

SUPPLEMENTARY MATERIAL

Two new biflavanoids from *Selaginella trichoclada* Alsto

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Abstract

Two new robustaflavones, (±)-trichocladabiflavone A (1) and uncinatabiflavone E (2), along with seven known biflavanoids (3-9) were isolated from the 70% EtOH extract of *Selaginella trichoclada*. Their structures and absolute configurations were established by extensive spectroscopic and circular dichroism (CD) analyses. Compound 1 was resolved into optically pure enantiomers (+)-1 and (–)-1 by chiral-phase HPLC. Moreover, compounds 1 and 2 exhibited moderate cytotoxicity against A549 and HepG2 human cancer cell lines.

Keywords: *Selaginella trichoclada*; robustaflavone; cytotoxicity

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Table S1 ^1H NMR and ^{13}C NMR spectroscopic data of compounds **1** and **2**

Position	1		2	
	δ_{H} (<i>J</i> in Hz)	δ_{C}	δ_{H} (<i>J</i> in Hz)	δ_{C}
2		163.9	5.48 (1H, dd, 2.8, 12.9)	79.5
3	6.97, 6.98 (1H, s)	104.1	3.32 (1H, m) 2.71 (1H, dd, 2.8, 17.1)	42.5
4		182.4		197.6
5	13.11 (1H, br s)	157.9		163.6
6		108.0	6.07 (1H, d, 2.2)	95.1
7		163.6		167.9
8	6.93, 6.95 (1H, s)	91.1	6.12 (1H, d, 2.2)	94.2
9		155.9		163.5
10		104.9		103.1
1'		122.7		127.7
2'	7.84 (1H, m)	130.7, 130.8	7.32 (1H, d, 2.0)	132.0
3'		123.3		116.9
4'		161.4		157.5
5'	7.23 (1H, d, 8.8)	112.1	6.81 (1H, d, 8.3)	115.6
6'	8.12 (1H, d, 8.8)	128.3	7.24 (1H, dd, 2.0, 8.3)	126.8
2''	5.44-5.55 (1H, m)	78.7, 78.9	5.39 (1H, dd, 2.6, 12.5)	78.4
3''	3.16-3.29 (1H, m) 2.69-2.77 (1H, m)	42.3, 42.5	3.18 (1H, dd, 12.5, 16.9) 2.65 (1H, dd, 2.6, 16.9)	42.5
4''		196.9		195.2
5''	12.39 (1H, br s)	161.3	12.13 (1H, br s)	158.1
6''		106.1, 106.0		108.1
7''		162.4		161.7
8''	6.07 (1H, s)	95.1	5.81 (1H, s)	96.7
9''		162.4		161.8
10''		101.9, 102.0		100.5
1'''		129.3, 129.4		129.8
2'''/6'''	7.36 (2H, d, 8.3)	128.8	7.33 (2H, d, 8.7)	128.7
3'''/5'''	6.82 (2H, d, 8.3)	115.7	6.80 (2H, d, 8.7)	115.6
4'''	9.65 (1H, br s)	158.2		158.1
7-OCH ₃	3.90, 3.91 (3H, s)	56.8	3.78 (1H, s)	56.3
4'-OCH ₃	3.79, 3.80 (3H, s)	56.3		
6-CH ₃	2.01 (3H, s)	7.8		

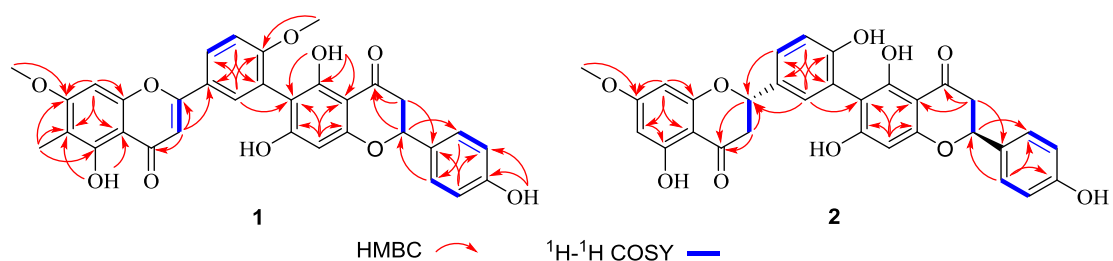


Figure S1. Key HMBC and ^1H - ^1H COSY correlations of compounds **1** and **2**

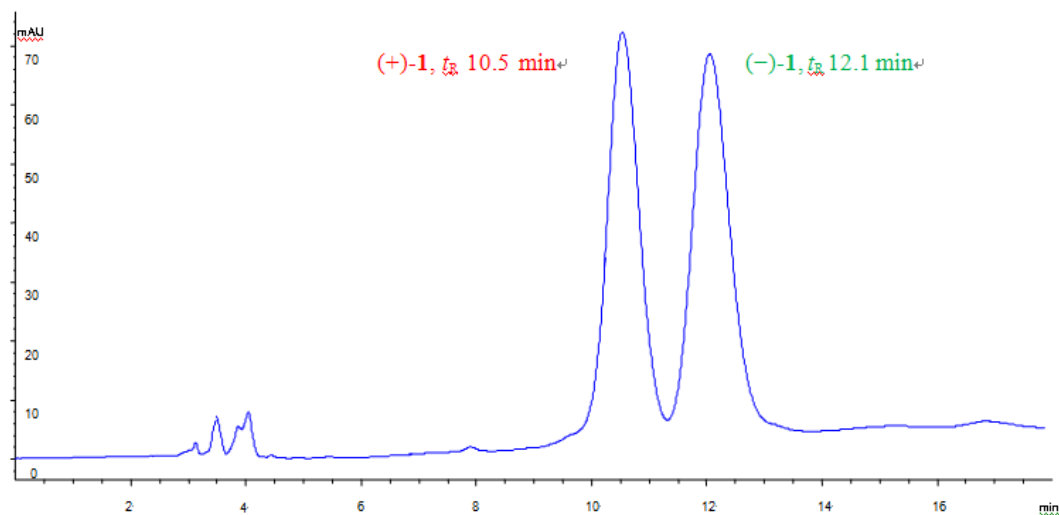


Figure S2. Chiral HPLC separation chromatograms of compound **1**.

