Supplementary Materials for

Cytotoxic Secondary Metabolites from Mangrove-Rhizosphere-Associated Fungus *Emericella* sp. Strain SWR1718

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ABSTRACT

Chemical exploration of mangrove-rhizosphere-associated fungus *Emericella* sp. strain SWR1718 (Aspergillaceae) was performed through various chromatographic workup procedures. The achieved results afforded one new natural compound, emericelactone E (**1**) in addition to known compounds (**2-7**). The planar structures of the purified compounds were unambiguously carried out using several spectroscopic methods. Both relative and absolute configurations of compound **1** were carefully determined based on NOESY experiments, coupling constants and comparing its optical rotation with related congeners. Moreover, a plausible biosynthetic pathway of **1** and its related derivatives is reported for the first time in our study. The cytotoxic potential of isolated metabolites was assessed toward three human tumor cell lines where some of them exhibited moderate activities compared to paclitaxel as a standard anticancer.

Keywords: *Emericella*; mangrove; emericelactone; cytotoxic activity.

Cytotoxicity assay

Cytotoxicity of all isolated compounds were measured against the following cell lines; HTB-176 human lymphoma (ATCC, HTB-176), SW-620 human colon cancer (ATCC, CCL-227), and HT-29 human colorectal adenocarcinoma (ATCC, HTB-38) employing Microculture Tetrazolium (MTT) assay. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay utilizes the formation of formazan crystals from MTT by living cells, and that this conversion is mitochondrial activity which is used as an indicator for the cell viability. This means that the total mitochondrial activity is proportional to viable cells. Therefore, the MTT assay is used efficiently and broadly to measure the drug in vitro cytotoxicity potential on cell lines. Thus, measuring formazan concentration reflected in optical density (OD) using a plate reader at 520 nm indicates the increase or decrease in viable cell number and can show clearly whether the test compound possesses a cytotoxic activity or not. The (OD) of the wells with cells that are mixed with compounds are compared to the OD of wells with cells without the test compounds. The drug concentration which is required to inhibit 50% cell-growth compared to the growth of the untreated control is referred to IC_{50} (50% inhibitory concentration). The detailed MTT assay can be summarized as follows:

a. A solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was prepared at concentration of 5 mg/mL in phosphate buffered saline (6.5 mM Na_2HPO_4, PBS; 1.5 mM KH_2PO_4,137 mM NaCl, 2.7 mM KCl; pH 7.4). This is followed by stirring with a magnetic stirrer for 1 h in dark then filtering the solution with a 0.22 mm filter and storage in 10-mL aliquots at −20°C. Acidified isopropanol (50 mL 2 M HCl to 2.5 L isopropanol) is used to dissolve formed formazan crystals, the solution is stored at least for a month at room temperature before it has been used.
b. These cell lines were cultured in Eagle's minimal essential medium supplement with 10% horse serum in roller tube culture. The medium contained 100 μg/mL streptomycin and 100 units/mL penicillin. The cells were maintained in a humidified atmosphere at 37° C with 5% CO_2. For the tested compounds **1**–**7**, stock solutions in ethanol 96% (v/v) were prepared. Exponentially growing cells were harvested, counted and diluted appropriately. In order to culture CCD-18 cells, complete growth media was used which consisted of:- high glucose (4.5 g/L) DMEM supplemented with 2 μM L-glutamine, 10 % v/v FBS, 0.1 mM non-essential amino acids and 100 U/ml Penicillin/Streptomycin.
c. Experiments have been performed in triplicates in order to minimize the results-variability. A round bottom plates were used and volumes of 80 μL was used, respectively. Blank wells (without cells) as well as control wells (without drugs) are included.
d. 50 μL containing 3750 cells of the cell suspension, were pipetted into 96-well microtiter plates. Subsequently, 50 μL of each solution of the tested compounds with different concentrations (6.25, 25, 50, 100 and 250 μM) were added to each well rendering the total

volume of cell and drug suspension to be 100 µL. The positive control well of Taxol was prepared as well.

e. The test plates were incubated in the presence of 5% CO_2 at 37 ºC for 72 h. From the MTT solution, 20 µL was added into each well. The blue formazan complex will appear as a result of the penetration of the yellow MTT in the healthy living cells with the presence of mitochondrial dehydrogenases. After an incubation period of 3 h 45 min at 37° C in a 90% humidified incubator with 5% CO_2, the medium was centrifuged (15 min, 20 °C, 210 × g) with 200 µL DMSO, the cells were lysed to liberate the formed formazan product. After shaking the mixture, the absorbance was measured at 520 nm using a scanning microtiter-well spectrophotometer to determine the concentration that killed 50% of cells (IC_{50}).
f. Acidified isopropanol (50 mL 2 M HCl to 2.5 L-isopropanol) is used to dissolve formed formazan crystals.
g. The OD is measured at 520 nm and the percentage of living cells can be calculated as in (Elissawy et al., 2019).
h. In this assay the 0.1% EGMME-DMSO media were used as negative control whereas, paclitaxel (Taxol®) was used as a positive control and the inhibition of cell growth was calculated in terms of IC_{50} values (Kumari et al., 2018; Ashour et al., 2006).
i. In this assay, mathematical, nonlinear regression (4-parameter model) was performed using GraphPad Prism software for the statistical analysis.
j. During the MTT assay, any experiment in which a turbidity or color change (usually indicates microbial contamination in cell culture are noticed) is excluded. Moreover, the mycoplasma is detected periodically via the microbiological culture method. The supernatant of the cell culture is placed in a liquid medium for mycoplasma culture or a mycoplasma agar medium for few days aerobically at 37°C for two weeks. The appearance of fried-eggs-like small colonies indicated possible presence of mycoplasma. In all experiments, all materials must be free from microbial contamination as well as mycoplasma.
k. All compounds did not show any reduction of MTT which may interfere with the results.

References

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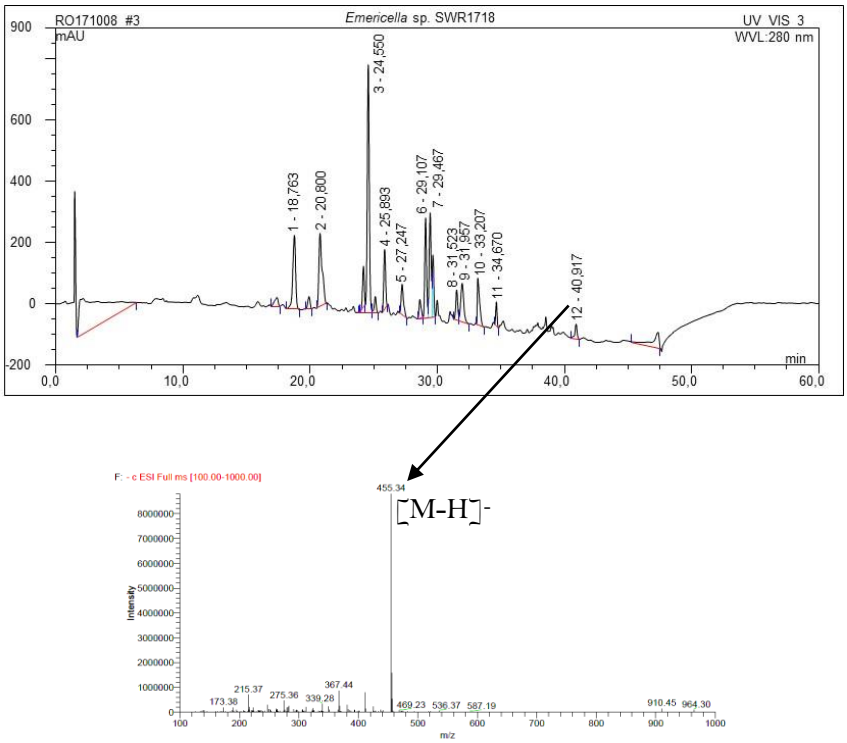

Figure S1. HPLC-MS of compound **1** in *Emericella* sp. SWR1718 extract.

