Supplementary Information

Discovery of highly functionalized 5,6-*seco*-grayanane diterpenoids as potent competitive PTP1B inhibitors

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Contents

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Table S1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Spectroscopic Data for **1**−**3** and **9** in Methanol-d_4.

	1		**2**		**3**		**9**	
no.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		148.4		143.2		143.4		143.9
2	7.13, brs	159.3[a]	7.31, d (2.5)	157.0	7.33, d (2.5)	157.1	7.46, d (2.6)	154.0
3α	4.47, brs	78.8	4.43, d (2.5)	77.8	4.43, d (2.5)	77.8	5.07, d (2.6)	83.1
4		49.7[b]		51.1		51.1		50.28
5		213.3[b]		211.7		211.7		210.3
6		177.8		178.6		178.0		178.5
7α		[c]	2.24, d (16.6)	37.9	2.26, d (16.5)	40.9	2.19, d (16.5)	37.9
7β			2.32, dd (16.6, 0.8)		2.33, d (16.5)		2.35, d (16.5)	
8		56.8		58.1		53.9		58.0
9β		140.4[b]	3.04, d (8.0)	40.0	3.12, d (7.8)	46.7	3.04, d (7.9)	40.0
10		120.0[b]		143.3		143.1		142.8
11α	2.17, overlap	24.4	1.76, m	25.8	1.73, m	24.6	1.77, m	25.7
11β	2.66, dd (15.9, 7.7)		2.02, m		2.01, m		2.03, m	
12α	1.92, ddd (15.9, 7.7, 3.3)	25.7	1.86, m	25.2	1.86, overlap	27.3	1.86, overlap	25.2
12β	1.67, m		1.86, m		1.92, overlap		1.86, overlap	
13α	2.44, m	51.5	2.82, t (2.7)	50.3	2.44, t (2.7)	52.6	2.81, m	50.27
14α	4.29, s	95.9	4.63, s	91.3	4.62, s	91.3	4.61, s	91.2
15α	2.24, overlap	55.1	5.30, q (1.6)	128.1	2.21, d (15.2)	55.1	5.31, s	128.1
15β	1.87, overlap				1.91, d (15.2)			
16		81.7		142.5		80.9		142.4
17	1.43, s	25.8	1.80, d (1.6)	15.3	1.42, s	25.1	1.80, d (1.5)	15.3
18	1.04, brs	20.6[a]	1.01, s	21.1	1.01, s	21.1	1.10, s	21.4
19	1.14, brs	23.7[a]	1.12, s	23.4	1.12, s	23.4	1.21, s	23.5
20	1.70, brs	19.9[a]	6.18, 5.57, s	120.5	6.20, 5.50, s	120.7	6.26, 5.61, s	121.2

[a] NMR data were assigned by HSQC spectrum (Figure S27). [b] NMR data were assigned by HMBC spectrum (Figure S28). [c] NMR data couldn't be assigned by any spectra.

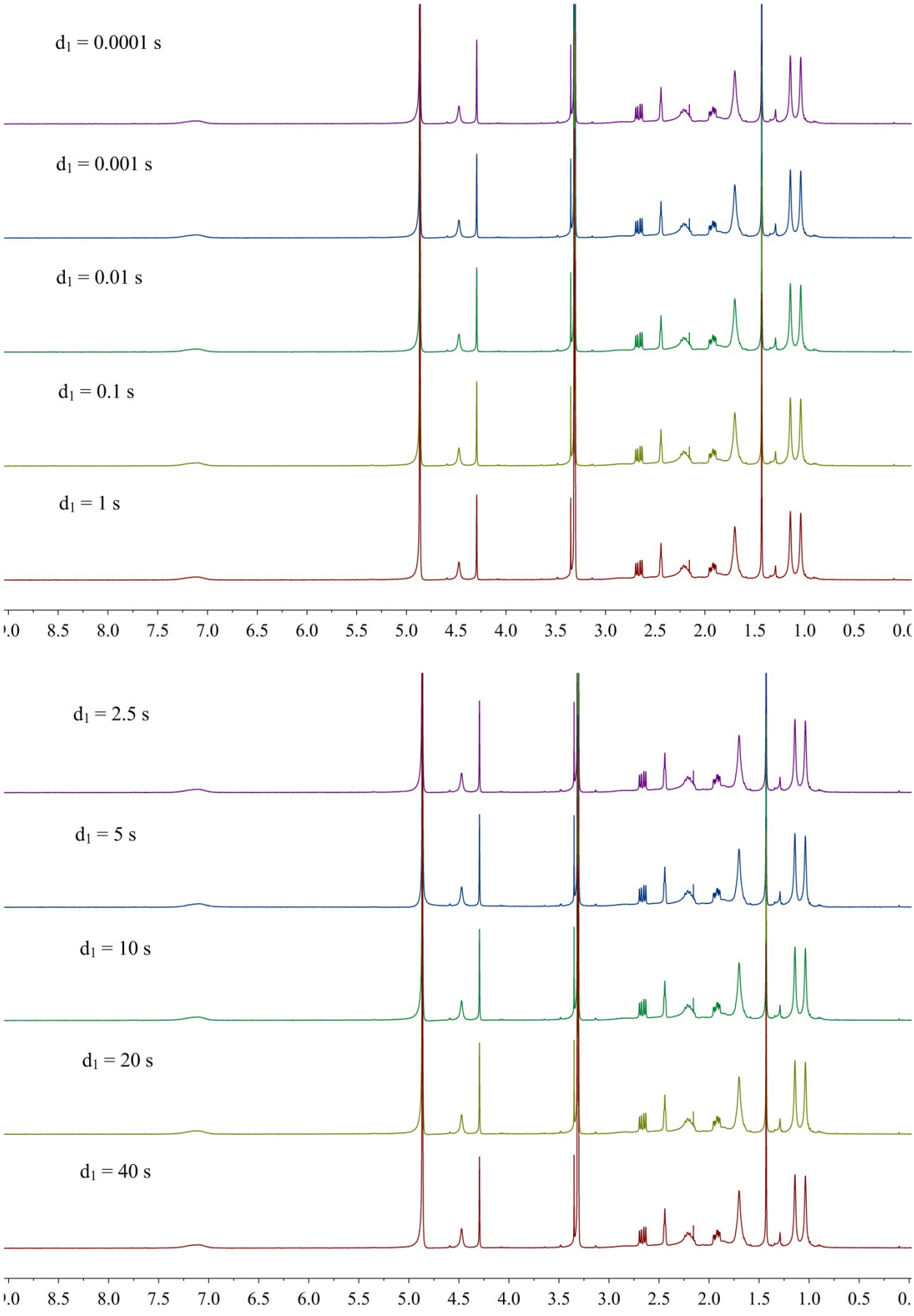


Figure S1. ^{1}H NMR spectra (400 MHz) of **1** in methanol-d_4 with different delay time.

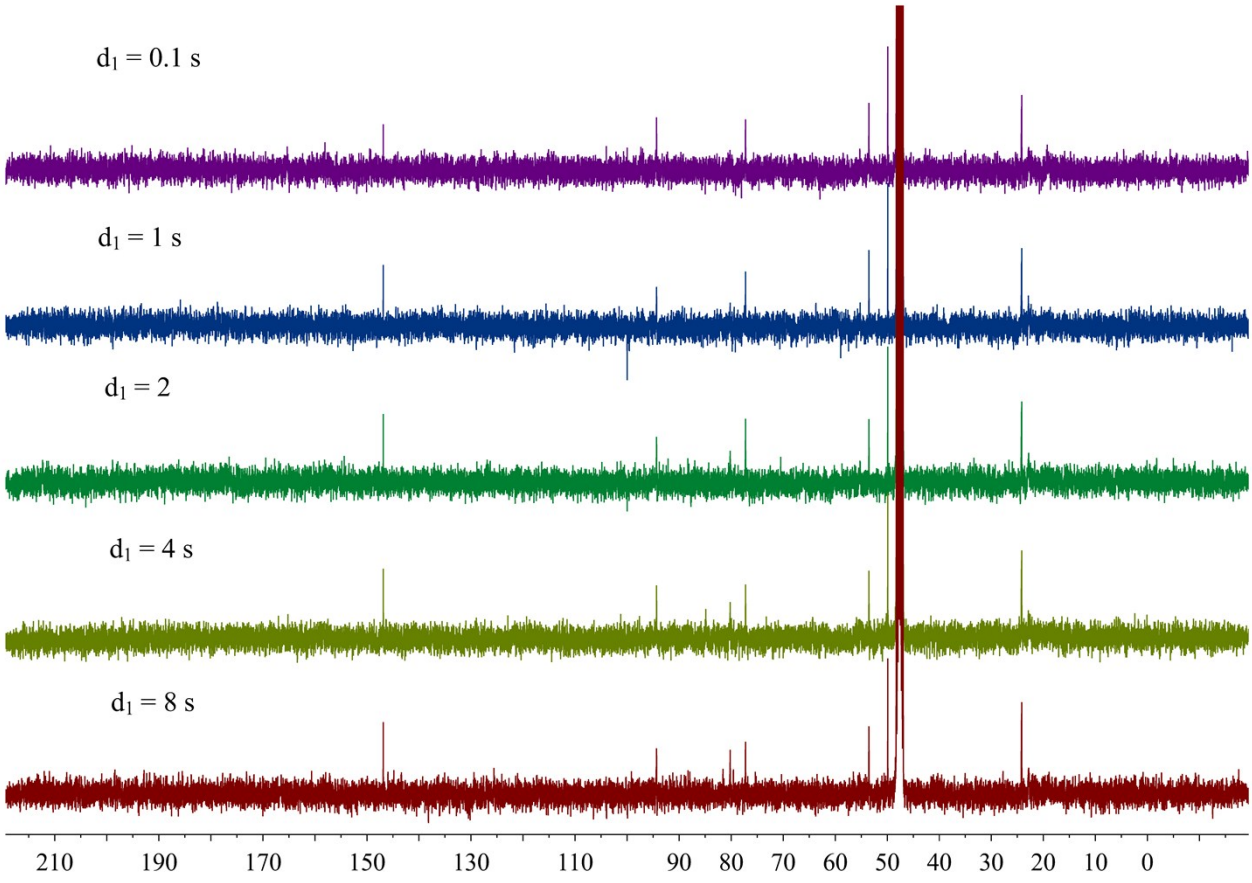


Figure S2. ^{13}C NMR spectra (100 MHz) of **1** in methanol-d_4 with different delay time.

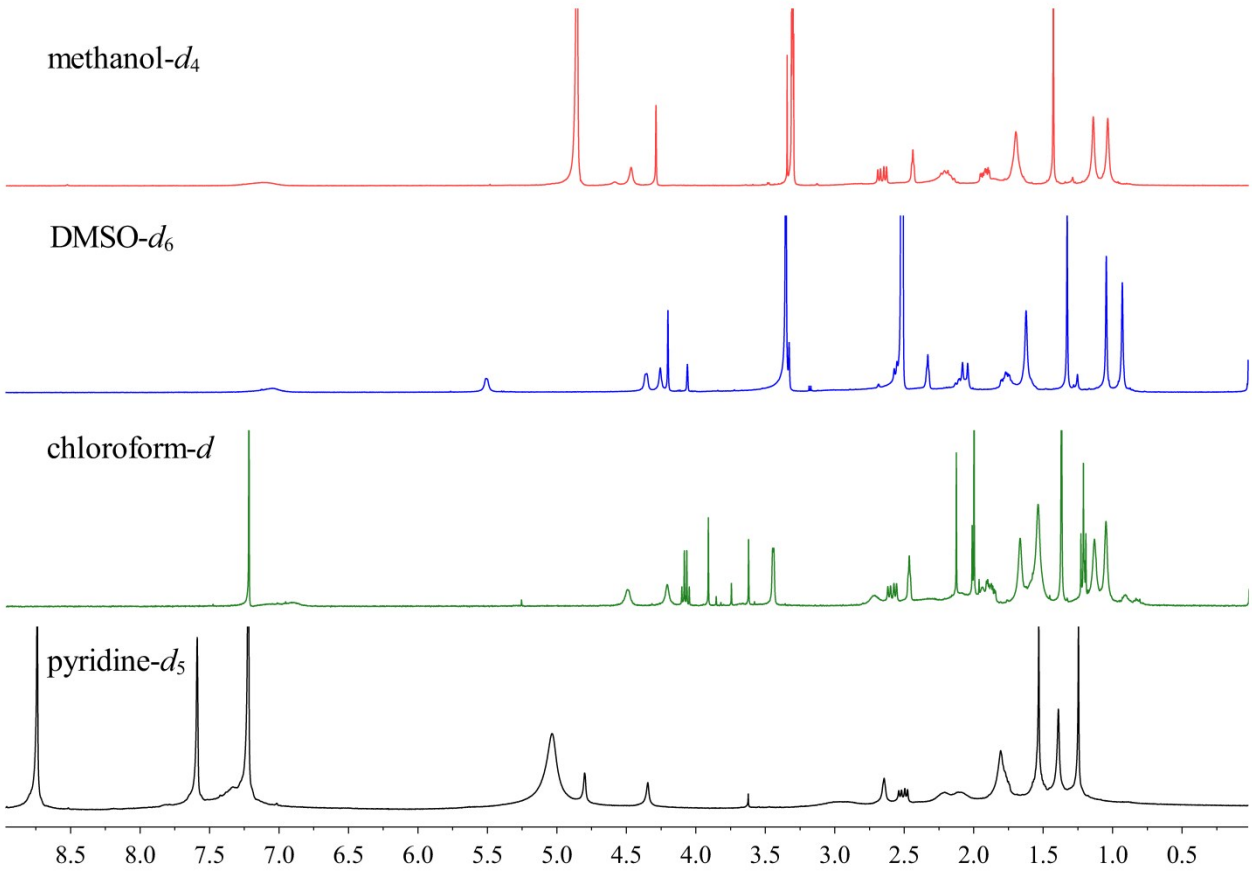


Figure S3. ^{1}H NMR spectra (400 MHz) of **1** in different solvents.

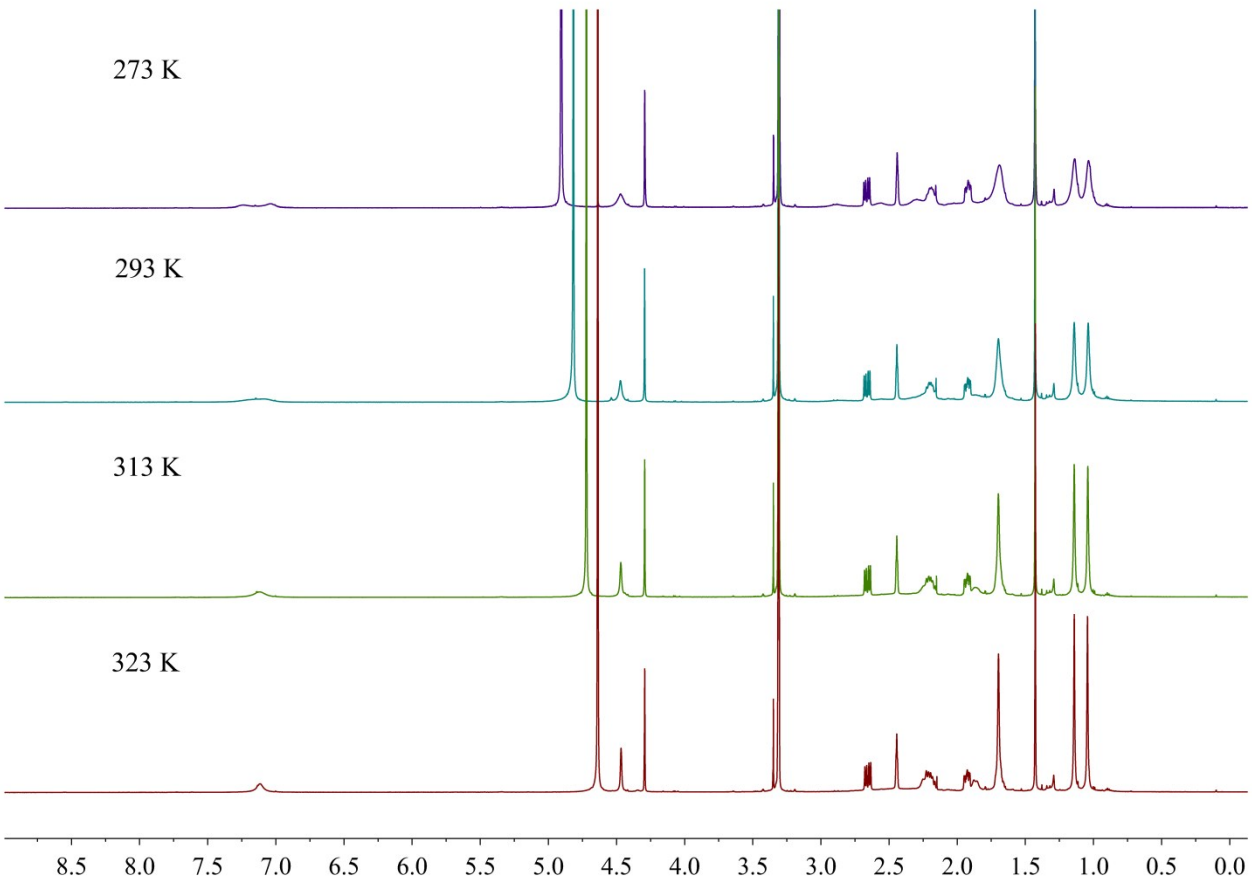


Figure S4. ^{1}H NMR experiments (600 MHz) of **1** in methanol-d_4 in different temperature.

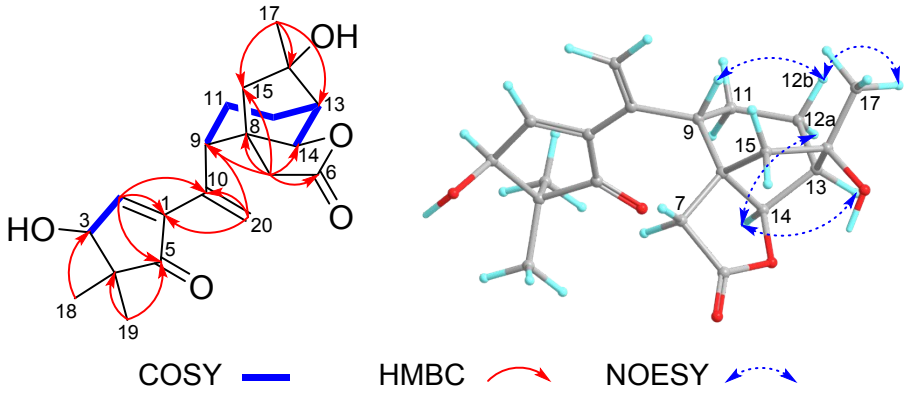


Figure S5. Selected ^{1}H–^{1}H COSY, HMBC, and NOESY correlations of **3**.

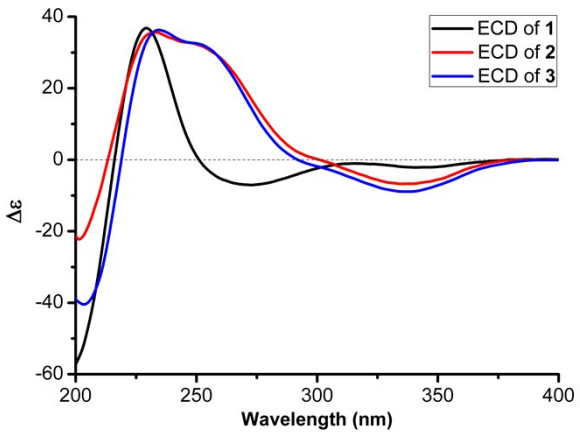
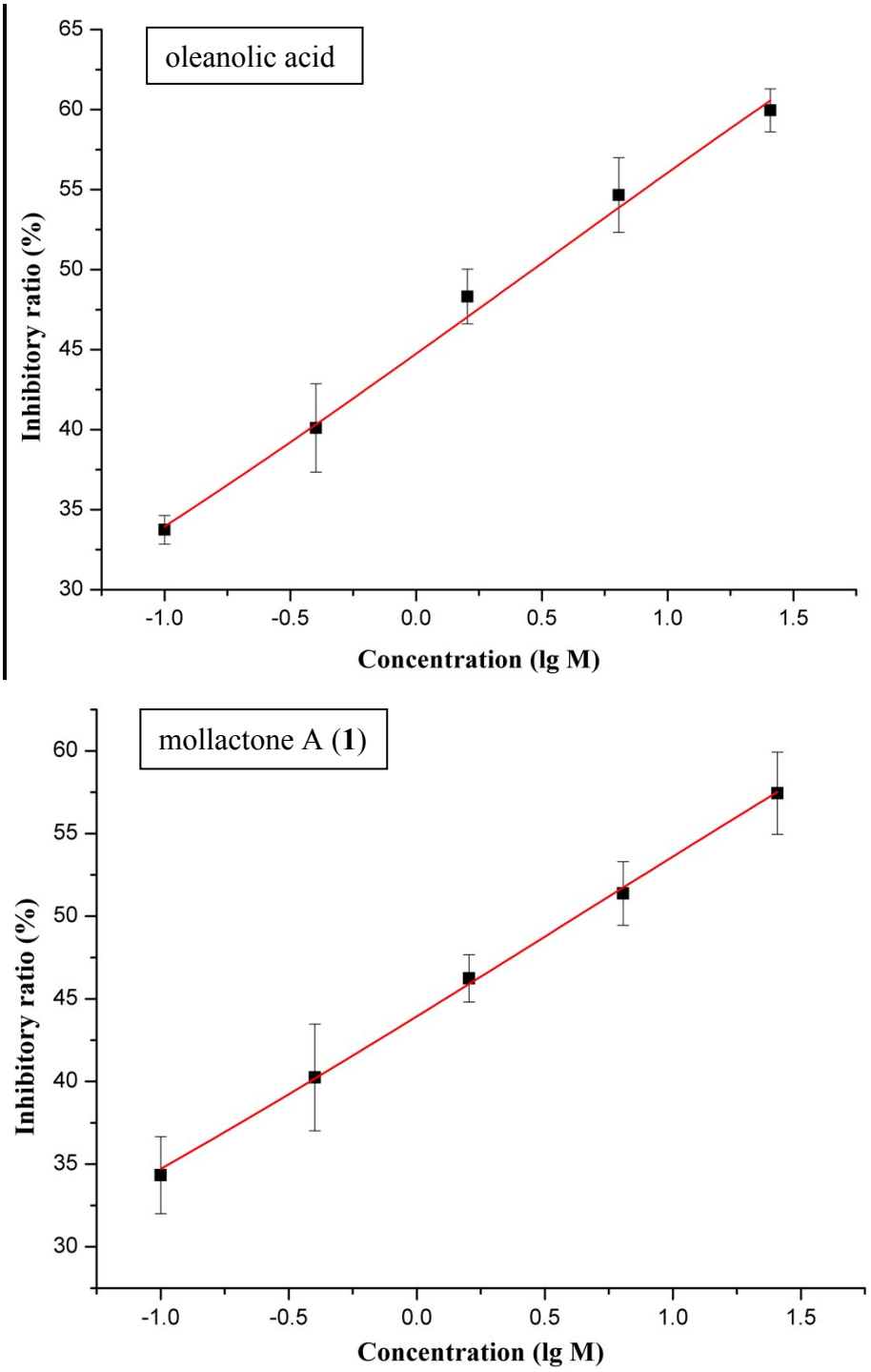
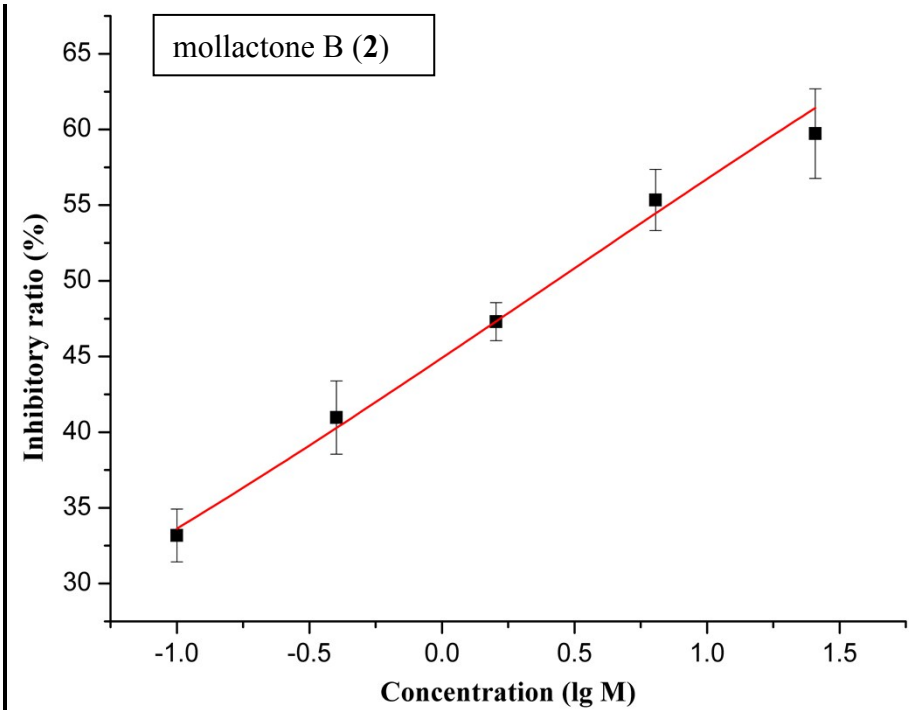
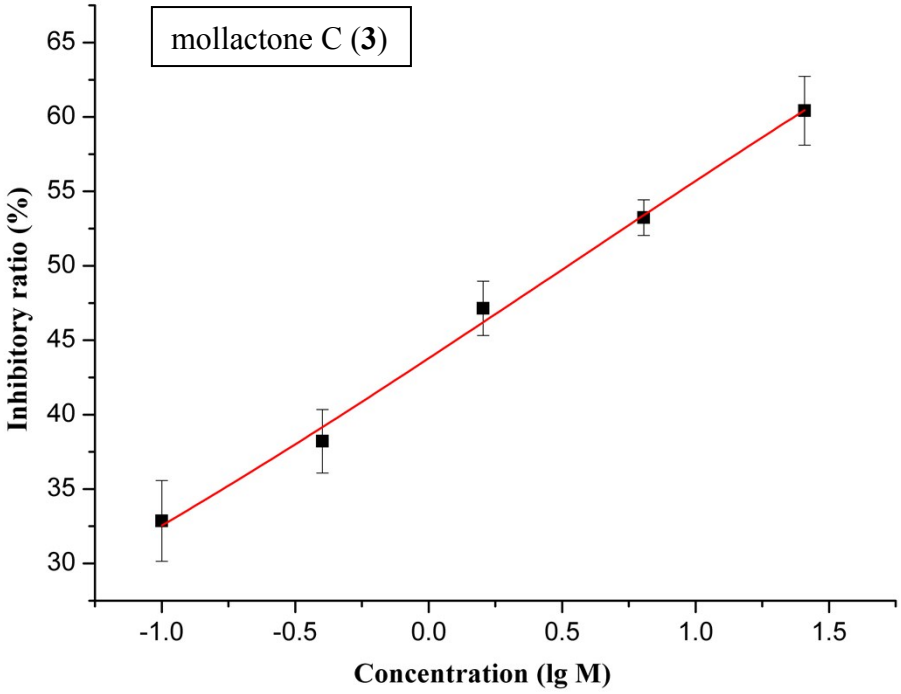


Figure S6. Experimental ECD spectra of **1**−**3**.




mollactone B (2)
Inhibitory ratio (%)
65
60
55
50
45
40
35
30
-1.0
-0.5
0.0
0.5
1.0
1.5
Concentration (lg M)


mollactone C (3)
Inhibitory ratio (%)
65
60
55
50
45
40
35
30
-1.0
-0.5
0.0
0.5
1.0
1.5
Concentration (lg M)

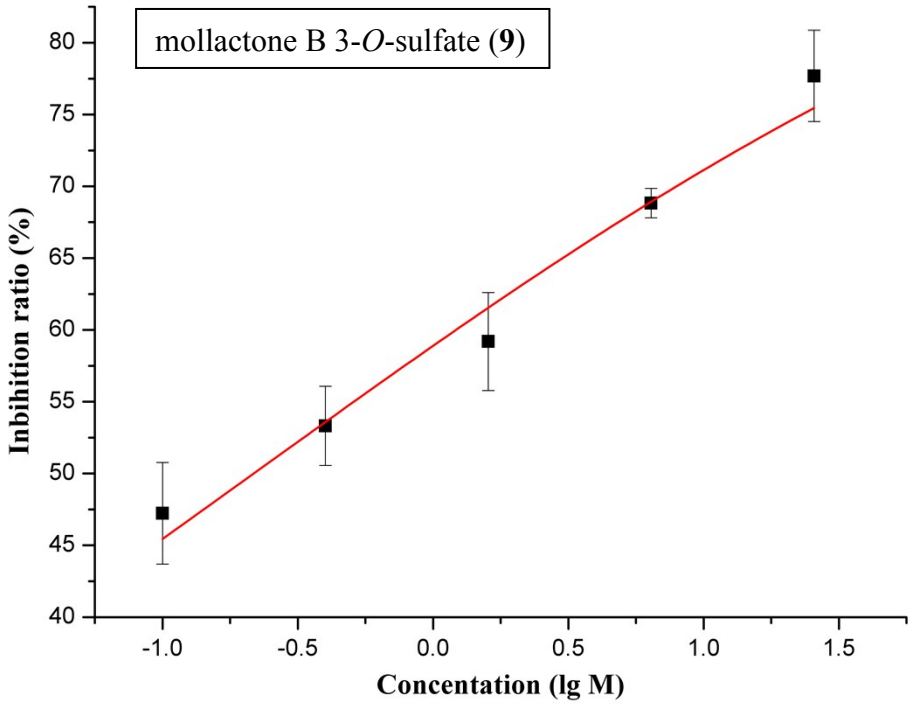


Figure S7. Concentration−inhibition ratio curves of oleanolic acid and compounds **1**−**3** and **9**.

The final concentration of oleanolic acid and compounds **1**−**3** and **9** were determined as 0.1, 0.4, 1.6, 6.4, and 25.6 μM.

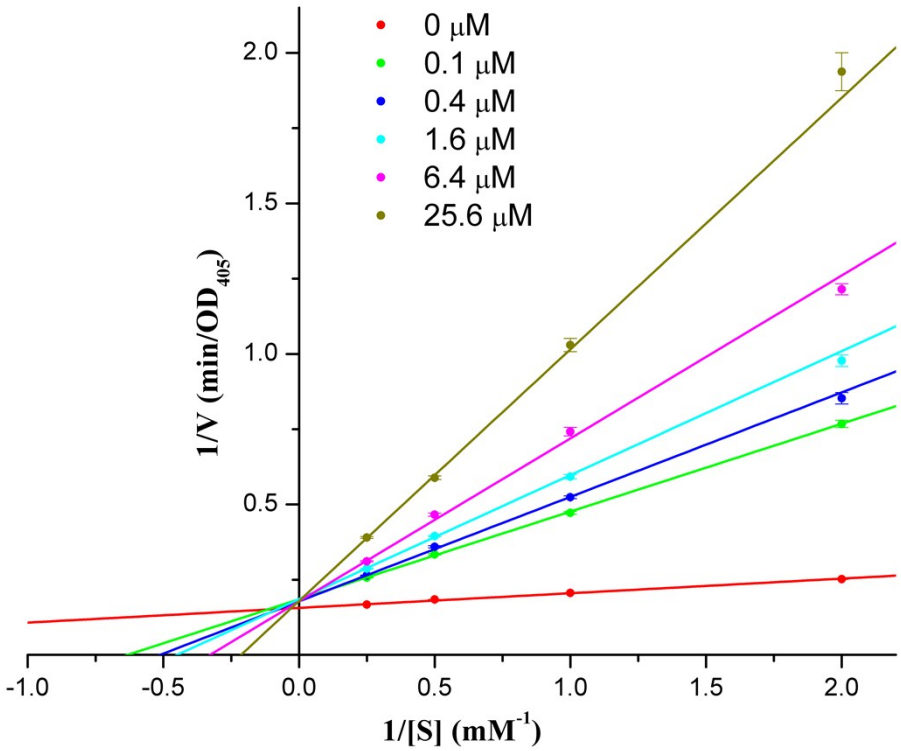


Figure S8. Lineweaver-Burk plot for the inhibition of PTP1B by mollactone A (**1**).

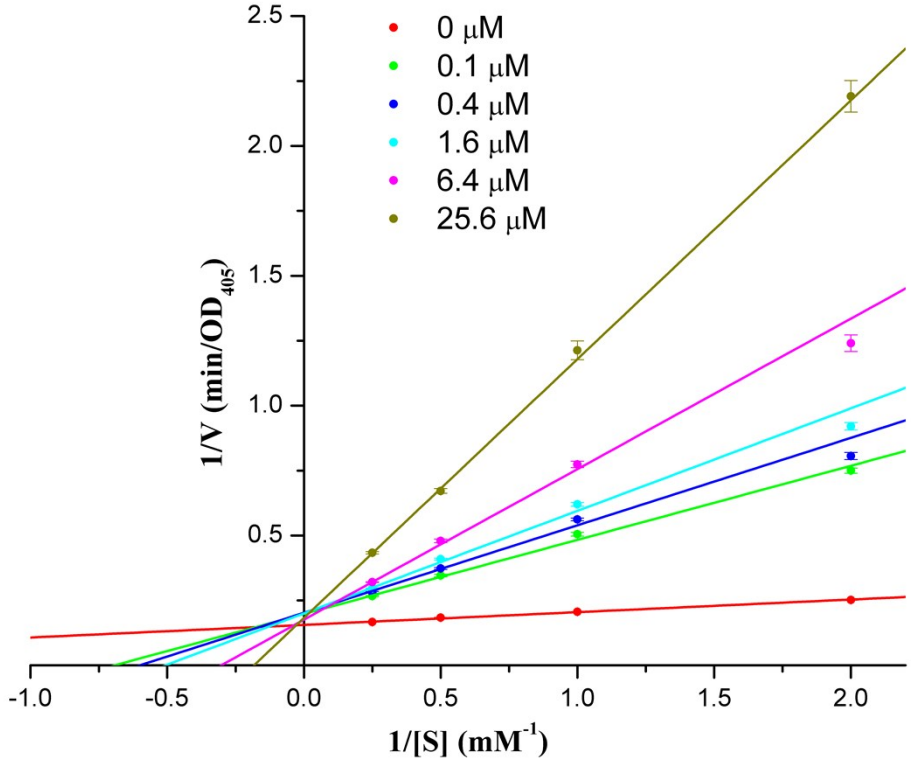


Figure S9. Lineweaver-Burk plot for the inhibition of PTP1B by mollactone B (**2**).

