SUPPLEMENTARY MATERIAL

Polyacetylene and phenolic constituents from the roots of *Codonopsis javanica*

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Abstract

Chemical investigation of the roots of *Codonopsis javanica* resulted in isolation of 12 compounds, including one new polyacetylene, codojavanyol (**1**), one new phenolic glycoside, codobenzyloside (**7**), and 10 known compounds, (2*E*,8*E*)-9-(tetrahydro-2*H*-pyran-2-yl)nona-2,8-diene-4,6-diyl-1-ol (**2**), lobetyol (**3**), lobetyolin (**4**), lobetyolinin (**5**), cordifolioidyne B (**6**), benzyl-α-L-arabinopyranosyl (1-6)-β-D-glucopyranoside (**8**), (*Z*)-8-β-D-glucopyranosyloxycinnamic acid (**9**), syringin (**10**), syringaresinol (**11**), and tryptophan (**12**). Their structures were elucidated by 1D and 2D NMR and MS spectroscopic analyses in comparison with the data reported in the literature. Of the isolates **1-9**, compounds **3-5** were shown to have weak cytotoxicity toward human carcinoma cell lines, including lung (A549), liver (HepG2), and breast (MCF7), with the induction of 41.4 to 55.6 % cell death at the concentration of 100 μM.

Keywords: *Codonopsis javanica*; *Campanumoea javanica*; Campanulaceae; polyacetylene; phenolic, cytotoxic

Experimental

1. General experimental procedures

Optical rotations were determined on a JASCO P-2000 polarimeter (Tokyo, Japan). UV spectra were recorded on a JASCO V-630 UV-VIS spectrophotometer (Jasco Inc., Japan). FT-IR spectra were obtained using a PerkinElmer spectrometer (L1600400 Spectrum Two DTGS, Perkin Elmer Inc., USA). HR-QTOF-MS were recorded on an Agilent 6530 Accurate-Mass spectrometer (CA, USA). The NMR spectra were recorded on a Bruker AVANCE III HD 500 FT-NMR spectrometer (Bruker, Germany) with tetramethylsilane (TMS) as an internal standard. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck), YMC*Gel (ODS-A, 12 nm, S-150 mm, YMC Co., Ltd.), Sephadex® LH-20 (25-100 μm, Merck), and Diaion® HP-20 (250-850 μm, 260 Å, Merck) resins. TLC was used on pre-coated silica gel 60 F_{254} (Merck) and RP-18 F_{254S} plates (Merck) and compounds were visualized by spraying with aqueous 10% H_2SO_4 and followed by heating over 3–5 min.

2. Acid hydrolysis and sugar identification

Compound **7** (1.0 mg) was hydrolysed with 1.0 M HCl (1.0 mL) for 3 h at 90 °C. After extracting the reaction mixture with ethyl acetate, the aqueous layer was obtained and evaporated *in vacuo* to give a dried and neutral residue. The residue was dissolved in pyridine (0.5 mL) containing L-cysteine methyl ester hydrochloride (3.0 mg) and the mixture was incubated at 60 °C for 1 h. Phenylisothiocyanate (15.0 μL) was then added and the reaction mixture was further heated at 60 °C for 1 h using hot plate stirrer. The reaction mixture was subsequently analysed by comparing the retention times of derivatives of sugars obtained from the reaction mixture with those of standard samples using reversed-phase HPLC under the following conditions: detection wavelength: 250 nm, mobile phase: 25% acetonitrile-water (0.1% formic acid); and Agilent Eclipse XDB-C18 column (5 μM, 4.6 × 250 mm, U.S.A). The sugar units of **7** were identified by comparison with the following authentic samples: D-glucose (t_R = 14.75 min) and L-arabinose (t_R = 17.15 min).

3. Cytotoxic assay

Cancer cells were cultivated in a humidified atmosphere of 5% CO_2 at 37°C for 48 h. Cell viability was examined by SRB assay for cell density determination, based on the measurement of cellular protein content (Monks et al. 1991). Viable cells were seeded in the growth medium (180 μL) into

96 well microwell plates (4×10^4 cells per well) and allowed to attach overnight. Test samples were added carefully into well of 96-well plates and the cultivation was continued under the same conditions for another 72 h. Thereafter, the medium was removed and the remaining cell monolayers are fixed with the cold 20% (w/v) TCA for 1 h at 4°C and stained by 1X SRB staining solution at room temperature for 30 min, after which the unbound dye is removed by washing repeatedly with 1% (v/v) acetic acid. The protein-bound dye is dissolved in 10 mM Tris base solution for OD determination at 515 nm on an ELISA Plate Reader (Bio-Rad). DMSO 10% was used as blank sample while ellipticine was used as positive control. The cytotoxicity was indicated as half inhibition concentration (IC_{50}), which was calculated by program TableCurve Version 4.0 from concentration range 100 µg/ml; 20 µg/ml; 4 µg/ml; 0.8 µg/ml. The experiment was prepared in triplicate. The inhibition rate (IR) of cells was calculated by the following formula IR% = [100% – [(absorbance$_t$ – absorbance$_0$)/(absorbance$_c$ – absorbance$_0$)] × 100], in which: IR: Inhibition rate of cell growth, absorbance$_t$: average optical density value at day 3; absorbance$_0$: average optical density value at time-zero; absorbance$_c$: average optical density value of the blank DMSO control sample.

Table S1. Cytotoxic effects of **1-9** (at 100 µM) toward A549, HepG2, and MCF7 cancer cells

Cell lines	Cell death (%)									Ellipticine* (IC_{50} = µM)
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	
A549	34.1	51.2	55.6	52.0	55.0	35.2	9.5	22.2	36.7	1.54 ± 0.02
HepG2	16.1	21.9	52.9	43.8	45.2	33.4	15.2	14.3	31.8	1.58 ± 0.04
MCF7	10.9	15.3	48.5	41.4	47.6	32.3	12.7	14.2	30.6	1.62 ± 0.03

* *Positive control*

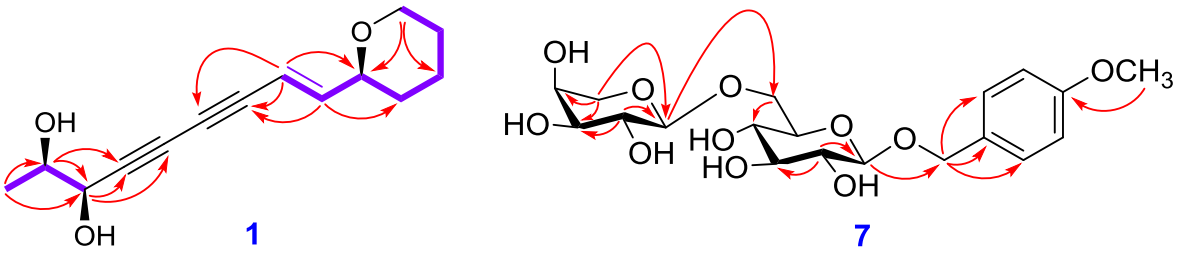

Figure S1. Selected HMBC and COSY correlations of **1** and HMBC cross-peaks of **7**

