

Supplementary Material

Isolation and identification of antioxidant constituents from the flowers of *Salvia miltiorrhiza*

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Abstract

A new salvianolic acid derivative, (7'E)-(7S, 8S)-salvianolic acid V (**1**), together with four previously described compounds (**2-5**) were obtained from the *n*-butanol fraction of *Salvia miltiorrhiza* flowers. Their structures were established by a series of spectroscopic methods, and the absolute configuration of **1** was determined by ECD calculation. Salvianolic acids (**1**) and phenolic acids (**2-4**) showed stronger DPPH free radical scavenging abilities and certain protective effects against H₂O₂-induced HSF cells oxidative damage, in which compound **1** (IC₅₀ 7.12 μM) possessed more noticeable free radical scavenging activity than the positive control vitamin C (IC₅₀ 14.98 μM).

Keywords: *Salvia miltiorrhiza*; salvianolic acid; antioxidant activity

List of content

1. General Experimental Procedures

2. Plant Material

3. Extraction and isolation

4. ECD calculation of compound 1

5. DPPH radical scavenging assay

6. Protective effects against H₂O₂-induced HSF cells oxidative damage

7. A Chem3D molecular modeling study of compound 1

Table S1. ¹H (500 MHz) and ¹³C NMR (125 MHz) spectrum data of compound **1** in MeOD (δ in ppm, J in Hz).

Table S2. ¹³C NMR (125 MHz) spectrum data of compounds **2-5** in MeOD (δ in ppm).

Table S3. ¹H NMR (500 MHz) spectrum data of compounds **2-5** in MeOD (δ in ppm, J in Hz).

Table S4. ¹H (500 MHz) and ¹³C NMR (125 MHz) spectrum data of compound **1** and salvianolic acid V in MeOD (δ in ppm, J in Hz).

Table S5. The antioxidant activity of the isolated compounds by DPPH assay.

Table S6. The optimized lowest energy 3D conformers and energy analysis of compound **1**.

Table S7-16. Cartesian coordinates for the low-energy optimized conformers of **1A-1J**.

Figure S2. Key ¹H-¹H COSY, HMBC and NOESY correlations of compound **1**.

Figure S3. Internuclear distances of H-7 and H-2'/H-7' in isomers **1a** (*E*-configuration of $\Delta^{7',8'}$) and **1b** (*Z*-configuration of $\Delta^{7',8'}$).

Figure S4. Experimental and calculated ECD spectra for compound **1**.

Figure S5. Protective effects of compounds **1-5** treated on H₂O₂-induced oxidative damaged HSF cells.

Figure S6. HR-ESI-MS spectrum of compound **1** in MeOH.

Figure S7. IR spectrum of compound **1** in MeOH.

Figure S8. UV spectrum of compound **1** in MeOH.

Figure S9. ¹H NMR (500 MHz) spectrum of compound **1** in MeOD.

Figure S10. ¹³C NMR (125 MHz) spectrum of compound **1** in MeOD.

Figure S11. ¹H-¹H COSY spectrum of compound **1** in MeOD.

Figure S12. HSQC spectrum of compound **1** in MeOD.

Figure S13. HMBC spectrum of compound **1** in MeOD.

Figure S14. NOESY spectrum of compound **1** in MeOD.

1. General Experimental Procedures

UV spectrum was recorded on a Thermo Scientific Evolution 201 spectro-photometer. Optical rotation was measured using a Rudolph AP-IV polarimeter. IR spectrum was measured on a Thermo Nicolet iS5 spectrometer. The NMR spectra were obtained using a Bruker Avance III 500-NMR spectrometer. HR-ESI-MS data was obtained using a Thermo UPLC-LTQ orbitrap XL spectrometer. ECD spectrum was recorded on a Chirascan photospectrometer. Silica gel (100-200 and 200-300 mesh, Shanghai Shengya Chemical Co., Ltd., Shanghai, China), MCI gel CHP20P (75-150 μm , Mitsubishi Chemical Corp., Tokyo, Japan), ODS-C₁₈ (50 μm , YMC, Kyoto, Japan), semi-prep HPLC (SEP LC-52, Beijing Saipuruisi Technology Co., Ltd., Beijing) with a NanoChrom C18 column (5 μm , 250 mm \times 10 mm) were employed for chromatographic separations.

2. Plant Material

The flowers of *Salvia miltiorrhiza* were collected from Nanyang of Henan Province, China, in May 2017 and identified by Prof. Liping Dai of Henan University of Chinese Medicine. The voucher specimen (NO.20170107) was deposited at the Engineering Technology Research Center for Comprehensive Development and Utilization of Authentic Medicinal Materials in Henan Province.

3. Extraction and isolation

The air-dried flowers of *S. miltiorrhiza* (4.5 kg) were refluxed with 70% EtOH (3 \times 20 L \times 2 h). The crude extracts (350 g) were suspended in water and partitioned with petroleum ether (57.7 g), EtOAc (125.7 g), and *n*-BuOH (108.2 g), successively. The *n*-BuOH fraction was chromatographed on silica gel column (100-200 mesh, 1.5 kg, 40 cm \times 10 cm i.d) using the gradient DCM-MeOH (100:5 ~ 0:100, v/v) as eluent to yield 3 fractions (Fr. a ~ c). Fr. b (40.2 g) was purified by MCI column eluted with MeOH-0.1% formic acid water from 10:90 to 40:60, to yield subfractions (Fr. b1 ~ b7). The Fr. b5 (1.9 g) was subjected to ODS column (MeOH-0.1% formic acid water, 5:95 ~ 40:60) to afford Fr. b5-1 ~ b5-5. The Fr. b5-3 (160.2 mg) was separated by semi-prep HPLC with ACN-0.1% formic acid water (10:90, 3 mL/min), to yield compounds **1** (15.9 mg), **3** (7.6 mg) and **4** (9.2 mg). The Fr. b5-1 (31.5 mg) was purified by semi-prep HPLC (MeOH-0.1% formic acid water, 10:90) to yield compound **2** (3.6 mg). Fr. a (16.5 g) was separated using a silica gel column (200-300 mesh, 30 cm \times 5 cm i.d, DCM-MeOH, 50:1 ~ 5:1) to obtain compound **5** (4.2 mg).

(7'E)-(7S, 8S)-Salvianolic acid V (1): Yellow amorphous powder; $[\alpha]_{\text{D}}^{25} +135.638$ (c 0.02, MeOH); IR (KBr) ν_{max} : 3345.77, 2952.08, 1718.40, 1456.92, 1032.51 cm^{-1} ; ECD (MeOH): λ ($\Delta\epsilon$) 207 (-2.19), 219 (0.49), 234 (-1.13), 253 (1.02), 276 (0.24), 300 (1.46). UV

(MeOH) λ_{\max} (log ϵ): 203 (4.39), 337 (3.84) nm; HR-ESI-MS at m/z 359.07599 $[M+H]^+$ (calcd for $C_{18}H_{15}O_8$, 359.07614); 1H and ^{13}C NMR (500 MHz, MeOD) data (Table S1).

Syringic acid (2): White amorphous powder; 1H NMR (500 MHz, MeOD) δ_H : 7.34 (2H, s, H-2, 6), 3.90 (6H, s, 3, 5-OCH₃). ^{13}C NMR (125 MHz, MeOD) δ_C : 170.0 (COOH), 148.84 (C-3, 5), 141.7 (C-4), 122.0 (C-1), 108.3 (C-2, 6), 56.8 (3, 5-OCH₃).

Protocatechuic acid (3): White amorphous powder; 1H NMR (500 MHz, MeOD) δ_H : 7.43 (1H, overlapped, H-6), 7.41 (1H, d, $J = 2.1$ Hz, H-2), 6.79 (1H, d, $J = 8.1$ Hz, H-5). ^{13}C NMR (125 MHz, MeOD) δ_C : 170.5 (C-7), 151.4 (C-4), 146.0 (C-3), 123.8 (C-6), 123.4 (C-1), 117.7 (C-5), 115.7 (C-2).

3,4-Dihydroxybenzaldehyde (4): White amorphous powder; 1H NMR (500 MHz, MeOD) δ_H : 9.66 (s, -CHO), 7.28 (1H, overlapped, H-6), 7.27 (1H, d, $J = 2.0$ Hz, H-2), 6.88 (1H, d, $J = 7.9$ Hz, H-5). ^{13}C NMR (125 MHz, MeOD) δ_C : 193.1 (C-7), 153.7 (C-4), 147.2 (C-3), 130.8 (C-1), 126.4 (C-6), 116.2 (C-5), 115.4 (C-2).

Esculetin (5): White solid; 1H NMR (500 MHz, MeOD) δ_H : 7.78 (1H, d, $J = 9.4$ Hz, H-4), 6.94 (1H, s, H-8), 6.75 (1H, s, H-5), 6.18 (1H, d, $J = 9.4$ Hz, H-3). ^{13}C -NMR (125 MHz, MeOD) δ_C : 164.3 (C-2), 152.1 (C-7), 150.5 (C-9), 146.1 (C-4), 144.6 (C-6), 113.0 (C-5), 112.8 (C-10), 112.4 (C-3), 103.6 (C-8).

4. ECD calculation of compound 1

A Chirascan CD spectropolarimeter was used to obtain the spectrum of compound **1**. The energy-minimized conformers of compound **1** were carried out by means of the Spartan's 14 software using Merck Molecular Force Field (MMFF). The conformers with Boltzmann population of over 2% were chosen for ECD calculations. The predominant conformers of compound **1** were subjected to the theoretical calculation of ECD spectra at the RB3LYP/6-31G(d,p) level using the time dependent density functional theory (TDDFT) method. The ECD curve of compound **1** was drawn via SpecDic software and OriginPro 8.

5. DPPH radical scavenging assay

The DPPH free radical scavenging activity of compounds **1-5** were measured following Zhang et al. In brief, DPPH-ethanol solution (100 μ L, 0.2 mM) was added to 100 μ L of the isolates with different concentrations (1-100 μ M) in ethanol solution. The mixture was shaken vigorously and the absorbance was measured at 517 nm after 30 min in the dark at room temperature. Vitamin C was used as a positive control. The DPPH scavenging effect was calculated as follows:

$$\text{Scavenging rate (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where A_{control} is the absorbance of the ethanol solution of DPPH and A_{sample} is the absorbance of the sample or standard. The final DPPH radical scavenging activities of samples in terms of the IC_{50} concentrations were calculated.