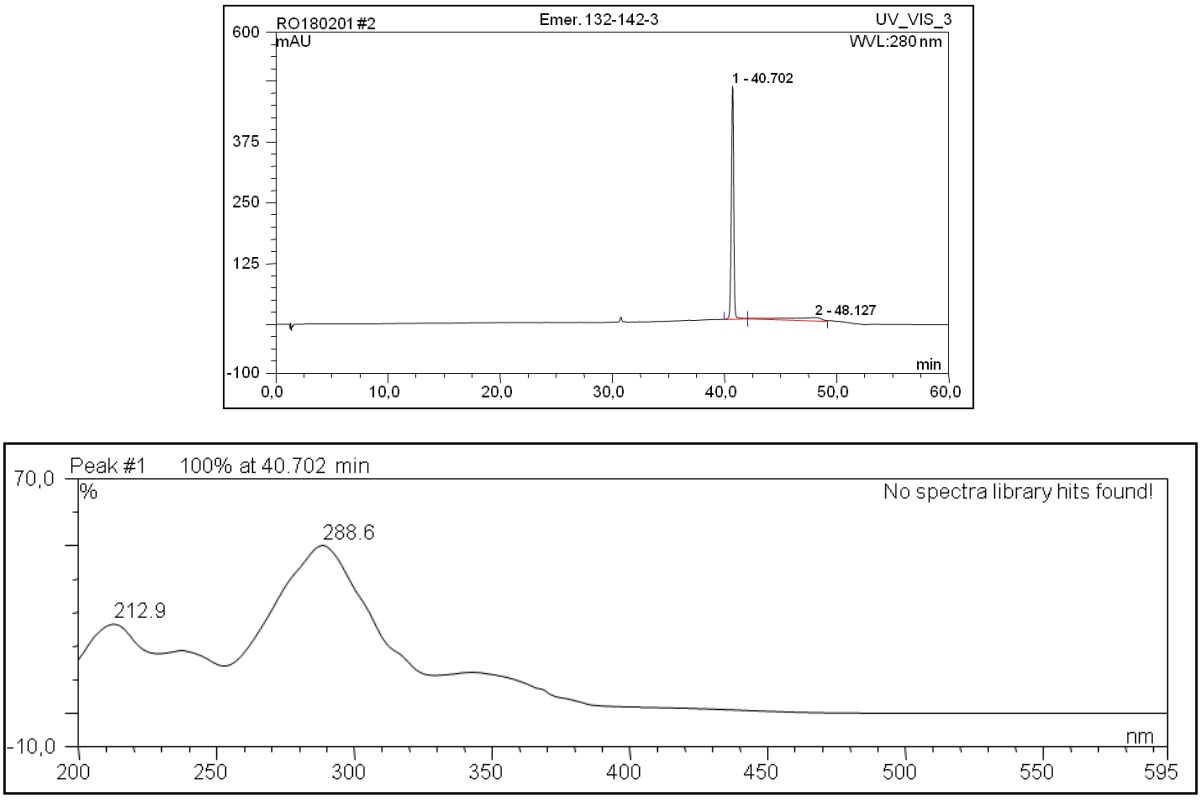

Figure S2. HPLC chromatogram of **1**

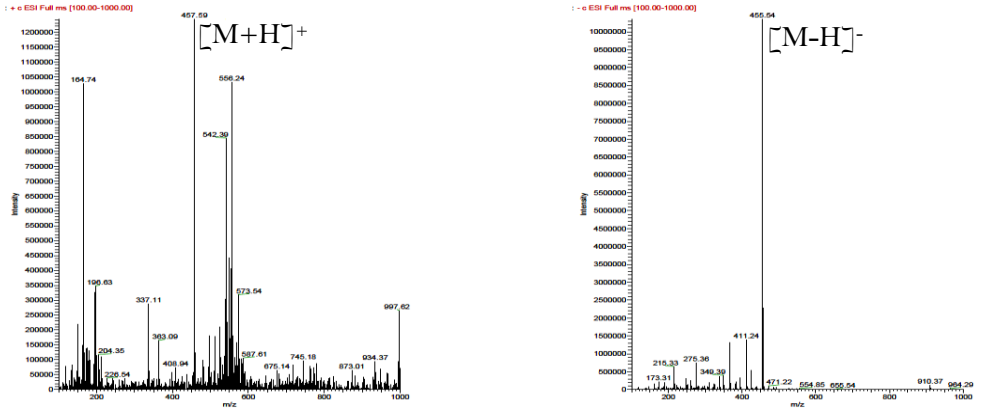

Figure S3. LRESIMS of **1**

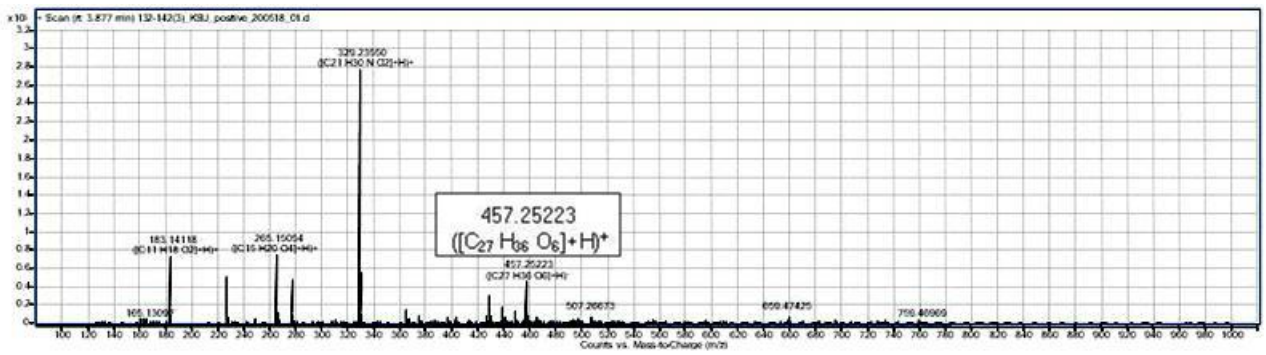

Figure S4. HRESIMS spectrum of **1**

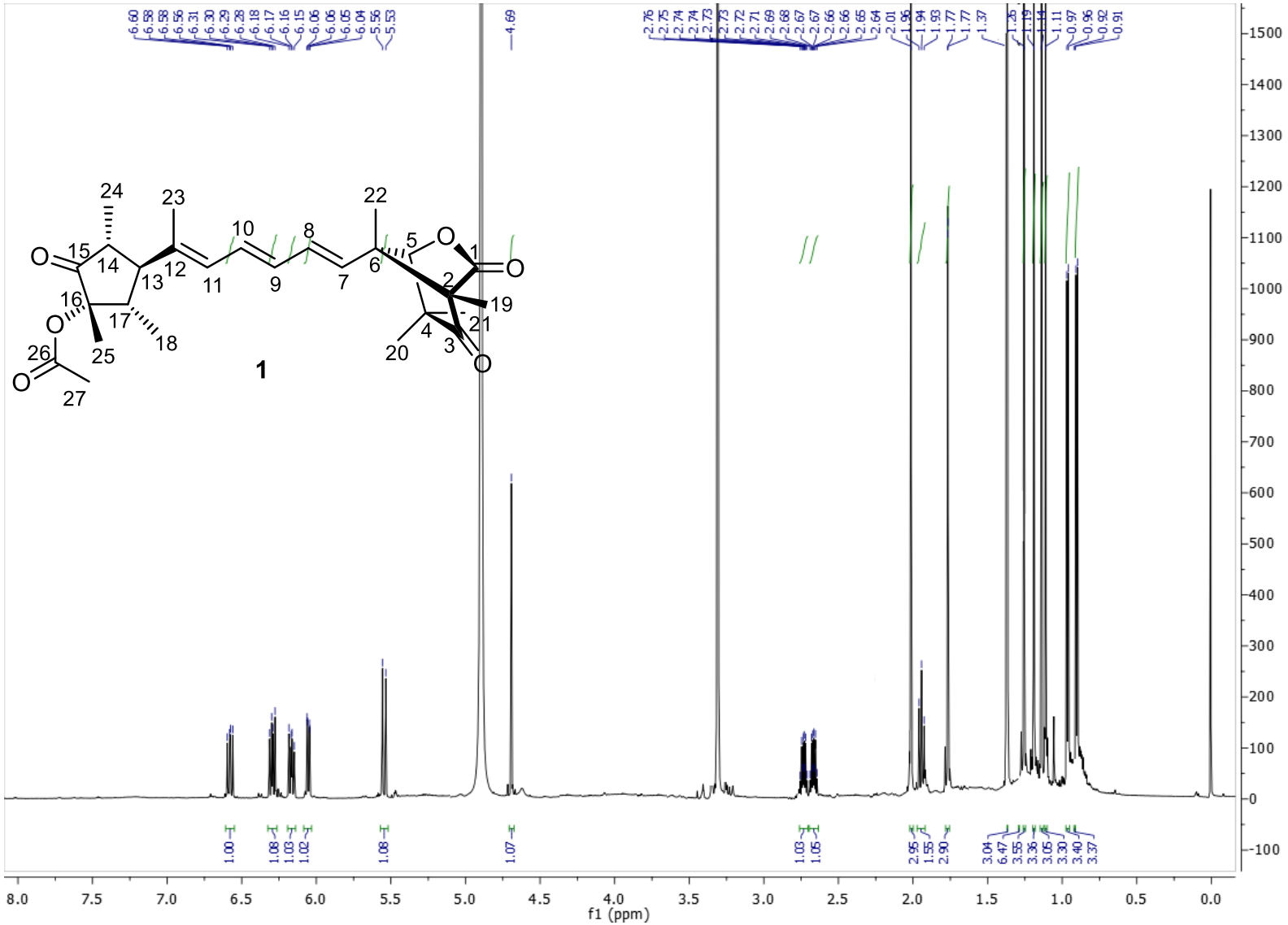

Figure S5. ^{1}H NMR spectrum of **1** in MeOH-d_4

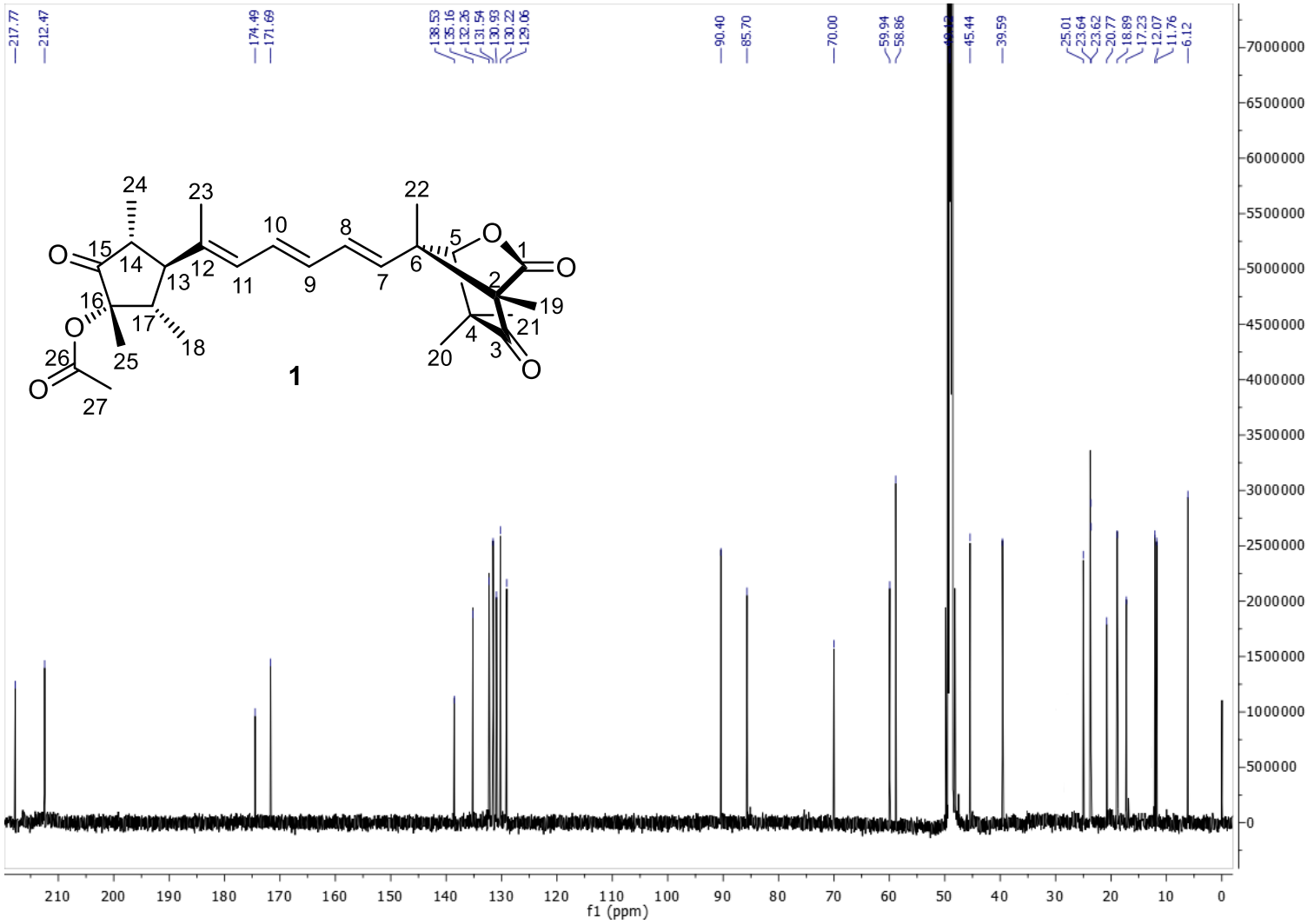

Figure S6. ^{13}C NMR spectrum of **1** in MeOH-d_4

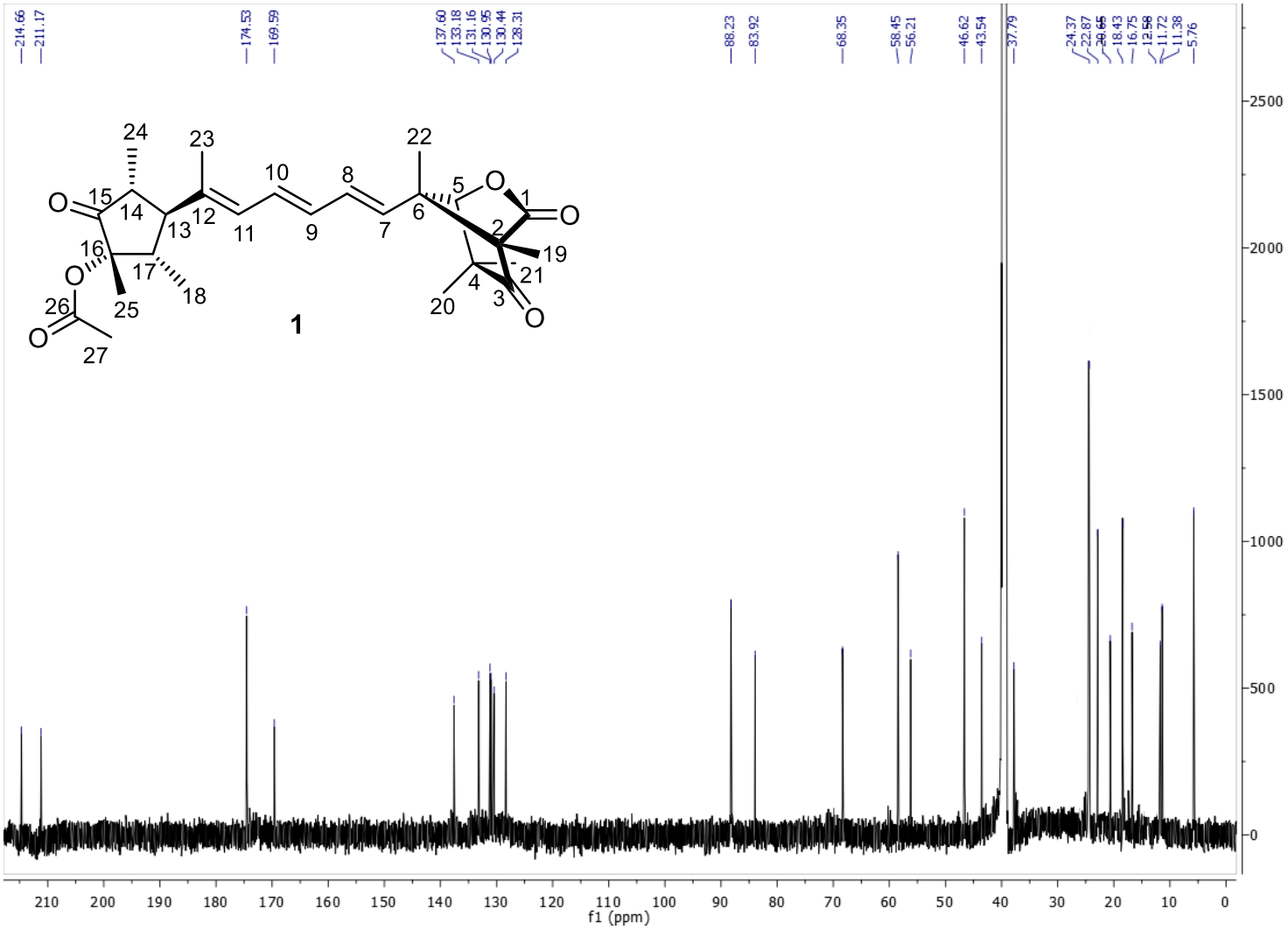

Figure S7. ^{13}C-NMR spectrum of **1** in DMSO-d_6