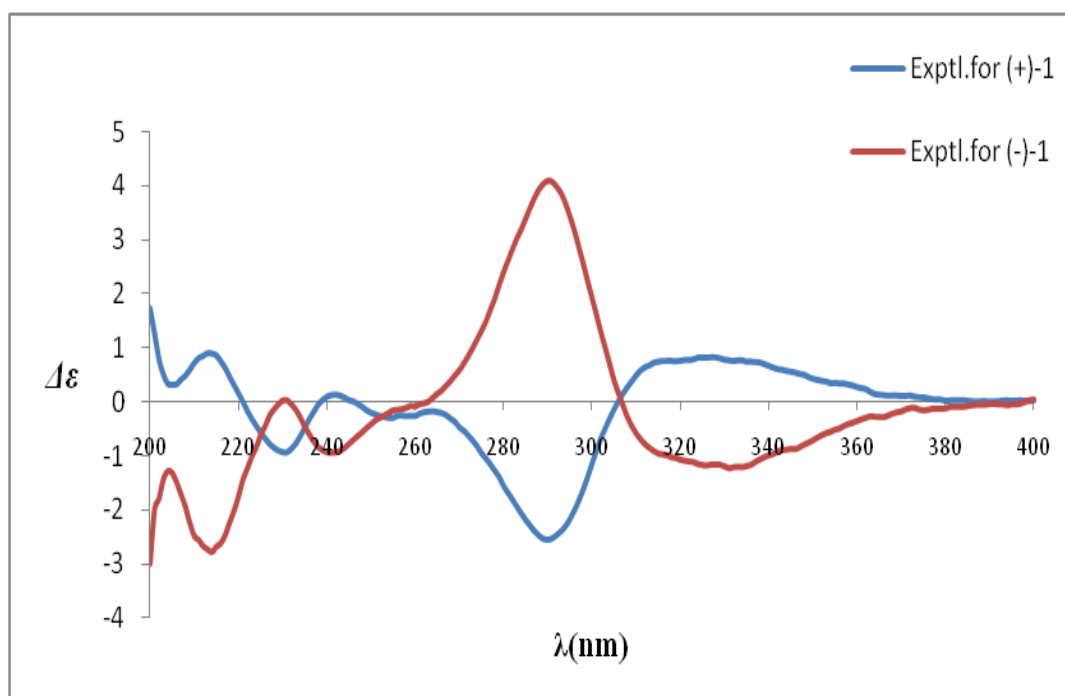


Figure S3. CD spectrum of compound $(\pm)\text{-1}$ in MeOH

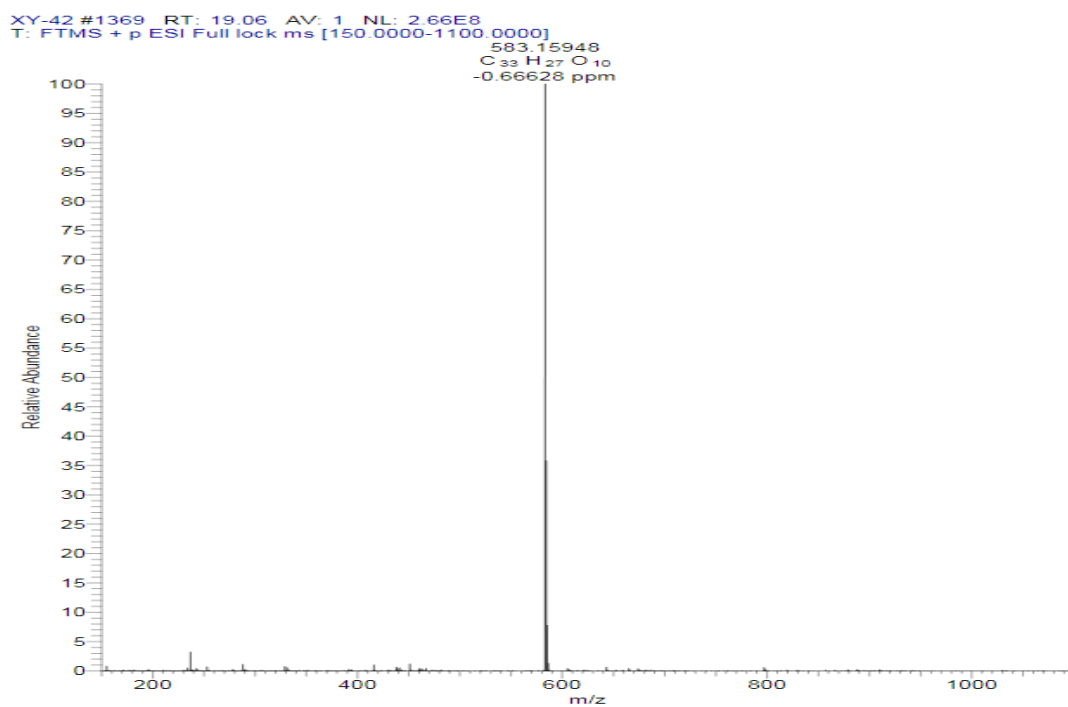


Figure S4. HRESIMS spectrum of compound **1**

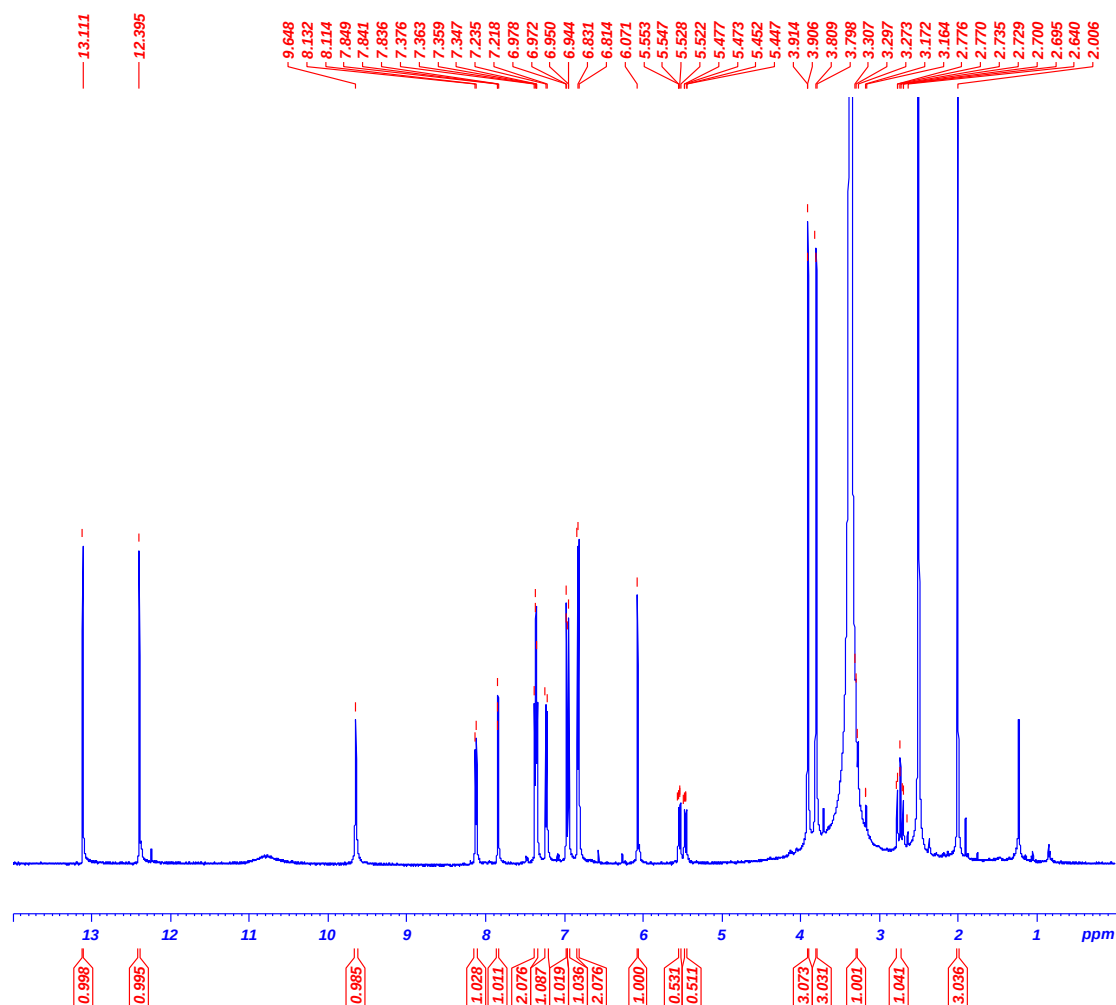


Figure S5. ¹H NMR spectrum (500 MHz, DMSO-*d*₆) of compound **1**

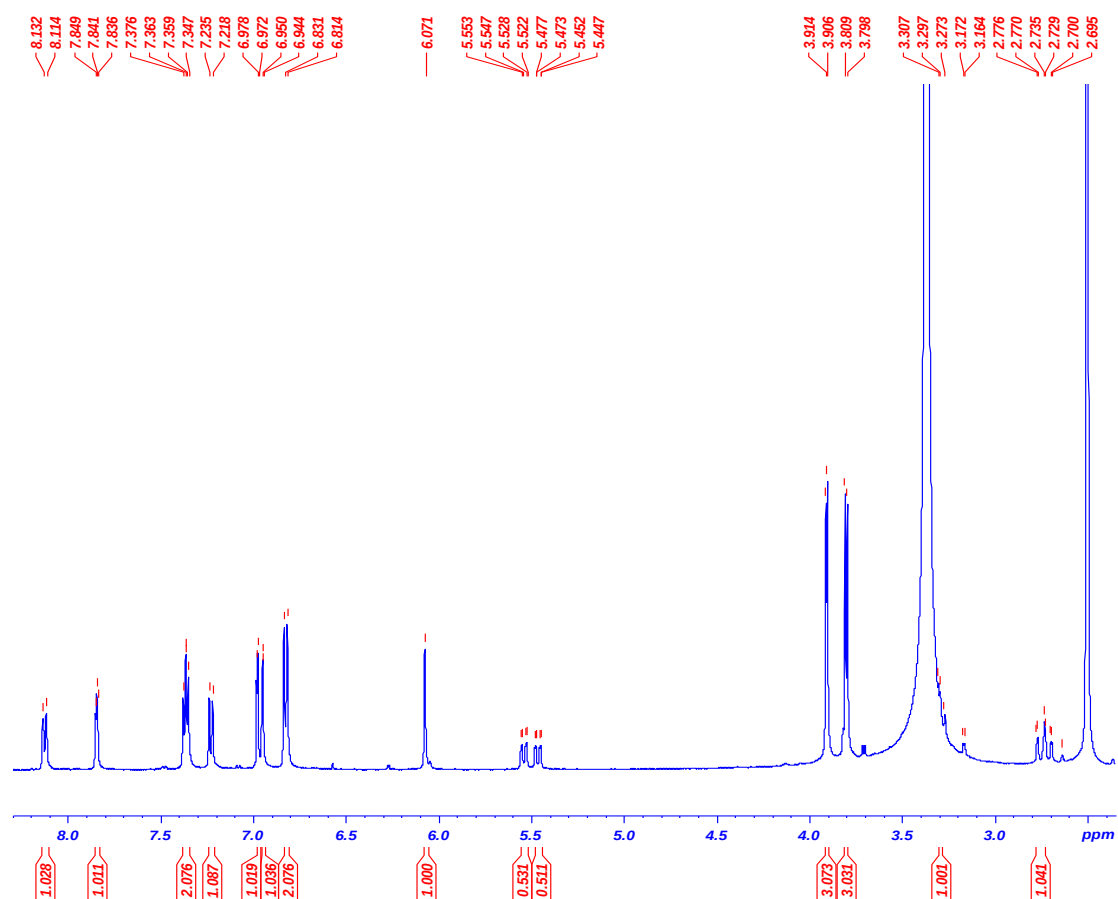


