SUPPLEMENTARY MATERIAL

Chemical Constituents from the Fruits of *Psoralea corylifolia* and Their Protective Effects on Ionizing Radiation Injury

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Abstract: Two new flavonoids, corylifol F (**1**) and corylifol G (**2**), together with 19 known compounds, were isolated from the fruits of *Psoralea corylifolia* L.. The structures of these compounds were determined by interpretation of spectroscopic data and comparison with literature properties. The radioprotective effects of the isolated compounds against ionizing radiation damage were also evaluated *in vitro*. The results showed that corylifol A exhibited radioprotective effects in both HBL-100 and MCF-7 cells, while psoralen, isopsoralen, corylifol C, and bakuchiol showed obvious selective action to protect HBL-100 cells against damage caused by ionizing radiation.

Keywords: corylifol F, corylifol G, *Psoralea corylifolia* L., anti-radiation.

Table of Contents

Experimental

General information:

Optical rotation data were determined on an Autopol IV digital polarimeter (Rudolph, Wilmington, MA, USA). UV spectra were carried out on a UV-2102PCS UV-Vis spectrophotometer (UNICO, Dayton, NJ, USA). IR spectra were recorded on a Spectrum 65 spectrometer (PerkinElmer, Waltham, MA, USA). NMR spectra were recorded on Bruker AVIII 400 MHz spectrometers (Bruker, Zurich, Switzerland). The HR-ESI-MS (Shimadzu, Kyoto, Japan) was used to detect the molecular weight of the compounds. Open column chromatography was carried out using silica gel (100-200 and 200-300 meshes, Qingdao Marine Chemical Co. Ltd., Qingdao, China), Sephadex LH-20 gel (GE healthcare bio-sciences AB, Uppsala, Sweden), and polyamide (Taizhou Luqiao Sijia biochemical plastic factory, Taizhou, China) as stationary phases. Preparative HPLC system was performed on an Agilent Technologies 1260 system (Agilent, Santa Clara, California) equipped with Symmetry Prep C18 column (19×150 mm, 7μm, Waters, Milford, MA, USA). TLC analysis was performed on silica gel 60 F254, (Merck KGaA, Darmstadt, Germany). The optical density was determined on a microplate reader (Flex Station 3®, Molecular Devices, USA).

Plant material:

The dried fruits of *Psoralea corylifolia* L. were purchased from Gansu province of China in November 2014, and authenticated by Prof. Tian-Xiang Li, Tianjin University of Traditional Chinese Medicine. The voucher specimen (B20523289) was deposited in herbarium of pharmacognosy, the Binhai Modern Chinese Medicine Laboratory of Tianjin University of Traditional Chinese Medicine, Tianjin, China.

Isolation of compounds from P. corylifolia:

The powdered fruits of *P. corylifolia* (15 kg) were macerated in 75% (v/v) ethanol solution for three times (24–36 h for each time) at room temperature successively. The combined extracts got the brown extractum by vacuum concentration. Then the extractum was fractionated on silica gel eluting with gradient petroleum ether-EtOAc (100:1→0:100, v/v) to obtain the 9:1 fraction (A, 0.300 kg), 8:2 fraction (B, 0.537 kg), and 1:1 fraction (C, 0.259 kg). Fraction A was subjected to silica gel column eluting

with petroleum ether-EtOAc (100:0→1:1, v/v) to gain 11 fractions (A1–A11). Fractions A4 and A5 were purified by Sephadex LH-20 column chromatography and further purified by silica gel column chromatography process repeatedly to give **9** (12.7 g) and **10** (6.5 mg), respectively.

Fraction B was also separated by silica gel column chromatography using petroleum ether–EtOAc (100:0→1:1, v/v) to yield 10 fractions (B1–B10). Fraction B6 was purified by silica gel column chromatographic process repeatedly to get **3** (21.3 g), **4** (17.9 g), and **5** (4.1 g). By using Sephadex LH-20 column chromatography and preparative HPLC, six compounds, **6** (24.3 mg), **7** (69.7 mg), and **21** (16.9 mg), were also obtain from fraction B6. Fraction B7 was subjected to Sephadex LH-20 column to afford **8** (45.7 mg). Fraction B9 was exposed to ODS column to give **1** (36.7 mg).

Fraction C was isolated through the silica gel column chromatography with the solvent system of CH_2Cl_2–EtOAc (95:5→8:2, v/v) to gain seven fractions (C1–C7). Fraction C1 was chromatographed on silica gel column by petroleum ether–EtOAc (8:2→7:3, v/v) and further submitted to silica gel column eluted with CH_2Cl_2–EtOAc to get **12** (16.0 mg). **11** (90.0 mg) was isolated from the fraction C2 by preparative HPLC. Fraction C3 was subjected to polyamide column chromatography with gradient CH_2Cl_2–MeOH (98:2→9:1, v/v) and further purified by preparative HPLC to yield **13** (805.5 mg), **14** (1.6 g), **16** (258.1 mg), and **18** (9.2 g). **20** (491.4 mg) was purified by silica gel column chromatography using petroleum ether–EtOAc (1:1, v/v) from fraction C4. Fraction C7 was chromatographed on polyamide column with CH_2Cl_2–MeOH (93:7→87:13, v/v) and further purified by preparative HPLC to obtained **2** (128.2 mg), **15** (72.0 mg), **17** (258.5 mg), and **19** (28.5 mg).

Cell culture:

Human breast cell line HBL-100 was obtained by Cell Bank of Chinese Academy of Sciences (Beijing, China). Human breast cancer cell line MCF-7 was supported by Tianjin Key Laboratory of Modern Chinese Medicine of Tianjin University of Traditional Chinese Medicine (Tianjin, China). HBL-100 and MCF-7 were cultured in RPMI-1640 medium (Gibco, New York, USA) and DMEM (High Gluose) (Gibco, New York, USA) supplemented with 10% fetal bovine serum and 1% (v/v)

penicillin-streptomycin in a humidified atmosphere with 5% CO_2 at 37℃, respectively. Then passaging the cells under the proportion of 1/2 when the adherence growth density reached 70–80% and took the cells in good condition to experiment.

Cytotoxic assay:

The cell viability effect was determined by the CCK-8 assay. Cells in good condition were seeded in 96 well plates (7×10^3 cells per well) after cell dissociation with 0.25% trypsin (Gibco, New York, USA). After culturing for 12 h to adherence growth, cells were treated with 100 μL solutions of various concentrations of the compounds (6.25, 12.5, 25, 50, 100 μmol/L) to culture for 48 h. Then the CCK-8 solution (100 μL) was added in per well to incubation for 3 h. The optical density at 450 nm was measured in a microplate reader to calculate the cell viability ($CV = (OD_{experiment}–OD_{blank}) / (OD_{control}–OD_{blank}) \times 100\%$) for the cytotoxity effects. The independent experiments were performed in triplicate.

Anti-radiation assay:

Choose the max concentration of the 21 compounds respectively that showed no significance difference ($P\geq0.05$) between the experiment group and model group in cytotoxic assay to test the anti-radiation effects in HBL-100 and MCF-7 cells individually. The experimental group, model group, and control group were set up here. Cells prepared to suspension by 0.25% trypsin in good condition were seeded in 96 well plates (7×10^3 cells per well) and cultured for 12 h. Every well was cultured for 24 h continually after injecting solutions (100 μL) with the maximum safe dose concentrations of the tested compounds above in cytotoxic assay. Cells in experimental group and model group were received radiation of $^{137}Cs\gamma$ for 8 min (1.00 Gy/min) and cultured for 24 h. Cells of the three groups were observed the OD at 450 nm in microplate reader which had handled with CCK-8 method for 3 h. The algorithm of cell viability was determined by the formula of $CV= (OD_{experiment}–OD_{blank}) / (OD_{model}–OD_{blank}) \times 100\%$ to obtain the anti-radiation effects.

Table S1. ^{1}H NMR and ^{13}C NMR spectroscopic data of compounds **1** and **2** (δ in ppm, J in Hz, DMSO-d_6).

	1		**2**	
Position	***δ*H**	***δ*C**	***δ*H**	***δ*C**
2	5.64, dd (12.8, 2.8)	79.7	8.24, s	152.7
3	3.28, dd (16.8, 12.8)	42.8	—	127.1
	2.78, dd (16.8, 2.8)			
4	—	190.2	—	174.7
5	8.15, s	128.4	7.96, d (8.8)	127.3
6	—	119.4	6.93, dd (8.8, 2.4)	115.1
7	—	167.1	—	162.5
8	6.84, s	100.8	6.86, d (2.4)	102.1
9	—	166.6	—	157.4
10	—	114.3	—	116.6
1′	—	128.5	—	123.7
2′	7.36, d (8.8)	128.5	7.25, d (2.4)	130.0
3′	6.81, d (8.8)	115.2	—	122.5
4′	—	157.9	—	154.8
5′	6.81, d (8.8)	115.2	6.83, d (8.0)	114.5
6′	7.36, d (8.8)	128.5	7.19, dd (8.0, 2.4)	127.4
1″			3.26, d (7.2)	28.1
2″			5.32, t (7.2)	122.3
3″			—	135.2
4″			1.97, m	35.3
5″			1.49, m	33.5
6″			3.84, t (6.8)	73.5
7″			—	148.2
8″			4.70, s	109.8
			4.83, s	
9″			1.68, s	16.1
10″			1.62, s	17.6
-OCH_3	3.95, s	56.8		
-CHO	10.19, s	187.6		
4′-OH	9.62, s	—		
6″-OH			4.65, br s,	—

