Electronic Supplementary Information (ESI)

Antiviral activity and mechanism of gossypols: the $O_2^{\cdot-}$ production rate is one fact, the chirality maybe the other

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Contents:

Data for key compounds

(-)-Gossypol ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.21 (s, 2H), 11.13 (s, 2H), 7.78 (s, 2H), 7.24 (s, 2H), 6.43 (s, 2H), 5.71 (s, 2H), 3.89 (s, 2H), 2.16 (s, 6H), 1.53 (d, J=6.8Hz, 12H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.4, 156.1, 150.5, 143.4, 134.1, 133.7, 129.7, 118.1, 115.9, 114.7, 111.8, 27.9, 20.4, 20.2.; $[\alpha]_D^{28}$ = -359.5 (*c* 0.26, $CHCl_3$). Chiral HPLC analysis: retention time: 4.87 min (major), 5.96 min (minor); ee: 97%, [analytical column using MeOH–2% phosphoric acid aqueous solutions= 90:5 as an eluent (1 ml/min), detected at 254 nm.].

(+)-Gossypol ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.21 (s, 2H), 11.13 (s, 2H), 7.78 (s, 2H), 7.24 (s, 2H), 6.43 (s, 2H), 5.71 (s, 2H), 3.89 (s, 2H)2.16 (s, 6H), 1.53 (d, J=6.8Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.4, 156.1, 150.5, 143.4, 134.1, 133.7, 129.7, 118.1, 115.9, 114.7, 111.8, 27.9, 20.4, 20.2.; $[\alpha]_D^{32}$ = +363.5 (*c* 0.105, $CHCl_3$), Chiral HPLC analysis: time: 4.91 min (minor), 6.09 min (major); ee: 99% [analytical column using MeOH–2% phosphoric acid aqueous solutions= 90:5 as an eluent (1 ml/min), detected at 254 nm.].

Compound 1 ^{1}H NMR (400 MHz, $DMSO_{-d6}$) δ 15.38 (d, J = 8.4 Hz, 2H), 10.38 (d, J = 8.4 Hz, 2H), 8.79 (s, 2H), 8.35 (s, 2H), 7.81 (d, J = 7.6 Hz, 2H), 7.75 (t, J = 7.2 Hz, 2H), 7.57 (J = 8.4 Hz, 2H), 7.42 (t, J = 7.6 Hz, 2H), 3.81-3.74 (m, 2H), 2.01 (s, 6H), 1.45-1.40 (m, 12H). ^{13}C NMR (100 MHz, $DMSO_{-d6}$) δ 171.4, 157.2, 151.0, 147.8, 146.2, 140.4, 134.9, 133.6, 131.0, 128.8, 127.3, 126.1, 125.7, 123.0, 121.7, 119.7, 117.6, 115.8, 108.1, 27.1, 20.7. HRMS(ESI) m/z calcd for $C_{44}H_{37}F_6N_2O_6$ $(M-H)^-$ 803.2561, found 803.2541. $[\alpha]_D^{25}$ = -1436.5 (*c* 0.105, CH_3OH).

Compound 2 ^{1}H NMR (400 MHz, $DMSO_{-d6}$) δ 15.38 (d, J=8.4 Hz, 2H), 10.38 (d, J=8.4 Hz, 2H), 8.78 (s, 2H), 8.35 (s, 2H), 7.82 (d, J=7.6Hz, 2H), 7.74 (t, J=7.2Hz, 2H), 7.57 (s, 2H), 7.42 (t, J=7.6Hz, 2H), 3.81-3.74 (m, 2H), 2.01 (s, 6H), 1.47-1.42 (m, 12H).^{13}C NMR (100 MHz, $DMSO_{-d6}$) δ 171.4, 157.2, 151.0, 146.2, 140.4, 135.0, 133.6, 131.0, 128.8, 127.3, 126.1, 125.7, 123.0, 121.7, 119.7, 119.3, 117.6, 115.8, 108.0, 27.1, 20.6. HRMS(ESI) m/z calcd for $C_{44}H_{37}F_6N_2O_6$ $(M-H)^-$ 803.2561, found 803.2537. $[\alpha]_D^{23}$ = +1430.5 (*c* 0.105, CH_3OH).

Compound 3 ^{1}H NMR (400 MHz, $DMSO_{-d6}$) δ 13.29 (d, J=12 Hz, 2H), 9.76 (d, J=12 Hz, 2H), 8.41 (s, 2H), 7.84 (s, 2H), 7.44 (s, 2H), 3.72-3.68 (m, 2H), 3.52-3.45 (m, 4H), 1.93 (s, 6H), 1.66-1.63 (m, 4H), 1.45-1.42 (m, 12H), 0.96-0.92 (m, 6H). ^{13}C NMR (100 MHz, $DMSO_{-d6}$) δ 172.1, 163.0, 150.1, 146.7, 131.6, 127.3, 126.8, 120.6, 117.0, 116.4, 103.5, 52.0, 27.0, 23.8, 20.8, 20.8, 20.7, 11.4. HRMS(ESI) m/z calcd for $C_{36}H_{43}N_2O_6$ $(M-H)^-$ 599.3127, found 599.3117. $[\alpha]_D^{25}$ = -698.4 (*c* 0.105, CH_3OH).

Compound 4 ^{1}H NMR (400 MHz, $DMSO_{-d6}$) 13.29 (d, J=12.8 Hz, 2H), 9.77 (d, J=12.8 Hz, 2H), 8.41 (s, 2H), 7.84 (s, 2H), 7.44 (s, 2H), 3.72-3.68 (m, 2H), 3.51-3.47 (m, 4H), 1.93 (s, 6H), 1.66-1.62 (m, 4H), 1.45-1.42 (m,12H), 0.95-

0.91 (m, 6H). ^{13}C NMR (100 MHz, DMSO$_{-d6}$) δ 171.5, 162.4, 149.5, 146.2, 131.1, 126.7, 126.3, 120.1, 116.4, 115.8, 103.1, 51.5, 26.5, 23.3, 20.3, 20.3, 20.2, 10.8. HRMS(ESI) m/z calcd for $C_{36}H_{43}N_2O_6$ $(M-H)^-$ 599.3127, found 599.3103. $[\alpha]_D^{23}$ = +671.2 (*c* 0.105, CH_3OH).

Compound 5 ^{1}H NMR (400 MHz, DMSO$_{-d6}$) δ 13.37 (s, 2H), 9.68 (d, J=8Hz, 2H), 8.42 (s, 2H), 7.43 (s, 2H), 4.22(s, 4H), 3.71-3.64 (m, 2H), 1.93 (s, 6H), 1.44 (s, 12H) ^{13}C NMR (100 MHz, DMSO$_{-d6}$) δ 172.3, 162.8, 149.9, 146.3, 131.3, 127.0, 126.7, 120.2, 116.4, 115.8, 103.7, 65.6, 26.5, 20.2. HRMS(ESI) m/z calcd for $C_{32}H_{35}N_2O_{12}S_2$ $(M-2Na+H)^-$ 703.1637, found 703.1607. $[\alpha]_D^{25}$ = -385.7 (*c* 0.112, CH_3OH).

Compound 6 ^{1}H NMR (400 MHz, DMSO$_{-d6}$) δ 13.38 (d, J=12Hz, 2H), 9.69(d, J=12Hz, 2H), 8.44 (s, 2H), 7.43(s, 2H), 4.20 (s, 4H), 3.73-3.68 (m, 2H), 1.92 (s, 6H), 1.43 (s, 12H). ^{13}C NMR (100 MHz, DMSO$_{-d6}$) δ 172.3, 162.8, 149.8, 146.3, 137.6, 131.3, 127.0, 126.7, 120.1, 116.5, 115.8, 103.7, 65.5, 26.5, 20.3. HRMS(ESI) m/z calcd for $C_{32}H_{35}N_2O_{12}S_2$ $(M-2Na+H)^-$ 703.1637, found 703.1620. $[\alpha]_D^{25}$ = +383.6 (*c* 0.112, CH_3OH).

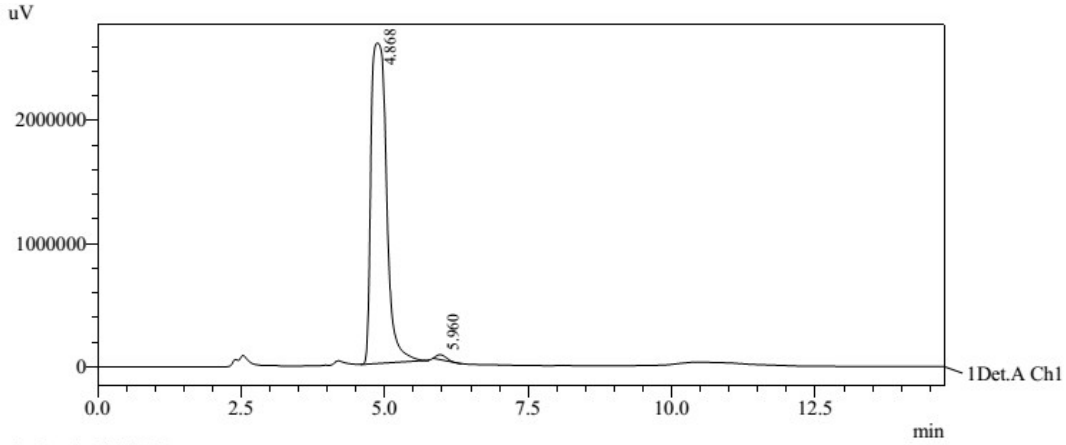
Compound 7 ^{1}H NMR (400 MHz, DMSO$_{-d6}$)δ 13.03 (d, J=9.6Hz, 2H), 9.76 (d, J=9.6Hz, 2H), 8.54 (s, 2H), 7.42 (s, 2H), 3.78 -3.77 (m, 4H), 3.71-3.69 (m, 2H), 2.80-2.84 (m, 4H), 1.93 (s, 6H), 1.44 (s, 12H). ^{13}C NMR (100 MHz, DMSO$_{-d6}$) δ 172.1, 162.2, 150.0, 147.1, 131.5, 127.4, 127.0, 120.7, 116.9, 116.6, 103.2, 50.9, 47.6, 26.5, 20.3.. HRMS(ESI) m/z calcd for $C_{34}H_{39}N_2O_{12}S_2$ $(M-2Na+H)^-$ 731.1950, found 731.1915. $[\alpha]_D^{27}$ = -138.48(*c* 0.111, CH_3OH).

Compound 8 ^{1}H NMR (400 MHz, DMSO$_{-d6}$) δ 13.03 (d, J=9.6Hz, 2H), 9.76 (d, J=9.6Hz, 2H), 8.50 (s, 2H), 7.41 (s, 2H), 3.77 -3.76 (m, 4H), 3.71-3.67 (m, 2H), 2.80-2.84 (m, 4H), 1.93 (s, 6H), 1.44 (s, 12H). ^{13}C NMR (100 MHz, DMSO$_{-d6}$) δ 172.1, 162.2, 150.0, 147.1, 131.5, 127.4, 126.9, 120.7, 116.9, 116.6, 103.7, 51.3, 48.1, 27.0, 20.8. HRMS(ESI) m/z calcd for $C_{34}H_{39}N_2O_{12}S_2$ $(M-2Na+H)^-$ 731.1950, found 731.1929. $[\alpha]_D^{27}$ = +137.38 (*c* 0.111, CH_3OH).

Compound 9 ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.84 (d, J=12Hz, 2H), 10.13 (d, J=12Hz, 2H), 8.62 (s, 2H), 8.25-8.23 (m, 4H) , 7.69 (s, 2H), 7.38-7.35 (m,4H), 5.84 (s, 2H), 3.73-3.70 (m, 2H), 2.17 (s, 6H), 1.55-1.51(m, 12H). ^{13}C NMR (100 MHz, DMSO$_{-d6}$) δ 155.7, 152.8, 147.2, 139.7, 138.6, 136.0, 126.3, 125.9, 124.2, 121.3, 117.0, 115.0, 113.6, 113.2, 108.9, 90.1, 26.6, 21.4, 21.3. HRMS(ESI) m/z calcd for $C_{42}H_{37}N_4O_{10}$ $(M-H)^-$ 757.2515, found 757.2486. $[\alpha]_D^{26}$ = -563.5 (*c* 0.109, DMF).

Compound 10 ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.84 (d, J=12Hz, 2H), 10.12 (d, J=12Hz, 2H), 8.62 (s, 2H), 8.25-8.23 (m, 4H) , 7.69 (s, 2H), 7.38-7.35 (m,4H), 5.81 (s, 2H), 3.73-3.70 (m, 2H), 2.17 (s, 6H), 1.55-1.51(m, 12H). ^{13}C NMR (100 MHz, DMSO$_{-d6}$) .δ 155.3, 152.3, 146.7, 139.1, 138.1, 138.0, 135.7, 125.8, 125.6, 123.7, 120.9, 114.6, 113.2, 112.7, 108.4, 89.7, 26.1, 20.8, 20.7. HRMS(ESI) m/z calcd for $C_{42}H_{37}N_4O_{10}$ $(M-H)^-$ 757.2515, found 757.2426. $[\alpha]_D^{27}$ = +557.8 (*c* 0.109, DMF).

Spectra of key compounds



1 Det.A Ch1 / 235nm

PeakTable

Detector A Ch1 235nm

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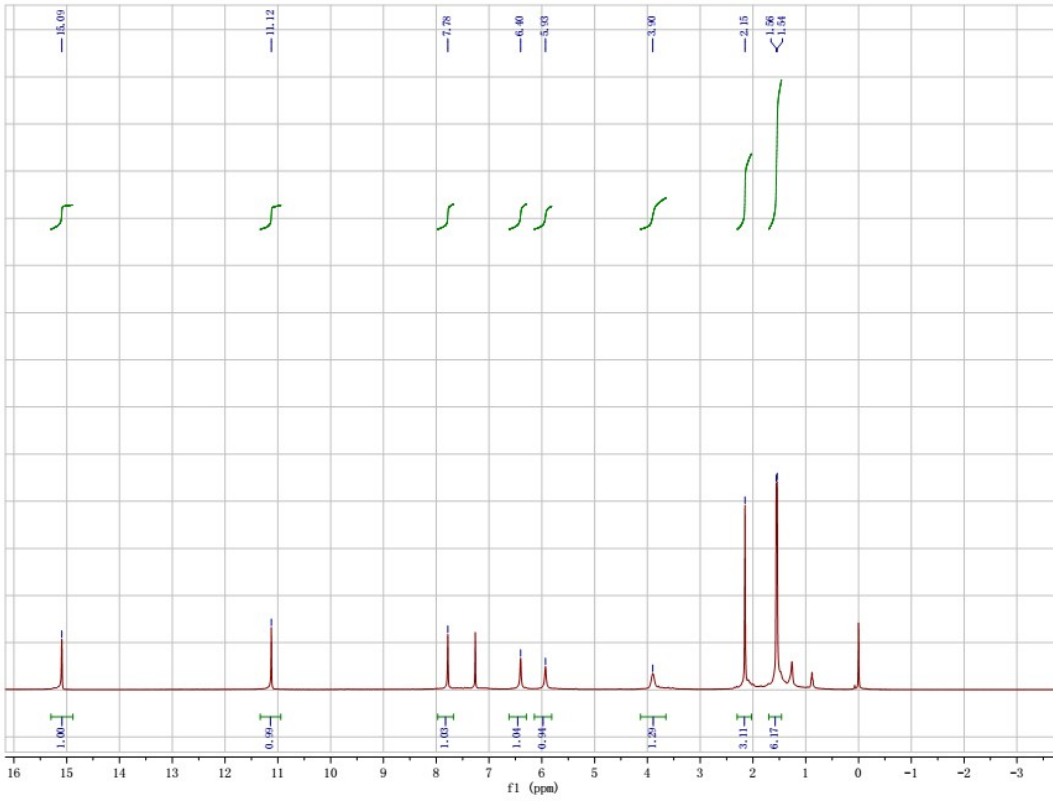
2	5.960	466813	39444	0.940	1.492
Total		49642737	2642984	100.000	100.000



