

Supporting Information

Genome Mining Discovery of a New Benzazepine Alkaloid Pseudofisinin A from the Marine Fungus *Neosartorya pseudofischeri* F27-1

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SUPPLEMENTART TABLES

Table S1. Bioinformatics analysis of the *pse* gene cluster.

Genes	Proteins	Gene size (bp/aa)	Putative Function
<i>pseA</i> (XP_026618556)	PseA	7523/2381	NRPS
<i>pseB</i> (XP_026618558)	PseB	1315/415	Indoleamine 2,3-dioxygenase
<i>pseC</i> (XP_026618557)	PseC	1407/384	Methyltransferase
<i>pseD</i> (XP_026618560)	PseD	1236 /411	flavine-dependent monooxygenase
<i>pseE</i> (XP_026618559)	PseE	879/292	Hypothetical protein
<i>pseF</i> (XP_026618554)	PseF	387/128	Blasticidin-S deaminase
<i>pseG</i> (XP_026618553)	PseG	1185/394	UbiH type hydroxylase

Table S2. Primers used in this study.

Primer name	Primer sequence (5'→3')
AmyB_5'- <i>pseA</i> -F	CCCTTCTCTGAACAATAAACCCACAGAAGGCATTTATGGACAAC TGTAAGATGCCG
<i>pseA</i> -1-R	CAGACCACTCTGCACTGATAAG
<i>pseA</i> -2-F	CATCCAAGACGCATATCCTTG
<i>pseA</i> -2-R	AATATCGTCACACACCACGAC
<i>pseA</i> -3-F	CCATCATCGCATCTACAAGACG
<i>glaA-pseA</i> -R	CGACCAGTTCGGAAGATCAGGCTTGGTCCACTGAGGAAGATG
<i>pseA-glaA</i> -F	CATCTTCCTCAGTGGACCAAGCCTGATCTTCCGAAGTGGTCG
<i>pseF-glaA</i> -R	CCTCGGGGGTGAGGGGCATTGCTGAGGTGTAATGATGCTG
<i>glaA-pseF</i> -F	CAGCATCATTACACCTCAGCAATGCCCCTCACCCCCGAGG
<i>pseF</i> -pYTP-R	GTGATGAGACCCAACAACCATGATACCAGGGGATTTGTTCTTCTTG AGCGTTGCACAG
<i>glaA-pseD</i> -F	CCTGAGCTTCATCCCCAGCATCATTACACCTCAGCAATGACGGTCA TCGACCAGGTC
AmyB- <i>pseD</i> _5'-R	GCTCGTTCGGCACCTTTAATCTCTCTGGAATGGACATACGCTC
<i>pseD</i> -AmyB_5'-F	GAGCGTATGTCCATTCCAGAGAGATTAAAGGTGCCGAACGAGC
<i>pseB</i> -AmyB_5'-R	GATCCCTCCGAGTCCTGGCATAAATGCCTTCTGTGGGGTTTATTG
AmyB_5'- <i>pseB</i> -F	CAATAAACCCACAGAAGGCATTTATGCCAGGACTCGGAGGGATC
<i>pseB</i> -pYTU-R	ACAGTGGAGGACATACCCGTAATTTTCTGGGCATTGGTTCGACGAT GCCATTGATGTG
<i>gpdA-pseE</i> -F	ctaaccattacccgccacatagacacatctaaacaATGTCGACAACTCCCACCACG
AmyB_5'- <i>pseE</i> -R	GCTCGTTCGGCACCTTTAATCAGCCAGCCTGCTTGAGTTGAAG
<i>pseE</i> -AmyB_5'-F	CTTCAACTCAAGCAGGCTGGCTGATTAAAGGTGCCGAACGAGC
<i>pseC</i> -AmyB_5'-R	CTGGCTAATTTCCCGTGTCTATAAATGCCTTCTGTGGGGTTTATTG
AmyB_5'- <i>pseC</i> -F	CAATAAACCCACAGAAGGCATTTATGACACGGGAAATTAGCCAG
<i>glaA-pseC</i> -F	CGACCAGTTCGGAAGATCAGGAGAAACATATGGTCATGGCTGC
<i>pseC-glaA</i> -F	GCAGCCATGACCATATGTTTCTCCTGATCTTCCGAAGTGGTCG
<i>pseG-glaA</i> -R	CCTCGGGGGTGAGGGGCATTGCTGAGGTGTAATGATGCTG

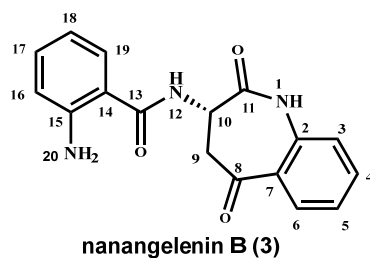
<i>glaA-pseG-F</i>	CCAGCATCATTACACCTCAGCAATGCCCCAACCCCGAATCG
<i>pseG-pYTR-R</i>	CTAAAGGGTATCATCGAAAGGGAGTCATCCAATTTGTTGATCTGCG GTGAGAGATTG
<i>pseA--pYTP-R</i>	TGATGAGACCCAACAACCATGATACCAGGGGATTTCTTGGTCCAC TGAGGAAGATG
<i>glaA-pseB-F</i>	CTGAGCTTCATCCCCAGCATCATTACACCTCAGCAATGCCAGGACT CGGAGGGATC
<i>pseB-pYTR-R</i>	ACAGTGGAGGACATACCCGTAATTTTCTGGGCATTTGGTCGACGAT GCCATTGATGTG
<i>gpdA-pseC-F</i>	taaccattaccccgccacatagacacatctaacaATGACACGGGAAATTAGCCAG
<i>pseC-pYTU-R</i>	CTAAAGGGTATCATCGAAAGGGAGTCATCCAATTTAGAAACATATG GTCATGGCTGC
<i>pET28a-pseC-F</i>	GTGCCGCGCGGCAGCCATATGATGACACGGGAAATTAGCCAG
<i>pseC-1-R</i>	CCTCCCGCAACGCACATCTGAGTAGGGACCTTGAAGCC
<i>pseC-2-F</i>	GGCTTCAAGGTCCCTACTCAGATGTGCGTTGCGGGAGG
<i>pseC-2-R</i>	GTCATGAGACCAACATCGGACATATGATGGACCCACGCGCCTC
<i>pseC-3-F</i>	GAGGCGCGTGGGTCCATCATATGTCCGATGTTGGTCTCATGAC
<i>pseC-3-R</i>	GAAAGCGTAGAAAGATTGGTCCGTCCGGTACGCCATCTGAAACG
<i>pseC-4-F</i>	CGTTTCAGATGGCGTACCGGACGGACCAATCTTTCTACGCTTTC
<i>pseC-4-R</i>	CCTAGGATGGTGCAACAGTCCCTCTGAAGTATGGTCCATCAGGATG
<i>pseC-5-F</i>	CATCCTGATGGACCATACTTCAGAGGACTGTTGCACCATCCTAGG
<i>pET28a-pseC-R</i>	ACGGAGCTCGAATTCGGATCCCTAGCCCACTCTCTTCAGAGC

Table S3. Plasmids used in this study.

