
SUPPLEMENTARY MATERIAL

New adenine analogues and a pyrrole alkaloid from *Selaginella delicatula*

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Abstract

Phytochemical study on the *n*-BuOH extract of *Selaginella delicatula* lead to the isolation, characterization and structure elucidation of two new adenine analogues, delicatulines A (**1**) and B (**2**), one new pyrrole alkaloid (**4**), and five known compounds (**3**, **5-8**). These new substances all contain an aliphatic chain in their parent nucleus, which were unusual to find in plants. In the present study, they were identified from Selaginellaceae for the first time. The structures and absolute configurations of these new compounds were determined by a combination of NMR and CD spectroscopic analyses. Compounds **1**, **3** and **4** were evaluated for their inhibitory activities on HBV surface antigen and HBV

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DNA in HepAD38 cells. The results showed that these compounds had only weak or no inhibitive effects on HBV.

Keywords: *Selaginella*, *Selaginella delicatula*, adenine analogues, alkaloid

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Table S1.

¹H and ¹³C NMR data of compound **1** (400 and 100 MHz, DMSO-*d*₆, *J* in Hz) and **2** (500 and 125 MHz, DMSO-*d*₆, *J* in Hz)

No.	1		2	
	δ_C	δ_H	δ_C	δ_H
1'	13.9	0.76 (3H, t, <i>J</i> = 8.0)	173.7	
2'	18.8	1.16 (2H, m)	44.0	2.52 (2H, m)
3'	30.4	1.34 (2H, m)	54.4	4.79 (1H, m)
4'	64.1	3.88 (2H, t, <i>J</i> = 4.0)	36.5	1.96 (1H, m)
				1.83 (1H, m)
5'	170.7		19.5	0.95 (1H, m)

				1.07 (1H, m)
6'	39.2	3.19 (1H, dd, $J = 8.0$)	14.0	0.78 (3H, t, $J = 8.0$)
		3.01 (1H, dd, $J = 8.0$)		
7'	52.4	4.82 (1H, m)		
8'	36.2	2.04 (1H, m)		
		1.78 (1H, m)		
9'	19.1	1.06 (2H, m)		
10'	13.7	0.81 (3H, t, $J = 8.0$)		
2	152.6	8.11 (1H, s)	152.3	8.09 (1H, s)
4	149.9		150.0	
5	119.4		119.5	
6	156.4		156.3	
8	140.6	8.21 (1H, s)	141.0	8.11 (1H, s)
10	NH ₂	7.18 (2H, s)	NH ₂	7.09 (2H, s)

Table S2.

¹H and ¹³C NMR data of compound **4** (500 and 125 MHz, DMSO-*d*₆, J in Hz)

No.	δ_C	δ_H
2	132.0	
3	124.5	6.98 (1H, d, $J = 3.5$)
4	110.2	6.21 (1H, d, $J = 3.5$)
5	143.9	
1'	179.5	9.45 (1H, s)
1'''	55.2	4.50 (2H, d)

1"	44.5	4.28 (2H, t)
2"	26.7	1.90 (2H, m)
3"	31.1	2.29 (2H, t)
4"	172.8	
5"	64.1	4.00 (2H, t)
6"	30.6	1.54 (2H, m)
7"	19.1	1.32 (2H, m)
8"	14.0	0.88 (3H, m)

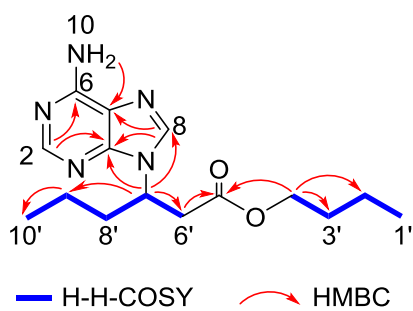


Figure S1. Key ^1H - ^1H COSY and HMBC correlations of compound **1**

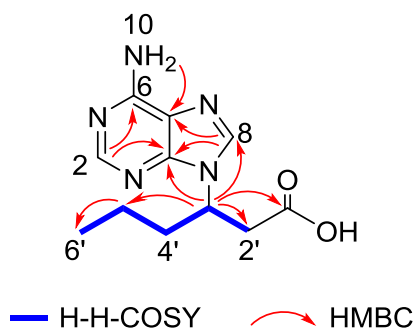


Figure S2. Key ^1H - ^1H COSY and HMBC correlations of compound **2**

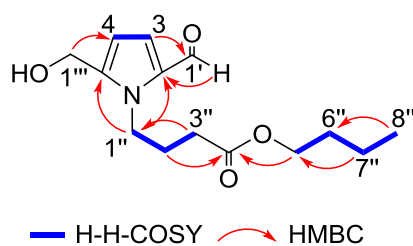


Figure S3. Key ^1H - ^1H COSY and HMBC correlations of compound **4**

Figure S4. CD spectrum of compounds **1** and **2** (in MeOH)

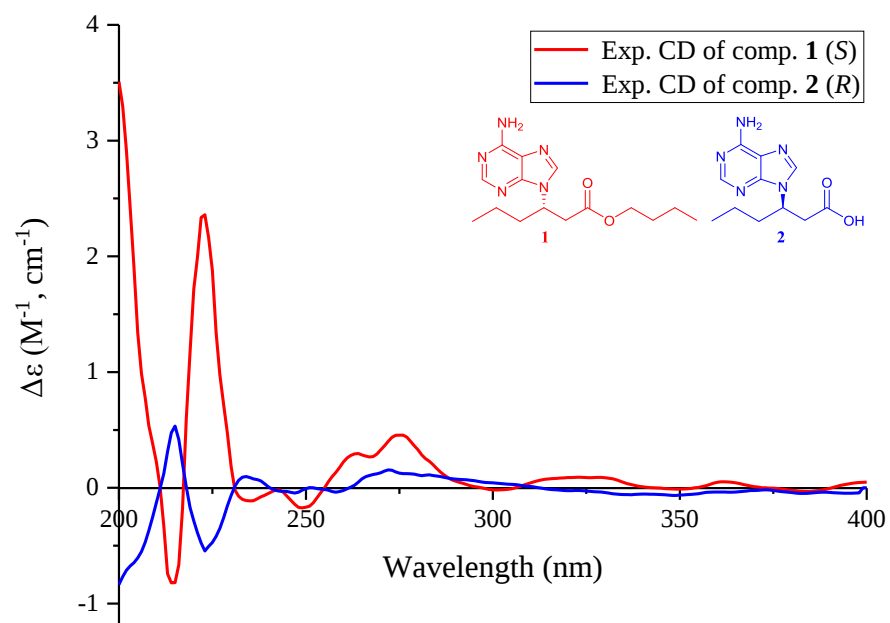


Figure S5. HRESIMS of compound 1

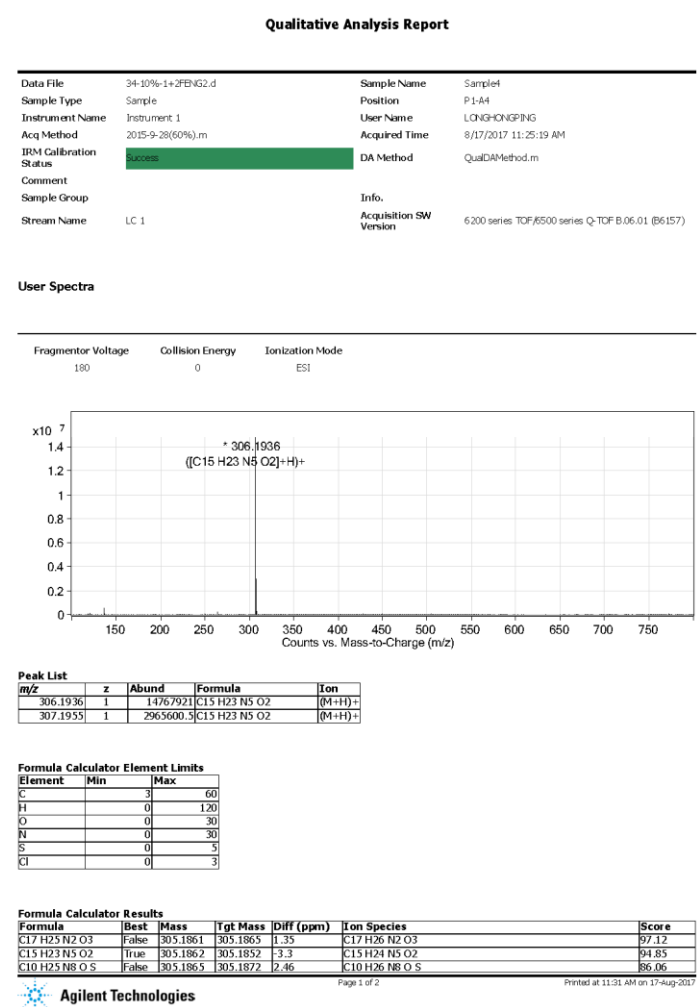


Figure S6. ^1H NMR spectrum of compound **1** (DMSO- d_6 , 400 MHz)

