SUPPLEMENTARY MATERIAL

Isolation and characterization of 2-pyridone alkaloids and alloxazines from *Beauveria bassiana*

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Abstract: Two novel compounds bearing heterocyclic nitrogen, 2-pyridone alkaloid (**1**) and alloxazine derivative (**2**), along with the known pretenellin B (**3**), pyridovericin (**4**) and lumichrome (**5**) were isolated from a culture of the entomopathogenic fungal strain *Beauveria bassiana*. The chemical structures of 2-pyridone alkaloid and alloxazine derivative were established on the basis of the interpretation of spectroscopic data. The isolated compounds were evaluated in a panel of five cancer cell lines and pyridovericin exhibited cytotoxicity (IC_{50}, μM) against cancer cell lines: HL-60 (25.9 ± 0.3), HCT8 (34.6 ± 3.6), MDA-MB435 (34.8 ± 3.8) and SF295 (31.1 ± 0.6). Considering that other pyridone compounds display good cytotoxic activity, it would be suggested to obtain new semi synthetic derivatives of pyridovericin, for the development of new cytotoxic chemical entities.

Keywords: Alloxazines, *Beauveria bassiana,* entomopathogenic fungus, pyridone alkaloids

Table of contents

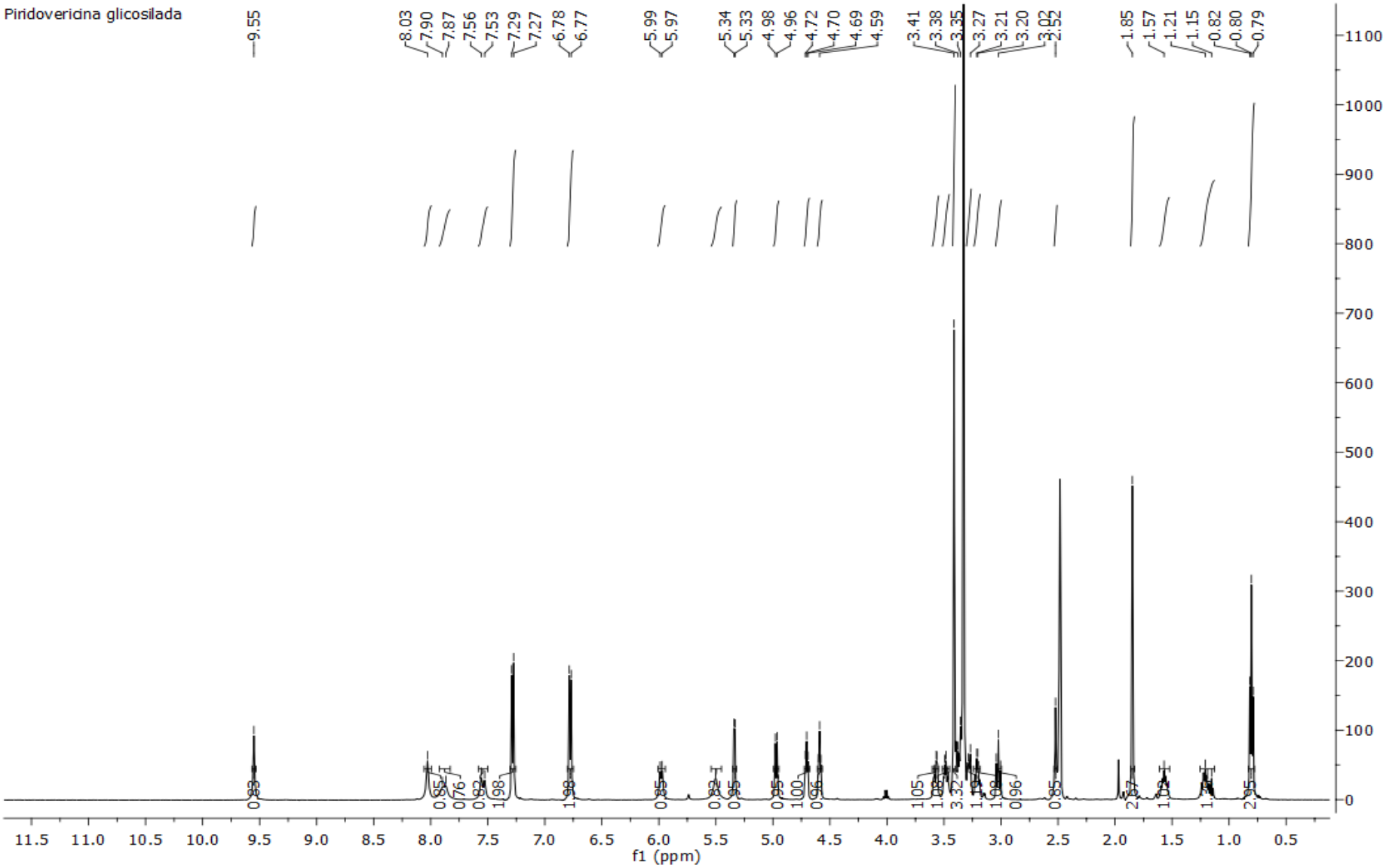


Figure S1. [1]H NMR (500 MHz, DMSO) spectrum of the new compound **1**

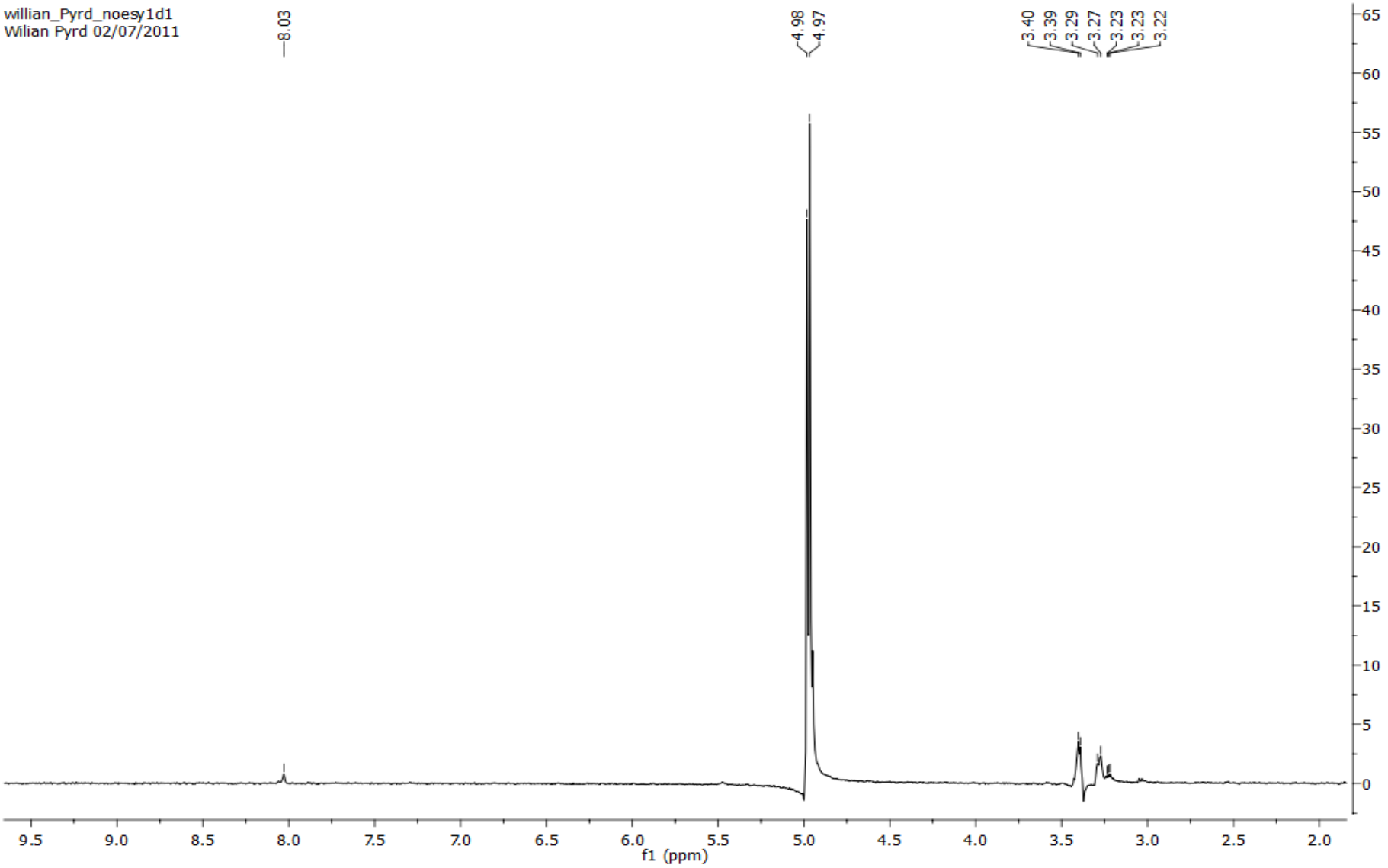


Figure S2. NOESY 1D NMR spectrum of the new compound **1** in DMSO.

