

Supplementary Materials

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Table S1. The antiSMASH-predicted BGCs for *Malbranchea circinata* SDU050.

BGC	Region	Type	Position		Product(SI>50%)
			From	To	
Cluster 1	1.1	NRPS, T1PKS	1	50,535	
Cluster 2	1.2	T1PKS	121,431	168,808	
Cluster 3	1.3	T1PKS	794,692	844,560	
Cluster 4	1.4	NRPS-like	1,022,514	1,065,976	
Cluster 5	1.5	T1PKS	1,397,638	1,450,769	
Cluster 6	1.5	fungal-RiPP-like	1,450,770	1,511,746	
Cluster 7	1.6	T1PKS	1,773,941	1,821,995	trichoxide
Cluster 8	1.7	NRPS, T1PKS	1,945,159	2,001,432	
Cluster 9	1.8	indole	2,198,180	2,219,523	
Cluster 10	1.9	T1PKS	2,573,081	2,620,957	AKML A/B/C/D
Cluster 11	1.10	T1PKS	2,763,863	2,810,887	
Cluster 12	1.11	terpene	2,909,815	2,931,766	
Cluster 13	1.12	NRPS	3,193,707	3,248,244	
Cluster 14	1.13	fungal-RiPP-like	3,340,401	3,402,658	
Cluster 15	1.14	T1PKS	4,878,820	4,926,029	
Cluster 16	1.15	T1PKS	4,964,161	5,010,815	
Cluster 17	1.16	T1PKS	5,063,751	5,110,489	
Cluster 18	1.17	T1PKS	5,660,052	5,708,440	
Cluster 19	1.17	NRPS-like	5,685,708	5,728,140	
Cluster 20	1.18	T1PKS	5,792,601	5,844,816	
Cluster 21	2.1	T1PKS	129,484	175,036	
Cluster 22	2.2	terpene	892,434	914,449	
Cluster 23	2.3	fungal-RiPP-like	1,173,957	1,238,502	
Cluster 24	2.4	T1PKS	1,630,963	1,678,916	
Cluster 25	2.4	T1PKS	1,677,462	1,729,479	
Cluster 26	2.5	NRPS-like	2,287,944	2,331,353	

Cluster 27	2.6	fungal-RiPP-like	2,701,543	2,762,459	
Cluster 28	2.7	terpene	2,893,577	2,914,761	
Cluster 29	2.8	fungal-RiPP-like	3,306,992	3,388,685	
Cluster 30	2.8	NRPS	3,343,343	3,408,685	
Cluster 31	3.1	NRPS-like, T1PKS	192,668	240,191	swainsonine
Cluster 32	3.2	T1PKS	1,208,456	1,257,041	
Cluster 33	3.3	T1PKS	3,555,328	3,616,474	
Cluster 34	4.1	T1PKS	13,556	61,375	
Cluster 35	4.1	T1PKS	57,452	127,824	
Cluster 36	4.2	indole	401,825	423,255	
Cluster 37	4.3	NRPS-like	743,573	789,156	
Cluster 38	4.3	NRPS-like	752,259	796,183	
Cluster 39	4.4	fungal-RiPP-like	1,073,005	1,135,667	
Cluster 40	4.5	T1PKS	1,175,205	1,224,695	
Cluster 41	4.6	T1PKS	1,487,335	1,536,244	
Cluster 42	4.7	terpene	1,799,121	1,821,407	clavaric acid
Cluster 43	5.1	fungal-RiPP-like	1	39,765	
Cluster 44	5.1	fungal-RiPP-like	1,545	62,033	
Cluster 45	5.2	fungal-RiPP-like	251,540	313,918	
Cluster 46	5.3	NRPS-like	1,468,983	1,517,847	
Cluster 47	5.4	NRPS	2,395,753	2,459,739	
Cluster 48	5.5	T1PKS	2,524,143	2,572,480	
Cluster 49	5.6	NRPS-like	4,066,662	4,110,077	
Cluster 50	5.6	T1PKS	4,078,883	4,126,784	
Cluster 51	5.7	T1PKS	4,353,201	4,400,379	
Cluster 52	5.8	fungal-RiPP-like	4,867,925	4,928,903	
Cluster 53	5.9	T1PKS	4,941,967	4,988,605	fujikurin A/B/C/D
Cluster 54	6.1	T1PKS	197,820	246,822	
Cluster 55	6.2	NRPS-like	477,497	521,107	

Cluster 56	6.3	fungal-RiPP-like	579,264	640,216	
Cluster 57	6.4	T1PKS	689,938	737,480	
Cluster58	6.5	NRPS	1,456,143	1,510,753	
Cluster 59	6.6	T1PKS	1,775,183	1,823,550	cichorine
Cluster 60	6.7	T1PKS	2,370,832	2,419,010	
Cluster 61	7.1	terpene	48,162	68,962	
Cluster 62	8.1	indole	388,727	410,602	
Cluster 63	8.1	T1PKS	407,085	454,116	
Cluster 64	8.1	NRPS-like	443,558	486,402	
Cluster 65	8.2	NRPS, T1PKS	597,132	648,233	
Cluster 66	8.3	T1PKS	898,191	956,862	Sorbicillin/ bisvertinolone
Cluster 67	8.3	NRPS	932,687	989,242	
Cluster 68	9.1	fungal-RiPP-like	468,915	528,215	
Cluster 69	9.1	NRPS-like	487,893	532,312	

Table S2. Deduced functions of *orfs* in the *scl* BGC (accession number: PQ824967).

<i>orfs</i>	Size (AA)	Proposed function	ID/SI	Origin and protein homologue
1	1147	transcription factor Cmr1	44/74	<i>Bombardia bombardia</i> /KAK0615866.1
2	275	versicolorin reductase	77/99	<i>Lachnellula suecica</i> /TVY85119.1
3	265	transcription factor	47/26	<i>Diplocarpon rosae</i> /PBP24182.1
A	2142	BcPKS12, polyketide synthase	65/97	<i>Calycina marina</i> /KAG9246027.1
5	543	cytochrome P450 monooxygenase psoD	74/89	<i>Hyphodiscus hymeniophilus</i> /KAG0648686.1
6	246	4-coumarate-coA ligase 1	75/99	<i>Physcia stellaris</i> /KAG7007251.1
7	245	acetyl-CoA synthetase-like protein	82/100	<i>Stipitochalara longipes</i> /KAE9381253.1
8	854	fungal-specific transcription factor	60/100	<i>Hyaloscypha finlandica</i> /KAH8770291.1
9	600	peptidyl-prolyl cis-trans isomerase cyp15	89/92	<i>Lachnellula cervine</i> /TVY53092.1
10	410	cyclin-domain-containing protein	60/99	<i>Bisporella</i> sp./KAH8602979.1
11	275	nucleophile aminohydrolase	57/69	<i>Calycina marina</i> /KAG9245827.1
12	204	FAD dependent sulfhydryl oxidase Erv2	72/88	<i>Halenospora varia</i> /KAH6671069.1

Table S3. Strains used and constructed in this study.

Strains	Description	Reference or source
Bacteria Strains		
<i>E. coli</i> DH5 α	Host strain of general cloning	Sangon Biotech
Fungal Strains		
<i>M. circinata</i> SDU050	Wild strain	This study
<i>M. circinata</i> SDU050/ Δ <i>sclA</i>	<i>sclA</i> disruption mutant strain originated from <i>M. circinata</i> SDU050	This study

Table S4. Plasmids used in this study.

Plasmid	Vector	Genes/Description
pBSKII-cas9-hph	-	The plasmid can be BsaI digested as CRISPR-Cas9 plasmid vector
pFC332	-	Template for sgDNA
pCas9- <i>sclA</i>	pBSKII-cas9-hph	<i>P_{gpdA}-HH-repeat-protospacer-sgDNA-HDV-T_{trpC}</i>

Table S5. Primers used in this study.

Primer	Sequence (5'-3')
For plasmids construction	
pCas9-ligase-F	TCAGGGCGATGGCCCACTACGCGTAAGCTCCCTAATTGGCC
pCas9-ligase-R	TGATTAGGGTGATGGTTCACGAGCCAAGAGCGGATTCCTCAG
pCas9- <i>sclA</i> -F	ACGAGTAAGCTCGTCGATCTGTCTGATAAGCCTCTGTTTTAGAGCTAGAAATAGCAAG
pCas9- <i>sclA</i> -R	GACGAGCTTACTCGTTTCGTCCTCACGGACTCATCAGGATCTGCGGTGATGTCTGCTC
For confirmation	
ID-Cas9-ligase-F	TCAGGGCGATGGCCCACTACGCGTAAGCTCCCTAATTGGCC
ID-Cas9-ligase-R	TGATTAGGGTGATGGTTCACGAGCCAAGAGCGGATTCCTCAG
ID- <i>sclA</i> -up-F1	GATCTGTCTGATAAGCCTCT
ID- <i>sclA</i> -up-F2	GCGCATGGGCCTTTCTTTGATAG
ID- <i>sclA</i> -down-R	GCCCAAGGGTATGGATCATCGAA

Table S6. Media and stock used in this study.

Medium name	Ingredient
Potato Dextrose Broth (PDB) Medium (1 L) (made by HUANKAI MICROBIAL SCL&TECH.CO., LTD, China)	24 g PDB power
Potato Dextrose Agar (PDA) Medium (1 L) (made by HUANKAI MICROBIAL SCL&TECH.CO., LTD, China)	39 g PDA power
	5 g Yeast extract
	10 g Tryptone
LB Medium (1 L)	10 g NaCl
	Adjust pH to 7.2–7.4 with 1M NaOH
	1 M sucrose
Protoplast Medium (1 L)	10 g Dextrose/Glucose
	20 mL Salt solution
	50 mM EDTA, 3% SDS, 50 mM Tris-HCl, pH
Lysis Buffer	7.2, 1% 2-mercaptoethanol
Osmotic Buffer	1.2 M MgSO ₄ ·7H ₂ O, 10 mM NaPhosphate buffer
Trapping Buffer	0.6 M sorbitol, 0.1 M Tris HCl, pH 7.0
STC Buffer	1.2 M sorbitol, 10 mM CaCl ₂ , 10 mM Tris-HCl pH 7.5
PEG solution	60% PEG, 50 mM CaCl ₂ , and 50 mM Tris-HCl, pH 7.5
	26 g KCl
	76 g KH ₂ PO ₄
Salt solution (1L)	26 g MgSO ₄
	50 mL Trace element
	Add 2 mL chloroform per L of solution
	2.20 g ZnSO ₄ ·7H ₂ O
	1.10 g H ₃ BO ₃
	0.50 g MnCl ₂ ·4H ₂ O
	0.16 g FeSO ₄ ·7H ₂ O
Trace elements (100 mL)	0.16 g CoCl ₂ ·5H ₂ O
	0.16 g CuSO ₄ ·5H ₂ O
	0.11 g (NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O
	Adjusted pH to 6.5 with 1 M KOH

Table S7: Summary of ¹H and ¹³C NMR data for compounds **7-9** in CD₃OD and CDCl₃ (δ in ppm).

no.	7 (CDCl ₃)			8 (CD ₃ OD)			9 (CD ₃ OD)		
	δ _H (mult., J)	type	δ _C	δ _H (mult., J)	type	δ _C	δ _H (mult., J)	type	δ _C
1		C	174.3		C	175.9		C	177.4
2		NH			NH			NH	
3	3.51, m	CH	58.1	3.33, m (overlap)	CH	55.8	3.46, m	CH	54.9
4	3.56, m (overlap)	CH	49.8	3.12, dd (5.2, 3.5)	CH	49.1	2.75, dd (5.8, 2.5)	CH	50.7
5		C	147.7	2.30, m	CH	36.1	2.43, m	CH	36.4
6		C	133.4		C	142.2		C	140.7
7		C	195.8	5.38, m	CH	126.5	5.37, m	CH	126.7
8	3.26, dd (10.0, 2.6)	CH	54.5	2.43, d (9.9)	CH	50.2	2.75, m (overlap)	CH	51.0
9		C	61.6		C	69.6		C	68.5
10	2.76, dd (13.3, 6.6)	CH ₂	42.2	2.72, dd (13.5, 3.7)	CH ₂	44.5	2.71, dd (13.5, 3.7)	CH ₂	43.5
	2.50, dd (13.3, 8.6)			2.63, dd (13.5, 9.0)			2.61, dd (13.5, 9.0)		
11	1.56, s	CH ₃	19.0	1.06, d (7.3)	CH ₃	14.0	1.11, d (7.3)	CH ₃	13.6
12	1.70, s	CH ₃	12.1	1.77, s	CH ₃	20.1	1.75, s	CH ₃	20.0
13	6.36, dd (15.6, 9.9)	CH	124.2	5.76, dd (15.5, 10.0)	CH	132.4	6.18, dd (15.2, 10.3)	CH	130.5
14	5.50, ddd (15.0, 10.4, 4.1)	CH	134.6	5.29, ddd (15.5, 10.0, 5.6)	CH	135.8	5.44, ddd (15.2, 10.3, 4.7)	CH	131.8
15	2.37, m	CH ₂	40.7	2.02, m	CH ₂	41.7	2.17, m	CH ₂	41.5
	2.01, m			1.67, m			1.88, m		
16	2.00, m	CH	30.4	1.59, m	CH	39.0	1.93, m	CH	32.1
17	1.44, m	CH ₂	32.2	1.53, m	CH ₂	35.6	1.35, m	CH ₂	33.1
	1.32, m			1.42, m			1.25, m		
18	1.36, m	CH ₂	21.8	2.33, m	CH ₂	33.8	1.17, m	CH ₂	23.0
	1.21, m			1.87, m			1.13, m		
19	2.05, m	CH ₂	22.3	6.29, ddd (16.2, 10.8, 5.8)	CH	147.3	1.66, m	CH ₂	22.9
20	3.56, m (overlap)	CH ₂	39.7	6.82, d (16.2)	CH	132.8	3.24, m	CH ₂	39.6
	2.28, ddd (18.5, 6.1, 2.7)						1.13, m		
21		C	209.0			201.1		C	213.5
22	0.90, d (6.3)	CH ₃	18.7	1.02, d (7.2)	CH ₃	25.2	0.87, d (7.2)	CH ₃	19.2
1'		C	136.5		C	138.0		C	137.3
2', 6'	7.16, d (7.3)	CH	129.3	7.14, d (7.2)	CH	131.2	7.10, d (7.2)	CH	131.9
3', 5'	7.35, t (7.3)	CH	129.1	7.25, t (7.2)	CH	129.5	7.24, t (7.2)	CH	129.4
4'	7.28, t (7.3)	CH	127.4	7.17, t (7.2)	CH	127.7	7.18, t (7.2)	CH	127.6

Recorded at 700 (for ¹H) or 175 (for ¹³C) MHz. Coupling constant *J* is in Hz and overlapped signals are reported without designating multiplicity.