Plasmids	Vector	Genes
pXXX1-1	pYTP	<i>AmyB_5'-pseA</i> ; <i>glaA-pseF</i>
pXXX1-2	pYTU	<i>AmyB_5'-pseB</i> ; <i>glaA-pseD</i>
pXXX1-3	pYTR	<i>GpdA-pseE</i> ; <i>AmyB_5-pseC</i> ; <i>GlaA-pseG</i>
pXXX1-4	pYTP	<i>AmyB_5'-pseA</i>
pXXX1-5	pYTU	<i>glaA-pseB</i>
pXXX1-6	pYTR	<i>GpdA-pseC</i>
pXXX1-7	pET28a	T7p-N-his- <i>pseC</i> -T7t

Table S4. NMR data of nanangelenin B (**3**) in DMSO-*d*₆.

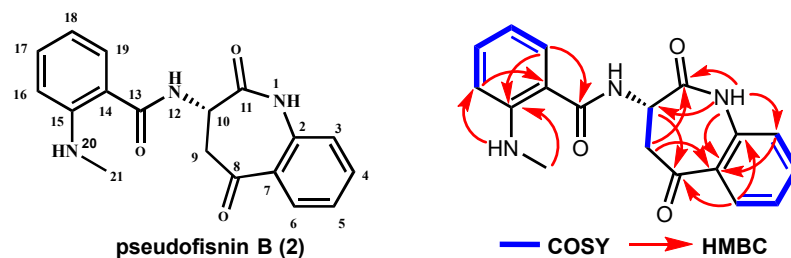
¹H NMR spectrum (600 MHz), ¹³C NMR spectrum (150 MHz)



No.	δ_{H} (<i>J</i> in Hz)	δ_{C} , type
1	10.36, s	
2		137.7, C
3	7.26, t (7.8)	124.3, CH
4	7.61, m	134.2, CH
5	7.19, d (8.1)	122.2, CH
6	7.75, dd (7.9, 1.7)	130.0, CH
7		128.4, C
8		197.8, C
9	3.02, dd (18.7, 2.7) 3.26, dd (18.7, 13.3)	45.8, CH ₂
10	5.00, ddd (13.3, 7.5, 2.7)	46.1, CH
11		171.2, C
12	8.42, d (7.4)	
13		168.6, C
14		113.9, C
15		149.7, C
16	6.70, d (8.2)	116.4, CH
17	7.16, m	132.1, CH
18	6.54, t (7.5)	114.6, CH
19	7.58, dd (8.0, 1.6)	128.4, CH
20	6.36, brs	

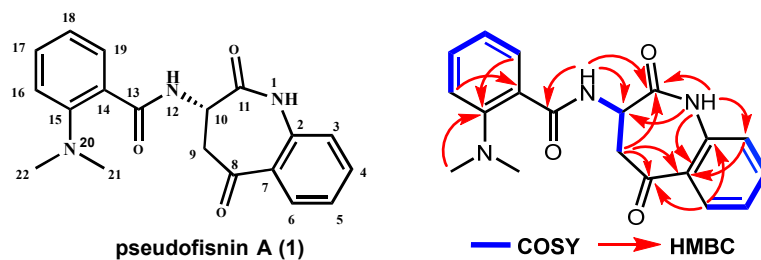
“m” means overlapped or multipet with other signals.

The NMR data of **1** is identical to the data reported in *J. Am. Chem. Soc.* 2020, 142(15), 7145-7152.

Table S5. NMR data of pseudofisnin B (**2**) in CDCl₃.¹H NMR spectrum (600 MHz), ¹³C NMR spectrum (150 MHz)

No.	δ_{H} (J in Hz)	δ_{C} , type
1	7.70, s	
2		135.4, C
3	7.00, d (8.0)	122.1, CH
4	7.56, t (7.7)	134.4, CH
5	7.32, t (7.7)	126.2, CH
6	7.93, d (8.0)	131.2, CH
7		129.8, C
8		196.6, C
9	3.04, dd (18.9, 12.9)	47.3, CH ₂
	3.44, m	
10	5.20, m	46.5, CH
11		171.7, C
12		
13		169.0, C
14		113.5, C
15		150.9, C
16	6.67, d (8.47)	111.3, CH
17	7.35, t (7.70)	133.5, CH
18	6.63, t (7.70)	114.6, CH
19	7.49, d (8.07)	127.7, CH
20	6.80, s	
21	2.85, d (4.30)	29.6, CH ₃

“m” means overlapped or multipet with other signals.

Table S6. NMR data of pseudofisnin A (**1**) in CDCl₃.¹H NMR spectrum (600 MHz), ¹³C NMR spectrum (150 MHz)

No.	δ_{H} (<i>J</i> in Hz)	δ_{C} , type
1	7.69, s	
2		135.7, C
3	6.99, d (8.0)	122.0, CH
4	7.54, t (7.7)	134.3, CH
5	7.31, t (7.7)	126.0, CH
6	7.93, d (8.0)	131.1, CH
7		130.1, C
8		197.5, C
9	3.07, dd (19.0, 12.9) 3.47, dd (19.0, 3.2)	47.4, CH ₂
10	5.39, m	47.0, CH
11		171.9, C
12	11.06, d (6.30)	
13		165.8, C
14		126.7, C
15		153.0, C
16	7.27, d (8.0)	120.3, CH
17	7.45, t (7.7)	132.4, CH
18	7.19, t (7.7)	124.3, CH
19	8.11, d (7.9)	131.4, CH
20		
21	2.79, s	45.4, CH ₃
22	2.79, s	45.4, CH ₃

“m” means overlapped or multipet with other signals.

SUPPLEMENTART FIGURES

Figure S1. SDS-PAGE analysis of purified PseC.

Left lane: protein marker; Right lane: the purified PseC (46.8 kDa).

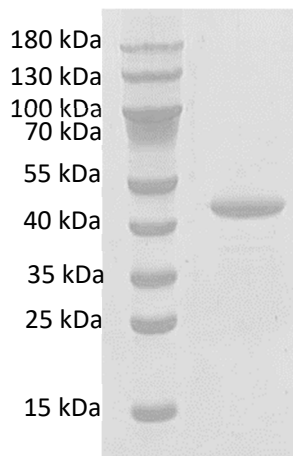


Figure S2. Kinetic analysis of the methylation reaction catalyzed by PseC.

(a) nanangelenin B (**3**) as the substrate; (b) pseudofisnin B (**2**) as the substrate.

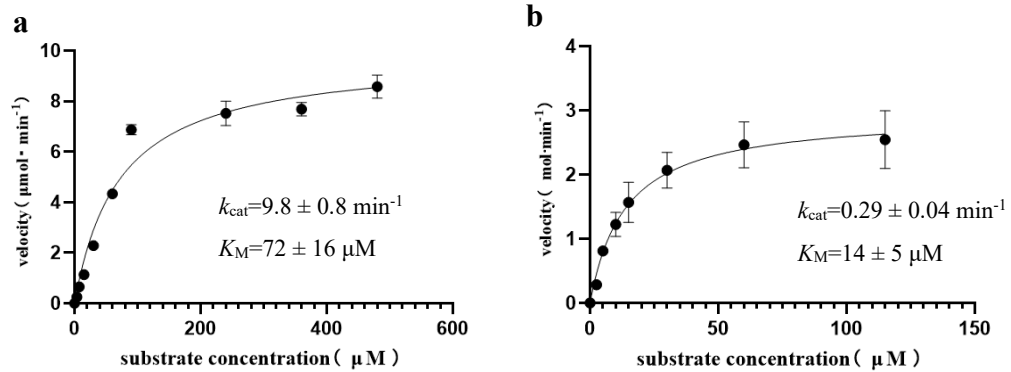


Figure S3. HR-ESIMS data of (a) pseudofisnin B and (b) pseudofisnin A.

