Supplementary Material

Picraquanines A－C, three new phenolic derivatives from the stems of *Picrasma quassioide*

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ABSTRACT: Three new phenolic derivatives, picraquanines A - C (**1** - **3**), along with 6 known ones **4** - **9** were obtained from the stems of *Picrasma quassioides* (D. Don) Benn. The new structures were determined by extensive spectroscopic data analysis, including IR, HRESIMS, ^{1}H-NMR, ^{13}C-NMR, HSQC, HMBC, ^{1}H-^{1}H COSY experiments.

The absolute configuration of **1** was determined by comparison of its experimental and calculated ECD spectra. Furthermore, all the compounds were tested for their nitric oxide (NO) inhibitory effects against LPS-stimulated RAW 264.7 cells, however, none of them exhibited inhibitory effects (IC_{50} >100 μM).

Key words: *Picrasma quassioides*; structure determination; Picraquanines A─C; NO inhibitory activity

Extraction and isolation

The air-dried stems of *Picrasma quassioides* (D. Don) Benn. (9.3 Kg) were extracted with 80% EtOH under reflux two times. After removing the solvent under reduced pressure, the concentrated residue was dissolved with EtOH, and then adjust pH 9 with NaOH. After filtered and concentrated, the concentration was extract with 2% HCl for four times, and then adjusted pH 5.0 ~ 6.0 with NaOH, finally, the concentrated extract (200 g) was obtained. The extract (200 g) was subjected to column chromatography (CC, 10*110 cm) on silica gel (2.0 Kg), eluting with a gradient solvent system (CH_2Cl_2-MeOH, 20:1 − 1:1) to give eleven fractions (F1 − F11); F1 (16 g) was subjected to CC (5*60 cm) on RP-C_{18} (100 g) eluting with MeOH-H_2O (5% − 60%) to give fourteen subfractions (F1-1 − F1-14). F1-3 (1.8 g) was purified by HPLC with MeCN-0.02%TFA/H_2O (13:87) to afford **5** (9.5 mg, t_R = 11.5 min), **2** (12 mg, t_R = 20.5 min), **8** (12 mg, t_R = 21.8 min) and **9** (10 mg, t_R = 23.5 min). F1-5 (2.2 g) was purified by HPLC with MeCN-0.02%TFA/H_2O (30:70) to afford **3** (2 mg, t_R = 14.2 min). F2 (24 g) was subjected to CC (5*60 cm) on RP-C_{18} (100 g) eluting with MeOH-H_2O (5% − 60%) to give eight subfractions (F2-1 − F2-8). F2-2 (2.0 g) was purified by HPLC with MeCN-0.02%TFA/H_2O (8:92) to afford **4** (4.0 mg, t_R = 15.6 min), **6** (1.7 mg, t_R = 16.5 min), **1** (3.2 mg, t_R = 21.8 min) and **7** (3.0 mg, t_R = 36.1 min).

Inhibitory assay of NO production

Cytotoxicity was examined using the Cell Counting Kit-8 (CCK-8). The absorbance at 450 nm was measured using a microplate reader (Thermo Fisher Scientific).

Viability was defined as the ratio (expressed as a percentage) of absorbance values of treated cells to untreated cells. Compounds 1–9 were dissolved in dimethyl sulfoxidediluted (DMSO) with complete medium to 6 degrees of concentration (0.1 μmol•L^{-1}, 1 μmol•L^{-1}, 10 μmol•L^{-1}, 25 μmol•L^{-1}, 50 μmol•L^{-1}, 100 μmol•L^{-1}) for inhibition rate determination. RAW 264.7 cells were maintained in Dulbecco's modified Eagle's medium (high-glucose condition) supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 lg/mL streptomycin at 37 ℃ in 5% CO_2. RAW 264.7 cells were pre-treated with each tested compound for 30 min, and then stimulated with lipopolysaccharide (LPS) (100 ng/mL) for 24 h. Aminoguanidine hydrochloride (100 μM) was used as a positive control. The NO production was measured using the Griess reagent. Briefly, cell culture supernatant was reacted with equal volumes of Griess reagent in a 96-well plate for 10 min, and then the absorbance at 540nm was measured by a plate reader. All experiments were performed in triplicate. All of the tested compounds were prepared as stock solutions with a concentration of 100 mM in DMSO.

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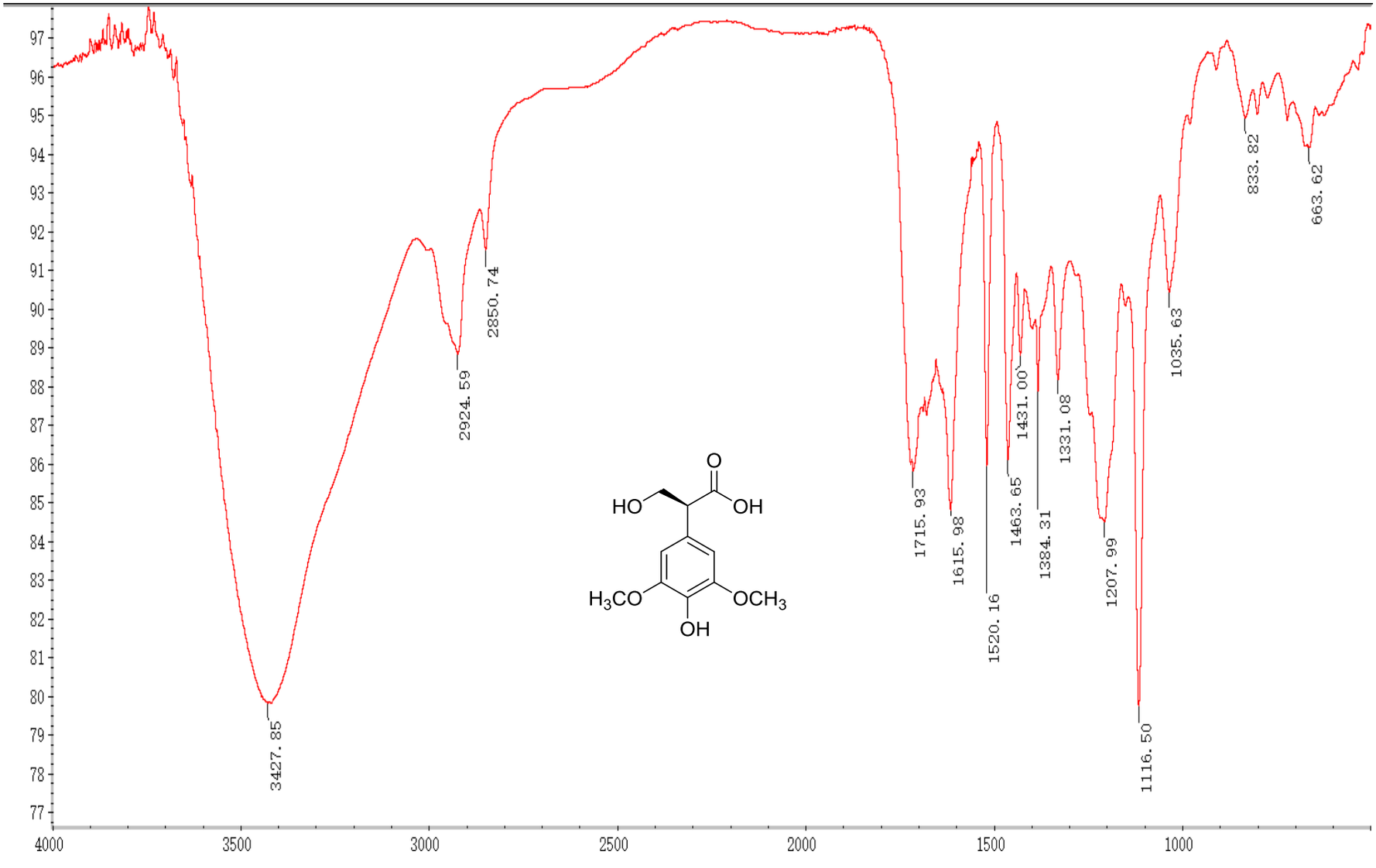


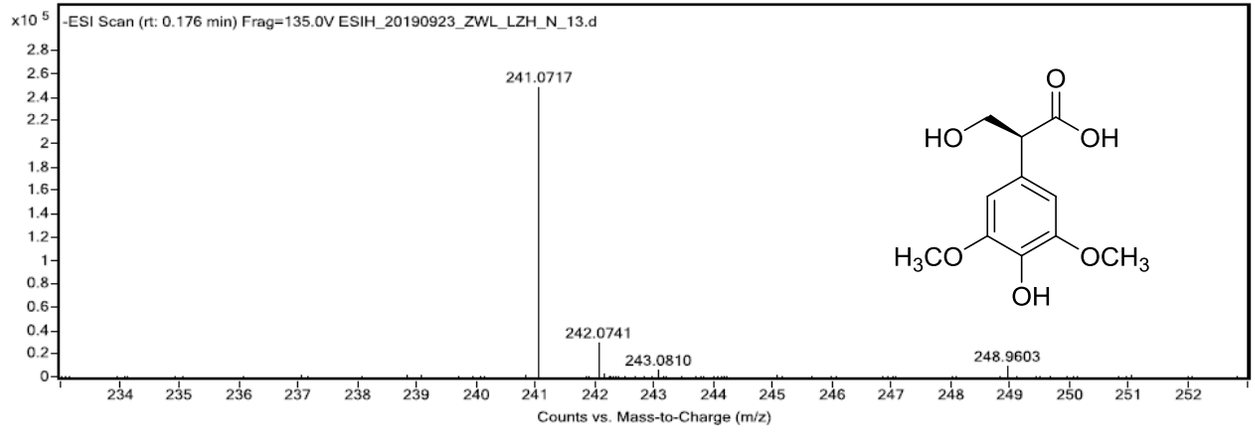
Figure S1. The IR spectrum of **1** (in KBr)

Qualitative Analysis Report

Data Filename	ESIH_20190923_ZWL_LZH_N_13.d	**Sample Name**	KM-22
Sample Type	Sample	**Position**	P1-C3
Instrument Name	Agilent G6520 Q-TOF	**Acq Method**	20160324_MS_ESIH_NEG_1min.m
Acquired Time	9/23/2019 16:03:45	**IRM Calibration Status**	Success
DA Method	small molecular data analysis method.m	**Comment**	ESIH by ZZY

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	ESI



Formula Calculator Results

m/z	Calc m/z	Diff (mDa)	Diff (ppm)	Ion Formula	Ion
241.0717	241.0718	0.05	0.2	C11 H13 O6	(M-H)-

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Figure S2. The HRESIMS spectrum of **1** (in MeOH)

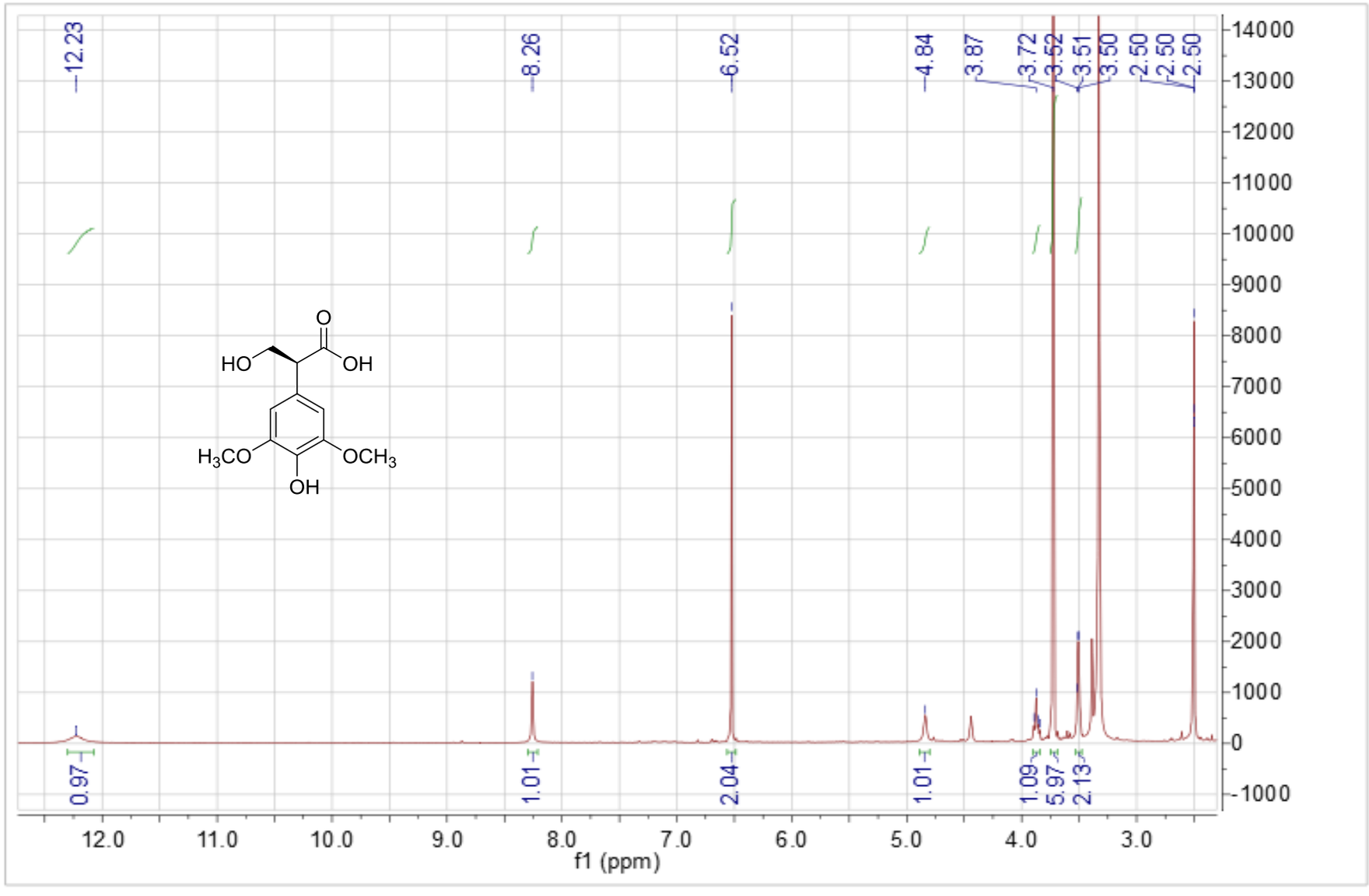


Figure S3. The ^{1}H NMR spectrum of **1** (in DMSO-d_6)

