Supplementary Material

Monarubins A–C from the Marine Shellfish-Associated Fungus *Monascus ruber* BB5

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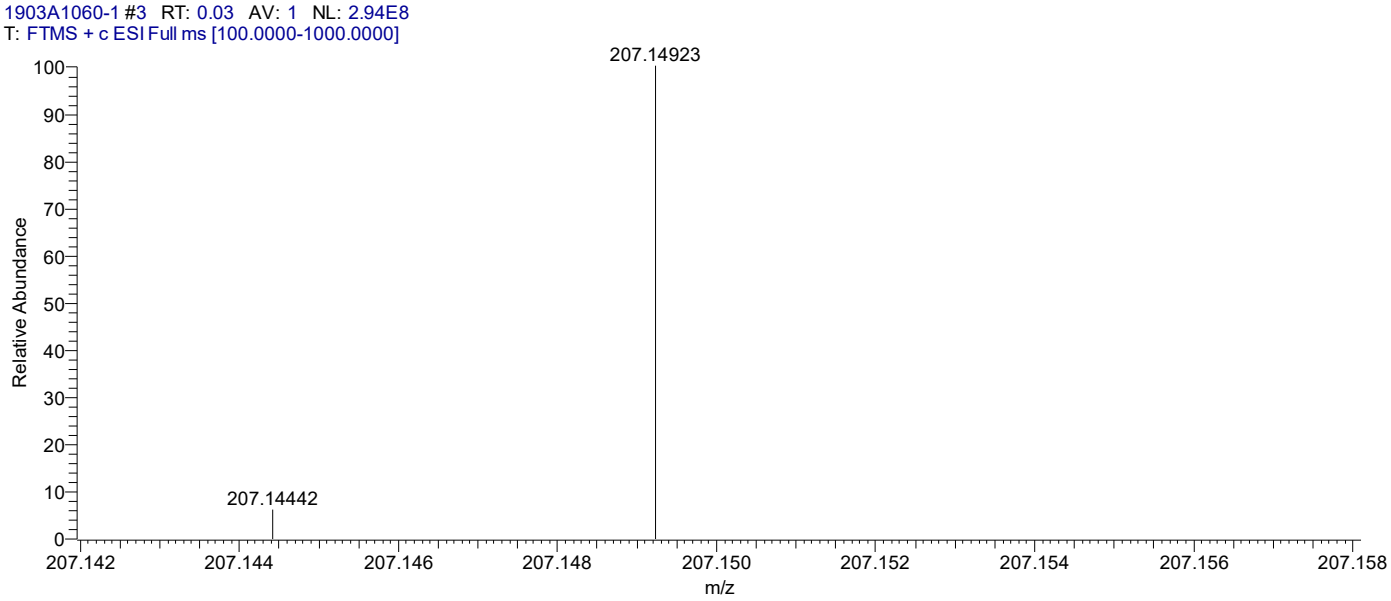
List of contents:



SPECTRUM-simulation :

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
207.14923	207.14919	0.19	4.5	C12 H19 O N2

Figure S1. HR-(+) ESI-MS spectrum of monarubin A (**1**).

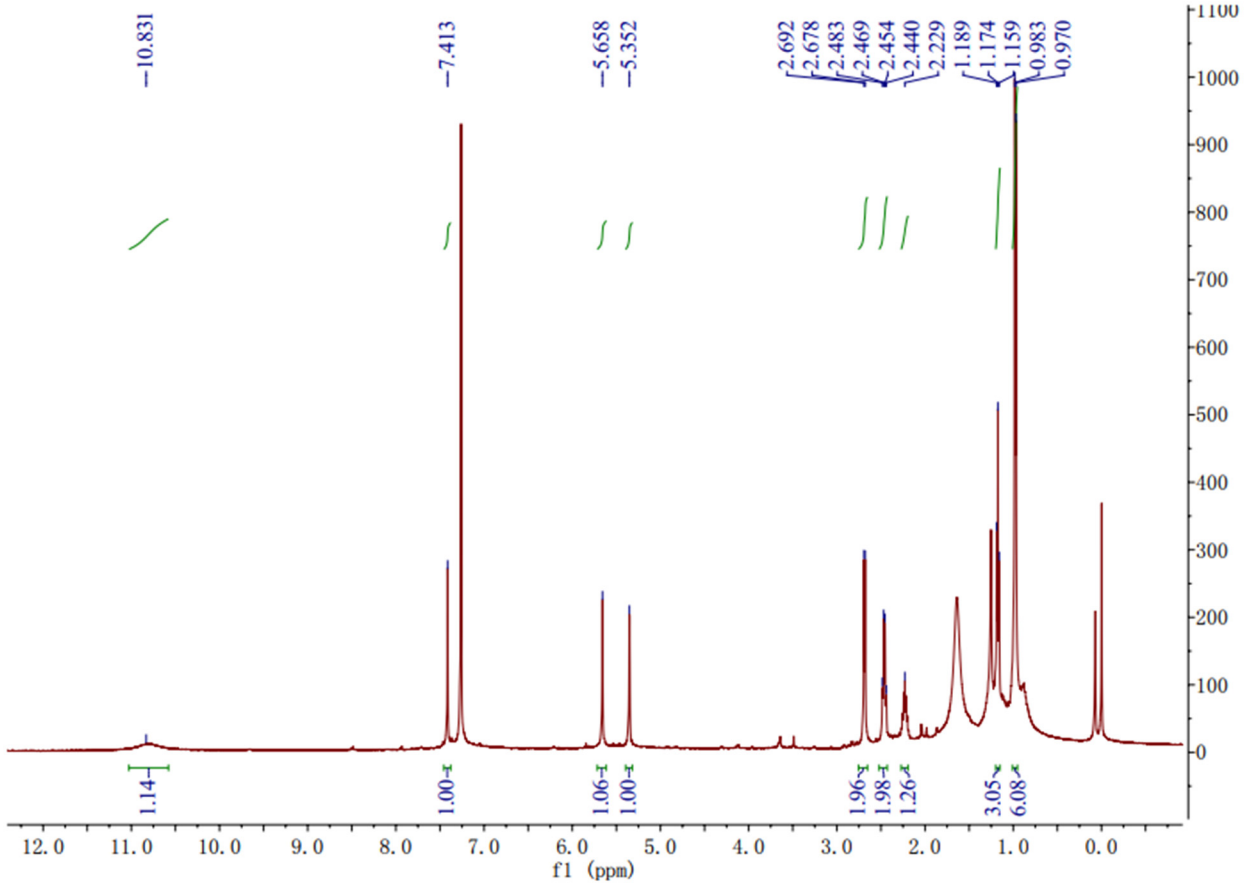


Figure S2. ^{1}H NMR spectrum of monarubin A (**1**) in $CDCl_3$ (500 MHz).

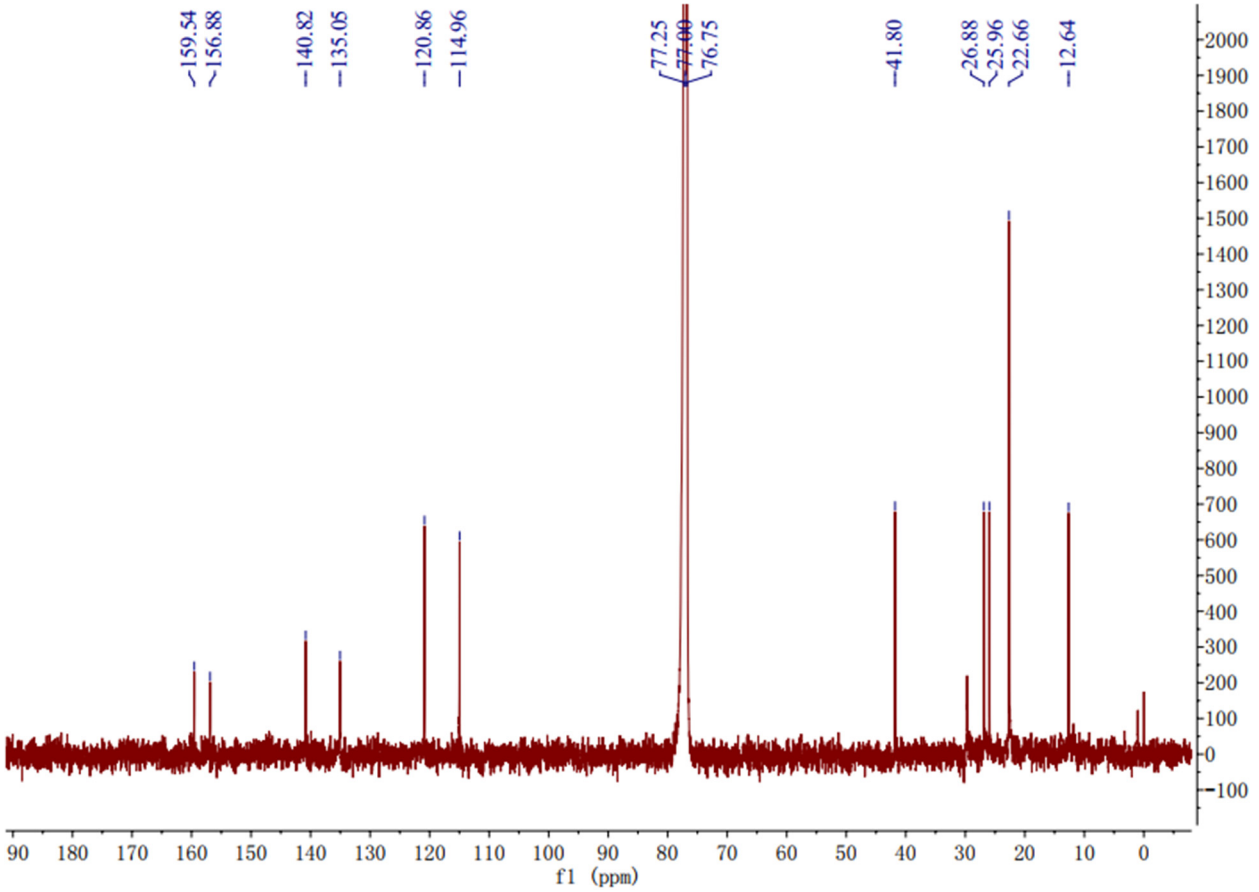


Figure S3. ^{13}C NMR spectrum of monarubin A (**1**) in $CDCl_3$ (125 MHz).

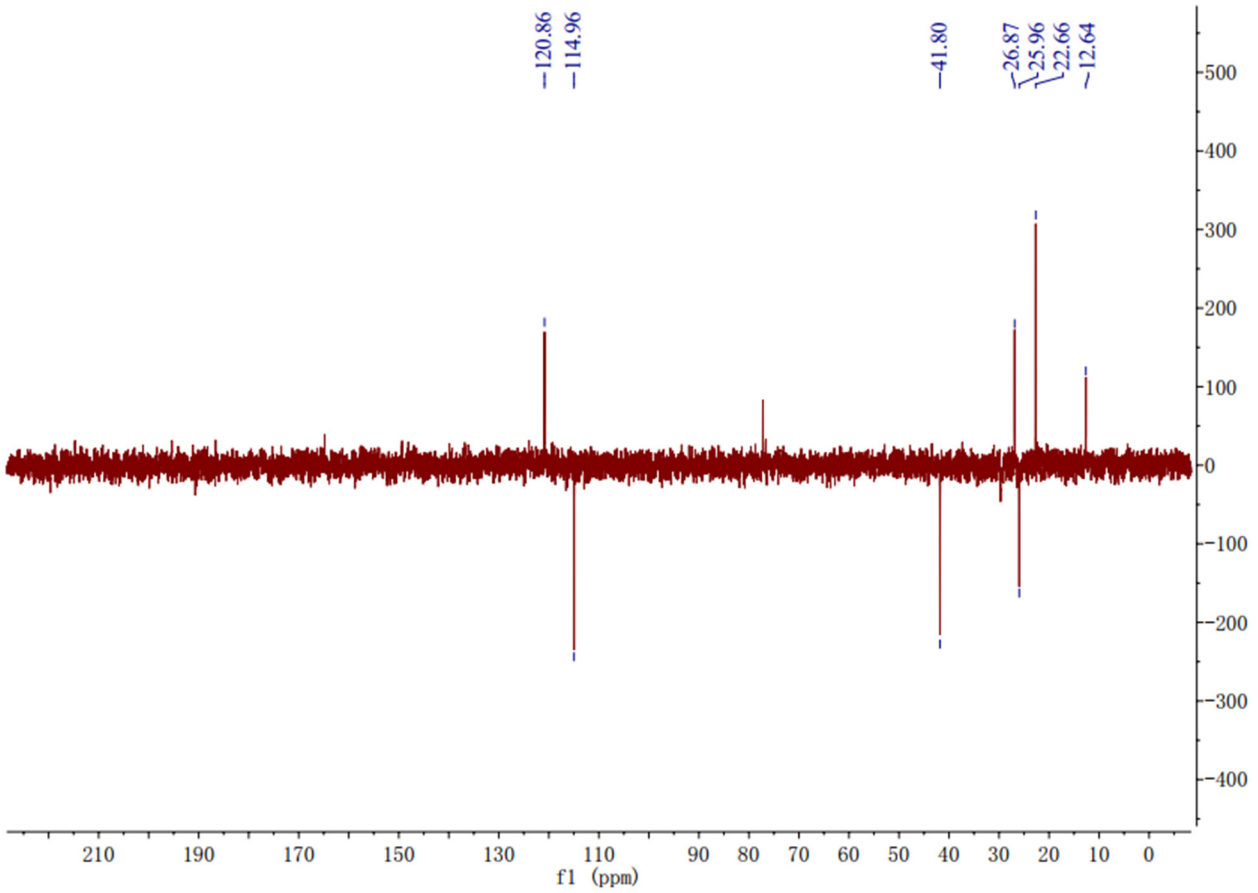


Figure S4. DPET 135 spectrum of monarubin A (**1**) in $CDCl_3$ (125 MHz).

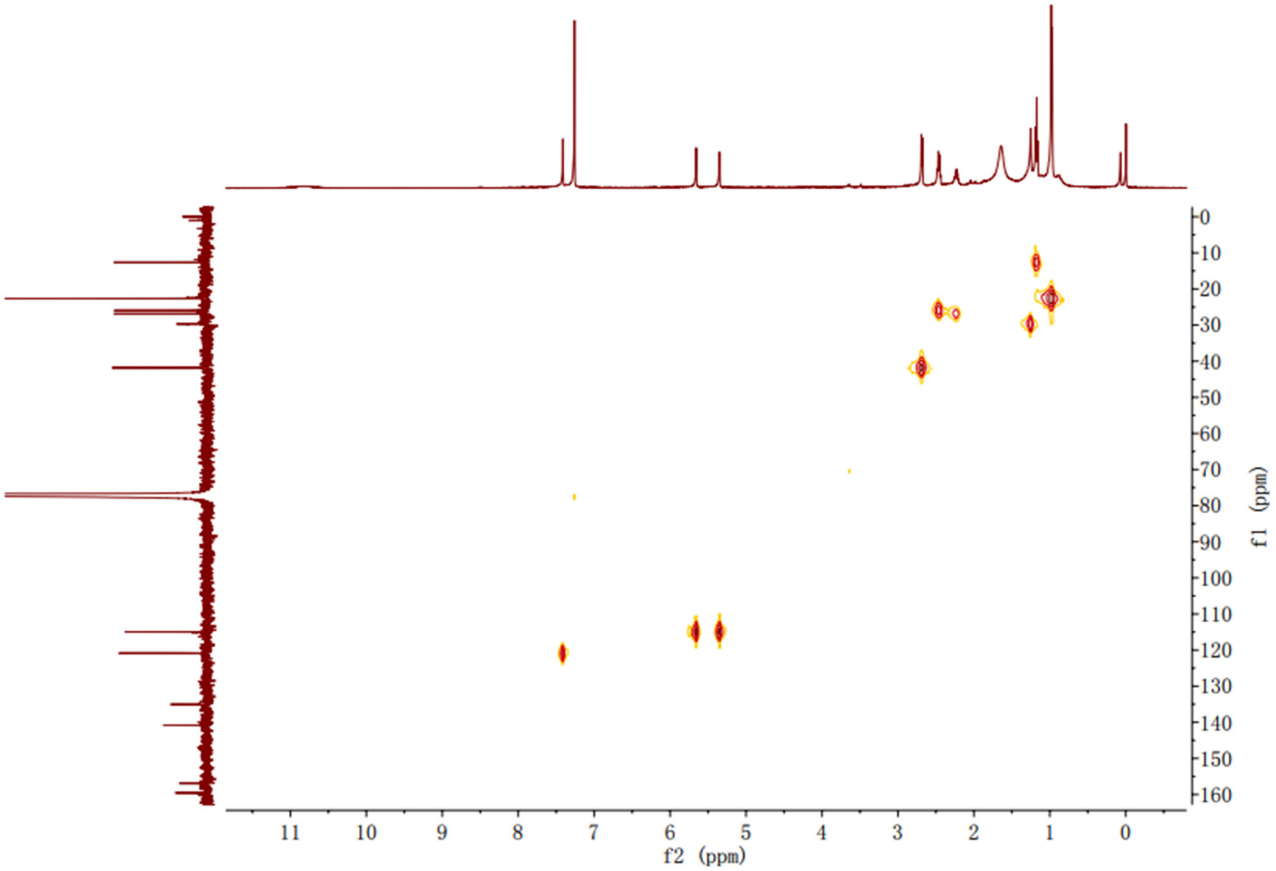


Figure S5. HMQC spectrum of monarubin A (**1**) in $CDCl_3$.

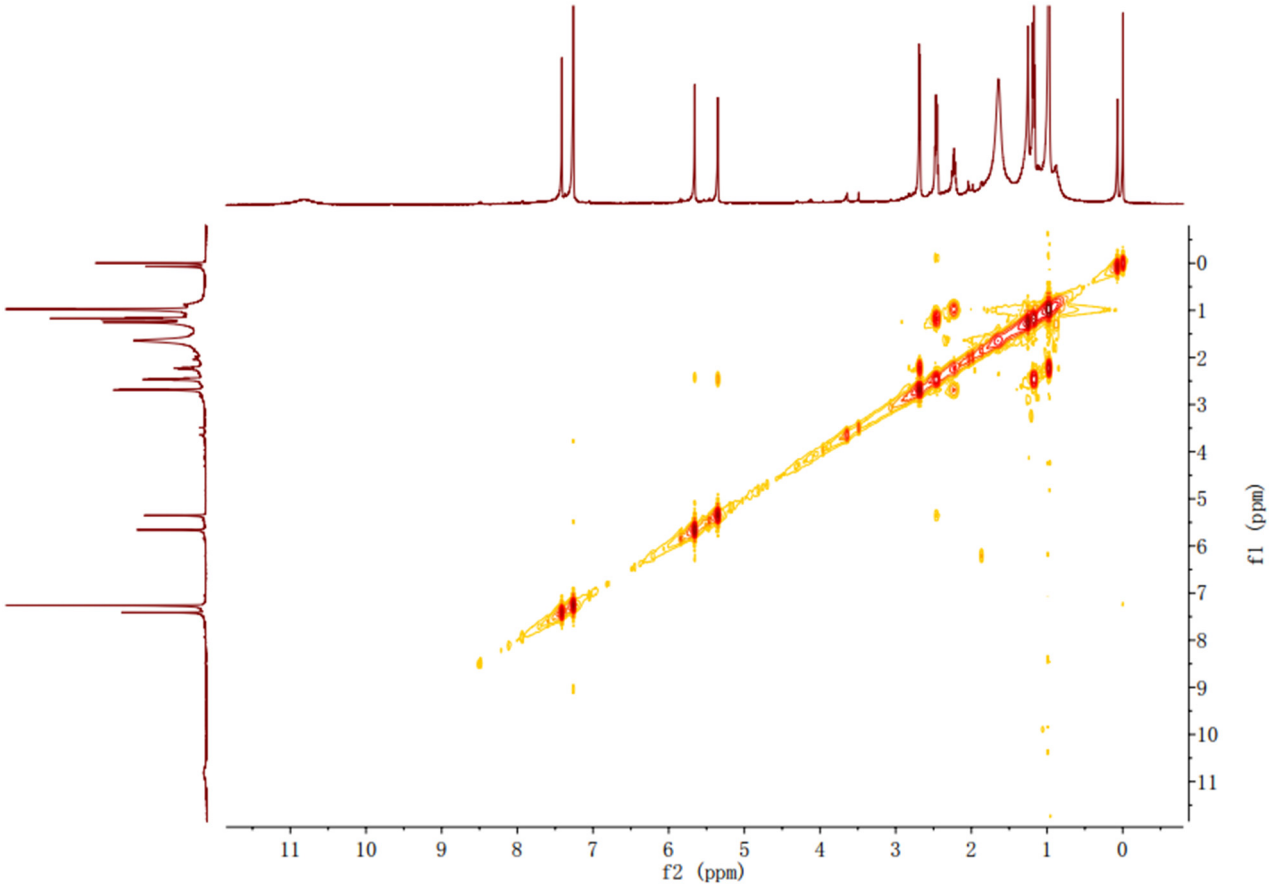


Figure S6. ^{1}H–$^{1}HCOSY$ spectrum of monarubin A (**1**) in $CDCl_3$.

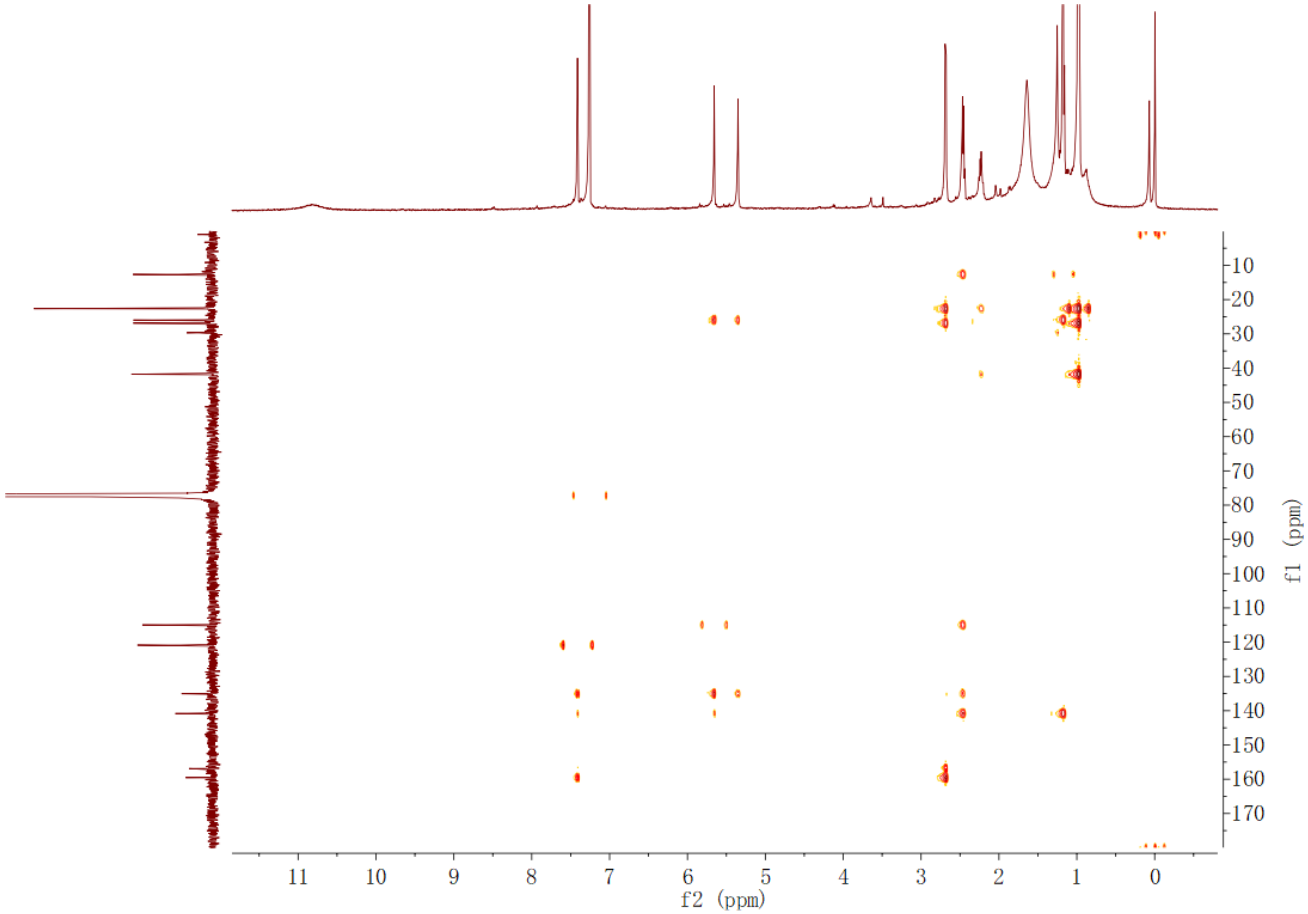


Figure S7. HMBC spectrum of monarubin A (**1**) in $CDCl_3$.