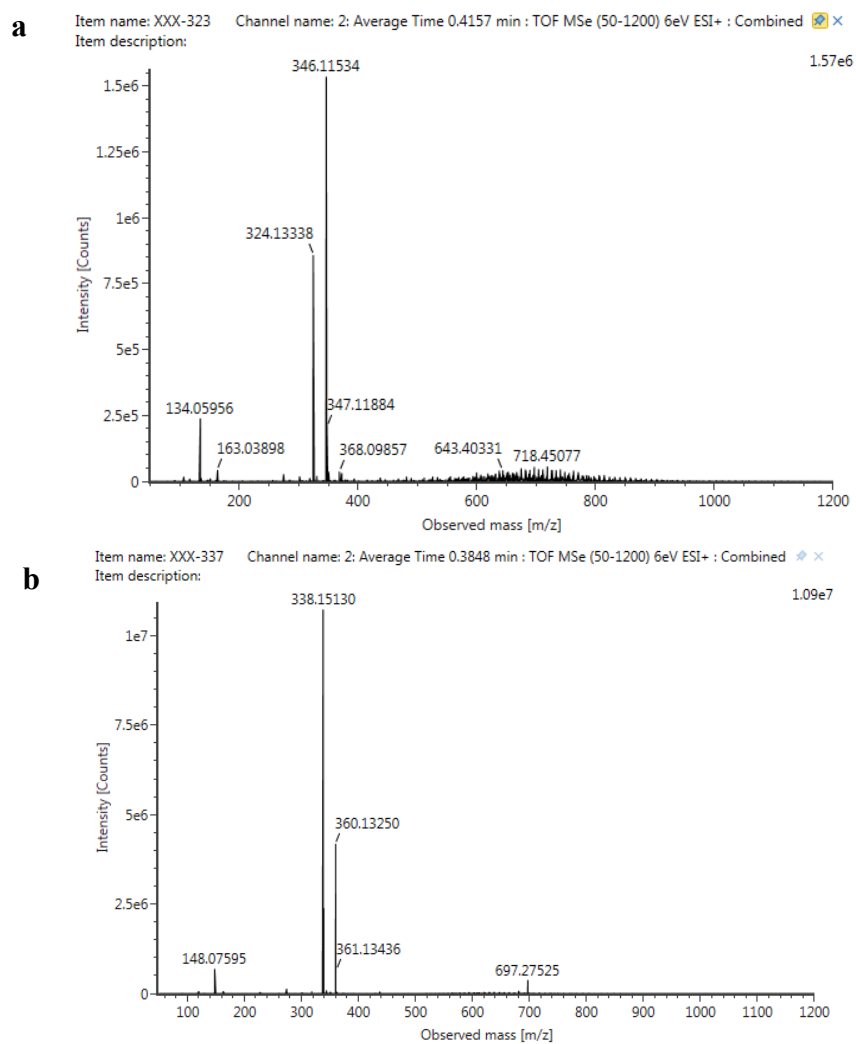


Figure S4. ^1H NMR spectrum of nanangelenin B (**3**) in $\text{DMSO}-d_6$.

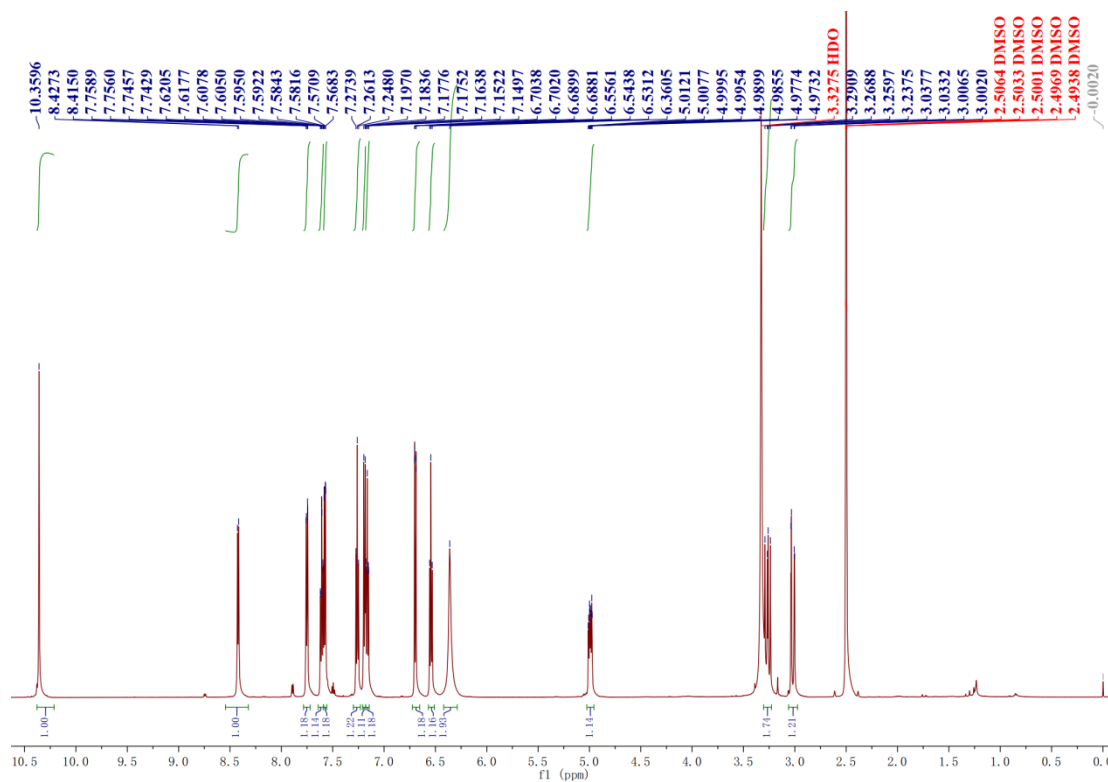


Figure S5. ^{13}C NMR spectrum of nanangelenin B (**3**) in $\text{DMSO}-d_6$.