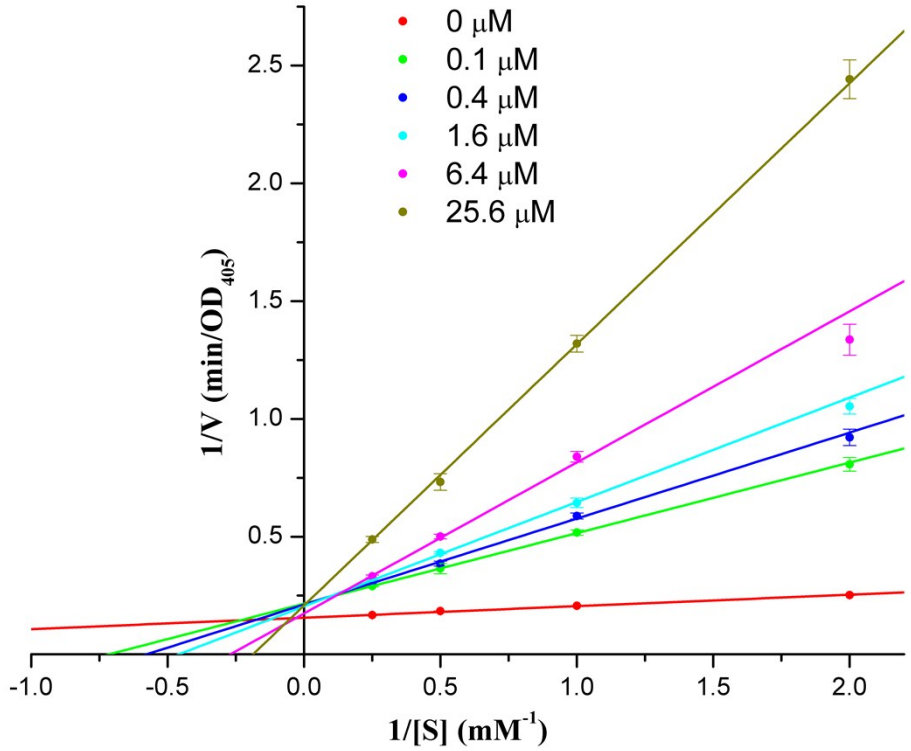


Figure S10. Lineweaver-Burk plot for the inhibition of PTP1B by mollactone C (**3**).

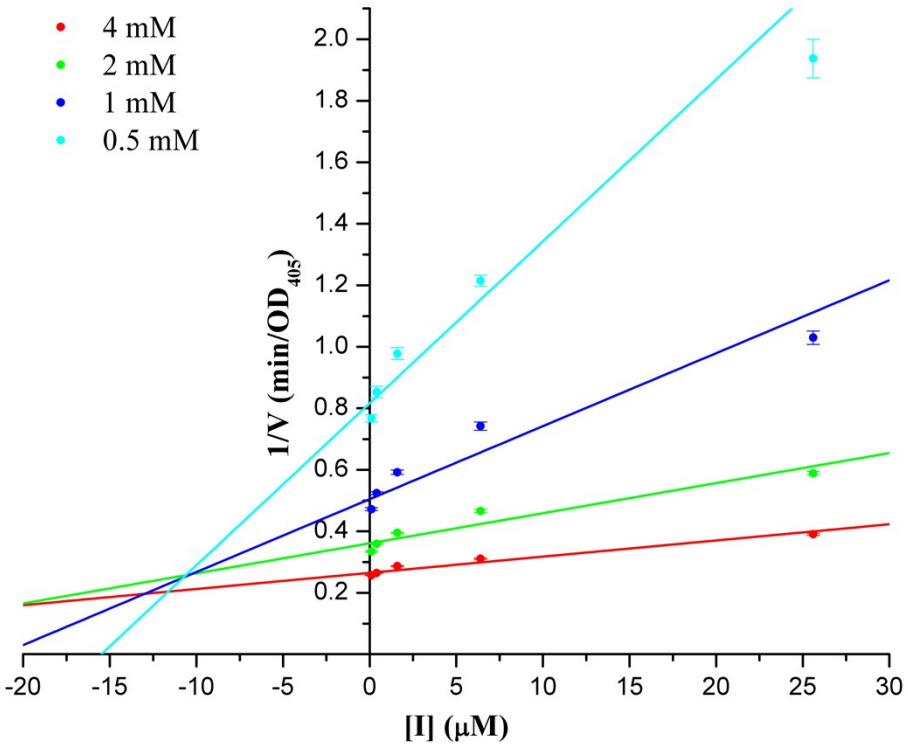


Figure S11. Dixon plot for the inhibition of the PTP1B by mollactone A (**1**).

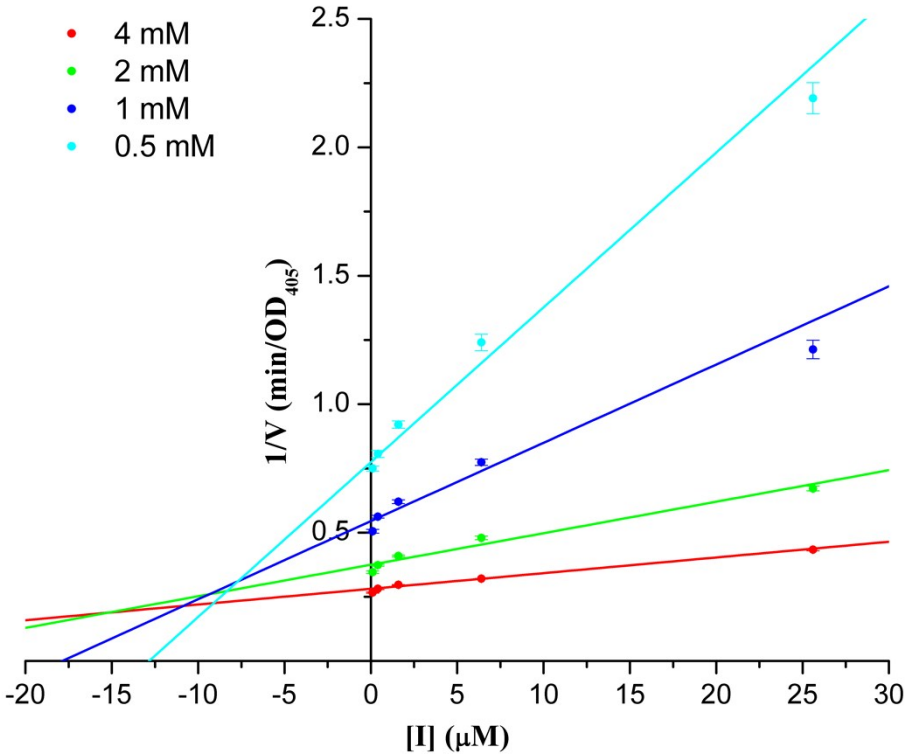


Figure S12. Dixon plot for the inhibition of the PTP1B by mollactone B (**2**).

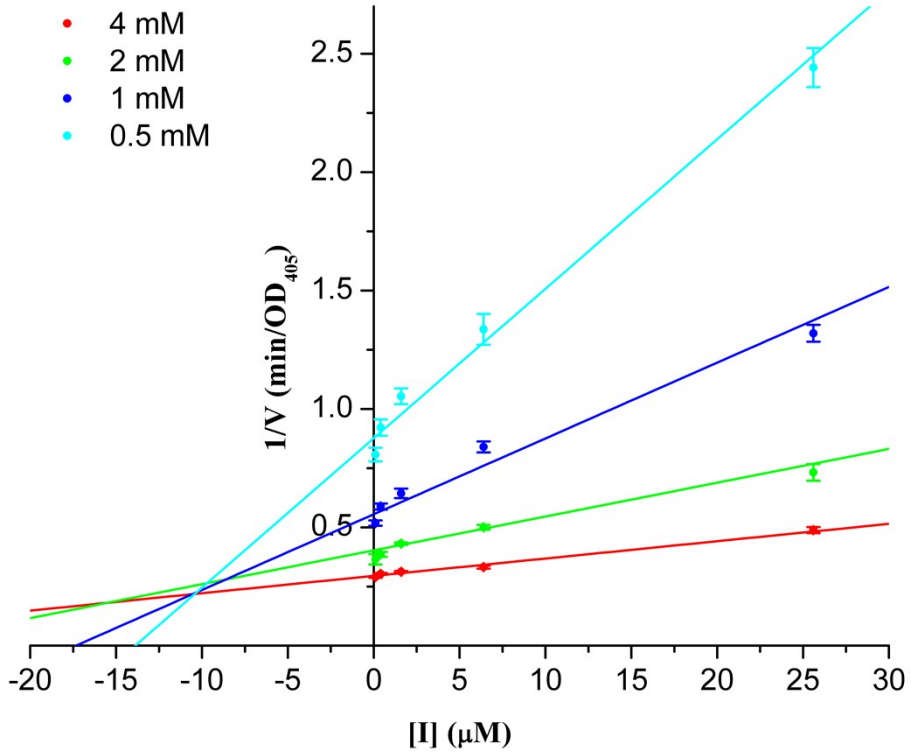


Figure S13. Dixon plot for the inhibition of the PTP1B by mollactone C (**3**).

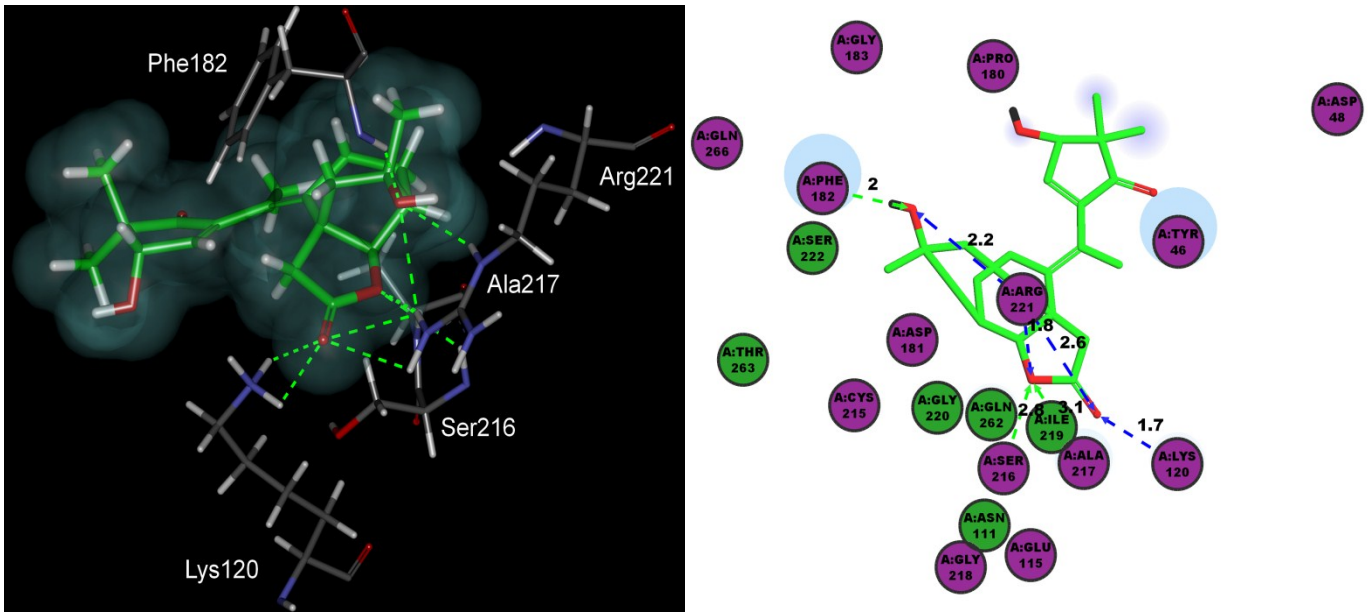


Figure S14. Molecular docking models for PTP1B inhibition of mollactone A (**1**).

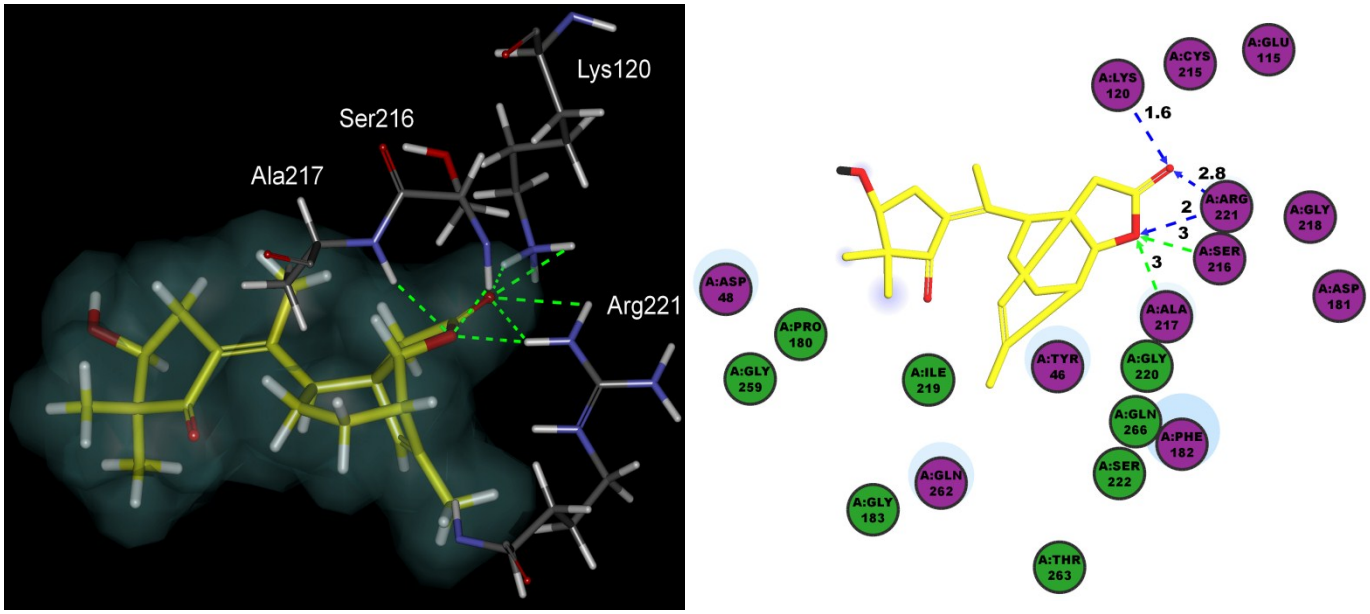


Figure S15. Molecular docking models for PTP1B inhibition of mollactone B (**2**).

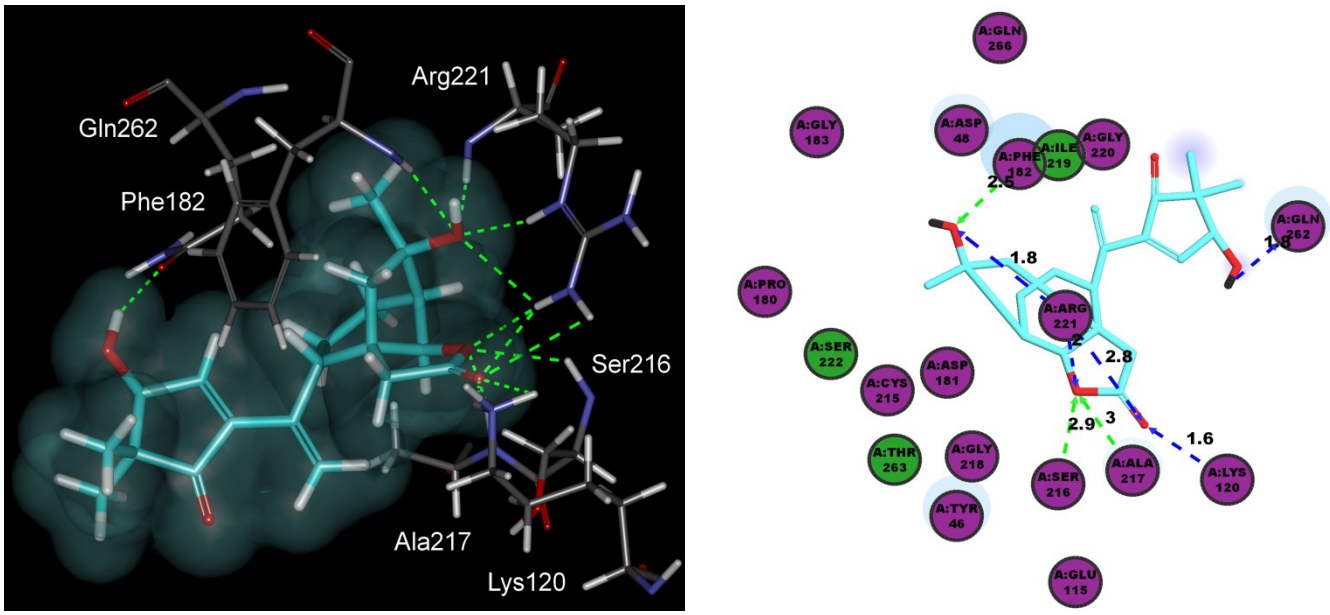


Figure S16. Molecular docking models for PTP1B inhibition of mollactone C (**3**).

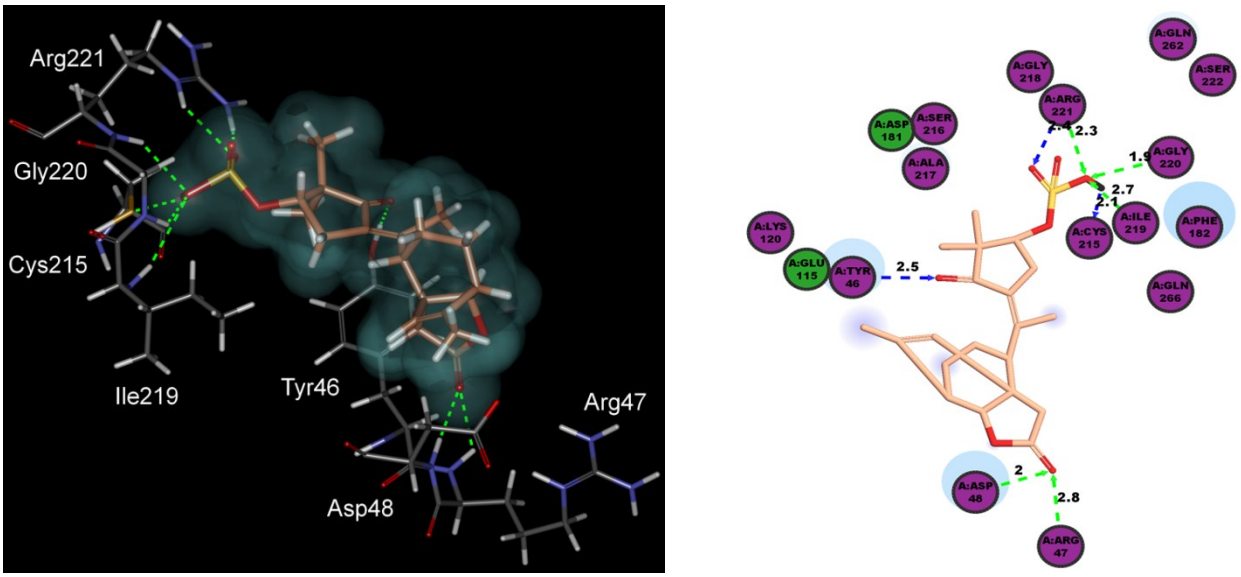


Figure S17. Molecular docking models for PTP1B inhibition of mollactone B 3-*O*-sulfate (**9**).

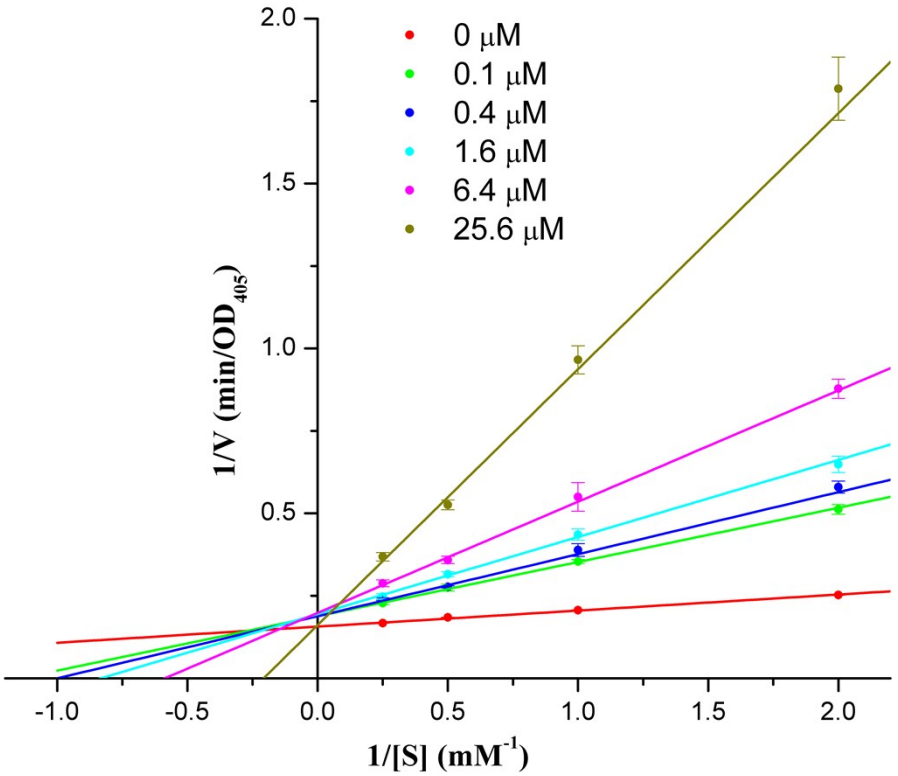


Figure S18. Lineweaver-Burk plot for the inhibition of PTP1B by mollactone B 3-*O*-sulfate (**9**).

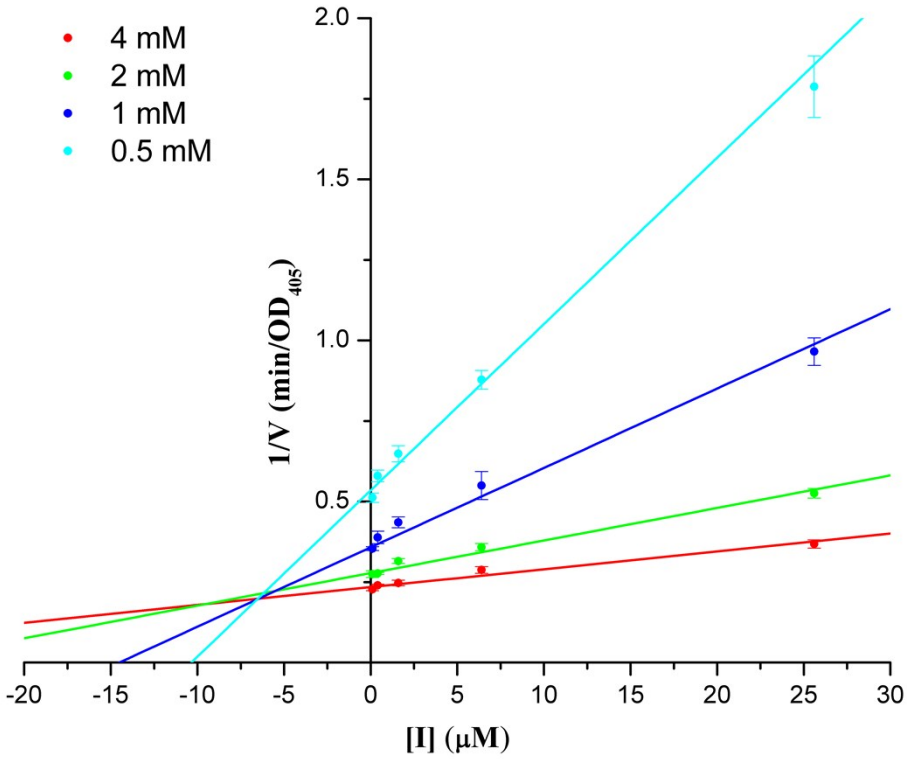


Figure S19. Dixon plot for the inhibition of the PTP1B by mollactone B 3-*O*-sulfate (**9**).

Table S2. Effect of different concentrations of mollactone A (**1**) on V_{max}, K_m, and the K_{ik} to K_{iv} ratio using *p*NPP as a substrate.

V_{max} and K_m values were calculated according to Lineweaver-Burk from the data shown in Fig. S10. K_{ik}/K_{iv} ratio was calculated according to Yang et al.

Inhibitor (**1**)	Substrate (*p*NPP)		
[I] (μM)	V_{max}	K_m	K_{ik}/K_{iv}
0	6.39	0.31	-
0.1	5.41	1.57	22.44
0.4	5.61	1.89	36.66
1.6	5.38	1.97	28.52
6.4	5.61	2.03	39.91
25.6	5.57	1.56	27.39

Table S3. Effect of different concentrations of mollactone B (**2**) on V_{max}, K_m, and the K_{ik} to K_{iv} ratio using *p*NPP as a substrate.

V_{max} and K_m values were calculated according to Lineweaver-Burk from the data shown in Fig. S12. K_{ik}/K_{iv} ratio was calculated according to Yang et al.

Inhibitor (**2**)	Substrate (*p*NPP)		
[I] (μM)	V_{max}	K_m	K_{ik}/K_{iv}
0	6.39	0.31	-
0.1	5.04	1.42	13.37
0.4	4.93	1.60	14.05
1.6	4.98	1.70	15.84
6.4	5.67	2.01	43.19
25.6	5.46	1.54	23.29

Table S4. Effect of different concentrations of mollactone C (**3**) on V_{max}, K_m, and the K_{ik} to K_{iv} ratio using *p*NPP as a substrate.

V_{max} and K_m values were calculated according to Lineweaver-Burk from the data shown in Fig. S14. K_{ik}/K_{iv} ratio was calculated according to Yang et al.

Inhibitor (**3**)	Substrate (*p*NPP)		
[I] (μM)	V_{max}	K_m	K_{ik}/K_{iv}
0	6.39	0.31	-
0.1	4.64	1.38	9.15
0.4	4.72	1.66	12.31
1.6	4.89	1.89	16.62
6.4	5.74	2.32	57.26
25.6	4.80	1.58	12.37

Table S5. Effect of different concentrations of mollactone B 3-*O*-sulfate (**9**) on V_{max}, K_m, and the K_{ik} to K_{iv} ratio using *p*NPP as a substrate.

V_{max} and K_m values were calculated according to Lineweaver-Burk from the data shown in Fig. S16. K_{ik}/K_{iv} ratio was calculated according to Yang et al.

Inhibitor (**9**)	Substrate (*p*NPP)		
[I] (μM)	V_{max}	K_m	K_{ik}/K_{iv}
0	6.39	0.31	-
0.1	5.32	0.86	8.82
0.4	5.32	0.95	10.26
1.6	5.14	0.97	8.75
6.4	5.05	0.88	6.93
25.6	6.22	1.01	82.62

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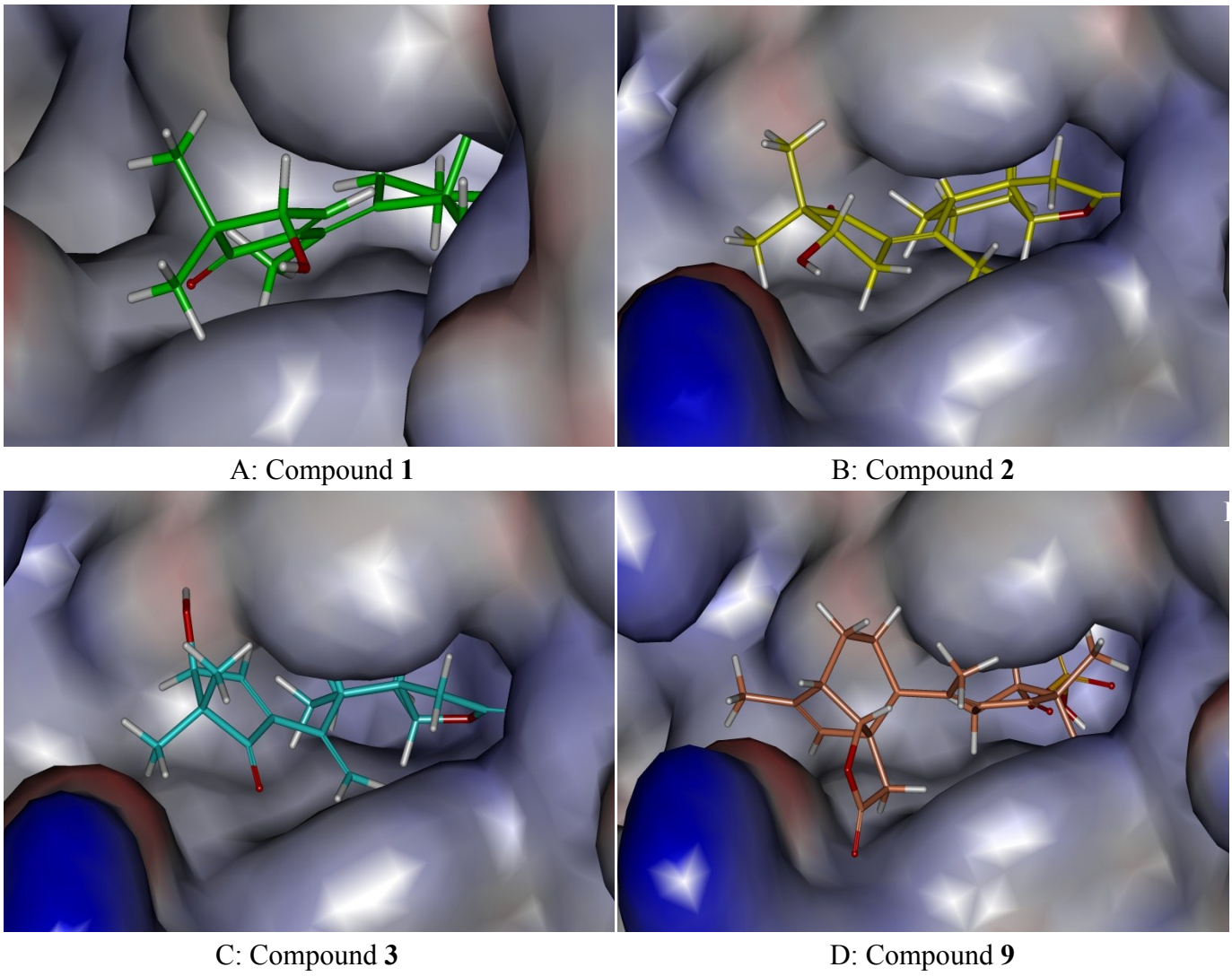


A: Compound **1** B: Compound **2**

C: Compound **3** D: Compound **9**

Figure S20. The docked conformation of mollactones A−C (**1**−**3**) and mollactone B 3-*O*-sulfate (**9**) in the binding pocket of PTP1B.

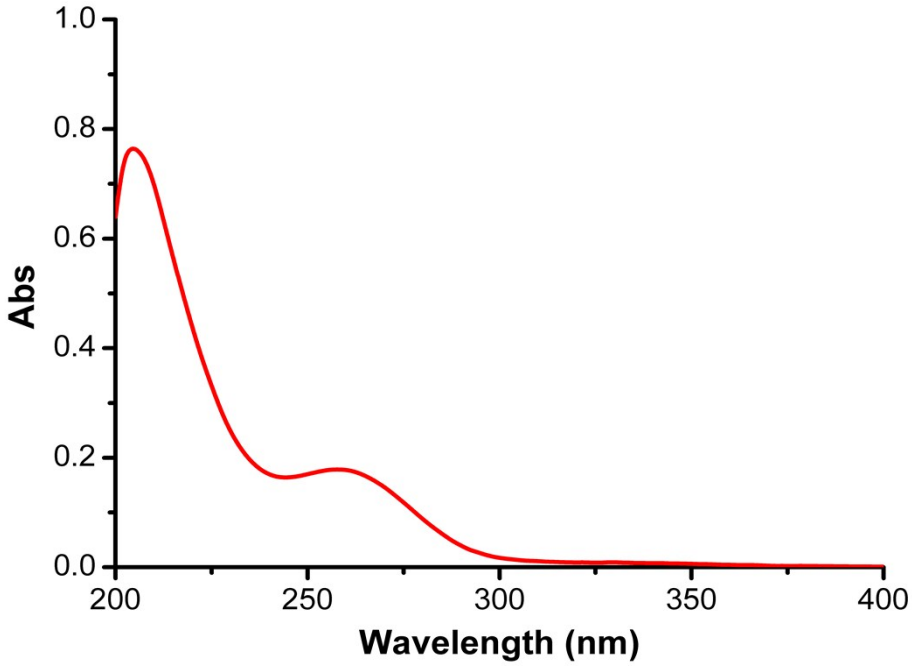


Figure S21. The UV spectrum of **1** in methanol.

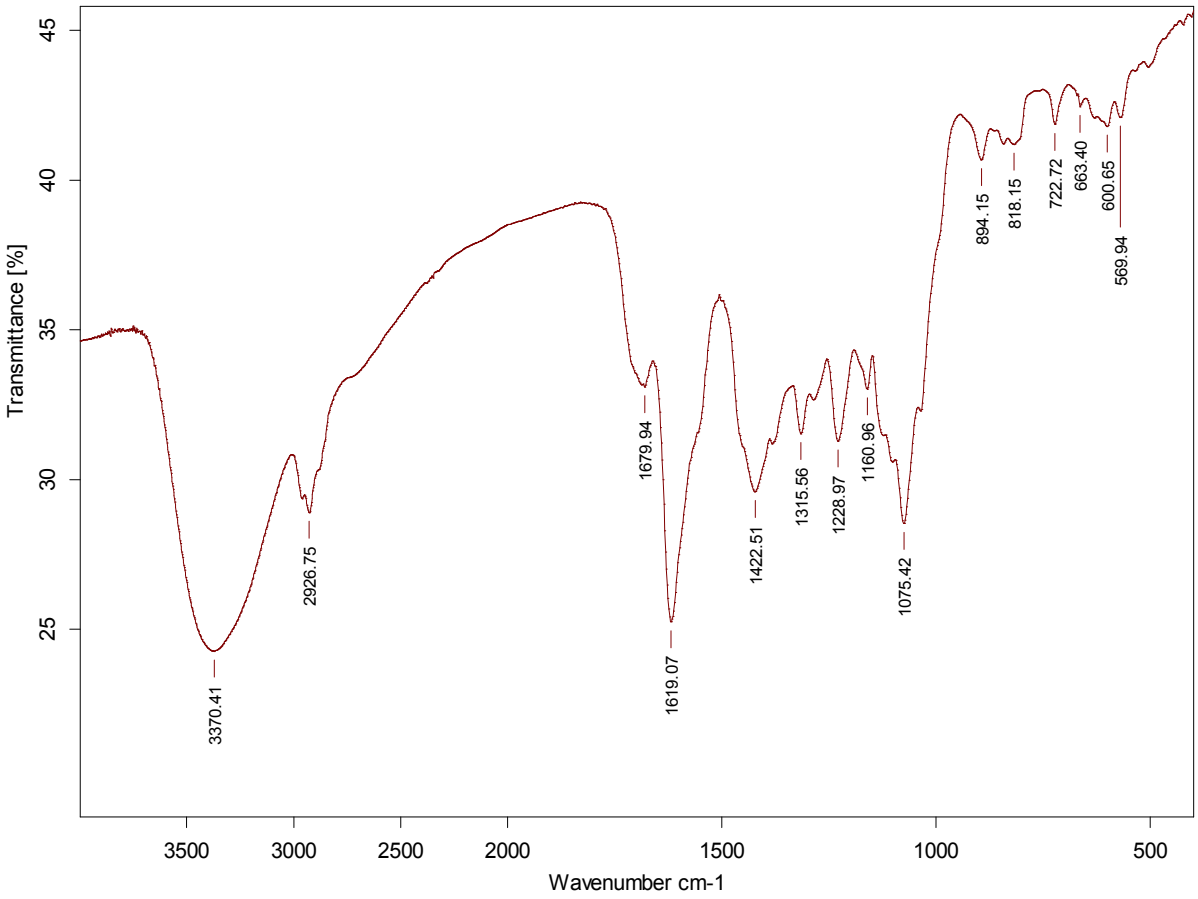


Figure S22. The IR spectrum of **1**.