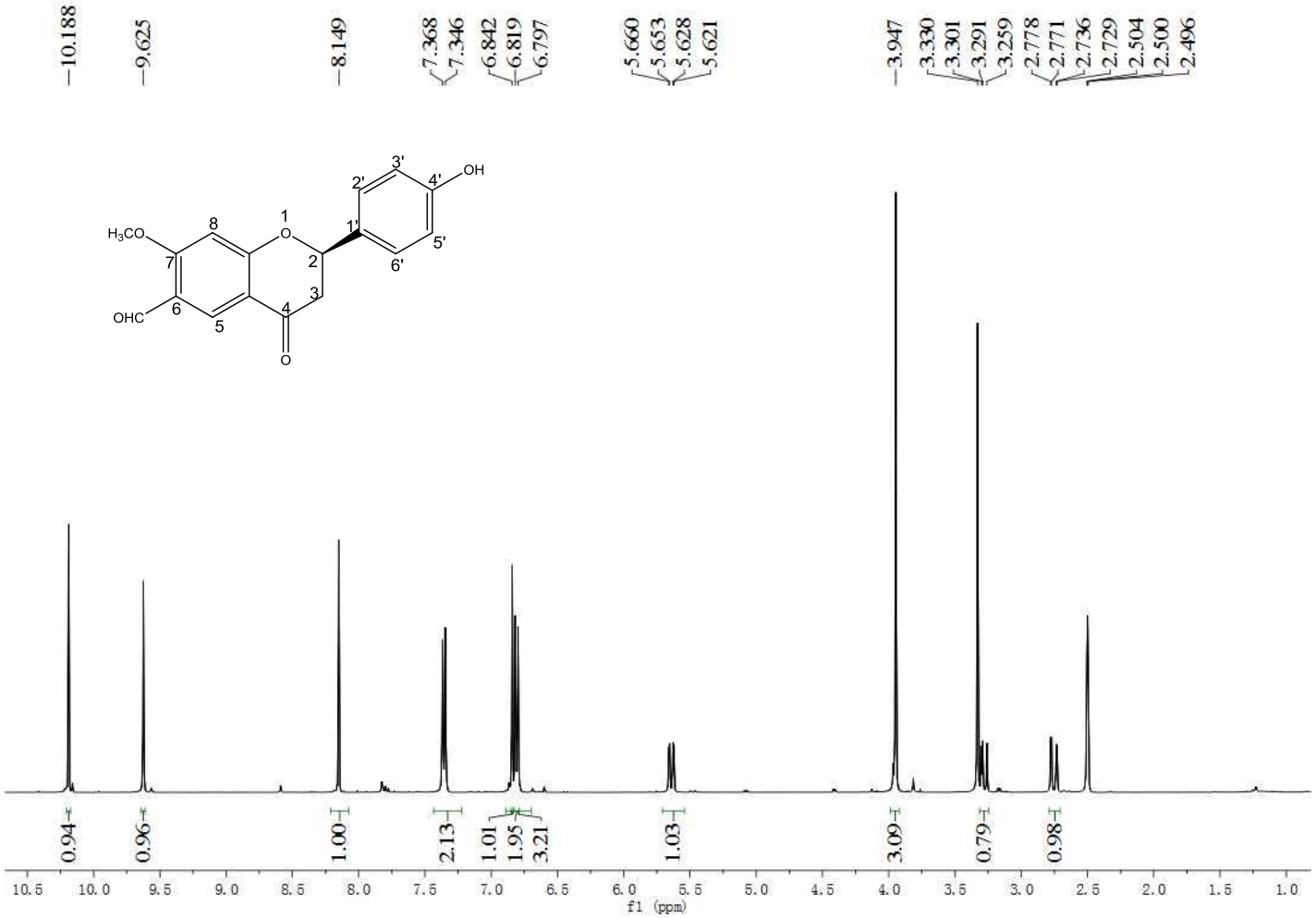


Figure S1. ^{1}H NMR spectrum of compound **1** in DMSO-d_6 (400 MHz)

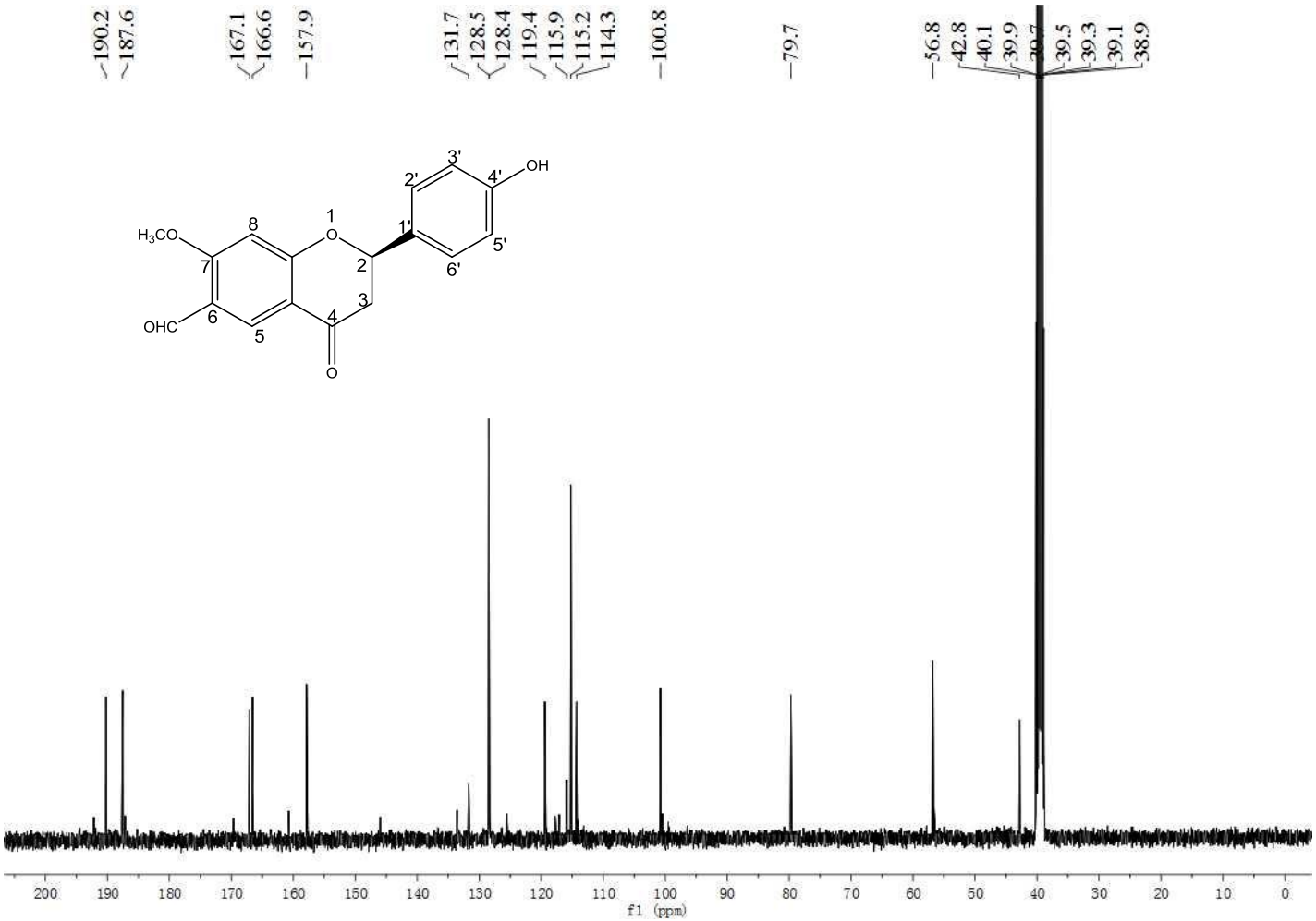


Figure S2. ^{13}C NMR spectrum of compound **1** in DMSO-d_6 (100 MHz)