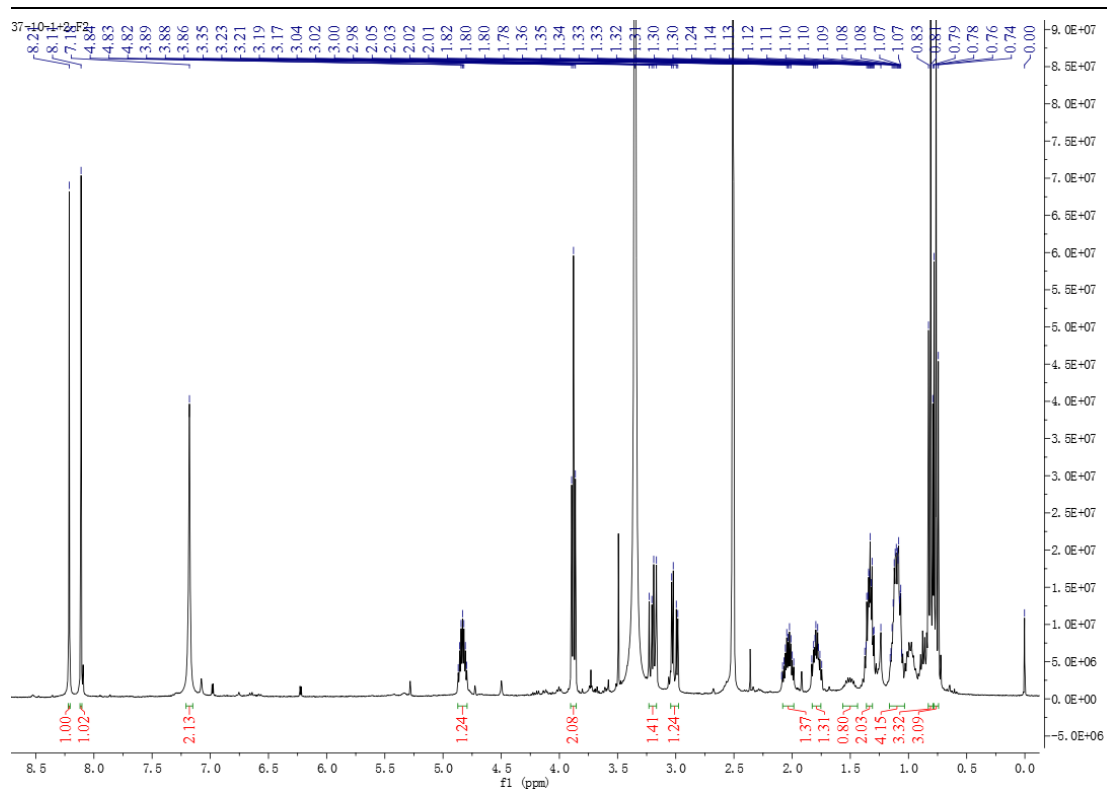


Figure S7. ^{13}C NMR spectrum of compound **1** ($\text{DMSO}-d_6$, 100 MHz)

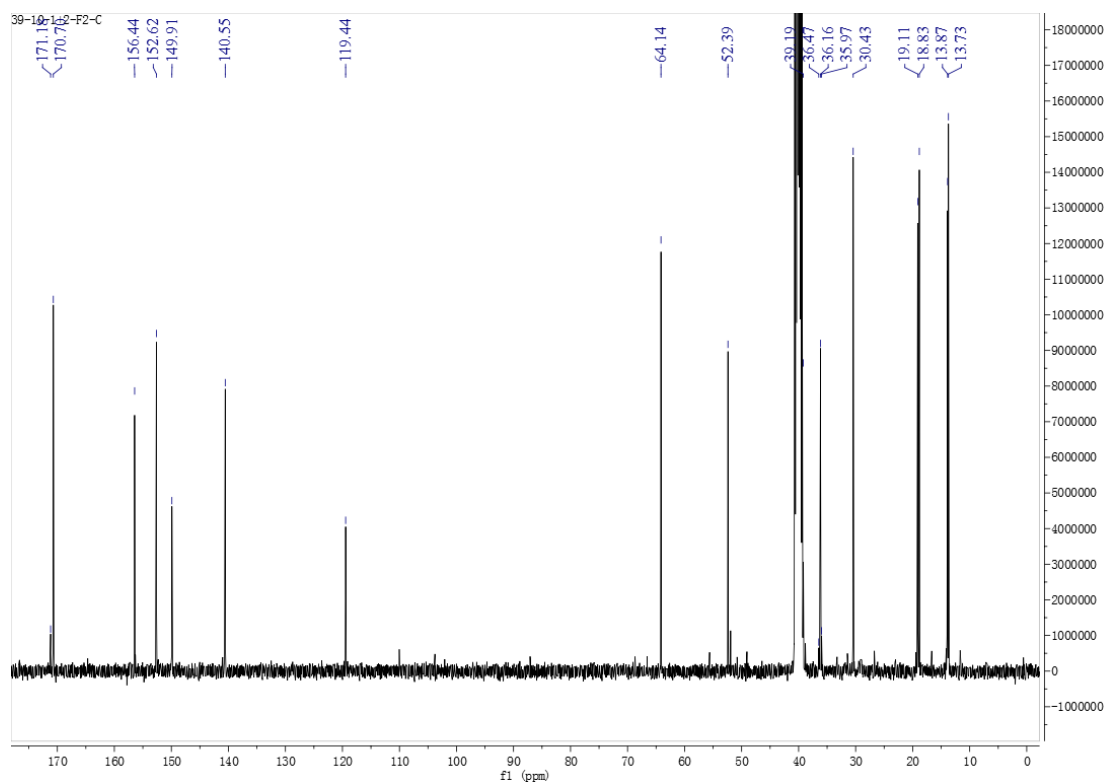


Figure S8. DEPT135 spectrum of compound **1** (DMSO-*d*₆, 100 MHz)

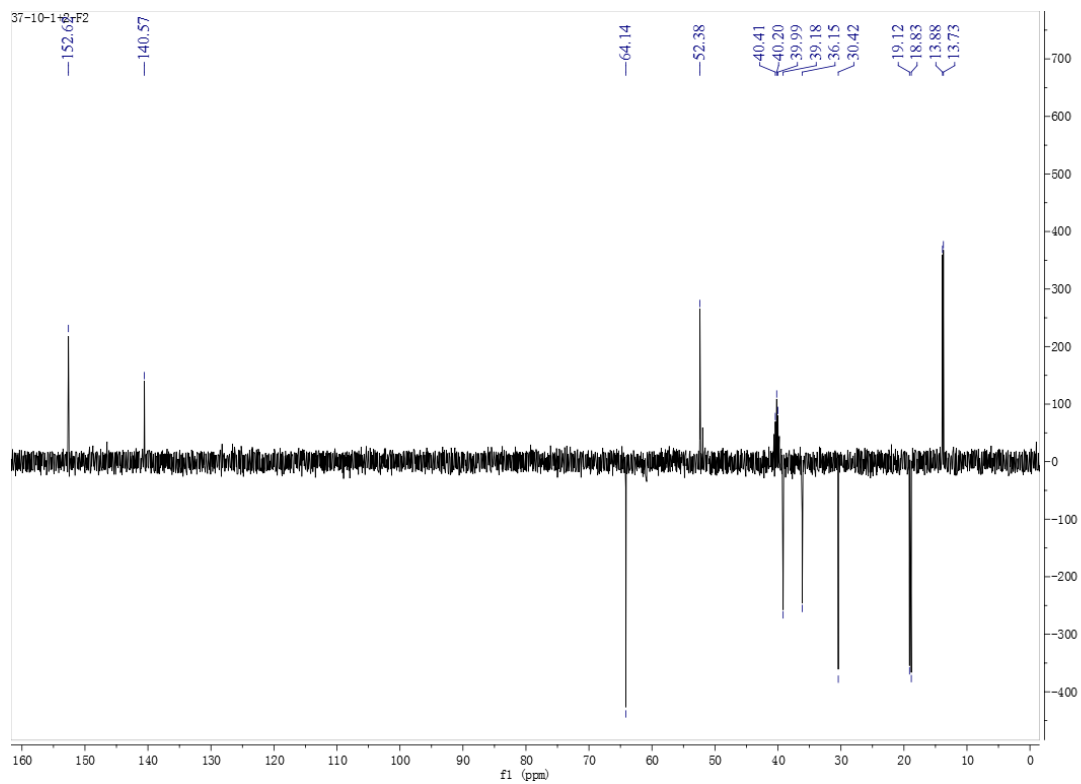


Figure S9. ¹H-¹H COSY spectrum of compound **1** (DMSO-*d*₆, 400 MHz)