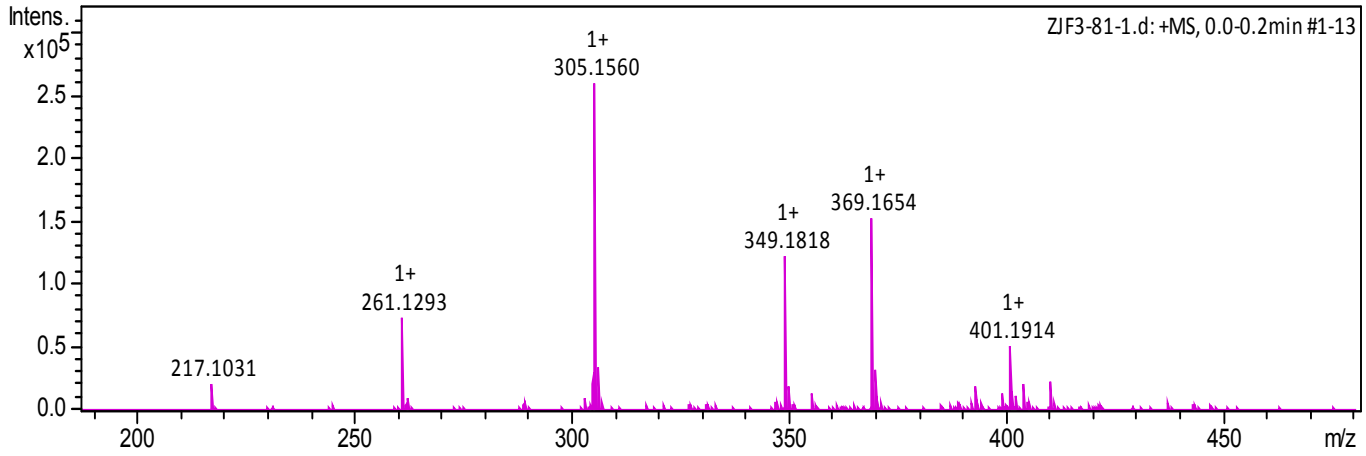


Figure S23. HR-ESI-MS spectrum of **1**.

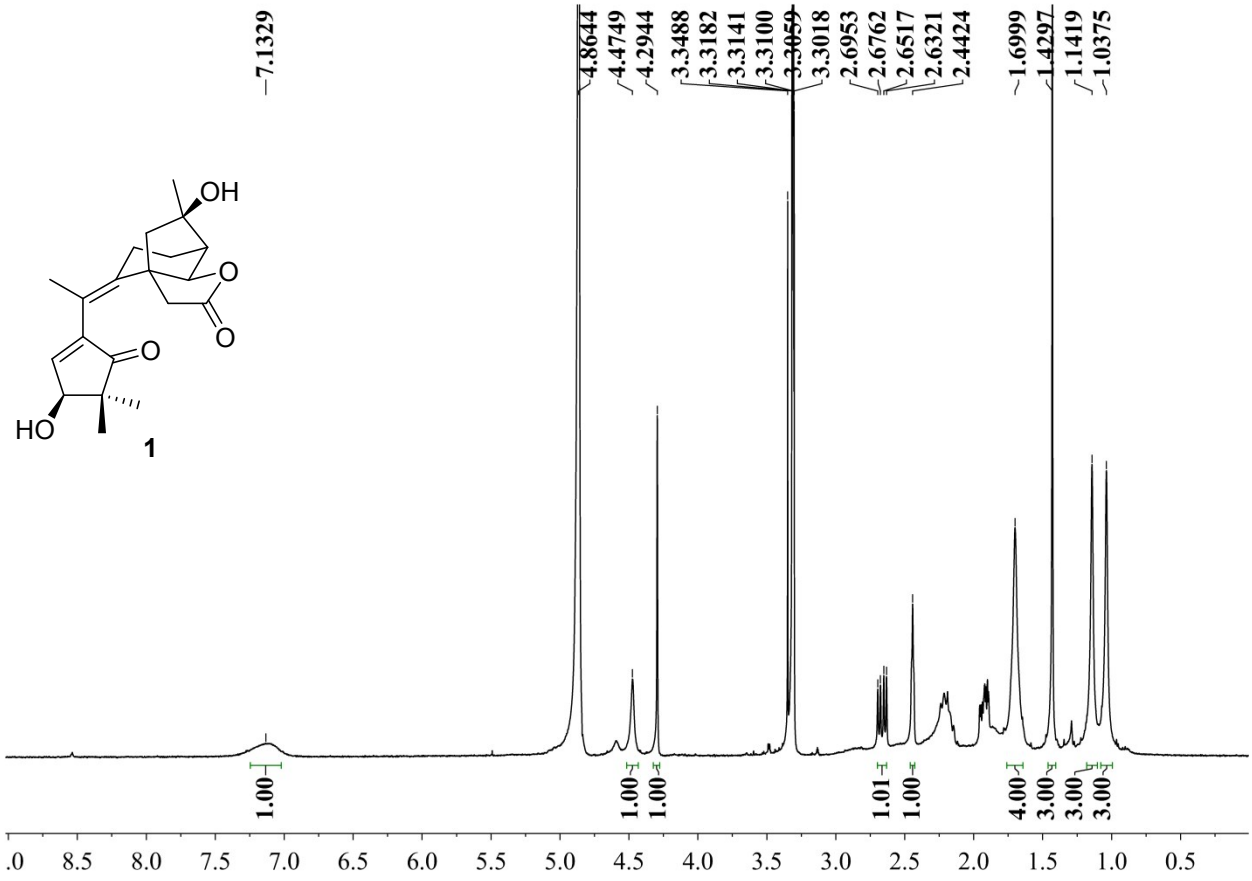


Figure S24. ^{1}H NMR spectrum of **1** (400 MHz, methanol-d_4)

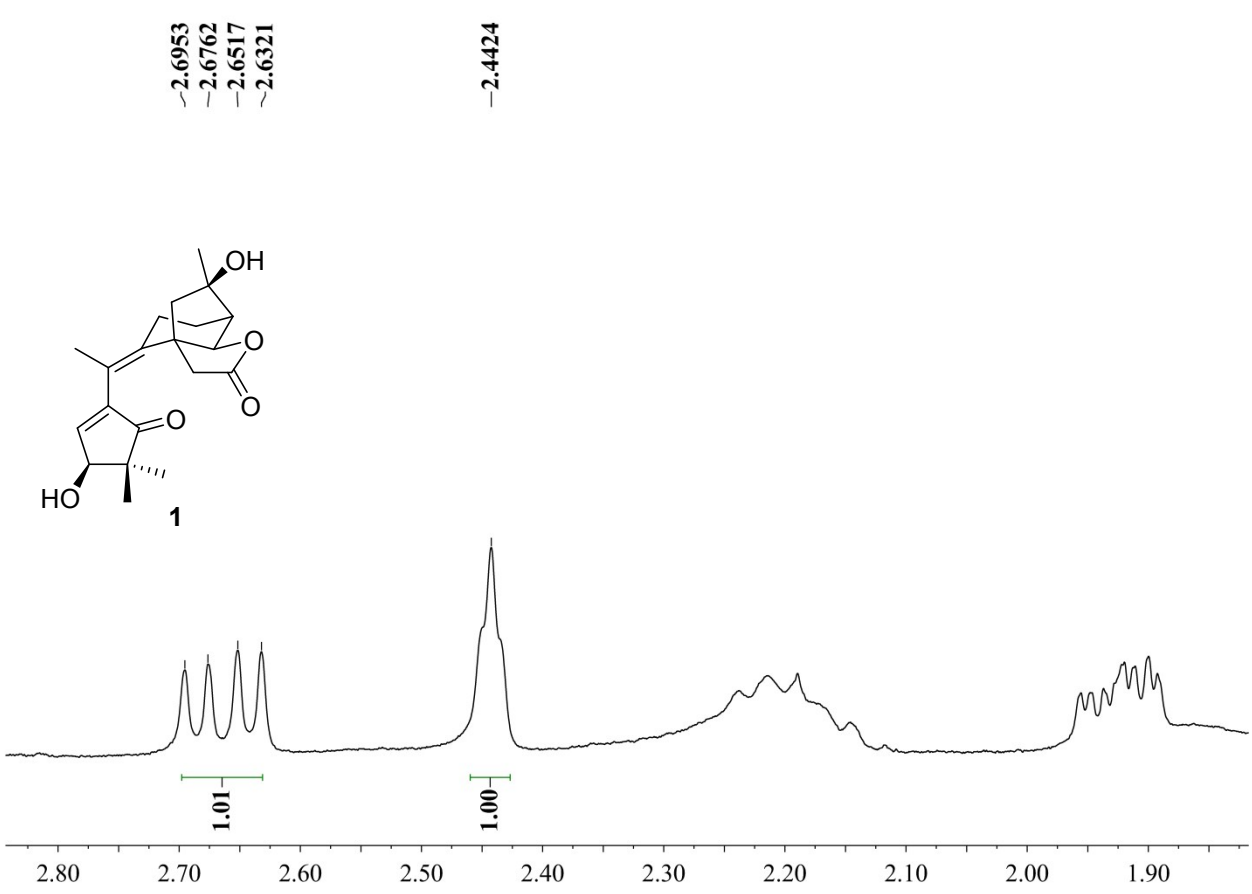


Figure S25. ^{1}H NMR spectrum of **1** (400 MHz, methanol-d_4, amplified)

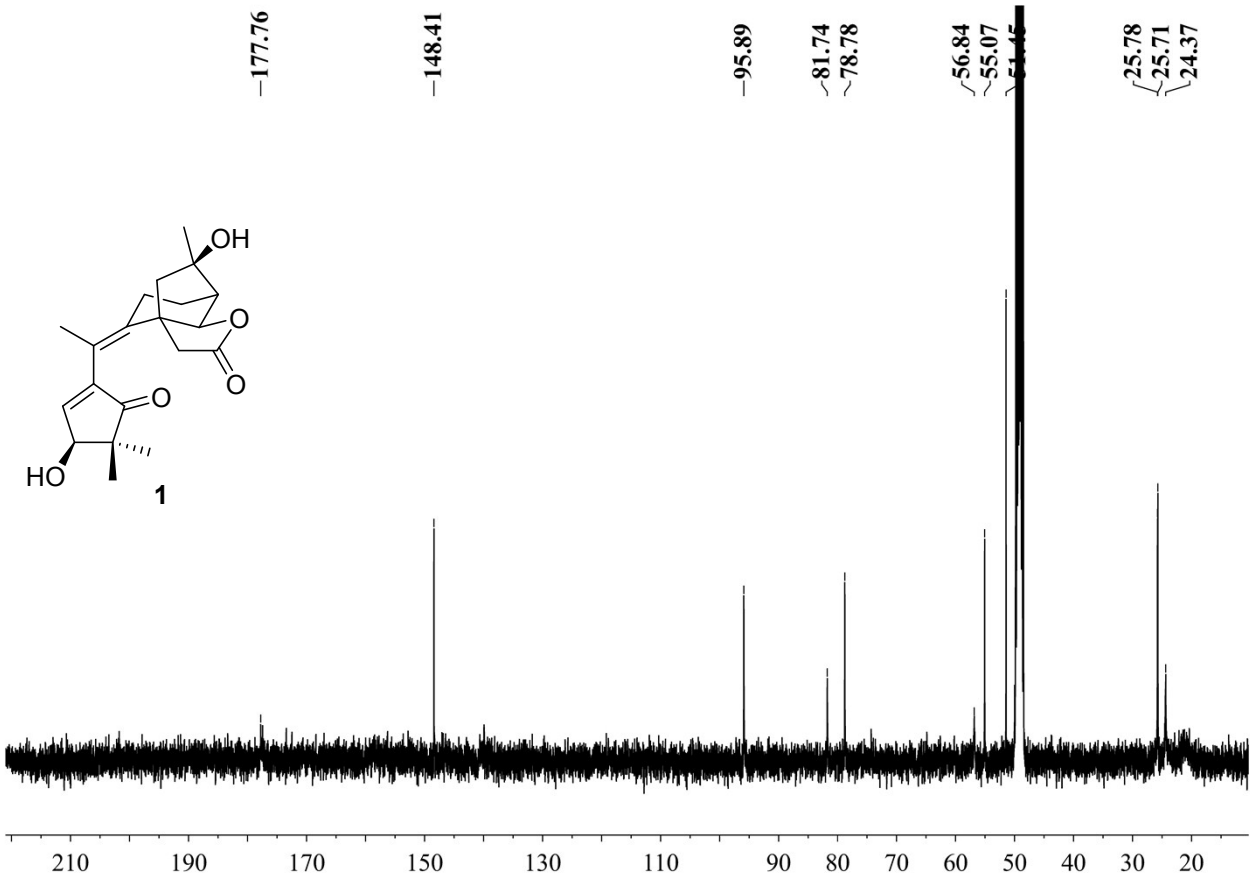


Figure S26. ^{13}C NMR spectrum of **1** (100 MHz, methanol-d_4)

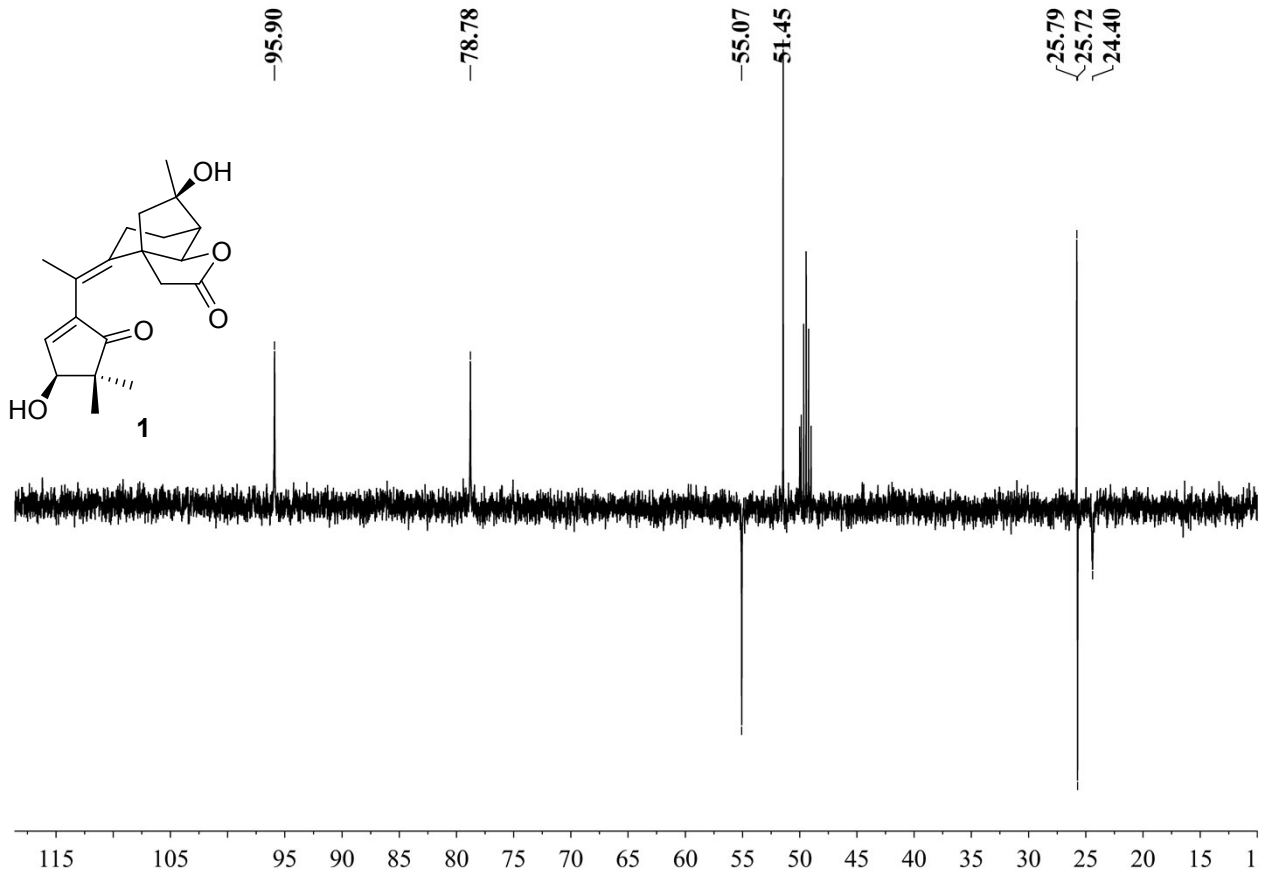


Figure S27. DEPT spectrum of **1** (100 MHz, methanol-d_4)

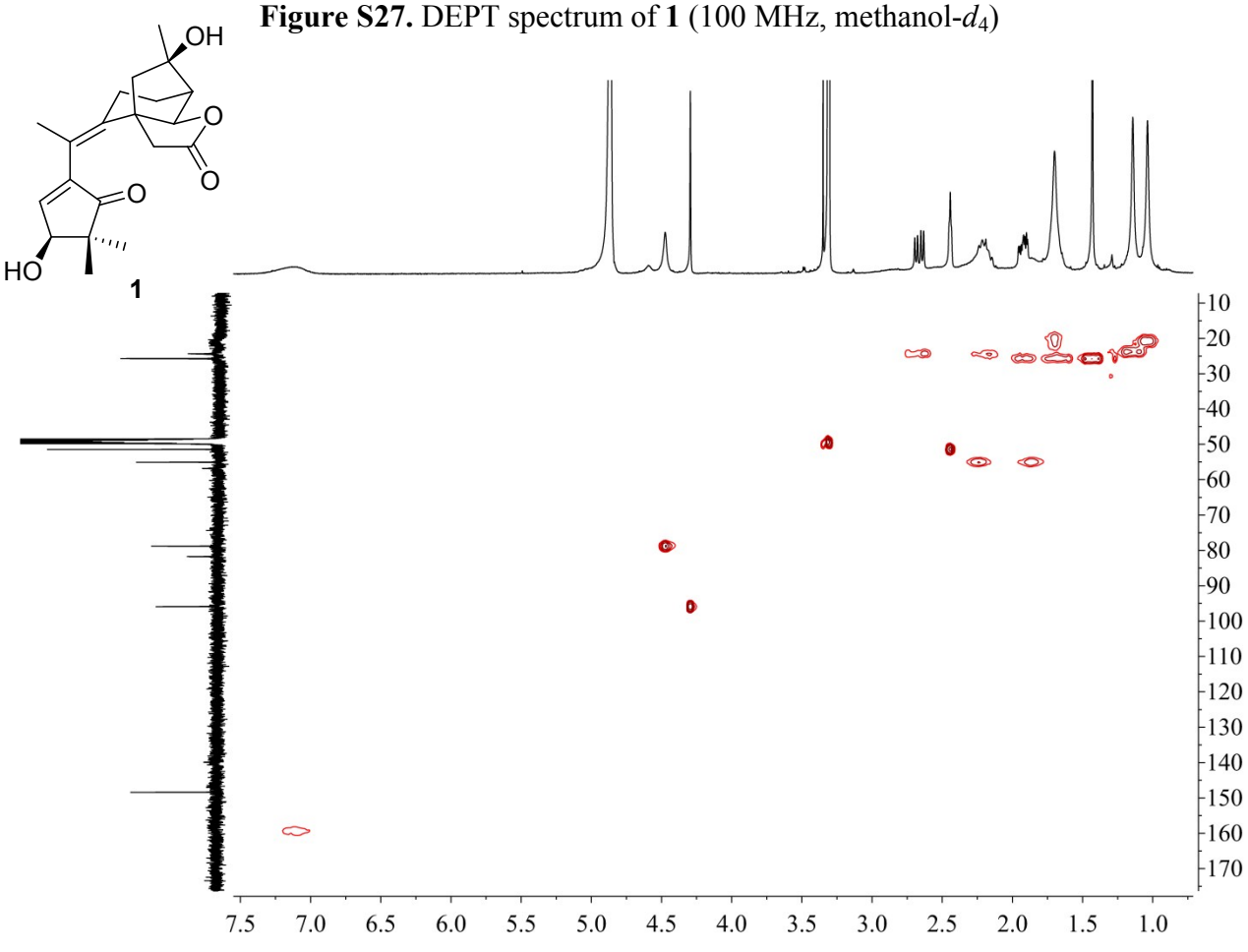


Figure S28. HSQC spectrum of **1** (^{1}H: 400 MHz, ^{13}C: 100 MHz, methanol-d_4)

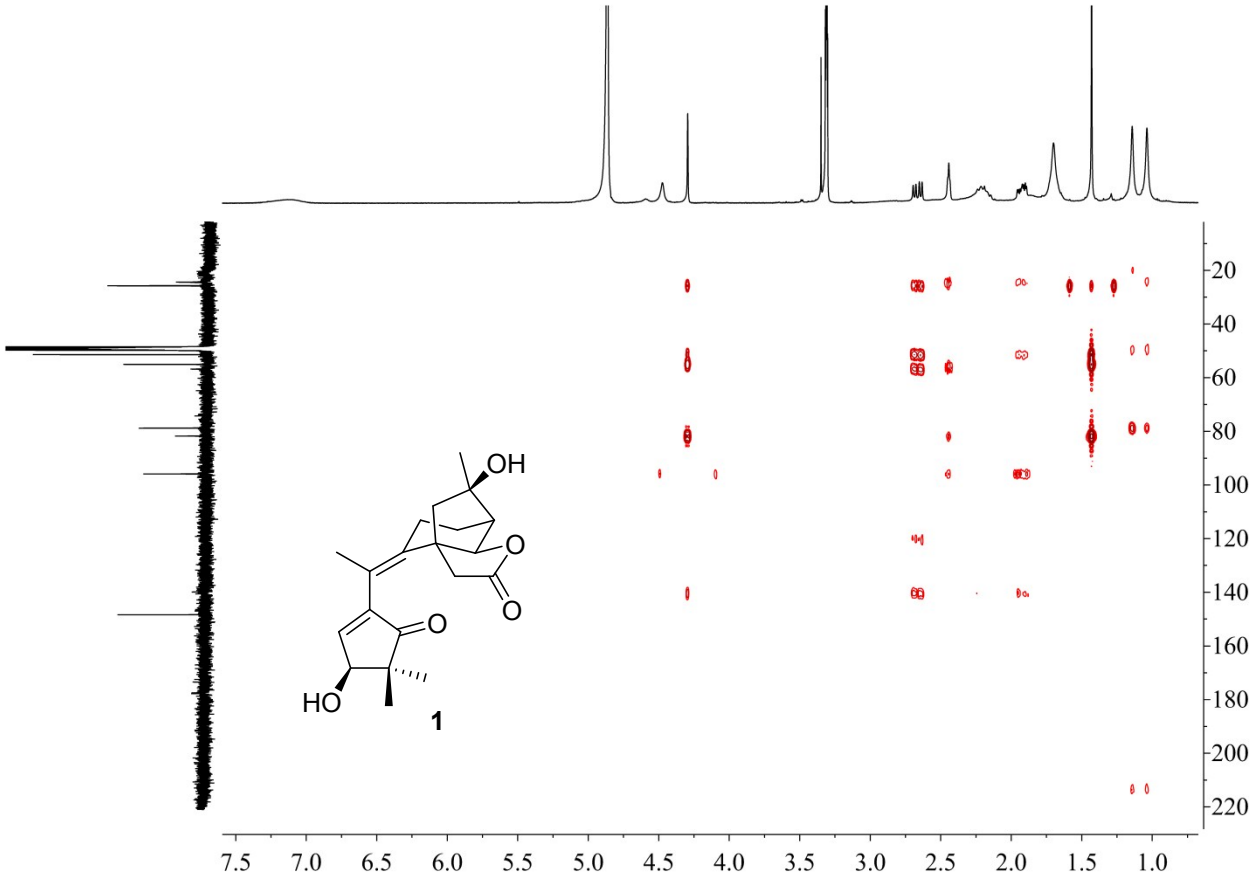


Figure S29. HMBC spectrum of **1** (^{1}H: 400 MHz, ^{13}C: 100 MHz, methanol-d_4)

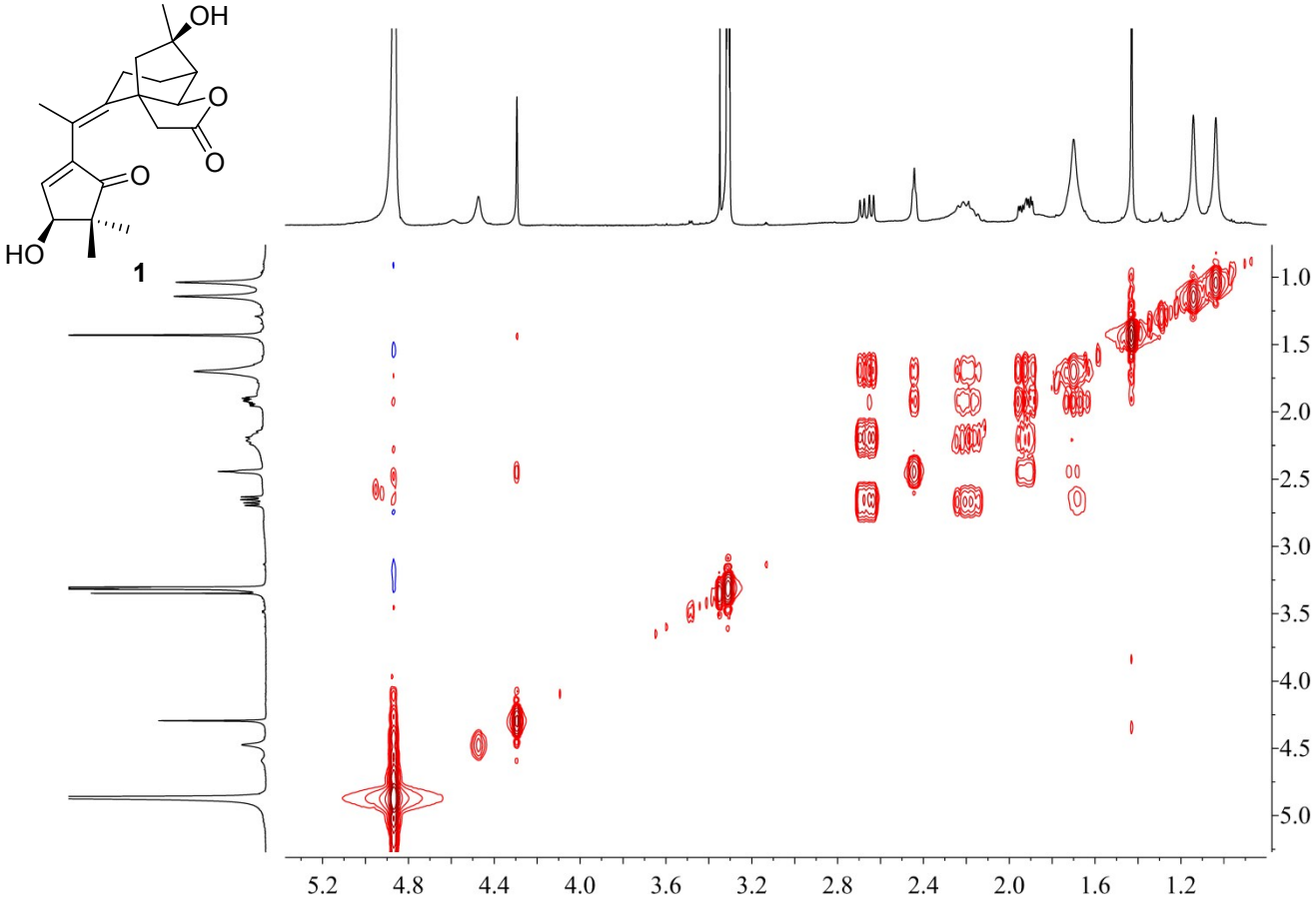


Figure S30. ^{1}H–^{1}H COSY spectrum of **1** (400 MHz, methanol-d_4)

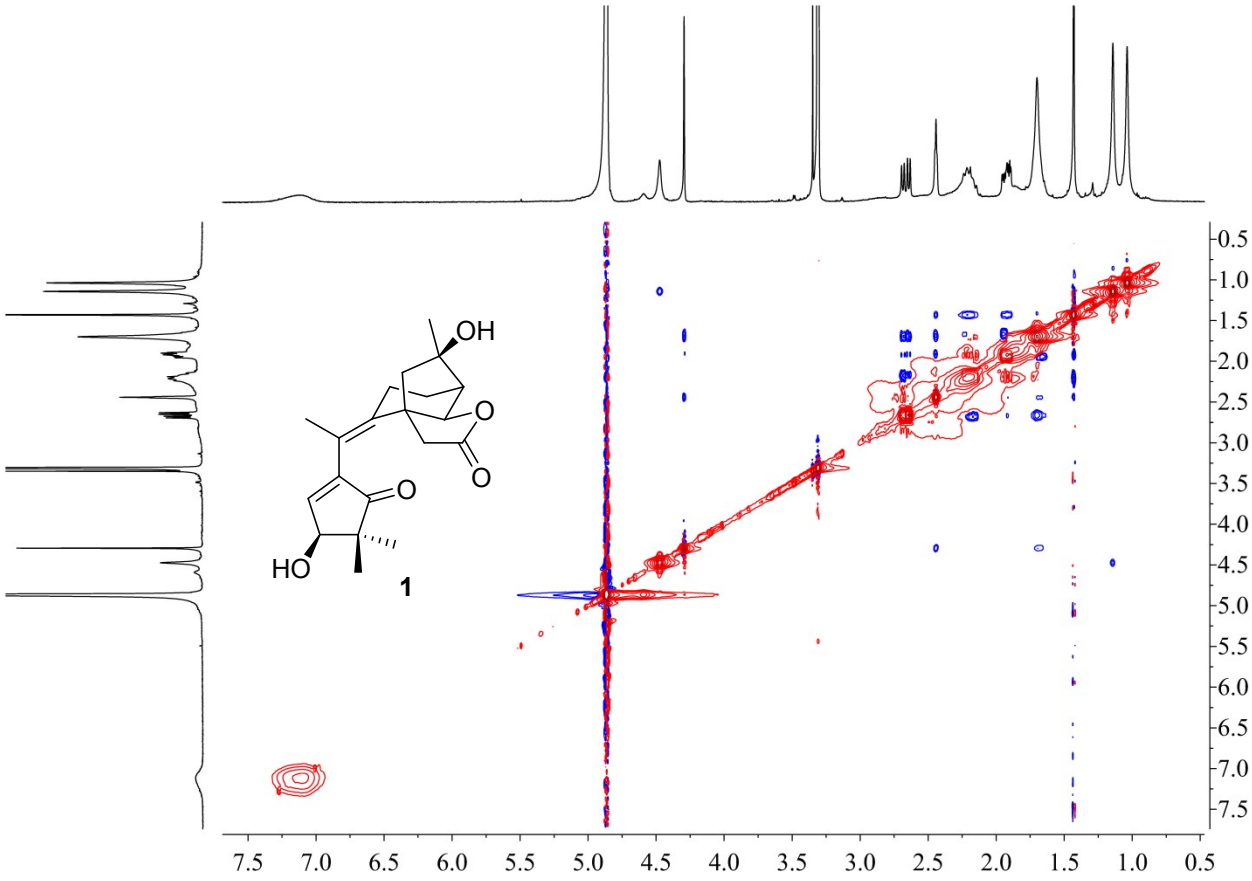


Figure S31. NOESY spectrum of **1** (400 MHz, methanol-d_4)

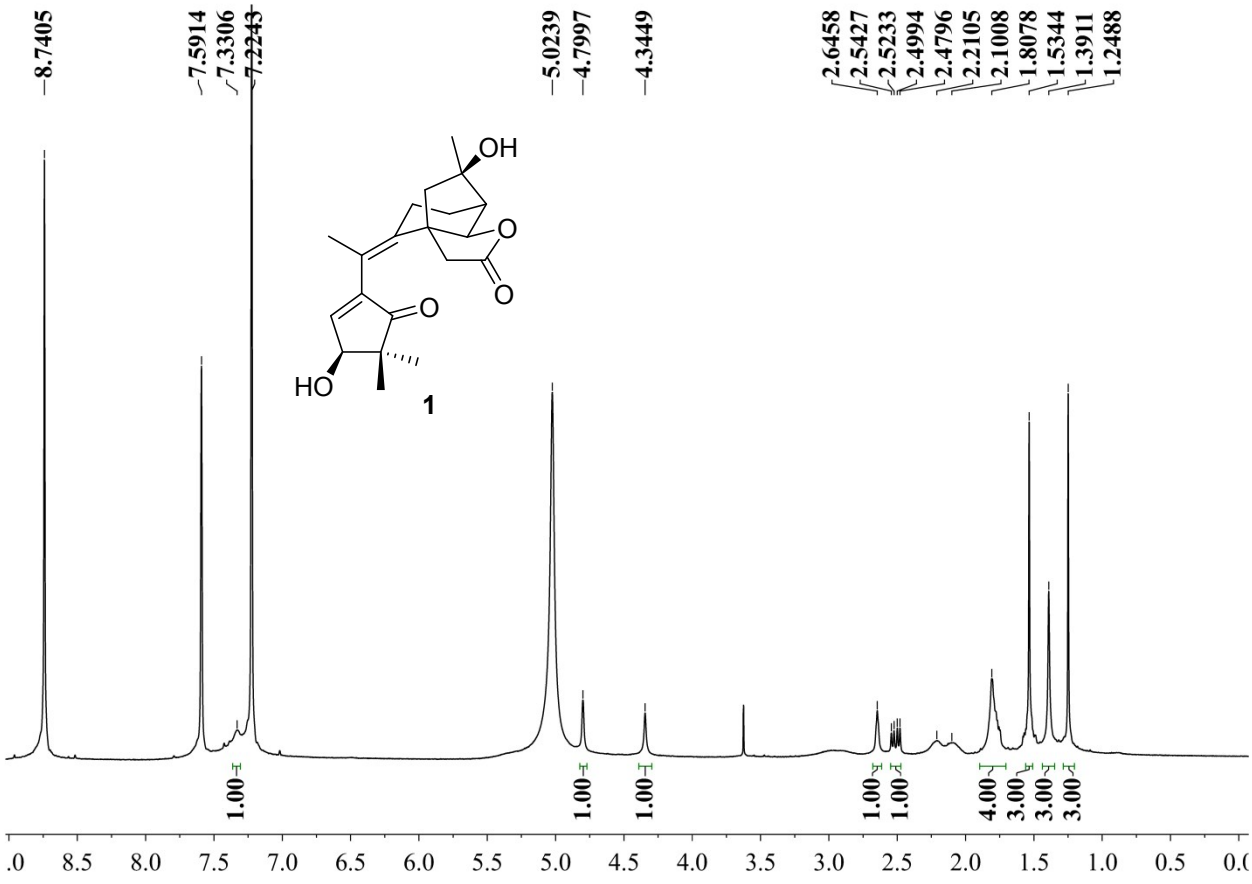


Figure S32. ^{1}H NMR spectrum of **1** (400 MHz, pyridine-d_5)

