

## **Diterpenoids with Potent Anti-Psoriasis Activity from *Euphorbia helioscopia* L.**

Zhen-Zhu Zhao<sup>1</sup>, Xu-Bo Liang<sup>1</sup>, Hong-Juan He<sup>2</sup>, Gui-Min Xue<sup>1</sup>, Yan-Jun Sun<sup>1</sup>, Hui Chen<sup>1</sup>, Yin-Sheng Zhao<sup>1</sup>, Li-Na Bian<sup>1</sup>, Wei-Sheng Feng<sup>1,\*</sup>, Xiao-Ke Zheng<sup>1,\*</sup>

<sup>1</sup>School of Pharmacy, Henan University of Chinese Medicine, Zhengzhou 450046, China

<sup>2</sup>Academy of Chinese Medical Sciences, Henan University of Chinese Medicine, Zhengzhou 450046, China

### **Corresponding Authors**

\*Tel/fax: 86-27-67842267. E-mail: fwsh@hactcm.edu.cn (W. -S. Feng)

\*Tel/fax: 86-27-67842267. E-mail: zhengxk.2006@163.com (X. -K. Zheng)

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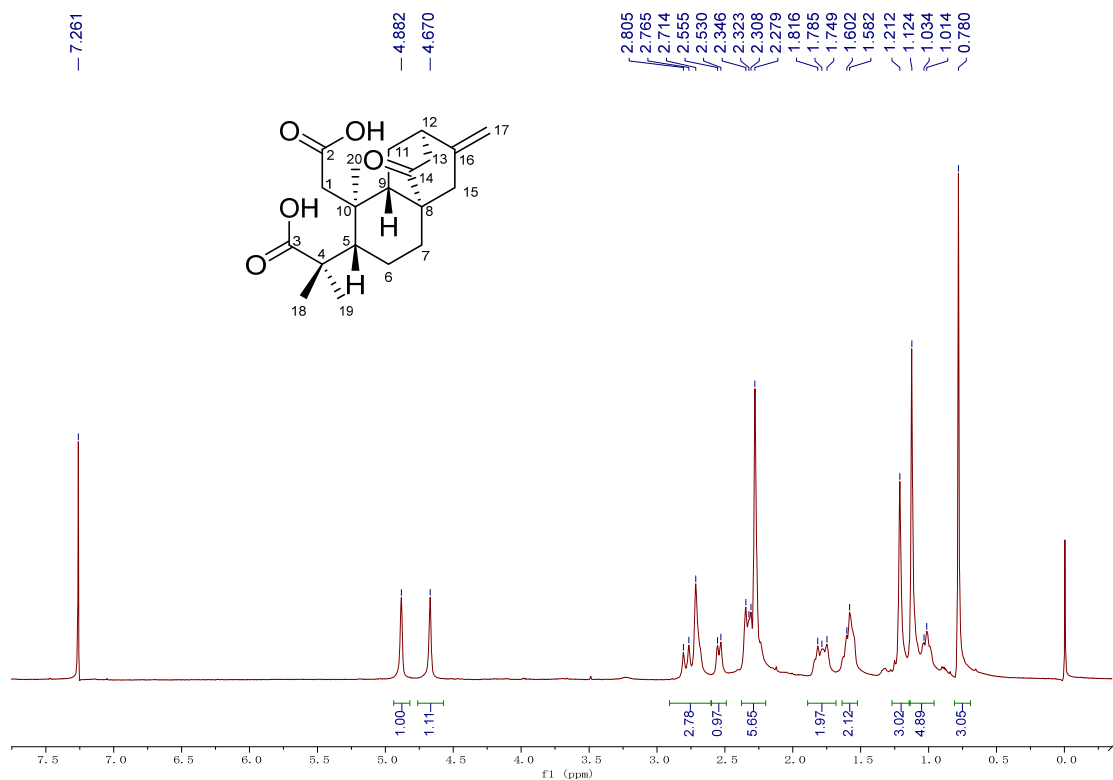
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## 1. Spectroscopic data

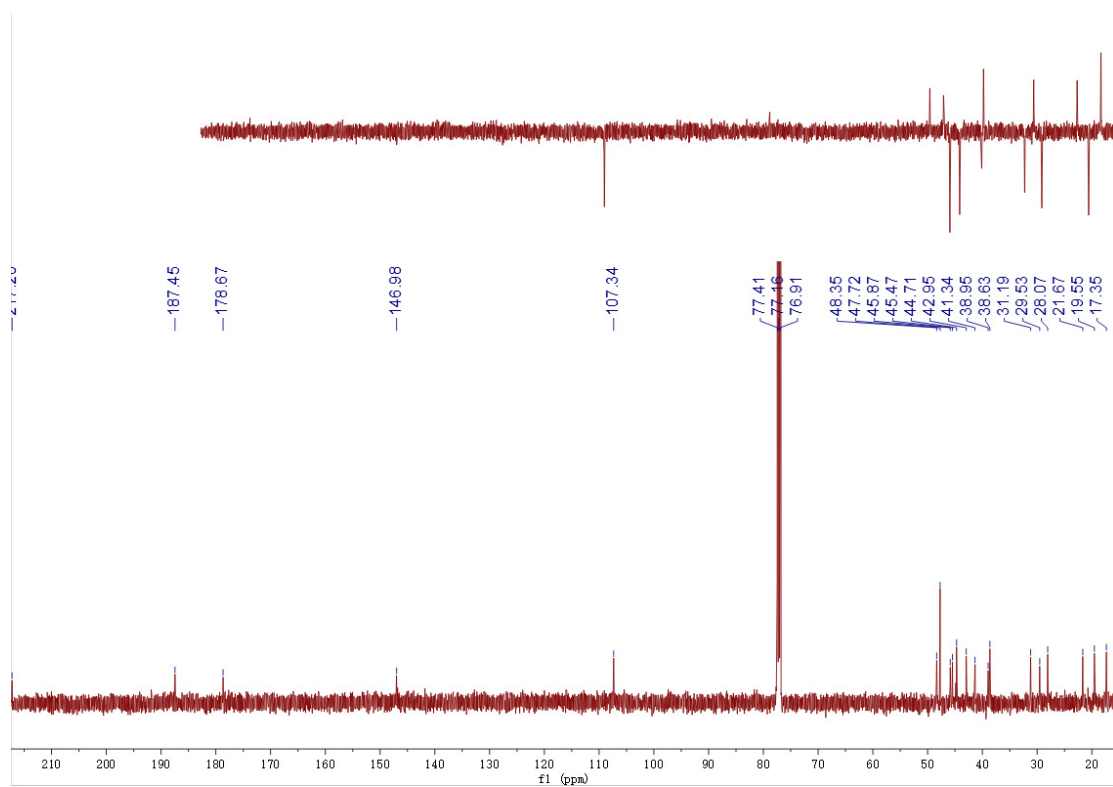
**Table S1 <sup>1</sup>H (500 MHz) and <sup>13</sup>C NMR (125 MHz) data of compounds 13, 17 and 20 (CDCl<sub>3</sub>,  $\delta$  in ppm, *J* in Hz)**

no.	13		17		20	
	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$	$\delta_C$	$\delta_H$
1	42.6	1.99, dd (13.6, 7.2) 1.32, dd (13.6, 11.1)	46.6	3.10, dd (15.1, 7.9) 1.41, dd (15.1, 10.3)	49.2	2.34, overlapped; 1.70, dd (16.3, 14.0)
2	36.7	2.49, m	38.6	2.19, m	38	2.33, overlapped
3	83.3	4.92, dd (7.6, 6.9)	82.8	5.30, d (10.3)	80.9	5.60, dd (4.3, 3.7)
4	44.9	3.20, dd (9.6, 7.6)	43.1	3.26, dd (10.3, 7.6)	50.2	2.90, dd (8.6, 4.3)
5	121.2	5.54, d (9.6)	137.0	6.76, d (7.6)	140.4	7.00, d (8.6)
6	138.6	/	134.5	/	138	/
7	73.8	5.02, dd (9.1, 3.2)	195.7	/	199.5	/
8	41.4	2.89, dd (13.8, 9.1) 2.76, dd (13.8, 3.4)	50.2	4.50, d (15.1) 3.02, d (15.1)	36.3	3.28, dd (12.7, 1.8) 2.44, dd (12.7, 10.1)
9	207.7	/	205.2	/	77.5	4.87, dd (9.9, 1.8)
10	51.2	/	49.9	/	40.1	/
11	132.8	5.33, d (15.8)	137	5.51, d (15.9)	138.4	5.13, d (16.0)
12	132.9	5.56, dd (15.8, 8.8)	133.2	5.30, dd (15.9, 10.1)	128.8	5.67, dd (15.9, 8.9)
13	39.6	2.35, m	39.1	2.49, m	39.8	2.35, overlapped
14	79.2	4.99, d (8.7)	75.7	5.89, d (1.2)	81.5	5.01, d (2.9)
15	84.8	/	92.4	/	84.5	/
16	18.3	1.13, d (6.8)	19.2	0.91, d (7.0)	14.5	0.98, d (7.3)
17	17.8	1.71, d (1.1)	12.3	1.83, s	12.4	1.84, s
18	21.4	1.11, s	25.8	1.15, s	19.9	0.93, s
19	24.3	1.18, s	22.1	1.32, s	24.0	1.17, s
20	19.3	0.98, d (6.8)	21.1	1.18, d (7.1)	26.1	0.93, d (6.1)
1'	165.9	/	165.6	/	165.9	/
2'	130.4	/	130.3	/	130.1	/
3',7'	129.5	7.91, dd (7.1, 1.3)	129.4	7.91, d (7.3)	129.9	8.04, d (7.3)
4',6'	128.4	7.38, t (7.8)	128.6	7.42, t (7.7)	128.7	7.45, t (7.7)
5'	133.1	7.52, t (7.4)	134.4	7.55, t (7.4)	133.2	7.56, t (7.5)
7-	169.7	/				
OAc	20.7	1.63, s				
9-					170.5	/
OAc					21.2	2.06, s
14-	171.0	/	170.1		170.7	/
OAc	21.2	2.17, s	21.6	2.23, s	21.0	2.23, s
15-			170.1			
OAc			25.2	2.15, s		

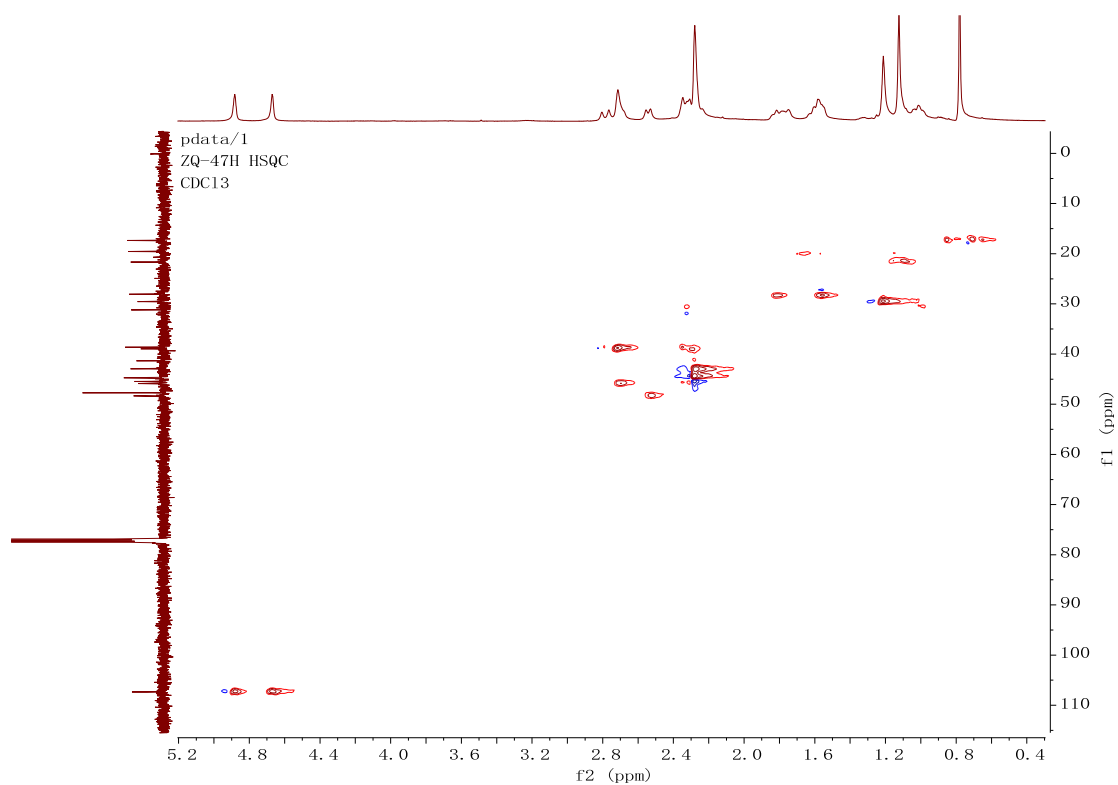
**Figure S1  $^1\text{H}$  NMR spectrum of 1 (500 MHz,  $\text{CDCl}_3$ )**



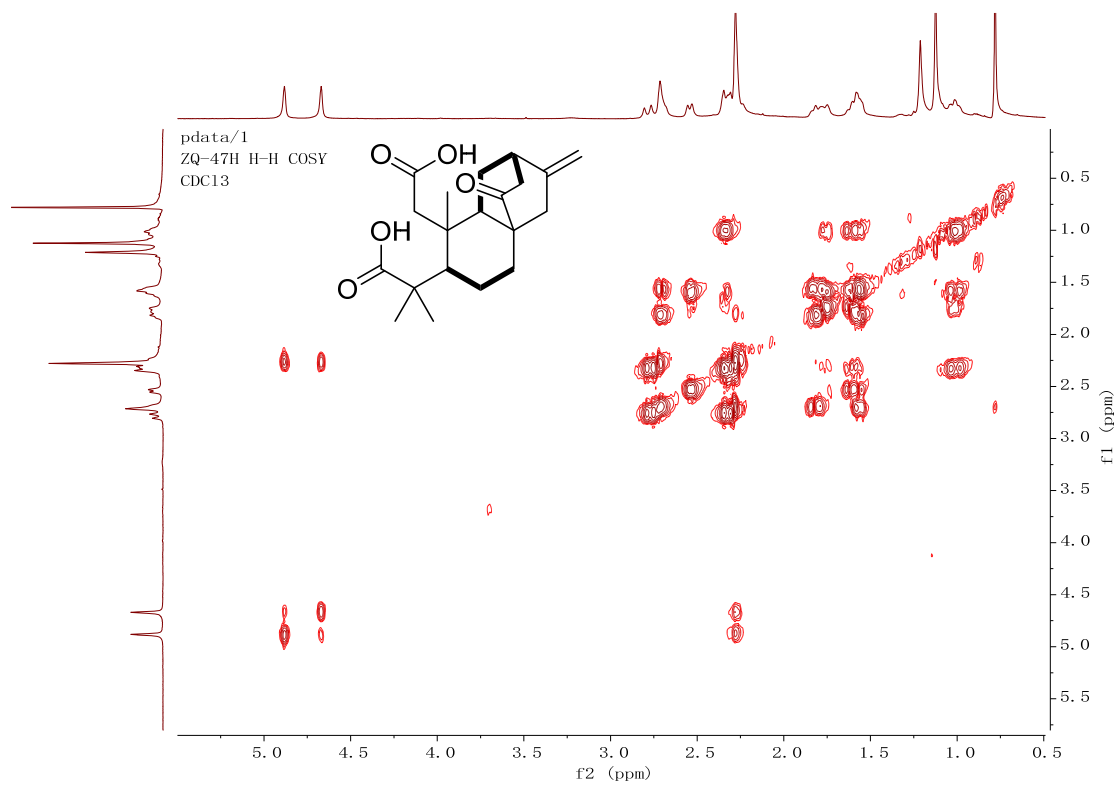
**Figure S2  $^{13}\text{C}$  and DEPT 135 NMR spectra of 1 (125 MHz,  $\text{CDCl}_3$ )**



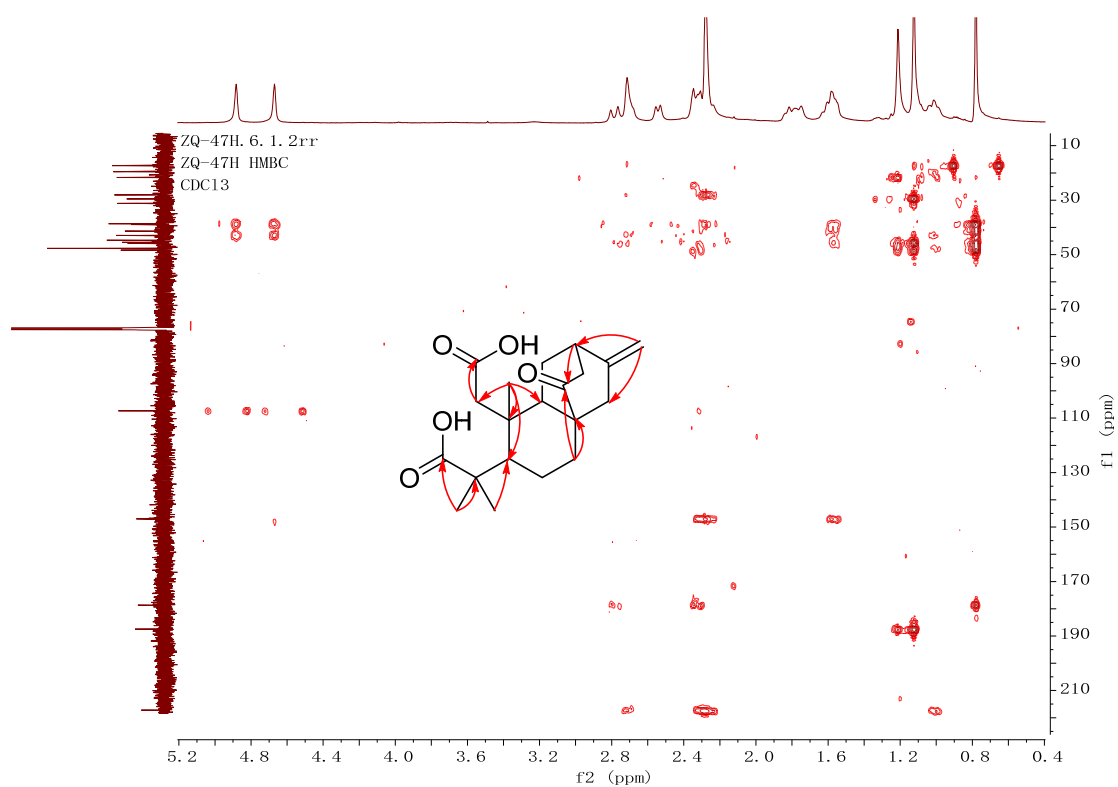
**Figure S3 HSQC spectrum of 1**



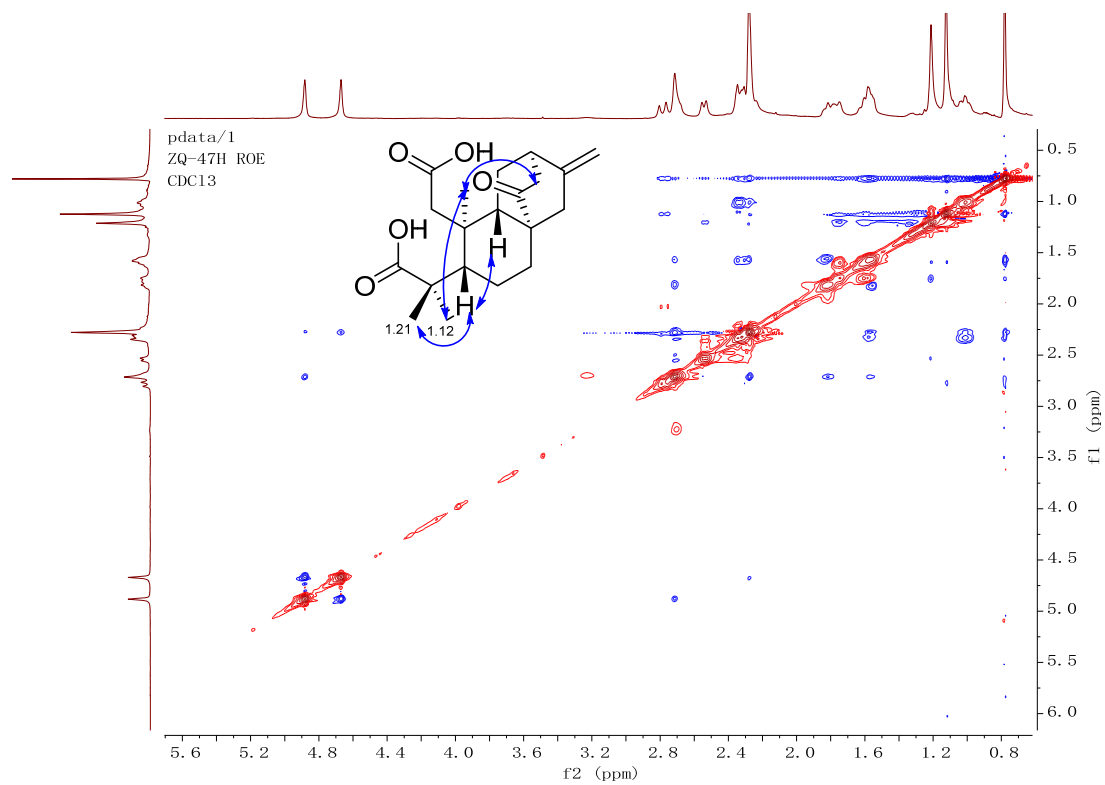
**Figure S4  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of 1**



