SUPPLEMENTARY MATERIAL

A new sexangularetin derivative from *Camellia hakodae* Ninh.

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A new flavonoid, sexangularetin 3-*O*-(2”-*O*-(*E*)-*p*-coumaroyl-*β*-D-glucopyranoside) (**1**), together with nine known flavonoid compouds (**2-10**), were isolated from flowers of *Camellia hakodae* Ninh. Their structured were established based on physicochemical and spectroscopic analyses. The new compound displayed moderate to weak cytotoxic activities against HepG2 (IC_{50} 192.00 μg/mL), Lu (IC_{50} 90.18 μg/mL), and KB (IC_{50} 72.72 μg/mL) cell lines, and inactivity against MCF7 (IC_{50} 256.00 μg/mL).

Keywords: sexangularetin 3-*O*-(2”-*O*-(*E*)-*p*-cumaroyl-*β*-D-glucopyranoside), *Camellia hakodae* Ninh., flavonoid, cytotoxic activity.

Spectroscopy of the compound **1**

Figure S1. HRESIMS of Compound **1**

Figure S2. ^{1}H NMR Spectrum of Compound **1** (500 MHz, MeOD)

Figure S3. ^{13}C NMR Spectrum of Compound **1** (125 MHz, MeOD)

Figure S4. DEPT Spectra of Compound **1** (125 MHz, MeOD)

Figure S5. COSY Spectrum of Compound **1** (500 MHz, MeOD)

Figure S6. HSQC Spectrum of Compound **1** (500 MHz, MeOD)

Figure S7. HMBC Spectrum of Compound **1** (500 MHz, MeOD)

Figure S8. NOESY Spectrum of Compound **1** (500 MHz, MeOD)

Biological assay

Table S1. *In vitro* cytotoxic activity of compound **1** against four human cell lines [Lung (Lu), breast (MCF7), epidermoid (KB) and hepatoma (HepG2)].