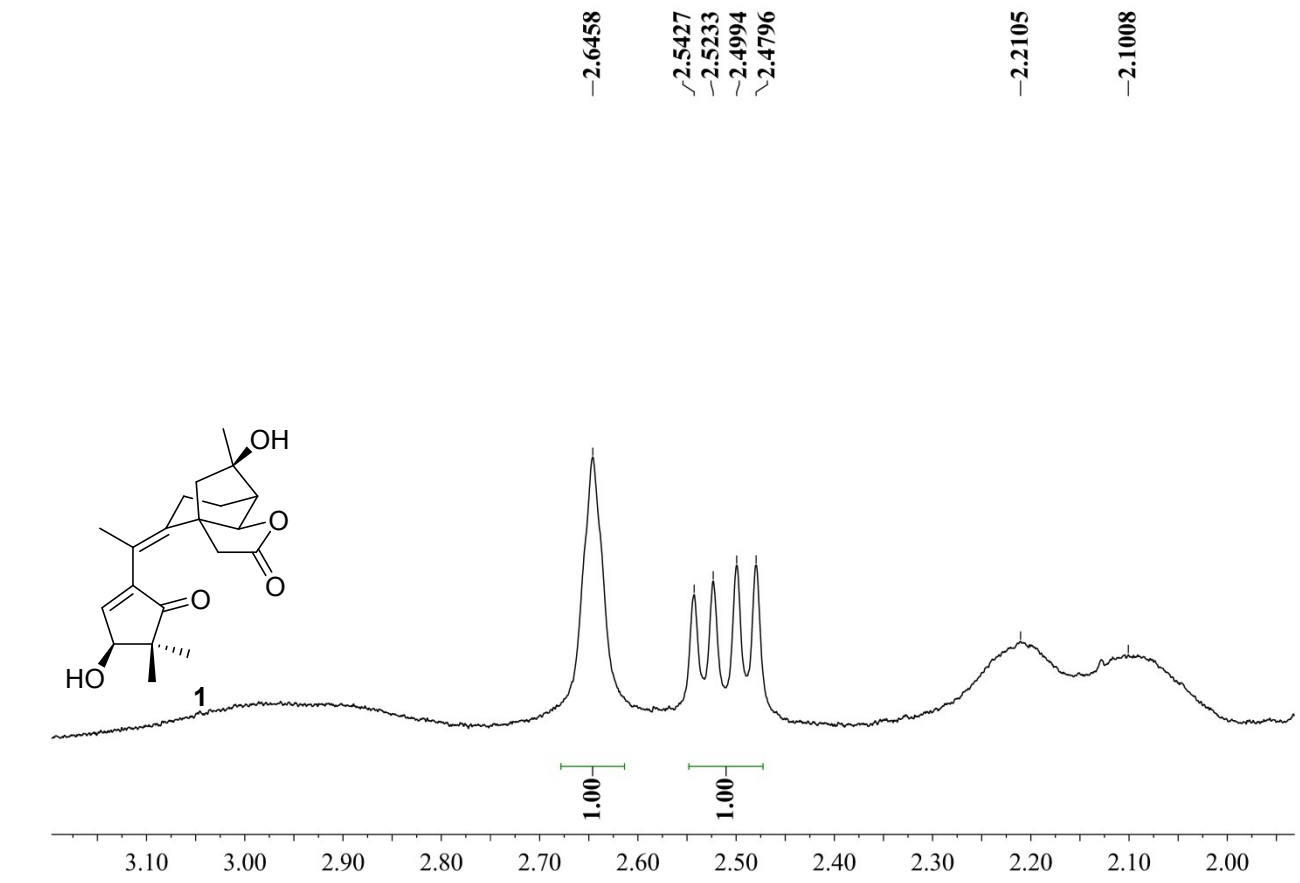


Figure S33. ^{1}H NMR spectrum of **1** (400 MHz, pyridine-d_5, amplified)

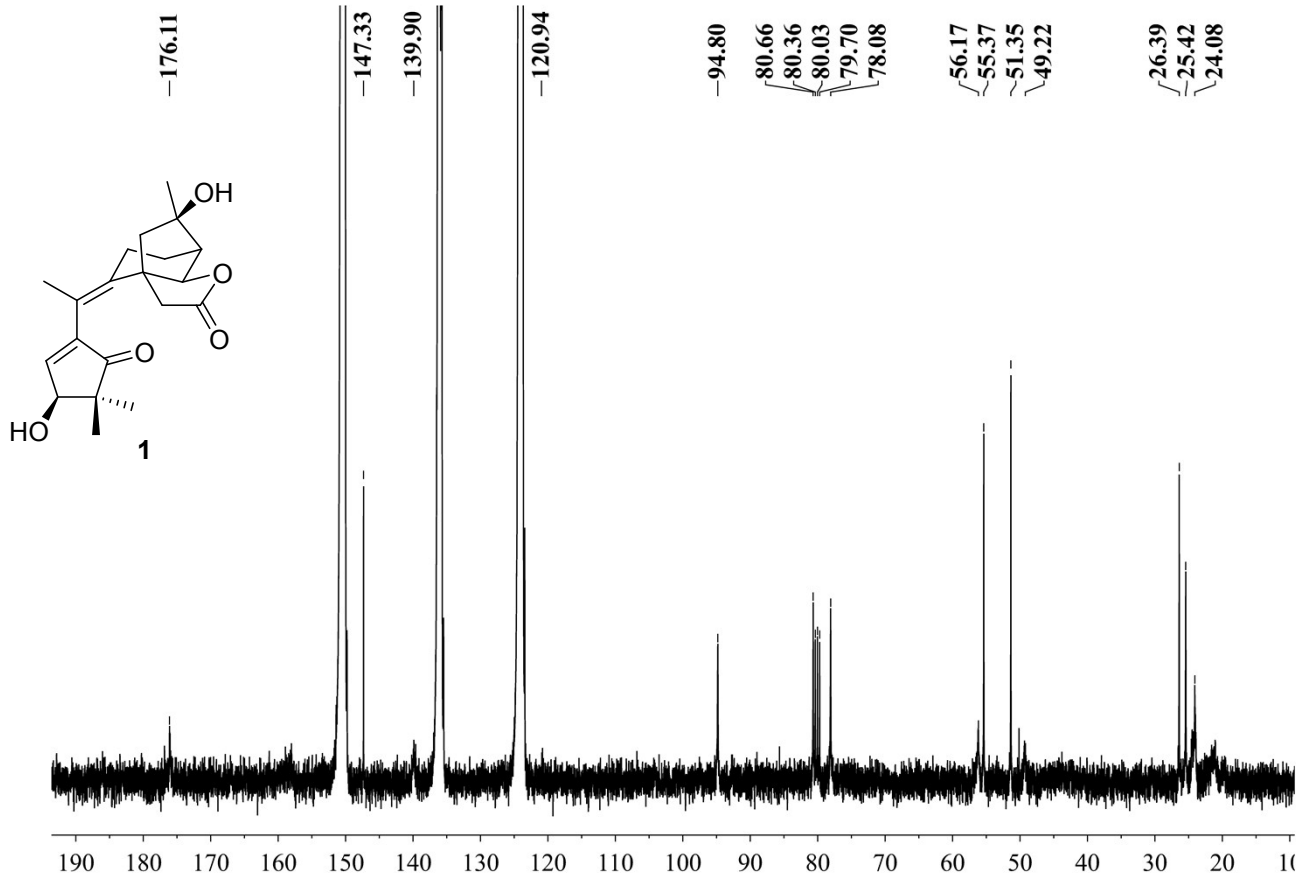


Figure S34. ^{13}C NMR spectrum of **1** (100 MHz, pyridine-d_5)

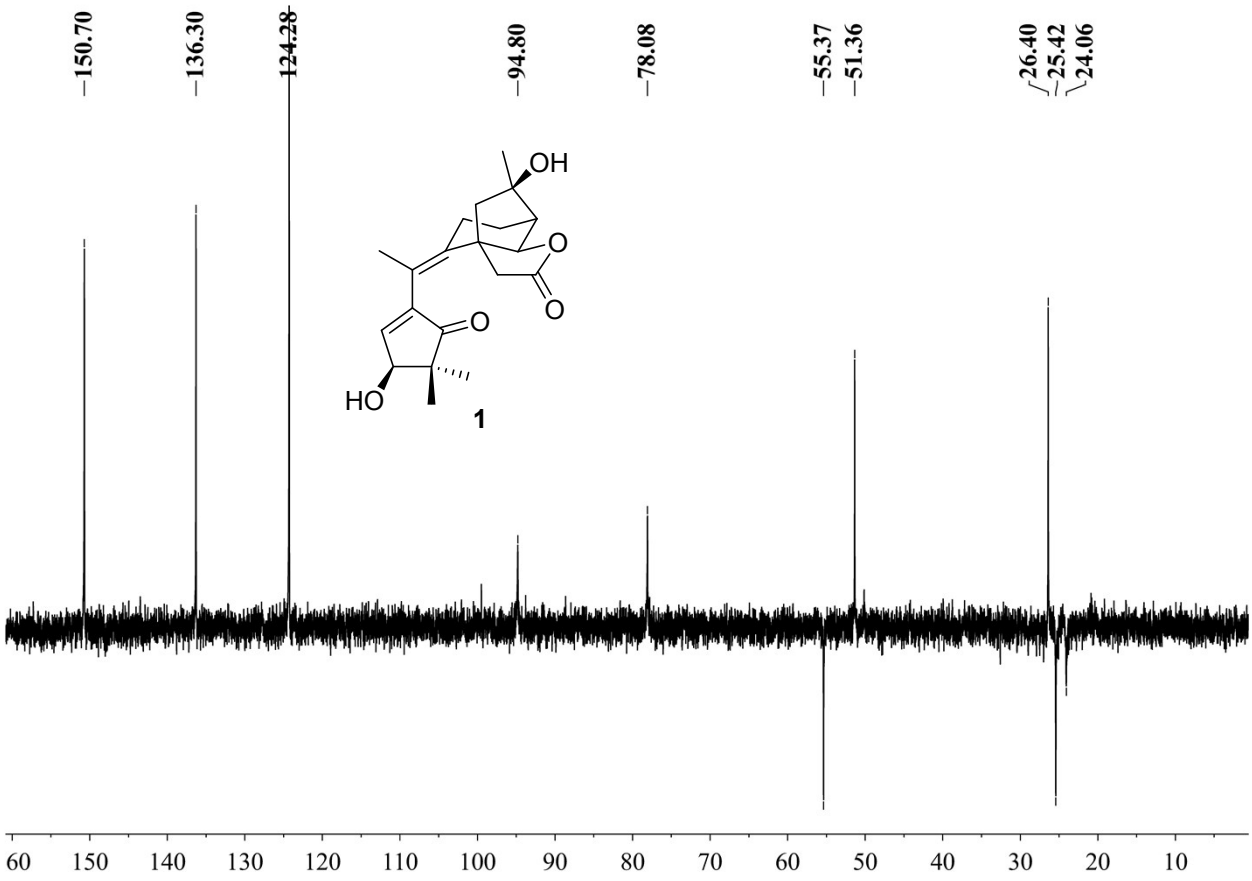


Figure S35. DEPT spectrum of **1** (100 MHz, pyridine-d_5)

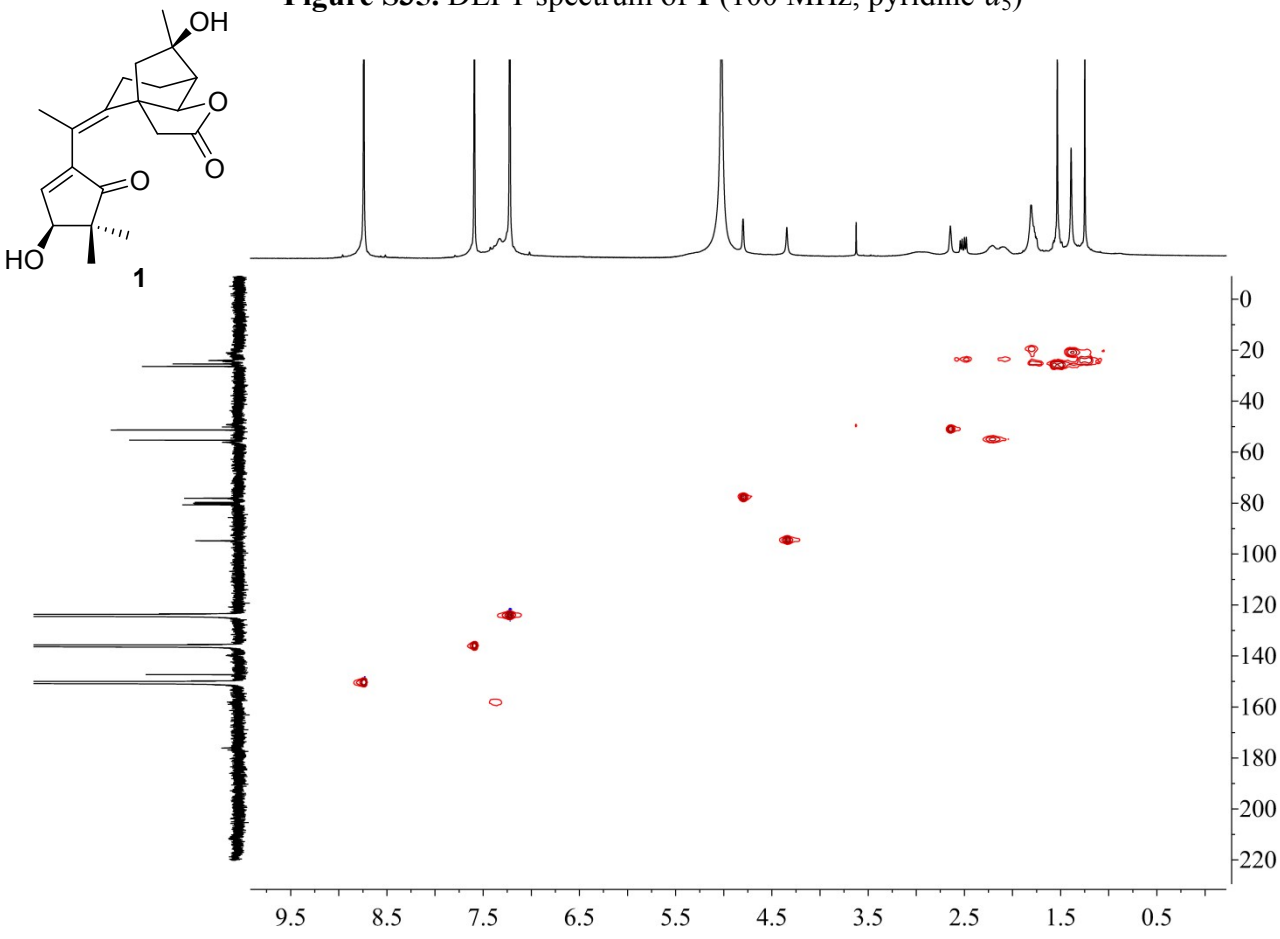


Figure S36. HSQC spectrum of **1** (^{1}H: 400 MHz, ^{13}C: 100 MHz, pyridine-d_5)

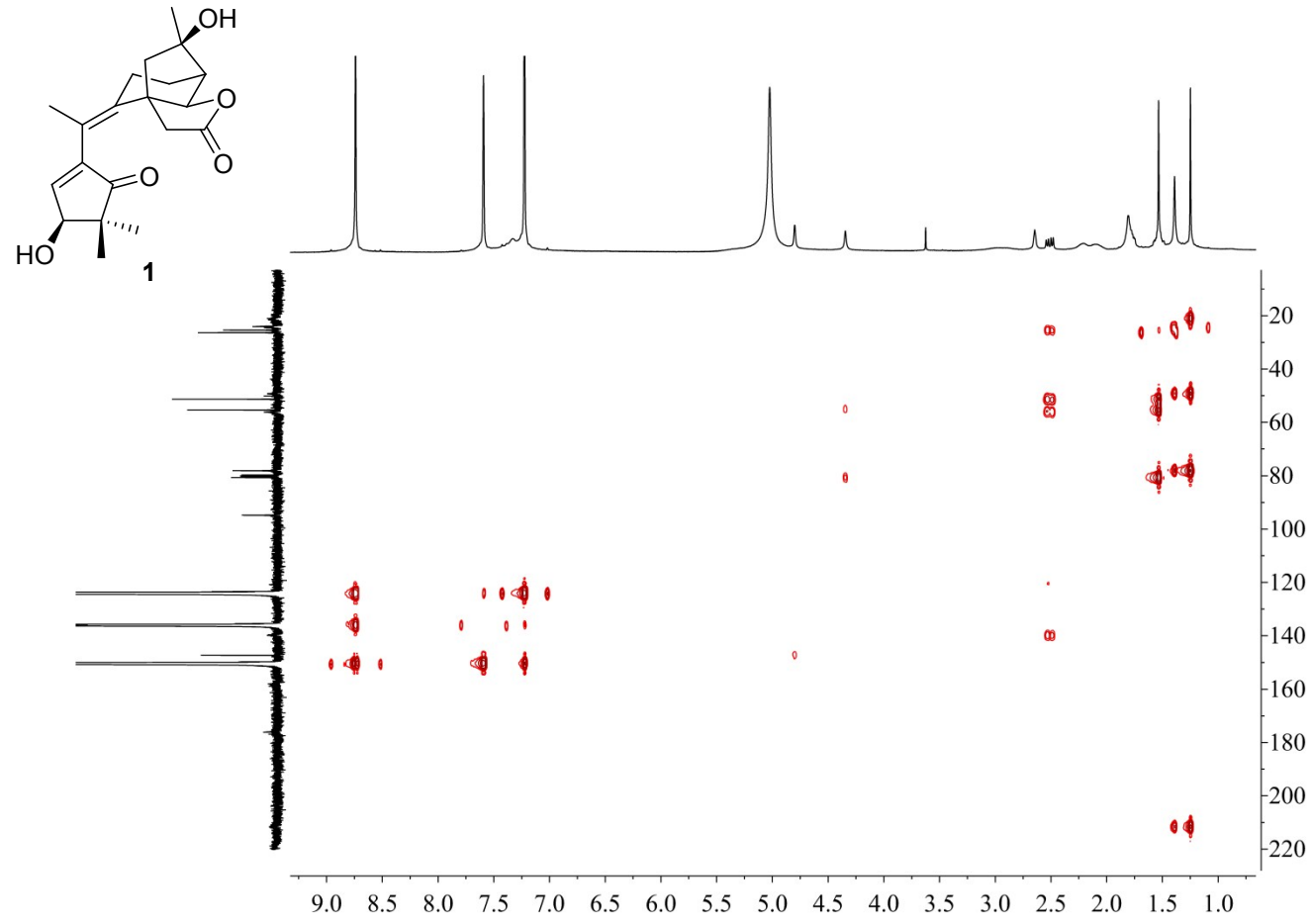


Figure S37. HMBC spectrum of **1** (^{1}H: 400 MHz, ^{13}C: 100 MHz, pyridine-d_5)

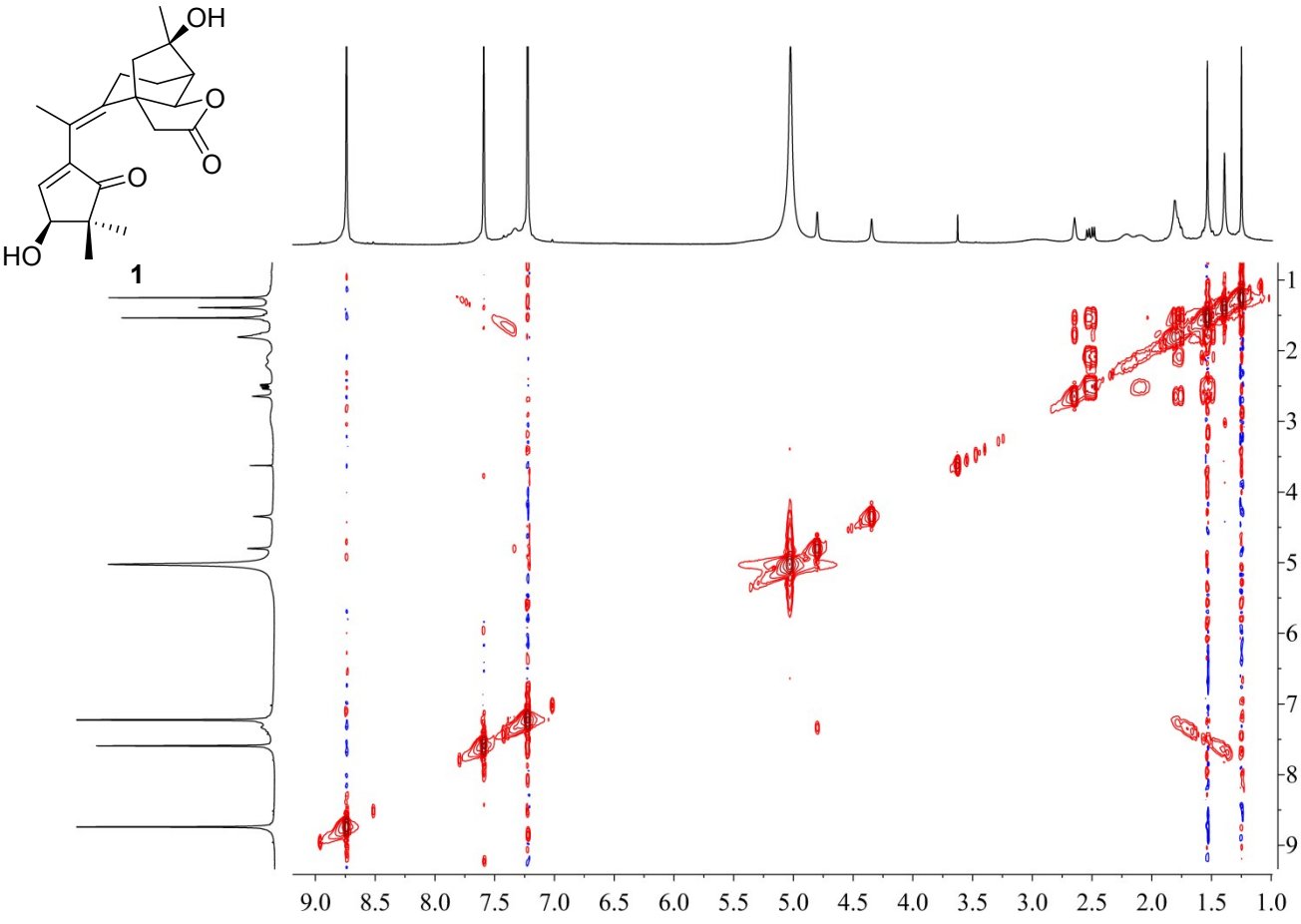


Figure S38. ^{1}H–^{1}H COSY spectrum of **1** (100 MHz, pyridine-d_5)

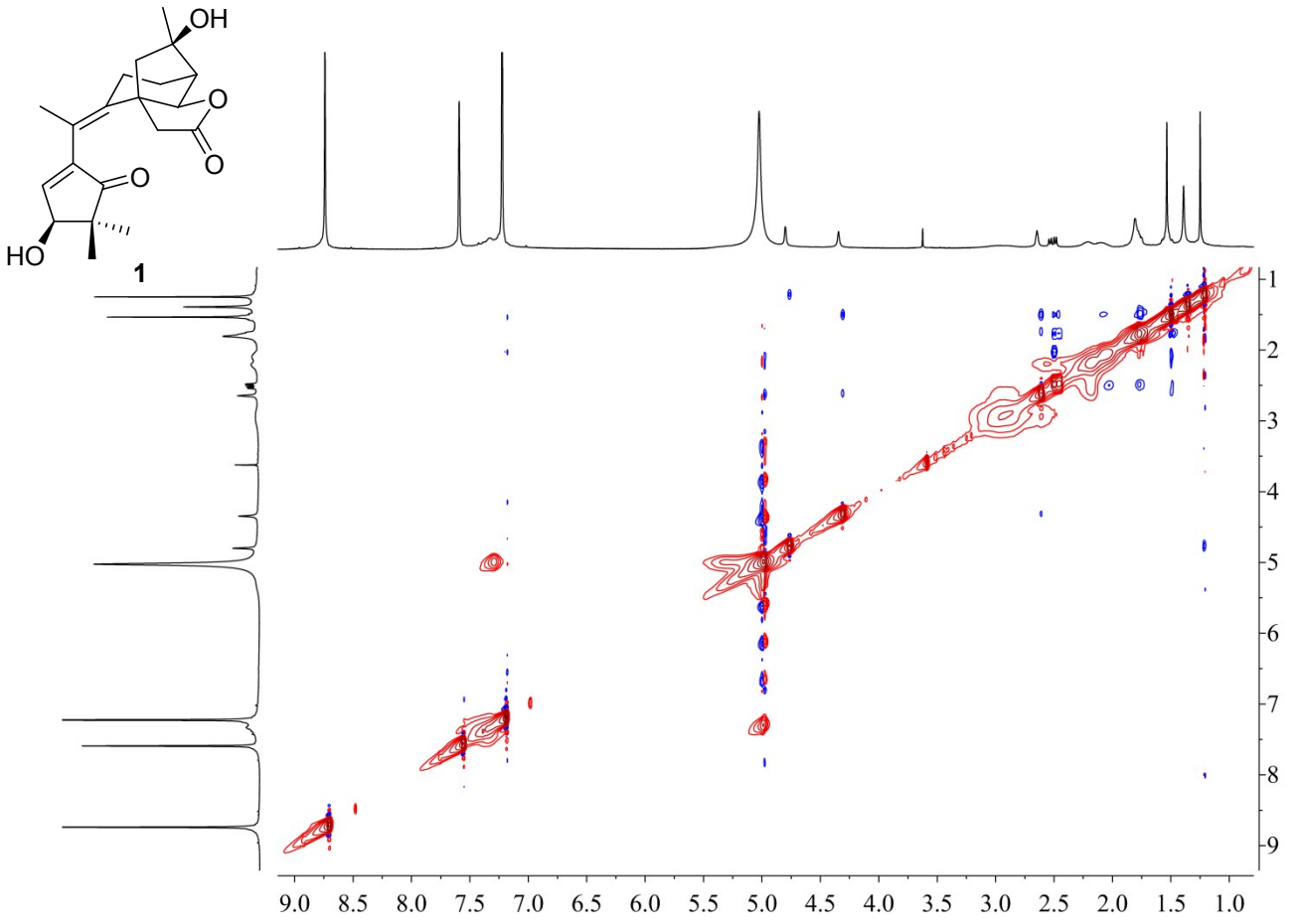


Figure S39. NOESY spectrum of **1** (100 MHz, pyridine-d_5)

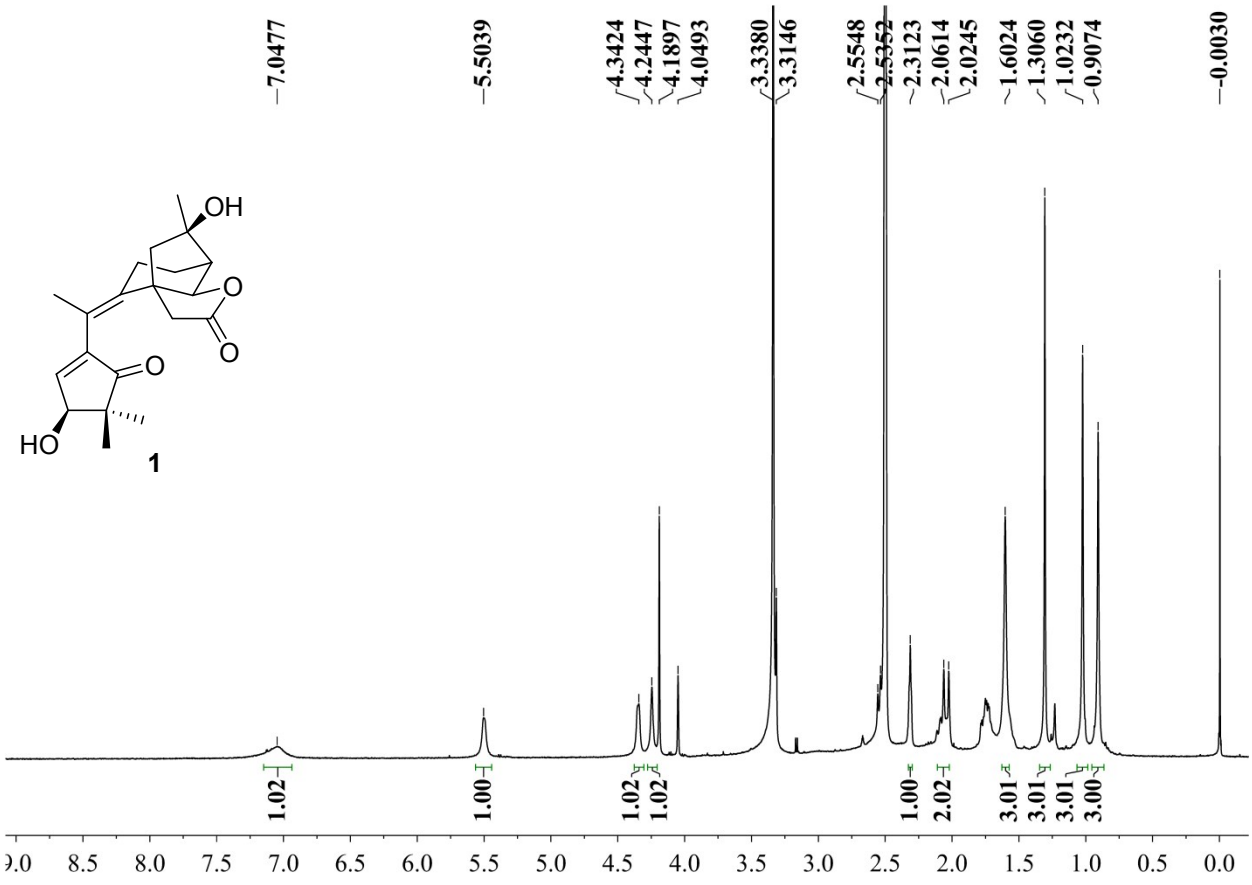


Figure S40. ^{1}H NMR spectrum of **1** (400 MHz, DMSO-d_6)

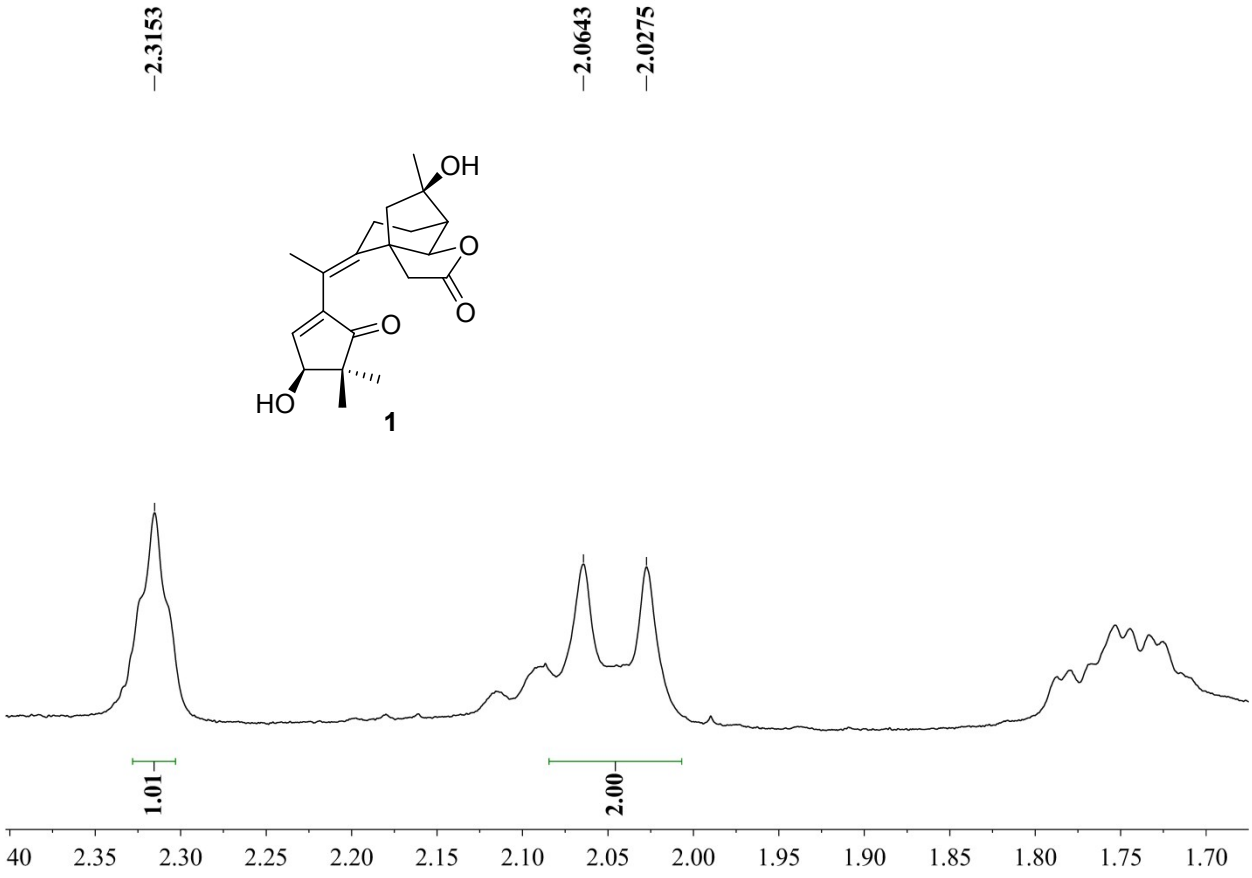


Figure S41. ^{1}H NMR spectrum of **1** (400 MHz, DMSO-d_6, amplified)