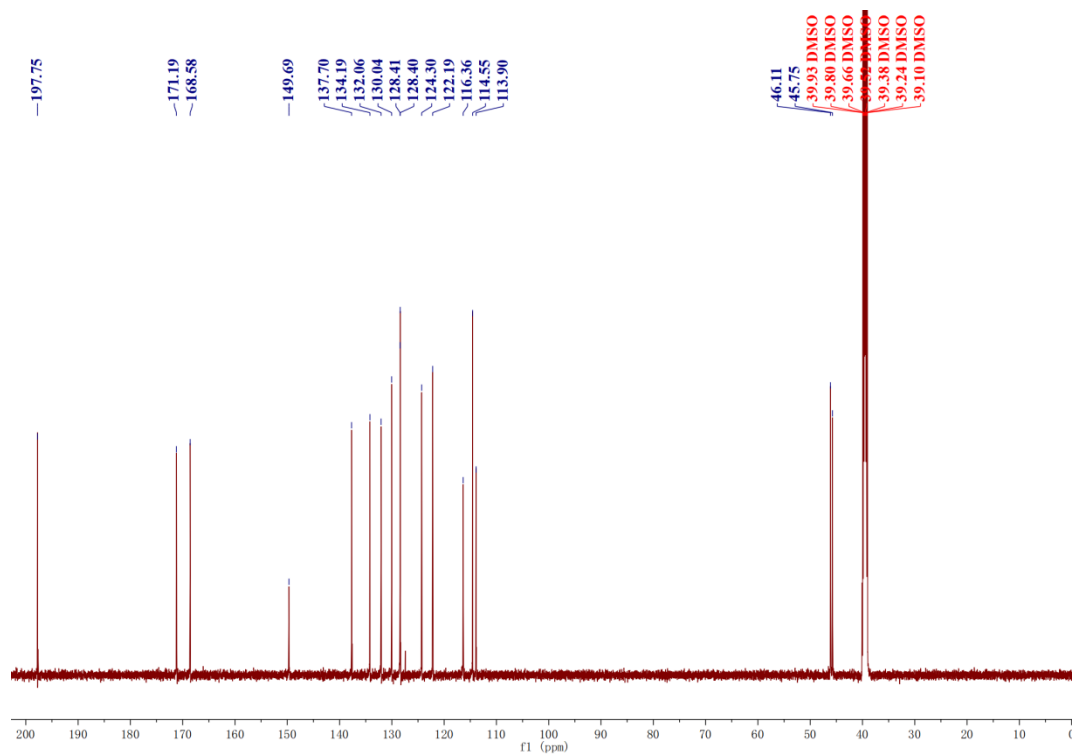


Figure S6. DEPT 135 spectrum of nanangelenin B (**3**) in DMSO-*d*₆.

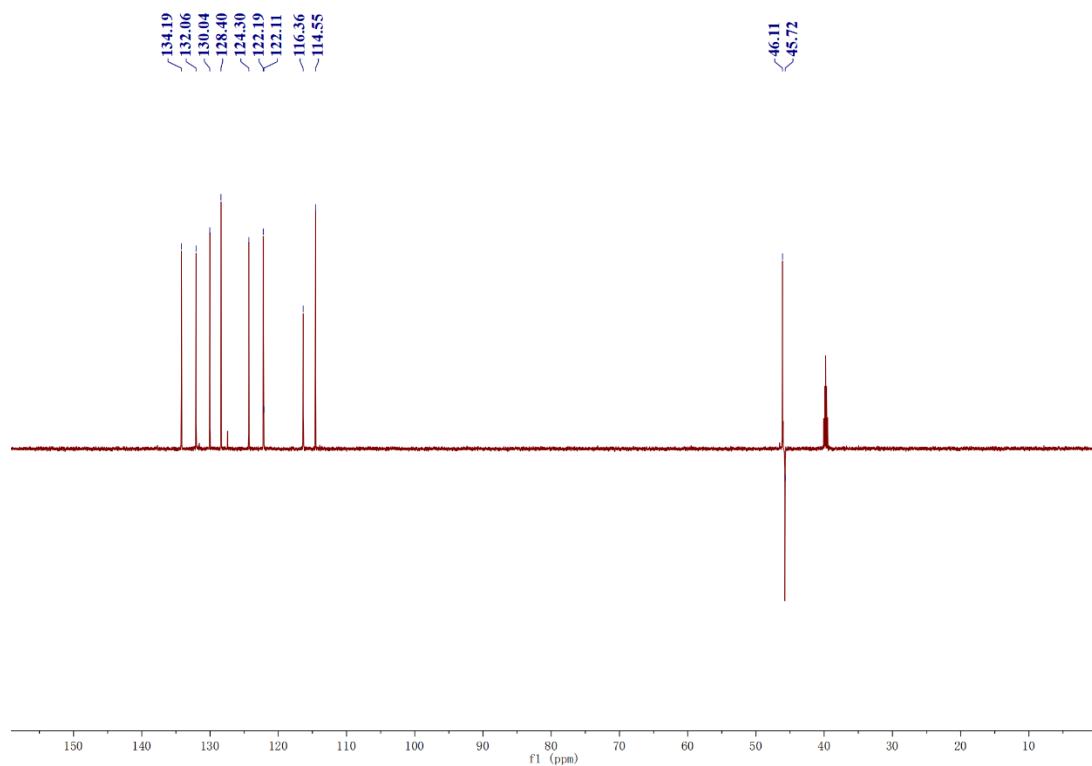


Figure S7. ¹H NMR spectrum of pseudofisnin B (**2**) in CDCl₃.

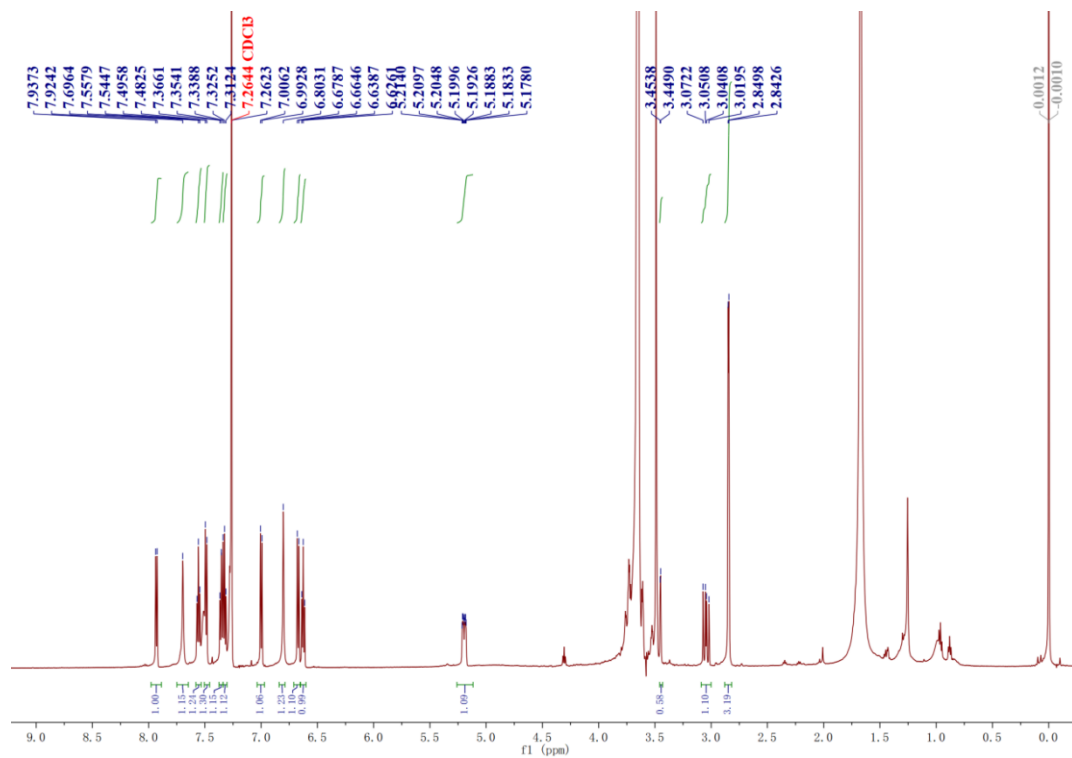


Figure S8. ^{13}C NMR spectrum of pseudofisnin B (**2**) in CDCl_3 .