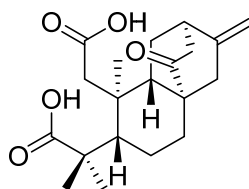
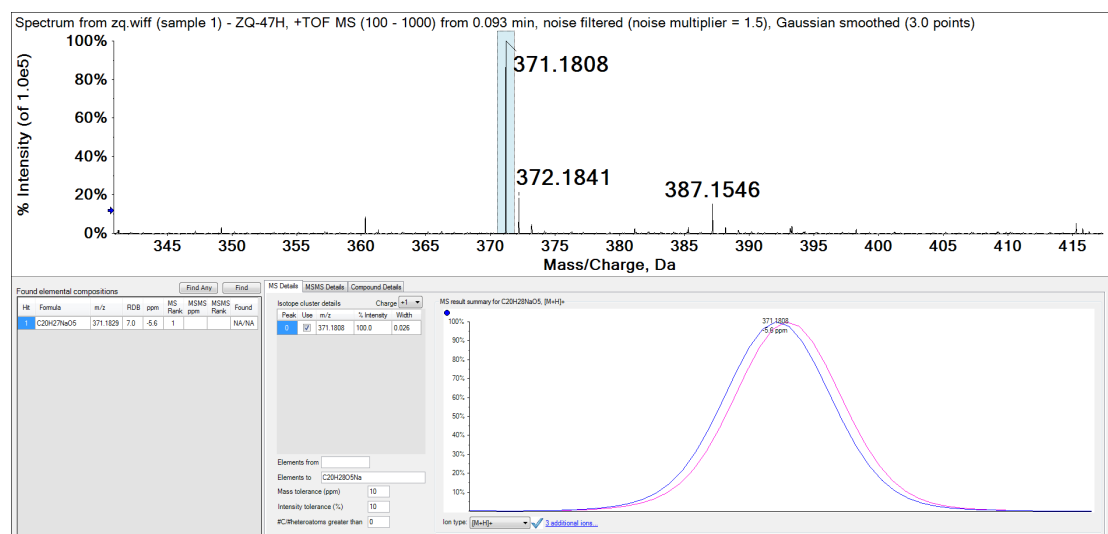
**Figure S5. HMBC spectrum of 1**



**Figure S6 ROESY spectrum of 1**



**Figure S7 HRESIMS report of 1**



Chemical Formula: C<sub>20</sub>H<sub>28</sub>O<sub>5</sub>  
Exact Mass: 348.19



Figure S8  $^1\text{H}$  NMR spectrum of 2 (500 MHz,)

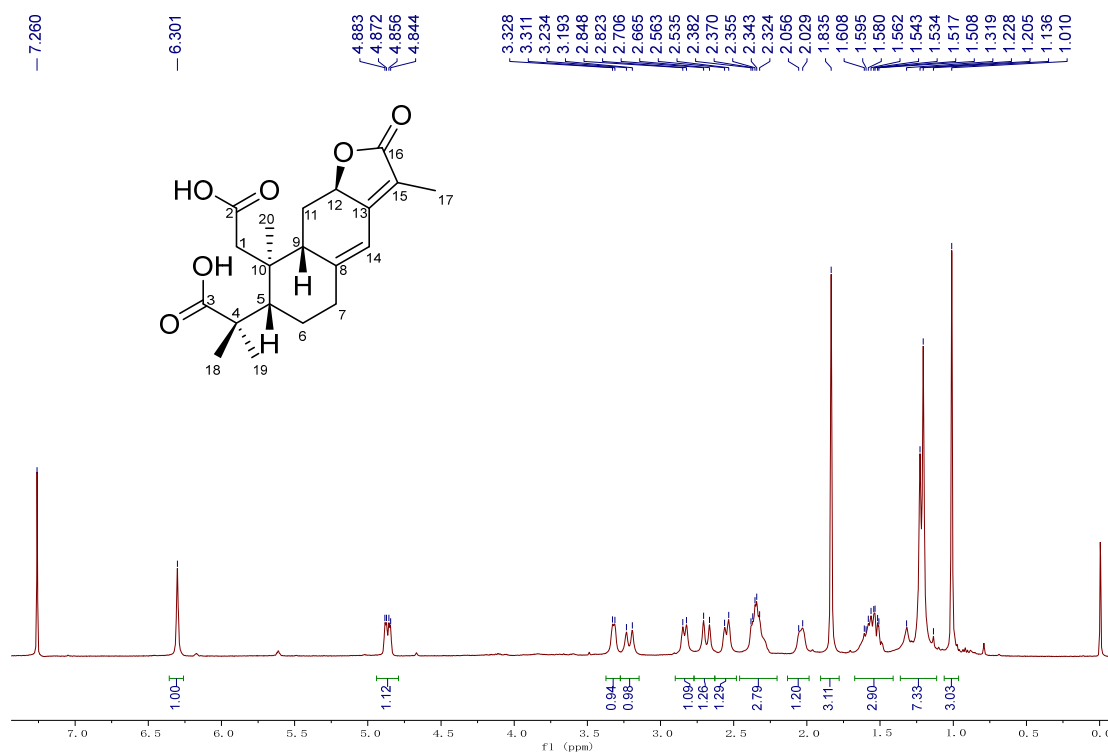
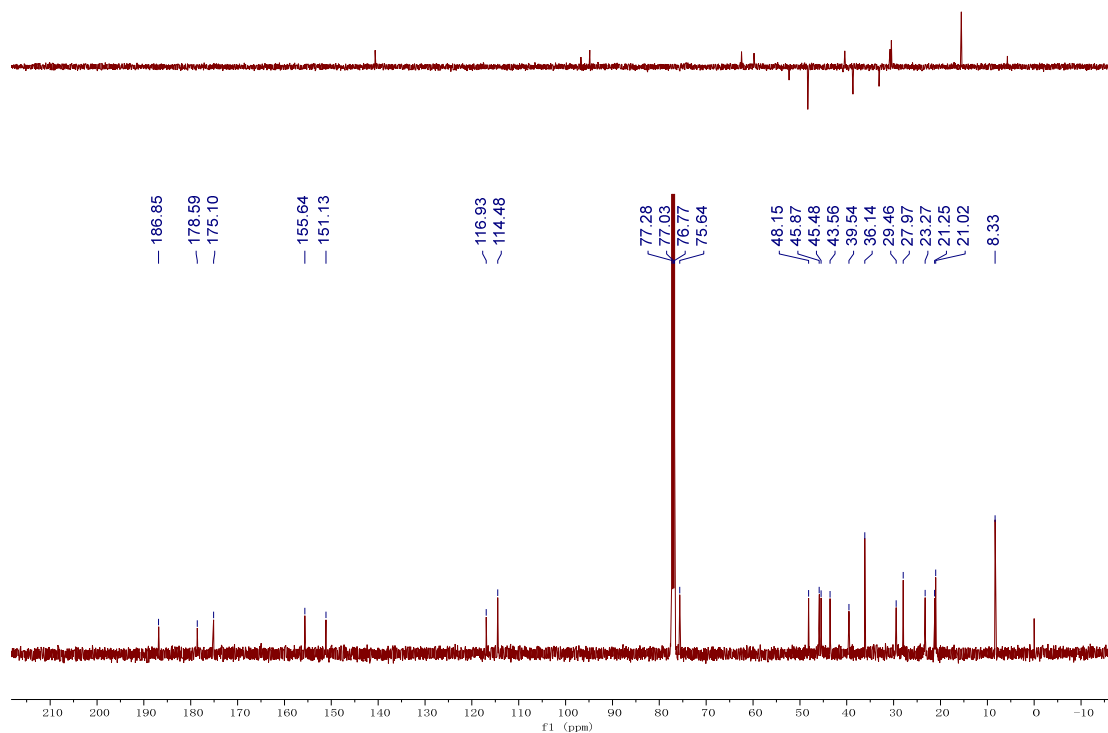
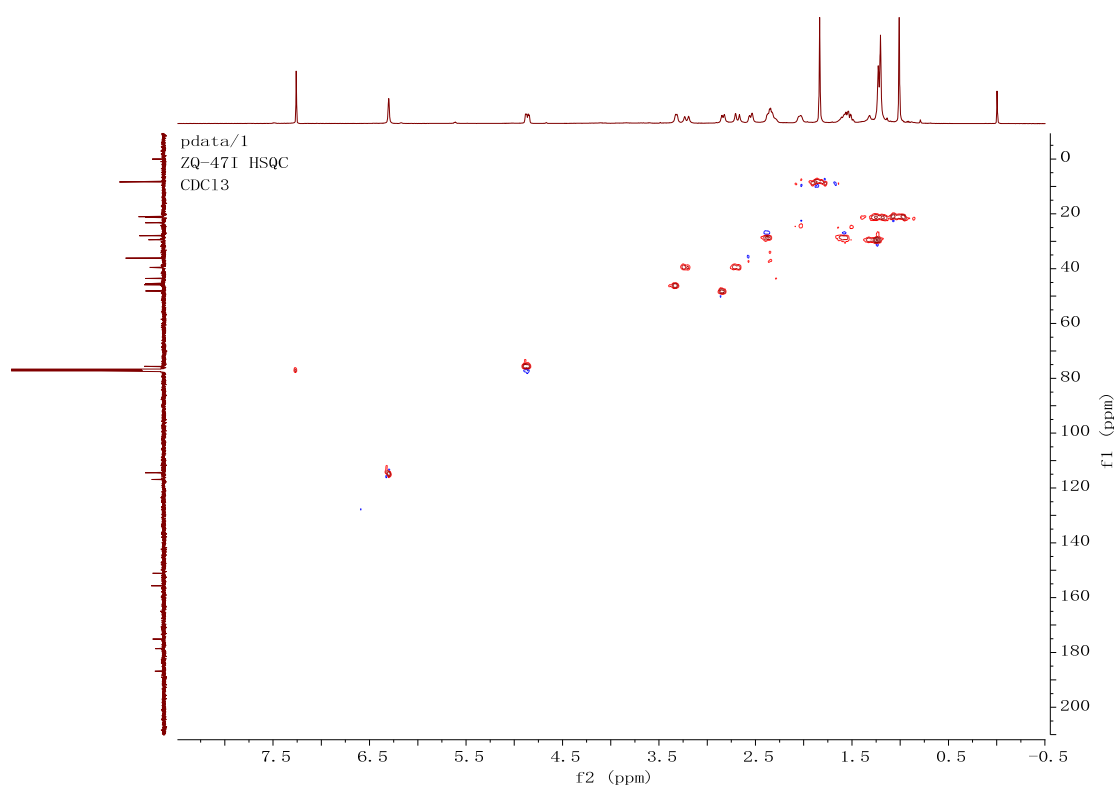


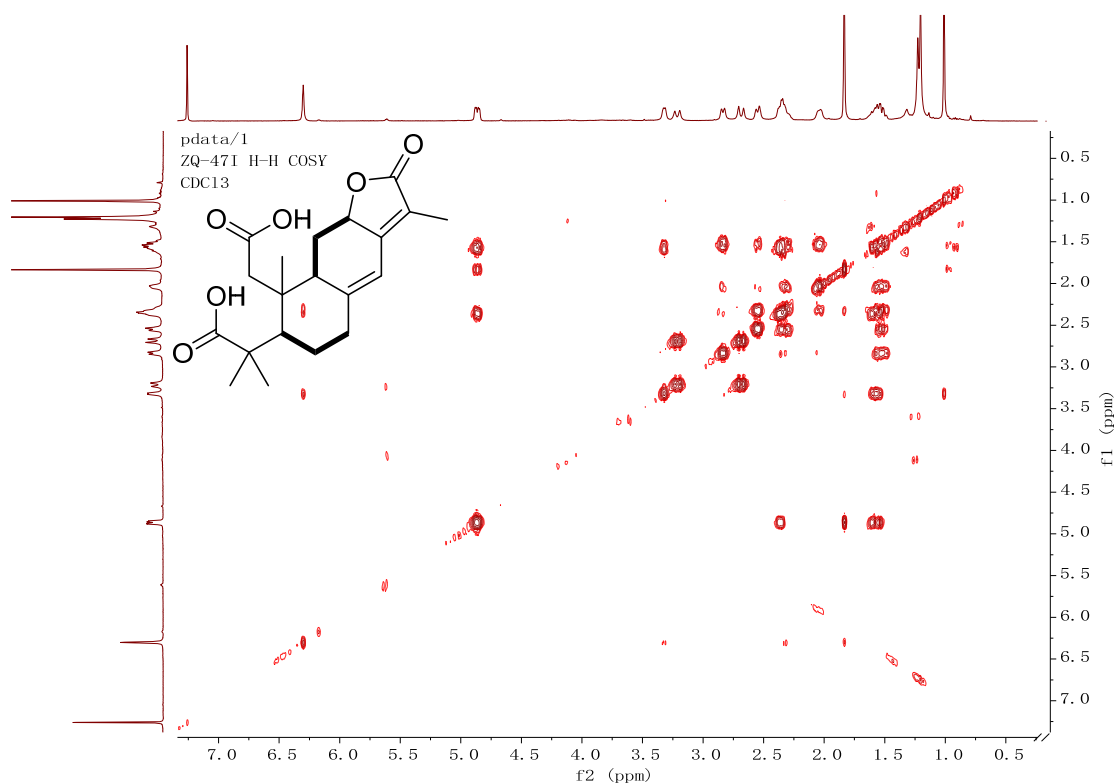
Figure S9  $^{13}\text{C}$  and DEPT 135 NMR spectra of 2 (125 MHz,  $\text{CDCl}_3$ )



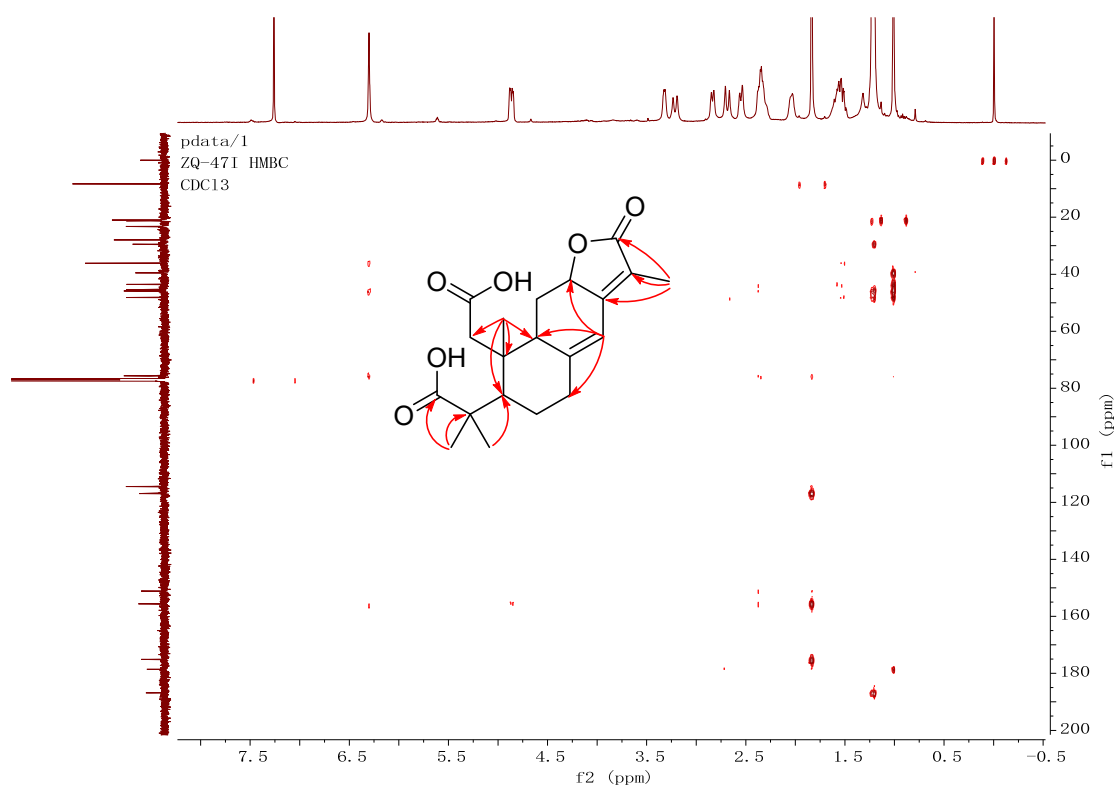
**Figure S10 HSQC spectrum of 2**



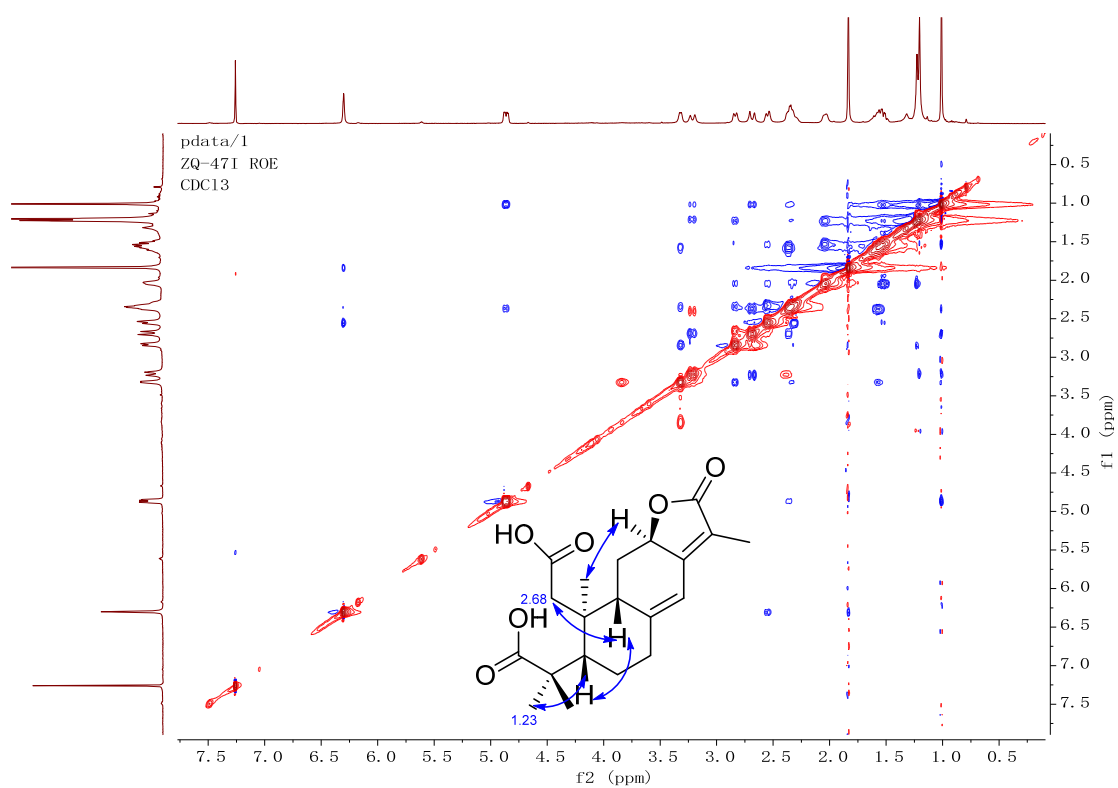
**Figure S11  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of 2**



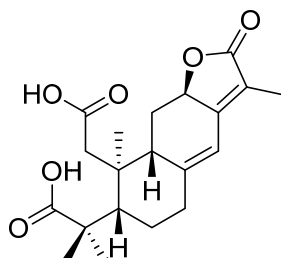
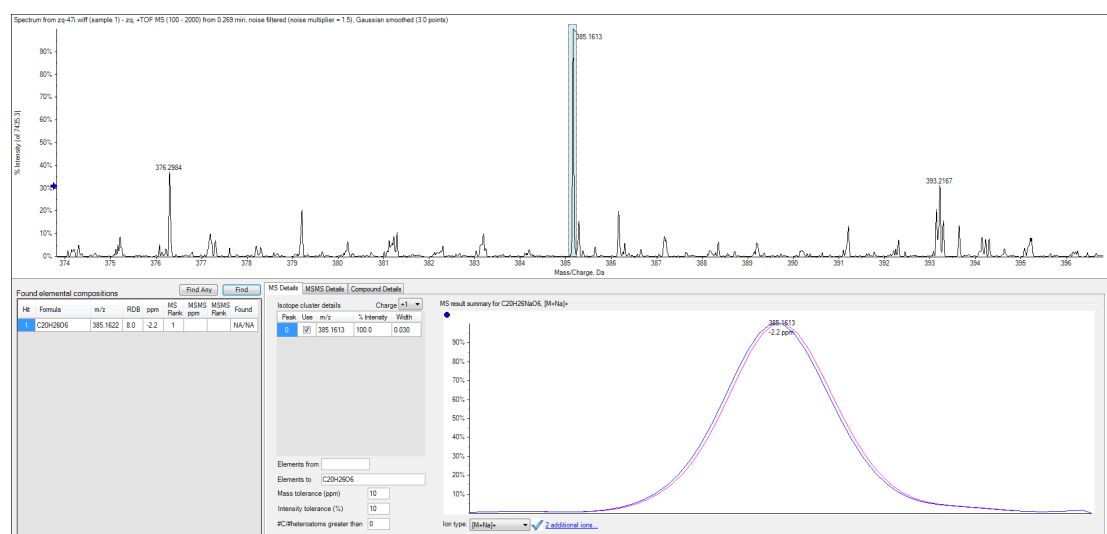
**Figure S12 HMBC spectrum of 2**