6. Protective effects against H_2O_2 -induced HSF cells oxidative damage

HSF cells (1.0×10^4 cells/well) were seeded in 96-well plates incubation period at 37 °C for 24h. 10, 50 and 100 μM of isolated compounds were added to designated wells. After 24 h, the cell viabilities were measured by MTT assay. The antioxidant activities of all the isolated compounds were determined through assessing the cell survival rate on the oxidative damage of HSF cells induced by H_2O_2 . HSF cells (1.0×10^4 cells/well) were pre-incubated for 24 h with various concentrations of compounds (10, 50 and 100 μM), respectively. Then, 500 μM H_2O_2 was added into model and treatment groups and incubated for 3 h. Finally, cell viability was assessed by MTT method.

7. A Chem3D molecular modeling study of compound 1

1a/1a' and **1b/1b'** respectively represented the different conformations of two isomers of compound **1** (*E* or *Z*-configuration of $\Delta^{7',8'}$) rotating around C-1'-C-7' bond. In the energy minimized conformation of **1a**, the internuclear distances between H-7 and H-2' could reach the range within 3.0 Å, while the distances between H-7 and H-7' were nearly 4.0 Å. Similarly, the internuclear distances between H-7 and H-6' in **1a'** also reach the range within 3.0 Å, while the distances between H-7 and H-7' were nearly 4.0 Å. Conversely, the distances between H-7 and H-2' in **1b** or H-6' in **1b'** were greater than 4.0 Å and the distances between H-7 and H-7' were within 3.0 Å, which could not match the strong correlations of H-7 and H-2'/H-6' observed in the ROESY spectrum. As the strong cross-peaks of H-7 and H-2'/H-6' were observed in the NOESY spectrum, **1a** was selected with favorable structure. Therefore, the configuration of $\Delta^{7',8'}$ was assigned as *E*.

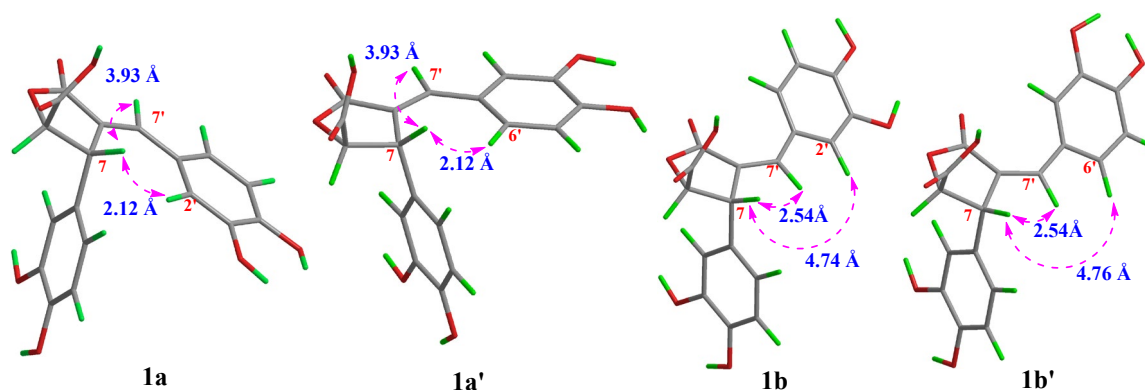


Figure S1. Internuclear distances of H-7 and H-2'/H-6'/H-7' in isomers **1a/1a'** (*E*-configuration of $\Delta^{7',8'}$) and **1b/1b'** (*Z*-configuration of $\Delta^{7',8'}$).

Table S1. ^1H (500 MHz) and ^{13}C NMR (125 MHz) data of compound **1** in MeOD (δ in ppm, J in Hz).

Position	δ_{H}	δ_{C}
1		133.8
2	6.78 (1H, d, 2.1)	115.1
3		145.7
4		146.9
5	6.75 (1H, d, 8.2)	116.8
6	6.68 (1H, dd, 8.2, 2.1)	119.5
7	4.52 (1H, s)	50.3
8	4.65 (1H, s)	85.8
9		167.2
1'		127.2
2'	6.91 (1H, d, 2.2)	118.6
3'		146.4
4'		149.3
5'	6.69 (1H, d, 8.2)	116.3
6'	6.83 (1H, dd, 8.2, 2.2)	125.4
7'	7.55 (1H, d, 1.7)	140.4
8'		124.2
9'		175.5

Table S2. ^{13}C NMR (125 MHz) data of compounds **2-5** in MeOD (δ in ppm).

Position	Compound 2	Compound 3	Compound 4	Compound 5
1	122.0	123.4	130.8	
2	108.3	115.7	115.4	164.3
3	148.8	146.0	147.2	112.4
4	141.7	151.4	153.7	146.1
5	148.8	117.7	116.2	113.0
6	108.3	123.8	126.4	144.6
7				152.1
8				103.6
9				150.5
10				112.8
COOH	170.0	170.5		
OCH ₃	56.8			
CHO	56.8		193.1	

Table S3. ^1H NMR (500 MHz) data of compounds **2-5** in MeOD (δ in ppm, J in Hz).

Position	Compound 2	Compound 3	Compound 4	Compound 5
2	7.34 (1H, s)	7.41 (1H, d, 2.1)	7.27 (1H, d, 2.0)	
3				6.18 (1H, d, 9.4)
4				7.78 (1H, d, 9.4)
5		6.79 (1H, d, 8.1)	6.88 (1H, d, 7.9)	6.75 (1H, s)
6	7.34 (1H, s)	7.43 (1H, m)	7.28 (1H, m)	
8				6.94 (1H, s)
OCH ₃	3.90 (6H, s)			
CHO			9.66 (s)	

Table S4. ^1H (500 MHz) and ^{13}C NMR (125 MHz) data of compound **1** and salvianolic acid V in MeOD (δ in ppm, J in Hz).

Position	Compound 1		Salvianolic acid V	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1		133.8		124.6
2	6.78 (1H, d, 2.1)	115.1	6.72 (1H, d, 2.0)	115.3
3		145.7		145.6
4		146.9		146.4
5	6.75 (1H, d, 8.2)	116.8	6.84 (1H, d, 8.0)	115.6
6	6.68 (1H, dd, 8.1, 2.0)	119.5	6.84 (1H, d, 2.5)	120.1
7	4.52 (1H, s)	50.3	4.33 (1H, dd, 4.5, 1.5)	52.9
8	4.65 (1H, s)	85.8	4.93 (1H, d, 4.5)	80.9
9		167.2		168.8
1'		127.2		127.3
2'	6.91 (1H, d, 2.1)	118.6	7.92 (1H, d, 2.0)	118.9
3'		146.4		145.3
4'		149.3		148.7
5'	6.69 (1H, d, 8.2)	116.3	6.81 (1H, d, 8.5)	116.5
6'	6.83 (1H, dd, 8.2, 2.2)	125.4	7.25 (1H, dd, 8.5, 2.0)	126.6
7'	7.55 (1H, d, 1.7)	140.4	6.74 (1H, m)	143.6
8'		124.2		134.4
9'		175.5		171.2

Table S5. The antioxidant activity of the isolated compounds by DPPH assay

Compounds	IC_{50} (μM)
1	7.12 ± 1.22
2	24.15 ± 2.29
3	33.47 ± 5.36
4	38.06 ± 5.14
5	> 100
Vitamin C	14.98 ± 2.43

Table S6. The optimized lowest energy 3D conformers and energy analysis of compound **1**.

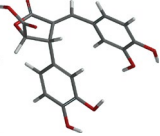
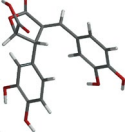
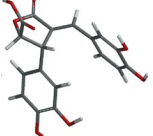
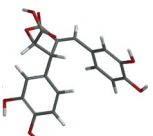
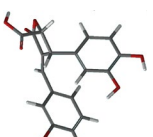
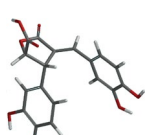
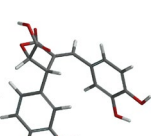
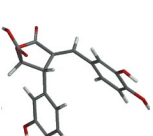
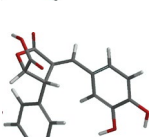
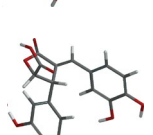
NO	3D conformers	rel. E (kcal/mol)	Boltzmann Dist (%)
1A		0.00	26.30
1B		0.32	15.30
1C		0.49	11.60
1D		0.49	11.60
1E		0.77	7.20
1F		0.80	6.80
1G		0.88	6.00
1H		1.09	4.20
1I		1.22	3.30
1J		1.48	2.20

Table S7. Cartesian coordinates for the low-energy optimized conformers of **1A**.

Conformer 1A Standard orientation					
Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	8	0	3.564342	0.396249	-0.697349
2	6	0	2.895374	0.524066	0.567894
3	6	0	1.449951	0.084263	0.360737
4	6	0	1.573388	-0.743431	-0.877034
5	6	0	0.729411	-1.639859	-1.411103
6	6	0	2.871624	-0.449684	-1.509464
7	6	0	-0.577313	-1.933089	-0.823186
8	6	0	0.473929	1.242756	0.198227
9	8	0	3.277321	-0.872074	-2.573953
10	6	0	0.69767	2.29485	-0.711469
11	6	0	-0.242632	3.316852	-0.866476
12	6	0	-1.410496	3.285419	-0.121487
13	6	0	-1.65199	2.253634	0.778111
14	6	0	-0.71701	1.239352	0.951037
15	6	0	-1.707578	-1.231139	-1.265799
16	6	0	-2.932118	-1.457423	-0.648755
17	6	0	-3.039021	-2.383309	0.385602
18	6	0	-1.930962	-3.094227	0.822239
19	6	0	-0.693223	-2.866914	0.216754
20	6	0	3.611612	-0.352114	1.590863
21	8	0	3.129057	-0.703532	2.656772
22	8	0	4.867496	-0.695297	1.214209
23	8	0	-4.061331	-0.776074	-1.009777
24	8	0	-4.245121	-2.591577	0.989836
25	8	0	-2.333761	4.276895	-0.286259
26	8	0	-2.835444	2.269129	1.462116
27	1	0	2.993756	1.560676	0.909282
28	1	0	1.115612	-0.537077	1.20009
29	1	0	0.970312	-2.169779	-2.330877
30	1	0	1.597969	2.332321	-1.321708
31	1	0	-0.068723	4.126726	-1.569254
32	1	0	-0.916228	0.430236	1.650163
33	1	0	-1.614056	-0.499803	-2.063572
34	1	0	-2.027994	-3.815103	1.62871
35	1	0	0.177146	-3.417947	0.567044
36	1	0	5.098629	-0.334703	0.329604
37	1	0	-3.820747	-0.128626	-1.696505
38	1	0	-4.871583	-2.029142	0.491351
39	1	0	-3.043651	4.060143	0.35122
40	1	0	-2.894604	1.452304	1.989545

Table S8. Cartesian coordinates for the low-energy optimized conformers of **1B**.

