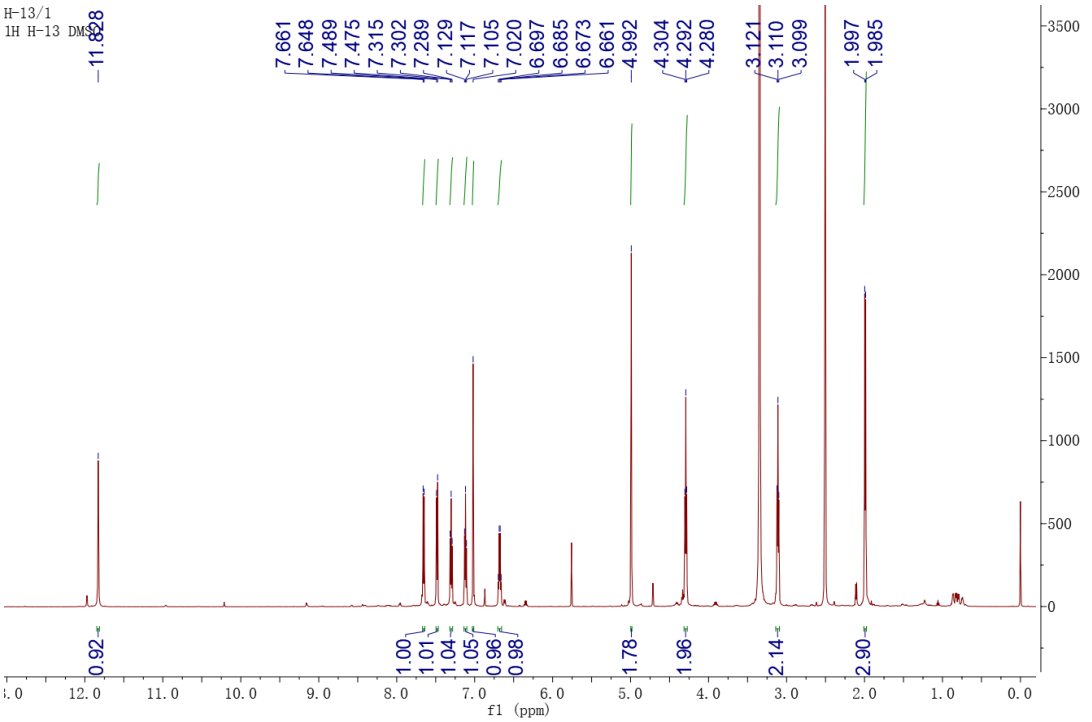


Figure S5. ^{1}H NMR spectrum of 17-oxo-19-(*Z*)-naucline in DMSO-d_6 (600MHz)

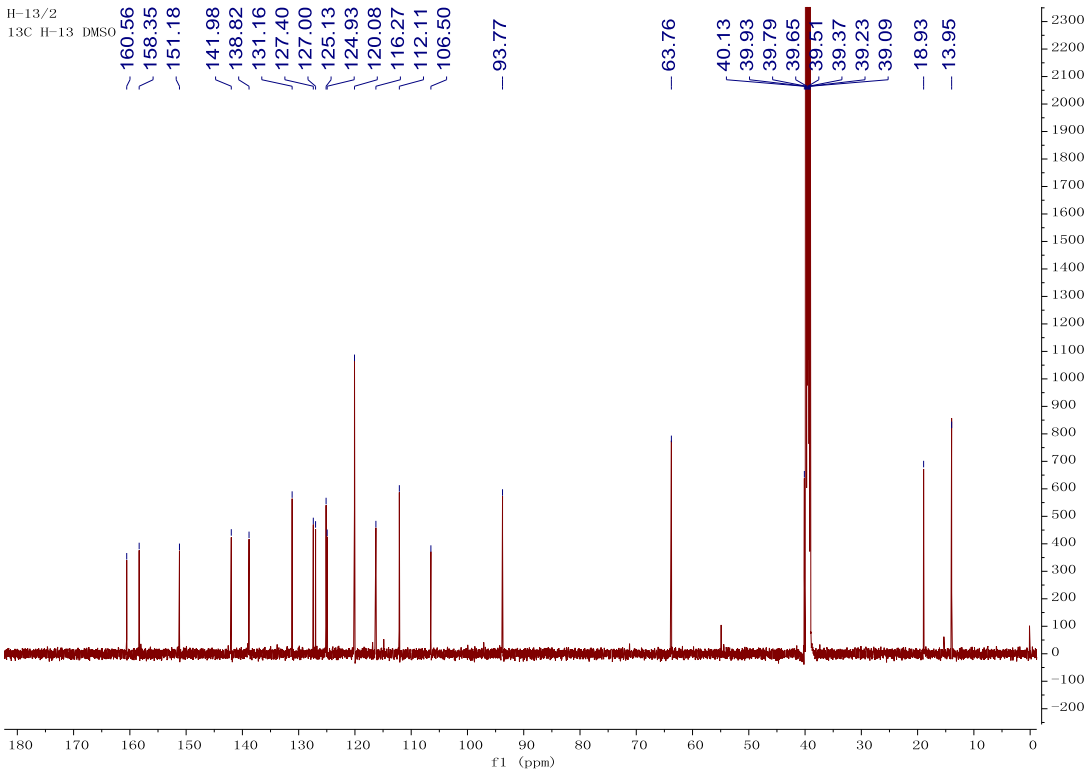


Figure S6. ^{13}C NMR spectrum of 17-oxo-19-(*Z*)-naucline in DMSO-d_6 (150MHz)

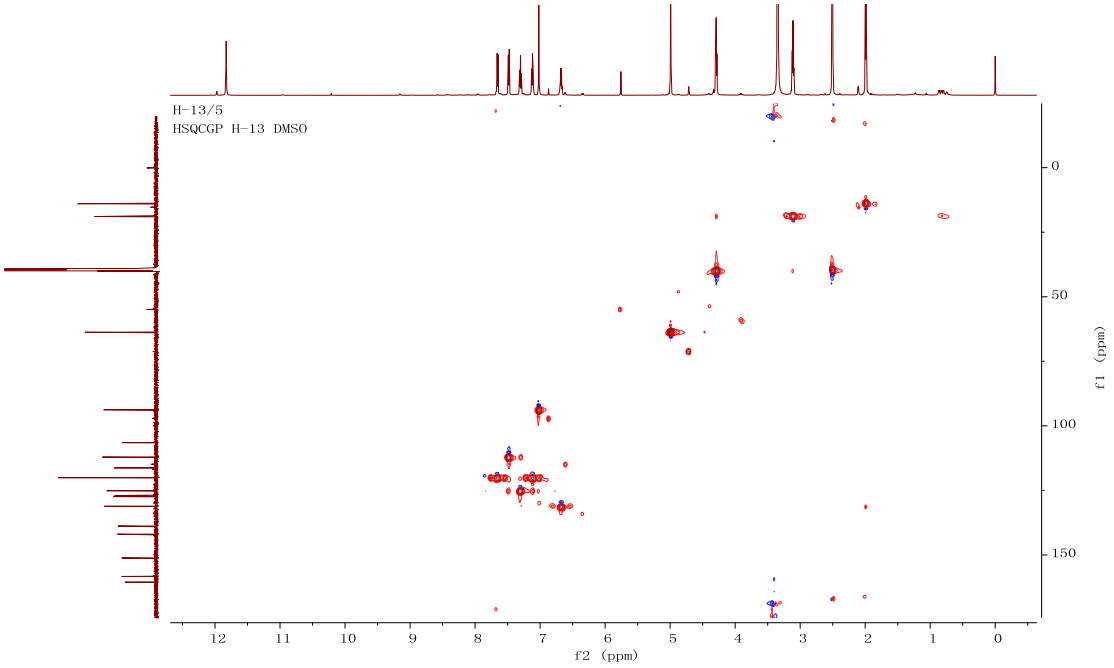


Figure S7. HSQC spectrum of 17-oxo-19-(*Z*)-naucline in DMSO-d_6 (600MHz)