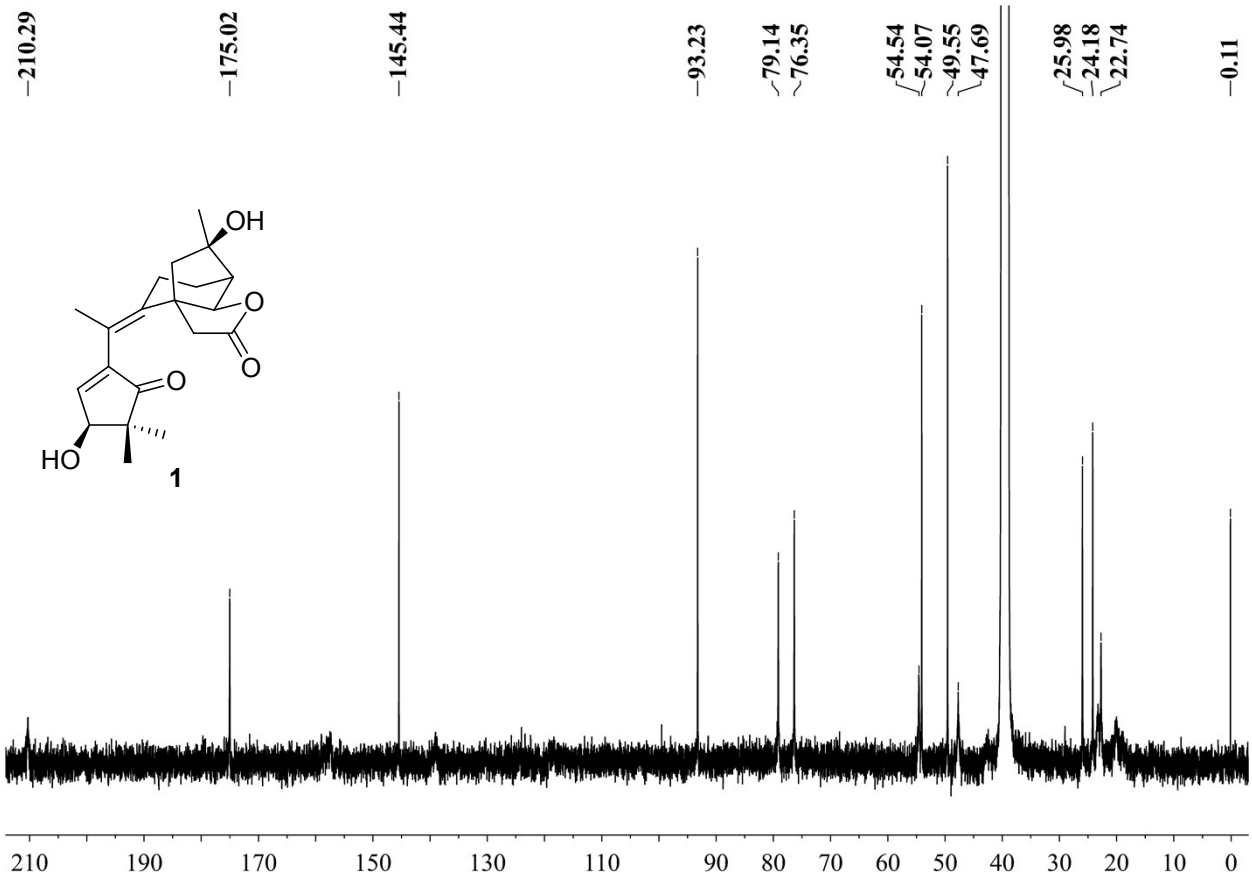


Figure S42. ^{13}C NMR spectrum of **1** (100 MHz, DMSO-d_6)

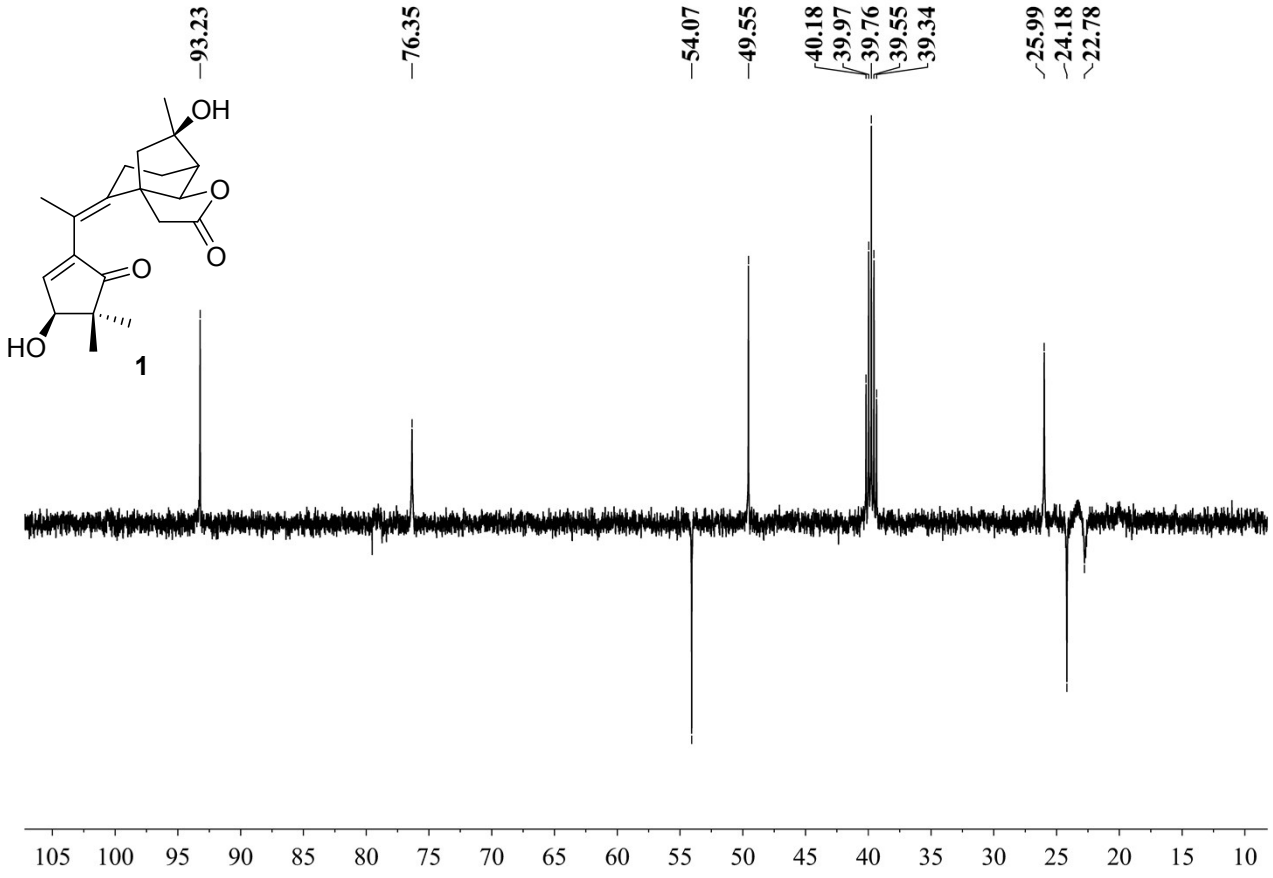


Figure S43. DEPT spectrum of **1** (100 MHz, DMSO-d_6)

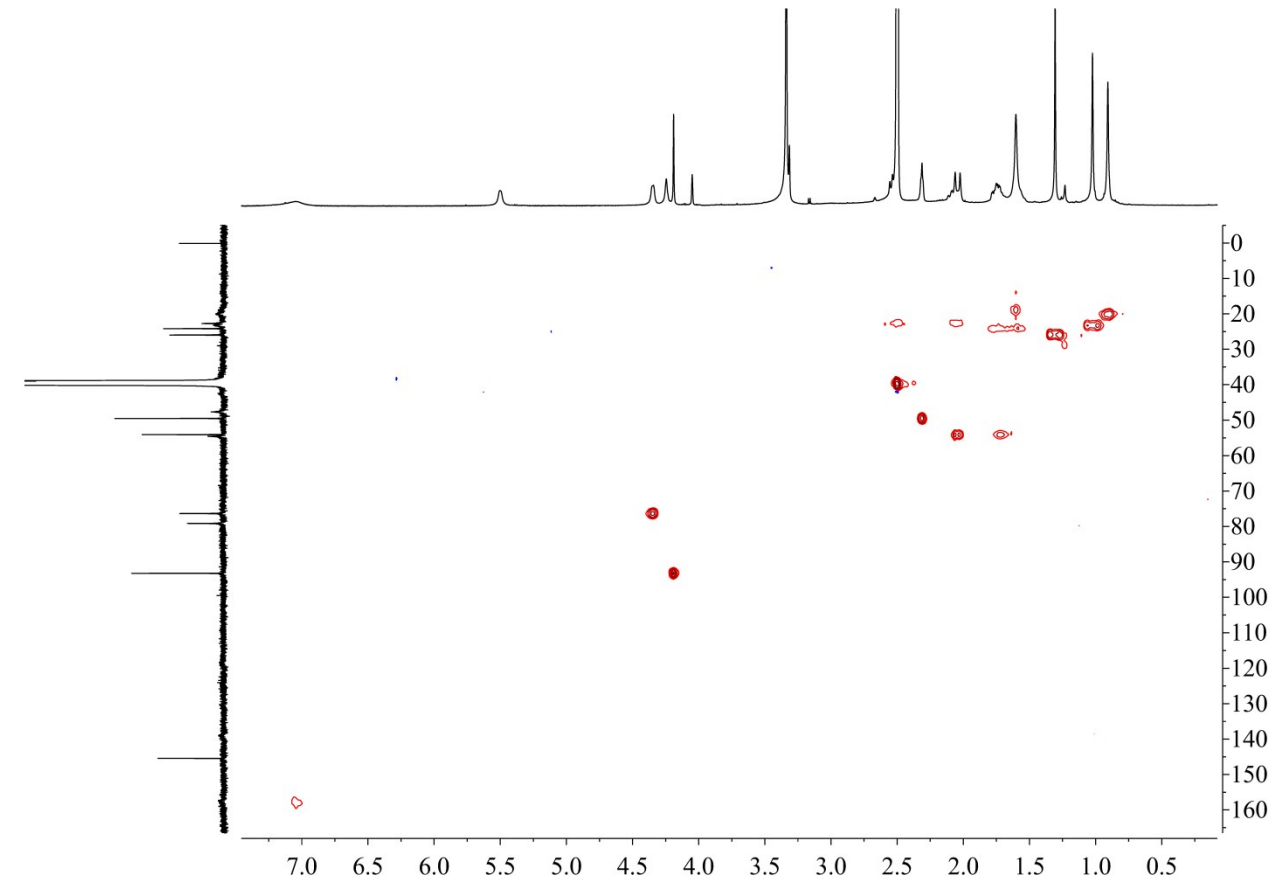


Figure S44. HSQC spectrum of **1** (^{1}H: 400 MHz, ^{13}C: 100 MHz, DMSO-d_6)

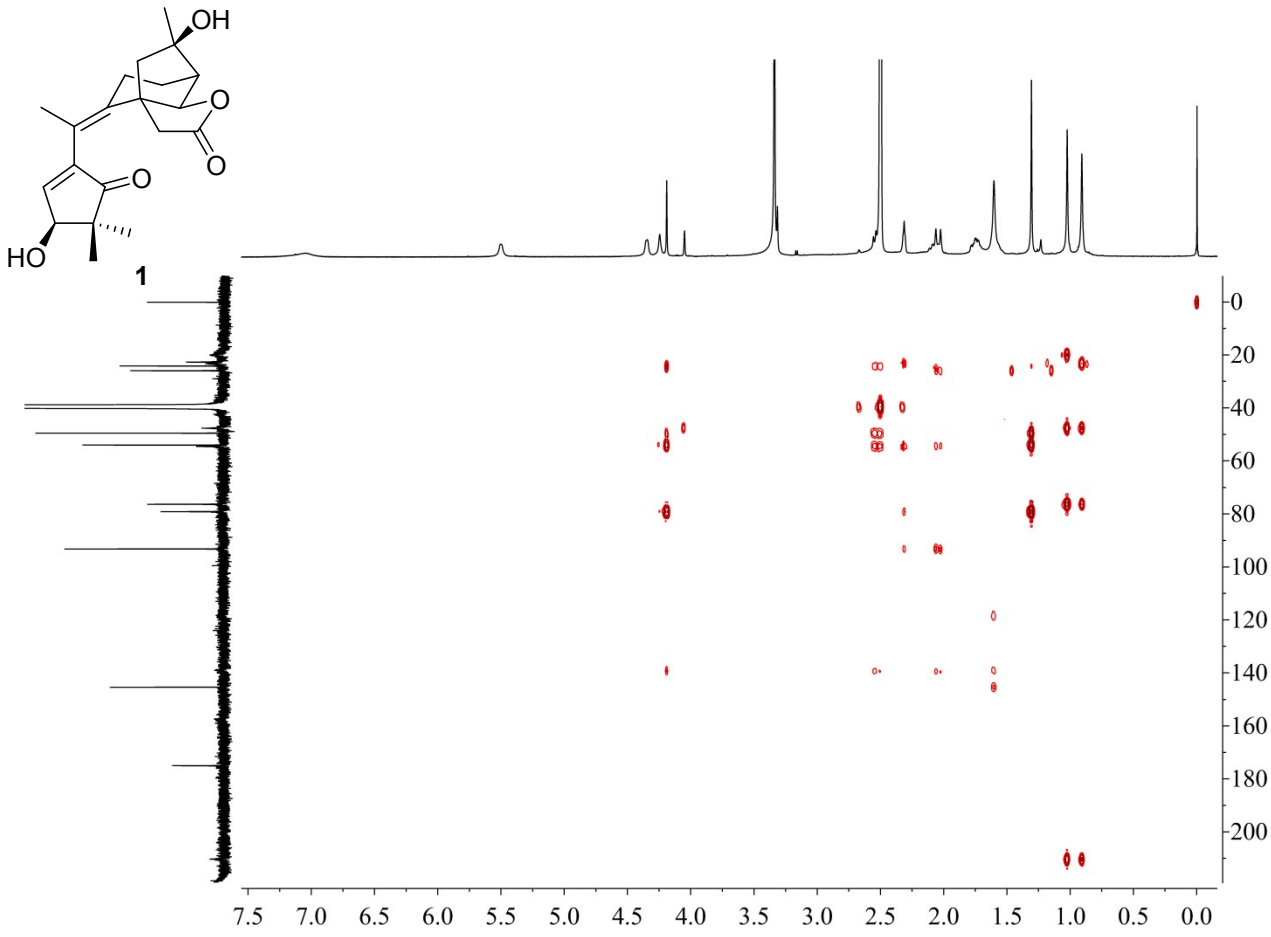


Figure S45. HMBC spectrum of **1** (^{1}H: 400 MHz, ^{13}C: 100 MHz, DMSO-d_6)

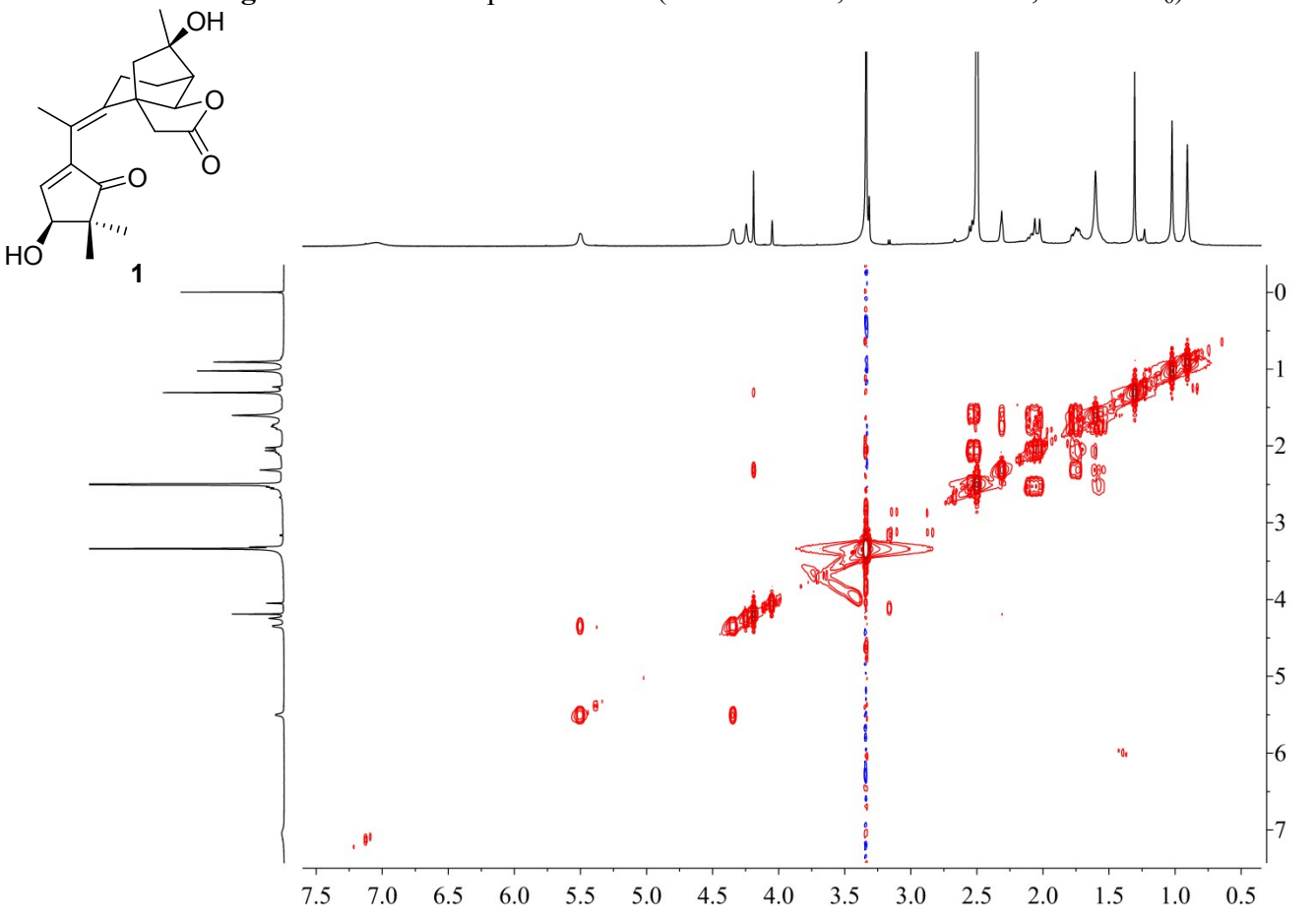


Figure S46. ^{1}H–^{1}H COSY spectrum of **1** (100 MHz, DMSO-d_6)

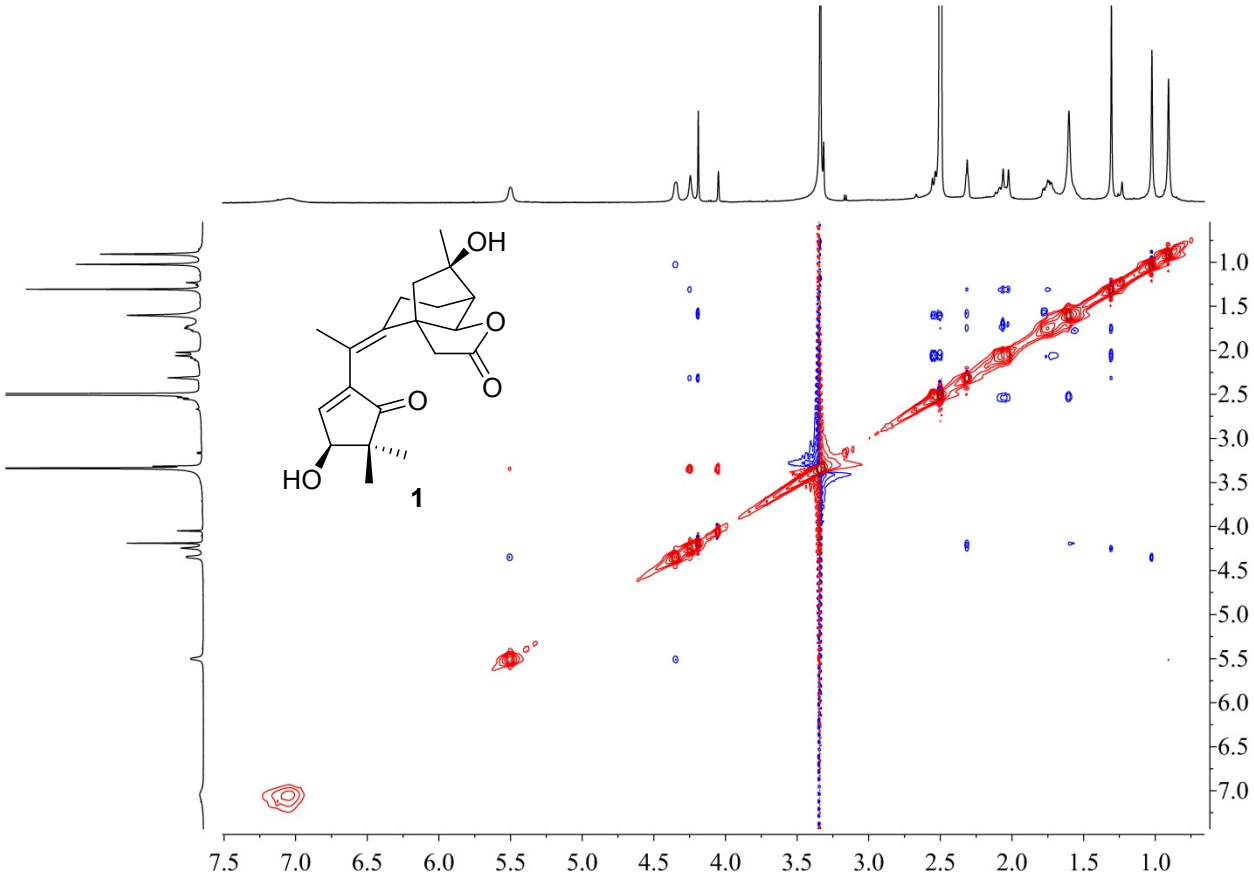


Figure S47. NOESY spectrum of **1** (100 MHz, DMSO-d_6)

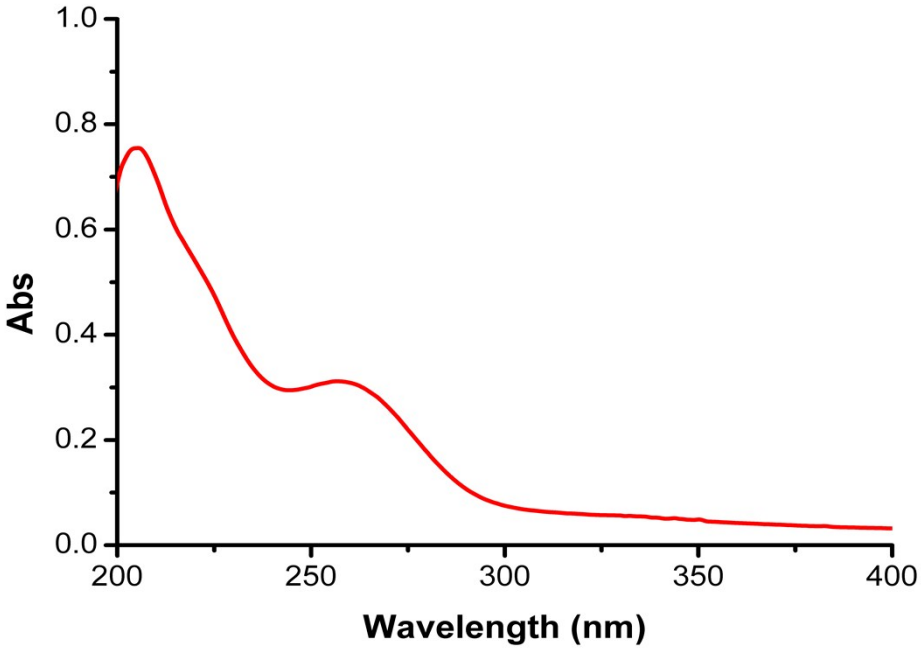


Figure S48. The UV spectrum of **2**.

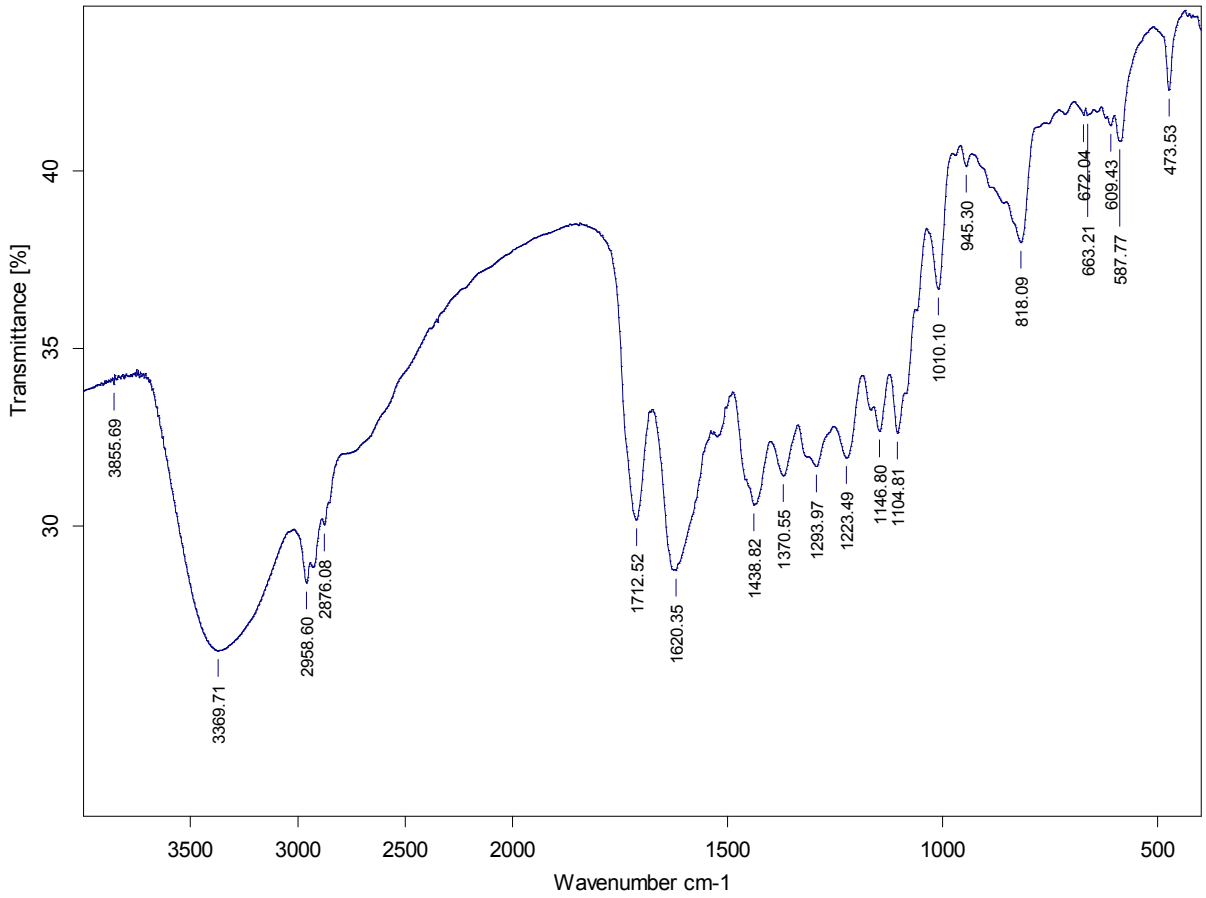


Figure S49. The IR spectrum of **2**

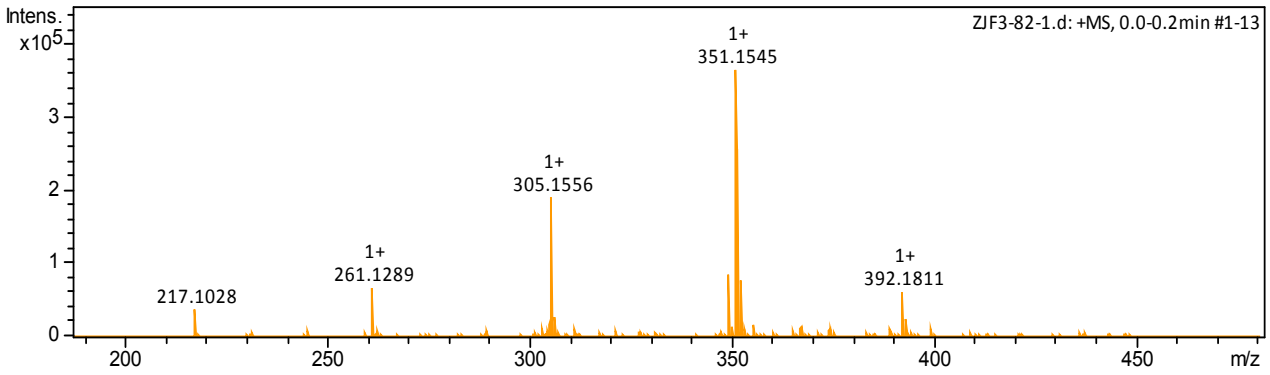


Figure S50. The HRESIMS spectrum of **2**

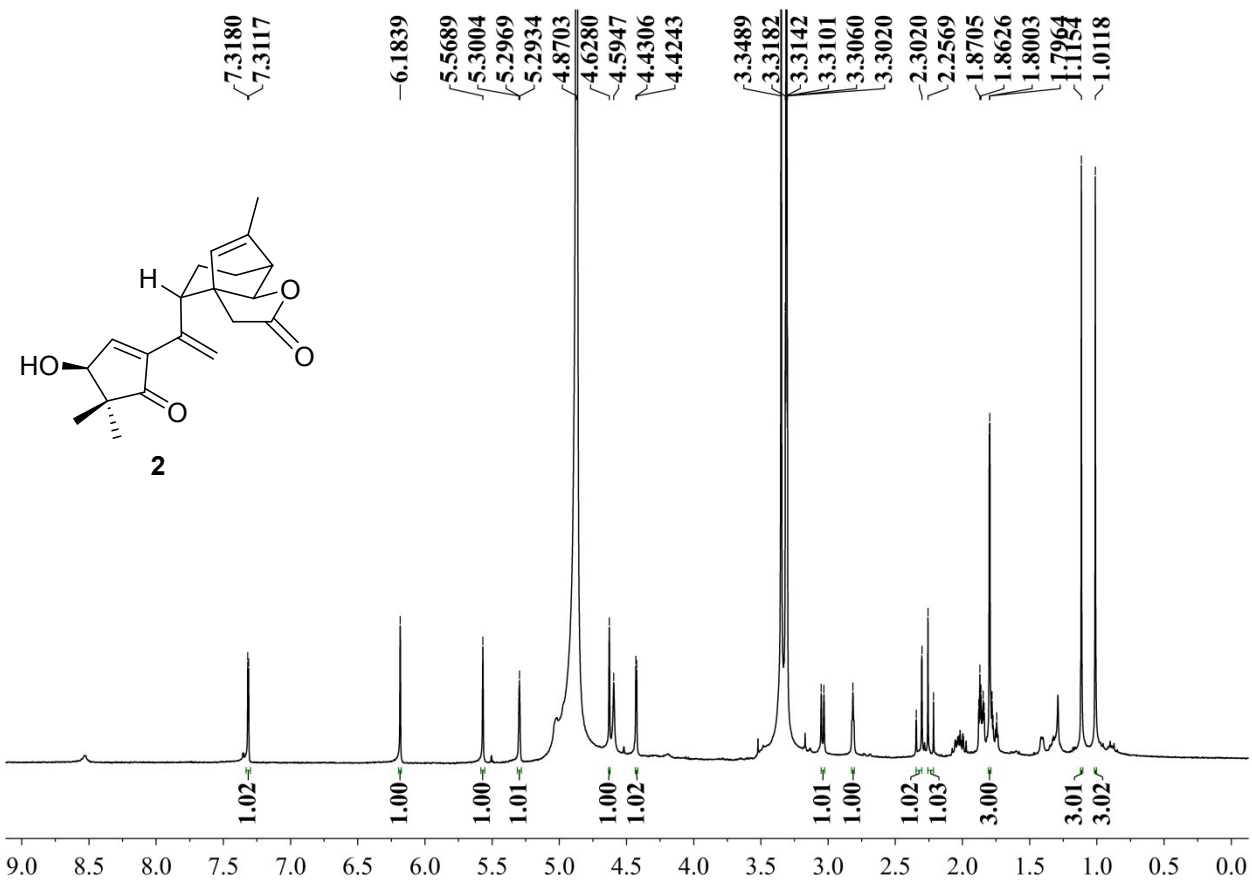


Figure S51. [1]H NMR spectrum of **2** (400 MHz, methanol-d_4)

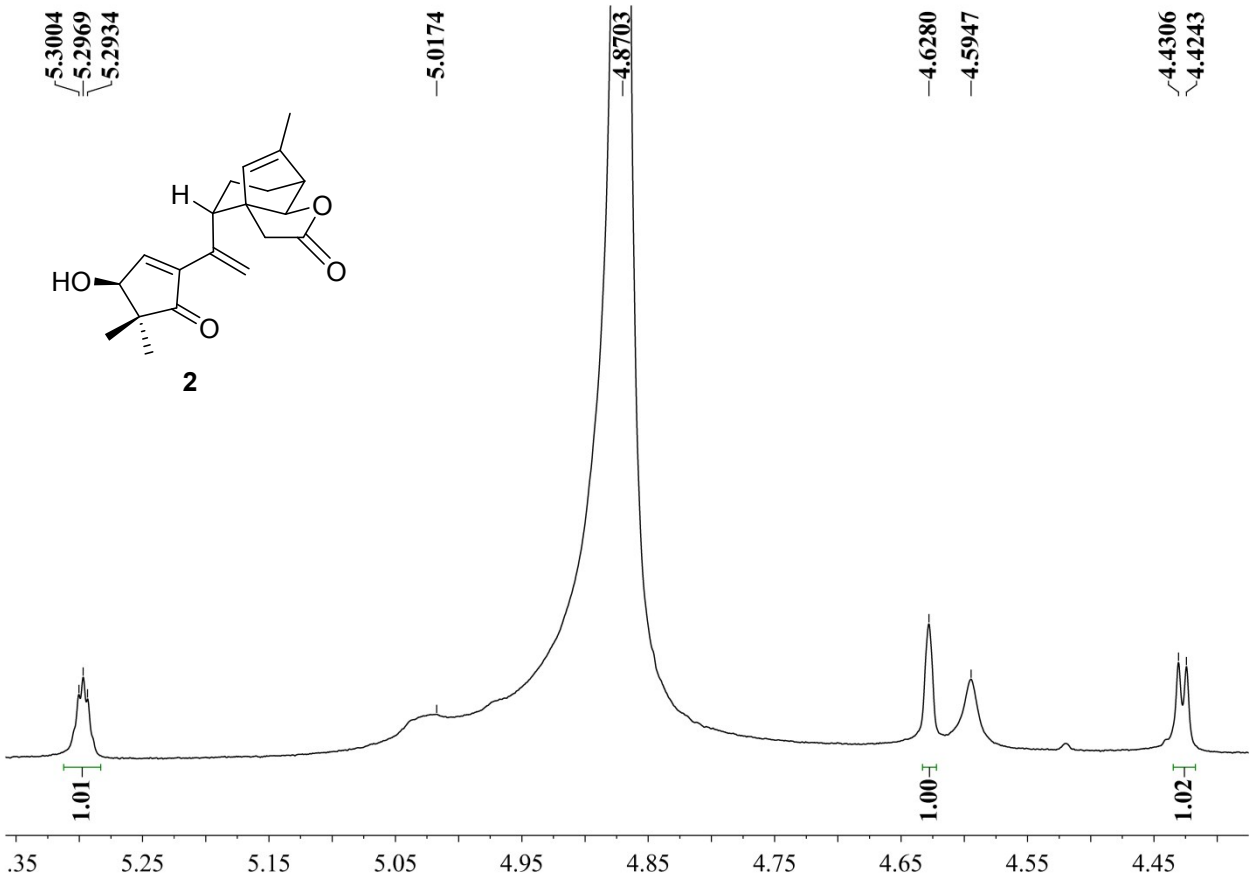


Figure S52. [1]H NMR spectrum of **2** (400 MHz, methanol-d_4, amplified)