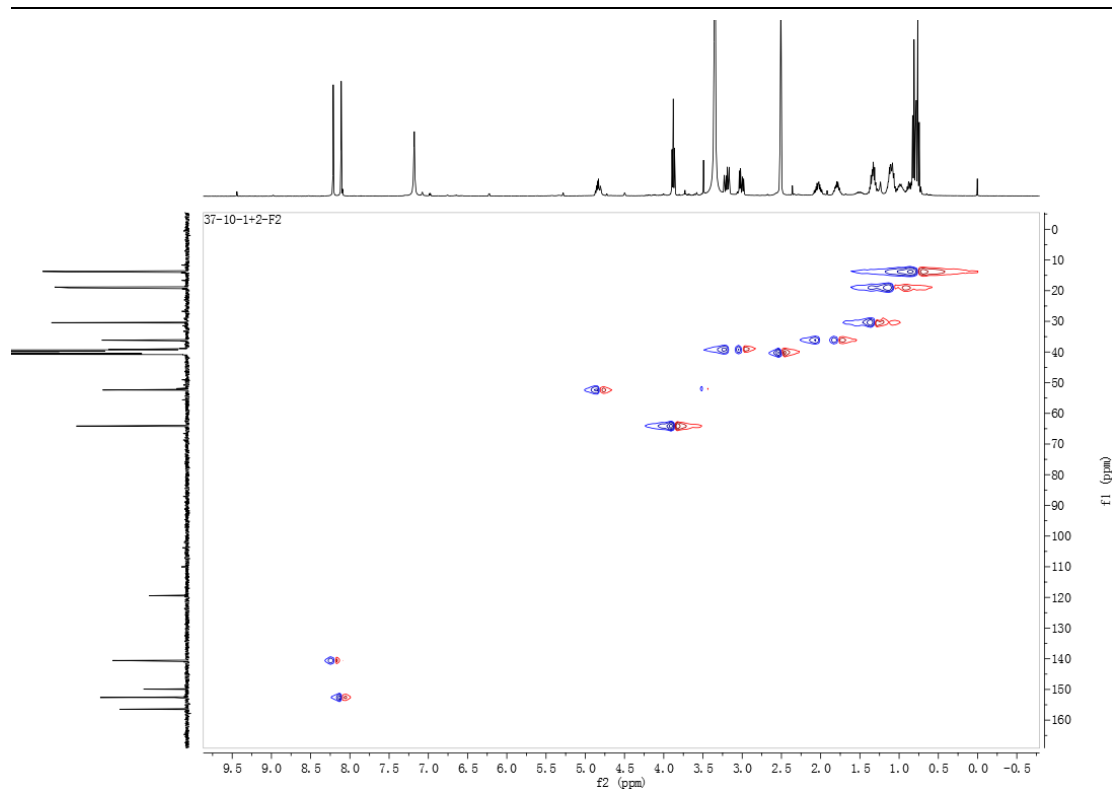


Figure S10. HSQC spectrum of compound **1** (DMSO- d_6 , 400 MHz)

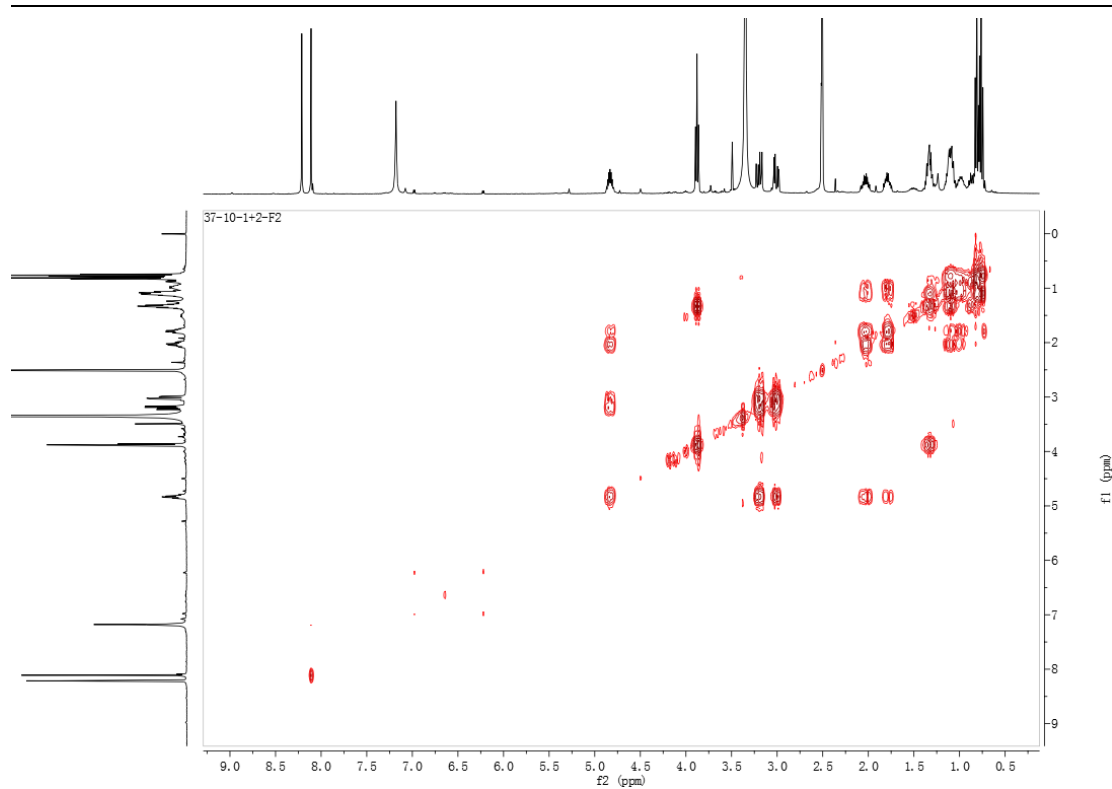


Figure S11. HMBC spectrum of compound **1** (DMSO-*d*₆, 400 MHz)