Conformer 1B Standard orientation					
Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	8	0	-3.452444	0.090555	0.723103
2	6	0	-2.844706	0.172397	-0.576402
3	6	0	-1.369792	-0.180274	-0.415346
4	6	0	-1.373349	-0.877537	0.905097
5	6	0	-0.432349	-1.634998	1.489227
6	6	0	-2.665561	-0.630219	1.567563
7	6	0	0.87444	-1.874979	0.87849
8	6	0	-0.462136	1.040668	-0.453907
9	8	0	-2.997938	-0.996188	2.677641
10	6	0	0.565143	1.101522	-1.41725
11	6	0	1.437471	2.192971	-1.47763
12	6	0	1.282664	3.232414	-0.572361
13	6	0	0.271261	3.185558	0.381711
14	6	0	-0.600676	2.106484	0.450389
15	6	0	1.947258	-1.027784	1.189445
16	6	0	3.172666	-1.225884	0.564455
17	6	0	3.335776	-2.260328	-0.353291
18	6	0	2.283659	-3.109769	-0.662812
19	6	0	1.046092	-2.915807	-0.045308
20	6	0	-3.558127	-0.799583	-1.509946
21	8	0	-3.112152	-1.181363	-2.581426
22	8	0	-4.769639	-1.193287	-1.046384
23	8	0	4.248513	-0.418428	0.813026
24	8	0	4.542813	-2.444979	-0.963674
25	8	0	2.103982	4.324834	-0.576829
26	8	0	0.12594	4.210684	1.27183
27	1	0	-3.015183	1.179899	-0.9726
28	1	0	-1.050164	-0.877922	-1.198936
29	1	0	-0.590615	-2.081639	2.469184
30	1	0	0.704592	0.284762	-2.12569
31	1	0	2.222693	2.203778	-2.227128
32	1	0	-1.371796	2.111142	1.2163
33	1	0	1.808189	-0.215145	1.896633
34	1	0	2.424231	-3.913378	-1.379488
35	1	0	0.220097	-3.578789	-0.29357
36	1	0	-4.973401	-0.794226	-0.171357
37	1	0	3.975646	0.253919	1.462105
38	1	0	5.120667	-1.756935	-0.577546
39	1	0	2.72867	4.230758	-1.317206
40	1	0	0.825367	4.850041	1.028261

Table S9. Cartesian coordinates for the low-energy optimized conformers of **1C**.

Conformer 1C Standard orientation					
Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	8	0	-3.52091	-0.173726	0.467955
2	6	0	-2.766156	-0.126603	-0.754099
3	6	0	-1.293837	-0.078646	-0.361512
4	6	0	-1.34954	-0.742017	0.975735
5	6	0	-0.396616	-1.376648	1.675143
6	6	0	-2.736619	-0.661057	1.472139
7	6	0	0.982196	-1.493345	1.200819
8	6	0	-0.702824	1.32401	-0.289499
9	8	0	-3.140052	-0.980112	2.57259
10	6	0	-1.396457	2.413482	0.272561
11	6	0	-0.805693	3.678387	0.343255
12	6	0	0.483099	3.855645	-0.133091
13	6	0	1.190984	2.79068	-0.677257
14	6	0	0.607246	1.531731	-0.76587
15	6	0	1.312881	-2.406917	0.189725
16	6	0	2.618175	-2.447257	-0.286916
17	6	0	3.587131	-1.594375	0.236456
18	6	0	3.275647	-0.698186	1.247602
19	6	0	1.967749	-0.648903	1.733546
20	6	0	-3.091386	-1.374423	-1.572047
21	8	0	-2.375977	-1.833857	-2.449673
22	8	0	-4.280027	-1.934012	-1.238814
23	8	0	2.99839	-3.302875	-1.283546
24	8	0	4.861178	-1.62966	-0.251856
25	8	0	1.059815	5.09002	-0.053798
26	8	0	2.467778	3.025753	-1.10587
27	1	0	-3.092693	0.740022	-1.339385
28	1	0	-0.697059	-0.663723	-1.070353
29	1	0	-0.606085	-1.81357	2.650051
30	1	0	-2.401375	2.300765	0.673918
31	1	0	-1.34359	4.516863	0.776333
32	1	0	1.169146	0.704054	-1.191867
33	1	0	0.551698	-3.064132	-0.220604
34	1	0	4.040072	-0.041229	1.651834
35	1	0	1.719352	0.065962	2.515923
36	1	0	-4.719801	-1.455417	-0.501283
37	1	0	2.230994	-3.84826	-1.531763
38	1	0	4.852946	-2.349774	-0.914217
39	1	0	1.950676	4.969128	-0.439501
40	1	0	2.868273	2.173727	-1.355118

Table S10. Cartesian coordinates for the low-energy optimized conformers of **1D**.

Conformer 1D Standard orientation					
Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	8	0	-2.587544	-2.114215	0.504727
2	6	0	-1.990403	-1.719756	-0.741755
3	6	0	-0.821073	-0.802005	-0.402362
4	6	0	-0.498123	-1.273691	0.976969
5	6	0	0.637056	-1.187231	1.686238
6	6	0	-1.67803	-1.979881	1.512029
7	6	0	1.84023	-0.52717	1.180323
8	6	0	-1.15969	0.681883	-0.45021
9	8	0	-1.829229	-2.399912	2.641938
10	6	0	-0.241952	1.57351	-1.043087
11	6	0	-0.49435	2.948461	-1.094436
12	6	0	-1.671819	3.439895	-0.550886
13	6	0	-2.58452	2.572644	0.039927
14	6	0	-2.340784	1.206115	0.100412
15	6	0	2.648568	-1.156174	0.222577
16	6	0	3.763186	-0.486563	-0.26945
17	6	0	4.07676	0.791497	0.186951
18	6	0	3.290362	1.419272	1.141006
19	6	0	2.167584	0.75665	1.640741
20	6	0	-1.527564	-2.977633	-1.472907
21	8	0	-0.674398	-2.997954	-2.347493
22	8	0	-2.173894	-4.097384	-1.065079
23	8	0	4.582625	-1.042102	-1.213102
24	8	0	5.169227	1.44293	-0.308762
25	8	0	-1.978025	4.771769	-0.568489
26	8	0	-3.741529	3.061899	0.575544
27	1	0	-2.755245	-1.244142	-1.365602
28	1	0	0.013828	-0.98482	-1.08819
29	1	0	0.704711	-1.592925	2.694355
30	1	0	0.6923	1.202599	-1.464514
31	1	0	0.236951	3.605814	-1.554155
32	1	0	-3.079714	0.57477	0.586274
33	1	0	2.398791	-2.152133	-0.131183
34	1	0	3.545864	2.414812	1.490777
35	1	0	1.543515	1.253744	2.380847
36	1	0	-2.813064	-3.90908	-0.341986
37	1	0	4.246158	-1.929938	-1.427999
38	1	0	5.569201	0.809122	-0.937471
39	1	0	-1.243499	5.245146	-0.996428
40	1	0	-3.690306	4.027858	0.428532

Table S11. Cartesian coordinates for the low-energy optimized conformers of **1E**.

Conformer 1E Standard orientation					
Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	8	0	3.602691	0.11042	-0.622209
2	6	0	2.878747	0.31901	0.600893
3	6	0	1.41136	0.009148	0.322533
4	6	0	1.507844	-0.789958	-0.935251
5	6	0	0.587587	-1.536096	-1.566081
6	6	0	2.867964	-0.646048	-1.482647
7	6	0	-0.79353	-1.661772	-1.099283
8	6	0	0.547271	1.253745	0.17042
9	8	0	3.287803	-1.10604	-2.526122
10	6	0	0.877347	2.294016	-0.720315
11	6	0	0.044577	3.40774	-0.856774
12	6	0	-1.120393	3.481454	-0.10997
13	6	0	-1.465556	2.462793	0.771041
14	6	0	-0.640704	1.353545	0.920413
15	6	0	-1.107369	-2.412365	0.038258
16	6	0	-2.424514	-2.461956	0.474387
17	6	0	-3.432947	-1.785962	-0.208513
18	6	0	-3.136441	-1.046234	-1.34544
19	6	0	-1.81212	-0.986203	-1.790207
20	6	0	3.455911	-0.597877	1.674196
21	8	0	2.898196	-0.861133	2.728798
22	8	0	4.680983	-1.084924	1.359296
23	8	0	-2.726281	-3.180961	1.595413
24	8	0	-4.705045	-1.875776	0.283714
25	8	0	-1.934996	4.5677	-0.250171
26	8	0	-2.63498	2.590534	1.468357
27	1	0	3.05546	1.348083	0.933674
28	1	0	0.994836	-0.608611	1.126847
29	1	0	0.820434	-2.04691	-2.499035
30	1	0	1.778849	2.250699	-1.328311
31	1	0	0.299692	4.208293	-1.545065
32	1	0	-0.920323	0.554927	1.603599
33	1	0	-0.341393	-2.951453	0.588452
34	1	0	-3.90477	-0.506962	-1.890094
35	1	0	-1.575373	-0.392206	-2.671361
36	1	0	4.983204	-0.775296	0.476814
37	1	0	-3.697227	-3.10419	1.686113
38	1	0	-5.298701	-1.399589	-0.323085
39	1	0	-2.670125	4.403824	0.373805
40	1	0	-2.741731	1.798947	2.025083

Table S12. Cartesian coordinates for the low-energy optimized conformers of **1F**.