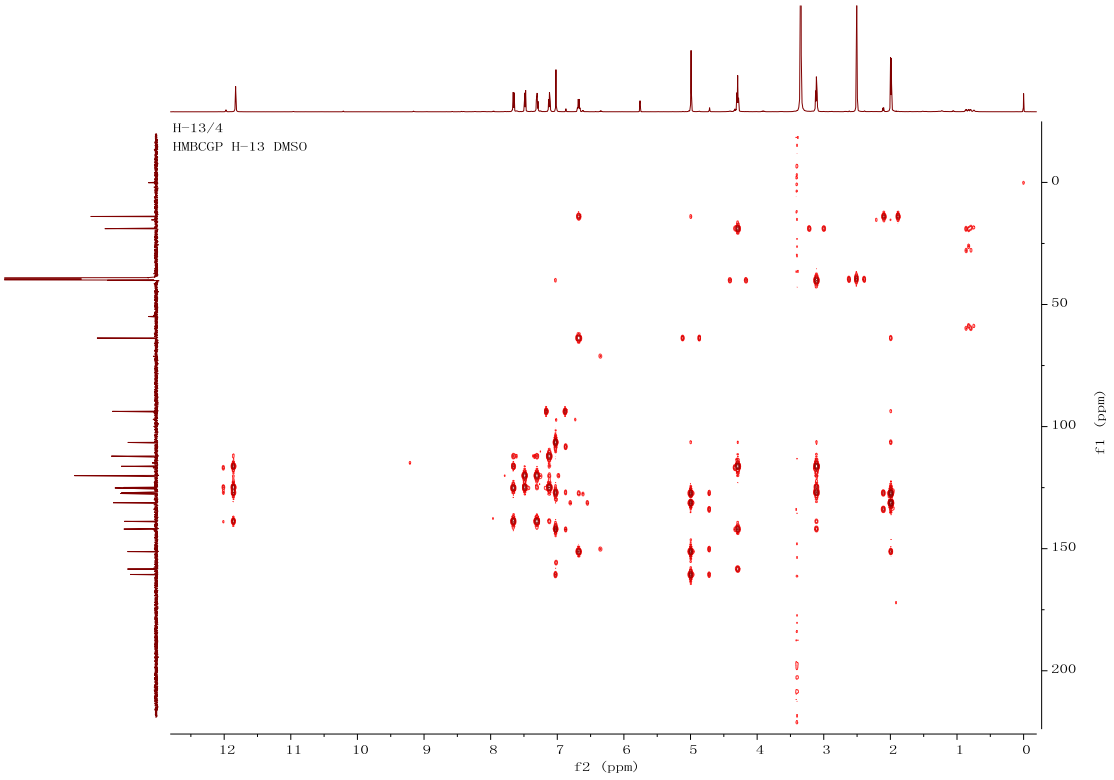


Figure S8. HMBC spectrum of 17-oxo-19-(*Z*)-naucline in DMSO-d_6 (600MHz)

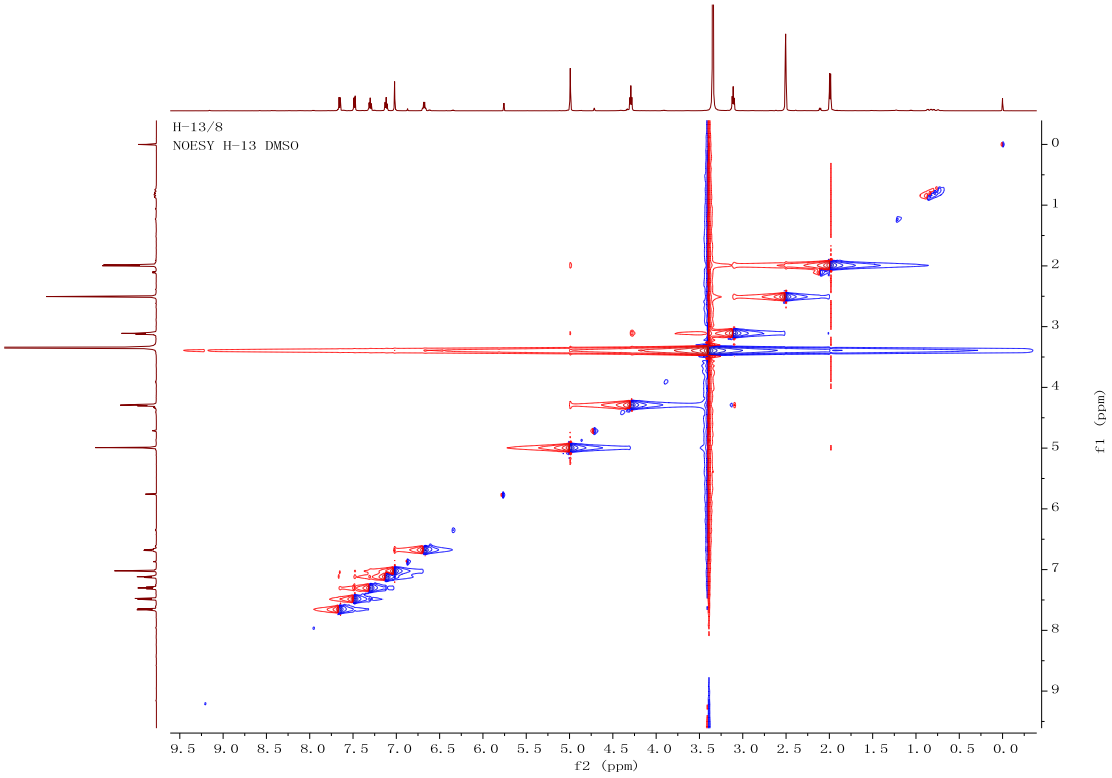


Figure S9. NOESY spectrum of 17-oxo-19-(*Z*)-naucline in DMSO-d_6 (600MHz)

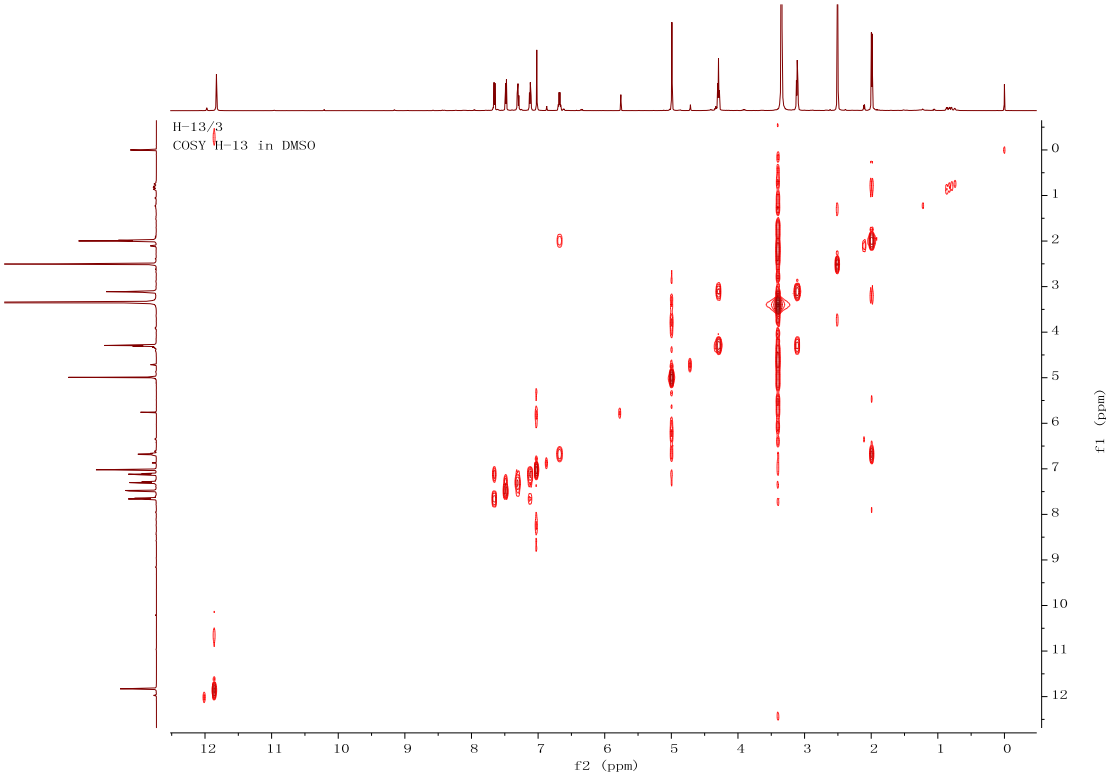


Figure S10. ^{1}H-^{1}H COSY spectrum of 17-oxo-19-(*Z*)-naucline in DMSO-d_6 (600 MHz)