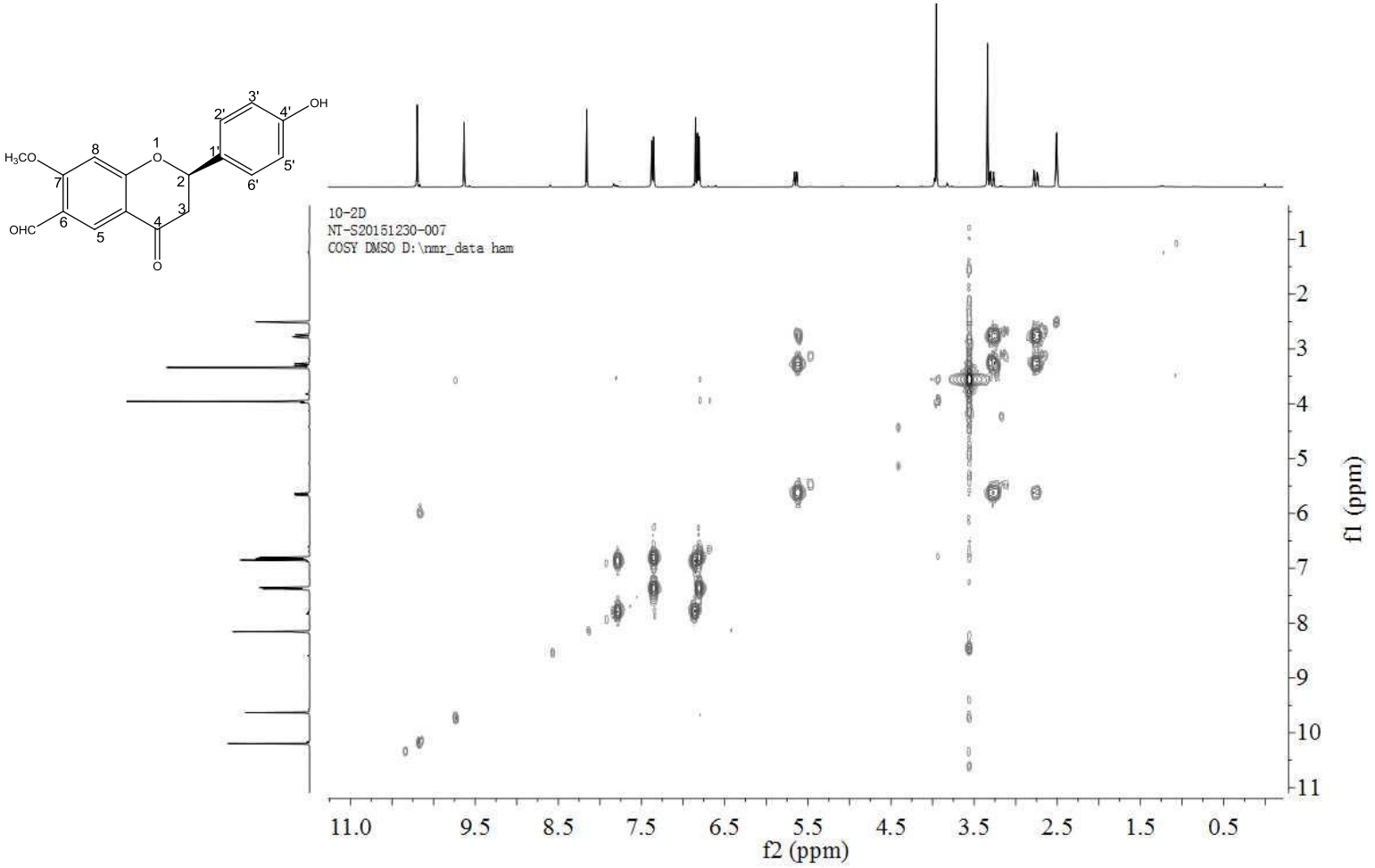


Figure S3. ^{1}H-^{1}H COSY spectrum of compound **1** in DMSO-d_6

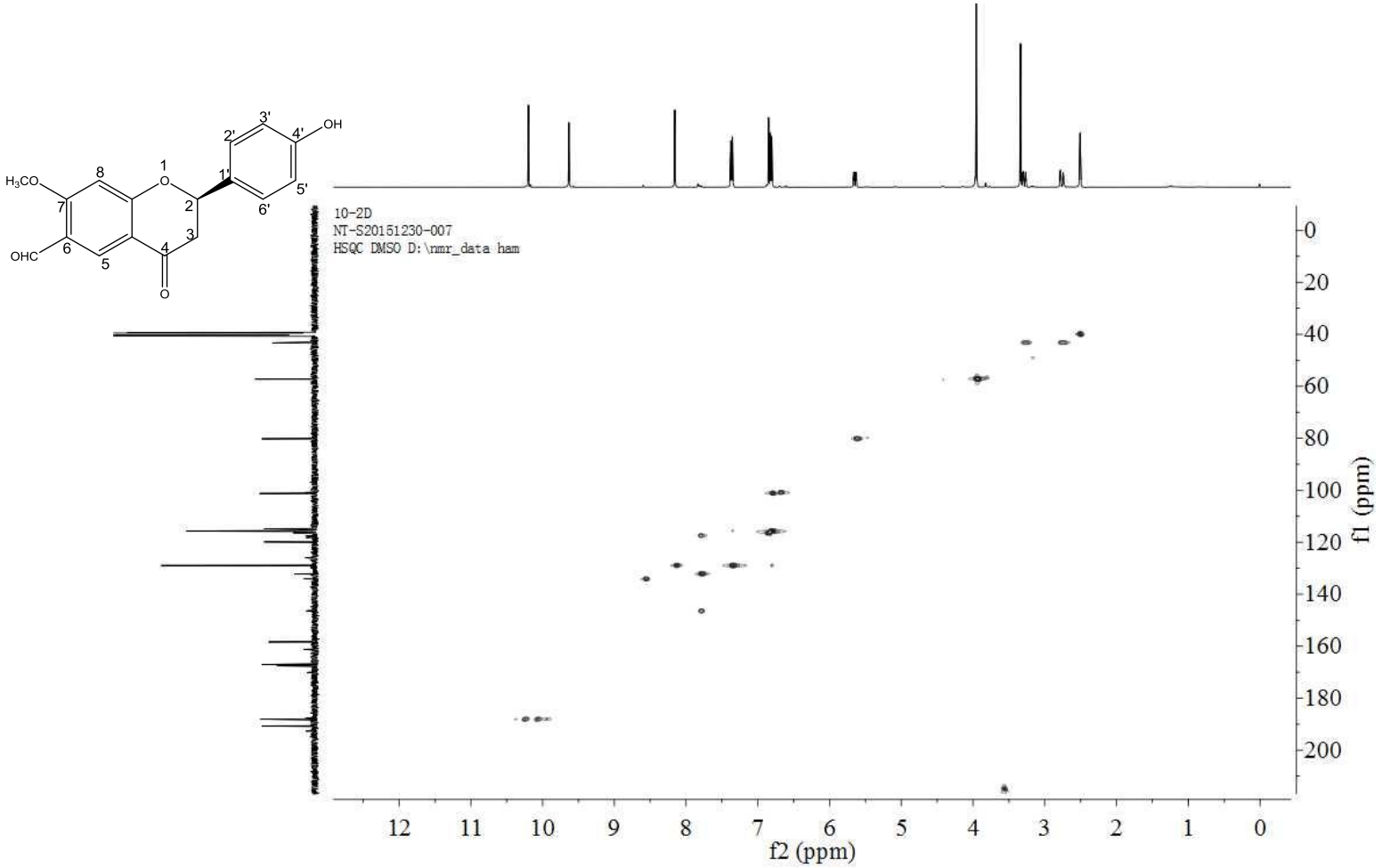


Figure S4. HSQC spectrum of compound **1** in DMSO-d_6

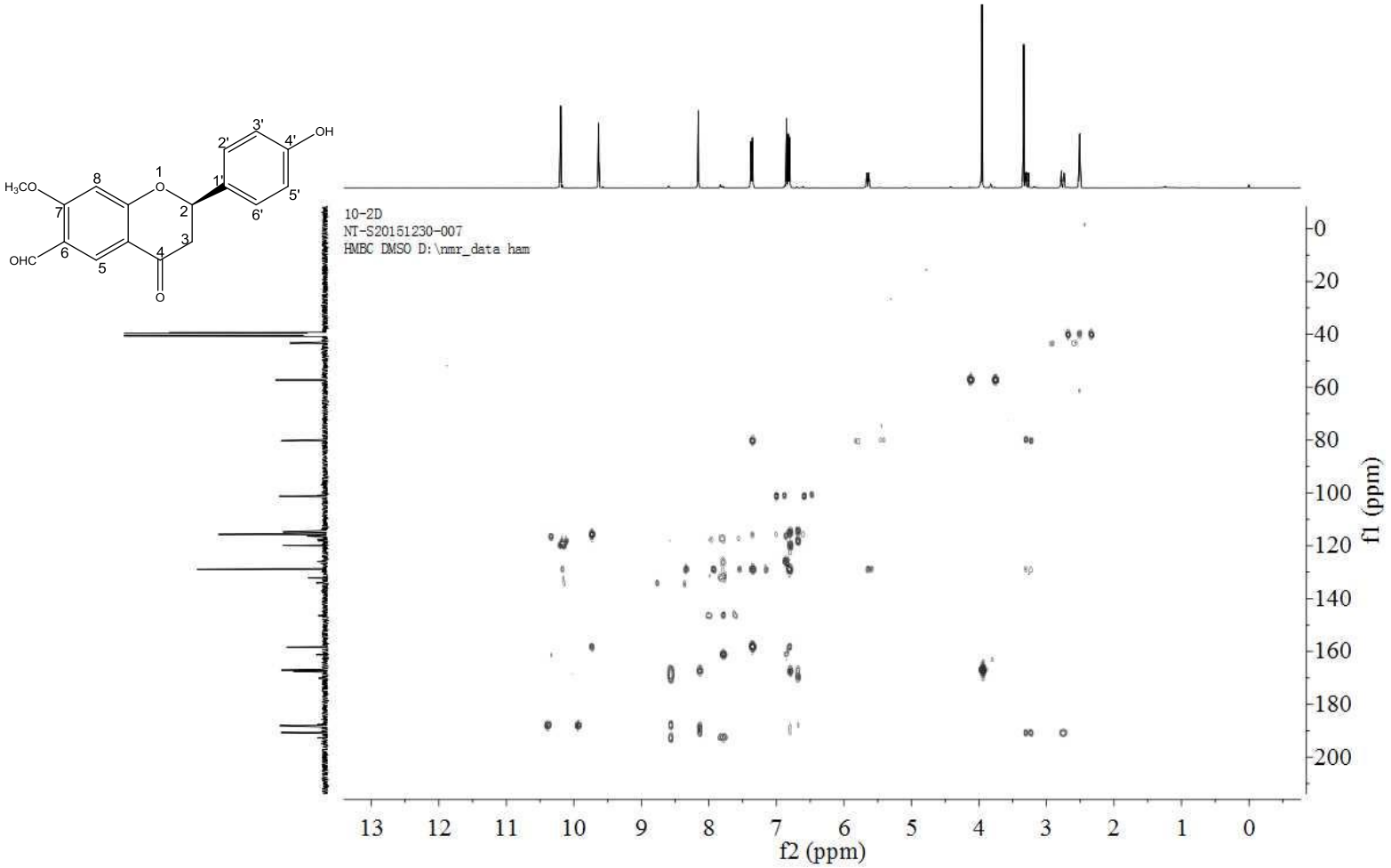


Figure S5. HMBC spectrum of compound **1** in DMSO-d_6