Figure S6. ^1H NMR spectrum of compound **1** (zoomed in)

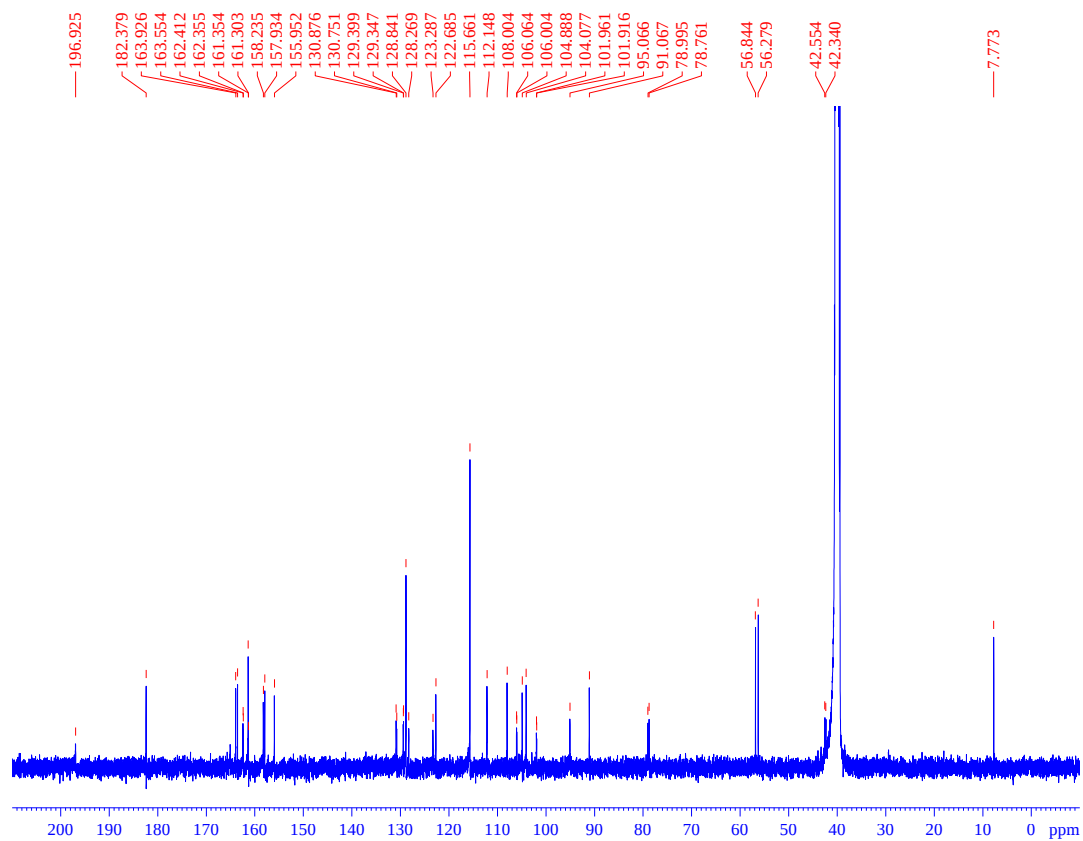


Figure S7. ^{13}C NMR spectrum (500 MHz, $\text{DMSO}-d_6$) of compound **1**

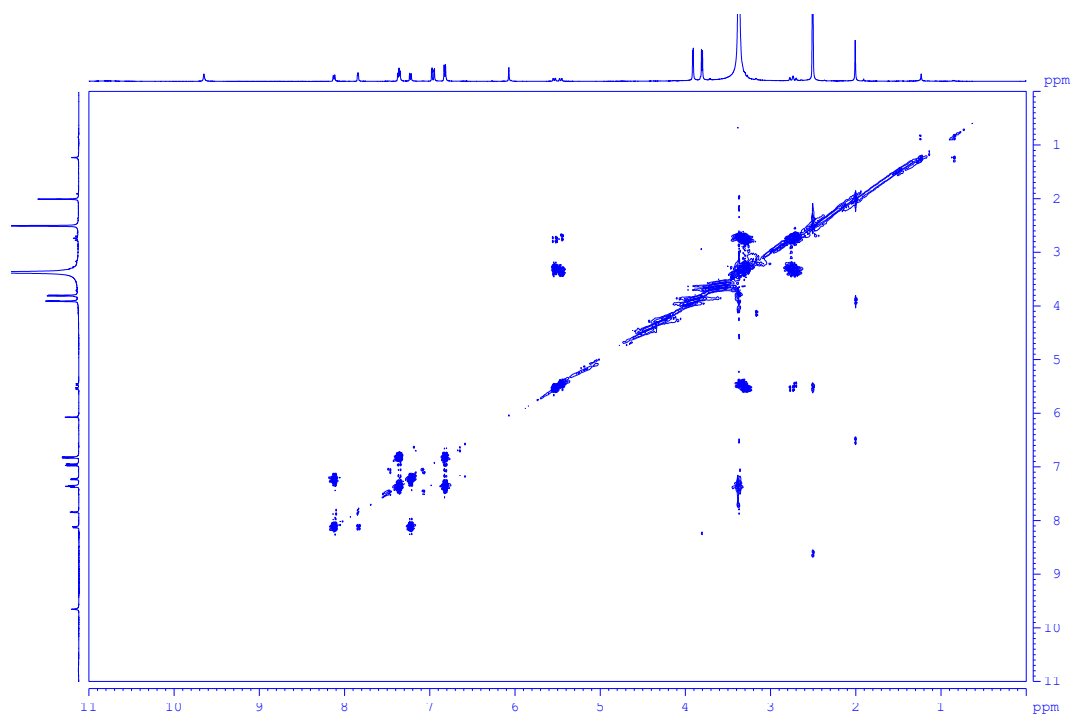


Figure S8. ^1H - ^1H COSY spectrum (500 MHz, $\text{DMSO-}d_6$) of compound **1**