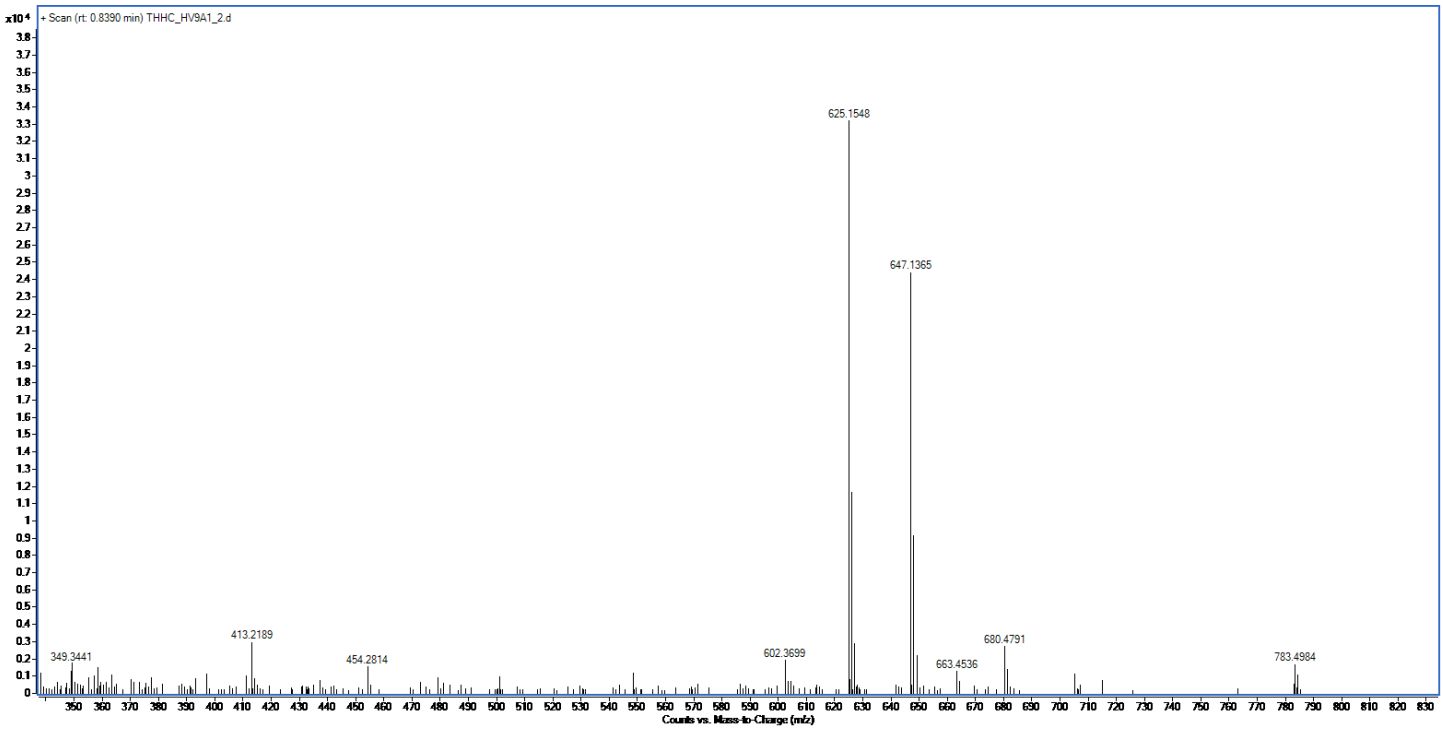

Figure S1**.** HRESIMS of Compound **1**

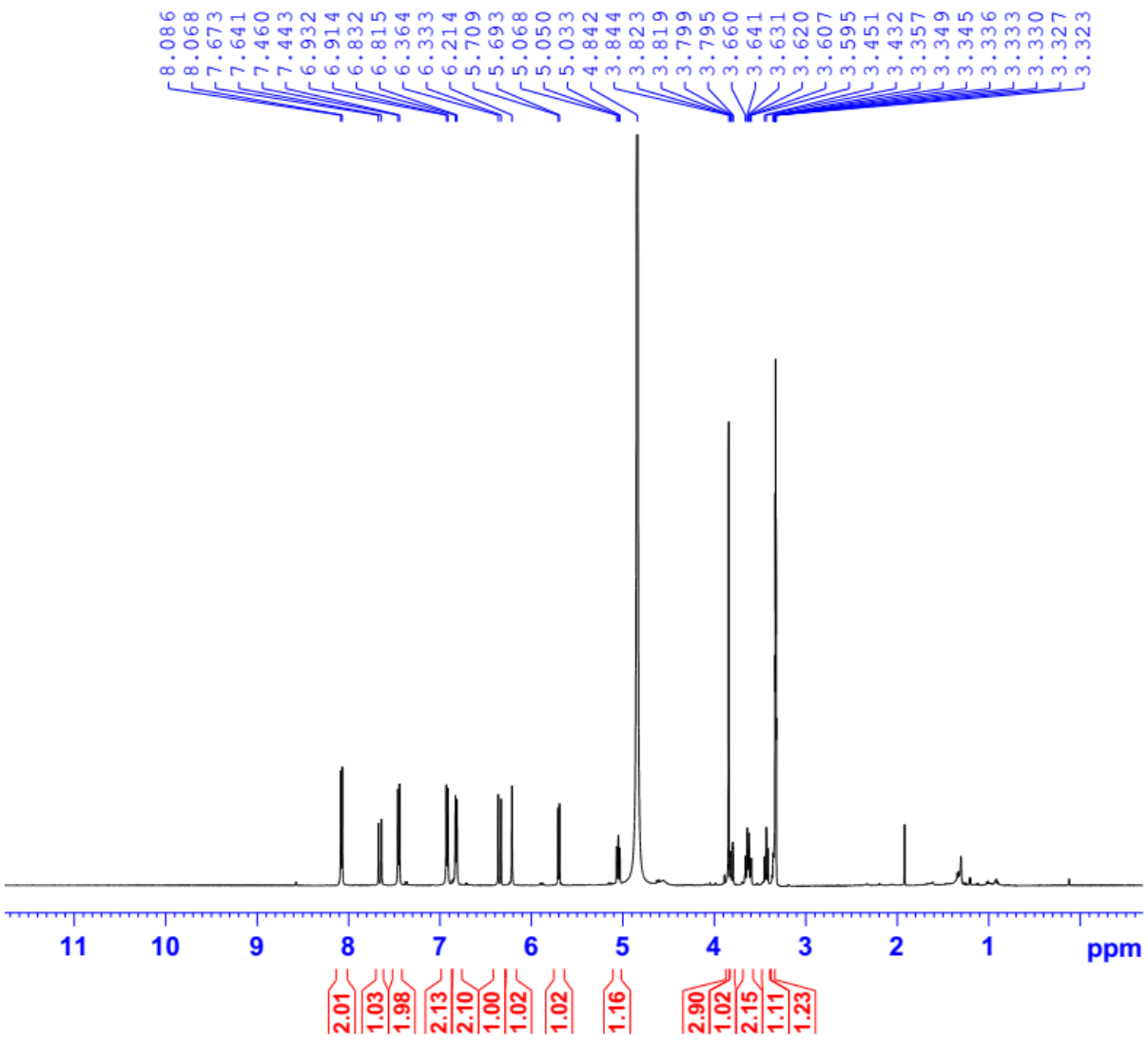

Figure S2. ^{1}H NMR