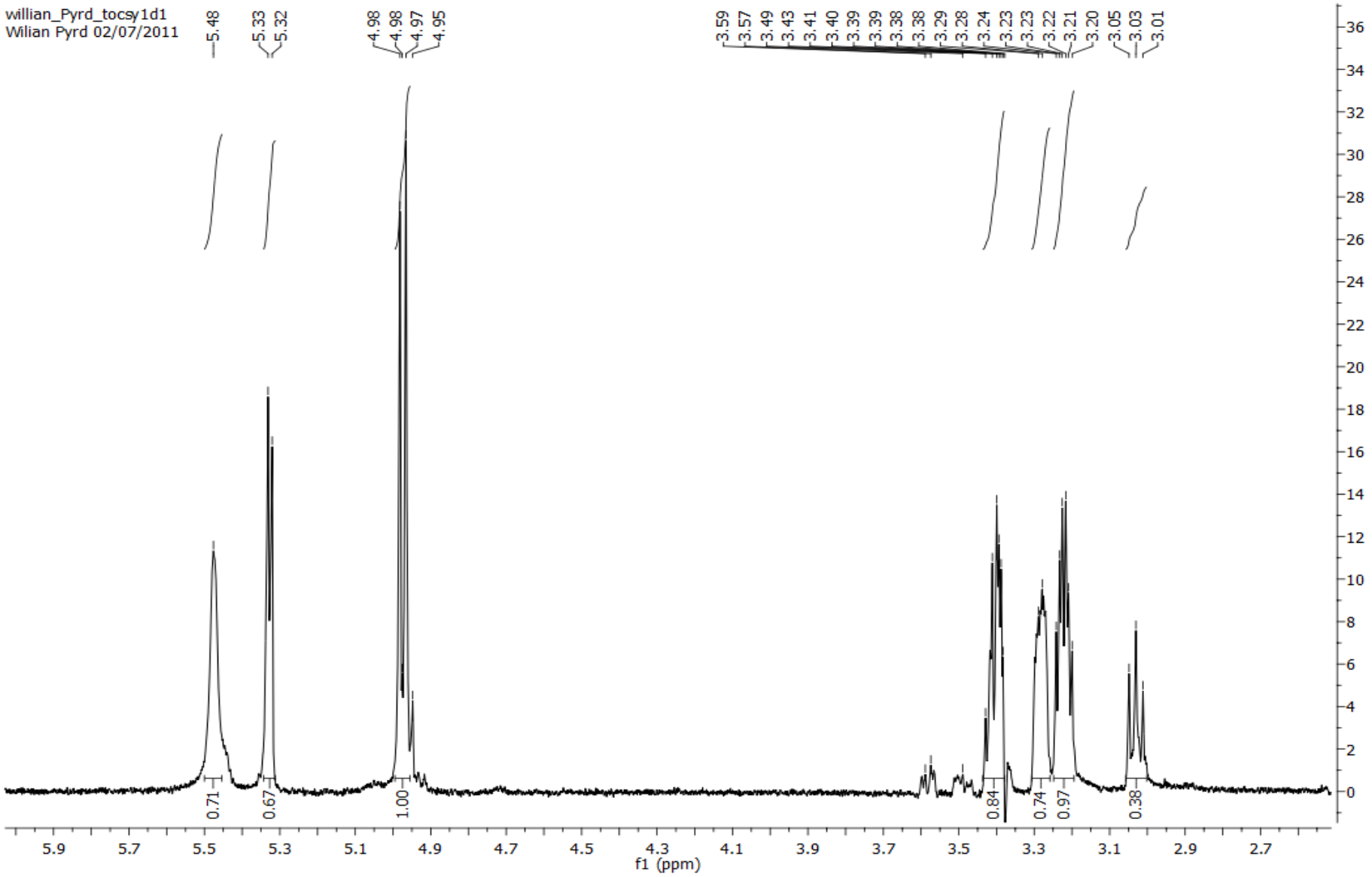


Figure S3. TOCSY 1D spectrum of the new compound **1** in DMSO.

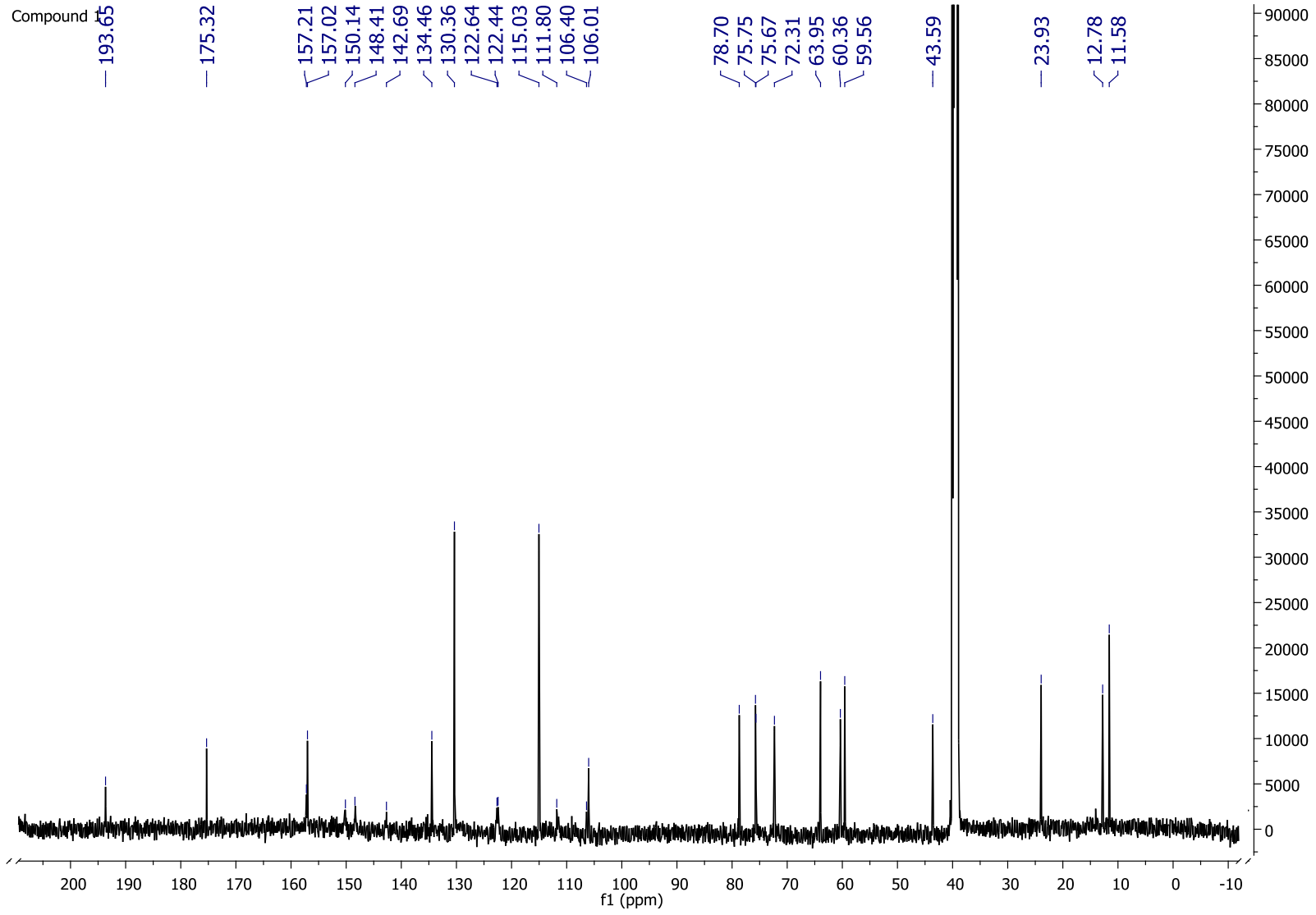


Figure S4. ^{13}C NMR (125 MHz, DMSO) spectrum of the new compound **1.**

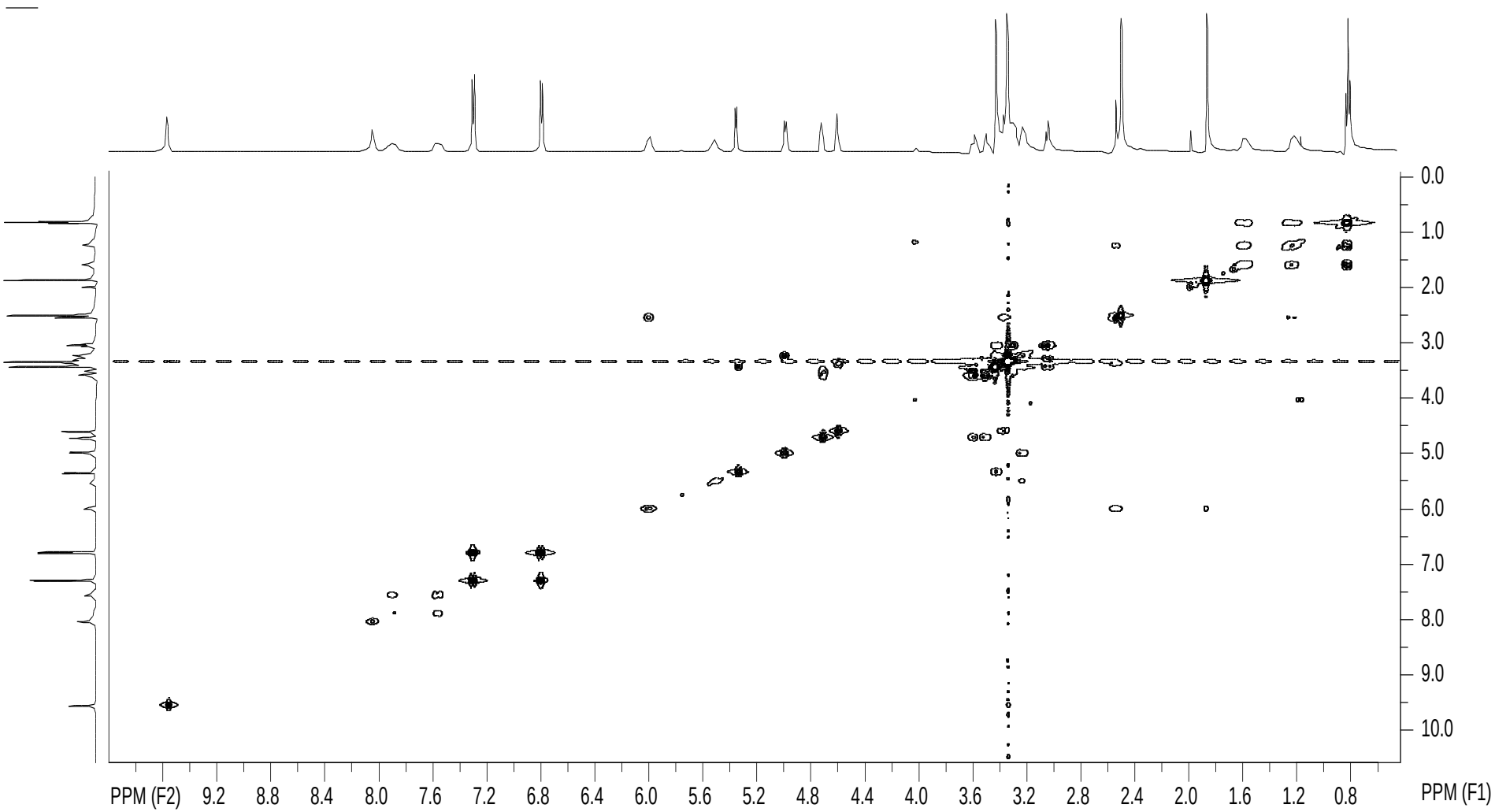


Figure S5. COSY NMR spectrum of the new compound **1** in DMSO.