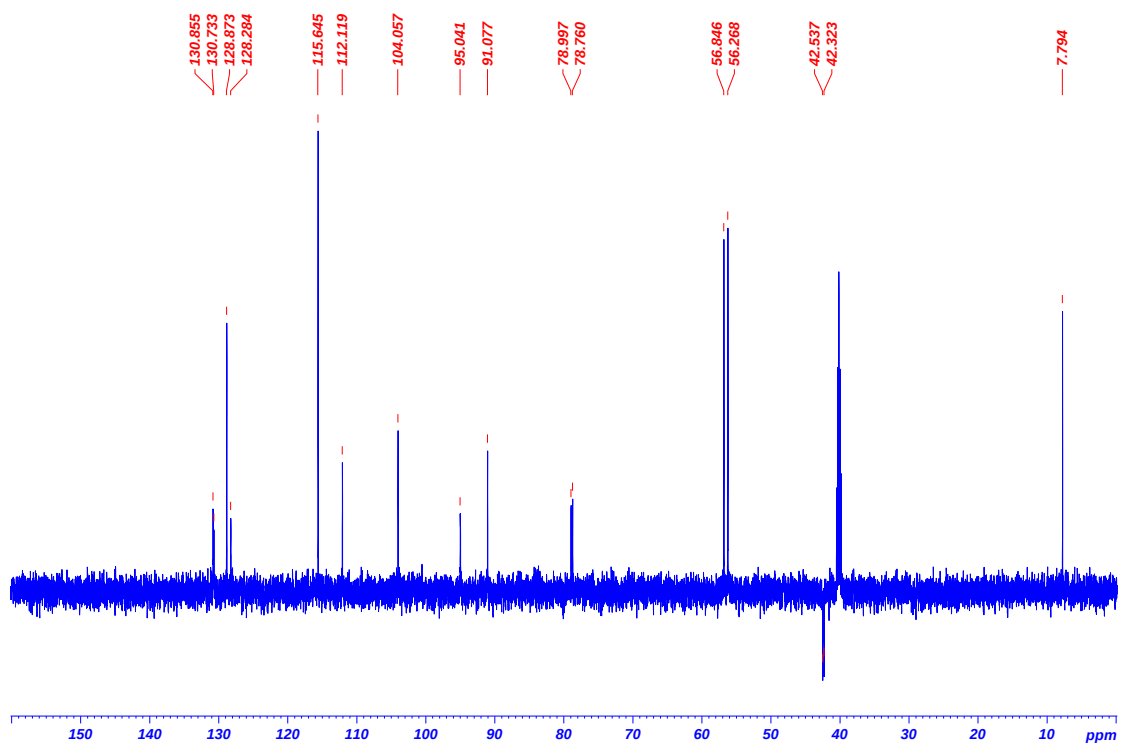


Figure S9. DEPT spectrum (500 MHz, $\text{DMSO-}d_6$) of compound **1**

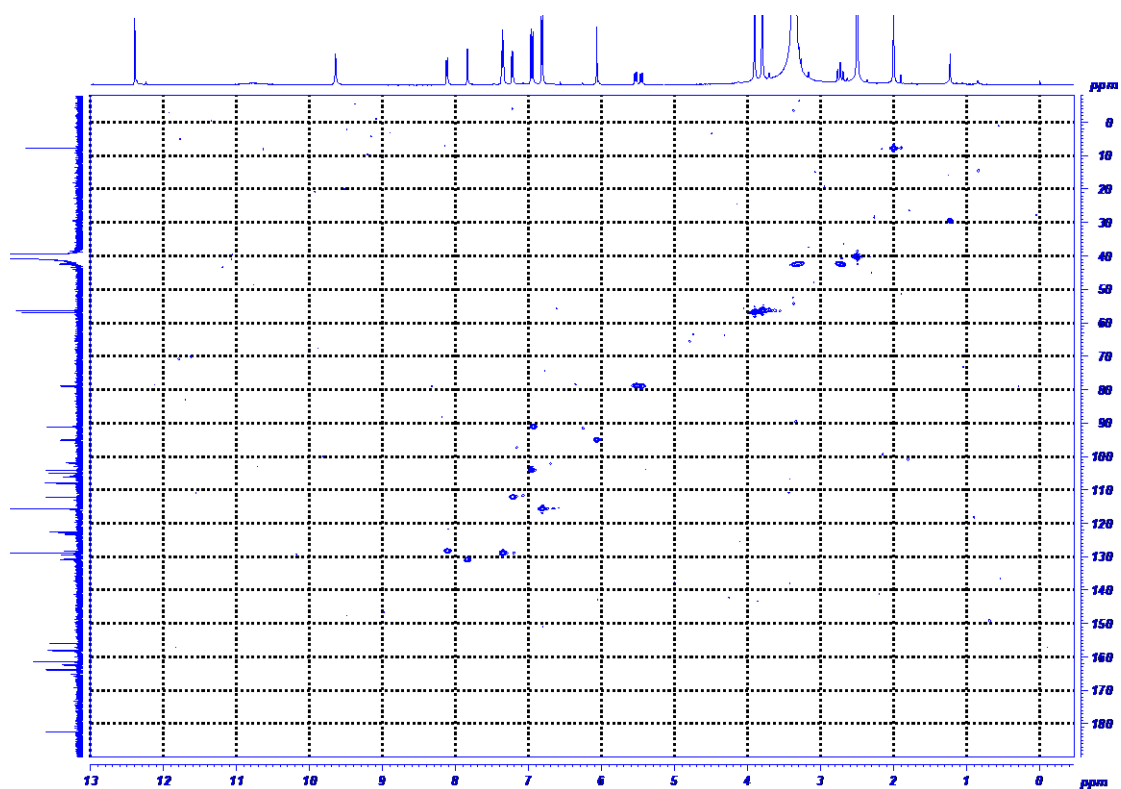


Figure S10. HSQC spectrum (500 MHz, DMSO-*d*₆) of compound **1**

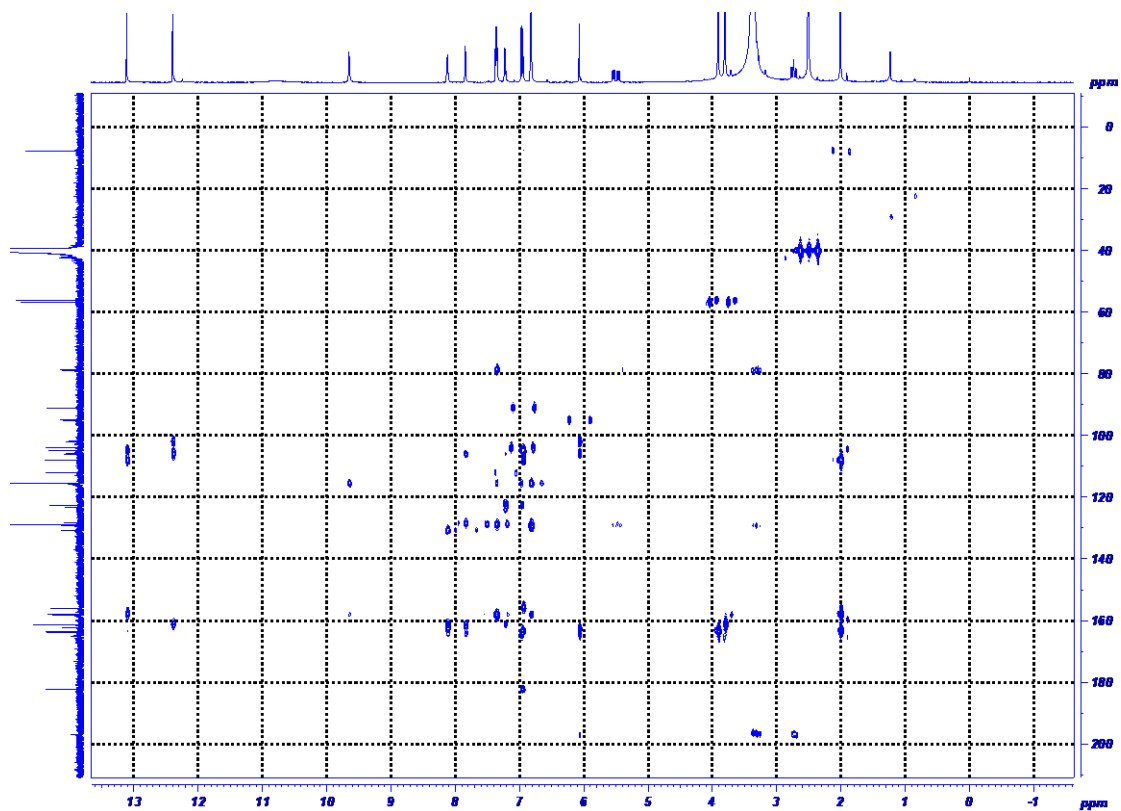


Figure S11. HMBC spectrum (500 MHz, DMSO-*d*₆) of compound **1**