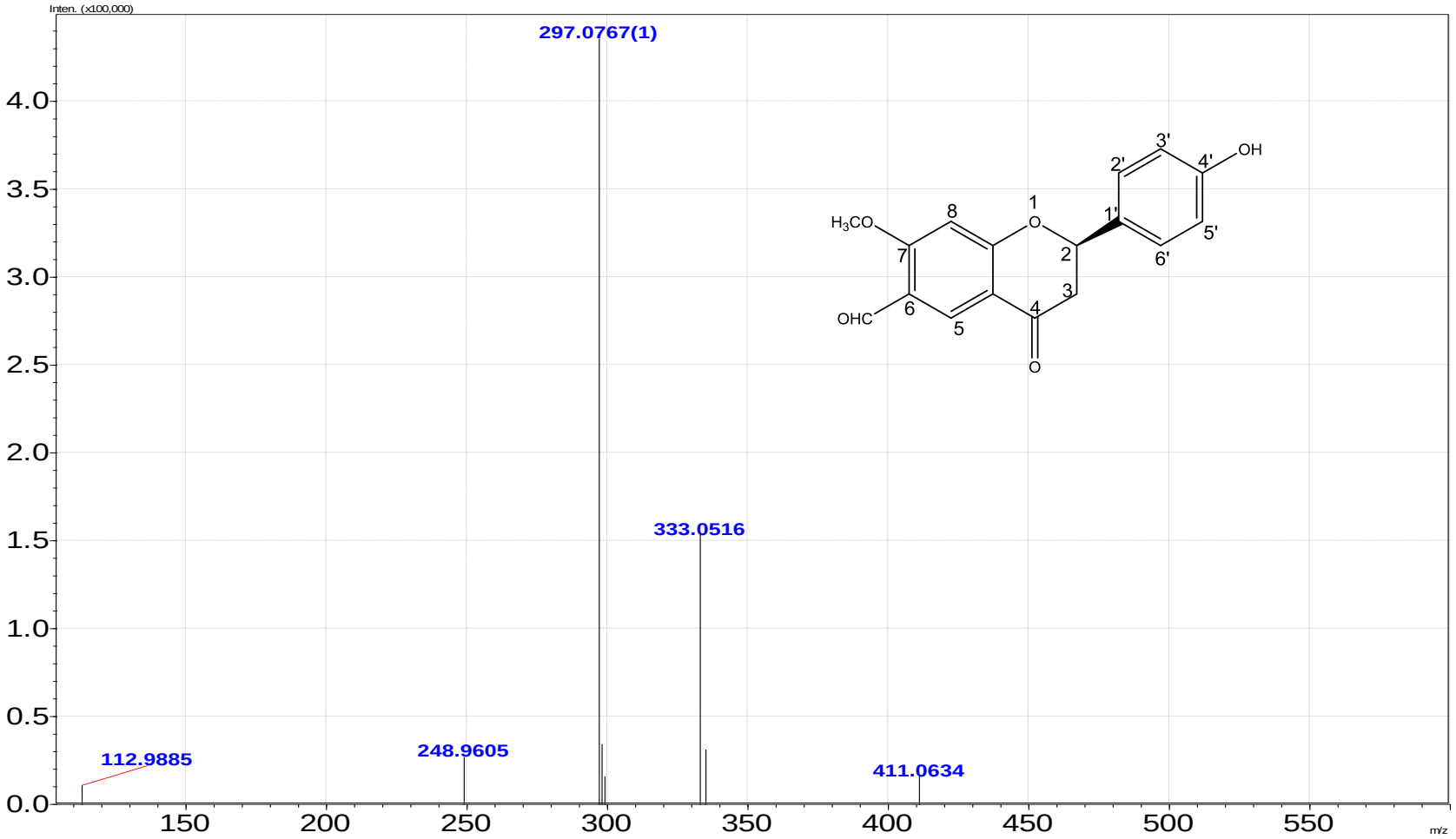


Figure S6. HR-ESI-MS spectrum of compound **1**

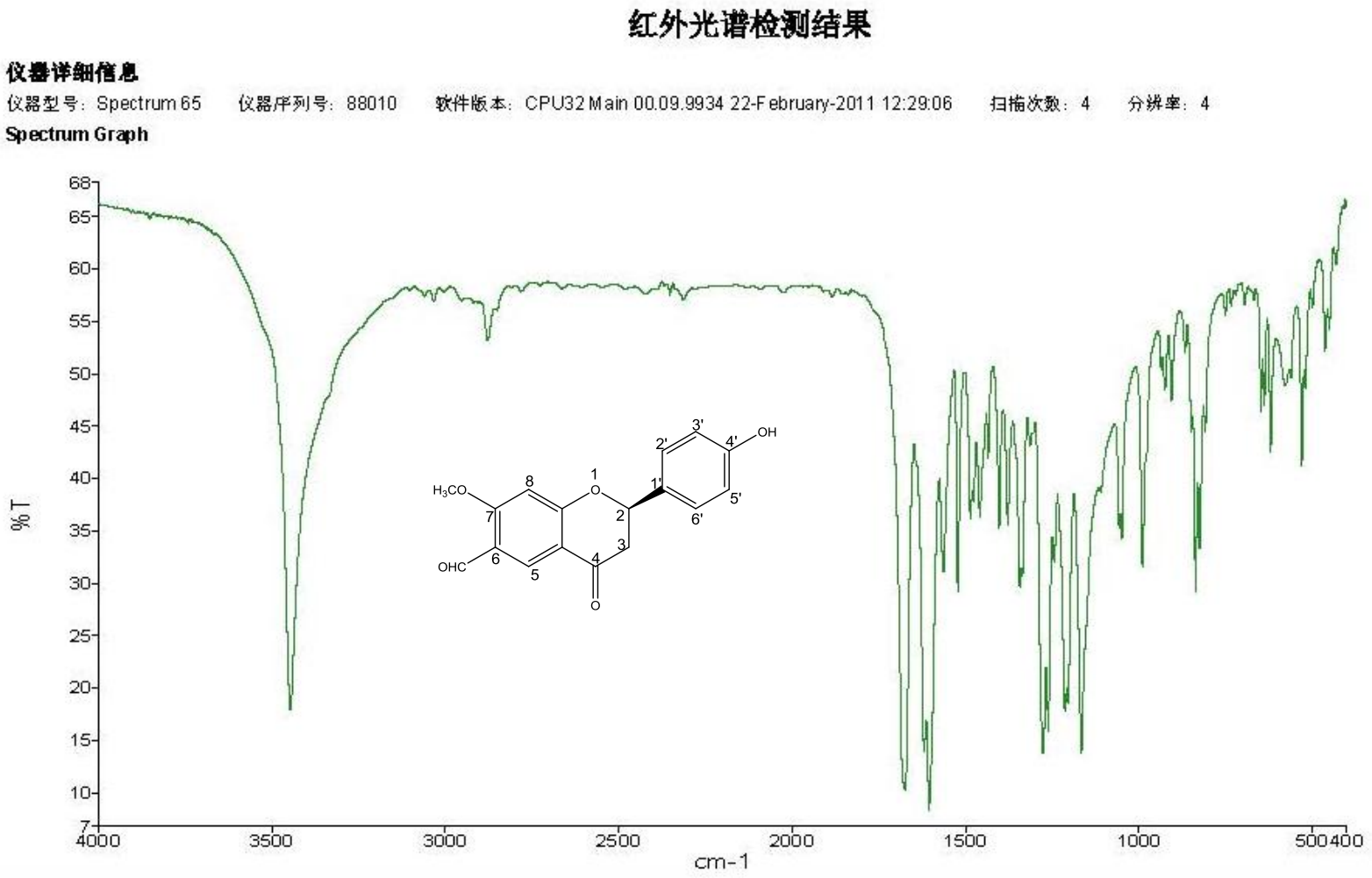


Figure S7. IR spectrum of compound **1**

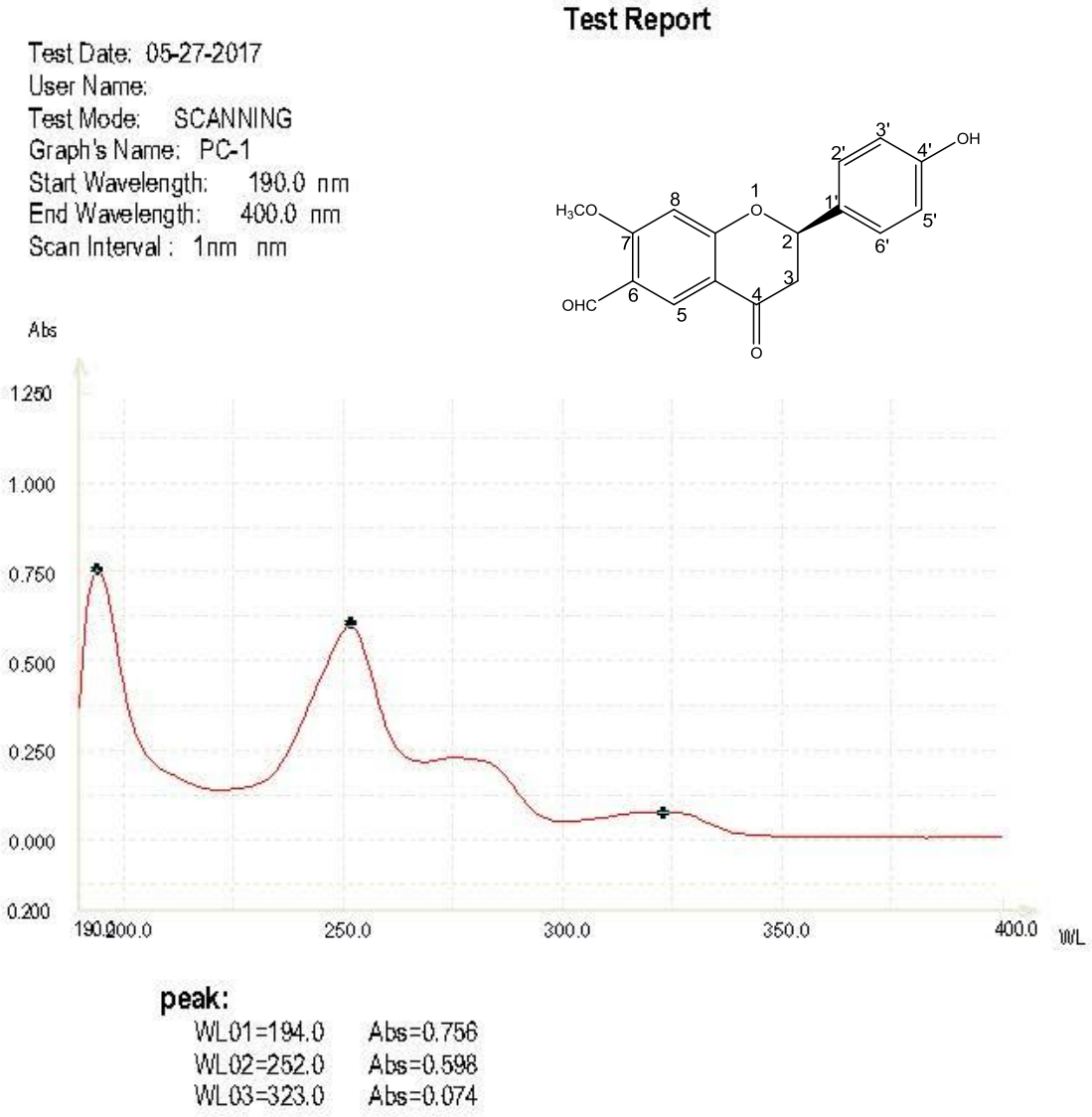


Figure S8. UV spectrum of compound **1**

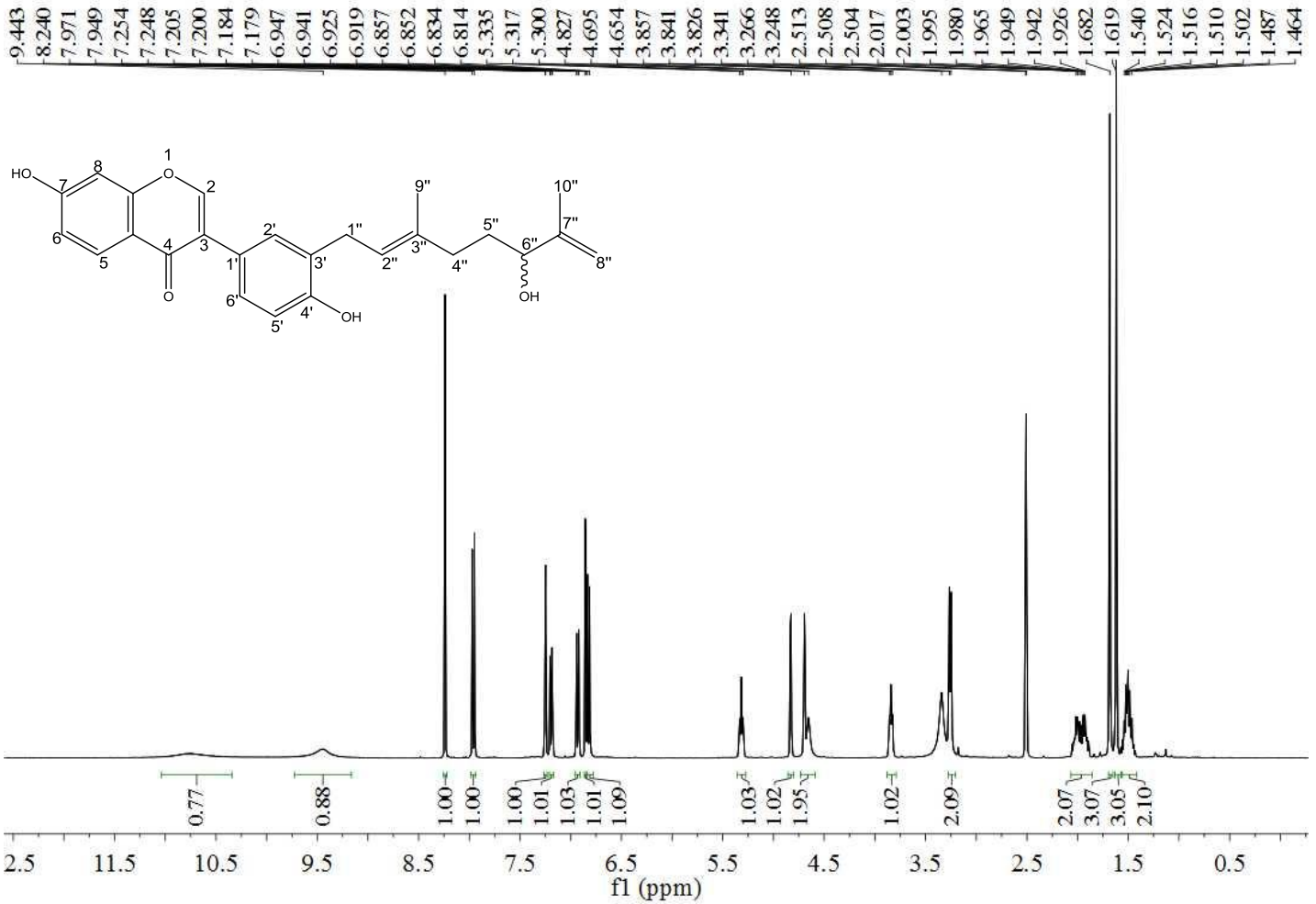