Spectrum of Compound **1** (500MHz, MeOD)

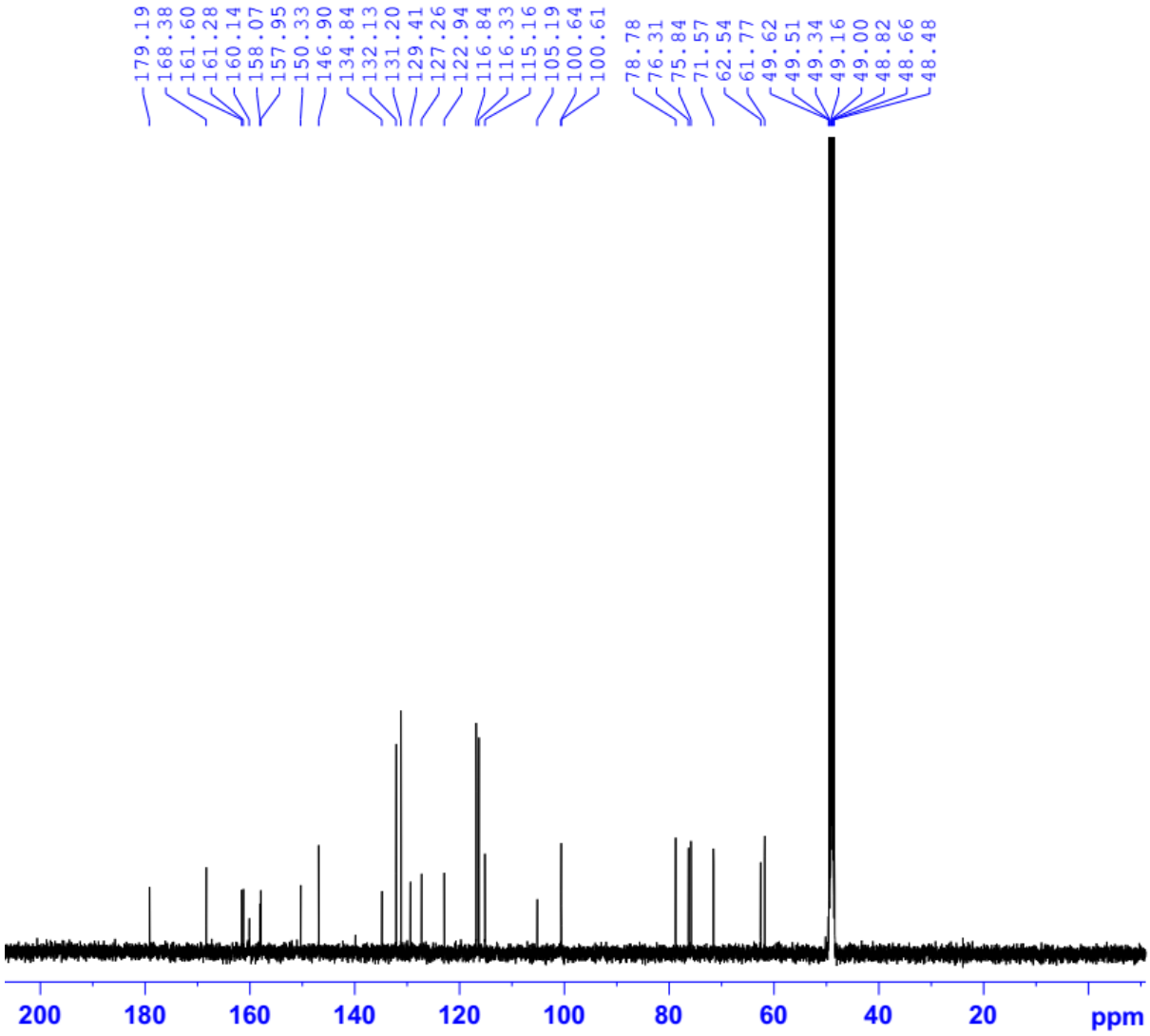

Figure S3. ^{13}C NMR Spectrum of Compound **1** (125 MHz, MeOD)

