

Supporting Information

Rec. Nat. Prod. 20:4 (2026):e25123770

Inhibition of the NLRP3 inflammasome by Callinudin

A: A novel 3,4-seco-labdane diterpenoid derived from

Callicarpa nudiflora

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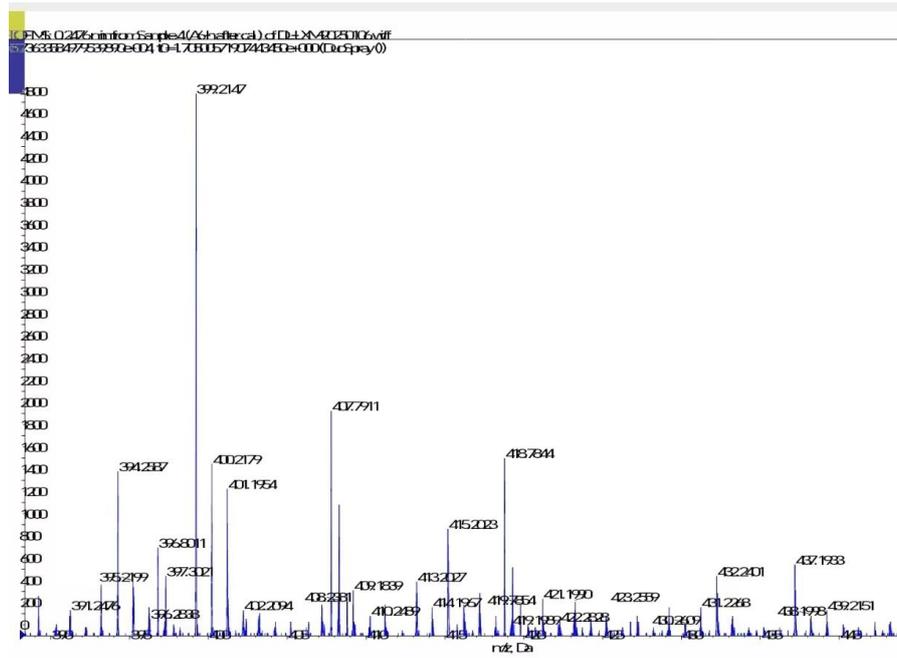