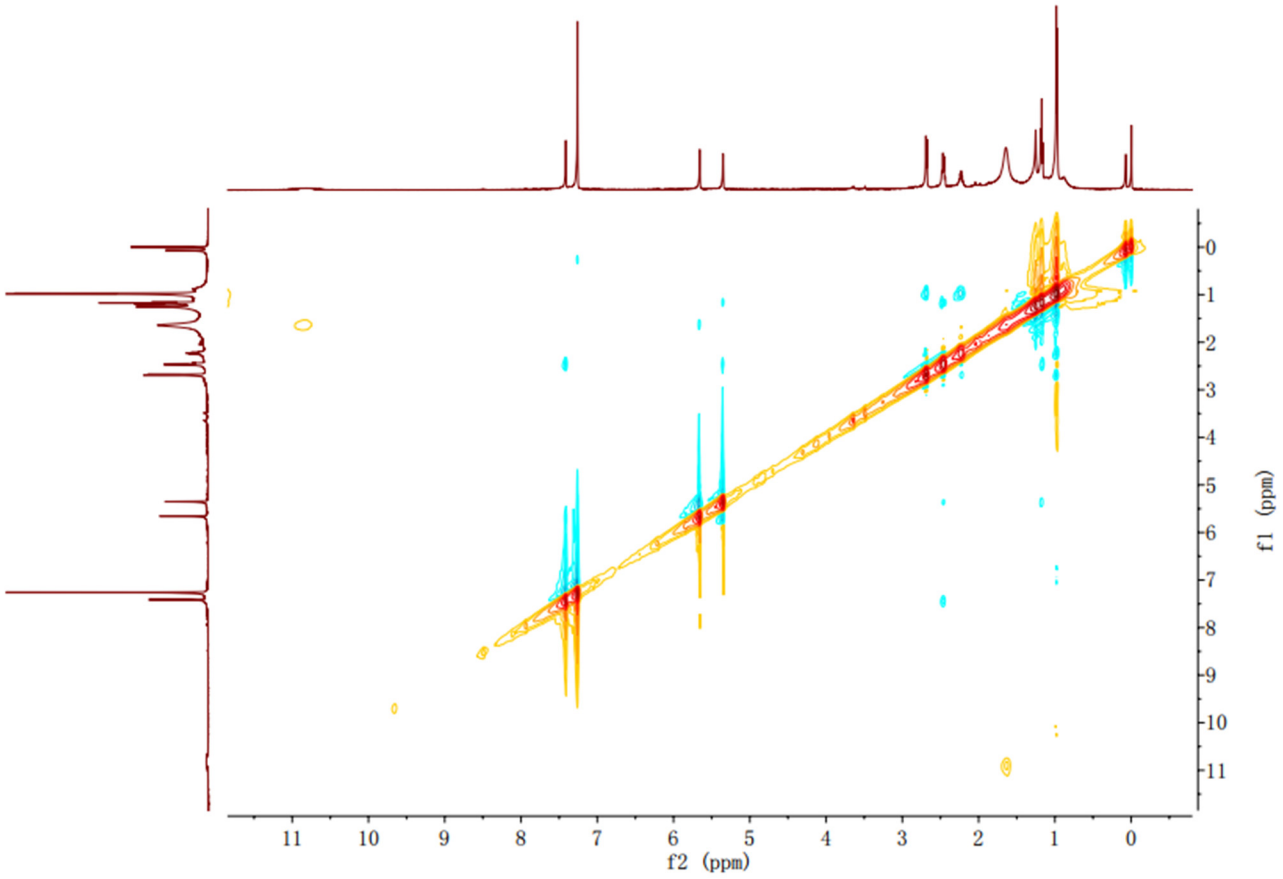


Figure S8. NOEY spectrum of monarubin A (**1**) in $CDCl_3$.

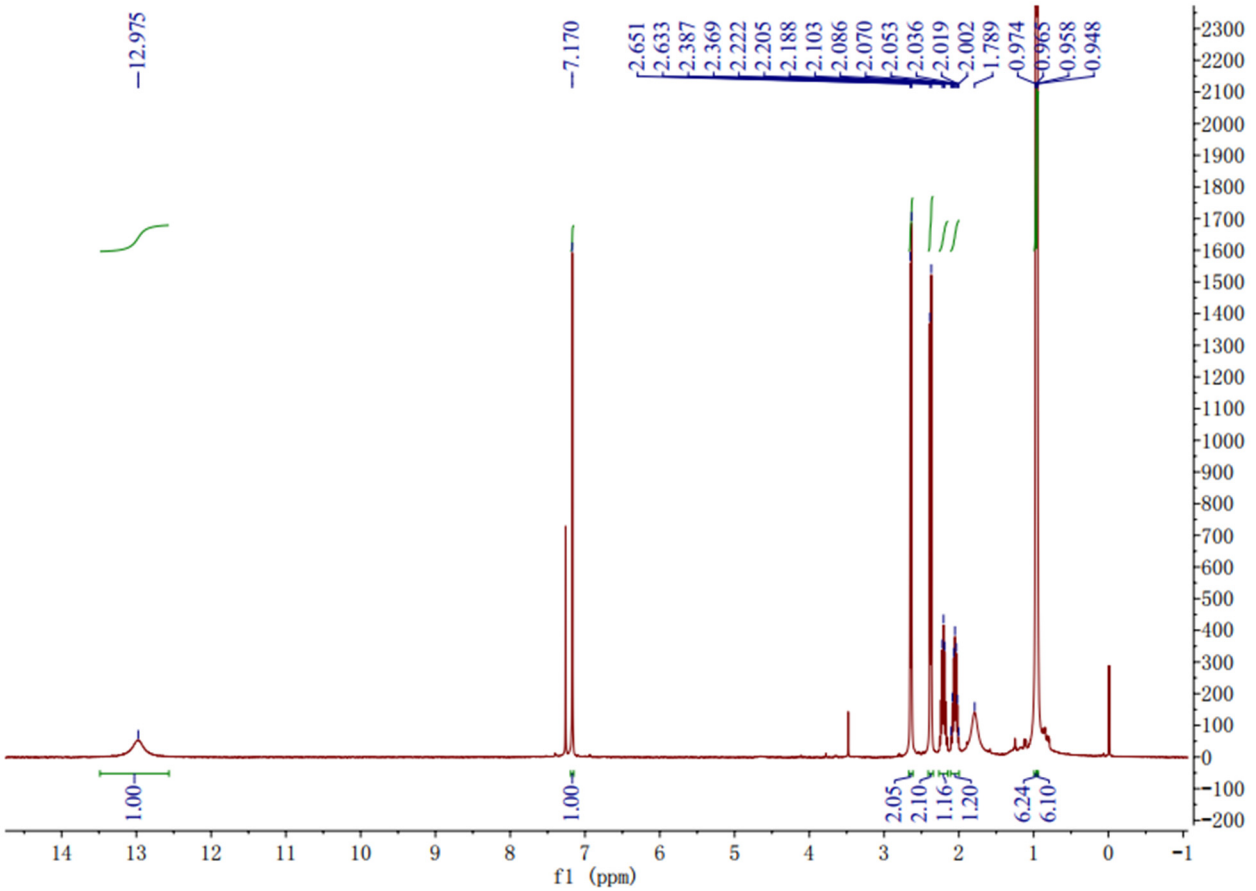


Figure S9. ^{1}H NMR spectrum of 3,6-diisobutyl-2(1*H*)-pyrazinone (**2**) in $CDCl_3$ (400 MHz).

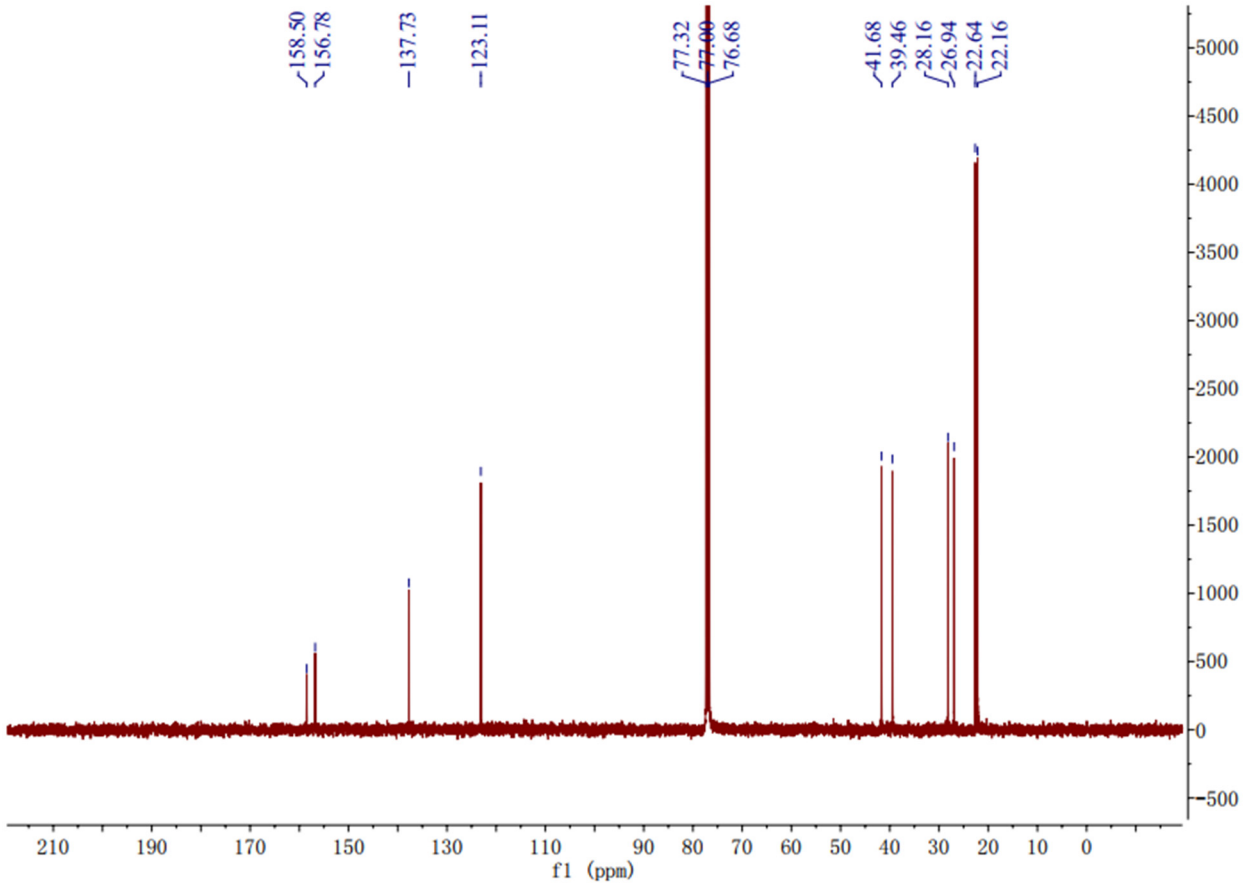
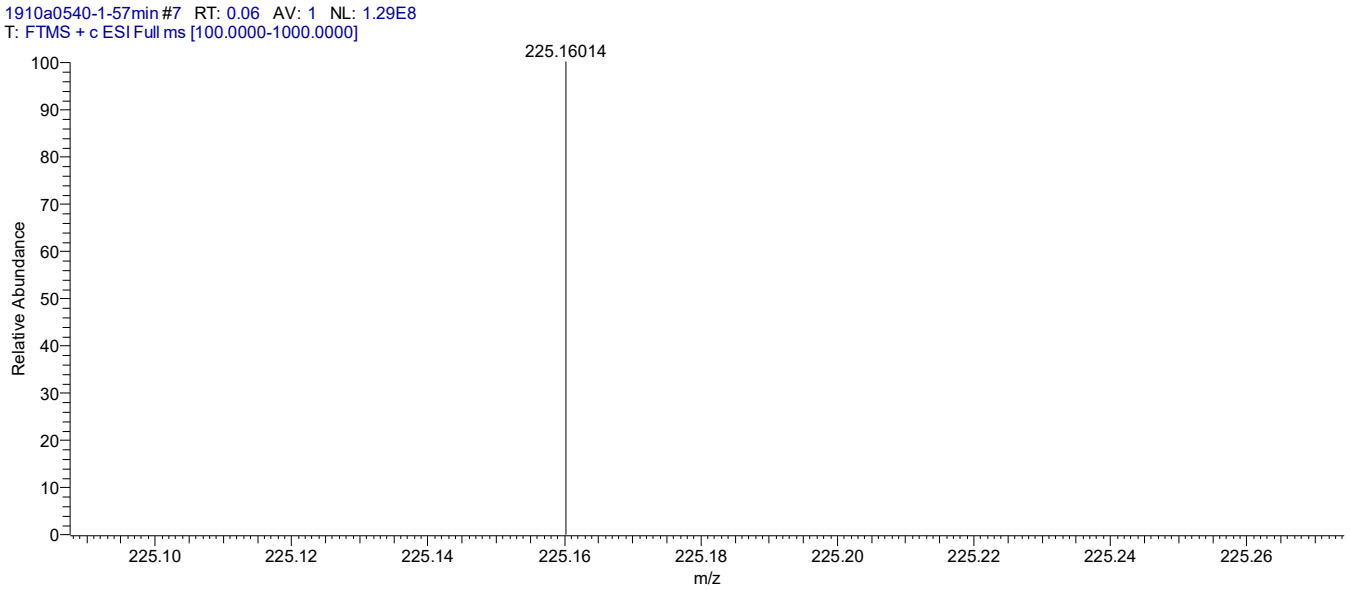


Figure S10. ^{13}C NMR spectrum of 3,6-diisobutyl-2(1*H*)-pyrazinone (**2**) in $CDCl_3$ (100 MHz).



SPECTRUM-simulation :

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
225.16019	225.15975	1.93	3.5	C12 H21 O2 N2

Figure S11. HR-(+) ESI-MS spectrum of deoxyhydroxyaspergillic acid (**3**).

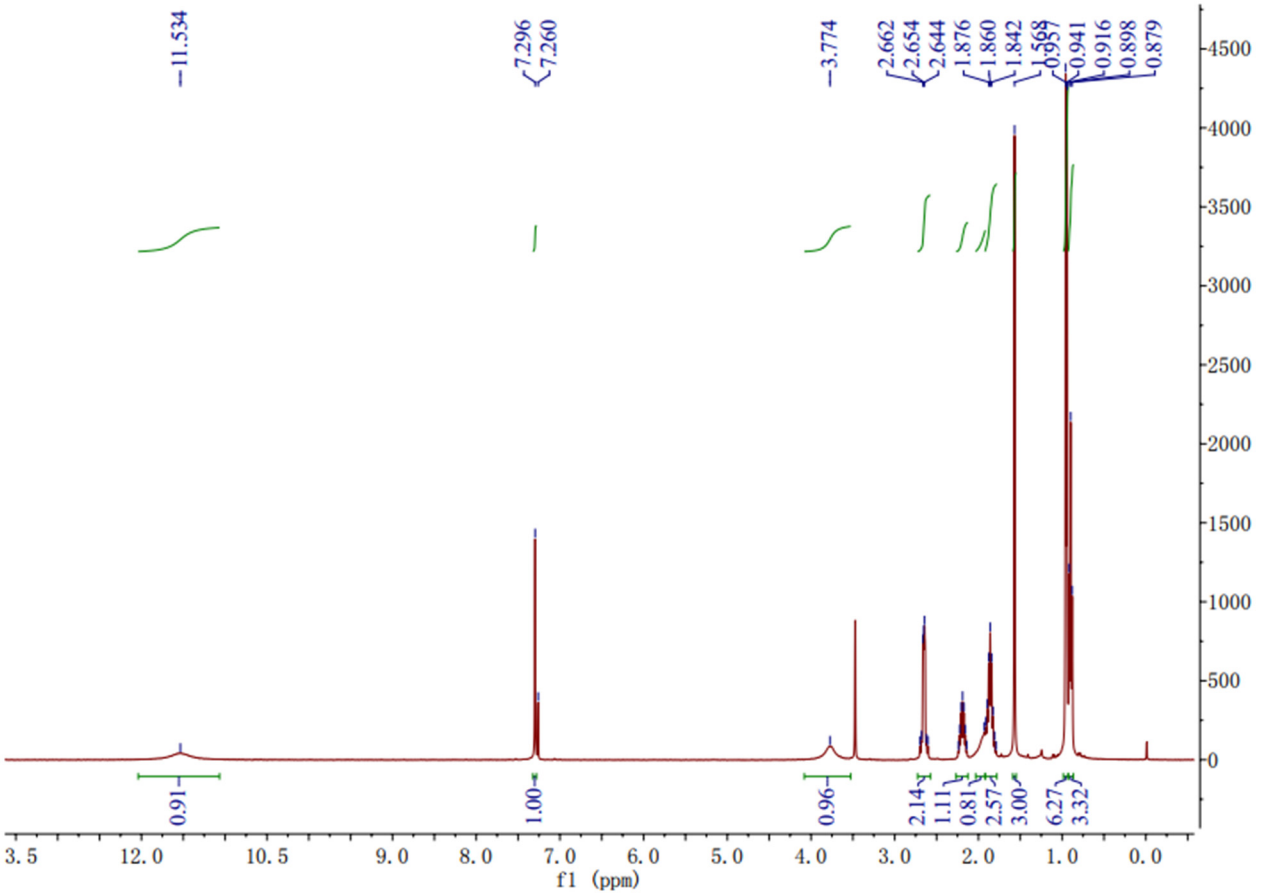


Figure S12. ^{1}H NMR spectrum of deoxyhydroxyaspergillic acid (**3**) in $CDCl_3$ (400 MHz).

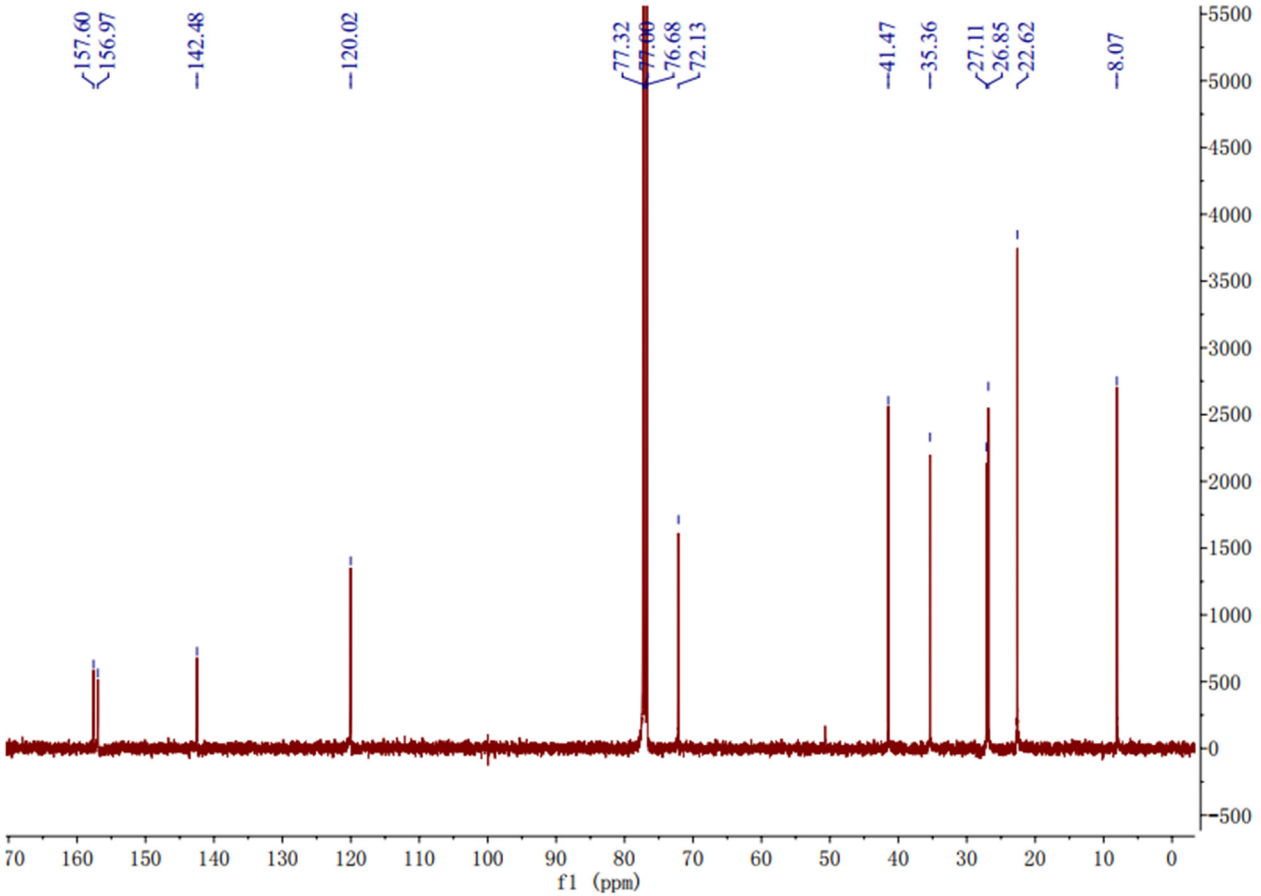
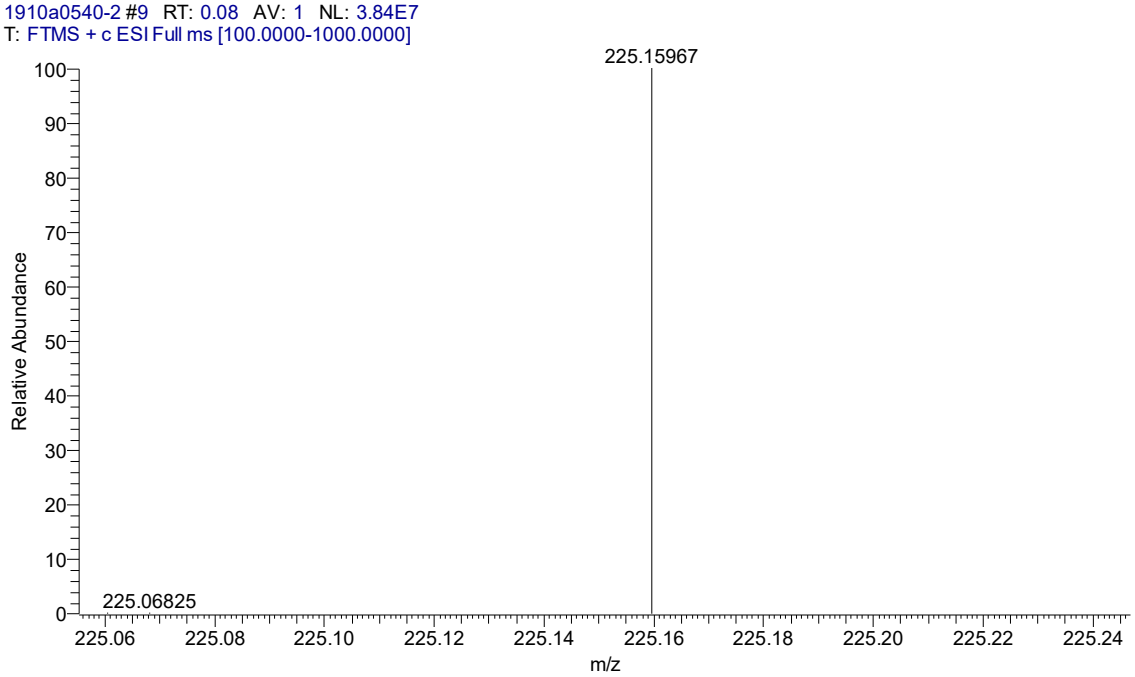


Figure S13. ^{13}C NMR spectrum of deoxyhydroxyaspergillic acid (**3**) in $CDCl_3$ (100 MHz).



SPECTRUM-simulation :

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
225.15967	225.15975	-0.37	3.5	C12 H21 O2 N2

Figure S14. HR-(+) ESI-MS spectrum of 2-hydroxy-6-(1-hydroxy-1-methylpropyl) 3-*sec*-buthylpyrazine (**4**).