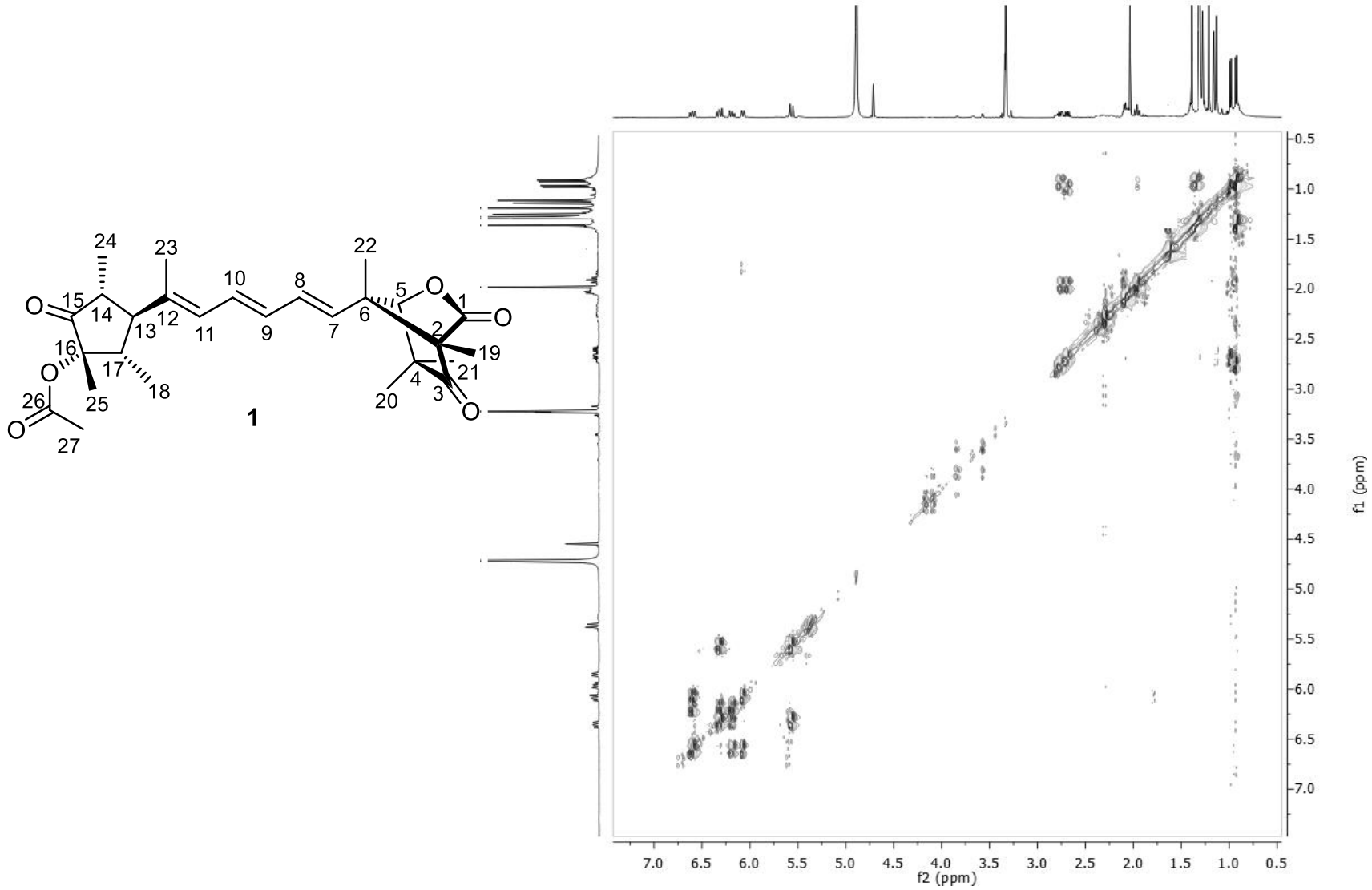

Figure S8. $^{1}H–^{1}H$ COSY spectrum of **1** in MeOH-d_4

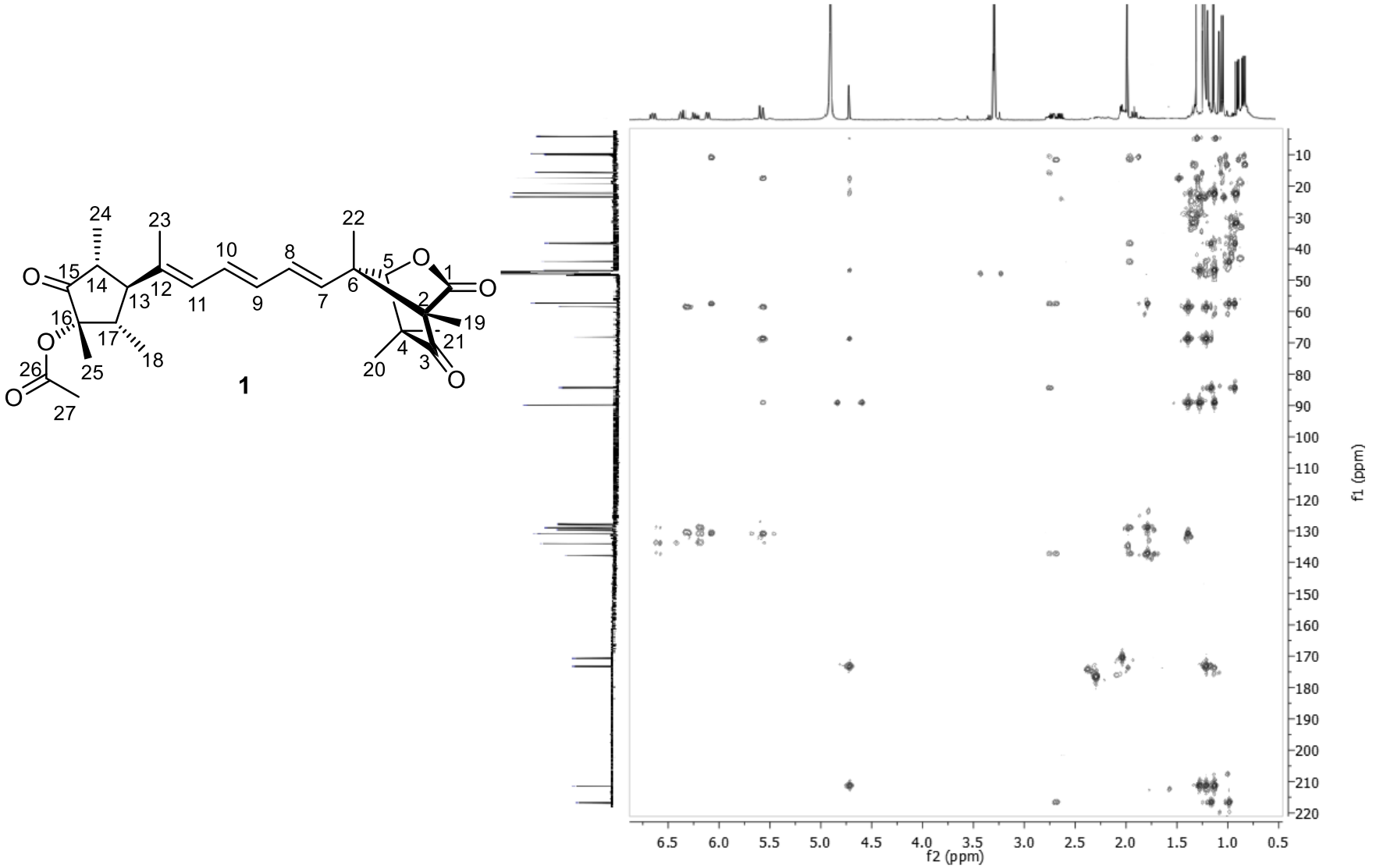

Figure S9. *g*HMBC spectrum of **1** in MeOH-d_4

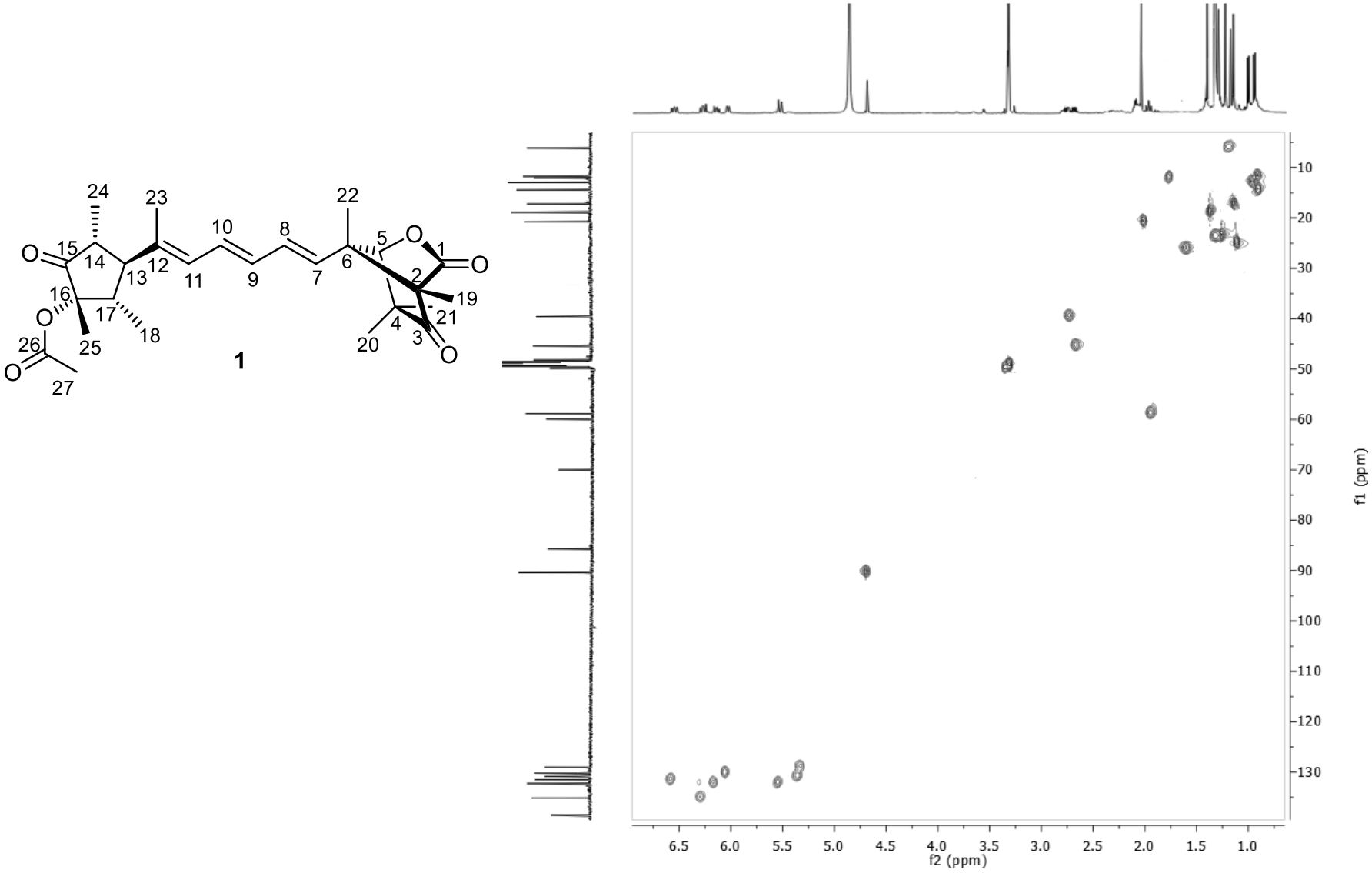

Figure S10. *g*HMQC spectrum of **1** in MeOH-d_4

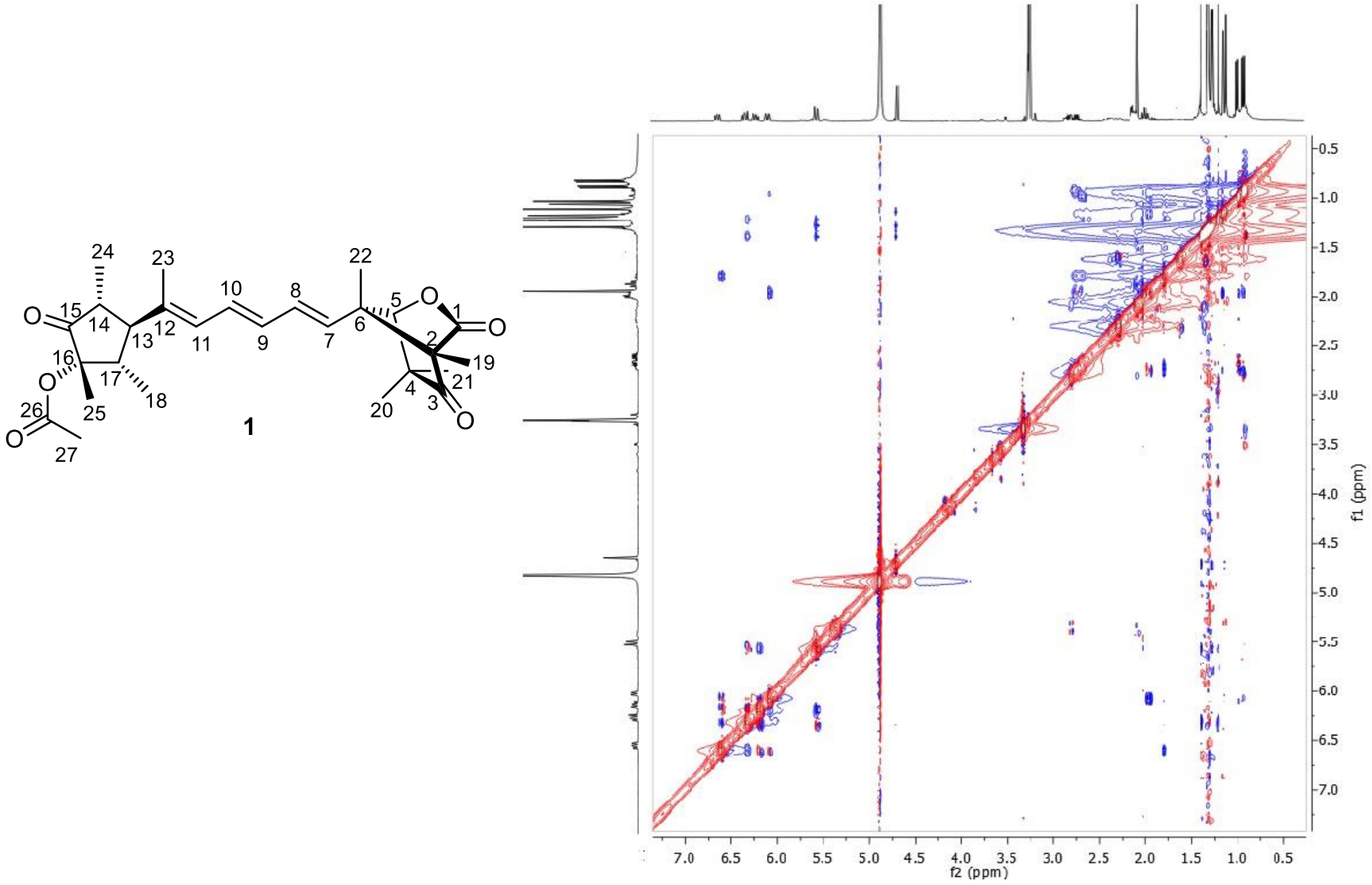

Figure S11. NOESY spectrum of **1** in MeOH-d_4

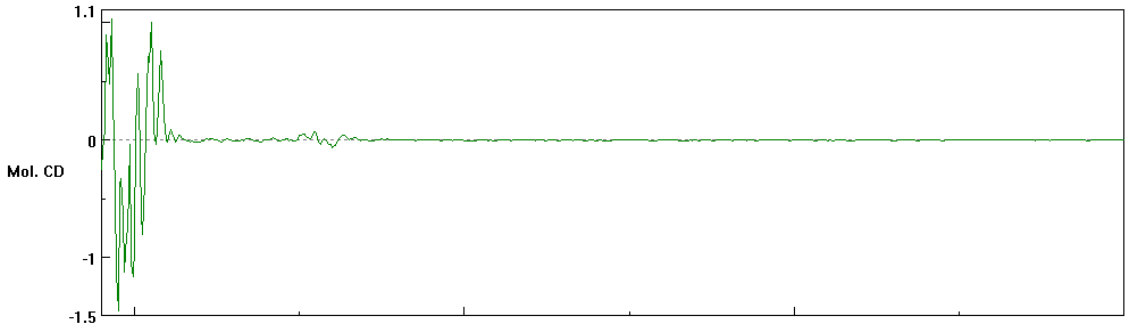

Figure S12. ECD spectrum of **1** showing decomposition of the compound.