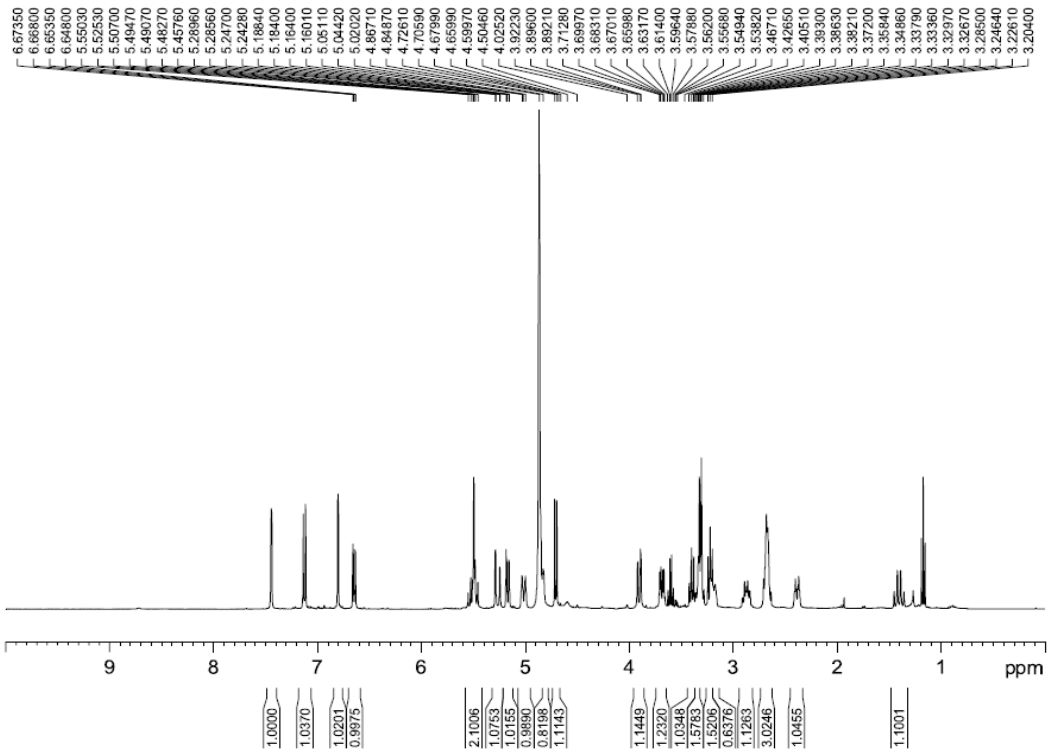


Figure S11. ^{1}H NMR spectrum of compound 2 in DMSO-d_6 (400MHz)

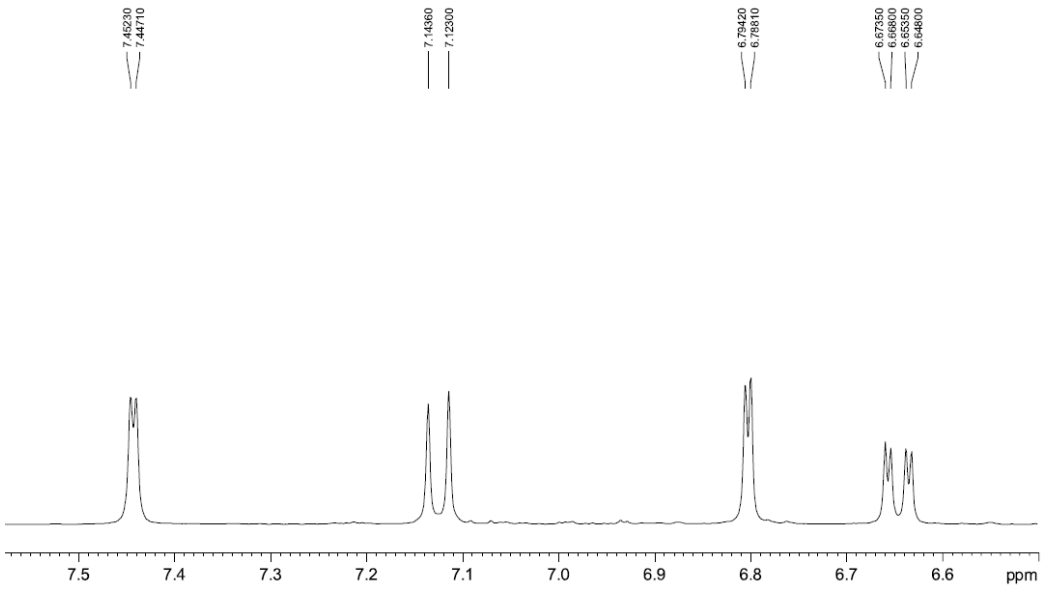


Figure S11-1. ^{1}H NMR spectrum of compound 2 in DMSO-d_6 (400MHz)

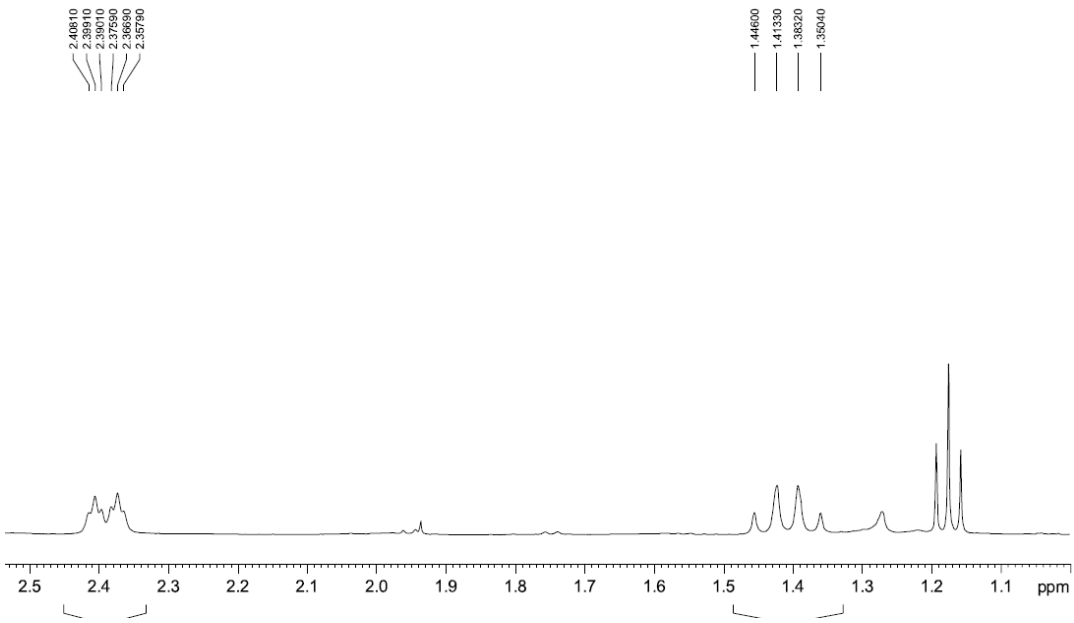


Figure S11-2. ^{1}H NMR spectrum of compound 2 in DMSO-d_6 (400MHz)