Figure S1: HR-ESI-MS spectrum of 1

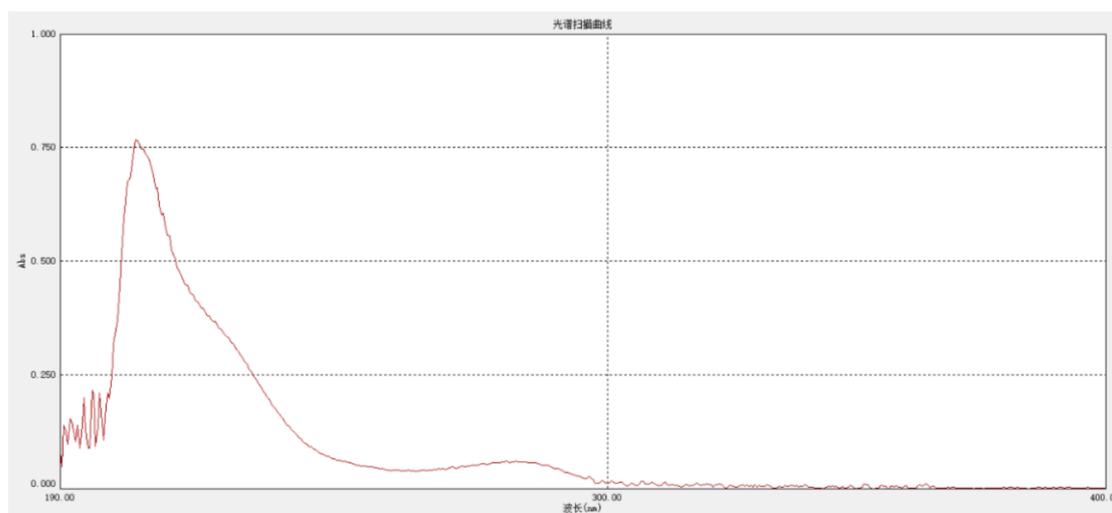


Figure S2: UV spectrum of 1

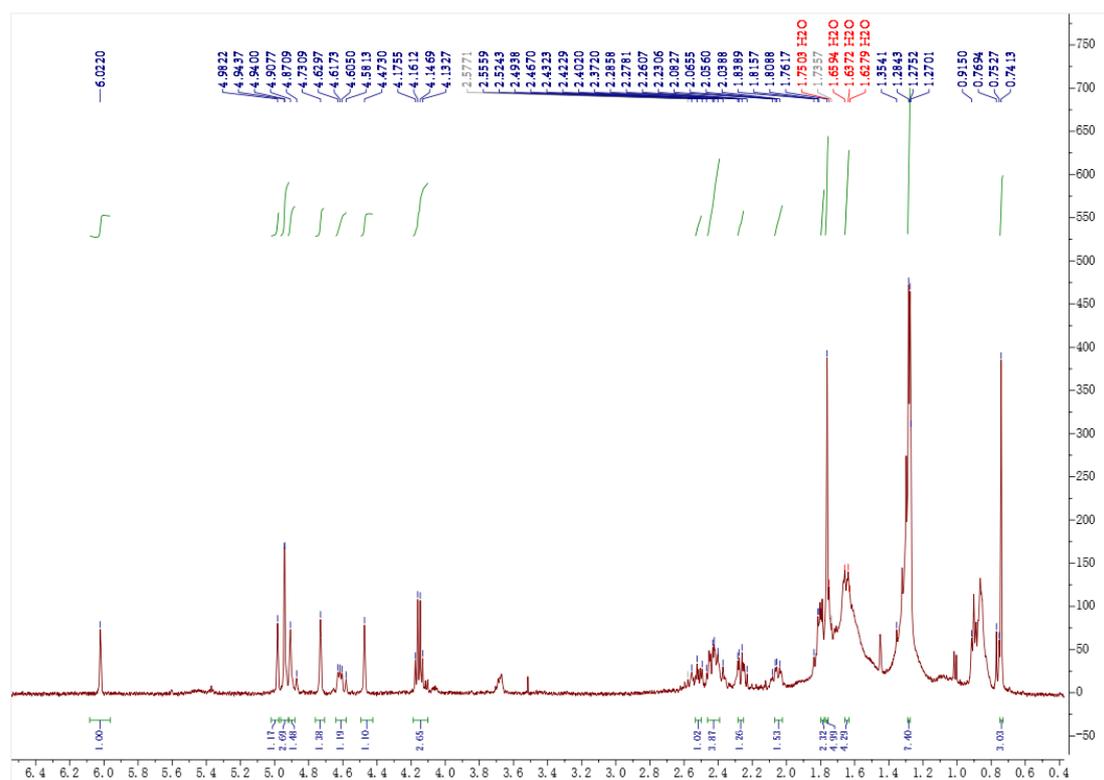


Figure S3: $^1\text{H-NMR}$ (600 MHz, CDCl_3) spectrum of **1**

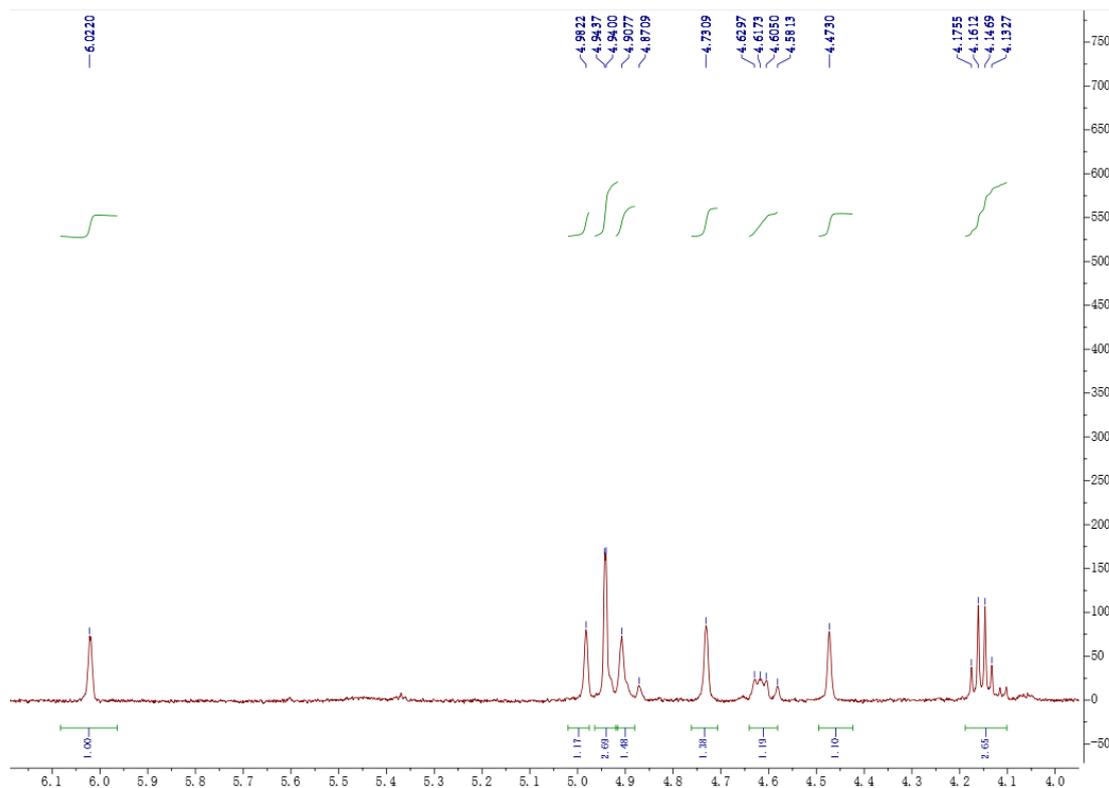


Figure S4: $^1\text{H-NMR}$ (600 MHz, CDCl_3) spectrum of **1** (from δ_{H} 4.0 ppm to δ_{H} 6.1 ppm)

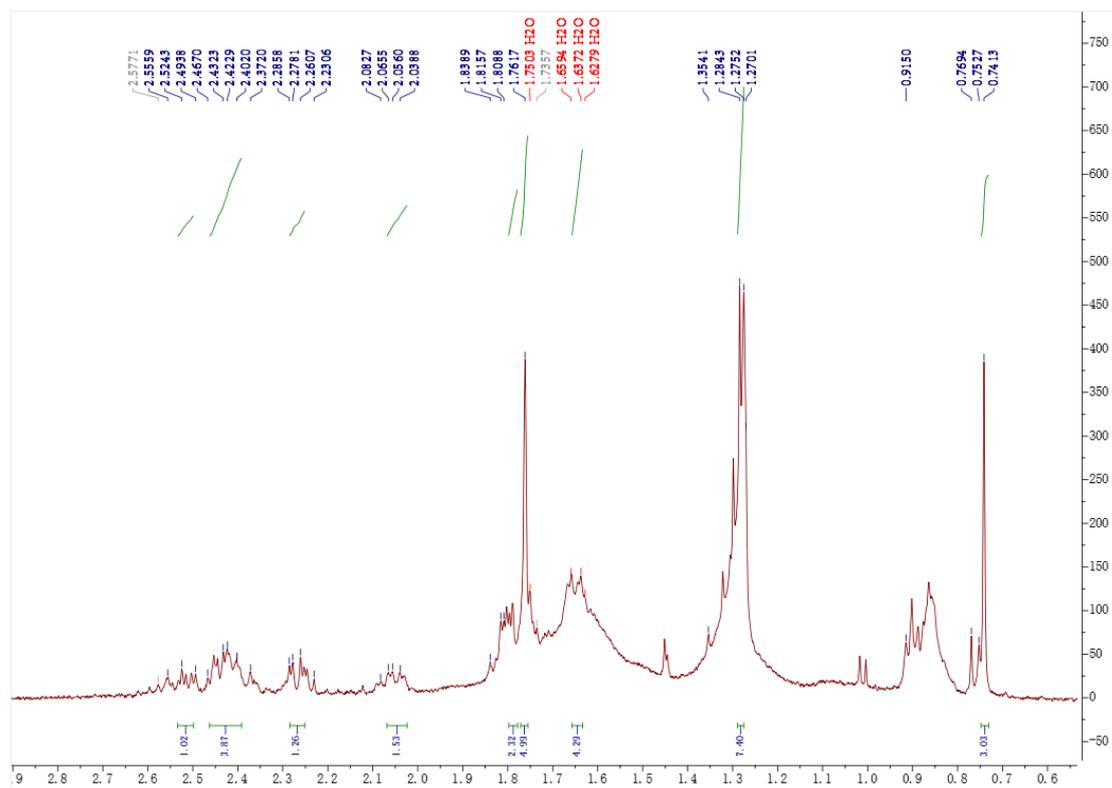


Figure S5: ¹H-NMR (600 MHz, CDCl₃) spectrum of **1** (from δ_H 0.6 ppm to δ_H 2.8 ppm)