Conformer 1F Standard orientation					
Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	8	0	-3.219858	-1.031152	0.603701
2	6	0	-2.604258	-0.869508	-0.685306
3	6	0	-1.13989	-0.523294	-0.438404
4	6	0	-0.941935	-1.126185	0.91312
5	6	0	0.173659	-1.543732	1.52953
6	6	0	-2.26581	-1.304404	1.538967
7	6	0	1.500429	-1.444615	0.923773
8	6	0	-0.84885	0.971756	-0.461122
9	8	0	-2.496169	-1.659891	2.677705
10	6	0	0.304791	1.433733	-1.126883
11	6	0	0.634936	2.792854	-1.150409
12	6	0	-0.194833	3.701077	-0.510034
13	6	0	-1.338073	3.260407	0.147628
14	6	0	-1.669723	1.911762	0.184242
15	6	0	2.317824	-0.344779	1.202691
16	6	0	3.561174	-0.253231	0.592391
17	6	0	4.004076	-1.242276	-0.28308
18	6	0	3.203119	-2.341607	-0.565043
19	6	0	1.946487	-2.440333	0.0405
20	6	0	-2.755798	-2.173806	-1.463316
21	8	0	-2.078	-2.487149	-2.430432
22	8	0	-3.748423	-2.965117	-0.986855
23	8	0	4.354891	0.827321	0.85352
24	8	0	5.23996	-1.084943	-0.84627
25	8	0	0.073139	5.041275	-0.493612
26	8	0	-2.151829	4.158918	0.776542
27	1	0	-3.150124	-0.098508	-1.240488
28	1	0	-0.501785	-1.011252	-1.184351
29	1	0	0.138886	-1.980095	2.526301
30	1	0	0.972125	0.729663	-1.62334
31	1	0	1.537282	3.111314	-1.662568
32	1	0	-2.563655	1.618898	0.72802
33	1	0	1.990765	0.44184	1.877252
34	1	0	3.528949	-3.122421	-1.244767
35	1	0	1.316338	-3.299709	-0.181894
36	1	0	-4.185956	-2.568941	-0.200456
37	1	0	5.173414	0.659106	0.345251
38	1	0	5.421625	-1.863214	-1.401387
39	1	0	0.92542	5.182685	-0.941138
40	1	0	-1.716944	5.023627	0.633701

Table S13. Cartesian coordinates for the low-energy optimized conformers of **1G**.

Conformer 1G Standard orientation					
Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	8	0	-3.529504	0.032484	0.537943
2	6	0	-2.834832	0.039142	-0.720995
3	6	0	-1.345598	-0.016202	-0.399082
4	6	0	-1.389294	-0.72105	0.917845
5	6	0	-0.486165	-1.505179	1.525618
6	6	0	-2.731868	-0.521088	1.497187
7	6	0	0.819961	-1.808682	0.942927
8	6	0	-0.670388	1.348005	-0.299208
9	8	0	-3.099024	-0.814735	2.617232
10	6	0	-1.300871	2.474643	0.265117
11	6	0	-0.630408	3.697742	0.364917
12	6	0	0.679337	3.794095	-0.074979
13	6	0	1.327614	2.689387	-0.612486
14	6	0	0.658906	1.477561	-0.746201
15	6	0	1.920278	-0.987389	1.208096
16	6	0	3.132605	-1.259157	0.588661
17	6	0	3.272094	-2.349776	-0.26644
18	6	0	2.19109	-3.182275	-0.525332
19	6	0	0.960253	-2.905635	0.078297
20	6	0	-3.286025	-1.170637	-1.535888
21	8	0	-2.663697	-1.645622	-2.473787
22	8	0	-4.478082	-1.671671	-1.128536
23	8	0	4.195046	-0.429421	0.808156
24	8	0	4.496782	-2.554802	-0.837739
25	8	0	1.33784	4.983618	0.041225
26	8	0	2.636173	2.830853	-0.982138
27	1	0	-3.129384	0.932998	-1.281915
28	1	0	-0.815274	-0.60777	-1.1545
29	1	0	-0.696559	-1.96956	2.487548
30	1	0	-2.318418	2.425774	0.646677
31	1	0	-1.121816	4.564201	0.798029
32	1	0	1.171539	0.624874	-1.184423
33	1	0	1.83443	-0.129973	1.870651
34	1	0	2.277249	-4.03515	-1.191183
35	1	0	0.108649	-3.549748	-0.134729
36	1	0	-4.838491	-1.182638	-0.355737
37	1	0	4.942693	-0.857375	0.343466
38	1	0	4.450204	-3.365042	-1.375237
39	1	0	2.229713	4.804421	-0.318595
40	1	0	3.021047	1.938339	-1.057217

Table S14. Cartesian coordinates for the low-energy optimized conformers of **1H**.

Conformer 1H Standard orientation					
Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	8	0	-3.332741	-0.819241	0.634002
2	6	0	-2.699497	-0.602543	-0.63735
3	6	0	-1.202246	-0.455246	-0.384555
4	6	0	-1.069319	-1.061769	0.972865
5	6	0	0.020885	-1.447568	1.653322
6	6	0	-2.413389	-1.209991	1.55814
7	6	0	1.382906	-1.291886	1.142662
8	6	0	-0.729066	0.990106	-0.445365
9	8	0	-2.684524	-1.61194	2.672576
10	6	0	0.330523	1.330131	-1.310323
11	6	0	0.802478	2.644022	-1.394981
12	6	0	0.214093	3.625571	-0.610662
13	6	0	-0.829702	3.302087	0.250146
14	6	0	-1.305659	2.000557	0.342118
15	6	0	1.862195	-2.099738	0.10645
16	6	0	3.152803	-1.901974	-0.364985
17	6	0	3.974714	-0.920565	0.184047
18	6	0	3.51317	-0.116011	1.217614
19	6	0	2.212884	-0.304466	1.696412
20	6	0	-3.00695	-1.789349	-1.544174
21	8	0	-2.405862	-2.043381	-2.576973
22	8	0	-4.048438	-2.539459	-1.107375
23	8	0	3.61525	-2.685292	-1.383562
24	8	0	5.234274	-0.785164	-0.330027
25	8	0	0.627664	4.927911	-0.648898
26	8	0	-1.403546	4.272372	1.020812
27	1	0	-3.155919	0.278859	-1.102064
28	1	0	-0.631588	-1.041346	-1.114404
29	1	0	-0.061954	-1.863465	2.656283
30	1	0	0.805682	0.565094	-1.924565
31	1	0	1.621178	2.871221	-2.070195
32	1	0	-2.118743	1.794232	1.033071
33	1	0	1.245525	-2.877213	-0.335379
34	1	0	4.134884	0.65645	1.658114
35	1	0	1.844534	0.332523	2.498774
36	1	0	-4.417081	-2.195071	-0.263671
37	1	0	4.533421	-2.38263	-1.531363
38	1	0	5.69911	-0.102552	0.184155
39	1	0	1.335202	5.000585	-1.312619
40	1	0	-0.9158	5.088663	0.791145

Table S15. Cartesian coordinates for the low-energy optimized conformers of **II**.

Conformer II Standard orientation					
Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms)		
			X	Y	Z
1	8	0	3.521036	0.042089	-0.531986
2	6	0	2.80414	0.16572	0.708237
3	6	0	1.324645	-0.007611	0.383115
4	6	0	1.410915	-0.811524	-0.873968
5	6	0	0.536223	-1.653338	-1.446117
6	6	0	2.762386	-0.635876	-1.439337
7	6	0	-0.791839	-1.938626	-0.900867
8	6	0	0.60174	1.322102	0.190922
9	8	0	3.158926	-1.017245	-2.522463
10	6	0	1.05197	2.310592	-0.708039
11	6	0	0.342043	3.504318	-0.884838
12	6	0	-0.828443	3.710772	-0.169263
13	6	0	-1.284202	2.744008	0.719458
14	6	0	-0.577512	1.565198	0.916529
15	6	0	-1.807559	-0.974367	-0.938472
16	6	0	-3.042035	-1.260546	-0.367097
17	6	0	-3.285744	-2.510223	0.194875
18	6	0	-2.298126	-3.483145	0.215963
19	6	0	-1.044564	-3.194421	-0.329079
20	6	0	3.306761	-0.898345	1.679244
21	8	0	2.716075	-1.245869	2.690023
22	8	0	4.509514	-1.415694	1.32726
23	8	0	-4.045726	-0.332749	-0.320817
24	8	0	-4.504912	-2.784623	0.743776
25	8	0	-1.574441	4.846811	-0.310088
26	8	0	-2.451011	2.939592	1.399977
27	1	0	3.042228	1.140428	1.149151
28	1	0	0.81634	-0.57645	1.170816
29	1	0	0.79664	-2.200564	-2.351321
30	1	0	1.957988	2.16644	-1.294063
31	1	0	0.713158	4.244895	-1.58682
32	1	0	-0.965766	0.831946	1.621328
33	1	0	-1.628419	-0.010383	-1.404764
34	1	0	-2.497962	-4.453104	0.661165
35	1	0	-0.268107	-3.955911	-0.298122
36	1	0	4.847074	-1.028927	0.489616
37	1	0	-3.653903	0.542549	-0.495422
38	1	0	-5.02153	-1.964129	0.614133
39	1	0	-1.123113	5.433635	-0.941701
40	1	0	-2.735092	3.842953	1.152962

Table S16. Cartesian coordinates for the low-energy optimized conformers of **1J**.