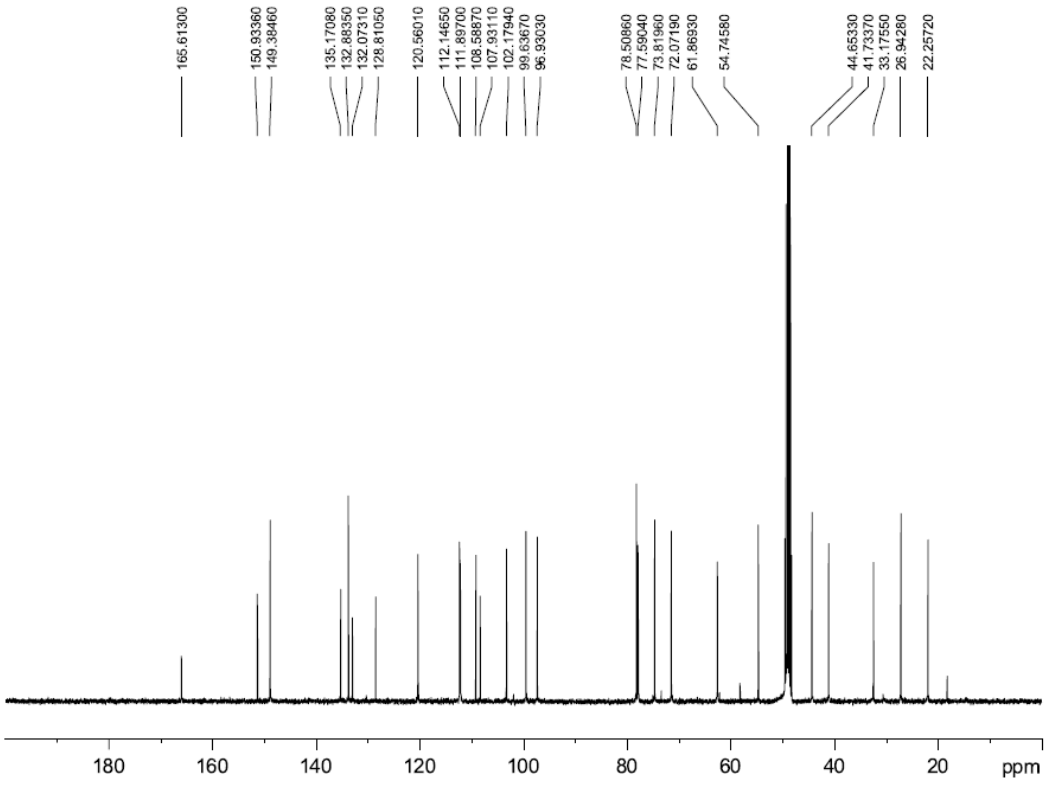


Figure S12. ^{13}C NMR spectrum of compound 2 in DMSO-d_6 (100MHz)

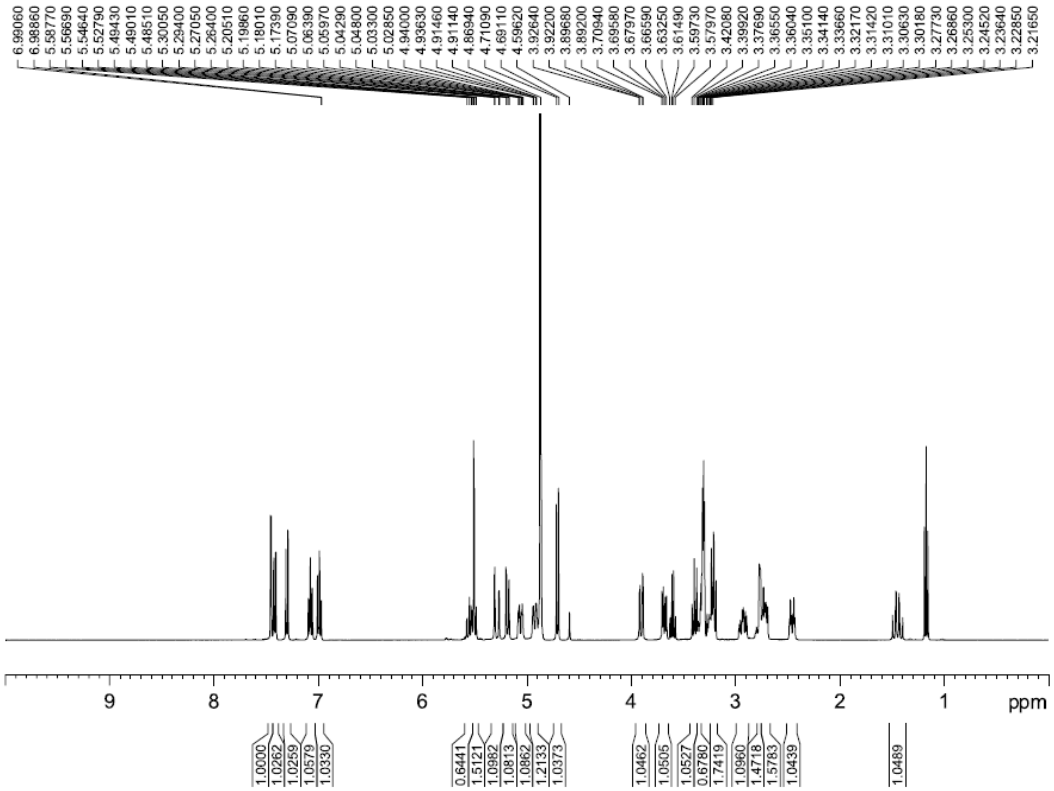


Figure S13. ^{1}H NMR spectrum of compound 3 in DMSO-d_6 (400MHz)

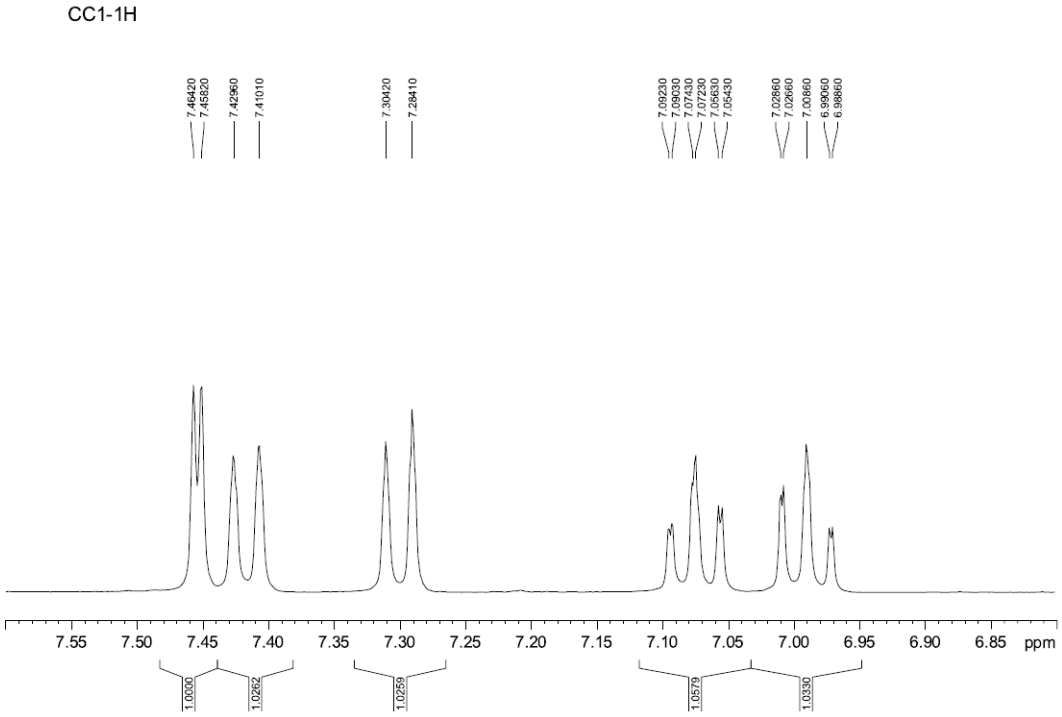


Figure S13-1. ^{1}H NMR spectrum of compound 3 in DMSO-d_6 (400MHz)