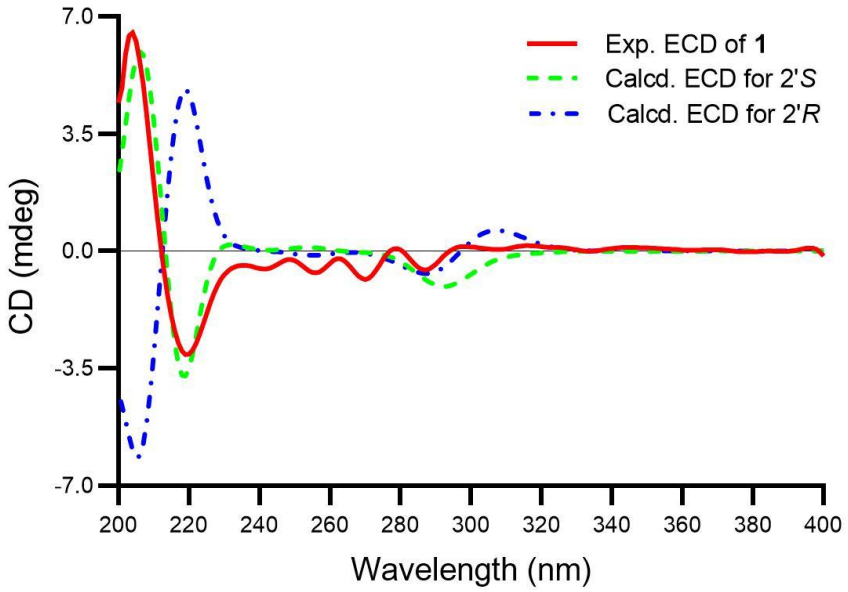

Figure S2. The experimental and calculated ECD spectra for compound **1**

3. HRESITOFMS and NMR spectra for compounds 1 and 7

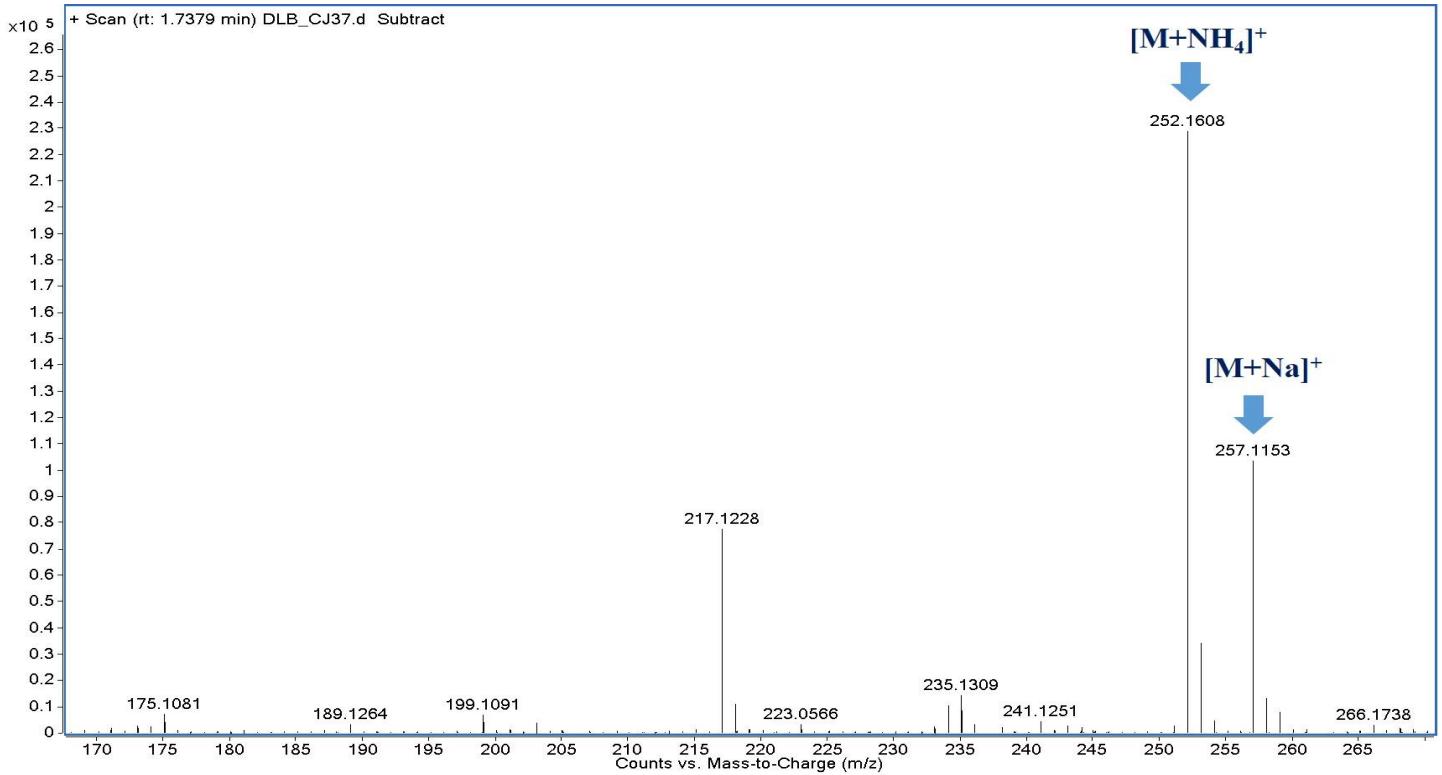