Center Number	Atomic Number	Atomic Type	Conformer 1J Standard orientation		
			Coordinates (Angstroms)		
			X	Y	Z
1	8	0	-3.383593	-0.37133	0.579432
2	6	0	-2.739102	-0.307404	-0.70388
3	6	0	-1.236367	-0.335424	-0.450172
4	6	0	-1.186575	-0.97509	0.898834
5	6	0	-0.180256	-1.595279	1.533285
6	6	0	-2.526459	-0.883475	1.506314
7	6	0	1.159367	-1.725096	0.960001
8	6	0	-0.609368	1.05408	-0.479952
9	8	0	-2.83695	-1.18525	2.641299
10	6	0	0.413569	1.31563	-1.4138
11	6	0	1.045366	2.559916	-1.458346
12	6	0	0.652881	3.549511	-0.573086
13	6	0	-0.362917	3.317265	0.348479
14	6	0	-0.999897	2.080686	0.402087
15	6	0	2.054599	-0.647005	1.002482
16	6	0	3.305094	-0.776955	0.409774
17	6	0	3.674374	-1.972917	-0.199781
18	6	0	2.802555	-3.050917	-0.236916
19	6	0	1.537972	-2.925867	0.342728
20	6	0	-3.196262	-1.493035	-1.547767
21	8	0	-2.623167	-1.887405	-2.551604
22	8	0	-4.335157	-2.067184	-1.088975
23	8	0	4.205752	0.252036	0.390599
24	8	0	4.90579	-2.091794	-0.776599
25	8	0	1.27837	4.761781	-0.60943
26	8	0	-0.697203	4.340173	1.192231
27	1	0	-3.078334	0.600934	-1.214769
28	1	0	-0.726094	-0.968805	-1.185796
29	1	0	-0.319484	-2.040194	2.517195
30	1	0	0.741517	0.542995	-2.109269
31	1	0	1.837445	2.751545	-2.176615
32	1	0	-1.785626	1.914289	1.132946
33	1	0	1.765135	0.28271	1.4832
34	1	0	3.101899	-3.97755	-0.717463
35	1	0	0.852848	-3.77006	0.303745
36	1	0	-4.667667	-1.631246	-0.27383
37	1	0	3.772242	1.043747	0.75603
38	1	0	5.333923	-1.225896	-0.62252
39	1	0	0.826256	5.295633	0.074131
40	1	0	-1.416235	4.041928	1.775683

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[1] Gaussian 16, Revision C.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V.

Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2019.

[2] Halgren, TA. 1999. MMFF VII. Characterization of MMFF94, MMFF94s, and other widely available force fields for conformational energies and for intermolecular-interaction energies and geometries. *J Comput Chem.* 20, 730–748.

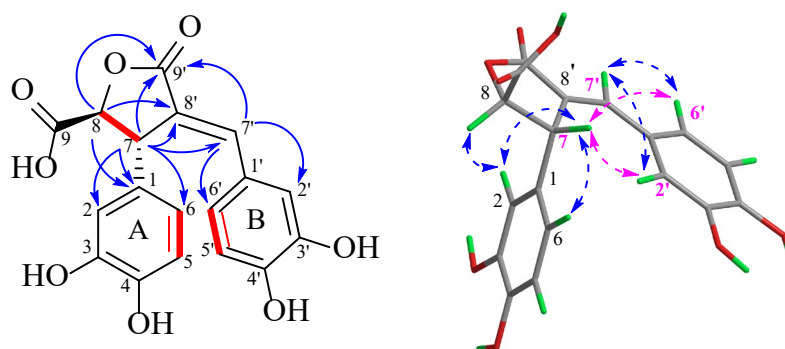


Figure S2. Key ^1H - ^1H COSY (bold lines), HMBC (arrows) and NOESY (double arrows) correlations of compound **1**.

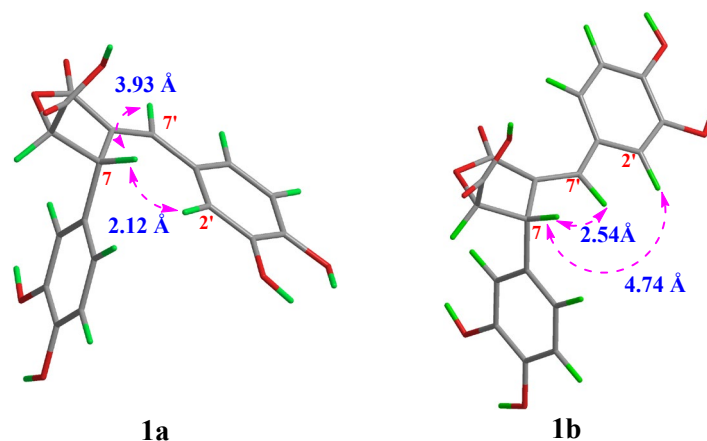


Figure S3. Internuclear distances of H-7 and H-2'/H-7' in isomers **1a** (*E*-configuration of $\Delta^{7',8'}$) and **1b** (*Z*-configuration of $\Delta^{7',8'}$).

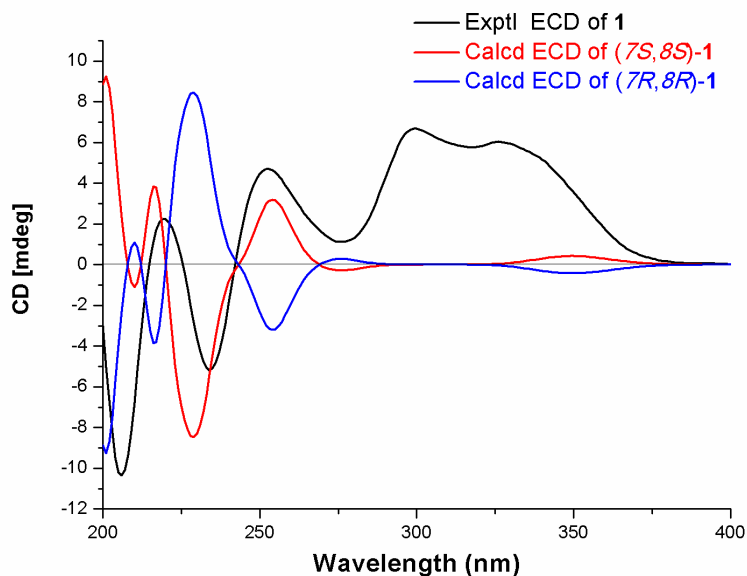


Figure S4. Experimental and calculated ECD spectra for compound **1**.

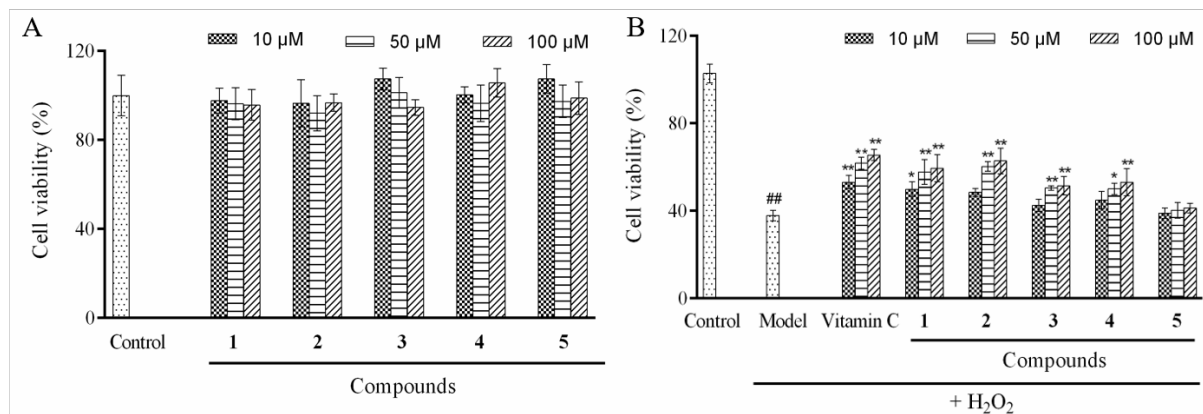


Figure S5. Protective effects of compounds **1-5** treated on H_2O_2 -induced oxidative damaged HSF cells. (A) Effects of compounds **1-5** on HSF cells proliferation; (B) Protective effects of compounds **1-5** on oxidative damaged HSF cells. Vitamin C was used as the positive control. All values were means \pm SD ($n = 3$). * $P < 0.05$, ** $P < 0.001$, # $P < 0.05$, compared with the H_2O_2 group.

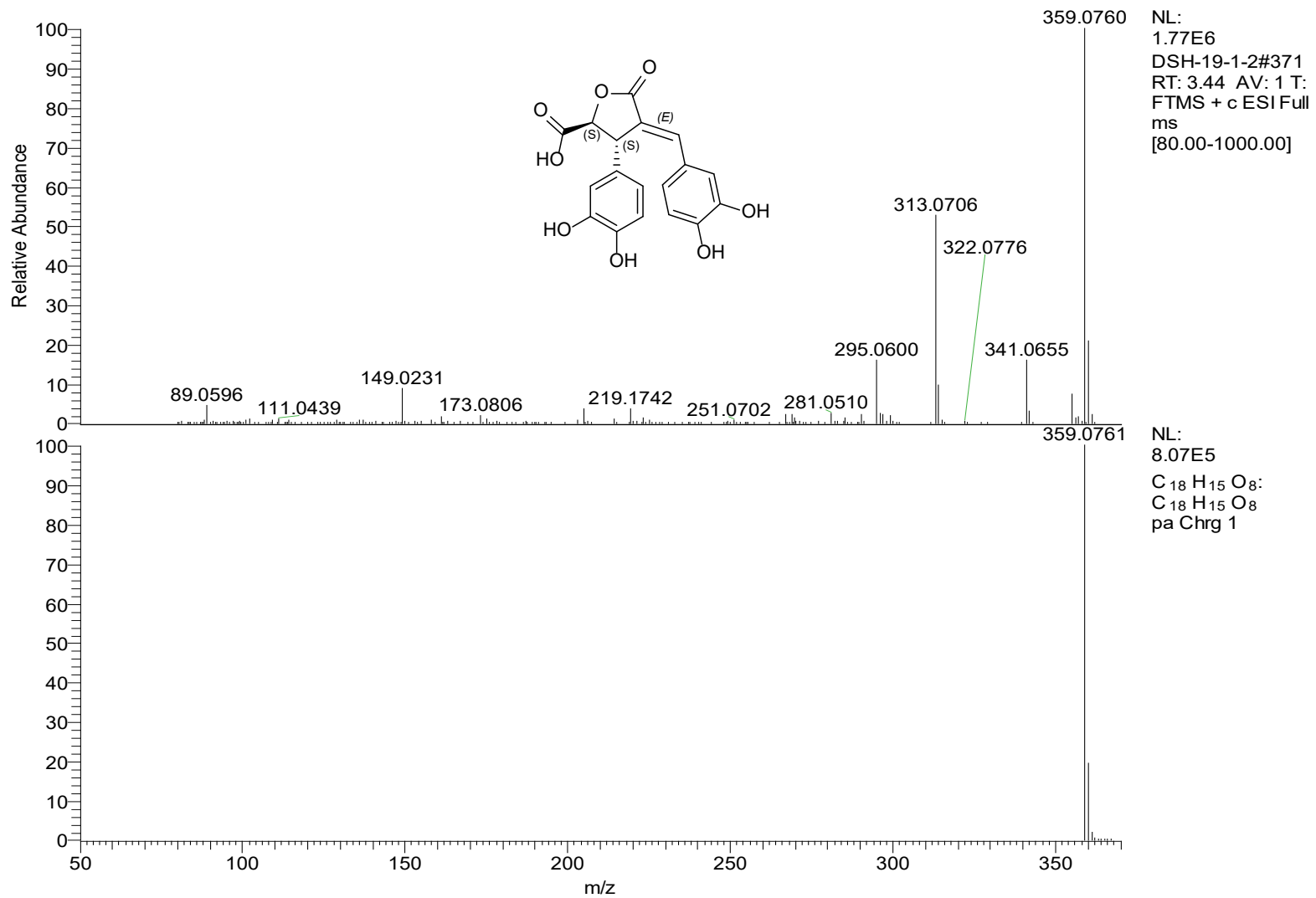


Figure S6. HR-ESI-MS spectrum of compound 1 in MeOH.