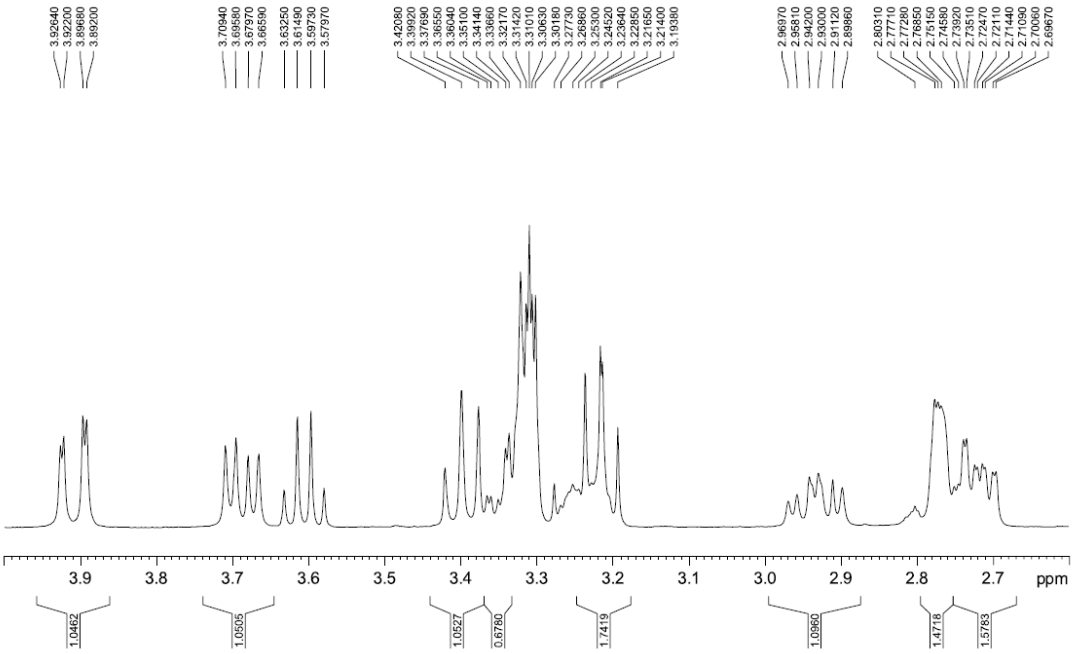


Figure S13-2. ^{1}H NMR spectrum of compound 3 in DMSO-d_6 (400MHz)

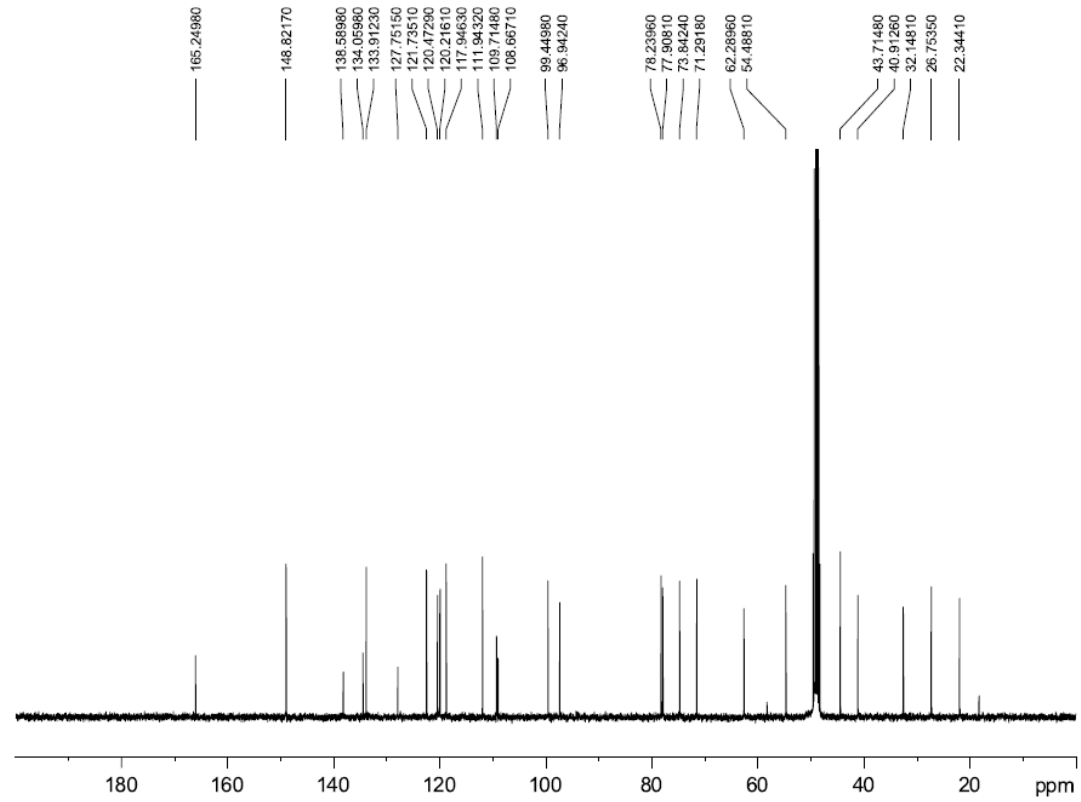


Figure S14. ^{13}C NMR spectrum of compound 3 in DMSO-d_6 (100MHz)

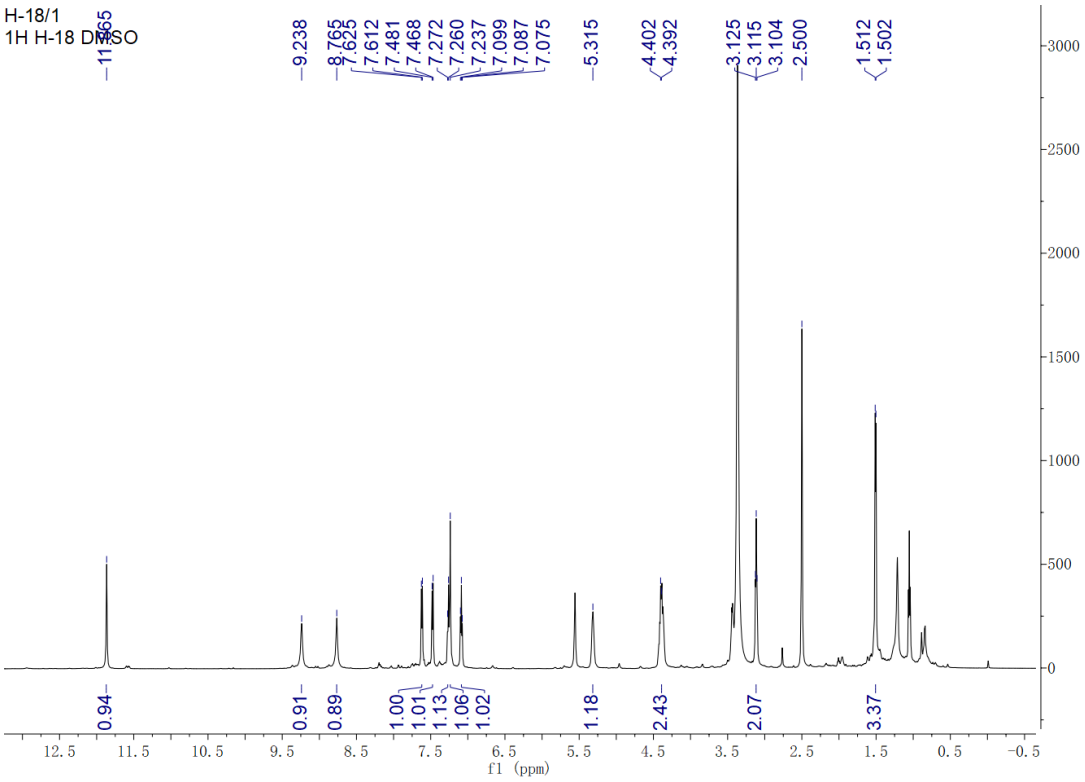


Figure S15. ^{1}H NMR spectrum of compound 4 in DMSO-d_6 (600MHz)