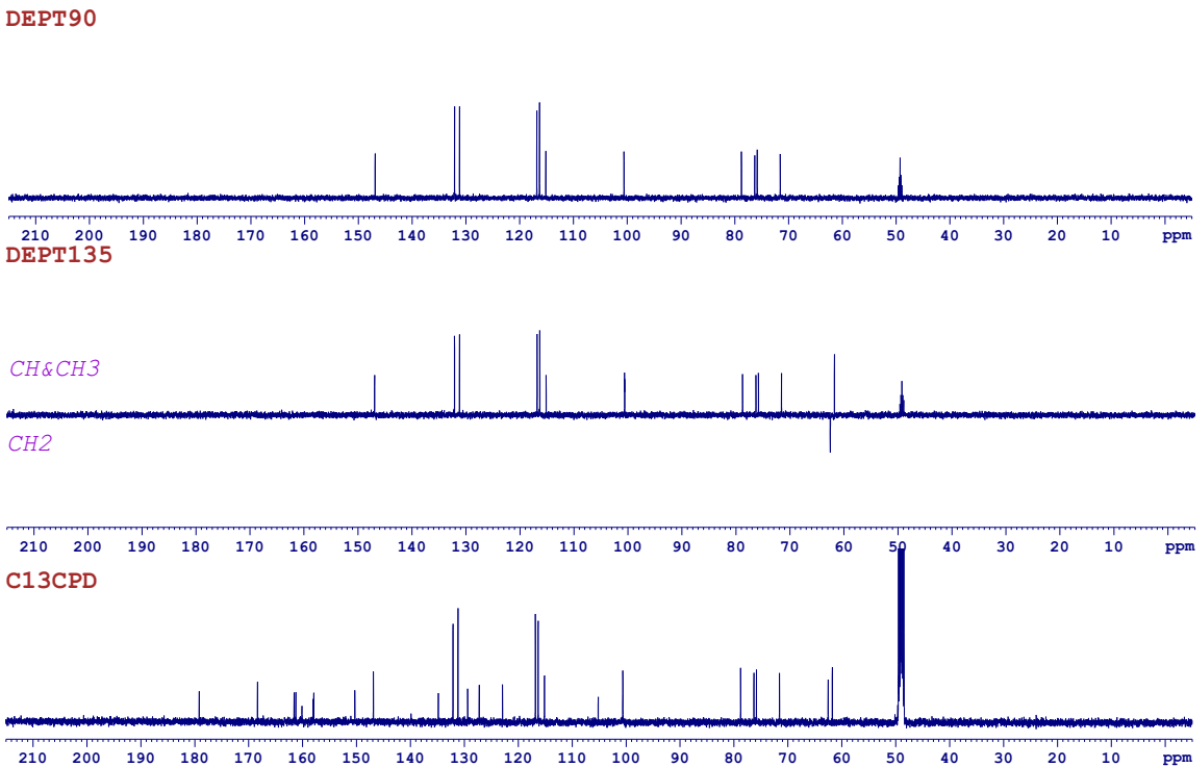

Figure S4. DEPT Spectra of Compound **1** (125 MHz, MeOD)