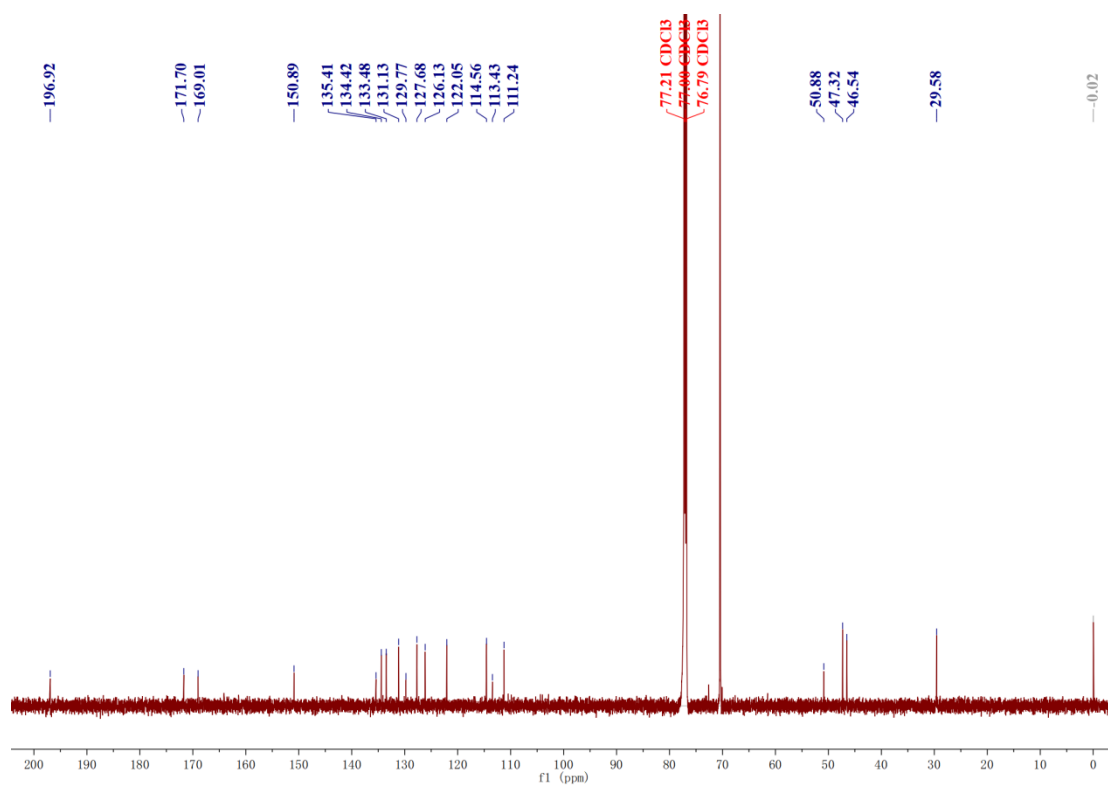


Figure S9. DEPT 135 spectrum of pseudofisnin B (**2**) in CDCl_3 .

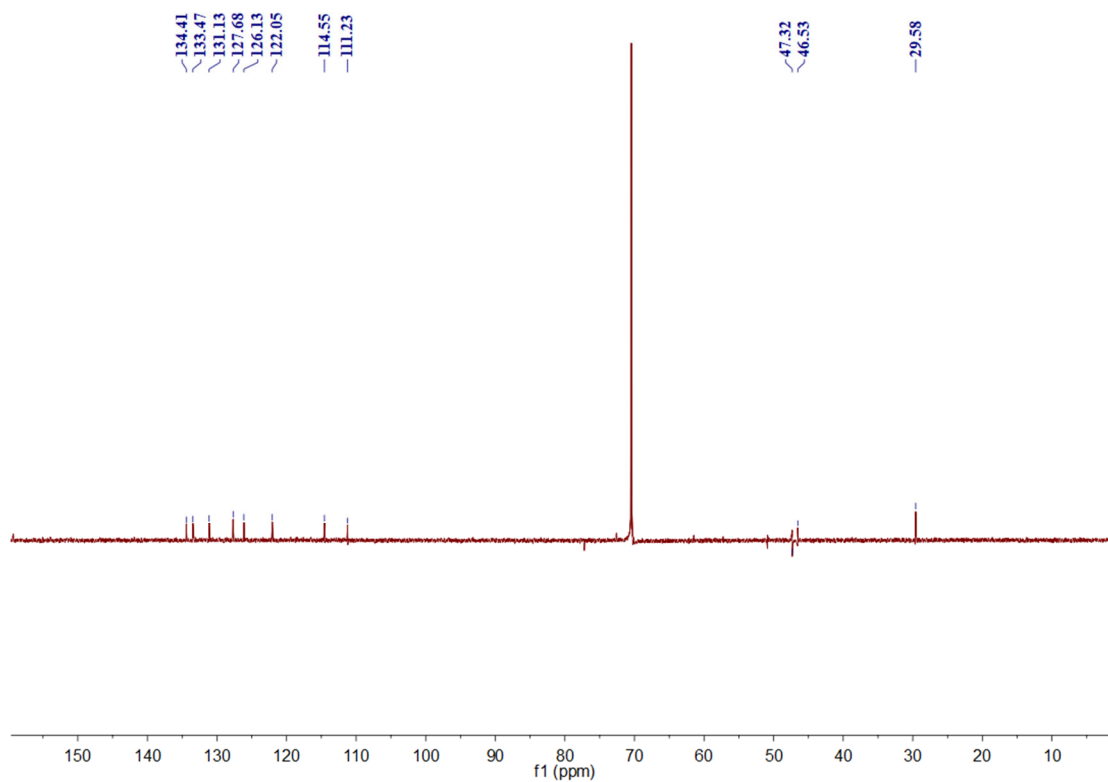


Figure S10. COSY spectrum of pseudofisinin B (**2**) in CDCl₃.