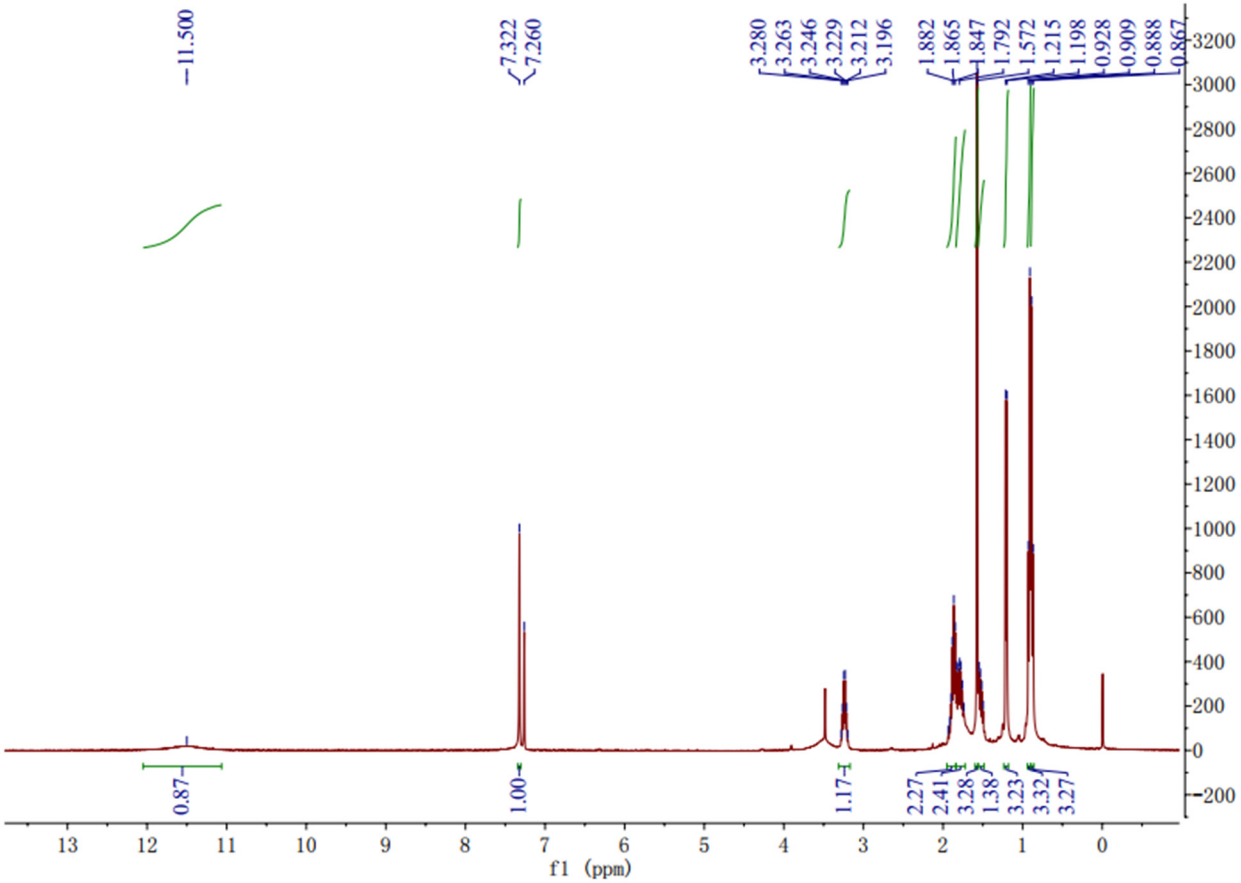


Figure S15. ^{1}H NMR spectrum of 2-hydroxy-6-(1-hydroxy-1-methylpropyl) 3-*sec*-buthylpyrazine (**4**) in $CDCl_3$ (400 MHz).

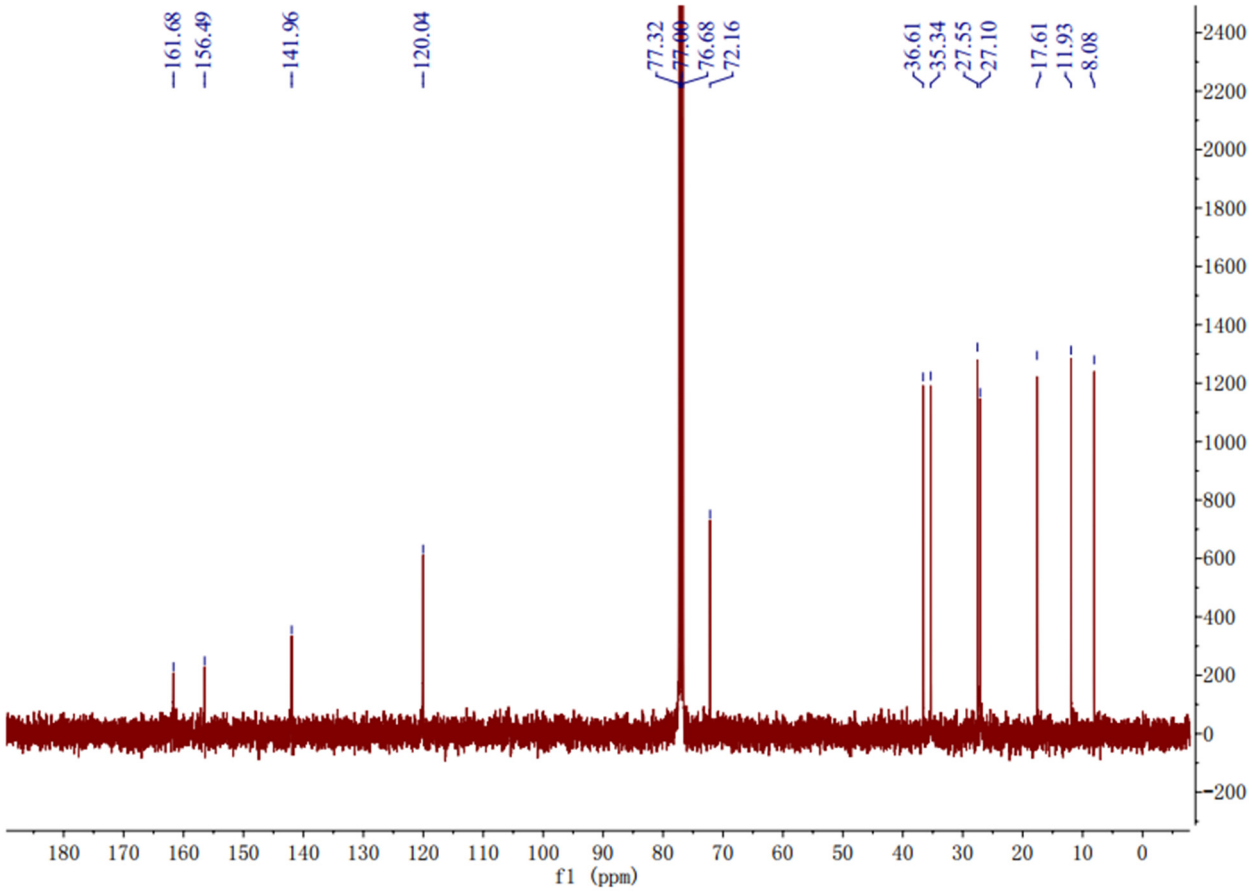


Figure S16. ^{13}C NMR spectrum of 2-hydroxy-6-(1-hydroxy-1-methylpropyl) 3-*sec*-buthylpyrazine (**4**) in $CDCl_3$ (100 MHz).

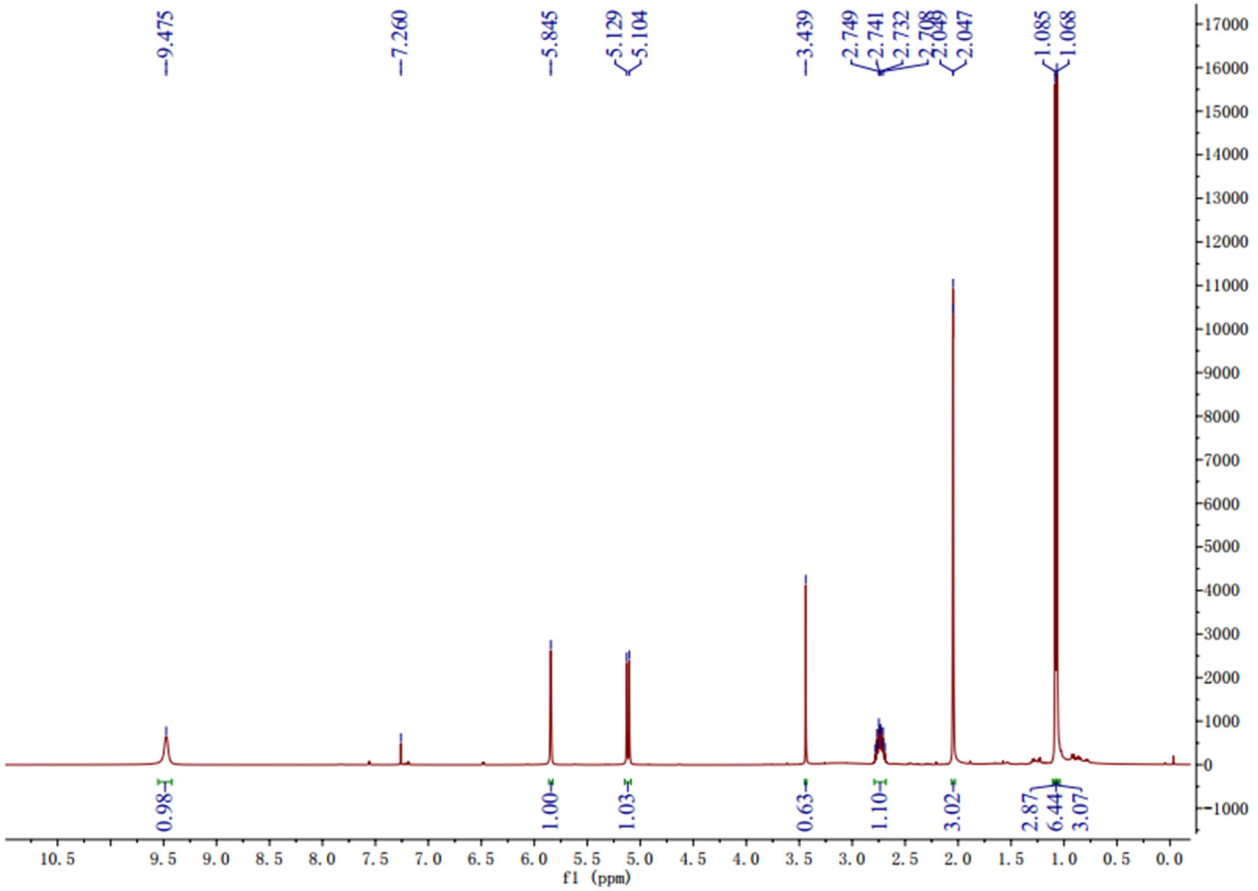


Figure S17. ^{1}H NMR spectrum of pulchellalactam (**5**) in $CDCl_3$ (400 MHz).

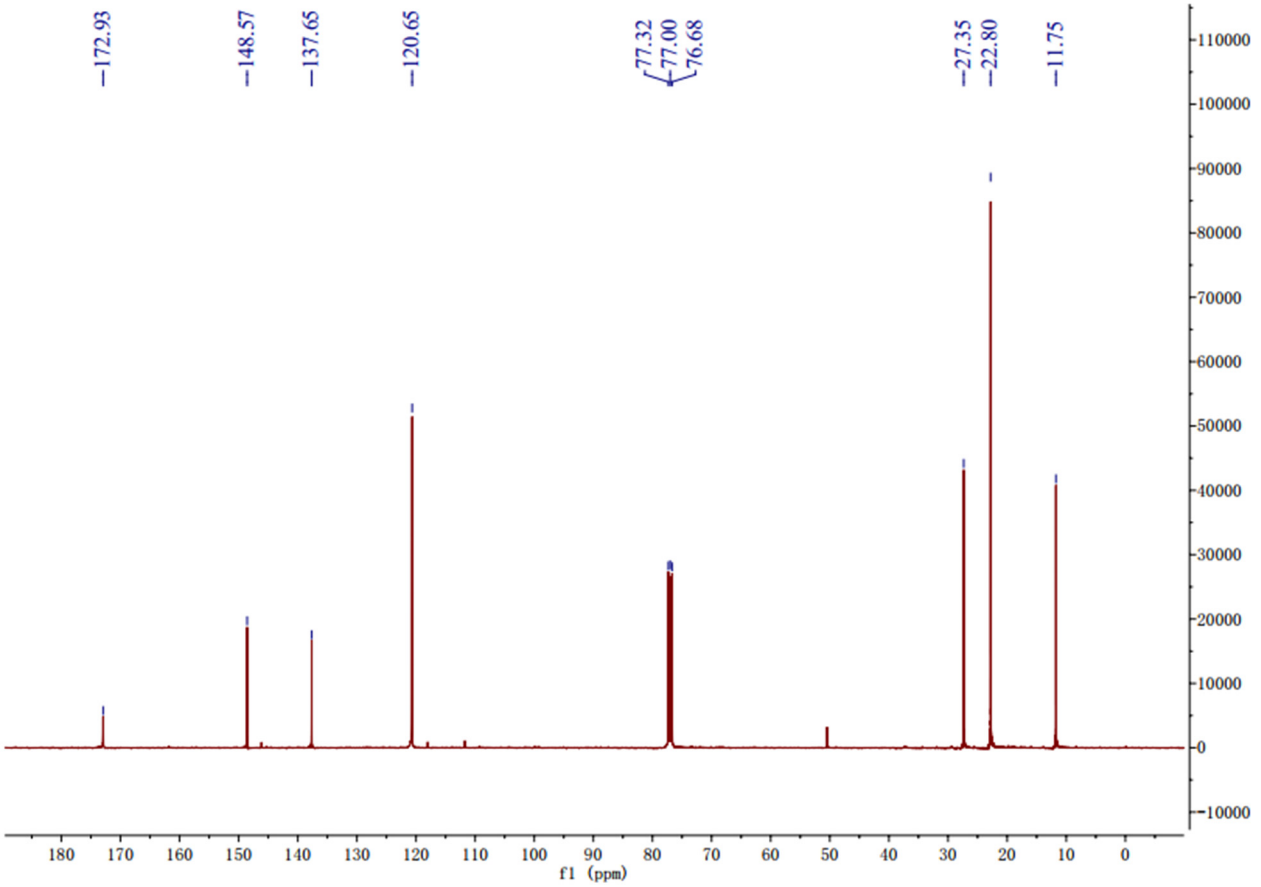
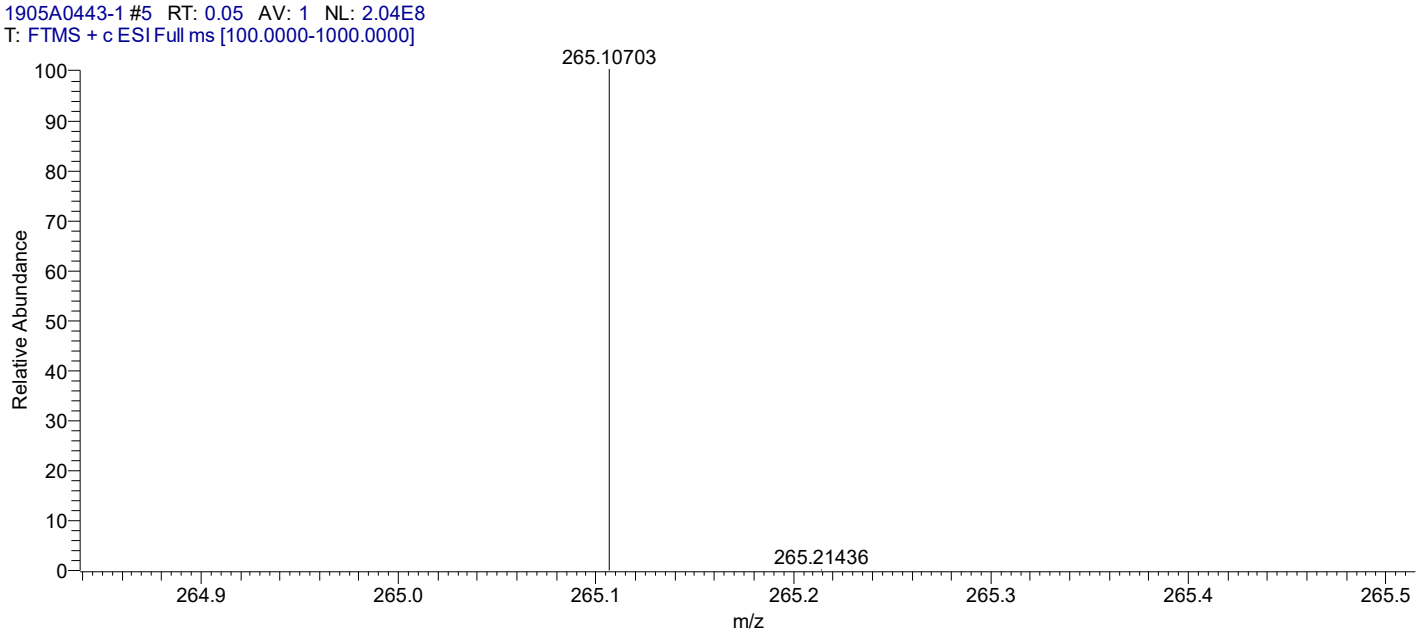


Figure S18. ^{13}C NMR spectrum of pulchellalactam (**5**) in $CDCl_3$ (100 MHz).



SPECTRUM - simulation :				
m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
265.10703	265.10705	-0.08	6.5	C14 H17 O5

Figure S19. HR-(+) ESI-MS spectrum of monarubin B (**6**).

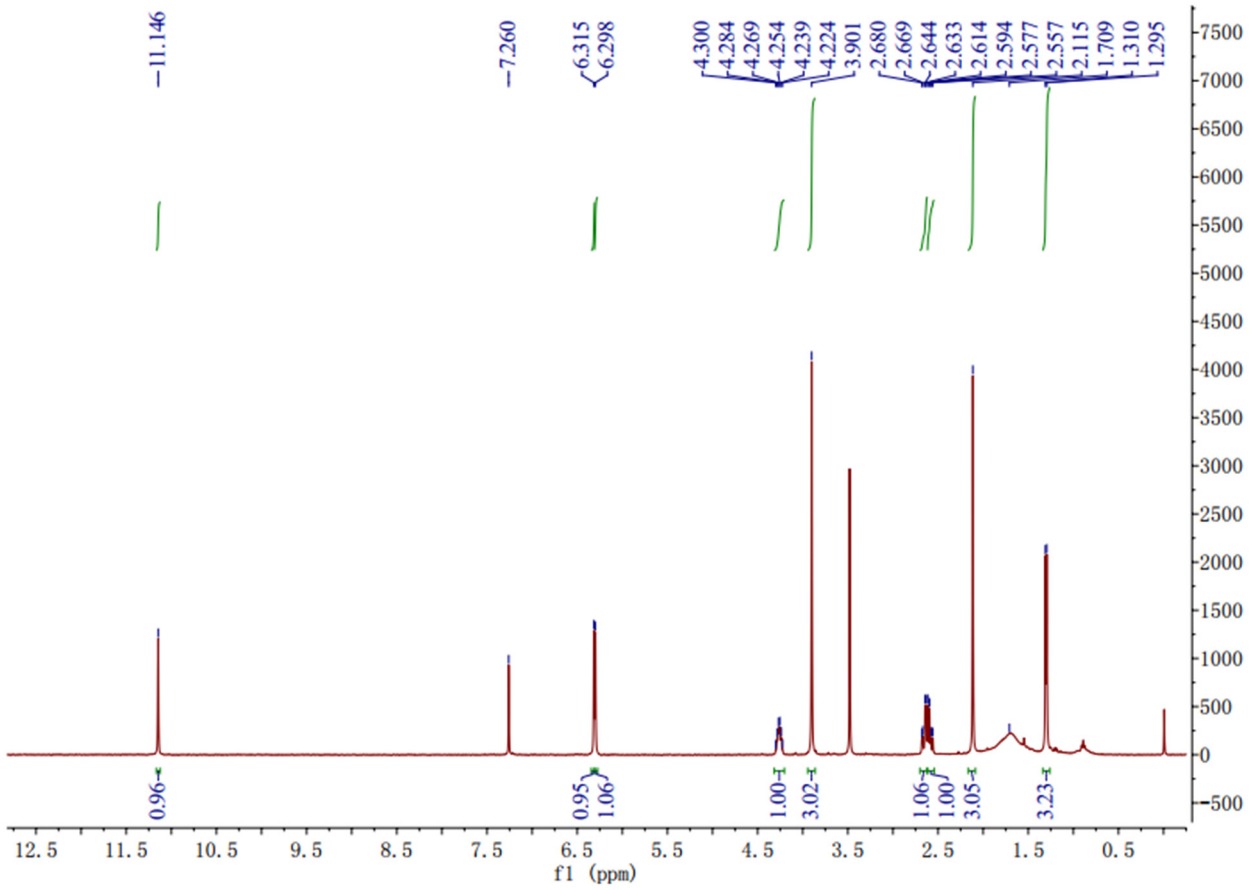


Figure S20. ^{1}H NMR spectrum of monarubin B (**6**) in $CDCl_3$ (400 MHz).

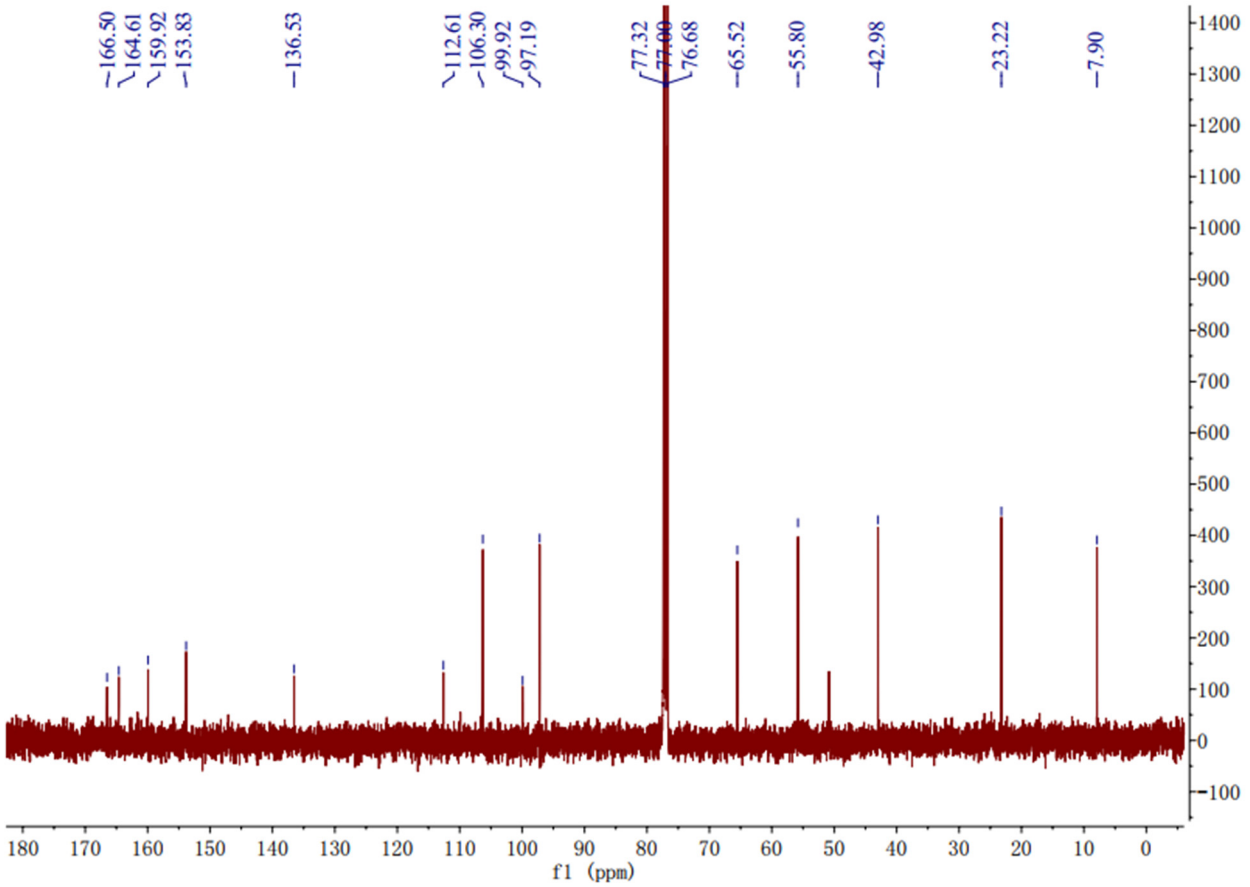


Figure S21. ^{13}C NMR spectrum of monarubin B (**6**) in $CDCl_3$ (100 MHz).