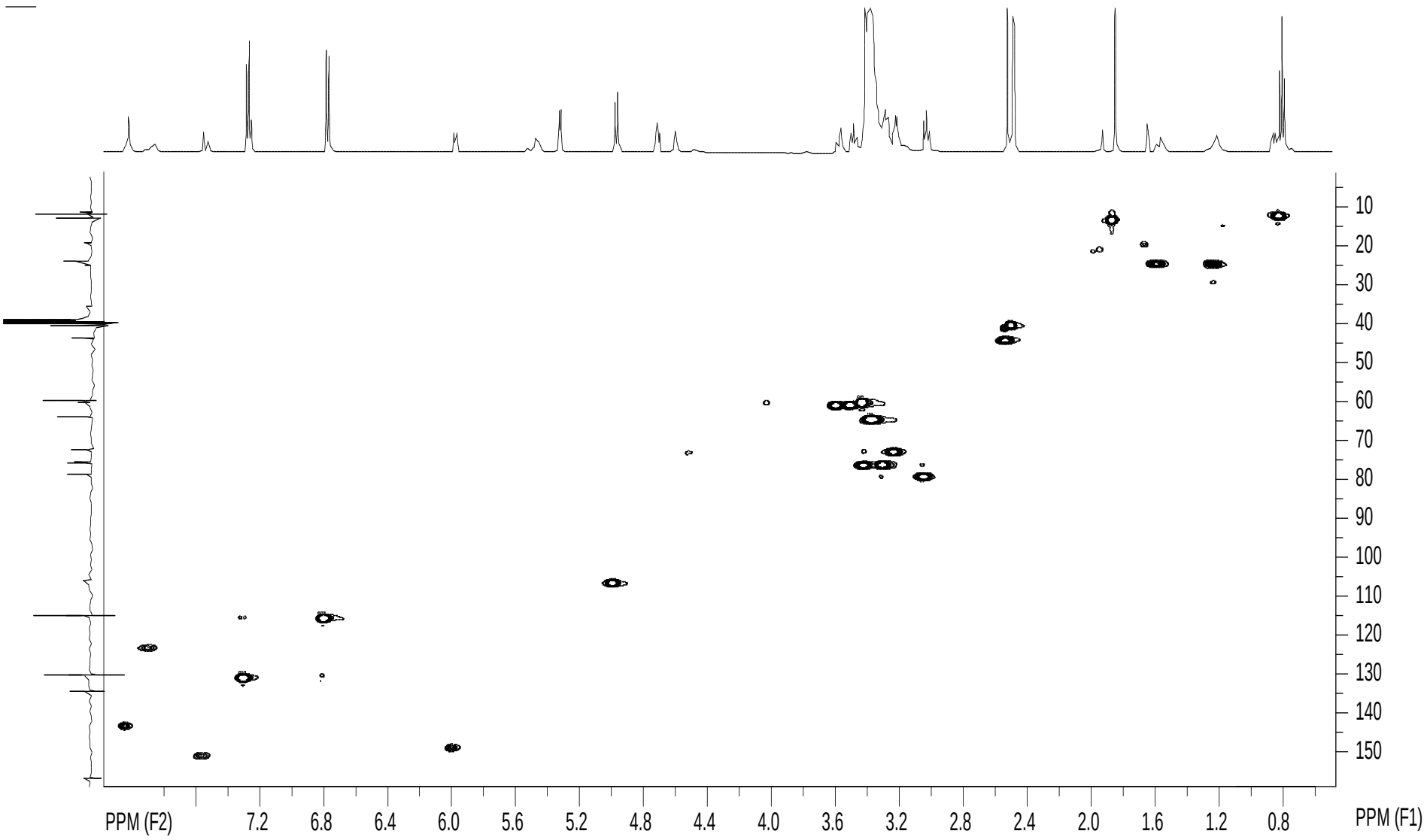


Figure S6. HMQC NMR spectrum of the new compound **1** in DMSO.

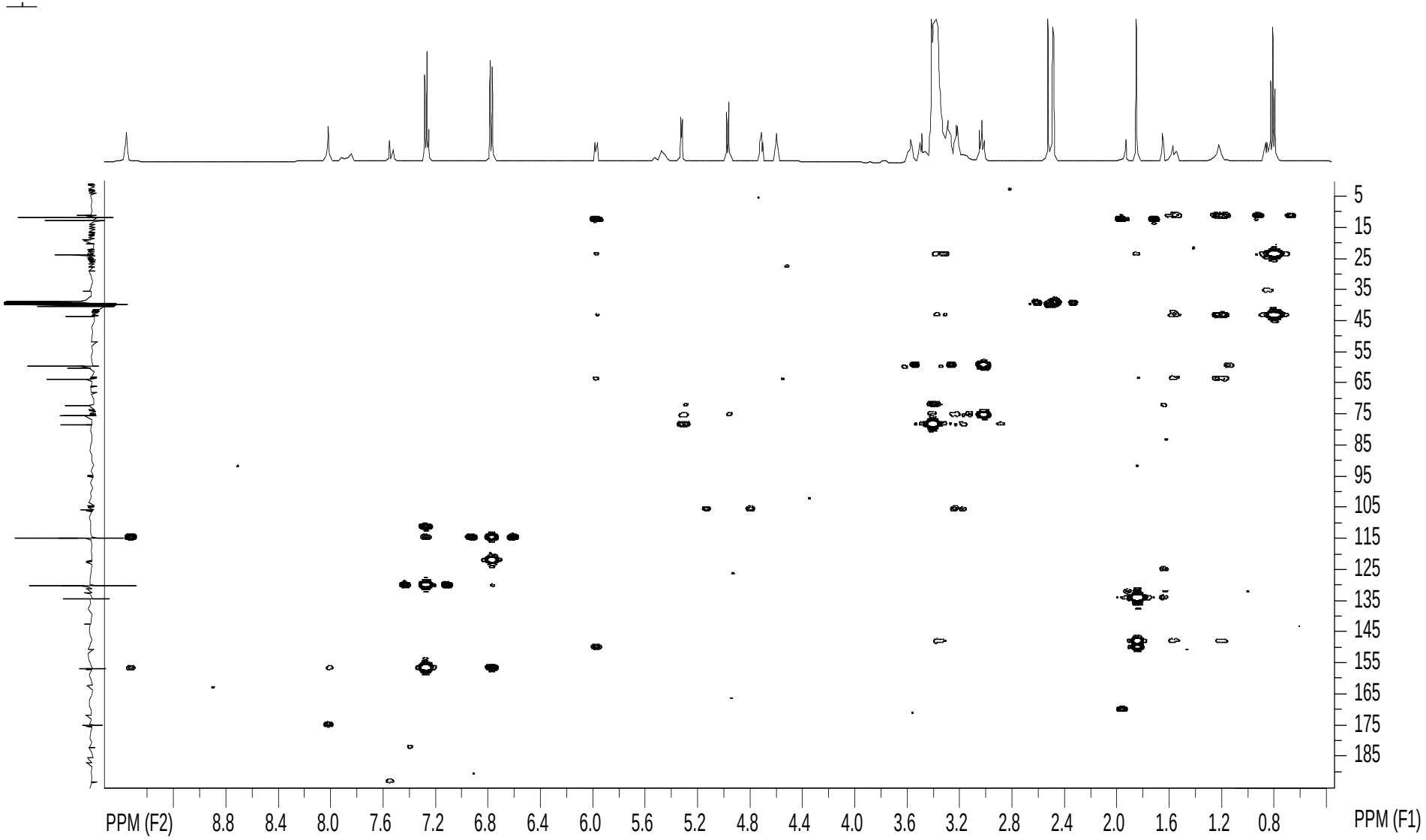


Figure S7. HMBC NMR spectrum of the new compound **1** in DMSO.