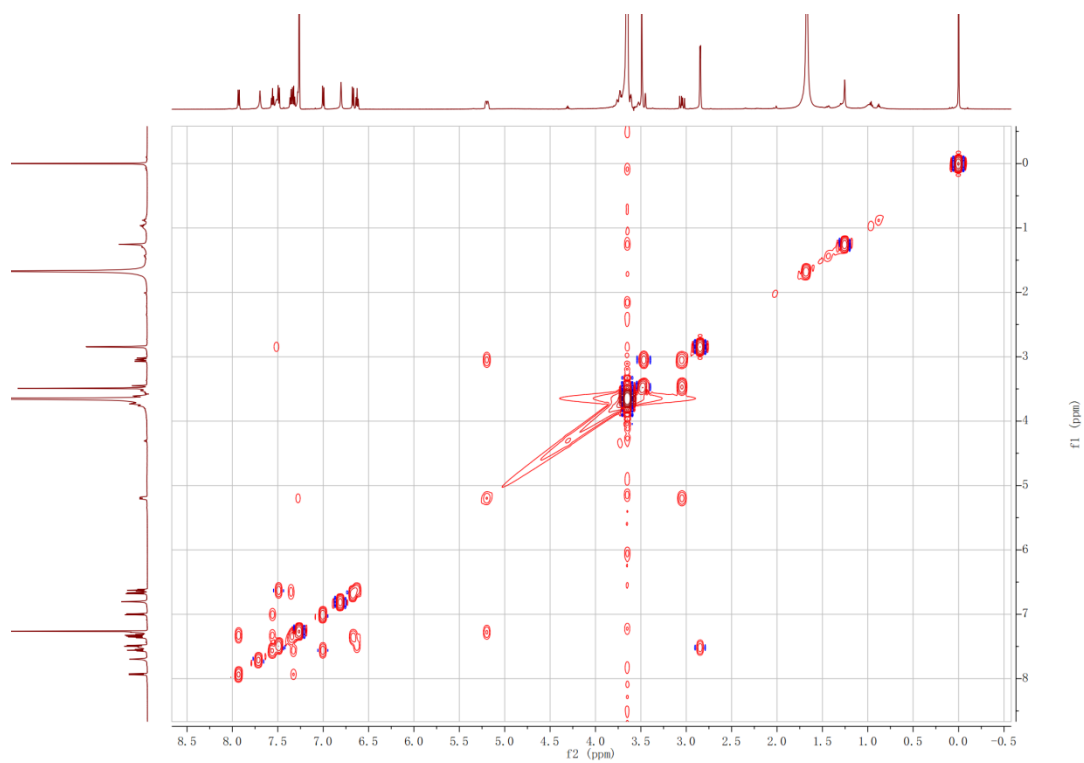


Figure S11. HSQC spectrum of pseudofisinin B (**2**) in CDCl₃.

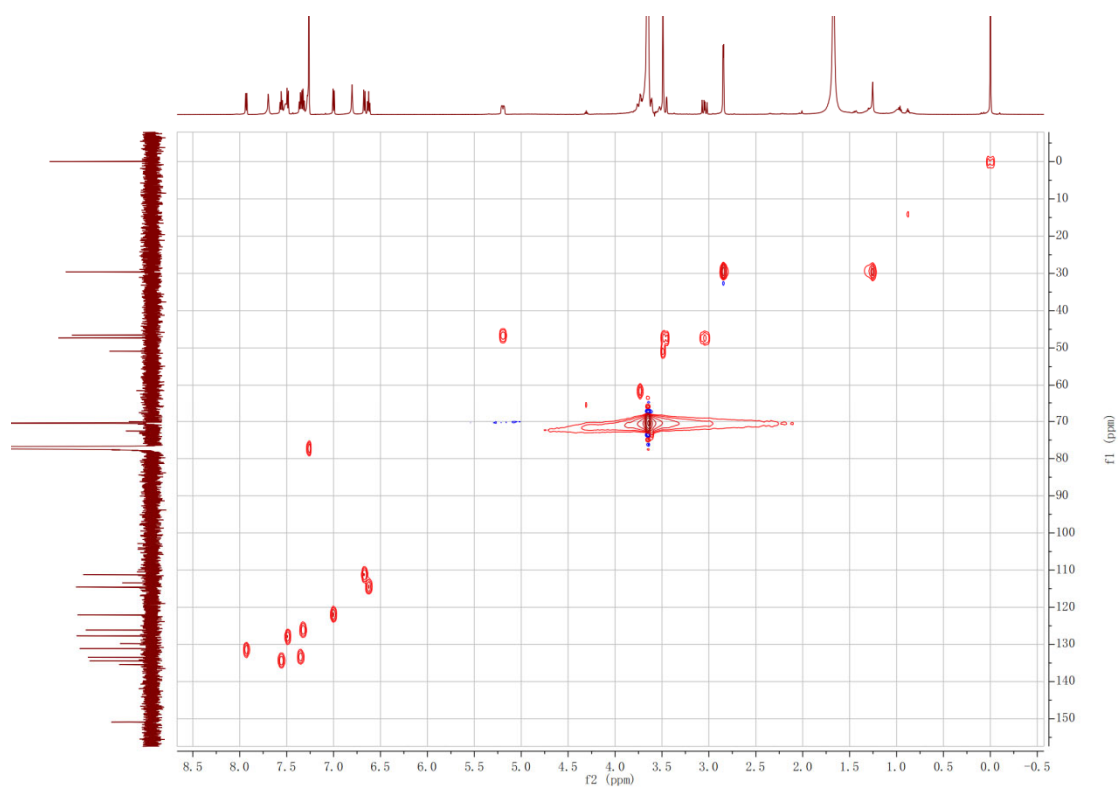


Figure S12. HMBC spectrum of pseudofisnin B (**2**) in CDCl₃.

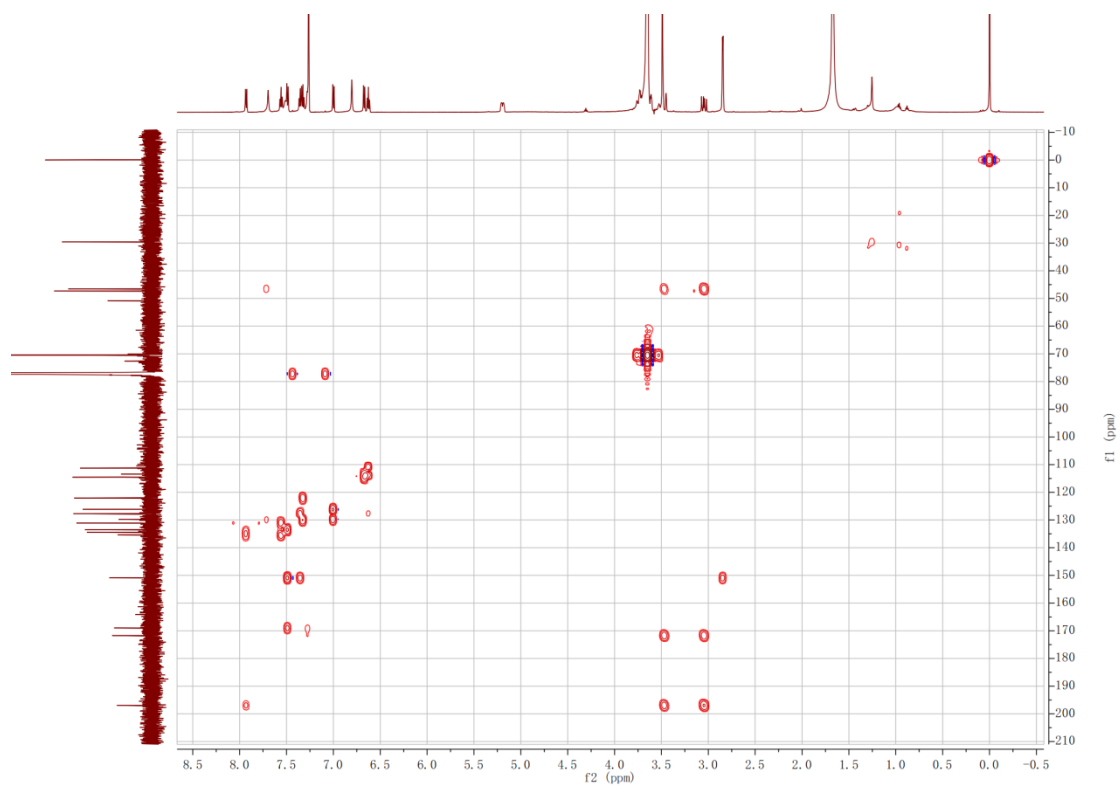


Figure S13. ¹H NMR spectrum of pseudofisnin A (**1**) in CDCl₃.