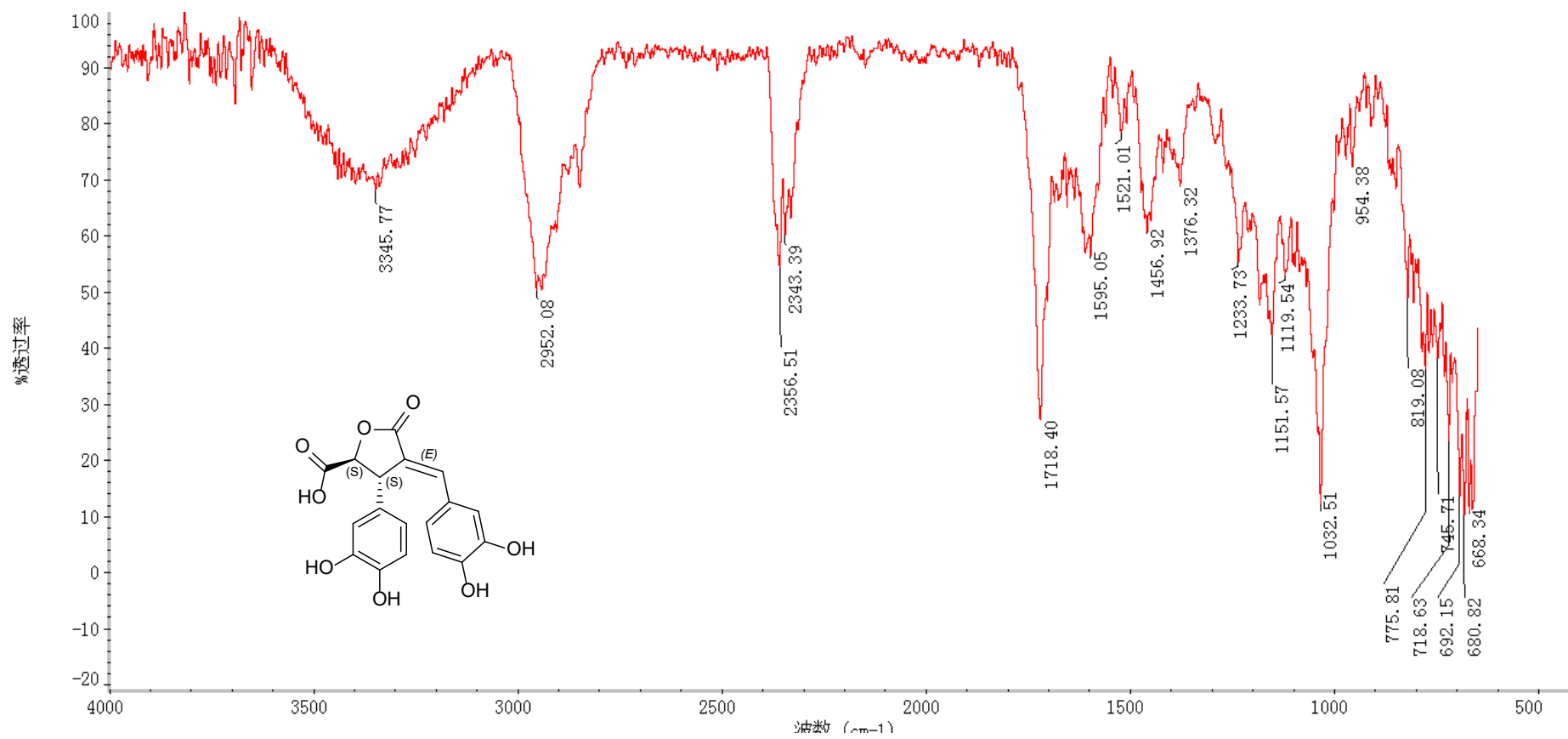


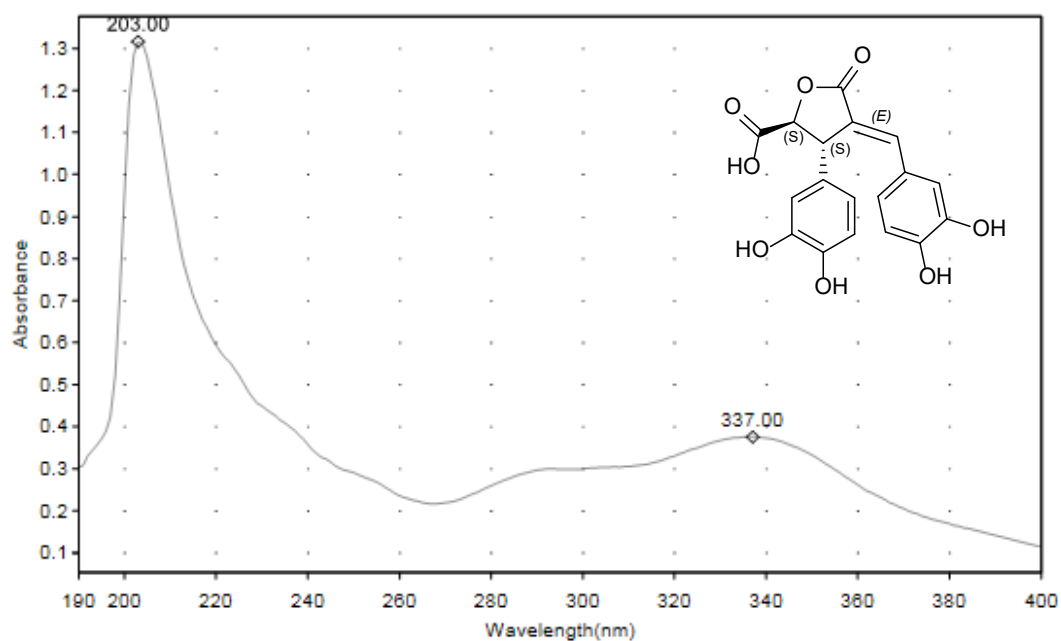
Figure S7. IR spectrum compound of 1 in MeOH.

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Operator Name (None Entered)
Department (None Entered)
Organization (None Entered)
Information (None Entered)

Date of Report 2021/1/6
Time of Report 16:42:13下午

Scan Graph



Results Table - scan013,DSH-DA-19-3,Cycle01

nm	A	Peak Pick Method
203.00	1.317	Find 8 Peaks Above -3.0000 A
337.00	.376	Start Wavelength190.00 nm
		Stop Wavelength400.00 nm
		Sort By Wavelength
Sensitivity	Medium	

Figure S8. UV spectrum of compound 1 in MeOH.

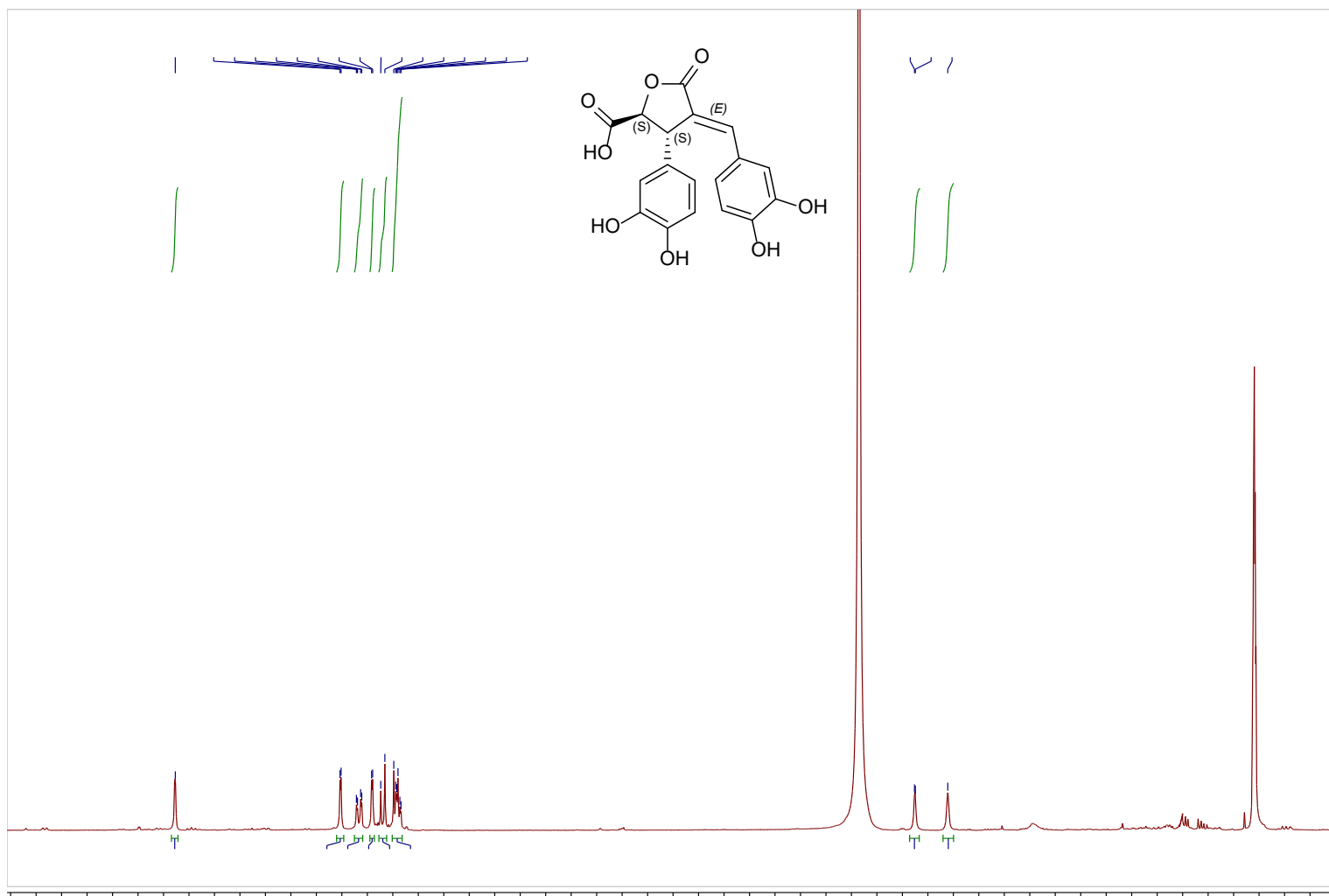


Figure S9. ¹H NMR (500 MHz) spectrum of compound **1** in MeOD.