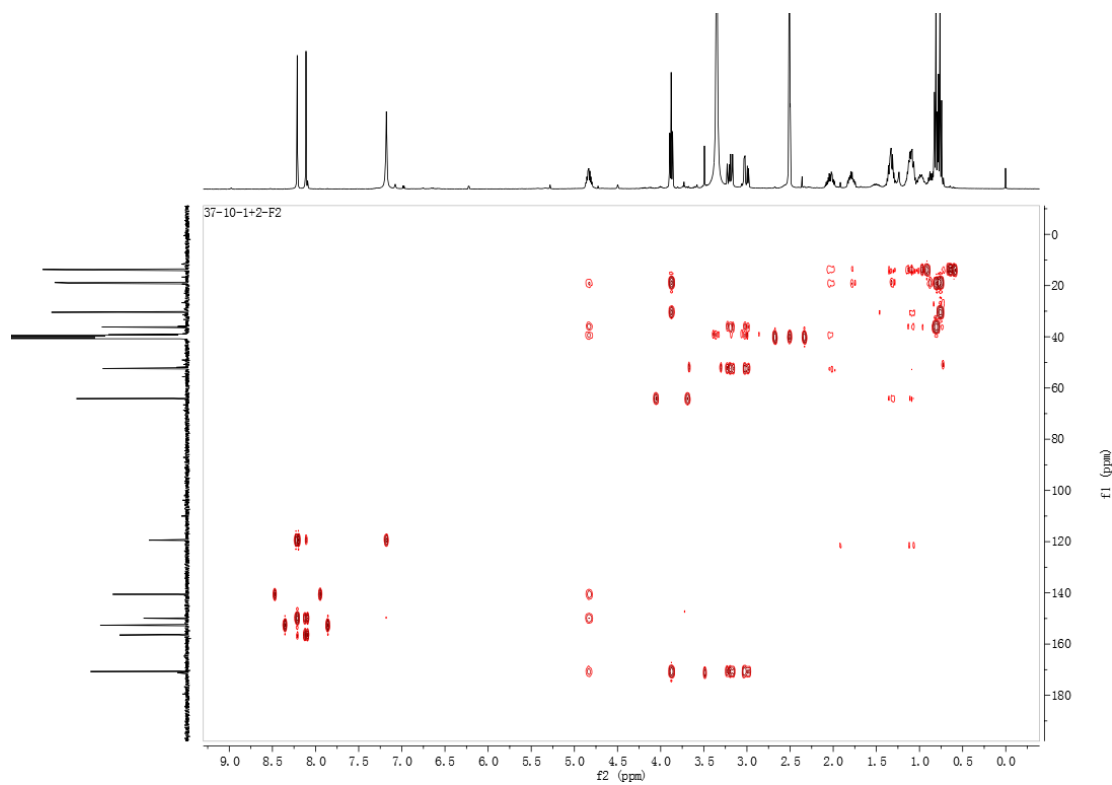


Figure S12. HRESIMS of compound 2

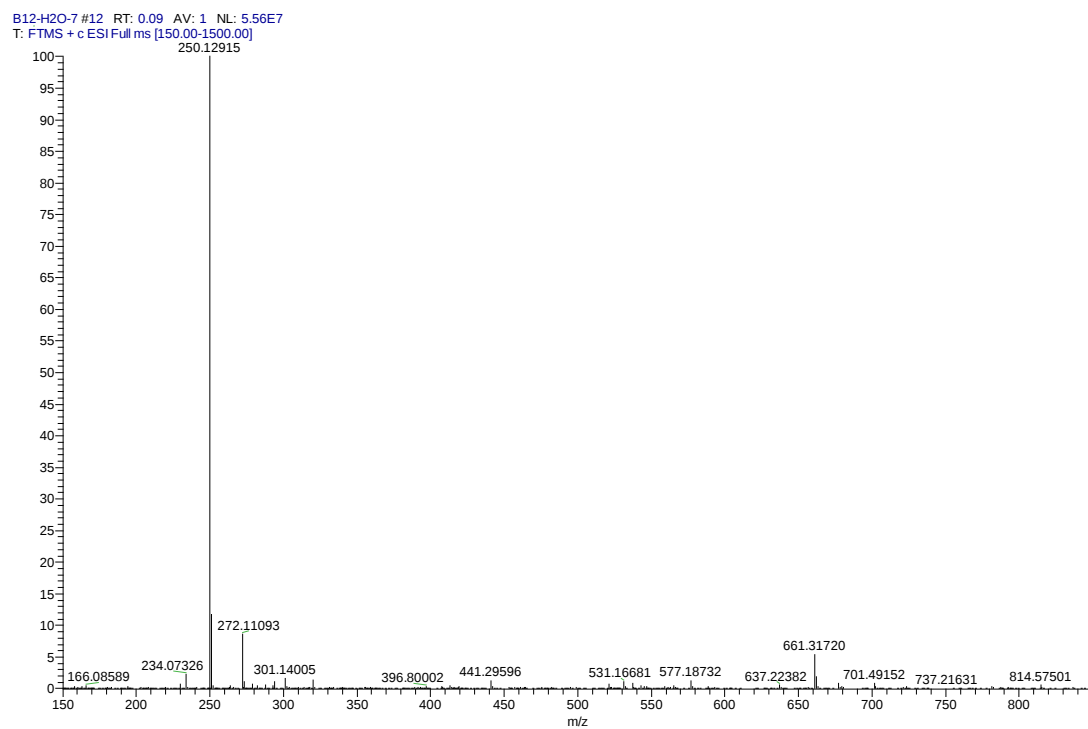


Figure S13. ^1H NMR spectrum of compound 2 (DMSO- d_6 , 500 MHz)

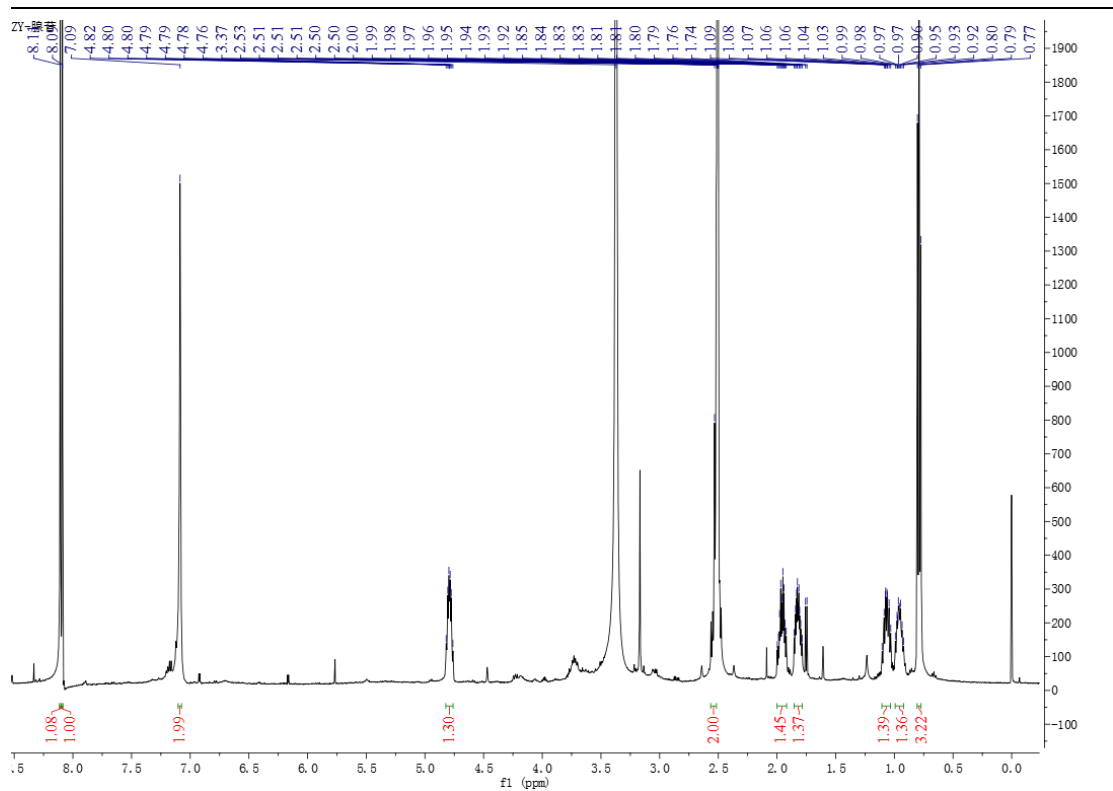


Figure S14. ^{13}C NMR spectrum of compound **2** ($\text{DMSO}-d_6$, 125 MHz)

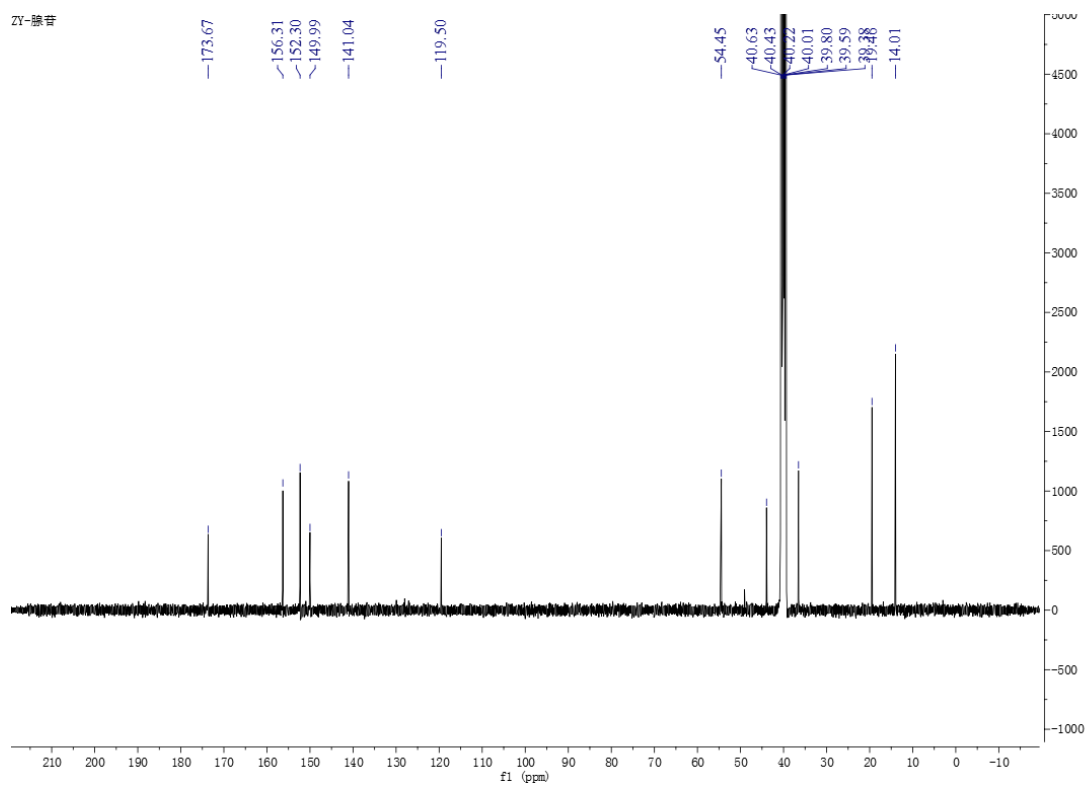


Figure S15. DEPT135 spectrum of compound **2** (DMSO-*d*₆, 125 MHz)