Figure S9. ^{1}H NMR spectrum of compound **2** in DMSO-d_6 (400 MHz)

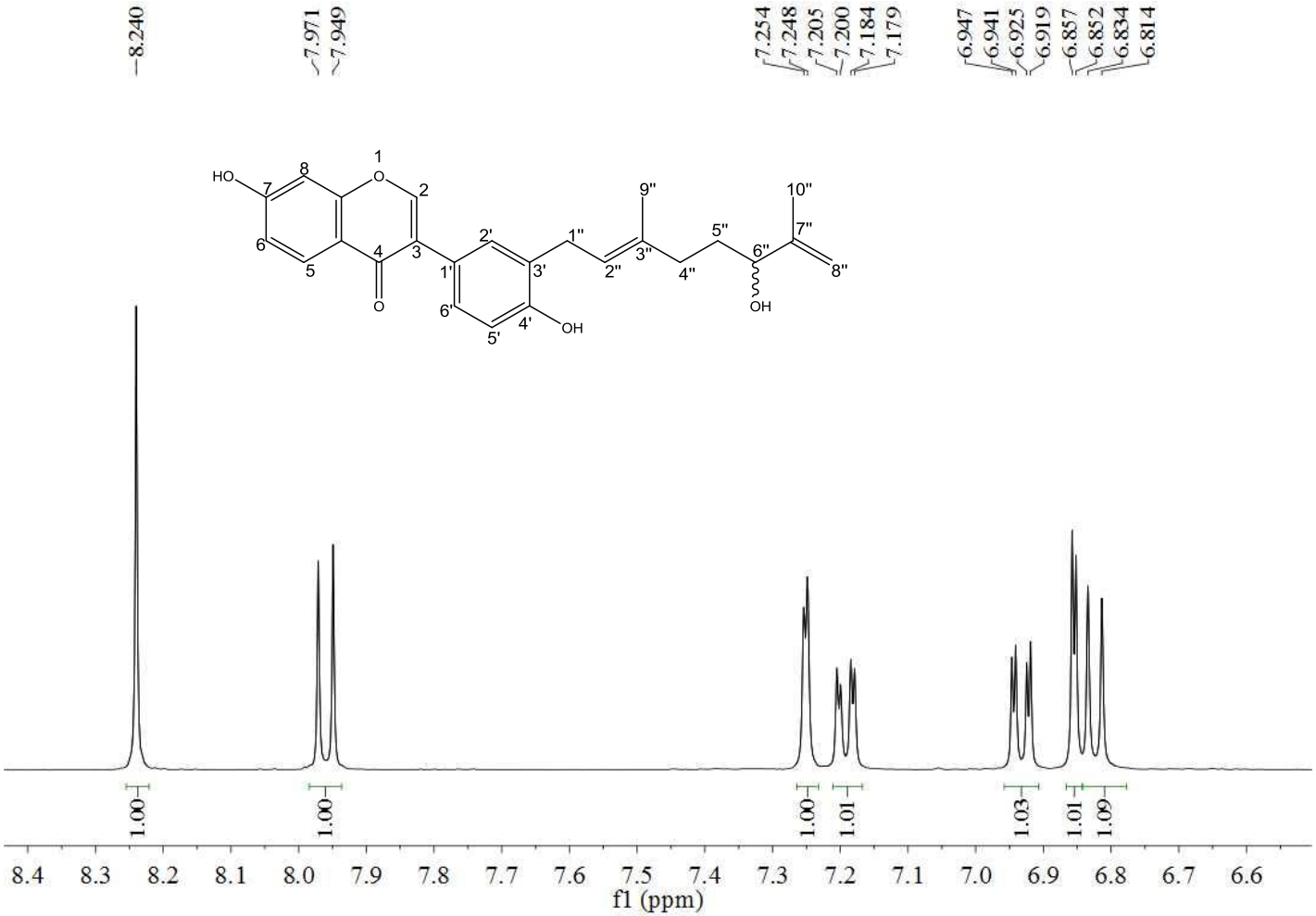


Figure S10. ^{1}H NMR spectrum of compound **2** in DMSO-d_6 (400 MHz)(Enlarge)

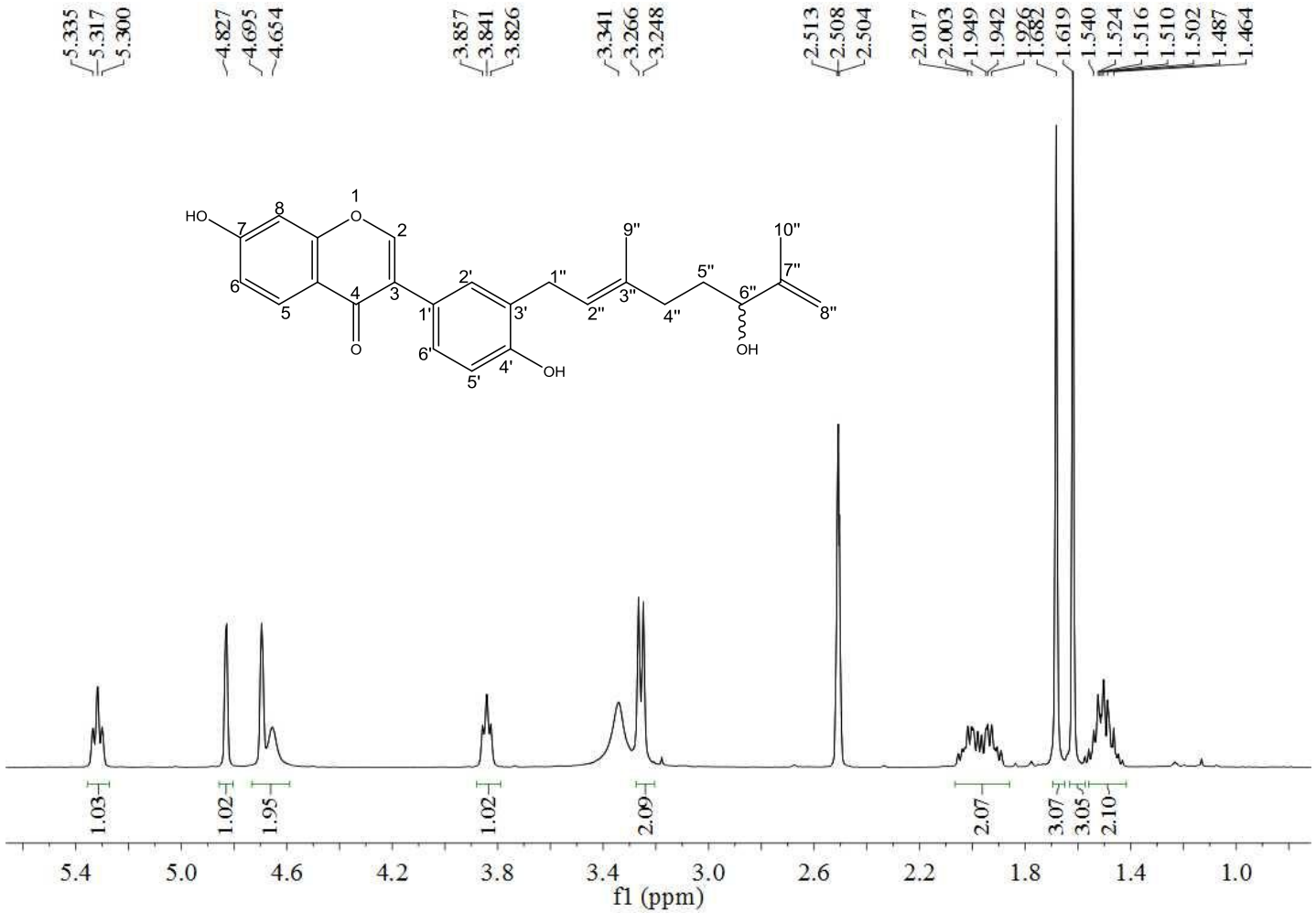


Figure S11. ^{1}H NMR spectrum of compound **2** in DMSO-d_6 (400 MHz) (Enlarge)