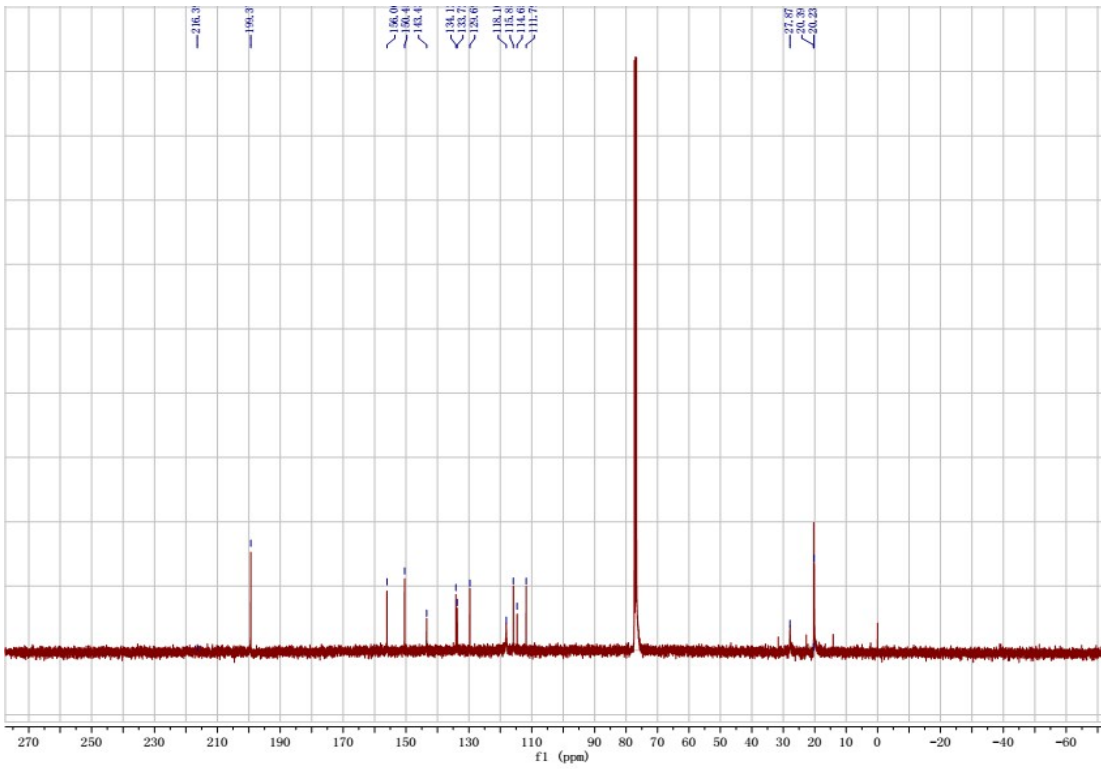
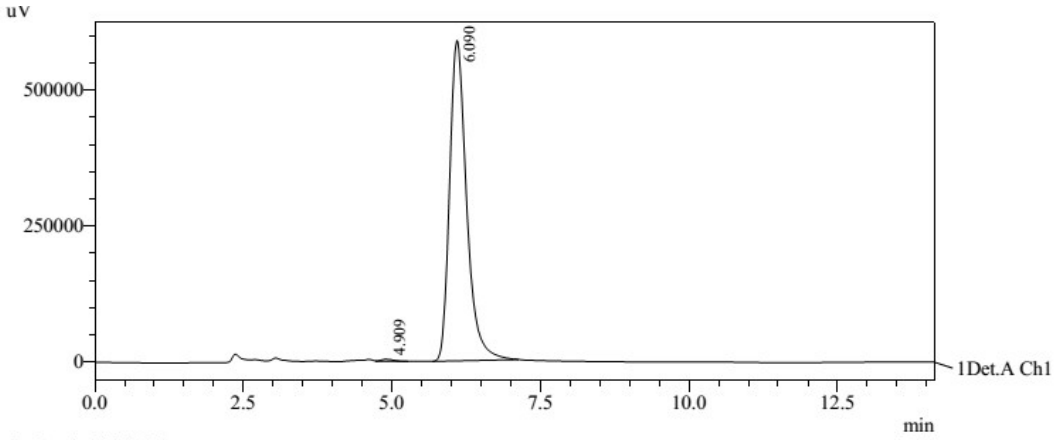


Figure S1. HPLC, ^{1}H NMR and ^{13}C NMR of (-)-gossypol.



1 Det.A Ch1 / 265nm

PeakTable

Detector A Ch1 265nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.909	48277	3527	0.411	0.596
2	6.090	11692765	588537	99.589	99.404
Total		11741041	592063	100.000	100.000

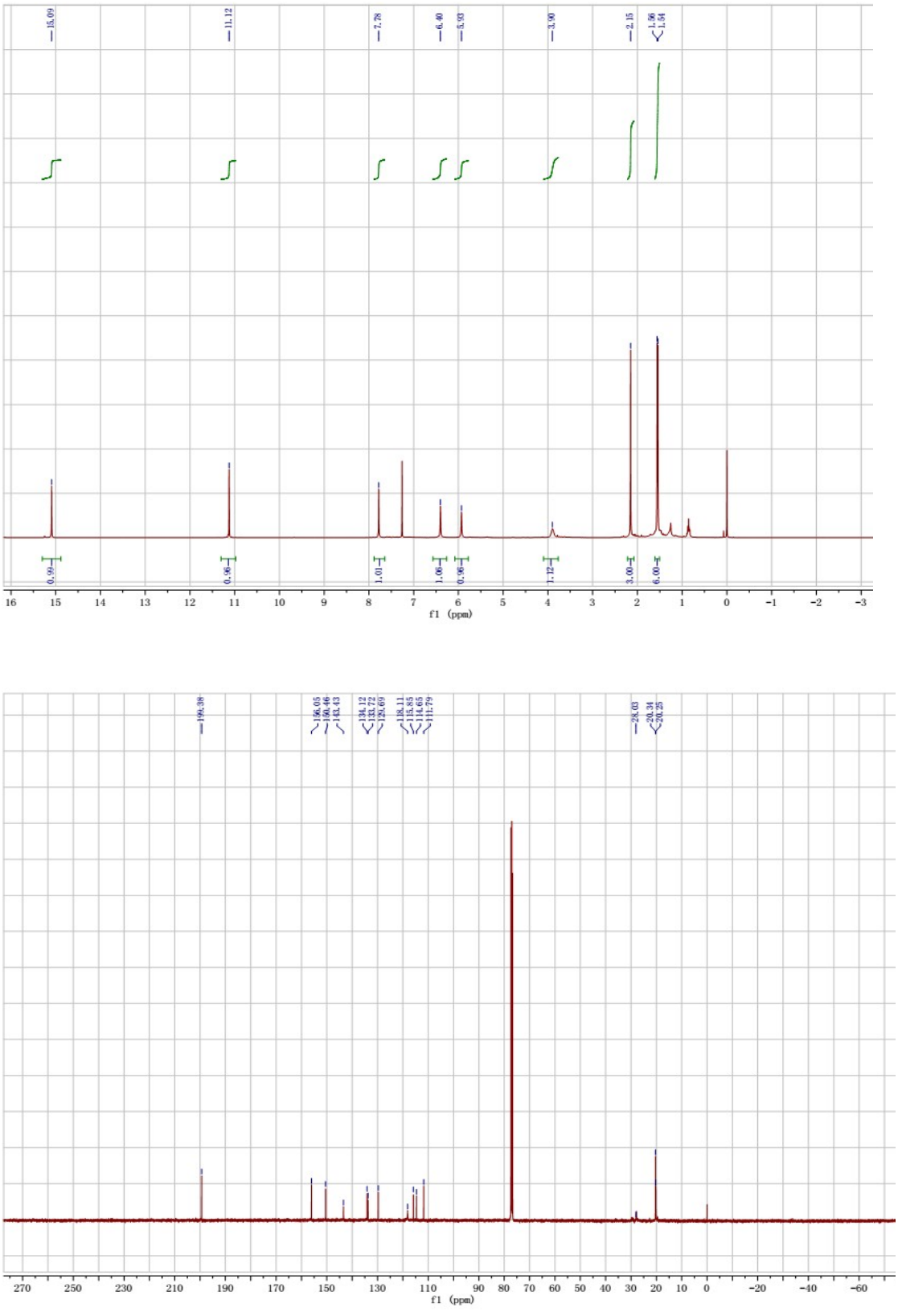
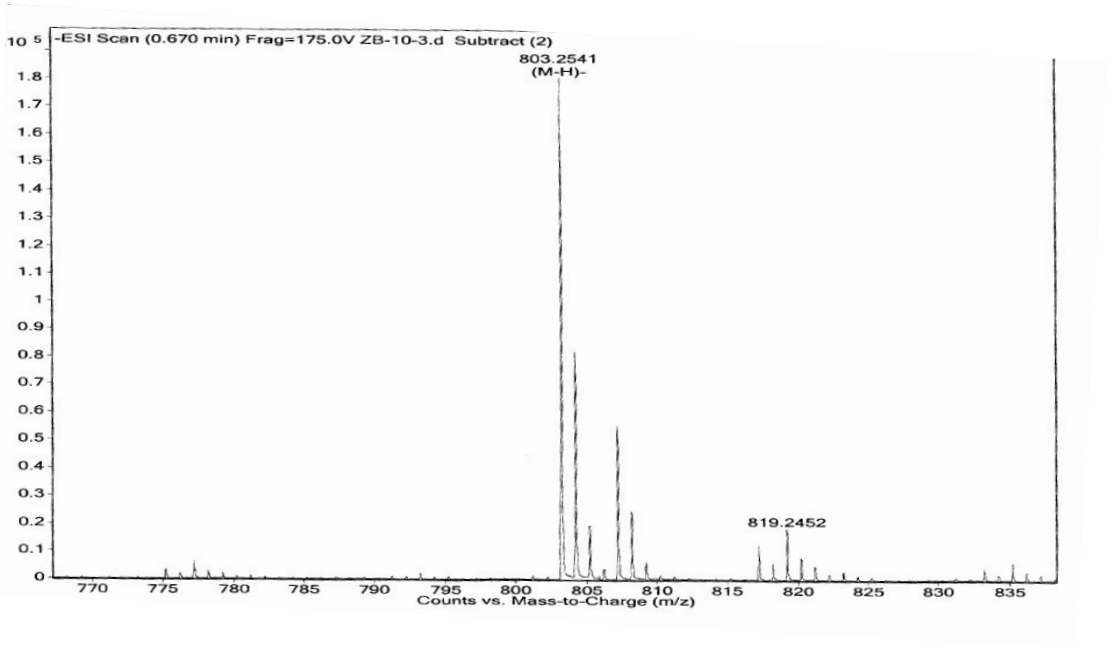
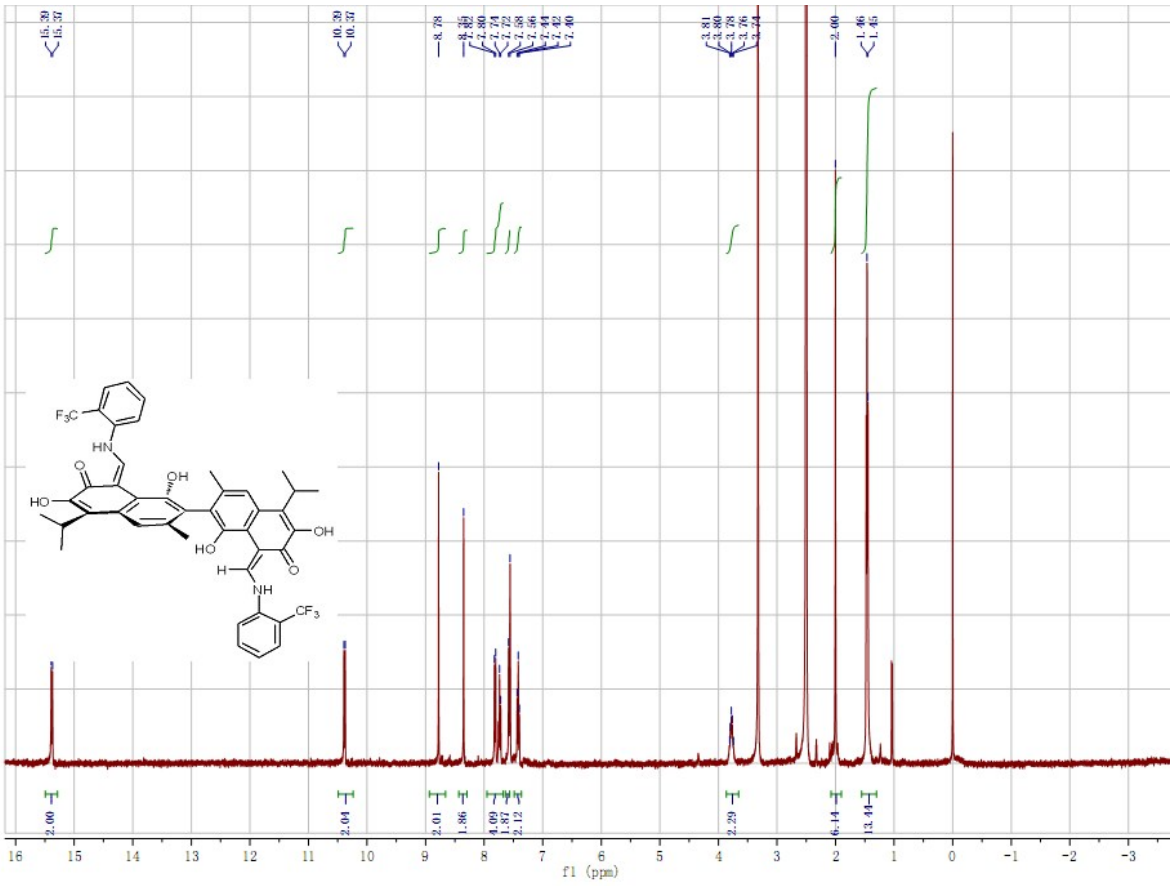


Figure S2. HPLC, ^{1}H NMR and ^{13}C NMR of (+)-gossypol.