Figure S3. HRESITOF mass spectrum of compound **1**

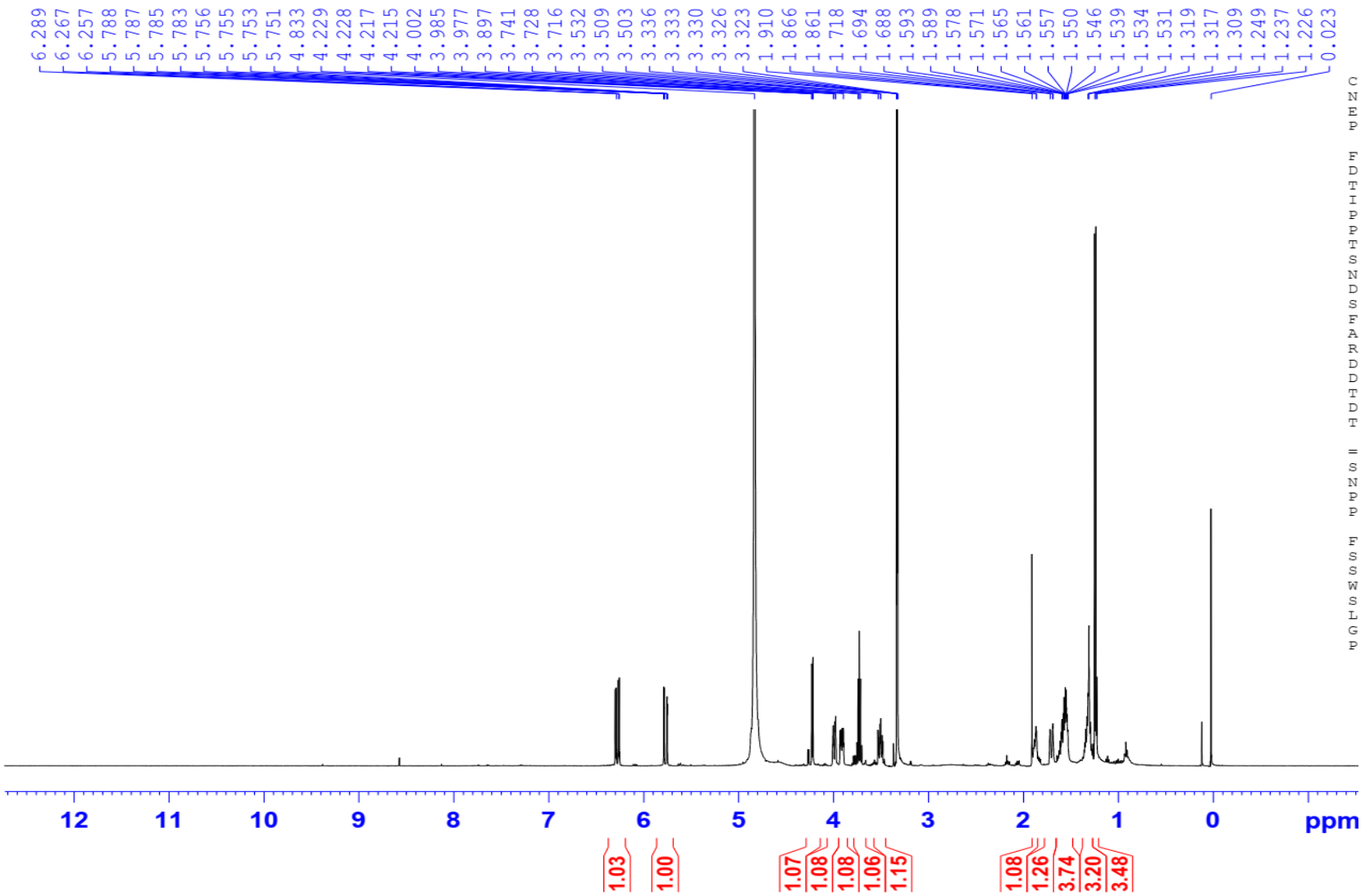

Figure S4. ^{1}H NMR spectrum (CD_3OD, 500 MHz) of compound **1**

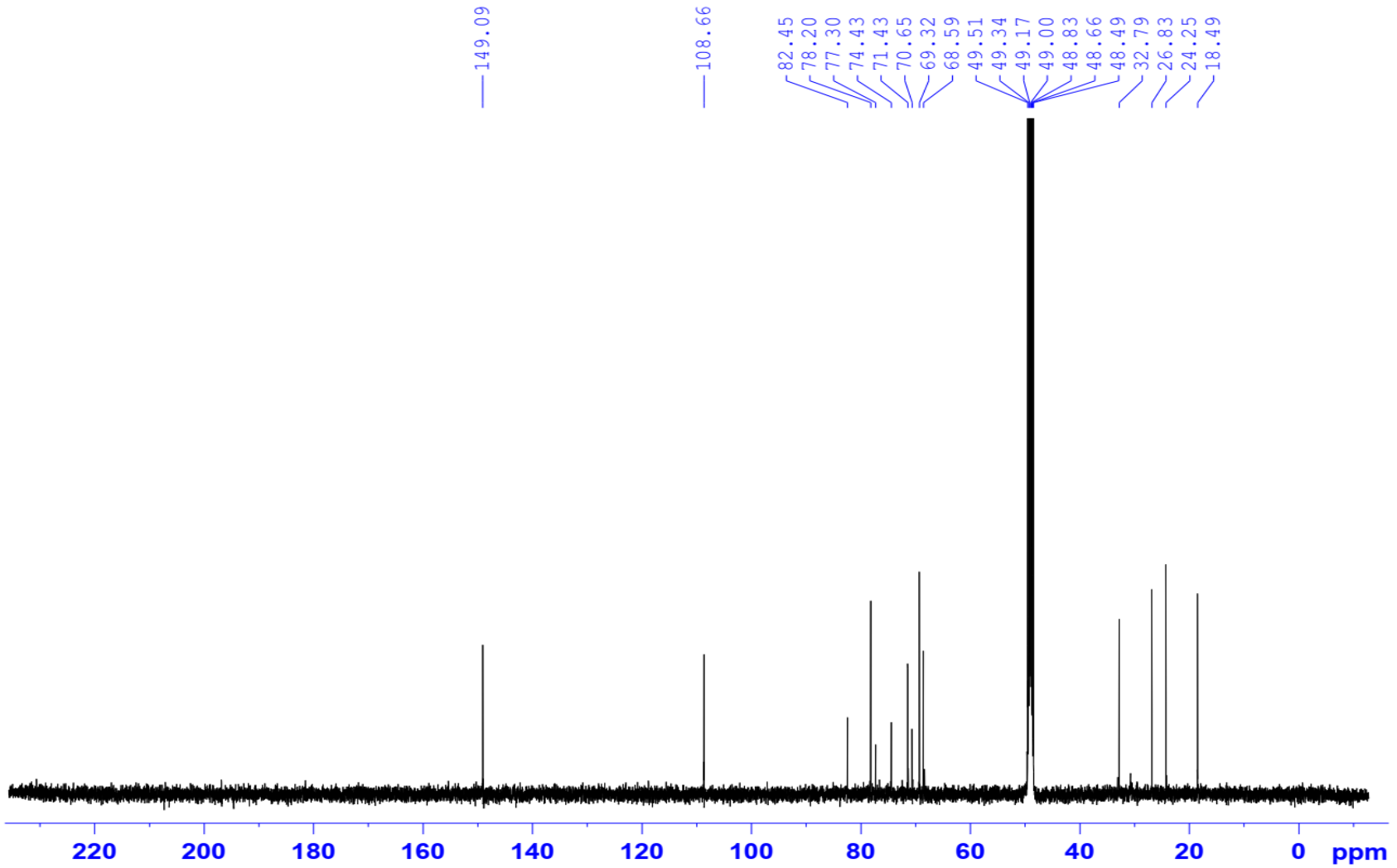