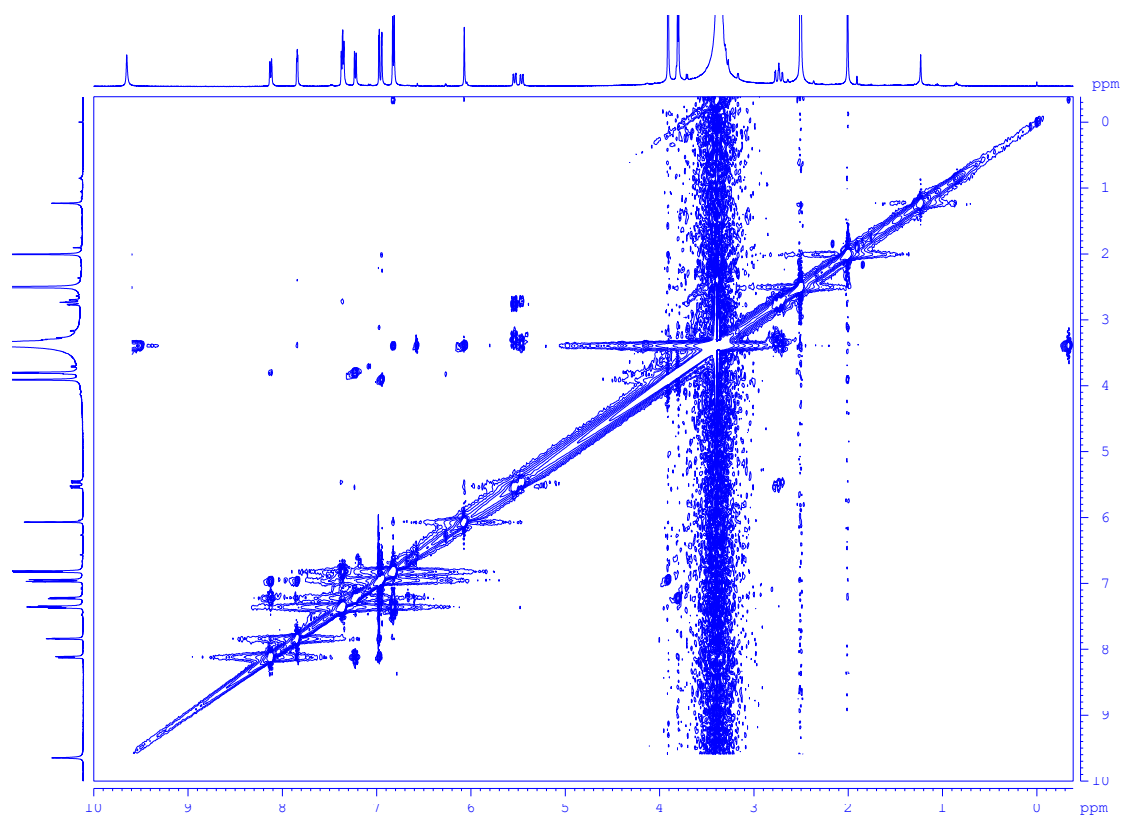


Figure S12. NOESY spectrum (500 MHz, DMSO- d_6) of compound **1**

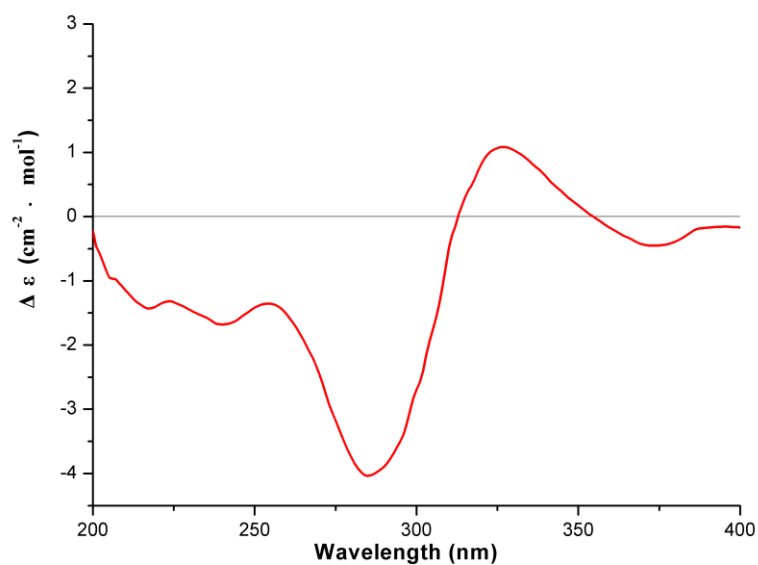


Figure S13. CD spectrum of compound **2** in MeOH

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
180	0	ESI