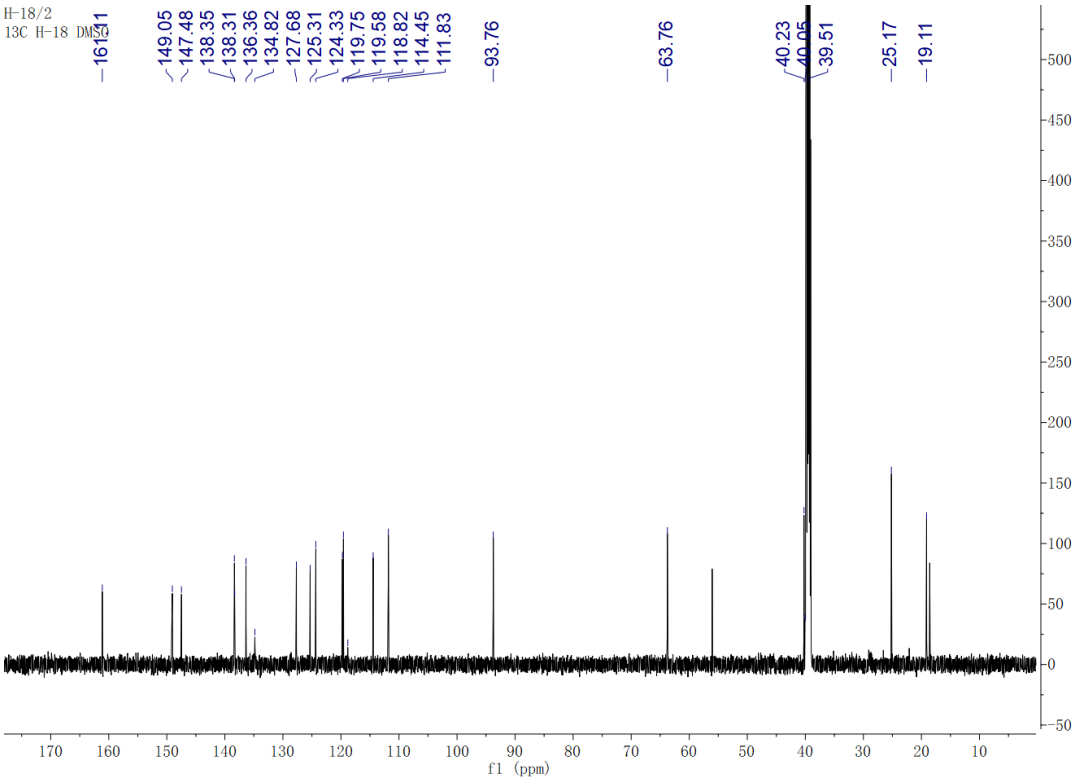


Figure S16. ^{13}C NMR spectrum of compound 4 in DMSO-d_6 (150MHz)

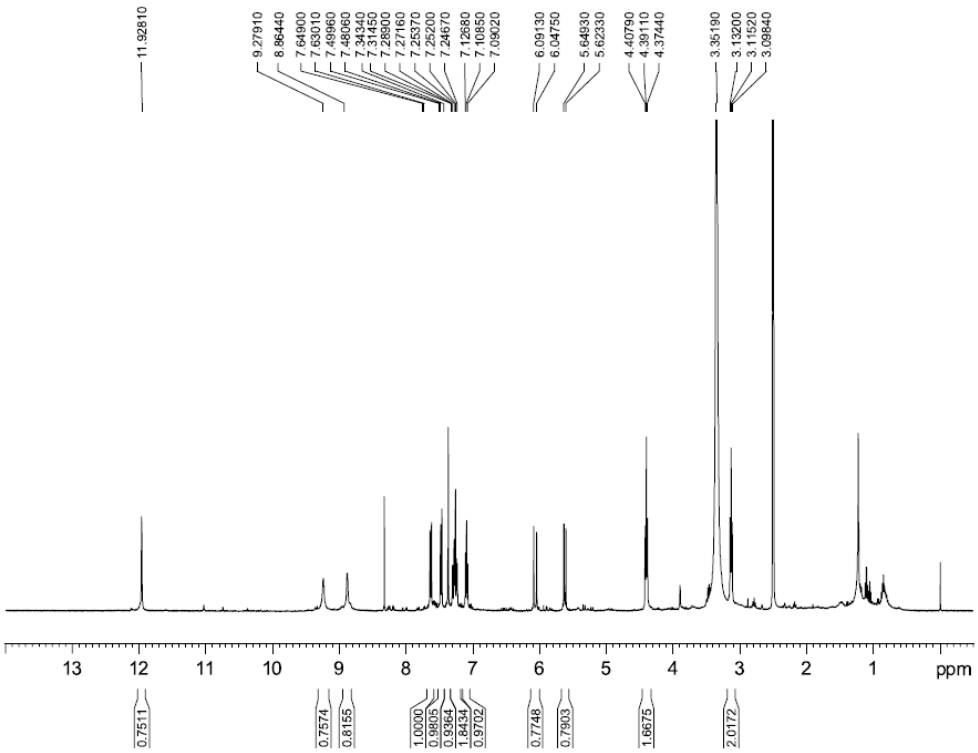


Figure S17. ^{1}H NMR spectrum of compound 5 in DMSO-d_6 (400MHz)

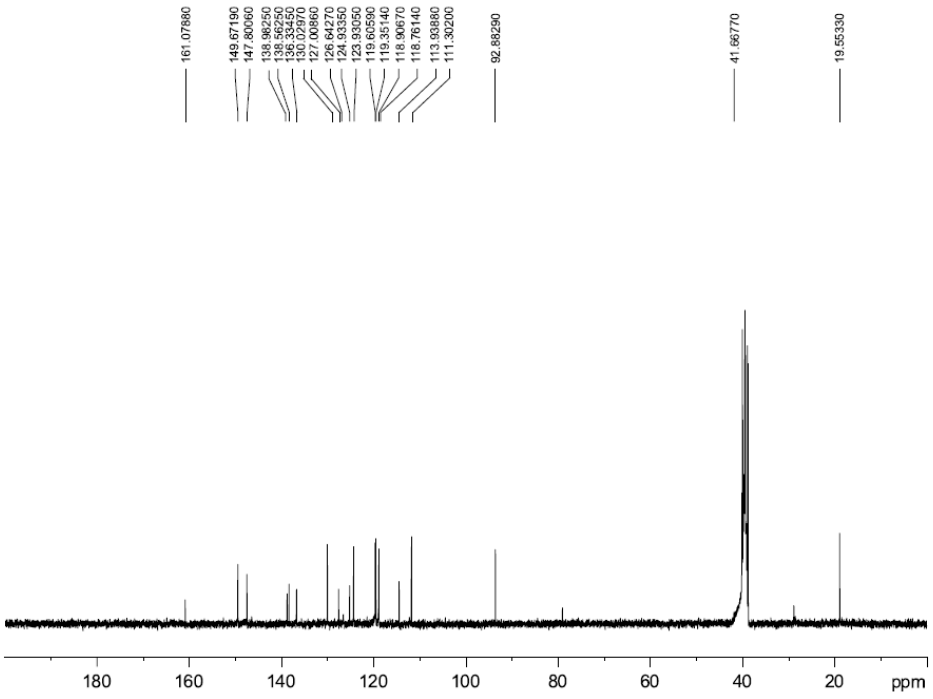


Figure S18. ^{13}C NMR spectrum of compound 5 in DMSO-d_6 (100MHz)