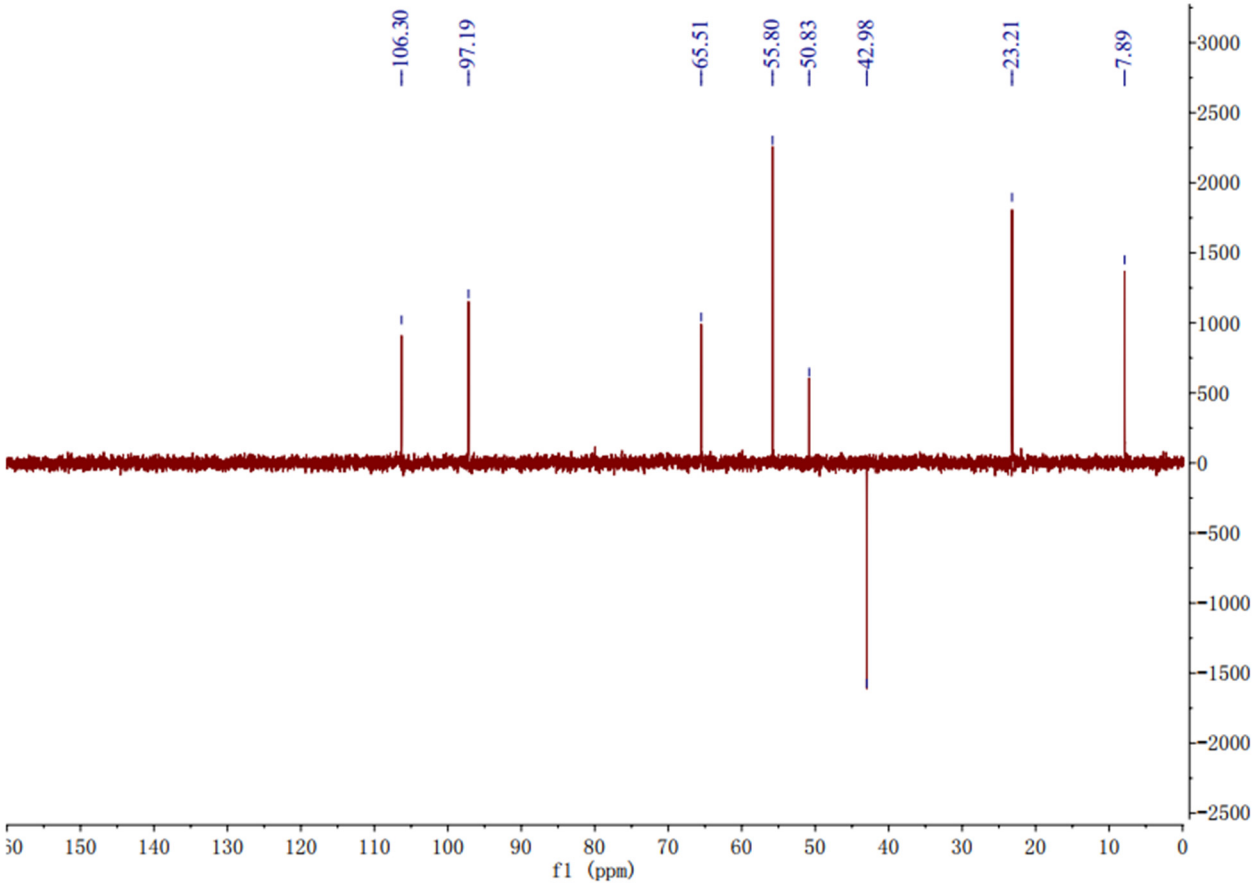


Figure S22. DEPT 135 spectrum of monarubin B (**6**) in $CDCl_3$ (100 MHz).

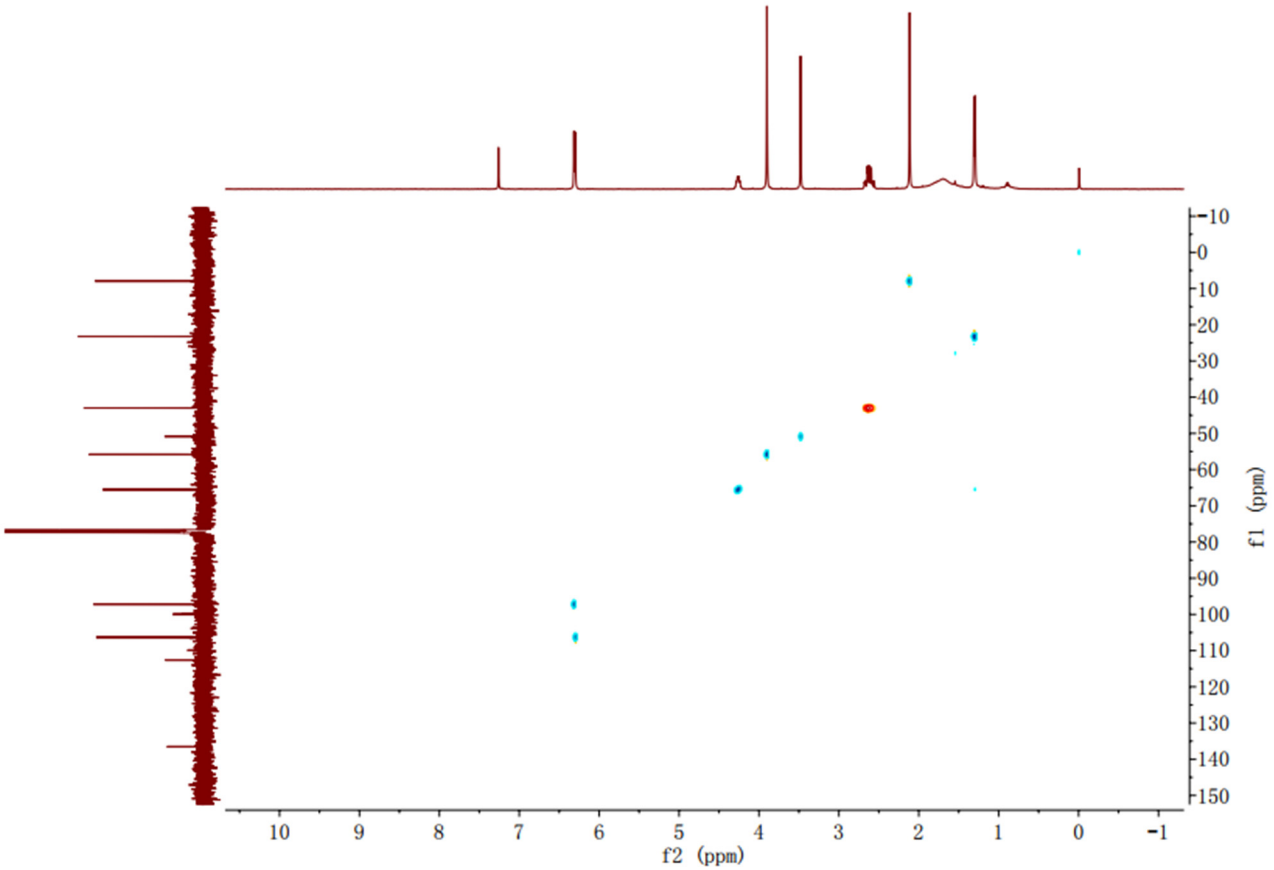


Figure S23. HMQC spectrum of monarubin B (**6**) in $CDCl_3$.

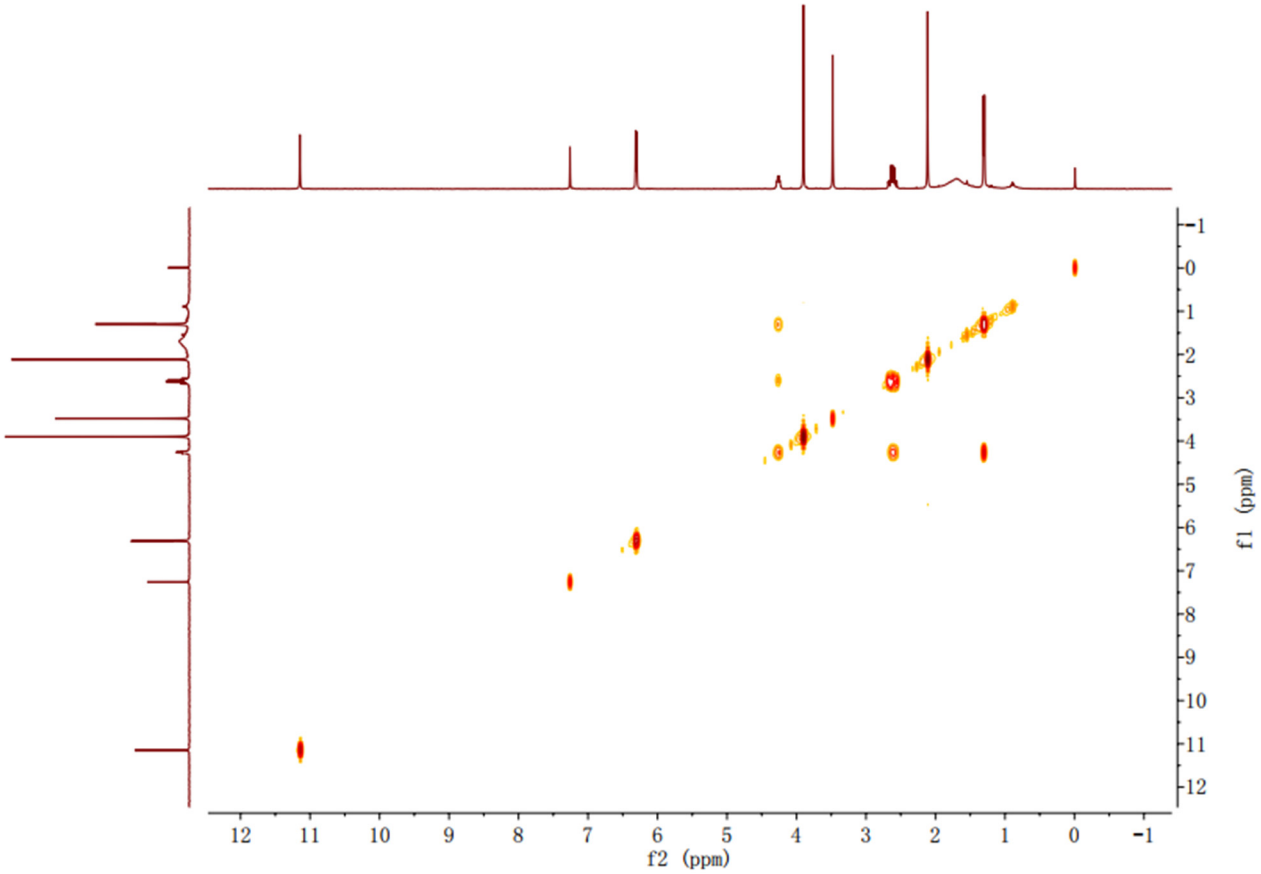


Figure S24. ^{1}H–^{1}H COSY spectrum of monarubin B (**6**) in $CDCl_3$.

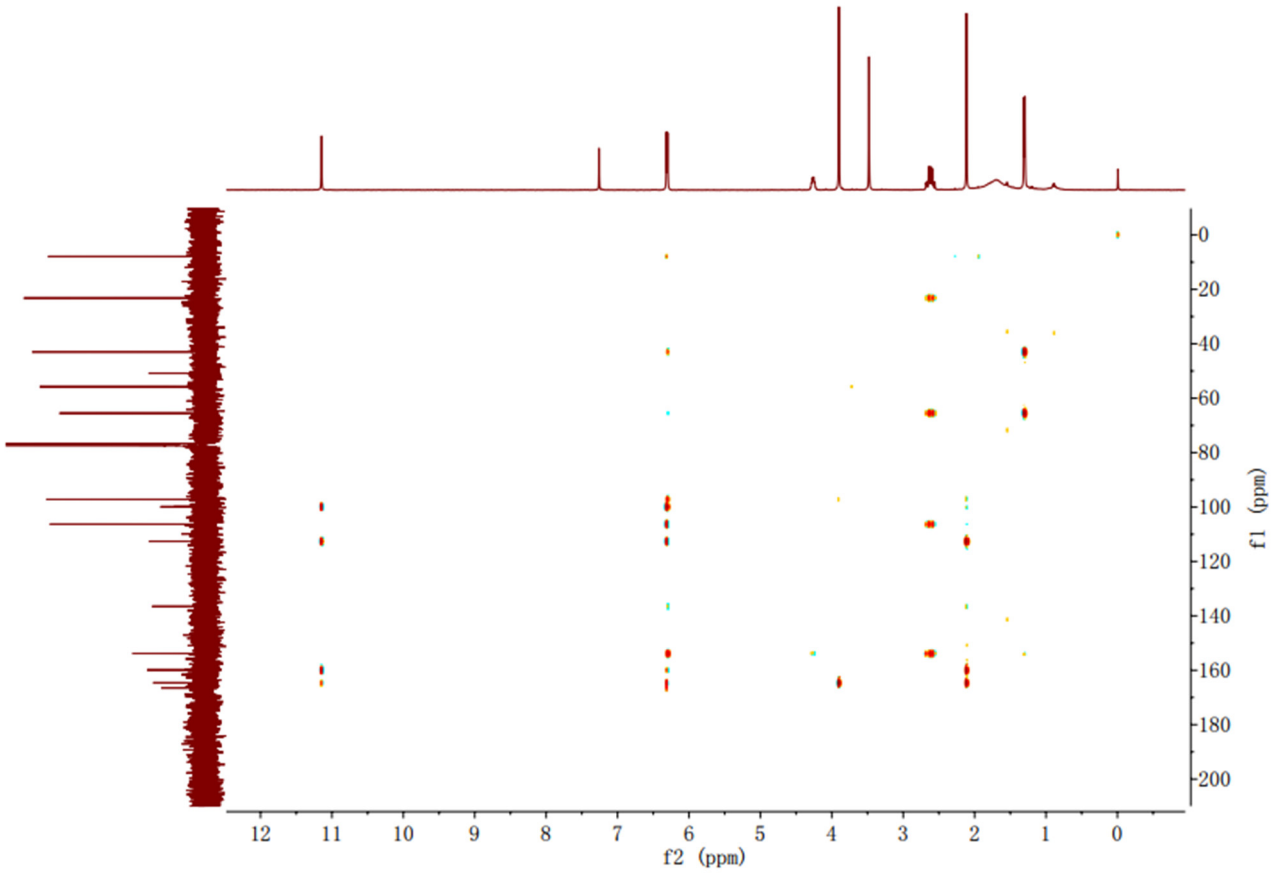


Figure S25. HMBC spectrum of monarubin B (**6**) in $CDCl_3$.

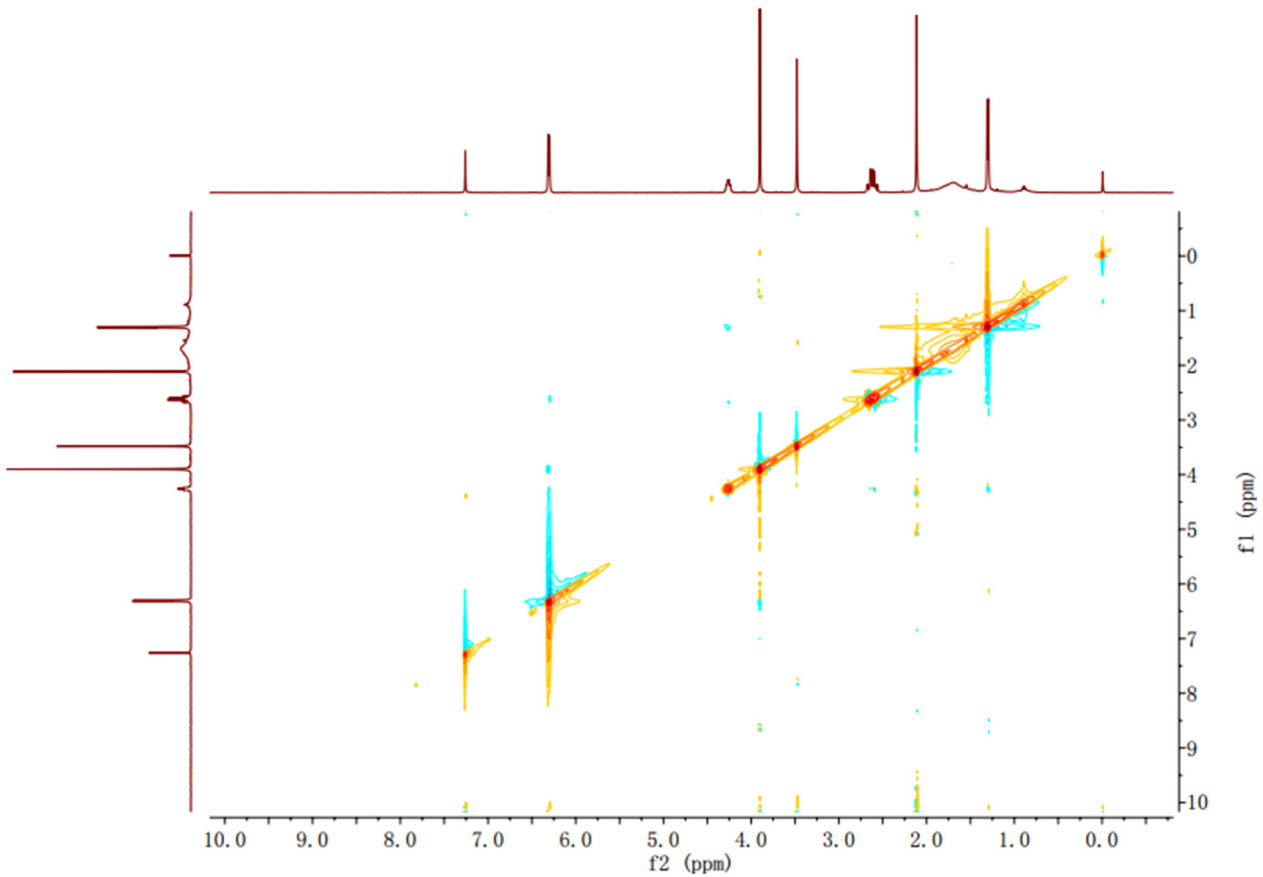


Figure S26. NOESY spectrum of monarubin B (**6**) in $CDCl_3$.

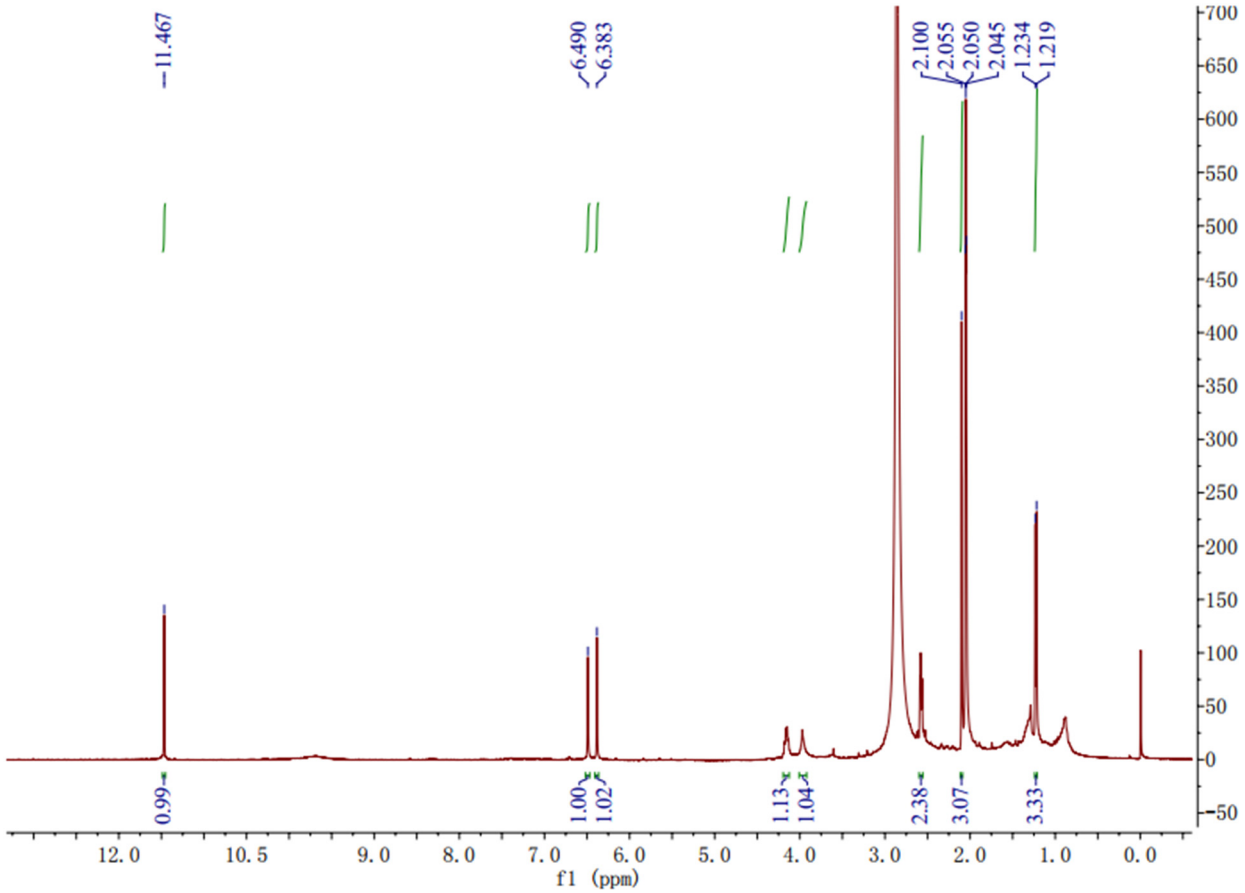


Figure S27. ^{1}H NMR spectrum of lunatinin (**7**) in actone-d_6 (400 MHz).

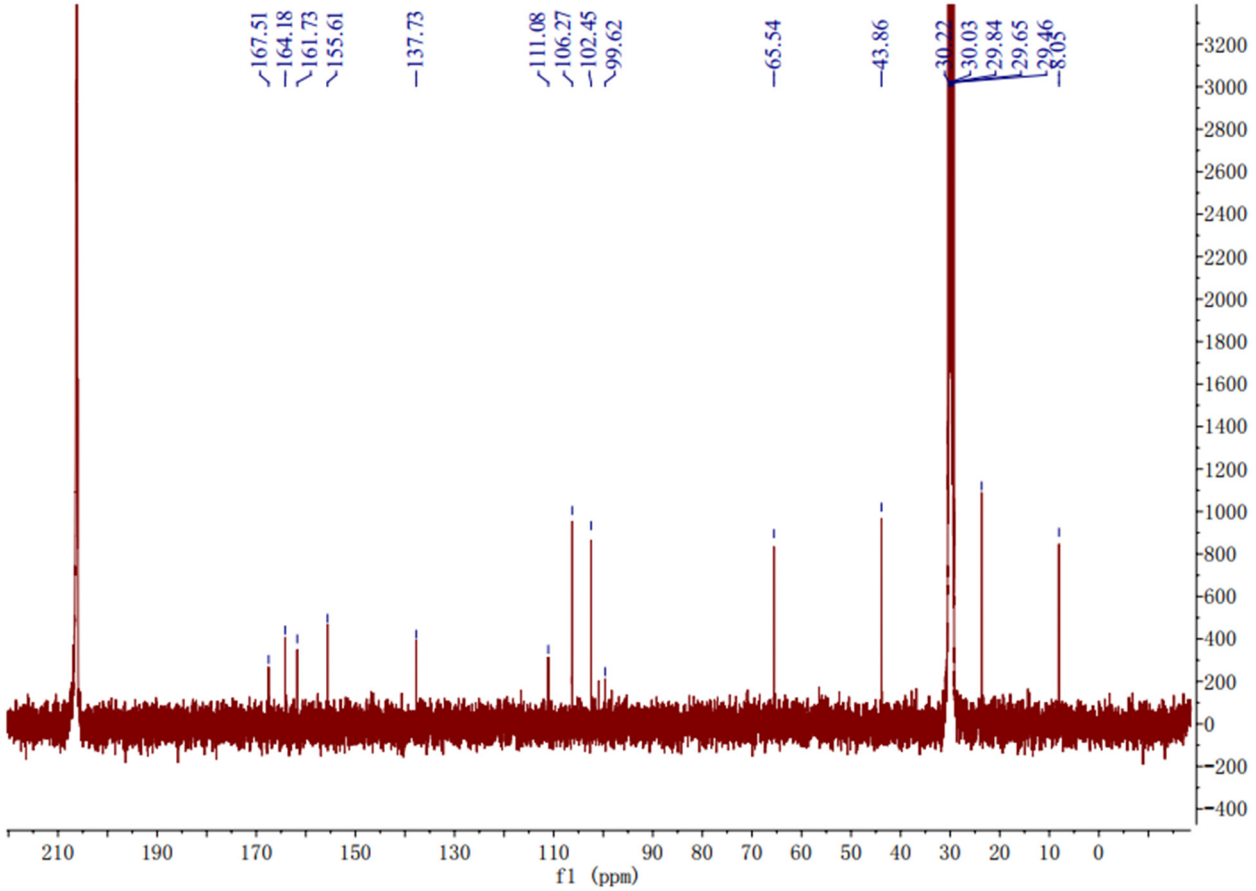


Figure S28. ^{13}C NMR spectrum of lunatinin (**7**) in actone-d_6 (100 MHz).