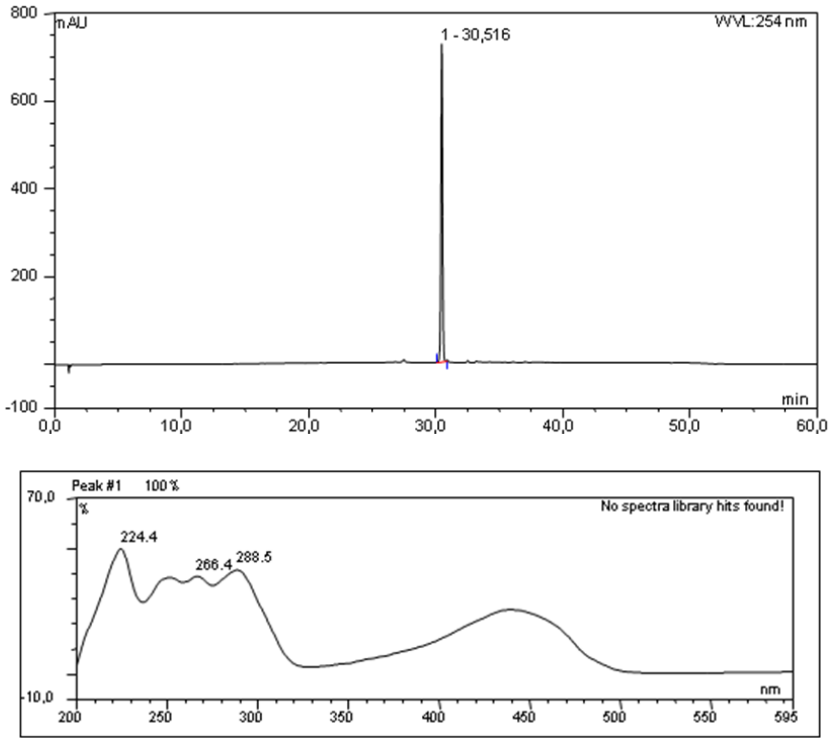

Figure S13. HPLC chromatogram of **2**

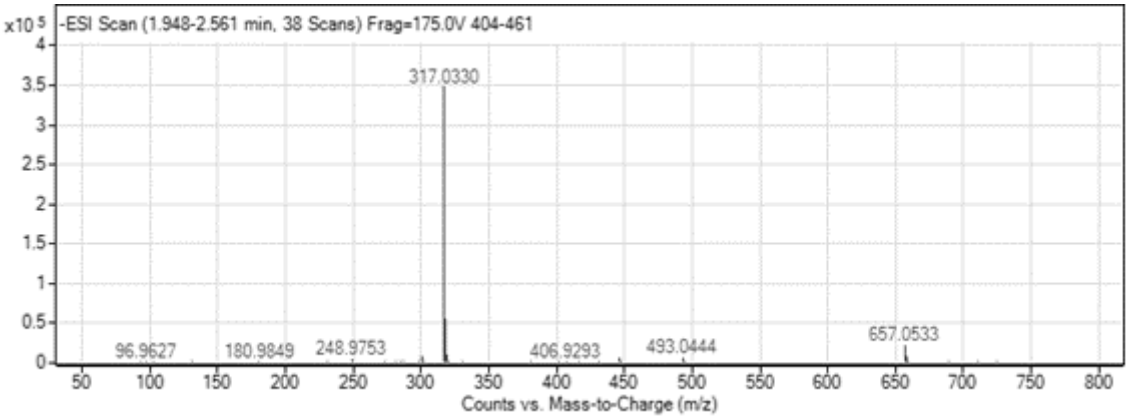

Figure S14. ESIMS spectrum of **2**

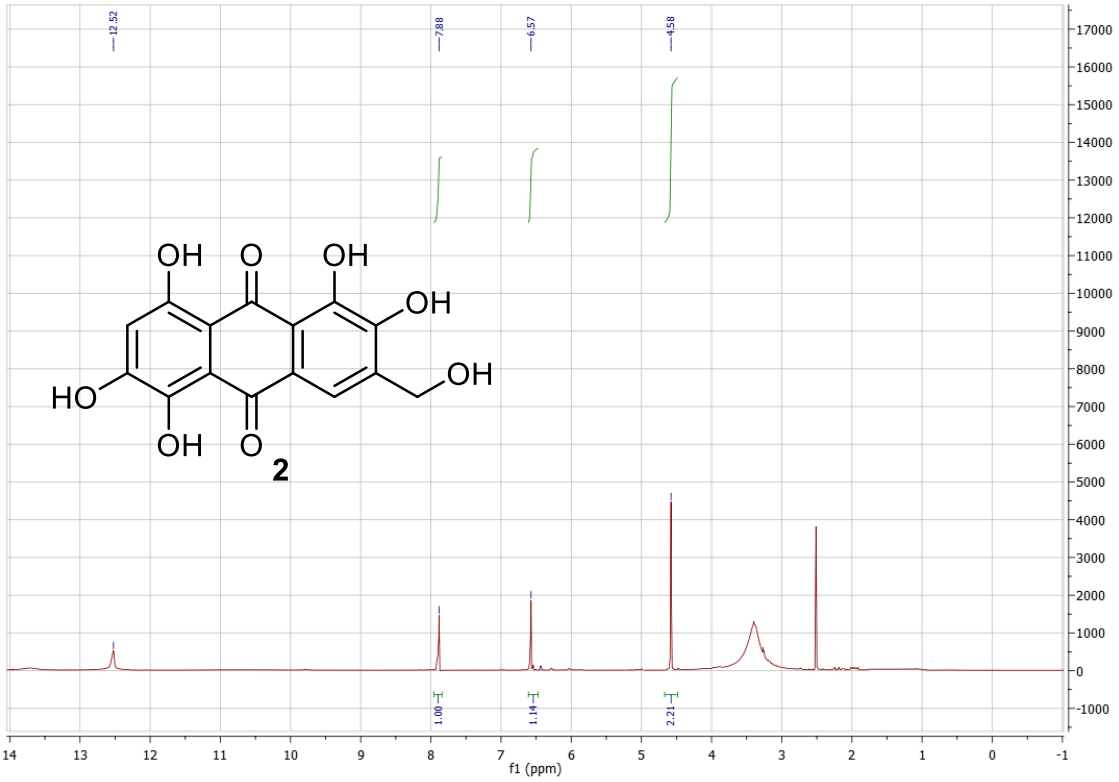

Figure S15. ^{1}H NMR spectrum of **2** in DMSO-d_6

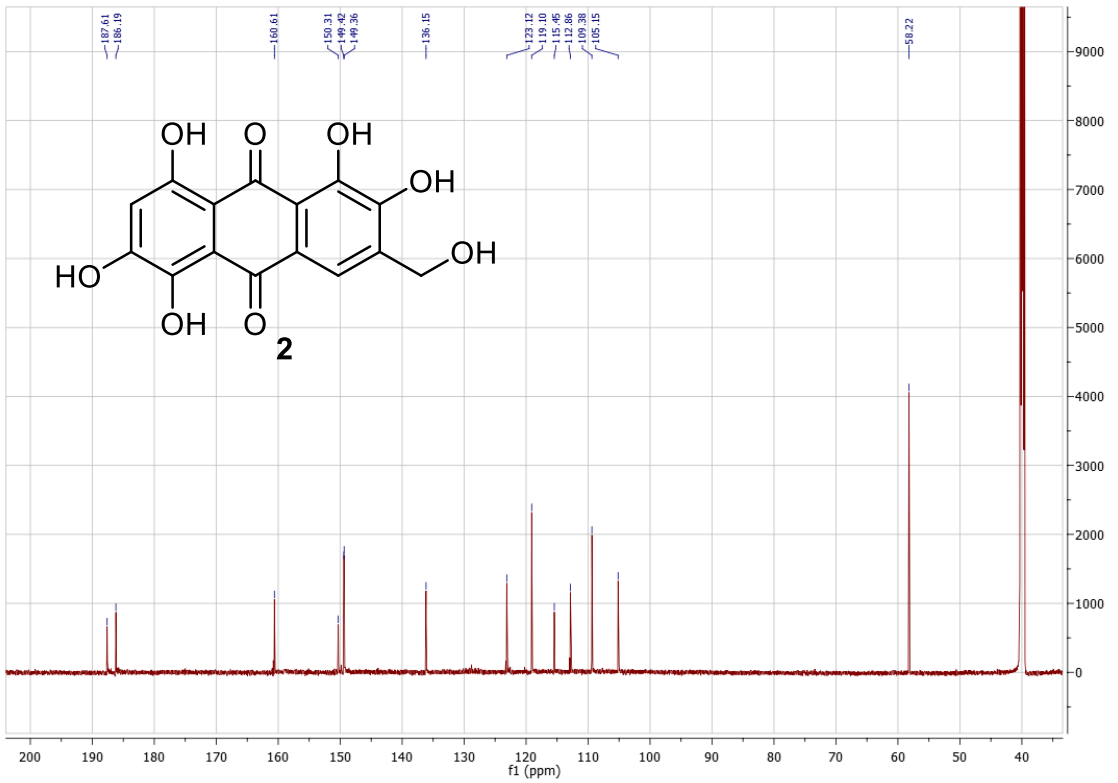

Figure S16. ^{13}C NMR spectrum of **2** in DMSO-d_6

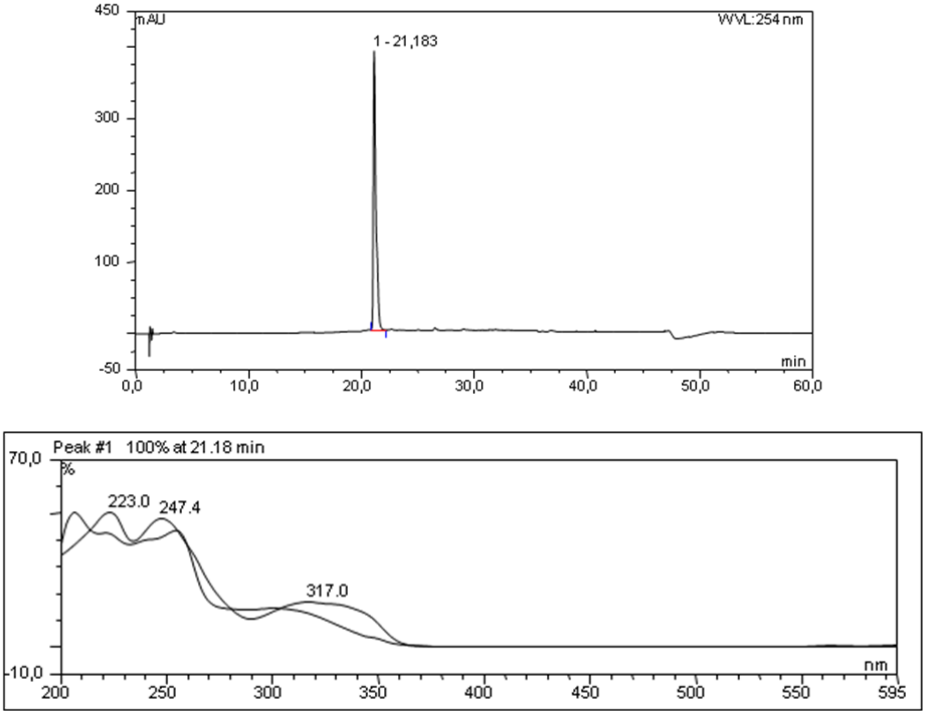

Figure S17. HPLC chromatogram of **3**

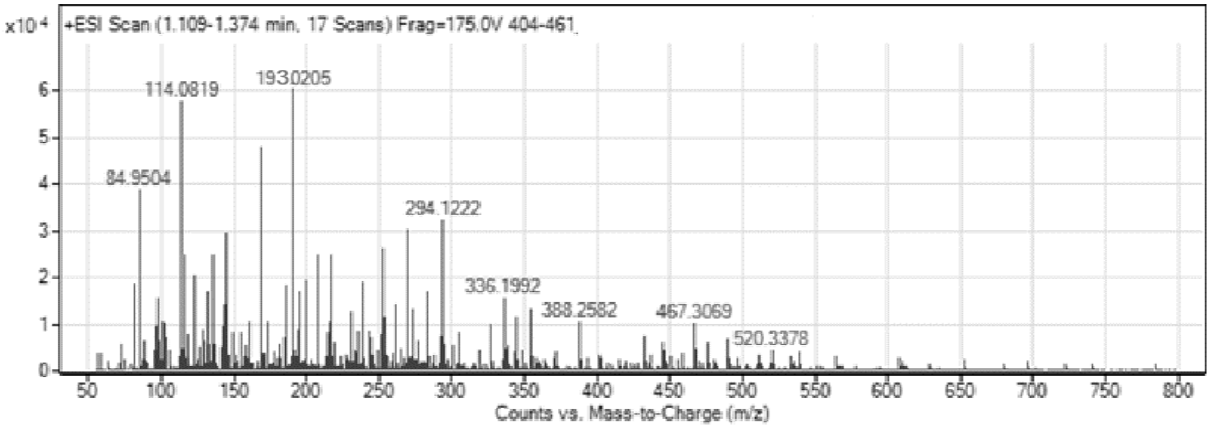

Figure S18. ESIMS spectrum of **3**