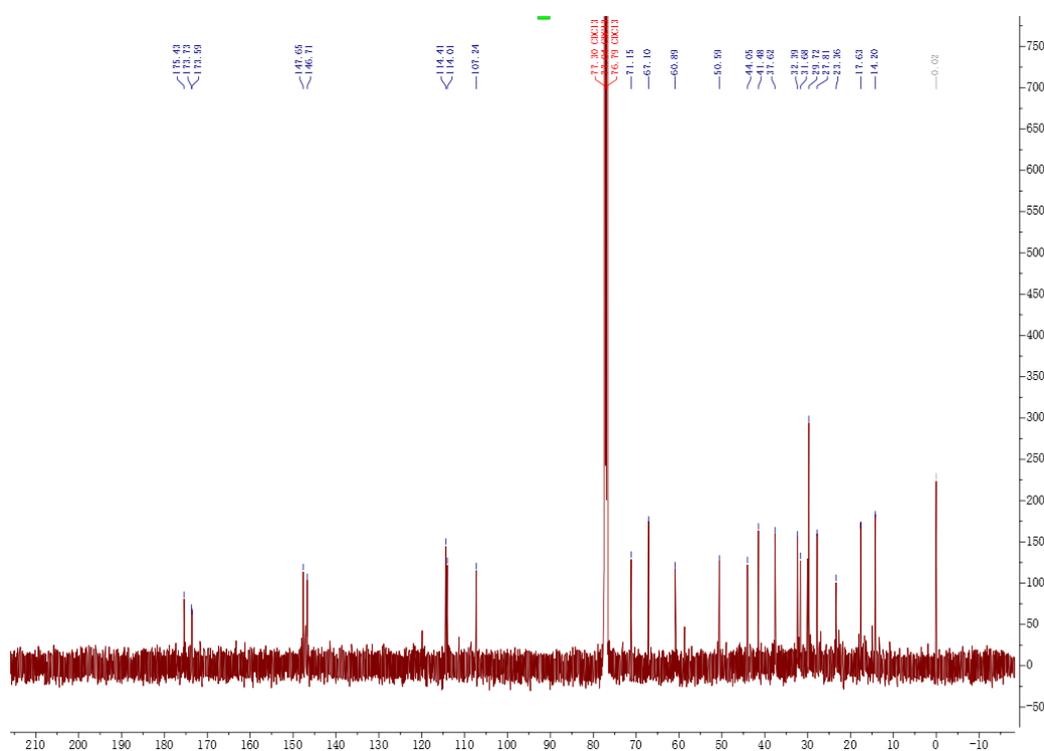


Figure S6: ¹³C-NMR (125 MHz, CDCl₃) spectrum of **1**

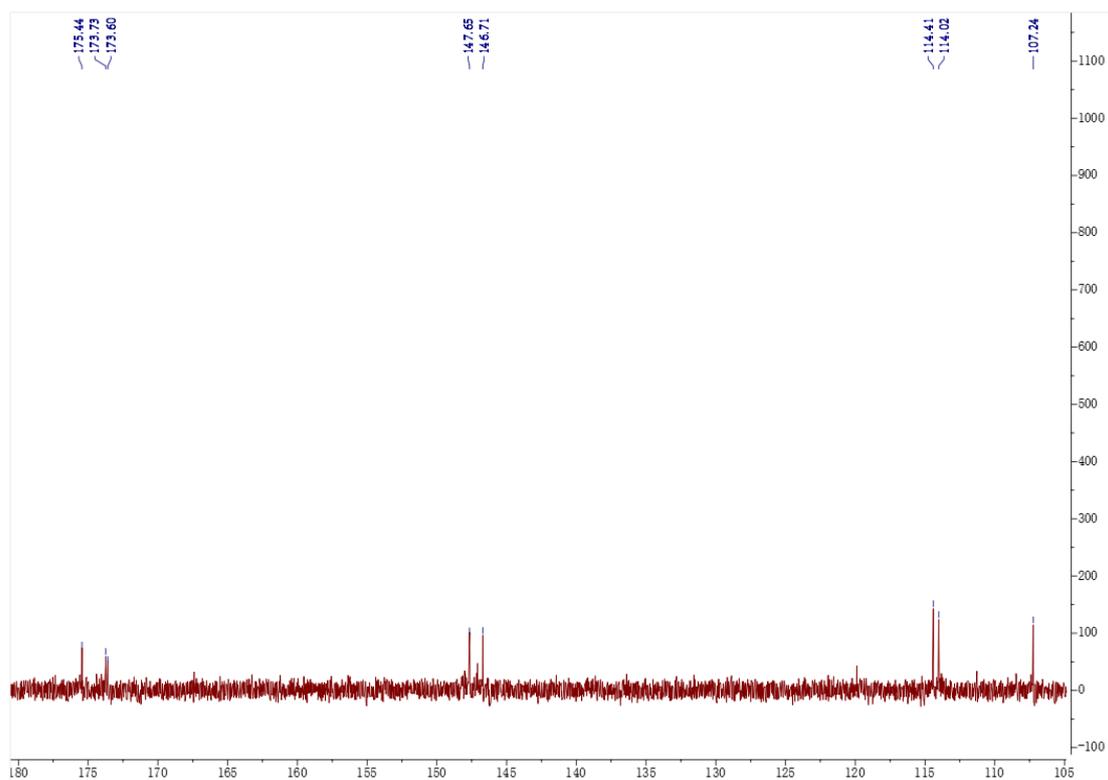


Figure S6: ^{13}C -NMR (125 MHz, CDCl_3) spectrum of **1** (from δ_{C} 105 ppm to δ_{C} 180 ppm)

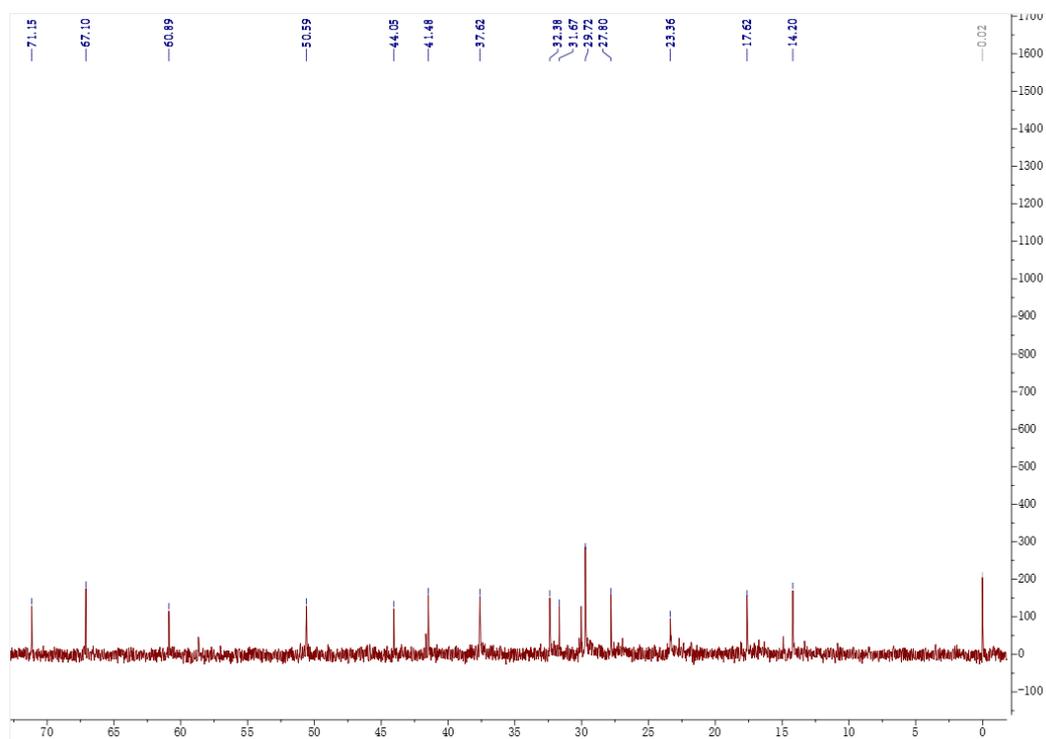


Figure S7: ^{13}C -NMR (125 MHz, CDCl_3) spectrum of **1** (from δ_{C} 0 ppm to δ_{C} 72 ppm)