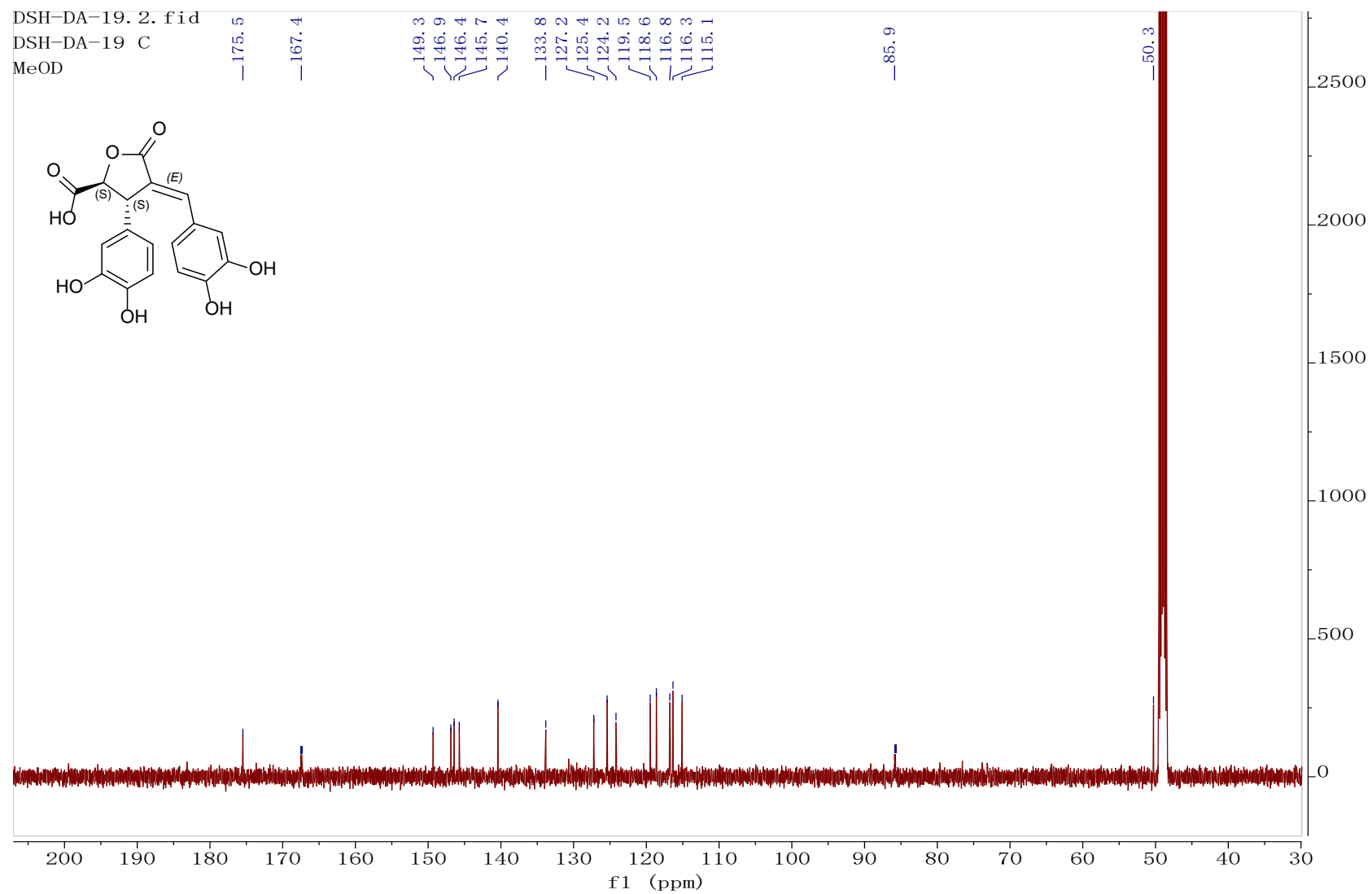


Figure S10. ^{13}C NMR (125 MHz) spectrum of compound **1** in MeOD.

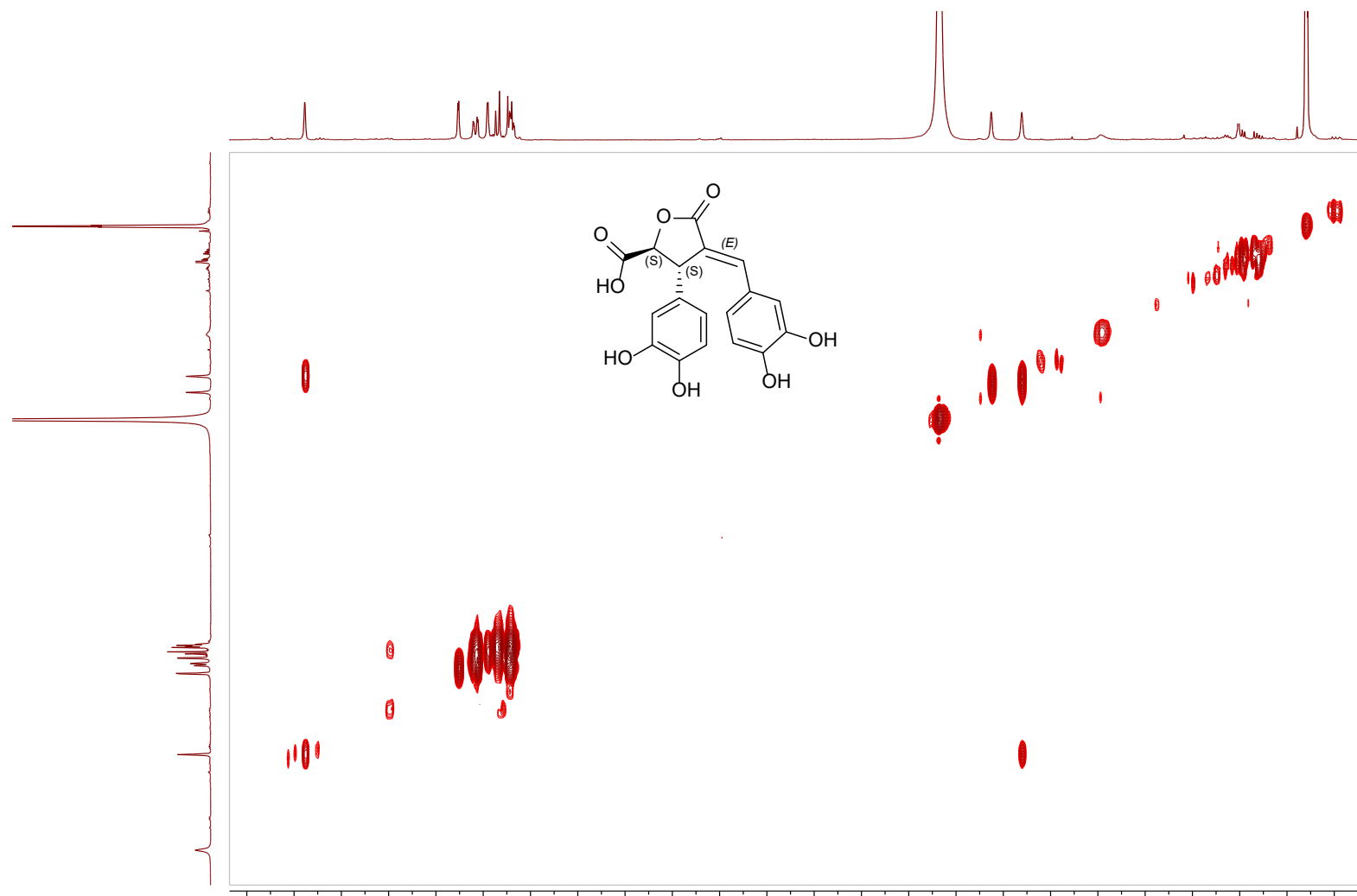
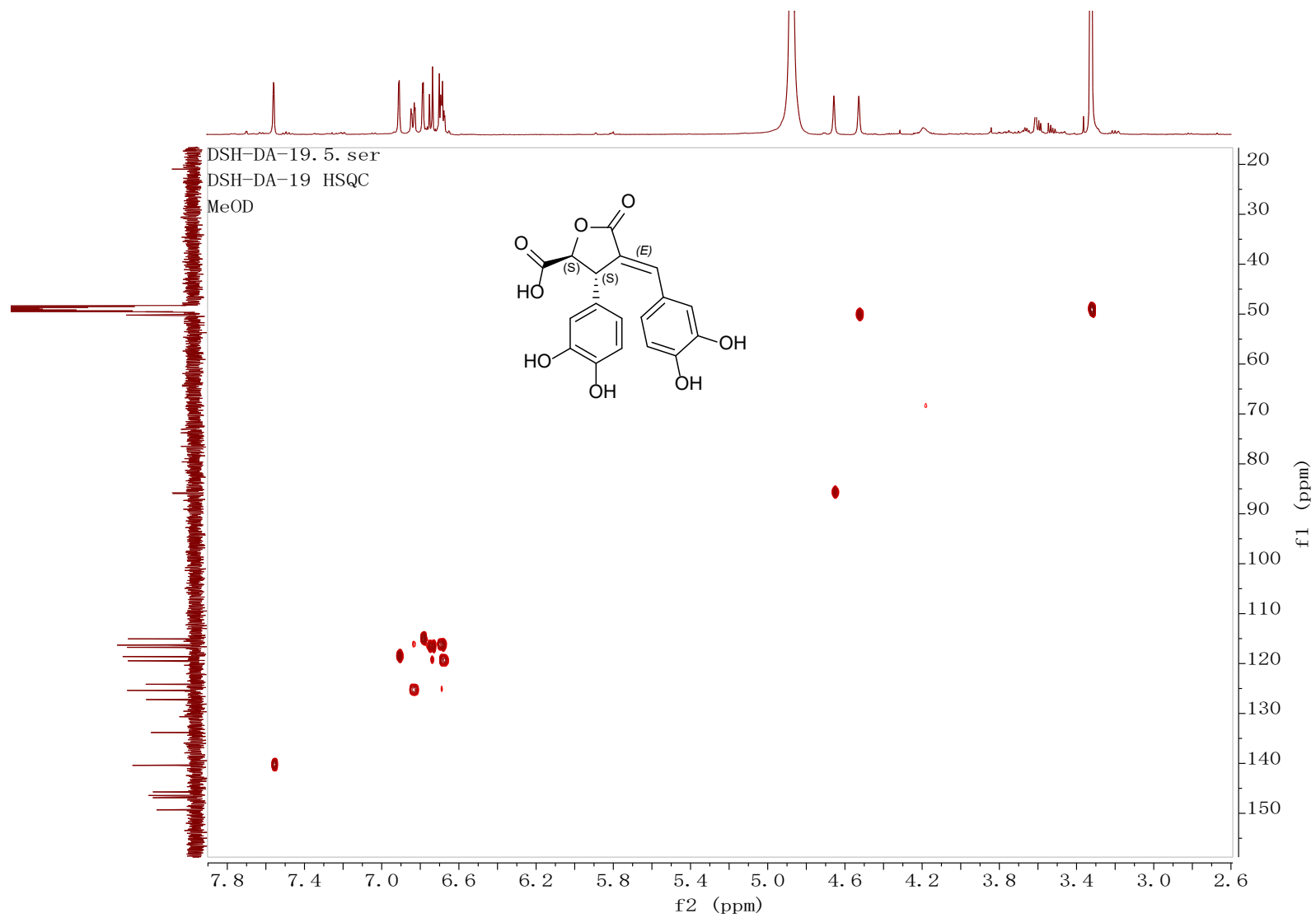