**Figure S13 ROESY spectrum of 2**

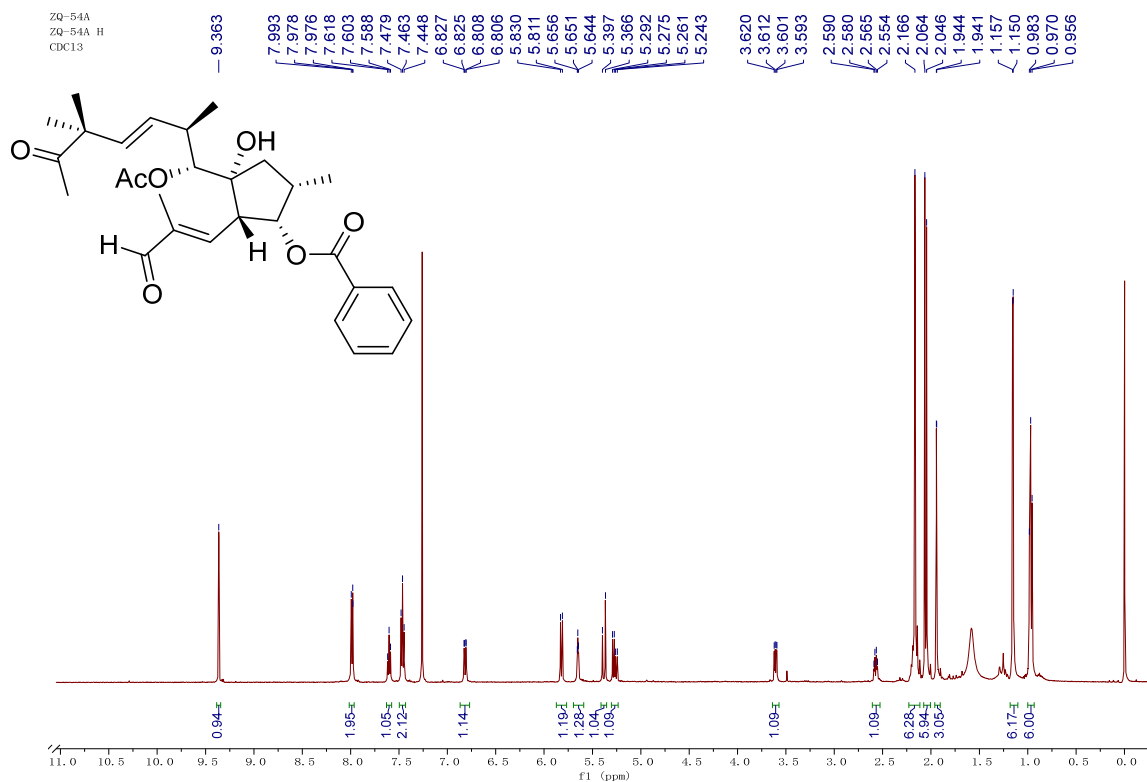


**Figure S14 HRESIMS report of 2**

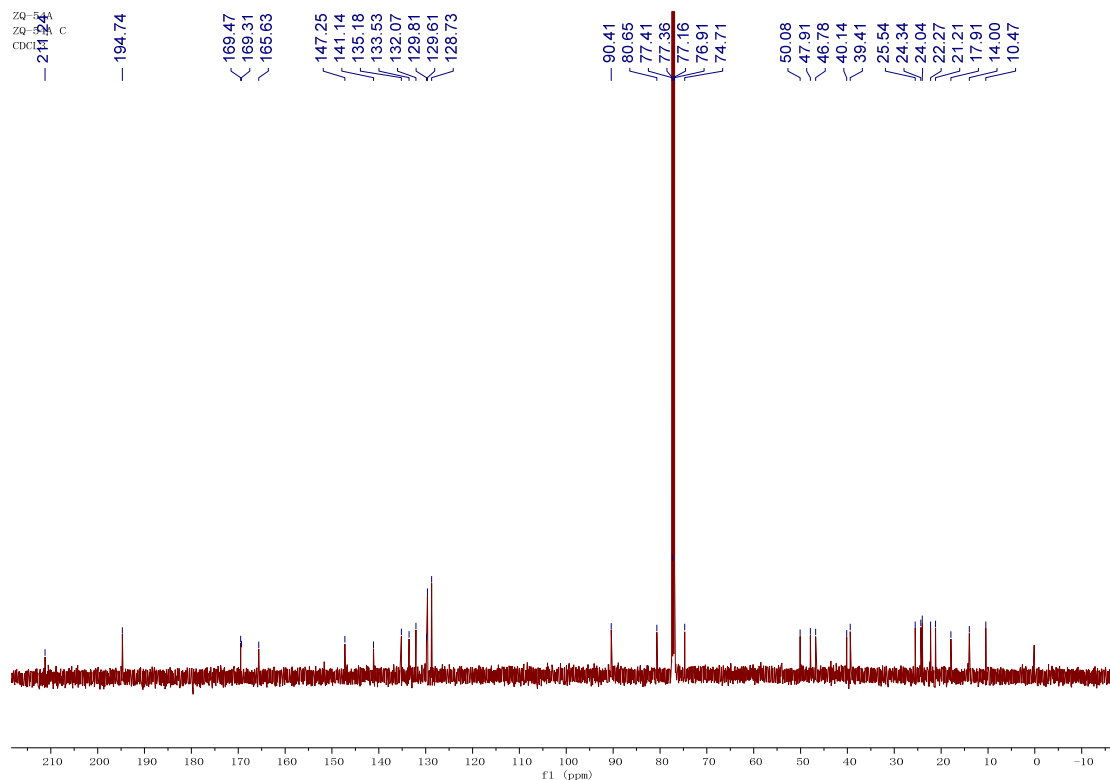


Chemical Formula: C<sub>20</sub>H<sub>26</sub>O<sub>6</sub>  
Exact Mass: 362.17

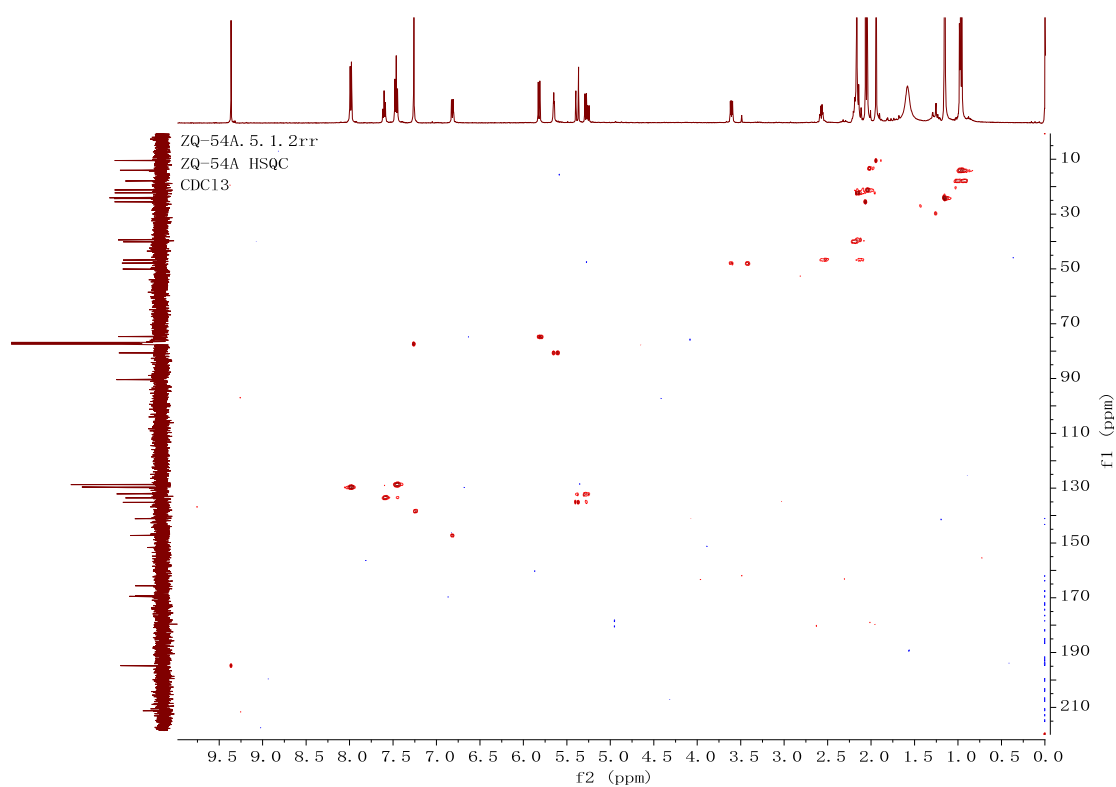
**Figure S15  $^1\text{H}$  NMR spectrum of 3 (500 MHz,  $\text{CDCl}_3$ )**



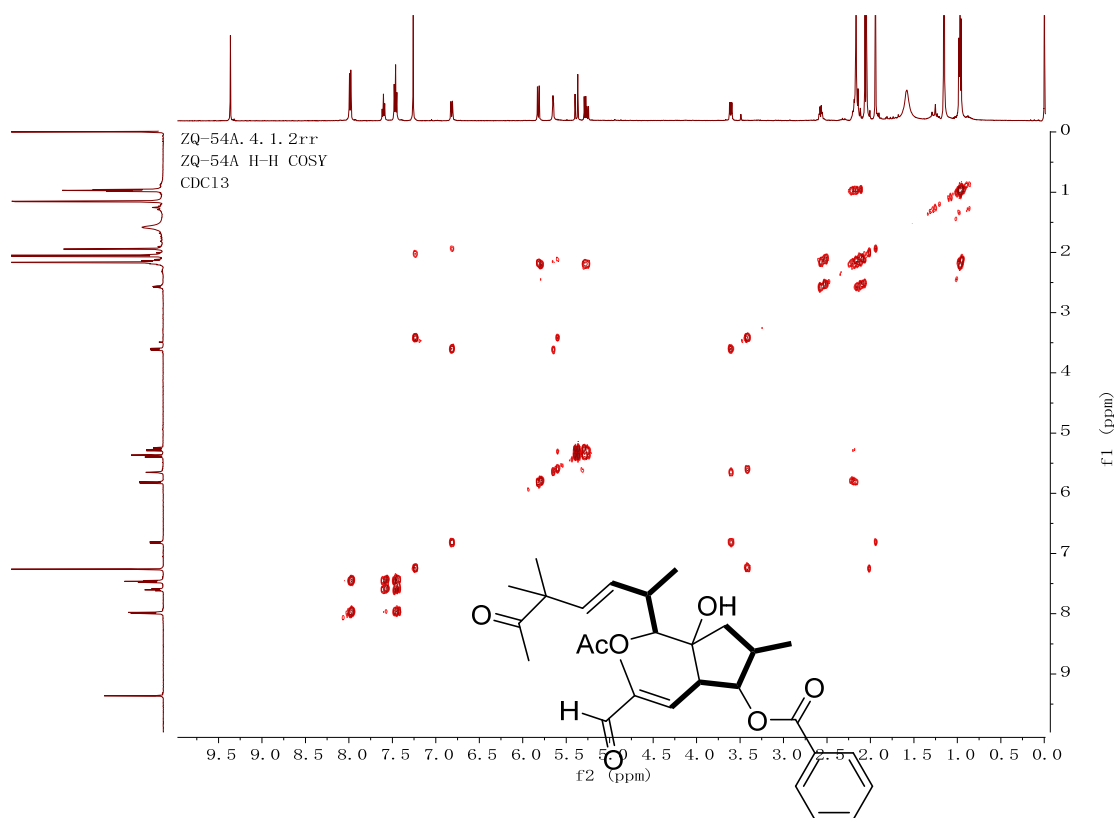
**Figure S16  $^{13}\text{C}$  NMR spectrum of 3 (125 MHz,  $\text{CDCl}_3$ )**



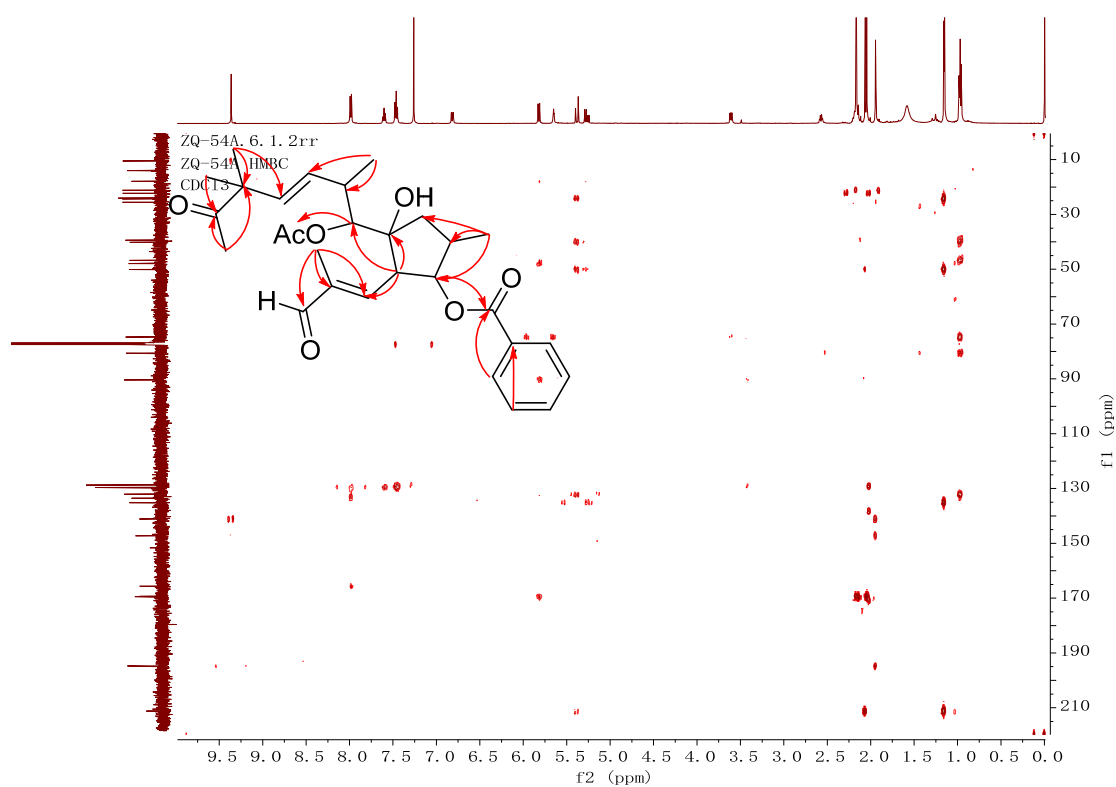
**Figure S17 HSQC spectrum of 3**



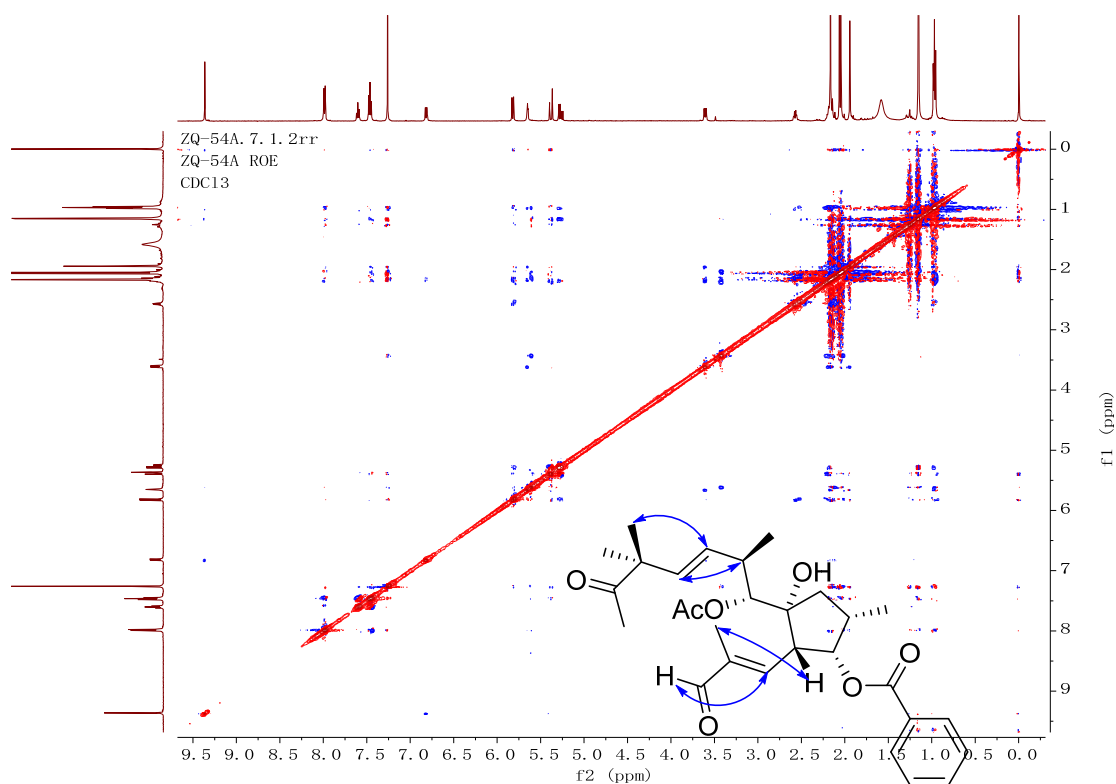
**Figure S18  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of 3**