Table S8. Single crystal X-ray diffraction analysis data of compound **7**.

Identification code	7
Empirical formula	C ₂₇ H ₃₃ NO ₃
Formula weight	419.54
Temperature/K	100(1)
Crystal system	trigonal
Space group	P3221
a/Å	12.2833(2)
b/Å	12.2833(2)
c/Å	27.4964(5)
α /°	90
β /°	90
γ /°	120
Volume/Å ³	3592.83(13)
Z	6
ρ calc/g/cm ³	1.163
μ /mm ⁻¹	0.590
F(000)	1356.0
Crystal size/mm ³	0.2 × 0.15 × 0.13
Radiation	Cu K α (λ = 1.54184)
2 Θ range for data collection/°	8.312 to 147.5
Index ranges	-14 ≤ h ≤ 14, -11 ≤ k ≤ 15, -32 ≤ l ≤ 33
Reflections collected	12496
Independent reflections	4724 [Rint = 0.0528, Rsigma = 0.0496]
Data/restraints/parameters	4724/267/347
Goodness-of-fit on F ²	1.066
Final R indexes [I > 2 σ (I)]	R1 = 0.0645, wR2 = 0.1637
Final R indexes [all data]	R1 = 0.0703, wR2 = 0.1670
Largest diff. peak/hole / e Å ⁻³	0.20/-0.19
Flack parameter	0.3(3)

Figure S1. ORTEP drawing of compound 7 with 50% ellipsoid contour probability.

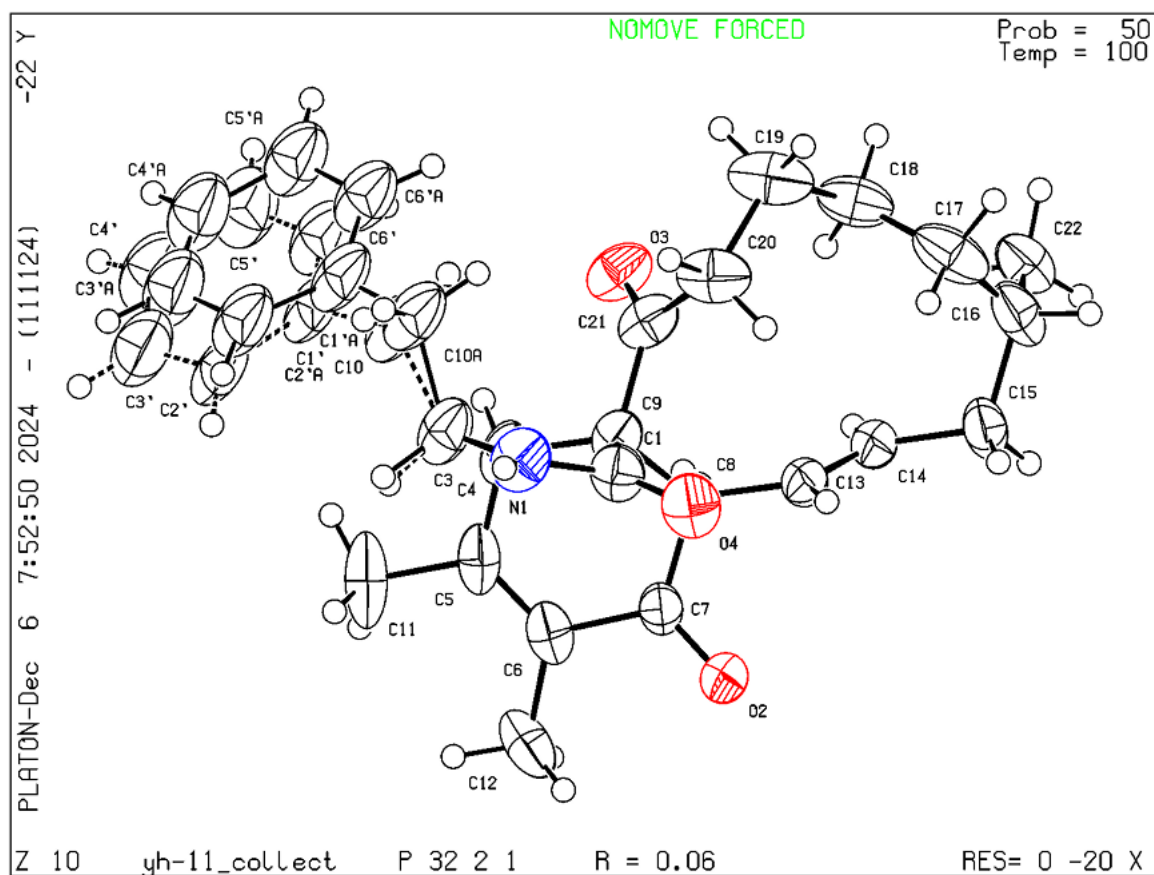


Figure S2. Construction and gel electrophoresis analyses of mutant $\Delta sclA$. (a) Construction of $\Delta sclA$ mutant and predicted PCR fragment size from wild-type and mutant. (b) Verification of the $\Delta sclA$ mutant by PCR DNA templates from WT (line 1 and 3) and $\Delta sclA$ mutant (line 2 and 4). Marker, DNA marker DL5000 (lane M). The long plasmid fragment inserted at the target site is difficult to be amplified.

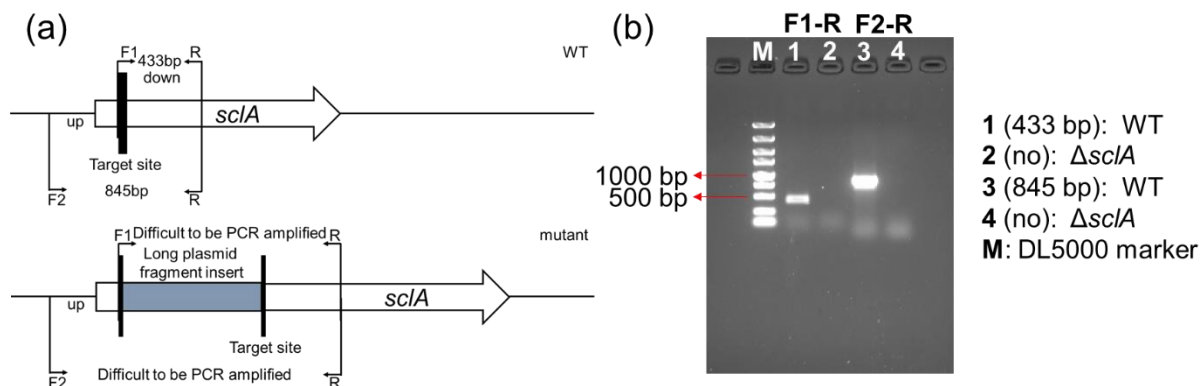


Figure S3. The organization of the PKS/NRPS BGC responsible for the biosynthesis of cytochalasins validated by the gene inactivation.

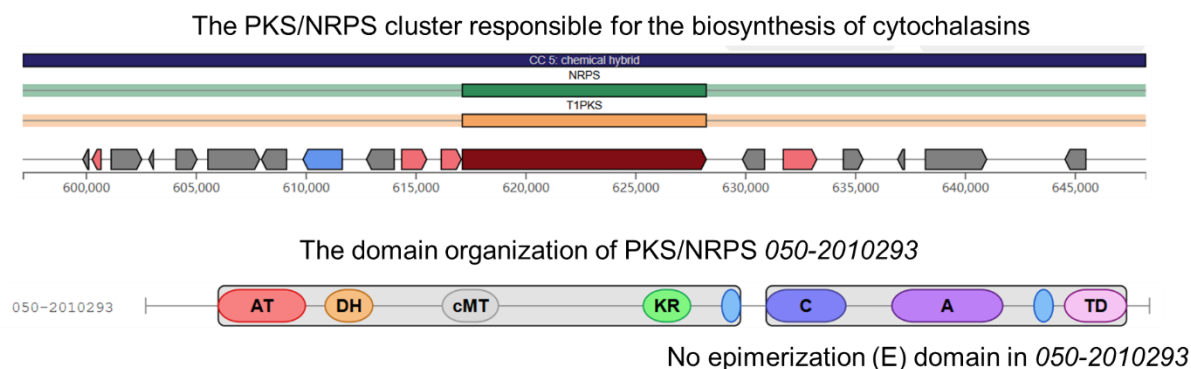


Figure S4. (+)-HRESIMS spectrum of Malcirchalsin A (7).

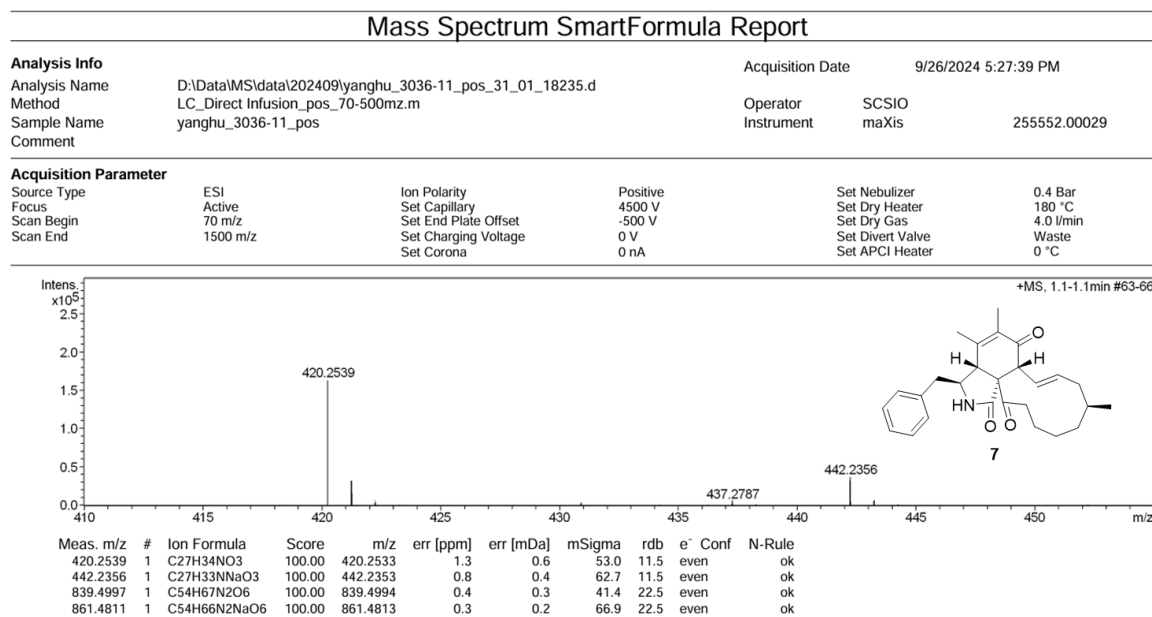


Figure S5. IR spectrum of Malcirchalsin A (7).

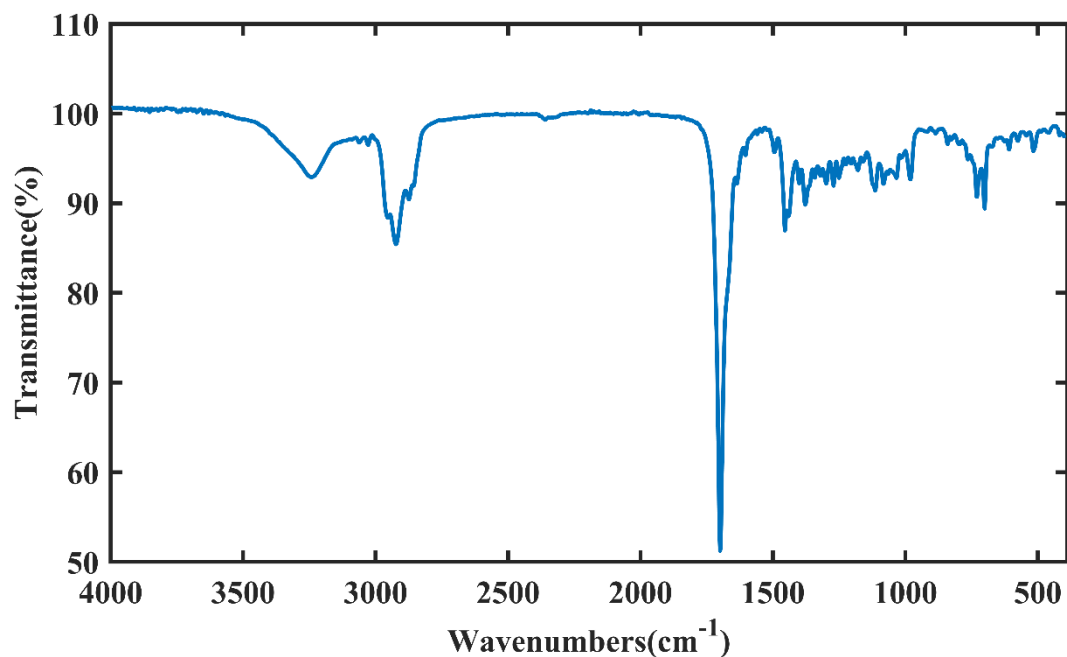


Figure S6. ^1H NMR (700 MHz, chloroform- d) spectrum of Malcirchalsin A (7).