-ESI Scan (0.670 min) Frag=175.0V ZB-10-3.d Subtract (2)
803.2541
(M-H)-
819.2452
Counts vs. Mass-to-Charge (m/z)


f1 (ppm)

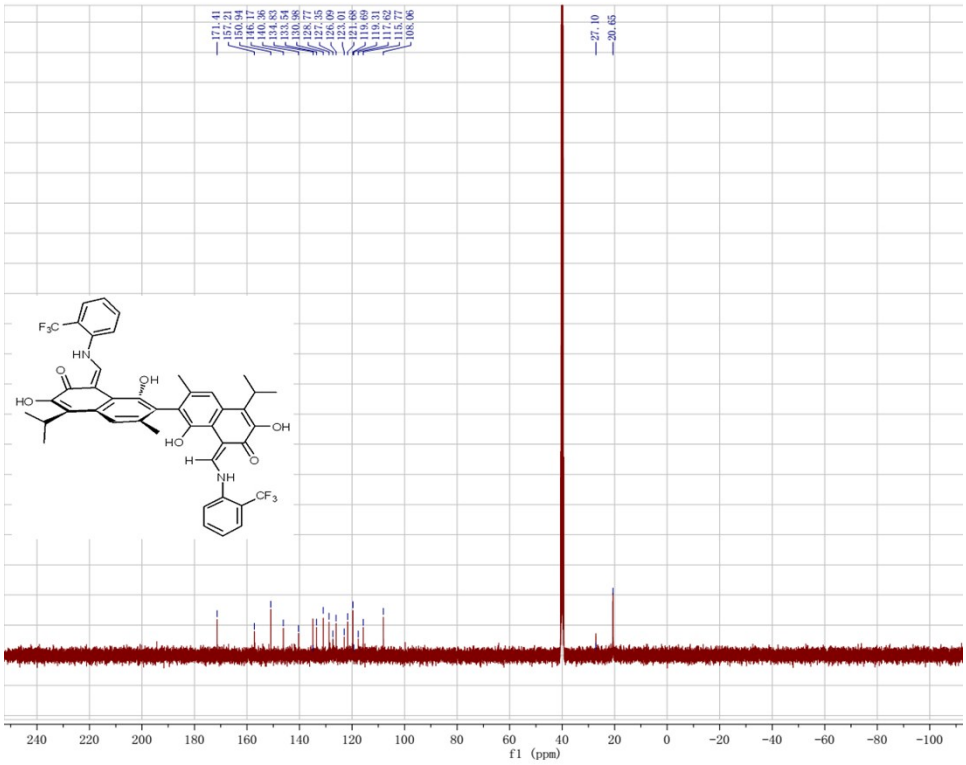
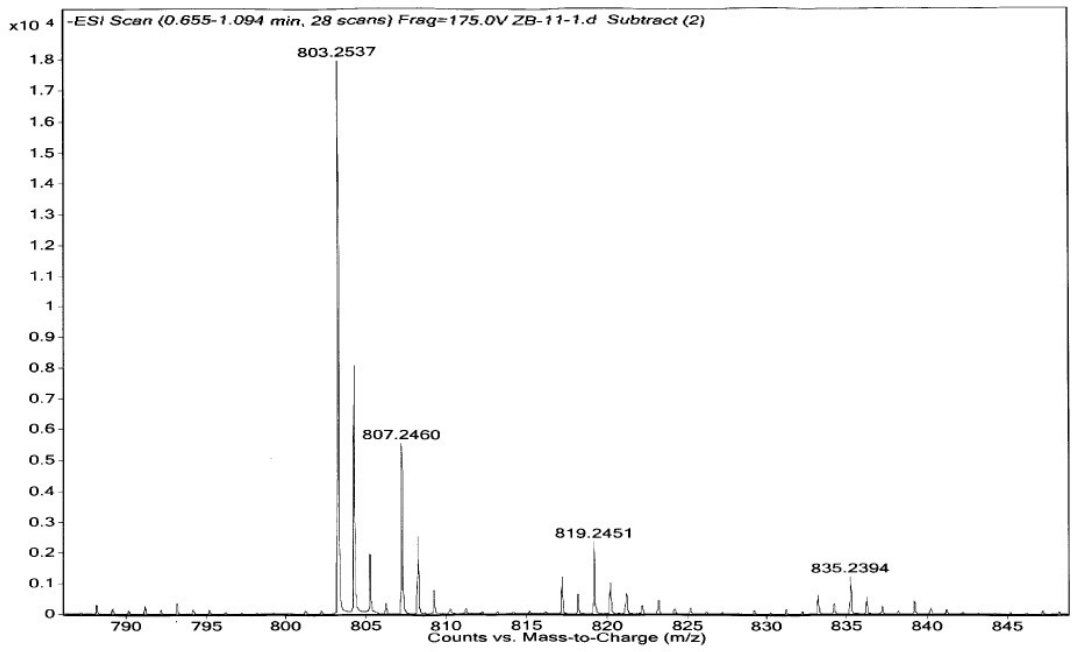
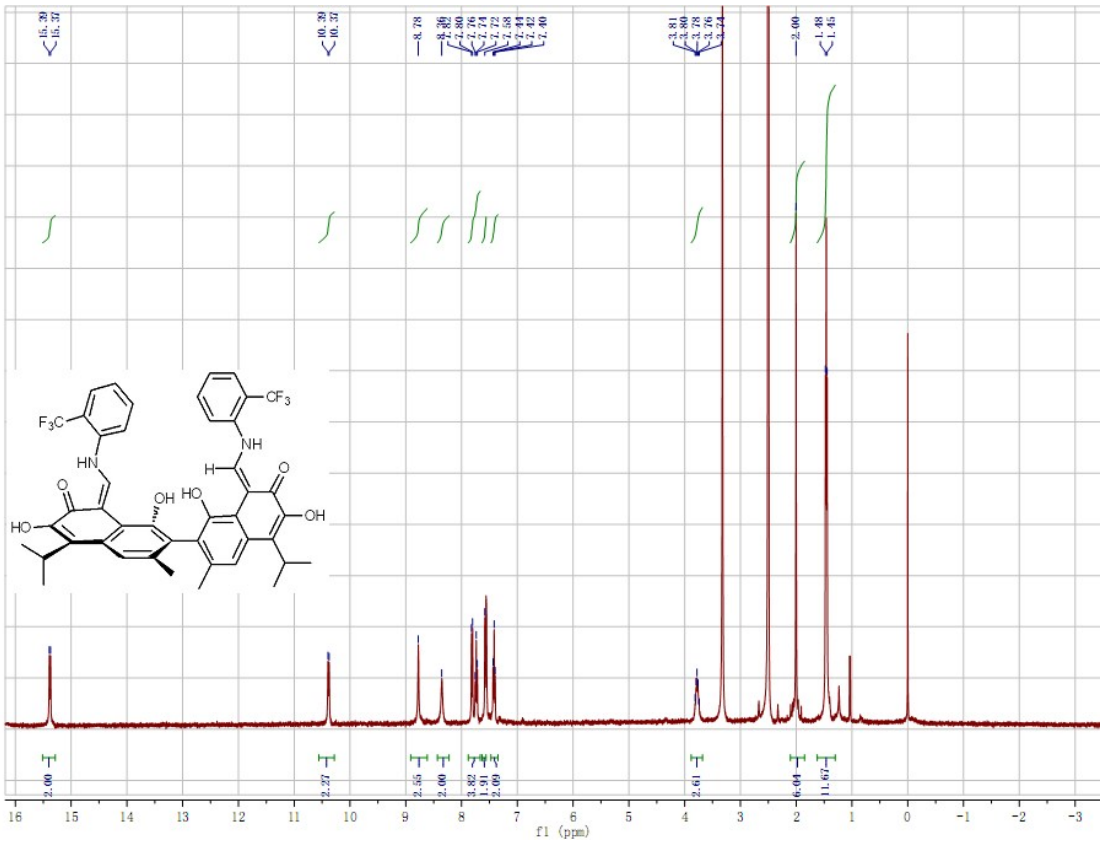


Figure S3. HRMS, ^{1}H NMR and ^{13}C NMR of compound **1**.




F3C
HN
O
OH
HO
CF3
NH
H
O
HO
OH
f1 (ppm)

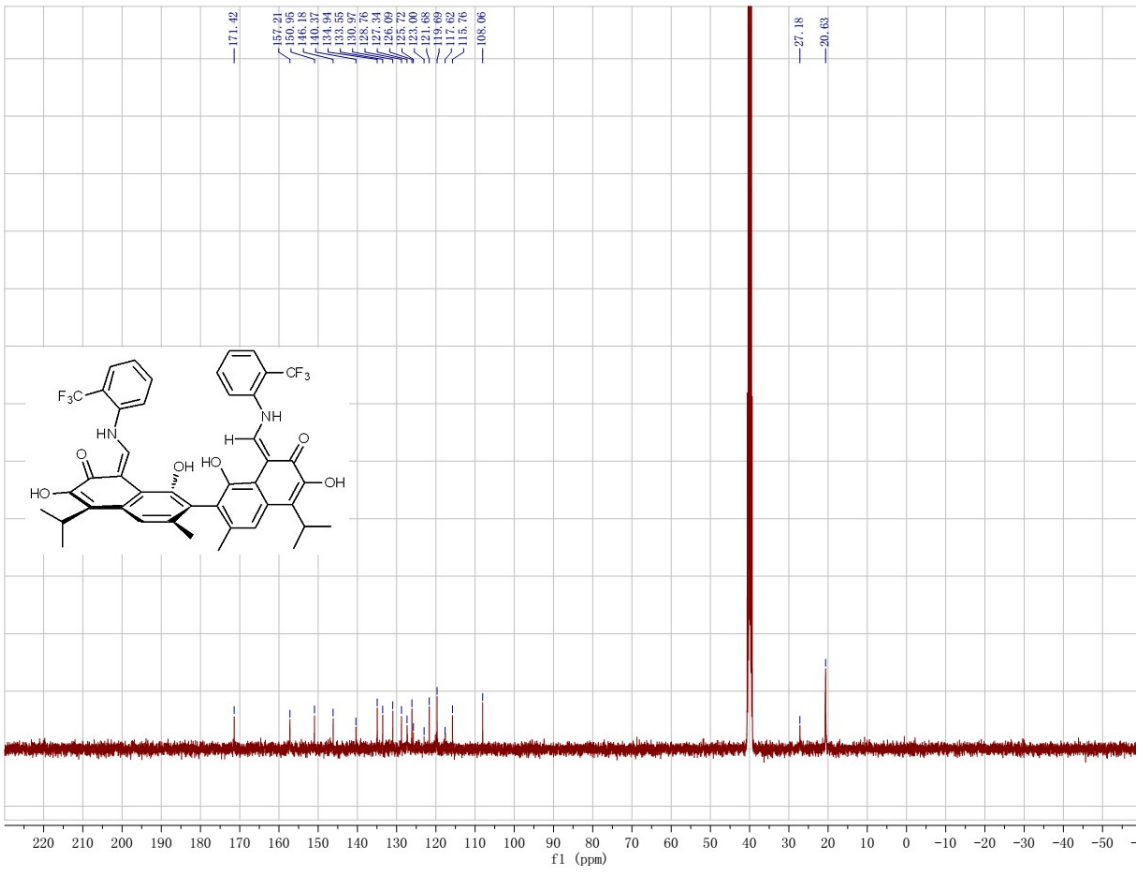
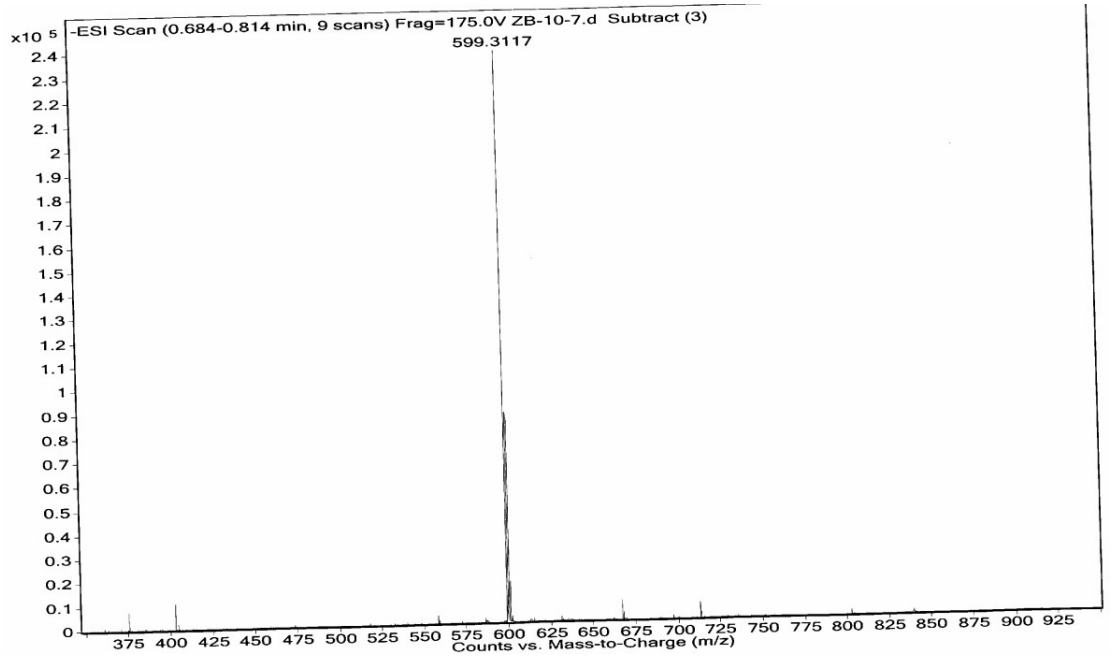
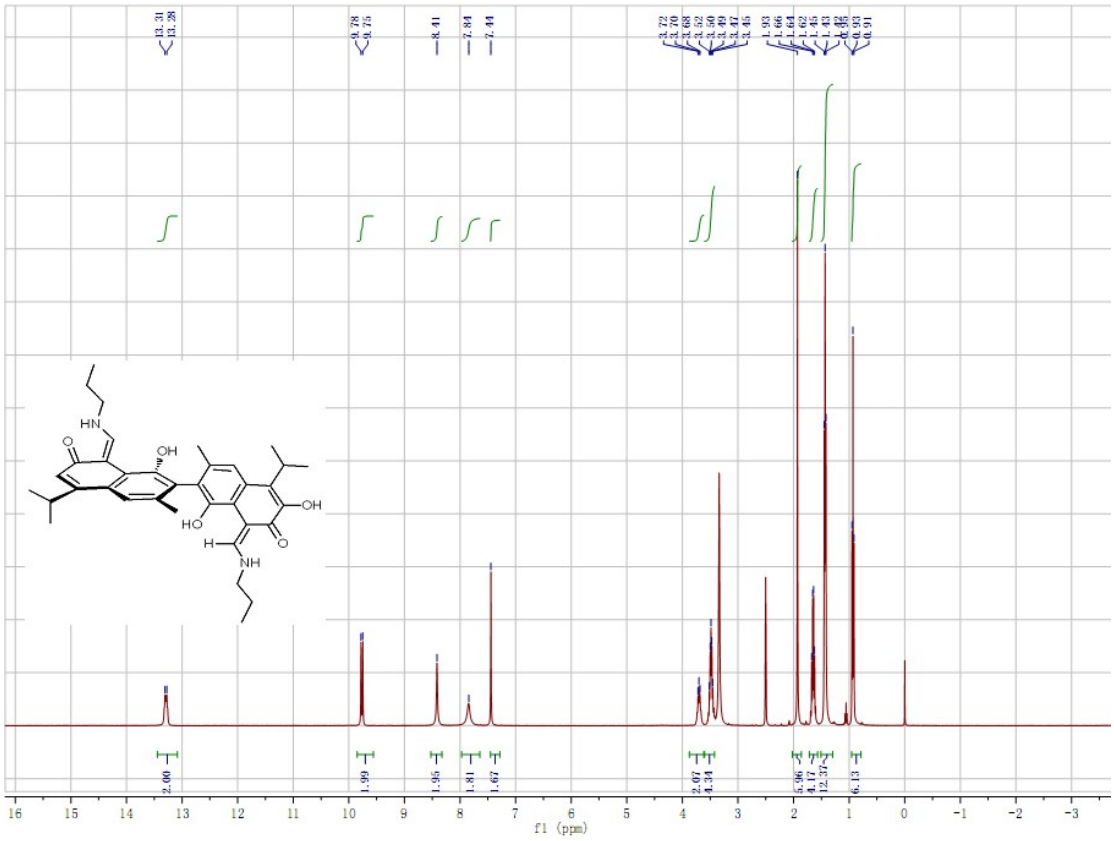


Figure S4. HRMS, ^{1}H NMR and ^{13}C NMR of compound **2**.