Figure S5. ^{13}C NMR spectrum (CD_3OD, 125 MHz) of compound **1**

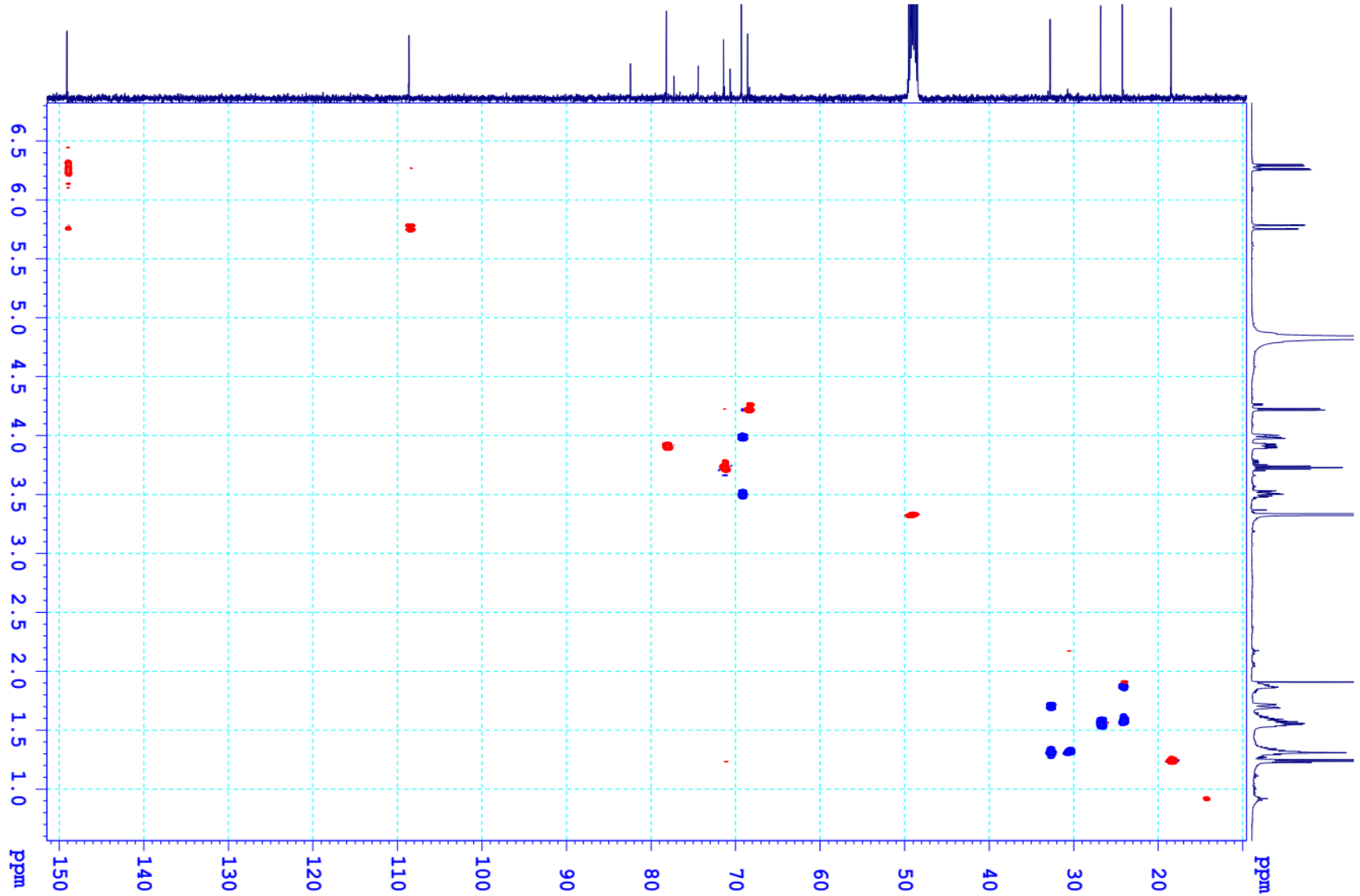

Figure S6. HSQC spectrum (CD_3OD, 500 MHz) of compound **1**

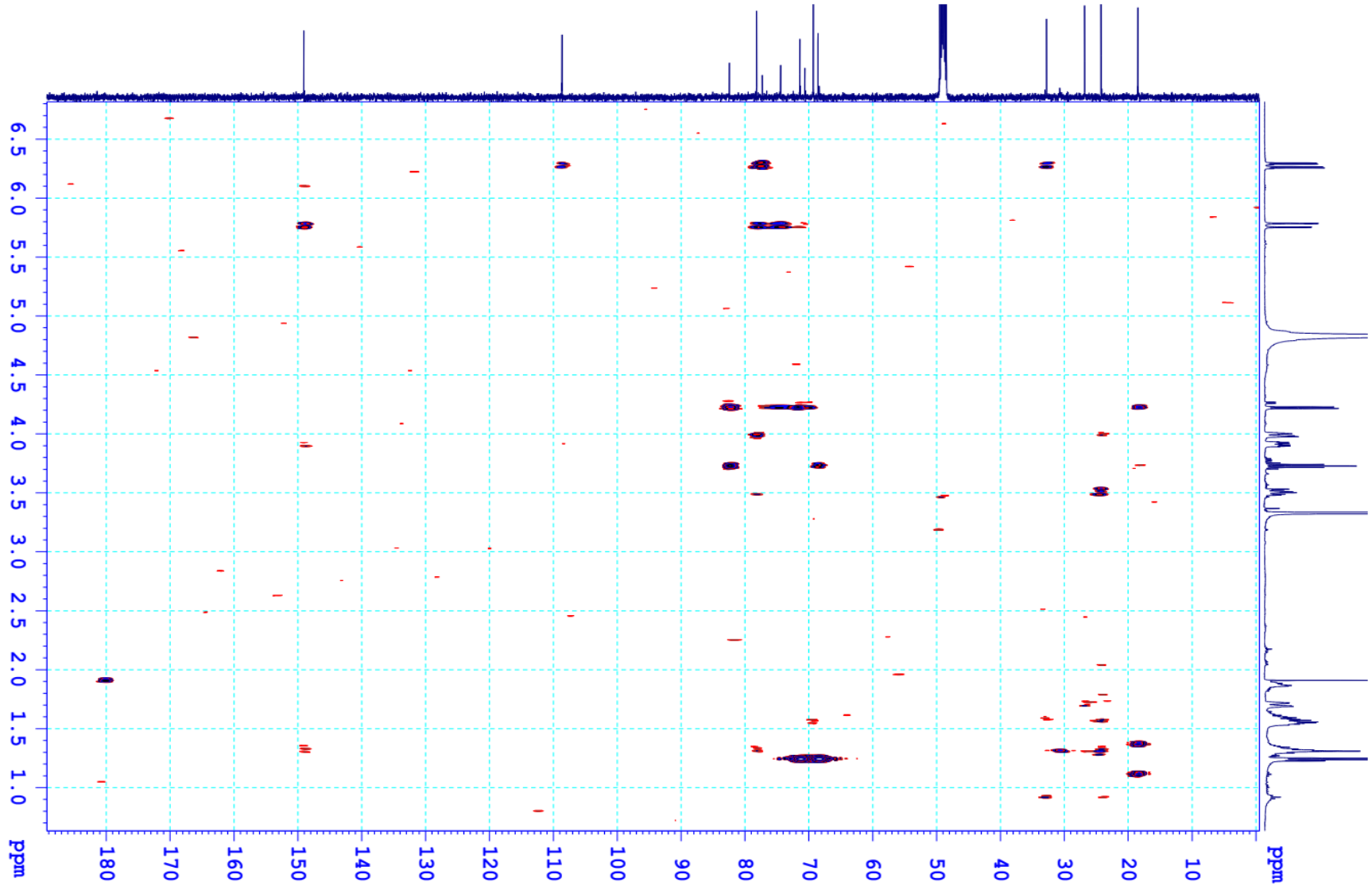