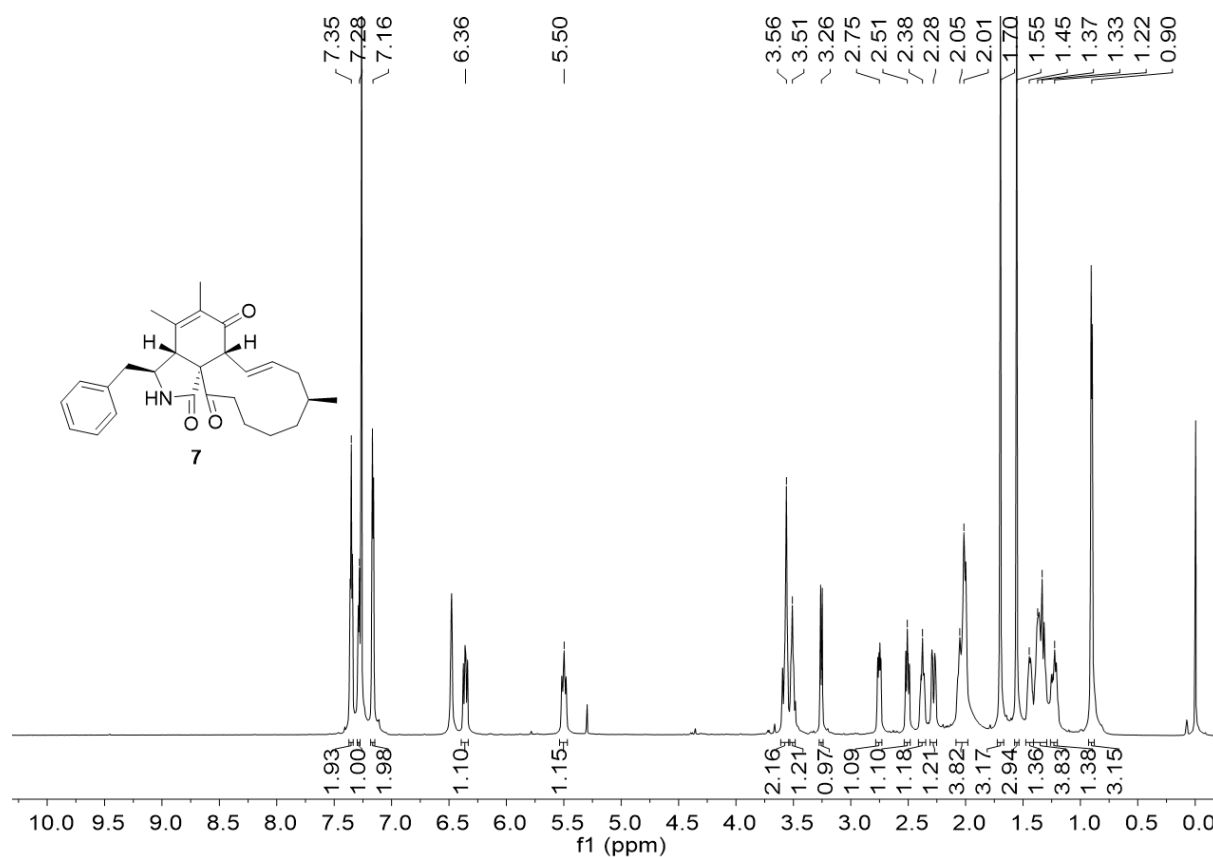


Figure S7. ^{13}C NMR (175 MHz, chloroform- d) spectrum of Malcirchalsin A (7).

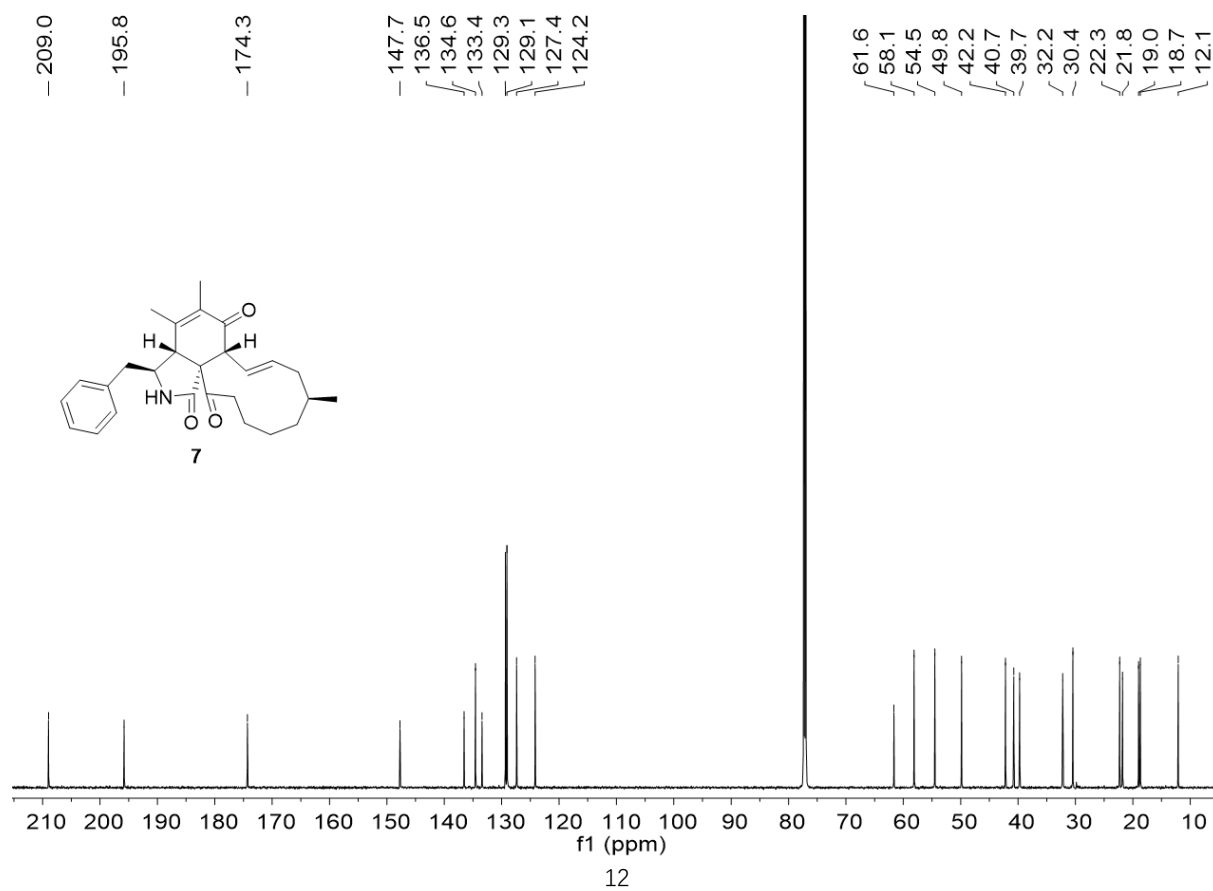


Figure S8. DEPT 135 (175 MHz, chloroform-*d*) spectrum of Malcirchalsin A (7).

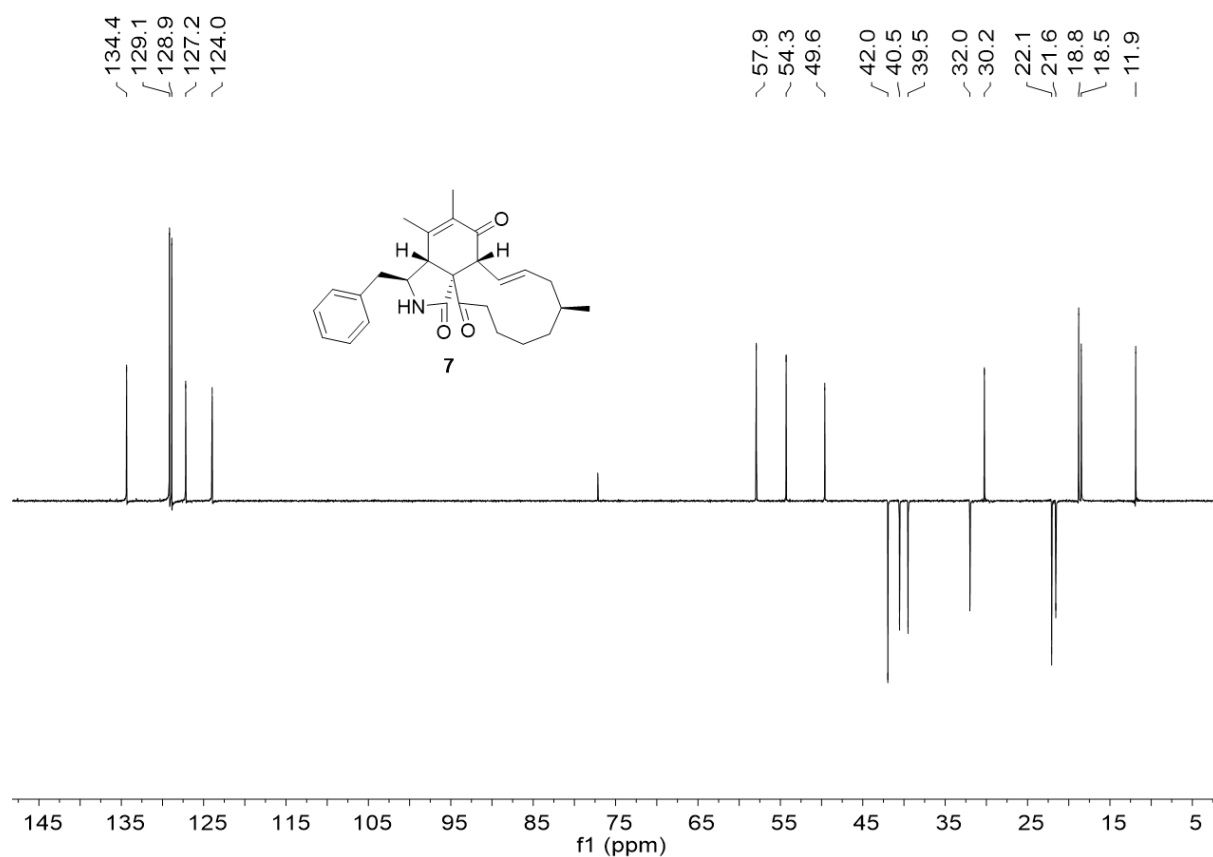


Figure S9. ^1H - ^1H COSY spectrum of Malcirchalsin A (7).

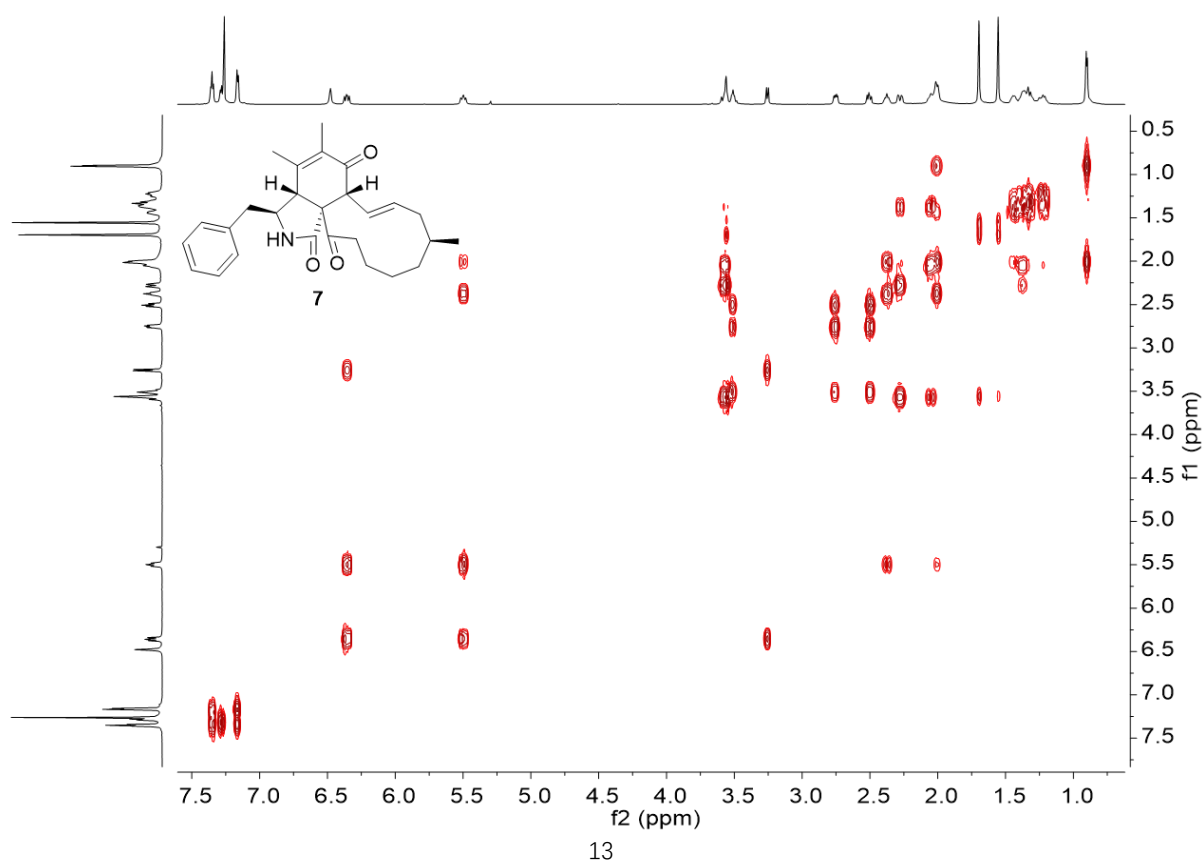


Figure S10. HSQC spectrum of Malcirchalsin A (7).