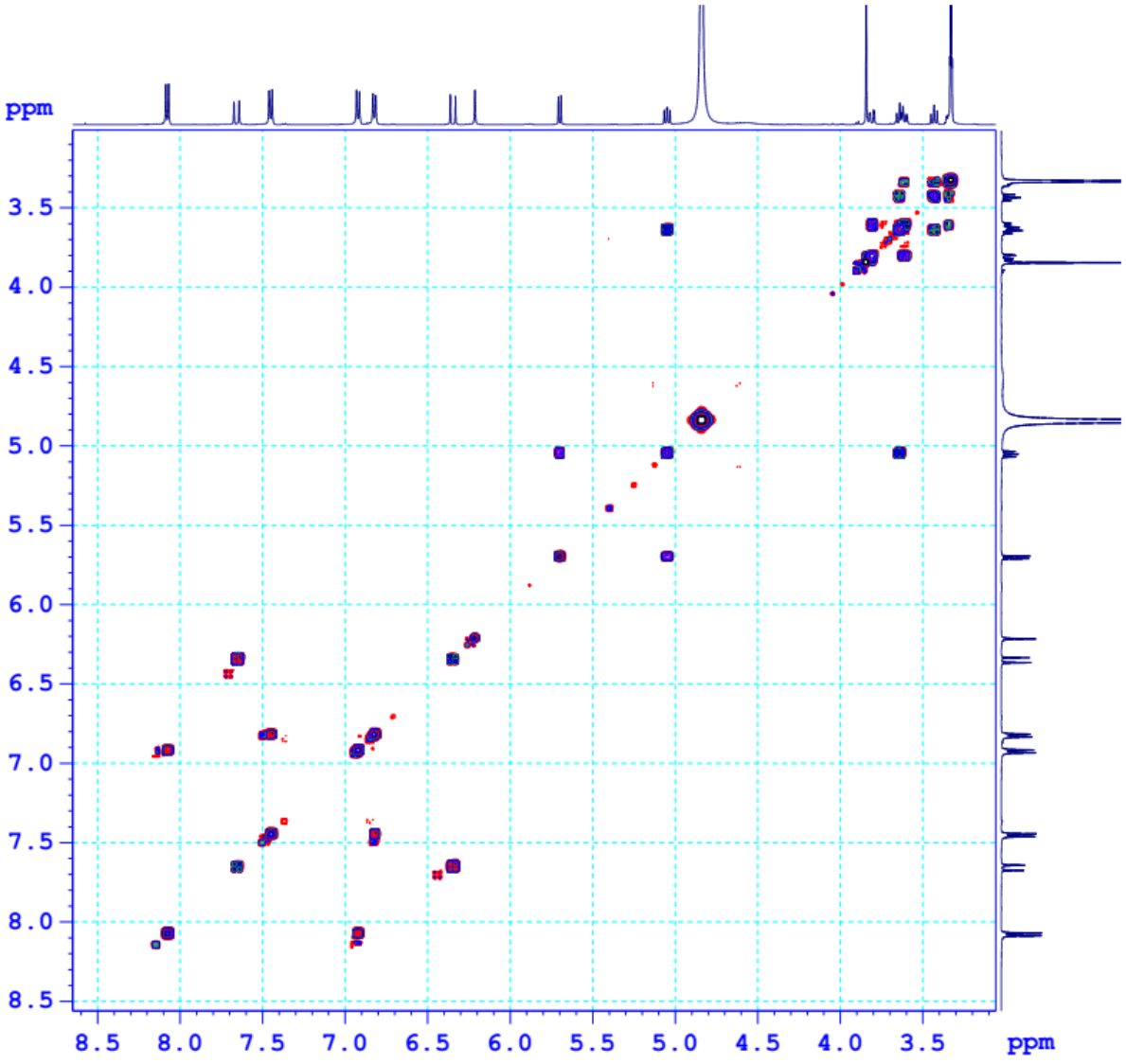

Figure S5. COSY Spectrum of Compound **1** (500MHz, MeOD)