Figure S7. HMBC spectrum (CD_3OD, 500 MHz) of compound **1**

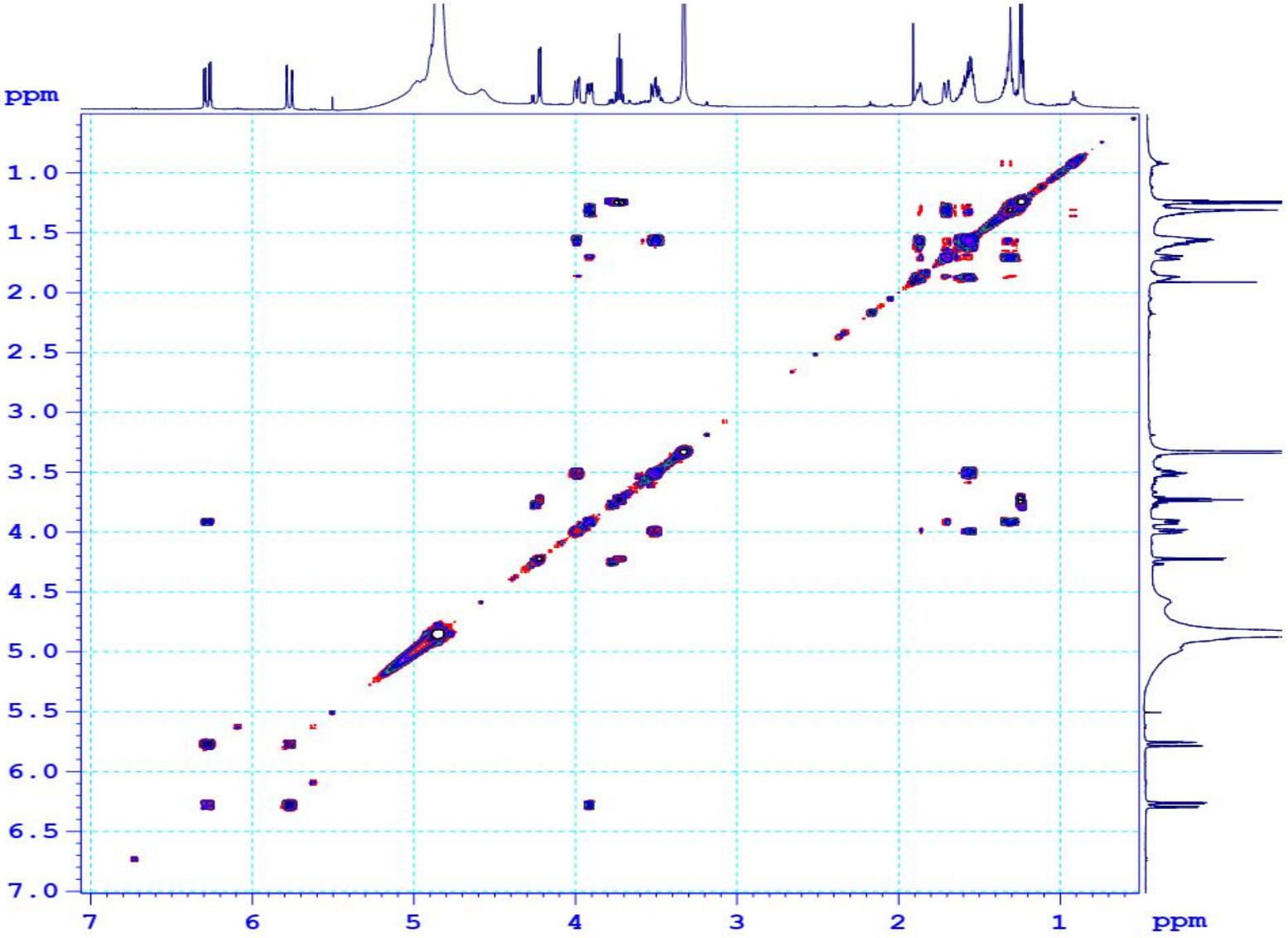

Figure S8. COSY spectrum (CD_3OD, 500 MHz) of compound **1**

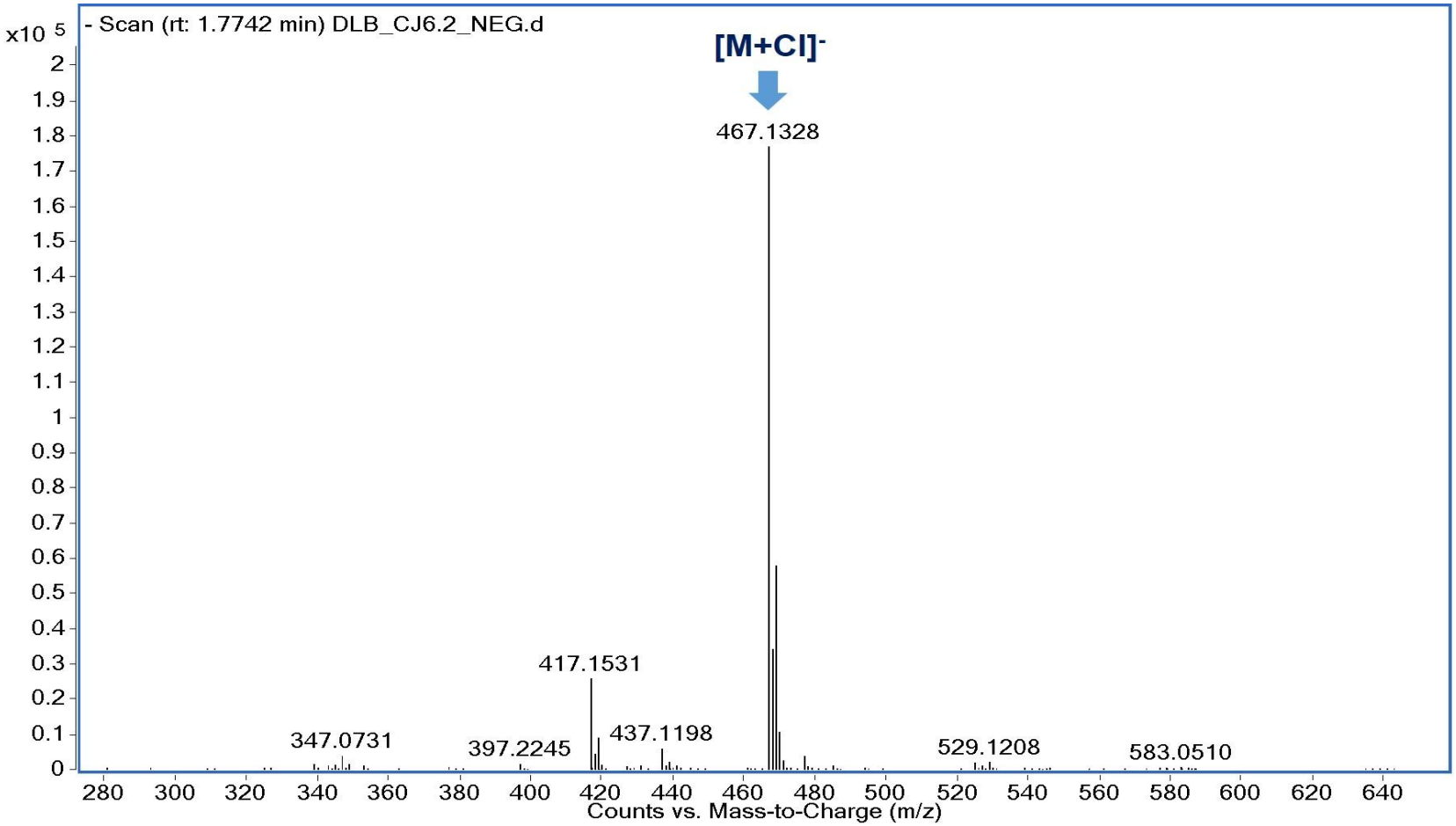