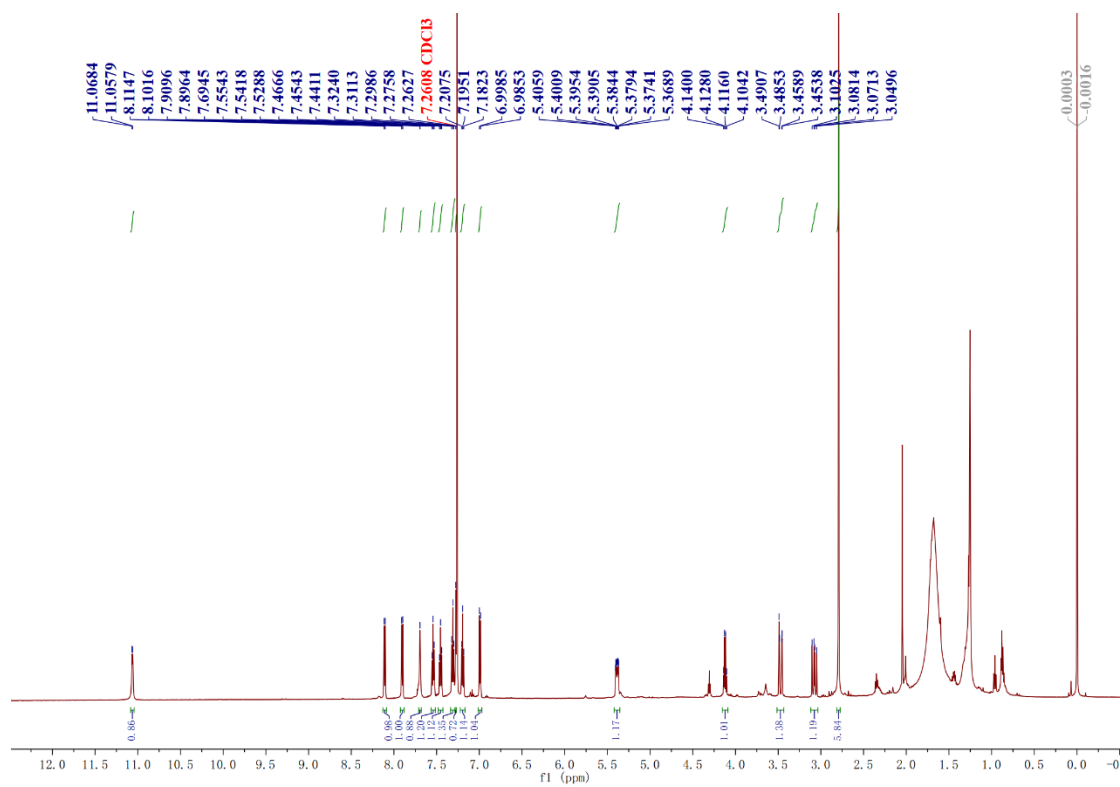


Figure S14. ^{13}C NMR spectrum of pseudofisnin A (**1**) in CDCl_3 .

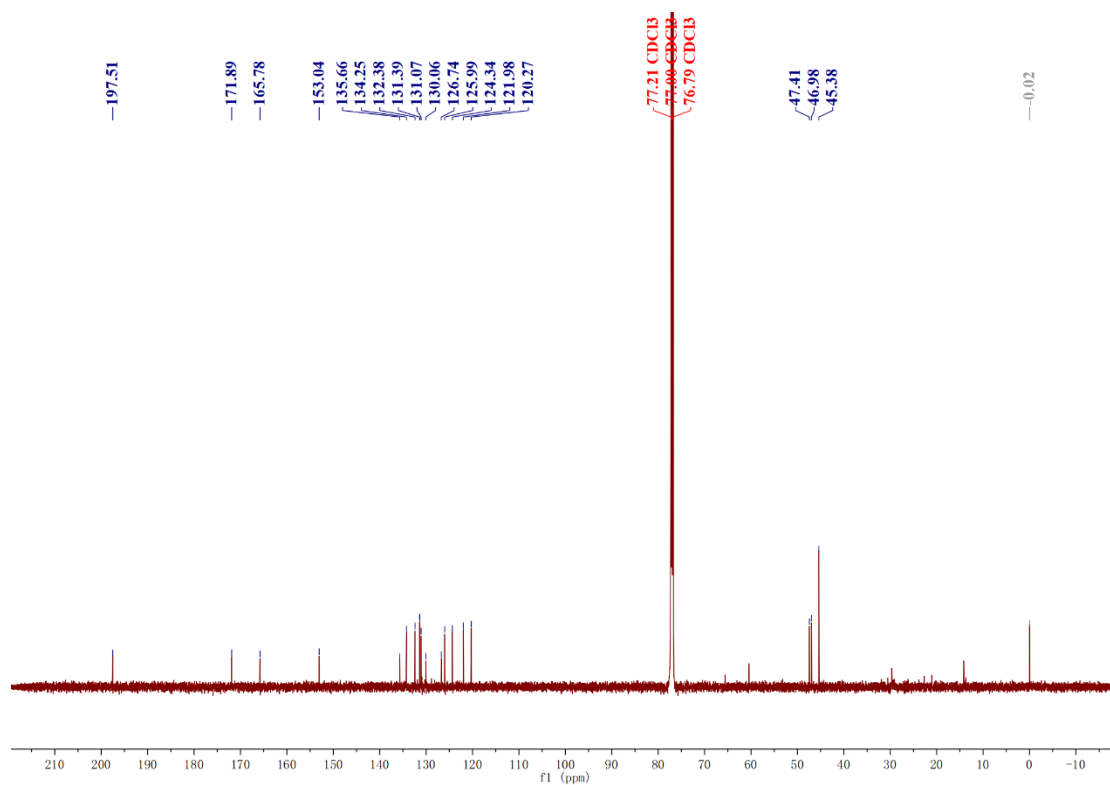


Figure S15. DEPT 135 spectrum of pseudofisnin A (**1**) in CDCl_3 .