13.31
13.28
9.78
9.75
8.41
7.84
7.44
3.72
3.70
3.68
3.52
3.50
3.49
3.47
3.45
1.93
1.66
1.64
1.62
1.45
1.43
0.95
0.93
0.91
HN
O
OH
HO
OH
O
H
NH
2.00
1.99
1.95
1.81
1.67
2.07
4.34
5.96
4.17
12.37
6.13
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
f1 (ppm)

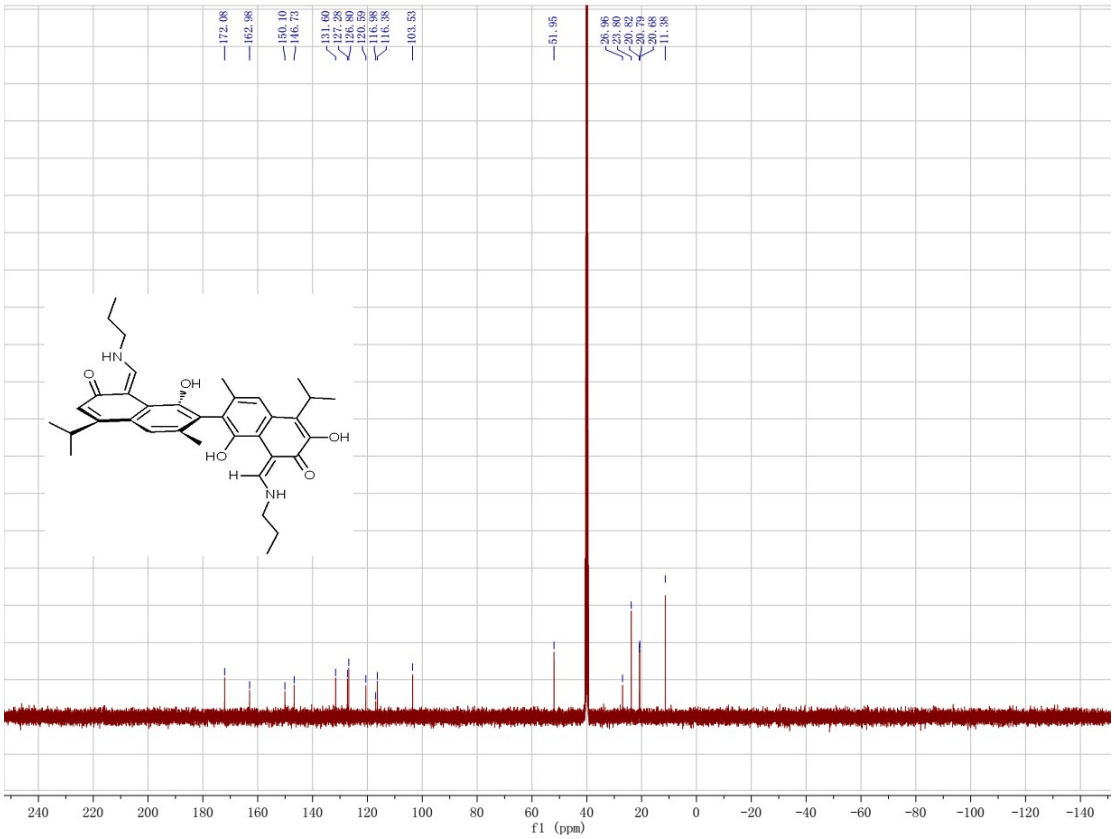
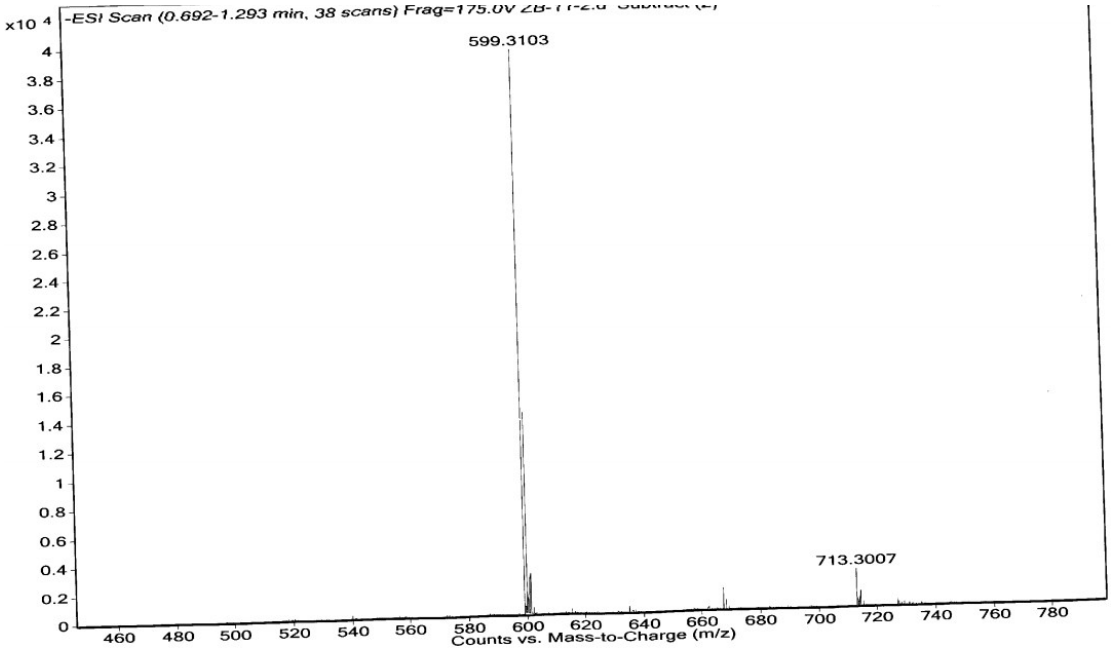
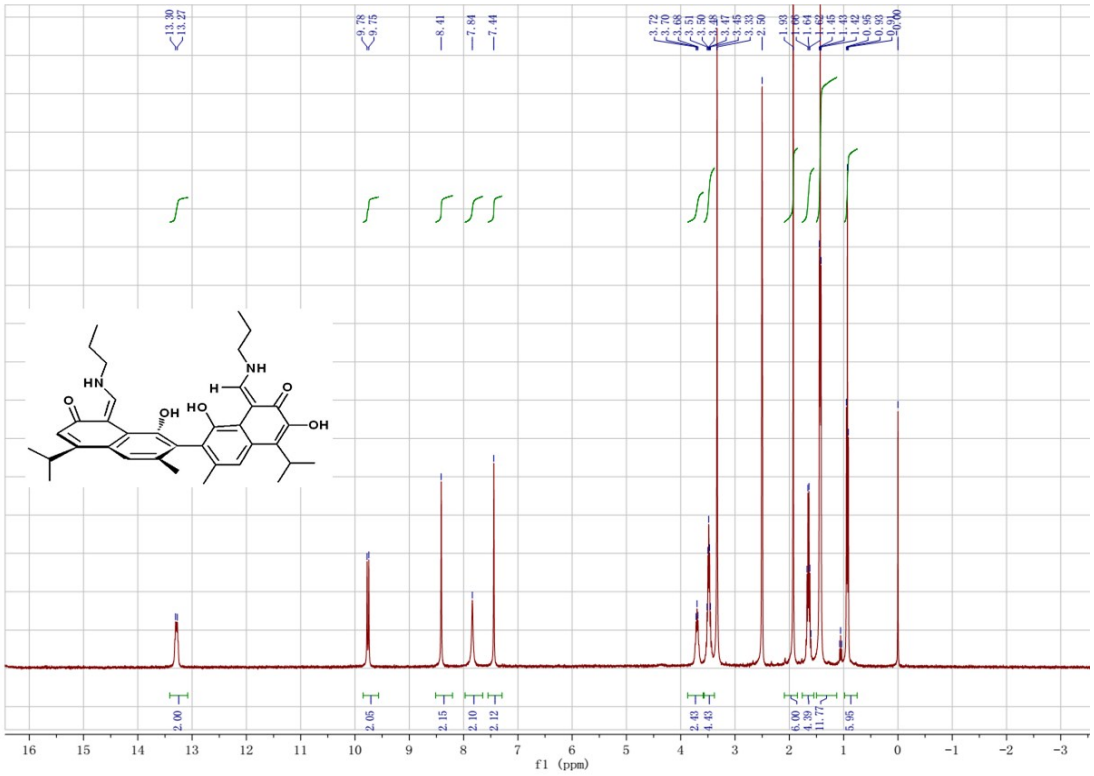


Figure S5. HRMS, ^{1}H NMR and ^{13}C NMR of compound **3**.




13.30
13.27
9.78
9.75
8.41
7.84
7.44
3.72
3.70
3.68
3.51
3.50
3.48
3.47
3.45
3.33
2.50
1.93
1.66
1.64
1.62
1.45
1.43
1.42
0.95
0.93
0.90
0.88
HN
O
OH
HO
H
NH
O
OH
2.00
2.05
2.15
2.10
2.12
2.83
4.43
6.00
4.90
11.77
5.46
f1 (ppm)

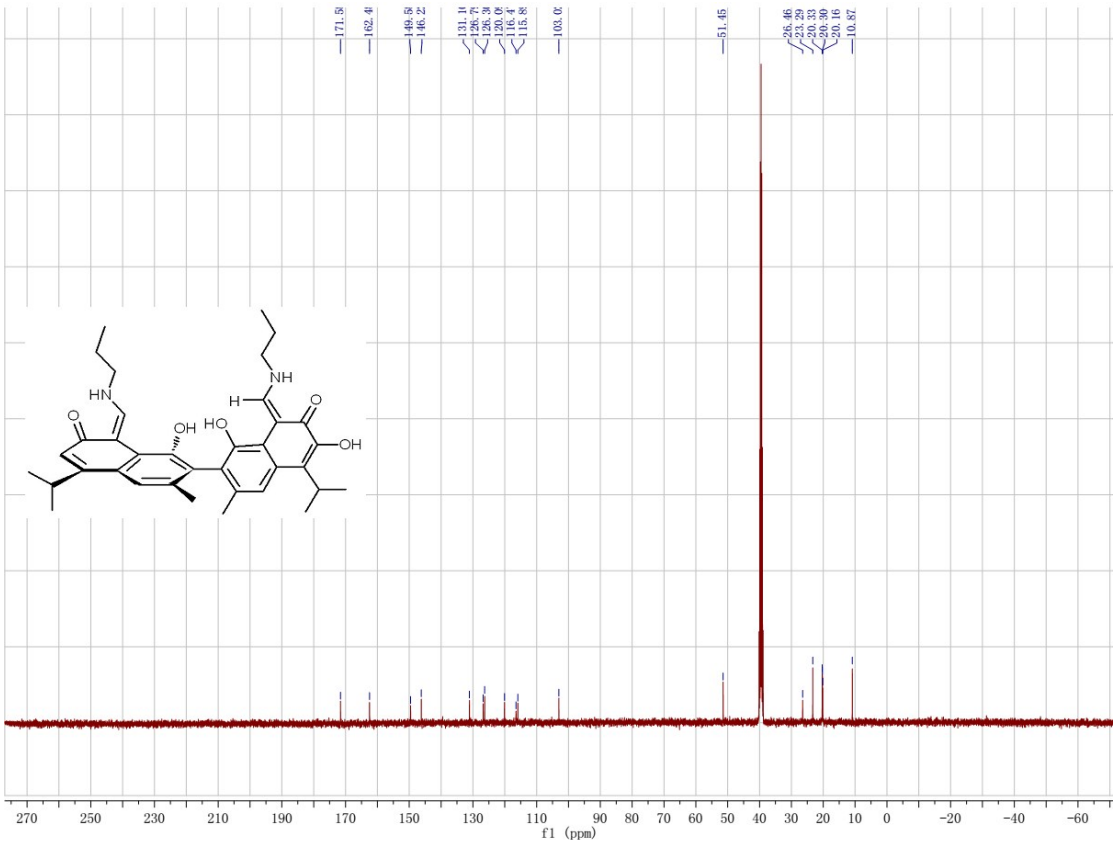
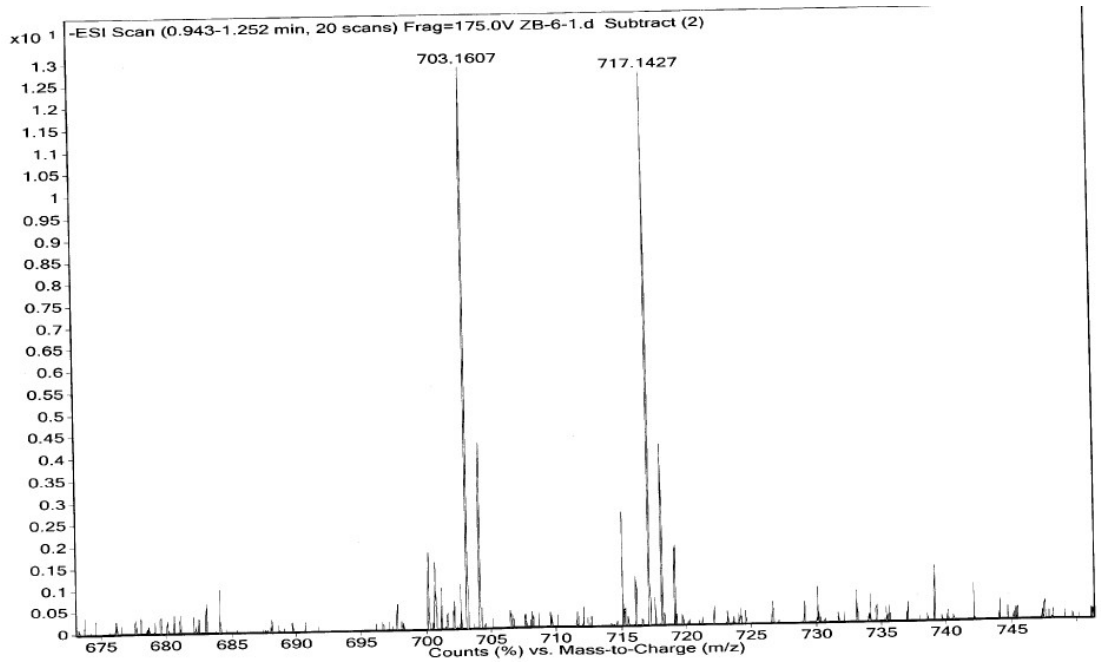
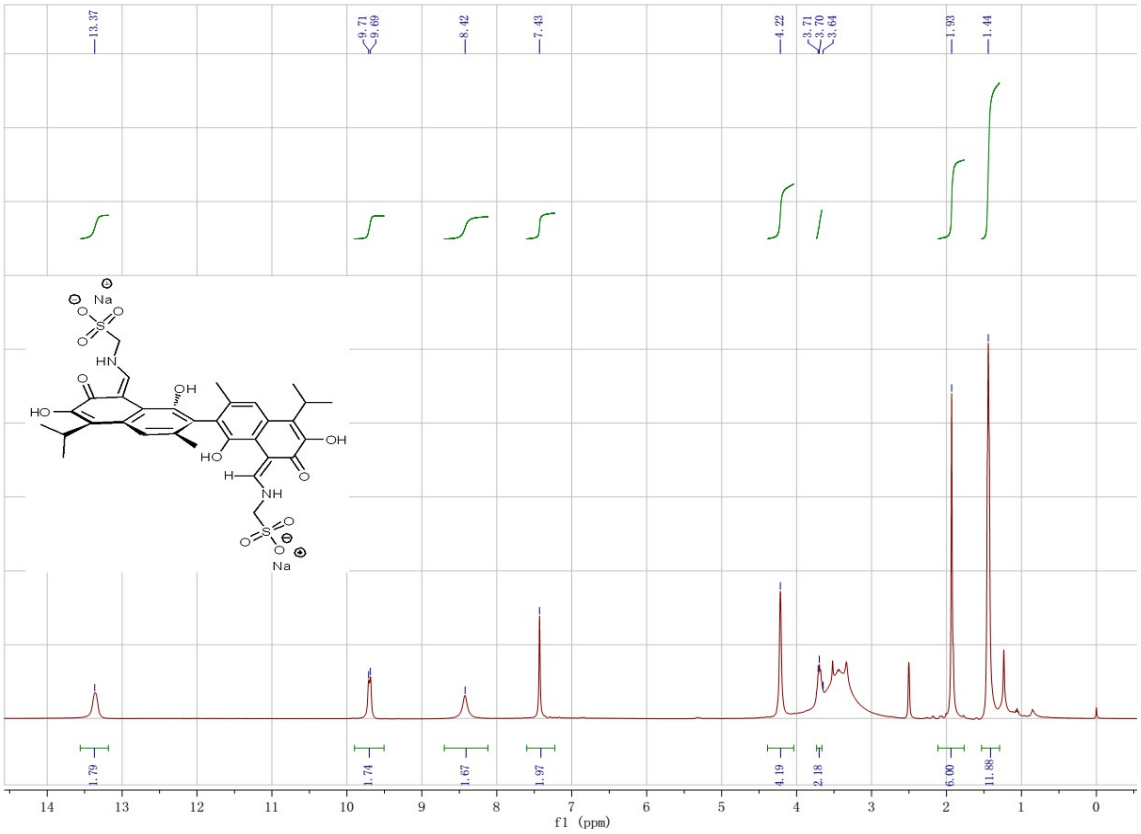

171.5
162.4
149.5
146.2
131.1
126.7
126.3
120.0
116.4
115.8
103.0
51.45
26.46
23.29
20.33
20.30
20.16
10.87
HN
O
OH
HO
H
NH
O
OH
f1 (ppm)

Figure S6. HRMS, ^{1}H NMR and ^{13}C NMR of compound **4**.