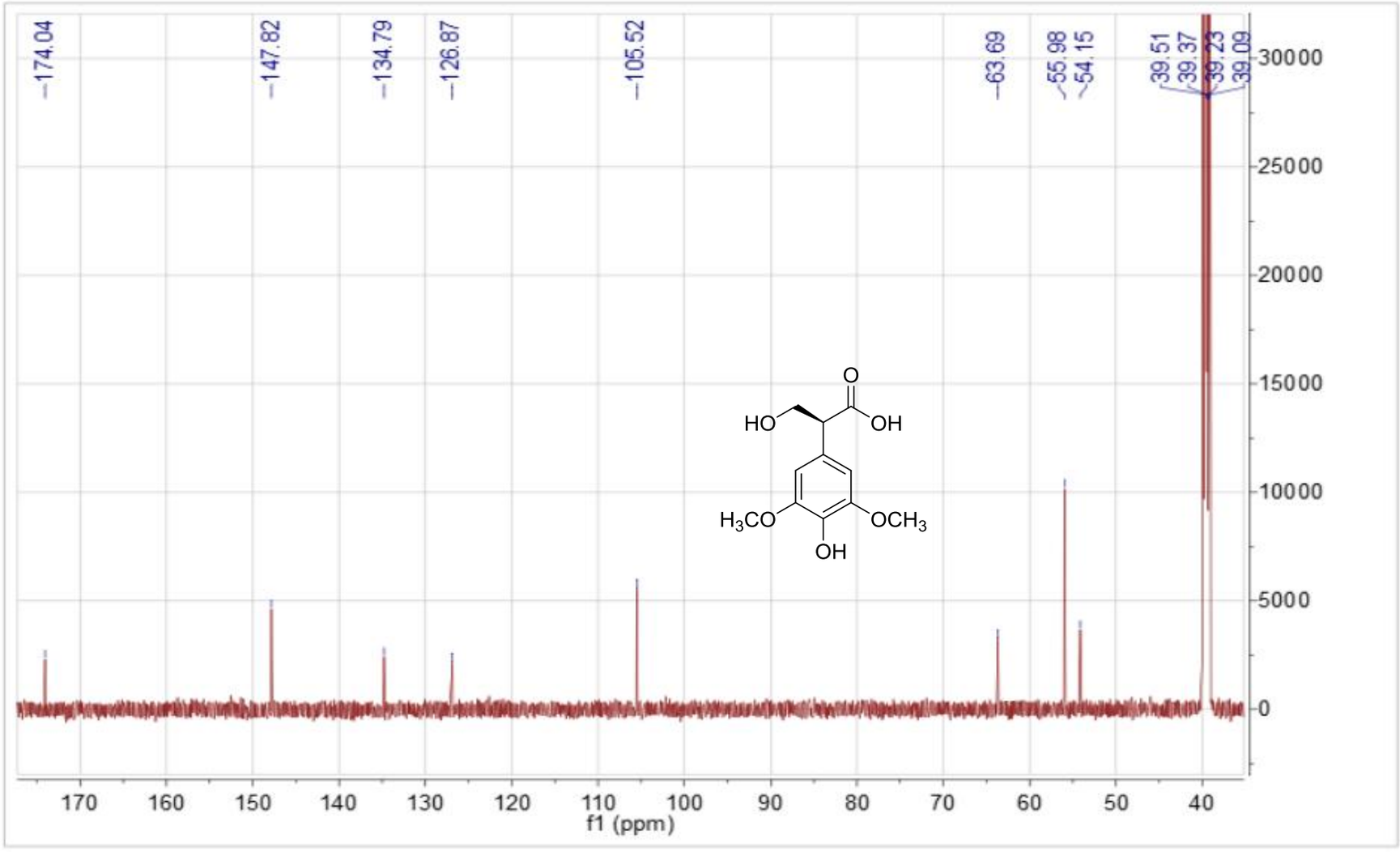


Figure S4. The ^{13}C NMR spectrum of **1** (in DMSO-d_6)

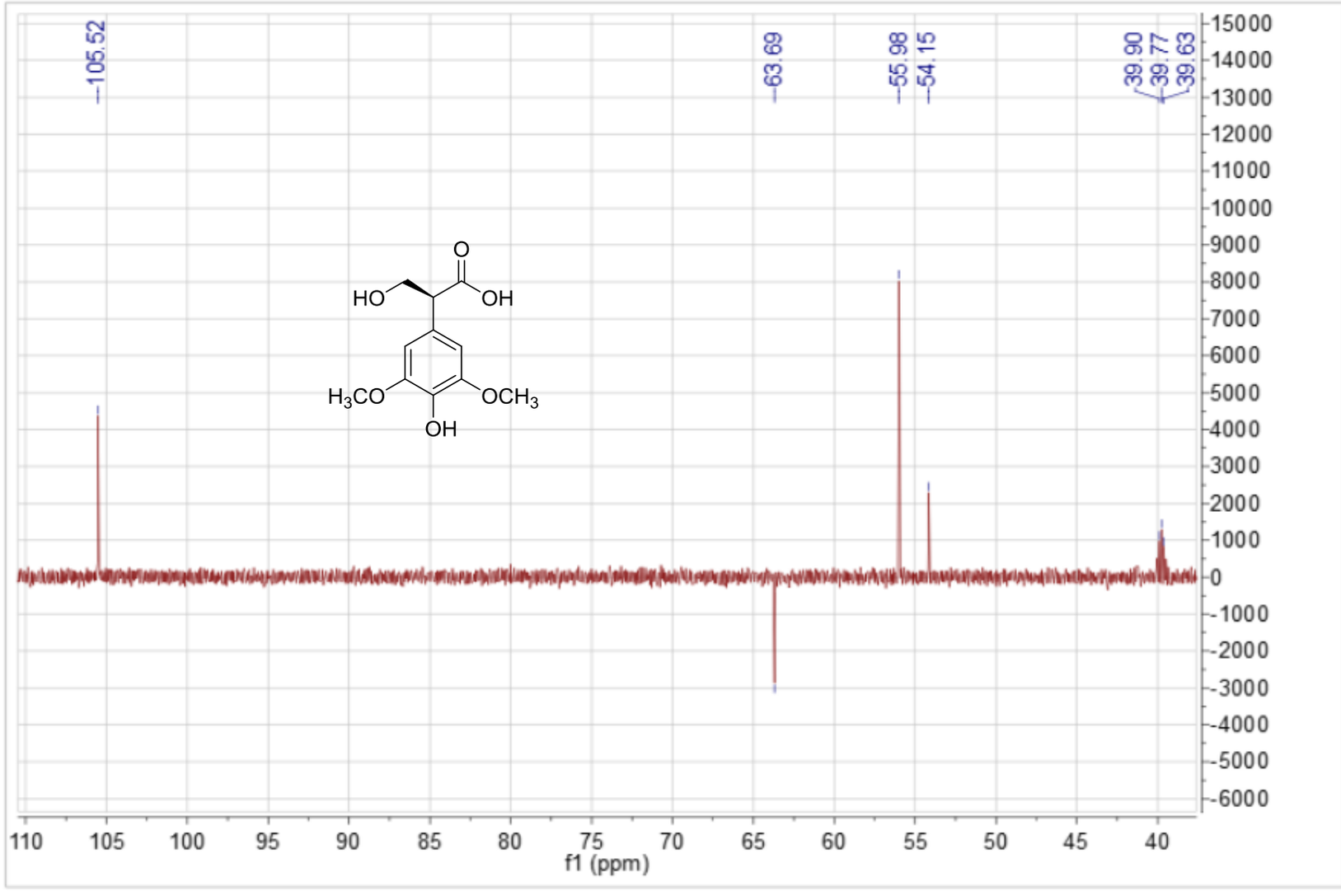


Figure S5. The DEPT 135 spectrum of **1** (in DMSO-d_6)

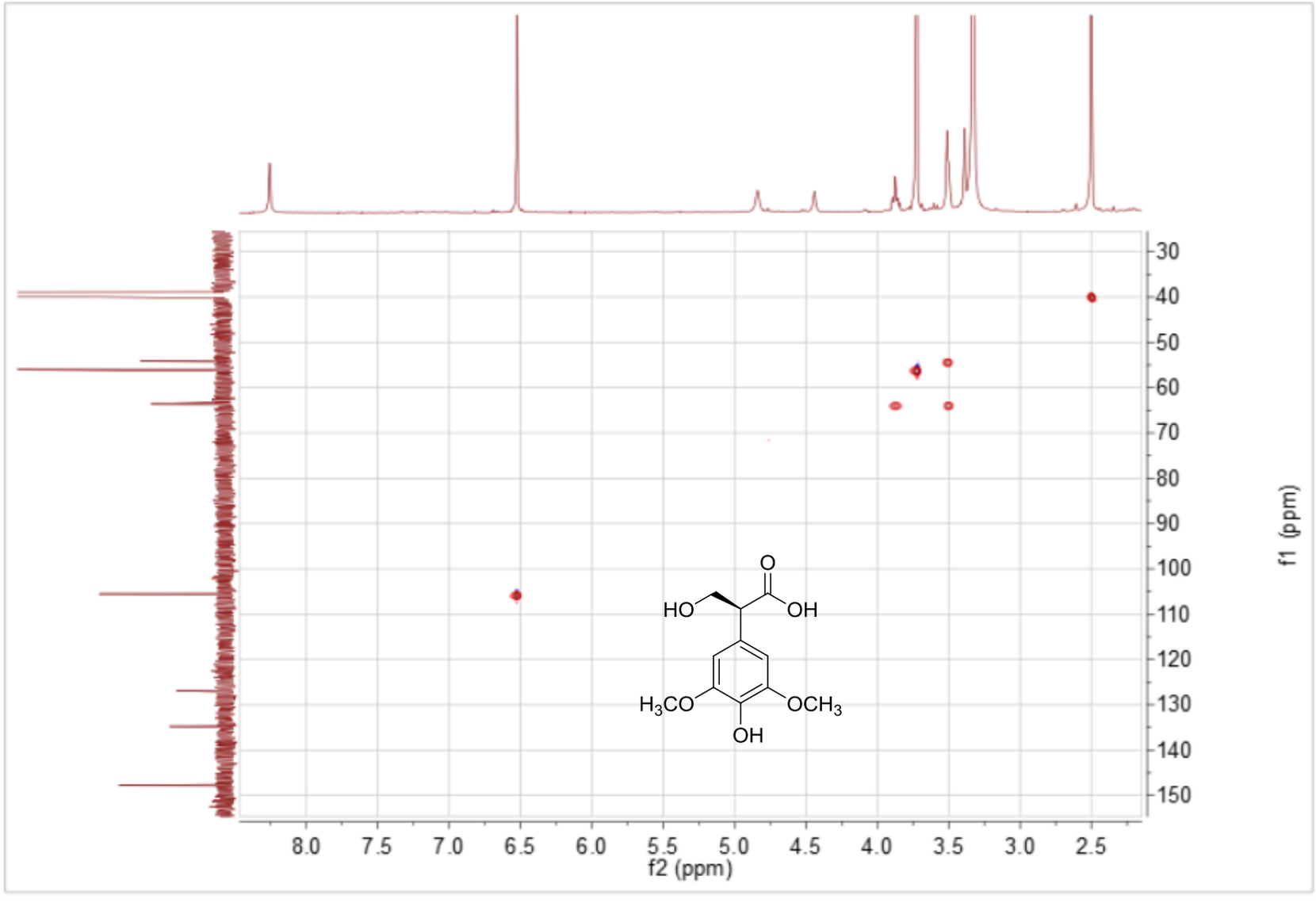


Figure S6. The HSQC spectrum of **1** (in DMSO-d_6)