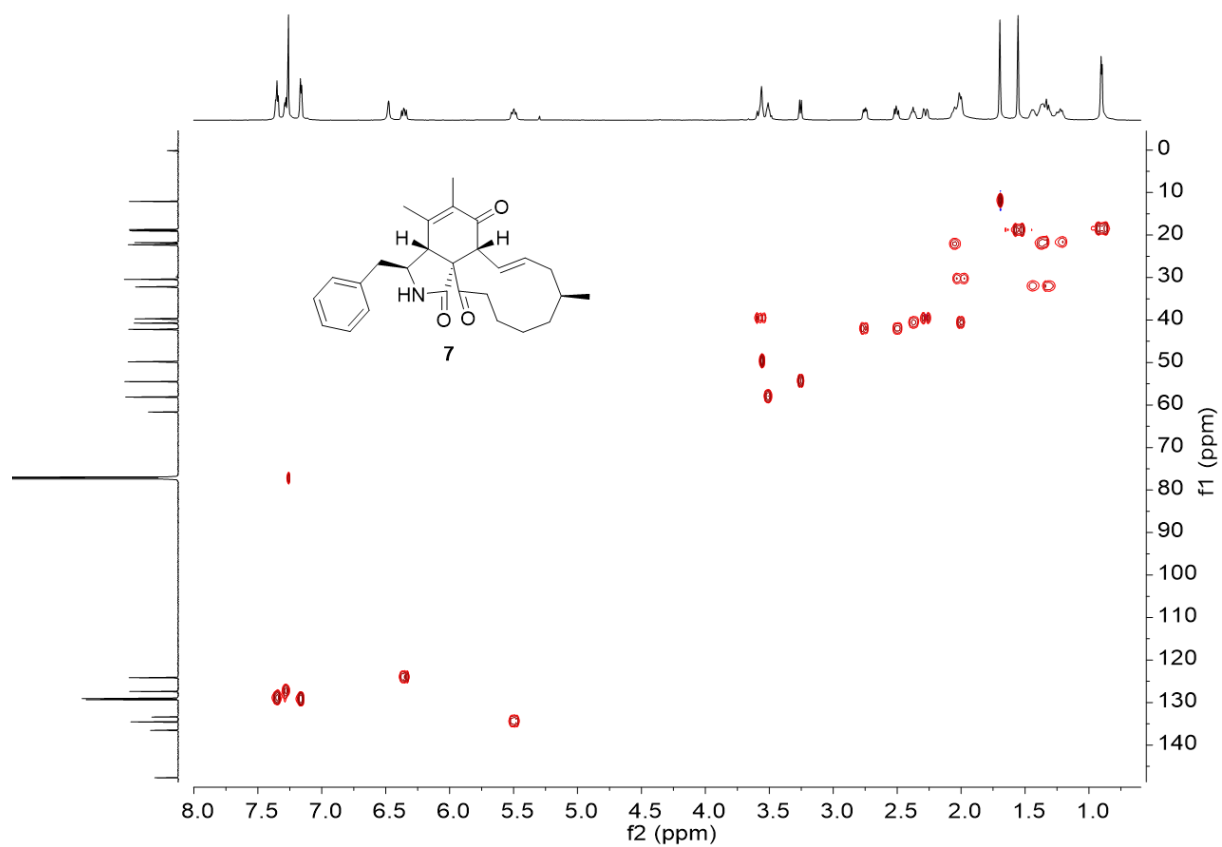


Figure S11. HMBC spectrum of Malcirchalsin A (7).

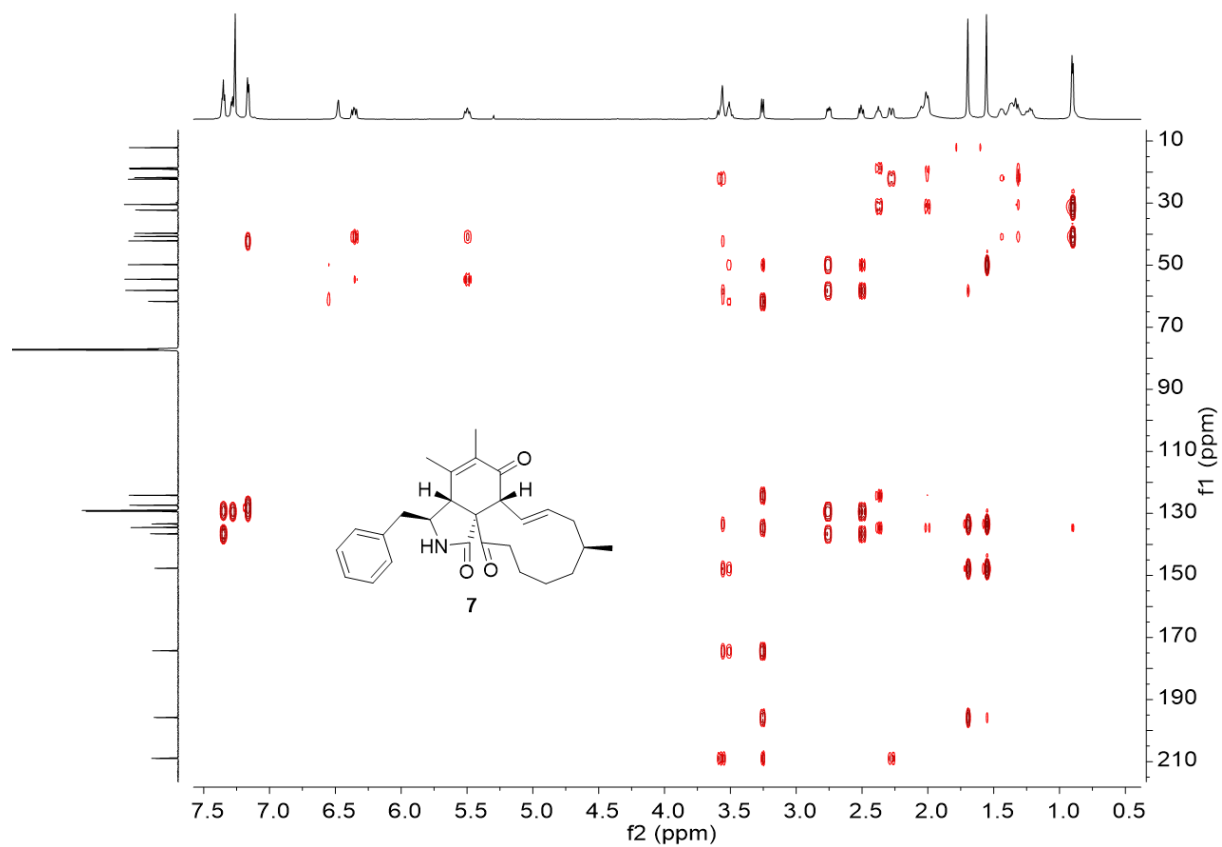


Figure S12. NOESY spectrum of Malcirchalsin A (7).

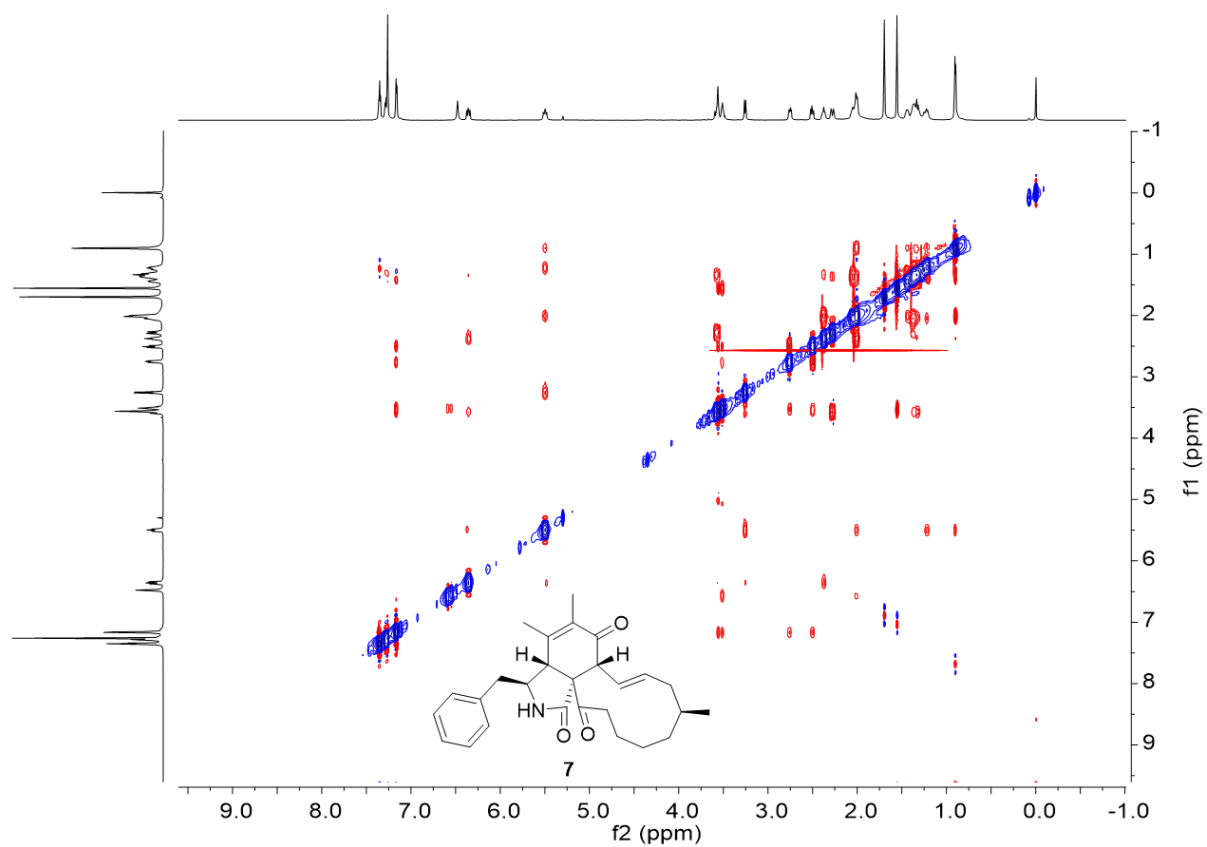


Figure S13. (+)-HRESIMS spectrum of Malcirchalsin B (8).

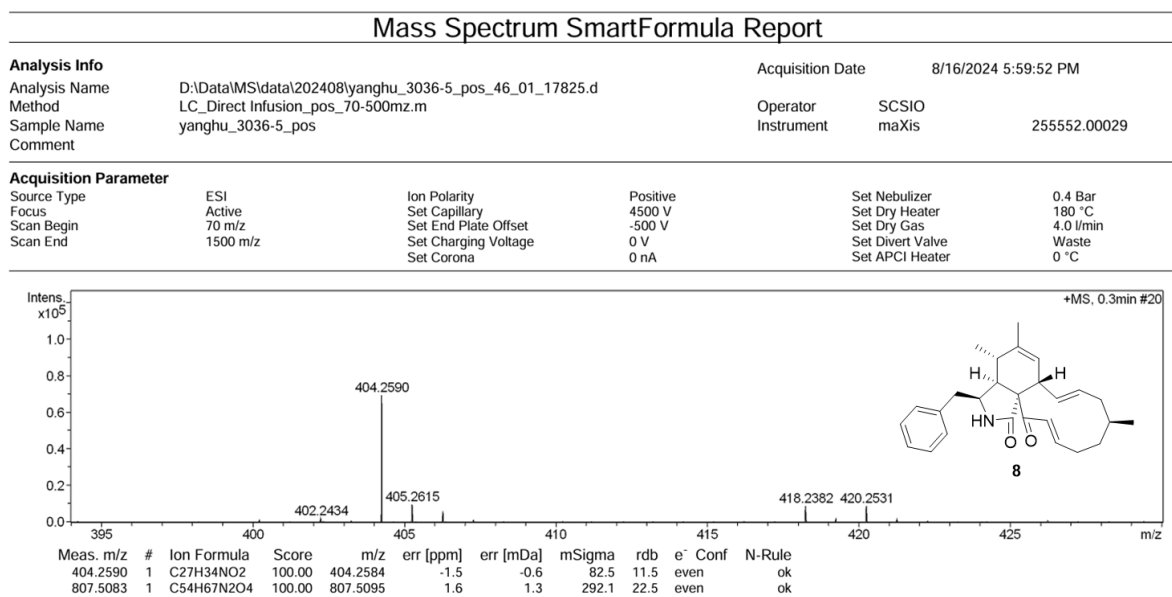


Figure S14. IR spectrum of Malcirchalsin B (8).

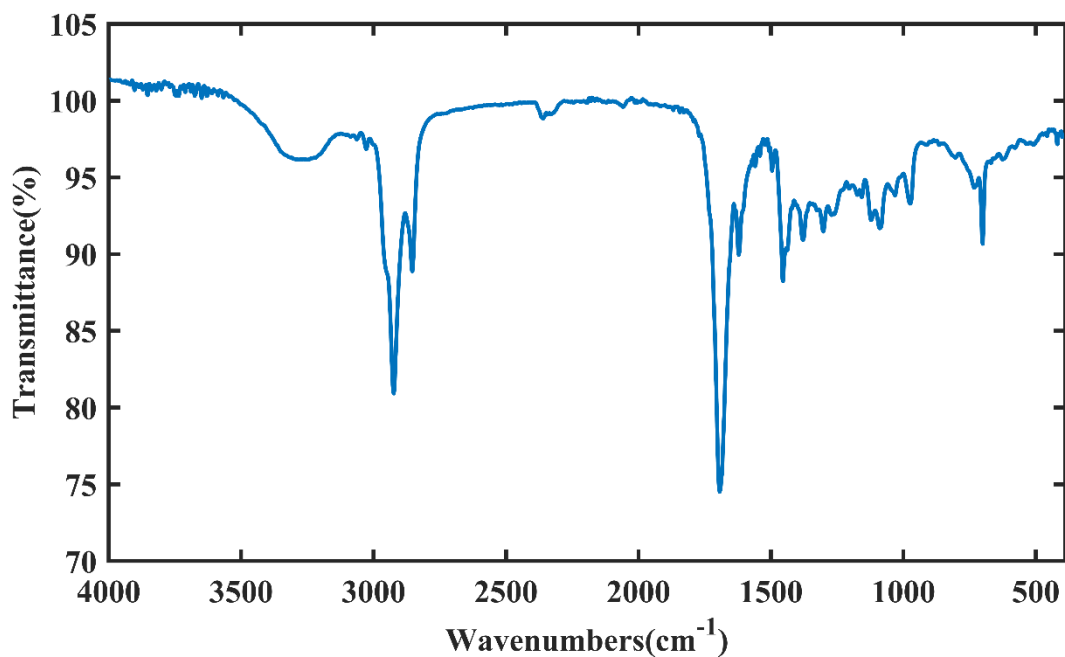


Figure S15. ^1H NMR (700 MHz, methanol- d_4) spectrum of Malcirchalsin B (**8**).