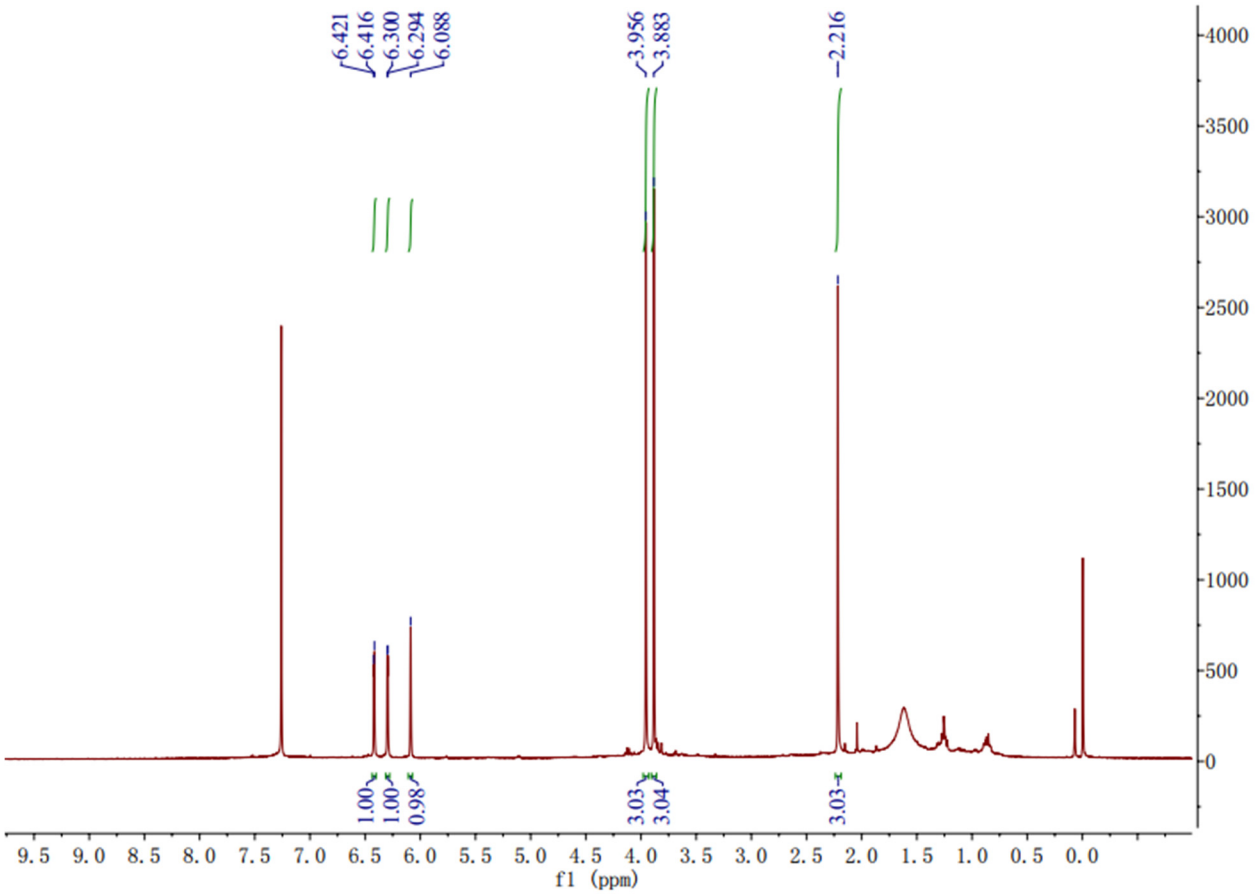


Figure S29. ^{1}H NMR spectrum of 6,8-dimethoxy-3-methylisocoumarin (**8**) in $CDCl_3$ (400 MHz).

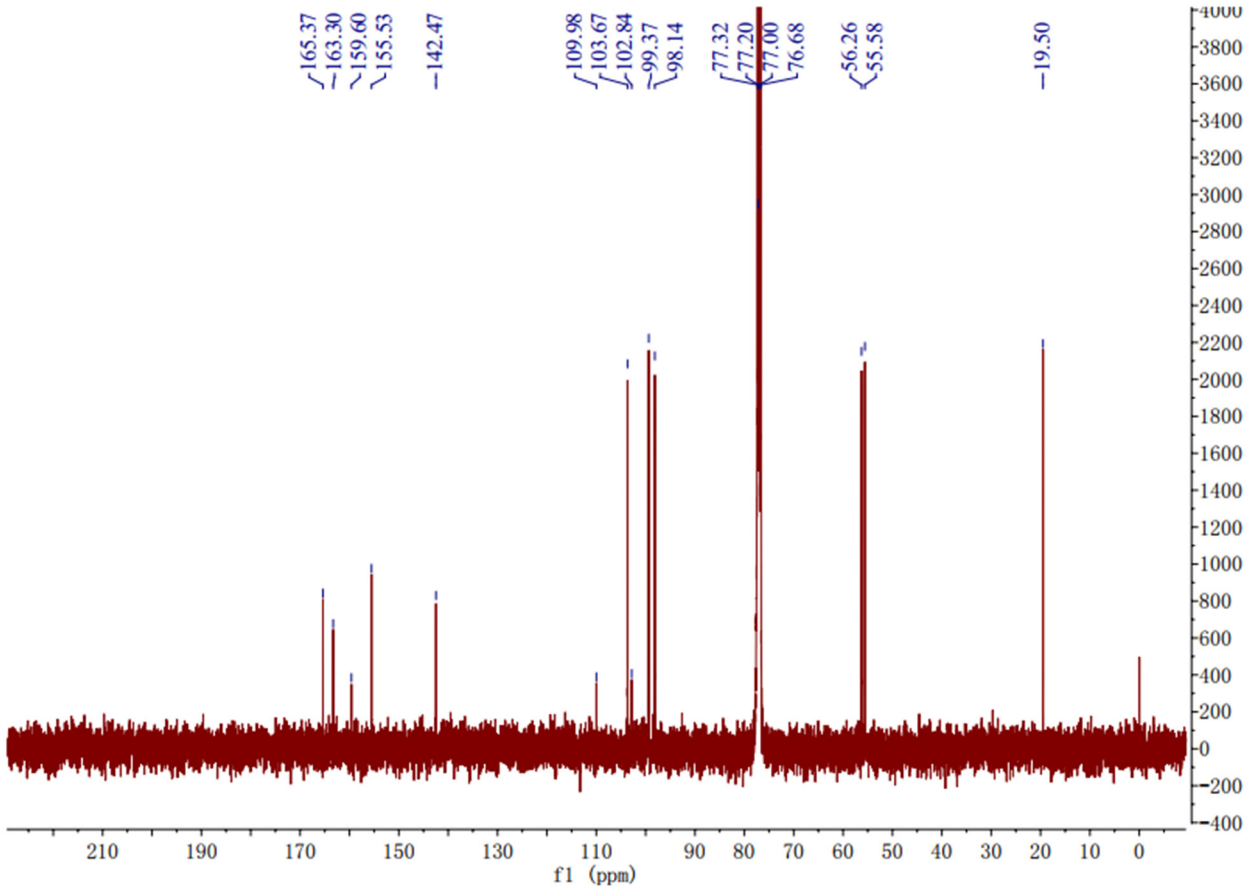


Figure S30. ^{13}C NMR spectrum of 6,8-dimethoxy-3-methylisocoumarin (**8**) in $CDCl_3$ (100 MHz).

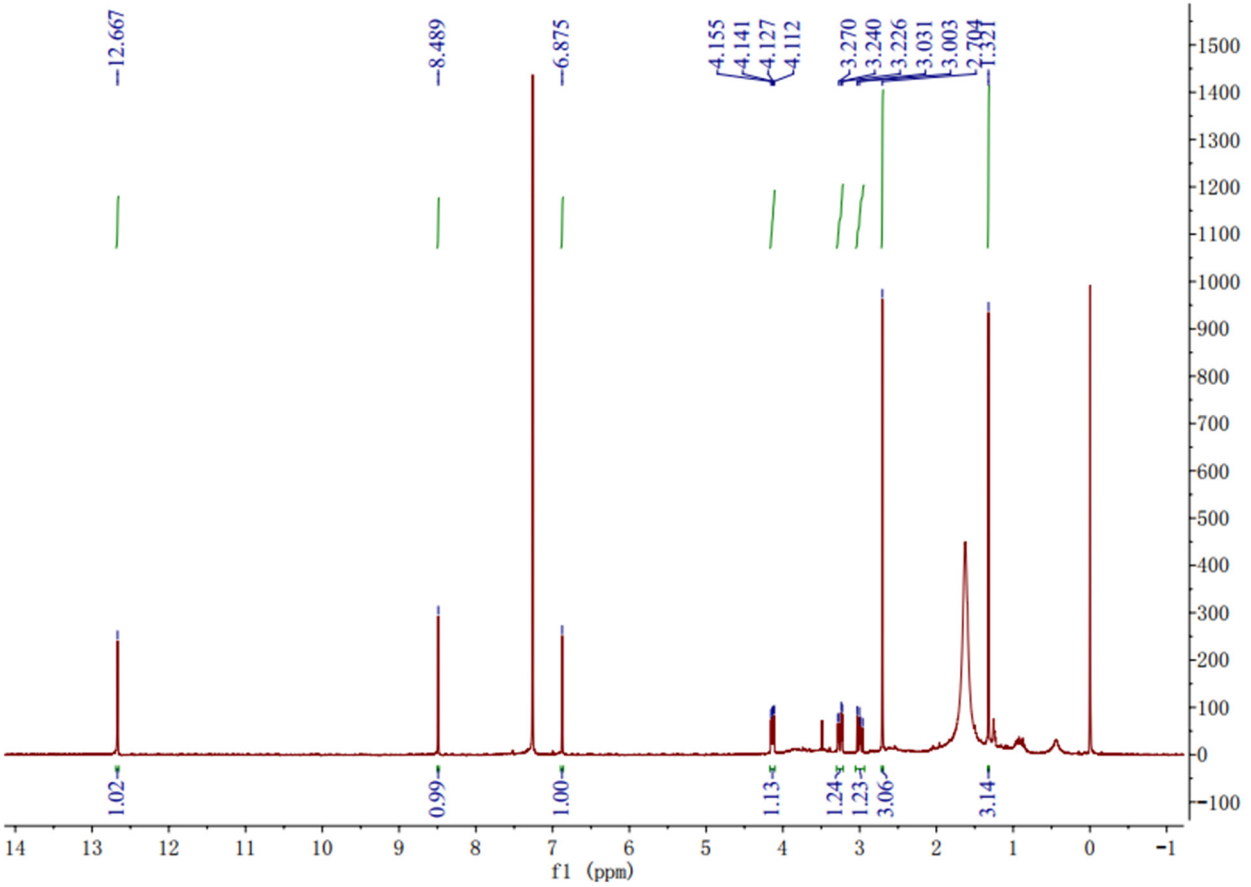


Figure S31. ^{1}H NMR spectrum of monaspurpurone (**9**) in $CDCl_3$ (400 MHz).

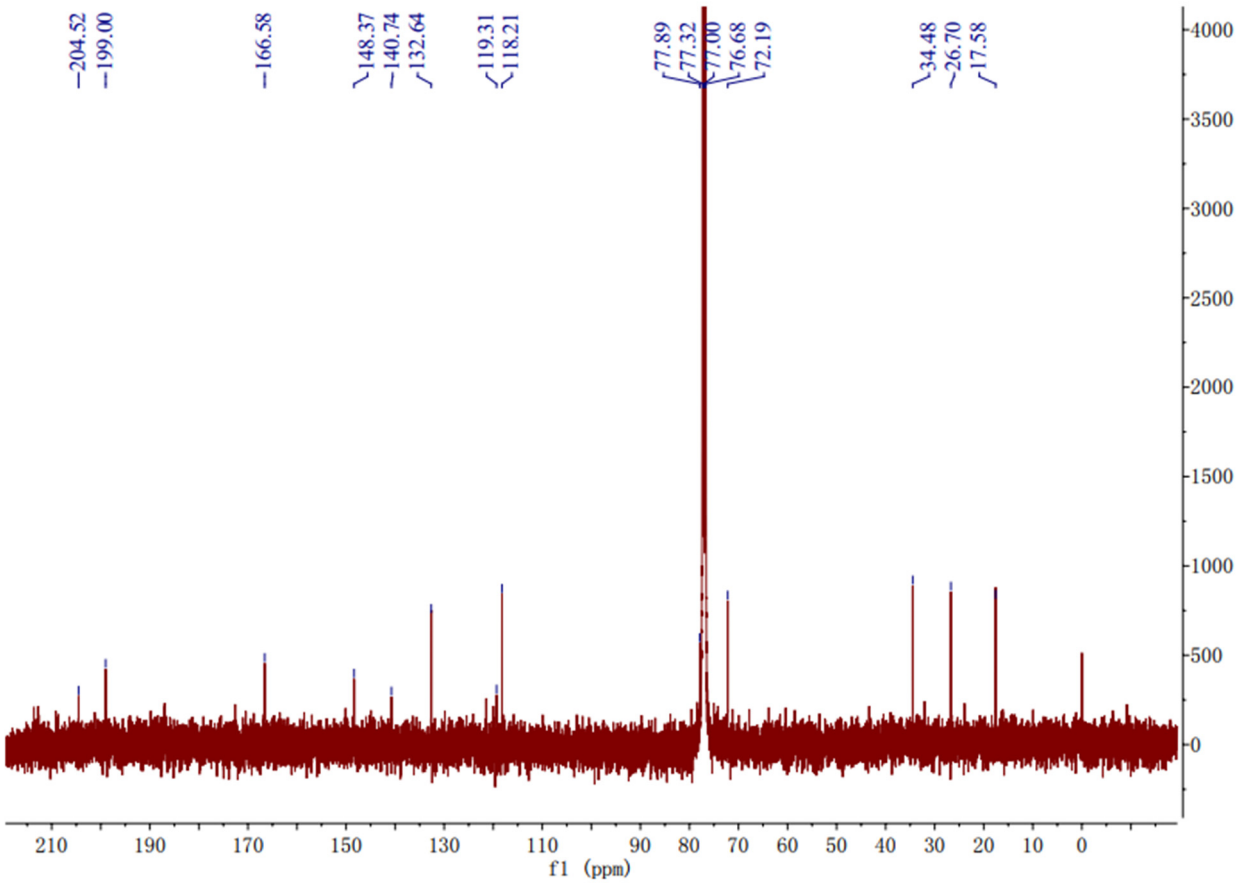


Figure S32. ^{13}C NMR spectrum of monaspurpurone (**9**) in $CDCl_3$ (100 MHz).

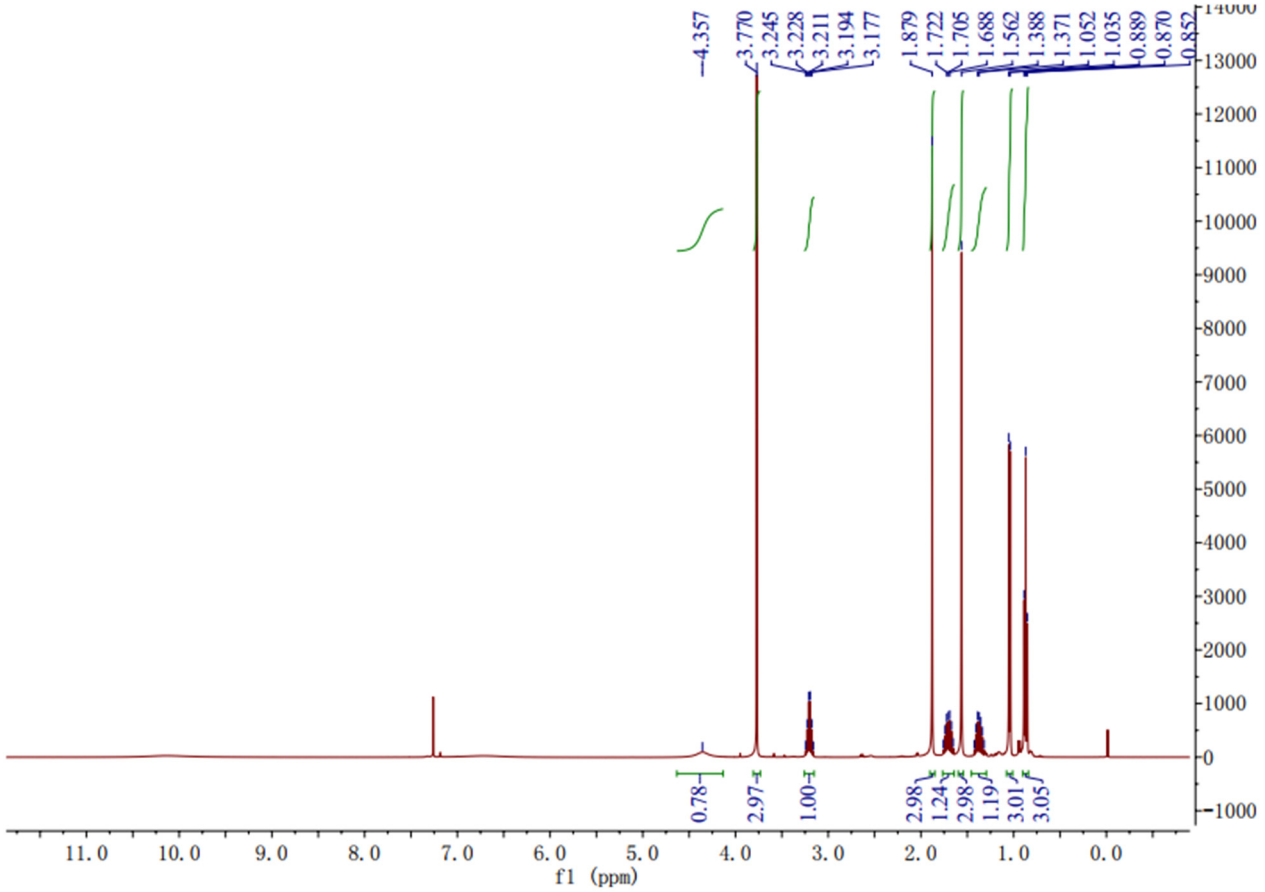


Figure S33. ^{1}H NMR spectrum of 5-amino-2,6-dimethyl-6-hydroxy-4-(2'-methyl-1-oxobutyl)-3-methoxy-2,4-cyclohexadien-1-one (**10**) in $CDCl_3$ (400 MHz).

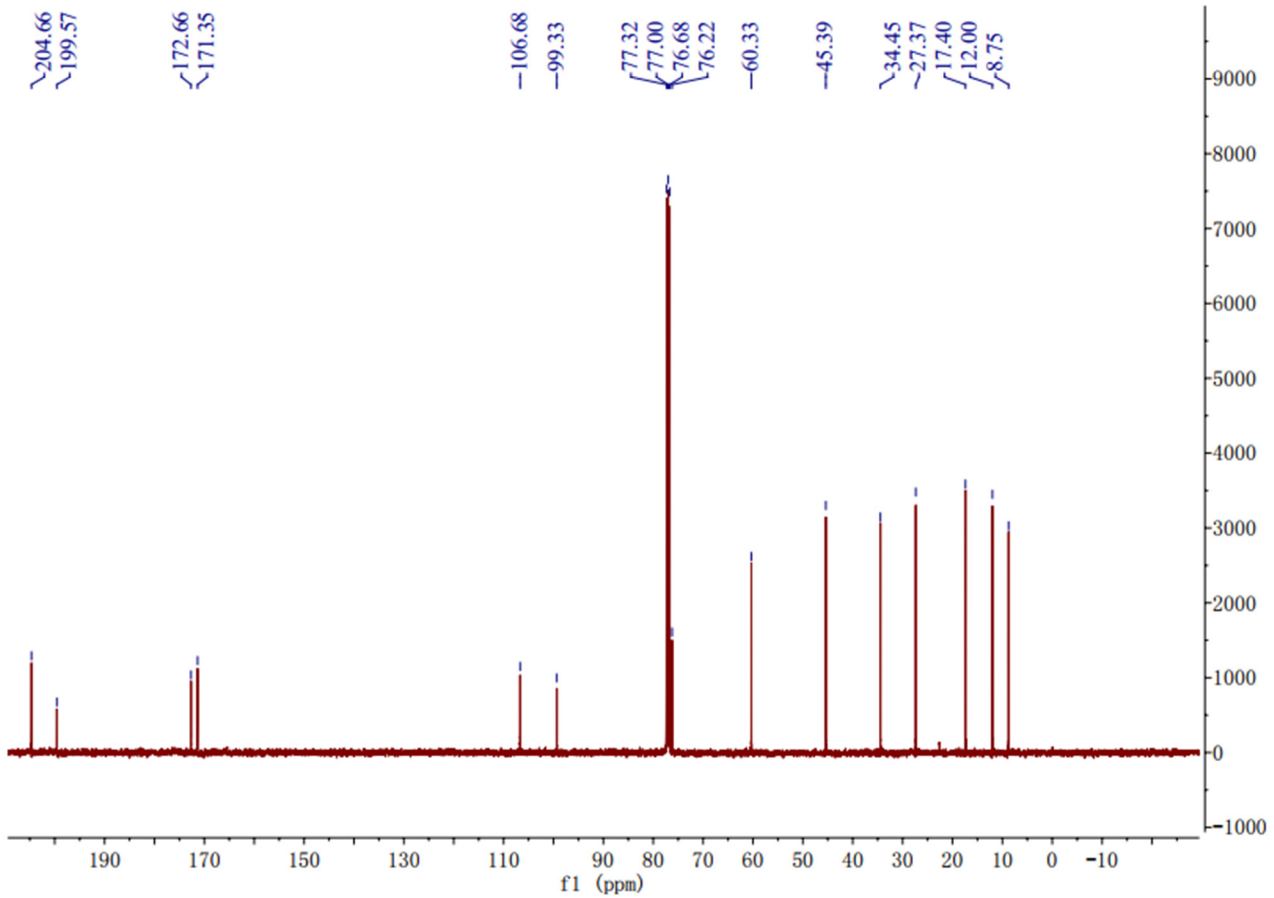


Figure S34. ^{13}C NMR spectrum of 5-amino-2,6-dimethyl-6-hydroxy-4-(2'-methyl-1-oxobutyl)-3-methoxy-2,4-cyclohexadien-1-one (**10**) in $CDCl_3$ (100 MHz).