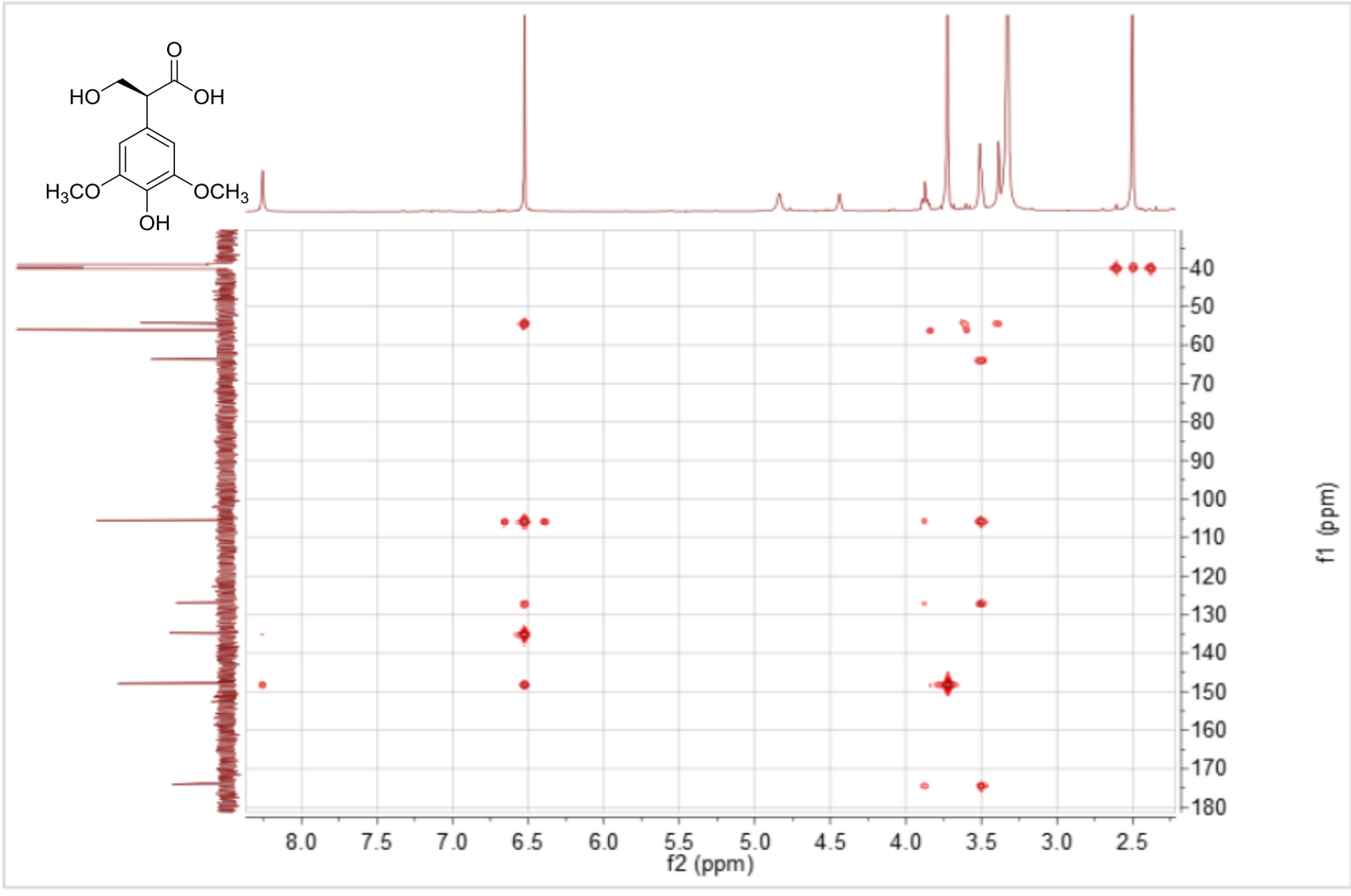


Figure S7. The HMBC spectrum of **1** (in DMSO-d_6)

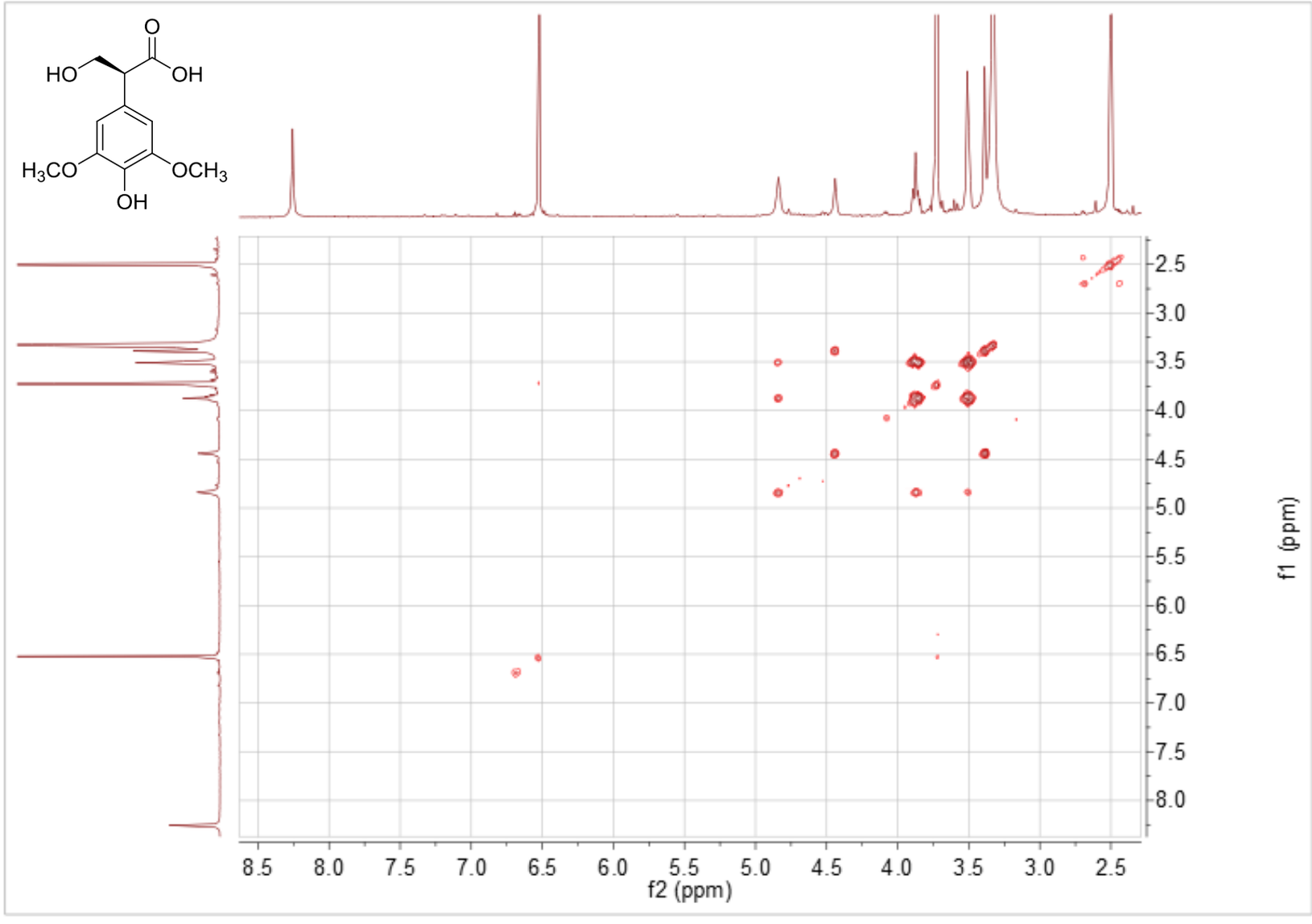


Figure S8. The ^{1}H-^{1}H COSY spectrum of **1** (in DMSO-d_6)

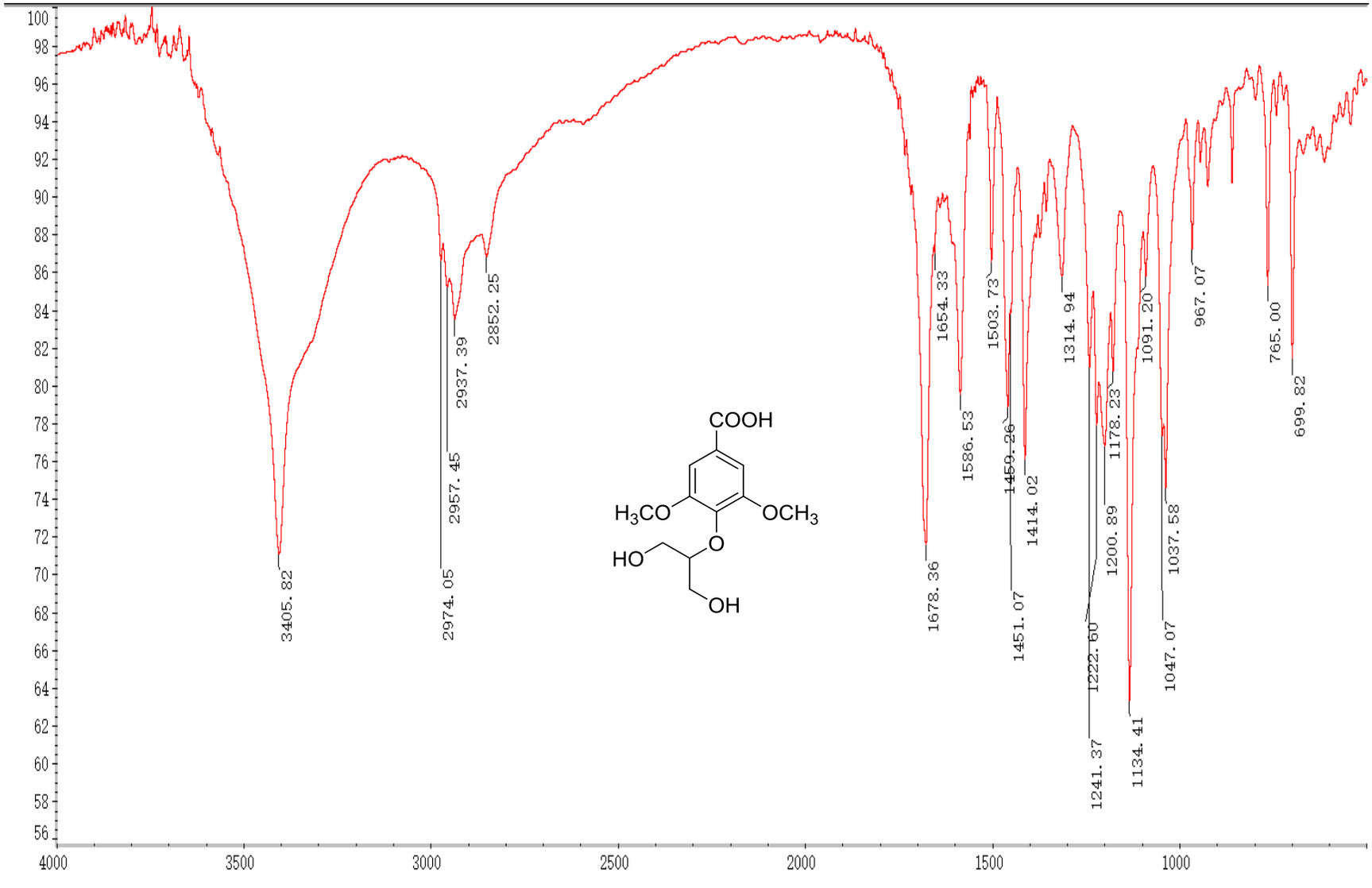


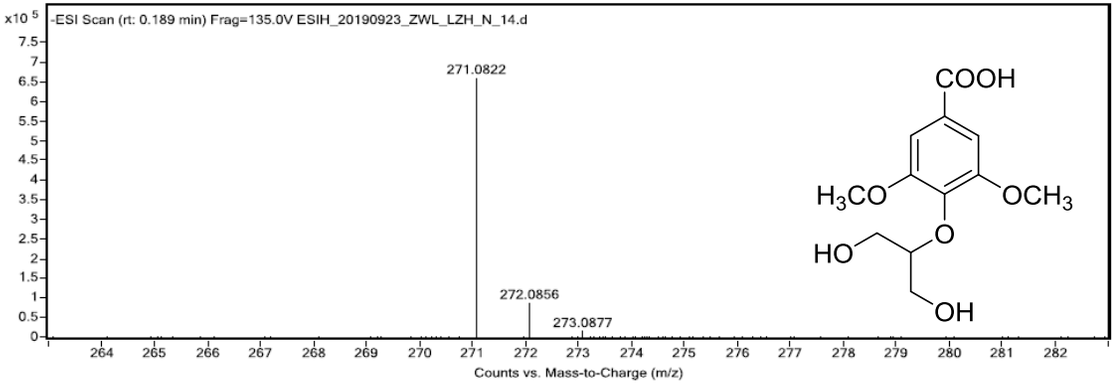
Figure S9. The IR spectrum of **2** (in KBr)

Qualitative Analysis Report

Data Filename	ESIH_20190923_ZWL_LZH_N_14.d	**Sample Name**	KM-29
Sample Type	Sample	**Position**	P1-C4
Instrument Name	Agilent G6520 Q-TOF	**Acq Method**	20160324_MS_ESIH_NEG_1min.m
Acquired Time	9/23/2019 16:05:15	**IRM Calibration Status**	Success
DA Method	small molecular data analysis method.m	**Comment**	ESIH by ZZY