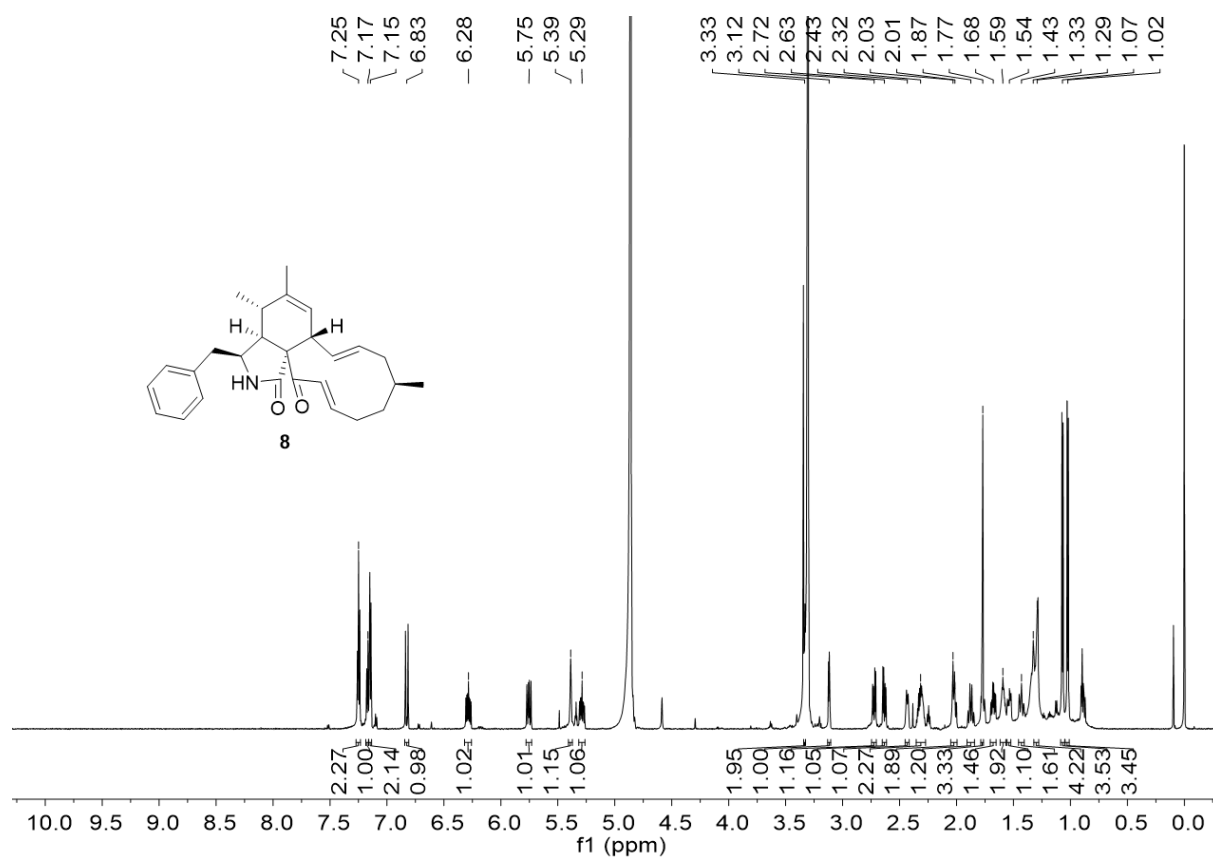


Figure S16. ^{13}C NMR (175 MHz, methanol- d_4) spectrum of Malcirchalsin B (**8**).

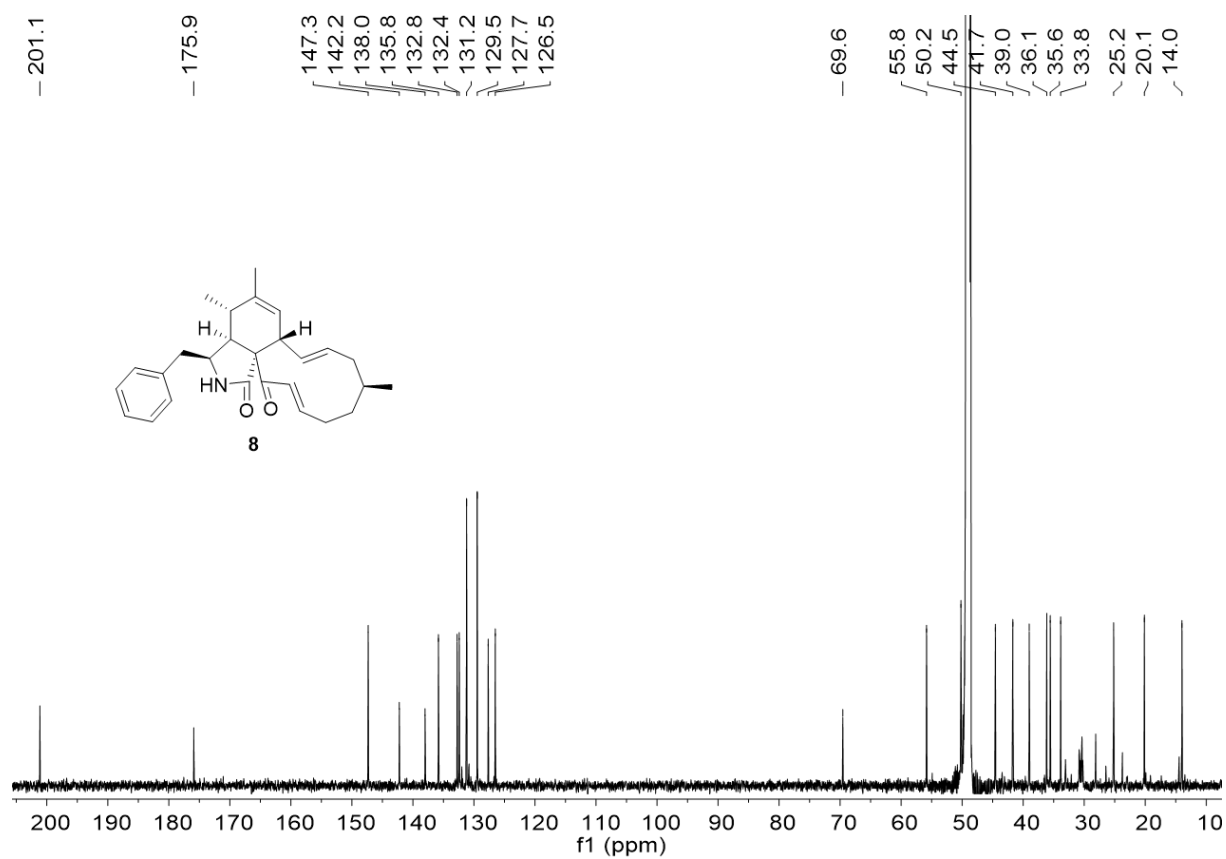


Figure S17. ^1H - ^1H COSY spectrum of Malcirchalsin B (**8**).

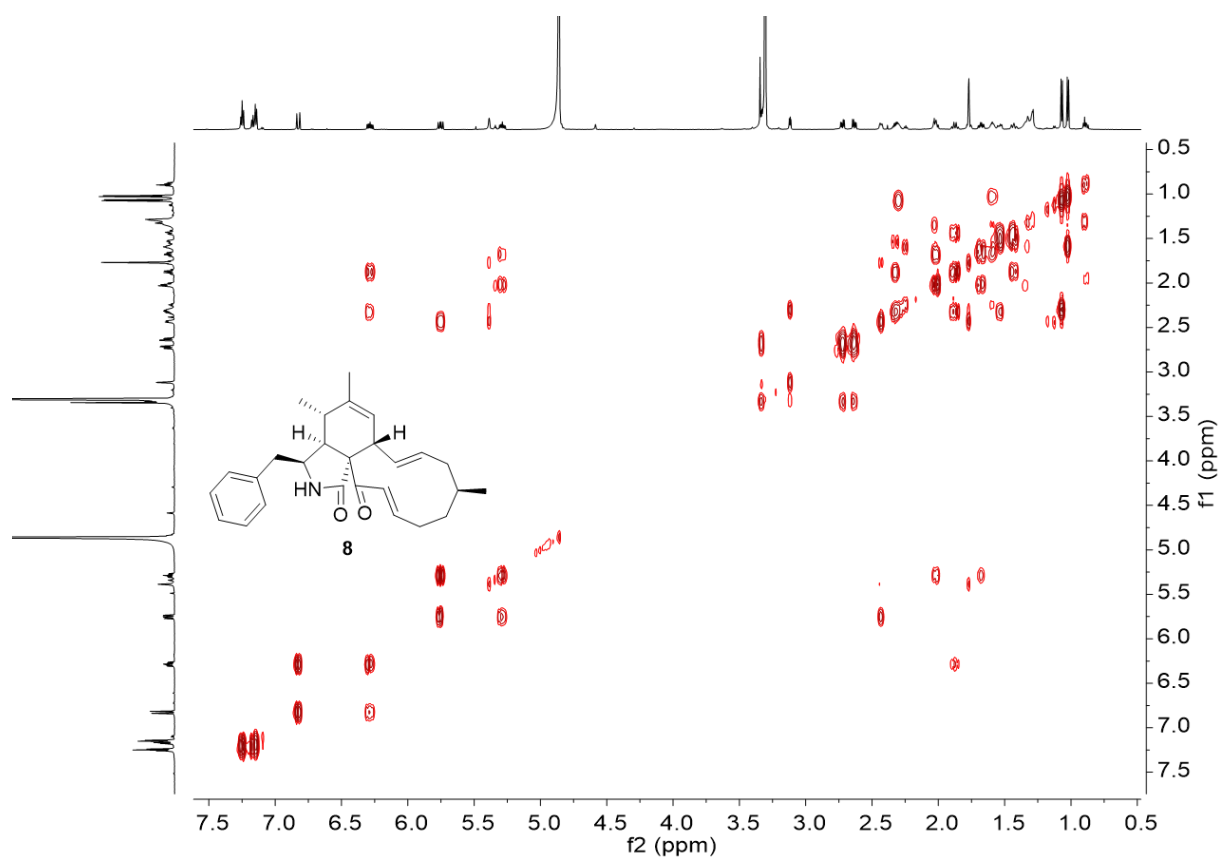


Figure S18. HSQC spectrum of Malcirchalsin B (**8**).

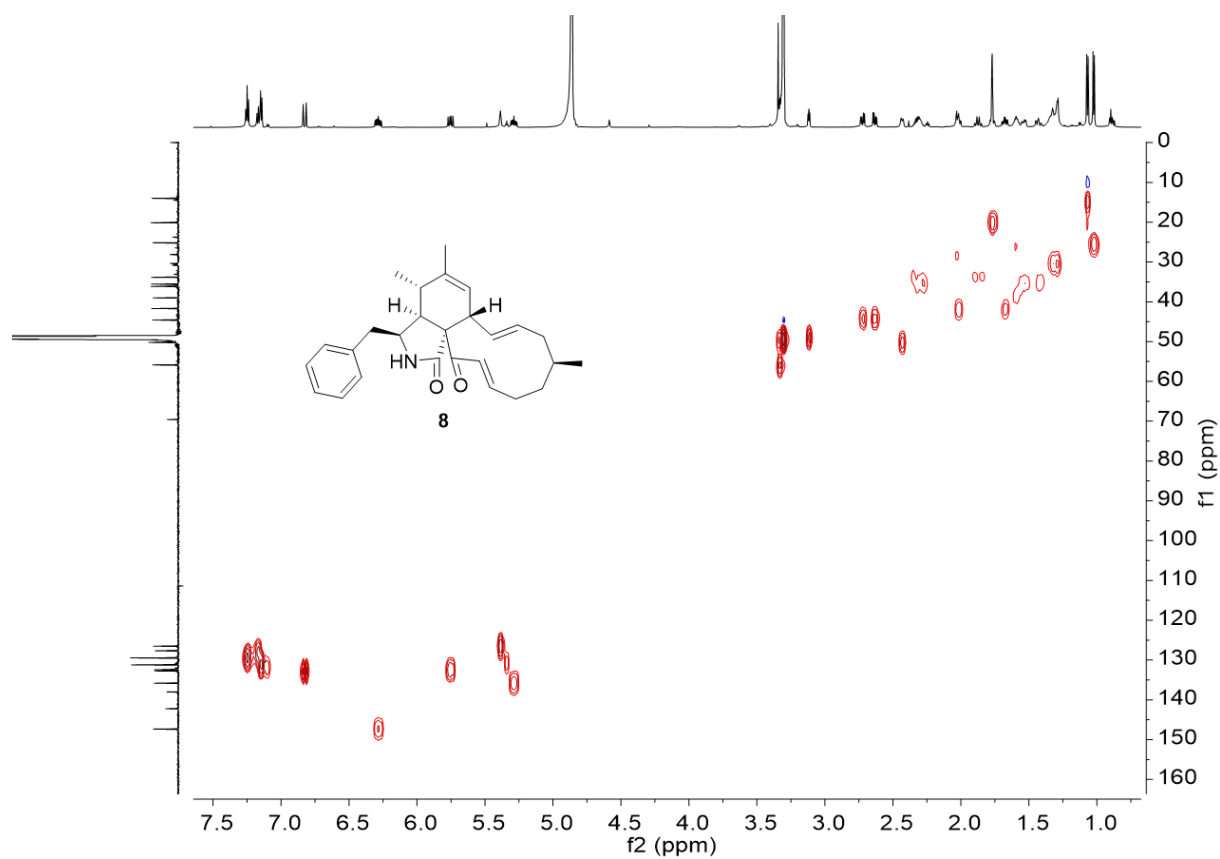


Figure S19. HMBC spectrum of Malcirchalsin B (**8**).