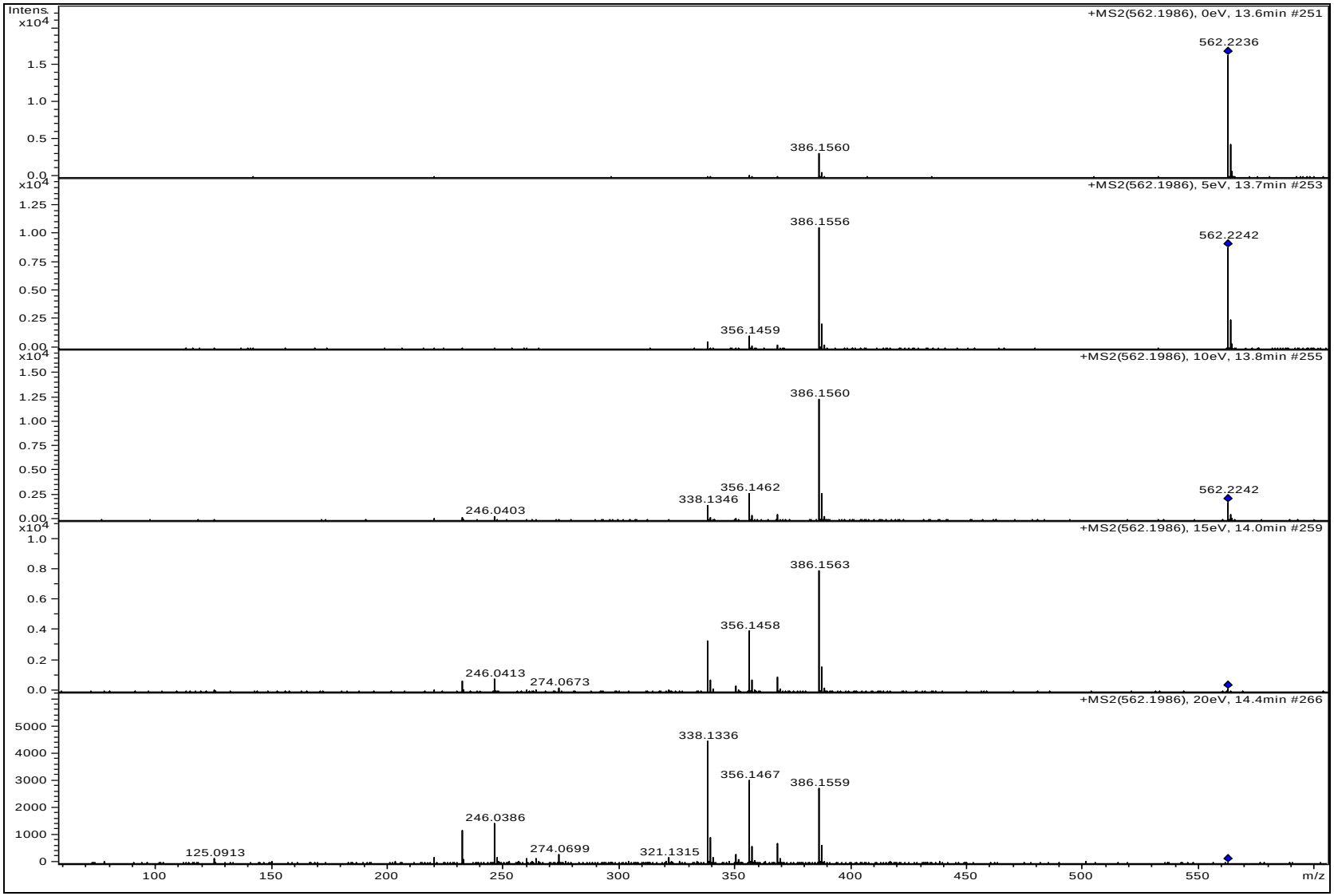


Figure S8. Mass spectra by HRMS of **1.**

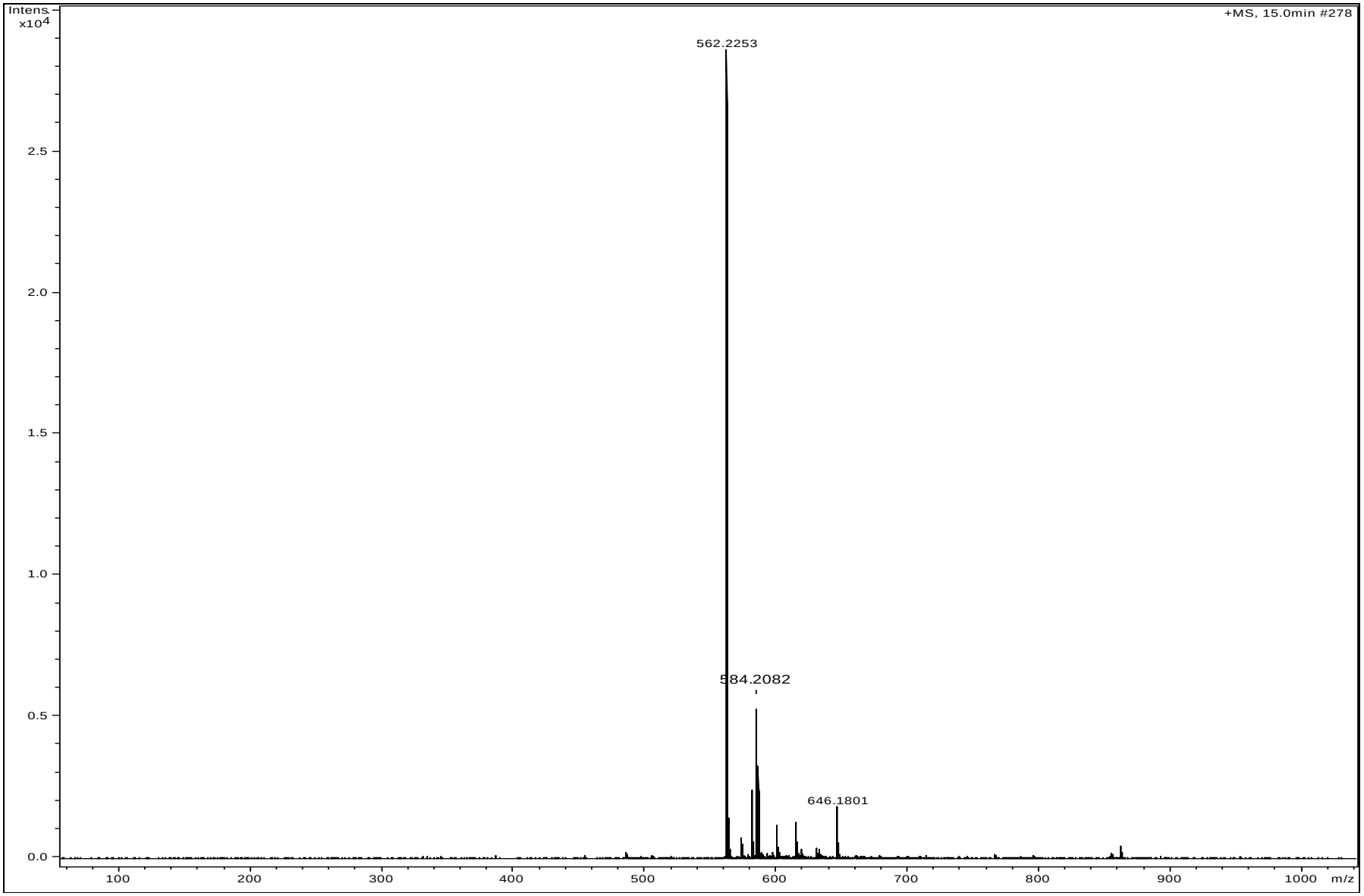


Figure S9. Mass spectra by HRMS of **1.**

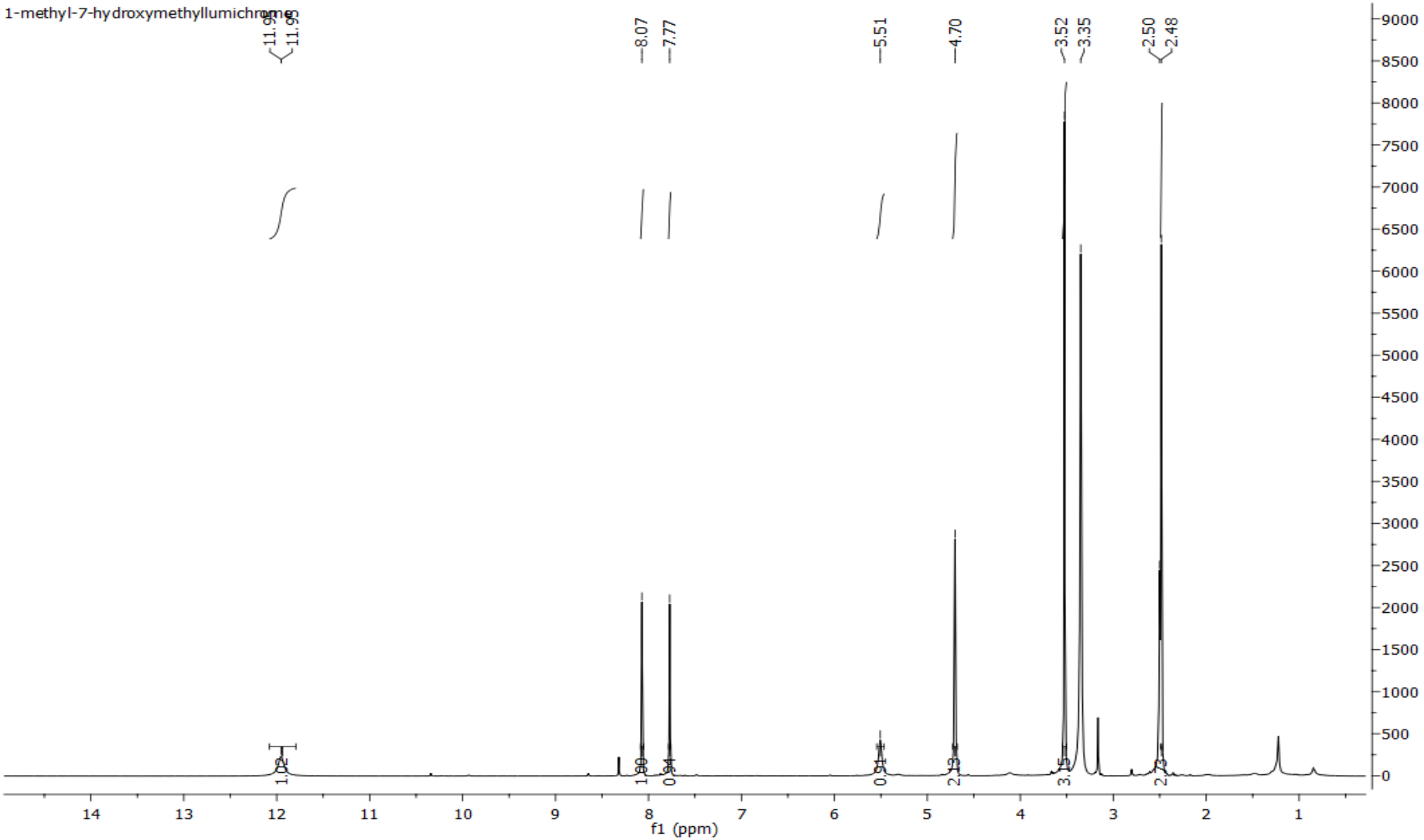


Figure S10. ^{1}H NMR (500 MHz, DMSO) spectrum of the new compound **2.**

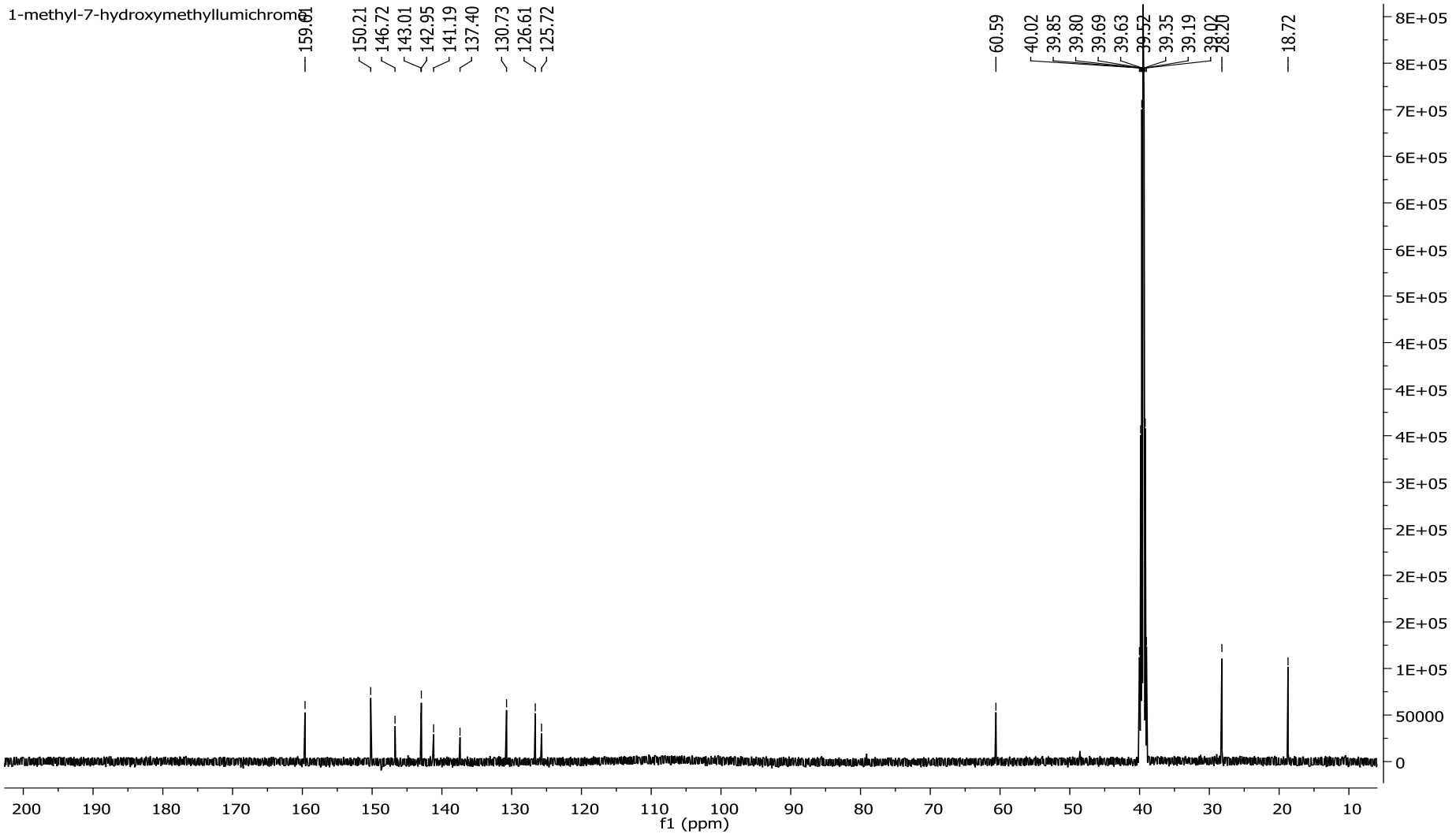