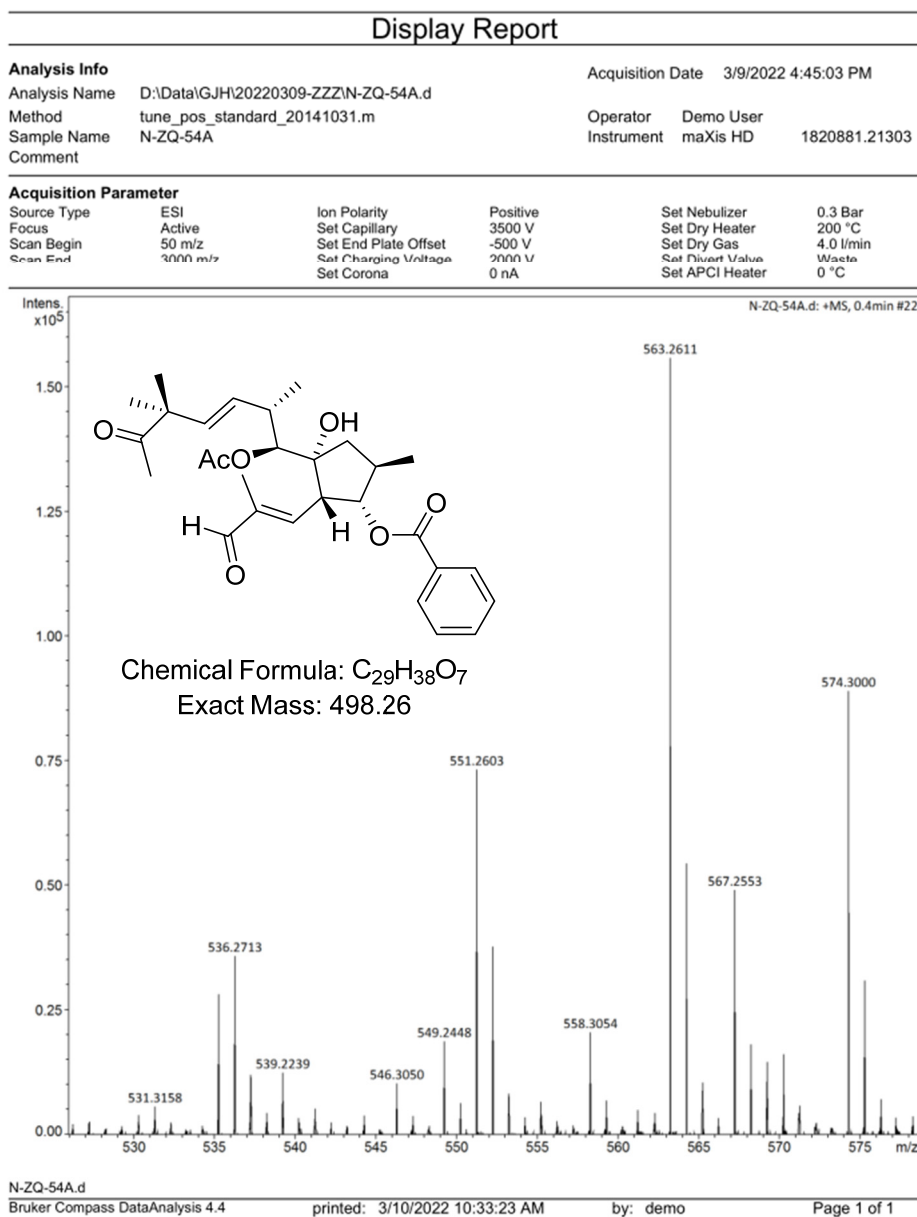
**Figure S19 HMBC spectrum of 3**



**Figure S20 ROESY spectrum of 3**

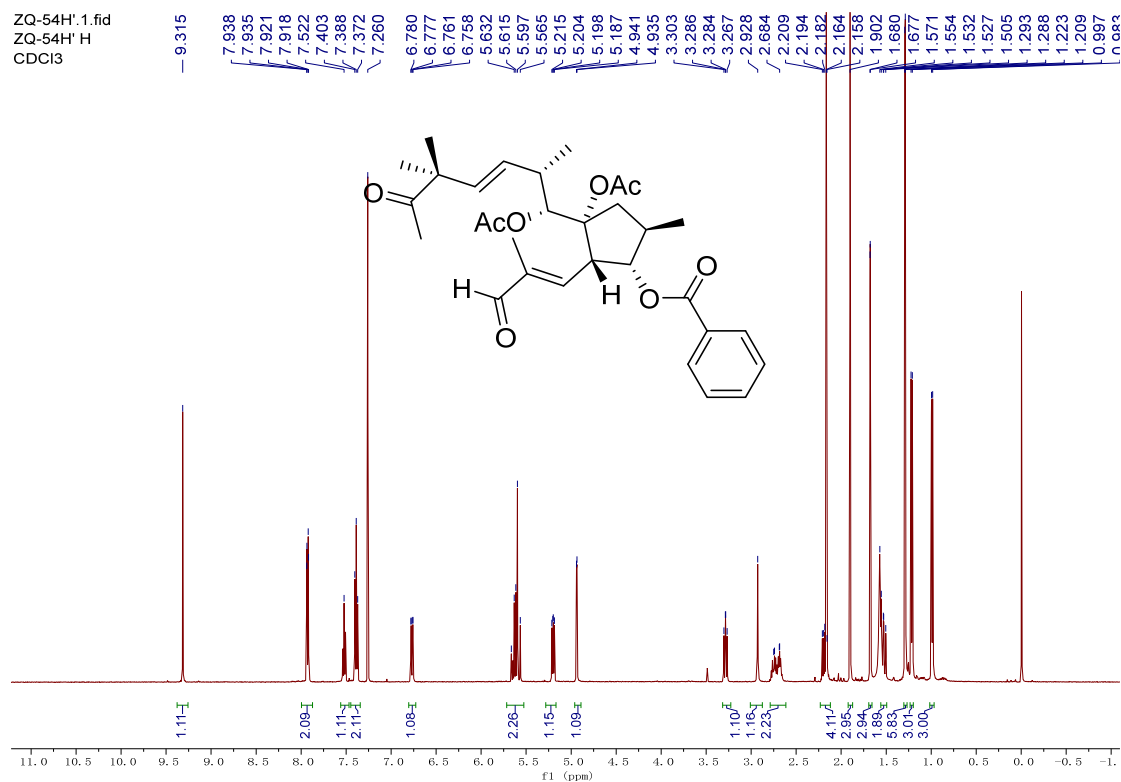


**Figure S21 HRESIMS report of 3**

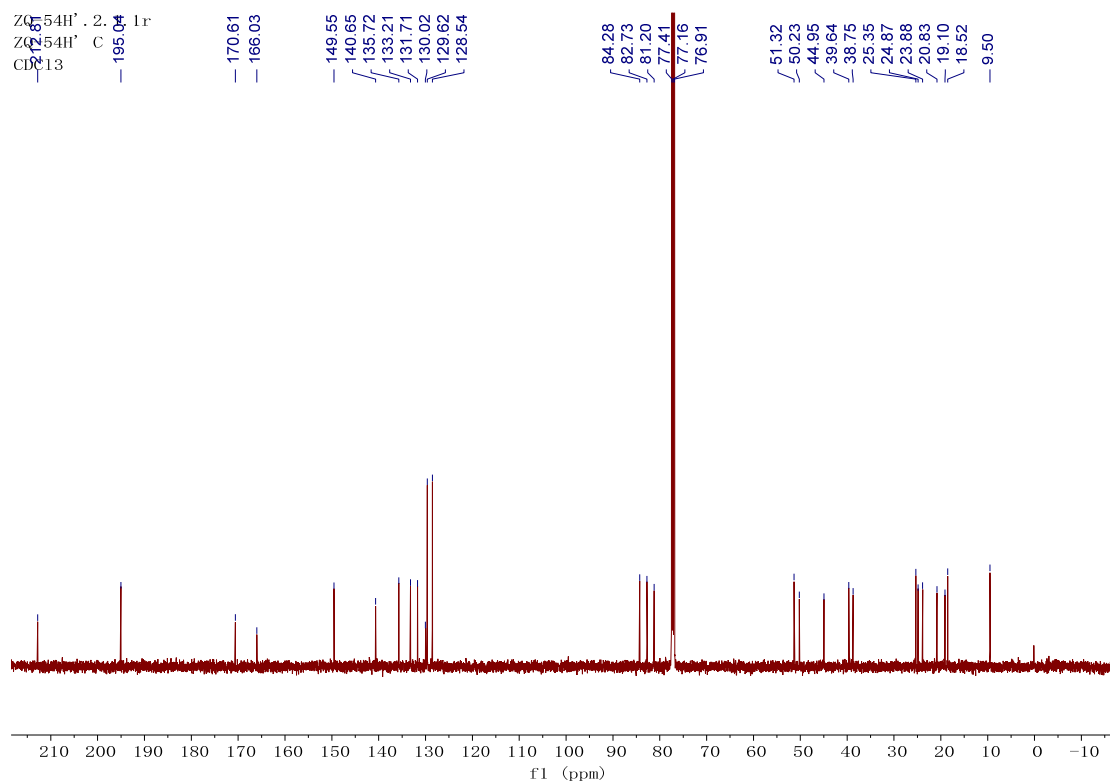




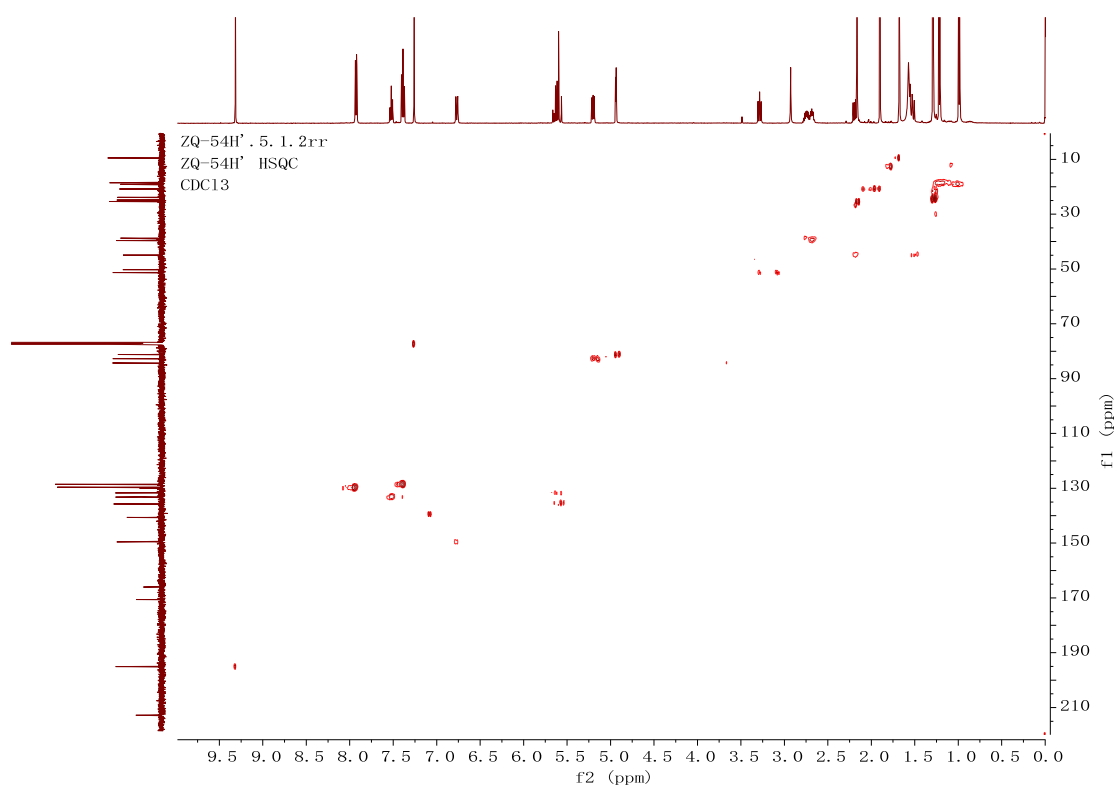
**Figure S22  $^1\text{H}$  NMR spectrum of 4 (500 MHz,  $\text{CDCl}_3$ )**



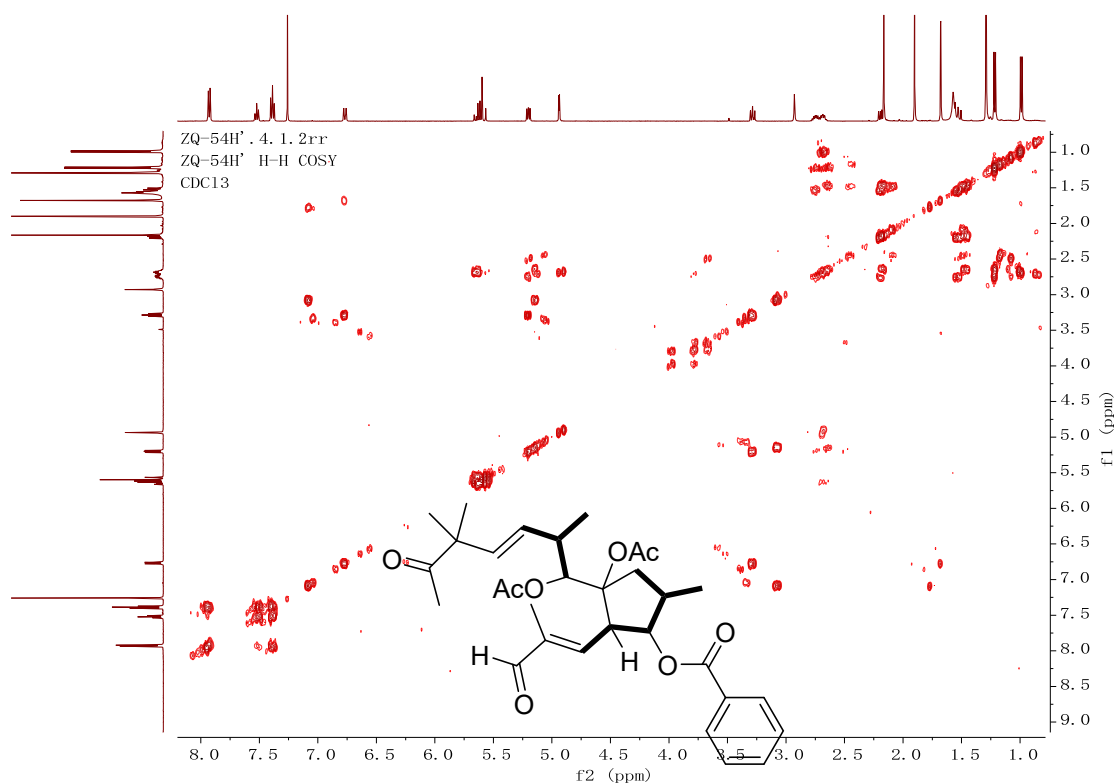
**Figure S23  $^{13}\text{C}$  NMR spectra of 4 (125 MHz,  $\text{CDCl}_3$ )**



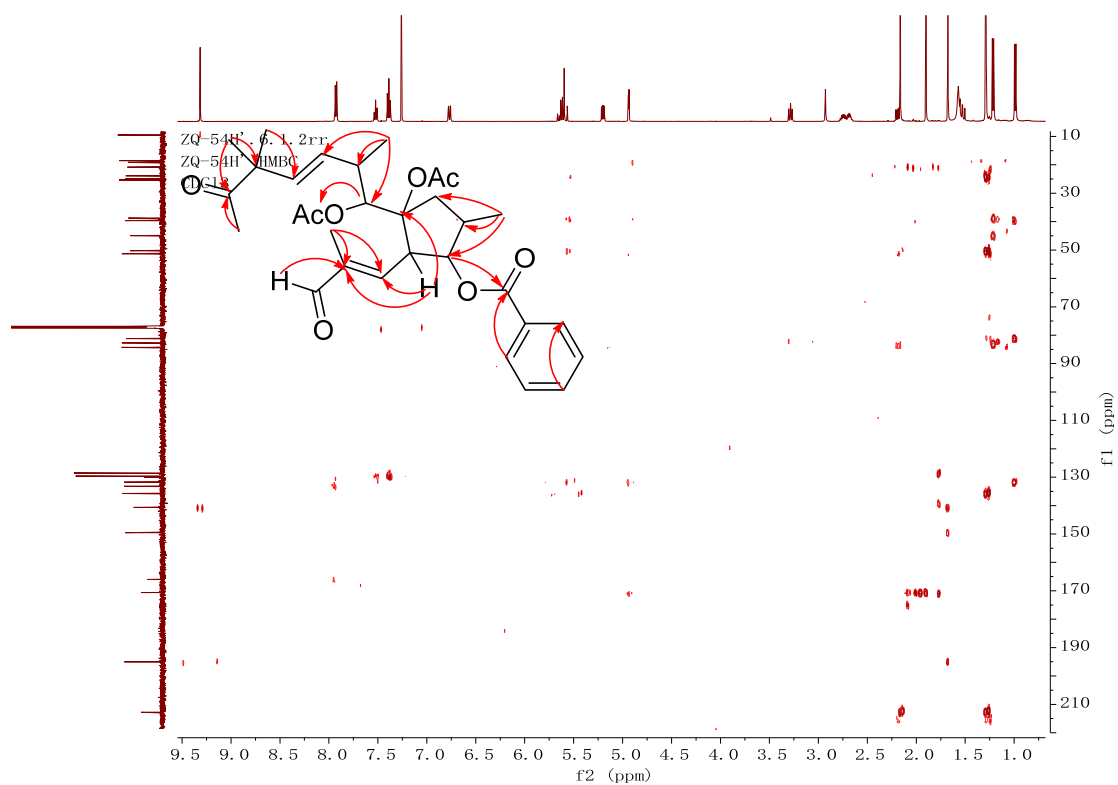
**Figure S24. HSQC spectrum of 4**



**Figure S25  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of 4**



**Figure S26 HMBC spectrum of 4**



**Figure S27 ROESY spectrum of 4**

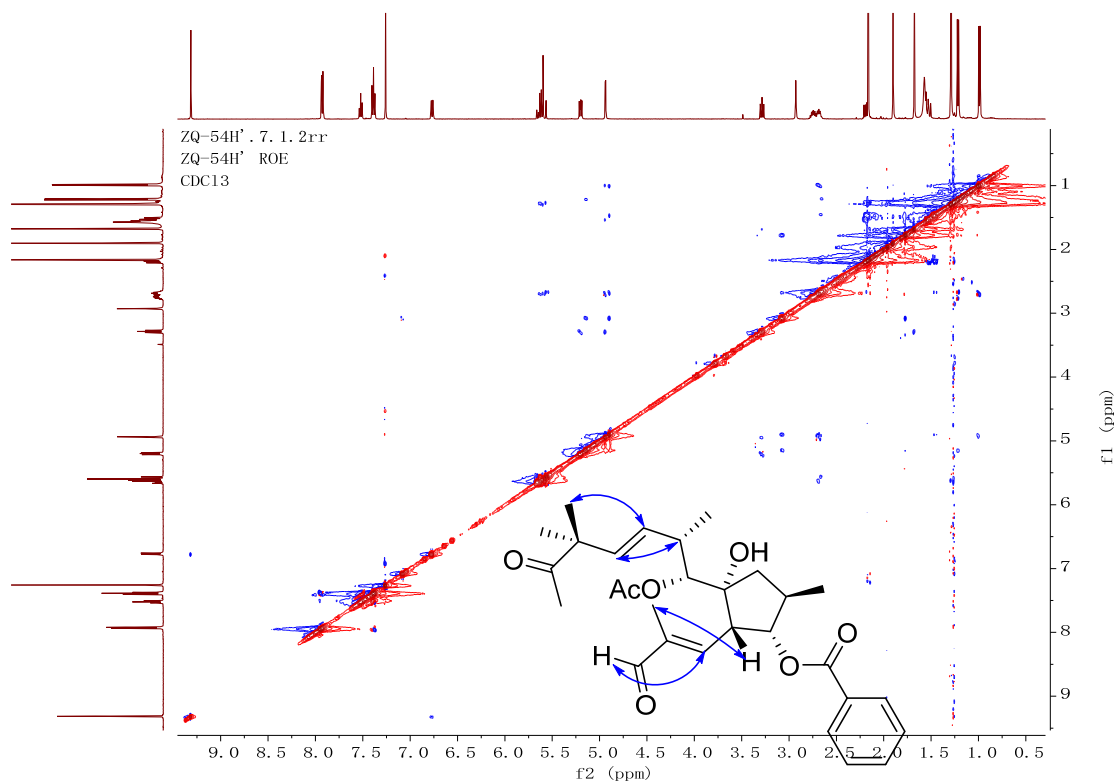
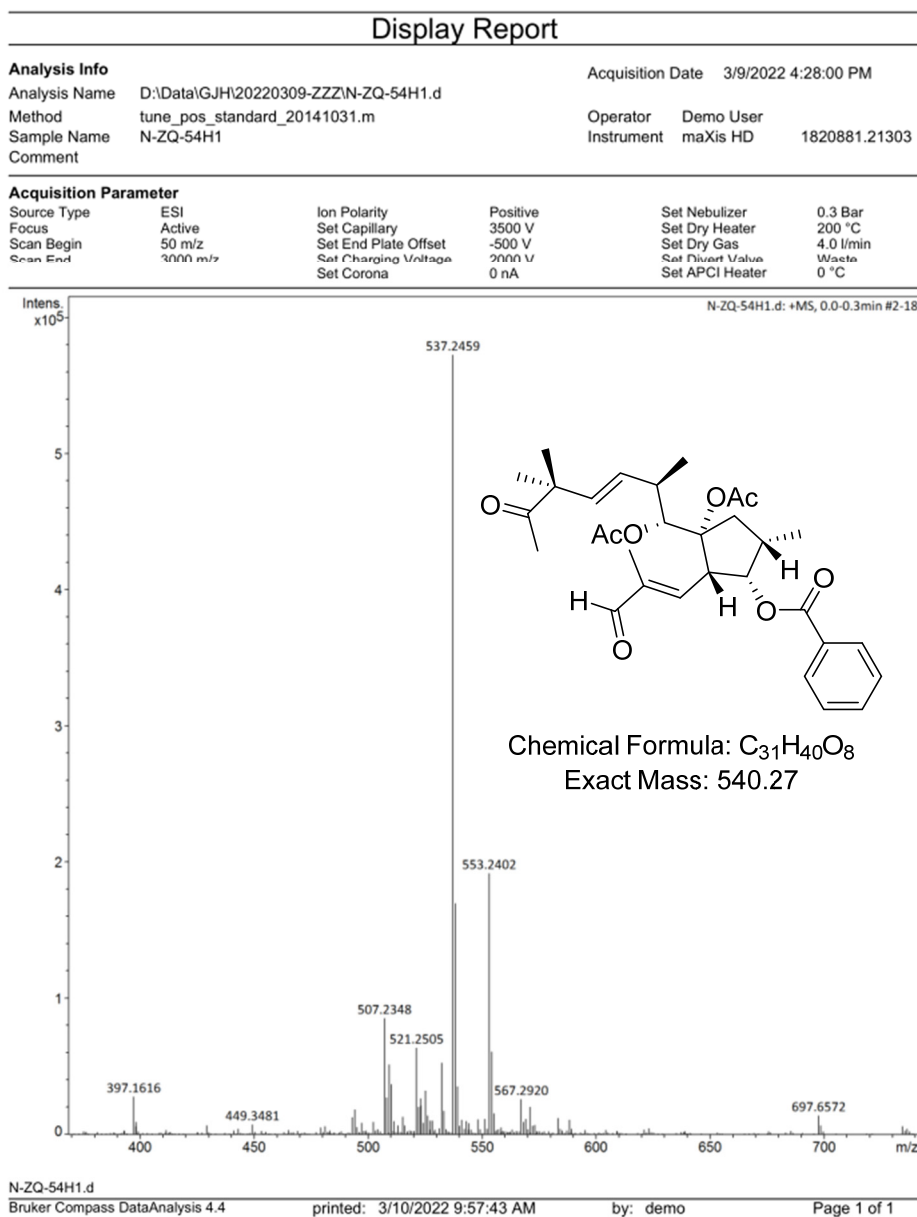


Figure S28 HRESIMS report of 4



<sup>1</sup>H NMR spectrum (400 MHz, CDCl<sub>3</sub>) of compound 1. The chemical structure of compound 1 is shown above the spectrum. The spectrum displays peaks from 0.913 to 7.958 ppm. Integration values are provided below the baseline.