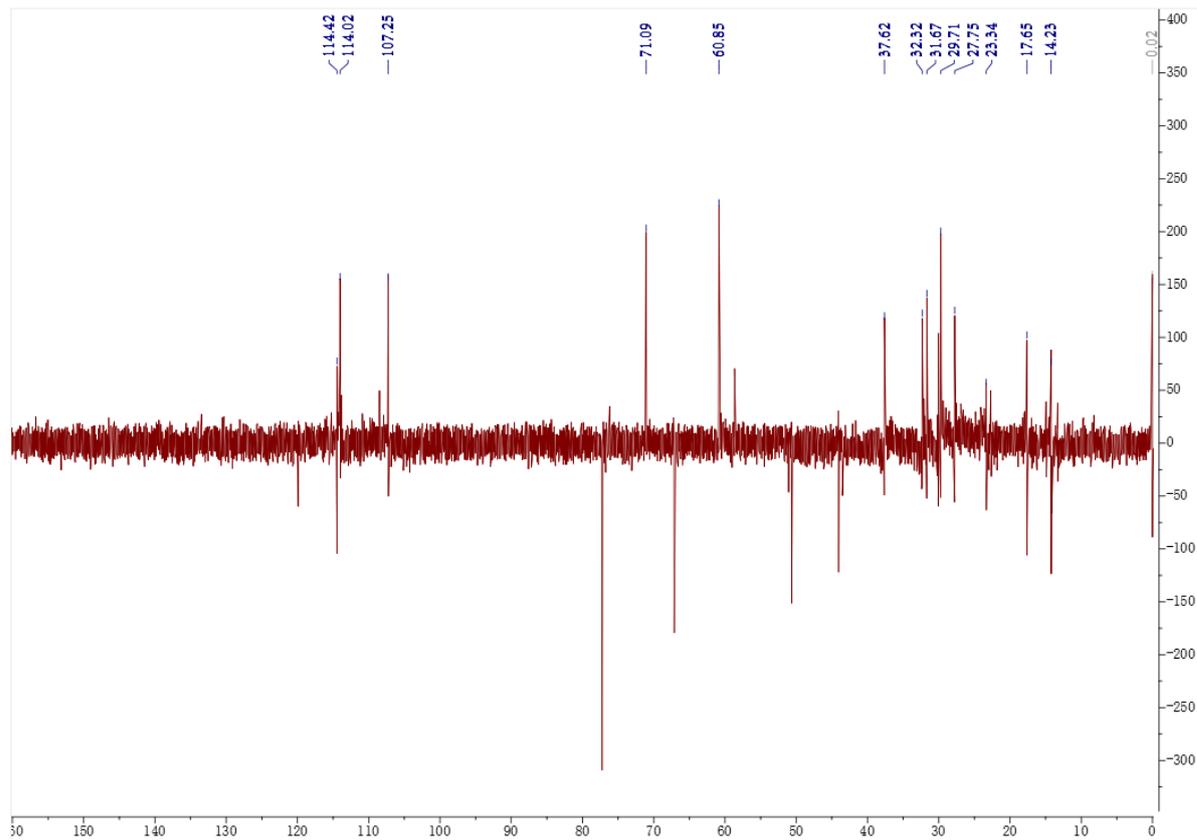


Figure S8: DEPT135 (125 MHz, CDCl_3) spectrum of **1**

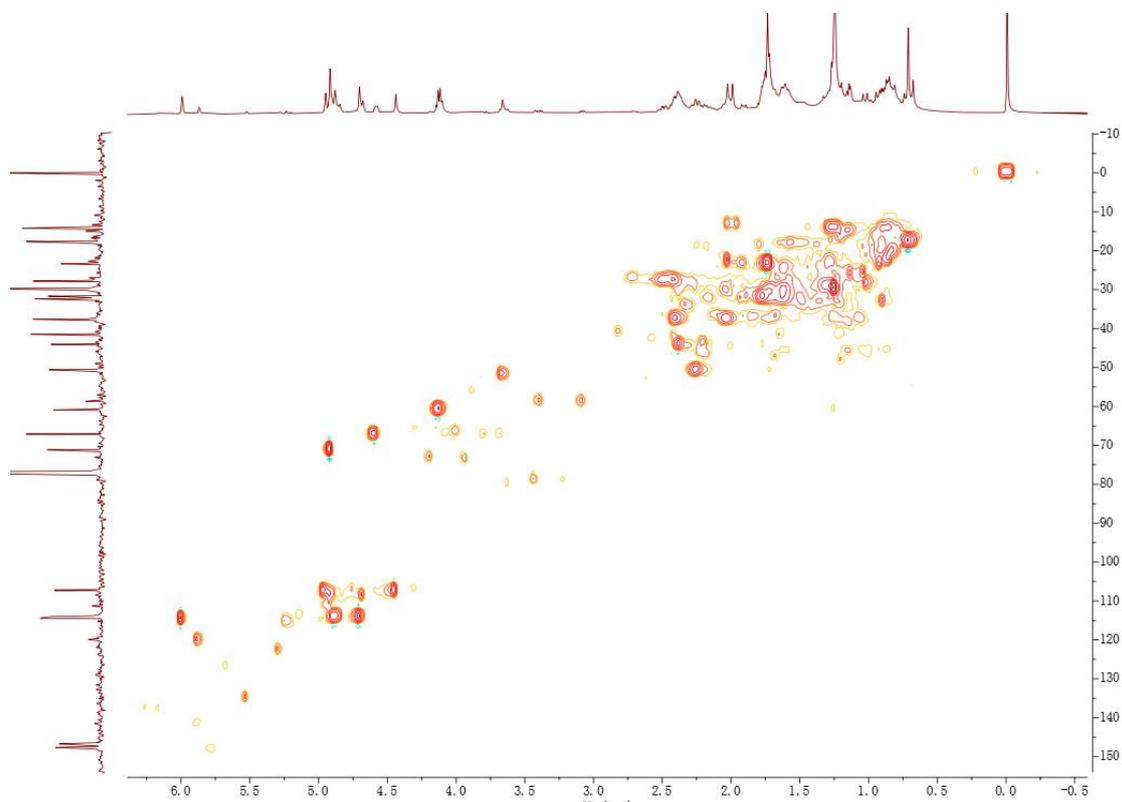


Figure S9: HSQC spectrum of **1**

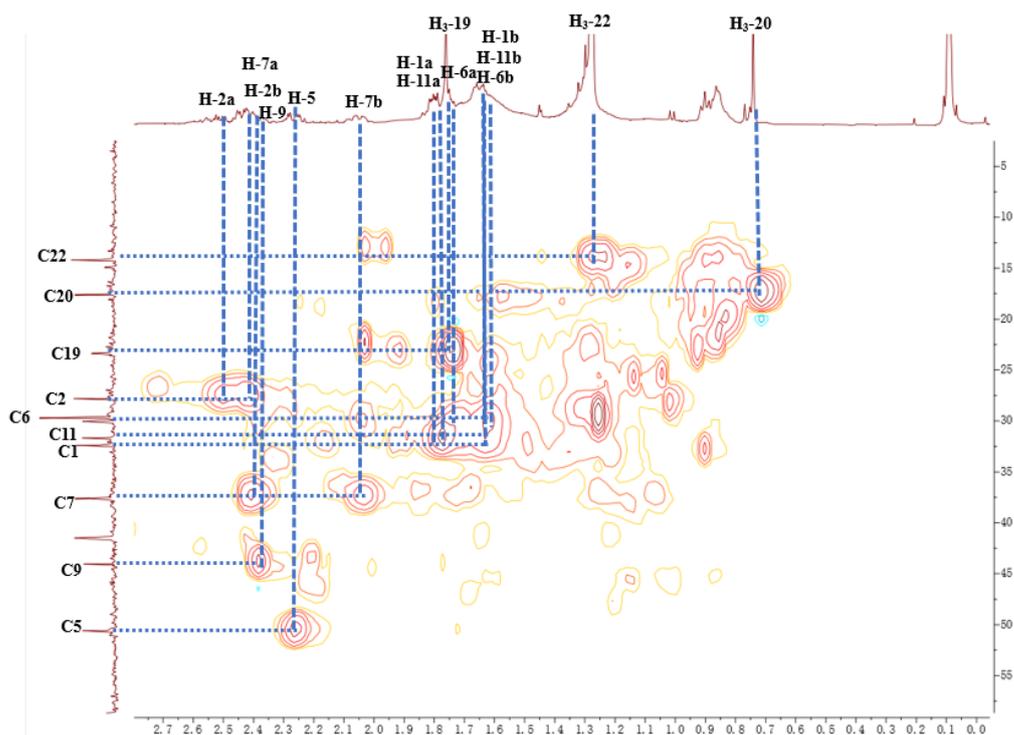


Figure S10: HSQC spectrum of **1** (from δ_H 0.6 ppm to δ_H 2.7 ppm)

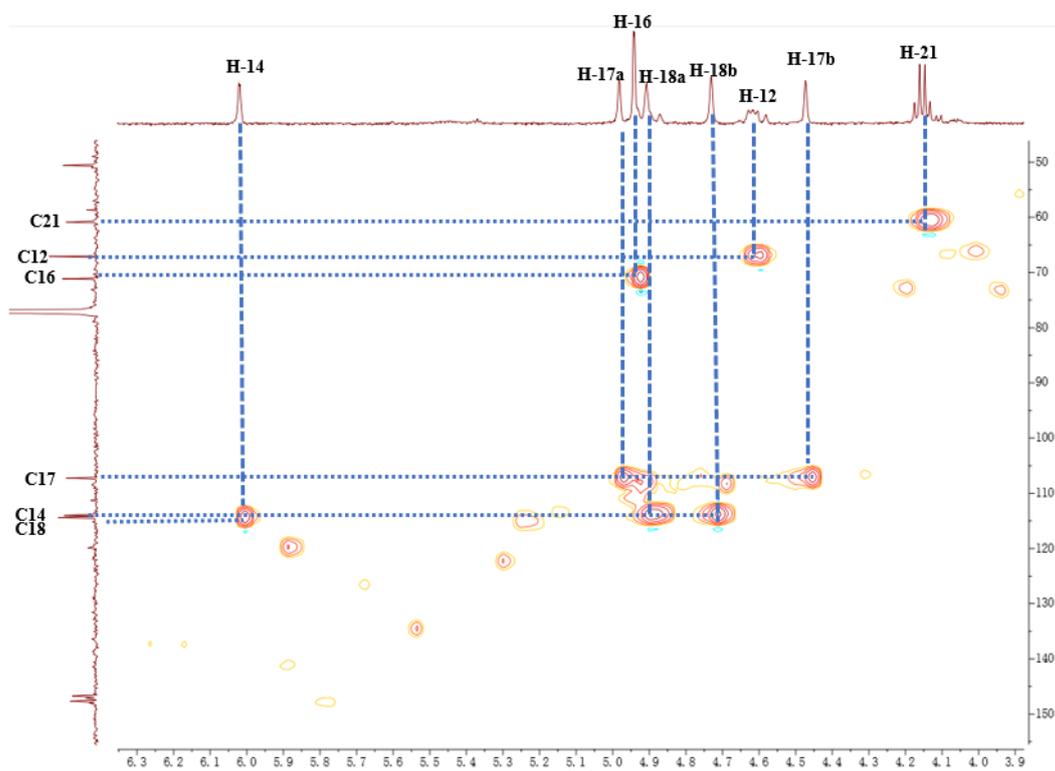


Figure S11: HSQC spectrum of **1** (from δ_H 4.0 ppm to δ_H 6.2 ppm)