Figure S11. ^{13}C NMR (125 MHz, DMSO) spectrum of the new compound **2.**

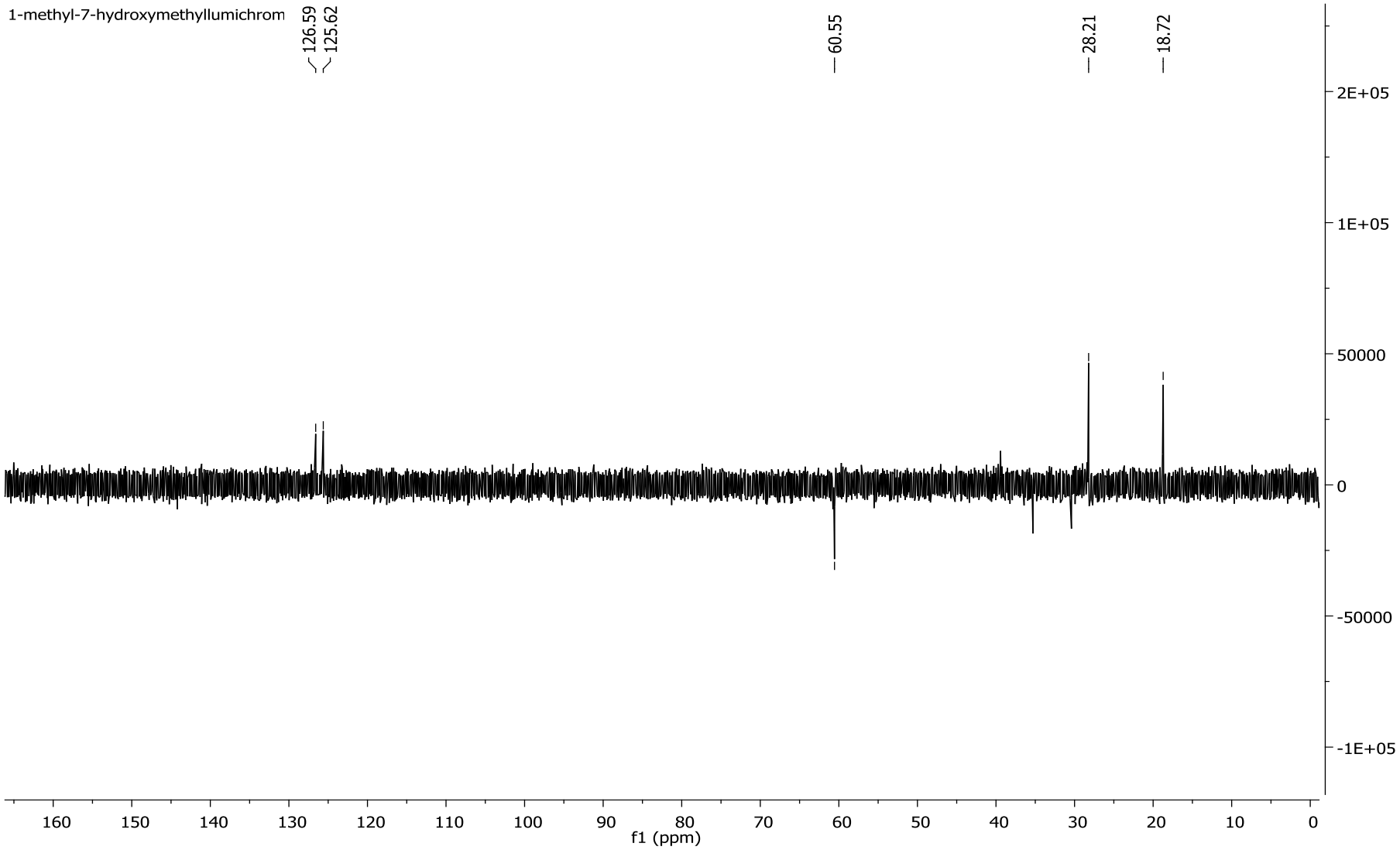


Figure S12. DEPT NMR spectrum of the new compound **2** in DMSO.

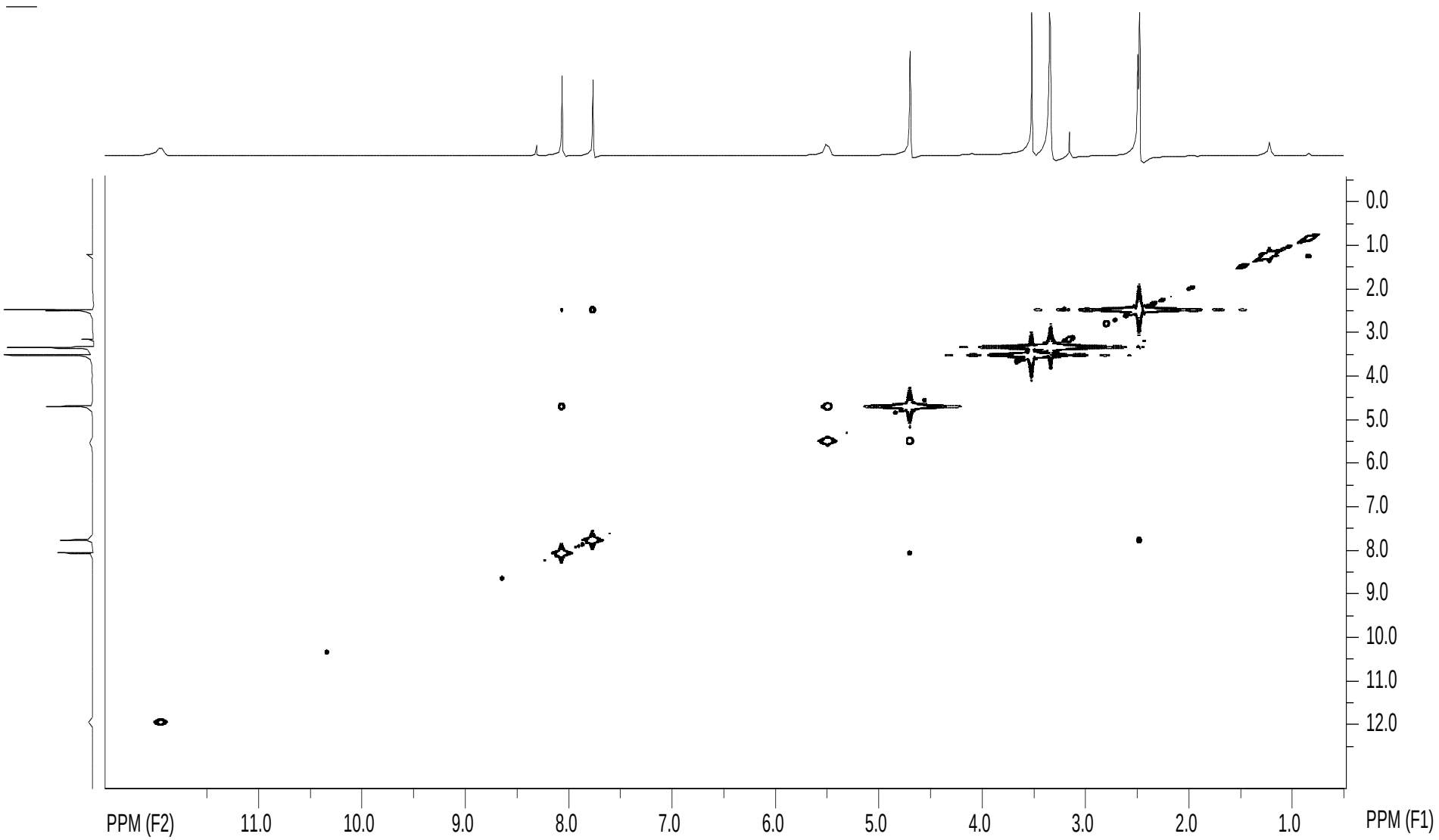


Figura S13. COSY NMR spectrum of the new compound **2** in DMSO.