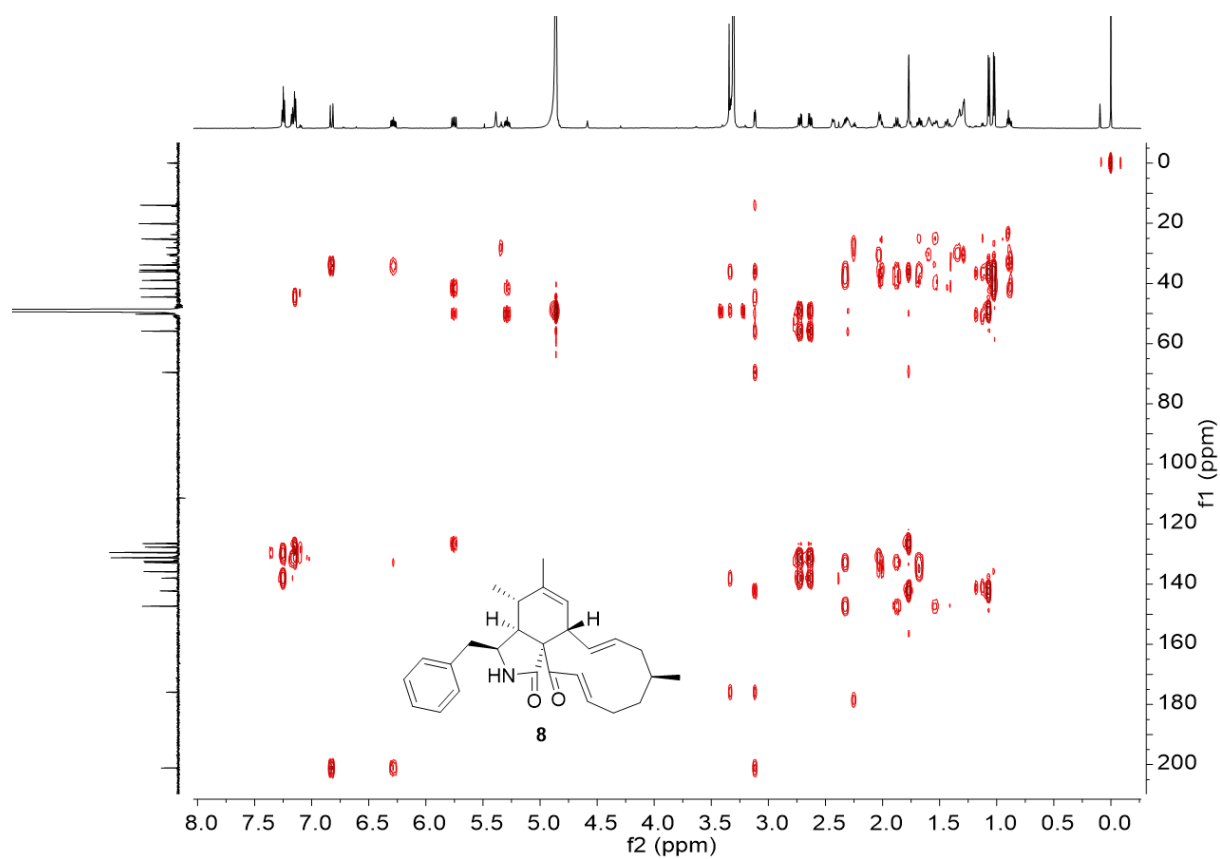


Figure S20. NOESY spectrum of Malcirchalsin B (**8**).

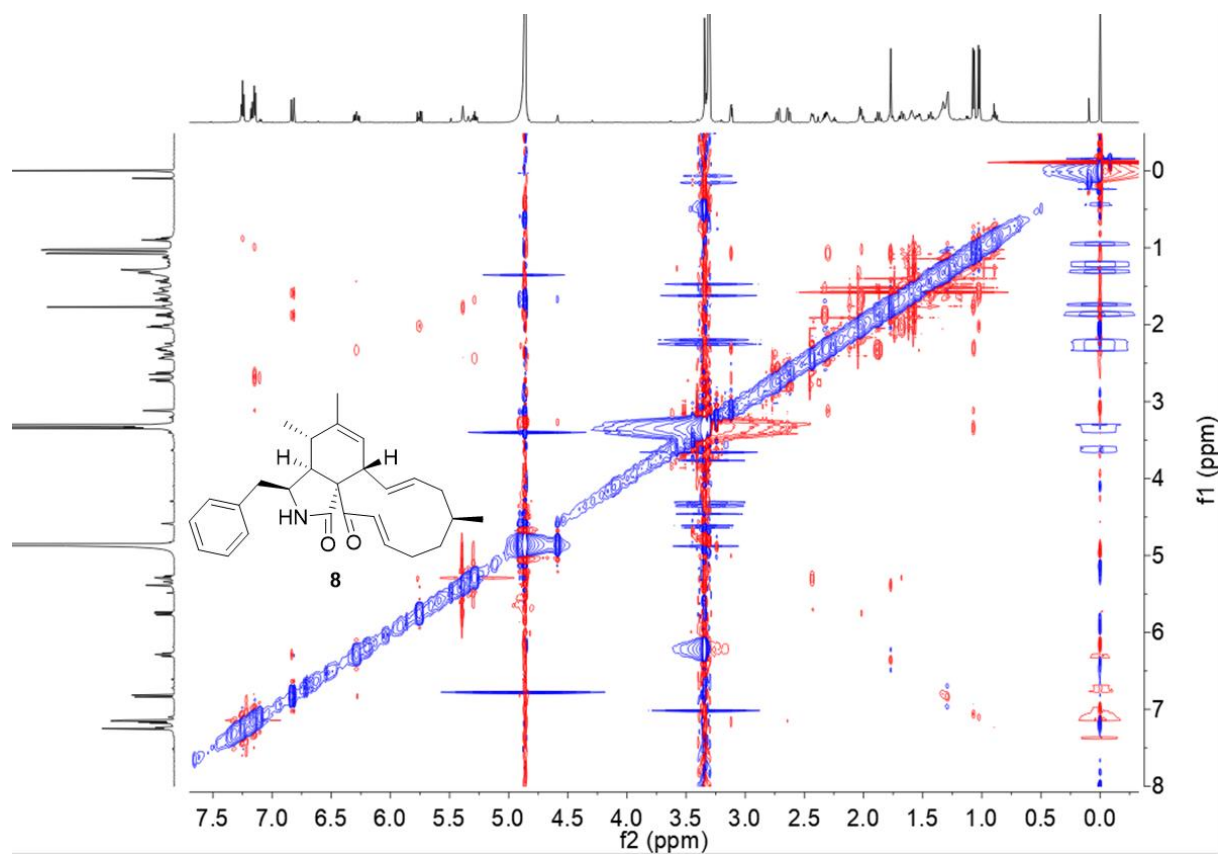


Figure S21. (+)-HRESIMS spectrum of Malcirchalsin C (9).

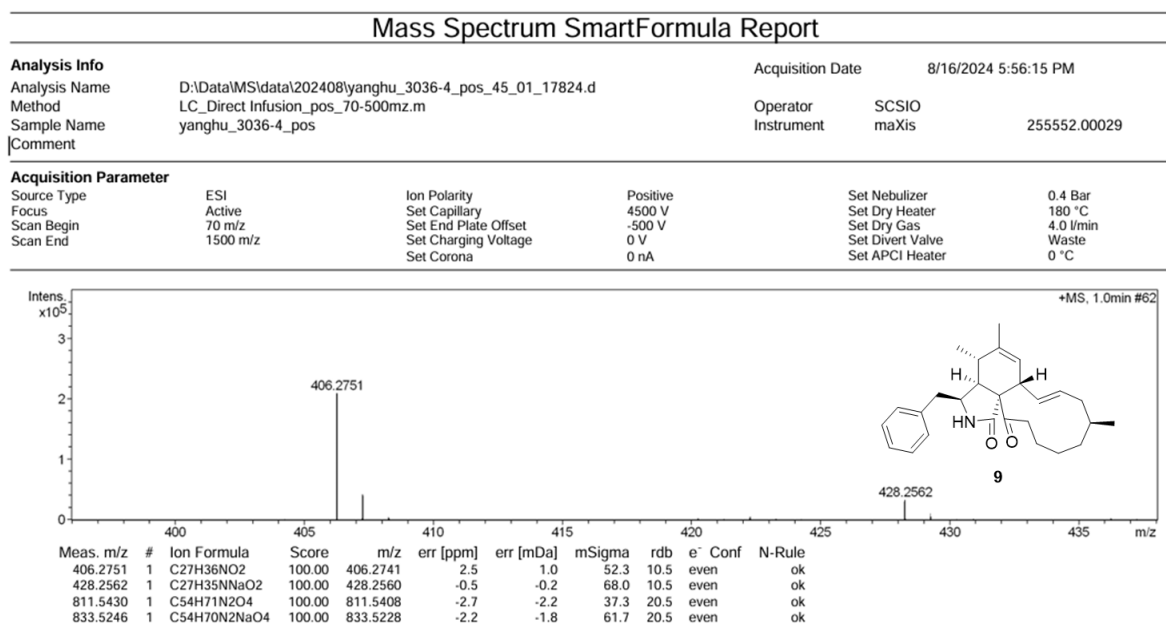


Figure S22. IR spectrum of Malcirchalsin C (9).

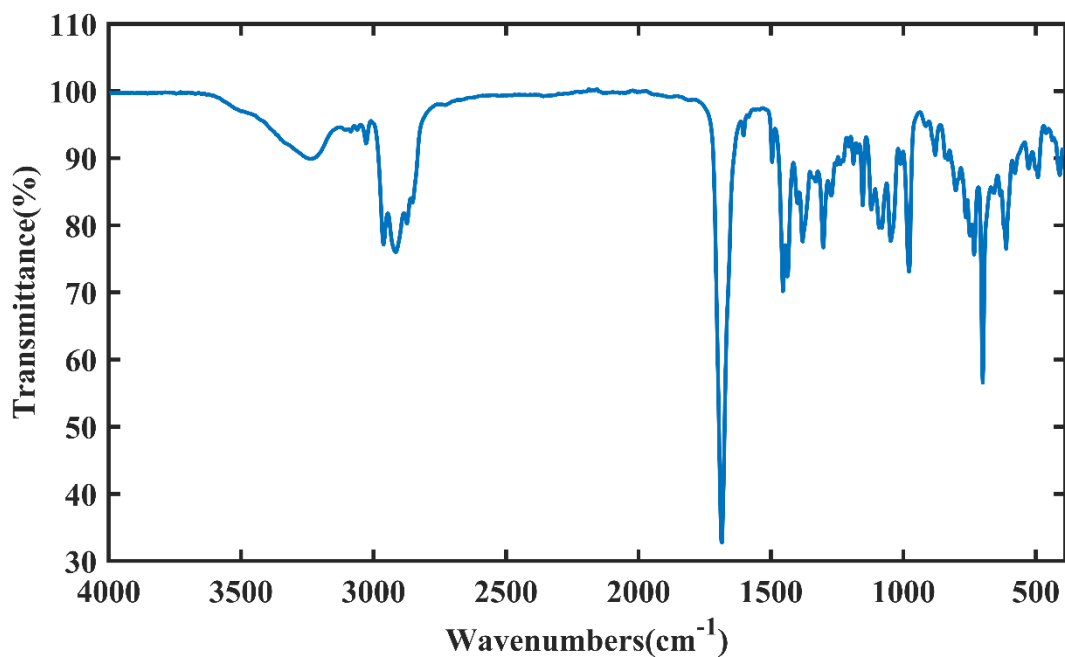


Figure S23. ^1H NMR (700 MHz, methanol- d_4) spectrum of Malcirchalsin C (**9**).