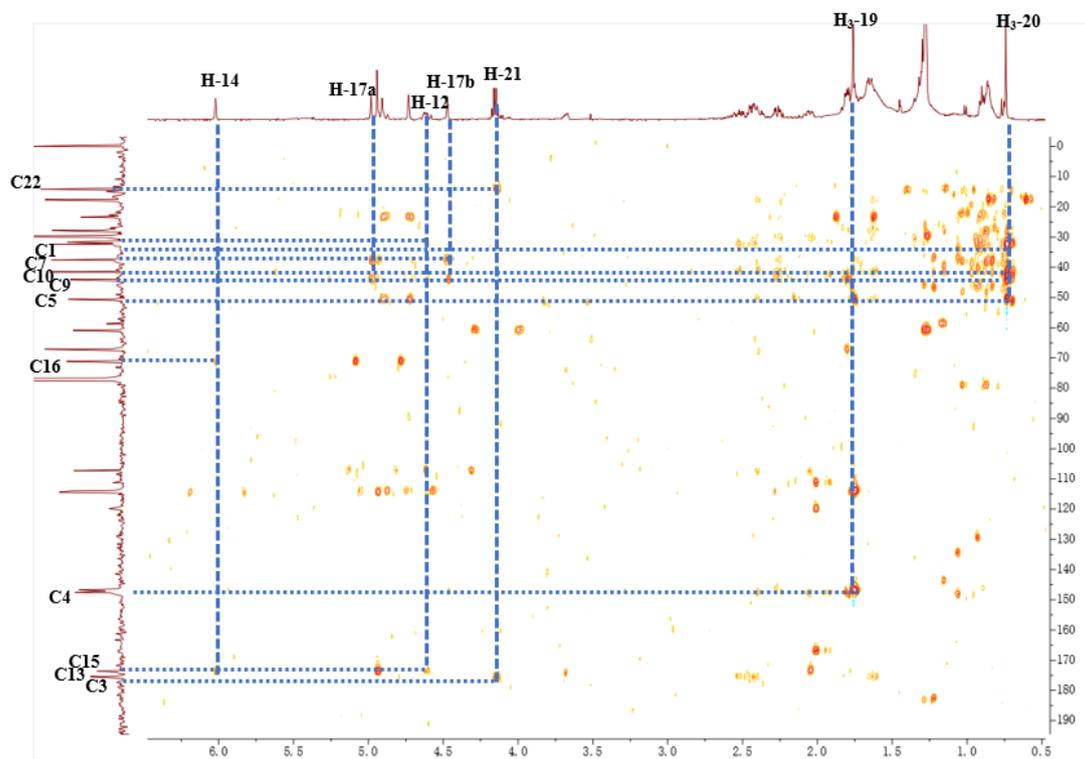


Figure S12: HMBC spectrum of 1

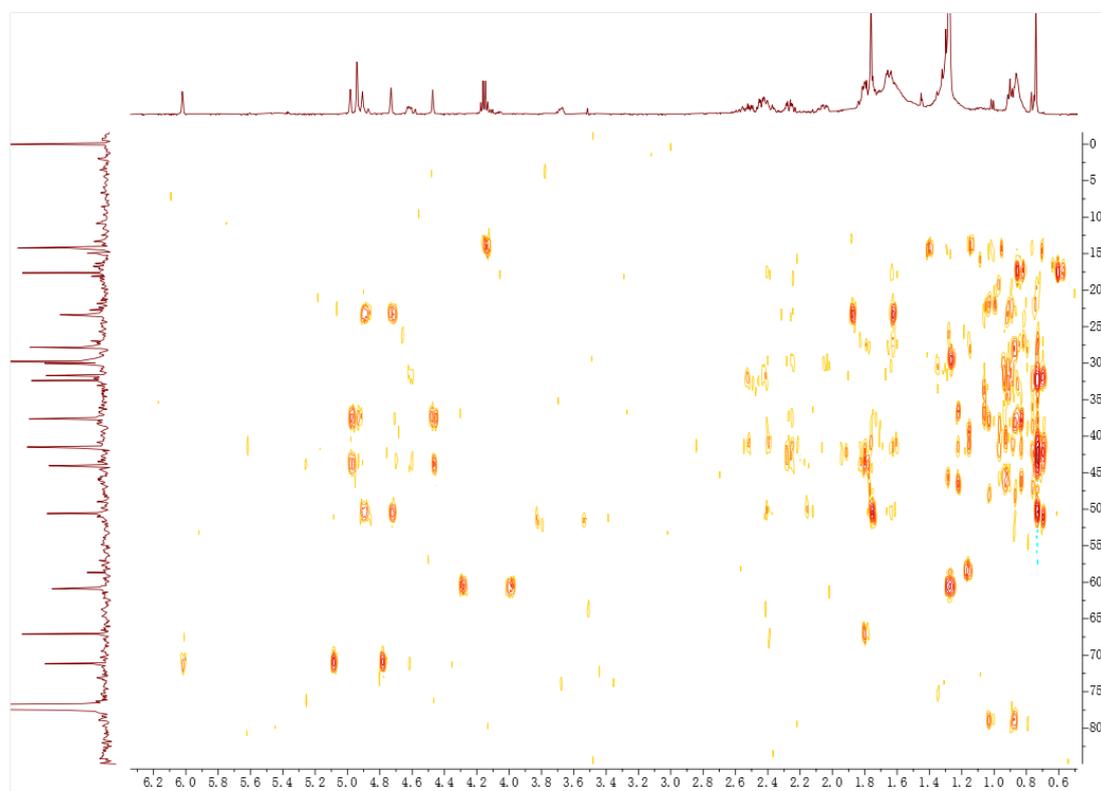


Figure S13: HMBC spectrum of 1 (from δ_C 10 ppm to δ_C 80 ppm)

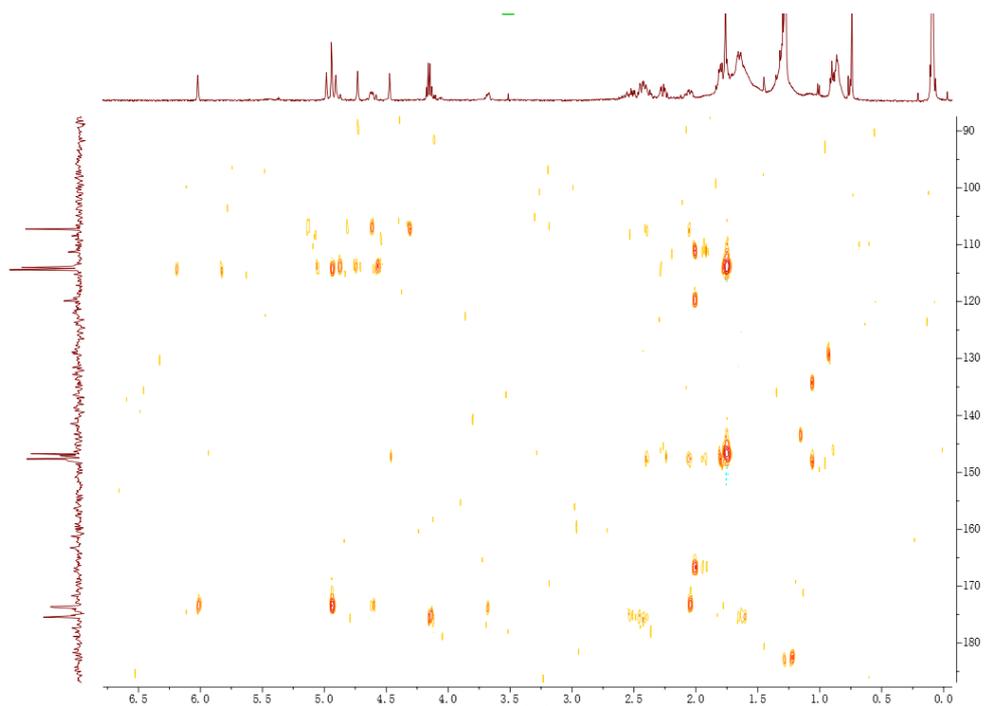


Figure S14: HMBC spectrum of **1** (from δ_c 100 ppm to δ_c 179 ppm)