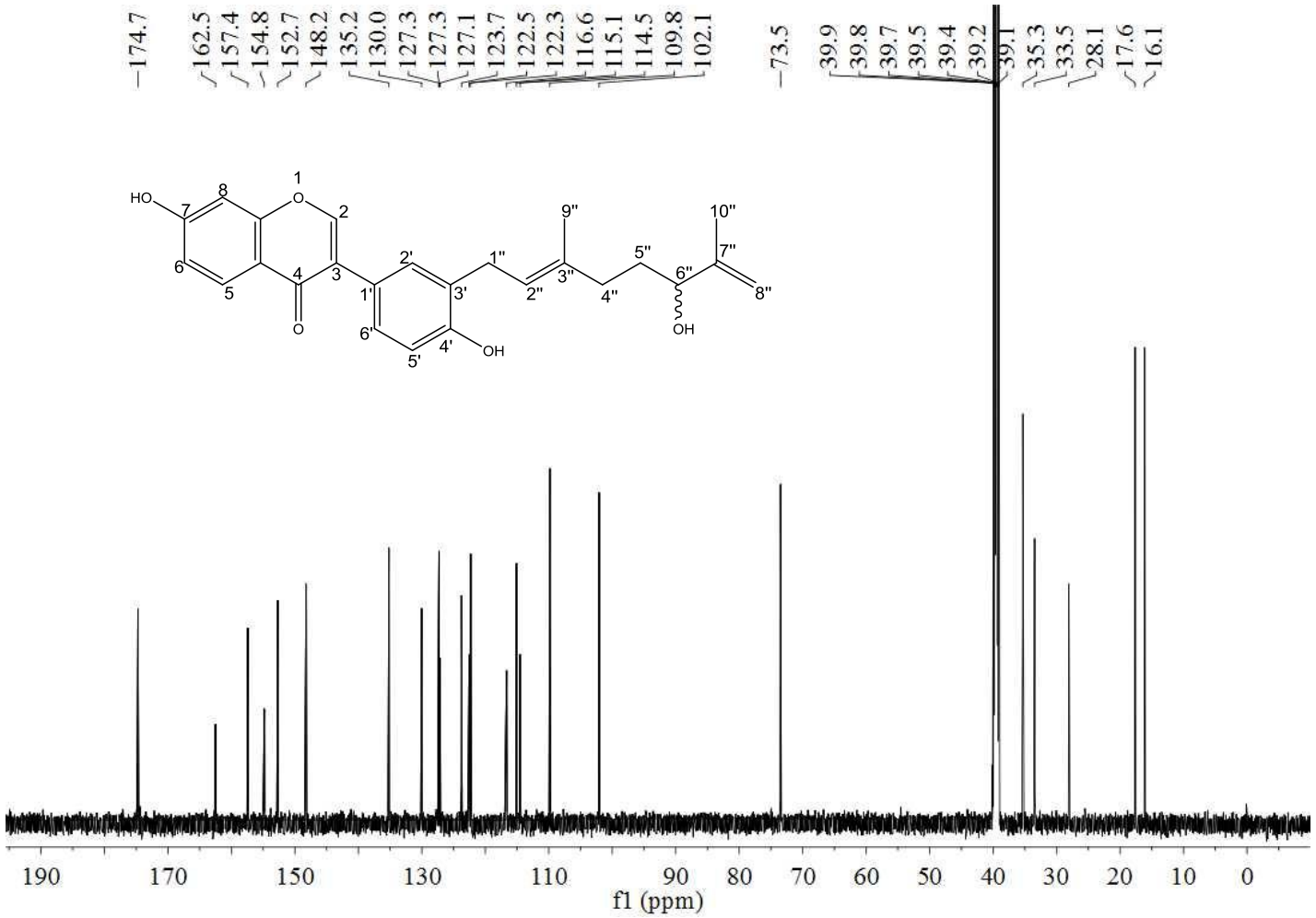


Figure S12. ^{13}C NMR spectrum of compound **2** in DMSO-d_6 (100 MHz)