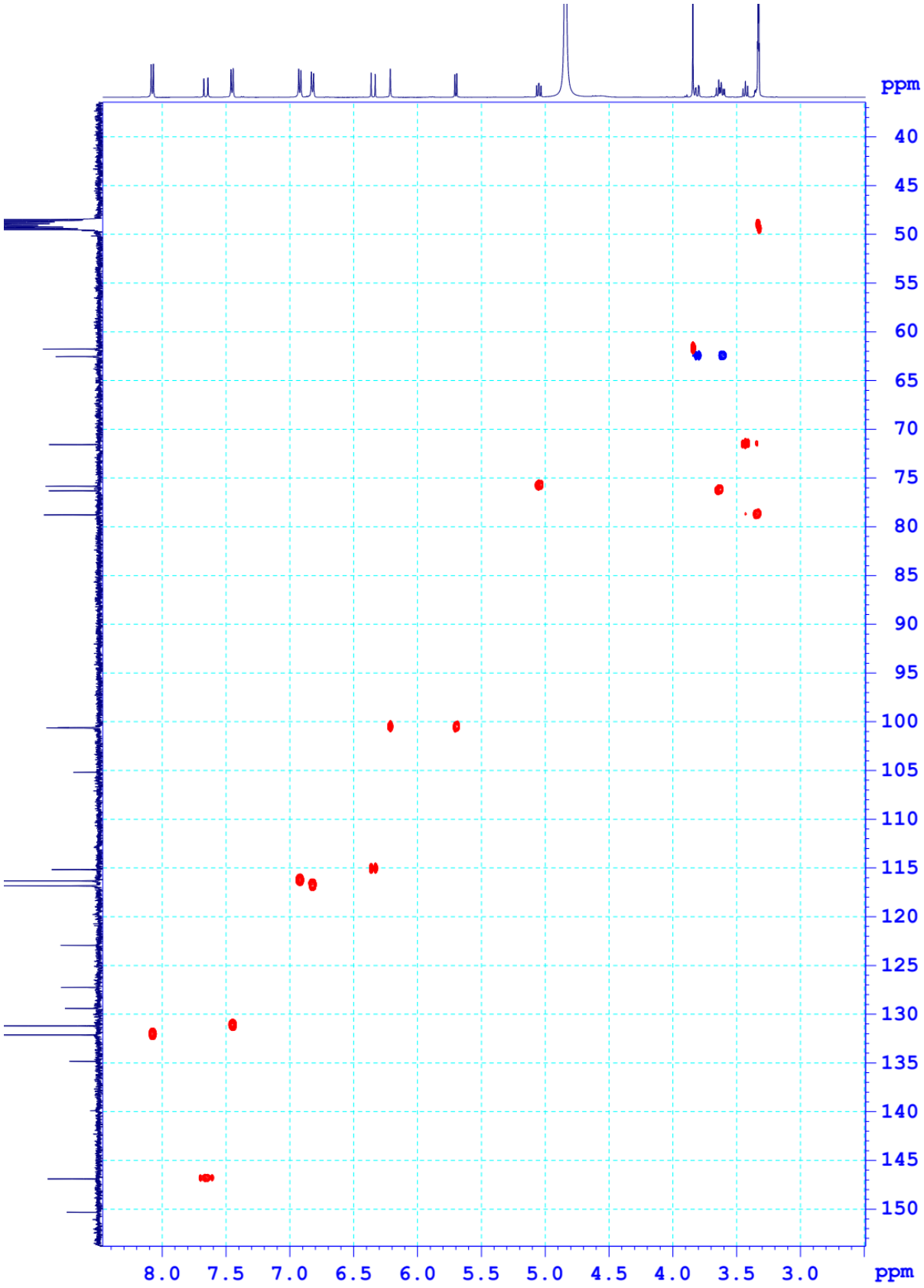

Figure S6. HSQC Spectrum of Compound **1** (500MHz, MeOD)