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	ESI



Formula Calculator Results

m/z	Calc m/z	Diff (mDa)	Diff (ppm)	Ion Formula	Ion
271.0822	271.0823	0.12	0.45	C12 H15 O7	(M-H)-

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Figure S10. The HRESIMS spectrum of **2** (in MeOH)

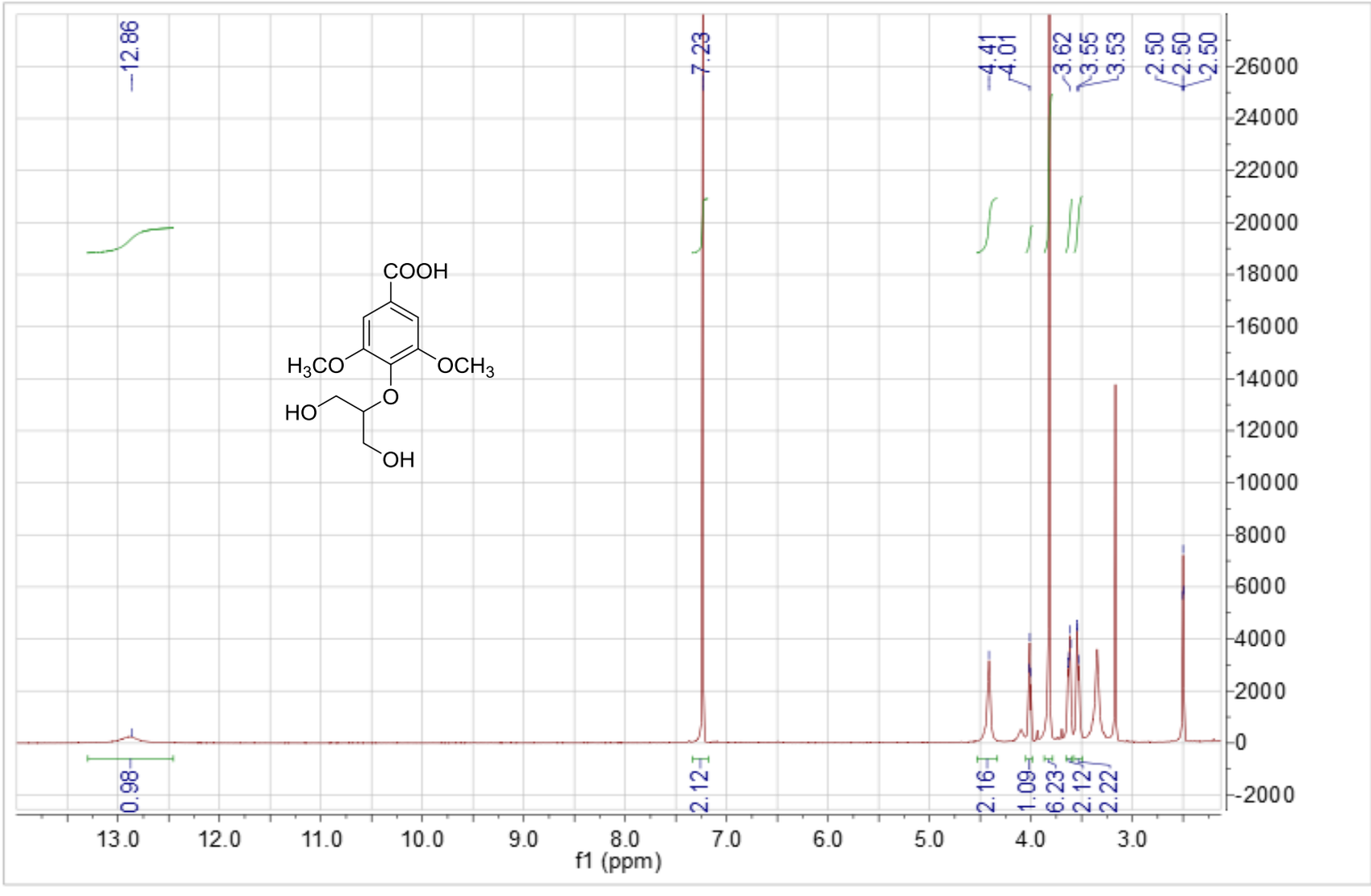


Figure S11. The ^{1}H NMR spectrum of **2** (in DMSO-d_6)

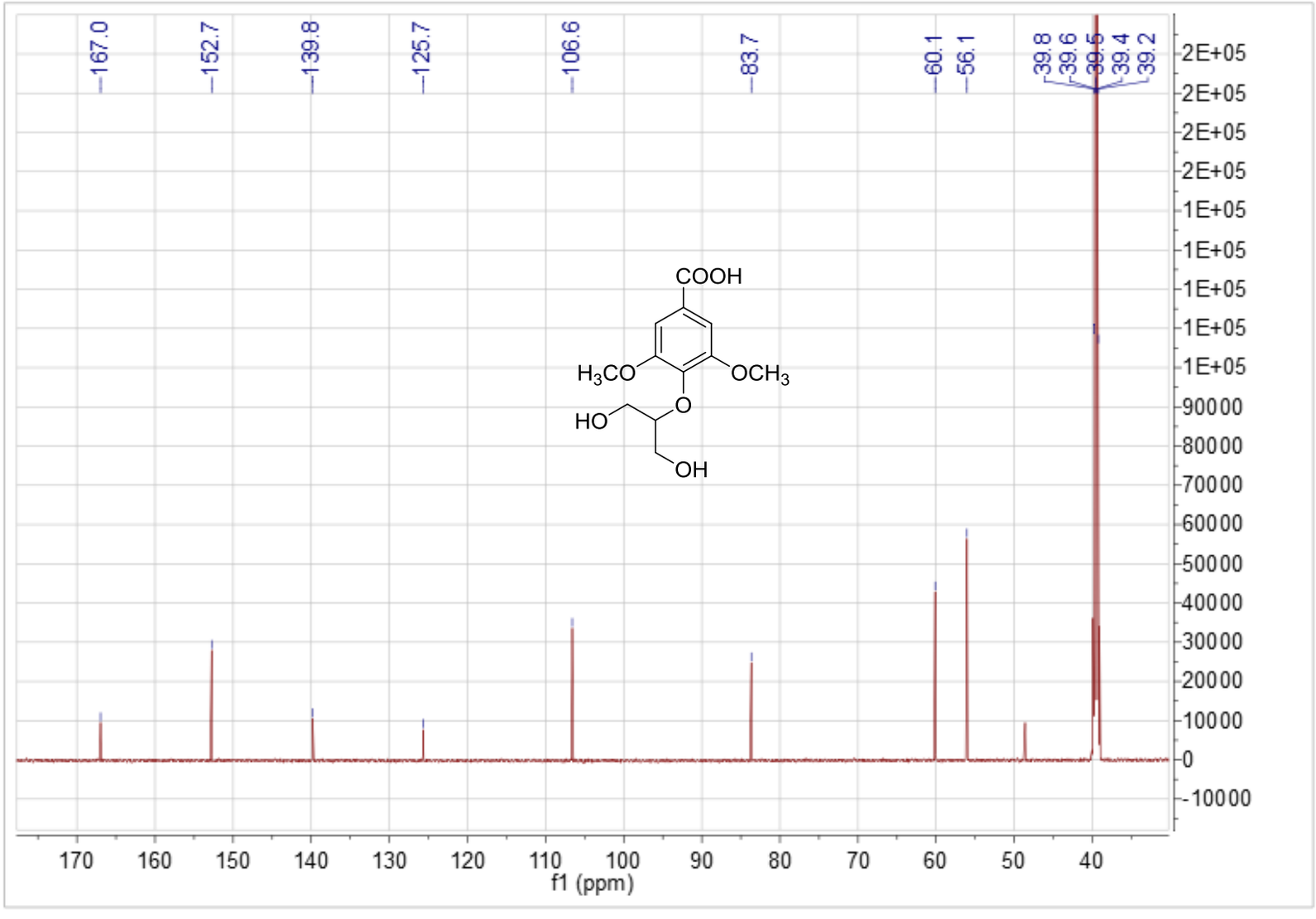


Figure S12. The ^{13}C NMR spectrum of **2** (in DMSO-d_6)

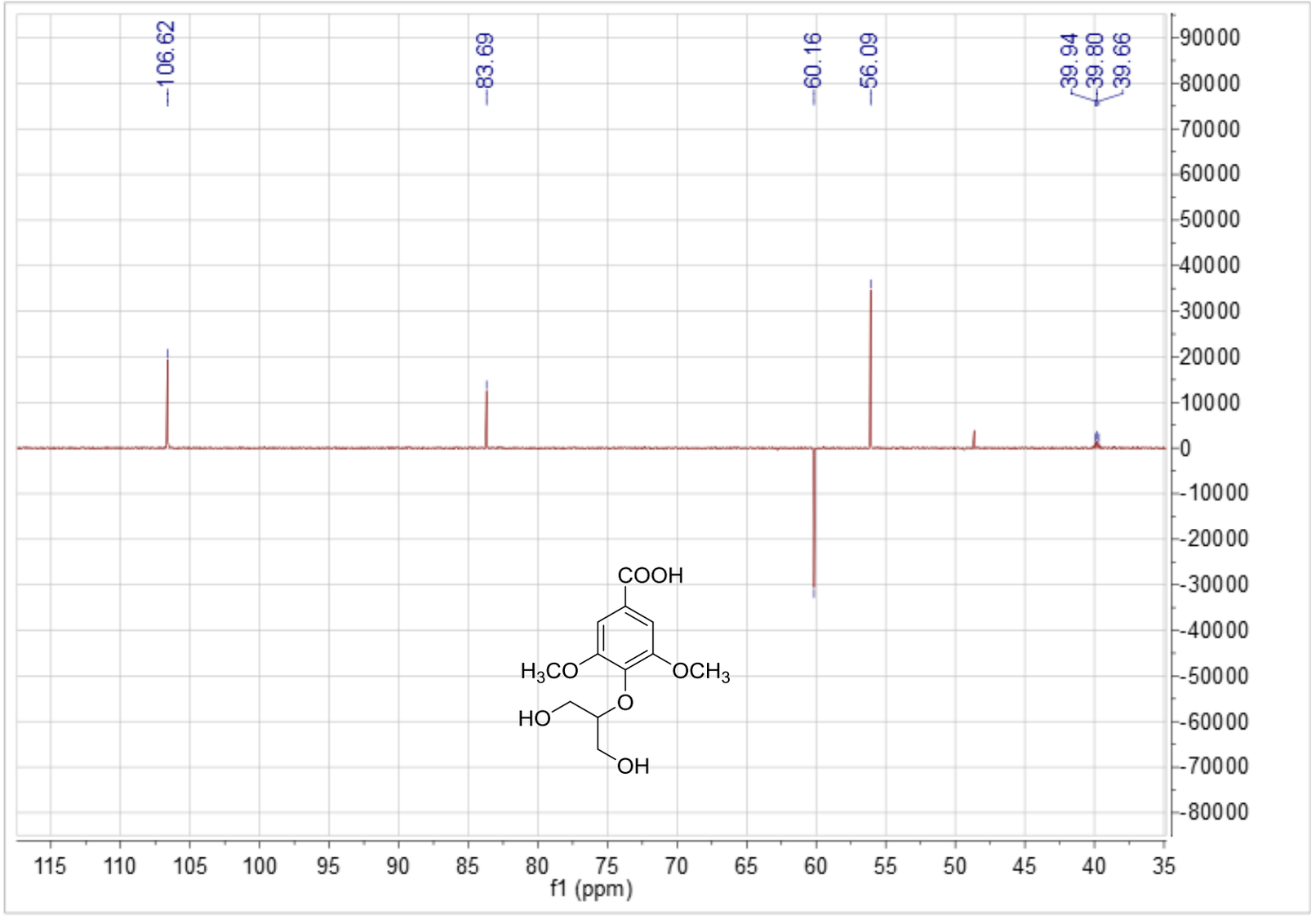


Figure S13. The DEPT 135 spectrum of **2** (in DMSO-d_6)