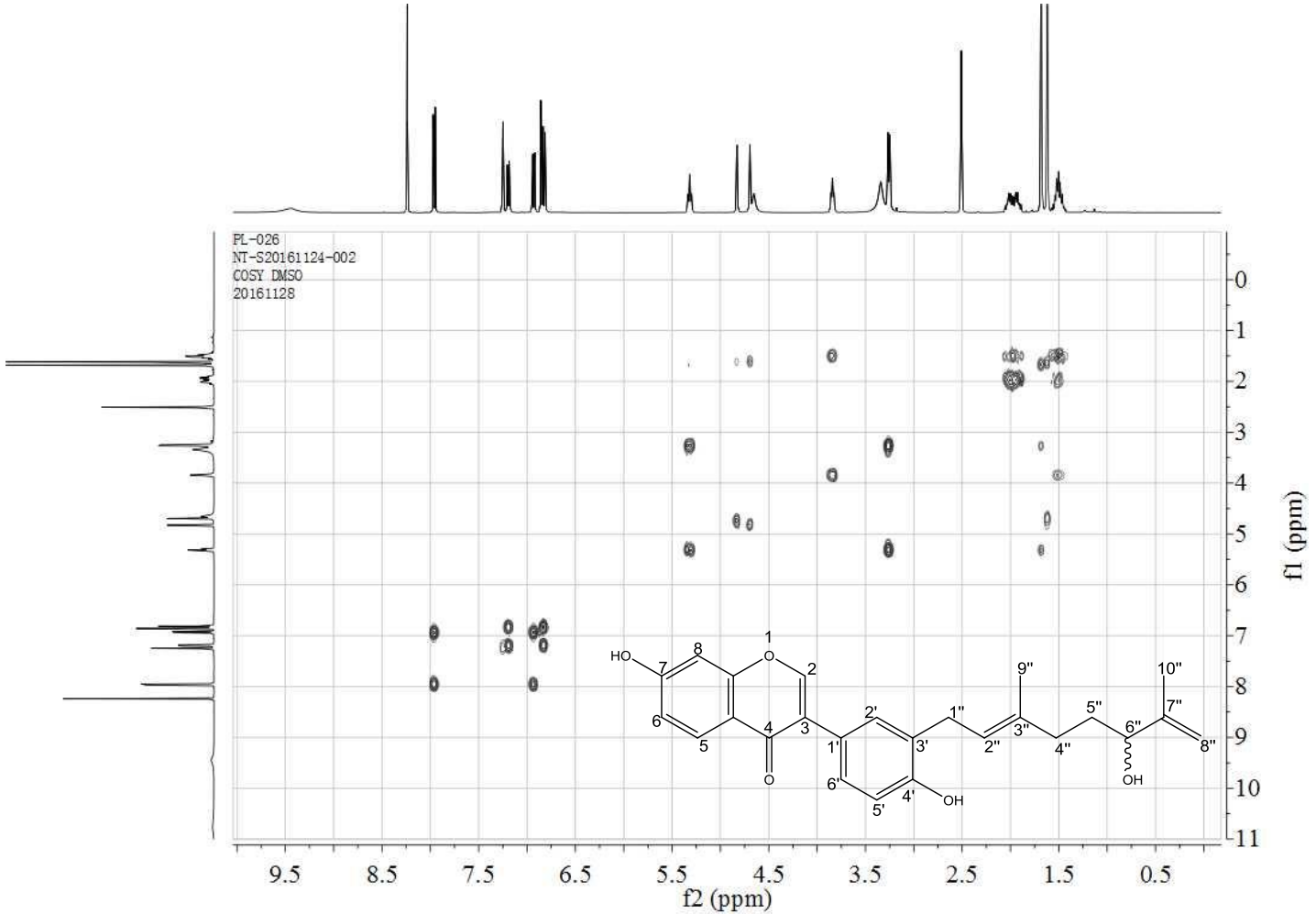


Figure S13. ^{1}H-^{1}H COSY spectrum of compound **2** in DMSO-d_6

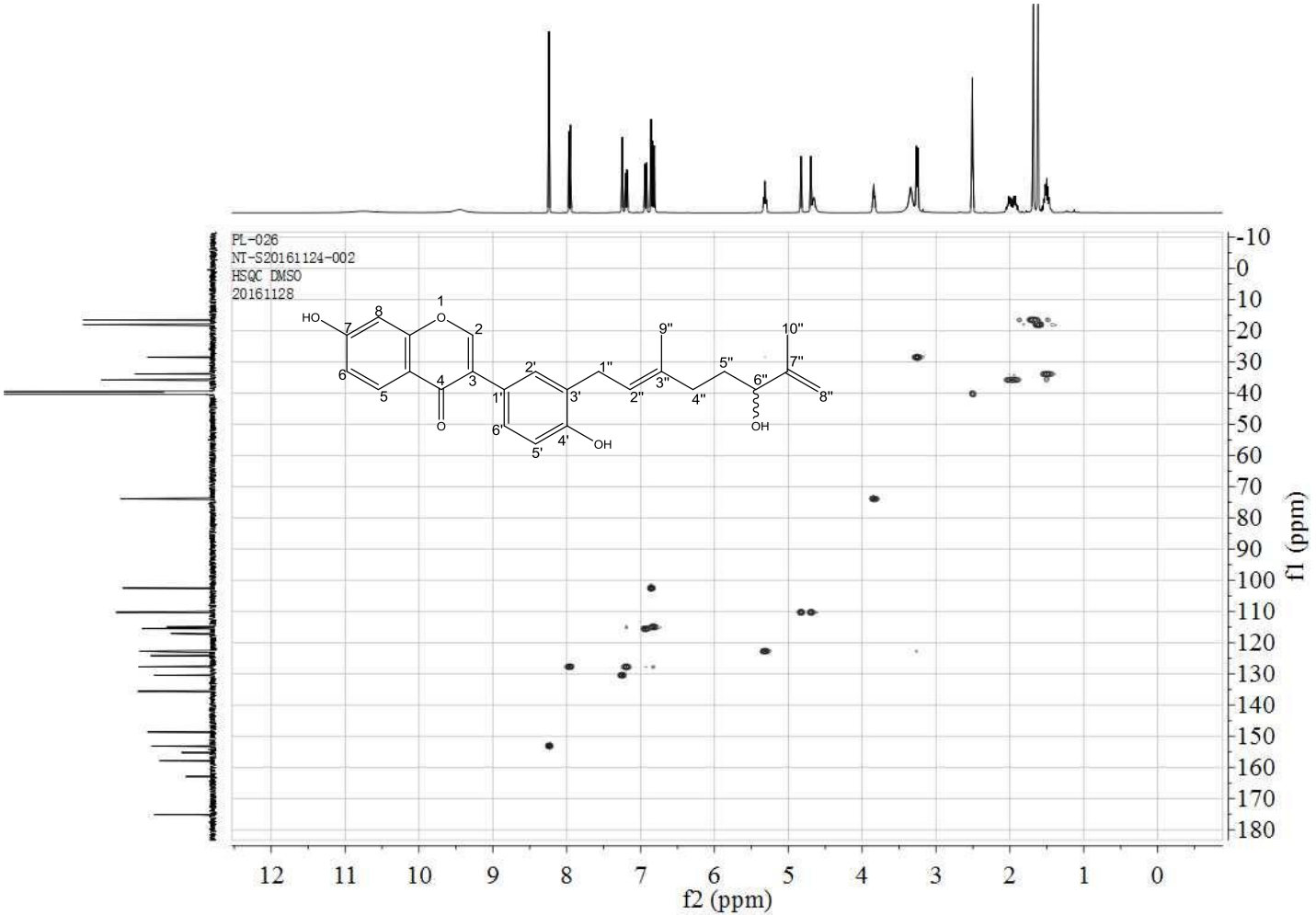


Figure S14. HSQC spectrum of compound **2** in DMSO-d_6

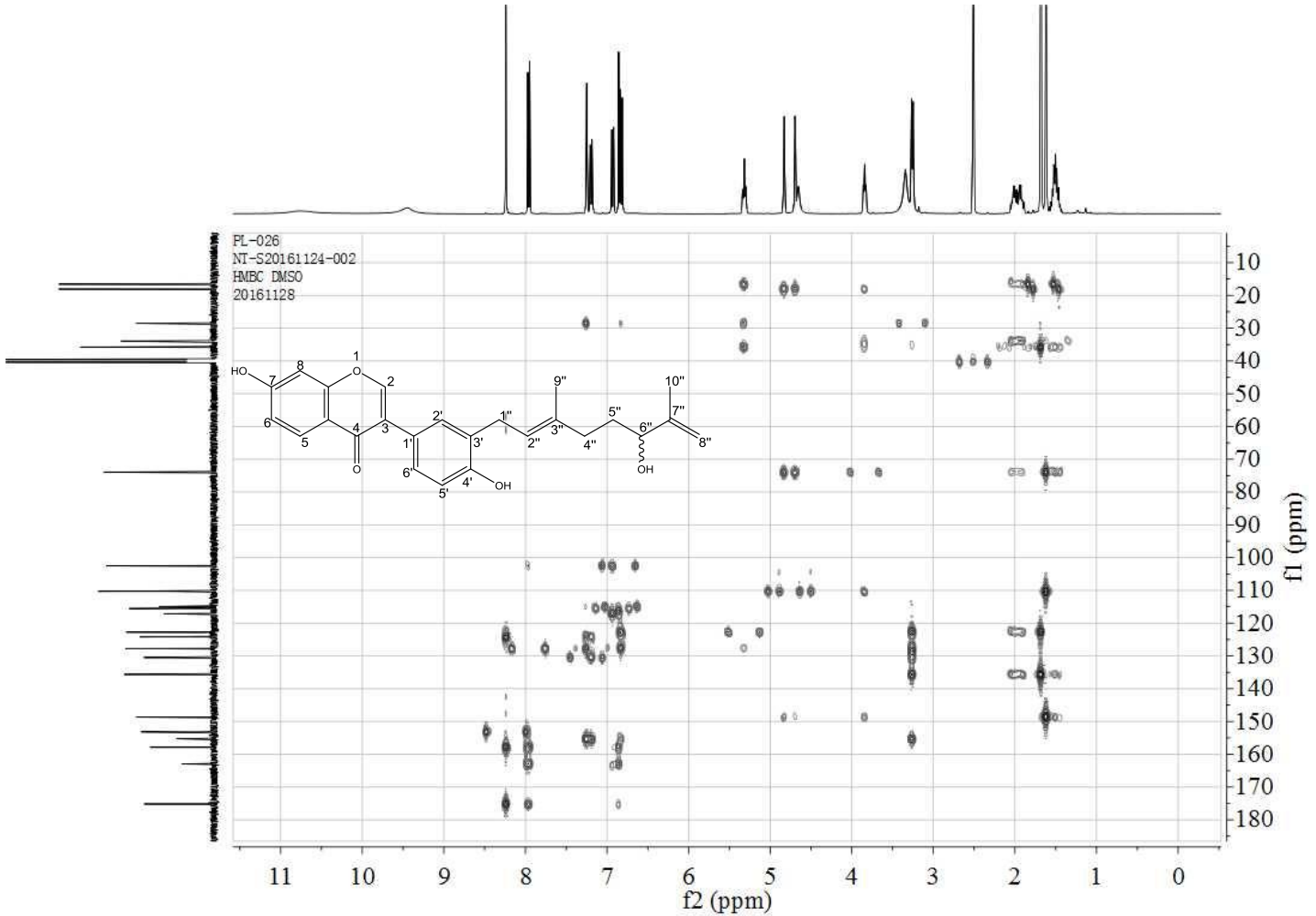


Figure S15. HMBC spectrum of compound **2** in DMSO-d_6

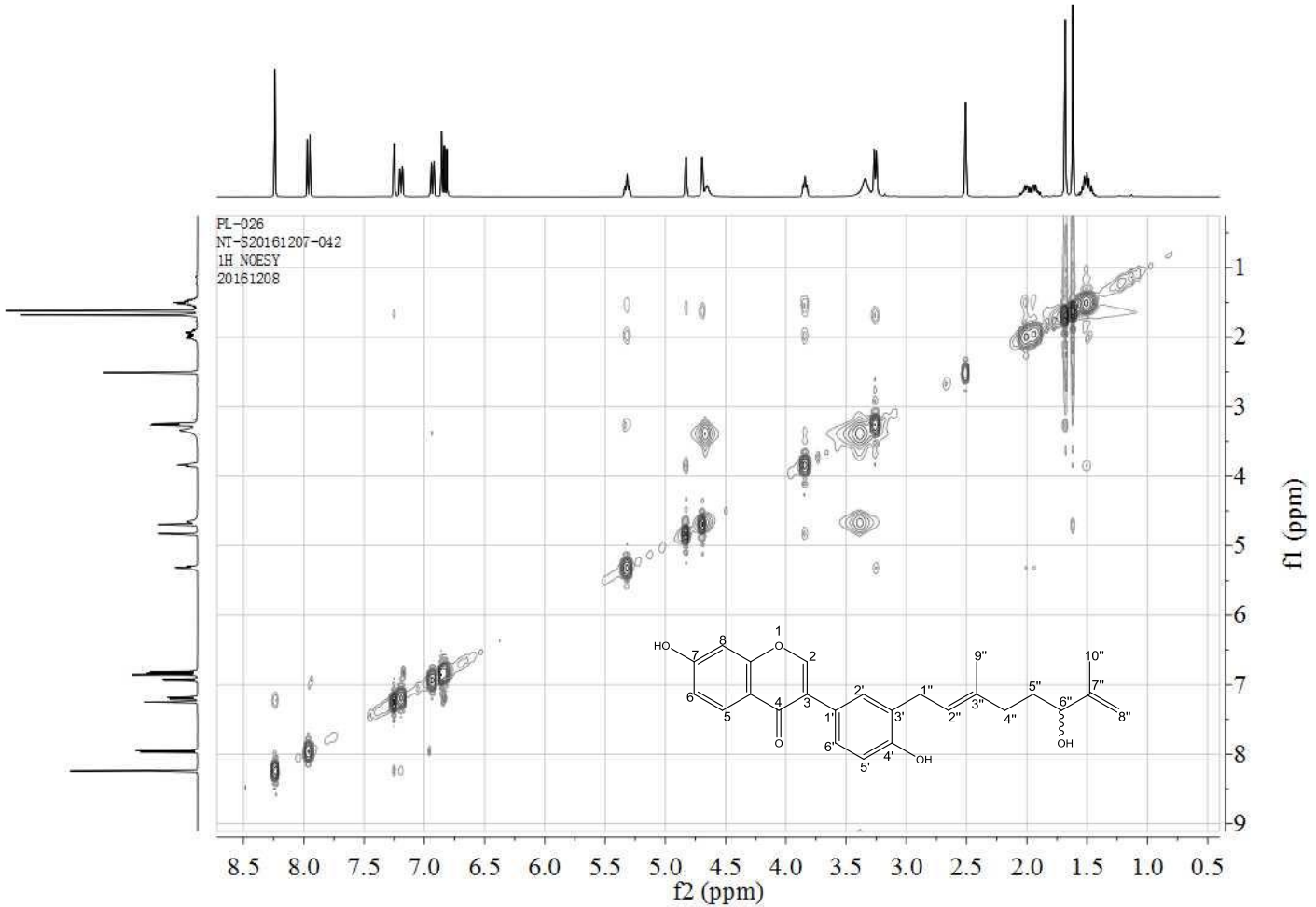


Figure S16. NOSEY spectrum of compound **2** in DMSO-d_6