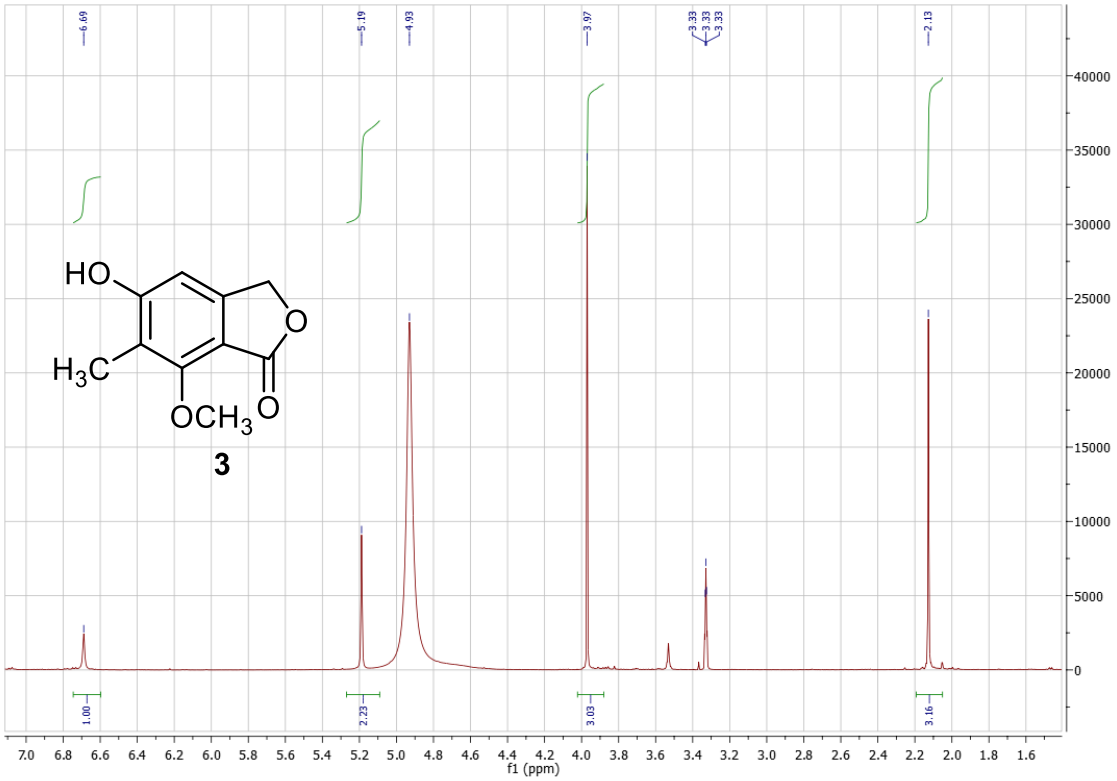

Figure S19. ^{1}H NMR spectrum of **3** in methanol-d_4

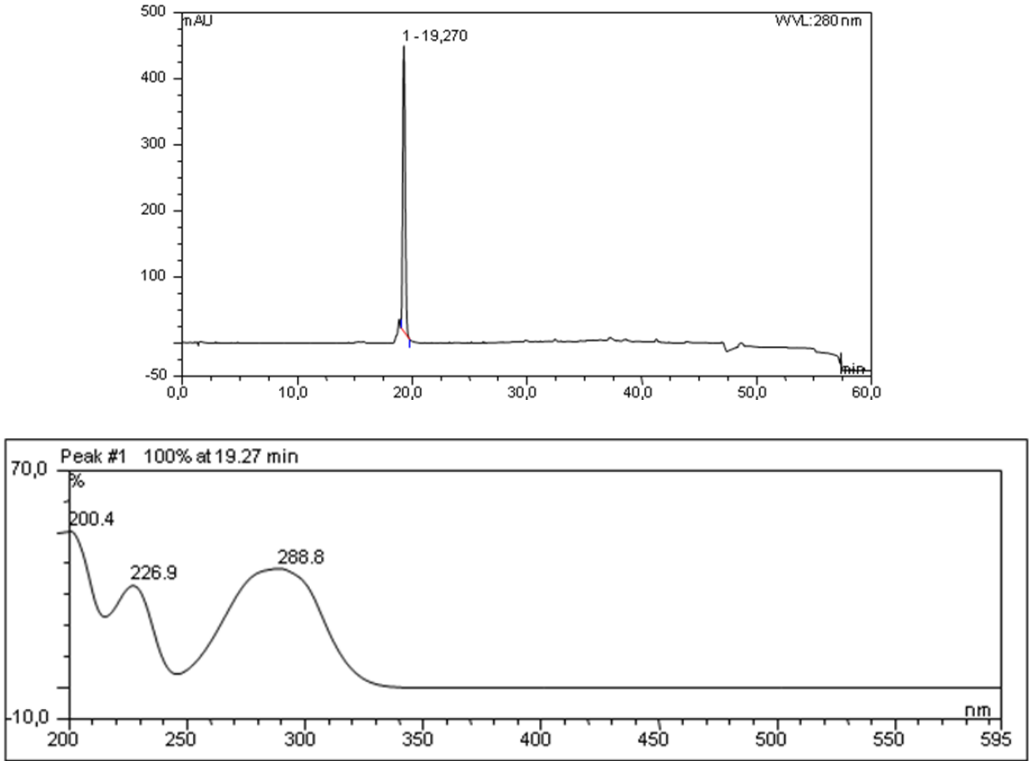

Figure S20. HPLC chromatogram of **4**

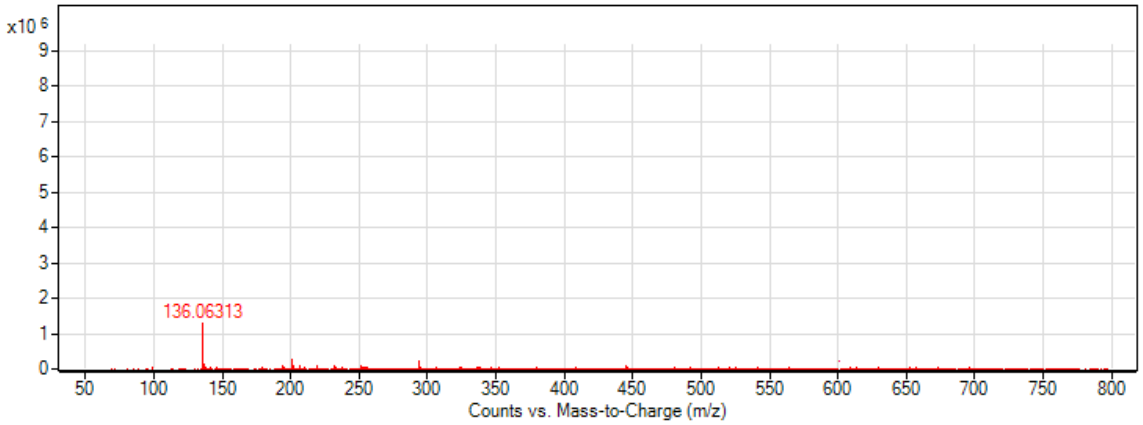

Figure S21. ESIMS spectrum of **4**

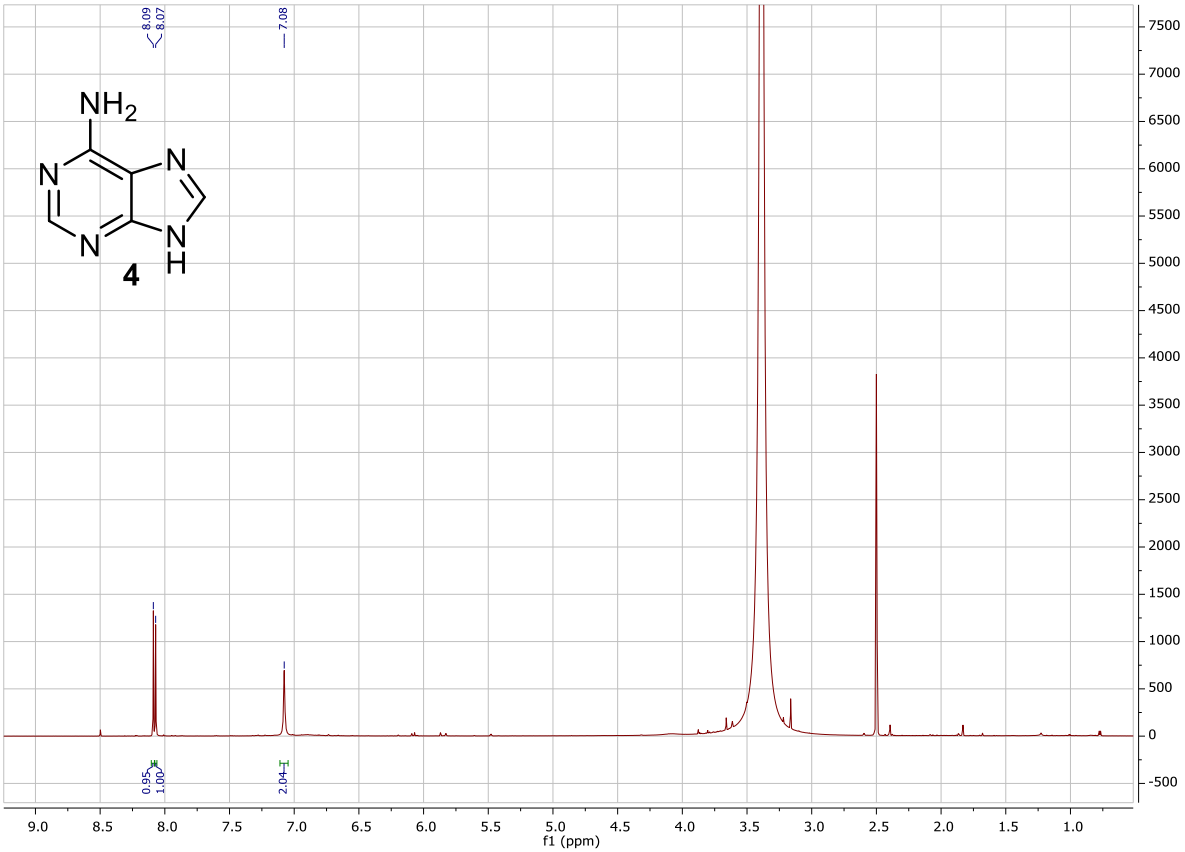

Figure S22. ^{1}H NMR spectrum of **4** in DMSO-d_6

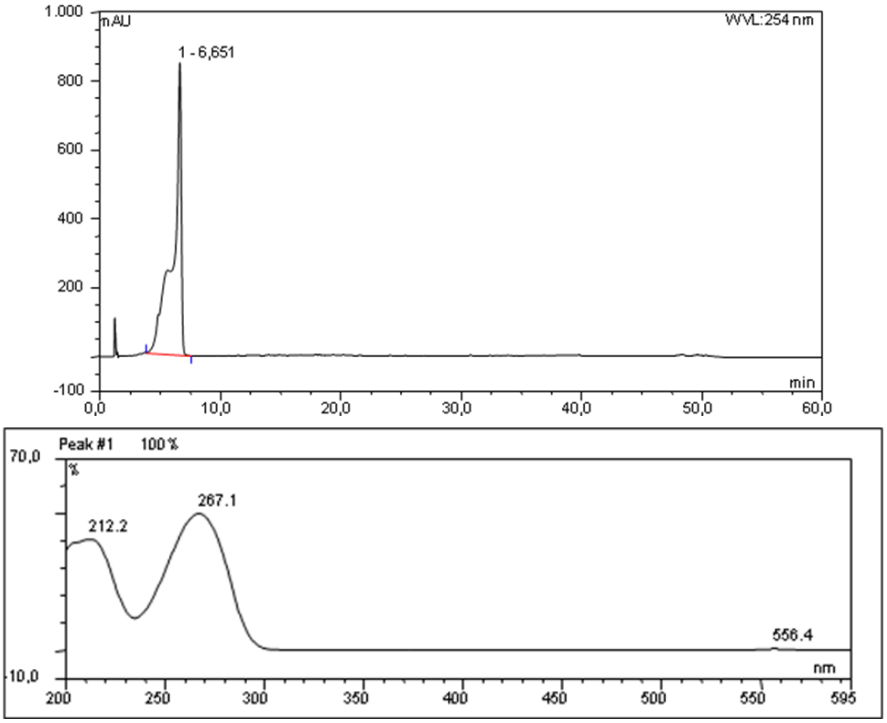

Figure S23. HPLC chromatogram of **5**

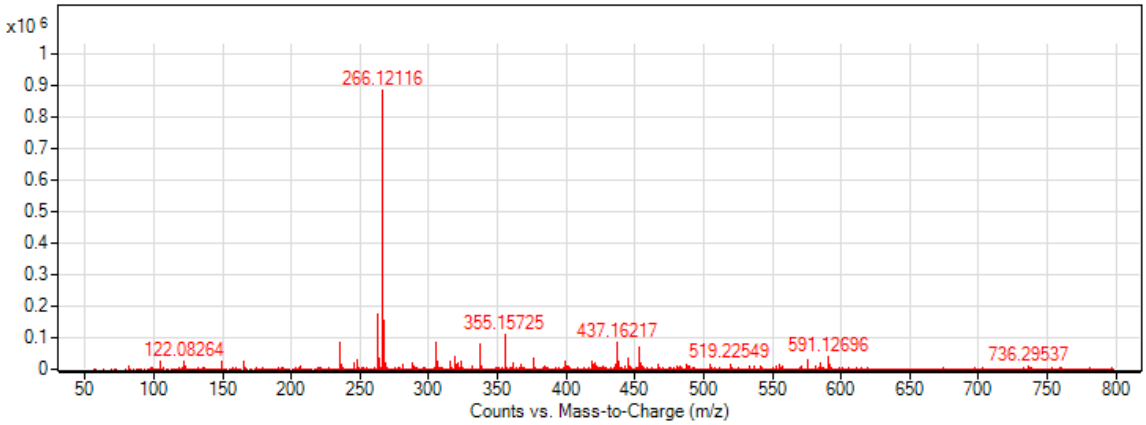