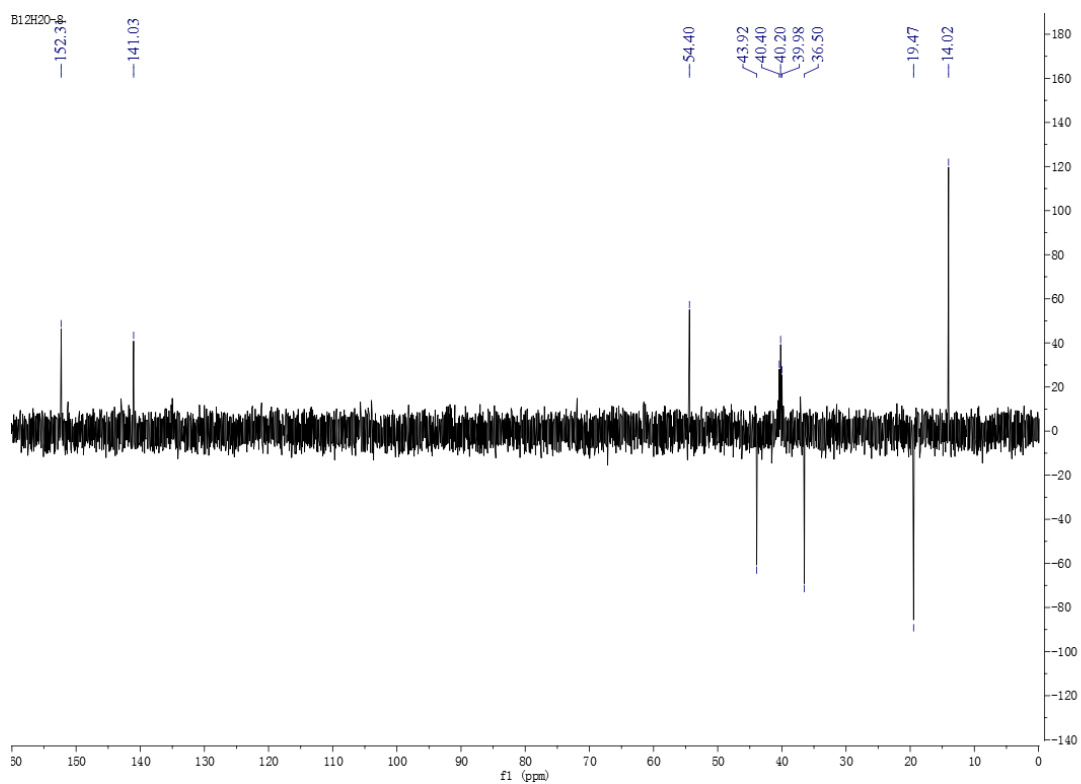


Figure S16. ¹H-¹H COSY spectrum of compound **2** (DMSO-*d*₆, 500 MHz)

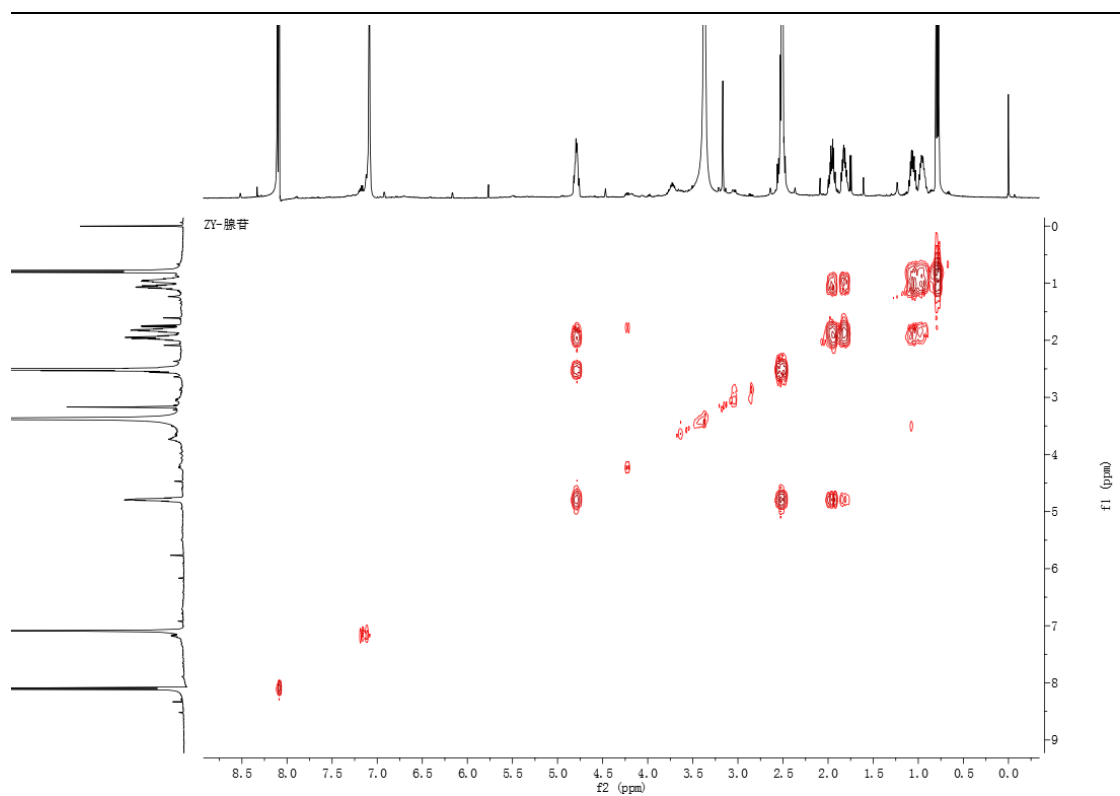


Figure S17. HSQC spectrum of compound 2 (DMSO-*d*₆, 500 MHz)

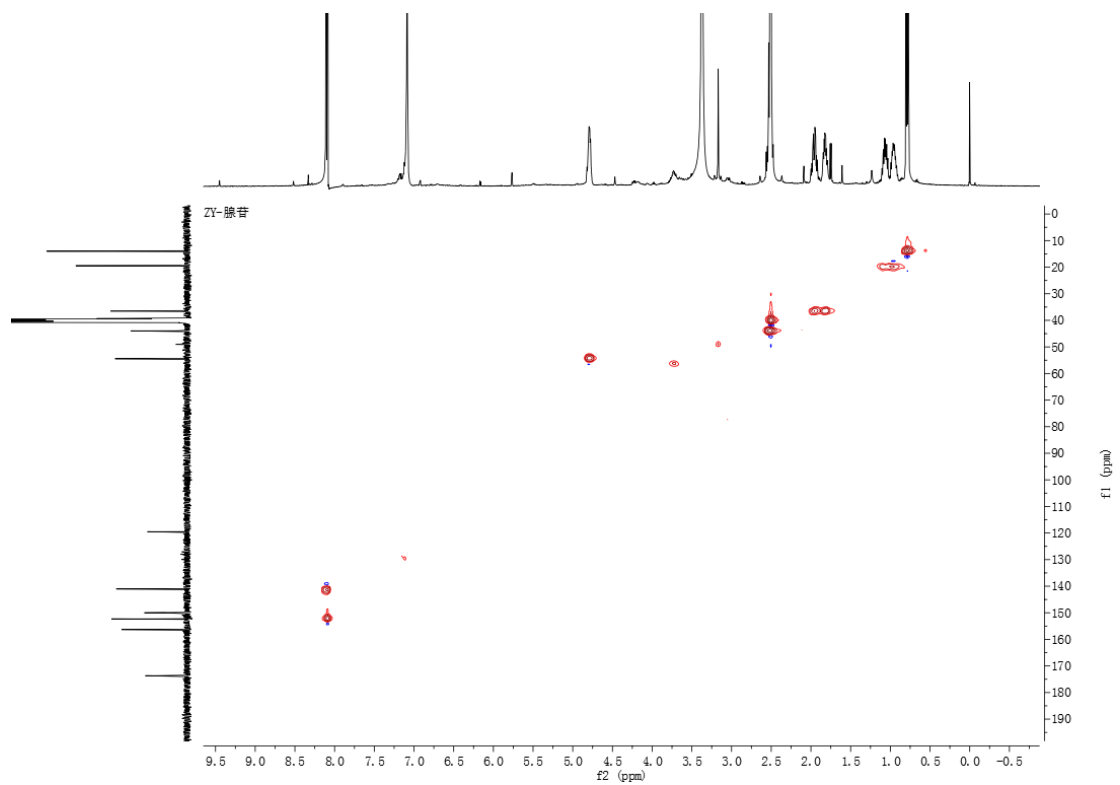


Figure S18. HMBC spectrum of compound **2** (DMSO-*d*₆, 500 MHz)