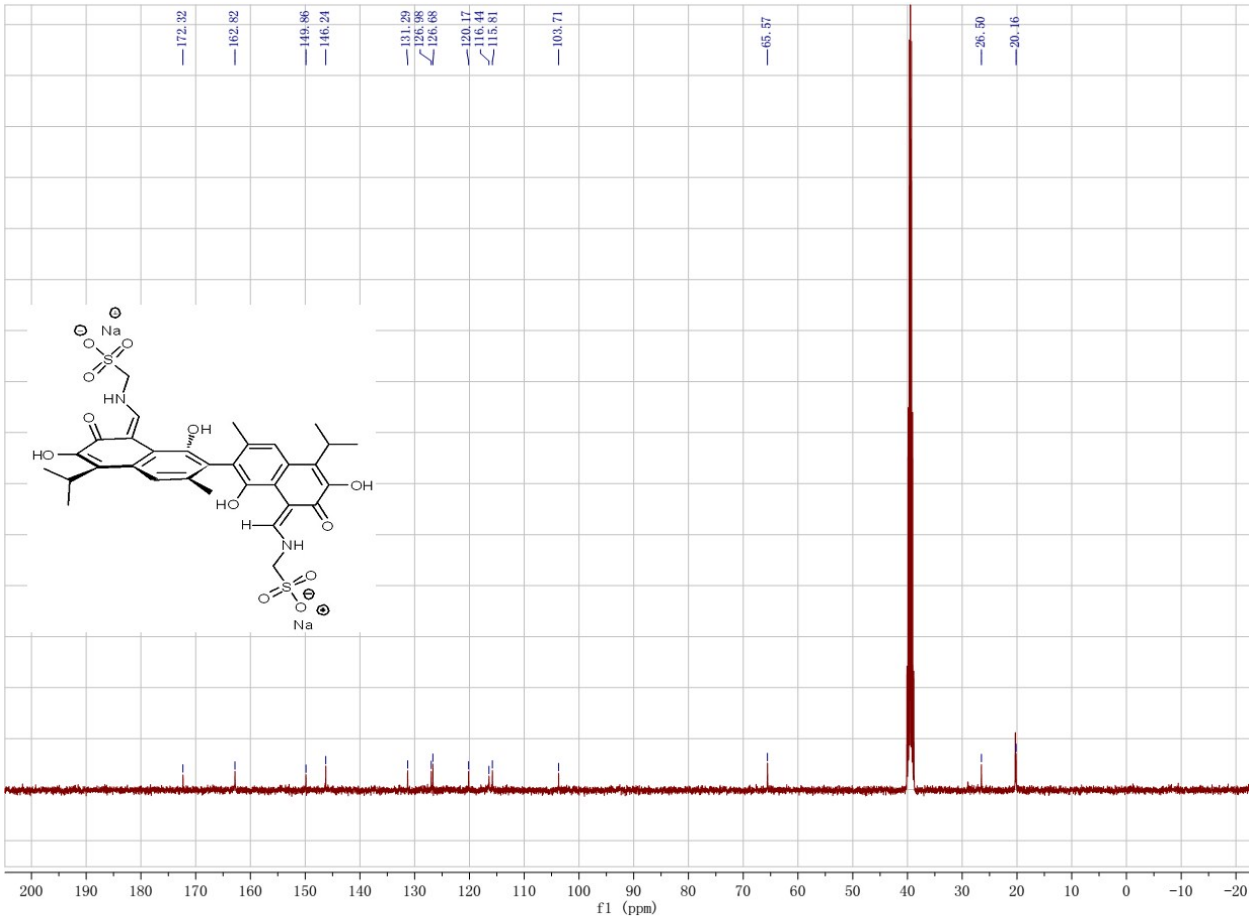
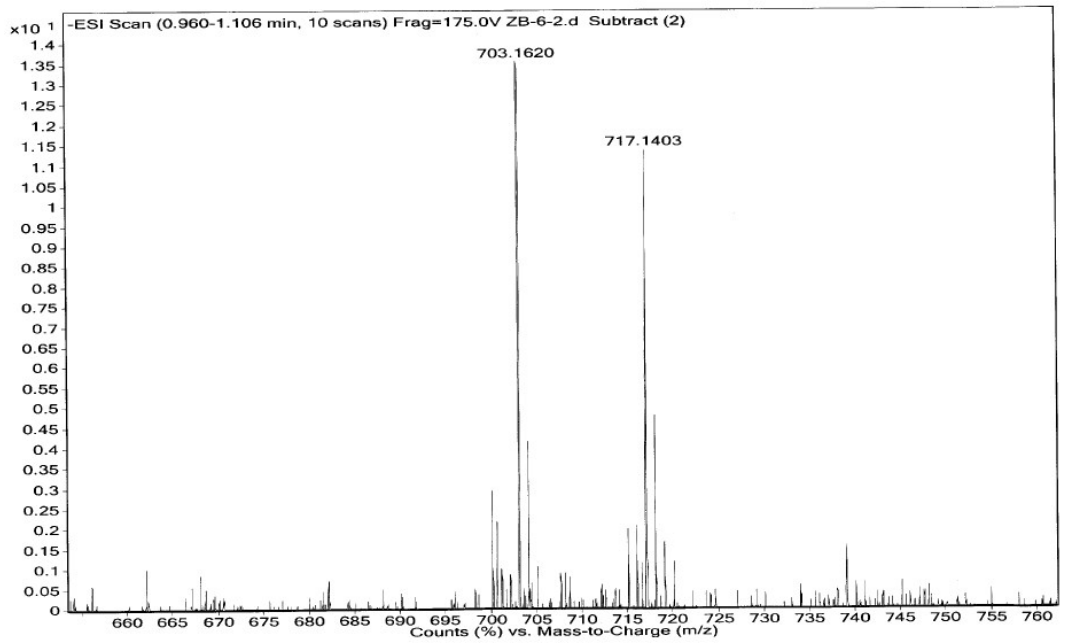
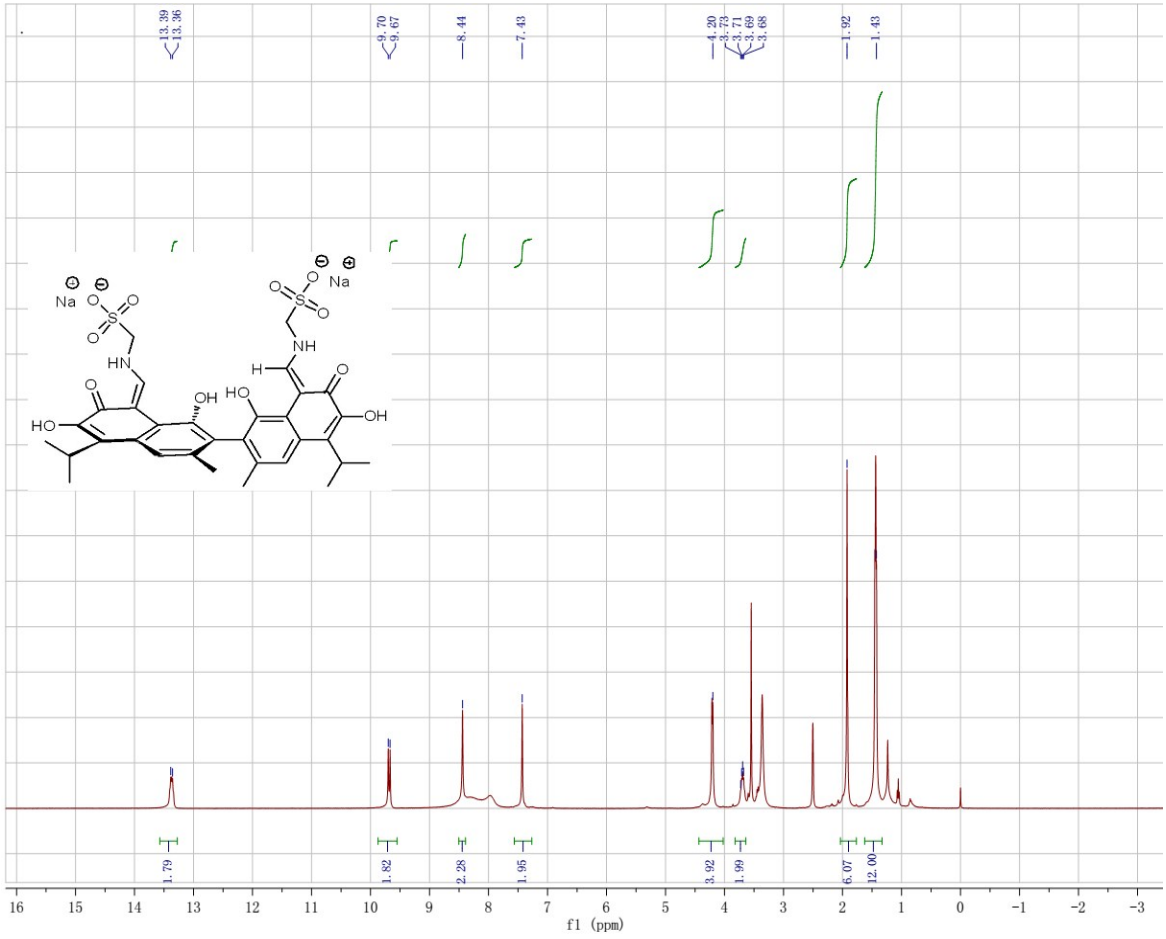


Figure S7. HRMS, ^{1}H NMR and ^{13}C NMR of compound **5**.




13.39
13.36
9.70
9.67
8.44
7.43
4.20
3.73
3.71
3.69
3.68
1.92
1.43
Na
Na
HN
NH
H
HO
OH
HO
OH
1.79
1.82
2.28
1.95
3.92
1.99
6.07
12.00
f1 (ppm)

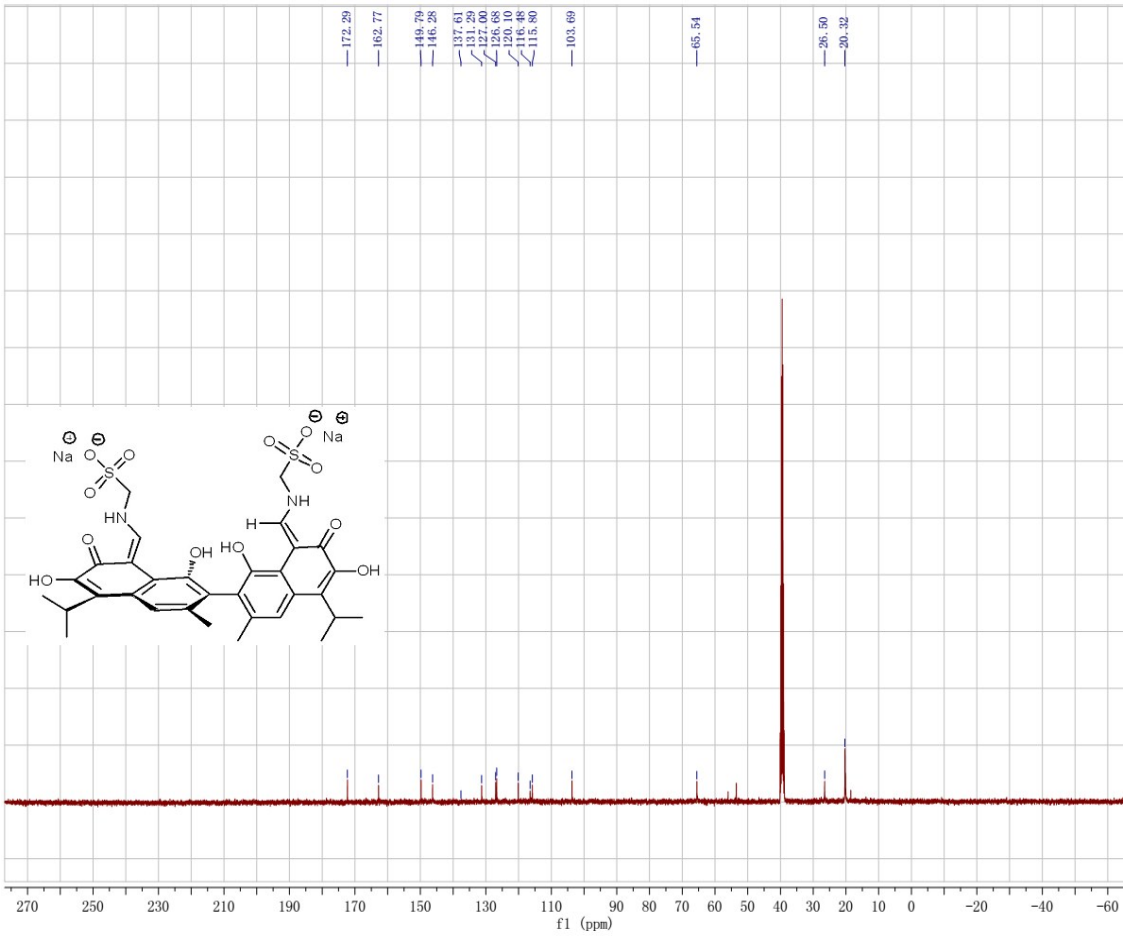
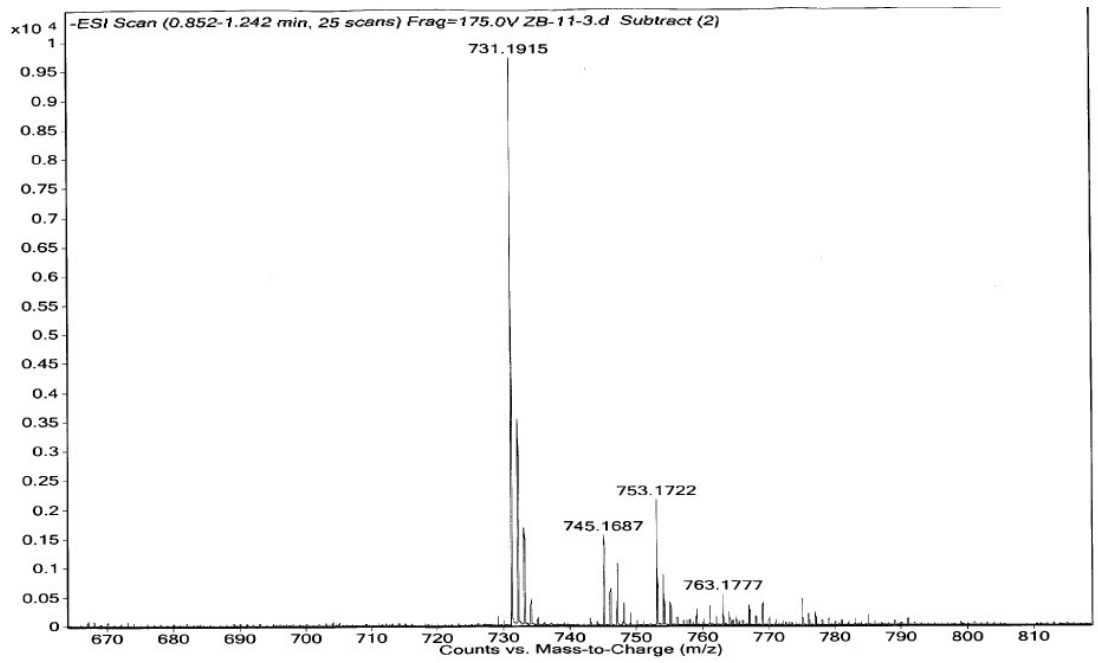
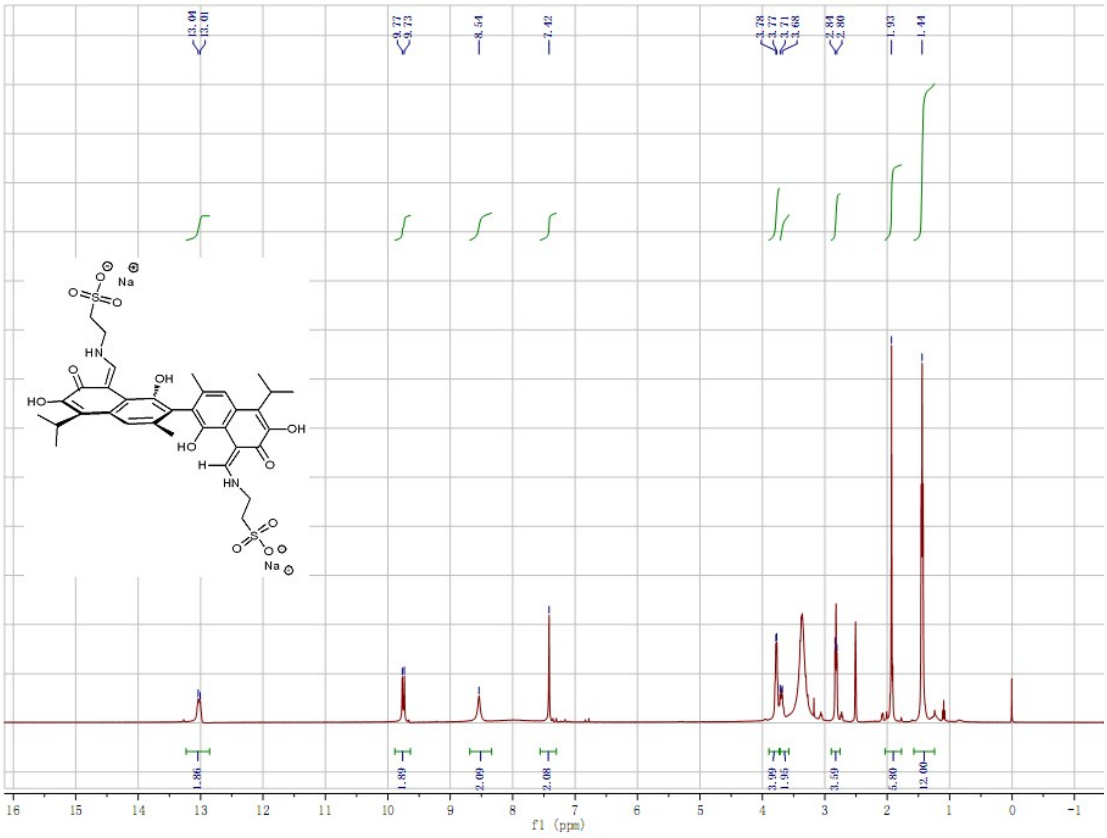