Figure S24. ESIMS spectrum of **5**

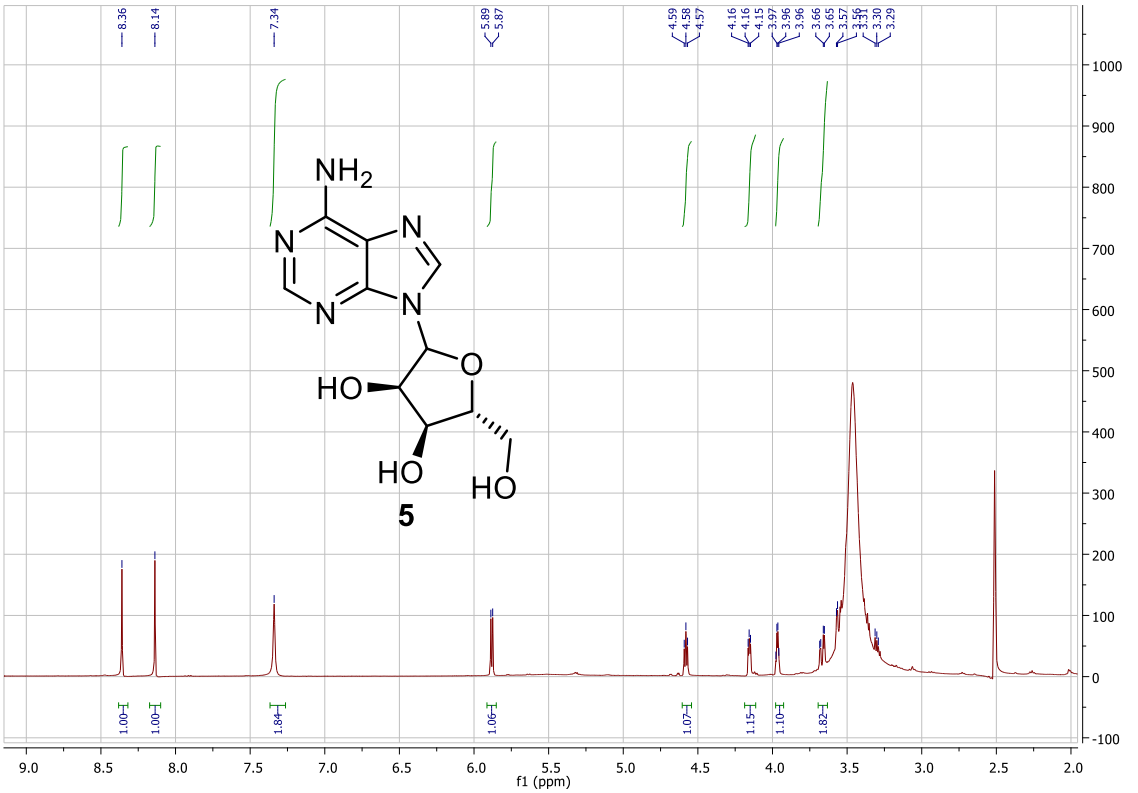

Figure S25. ^{1}H NMR spectrum of **5** in DMSO-d_6

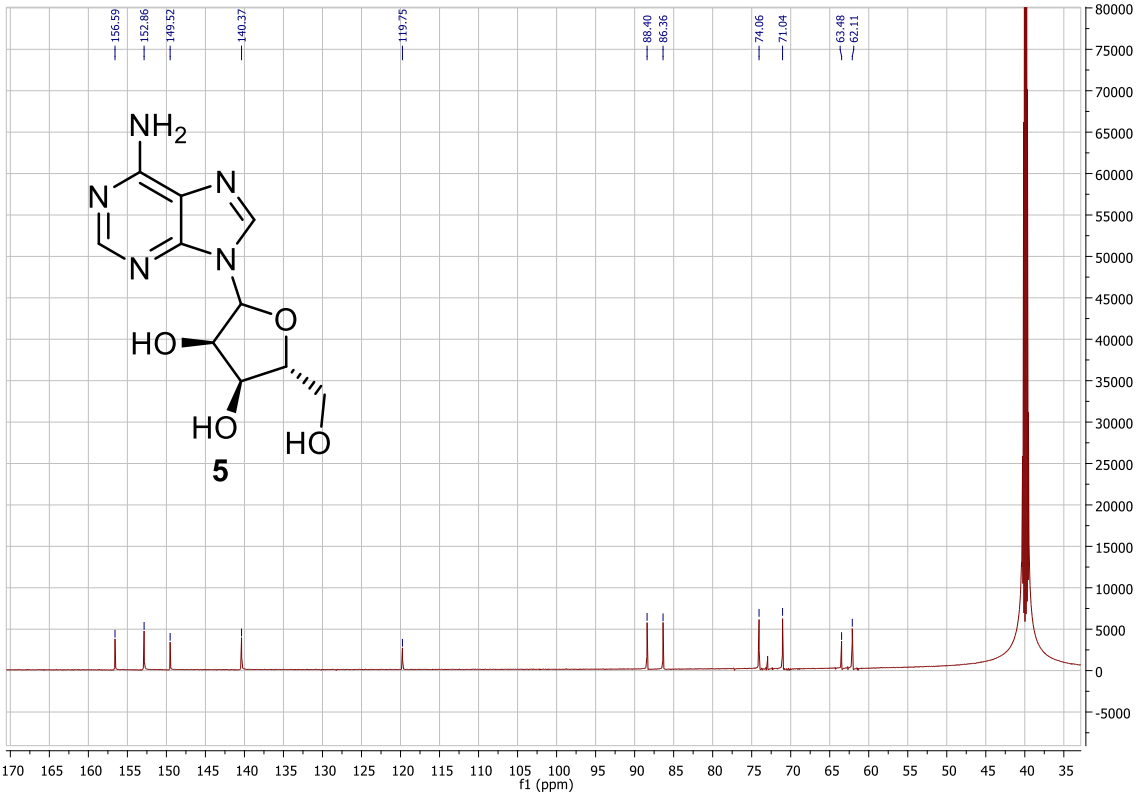

Figure S26. ^{13}C NMR spectrum of **5** in DMSO-d_6

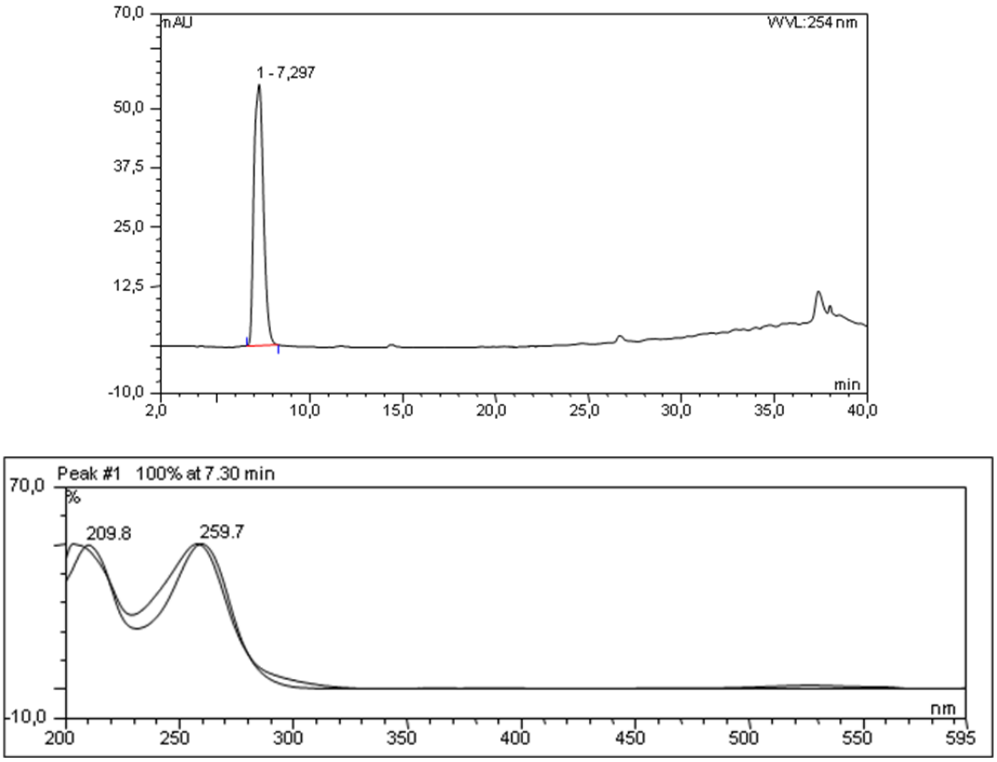

Figure S27. HPLC chromatogram of **6**

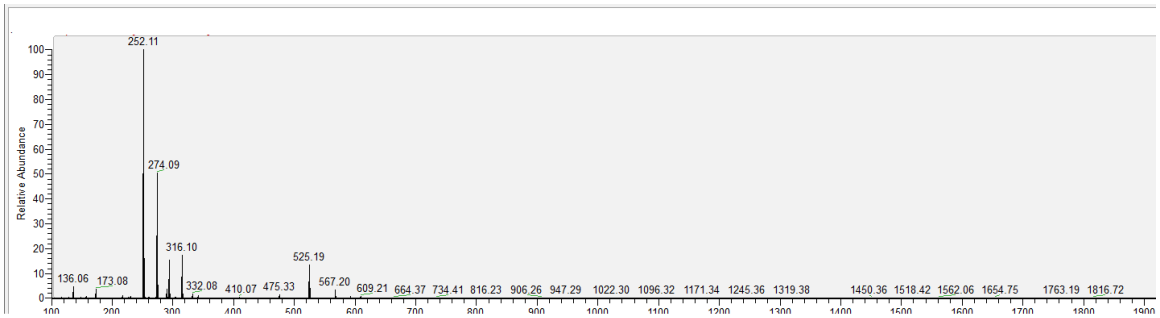

Figure S28. ESIMS spectrum of **6**

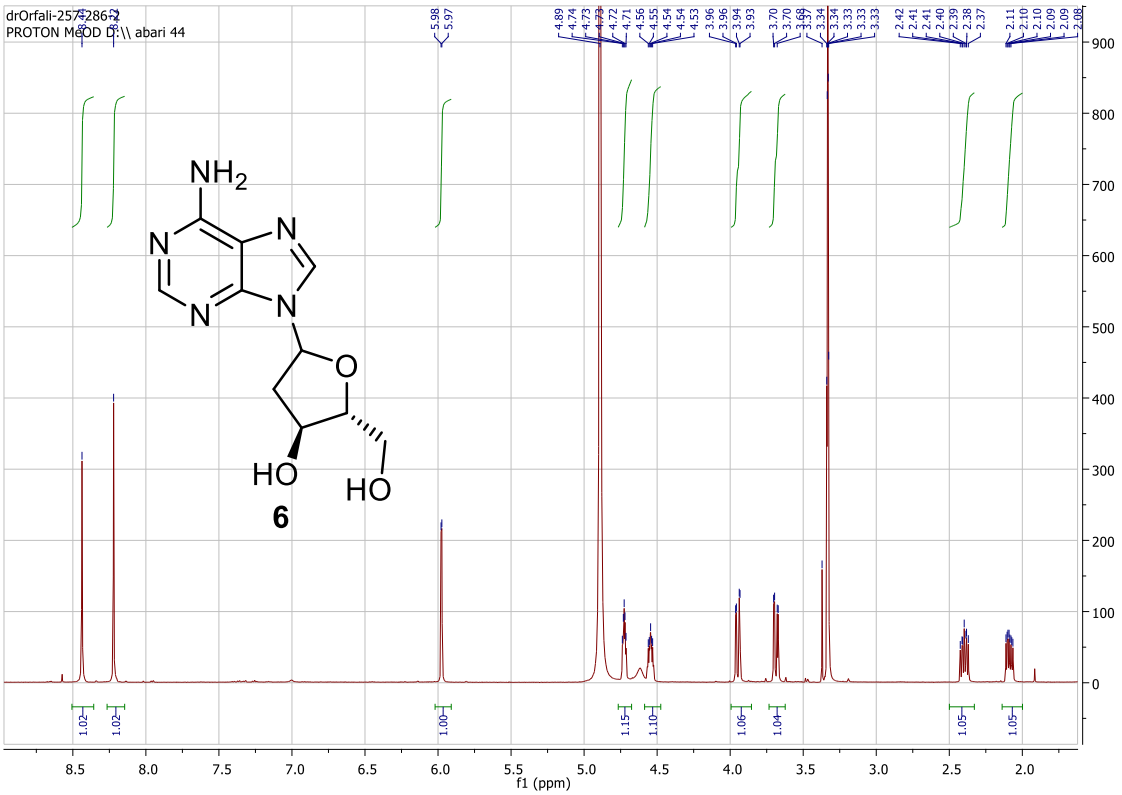

Figure S29. ^{1}H NMR spectrum of **6** in methanol-d_4