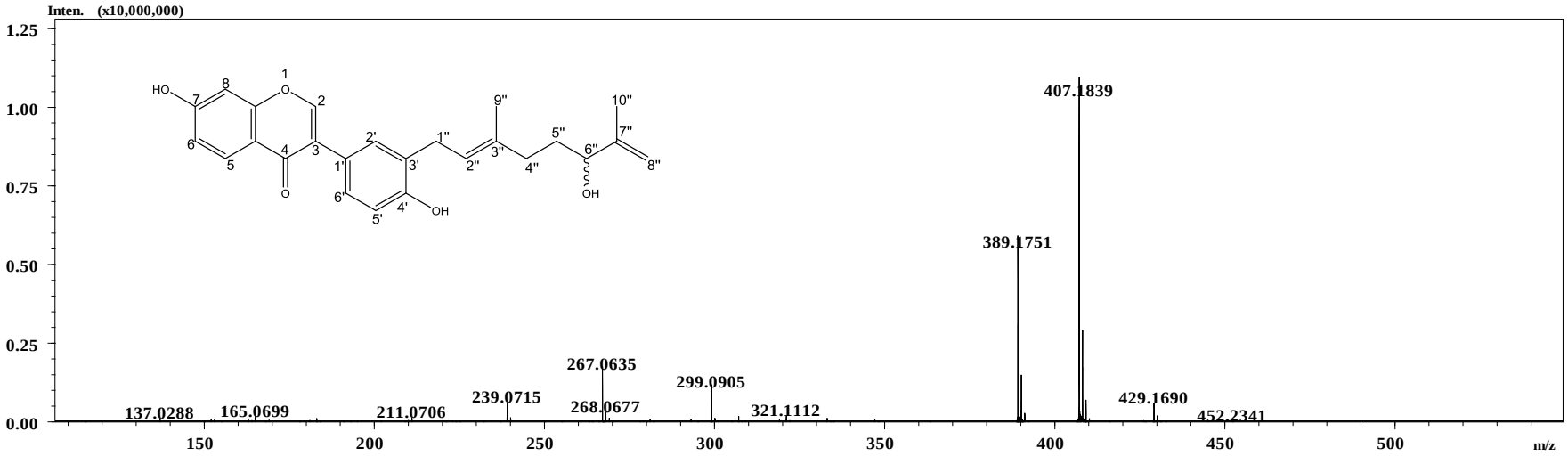


Figure S17. HR-ESI-MS Spectrum of compound **2**

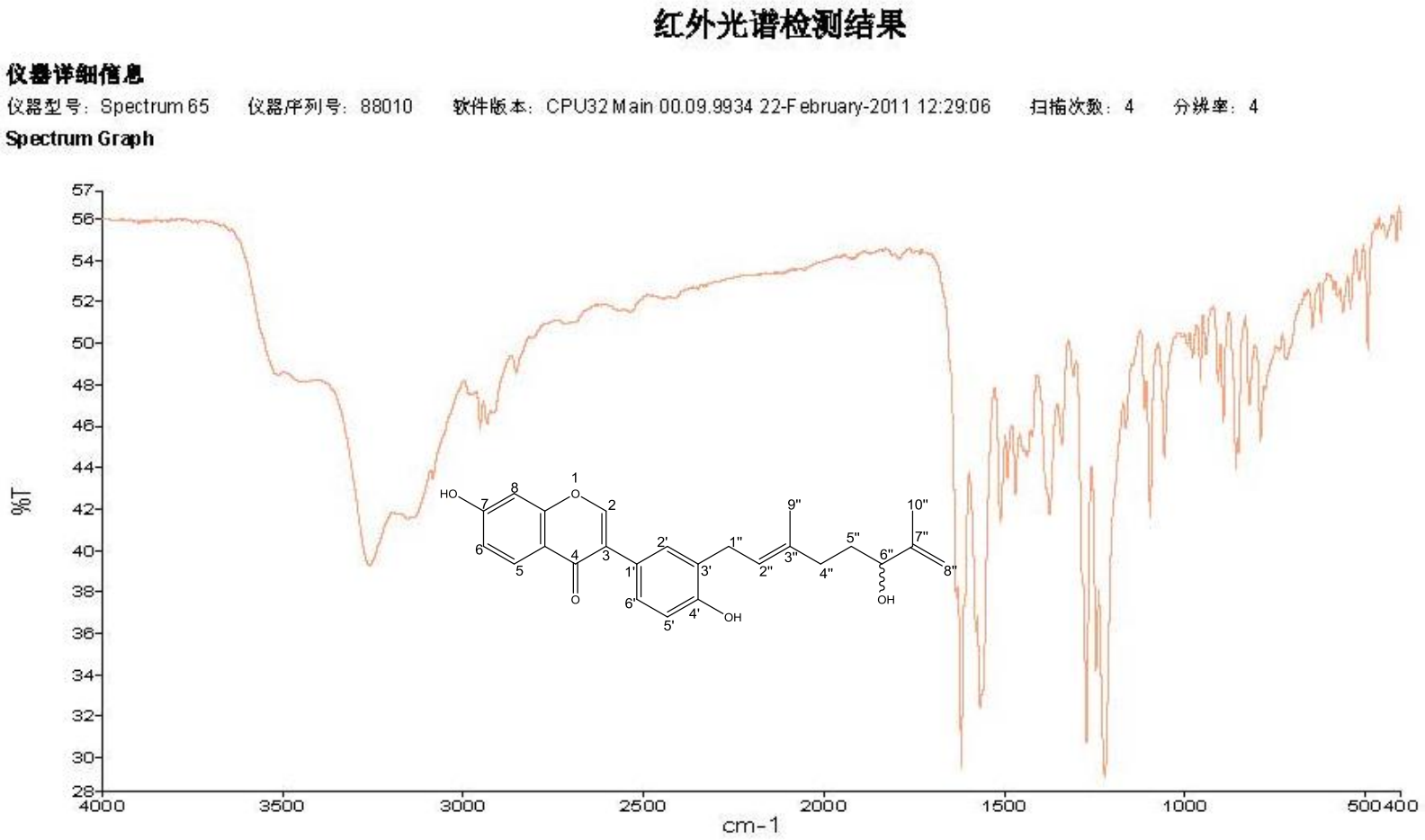


Figure S18. IR spectrum of compound **2**

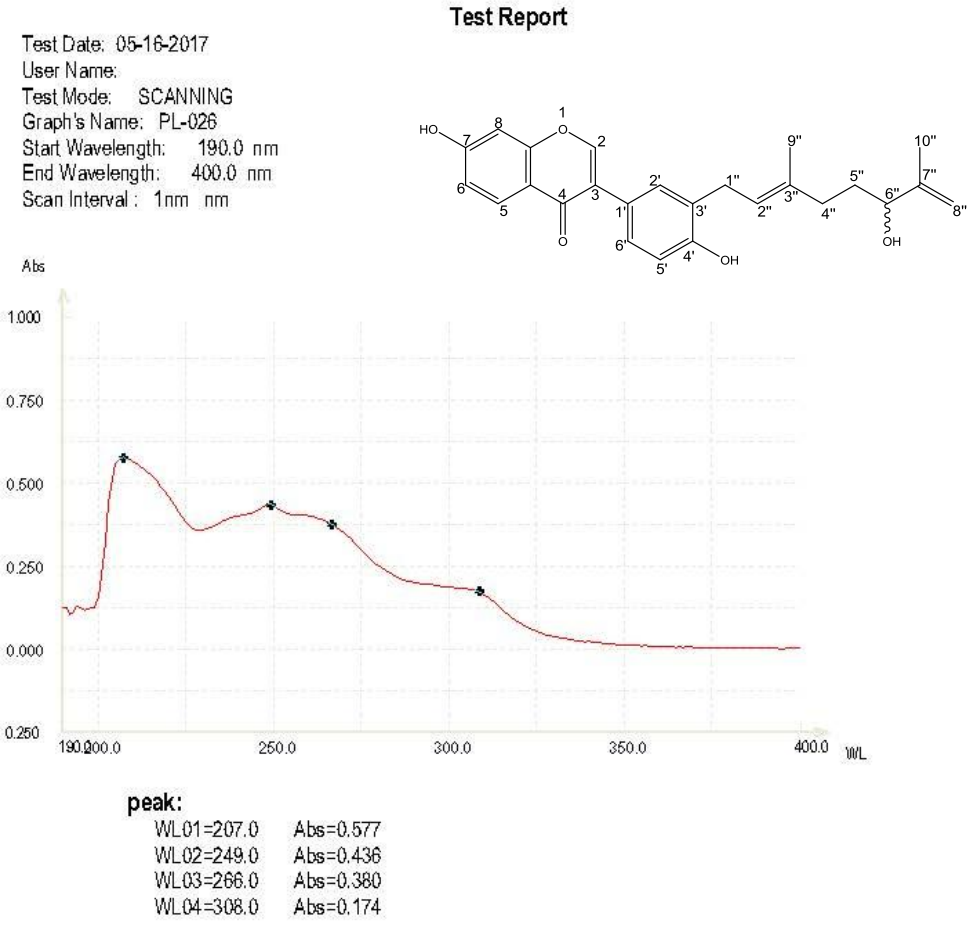
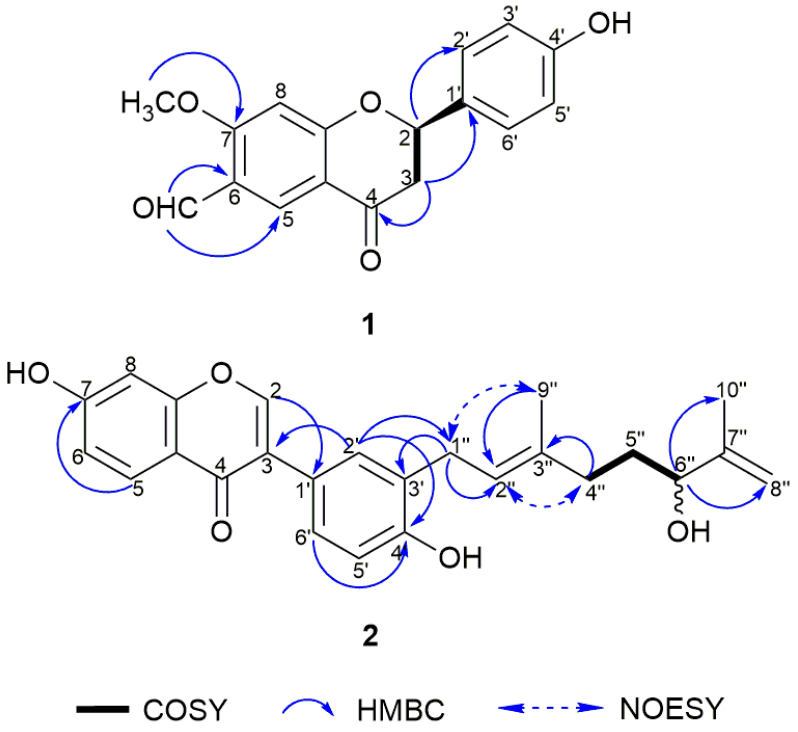


Figure S19. UV spectrum of compound **2**



1

2

Figure S20. Key ^{1}H-^{1}H COSY, HMBC, and NOESY correlations of compounds **1** and **2**.