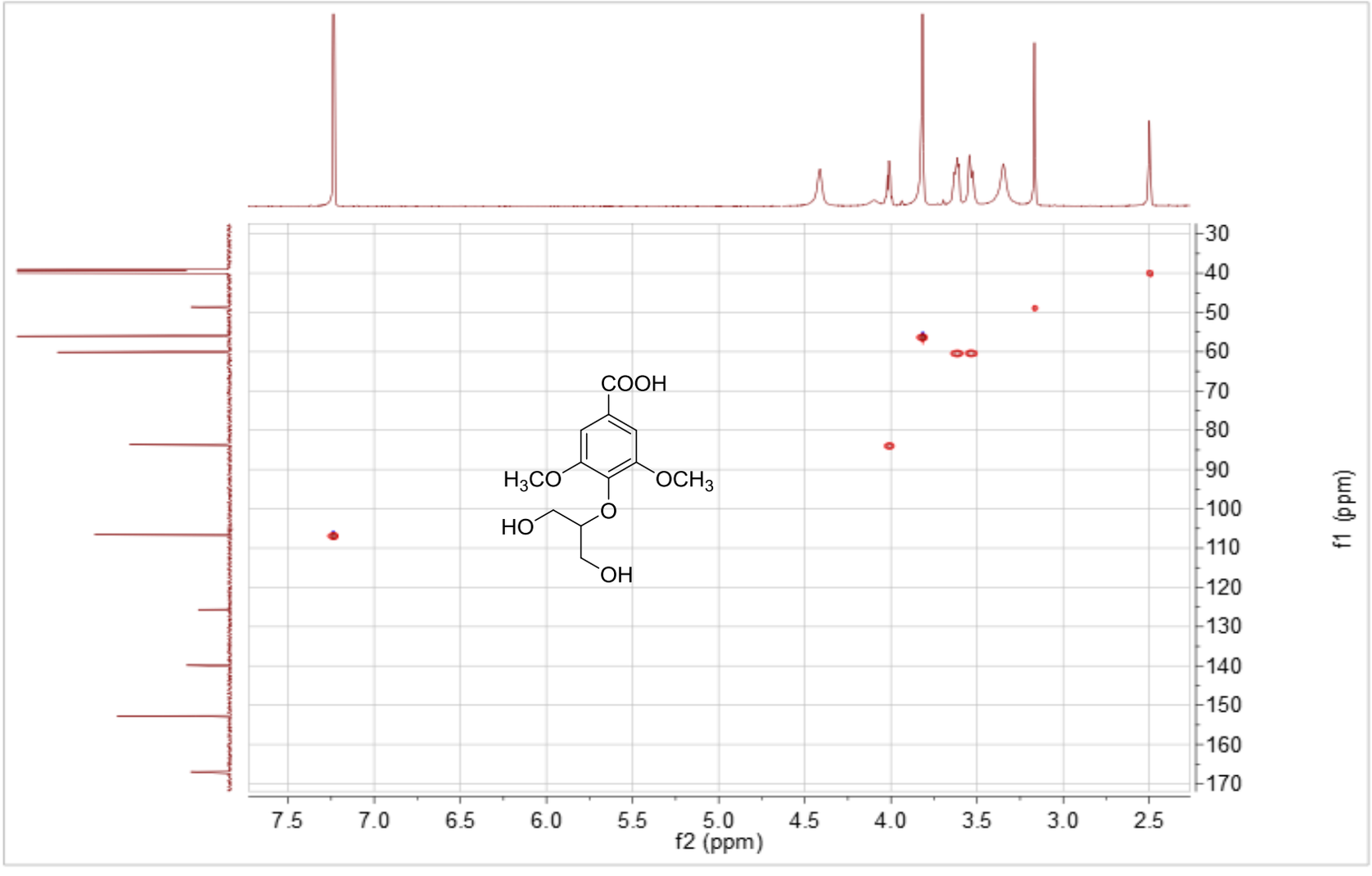


Figure S14. The HSQC spectrum of **2** (in DMSO-d_6)

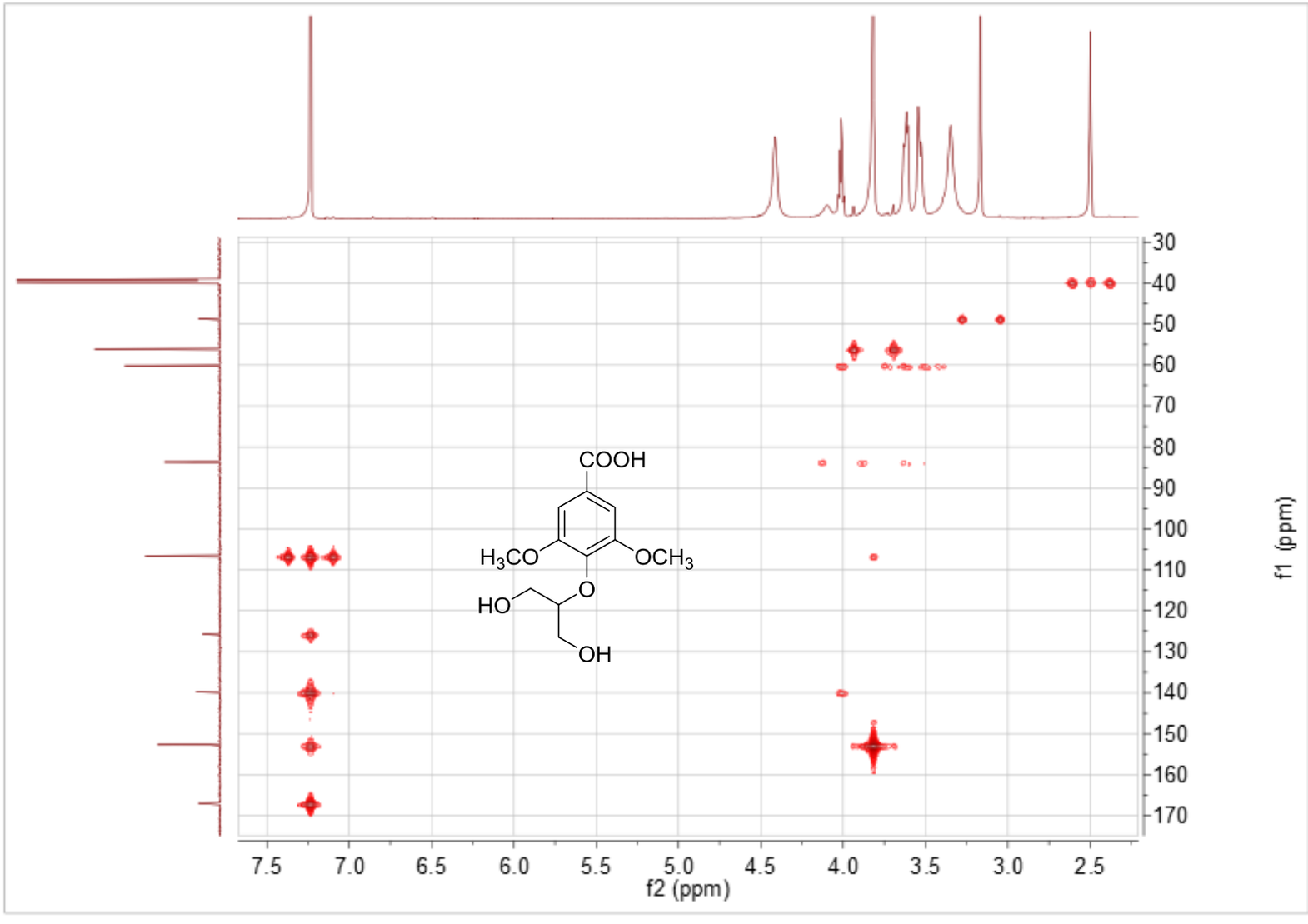


Figure S15. The HMBC spectrum of **2** (in DMSO-d_6)

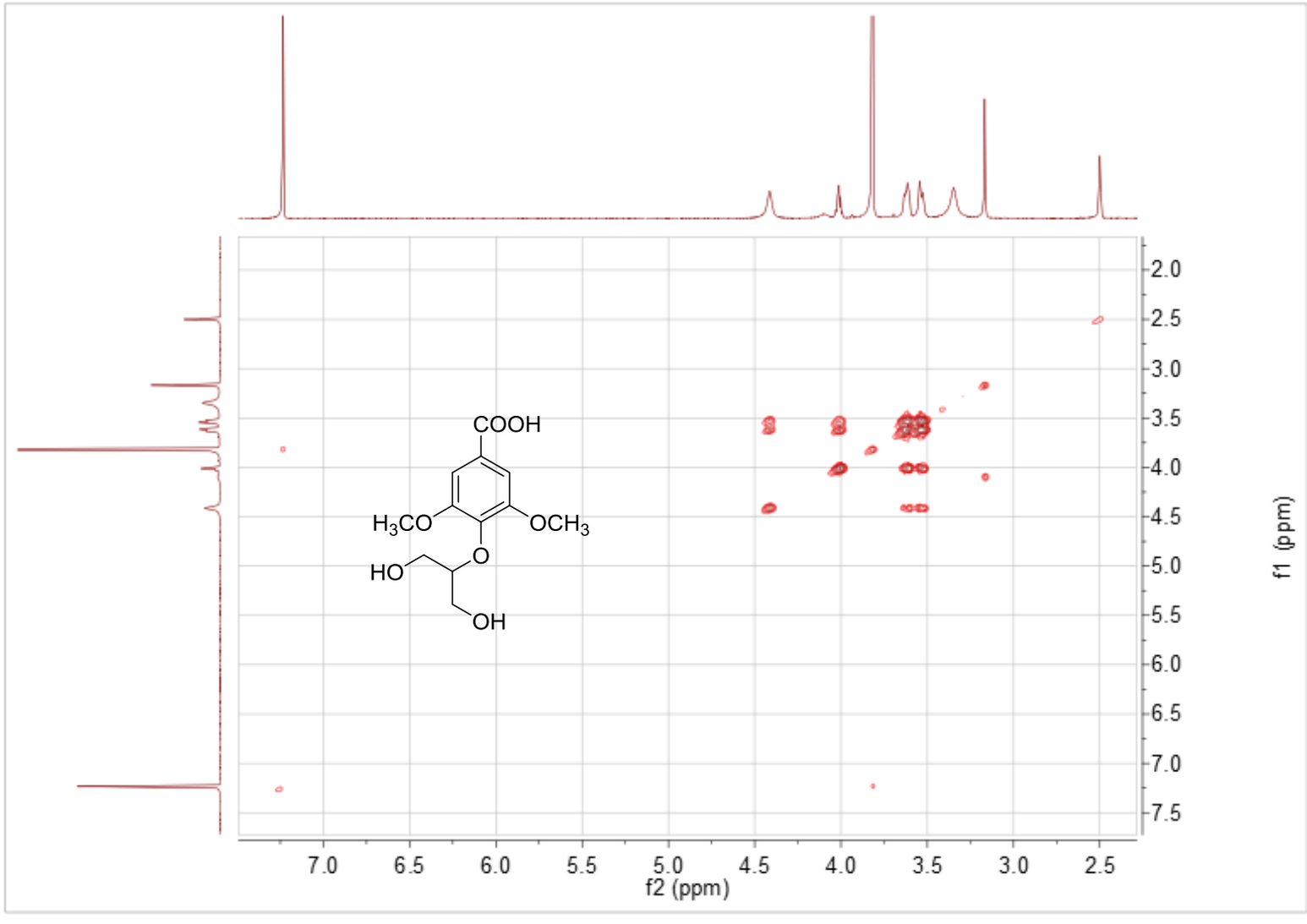


Figure S16. The ^{1}H-^{1}H COSY spectrum of **2** (in DMSO-d_6)