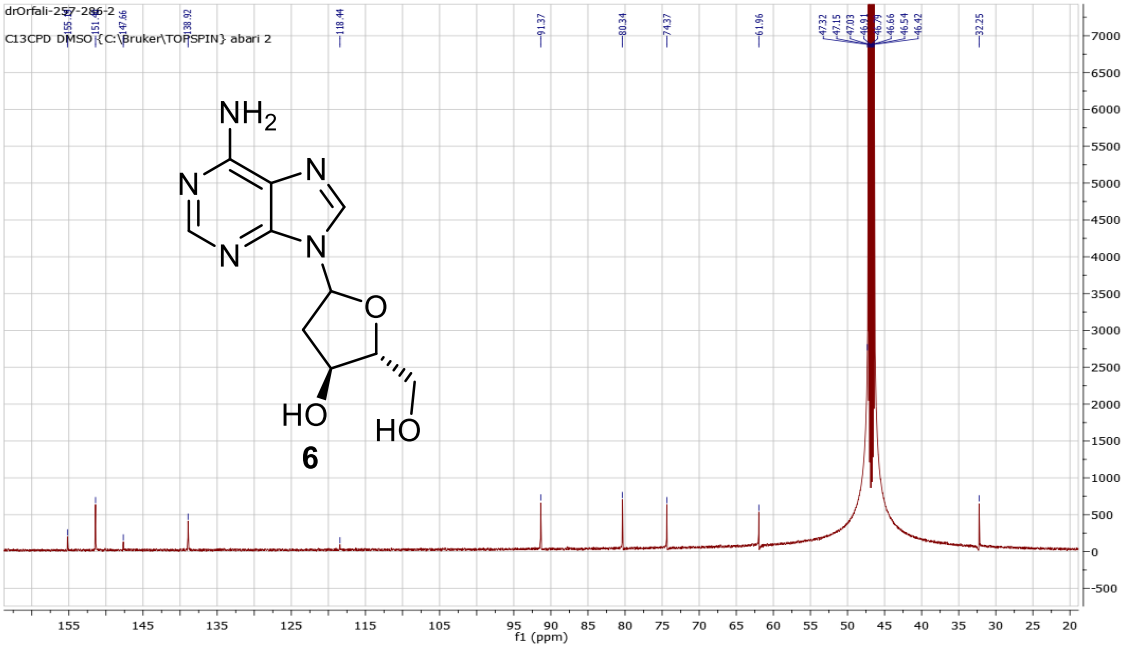

Figure S30. ^{13}C NMR spectrum of **6** in methanol-d_4

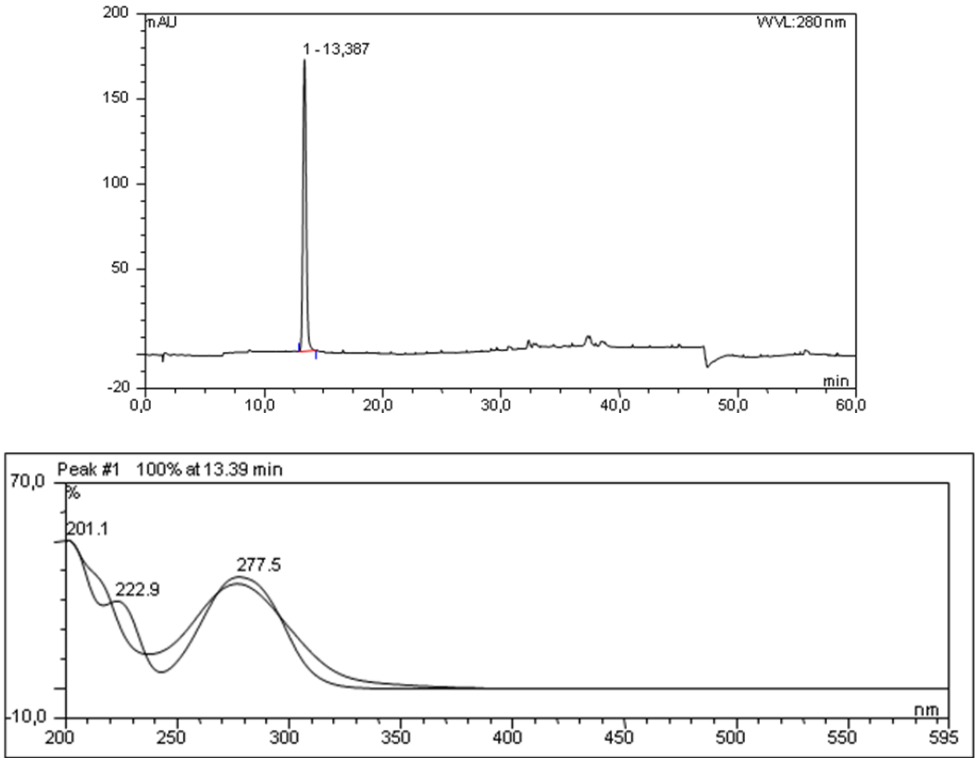

Figure S31. HPLC chromatogram of **7**

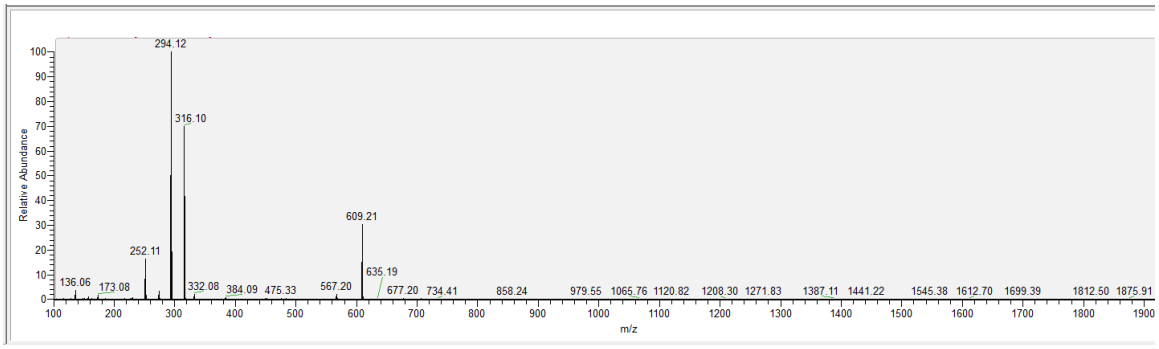

Figure S32. ESIMS spectrum of **7**

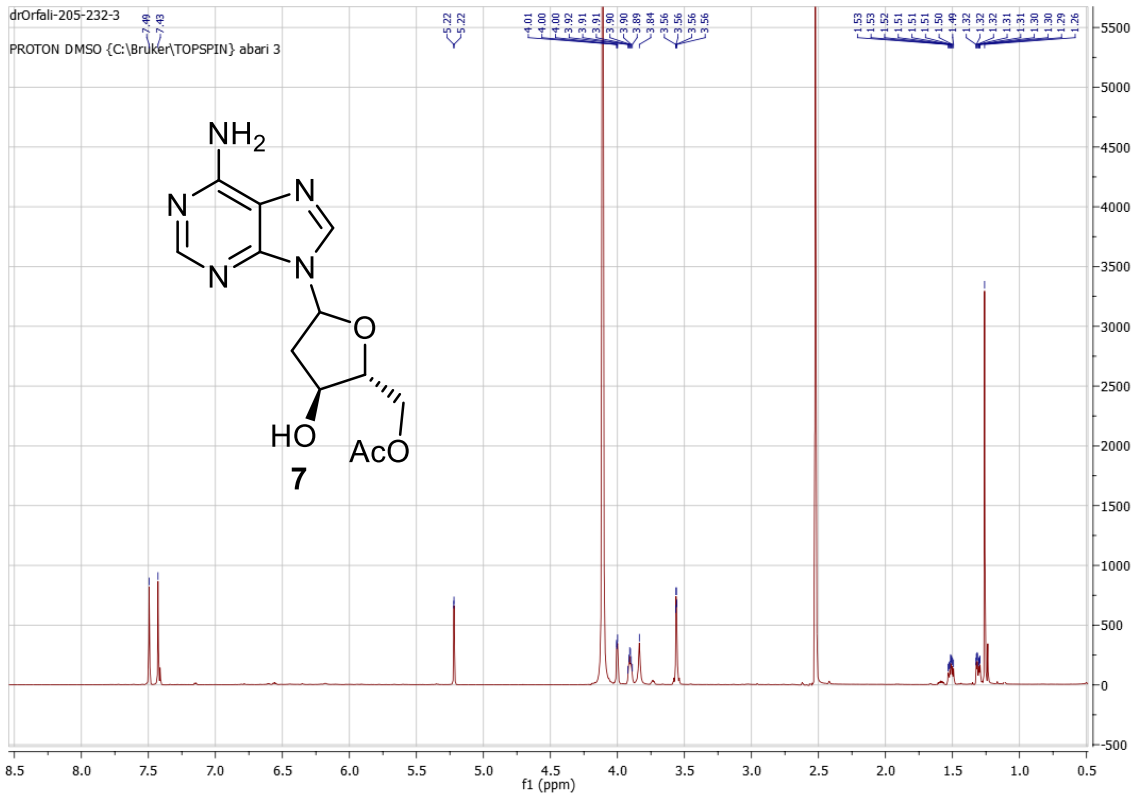

Figure S33. ^{1}H NMR spectrum of **7** in DMSO-d_6

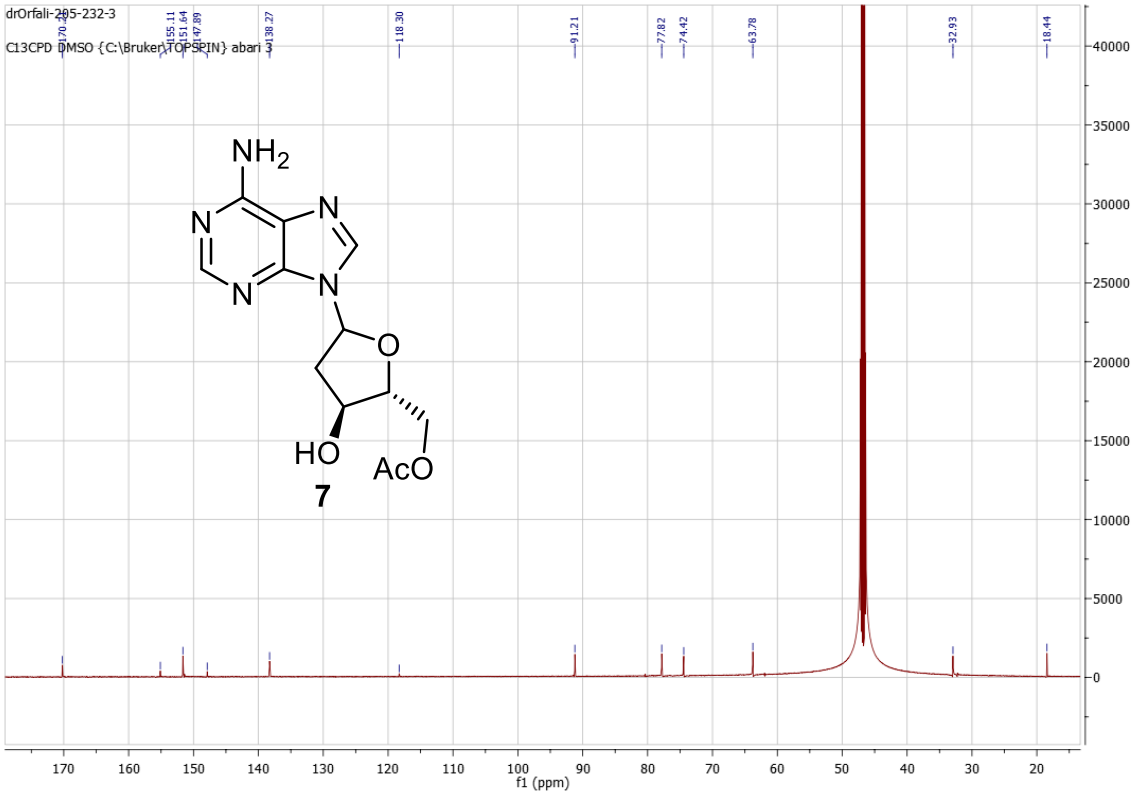

Figure S34. ^{13}C NMR spectrum of **7** in DMSO-d_6

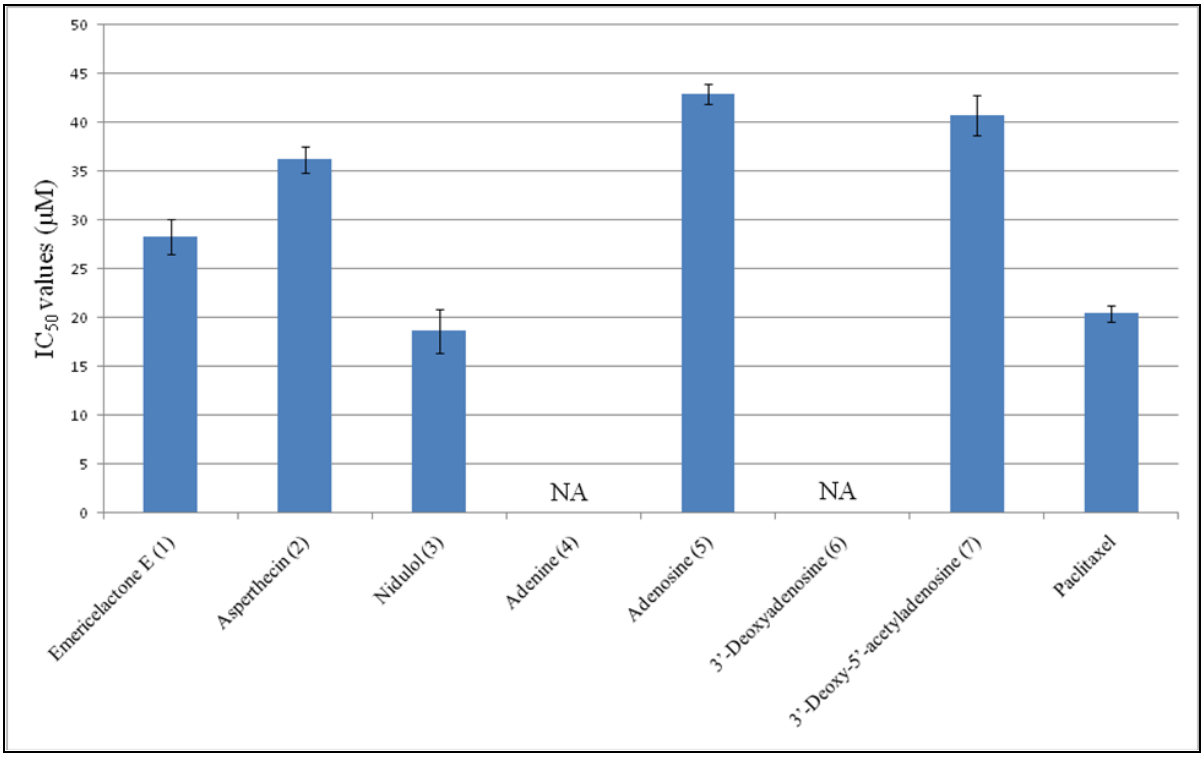