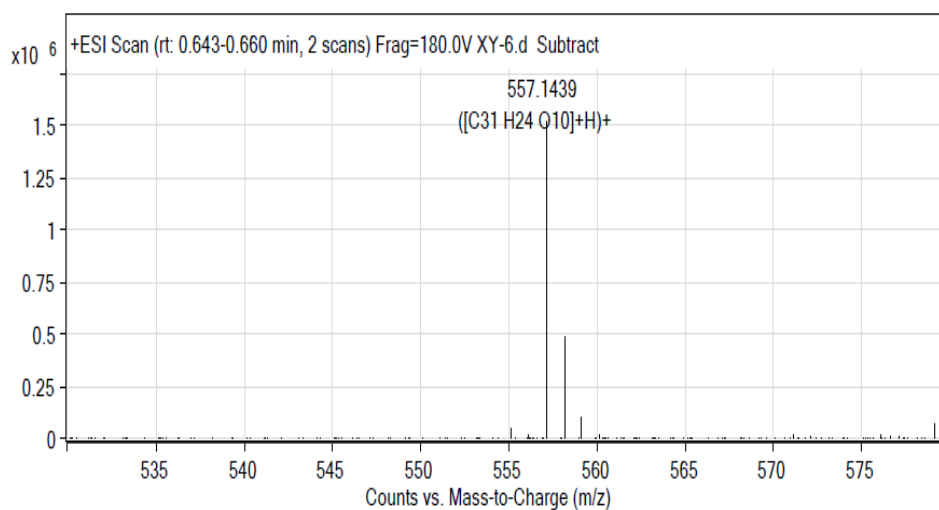


Figure S14. HRESIMS spectrum of compound **2**

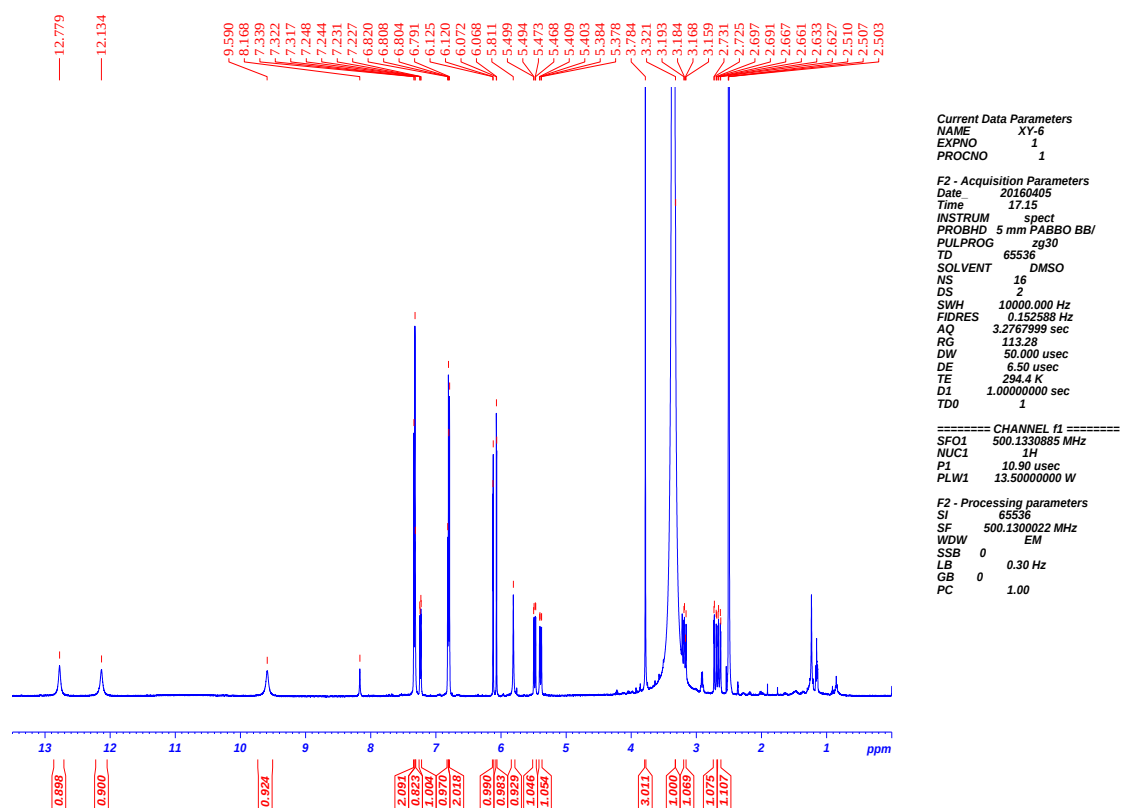


Figure S15. ^1H NMR spectrum (500 MHz, $\text{DMSO}-d_6$) of compound **2**

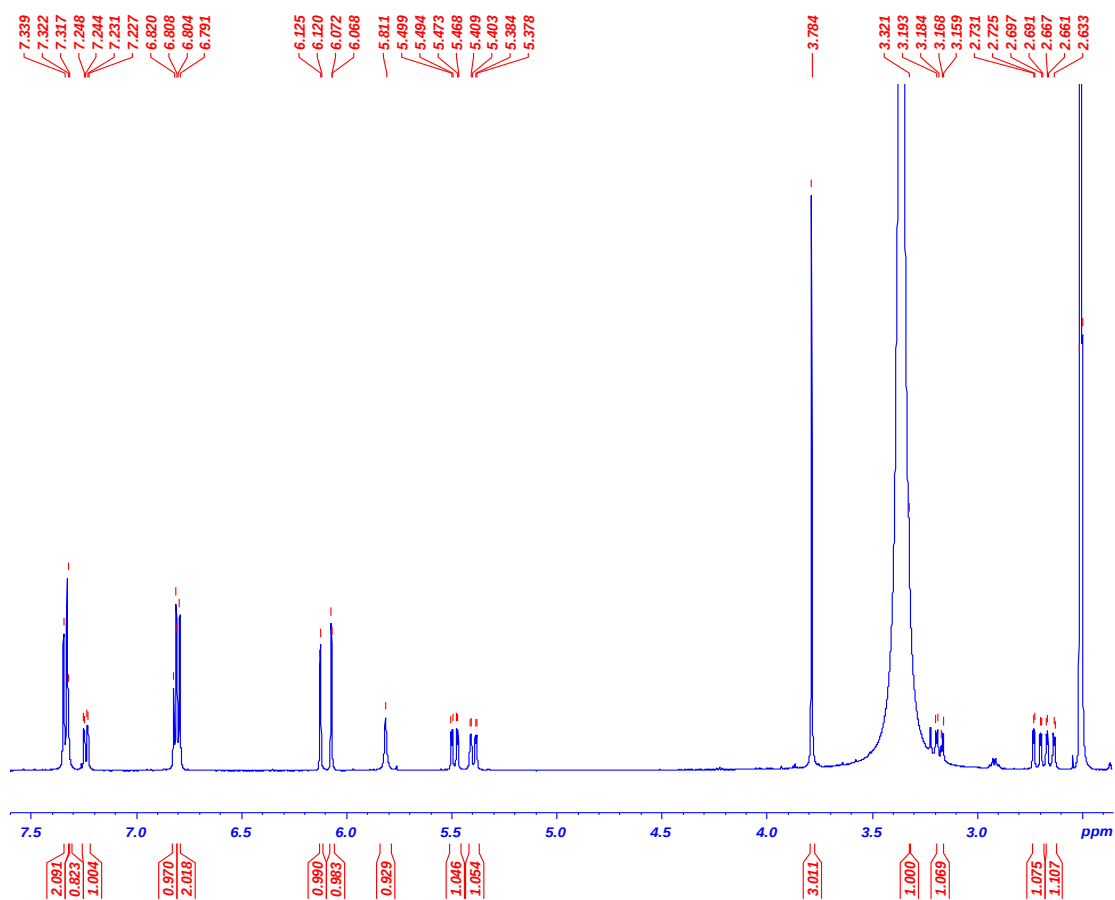


Figure S16. ^1H NMR spectrum of compound **2** (zoomed in)