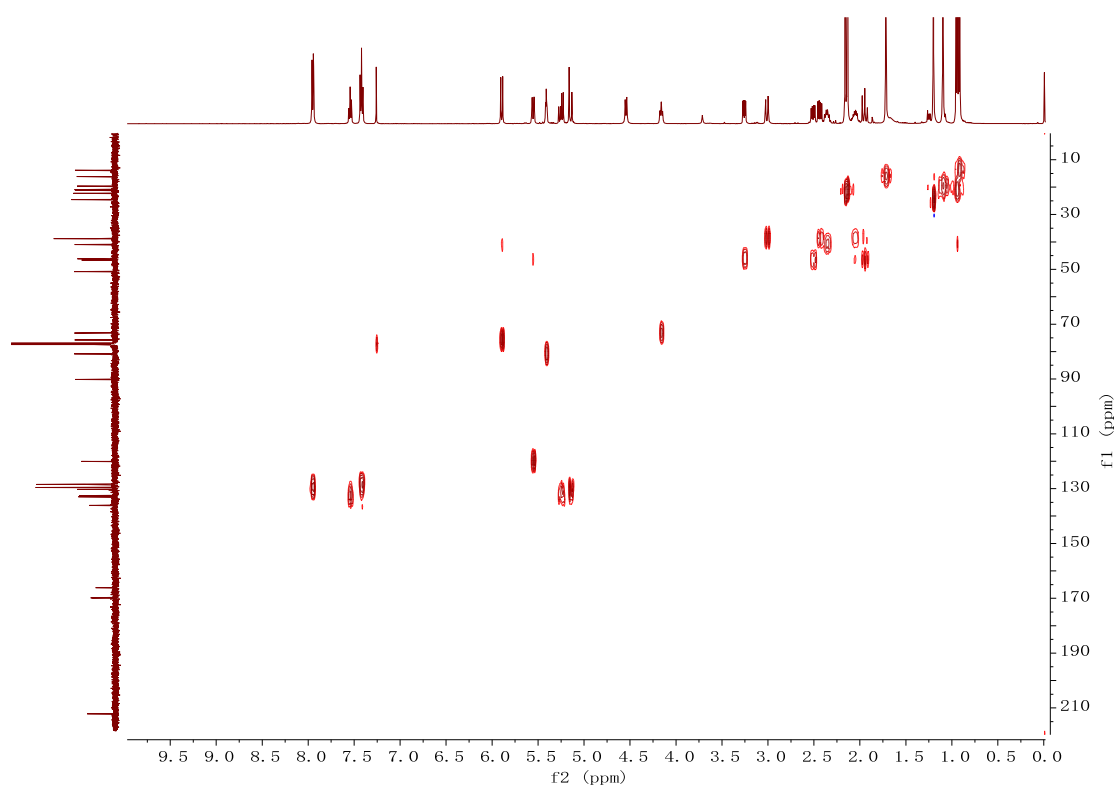
Chemical Shift (ppm)	Integration
7.958	1.99
7.944	1.00
7.941	2.03
7.559	0.97
7.544	0.96
7.529	0.96
7.435	0.96
7.419	1.02
7.404	0.95
5.906	0.98
5.885	0.97
5.565	0.96
5.544	0.95
5.411	0.96
5.403	0.96
5.275	0.96
5.257	0.96
5.244	0.93
5.226	0.95
5.162	0.95
5.132	0.96
4.553	0.96
4.535	0.96
4.160	0.97
3.272	0.99
3.263	0.96
3.251	0.95
3.242	0.95
3.028	0.93
3.024	1.06
2.999	1.02
2.995	0.96
2.530	0.95
2.518	5.93
2.502	1.06
2.480	1.02
2.457	0.96
2.444	3.04
2.428	0.96
2.415	1.02
2.352	0.96
2.159	3.17
2.136	6.00
1.975	
1.947	
1.920	
1.719	
1.717	
1.201	
1.097	
0.954	
0.941	
0.927	
0.913	

212.69  
ZQ-<sup>13</sup>C  
CDCl<sub>3</sub>

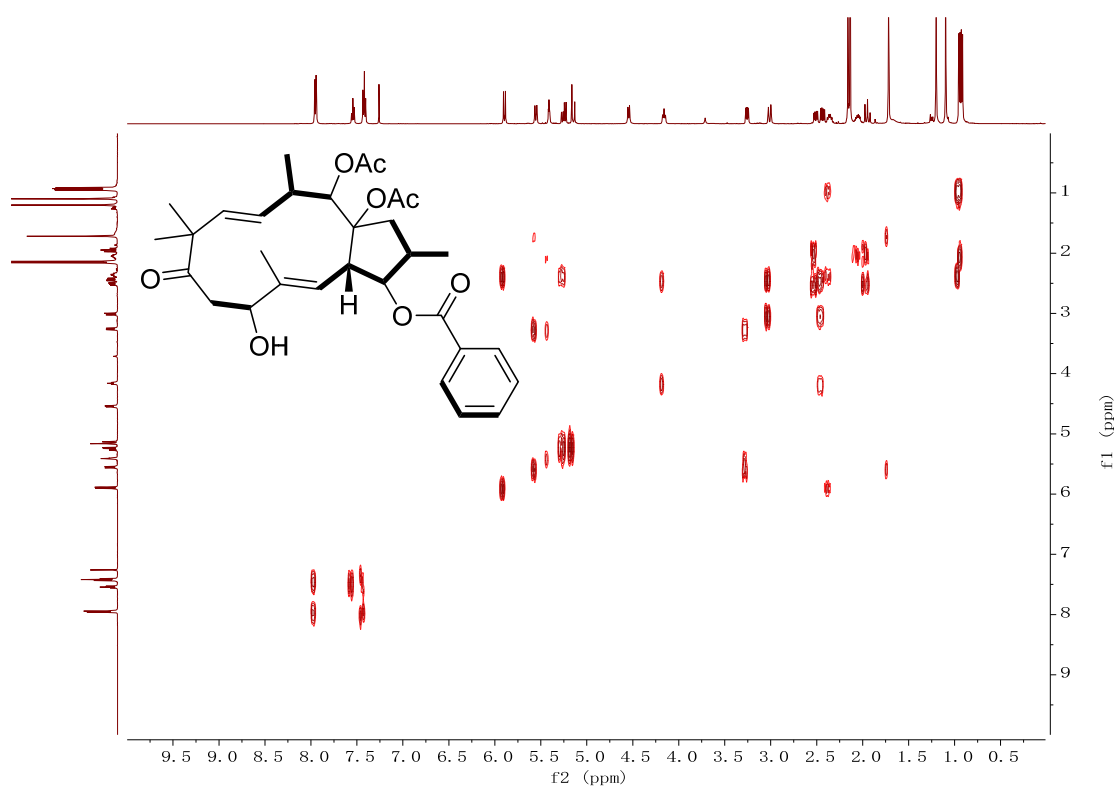
169.98  
169.82  
166.14  
136.11  
133.00  
132.57  
130.47  
130.25  
129.56  
128.49  
120.09  
90.17  
80.86  
77.41  
77.16  
76.91  
75.76  
73.21  
50.84  
46.70  
46.17  
40.94  
38.81  
24.55  
22.26  
21.09  
20.92  
19.64  
16.16  
13.88

f1 (ppm)

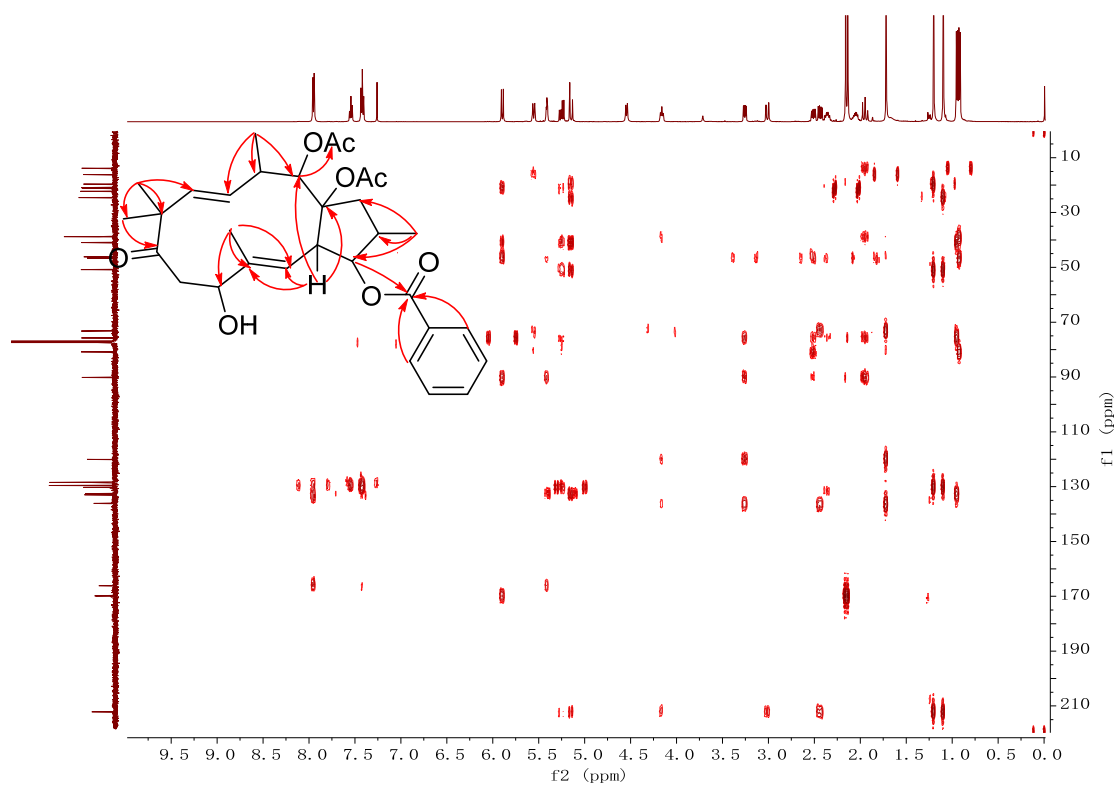
**Figure S31 HSQC spectrum of 5**



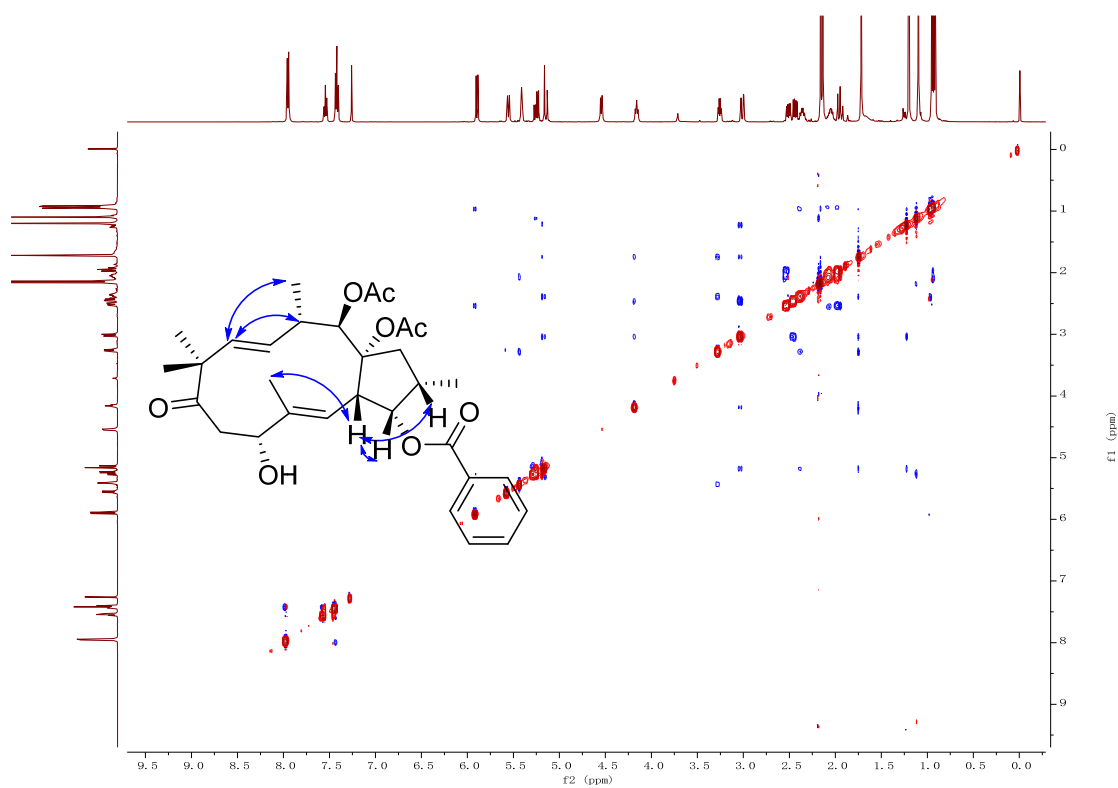
**Figure S32  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of 5**



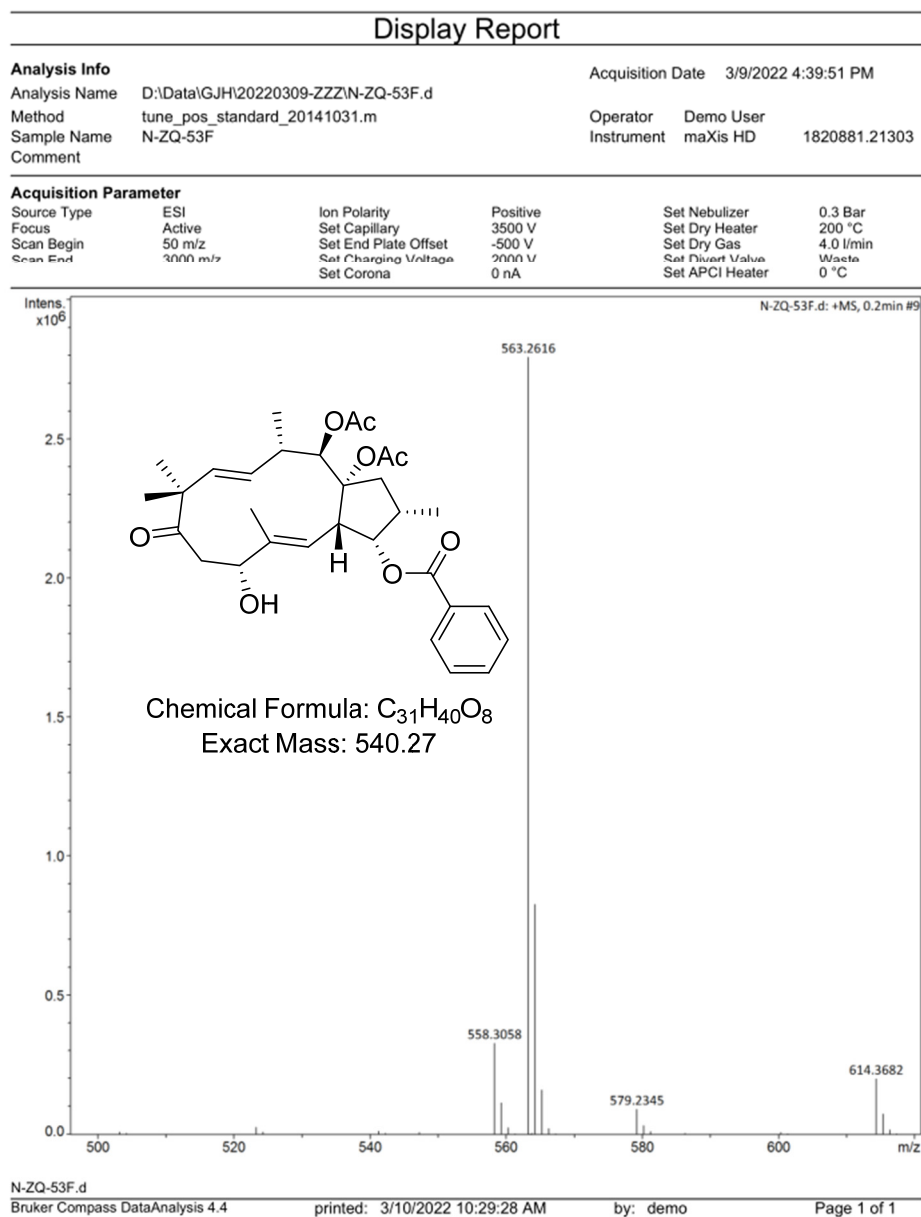
**Figure S33 HMBC spectrum of 5**



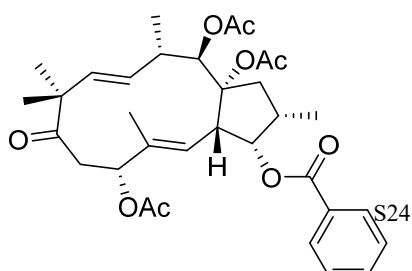
**Figure S34 ROESY spectrum of 5**



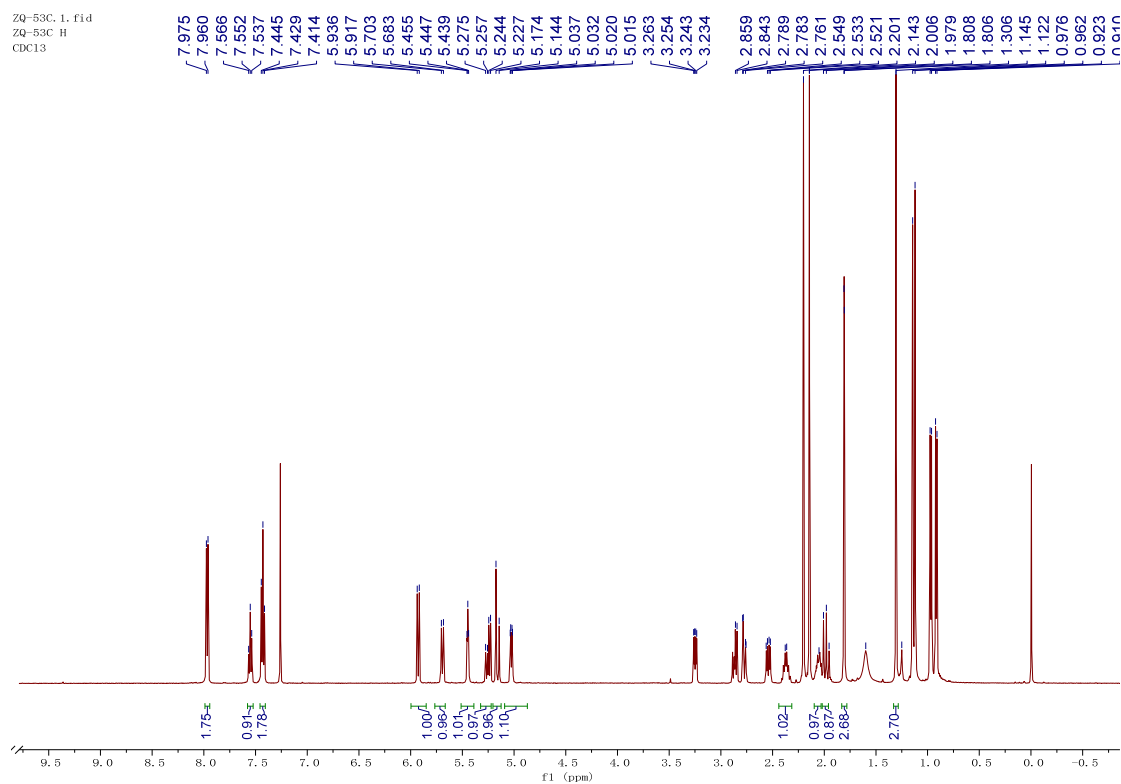
**Figure S35 HRESIMS report of 5**



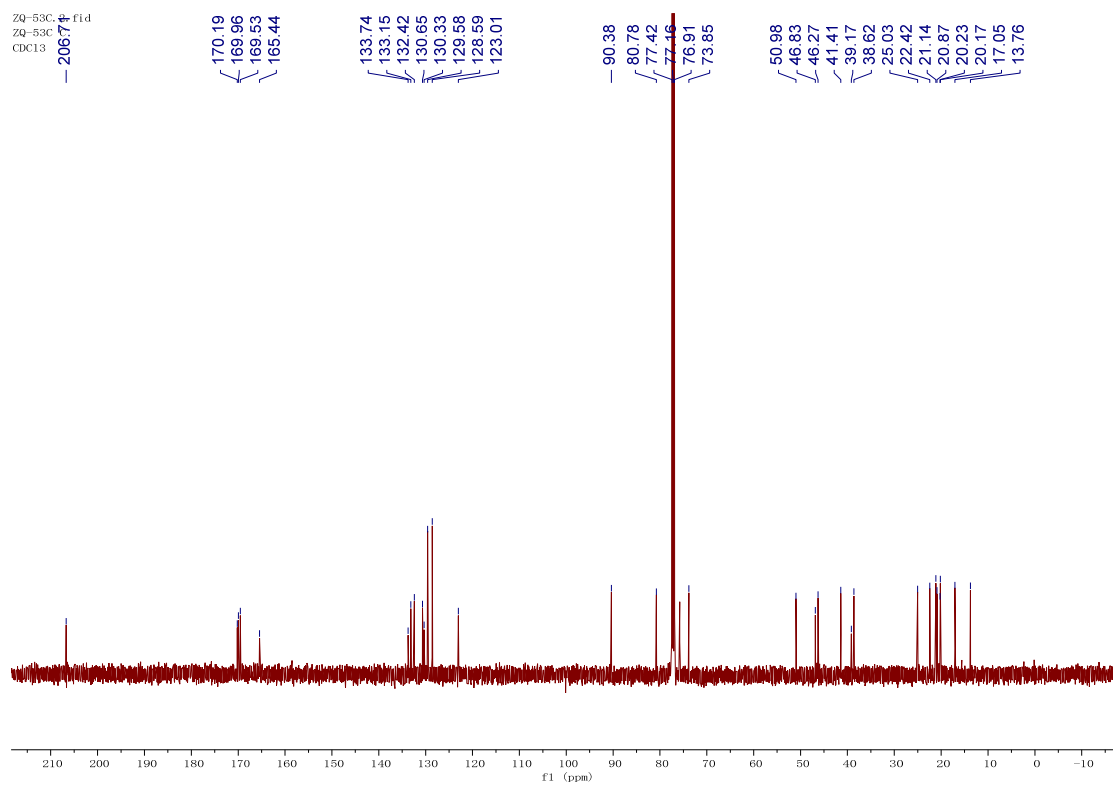
**Figure S36  $^1H$  NMR spectrum of 6 (500 MHz,  $CDCl_3$ )**