Figure S8. HRMS, ^{1}H NMR and ^{13}C NMR of compound **6**.




13.04
13.01
9.77
9.73
8.54
2.42
3.78
3.77
3.71
3.68
2.84
2.80
1.93
1.44
Na
HN
OH
HO
OH
HO
H
HN
Na
1.86
1.89
2.09
2.08
3.99
1.95
3.69
5.80
12.00
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)

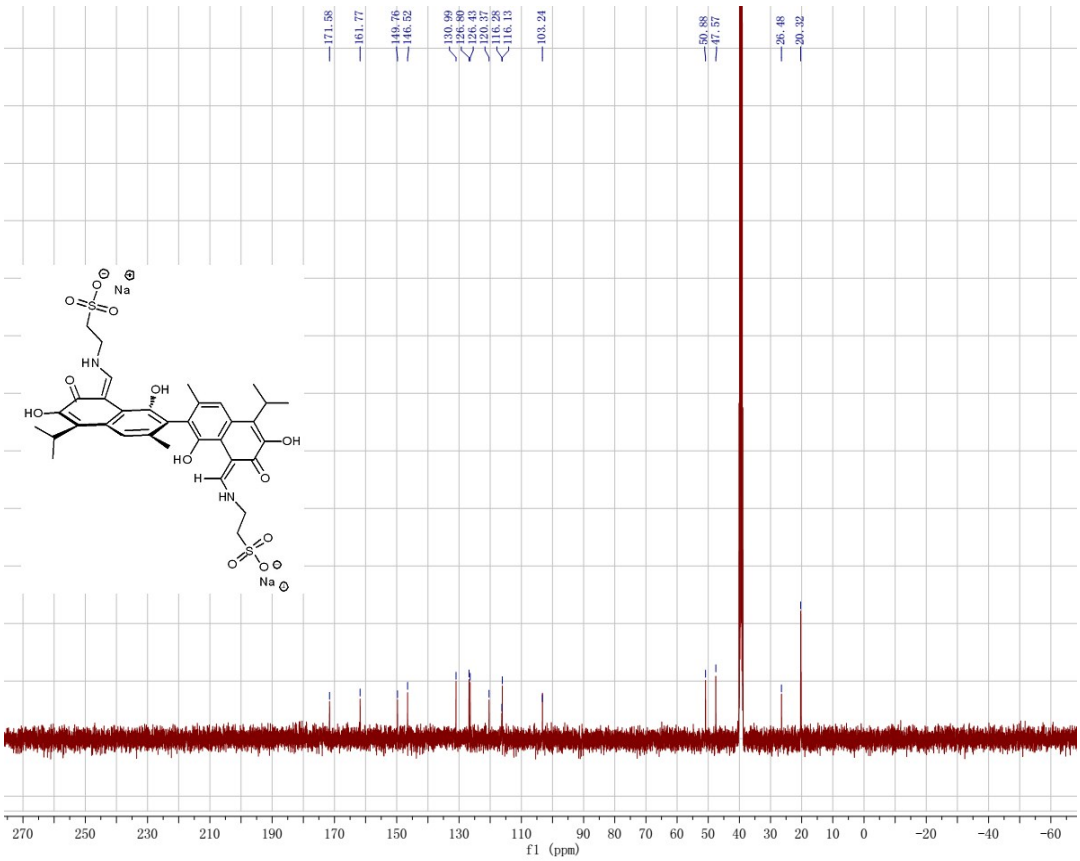
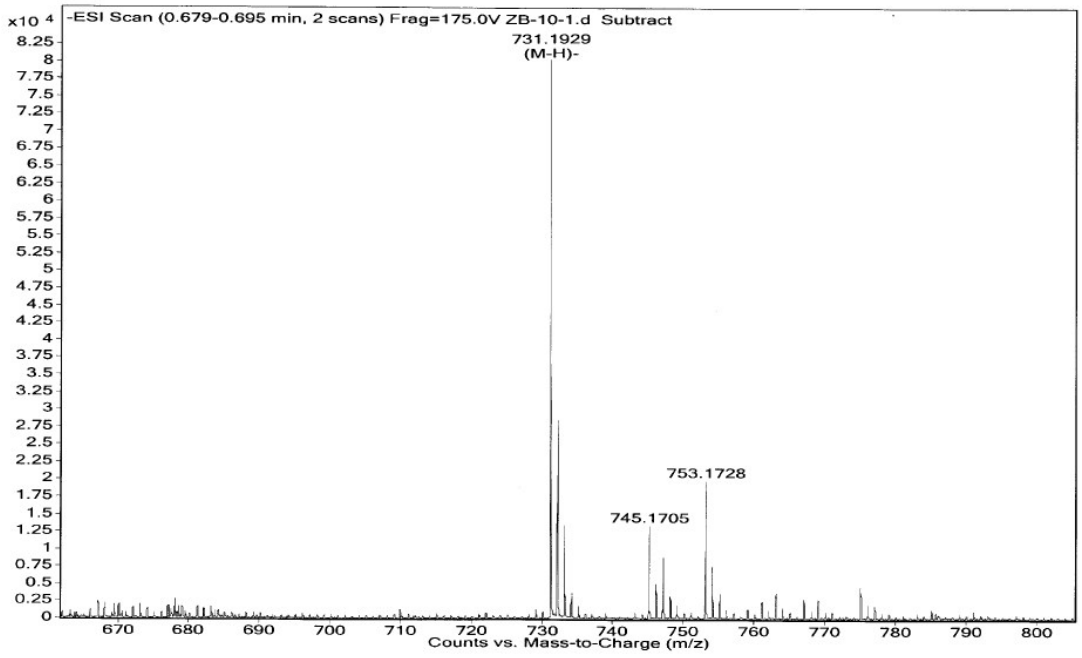
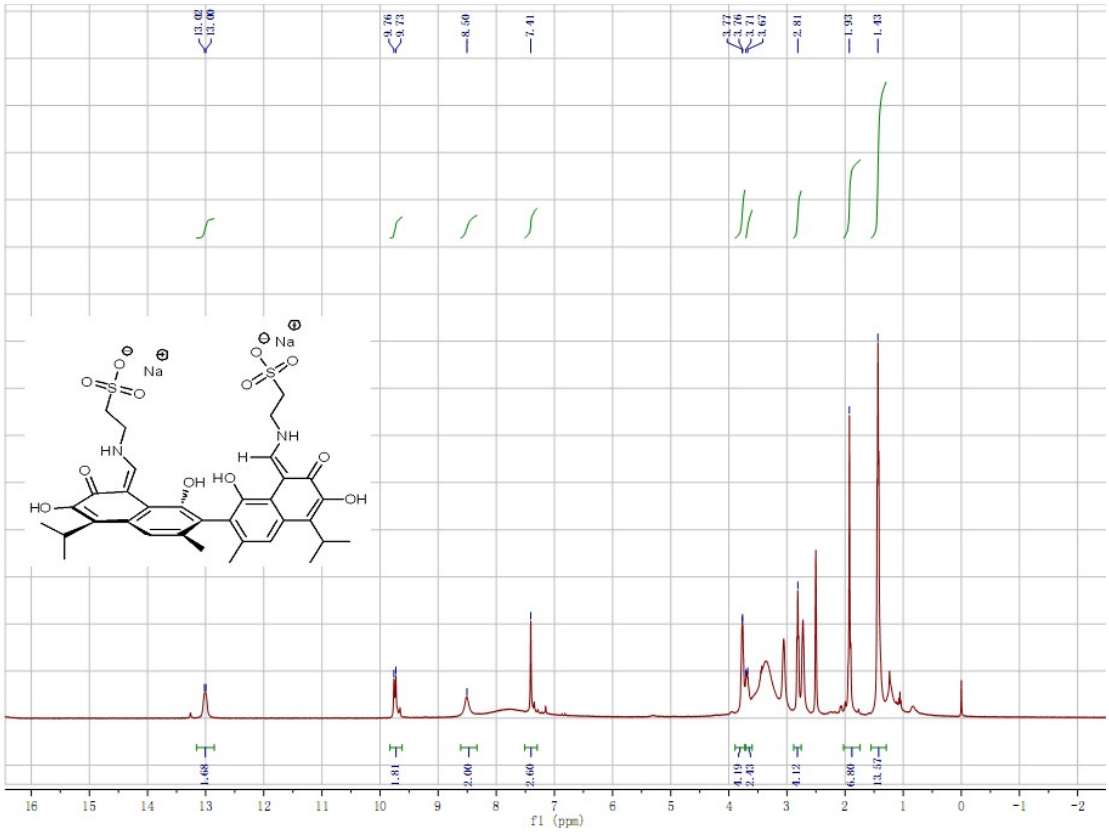


Figure S9. HRMS, ^{1}H NMR and ^{13}C NMR of compound **7.**




13.02
13.00
9.76
9.73
8.50
7.41
3.77
3.76
3.71
3.67
2.81
1.93
1.43
Na
Na
HN
NH
H
OH
HO
HO
OH
1.68
1.81
2.00
2.60
4.19
2.43
4.12
6.80
13.57
f1 (ppm)