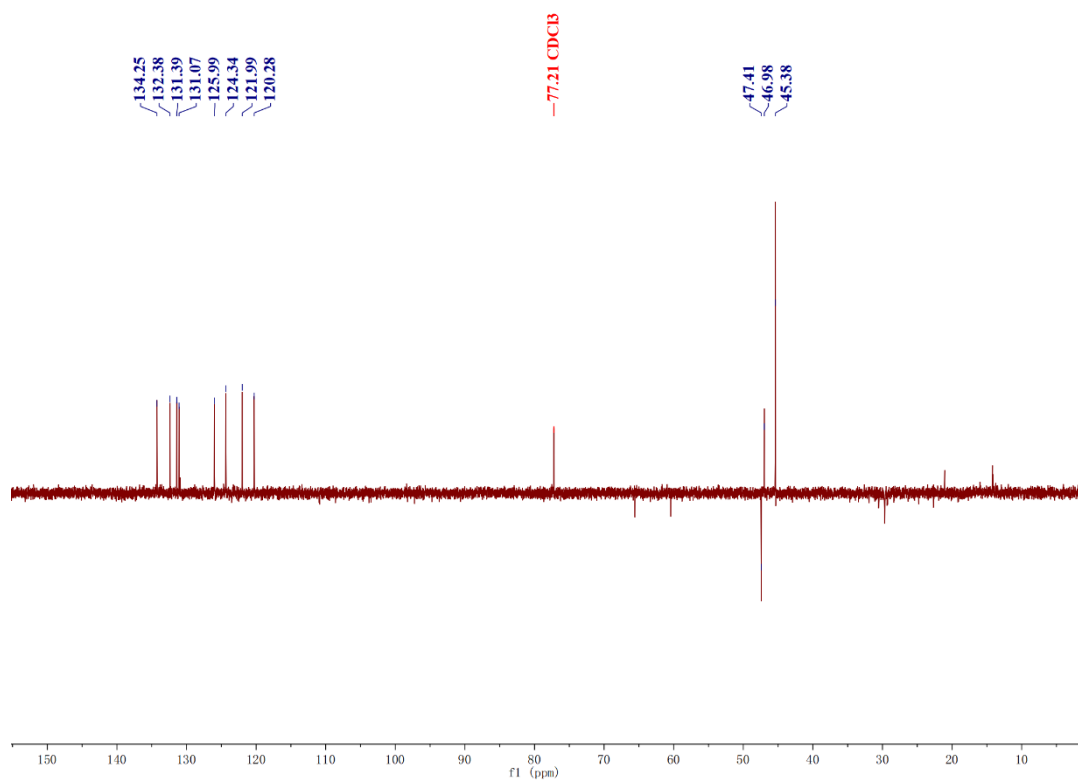


Figure S16. COSY spectrum of pseudofisnin A (**1**) in CDCl₃.

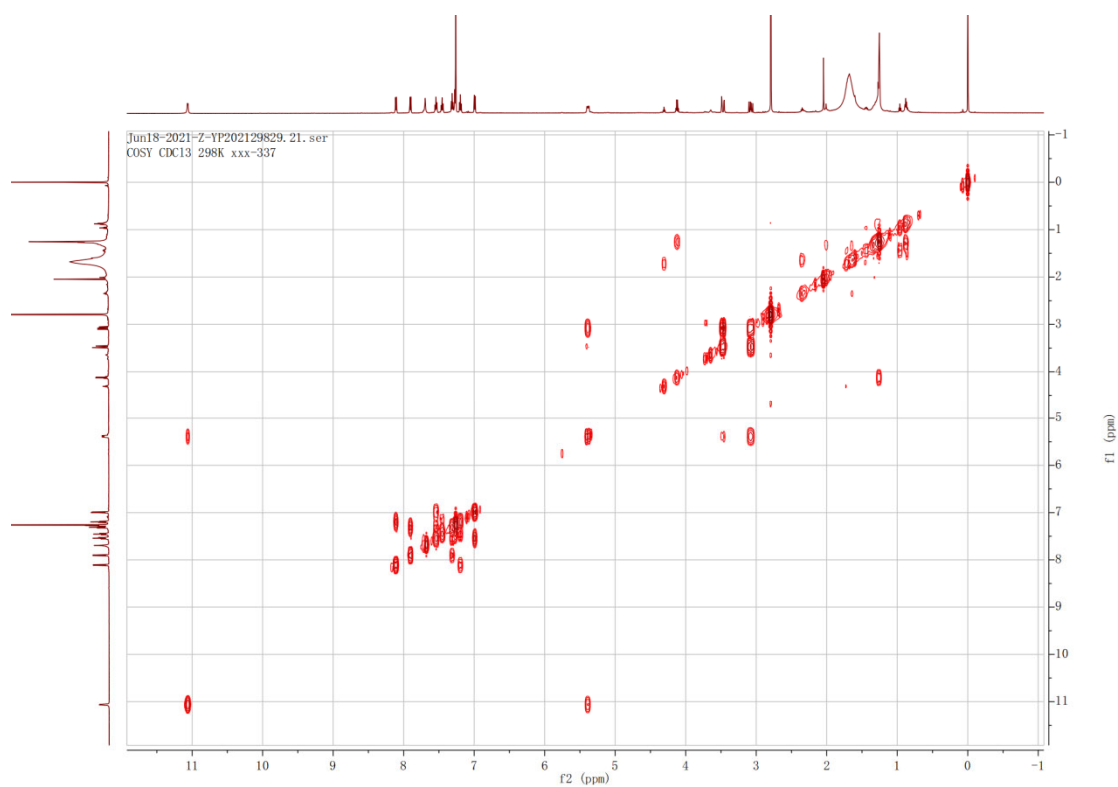


Figure S17. HSQC spectrum of pseudofisnin A (**1**) in CDCl₃.

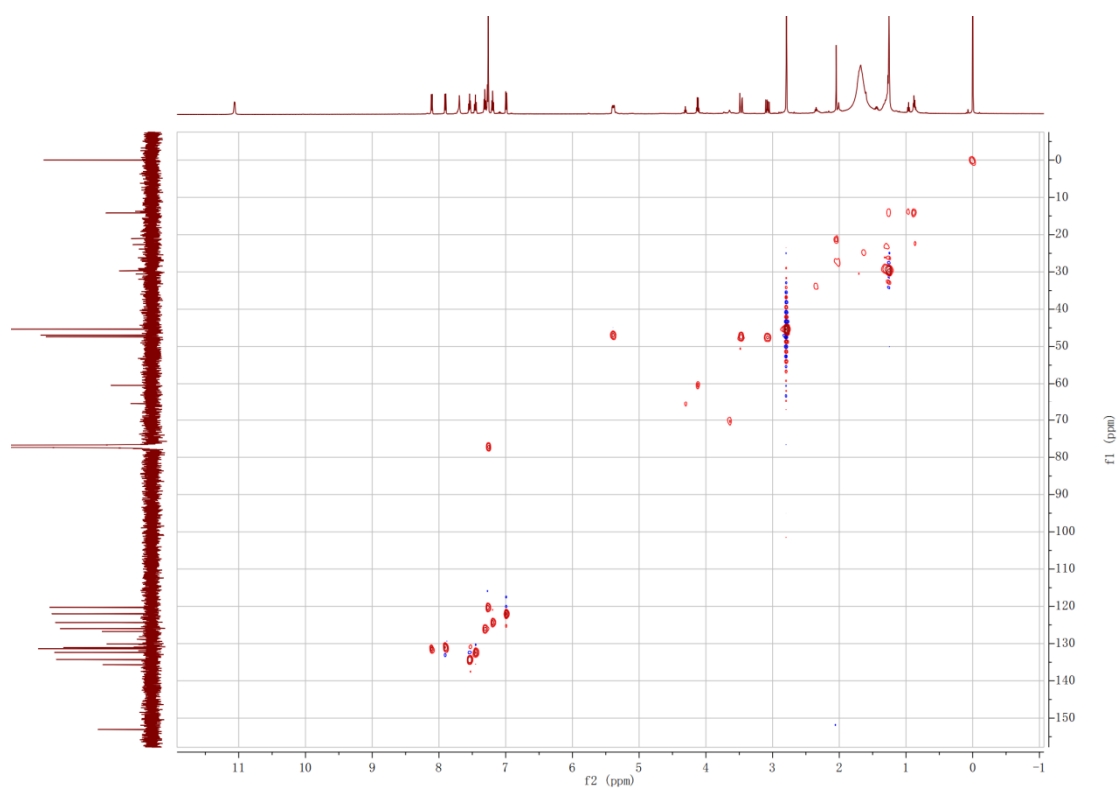


Figure S18. HMBC spectrum of pseudofisnin A (**1**) in CDCl₃.