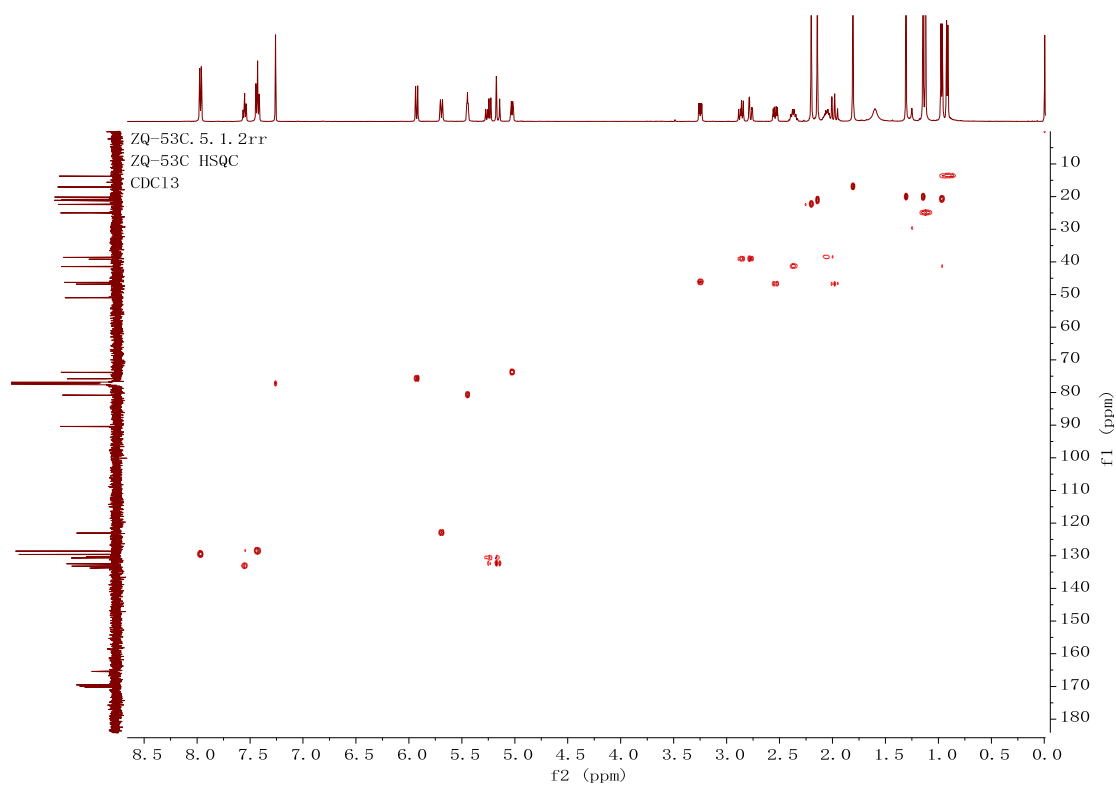




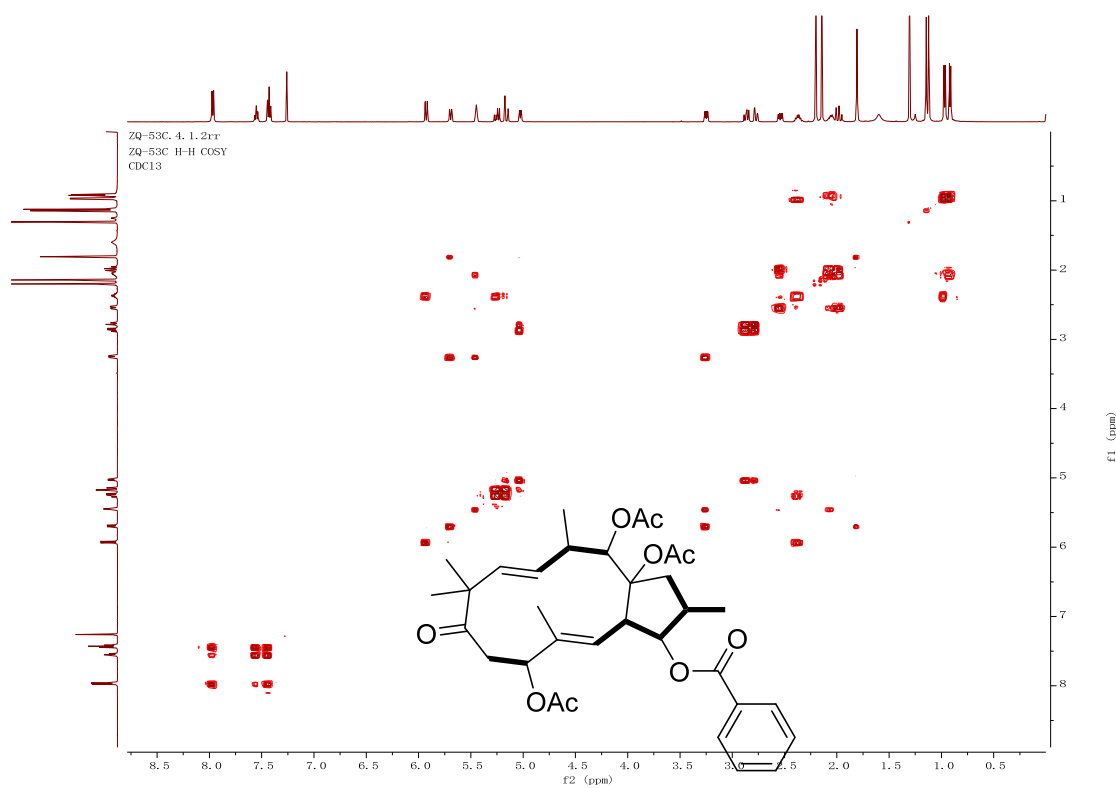
**Figure S37 <sup>13</sup>C NMR spectrum of 6 (125 MHz, CDCl<sub>3</sub>)**



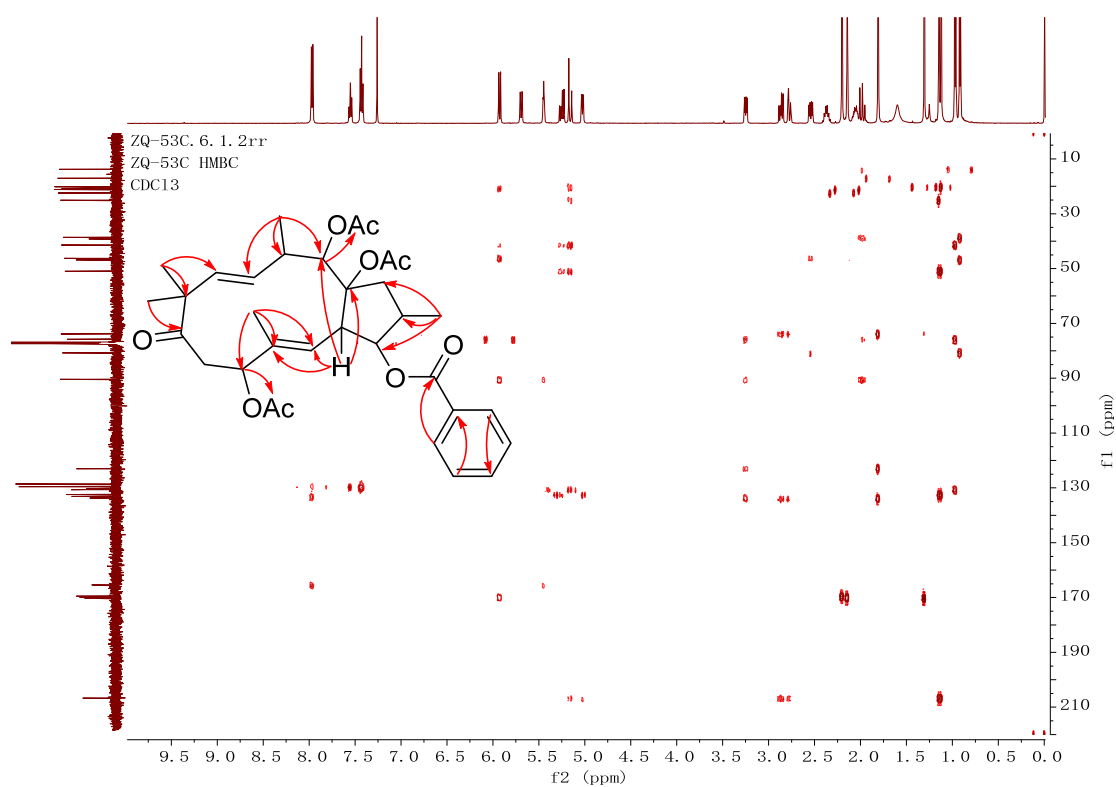
**Figure S38 HSQC spectrum of 6**



**Figure S39  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of 6**



**Figure S40 HMBC spectrum of 6**



**Figure S41 ROESY spectrum of 6**

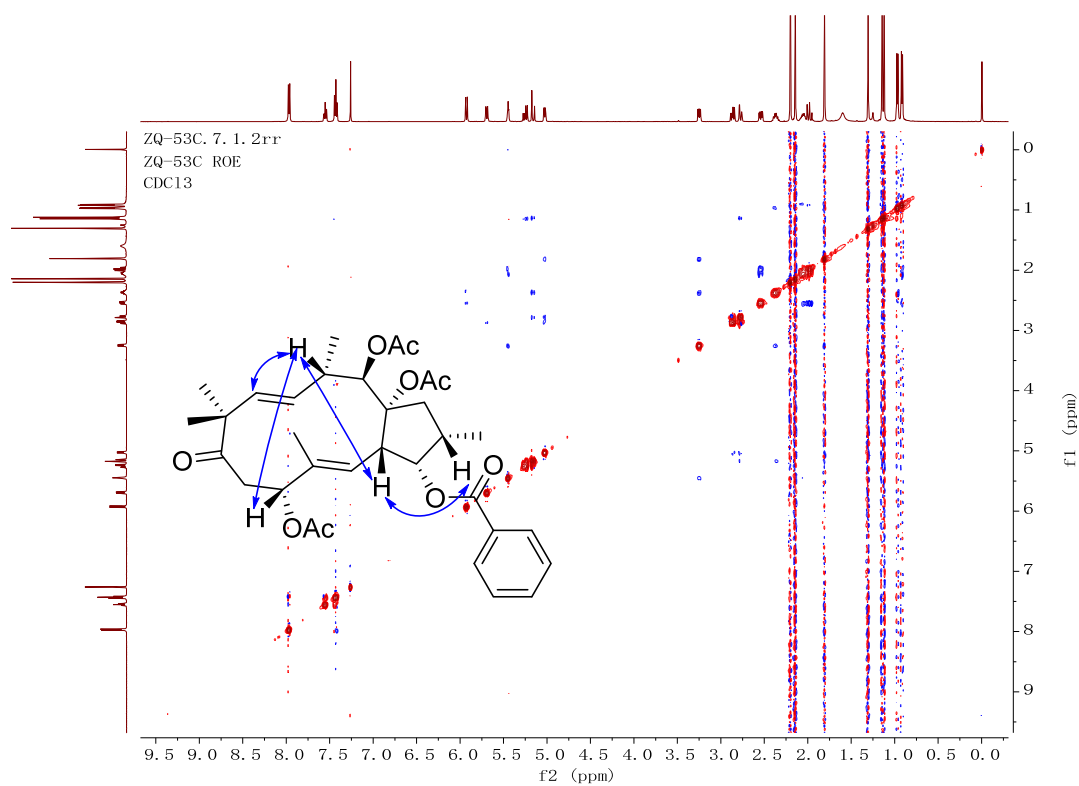


Figure S42 HRESIMS report of 6

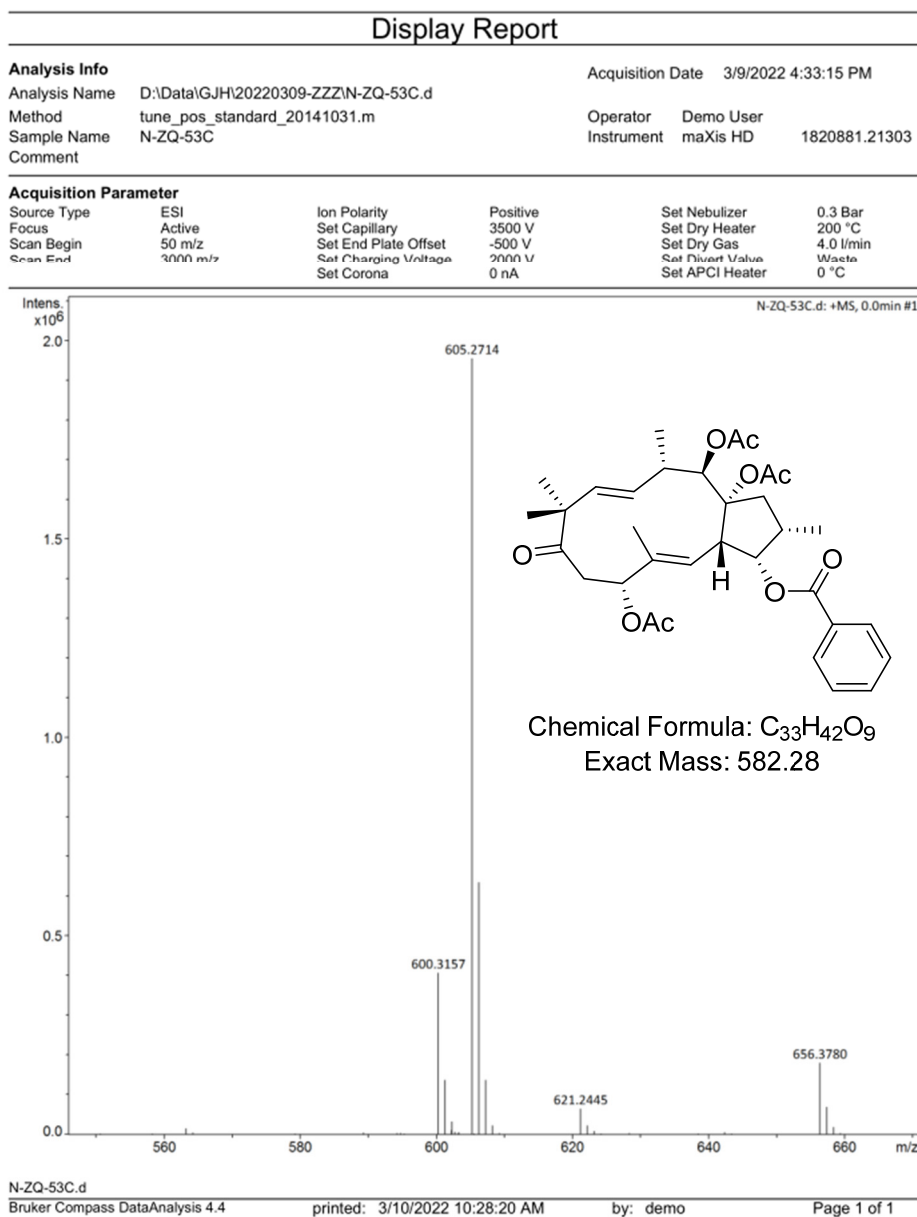


Figure S43  $^1\text{H}$  NMR spectrum of **7** (500 MHz,  $\text{CDCl}_3$ )

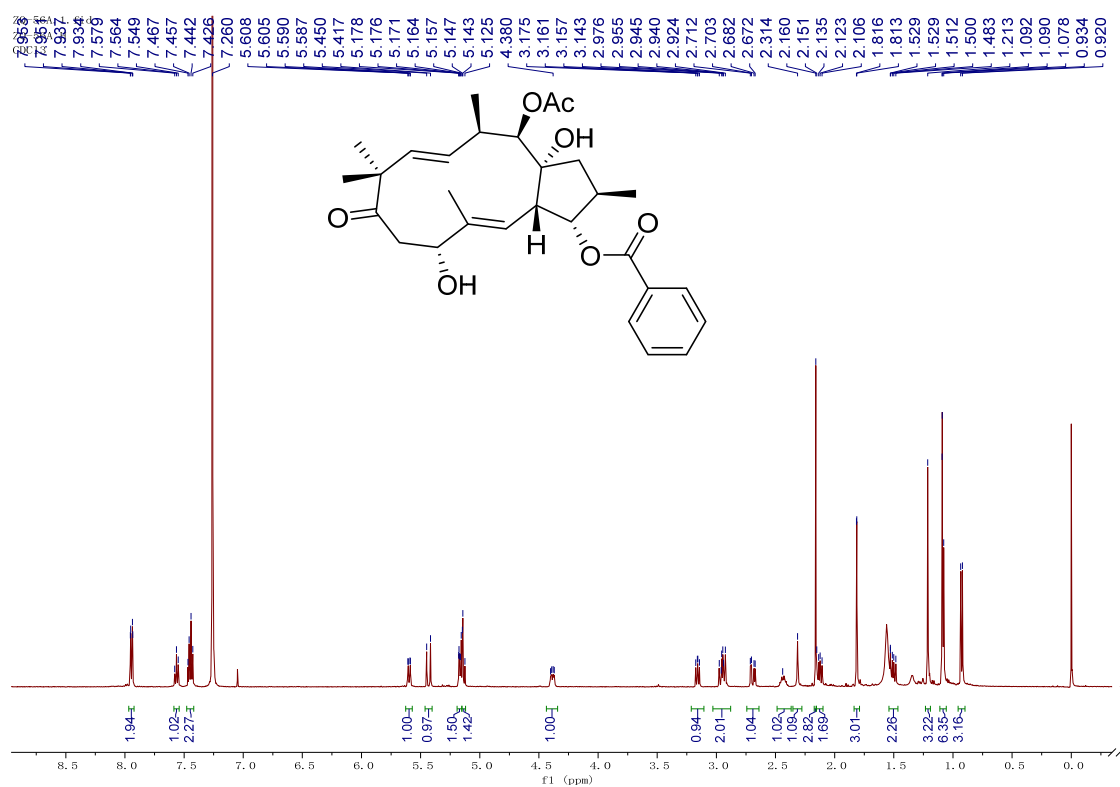
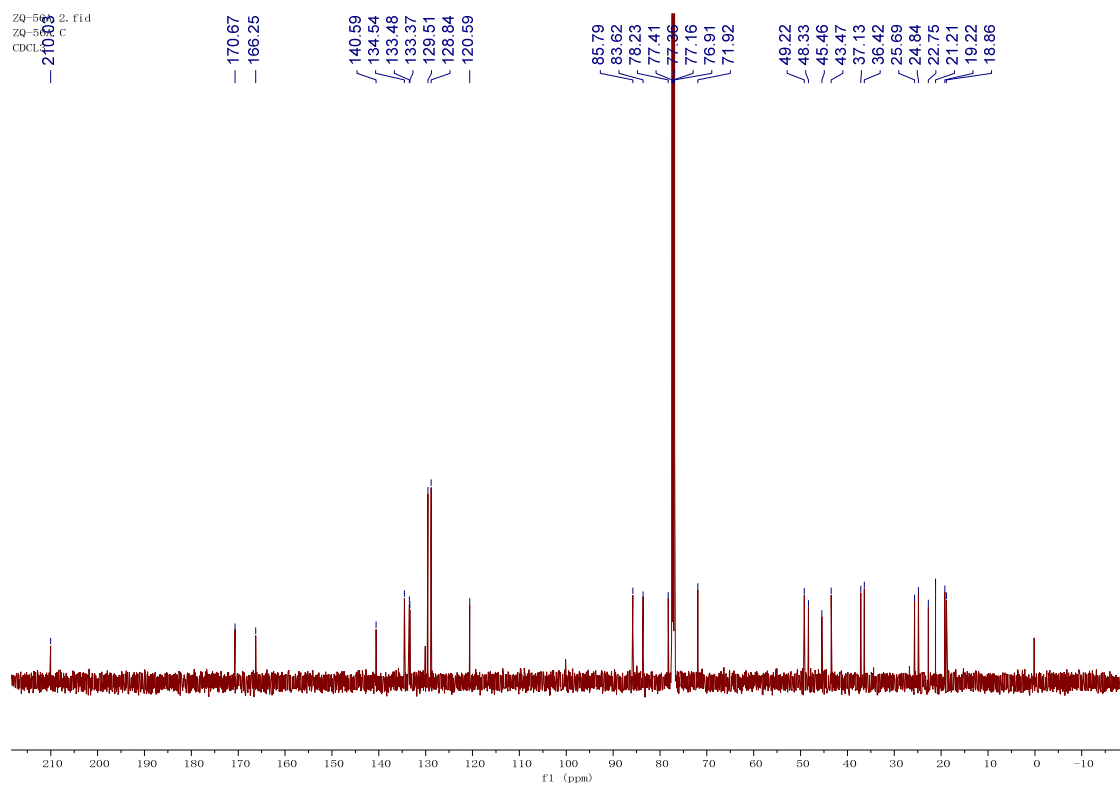
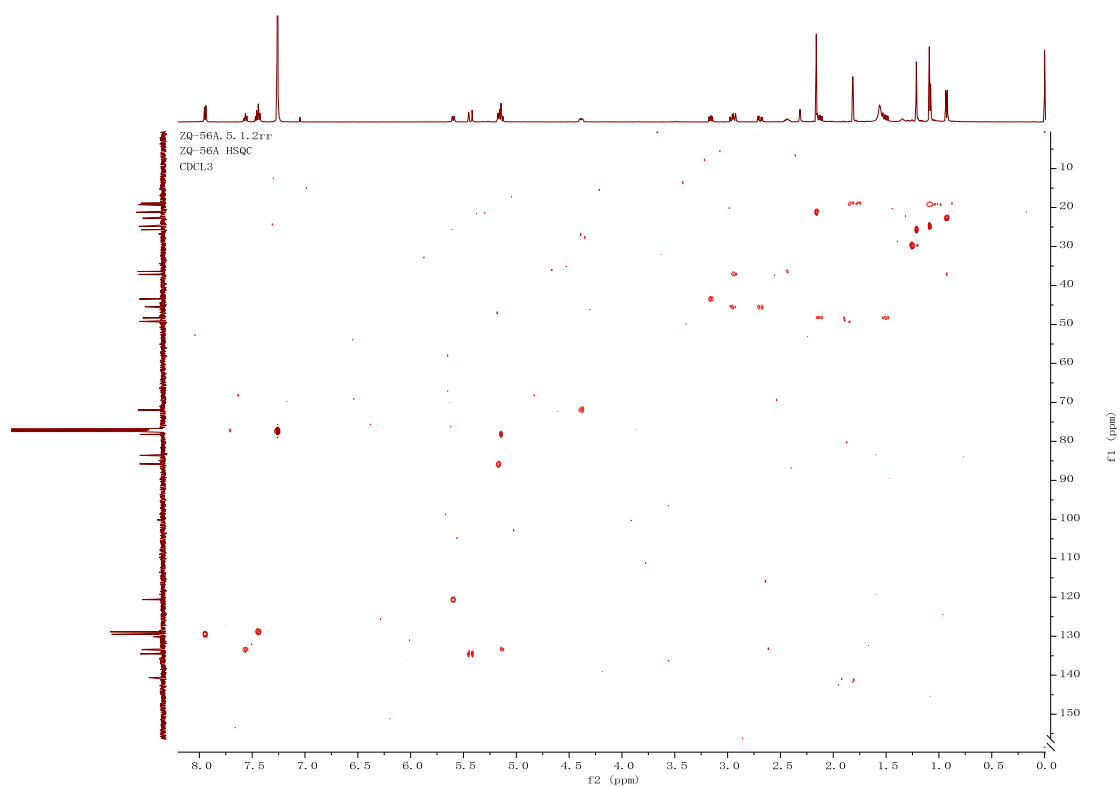