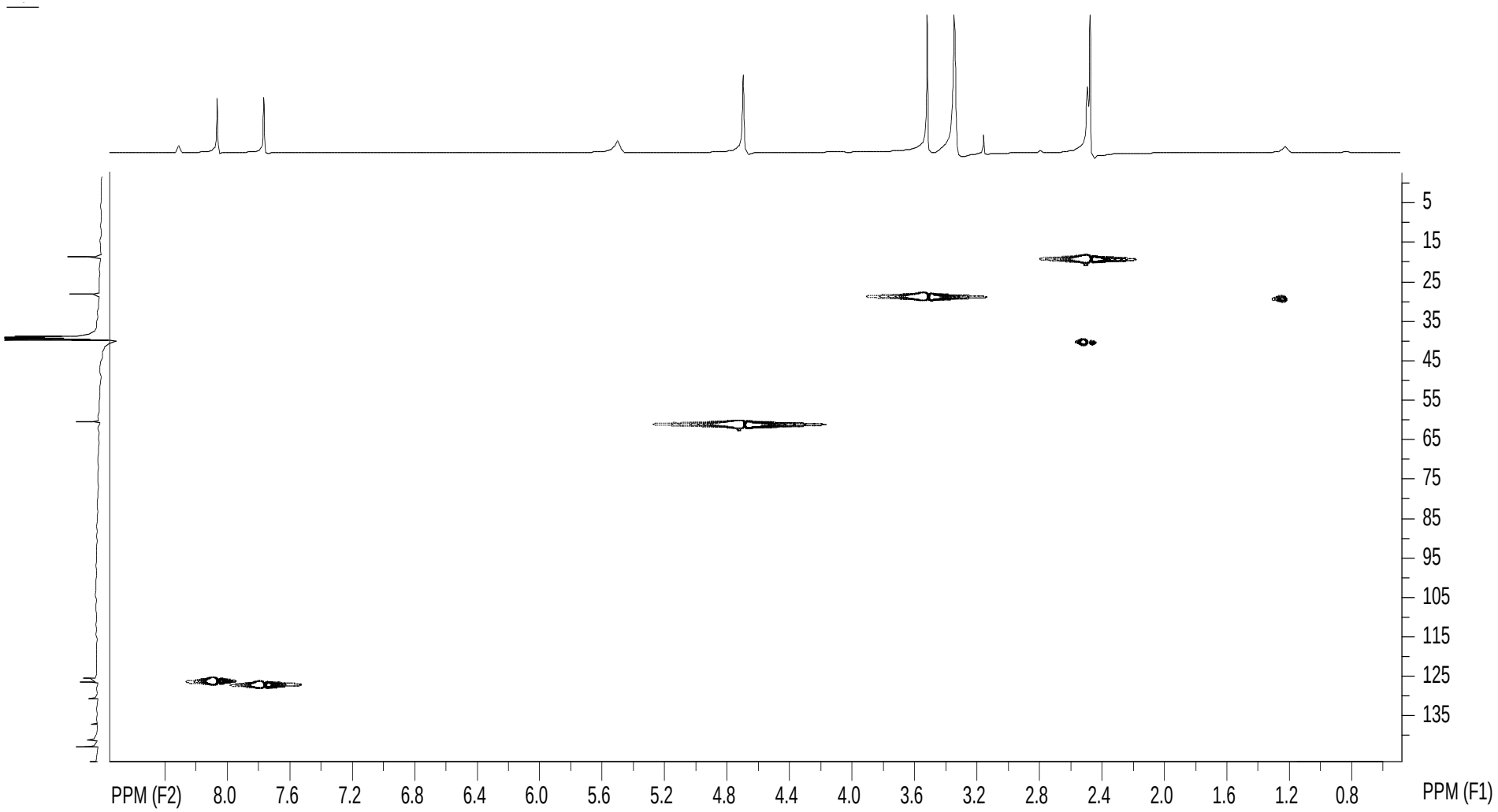


Figura S14. HMQC NMR spectrum of the new compound **2** in DMSO.

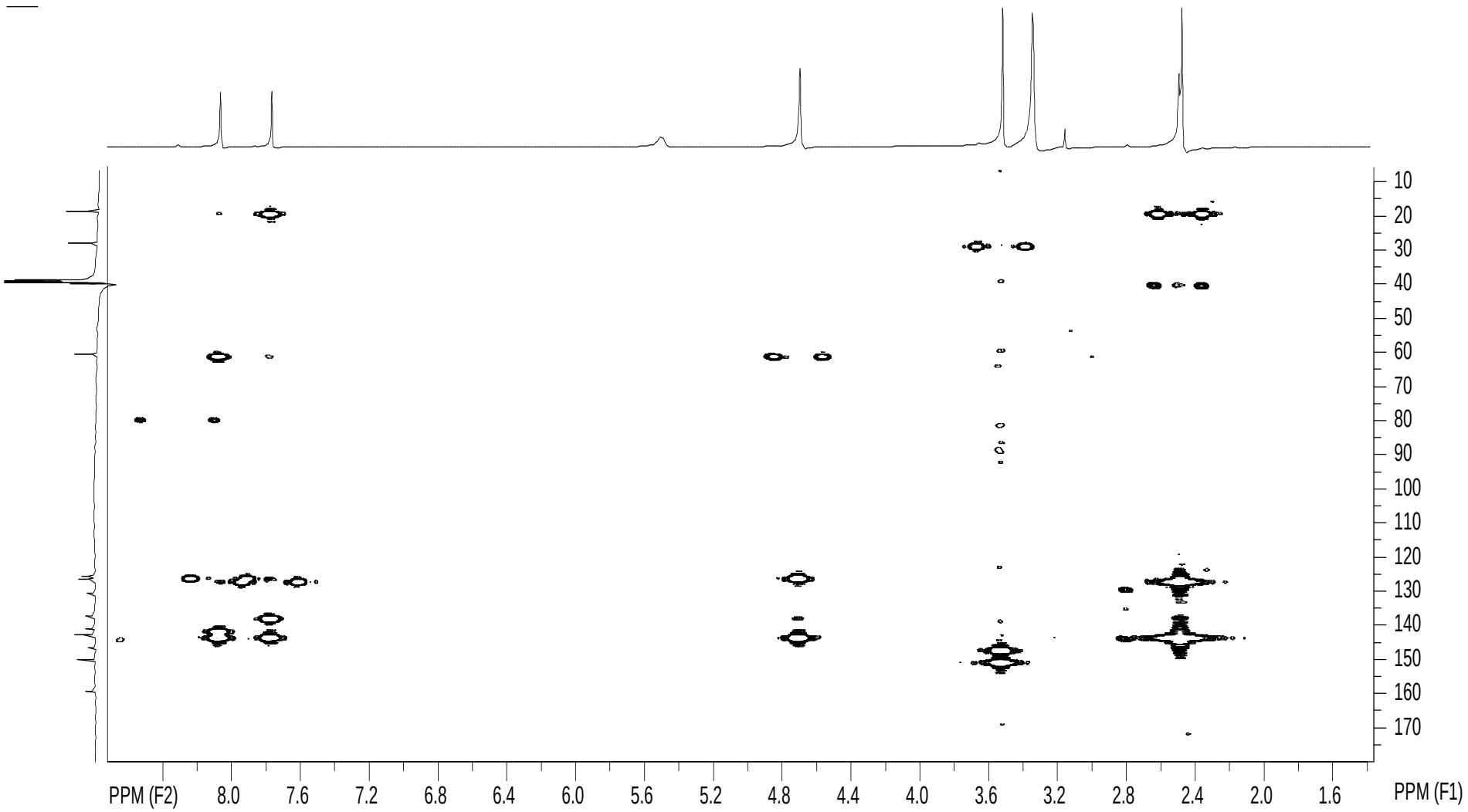


Figura S15. HMBC NMR spectrum of the new compound **2** in DMSO.

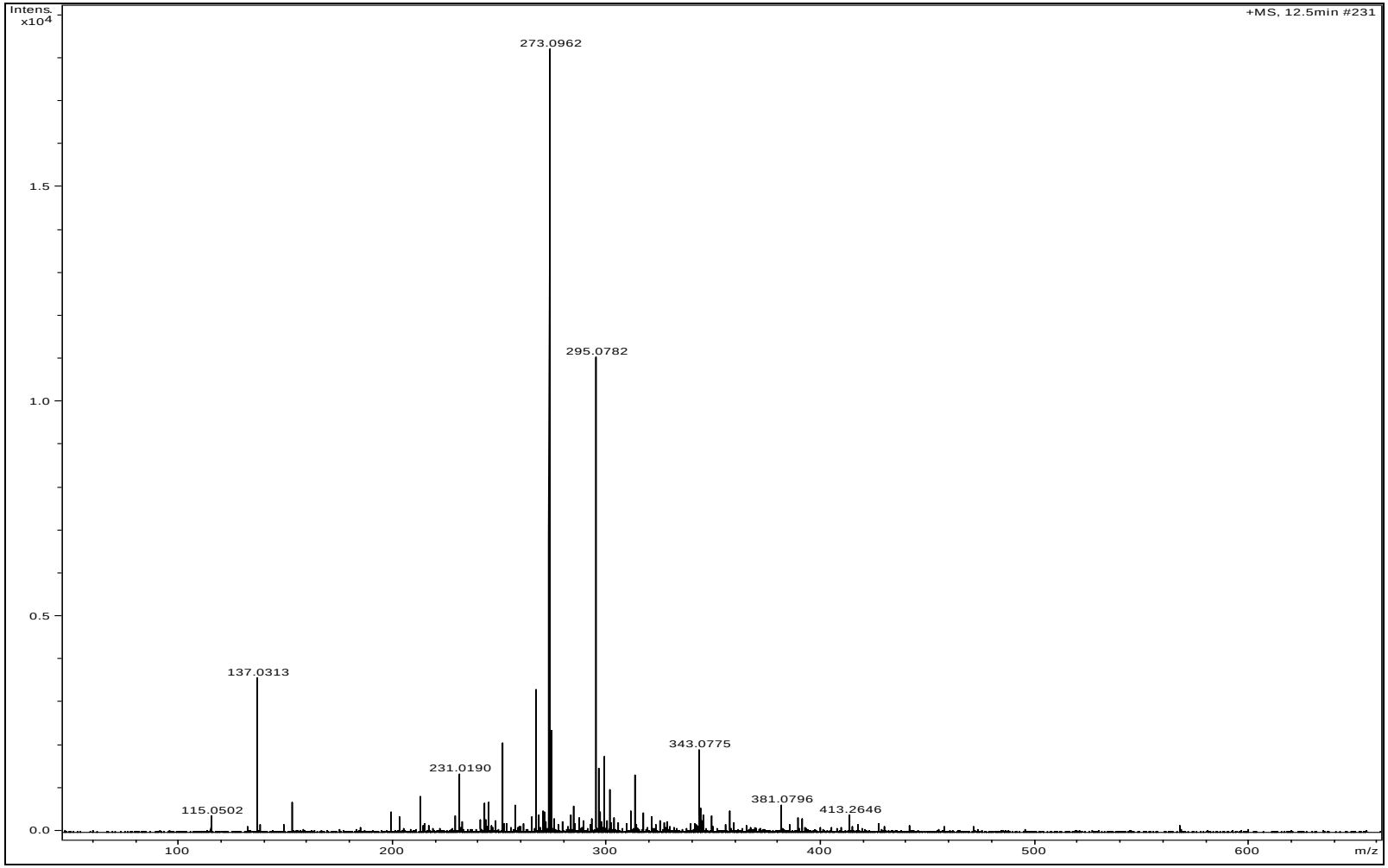


Figure S16. Mass spectra by HRMS of **2.**

Spectral data of new compounds.