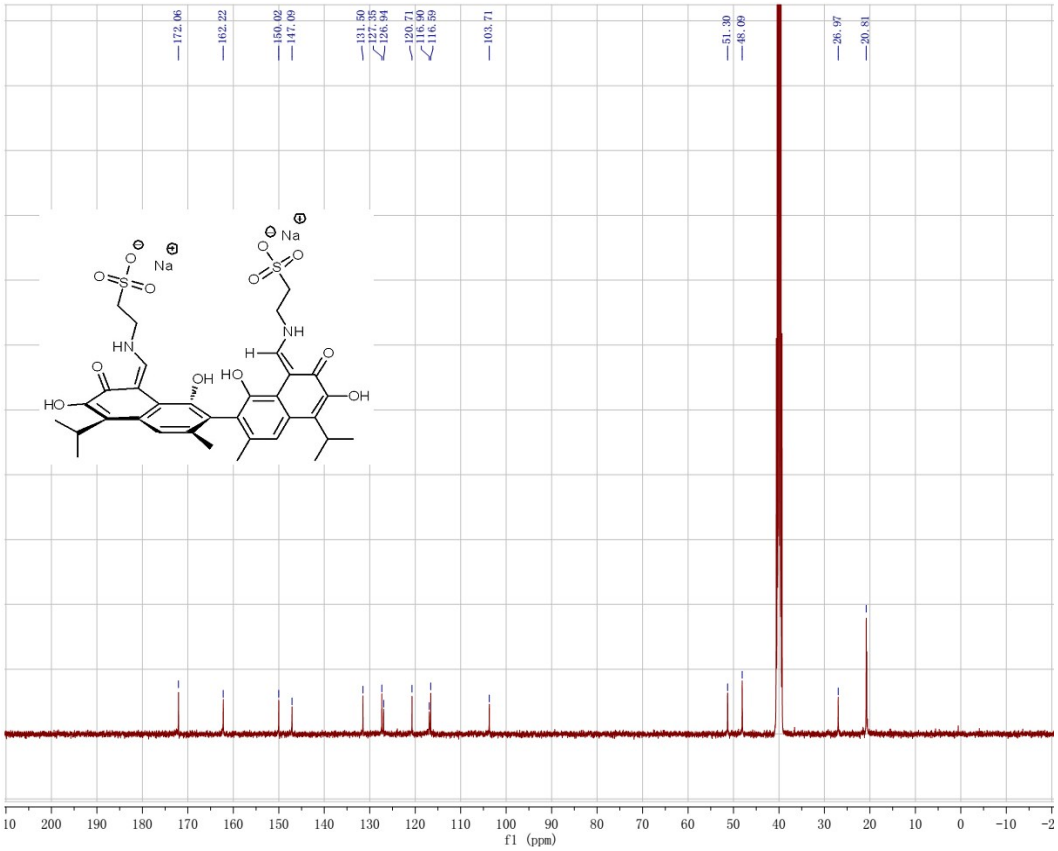
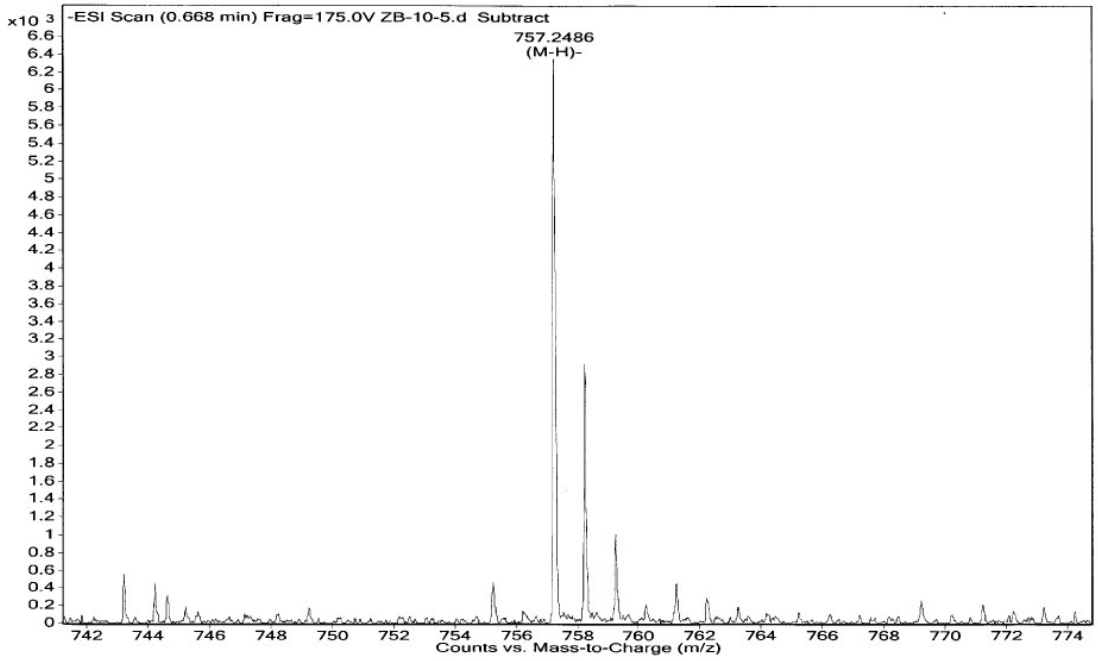
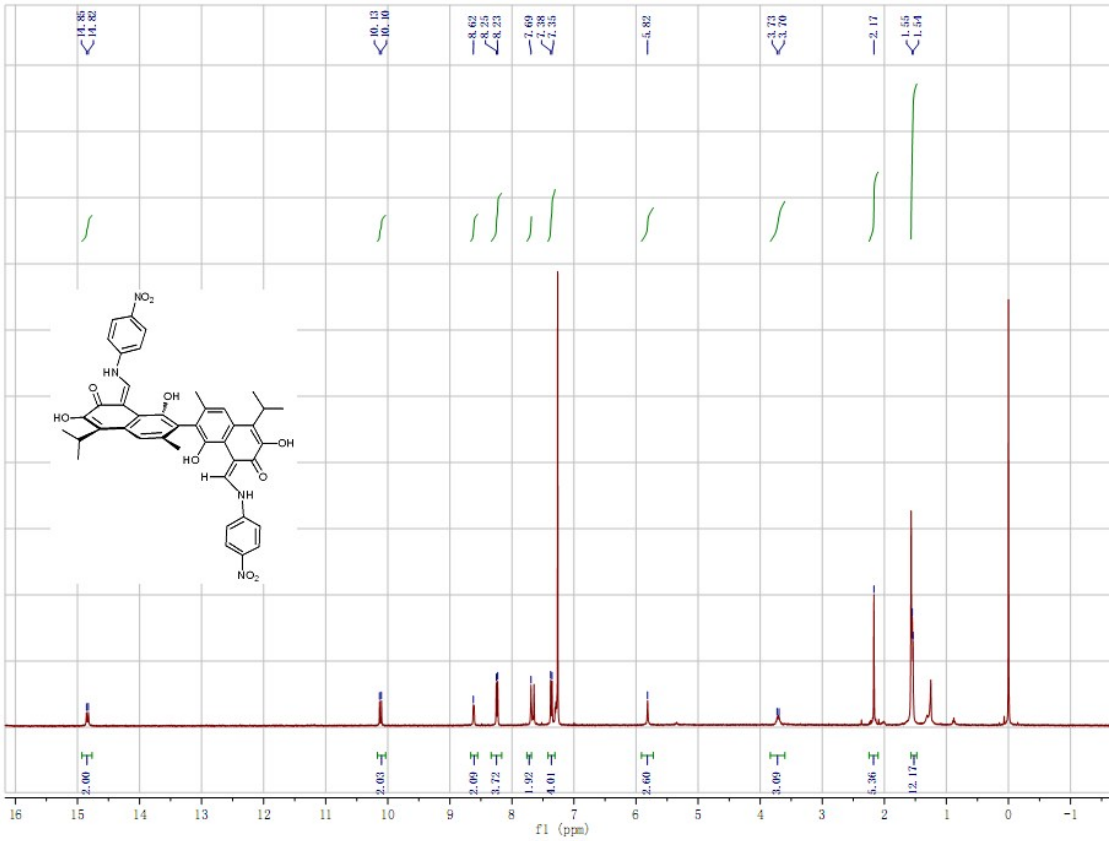


Figure S10. HRMS, ^{1}H NMR and ^{13}C NMR of compound 8.




14.85
14.82
10.13
10.10
8.62
8.25
8.23
7.69
7.38
7.35
5.82
3.73
3.70
2.17
1.56
1.54
2.00
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2.09
3.72
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4.01
2.60
3.09
5.36
12.17
f1 (ppm)

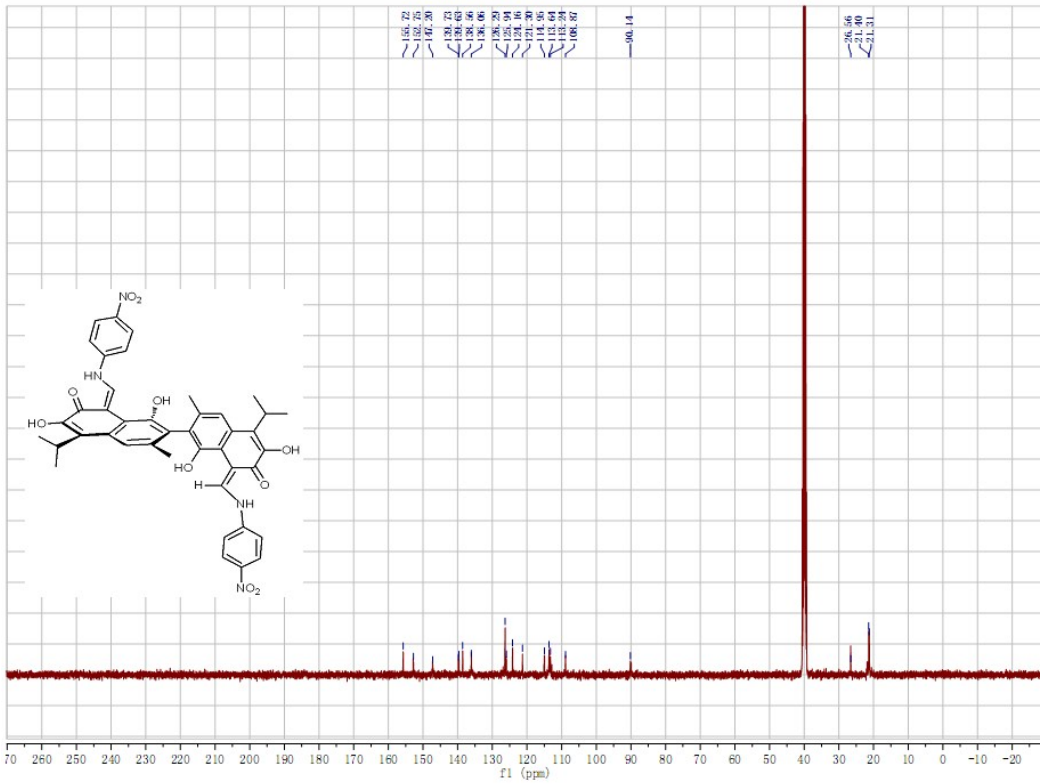
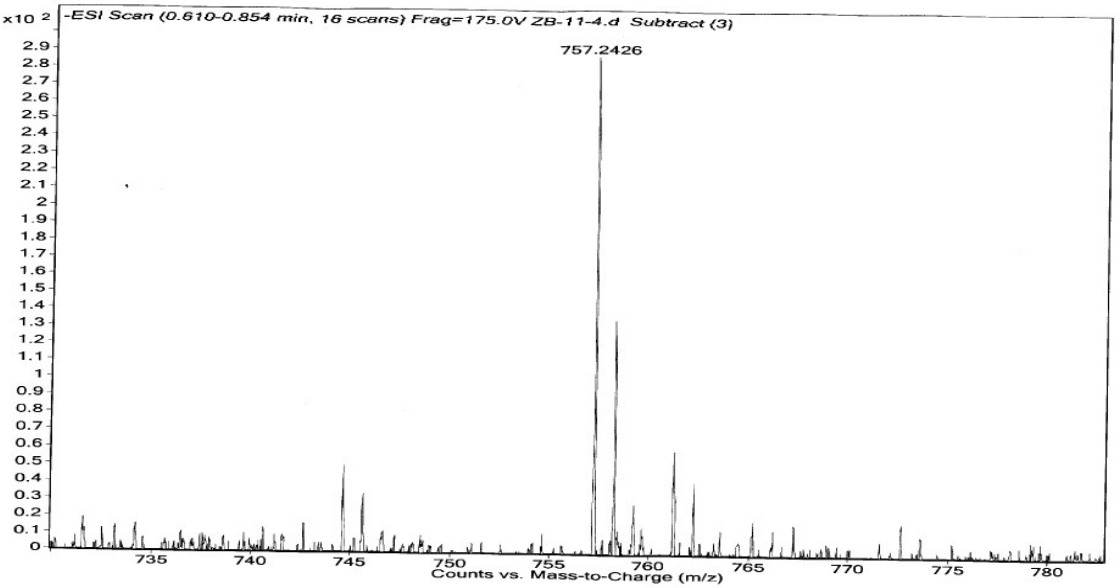
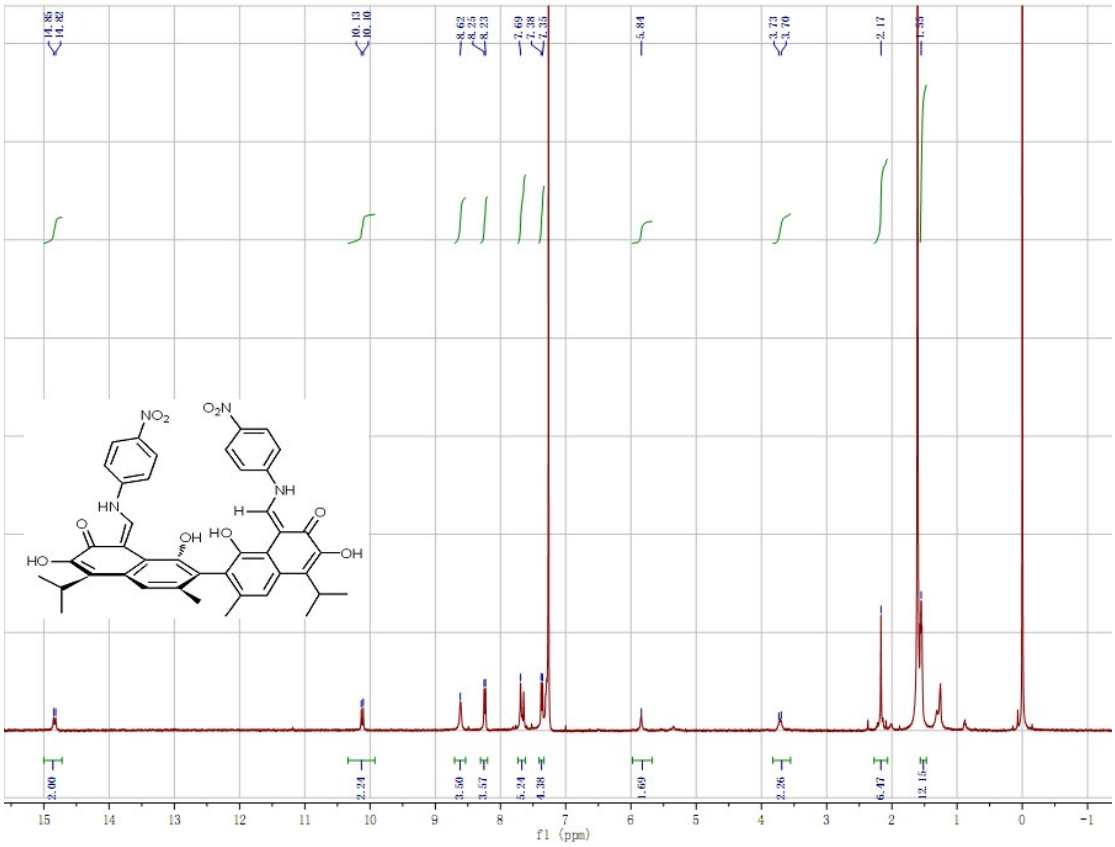


Figure S11. HRMS, ^{1}H NMR and ^{13}C NMR of compound 9.




14.85
14.82
10.13
10.10
8.62
8.25
8.23
7.69
7.38
7.35
5.84
3.73
3.70
2.17
1.35
NO2
O2N
HN
NH
H
O
OH
HO
OH
HO
2.00
2.24
3.50
3.57
5.24
4.38
1.69
2.26
6.47
12.14
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)