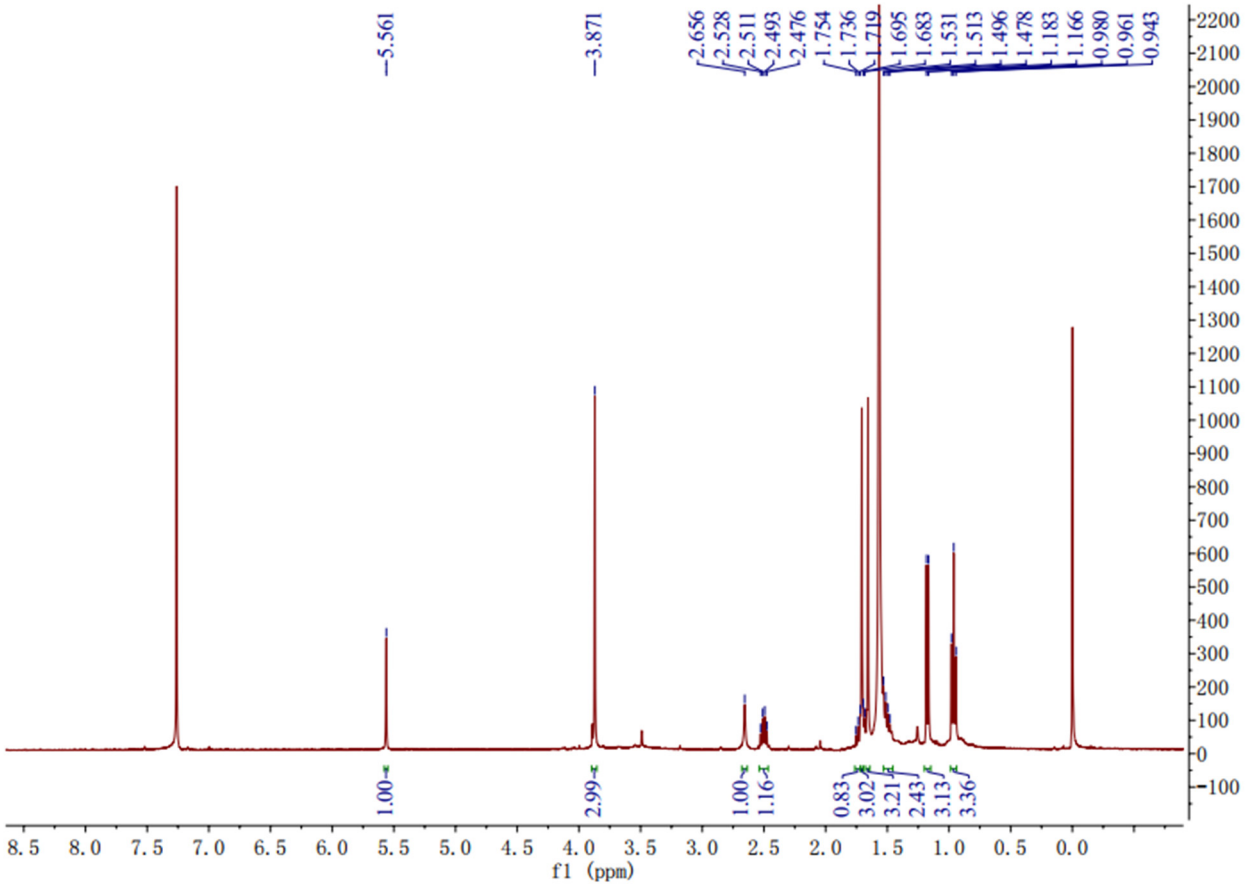


Figure S35. ^{1}H NMR spectrum of phomaligol A (**11**) in $CDCl_3$ (400 MHz).

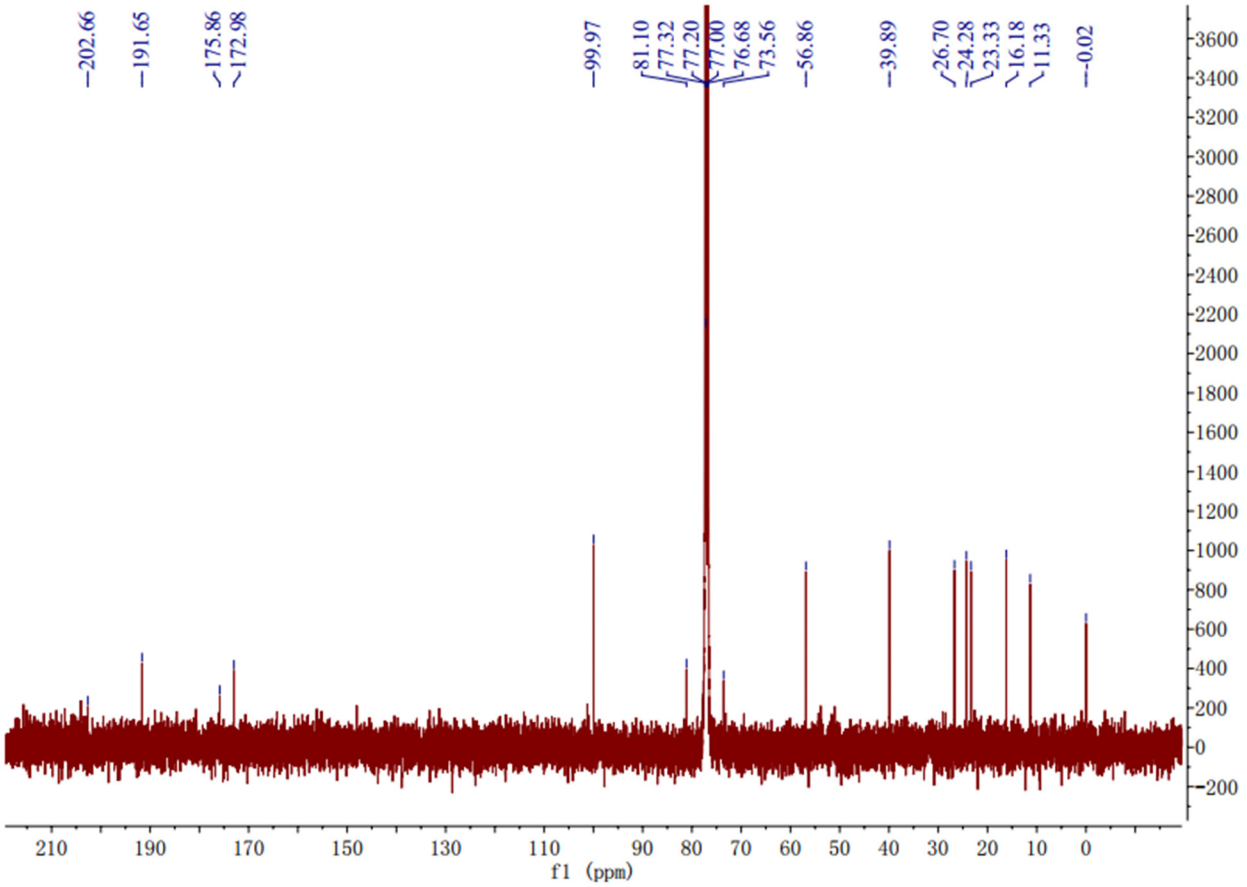


Figure S36. ^{13}C NMR spectrum of phomaligol A (**11**) in $CDCl_3$ (100 MHz).

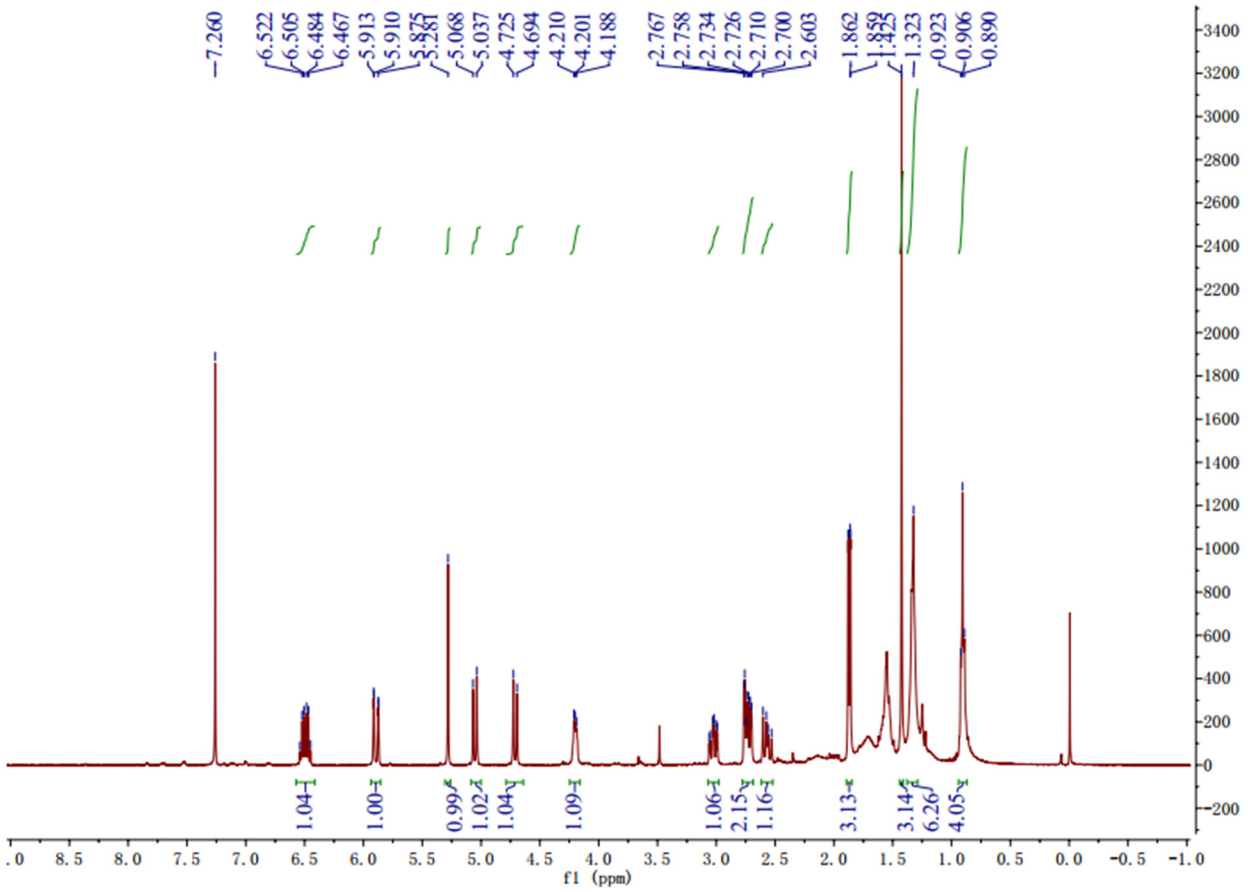


Figure S37. ^{1}H NMR spectrum of monascuspiloin (**12**) in $CDCl_3$ (400 MHz).

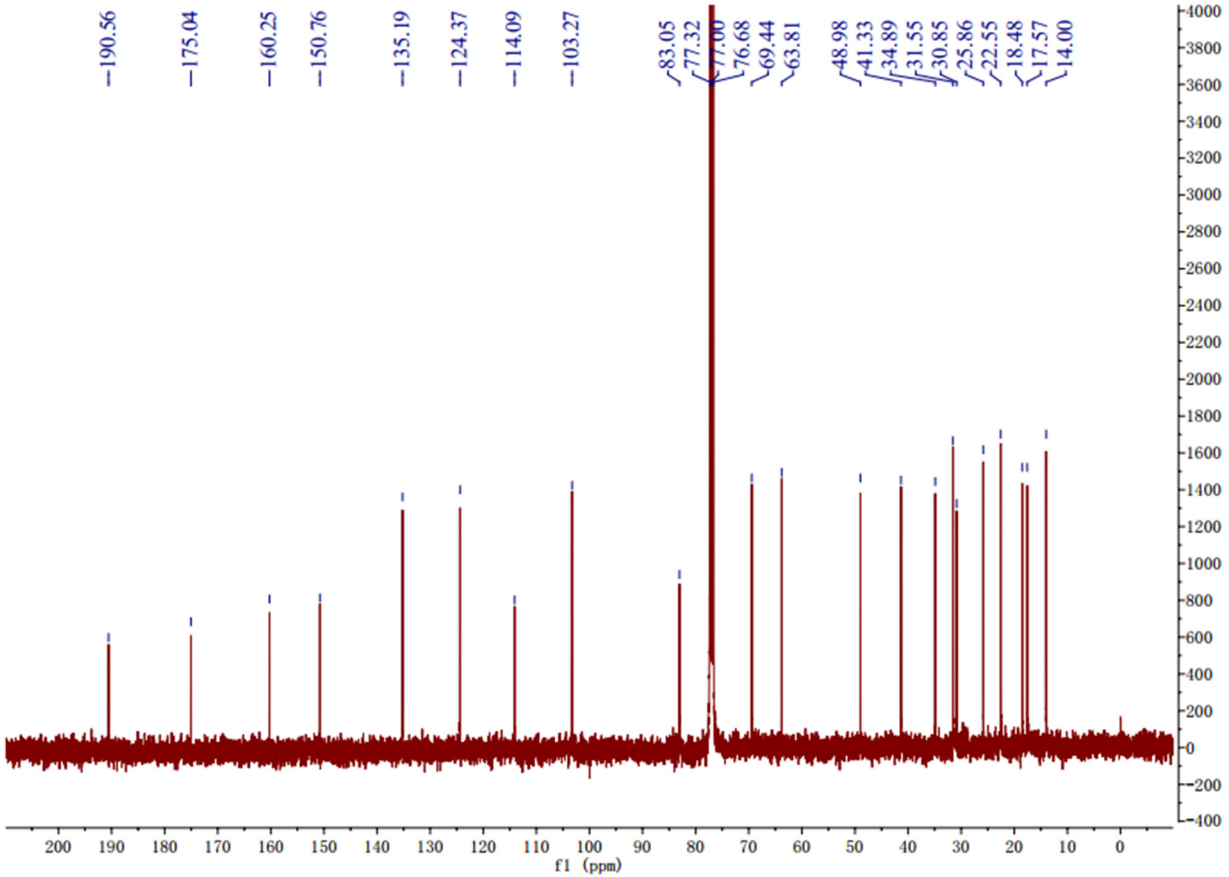
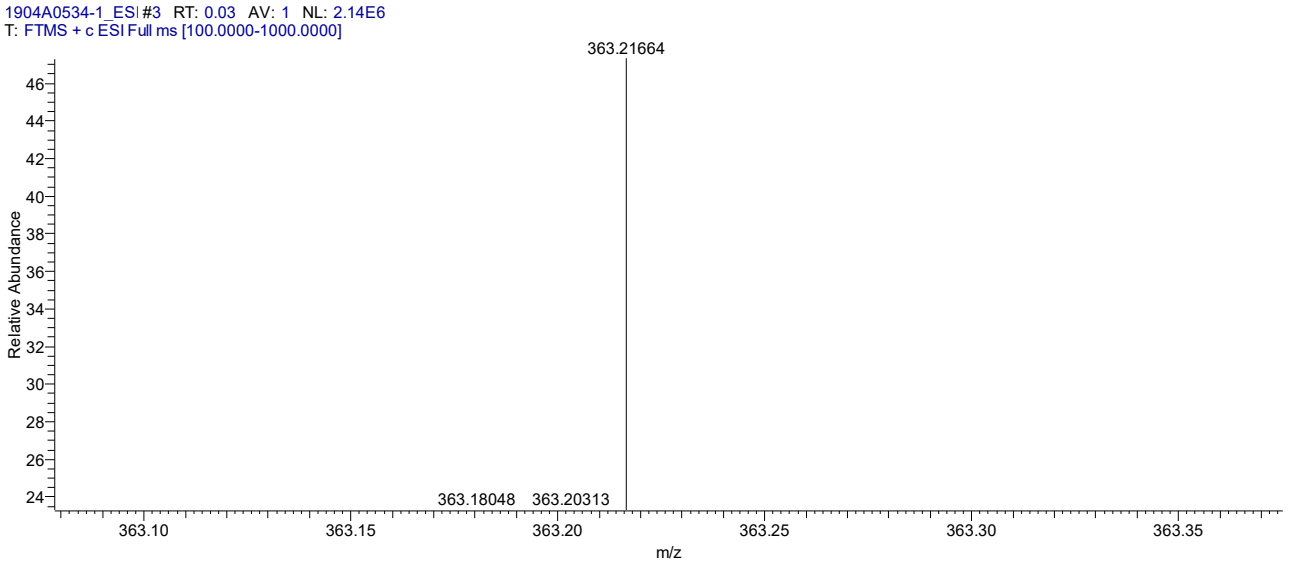


Figure S38. ^{13}C NMR spectrum of monascuspiloin (**12**) in $CDCl_3$ (100 MHz).



SPECTRUM -simulation :				
m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
363.21664	363.21664	0.11	6.5	C21 H31 O5

Figure S39. HR-(+) ESI-MS spectrum of monarubin C (**13**).

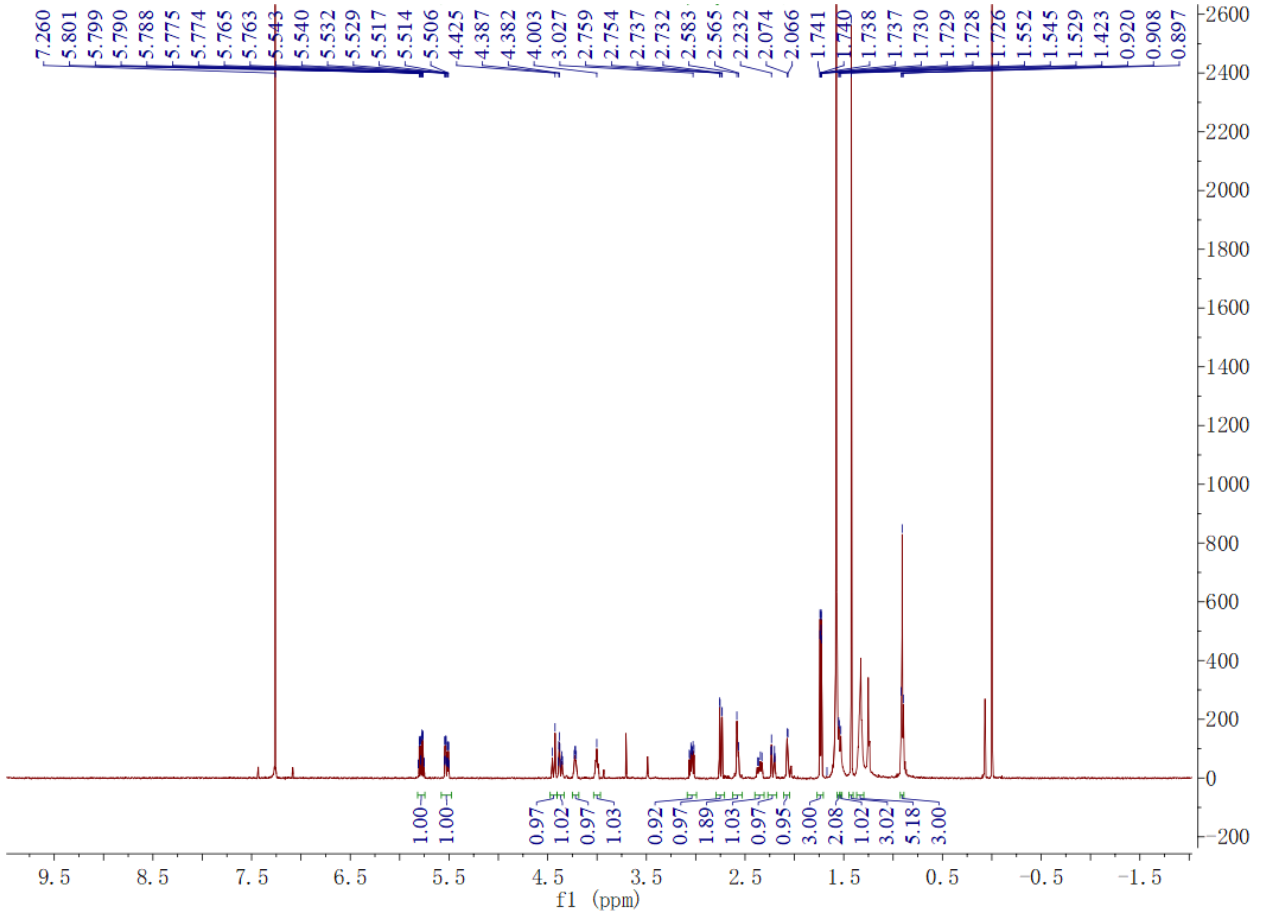


Figure S40. ^{1}H NMR spectrum of monarubin C (**13**) in $CDCl_3$ (600 MHz).

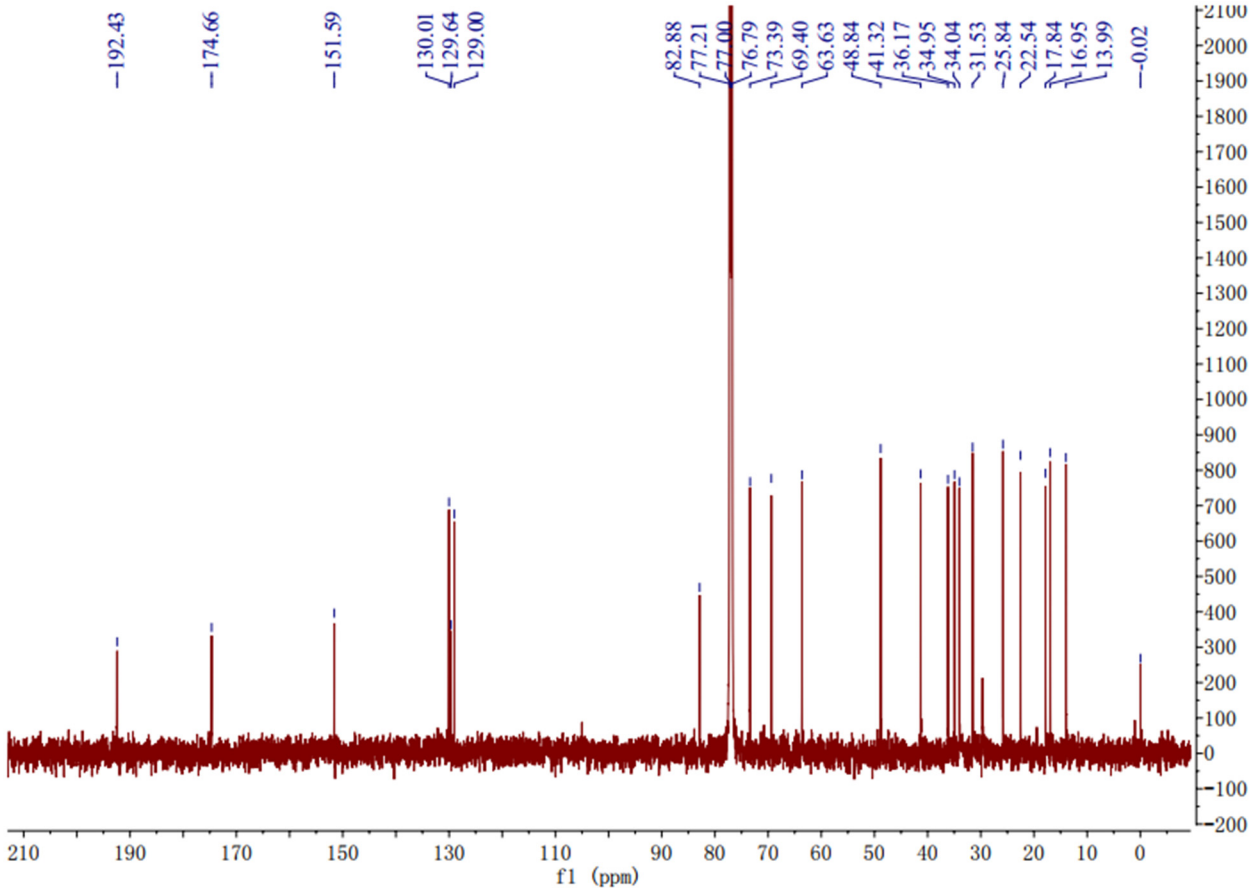


Figure S41. ^{13}C NMR spectrum of monarubin C (**13**) in $CDCl_3$ (150 MHz).

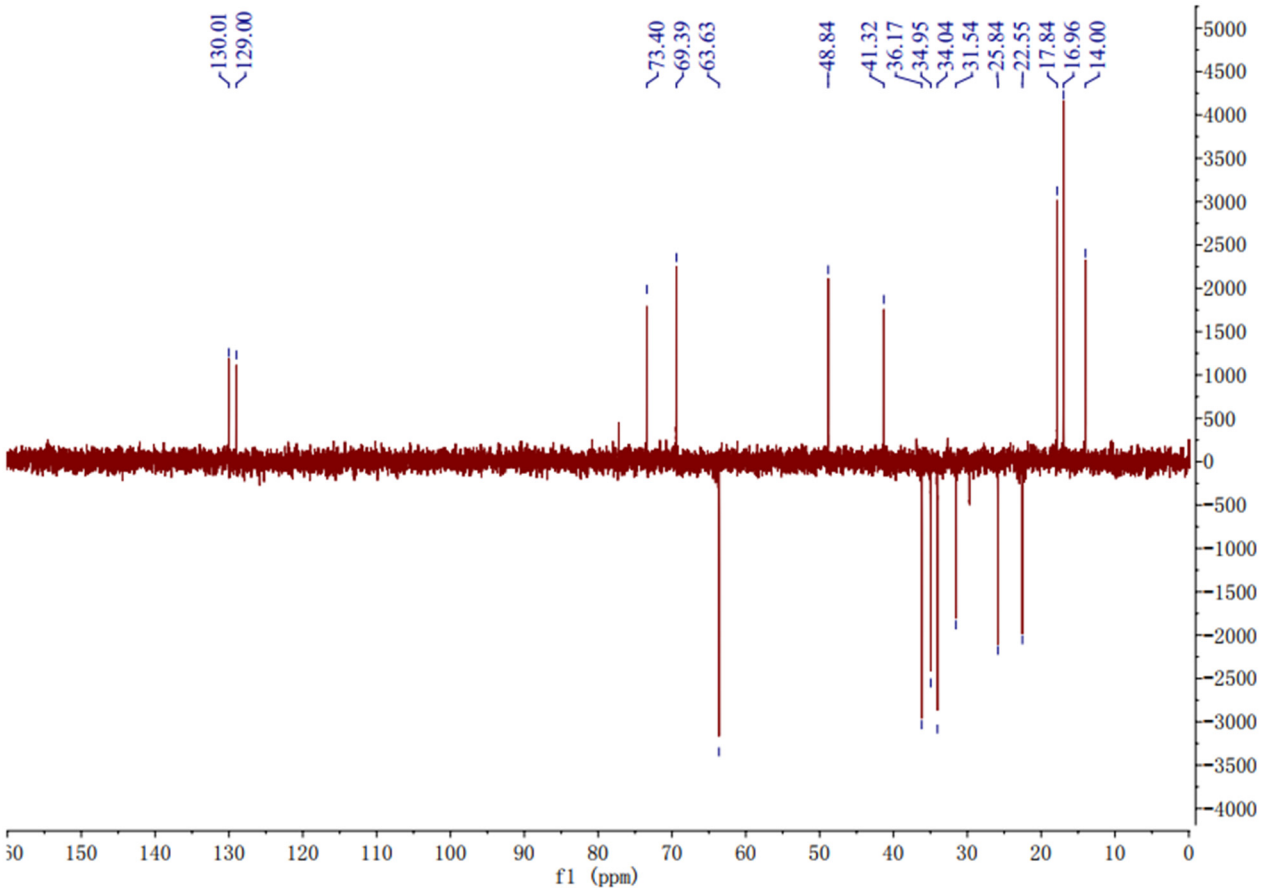


Figure S42. DEPT 135 spectrum of monarubin C (**13**) in $CDCl_3$ (150 MHz).