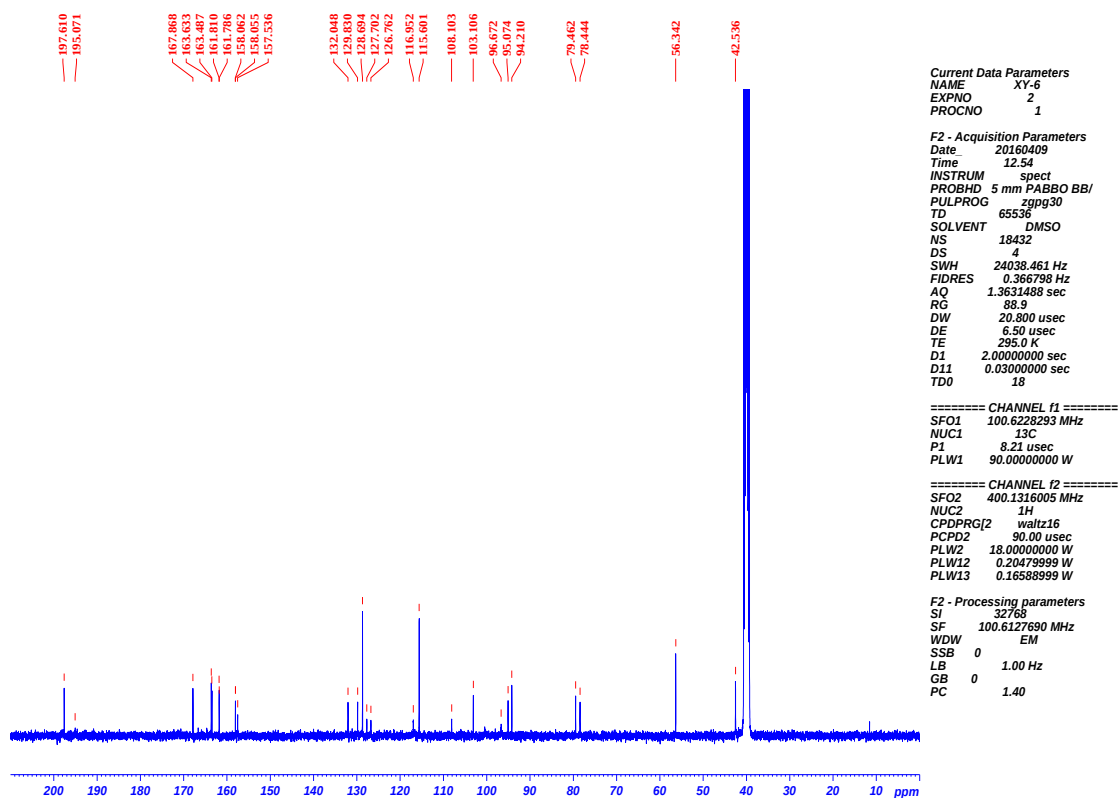


Figure S17. ^{13}C NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of compound 2

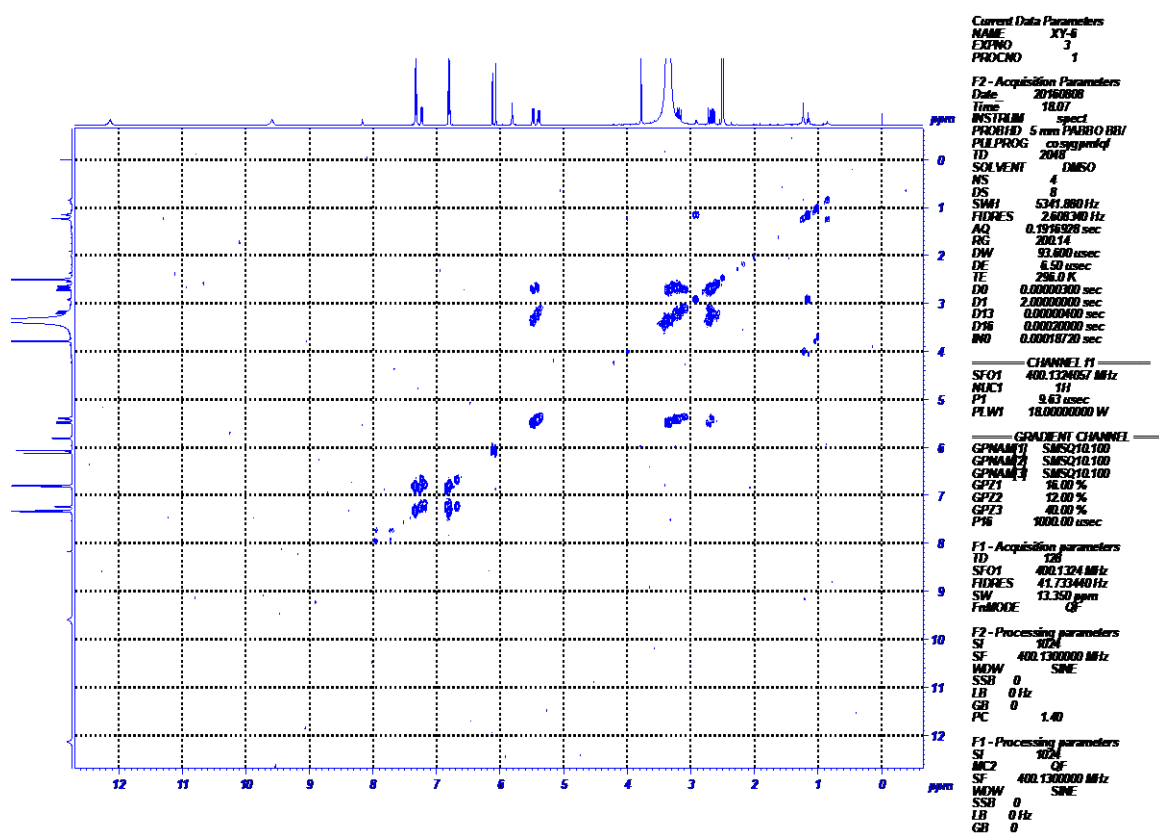


Figure S18. $^1\text{H}-^1\text{H}$ COSY (400 MHz, $\text{DMSO}-d_6$) spectrum of compound 2