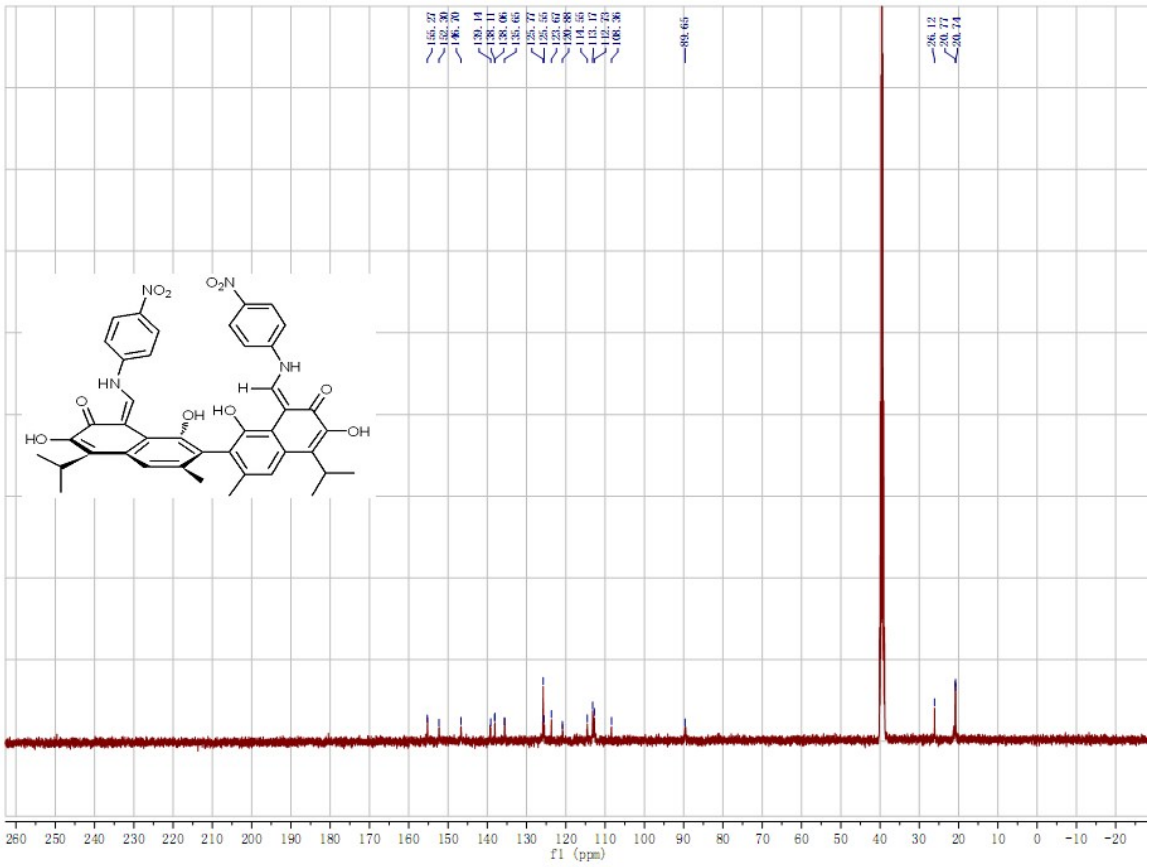


Figure S12. HRMS, ^{1}H NMR and ^{13}C NMR of compound 10

Data of experiments

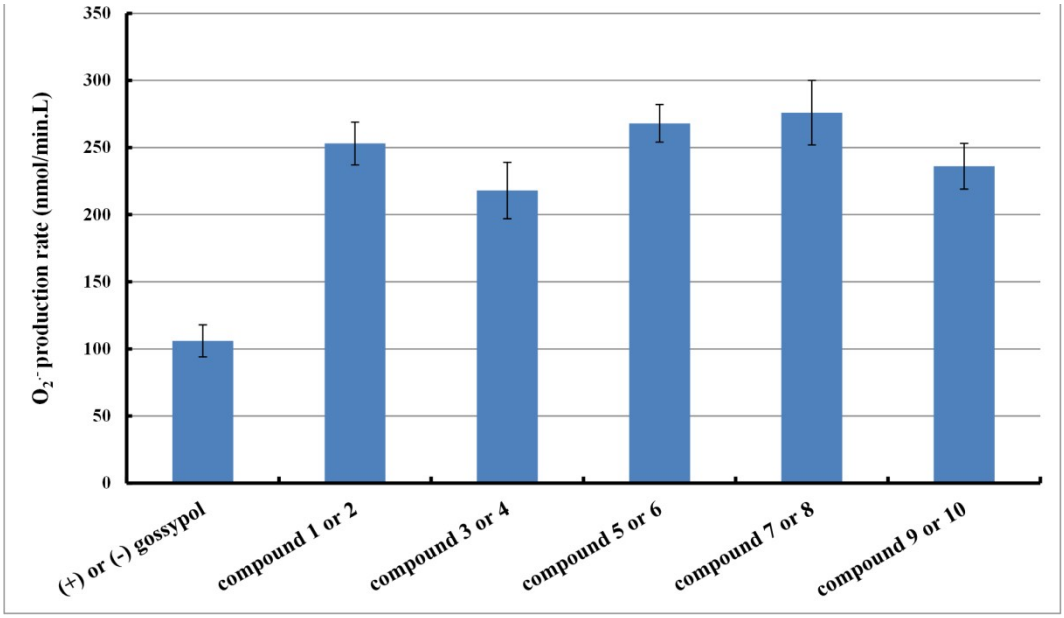


Figure S13. The $O_2^{\cdot-}$ production rate of (+), (-) -gossypol and their Schiff bases at a concentration of 500 μg/mL and time of 5 hs in the solution (DMF:H_2O=1:10).

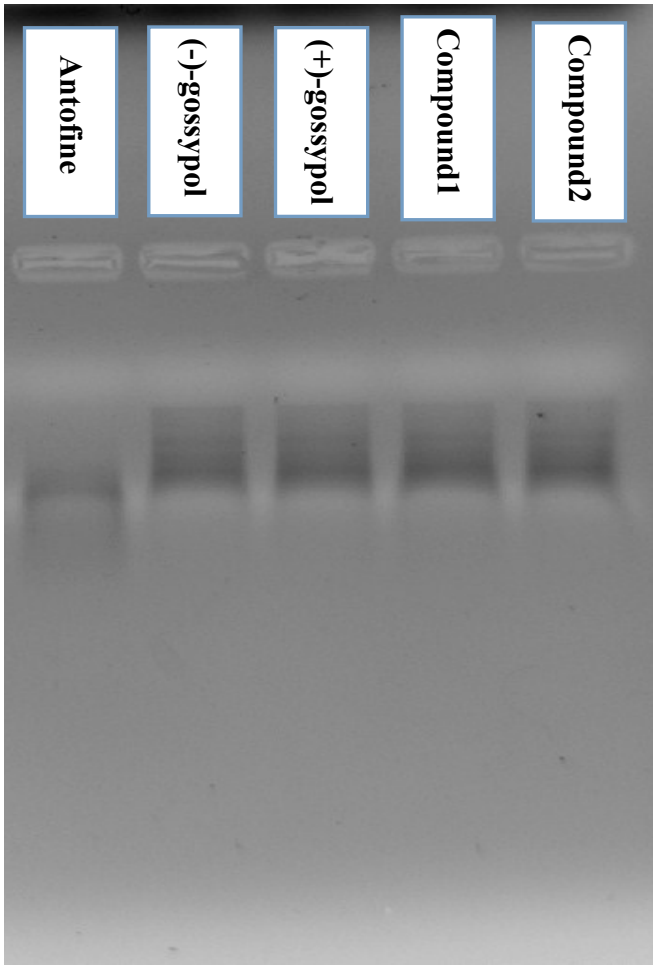


Figure S14 The TMV RNA extracted from the assemble solution, which mixed with the antofine, (-)-gossypol, (+)-gossypol, compound 1 and compound 2, separately.

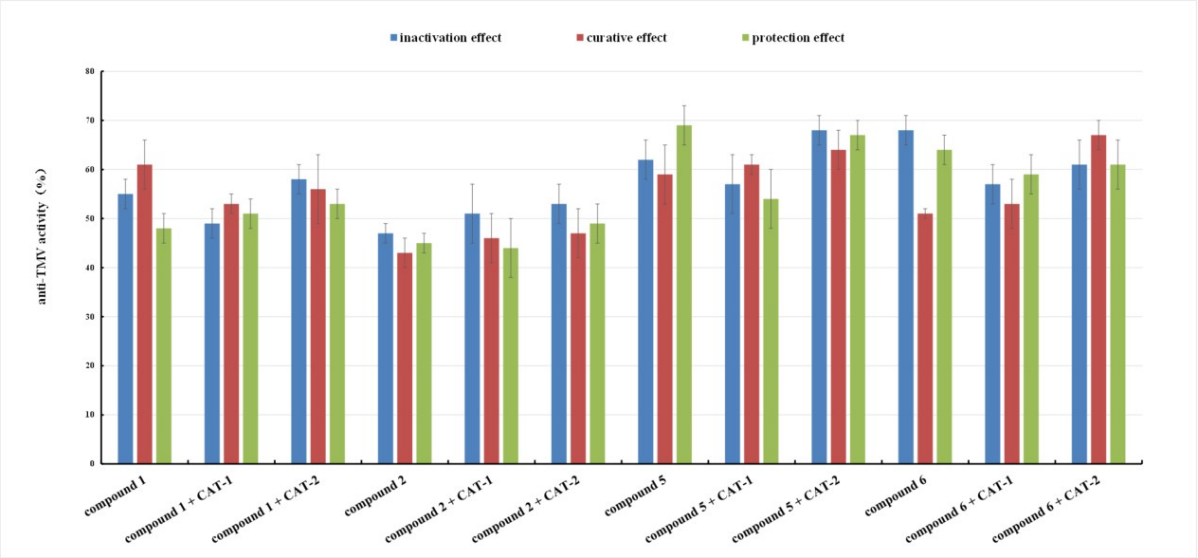


Figure S15. The anti-TMV activities of compound, compound + CAT-1, compound + CAT-2. Compound + CAT-1 refer to adding CAT 10 mins before the compound is applied to tobacco leaves, and compound + CAT-2 refer to adding CAT 10 mins after inoculation of tobacco leaves, which treated by compound for 24hs, with TMV. The means of three independent experiments ± SD are shown.

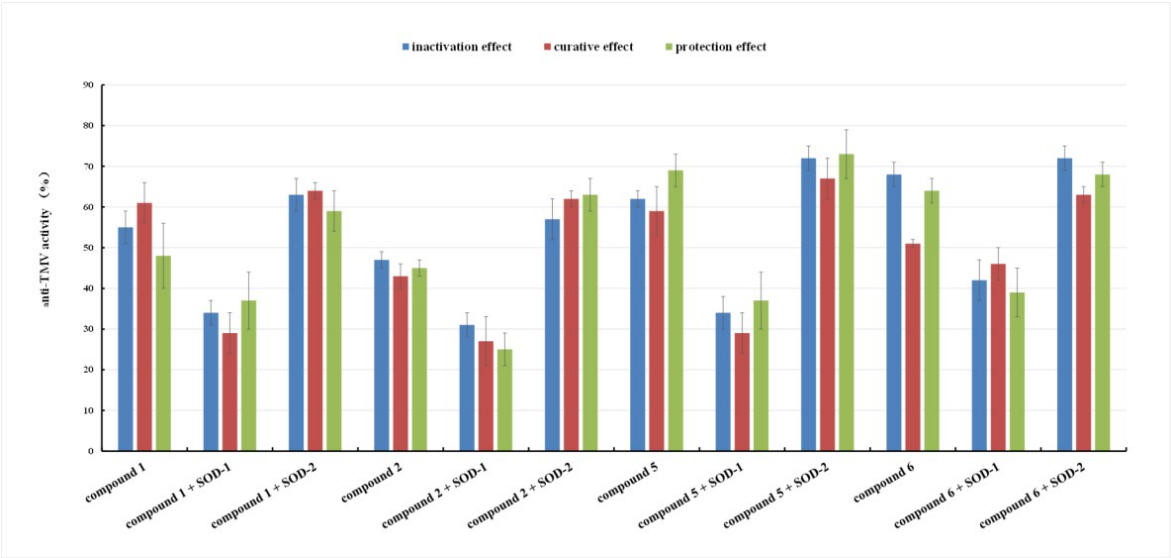
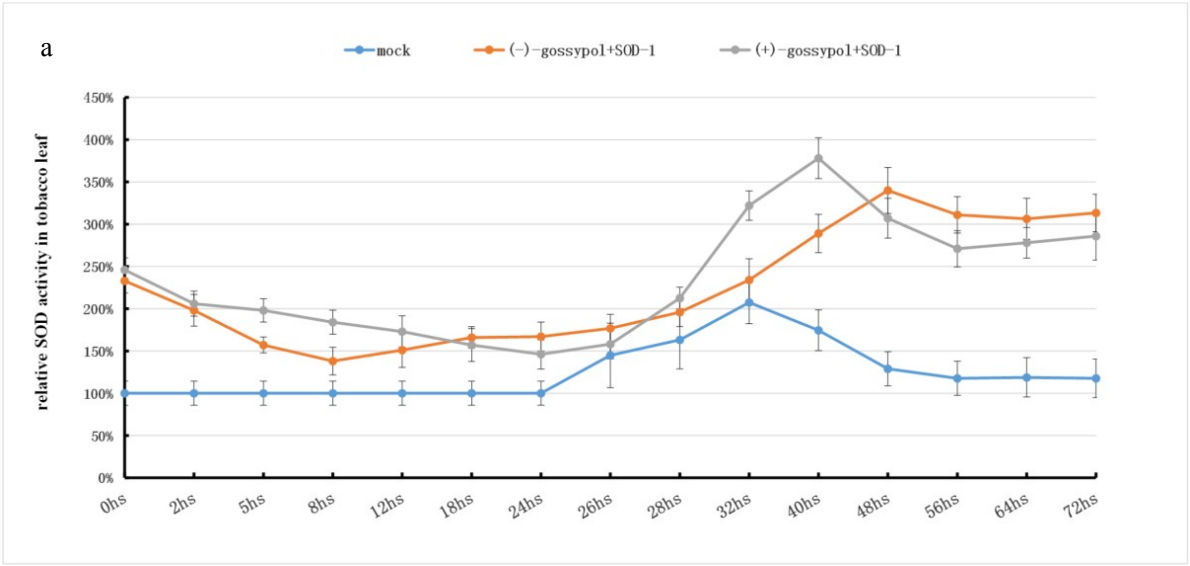


Figure S16. The anti-TMV activities of compound, compound + SOD-1, compound + SOD-2. Compound + SOD-1 refer to adding SOD 10 mins before the compound is applied to tobacco leaves, and compound + SOD-2 refer to adding SOD 10 mins after inoculation of tobacco leaves, which treated by compound for 24hs, with TMV. The means of three independent experiments ± SD are shown.



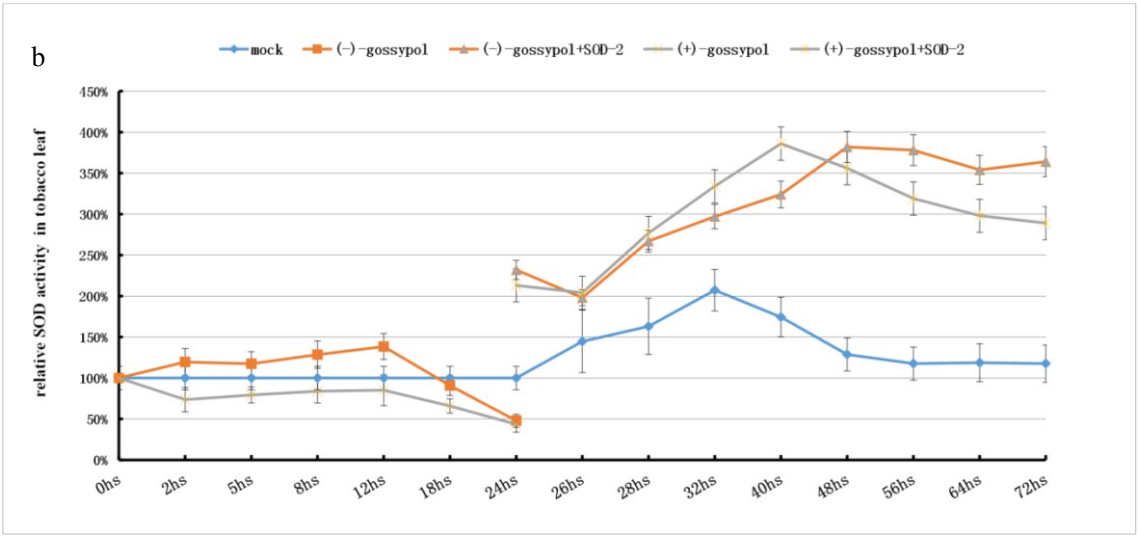


Figure S17. (a) The change of SOD activities in tobacco leaves treated by (-)-gossypol + SOD-1 and (+)-gossypol + SOD-1. (b) The change of SOD activities in tobacco leaves treated by (-)-gossypol + SOD-2 and (+)-gossypol + SOD-2. Compound + SOD-1 refer to adding SOD 10 mins before the compound is applied to tobacco leaves, and compound + SOD-2 refer to adding SOD 10 mins after inoculation of tobacco leaves, which treated by compound for 24hs, with TMV. The means of three independent experiments ± SD are shown.

Table S1. The $O_2^{\cdot-}$ production rate in tobacco leaf.

position of leaves	data	the $O_2^{\cdot-}$ production rate at the time of 2 h

		Plant A	Plant B	Plant C	Plant D	Plant E
Upper	primary data (nmol/mg. min)	232	392	337	324	384
	reference (nmol/mg. min)	134	213	208	177	233
	relative data (nmol/mg. min)	173%	184%	176%	183%	165%
Middle	primary data (nmol/mg. min)	190	320	327	279	313
	reference (nmol/mg. min)	125	192	185	166	210
	relative data	152%	167%	177%	168%	149%
Lower	primary data (nmol/mg. min)	177	276	266	236	264
	reference (nmol/mg. min)	113	179	164	151	193
	relative data	157%	154%	162%	156%	137%