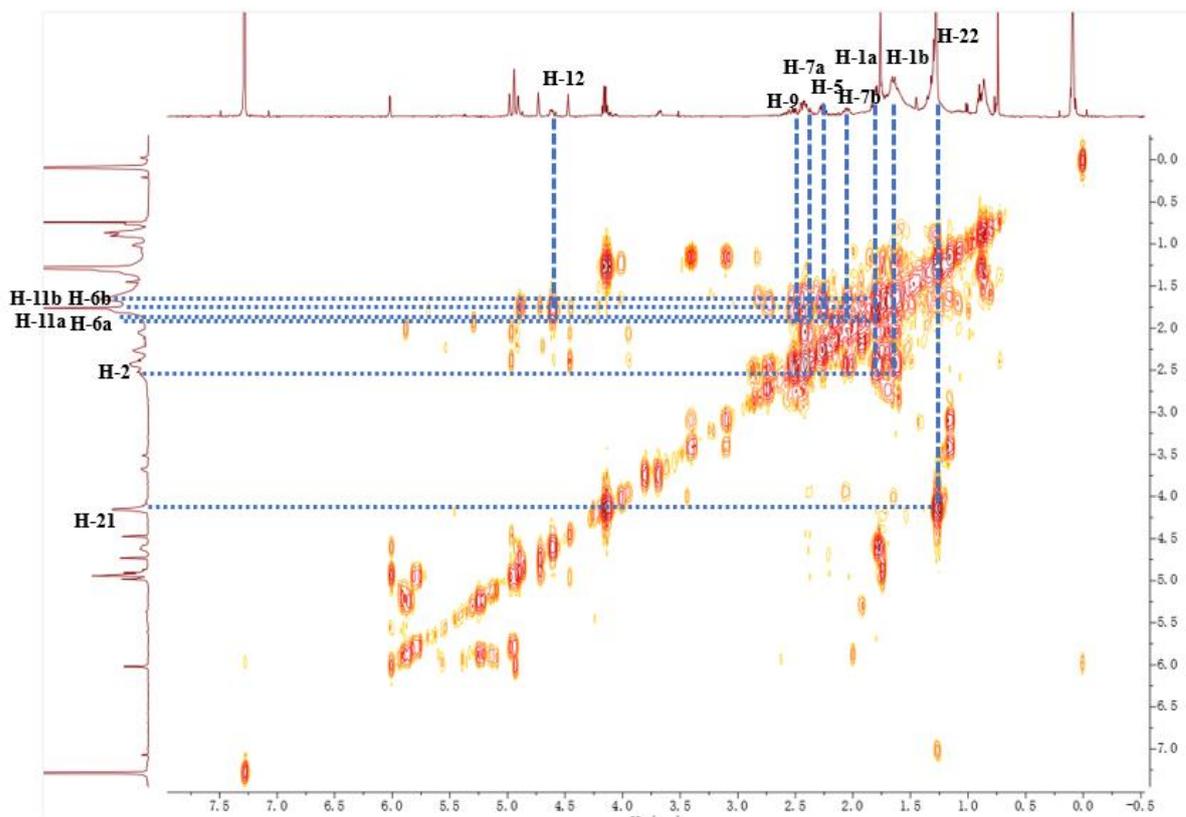


Figure S15: ^1H - ^1H COSY spectrum of **1**

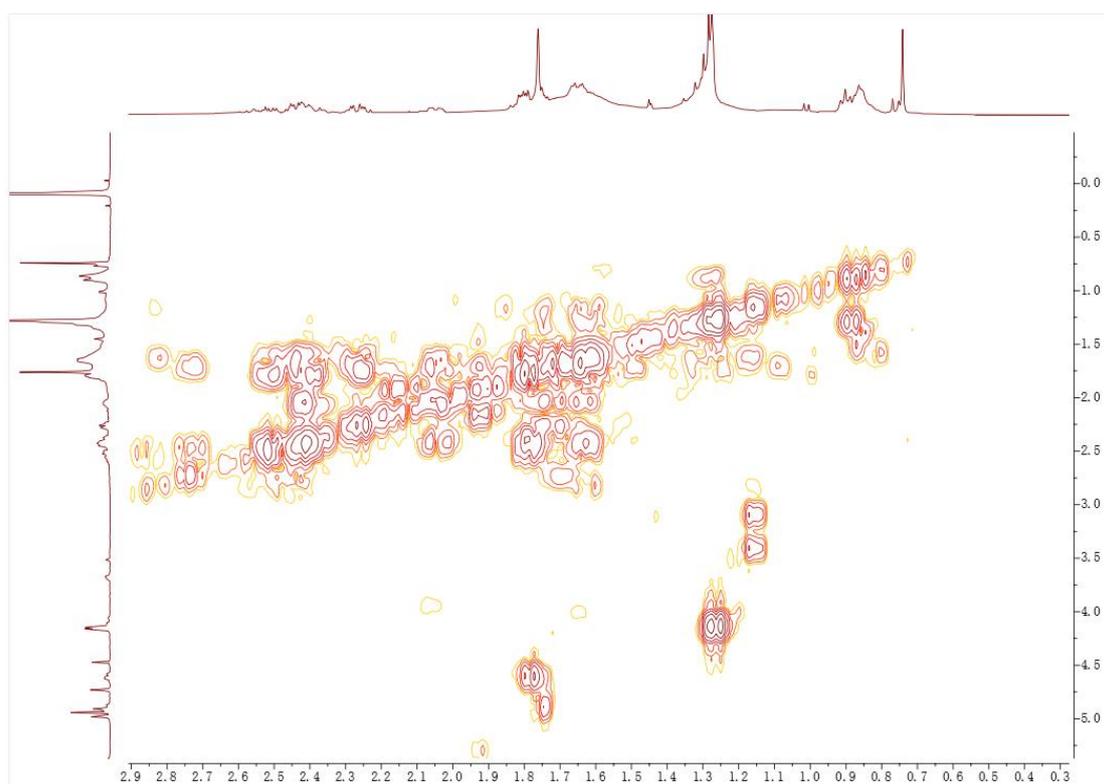


Figure S16: ^1H - ^1H COSY spectrum of **1** (from $\delta_{\text{H}}0$ ppm to δ_{H} 2.80 ppm)

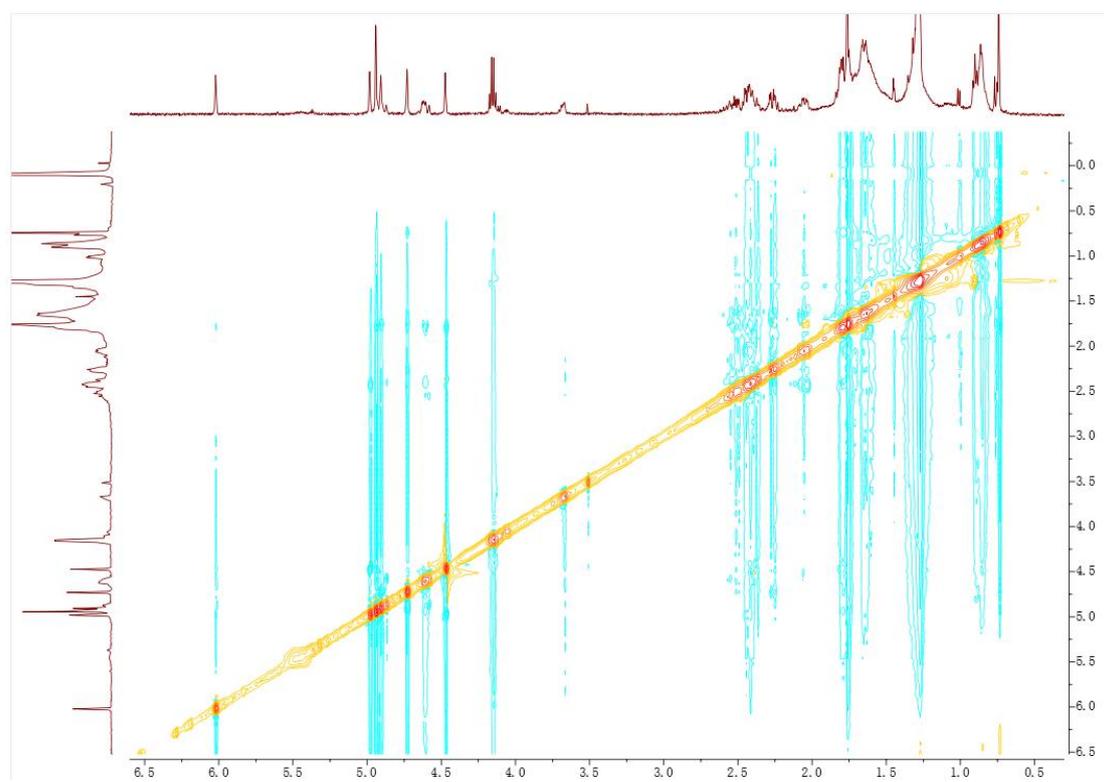


Figure S17: ROESY spectrum of **1**

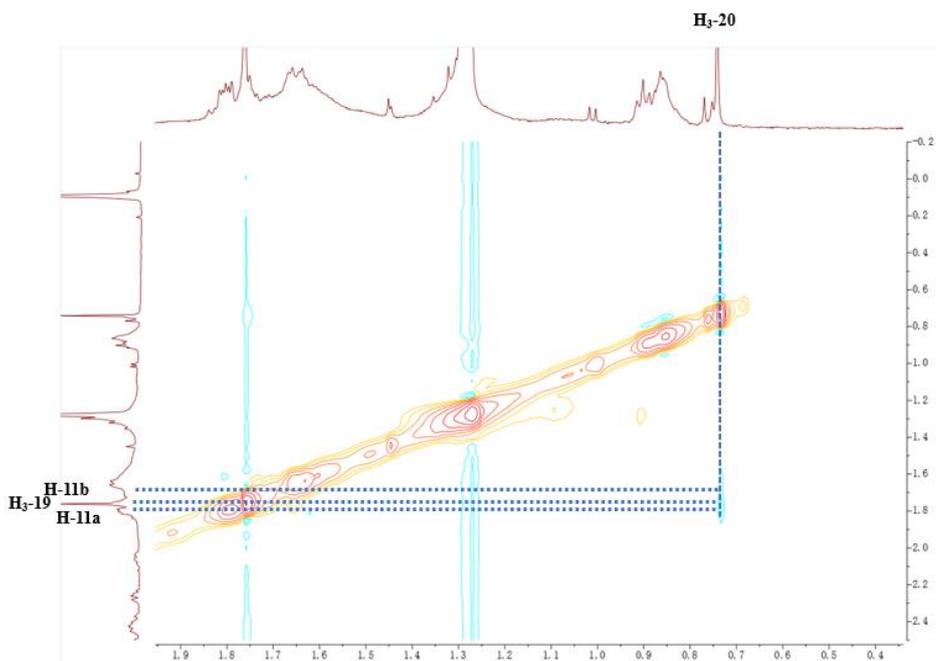


Figure S18: ROESY spectrum of **1**(from δ_{H} 0.6 ppm to δ_{H} 1.9 ppm)