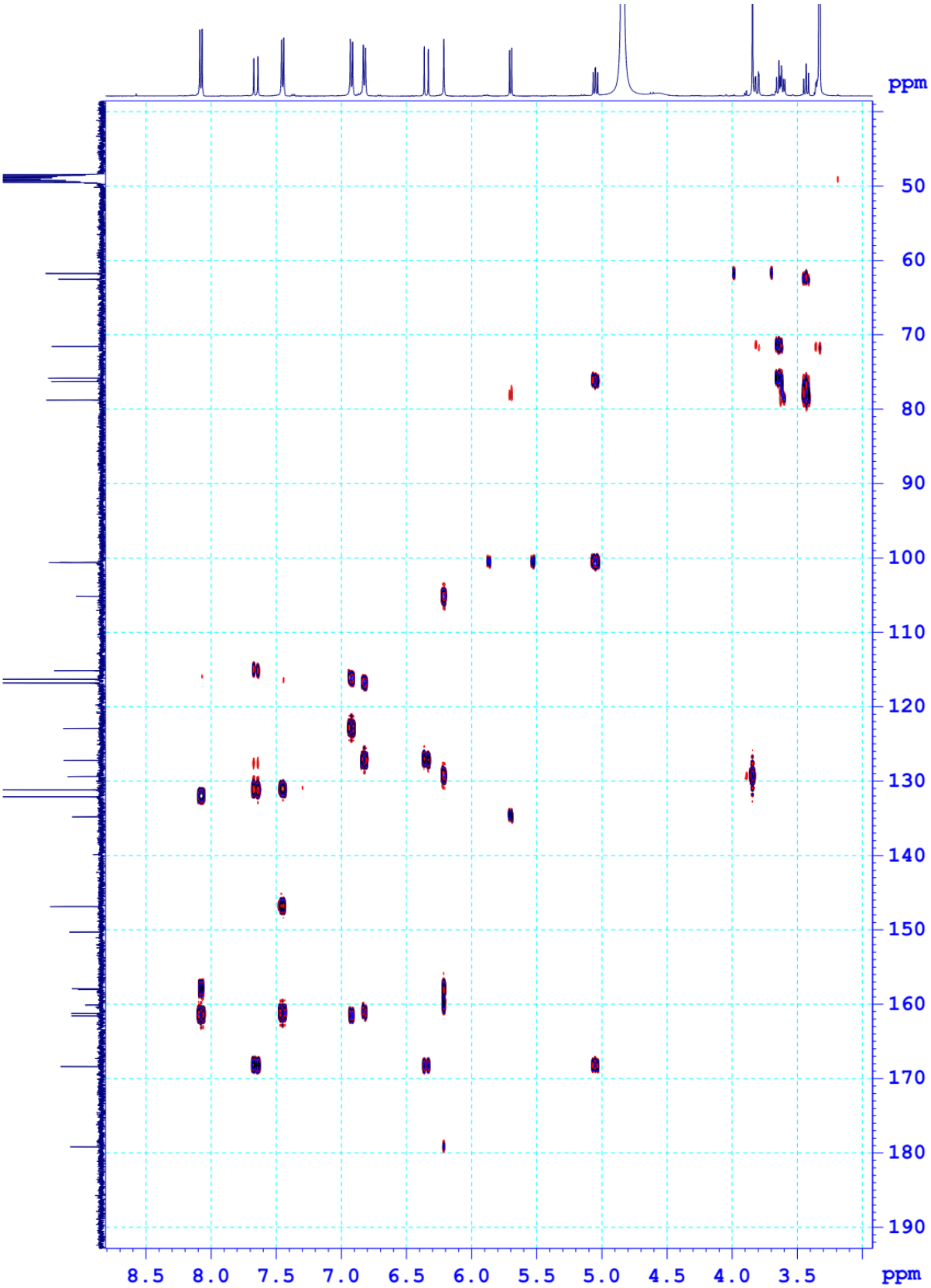

Figure S7. HMBC Spectrum of Compound **1** (500MHz, MeOD)