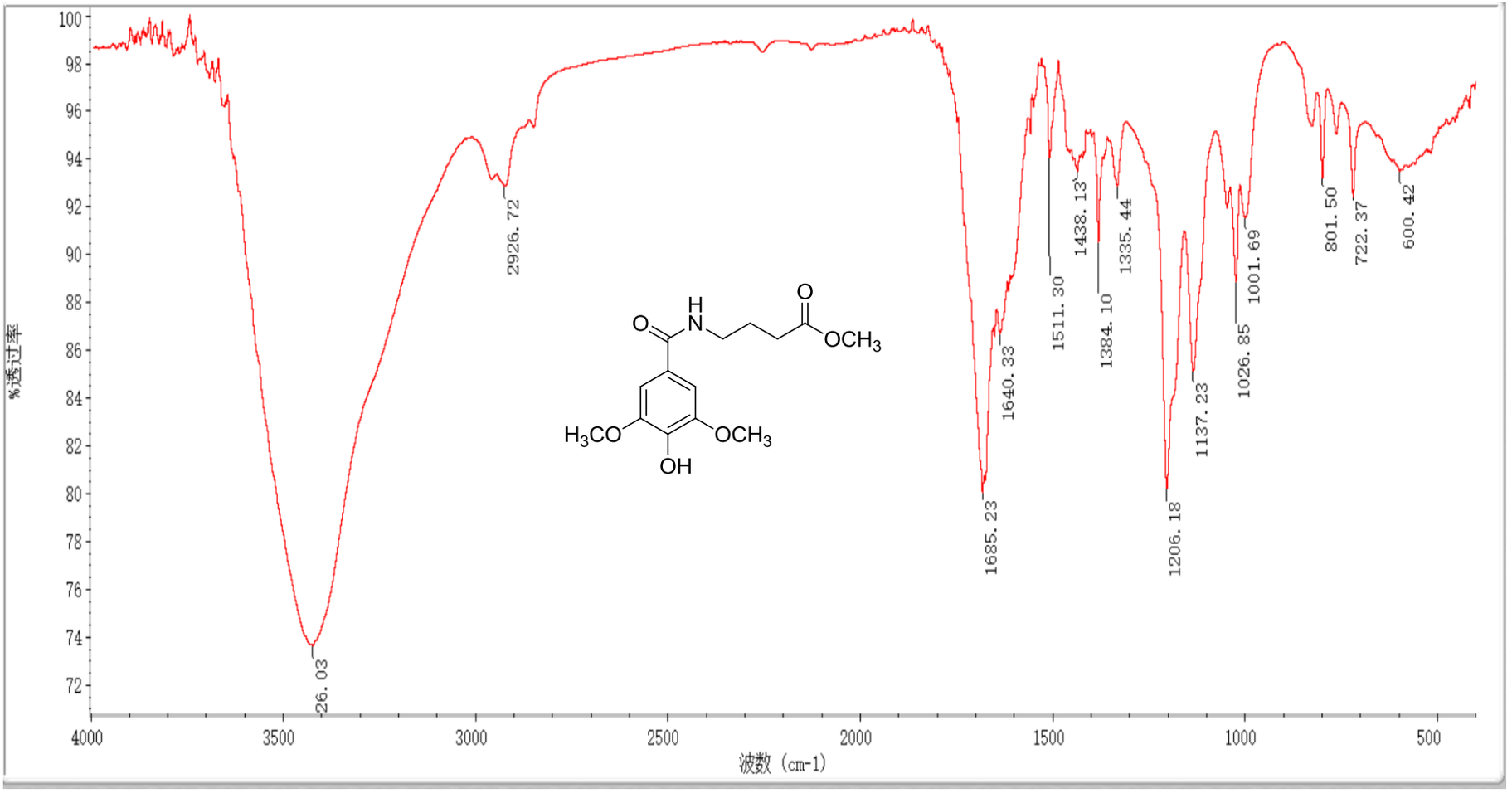


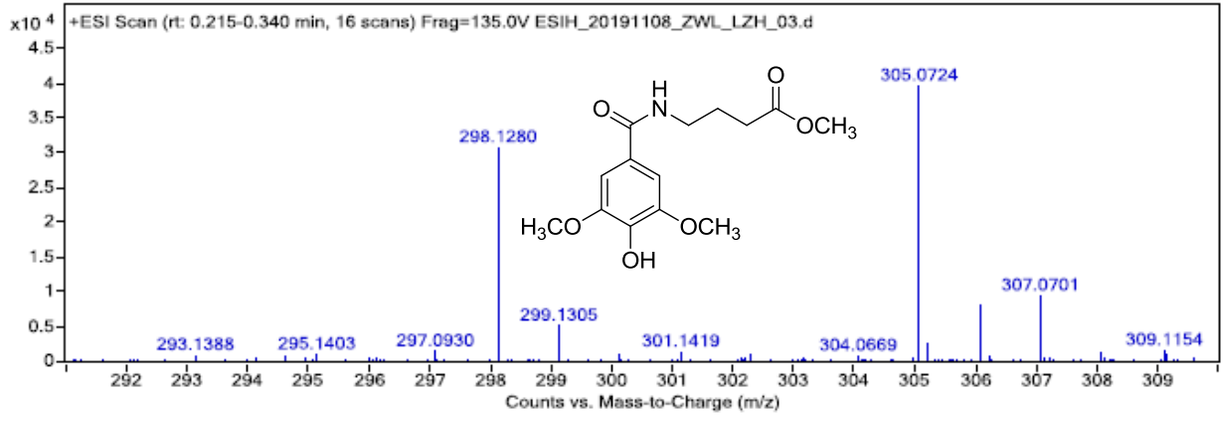
Figure S17. The IR spectrum of **3** (in KBr)

Qualitative Analysis Report

Data Filename	ESIH_20191108_ZWL_LZH_03.d	**Sample Name**	KM-48
Sample Type	Sample	**Position**	P1-A3
Instrument Name	Agilent G6520 Q-TOF	**Acq Method**	20160322_MS_ESIH_POS_1min.m
Acquired Time	11/8/2019 10:23:07	**IRM Calibration Status**	Success
DA Method	small molecular data analysis method.m	**Comment**	ESIH by ZZY

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
135	0	ESI



Formula Calculator Results

m/z	Calc m/z	Diff (mDa)	Diff (ppm)	Ion Formula	Ion
298.128	298.1285	0.52	1.73	C14 H20 N O6	(M+H)+

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Figure S18. The HRESIMS spectrum of **3** (in MeOH)

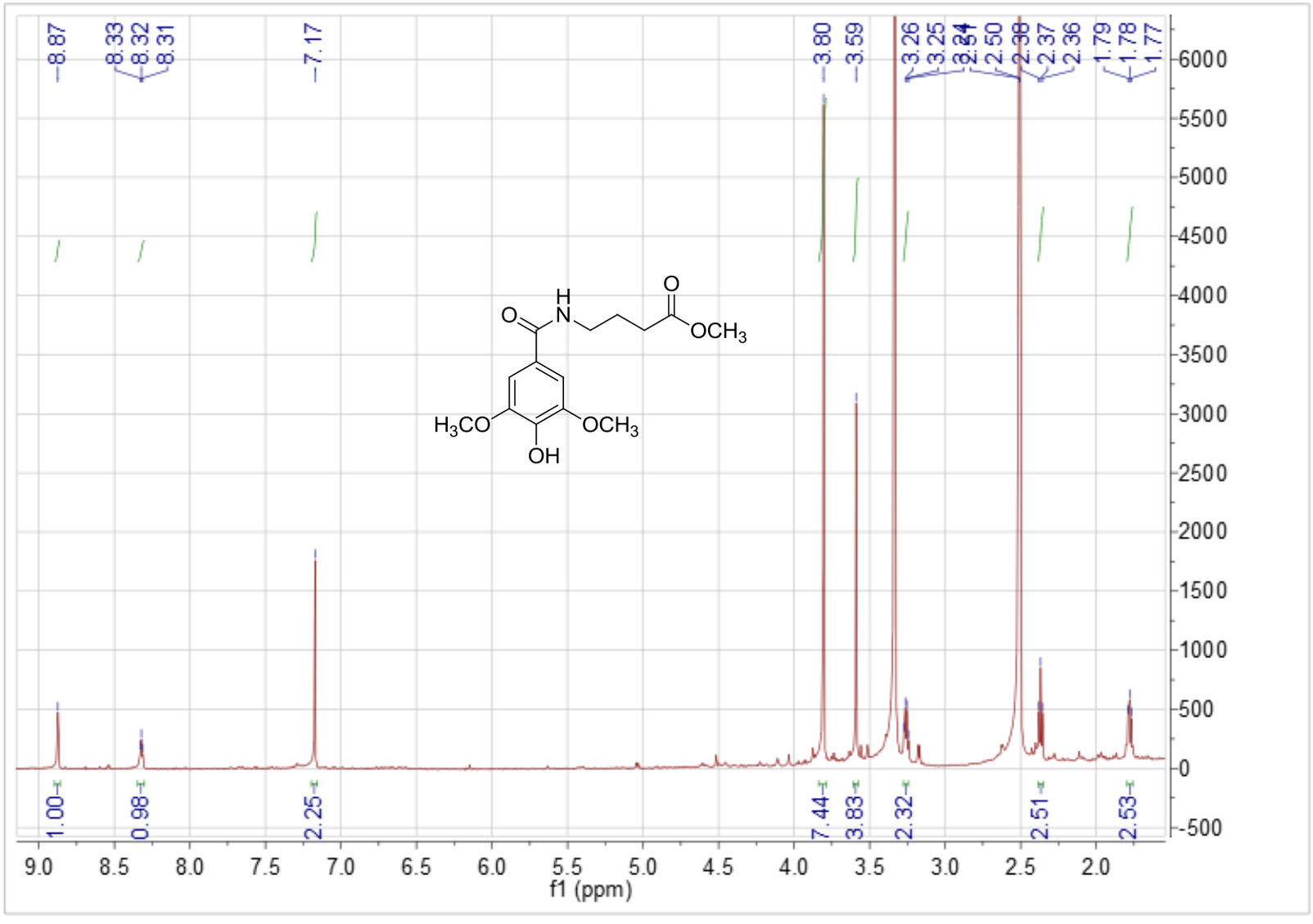

Figure S19. The ^{1}H NMR spectrum of **3** (in DMSO-d_6)

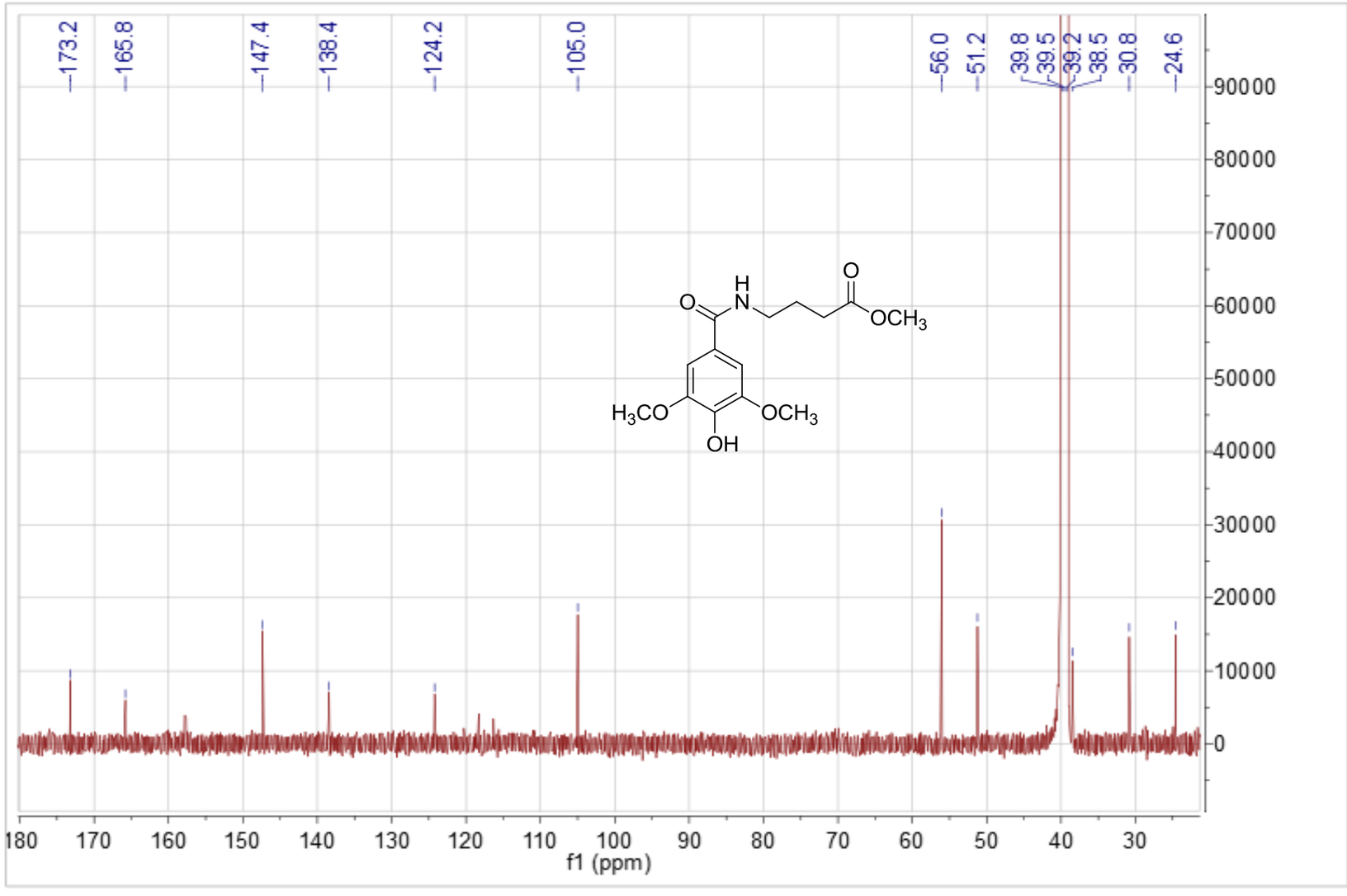

Figure S20. The ^{13}C NMR spectrum of **3** (in DMSO-d_6)