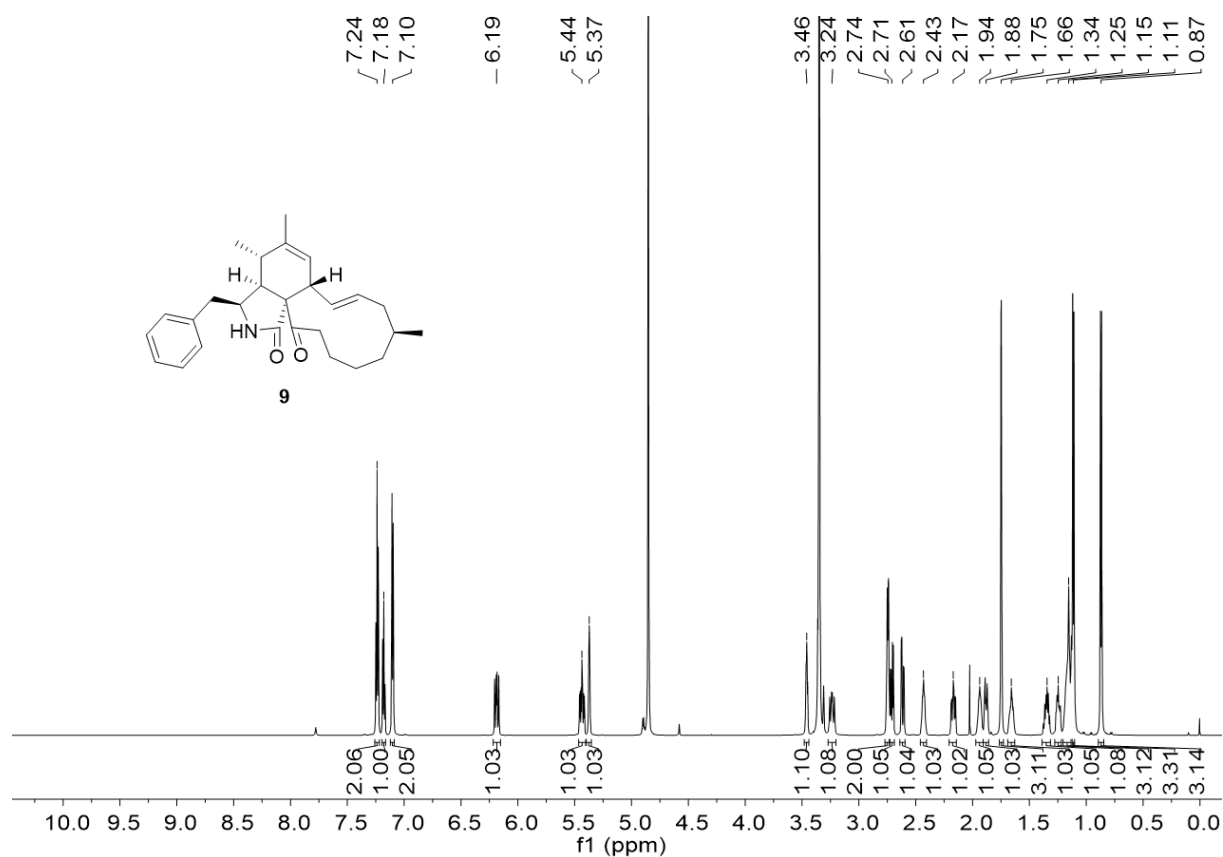


Figure S24. ^{13}C NMR (175 MHz, methanol- d_4) spectrum of Malcirchalsin C (**9**).

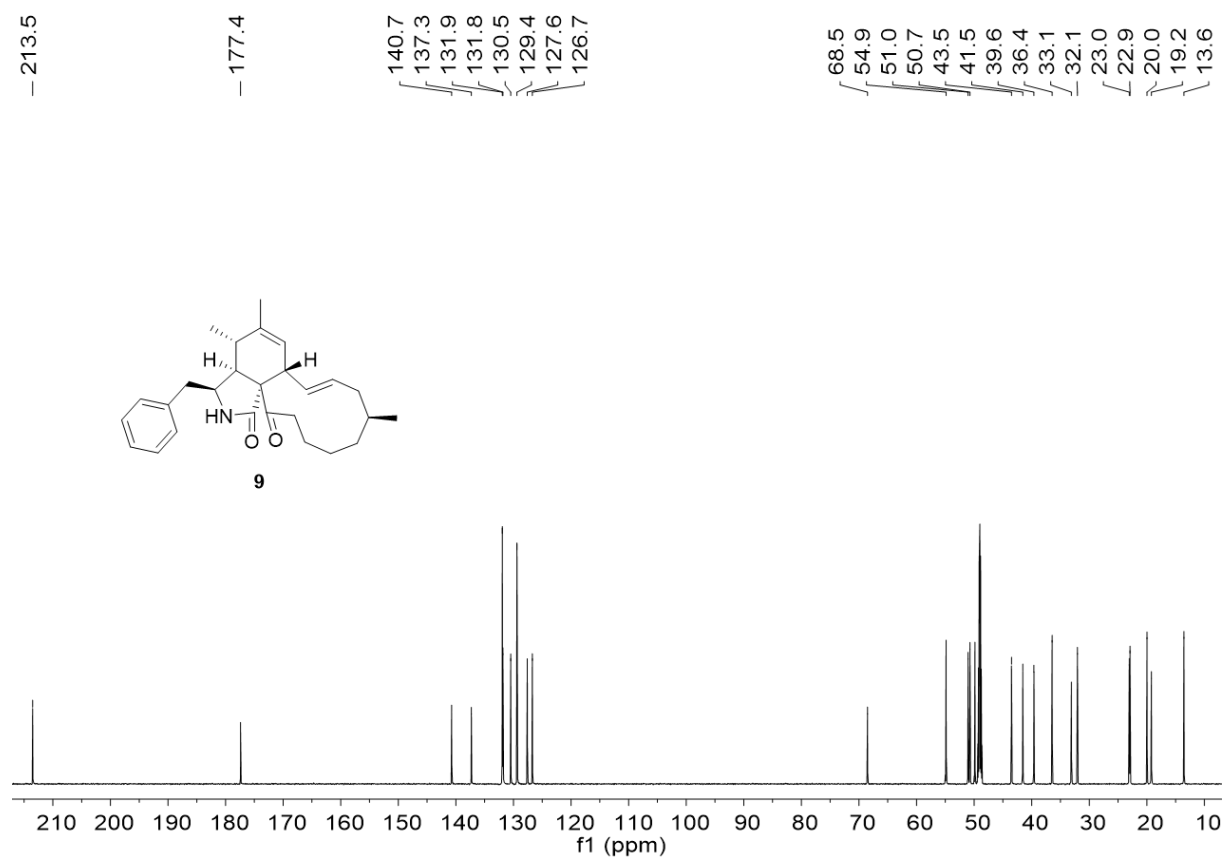


Figure S25. ^1H - ^1H COSY spectrum of Malcirchalsin C (**9**).

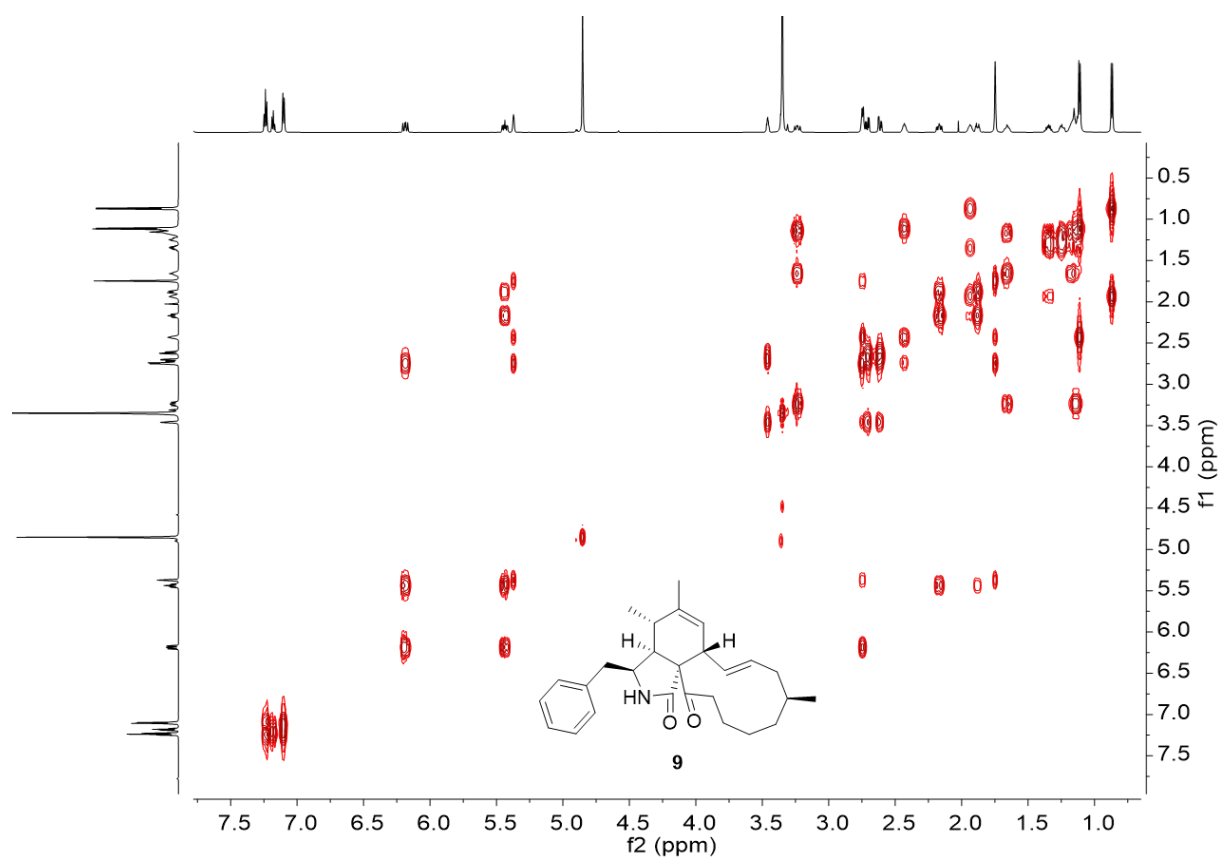


Figure S26. HSQC spectrum of Malcirchalsin C (**9**).

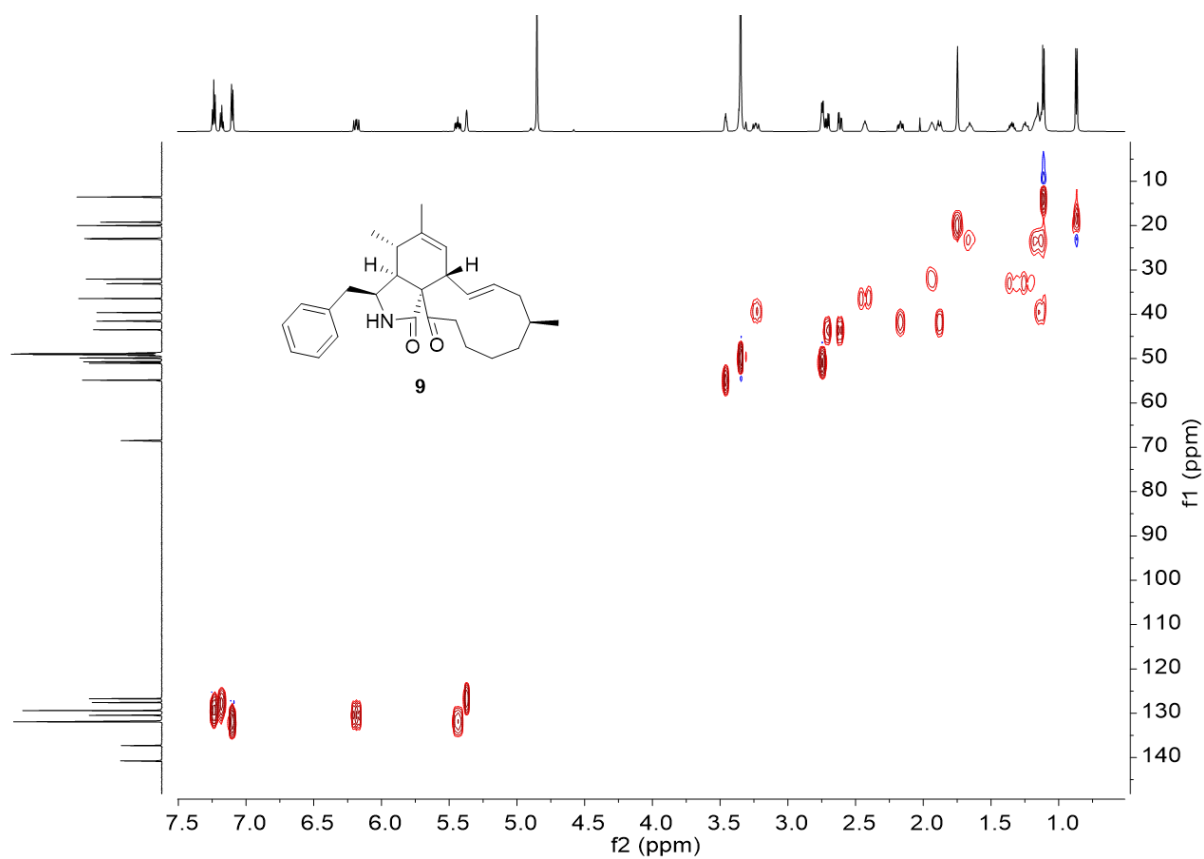


Figure S27. HMBC spectrum of Malcirchalsin C (9).