Pyridovericin-N-O-(4-O-methyl-β-D-glucopyranoside) (1): pale yellow oil; $[\alpha]^{25}_{D}$ -59.5 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 200 (2.92), 205 (2.94), 247 (2.74), 342 (2.59) nm; IR (KBr) ν_{max} 3360, 2875, 1672, 1569, 1320, 1034, 969 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.55 (1H, *s*, OH-4′), 8.03 (1H, *s*, H-6), 7.88 (1H, *d*, *J* = 15.0 Hz, H-8), 7.55 (1H, *d*, *J* = 15.0 Hz, H-9), 7.28 (2H, *d*, *J* = 8.4 Hz, H-2′,6′), 6.78 (2H, *d*, *J* = 8.4 Hz, H-3′,5′), 5.98 (1H, *d*, *J* = 9.4 Hz, H-11), 5.48 (1H, *s*, OH-2″), 5.33 (1H, *d*, *J* = 5.6 Hz, OH-3″), 4.97 (1H, *d*, *J* = 8.0 Hz, H-1″), 4.71 (1H, *t*, *J* = 5.6 Hz, OH-6″), 4.59 (1H, *t*, *J* = 5.4 Hz, OH-15), 3.57 (1H, *m*, H-6″), 3.48 (1H, *m*, H-6″), 3.41 (3H, *s*, H-4″ (- OCH_3)), 3.38 (1H, *m*, H-3″), 3.35 (2H, *m*, H-15), 3.28 (1H, *m*, H-5″), 3.21 (1H, *ddd*, *J* = 4.4, 8.0, 9.1 Hz, H-2″), 3.02 (1H, *t*, *J*= 9.1 Hz, H-4″), 2.52 (1H, *m*, H-12), 1.85 (3H, *s*, H-16), 1.57 (1H, *m*, H-13), 1.21 (1H, *m*, H-13), 0.80 (3H, *t*, *J* = 7.4 Hz, H-14) and ^{13}C NMR (125 MHz, DMSO-d_6): δ 157.2 (C-2), 106.4 (C-3) 175.3 (C-4), 111.8 (C-5), 142.7 (C-6), 193.6 (C-7), 122.4 (C-8), 150.1 (C-9), 134.5 (C-10), 148.4 (C-11), 43.6 (C-12), 23.9 (C-13), 11.6 (C-14), 63.9 (C-15), 12.8 (C-16), 122.6 (C-1′), 130.4 (C-2′,6′), 115.0 (C-3′,5′), 157.0 (C-4′), 106.0 (C-1″), 72.3 (C-2″), 75.7 (C-3″), 78.7 (C-4″), 59.6 (C-4″, - OCH_3), 75.7 (C-5″), 60.3 (C-6″); HRMS *m/z* 562.2253 $[M+H]^+$ (calcd for $C_{28}H_{35}NO_{11} + H^+$, 562.2283).

1-Methyl-11-hydroxylumichrome (2): yellow powder; mp 321–322 ºC; UV (MeOH) λ_{max} (log ε) 200 (2.57), 244 (2.59), 251 (2.60), 339 (2.10), 420 (2.23) nm; IR (KBr) ν_{max} 2931, 1722, 1425, 1375, 1267, 733 cm^{-1};^{1}H NMR (500 MHz, DMSO-d_6): δ 11.96 (1H, *s*, NH-2), 8.07 (1H, *s*, H-6), 7.77 (1H, *s*, H-7), 5.51 (1H, *s*, OH-11), 4.70 (2H, *s*, H-11), 3.52 (3H, *s*, –NCH_3-1) 2.48 (3H, *s*, H-12) and ^{13}C NMR (125 MHz, DMSO-d_6): δ 28.2 (-NCH_3-1), 150.2 (C-2), 159.6 (C-3), 130.7 (C-4a), 142.9 (C-5a), 125.7 (C-6), 141.2 (C-7), 143.0 (C-8), 126.6 (C-9), 137.4 (C-9a), 146.7 (C-10a), 60.6 (C-11), 18.7 (C-12); HRMS *m/z* 273.0962 $[M+H]^+$ (calcd for $C_{13}H_{13}N_4O_3 + H^+$, 273.0982).

Table S1. NMR Spectroscopic data (500 MHz) for alkaloid **1**.

position	pyridovericin-*N*-*O*-(4-*O*-methyl-β-D-glucopyranoside) (**1**)[a]		
	δ_C mult.	δ_H (*J* in Hz)	HMBC[b]
1	N-O	-	-
2	157.2, C	-	-
3	106.4, C	-	-
4	175.4, C	-	-
5	111.8, C	-	-
6	142.7, CH	8.03, *s*	4
7	193.7, C	-	-
8	122.4, CH	7.88, *d* (15.0)	
9	150.1, CH	7.55, *d* (15.0)	7
10	134.4, C	-	
11	148.4, CH	5.98, *d* (9.4)	9, 12, 16
12	43.6, CH	2.52, *m*	-
13	23.9, CH_2	1.21, *m*; 1.57, *m*	11, 12, 14, 15
14	11.6, CH_3	0.80, *t* (7.4)	12, 13
15	63.9, CH_2	3.35, *m*	11, 12, 13
		4.59, *t* (5.4) (OH)	15
16	12.8, CH_3	1.85, *s*	9, 10, 11
1′	122.6, C	-	-
2′,6′	130.3, C	7.28, *d* (8.4)	5, 2′, 3′, 4′
3′,5′	115.0, C	6.78, *d* (8.4)	1′, 2′, 3′, 4′
4′- OH	157.0, C	9.55, *s*	3′, 5′
1 –N*O*-sugar			
1″	106.0,CH	4.97, *d* (8.0)	3″
2″	72.3,CH	3.21, *ddd* (4.4, 8.0, 9.1)	1″
		5.48, *s* (OH)	
3″	75.7,CH	3.38, *m*	2″, 4″
		5.33, *d* (5.6) (OH)	4″
4″	78.7,CH	3.02, *t* (9.1)	5″, 6″
4-*O*CH_3	59.5,CH_3	3.41, *s*	4″
5″	75.6,CH	3.28, *m*	6″
6″	60.3,CH_2	3.48, *m*; 3.57 *m*	-
		4.71, *t* (5.6) (OH)	5″, 6″

[a]In DMSO-d_6. [b]HMBC correlations are from hydrogen(s) stated to the indicated carbon.

Table S2. NMR Spectroscopic data (500 MHz) for compound **2**.

position	1-methyl-11-hydroxylumichrome (**2**)[a]		
	δ_C mult.	δ_H (J in Hz)	HMBC[b]
1	28.2, N-CH_3	3.52, s	146.7, 150.2
2	150.2, C	-	-
3	NH	11.96, s	-
4	159.6, C	-	-
4a	130.7, C	-	-
5	N		
5a	142.9, C	-	-
6	125.7, CH	8.07, s	60.6, 141.2, 143.0
7	141.2, C	-	
8	143.0, C	-	
9	126.6, CH	7.77, s	18.7, 137.4, 143.0
9a	137.4, C	-	-
10	N		
10a	146.7, C	-	
11	60.6, CH_2	4.70, s	125.7, 143.0
11 – OH		5.51, s	
12	18.7, CH_3	2.48, s	126.6, 143.0

[a]In DMSO-d_6. [b]HMBC correlations are from hydrogen(s) stated to the indicated carbon.

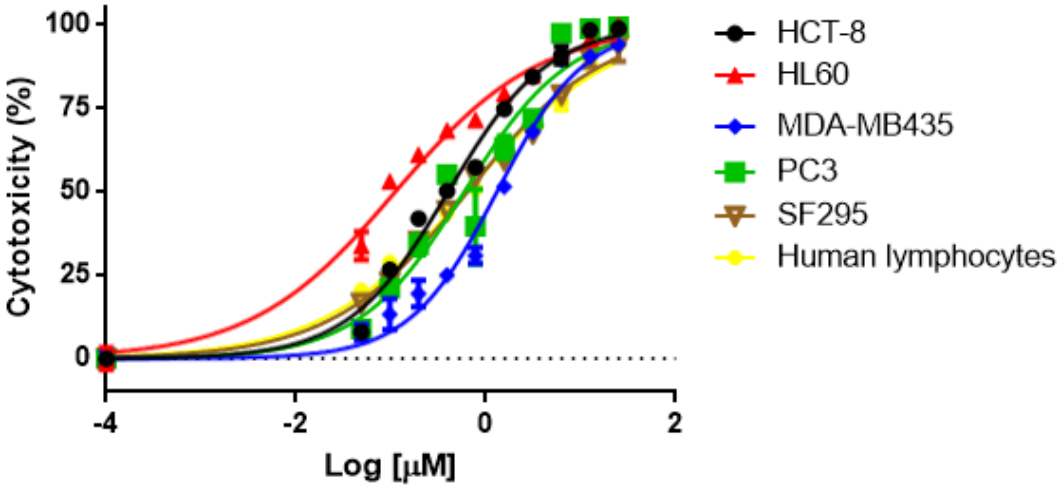
Spectral data of known compounds

Pretenellin B (3): yellow powder; mp 203–206 ºC; $[\alpha]^{25}_D$ -48.0 (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ε) 201 (3.02), 226 (2.61), 246 (2.75), 336 (2.62) nm; IR (KBr) ν_{max} 3403, 2963, 1661, 1611, 1578, 1325, 980. cm^{-1};^{1}H NMR (500 MHz, DMSO-d_6): δ 7.97 (1H, *d*, *J* = 15.0 Hz, H-8), 7.58 (1H, *d*, *J* = 15.0 Hz, H-9), 7.47 (1H, *s*, H-6), 7.28 (2H, *d*, *J* = 8.6 Hz, H-2′,6′), 6.81 (2H, *d*, *J* = 8.6 Hz, H-3′,5′), 5.85 (1H, *d*, *J* = 9.0 Hz, H-11), 2.56 (1H, *m*, H-12), 1.91 (3H, *s*, H-16), 1.46 (1H, *m*, H-13), 1.35 (1H, *m*, H-13), 1.03 (3H, *d*, *J* = 6.7 Hz, H-15), 0.88 (3H, *t*, *J* = 7.4 Hz, H-14) and ^{13}C NMR (125 MHz, DMSO-d_6): δ 164.1 (C-2), 105.9 (C-3), 177.7 (C-4), 112.8 (C-5), 140.1 (C-6), 195.8 (C-7), 123.6 (C-8), 150.8 (C-9), 134.3 (C-10), 151.1 (C-11), 36.1 (C-12), 30.8 (C-13), 11.9 (C-14), 20.2 (C-15), 12.5 (C-16), 124.1 (C-1′),

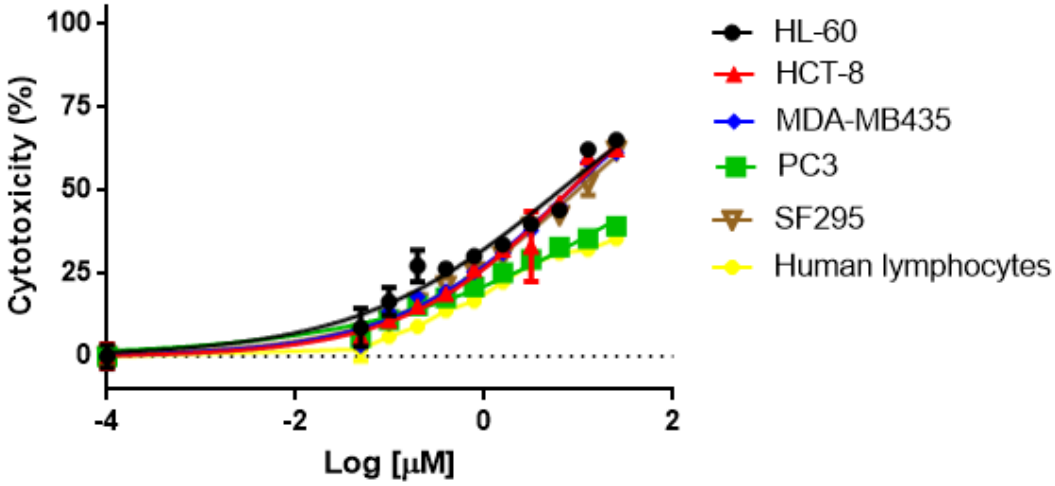
131.0 (C-2′,6′, 115.7 (C-3′,5′), 156.7 (C-4′); HRMS *m/z* 354.1702 $[M+H]^+$ (calcd for $C_{21}H_{24}NO_4 + H^+$, 354.1700).