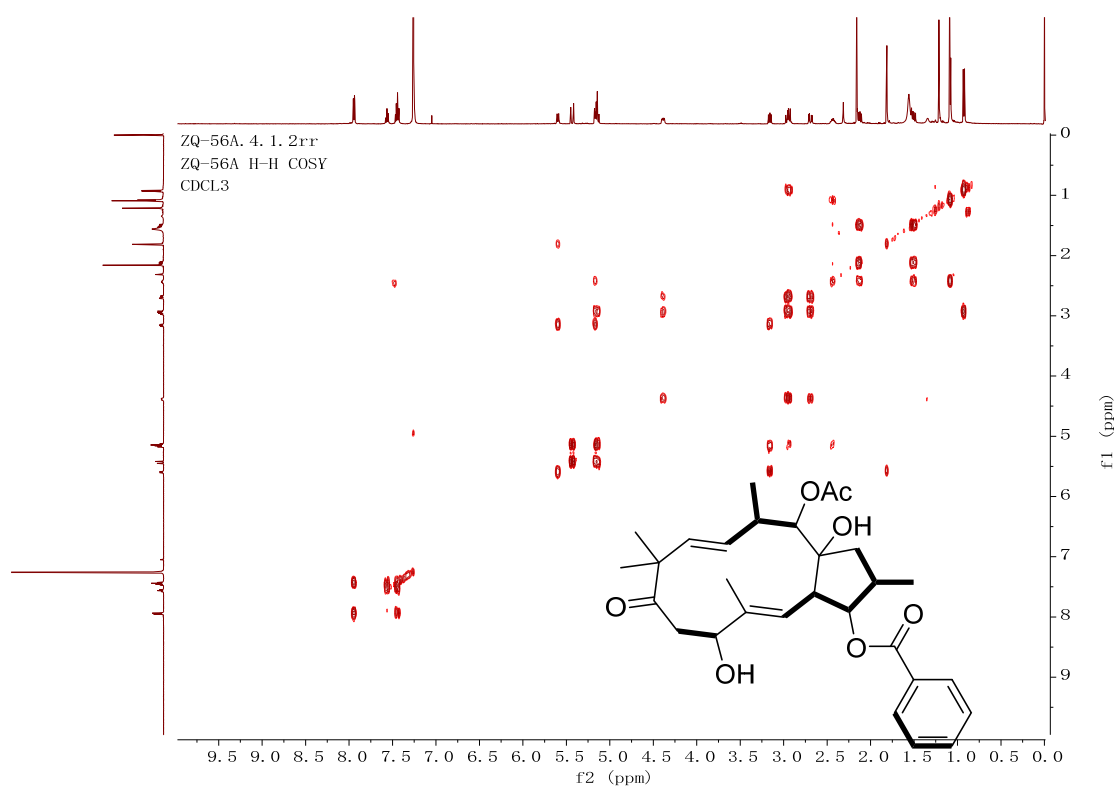
Figure S44  $^{13}\text{C}$  NMR spectrum of **7** (125 MHz,  $\text{CDCl}_3$ )



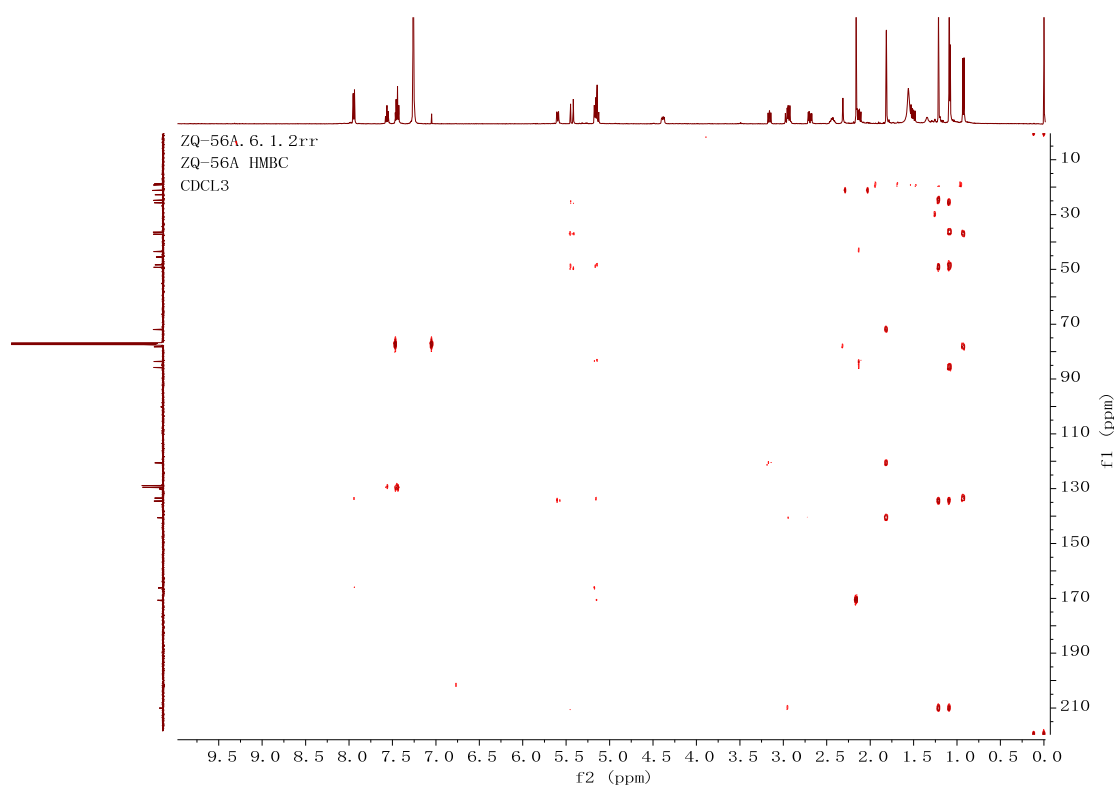
**Figure S45 HSQC spectrum of 7**



**Figure S46  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of 7**



**Figure S47 HMBC spectrum of 7**



**Figure S48 ROESY spectrum of 7**

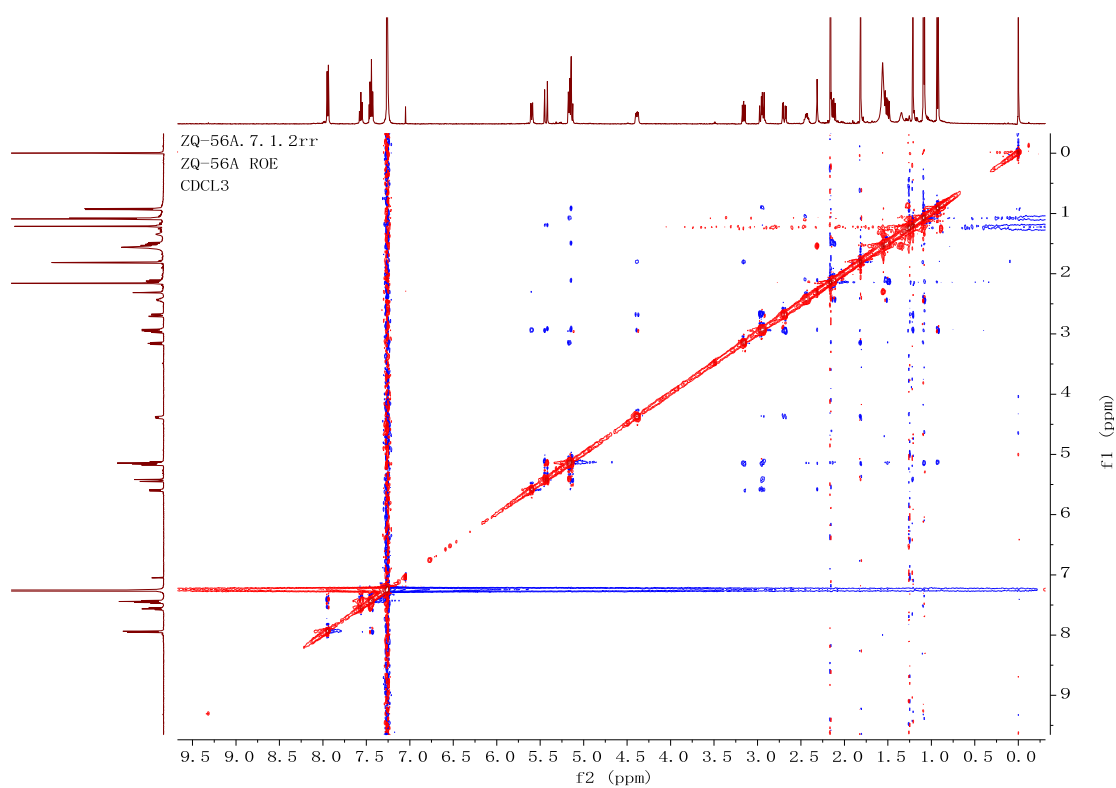


Figure S49 HRESIMS report of 7

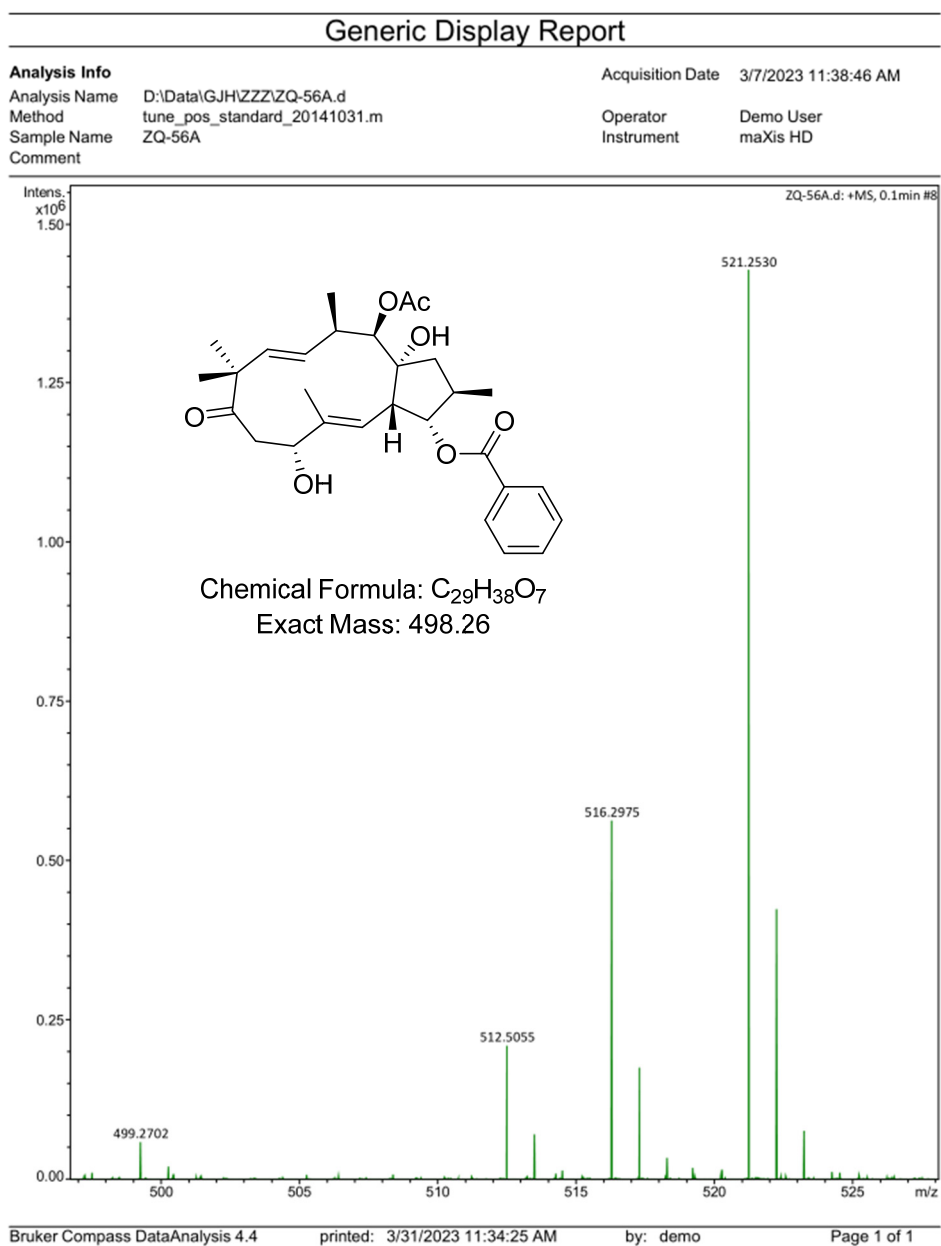




Figure S46  $^1\text{H}$  NMR spectrum of 13 (500 MHz,  $\text{CDCl}_3$ )

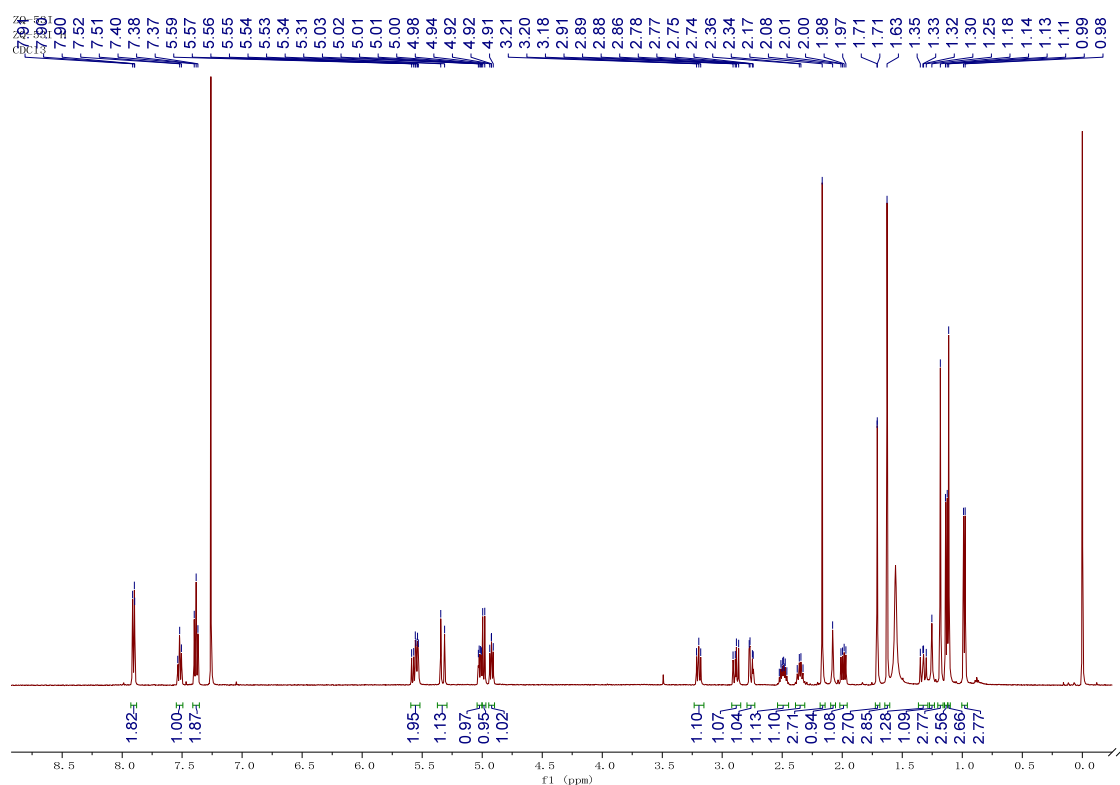
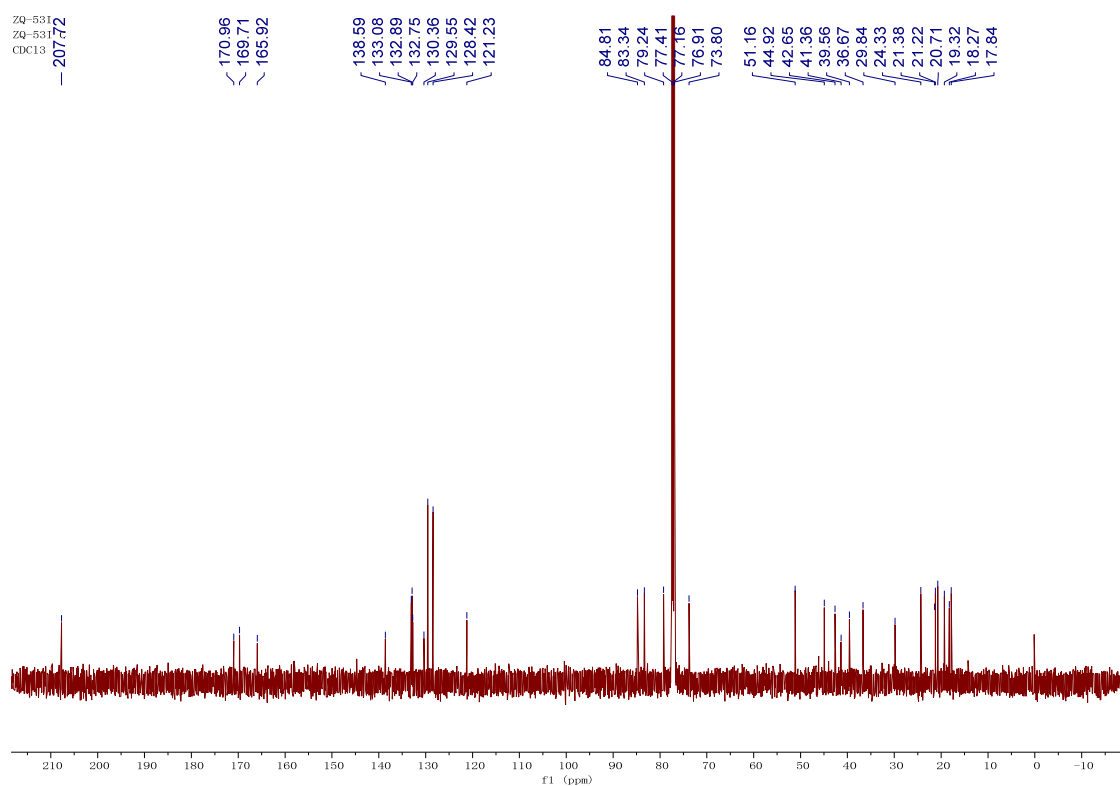
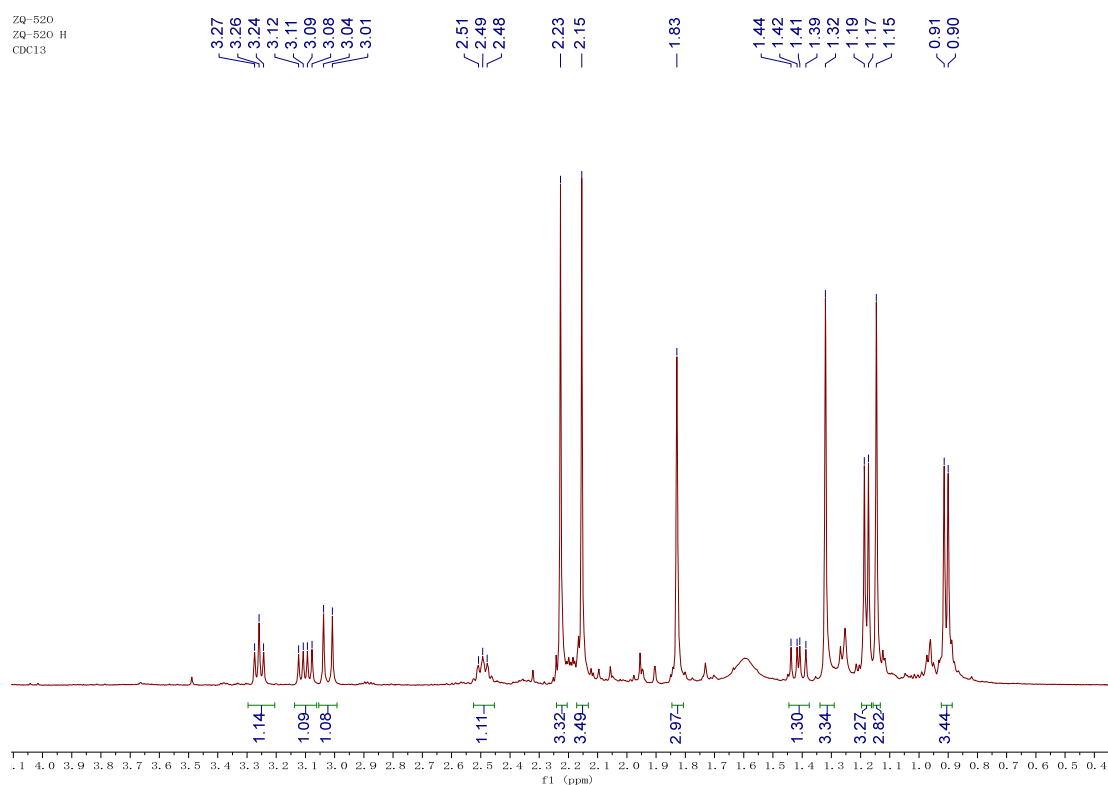