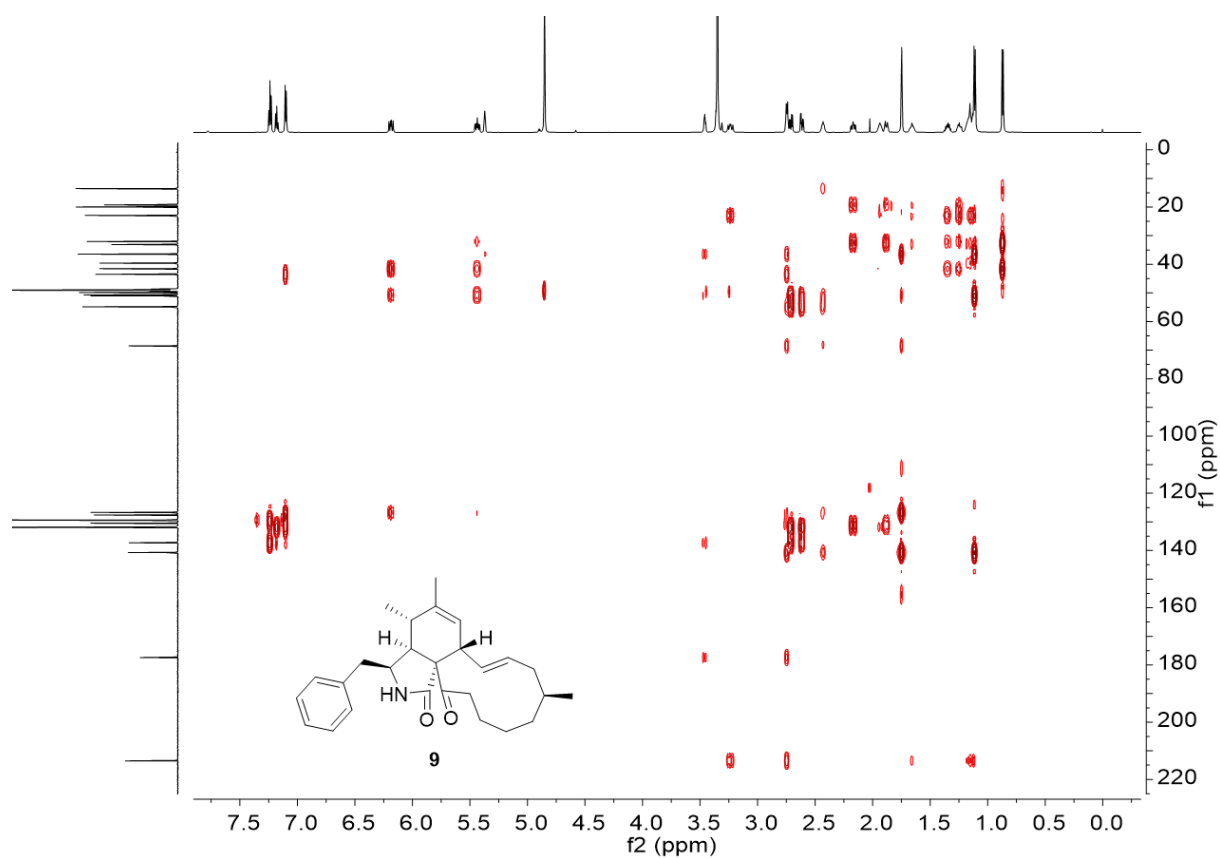


Figure S28. NOESY spectrum of Malcirchalsin C (9).

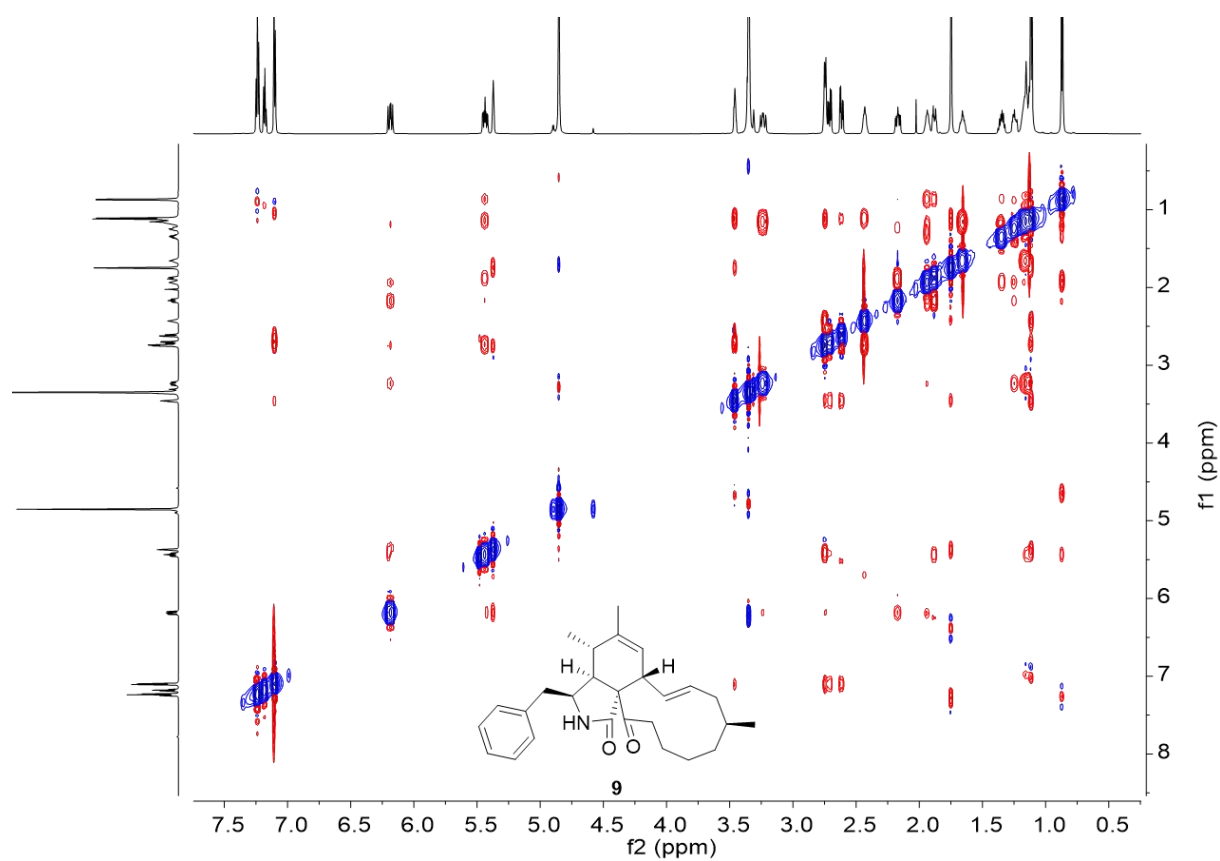
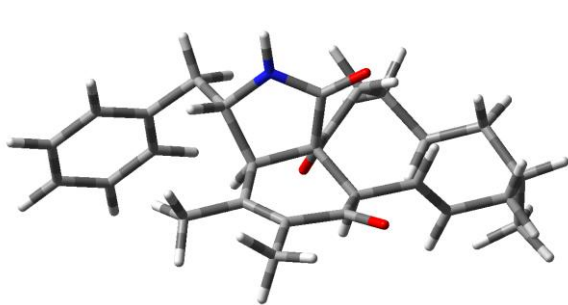
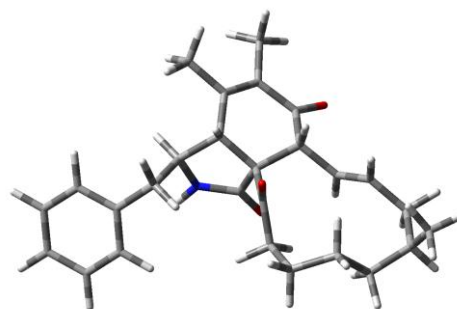


Figure S29. The optimized conformers and equilibrium populations of **7**.

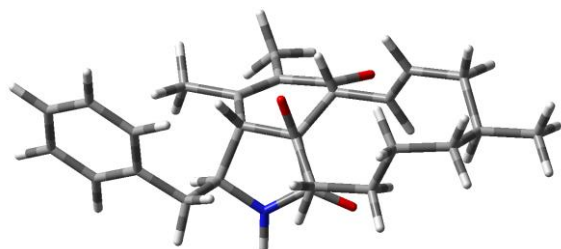
(3*S*, 4*R*, 8*R*, 9*R*, 16*S*)-**7**



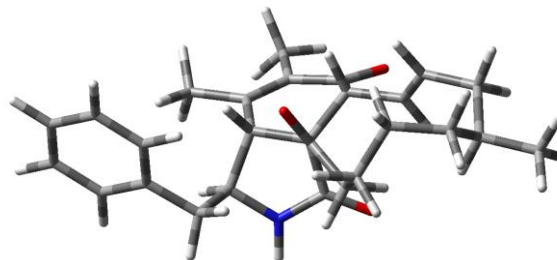
Conf.1 (53.35%)



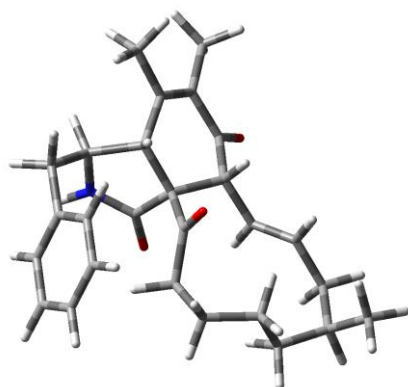
Conf.2 (10.19%)



Conf.3 (11.45%)



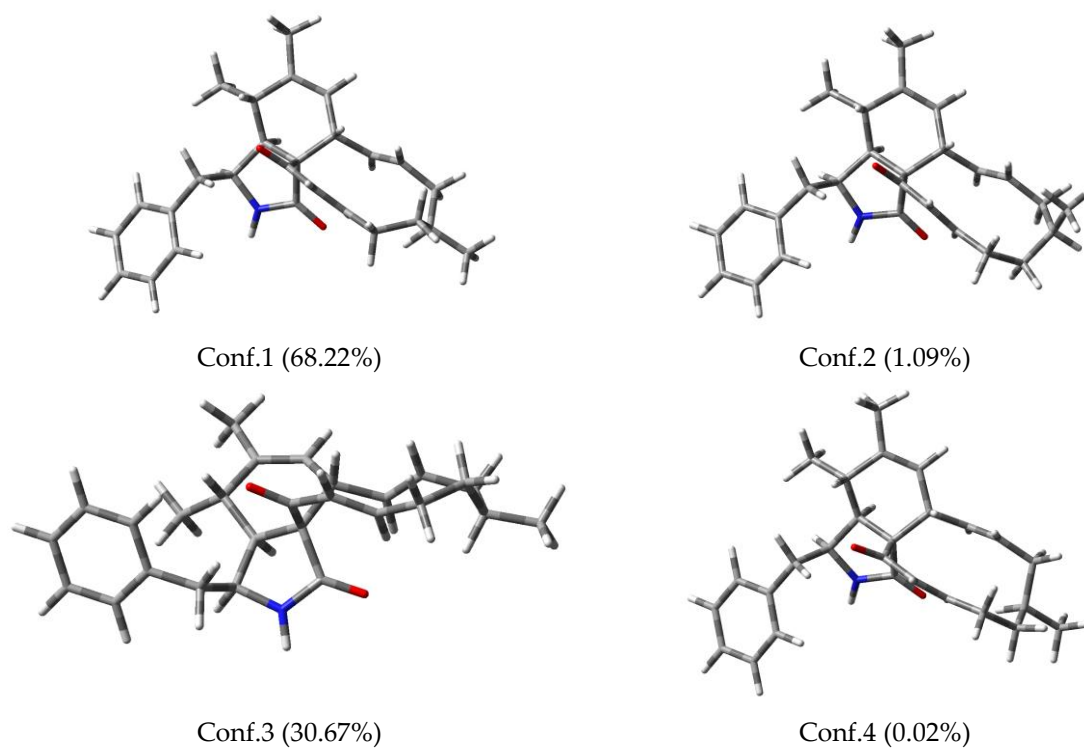
Conf.4 (6.42%)



Conf.5 (18.58%)

Figure S30. The optimized conformers and equilibrium populations of **8**.

(3*S*, 4*S*, 5*S*, 8*S*, 9*S*, 16*S*)-**8**



(3*S*, 4*R*, 5*S*, 8*S*, 9*S*, 16*S*)-**8**

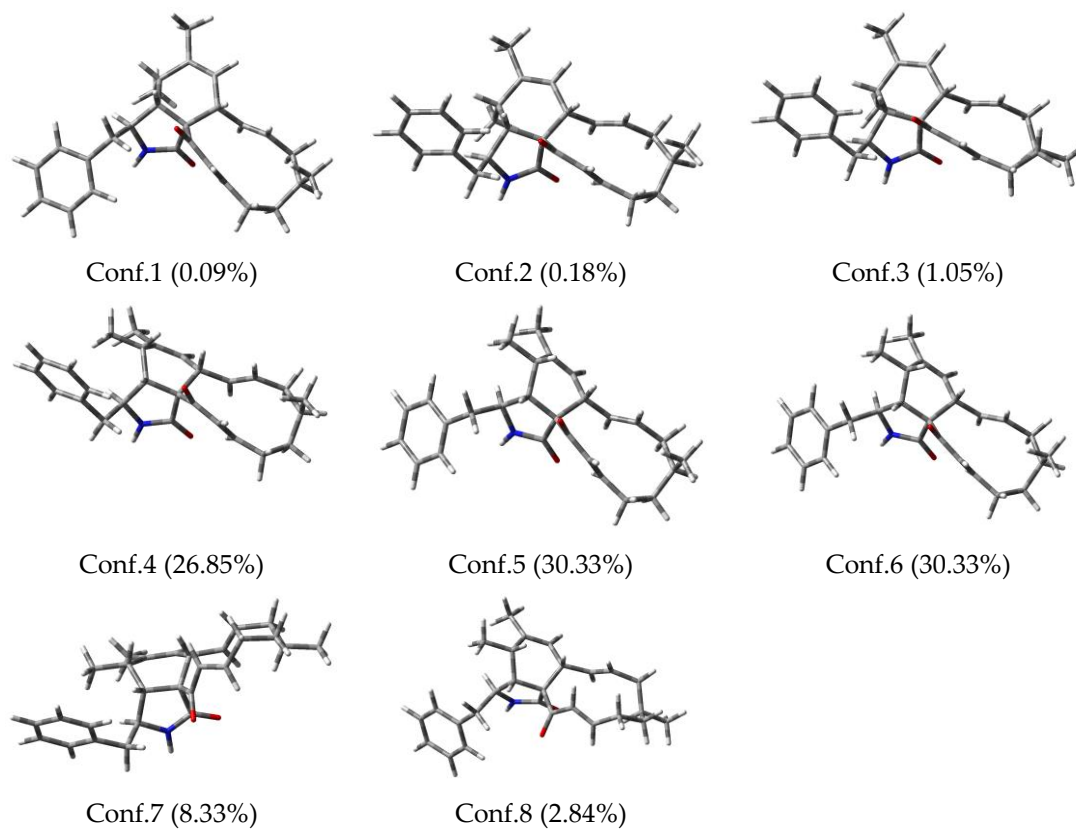


Figure S31. Molecular network of secondary metabolites in extracts of *Malbranchea circinata* SDU050.