Figure S11. ^1H - ^1H COSY spectrum of compound **1** in MeOD.



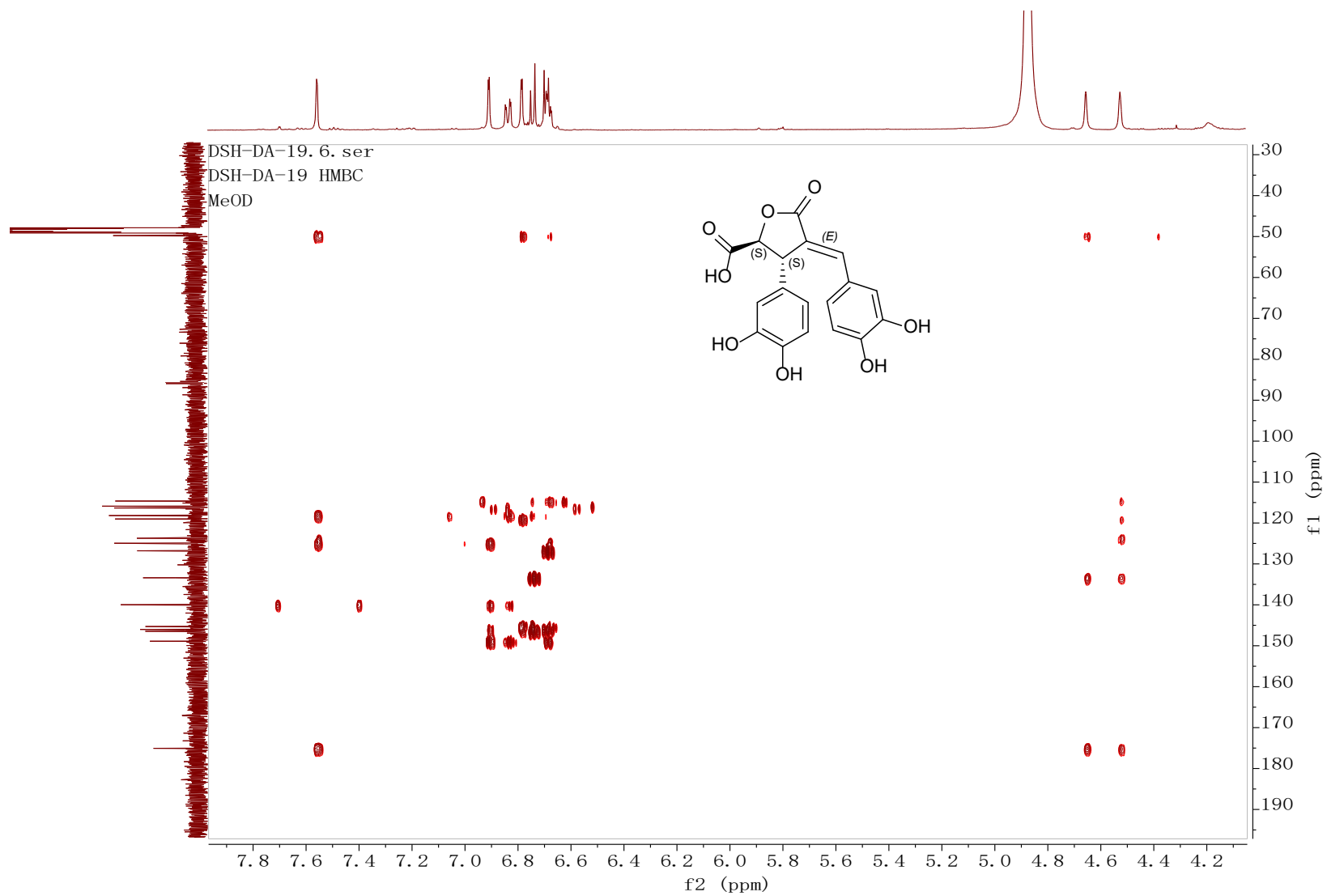


Figure S13. HMBC spectrum of compound **1** in MeOD.

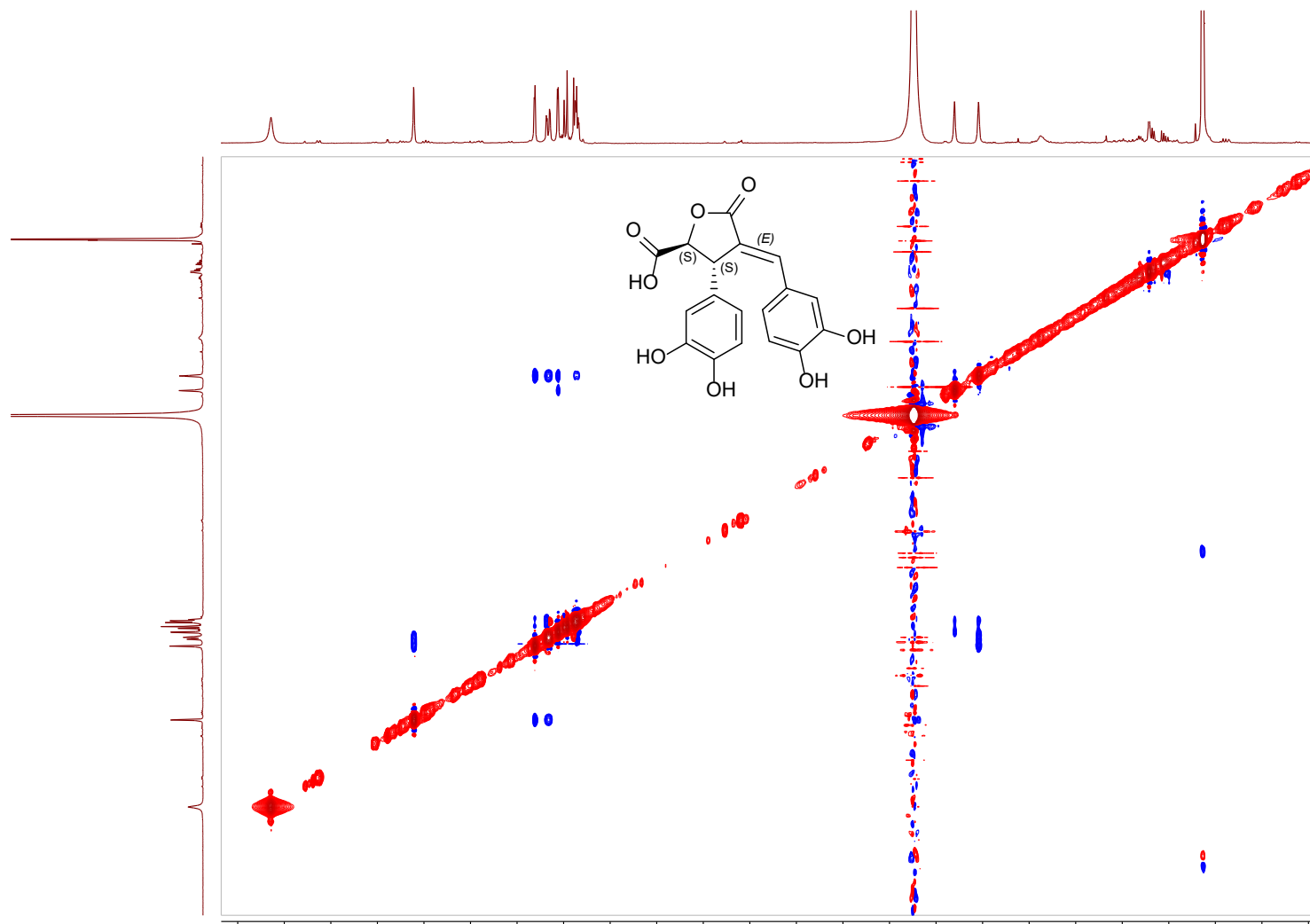


Figure S14. NOESY spectrum of compound 1 in MeOD.