Pyridovericin (4): pale yellow powder; mp 201–204 ºC; $[\alpha]^{25}_D$ -15.0 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 215 (2.73), 245 (2.56), 340 (2.38) nm; IR (KBr) ν_{max} 3470, 3373, 3100, 1679, 1604, 1471 992 cm^{-1};^{1}H NMR (500 MHz, DMSO-d_6): δ 11.60 (1H, *sl*, NH-1), 9.45 (1H, *s*, OH-4′), 7.98 (1H, *d*, *J* = 15.0 Hz, H-8), 7.53 (1H, *s*, H-6), 7.52 (1H, *d*, *J* = 15.0 Hz, H-9), 7.25 (2H, *d*, *J* = 8.3 Hz, H-2′,6′), 6.76 (2H, *d*, *J* = 8.3 Hz, H-3′,5′), 5.93 (1H, *d*, *J* = 10.0 Hz, H-11), 4.57 (1H, *t*, *J* = 5.4 Hz, OH-15), 3.35 (2H, *m*, H-15), 2.48 (1H, *m*, H-12), 1.88 (3H, *s*, H-16), 1.59 (1H, *m*, H-13), 1.20 (1H, *m*, H-13), 0.80 (3H, *t*, *J* = 7.7 Hz, H-14) and ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.7 (C-2), 105.9 (C-3), 176.9 (C-4), 112.7 (C-5), 140.6 (C-6), 193.8 (C-7), 123.0 (C-8), 149.3 (C-9), 134.5 (C-10), 147.5 (C-11), 43.6 (C-12), 24.0 (C-13), 11.6 (C-14), 64.0 (C-15), 12.8 (C-16), 123.4 (C-1′), 130.1 (C-2′,6′), 115.0 (C-3′,5′), 156.7 (C-4′); HRMS *m/z* 370.1646 $[M+H]^+$ (calcd for $C_{21}H_{23}NO_5 + H^+$, 370.1649).

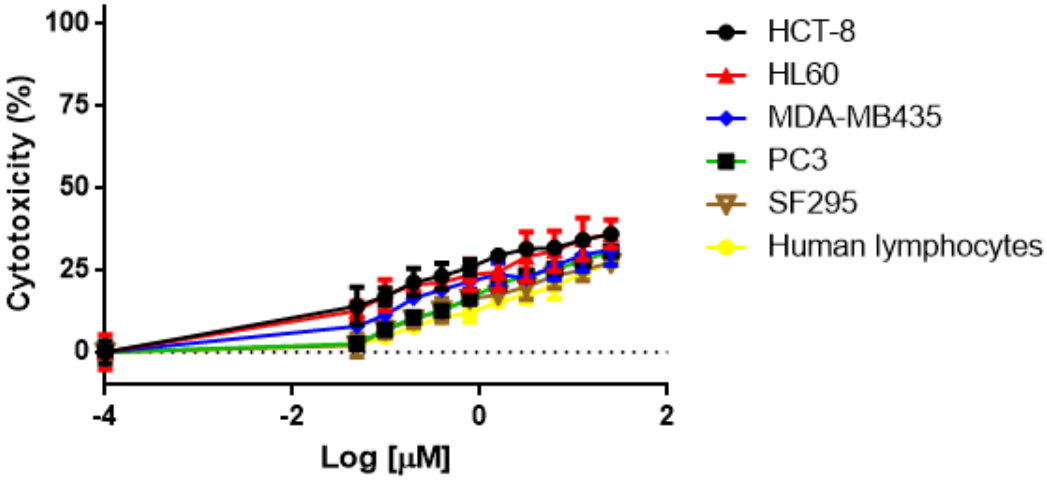
Lumichrome (5): yellow powder; mp 318–320 ºC; UV (MeOH) λ_{max} (log ε) 212 (2.17), 252 (2.54), 331 (2.55), 415 (2.21) nm; IR (KBr) ν_{max} 2920, 1710, 1432, 1390, 1280, 720 cm^{-1};^{1}H NMR (500 MHz, DMSO-d_6): δ 11.80 (1H, *s*, NH-1) 11.60 (1H, *s*, NH-2), 7.91 (1H, *s*, H-6), 7.70 (1H, *s*, H-7), 2.49 (3H, *s*, H-11), 2.47 (3H, *s*, H-12) and ^{13}C NMR (125 MHz, DMSO-d_6): δ 150.0 (C-2), 160.6 (C-3), 130.1 (C-4a), 138.9 (C-5a), 125.8 (C-6), 144.7 (C-7), 138.4 (C-8), 128.7 (C-9), 146.5 (C-9a), 141.7 (C-10a), 20.2 (C-11), 19.5 (C-12); HRMS *m/z* 243.0863 $[M+H]^+$ (calcd for $C_{12}H_{11}N_4O_2 + H^+$, 243.0877).



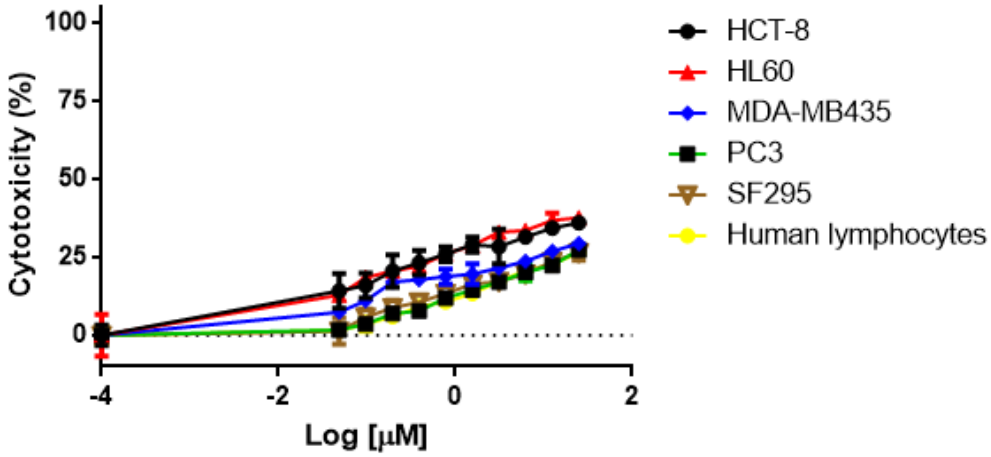
Doxorrubicin (reference compound)



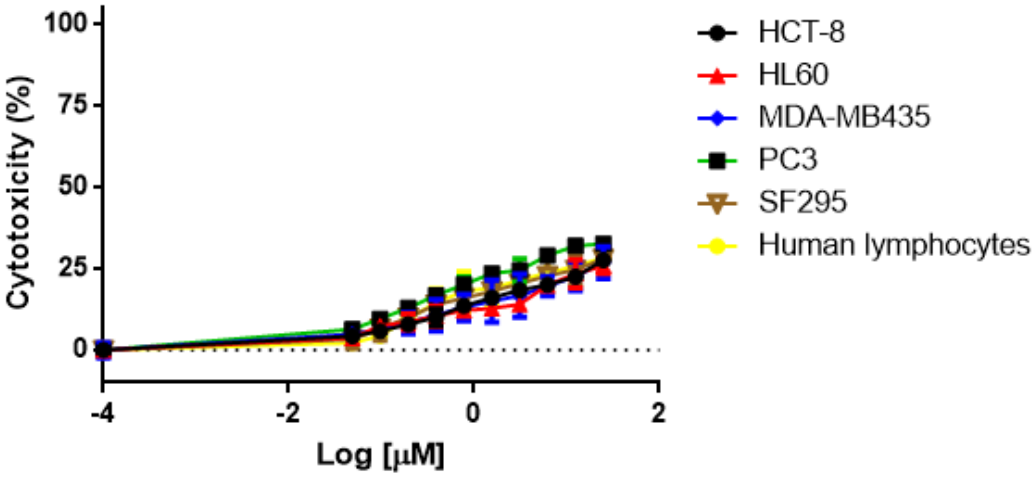
Pyridovericin (4)



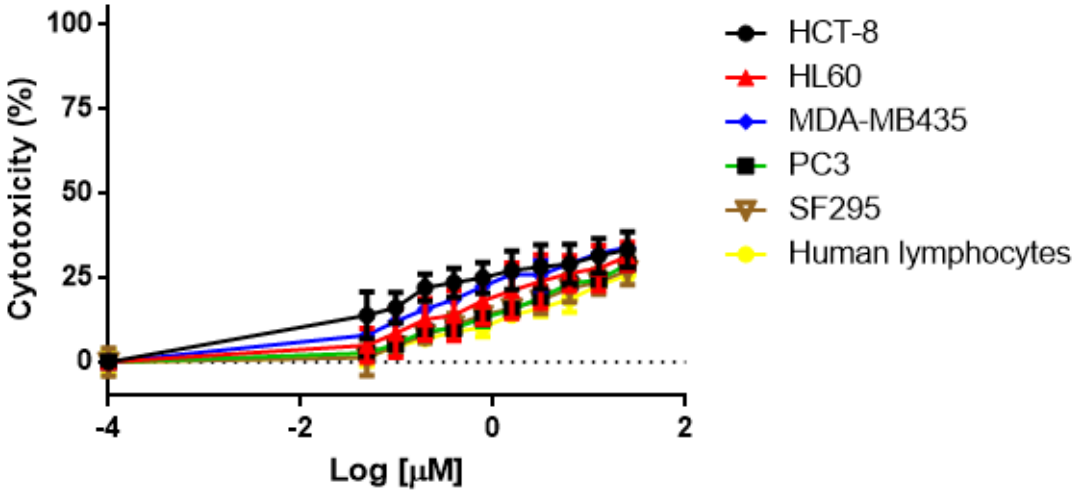
Pretenellin B (3)



Lumichrome (5)



1-Methyl-11-hydroxylumichrome (2)



Pyridovericin-*N*-*O*-(4-*O*-methyl-β-D-glucopyranoside) (1)

Figure S17. Graphics of cytotoxicity assay.