Figure S9. HRESITOF mass spectrum of compound **7**

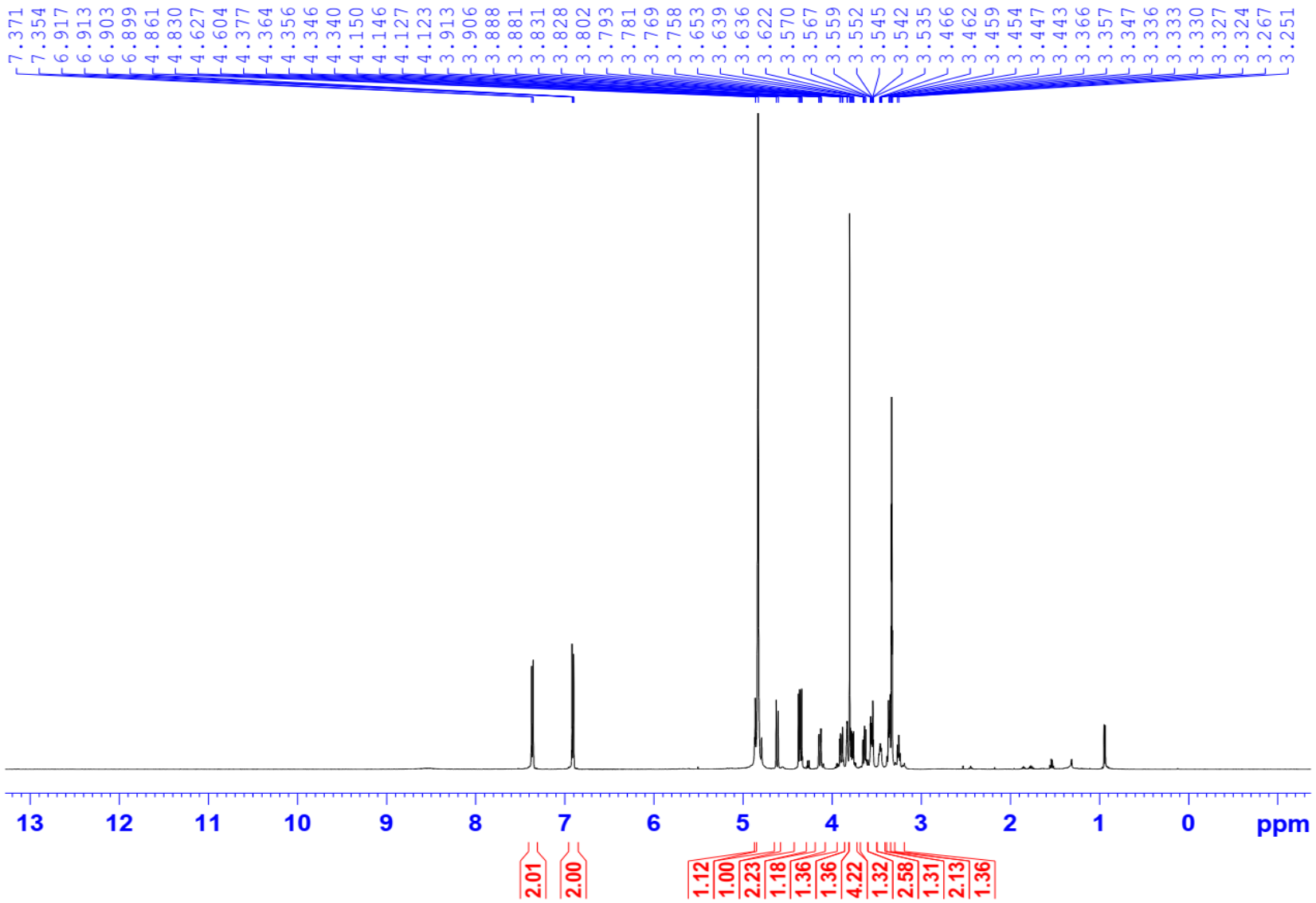

Figure S10. ^{1}H NMR spectrum (CD_3OD, 500 MHz) of compound **7**

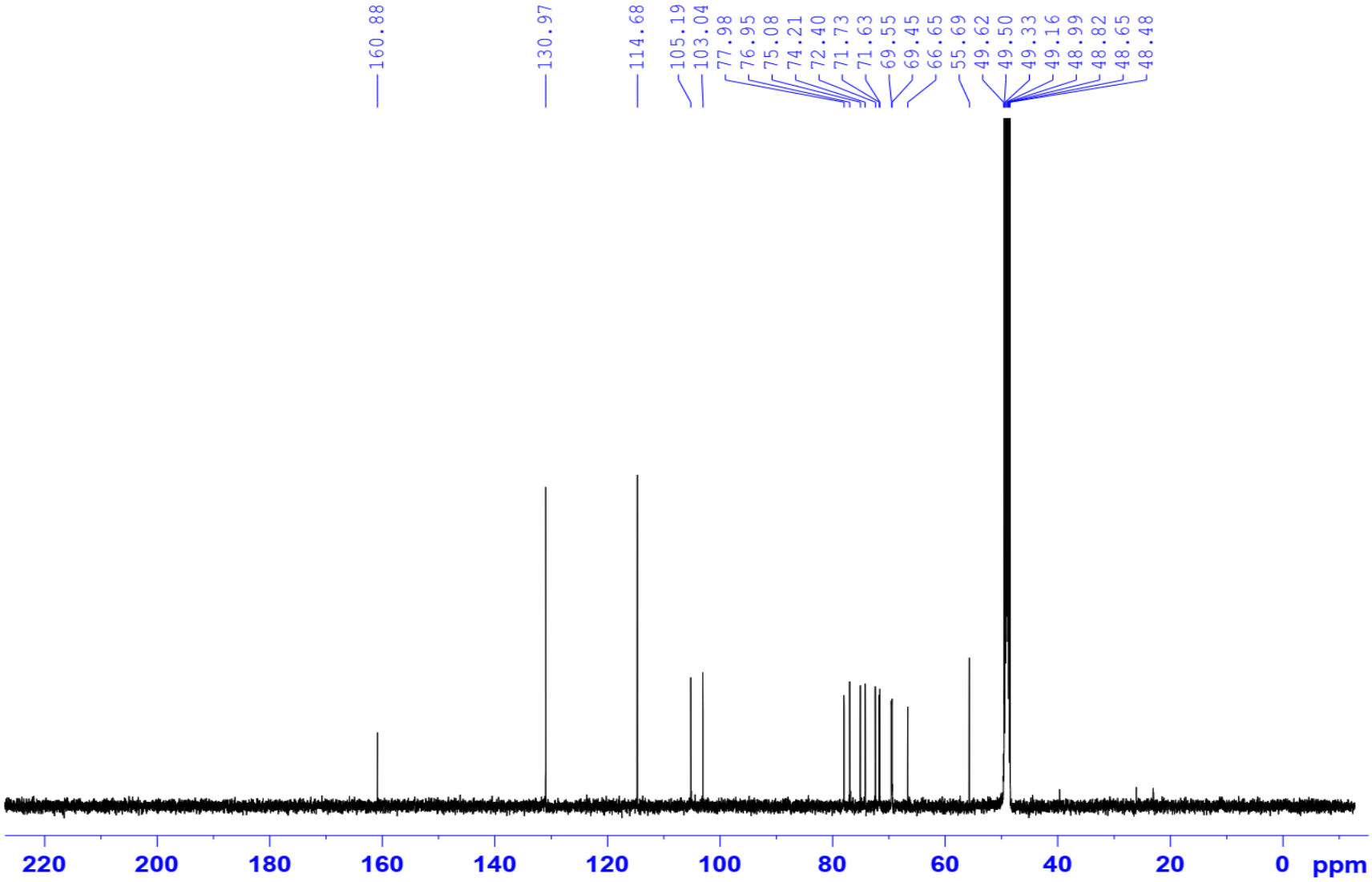