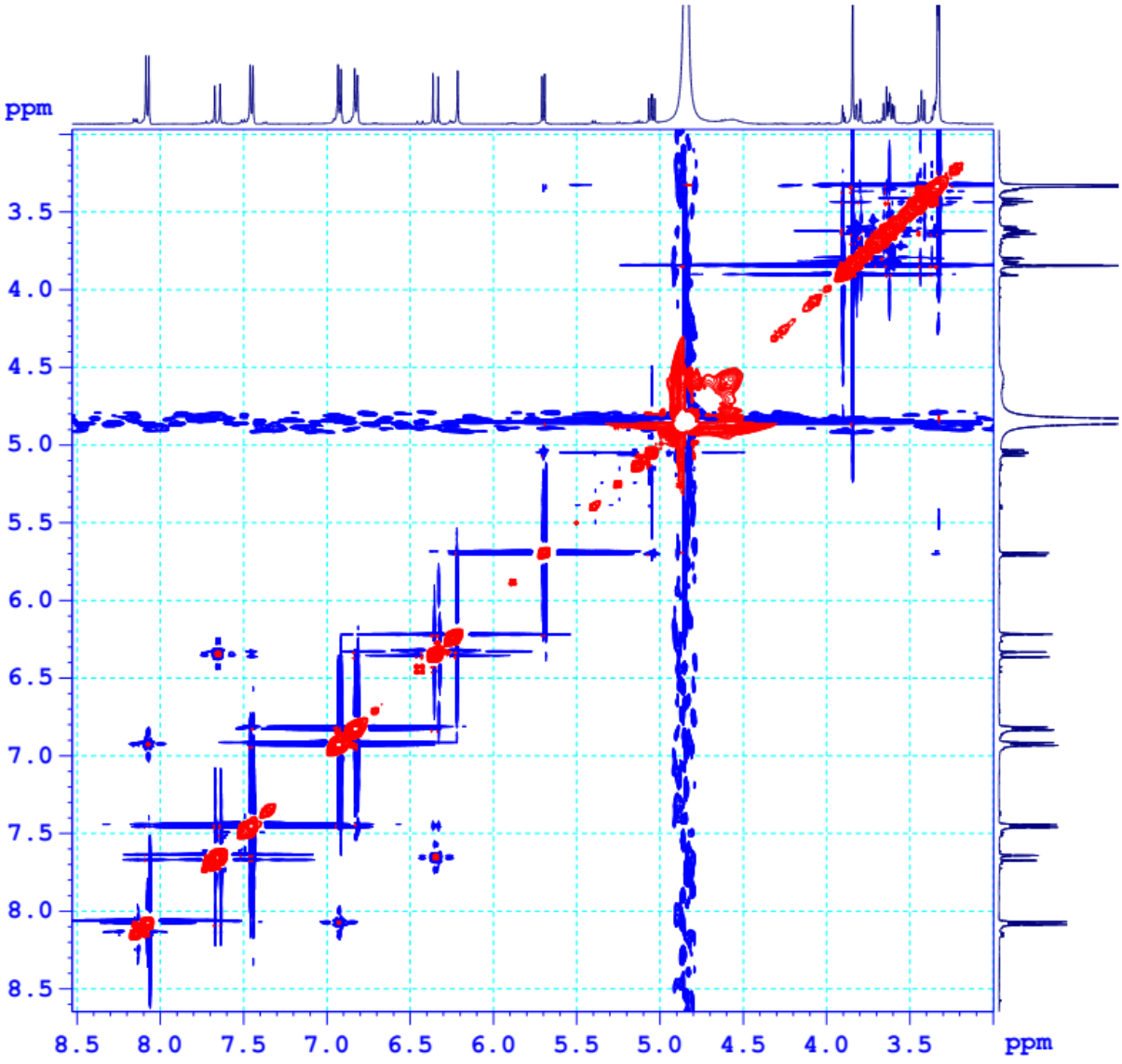

Figure S8. NOESY Spectrum of Compound **1** (500MHz, MeOD)

Biological assay

Cell lines and chemicals

Human cancer cell lines consisting of lung (Lu), breast (MCF7), epidermoid (KB) and hepatoma (HepG2) were obtained from American Type Culture Collection (ATCC), Manassas, United State of America. 2,2-Diphenyl-1-picryl-hydrazyl (DPPH), Ascorbic acid (AA), Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT salt) and Ellpiticine were purchased from Sigma and Fluka, Germany.Other chemicals were of analytical grade.

Cytotoxicity assay

Four human cancel cell lines including MCF7 (breast carcinoma cell line), KB (epidermoid carcinoma cell line), Lu (lung cancer cell line), HepG2 (hepatoma carcinoma cell line) were obtained from the ATCC (Manassas, VA). They were grown in medium RPMI 1640 supplemented with 10% FBS (Fetal bovine serum), 50 IU/ml penicillin and 50 µg/ml streptomycin. All the cell lines were maintained at 37°C in a 5% CO_2 atmosphere with 98% humidity.

The MTT assay is based on the protocol described by Tim Mossmann[1]. The test sample (crude extract and compound), dissolved in DMSO with five concentrations (1.0, 4.0, 16.0, 64.0, and 256.0 µg/mL), were added into the available culture cells and culture plates were incubated for 3 days. After the exposure times, the culture cells were treated with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] while OD was measured at 540 nm absorbance on microplate reader (TECAN GENIOUS). Ellpiticine dissolved in DMSO (2 mg/mL), were used as positive control. The inhibitory concentration (IC) values were calculated by formula:

$$IC_{50} = \frac{OD_{control} - OD_{test}}{OD_{control}} \times 100 \qquad (1)$$

IC_{50} is the concentration in µg/mL required for 50% inhibition of cell growth as compared to that of untreated control.

Table S1. *In vitro* cytotoxic activity of compound **1** against four human cell lines [Lung (Lu), breast (MCF7), epidermoid (KB) and hepatoma (HepG2)].

Sample	IC_{50}			
	KB	HepG2	Lu	MCF7
1	72.72	192	90.18	256
Ellipticine	0.27	0.30	0.35	0.48

References

[1] Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods*. 1983;65(1-2):55-63.