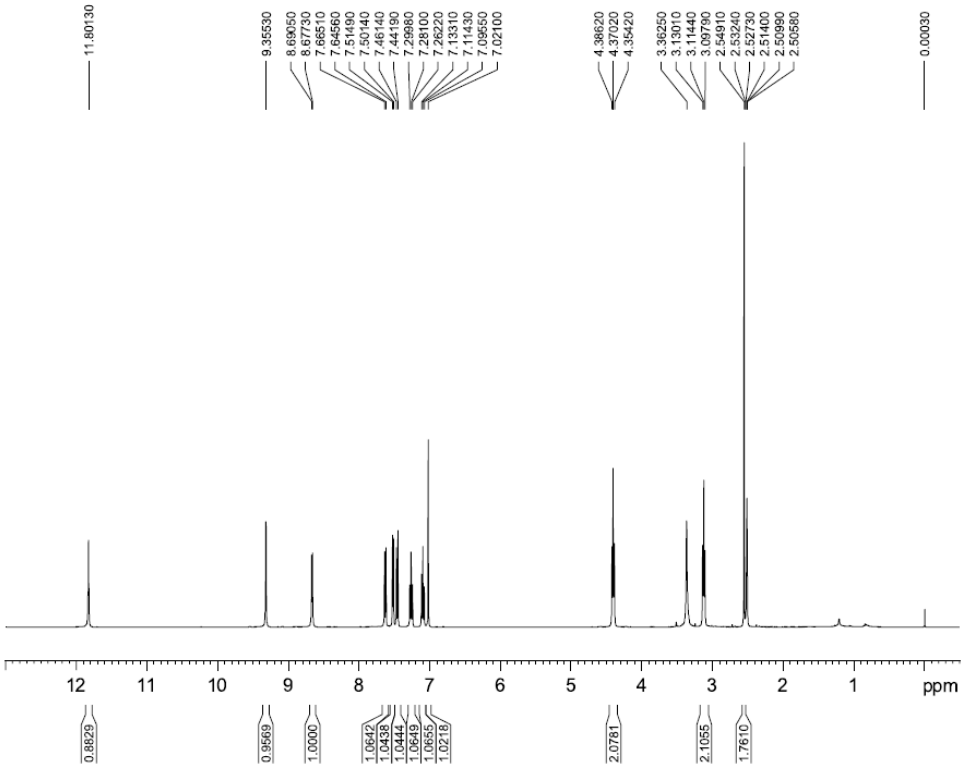


Figure S19. ^{1}H NMR spectrum of compound 6 in DMSO-d_6 (400MHz)

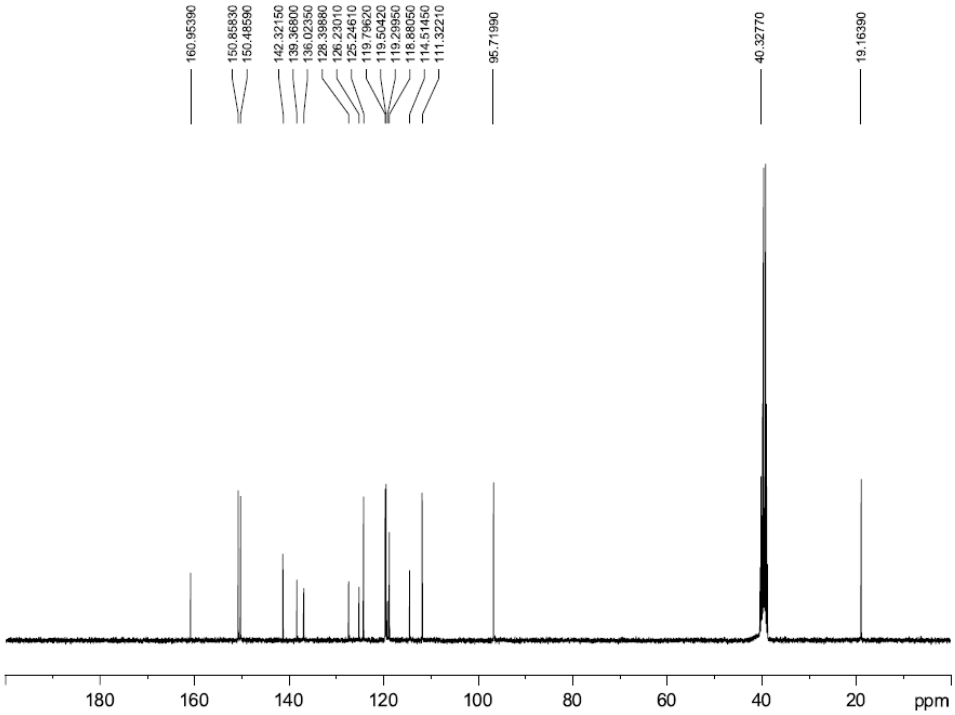


Figure S20. ^{13}C NMR spectrum of compound 6 in DMSO-d_6 (100MHz)

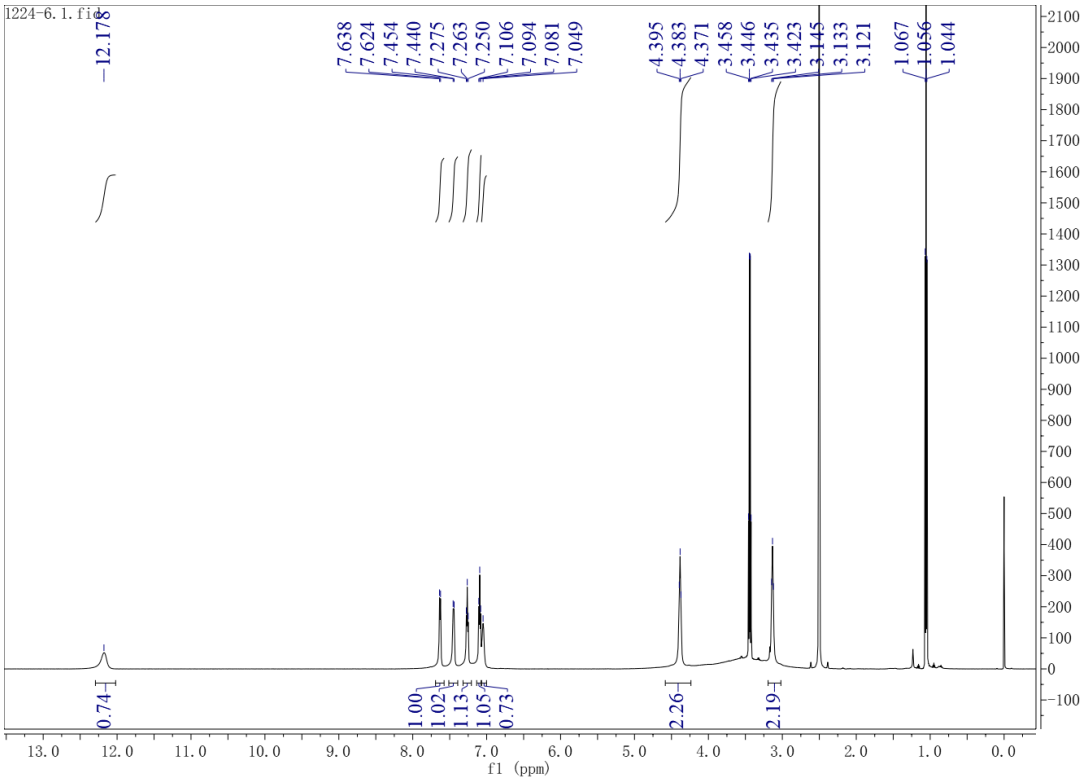


Figure S21. ^{1}H NMR spectrum of compound 7 in DMSO-d_6 (600MHz)