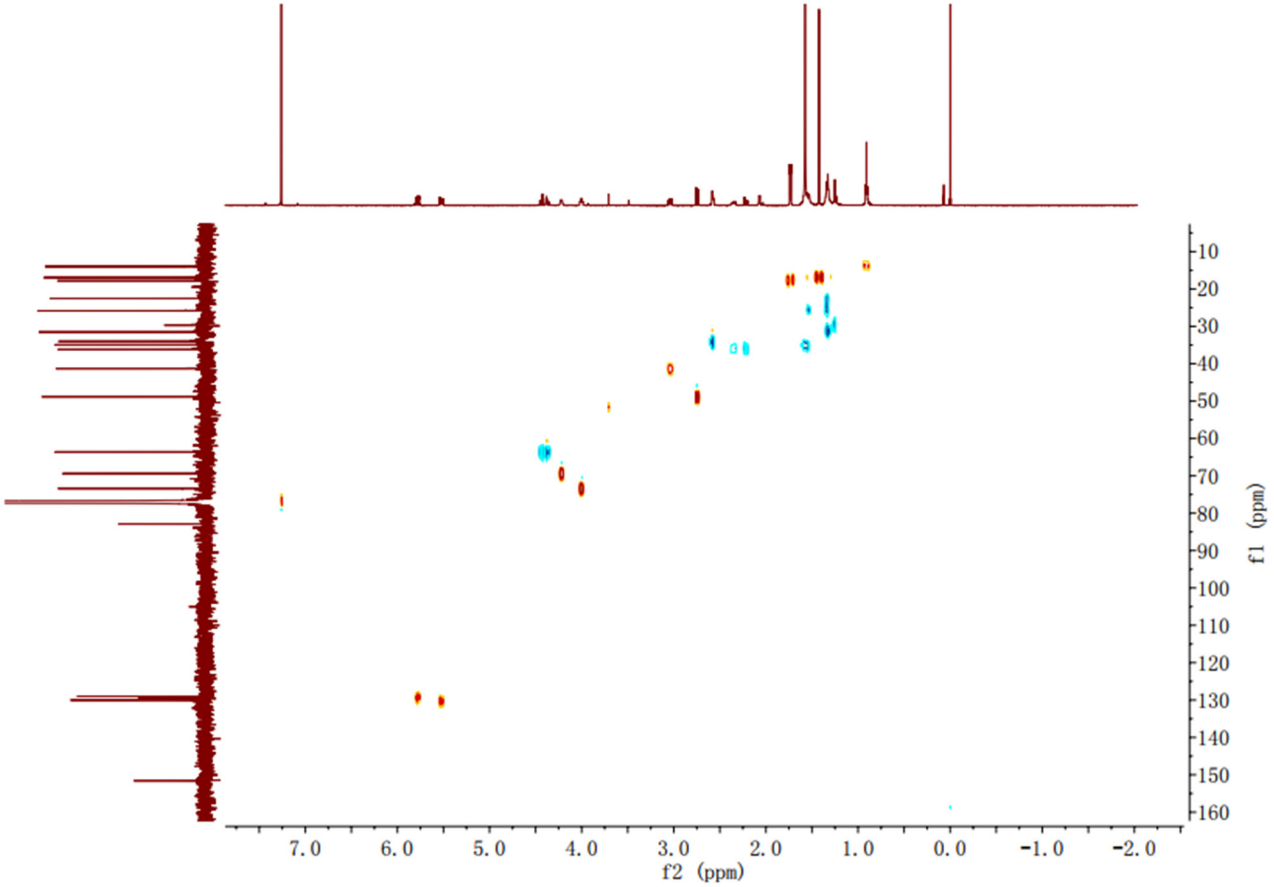


Figure S43. HMQC spectrum of monarubin C (**13**) in $CDCl_3$.

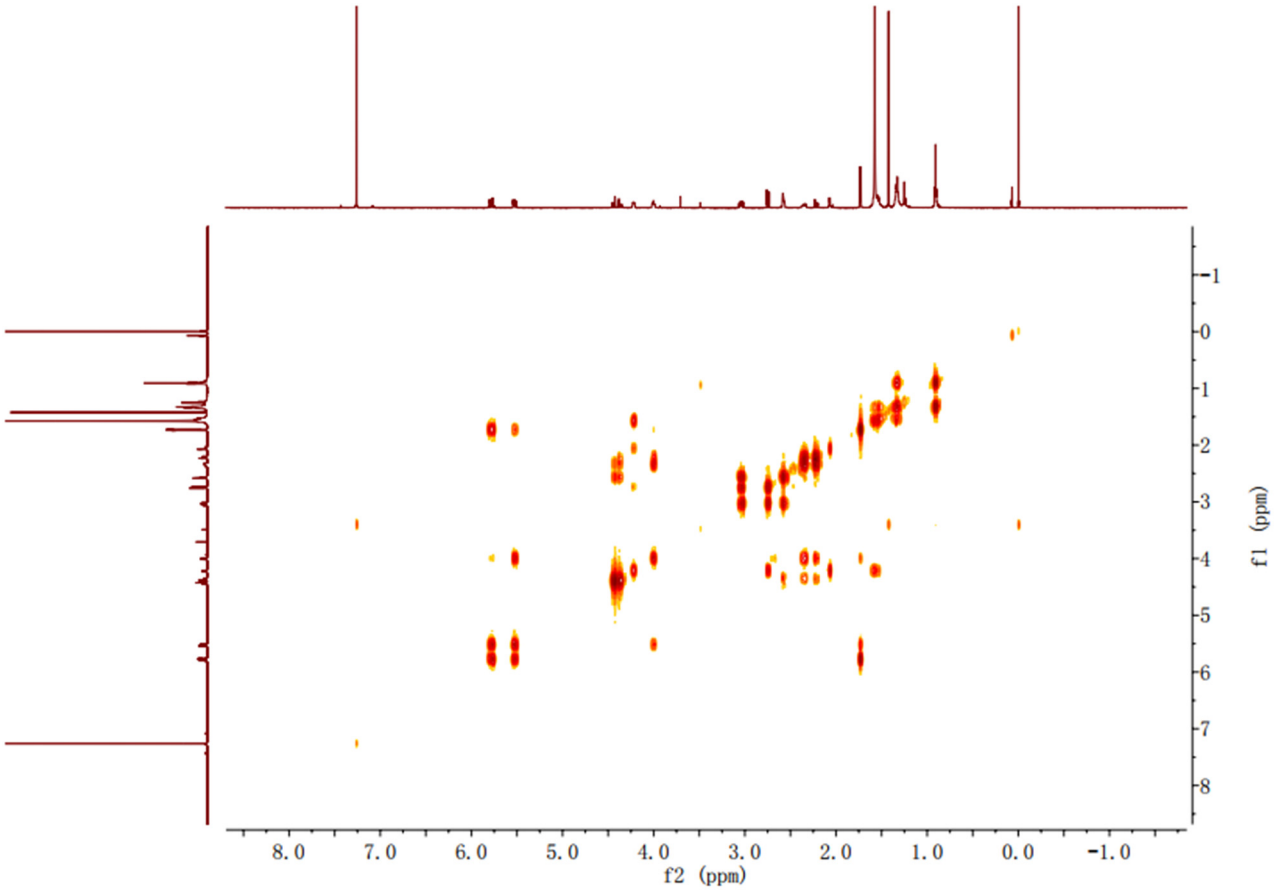


Figure S44. ^{1}H–^{1}HCOSY spectrum of monarubin C (**13**) in $CDCl_3$.

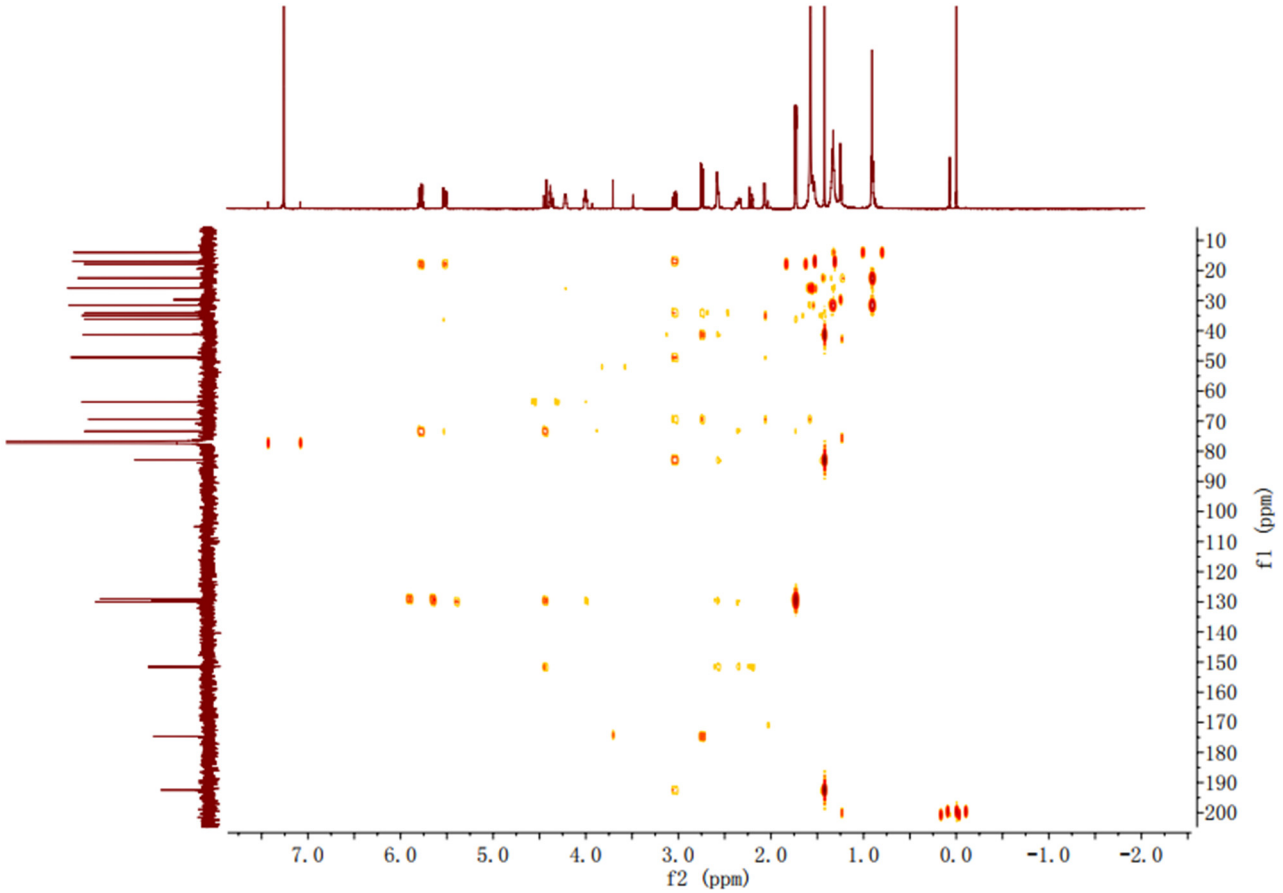


Figure S45. HMBC spectrum of monarubin C (**13**) in $CDCl_3$.

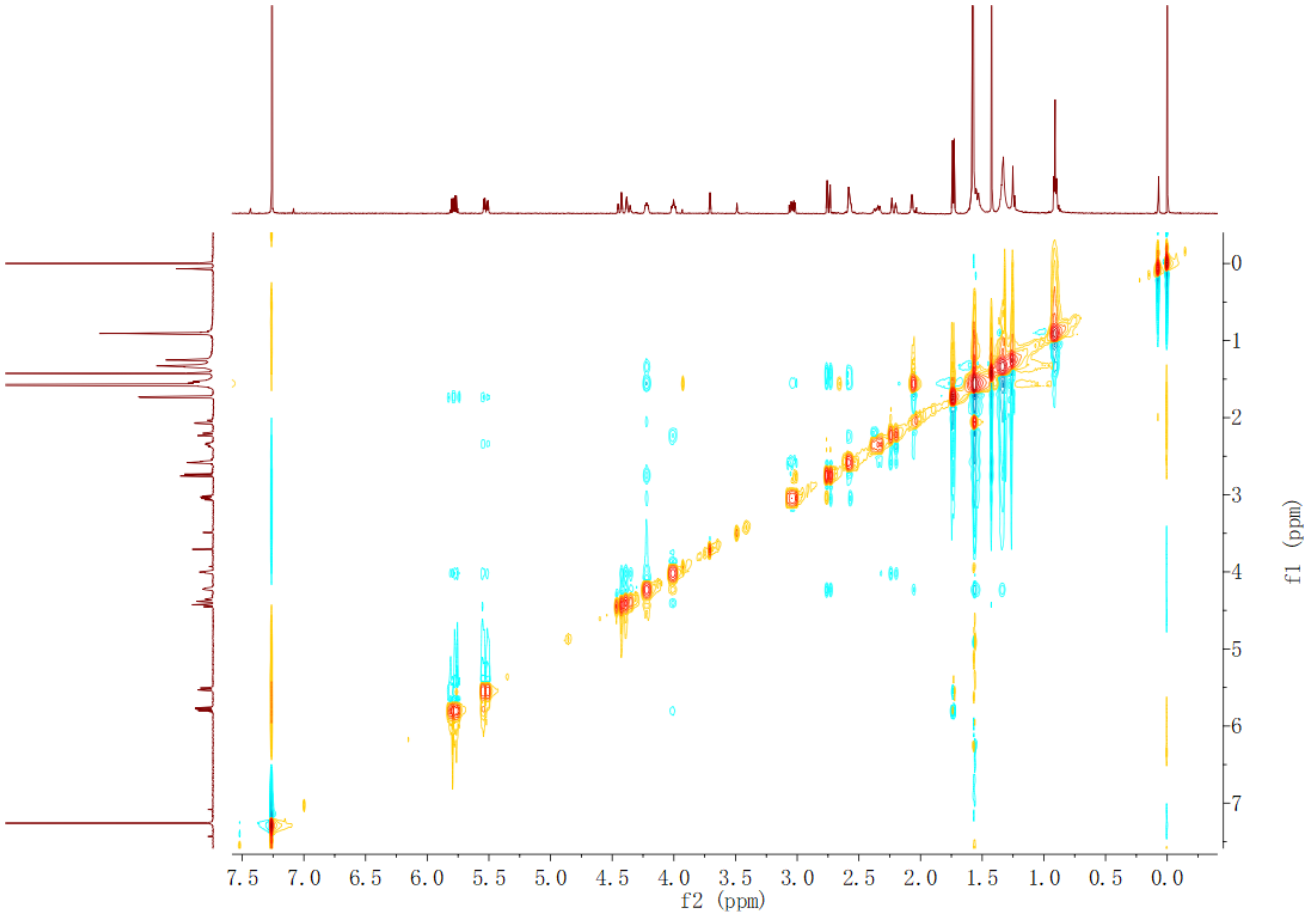


Figure S46. NOESY spectrum of monarubin C (**13**) in $CDCl_3$.

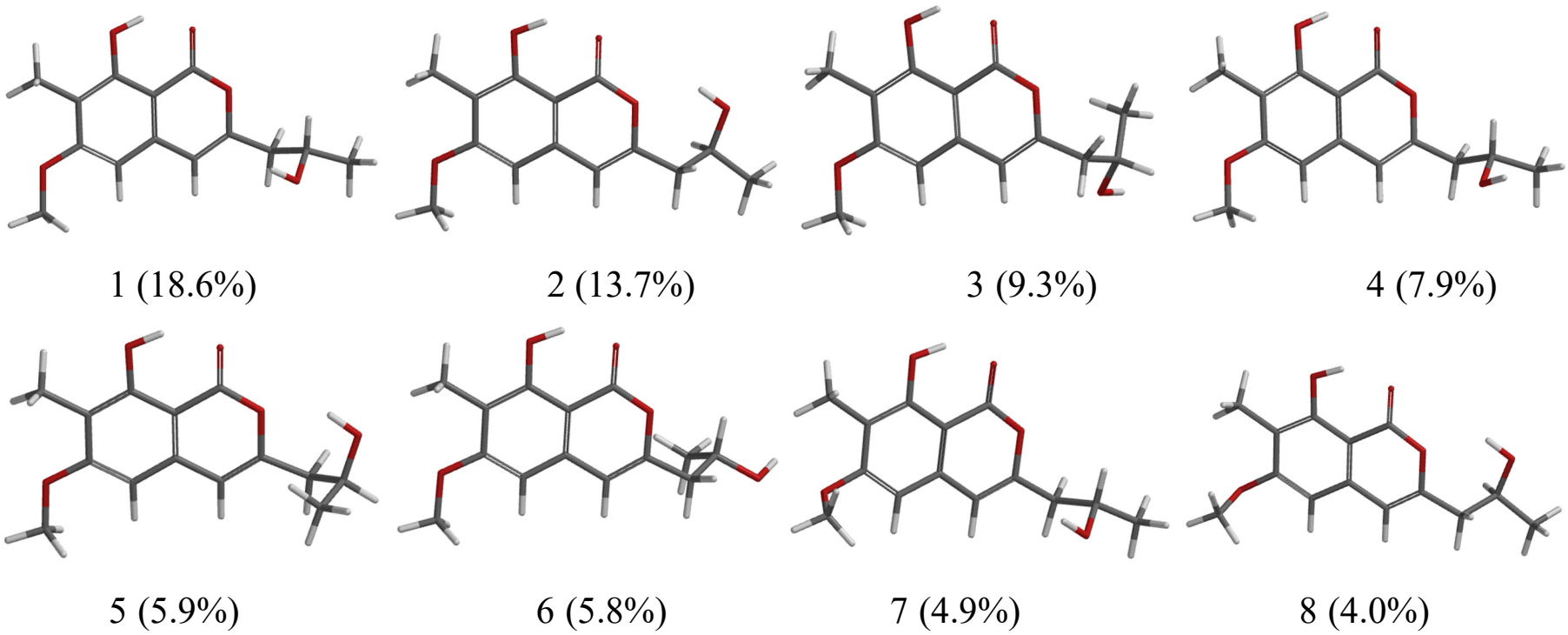


Figure S47. The most stable conformers of 11*S*-**6** calculated at the B3LYP/6-31+G(d) level. Relative populations are in parentheses.

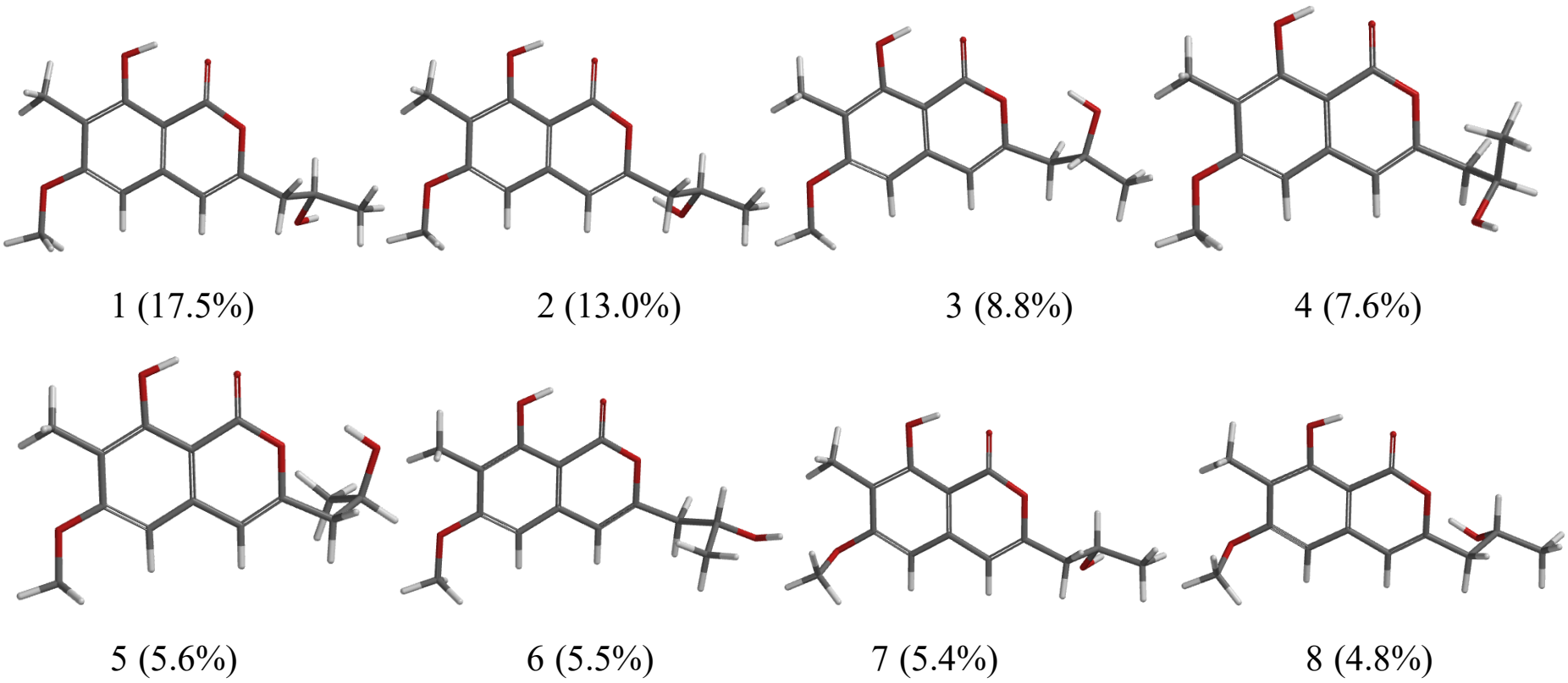


Figure S48. The most stable conformers of 11*R*-**6** calculated at the B3LYP/6-31+G(d) level. Relative populations are in parentheses.