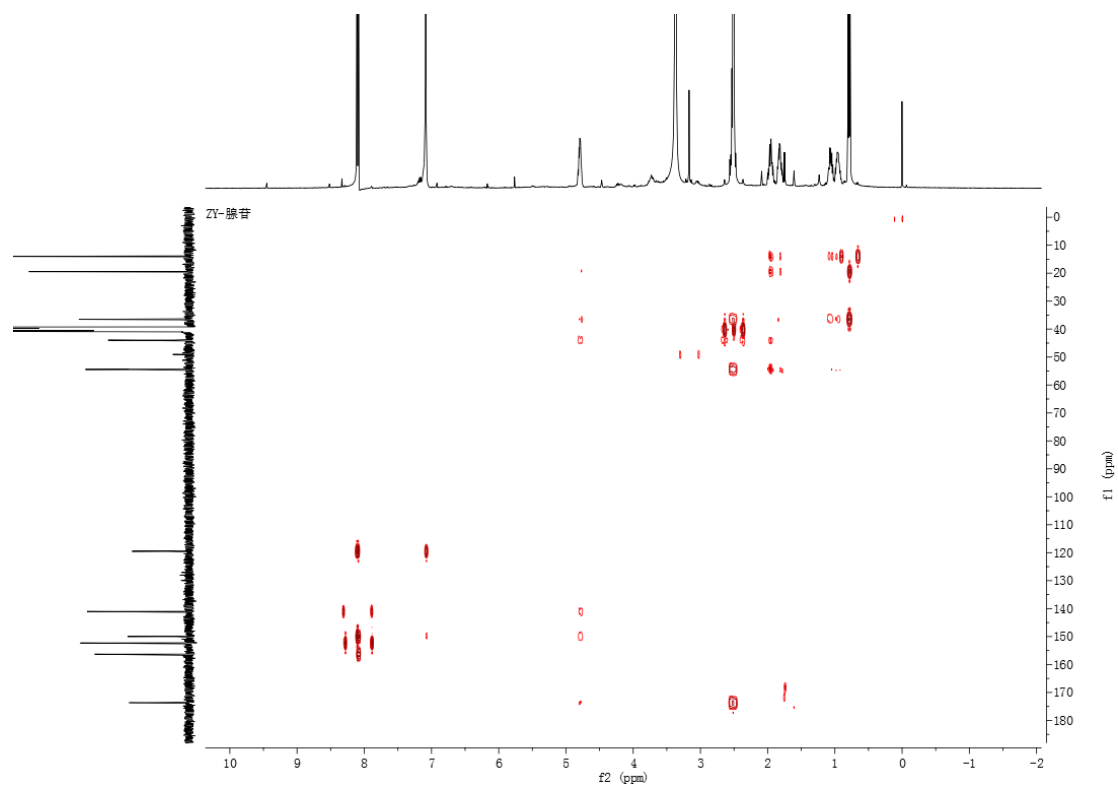


Figure S19. HRESIMS of compound **4**

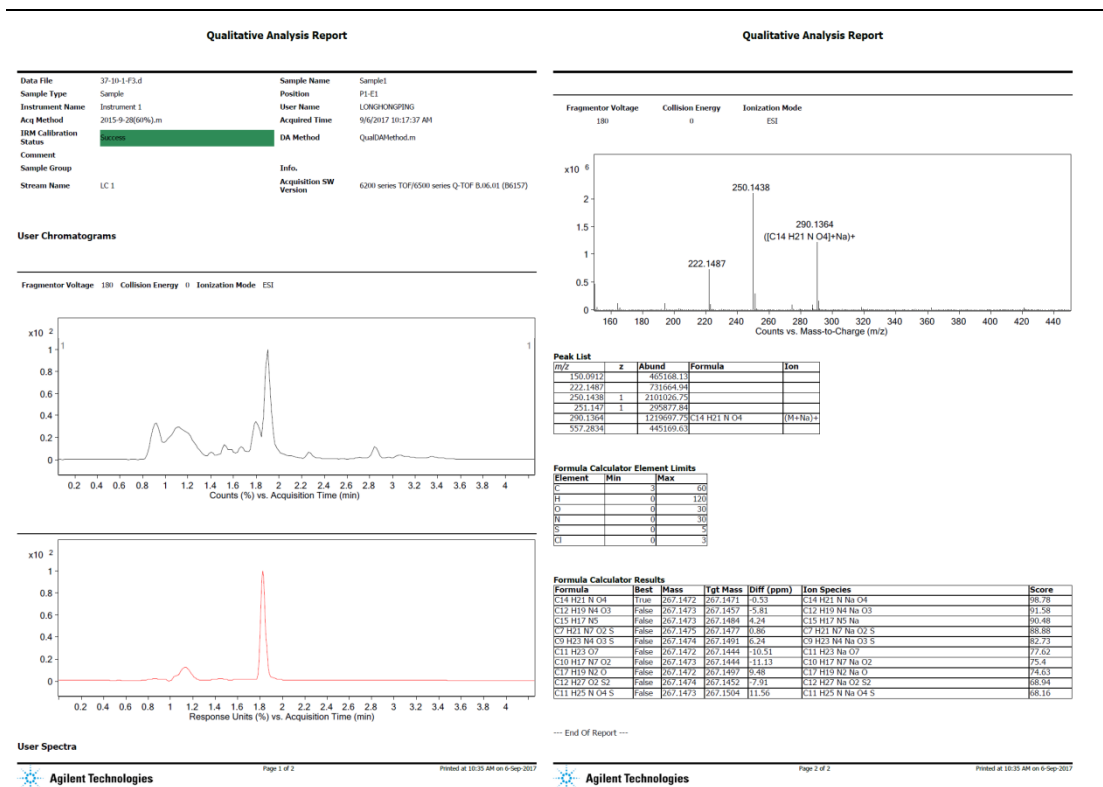


Figure S20. ^1H NMR spectrum of compound **4** (DMSO- d_6 , 500 MHz)

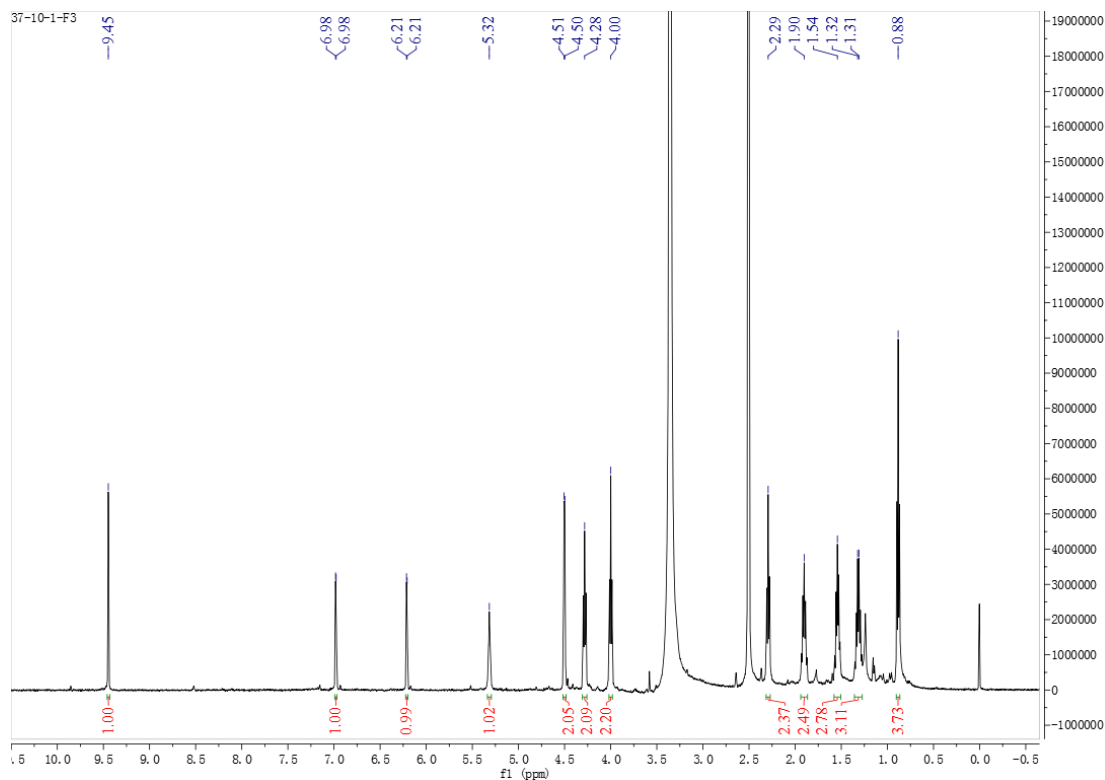


Figure S21. ^{13}C NMR spectrum of compound **4** (DMSO- d_6 , 125 MHz)