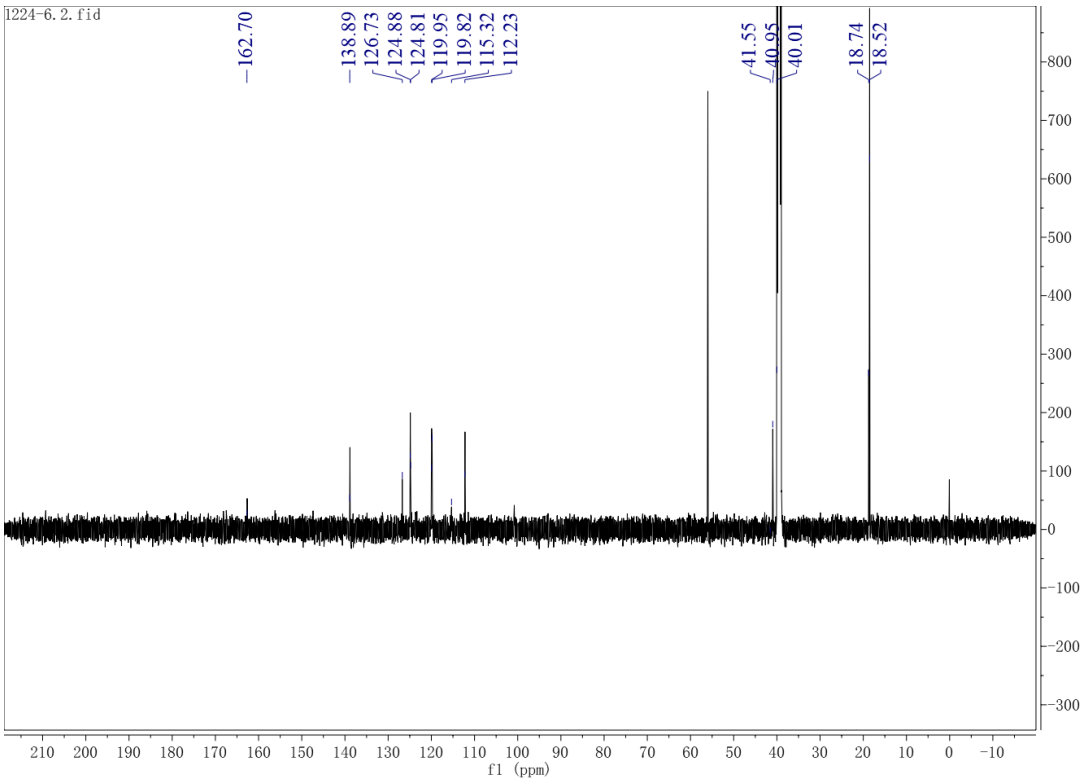
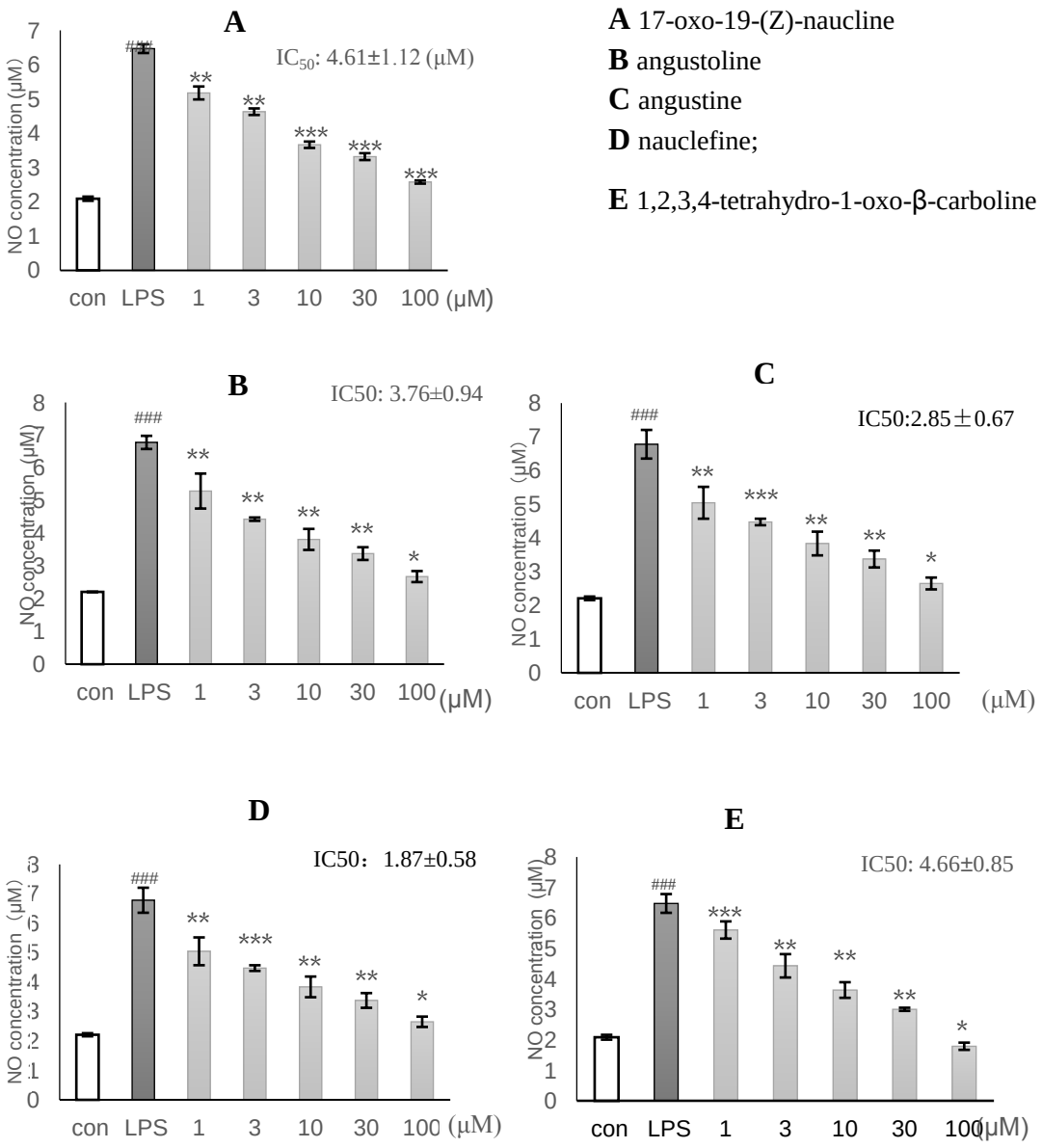


Figure S22. ^{13}C NMR spectrum of compound 7 in DMSO-d_6 (150MHz)



A 17-oxo-19-(Z)-naucline
B angustoline
C angustine
D nauclefine;
E 1,2,3,4-tetrahydro-1-oxo-β-carboline

Figure S23. Effects of compounds (**A**, 17-oxo-19-(Z)-naucline; **B**, angustoline; **C**, angustine; **D**, nauclefine; **E**, 1,2,3,4-tetrahydro-1-oxo-β-carboline) on NO production in LPS stimulated RAW264.7 macrophage cells. Comparing with LPS group, *p<0.05, **p<0.01, ***p<0.001; comparing with normal group, $^{###}p$<0.001. Data were presented as Mean ± SD, n = 3.