Figure S11. ^{13}C NMR spectrum (CD_3OD, 125 MHz) of compound **7**

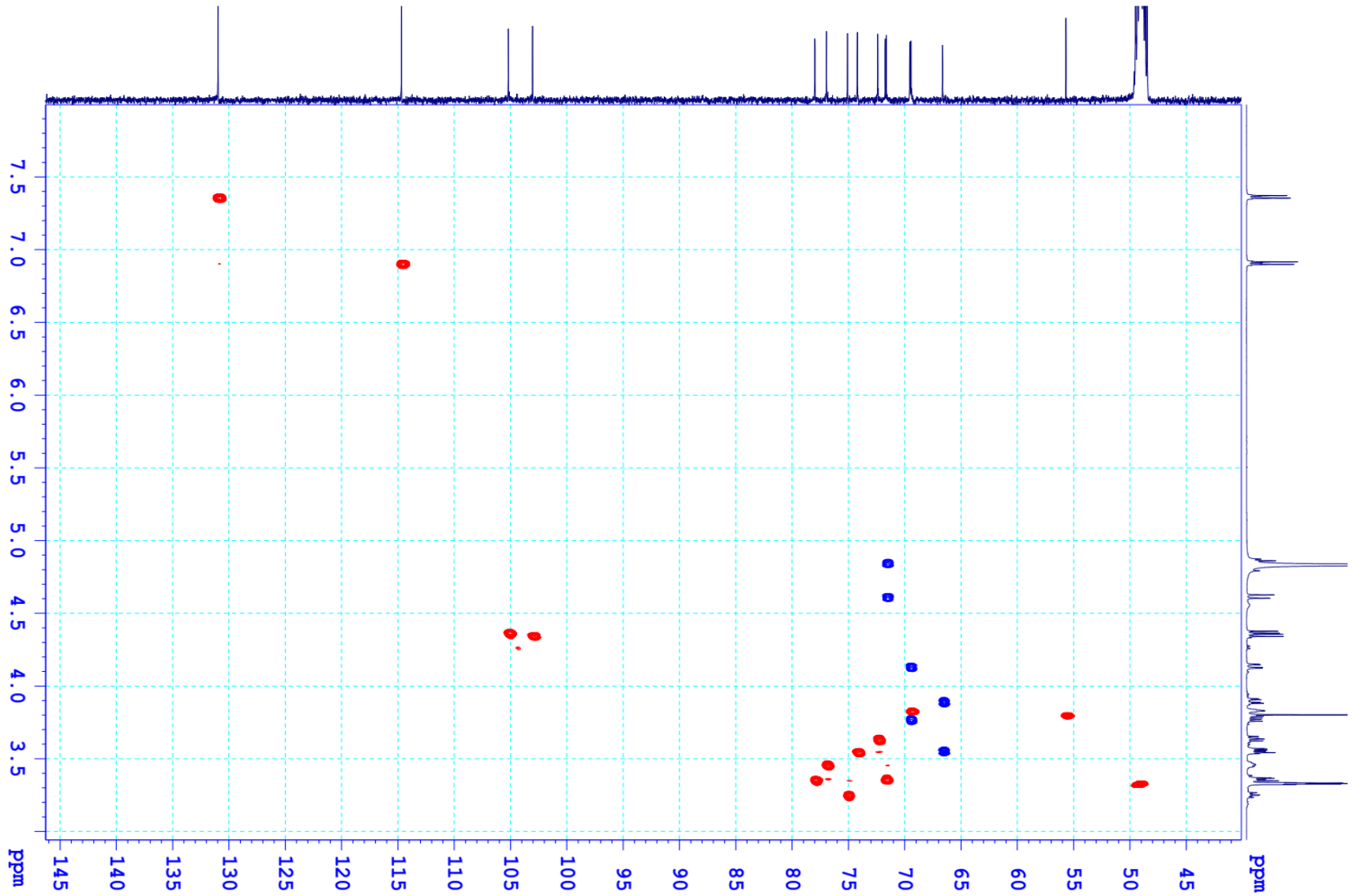

Figure S12. HSQC spectrum (CD_3OD, 500 MHz) of compound **7**

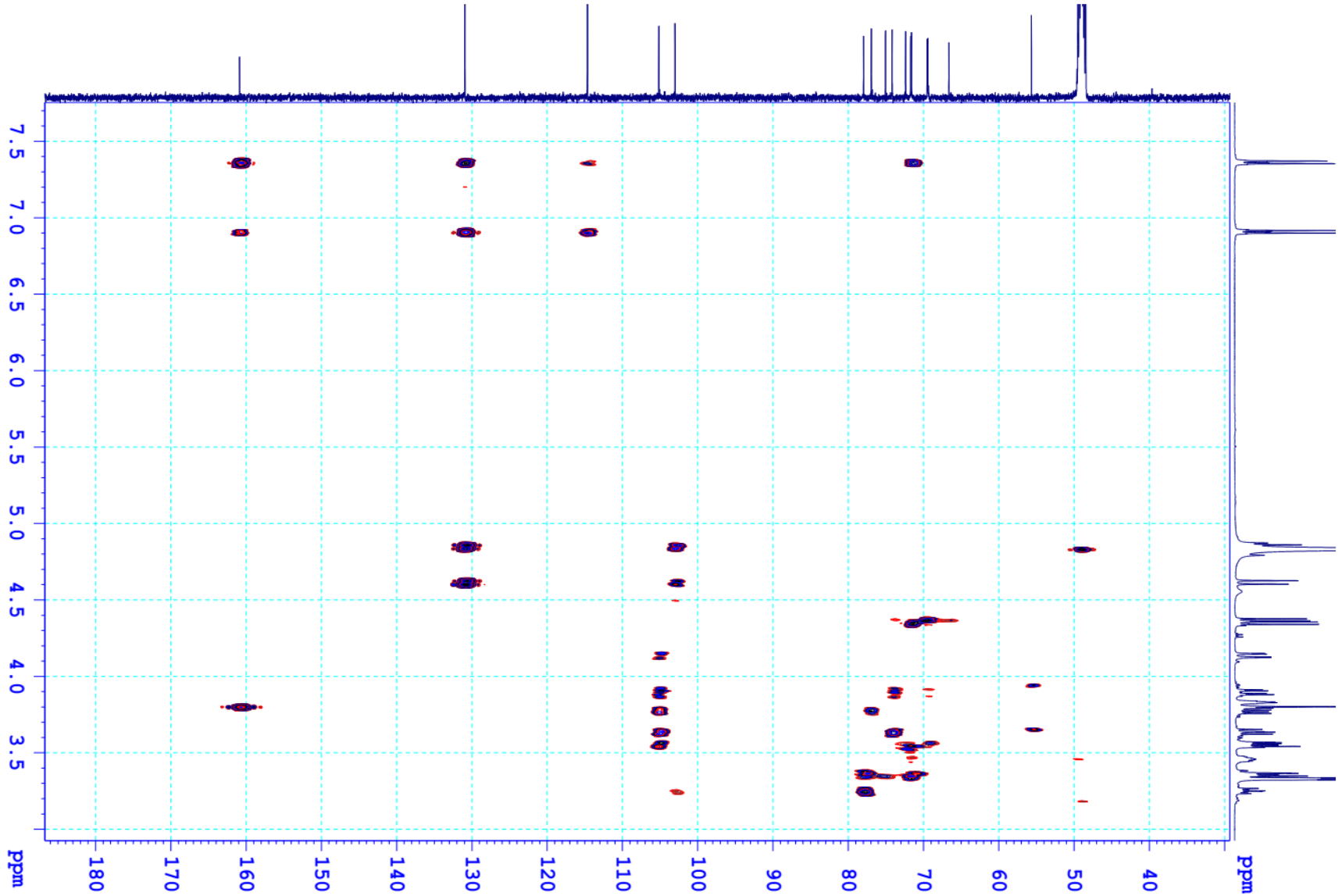

Figure S13. HMBC spectrum (CD_3OD, 500 MHz) of compound **7**

References

Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-Wolff A, et al. 1991. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. J Natl Cancer Inst. 83(11):757-766.