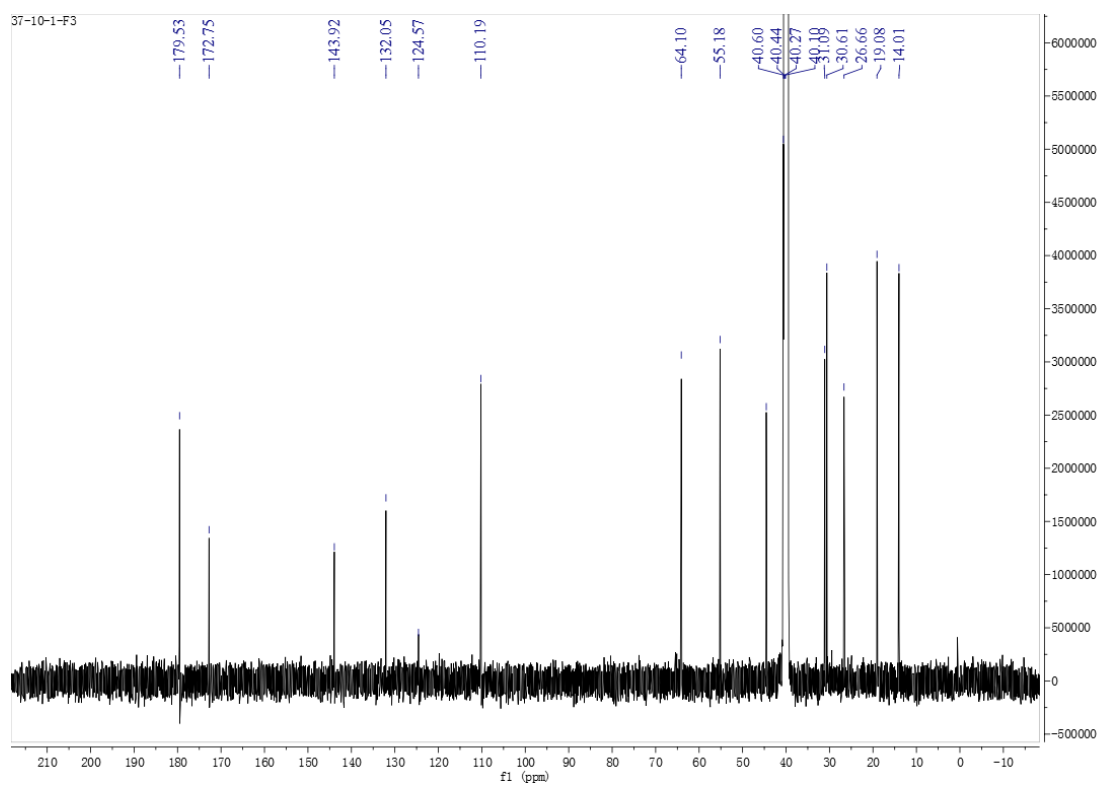


Figure S22. DEPT135 spectrum of compound **4** (DMSO- d_6 , 125 MHz)

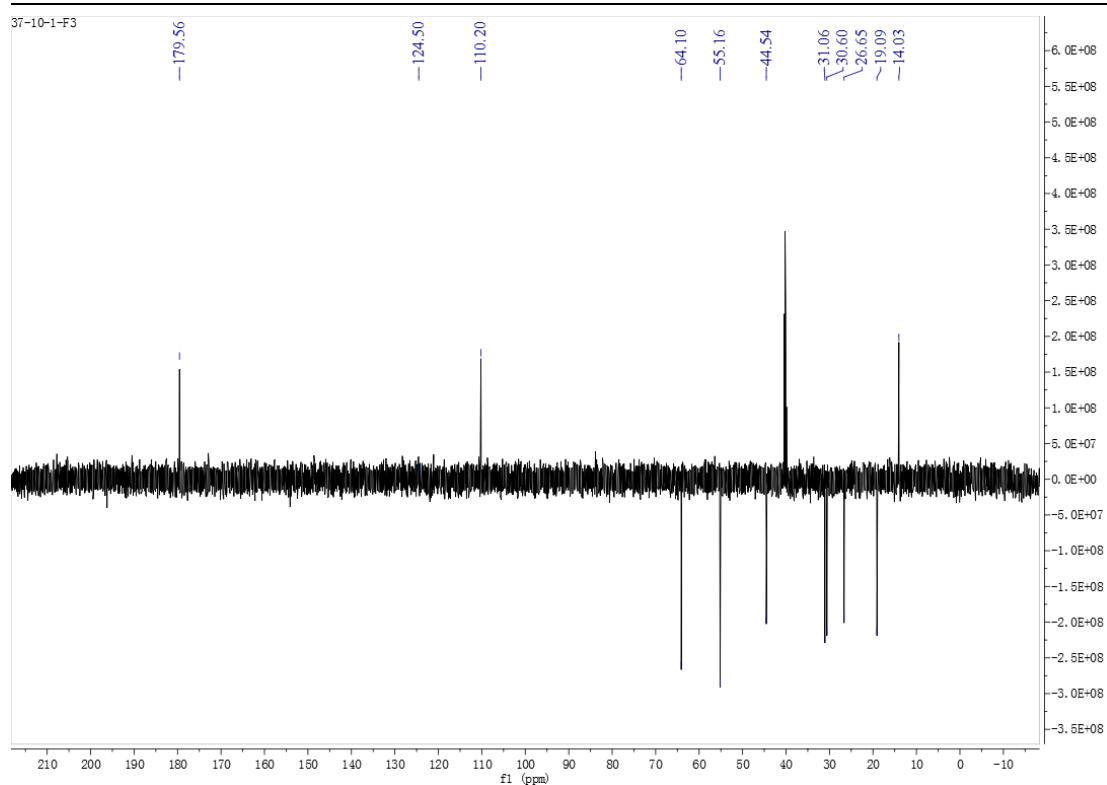


Figure S23. ^1H - ^1H COSY spectrum of compound **4** (DMSO- d_6 , 500 MHz)

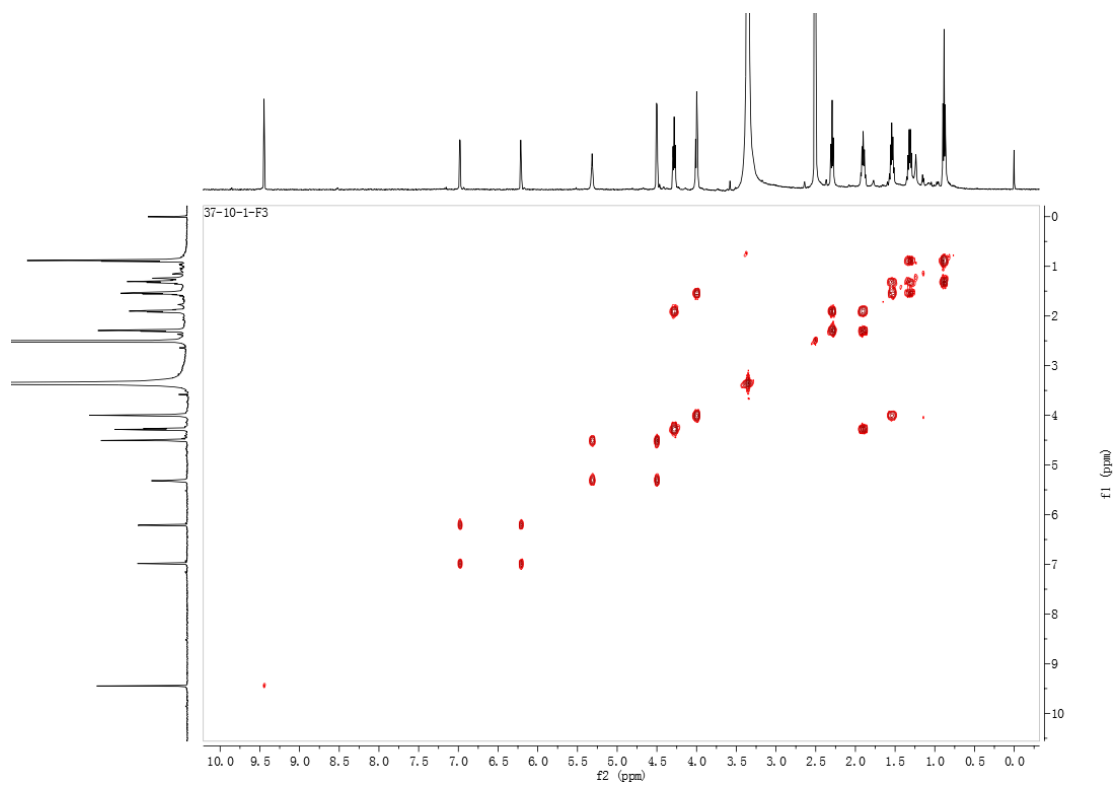


Figure S24. HSQC spectrum of compound **4** (DMSO-*d*₆, 500 MHz)