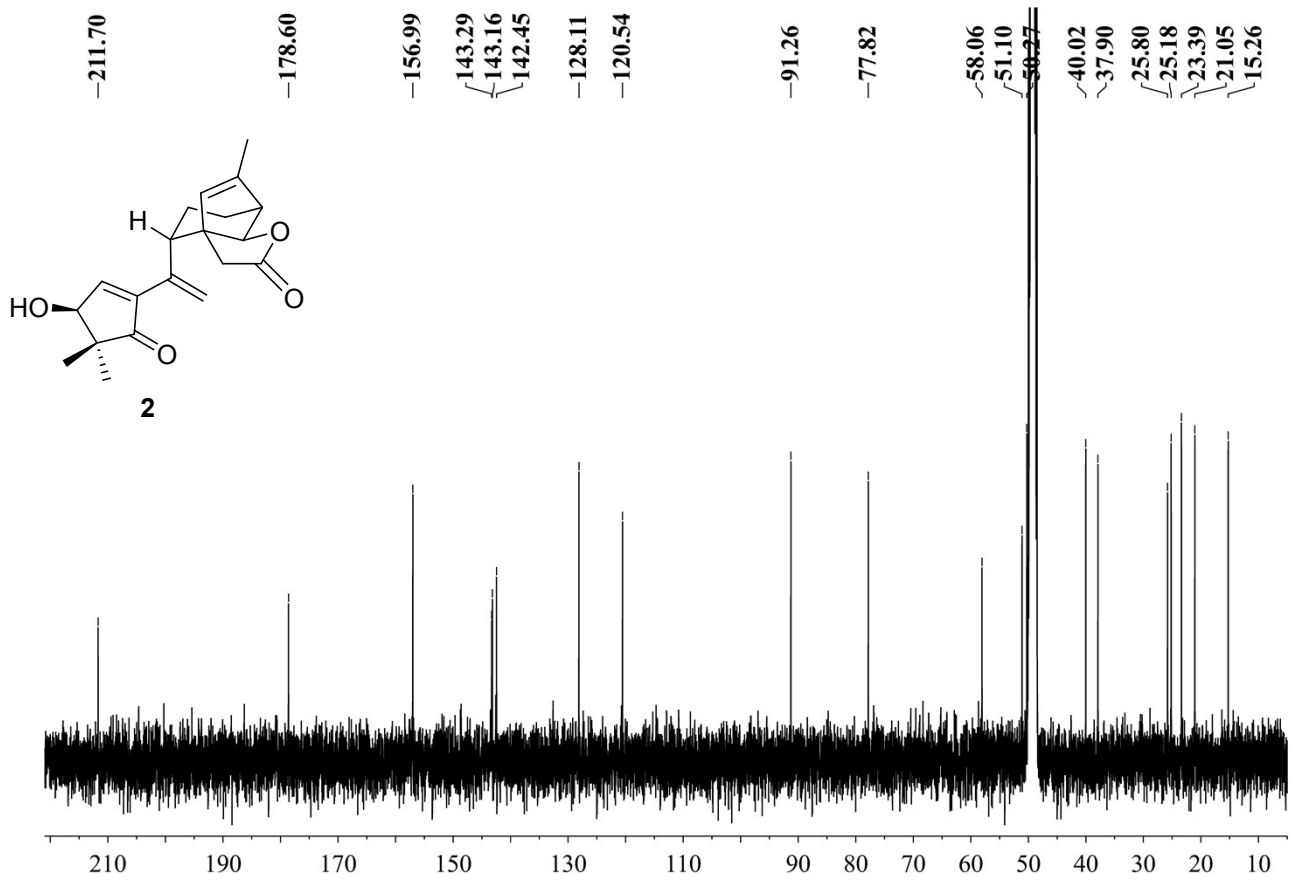


Figure S53. ^{13}C NMR spectrum of **2** (100 MHz, methanol-d_4)

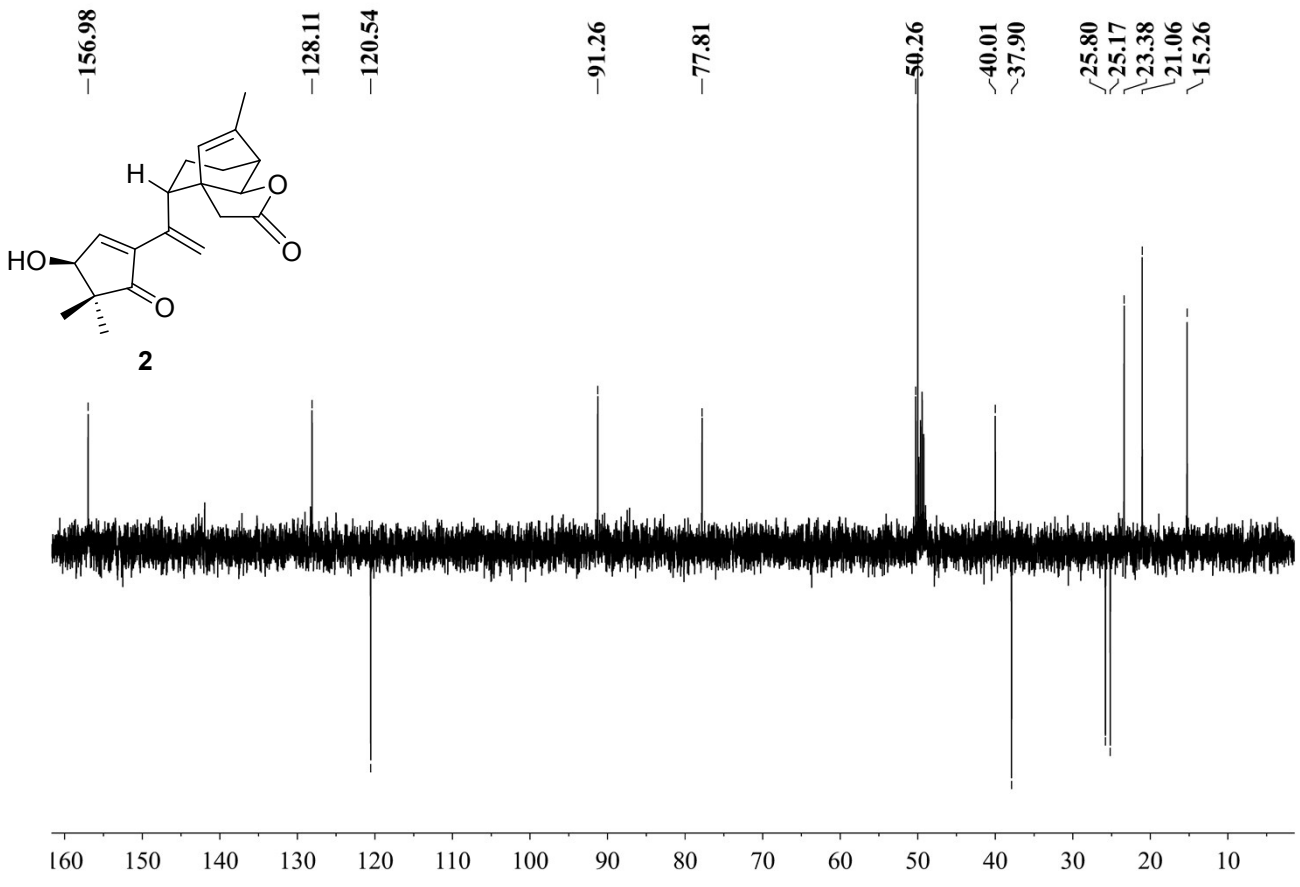


Figure S54. DEPT spectrum of **2** (100 MHz, methanol-d_4)

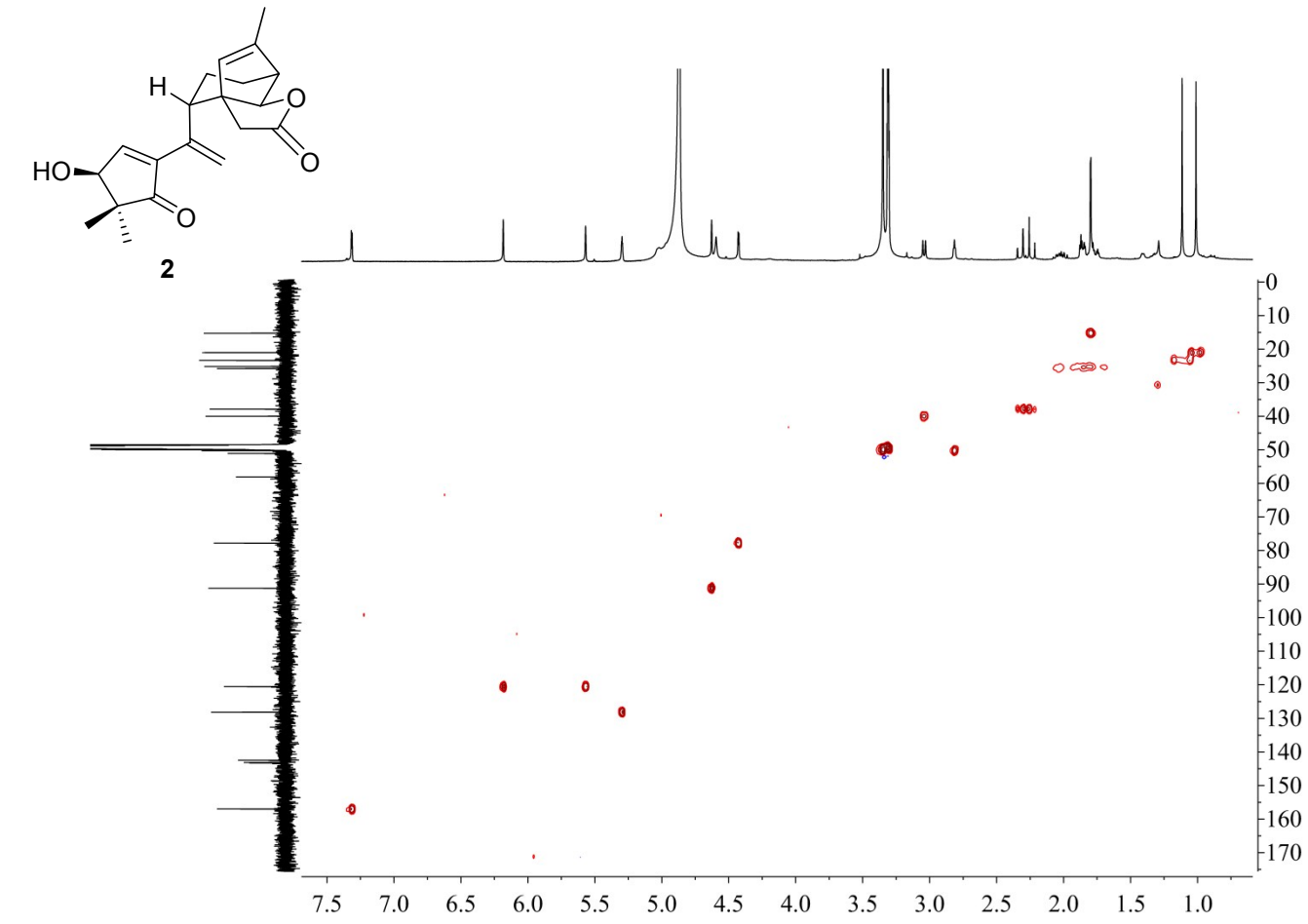


Figure S55. HSQC spectrum of **2** (^{1}H: 400 MHz, ^{13}C: 100 MHz, methanol-d_4)

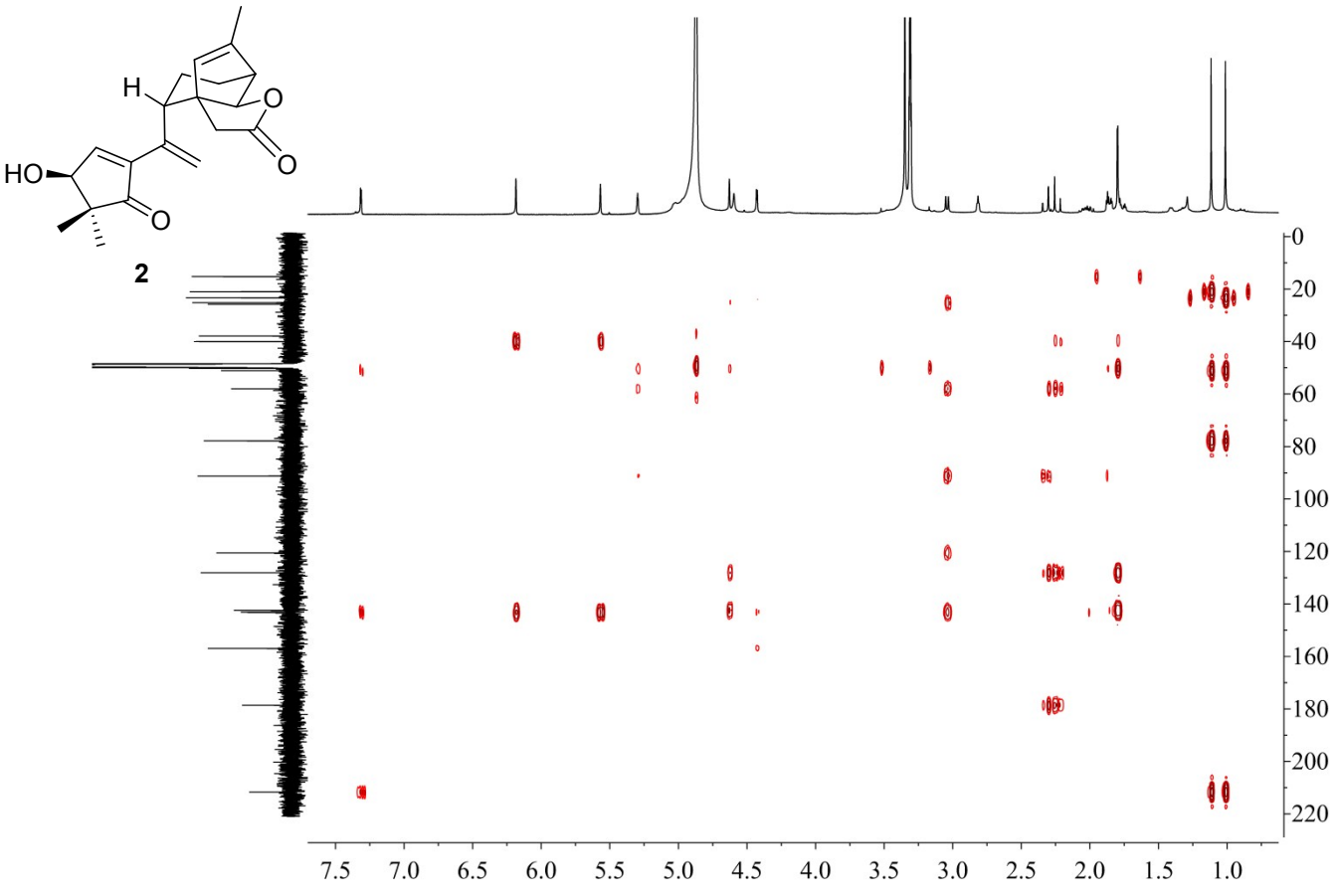


Figure S56. HMBC spectrum of **2** (^{1}H: 400 MHz, ^{13}C: 100 MHz, methanol-d_4)

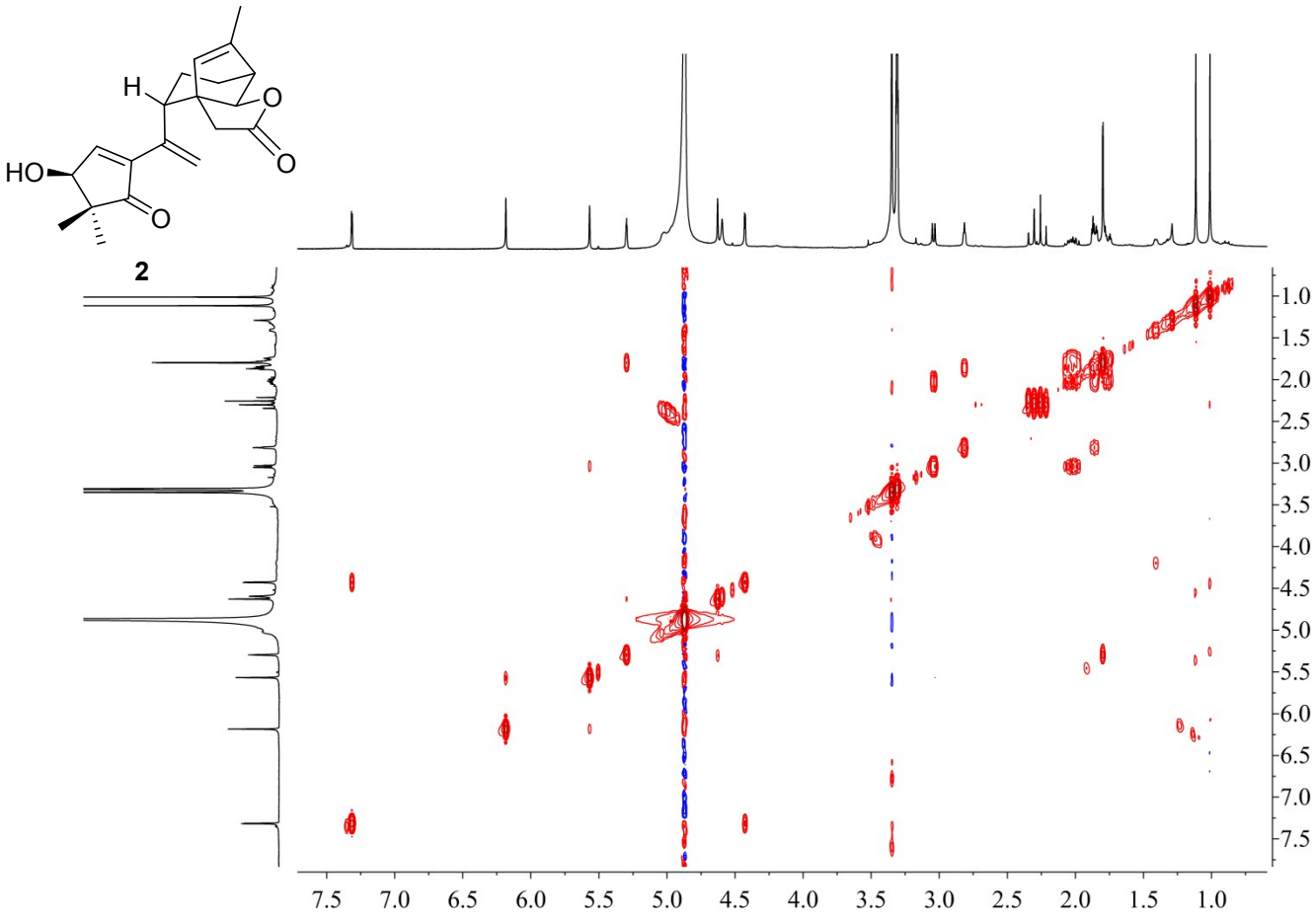


Figure S57. $^{1}H-^{1}H$ COSY spectrum of **2** (100 MHz, methanol-d_4)

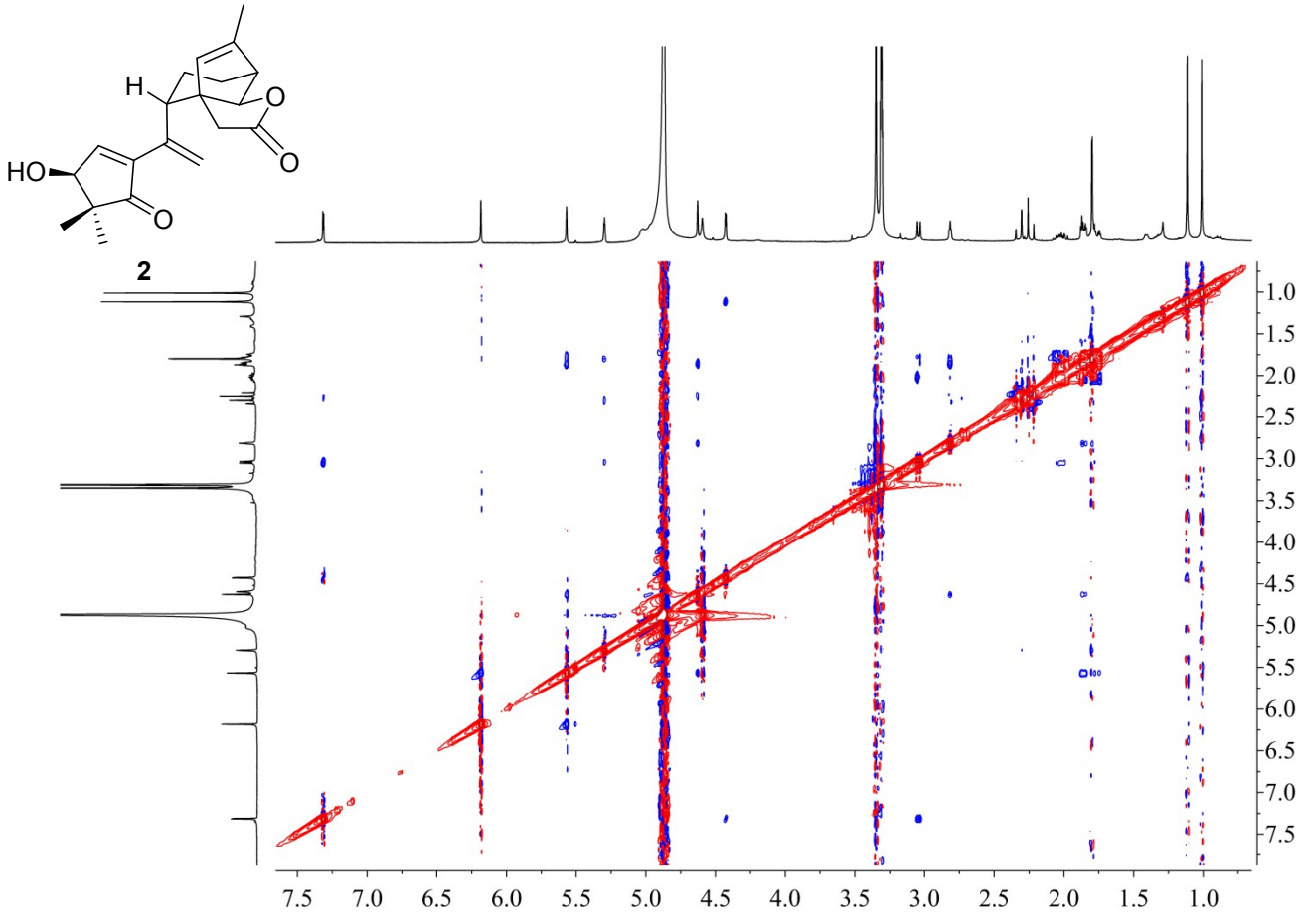


Figure S58. NOESY spectrum of **2** (100 MHz, methanol-d_4)

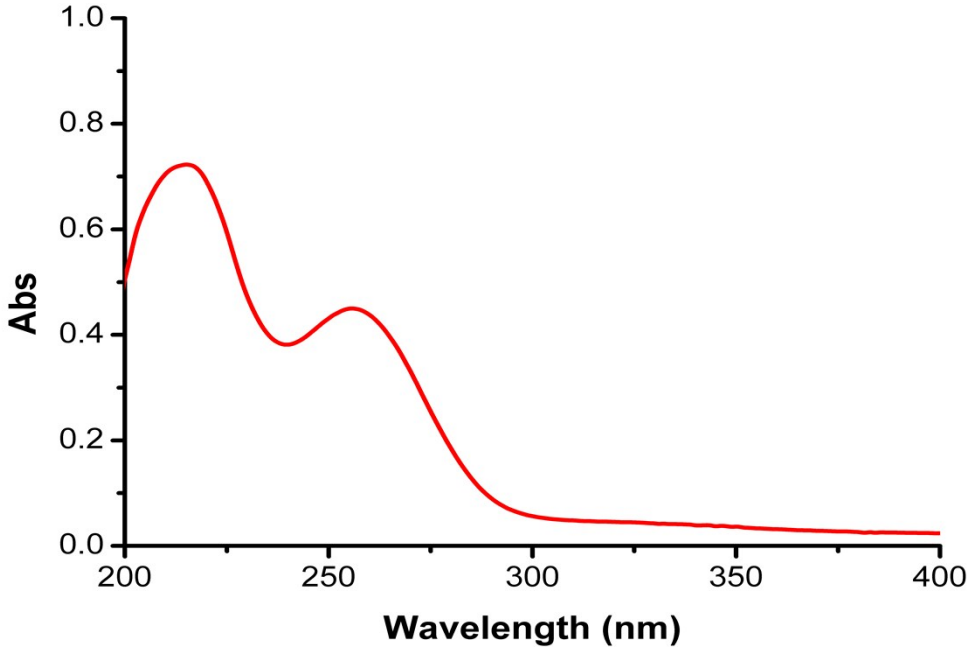


Figure S59. The UV spectrum of **3**.

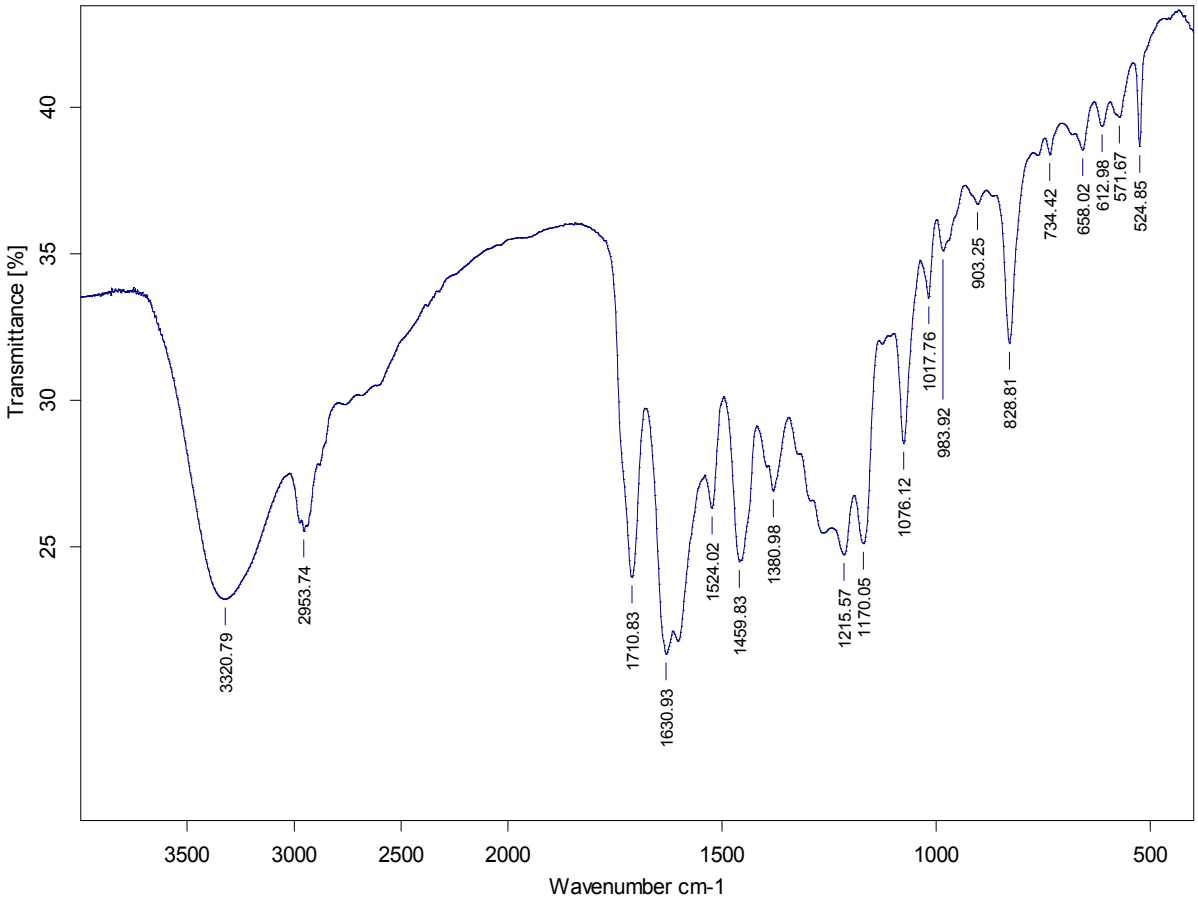


Figure S60. The IR spectrum of **3**

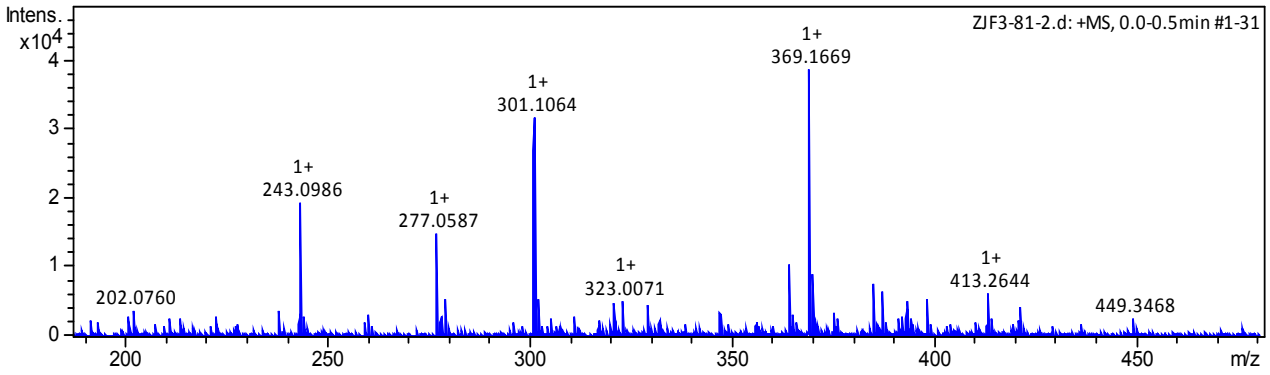


Figure S61. The HRESIMS spectrum of **3**

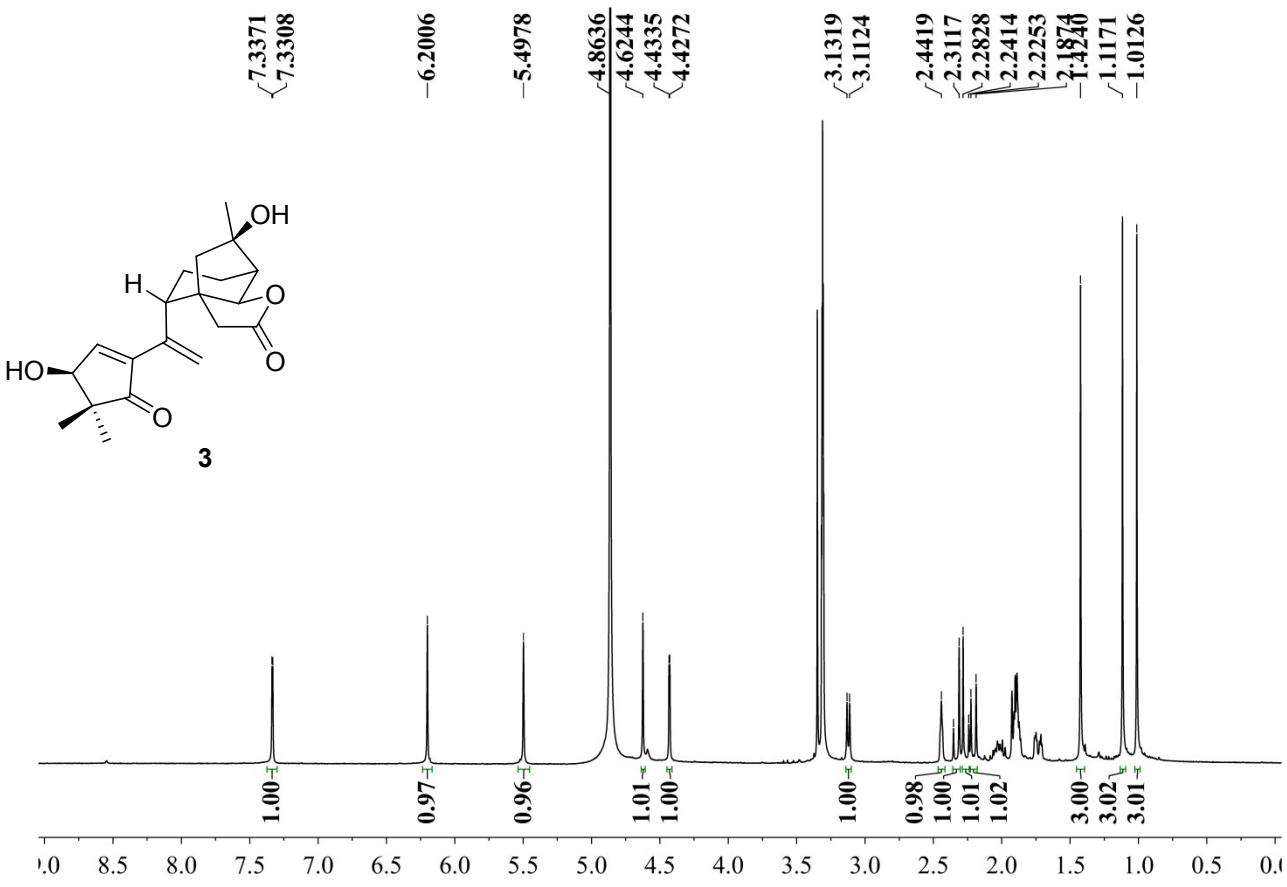


Figure S62. ^{1}H NMR spectrum of **3** (400 MHz, methanol-d_4)