Figure S35. IC_{50} values of compounds (**1**-**7**) against HTB-176 cells (lymphoma).

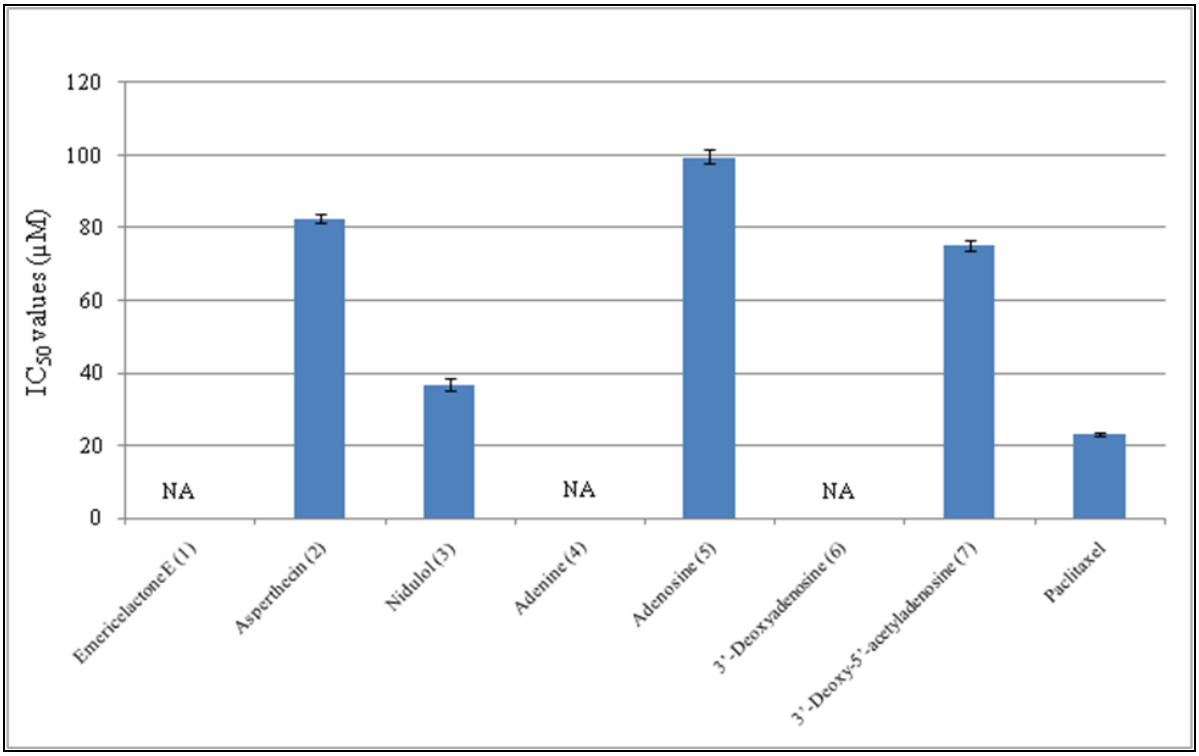

Figure S36. IC_{50} values of compounds (**1**-**7**) against SW-620 cells (colon).

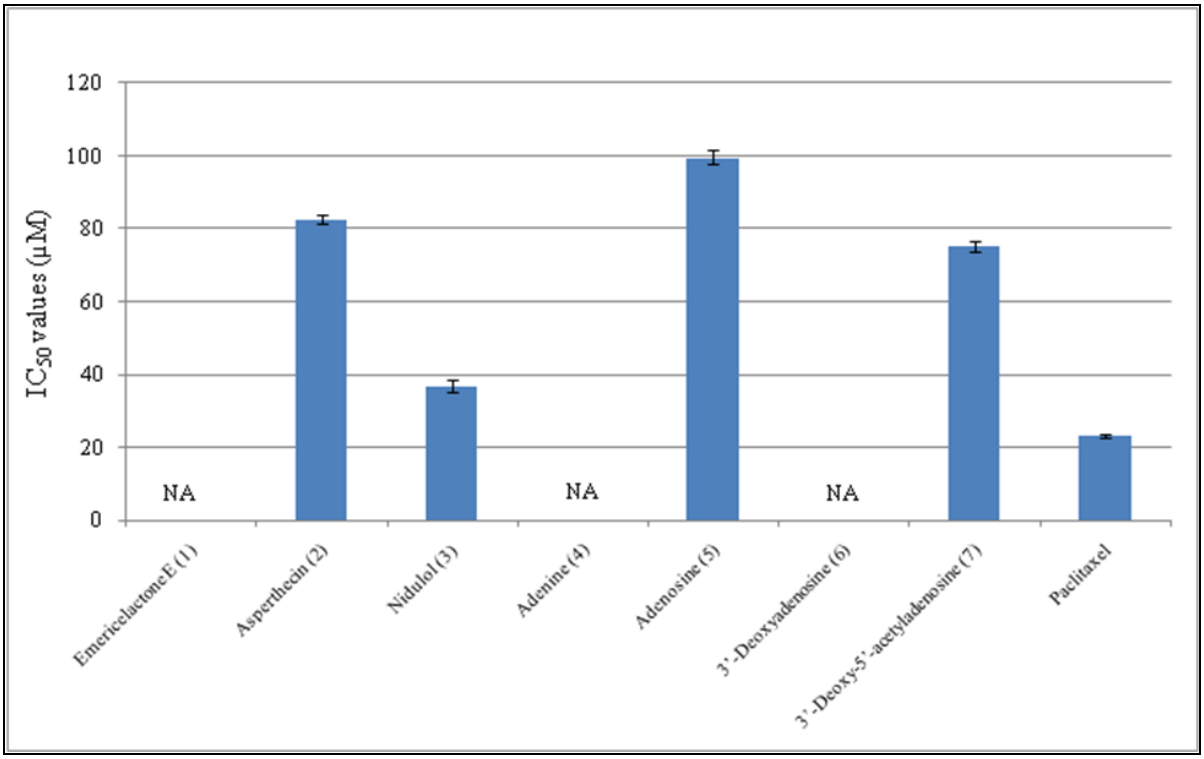

Figure S37. IC_{50} values of compounds (**1**-**7**) against HT-29 cells (colorectal adenocarcinoma).

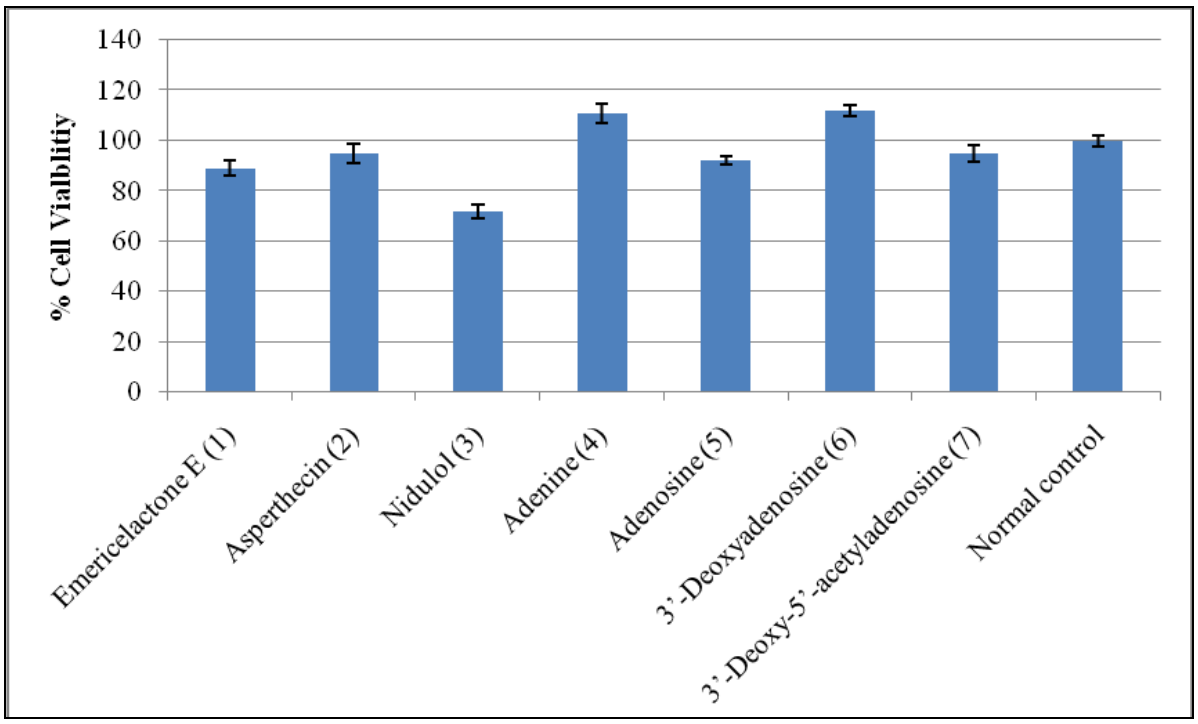

Figure S38. % Cell viability of compounds (**1**-**7**) against CCD-18 (normal colon) cell line.