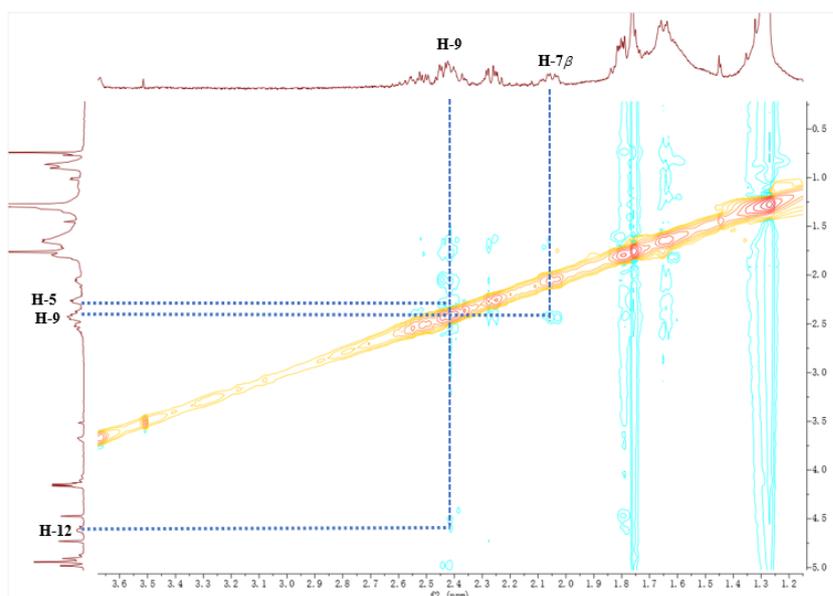


Figure S19: ROESY spectrum of **1**(from δ_{H} 1.2 ppm to δ_{H} 3.2 ppm)

Figure S20: Scifinder similarity report for compound 1

Initiating Search

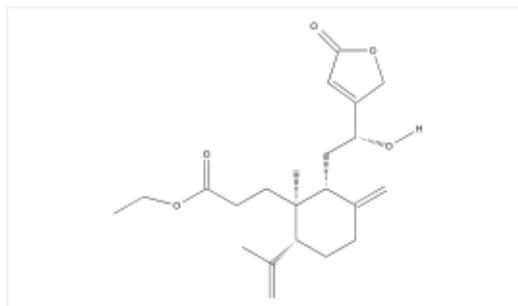
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Filtered By:

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Number of Components: **1**



Structure Match: **Similarity**

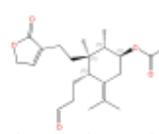
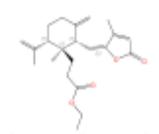
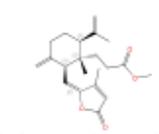
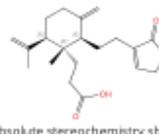
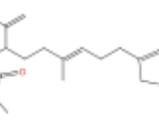
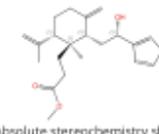
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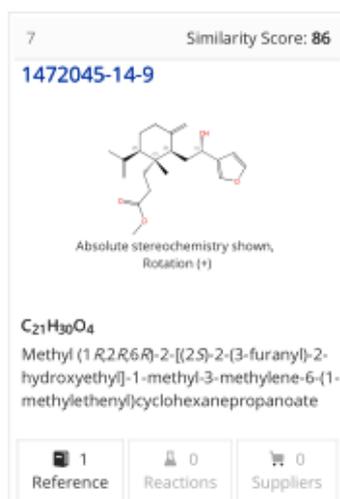
Task	Result Type	View
Exported: Returned Substance Results + Filters (7)	Substances	View Results

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 Substances (7)

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<p>1 Similarity Score: 89</p> <p>1620682-90-7</p>  <p>Absolute stereochemistry shown, Rotation (-)</p> <p>C₂₂H₃₂O₅ (1<i>R</i>,2<i>R</i>,3<i>S</i>,4<i>S</i>)-4-(Acetyloxy)-2-[2-(2,5-dihydro-2-oxo-3-furanyl)ethyl]-2,3-dimethyl-6-(1-methylethylidene)cyclohexanepropanal</p> <p> 1  0  0 Reference Reactions Suppliers</p>	<p>2 Similarity Score: 88</p> <p>3092410-96-0</p>  <p>Absolute stereochemistry shown, Rotation (-) Double bond geometry shown</p> <p>C₂₂H₃₀O₄ Cyclohexanepropanoic acid, 1-methyl-3-methylene-6-(1-methylethenyl)-2-[(<i>E</i>)-(3-methyl-5-oxo-2(5<i>H</i>)-furan-2-ylidene)methyl]-, ethyl ester, (1<i>S</i>,2<i>S</i>,6<i>S</i>)-</p> <p> 1  0  0 Reference Reactions Suppliers</p>	<p>3 Similarity Score: 87</p> <p>3092410-93-7</p>  <p>Absolute stereochemistry shown, Rotation (-) Double bond geometry shown</p> <p>C₂₁H₂₈O₄ Cyclohexanepropanoic acid, 1-methyl-3-methylene-6-(1-methylethenyl)-2-[(<i>E</i>)-(3-methyl-5-oxo-2(5<i>H</i>)-furan-2-ylidene)methyl]-, methyl ester, (1<i>S</i>,2<i>S</i>,6<i>S</i>)-</p> <p> 1  0  0 Reference Reactions Suppliers</p>
<p>4 Similarity Score: 86</p> <p>1605307-29-6</p>  <p>Absolute stereochemistry shown</p> <p>C₂₀H₂₈O₄ (1<i>R</i>,2<i>R</i>,6<i>R</i>)-2-[2-(2,5-Dihydro-2-oxo-3-furanyl)ethyl]-1-methyl-3-methylene-6-(1-methylethenyl)cyclohexanepropanoic acid</p> <p> 3  0  0 References Reactions Suppliers</p>	<p>5 Similarity Score: 86</p> <p>913691-26-6</p>  <p>Absolute stereochemistry shown</p> <p>C₂₁H₃₀O₄ Methyl 2-[6-(2,5-dihydro-5-oxo-3-furanyl)-3-methyl-3-hexen-1-yl]-1-methyl-3-methylenecyclohexanecarboxylate</p> <p> 0  0  0 References Reactions Suppliers</p>	<p>6 Similarity Score: 86</p> <p>2225891-19-8</p>  <p>Absolute stereochemistry shown</p> <p>C₂₁H₃₀O₄ Methyl (1<i>S</i>,2<i>S</i>,6<i>S</i>)-2-[(2<i>S</i>)-2-(3-furanyl)-2-hydroxyethyl]-1-methyl-3-methylene-6-(1-methylethenyl)cyclohexanepropanoate</p> <p> 3  0  0 References Reactions Suppliers</p>



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Table S1: The comparison table for the NMR chemical shifts of Callinudin A (**1**) and nudiflopene U [11]

Position	Callinudin A (1) ^a		nudiflopene U ^b	
	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}
1a	1.76, m	32.3	1.72 ^c	32.4
1b	1.62, m		1.62 ^c	
2a	2.51, m	27.8	2.54, m	27.6
2b	2.42, m		2.41 ^c	
3	-	175.4		175.5
4	-	146.7		146.8
5	2.26, m	50.6	2.25 ,(dd, 12.5, 3.8)	50.7
6a	1.72, m	29.7	1.70 ^c	30.2
6b	1.65, m		1.62 ^c	
7a	2.43, m	37.6	2.39 ^c	37.7
7b	2.04, m		2.09(ddd, 12.9, 12.9, 5.1),	
8	-	147.6		147.7
9	2.39, m	44.0	2.35 ^c	44.3
10	-	41.5		41.4
11a	1.77, m	31.7	1.74 ^c	31.7

11b	1.61, m		1.74 ^c	
12	4.61, m	67.1	4.59((dd, 8.6, 3.3)	67.0
13	-	173.6	-	174.0
14	6.02(s)	114.4	5.96 (s)	114.4
15	-	173.7	-	174.0
16	4.94(brs)	71.1	4.91 (brs)	71.4
17a	4.96(brs)	107.2	4.94 (s)	107.3
17b	4.45(brs)		4.43 (s)	
18a	4.89(brs)	114.0	4.87(s)-	114.0
18b	4.72(brs)		4.68(s)	
19	1.75 (s)	23.3	1.72(s)	23.5
20	0.73 (s)	17.6	0.70(s)	17.7
21	4.15(q, $J = 7.5$ Hz)	60.9	3.65(s)	52.0
22	1.28 (t, $J = 7.5$ Hz)	14.2	-	

a: ¹H (600 MHz in CDCl₃), b: ¹H (400 MHz in CDCl₃) c: overlapping signal.