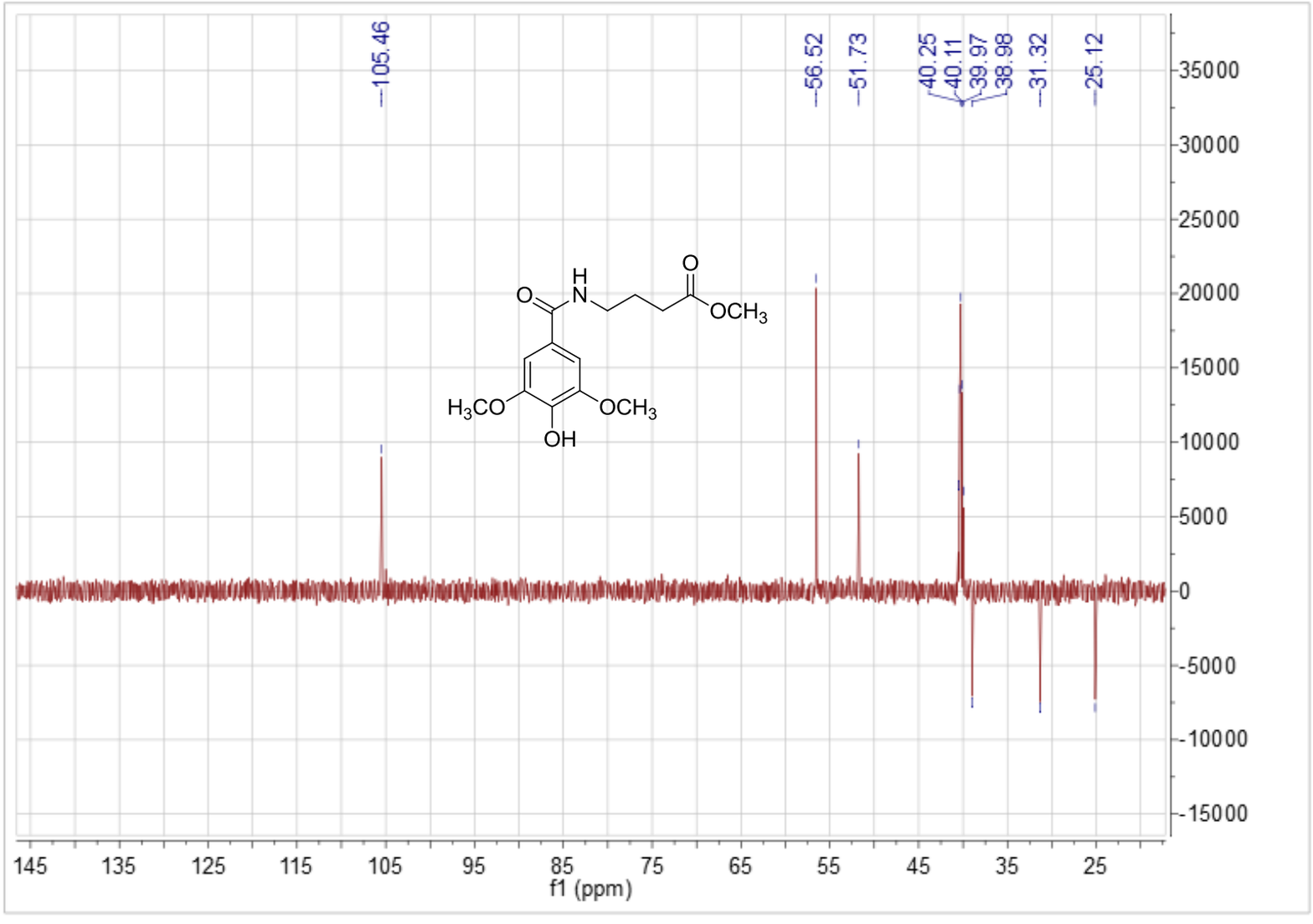

Figure S21. The DEPT 135 spectrum of **3** (in DMSO-d_6)

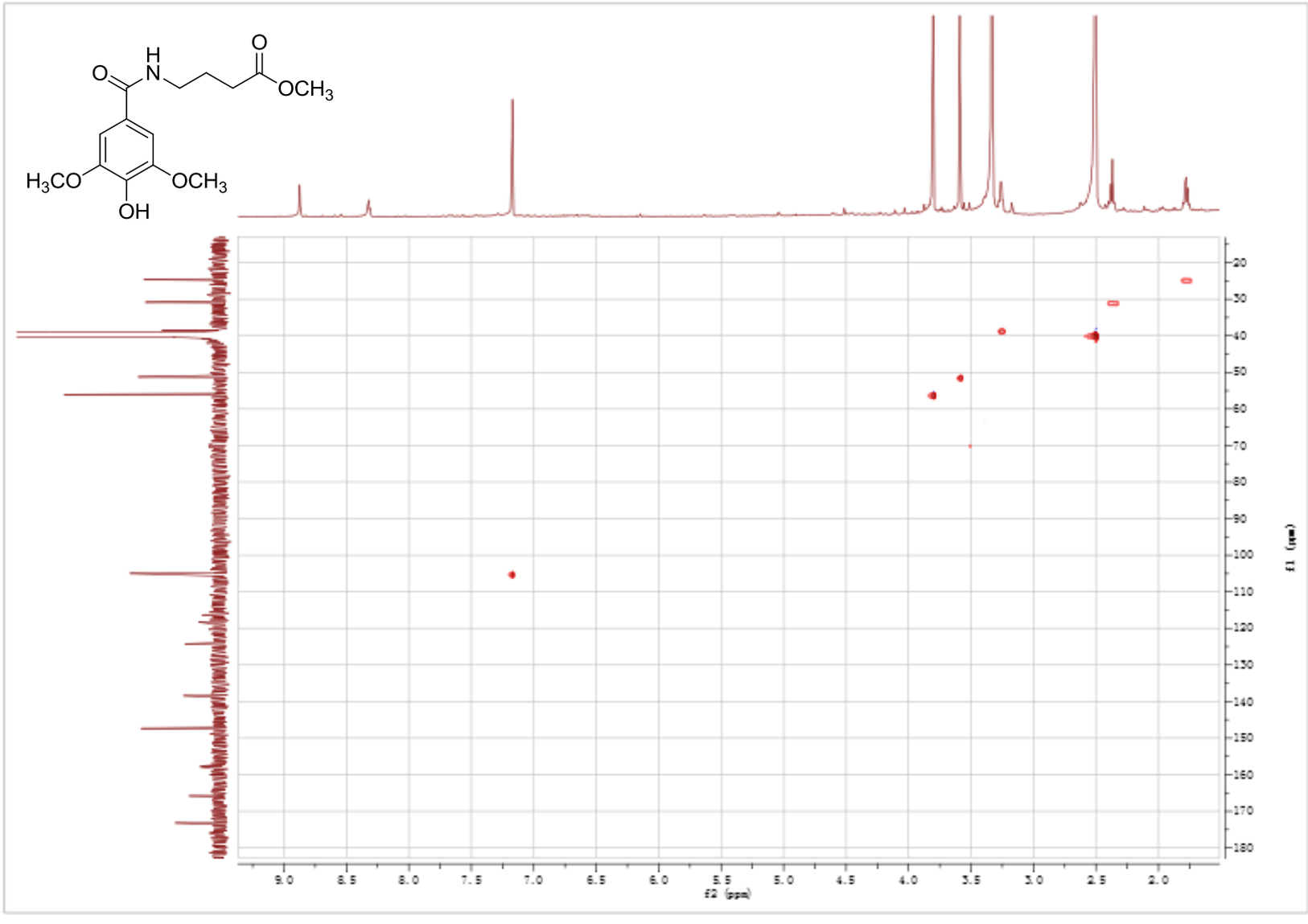

Figure S22. The HSQC spectrum of **3** (in DMSO-d_6)

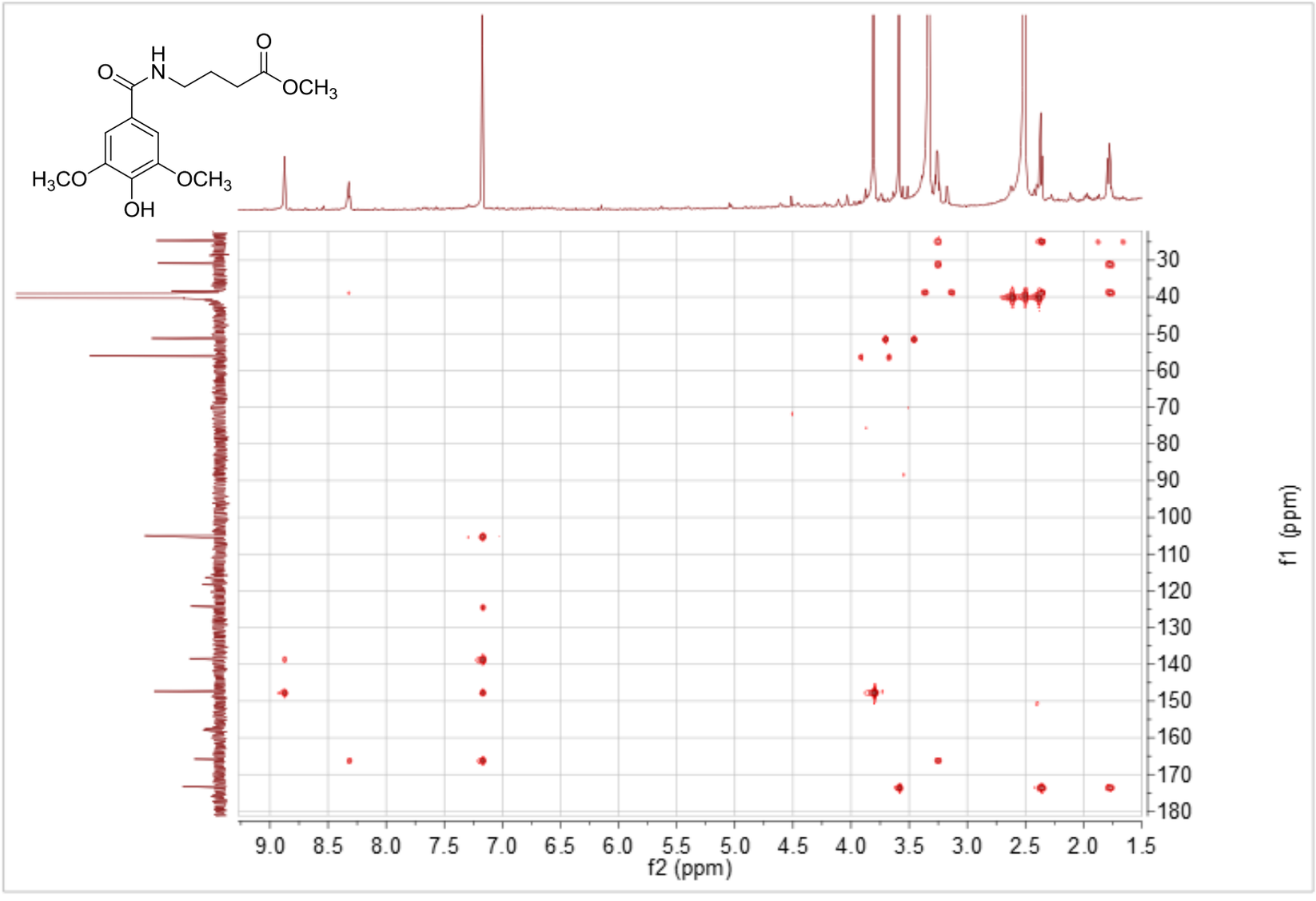

Figure S23. The HMBC spectrum of **3** (in DMSO-d_6)

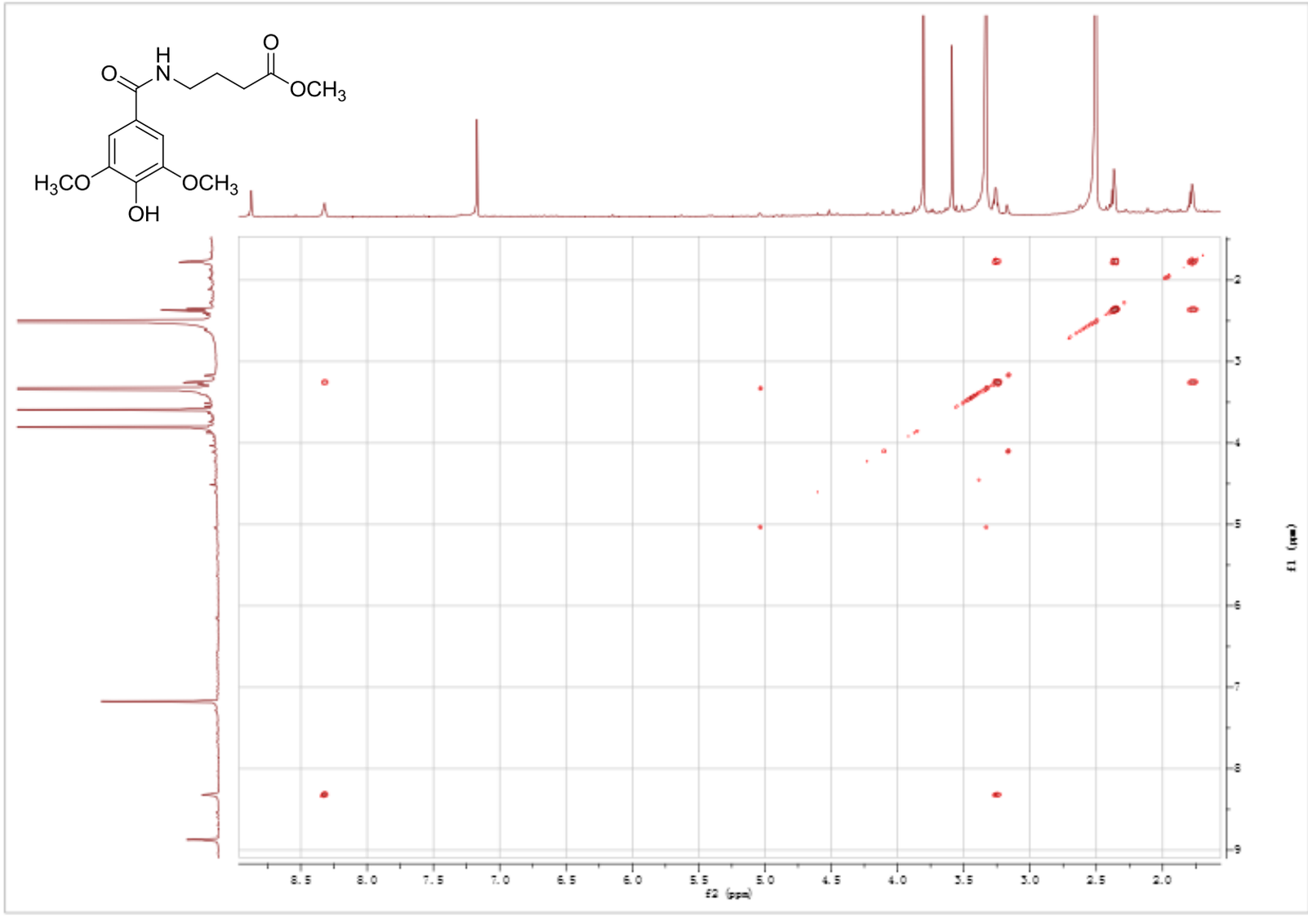

Figure S24. The ^{1}H-^{1}H COSY spectrum of **3** (in DMSO-d_6)