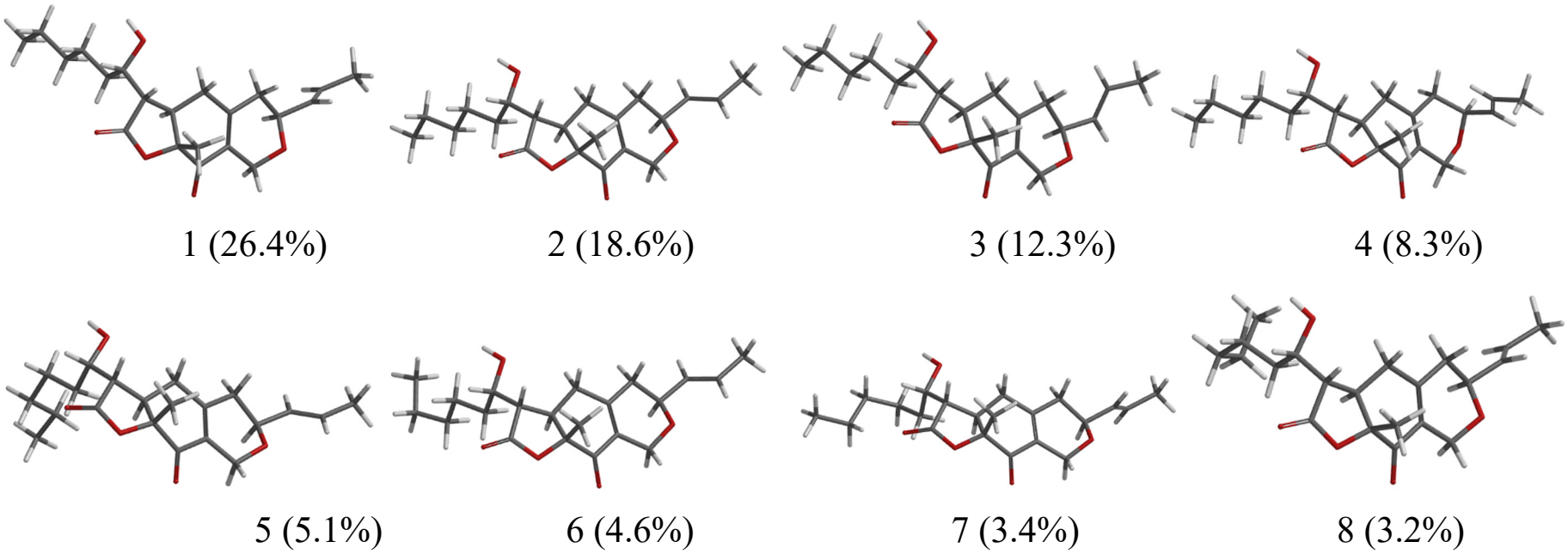


Figure S49. The most stable conformers of 3*S*,6*R*,7*S*,11*S*,17*R*-**13** calculated at the B3LYP/6-31+G(d) level. Relative populations are in parentheses.

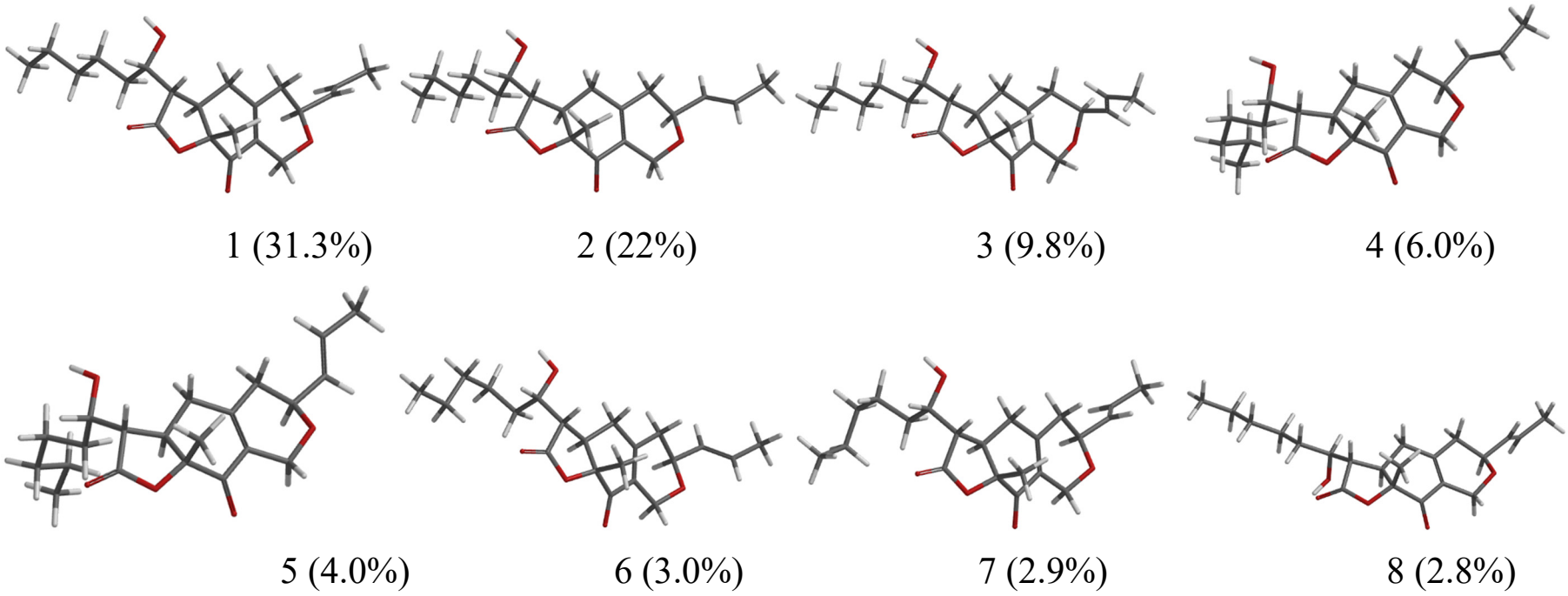


Figure S50. The most stable conformers of 3*S*,6*R*,7*S*,11*S*,17*S*-**13** calculated at the B3LYP/6-31+G(d) level. Relative populations are in parentheses.

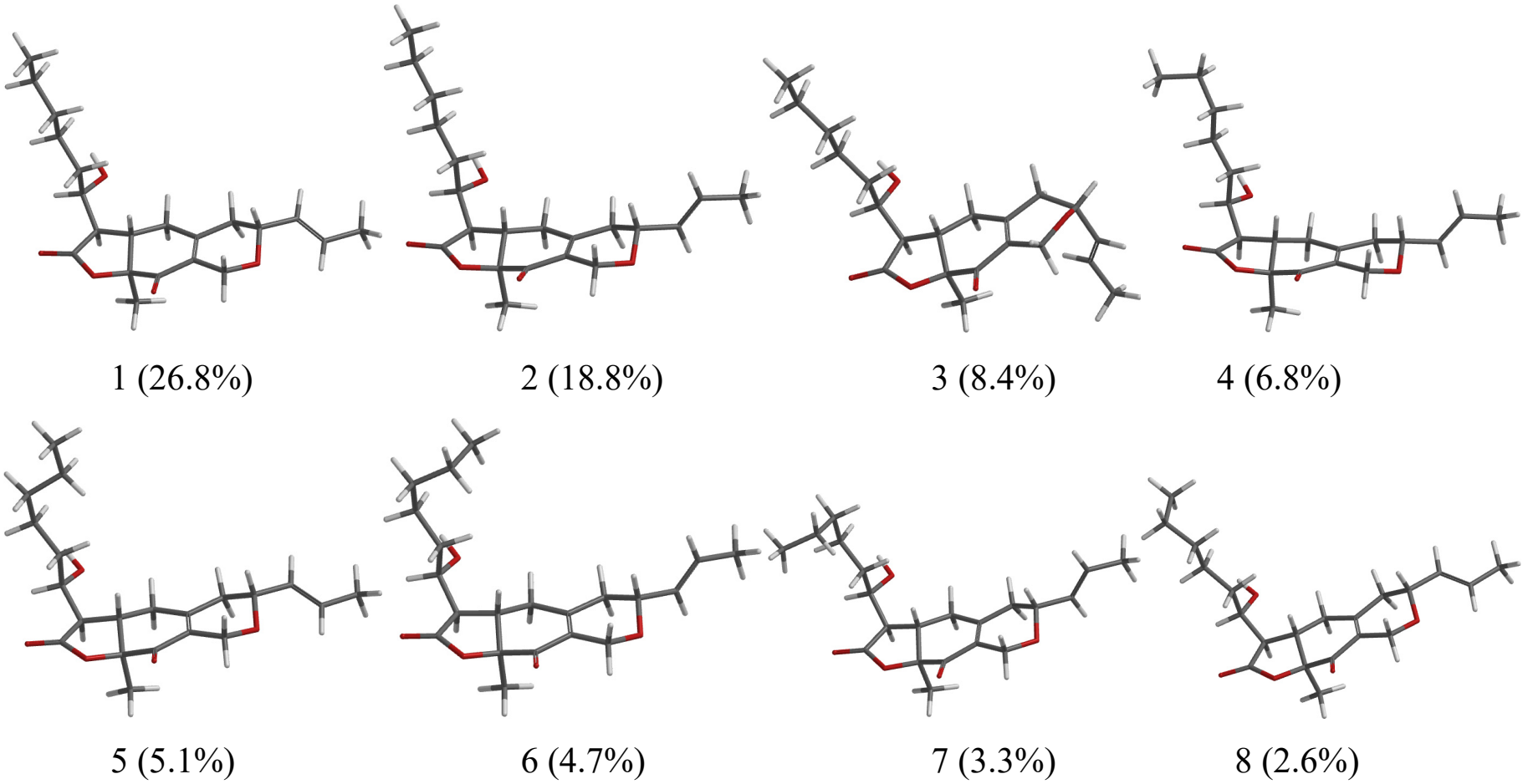


Figure S51. The most stable conformers of 3*R*,6*S*,7*R*,11*R*,17*S*-**13** calculated at the B3LYP/6-31+G(d) level. Relative populations are in parentheses.

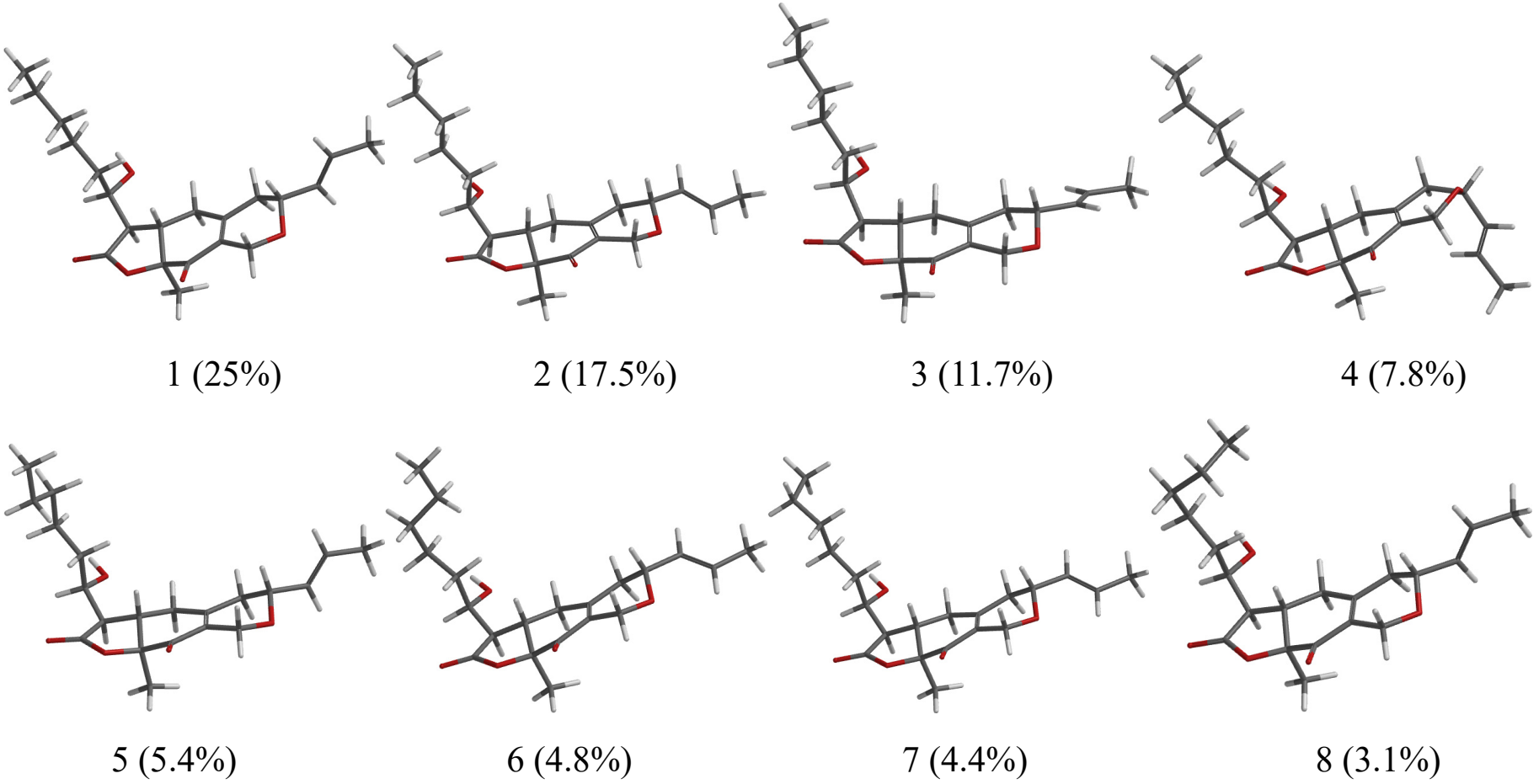


Figure S52. The most stable conformers of 3*R*,6*S*,7*R*,11*R*,17*R*-**13** calculated at the B3LYP/6-31+G(d) level. Relative populations are in parentheses.

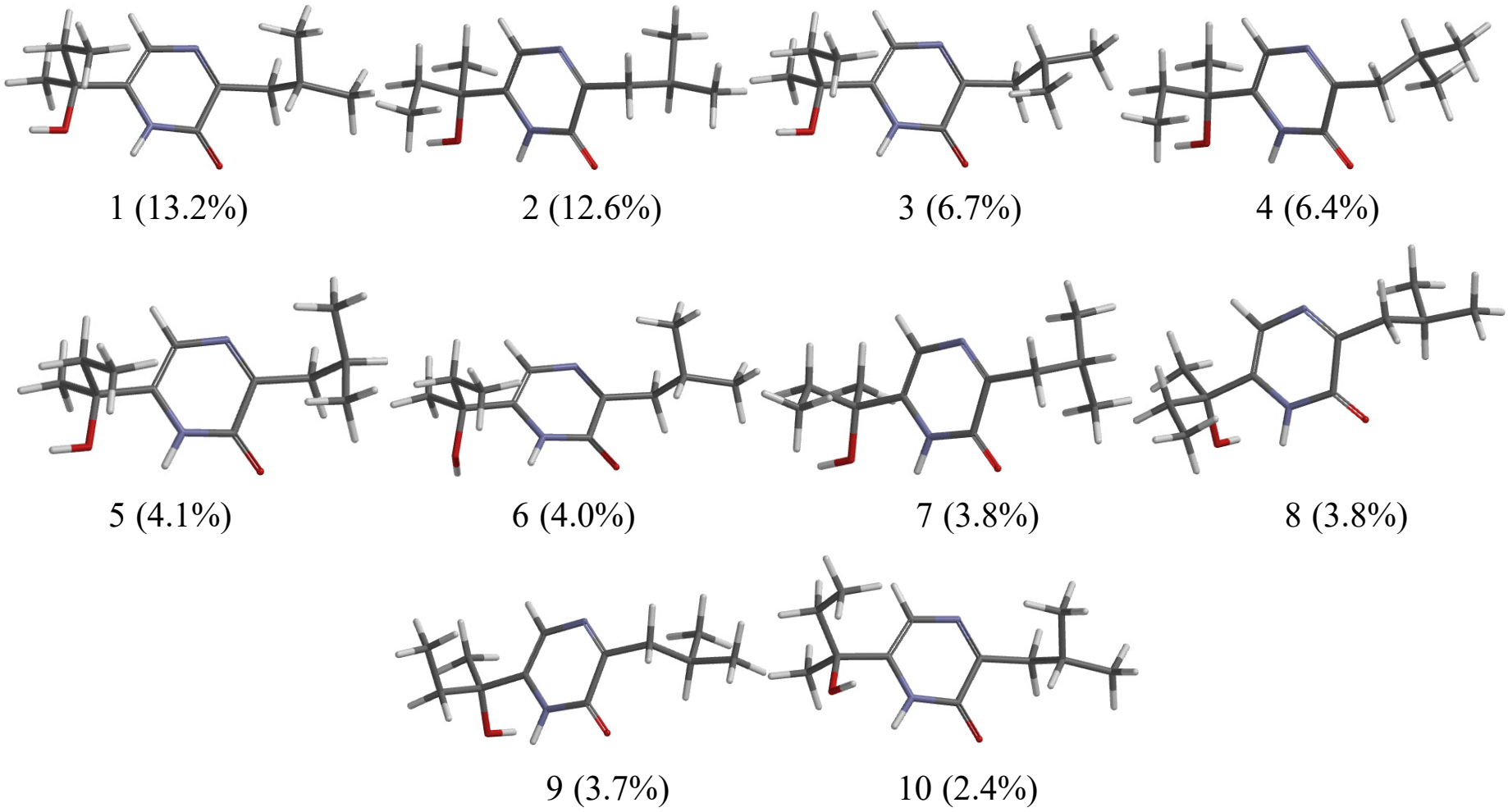


Figure S53. The most stable conformers of 11*S*-**3** calculated at the B3LYP/6-31+G(d) level. Relative populations are in parentheses.

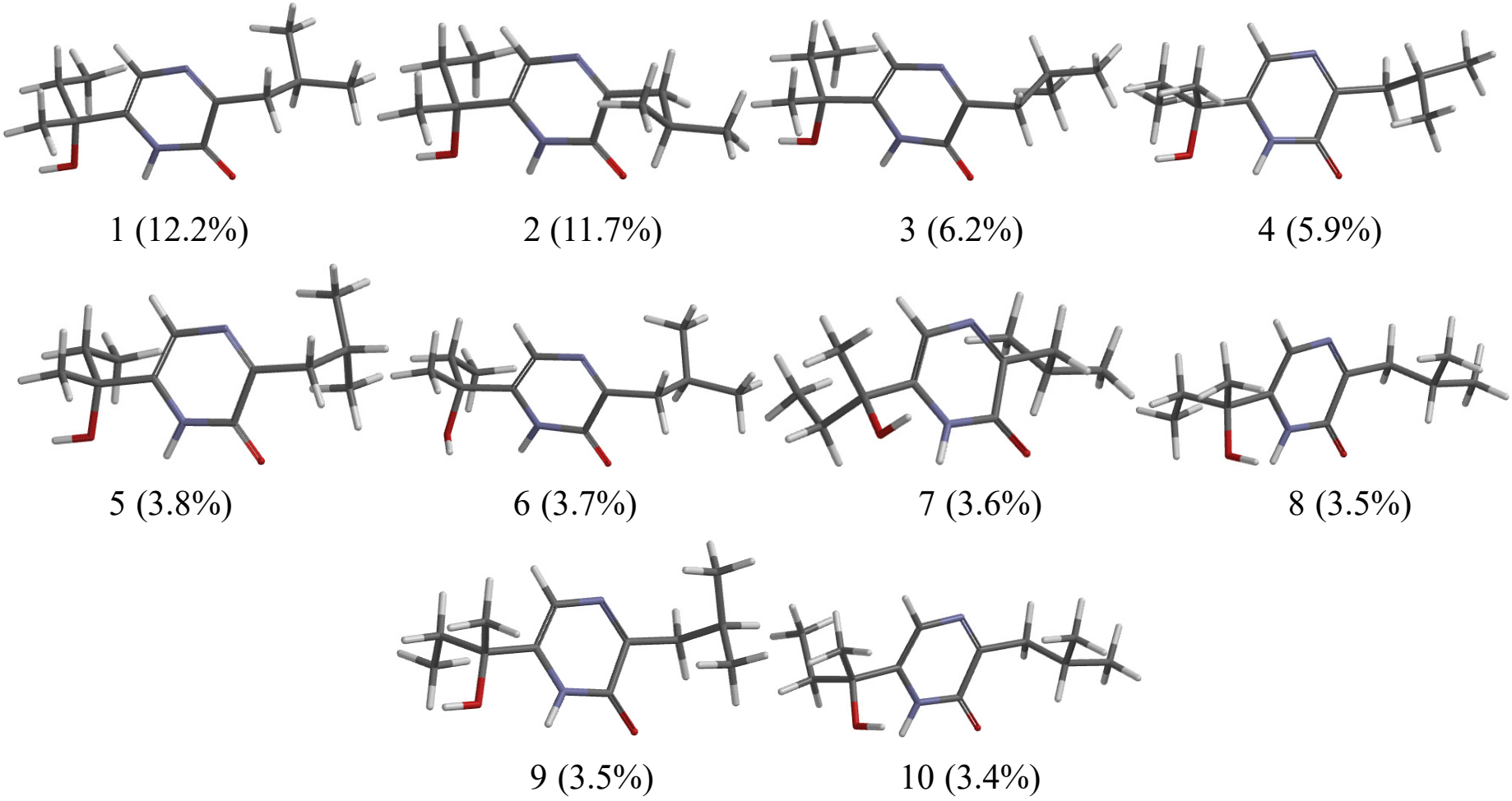


Figure S54. The most stable conformers of 11*R*-**3** calculated at the B3LYP/6-31+G(d) level. Relative populations are in parentheses.

Table S1. X-ray Crystallography Data for **4**.

Empirical formula	$2(C_{12}H_{20}N_2O_2)$
Formula weight	448.60
Temperature/K	249.99(10)
Crystal system	monoclinic
Space group	$P2_1$
a/Å	11.77370(10)
b/Å	6.91360(10)
c/Å	16.5776(2)
α/°	90
β/°	91.3700(10)
γ/°	90
Volume/Å^3	1349.01(3)
Z	2
ρ_{calc}g/cm^3	1.104
μ/mm^{-1}	0.607
F(000)	488.0
Crystal size/mm^3	0.2 × 0.15 × 0.08
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	7.51 to 148.142
Index ranges	$-14 \leq h \leq 14$, $-8 \leq k \leq 8$, $-14 \leq l \leq 19$
Reflections collected	29460
Independent reflections	5242 [R_{int} = 0.0525, R_{sigma} = 0.0221]
Data/restraints/parameters	5242/1/299
Goodness-of-fit on F^2	1.039

Final R indexes [I>=2σ (I)]	R_1 = 0.0570, wR_2 = 0.1619
Final R indexes [all data]	R_1 = 0.0588, wR_2 = 0.1647
Largest diff. peak/hole / e $Å^{-3}$	0.52/-0.22
Flack parameter	-0.04(7)

Table S2.The hepatocellular carcinoma cell line HepG2 activity of manorubin B (**6**)

HepG2								
	DMSO (μL)	manorubin B (**6**) (μmol/L)						
	0.054	0.037	0.111	0.333	1	3	9	27
OD (570nm) [a]	1.44	1.437	1.377	1.294	0.996	0.454	0.388	0.379
Cell viability (100%) [b]		96.3	94.7	91.2	66.3	32.4	24.5	27.7
Inhibition rate (100%) [c]		3.7	5.3	8.8	33.7	67.6	75.5	72.3

[a] Average of three measurements, OD: Optical density, [b] Cell viability = Measurements value (OD)/Control value DMSO (OD) × 100%,

[c] Inhibition rate = 100% – Cell viability.

Table S3. The hepatocellular carcinoma cell line QGY7701 activity of manorubin B (**6**)

QGY7701								
	DMSO (μL)	manorubin B (**6**) (μmol/L)						
	0.054	0.037	0.111	0.333	1	3	9	27
OD (570nm)	1.46	1.320	1.310	1.147	0.423	0.255	0.235	0.188
Cell viability（100%）		90.2	89.5	78.4	28.9	17.4	16.1	12.8
Inhibition rate（100%）		9.8	10.5	21.6	71.1	82.6	83.9	87.2

Table S4. The nasopharyngeal carcinoma cell line SUNE1 activity of lunatinin (**7**)

SUNE1								
	DMSO (μL)	lunatinin (**7**) (μmol/L)						
	0.2	1.5625	3.125	6.25	12.5	25	50	100
OD (570nm)	0.791	0.668	0.672	0.578	0.452	0.391	0.364	0.368
Cell viability（100%）		84.5	84.9	73	57.2	49.5	46.1	46.5
Inhibition rate（100%）		15.5	15.1	27	42.8	50.5	53.9	53.5

Table S5. The hepatocellular carcinoma cell line HepG2 activity of lunatinin (**7**)

HepG2								
	DMSO (μL)	lunatinin (**7**) (μmol/L)						
	0.2	1.5625	3.125	6.25	12.5	25	50	100
OD (570nm)	0.422	0.373	0.347	0.233	0.142	0.111	0.085	0.092
Cell viability（100%）		88.3	82.3	55.3	33.6	26.2	20.1	21.7
Inhibition rate（100%）		11.7	17.7	44.7	66.4	73.8	79.9	78.3

Table S6. The hepatocellular carcinoma cell line QGY7701 activity of lunatinin (**7**)

QGY7701								
	DMSO (μL)	lunatinin (**7**) (μmol/L)						
	0.2	1.5625	3.125	6.25	12.5	25	50	100
OD (570nm)	0.976	0.915	0.758	0.479	0.216	0.162	0.102	0.097
Cell viability（100%）		93.8	77.7	49.1	22.1	16.6	10.4	9.9
Inhibition rate（100%）		6.2	22.3	5.9	77.9	83.4	89.6	90.0