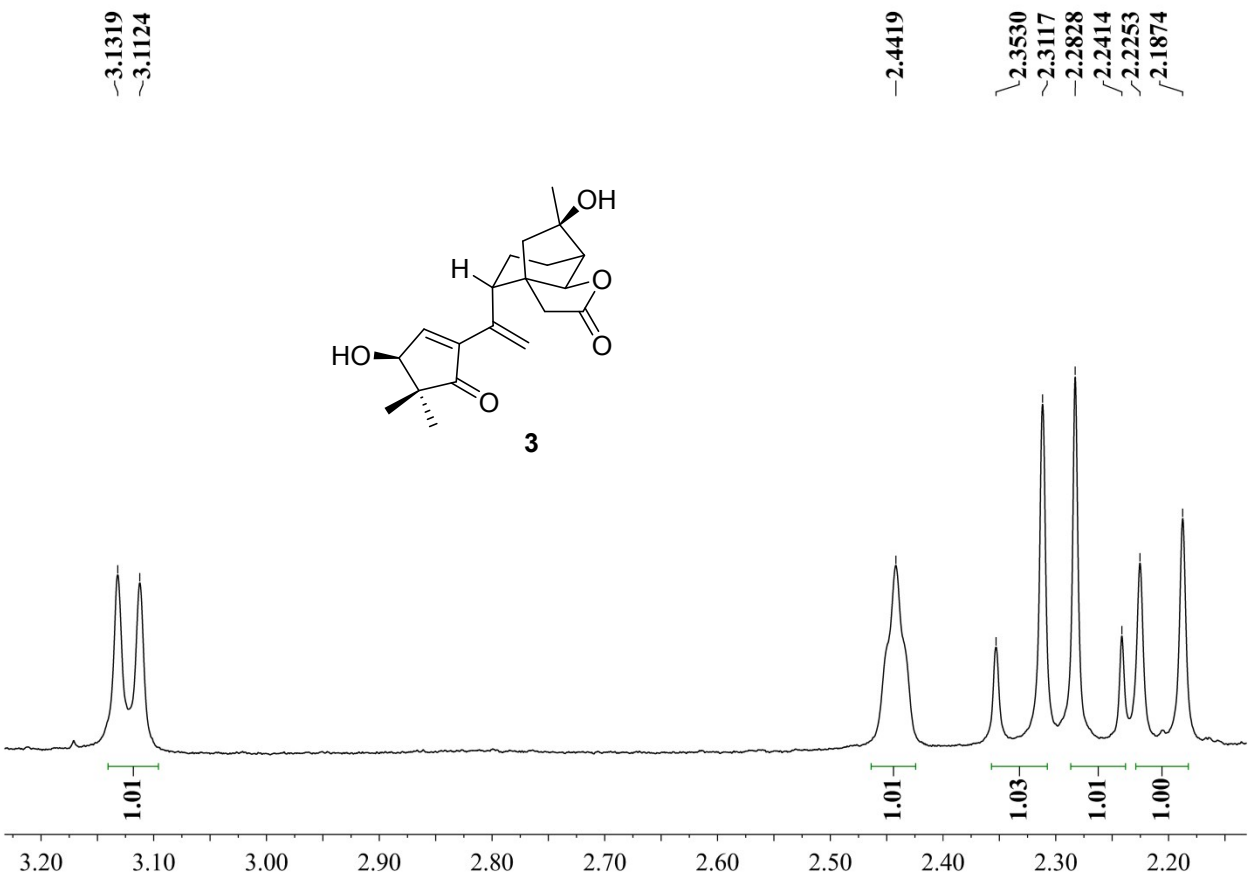


Figure S63. ^{1}H NMR spectrum of **3** (400 MHz, methanol-d_4, amplified)

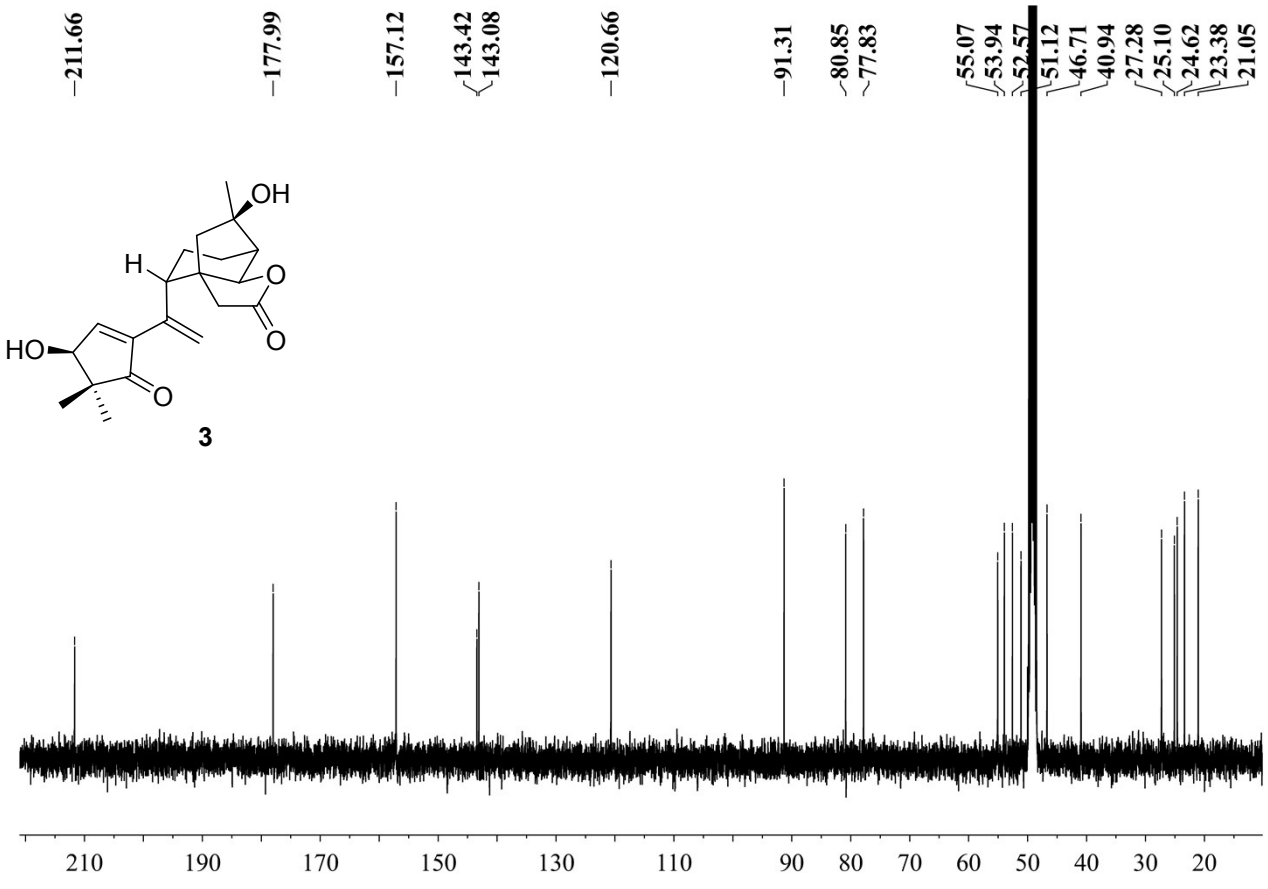


Figure S64. ^{13}C NMR spectrum of **3** (100 MHz, methanol-d_4)

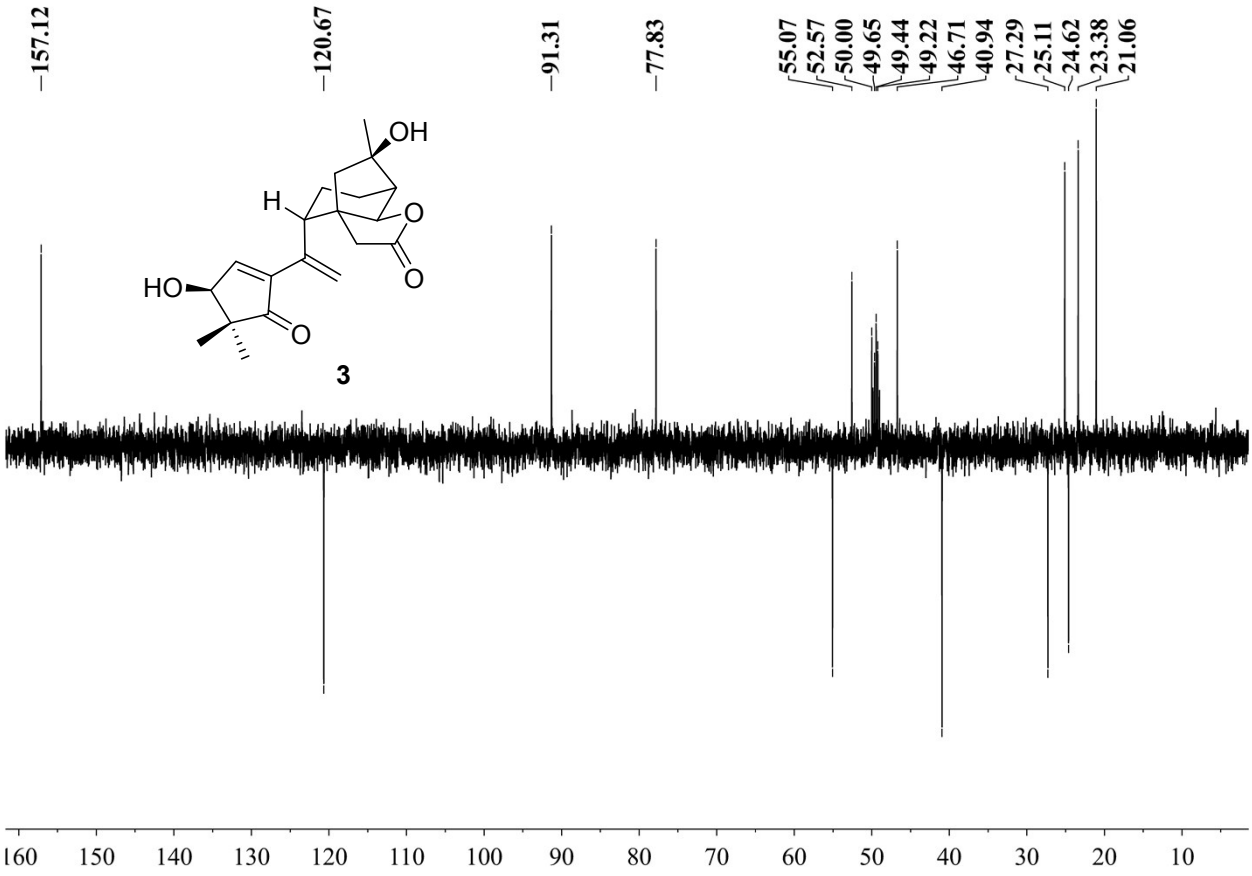


Figure S65. DEPT spectrum of **3** (100 MHz, methanol-d_4)

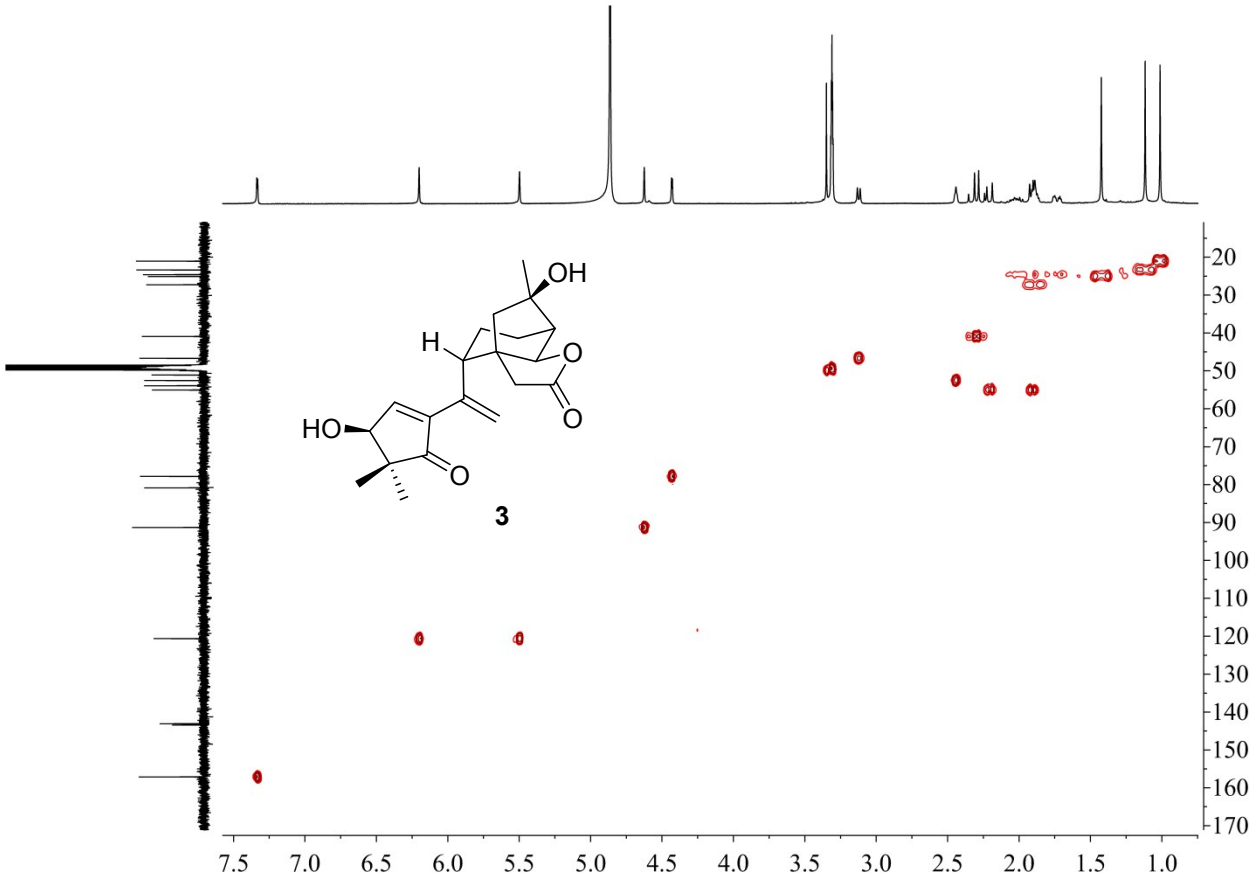


Figure S66. HSQC spectrum of **3** (^{1}H: 400 MHz, ^{13}C: 100 MHz, methanol-d_4)

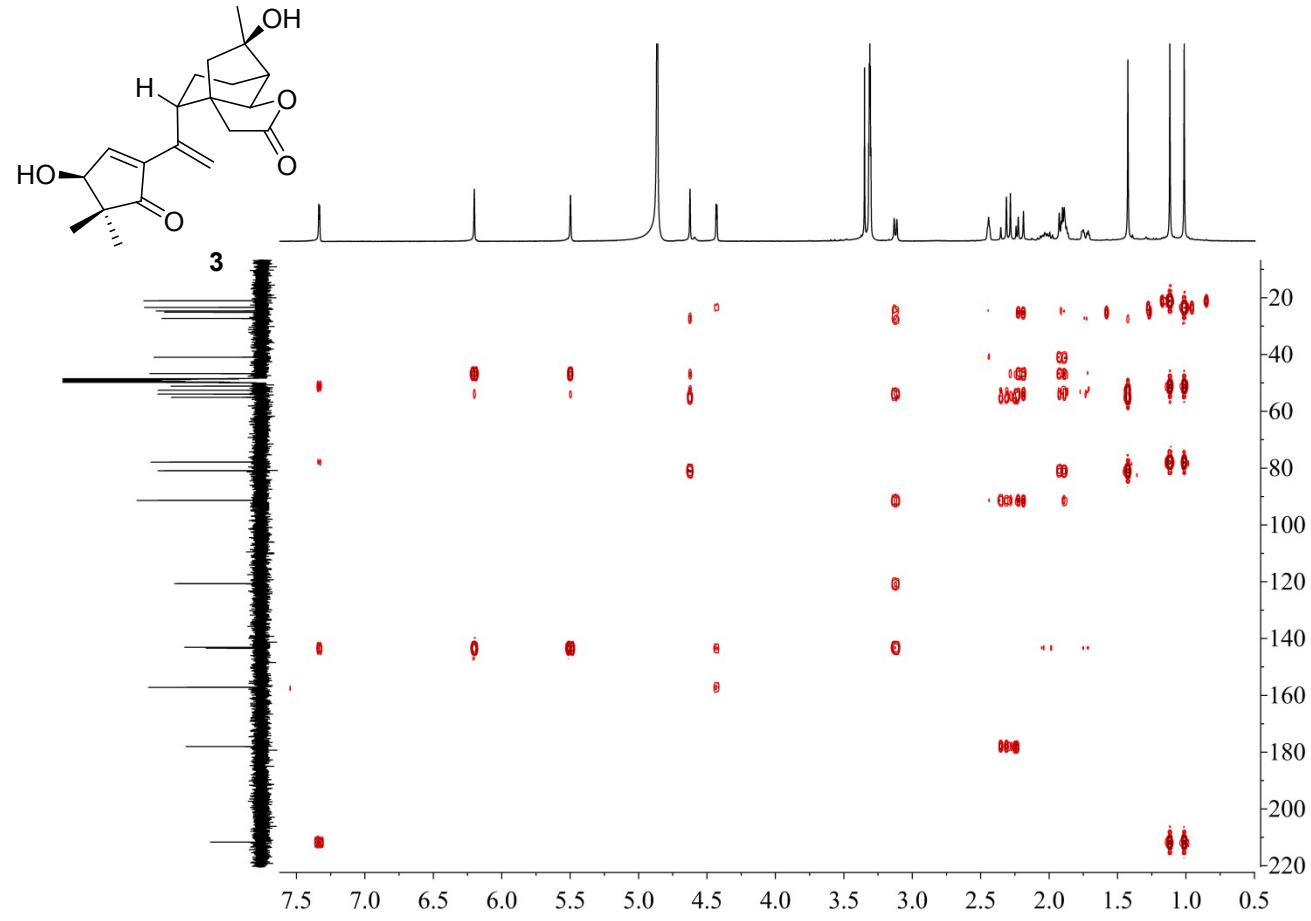


Figure S67. HMBC spectrum of **3** (^{1}H: 400 MHz, ^{13}C: 100 MHz, methanol-d_4)

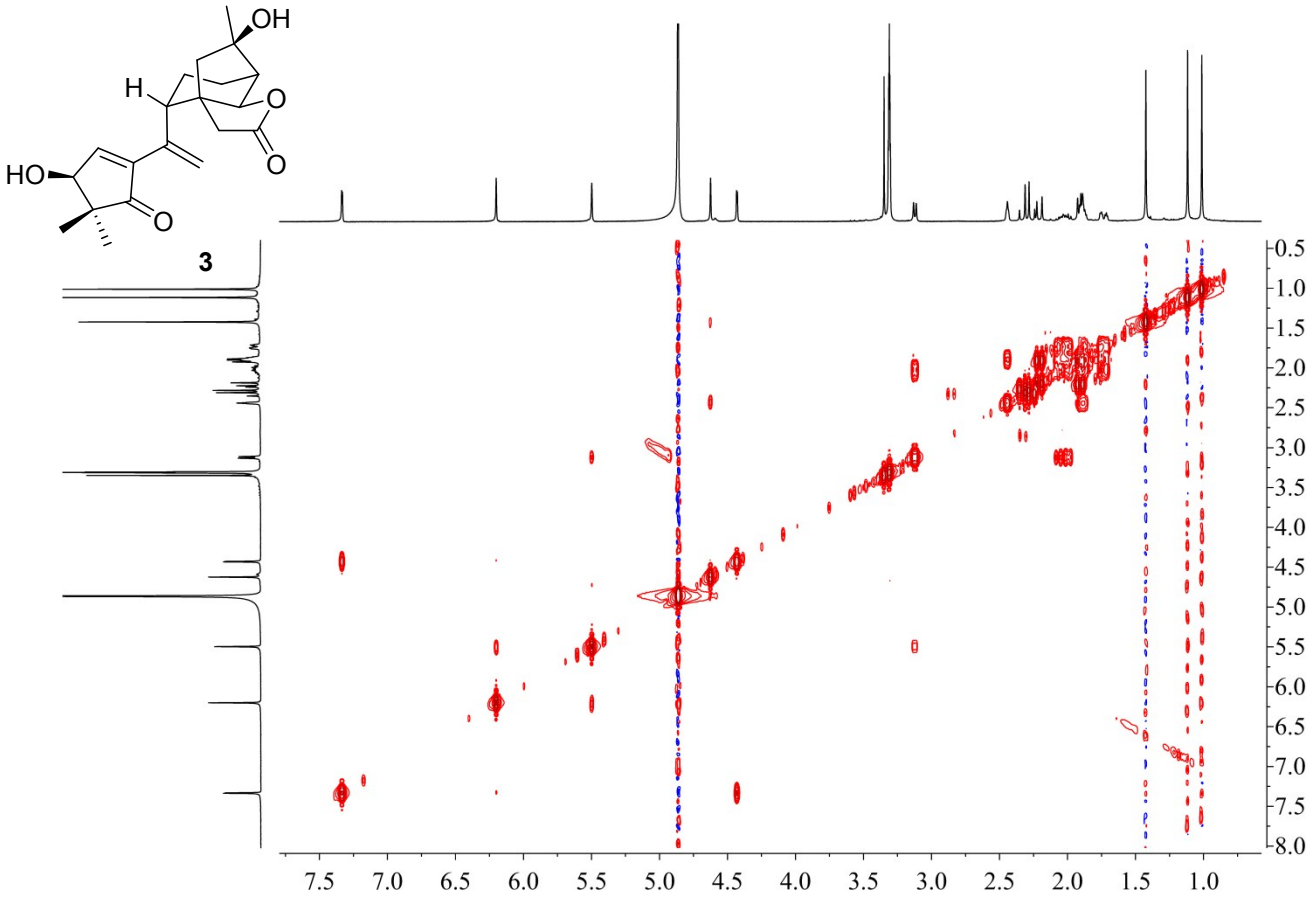


Figure S68. ^{1}H–^{1}H COSY spectrum of **3** (400 MHz, methanol-d_4)

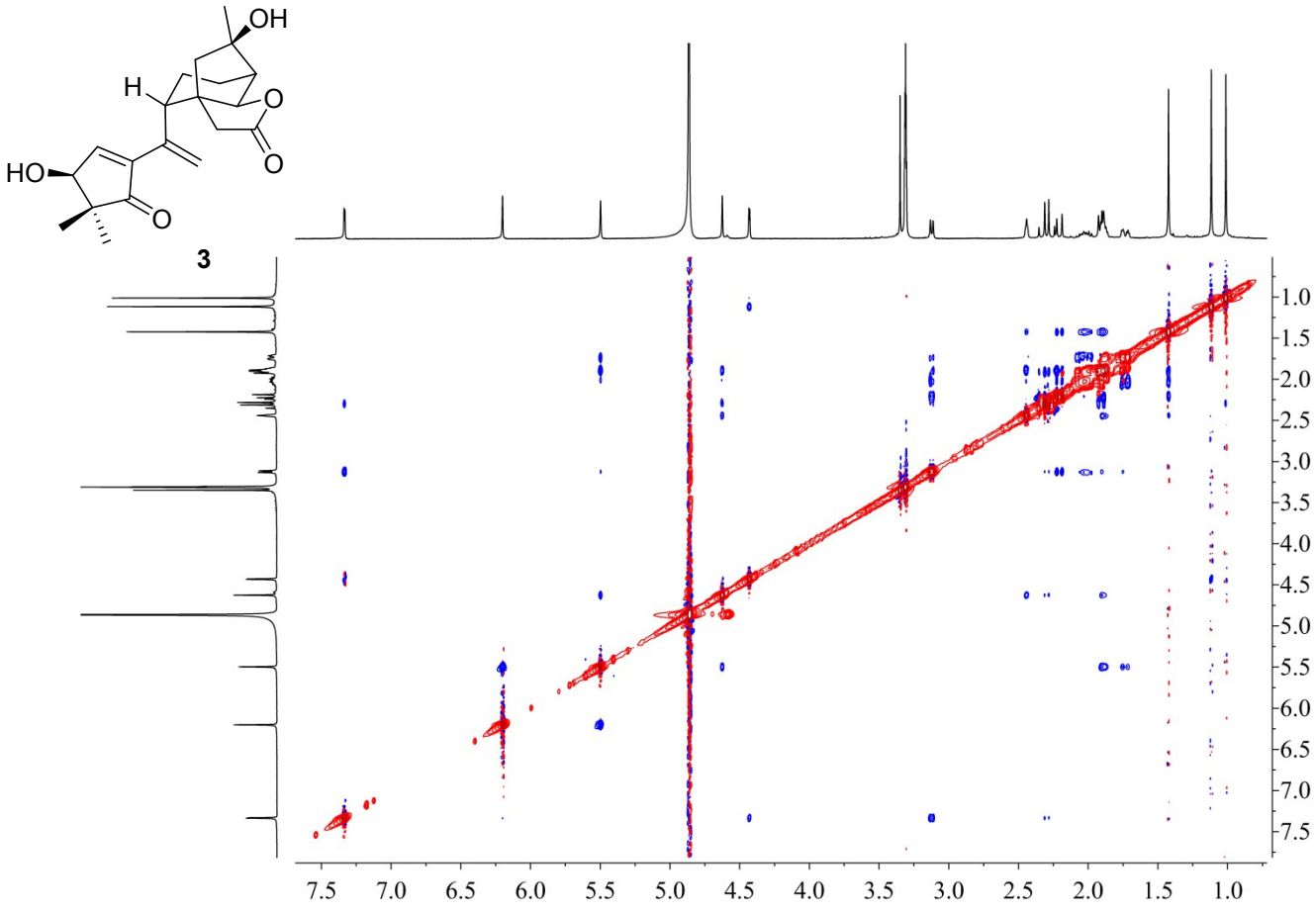


Figure S69. NOESY spectrum of **3** (400 MHz, methanol-d_4)

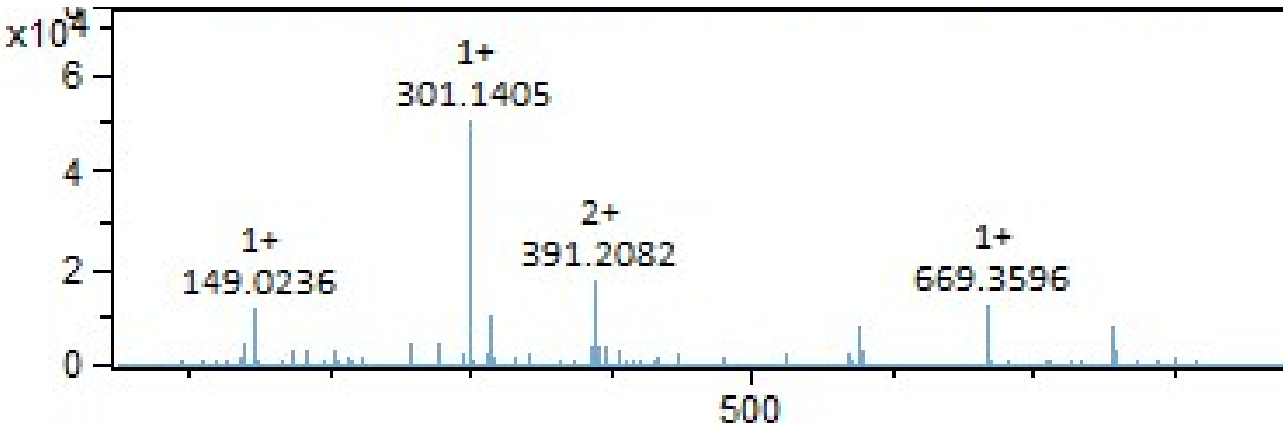
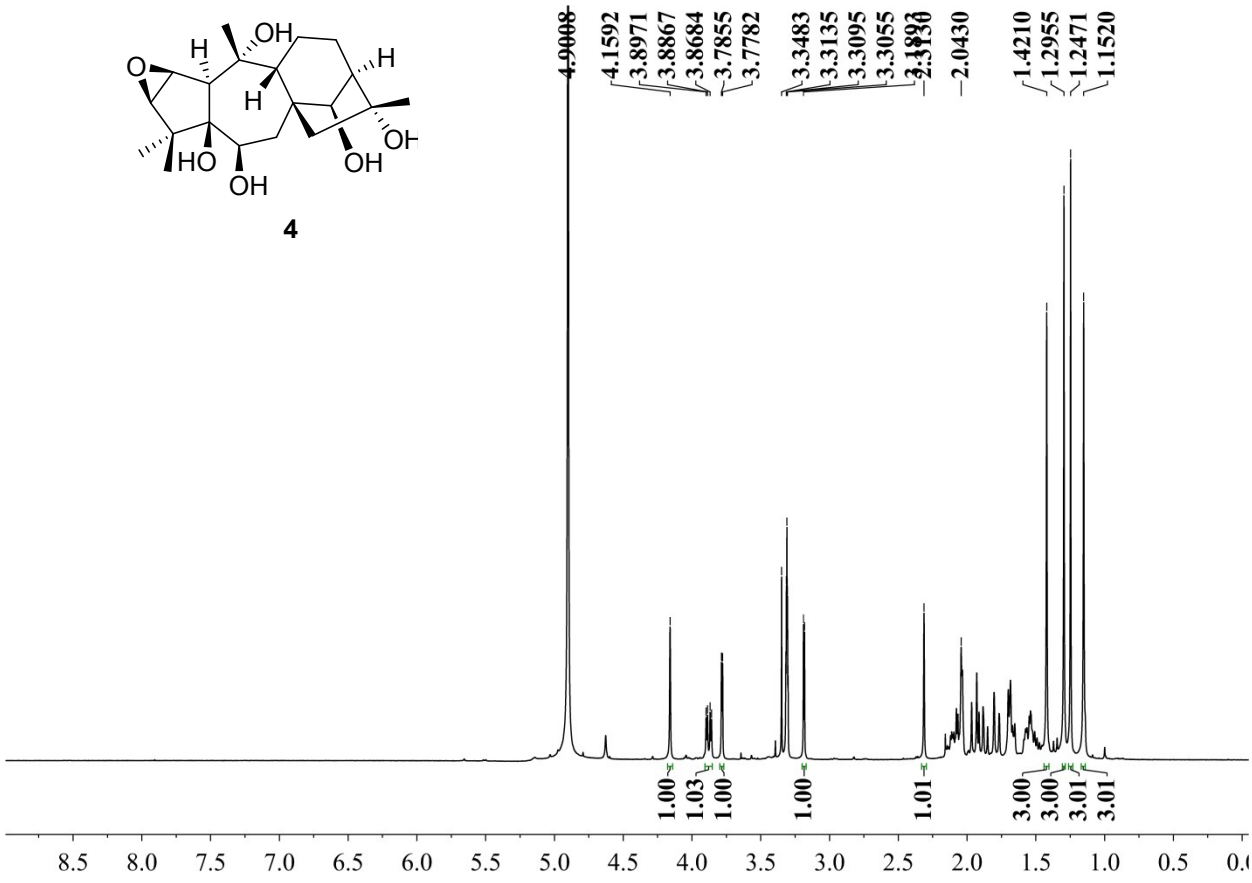
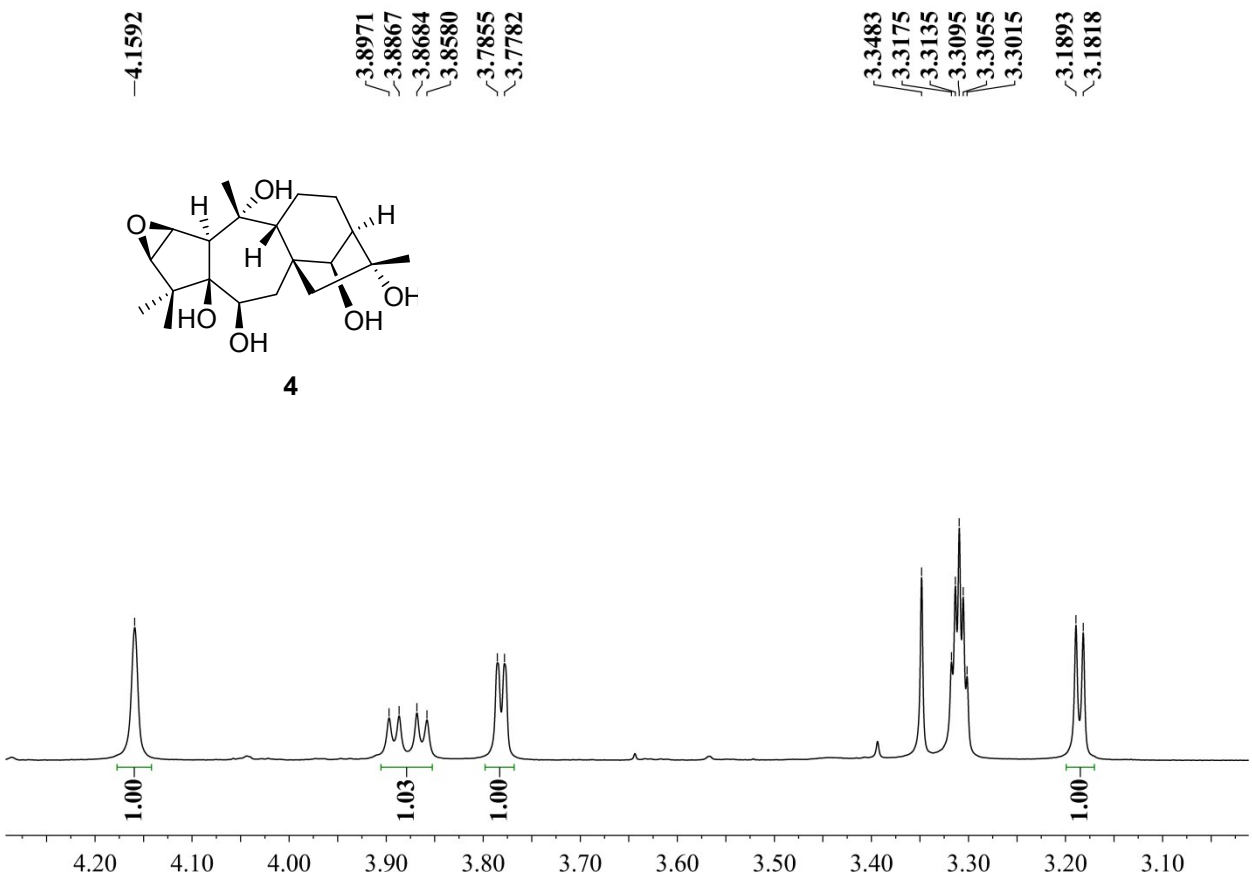


Figure S70. The HRESIMS spectrum of **4**



4

Figure S71. [1]H NMR spectrum of **4** (400 MHz, methanol-d_4)



4

Figure S72. [1]H NMR spectrum of **4** (400 MHz, methanol-d_4, amplified)