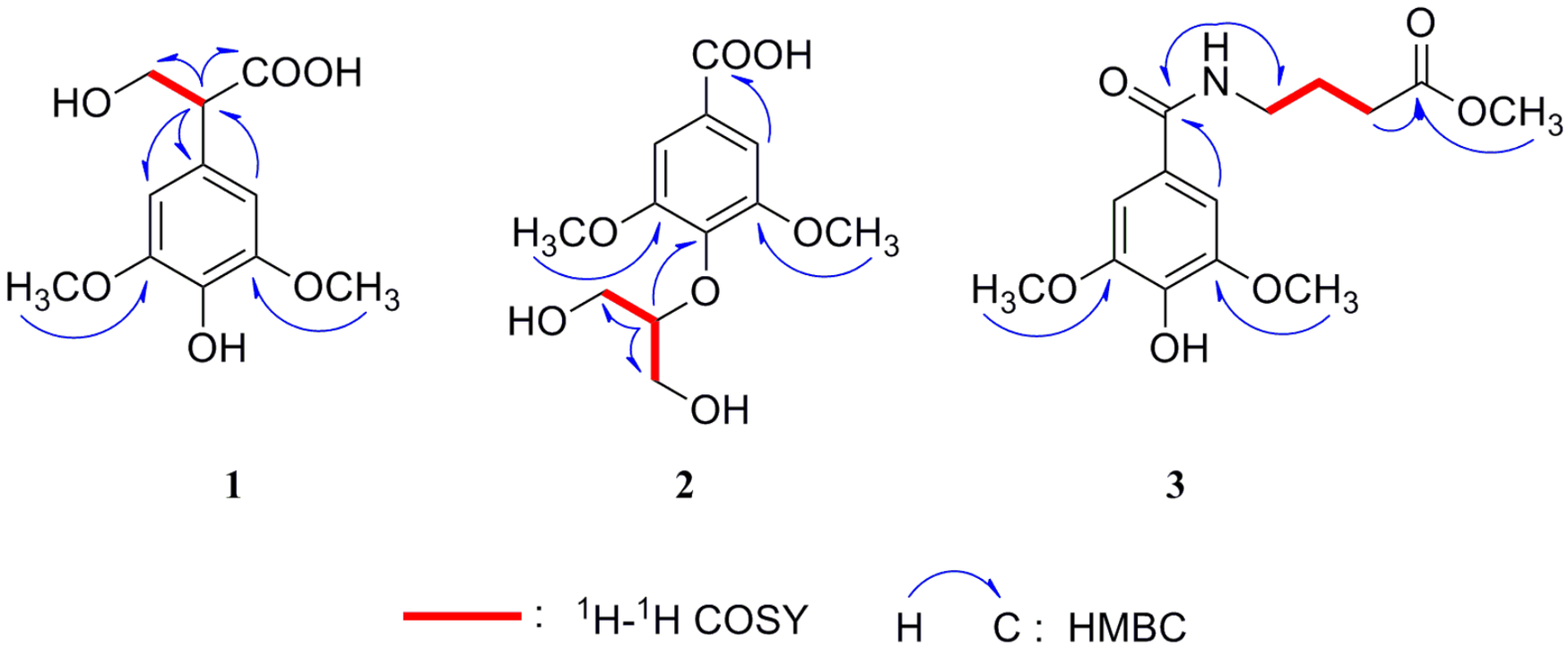

Figure S25. The Key ^{1}H-^{1}H COSY and HMBC correlations of **1** - **3**

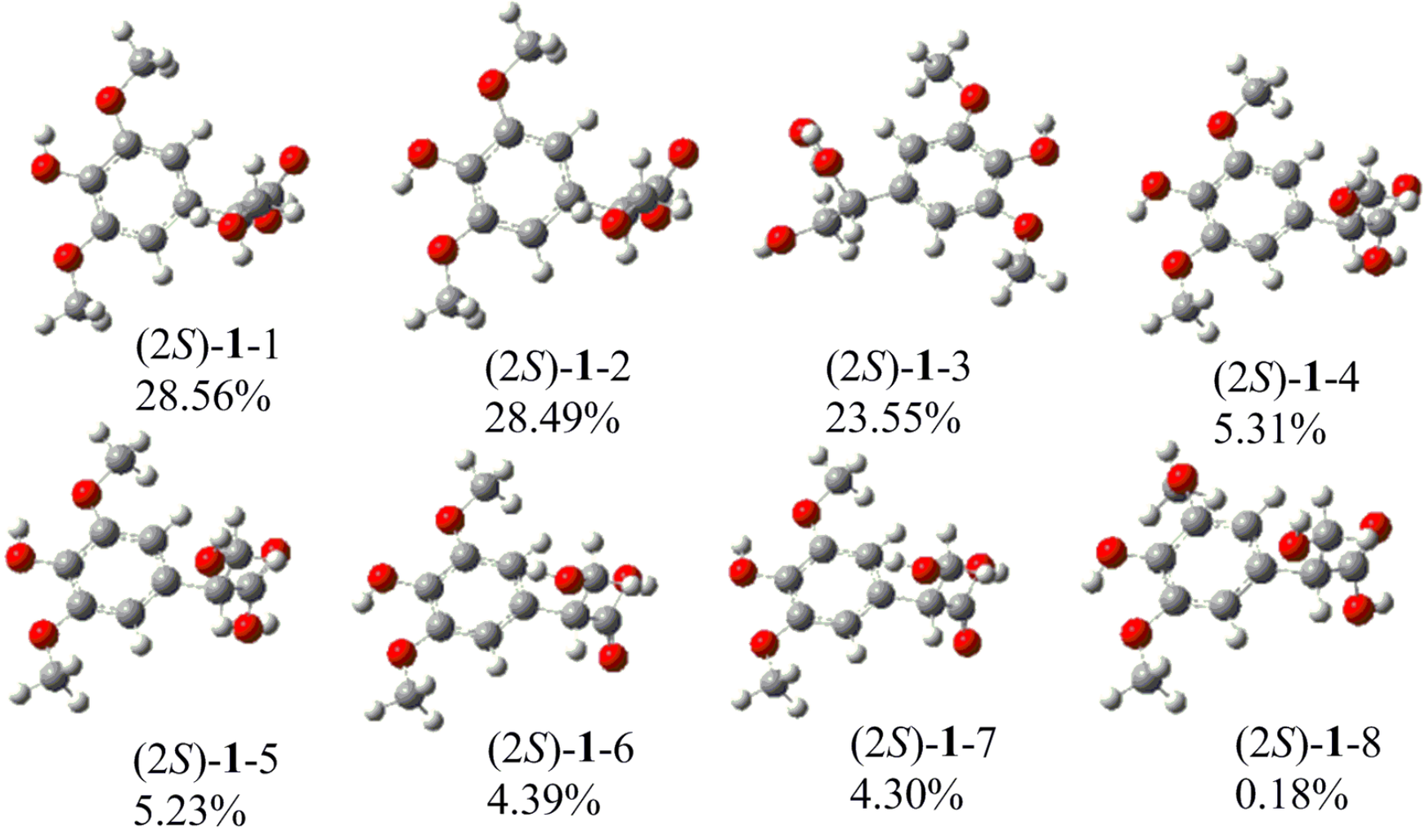

Figure S26. b3lyp/6-31g(d) optimized lowest energy conformers for (2*S*)-**1** and their equilibrium populations

The experimental ECD spectrum of **1** (red line) and the calculated ECD spectrum of (2*S*)-**1** (red short dash) and (2*R*)-**1** (blue short dash). The calculated ECD (excited states 30) spectrum were plotted as sums of Gaussians 09 with a 0.30 eV exponential half-width using the program Specdis 1.62, and the UV shifted was 17 nm.

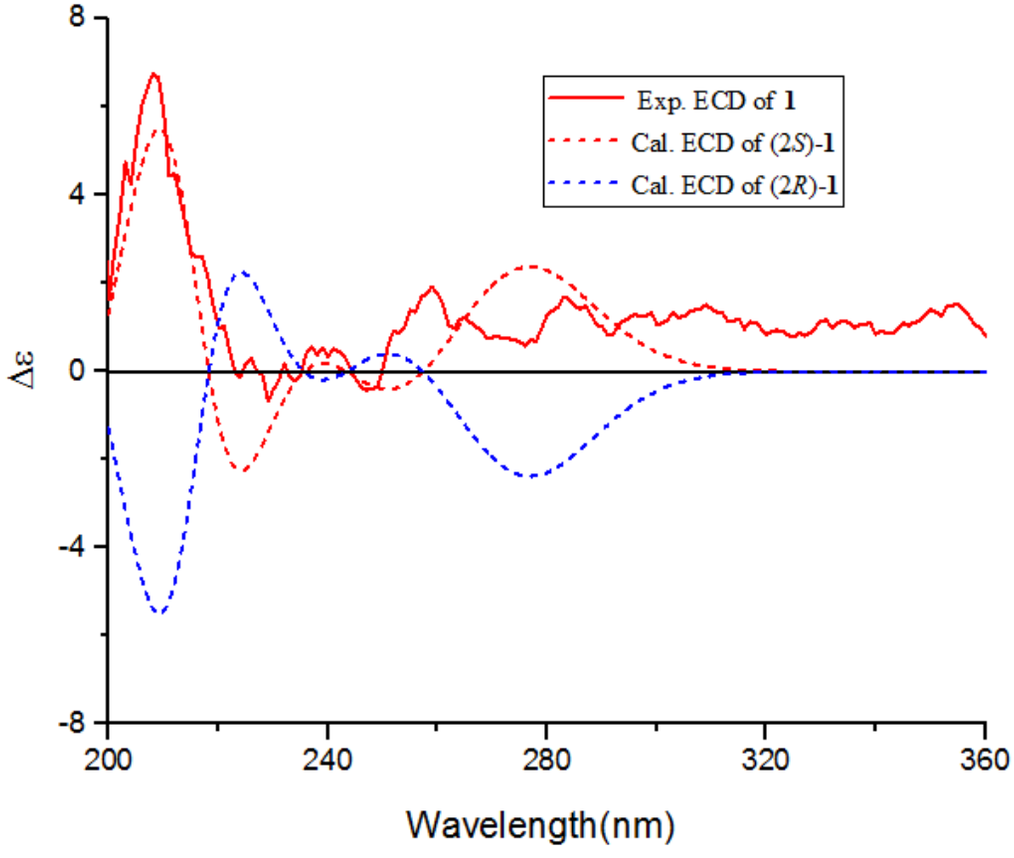

Figure S27. Experimental and calculated ECD spectra of **1**

Table S1 ^{1}H and ^{13}C NMR data of **1** (600 and 150 MHz in DMSO-d_6).

No.	δ_H (J in Hz)	δ_C
1'	3.89 (1H, dd, J = 10.5, 8.6 Hz), 3.52 (1H, overlap)	63.7
2'	3.50 (1H, overlap)	54.2
1		126.9
2, 6	6.52 (2H, s)	105.5
3, 5		147.8
4		134.8
2'-COOH	12.23 (1H, brs)	174.0
3, 5-OMe	3.72 (6H, s)	56.0
4-OH	8.26 (1H, s)	

Table S2 ^{1}H and ^{13}C NMR data of **2** (600 and 150 MHz in DMSO-d_6).

No.	δ_H (J in Hz)	δ_C
1		125.7
2, 6	7.23 (2H, s)	106.6
3, 5		152.7
4		139.8
1', 3'	3.53~3.63 (4H, overlap)	60.1
2'	4.01 (1H, m)	83.4
3, 5-OMe	3.82 (6H, s)	56.1
1-COOH	12.86 (1H, s)	167.0

1', 3'-OH	4.41 (2H, s)

Table S3 ^{1}H and ^{13}C NMR data of **3** (600 and 150 MHz in DMSO-d_6).

No.	δ_H (J in Hz)	δ_C
1		124.2
2, 6	7.17 (2H, s)	105.0
3, 5		147.4
4		138.4
7		173.2
1'		165.8
2'	2.36 (2H, m)	30.8
3'	1.78 (2H, m)	24.6
4'	3.25 (2H, m)	38.5
1'-OMe	3.59 (3H, s)	51.2
3, 5-OMe	3.80 (6H, s)	56.0
4-OH	8.87 (1H, s)	
NH	8.32 (1H, t, J = 5.5 Hz)	