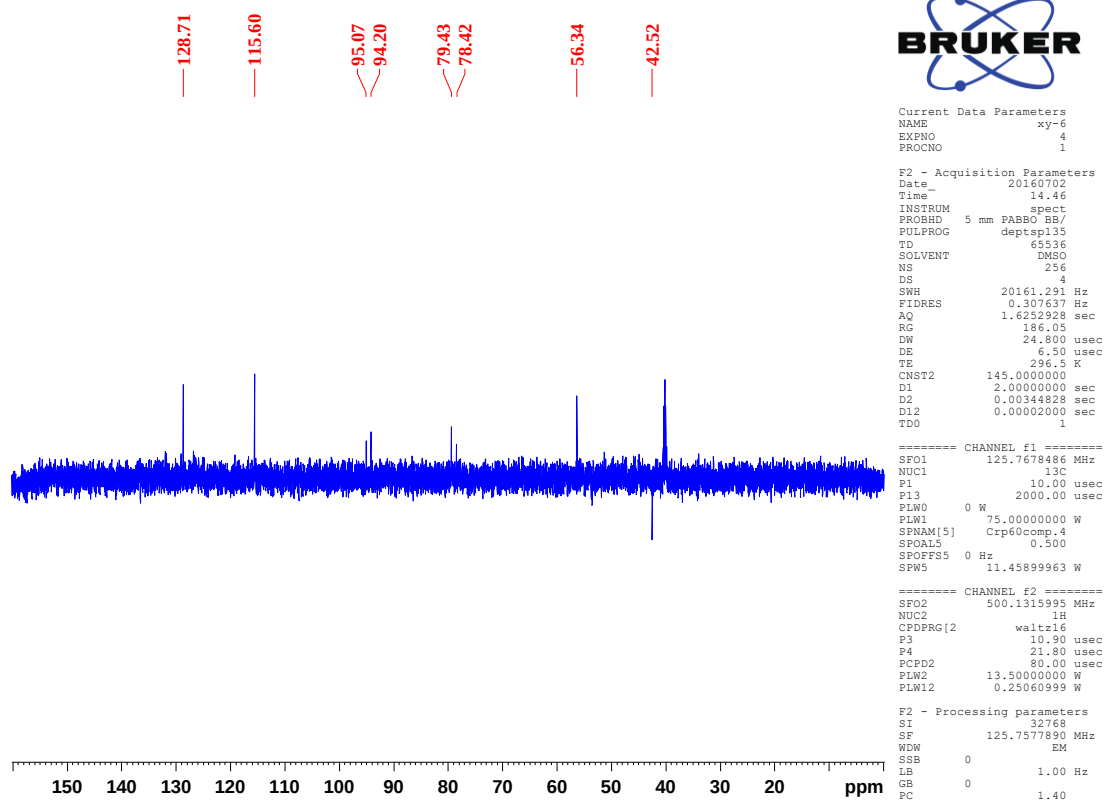


Figure S19. DEPT spectrum (500 MHz, DMSO- d_6) of compound 2

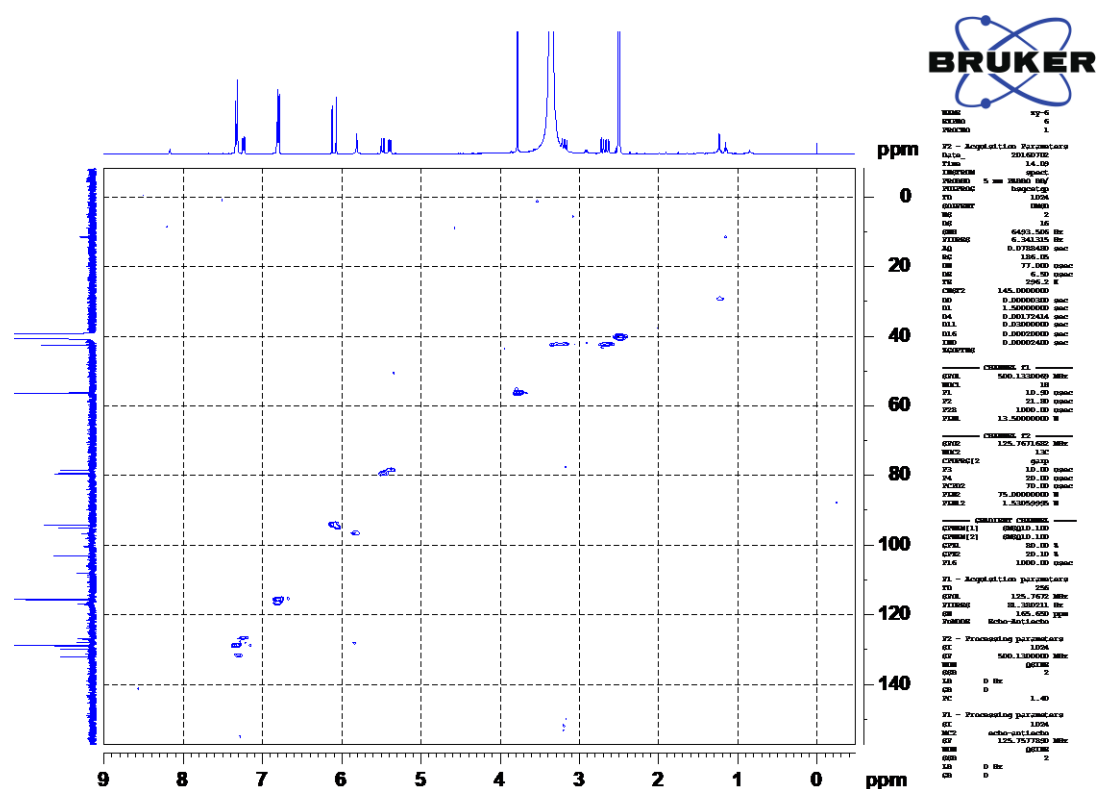


Figure S20. HSQC spectrum (500 MHz, DMSO- d_6) of compound 2

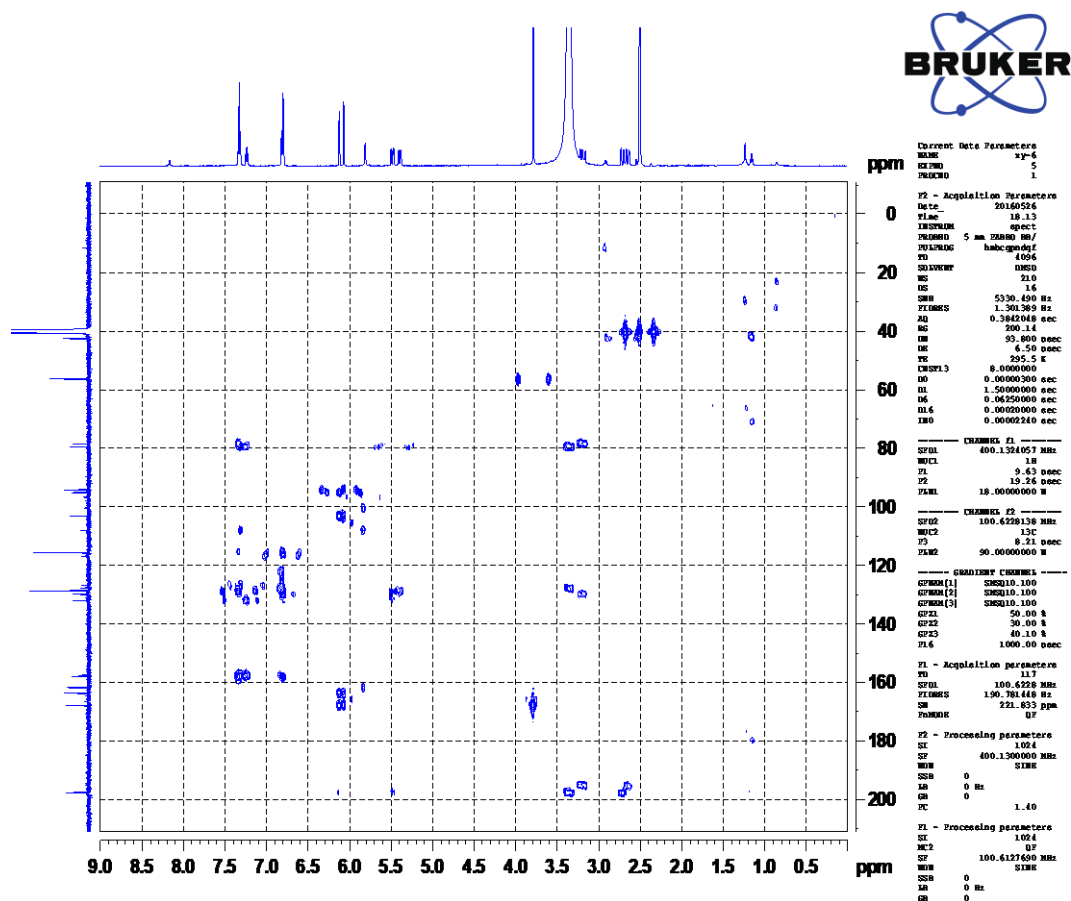


Figure S21. HMBC spectrum (400 MHz, DMSO-*d*₆) of compound 2

Table S2. Cytotoxicity of compounds 1- 9 against Human Cancer Cells^a

compound	A549	HepG2
1	34.8 ± 0.1	31.4 ± 0.2
2	32.2 ± 0.4	33.5 ± 0.8
3	54.5 ± 0.3	61.7 ± 0.4
4	62.8 ± 0.6	52.1 ± 0.6
5	69.6 ± 0.5	60.4 ± 0.2
6	> 80	73.9 ± 0.3
7	58.1 ± 0.9	> 80
8	> 80	> 80
9	> 80	> 80
Oxaliplatin	5.2 ± 0.2	6.5 ± 0.1

^a Results expressed as the mean IC₅₀ values in μM from triplicate measurements.