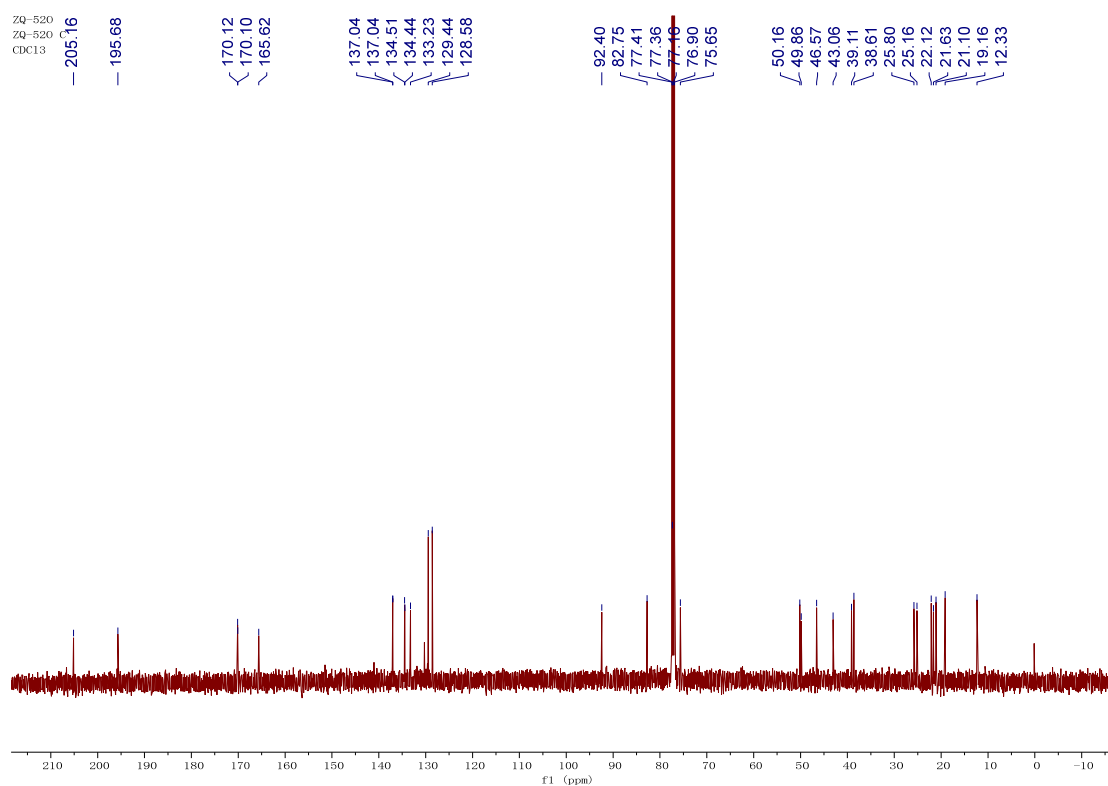
Figure S47  $^{13}\text{C}$  NMR spectrum of 13 (125 MHz,  $\text{CDCl}_3$ )



**Figure S48  $^1\text{H}$  NMR spectrum of 17 (500 MHz,  $\text{CDCl}_3$ )**

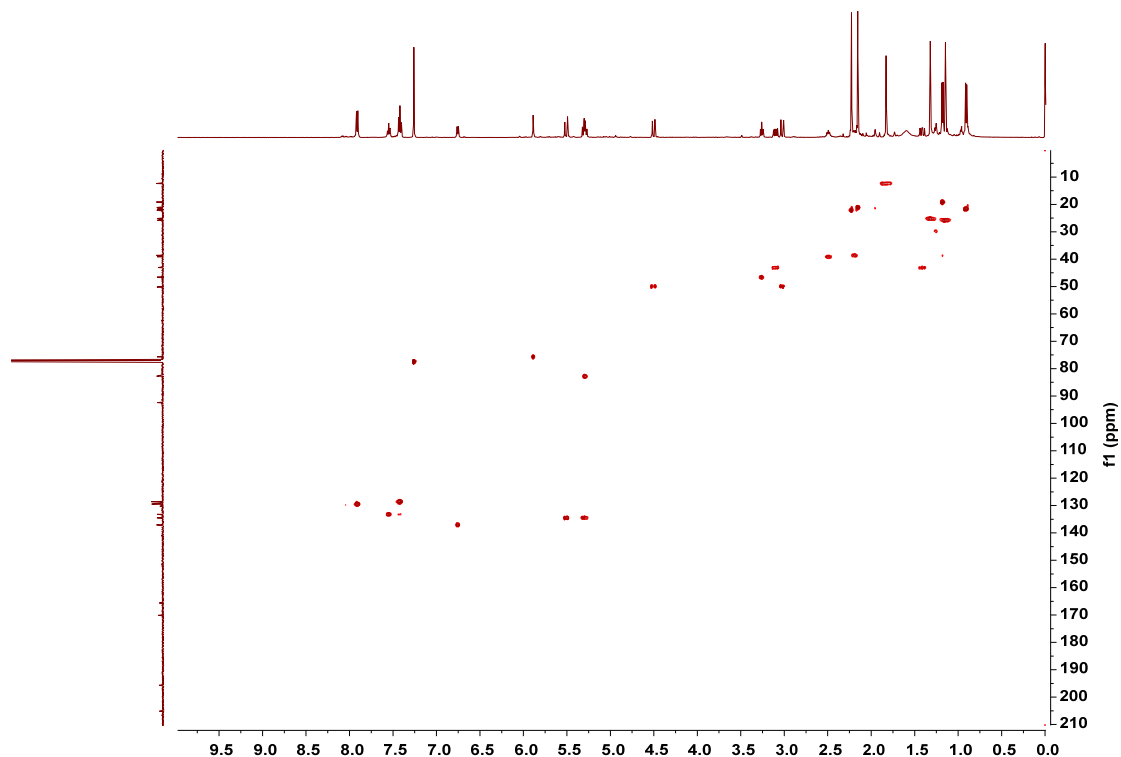


**Figure S49  $^{13}\text{C}$  NMR spectrum of 17 (125 MHz,  $\text{CDCl}_3$ )**



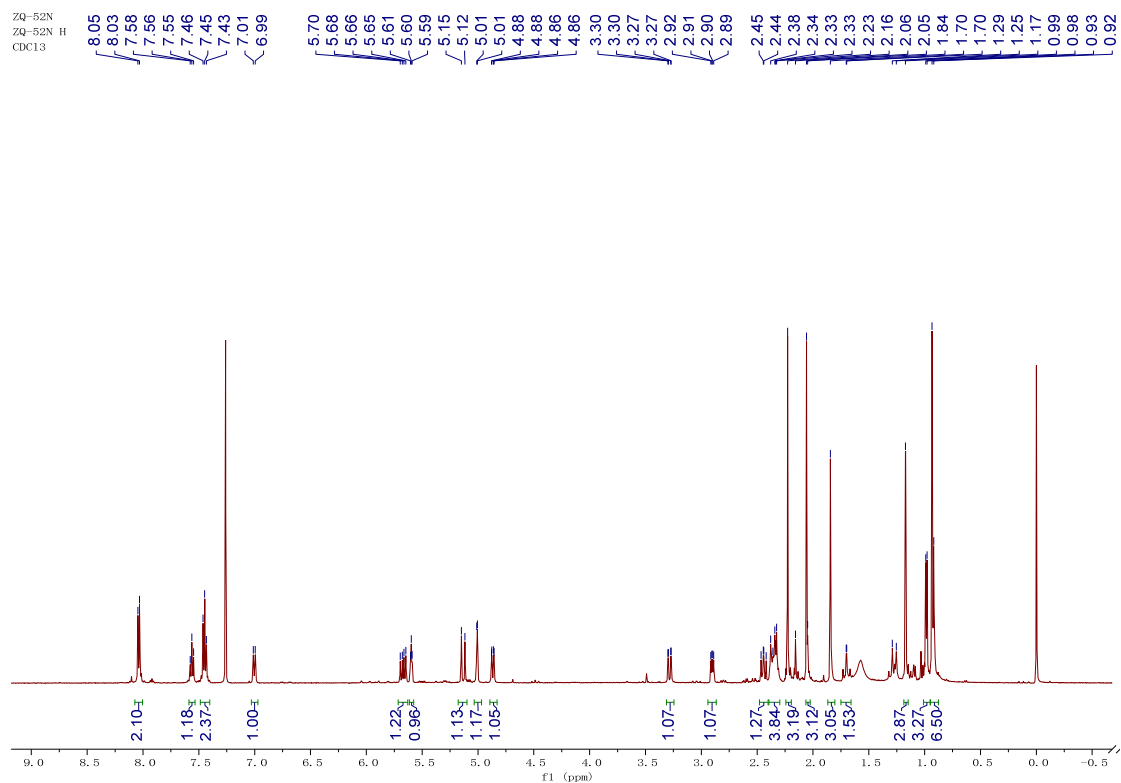
**Figure S50 HSQC spectrum of 17**

ZQ-520, 5, 1, 2rr — ZQ-520 HSQC — CDCl<sub>3</sub>

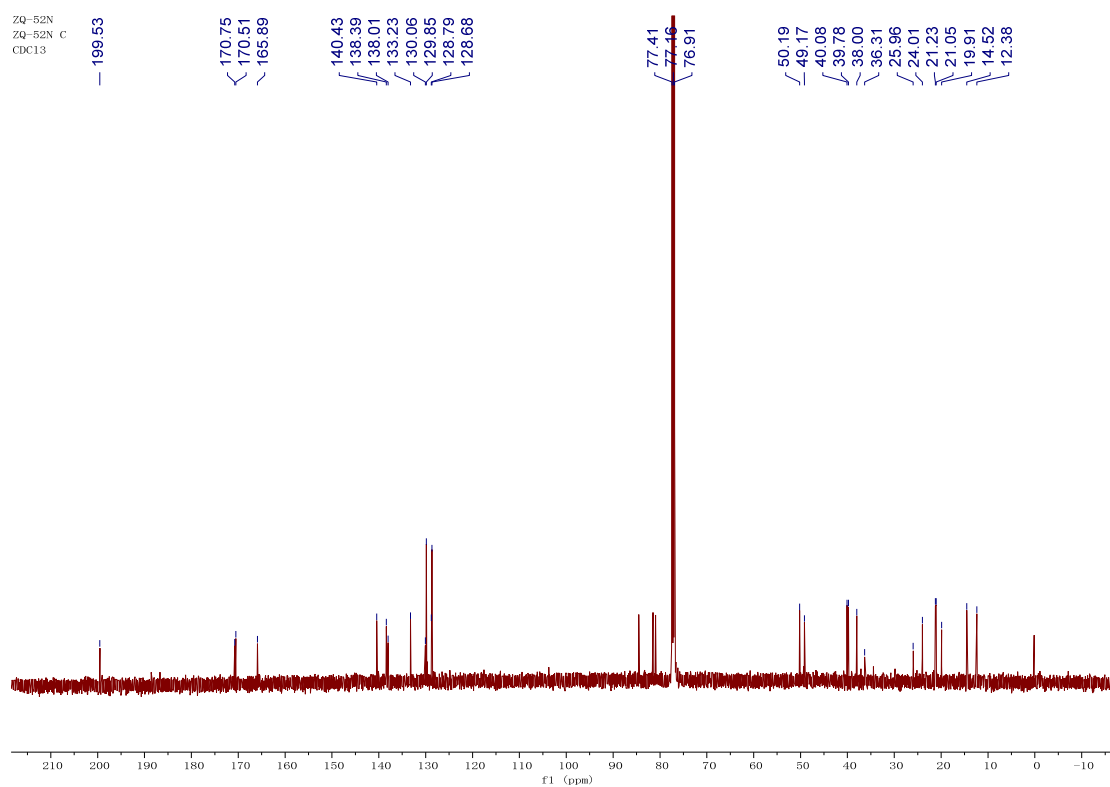


**Figure S51 <sup>1</sup>H NMR spectrum of 20 (500 MHz, CDCl<sub>3</sub>)**

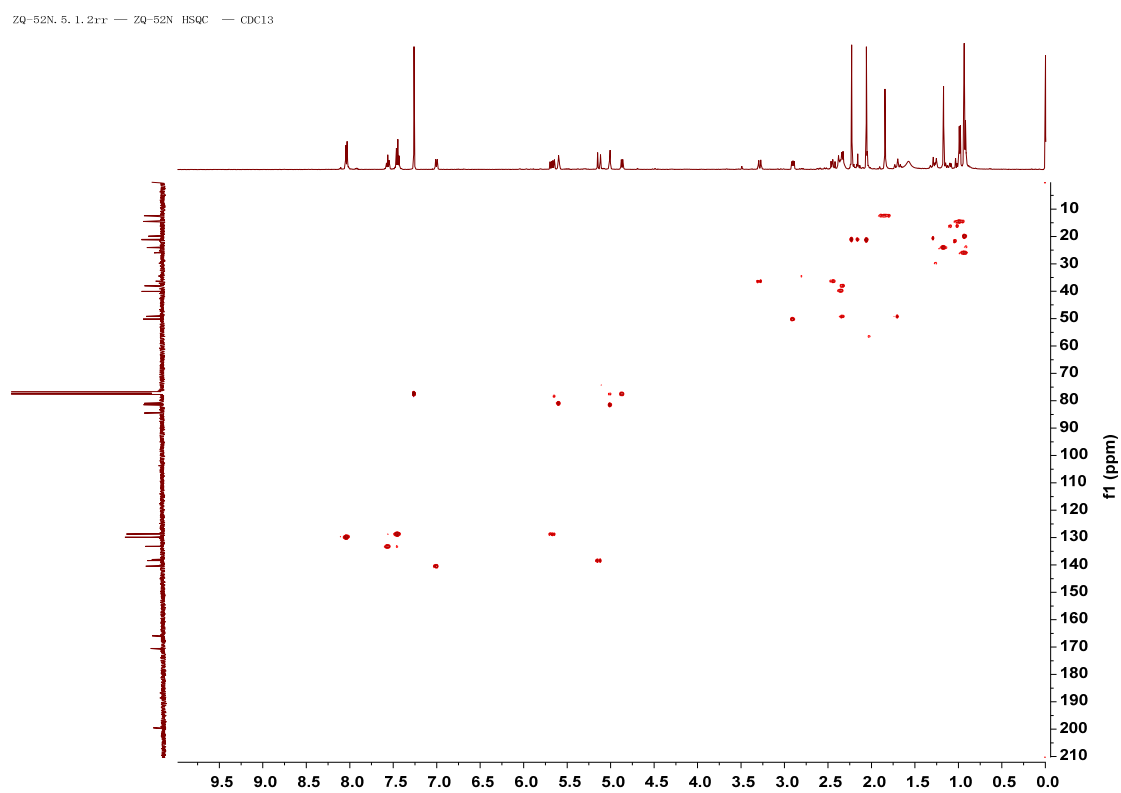
ZQ-52N  
ZQ-52N H  
CDCl<sub>3</sub>



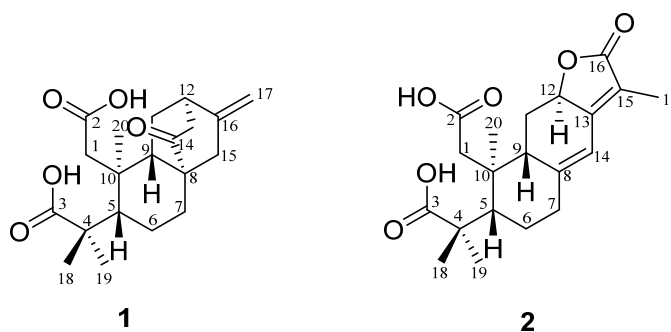
**Figure S52  $^{13}\text{C}$  NMR spectrum of 20 (125 MHz,  $\text{CDCl}_3$ )**



**Figure S53 HSQC spectrum of 20**



## 2. Computational details for **1** and **2**



Conformation search based on molecular mechanics with MMFF94s force fields were performed for **1** and **2** each affording seven and six stable conformers with distributions higher than 5%<sup>2,3</sup>, respectively. All these conformers were further optimized by the density functional theory method at the B3LYP/6-31G(d) level in Gaussian 09 program package<sup>3</sup>, leading to six conformers for **1** and four conformers for **2** within a 3 kcal/mol energy threshold from global minimum, respectively (Tables S2 and S3). Conformers were subjected to theoretical calculation of ECD using time-dependent density functional theory (TDDFT) at B3LYP/6-31G(d) level with CPCM model in methanol based on B3LYP/6-31G(d) optimized conformers. The calculated ECD curves for **1** and **2** were weighted using SpecDis<sup>4</sup>.

**Table S2.** Energy analysis for conformers of **1** at B3LYP/6-31G(d) level in the gas phase

Specie	Gibbs free energy	$\Delta E$ (kcal/mol)	$P_E\%$
<b>1-a</b>	-1154.702992	1.62	4.3
<b>1-b</b>	-1154.705568	0.00	66.4
<b>1-c</b>	-1154.702063	2.20	1.6
<b>1-d</b>	-1154.703669	1.19	8.9
<b>1-e</b>	-1154.704035	0.96	13.1
<b>1-f</b>	-1154.703232	1.47	5.6
<b>1-g</b>	-1154.699622	3.73	0

**Table S3.** Energy analysis for conformers of **2** at B3LYP/6-31G(d) level in the gas phase

Specie	Gibbs free energy	$\Delta E$ (kcal/mol)	$P_E\%$
<b>2-a</b>	-1228.762974	1.40	6.7
<b>2-b</b>	-1228.763845	0.86	17.0
<b>2-c</b>	-1228.759925	3.32	0.3
<b>2-d</b>	-1228.765210	0.00	72.1
<b>2-e</b>	-1228.762444	1.74	3.8
<b>2-f</b>	-1228.759062	3.86	0.1

### 3. Cytokine Analysis by ELISA of Induced T cells

**Table S4.** Concentrations of IFN- $\gamma$ /IL-2/IL-17A in different groups

Group s	c ( $\mu$ M)	Concentration (pg/mL)		
		IFN- $\gamma$	IL-2	IL-17A
<b>M</b>		22654.9 $\pm$ 1278.5	2638.4 $\pm$ 442.2	3395.2 $\pm$ 80.8
<b>7</b>	2.5	11984.4 $\pm$ 390.1****	1340.6 $\pm$ 370.6**	2027.9 $\pm$ 898.5 <sup>ns</sup>
<b>7</b>	1	10806.1 $\pm$ 921.6****	1469.6 $\pm$ 510.2**	2262.5 $\pm$ 784.7 <sup>ns</sup>
<b>21</b>	2.5	11601.4 $\pm$ 646.7****	1280.3 $\pm$ 139.9**	2150.3 $\pm$ 430.0 <sup>ns</sup>
<b>21</b>	1	15945.9 $\pm$ 695.9****	1774.7 $\pm$ 540.0 <sup>ns</sup>	2992.9 $\pm$ 684.7*
<b>Dex</b>	0.05	13914.5 $\pm$ 1764.1****	1387.5 $\pm$ 294.7**	1557.2 $\pm$ 432.5****
<b>Y</b>		309.4 $\pm$ 29.5****	untested****	untested****

Note: vs M, \*\*\*\*  $P < 0.0001$ , \*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$

### 4. EdU results for **7** and **21**

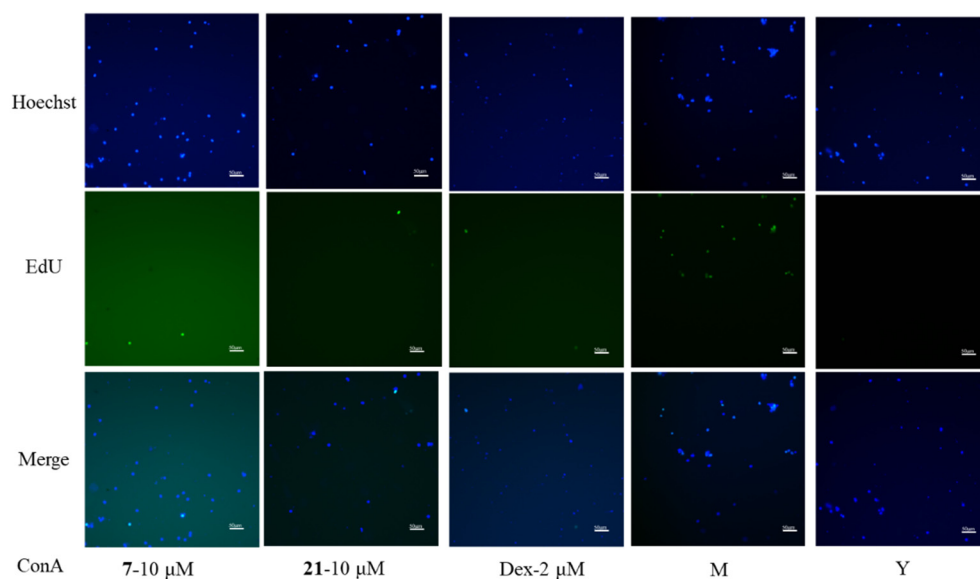


Figure S54. Inhibitory activities of **7** and **21** against induced T cells (ConA) measured by EdU methods.

## References:

- (1) Goto, H., Osawa, E., 1989. Corner flapping: a simple and fast algorithm for exhaustive generation of ring conformations. *J. Am. Chem. Soc.* *111*, 8950–8951.
- (2) Goto, H., Osawa, E., 1993. *J. Chem. Soc., Perkin Trans. 2*, 187–198.
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- (4) Bruhn, T., Schaumlöffel, A., Hemberger, Y., Bringmann, G., 2012. *Spec Dis*, version 1.71, University of Würzburg, Germany,