Table S2: The comparison table for the NMR chemical shifts of compound **2** and callnudooid F

Position	2^a		callnudooid F ^b	
	$\delta_{\text{H}}(J \text{ in Hz})$	δ_{C}	$\delta_{\text{H}}(J \text{ in Hz})$	δ_{C}
1a	1.77 ^c (m)	32.7	1.76 ^c (m)	32.4
1b	1.58 ^c (m)		1.59 ^c (m)	
2a	2.47(m)	27.9	2.47(m)	27.6
2b	2.34(m)		2.32(m)	
3	-	174.4		175.5
4	-	146.9		146.8
5	2.29 ^c (m)	50.2	2.36 ^c (m) overlap	50.7
6a	1.71 ^c (m)	29.8	1.75 ^c (m)	30.2
6b	1.61 ^c (m)		1.58 ^c (m)	
7a	2.37 ^c (m)	37.2	2.36 ^c (m)	37.7
7b	2.08, m		2.10(td, 13.6, 4.8),	
8	-	147.6		147.7
9	2.48 ^c	45.2	2.51 ^c	44.3
10	-	40.9		41.4
11a	2.49 ^c	30.9	2.52 ^c	31.7

11b	2.44 ^c		2.43 ^c	
12		179.1	4.59((dd, 8.6, 3.3)	67.0
17a	4.85(brs)	107.1	4.94 (s)	107.3
17b	4.64(brs)		4.43 (s)	
18a	4.89(brs)	114.2	4.87(s)-	114.0
18b	4.71(brs)		4.68(s)	
19	1.74(s)	23.6	1.72(s)	23.5
20	0.72 (s)	17.6	0.70(s)	17.7
21	3.66(s)	51.7	3.65(s)	52.0

a: ¹H (400 MHz in CDCl₃). b: ¹H (400 MHz in CD₃OD) c: overlapping signal.

Table S3: The comparison table for the NMR chemical shifts of compound **3** and nudiflopene H.

Position	3^a		nudiflopene H. ^b	
	$\delta_{\text{H}}(J \text{ in Hz})$	δ_{C}	$\delta_{\text{H}}(J \text{ in Hz})$	δ_{C}
1a	1.77 ^c (m)	34.0	1.69(m)	33.9
1b	1.58 ^c (m)		1.41 (m)	
2a	2.47(m)	27.9	2.42(m)	28.0
2b	2.34(m)		2.15(m)	
3	-	179.2		179.8
4	-	146.3		146.2
5	2.29 ^c (m)	50.6	2.29 (dd,12.6,3.6)	50.4
6a	1.71 ^c (m)	29.2	1.82 (m)	29.0
6b	1.61 ^c (m)		1.60 (m)	
7a	2.37 ^c (m)	36.2	2.41 (m)	36.0
7b	2.08, m		2.15(m)	
8	-	147.9	-	147.7
9	2.48 ^c	47.3	3.35(d,10.5)	47.1
10	-	41.5	-	41.3
11a	2.49 ^c	109.9	5.39(d,10.5)	109.9
12		151.7	-	151.5
13		154.4	-	154.3
14		116.9	5.94(s)	116.7
15		169.3	-	169.3
16		12.0	2.20(s)	11.9
17a	4.85(brs)	109.2	4.81 (s)	109.0
17b	4.64(brs)		4.46 (s)	
18a	4.89(brs)	114.5	4.90(s)-	114.3
18b	4.71(brs)		4.75(s)	

19	1.74(s)	23.8	1.76(s)	23.7
20	0.72 (s)	17.2	0.91(s)	17.0

a: ¹H (600 MHz in CDCl₃). b: ¹H (400 MHz in CDCl₃)

2. ECD calculation details

1. Methods

In general, conformational analyses were carried out via random searching in the Sybyl-X 2.0 using the MMFF94S force field with an energy cutoff of 5 kcal/mol.^[1] The results showed three lowest energy conformers. Subsequently, geometry optimizations and frequency analyses were implemented at the B3LYP-D3(BJ)/6-31G* level in CPCM methanol using ORCA5.0.3^[2] All conformers used for property calculations in this work were characterized to be stable point on potential energy surface (PES) with no imaginary frequencies. The excitation energies, oscillator strengths, and rotational strengths (velocity) of the first 60 excited states were calculated using the TD-DFT methodology at the PBE0/def2-TZVP level in CPCM methanol using ORCA5.0.3.^[2] The ECD spectra were simulated by the overlapping Gaussian function (half the bandwidth at 1/e peak height, sigma = 0.30 for all).^[3] Gibbs free energies for conformers were determined by using thermal correction at B3LYP-D3(BJ)/6-31G* level and electronic energies evaluated at the wB97M-V/def2-TZVP level in CPCM methanol using ORCA5.0.3^[2] To get the final spectra, the simulated spectra of the conformers were averaged according to the boltzmann distribution theory and their relative Gibbs free energy (ΔG). By comparing the experiment spectra with the calculated model molecules, the absolute configuration of the only chiral center was determined to be .

[1].Sybyl Software, version X 2.0; Tripos Associates Inc.: St. Louis, MO, 2013.

[2]. Neese, F. (2012) The ORCA program system, Wiley Interdiscip. Rev.: Comput. Mol. Sci., 2, 73-78.

[3]. Stephens, P. J.; Harada, N. ECD cotton effect approximated by the Gaussian curve and other methods. Chirality2010, 22, 229–233.

2. Results

Table S2. Gibbs free energies^a and equilibrium populations^b of low-energy conformers of **1**.

Conformers	ΔG (a.u.)	P(%)/100	G(a.u.)
1000003_tddft_	0.00000	85.13	-1233.260979
1000004_tddft_	0.00235	7.09	-1233.258633
1000005_tddft_	0.00226	7.78	-1233.258719

^awB97M-V/def2-TZVP, in a.u.

^bFrom ΔG values at 298.15K.

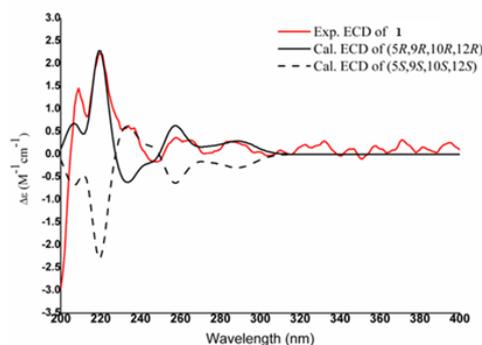


Figure . Calculated and experimental ECD spectra of compound 1

Cell culture

At 37 °C with 5% CO₂, THP-1 cells were grown in RPMI-1640 media with 10% FBS, 50 μM β-mercaptoethanol, and 1% penicillin–streptomycin. Cells were seeded at a density of 5×10⁵ cells/mL in six-well plates and differentiated into adherent macrophages by treatment with 100 ng/mL Phorbol 12-myristate 13-acetate (PMA). Subsequent experiments were performed after the cells had reached a stable and fully differentiated state.

CCK-8 assay

PMA-induced macrophages were seeded in 96-well plates at 3×10⁴ cells/well. Twenty four hours later, cells were incubated with test specimen for 12 h. Cells without any treatment were used as a control. CCK8 assay was used to assess the cytotoxicity of test specimen on PMA induced macrophages, based on the manufacturer's recommendation.

Western blot

Furthermore, after treatment with 1 at various doses (6 and 14 μM), we examined the influence of this compound on the expression levels of NLRP3, IL-1β, and IL-18 in LPS-induced THP-1 macrophages through Western blotting.

Total cellular proteins were extracted using RIPA lysis buffer, and protein concentrations were quantified using the BCA assay. Protein samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% non-fat milk for 1 hour, followed by incubation with antibodies overnight at 4°C, and then incubated with the HRP-coupled secondary antibody for 1

hour. Protein bands were visualized using a gel imaging system and a hypersensitive chemiluminescence (ECL) detection kit.

ELISA detection of cytokines in cell culture supernatan

The supernatant of THP-1 inflammatory cells after drug intervention was collected, and the contents of IL-18, IL-1 β and NLRP3 in the cell supernatant were determined according to the instructions of the ELISA kit.

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