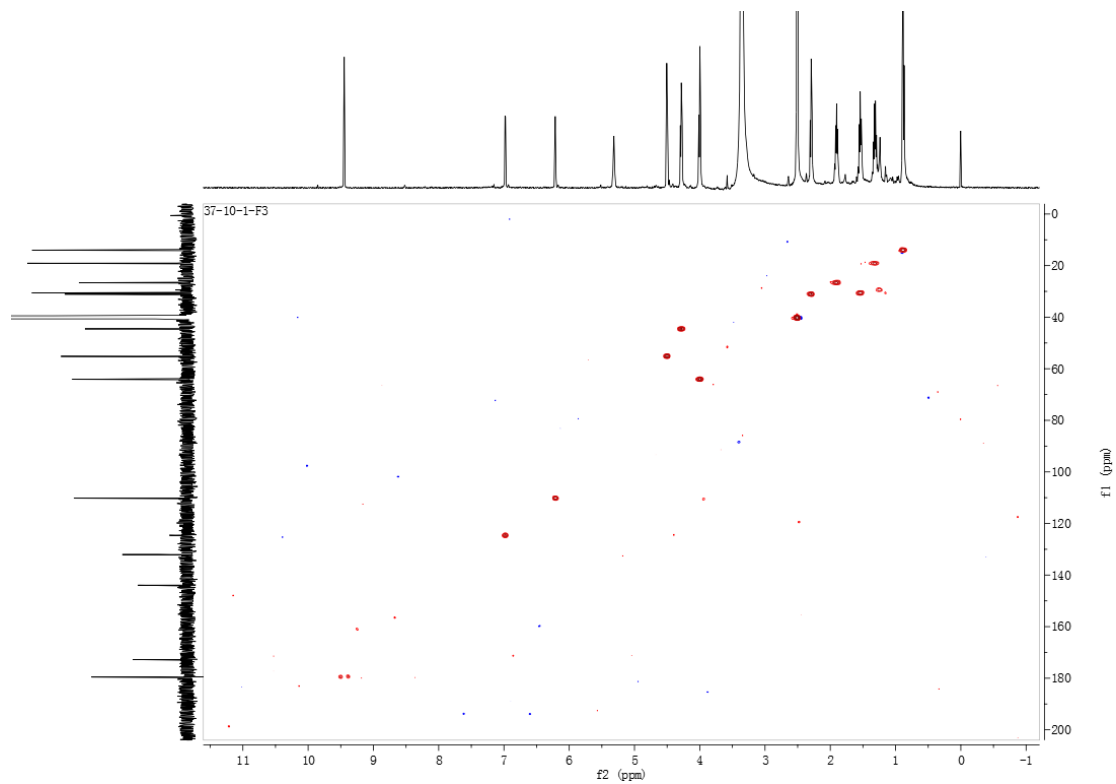


Figure S25. HMBC spectrum of compound **4** (DMSO-*d*₆, 500 MHz)

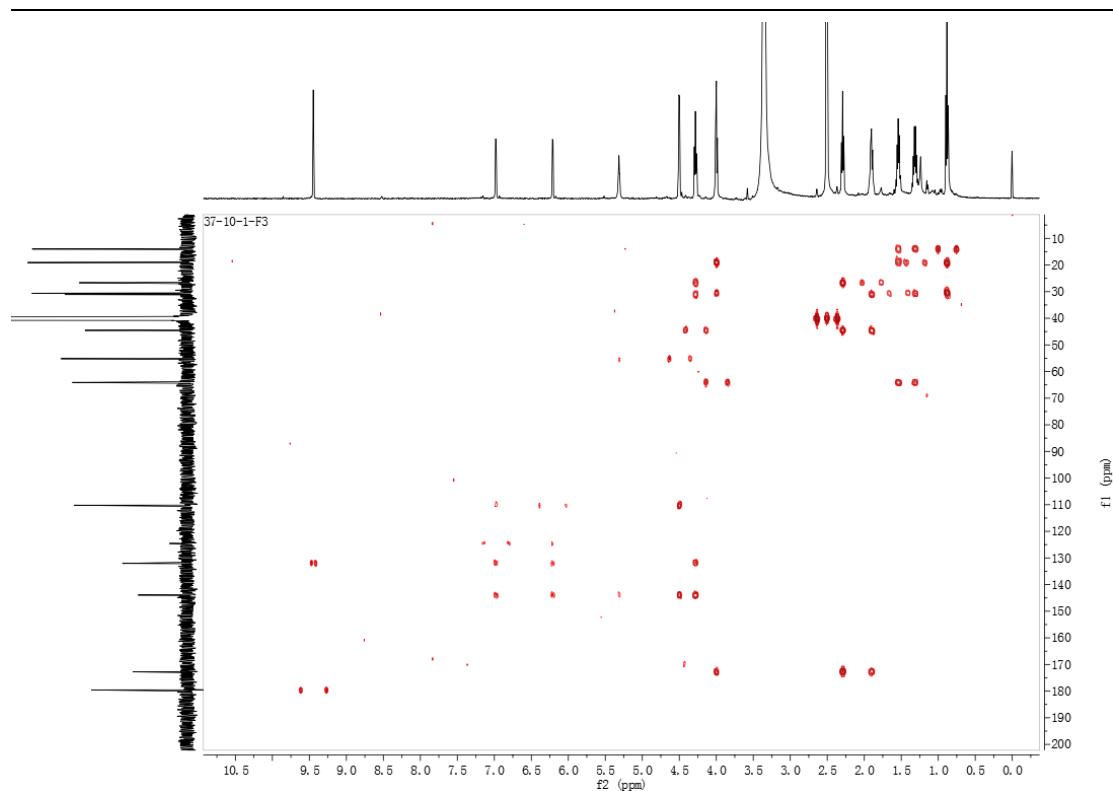


Table S3. Inhibition rates of compounds **1**, **3**, **4** and **GLS4** on HBsAg in HepAD38 cells

Inhibition rate %					
Concentration (μ M)	Comp. 1	Comp. 3	Comp. 4	Concentration (nM)	GLS4
50.000	20.50 $\pm$ 0.29	8.72 $\pm$ 0.21	11.08 $\pm$ 0.37	1000	46.59 $\pm$ 0.14
12.500	23.20 $\pm$ 0.11	5.56 $\pm$ 0.38	12.72 $\pm$ 0.20	250	52.61 $\pm$ 0.22
3.125	6.22 $\pm$ 0.43	5.67 $\pm$ 0.18	9.63 $\pm$ 0.50	62.5	49.53 $\pm$ 0.31
0.781	<5	<5	<5	15.625	35.54 $\pm$ 0.09
0.195	<5	<5	<5	3.906	10.16 $\pm$ 0.17

0.048	<5	<5	<5	0.976	9.92 ± 0.28
0.012	<5	<5	<5	0.244	0.77 ± 0.27
0.003	<5	<5	<5	0.061	2.19 ± 0.25

Table S4. Inhibition rates of compounds **1**, **3**, **4** and **GLS4** on HBV DNA in HepAD38 cells

Inhibition rate %					
Concentration (μM)	Comp. 1	Comp. 3	Comp. 4	Concentration (nM)	GLS4
50.000	10.40 ± 0.16	27.63 ± 0.18	17.26 ± 0.24	1000	90.95 ± 0.38
12.500	12.89 ± 0.22	20.40 ± 0.09	6.78 ± 0.17	250	90.05 ± 0.34
3.125	8.98 ± 0.16	5.35 ± 0.12	<5	62.5	89.56 ± 0.18
0.781	<5	<5	<5	15.625	78.57 ± 0.26
0.195	<5	<5	<5	3.906	30.84 ± 0.29
0.048	<5	<5	<5	0.976	7.80 ± 0.18
0.012	<5	<5	<5	0.244	8.08 ± 0.30
0.003	<5	<5	<5	0.061	10.55 ± 0.33