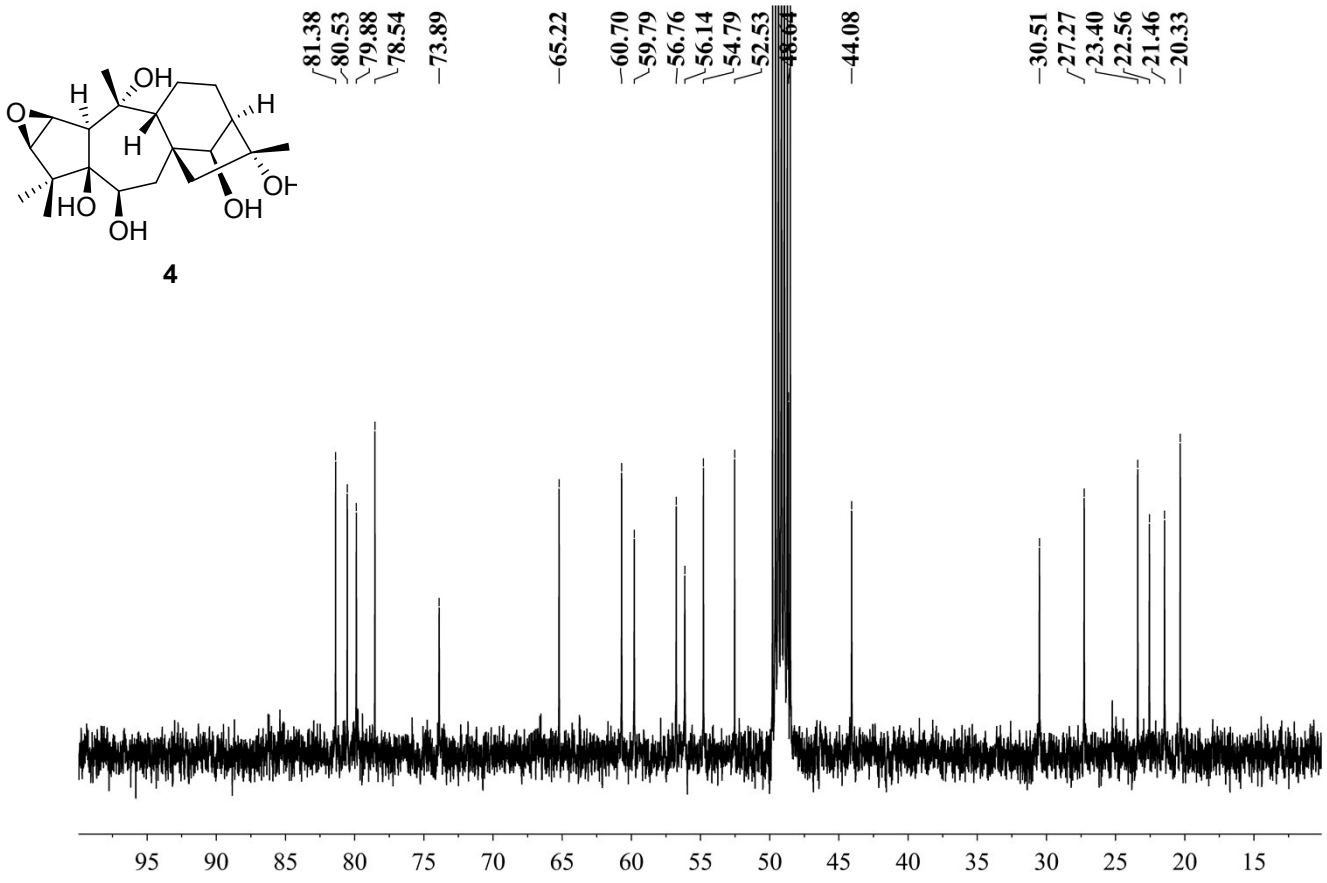


Figure S73. ^{13}C NMR spectrum of **4** (100 MHz, methanol-d_4)

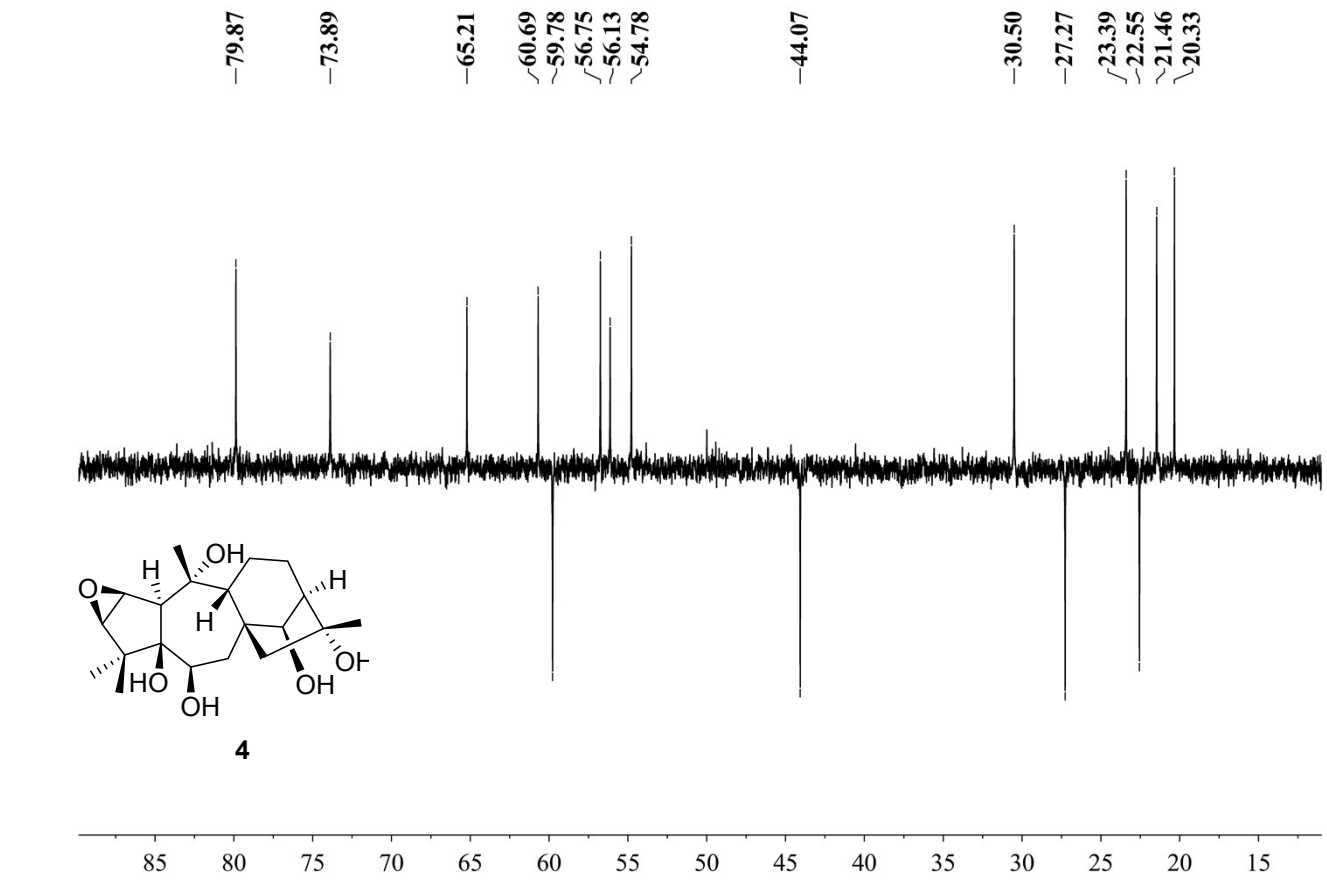


Figure S74. DEPT spectrum of **4** (100 MHz, methanol-d_4)

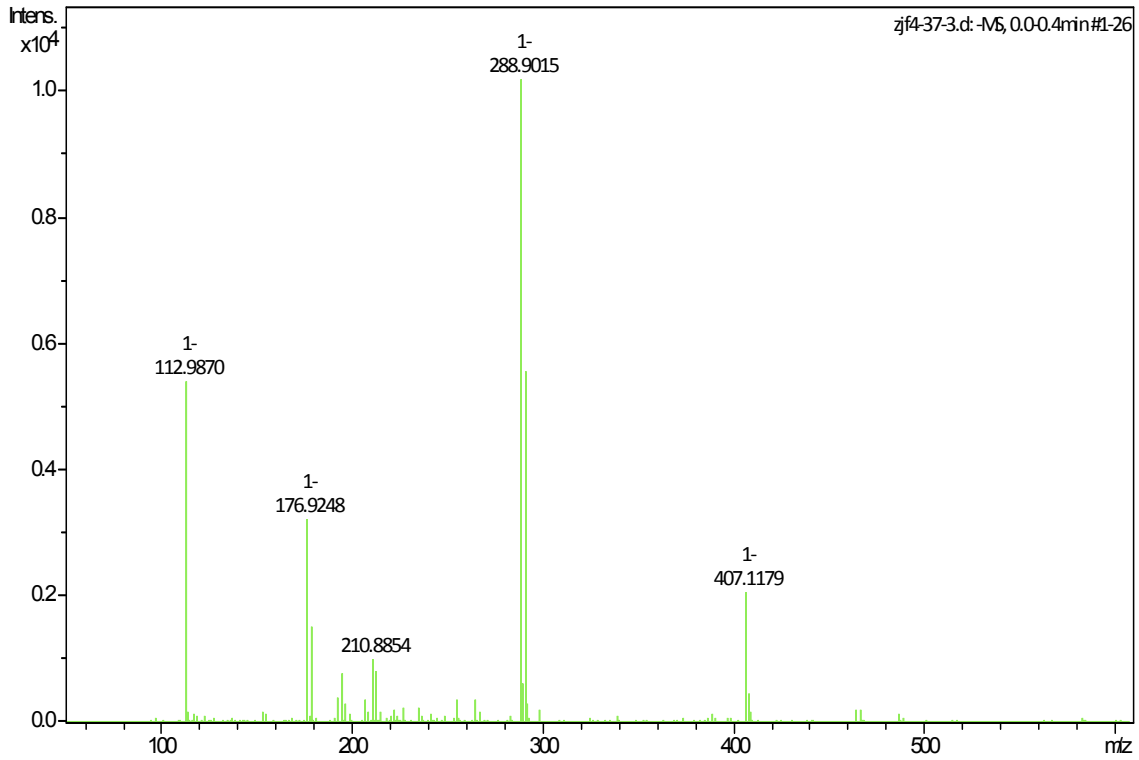


Figure S75. The HRESIMS spectrum of **9**

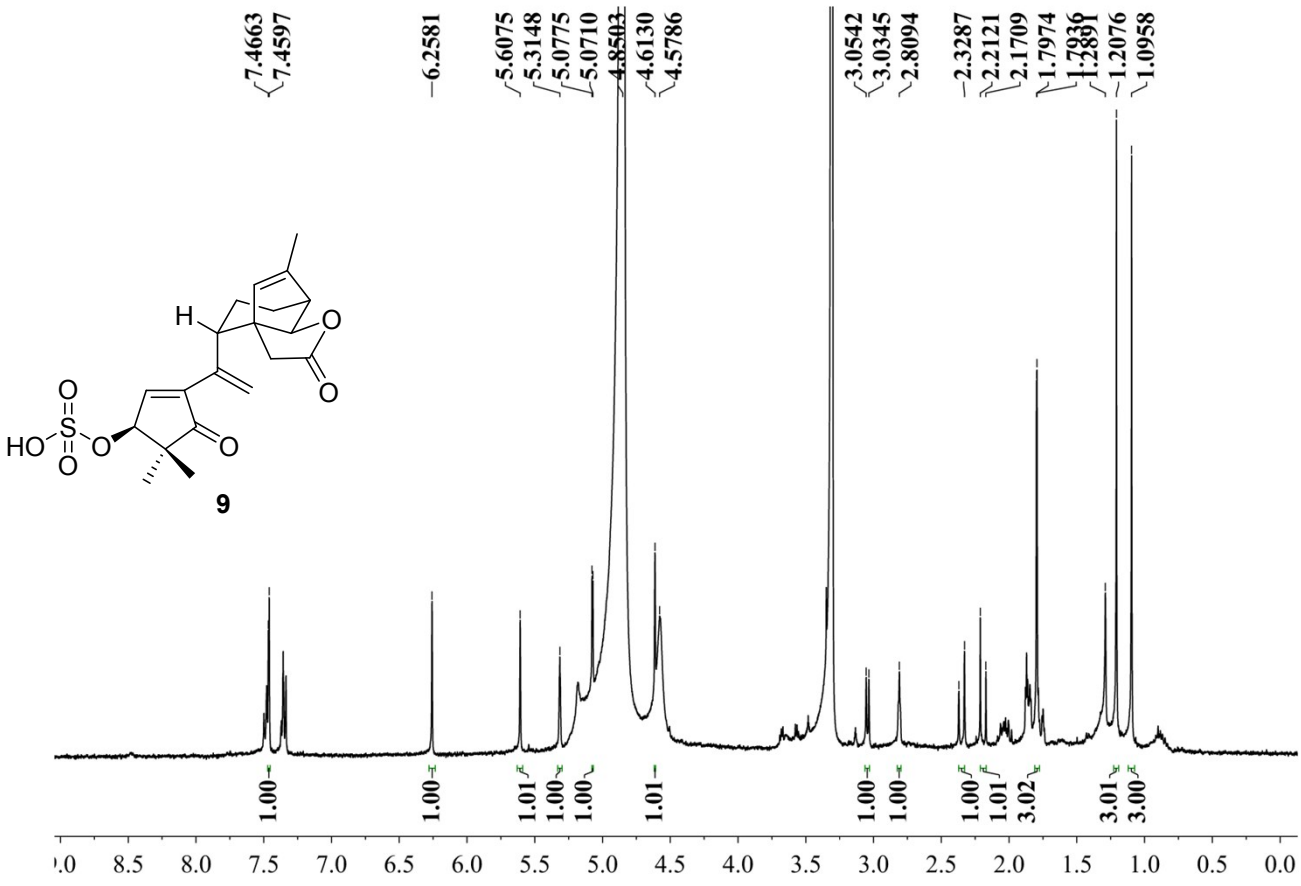


Figure S76. ^{1}H NMR spectrum of **9** (400 MHz, methanol-d_4)

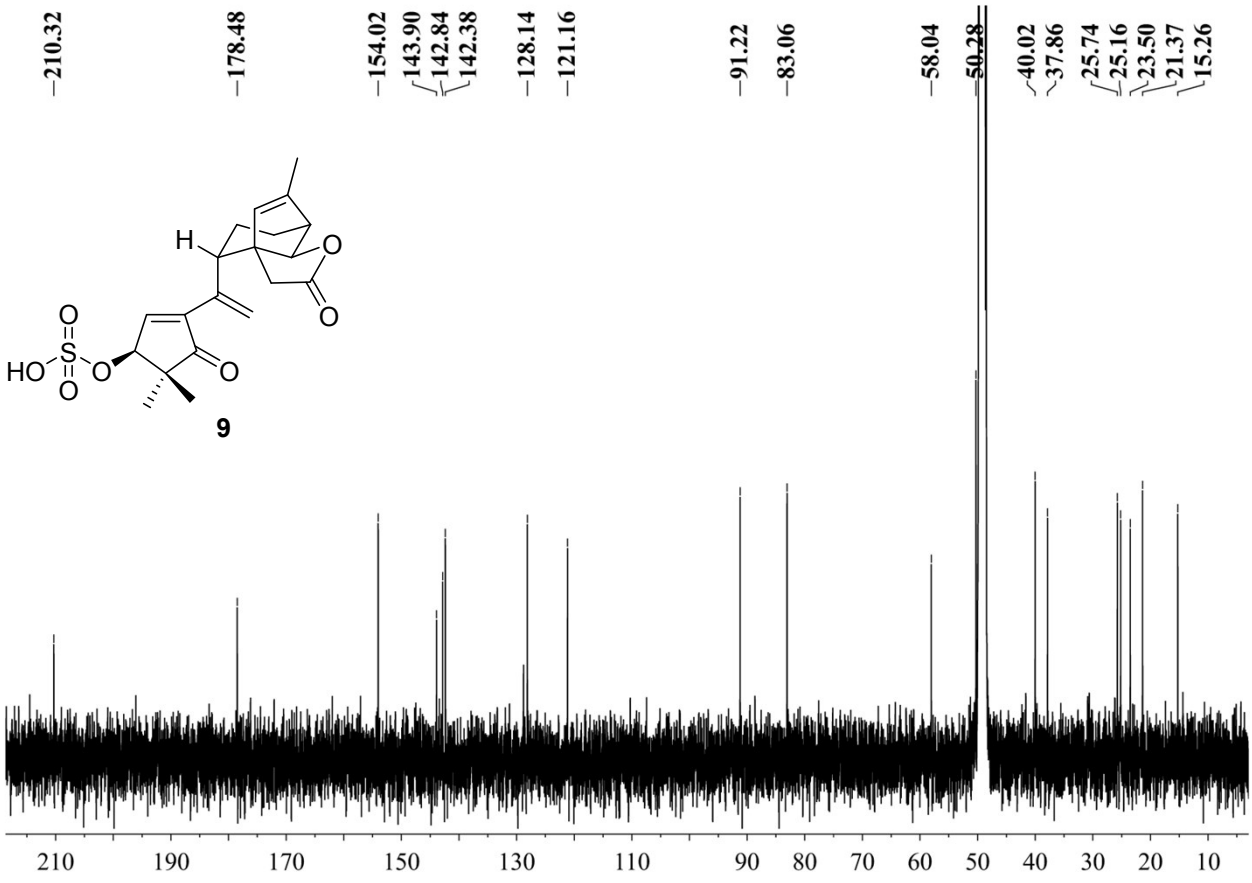


Figure S77. ^{13}C NMR spectrum of **9** (100 MHz, methanol-d_4)

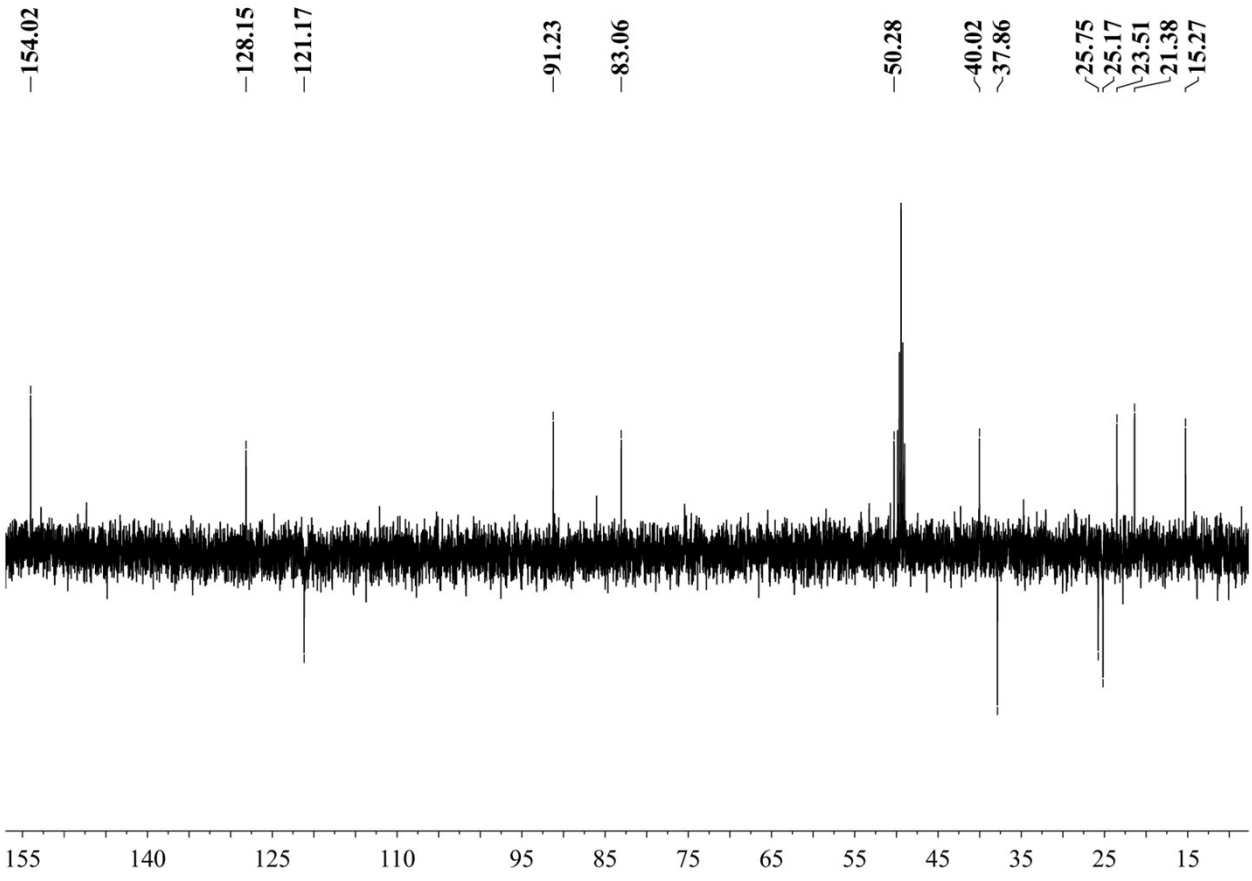


Figure S78. DEPT spectrum of **9** (100 MHz, methanol-d_4)

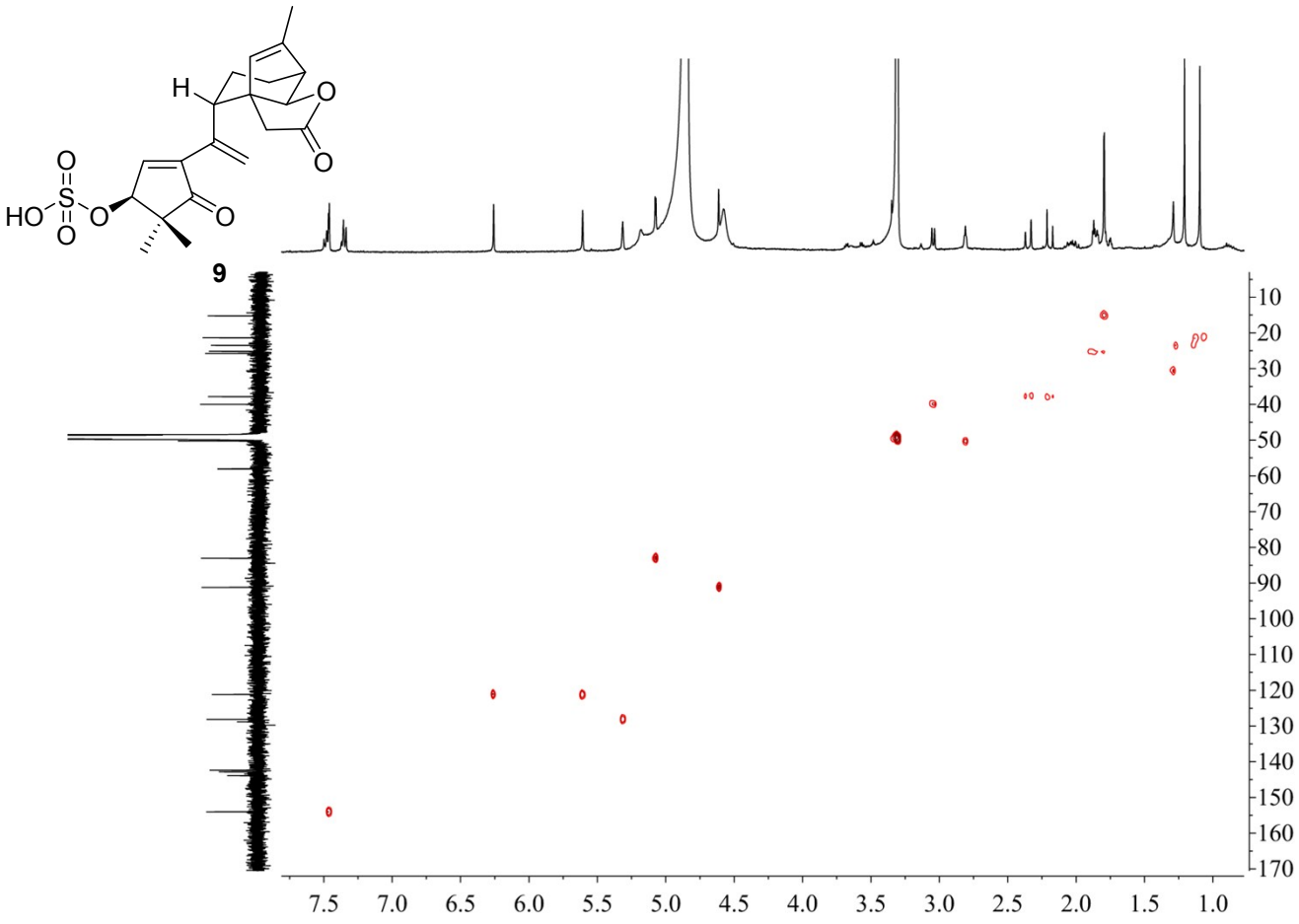


Figure S79. HSQC spectrum of **9** (^{1}H: 400 MHz, ^{13}C: 100 MHz, methanol-d_4)

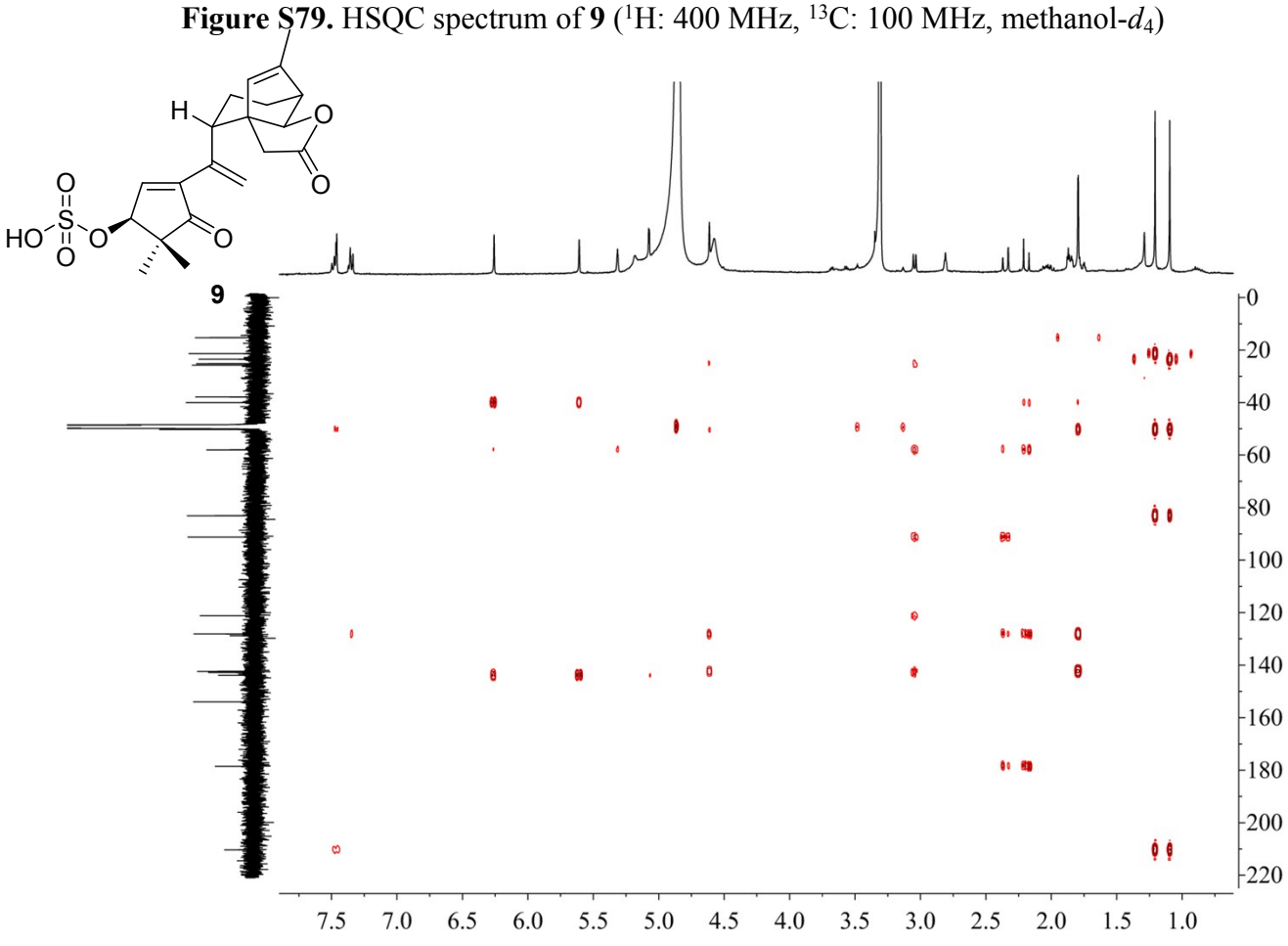


Figure S81. HMBC spectrum of **9** (^{1}H: 400 MHz, ^{13}C: 100 MHz, methanol-d_4)

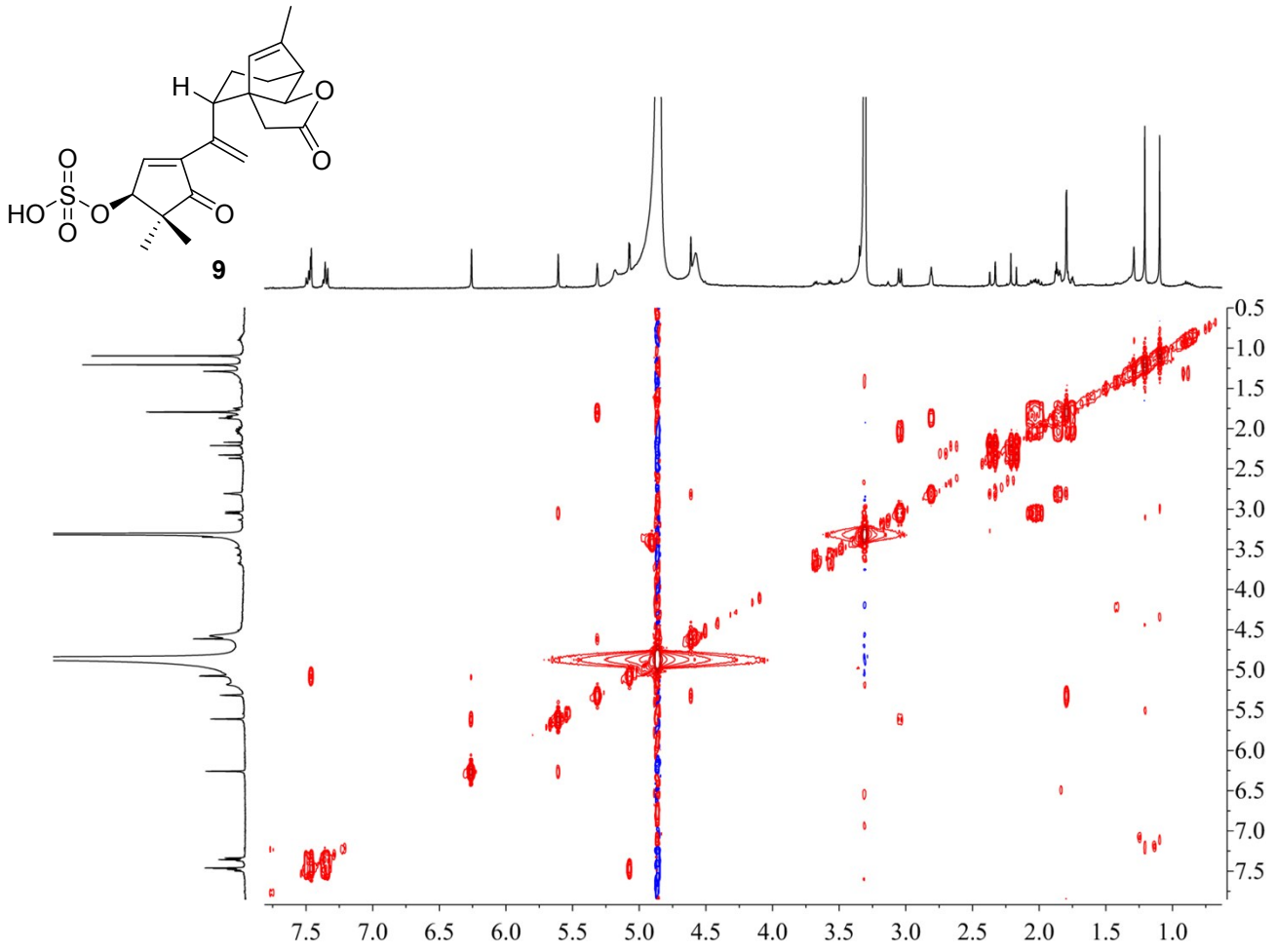


Figure S81. $^{1}H-^{1}H$ COSY spectrum of **9** (400 MHz, methanol-d_4)

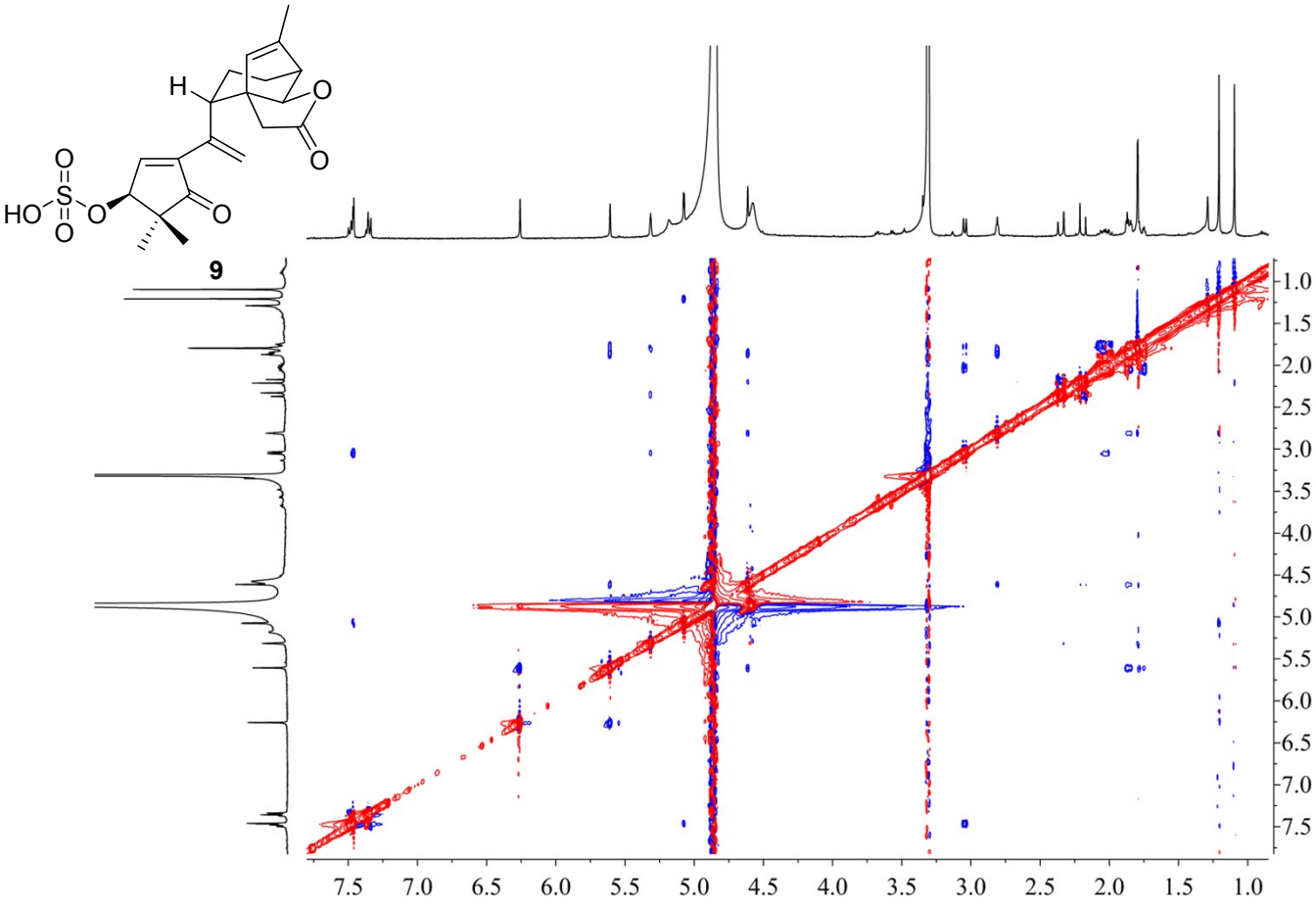


Figure S82. NOESY spectrum of **9** (400 MHz, methanol-d_4)