Supporting Information

Delitschiapyrone A, a Pyrone–naphthalenone Adduct Bearing a New Pentacyclic Ring System from the Leaf-associated Fungus *Delitschia sp*. FL1581

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Experimental Section

General Experimental Procedures. Optical rotations were measured with a Jasco Dip-370 polarimeter using MeOH as the solvent. UV spectra were recorded with Shimadzu UV 2601 spectrophotometer. 1D and 2D NMR spectra were recorded with a Bruker Avance III 400 NMR instrument at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Chemical shift values (δ) are given in parts per million (ppm), and the coupling constants are in Hz. Low resolution and high-resolution MS were recorded on Shimadzu LCMS-DQ8000α and JEOL HX110A spectrometers, respectively. HPLC purifications were carried out on a 10 × 250 mm Phenomenex Luna 5 μm C18 (2) column with a Waters Delta Prep system consisting of a PDA 996 detector. MM2 energy minimizations of possible conformations of emericellenes were performed using Cambridge Soft Chembio 3D Ultra.

Fungal Isolation and Identification. Strain *Delitschia* sp. FL1581 was isolated from a surface-sterilized, fallen leaf of saw palmetto (*Serenoa repens*) obtained from a pine-dominated forest in central Florida, USA.[1] The strain was accessioned as a living mycelial voucher at the Robert L. Gilbertson Mycological Herbarium (MYCO-ARIZ, FL1581). Total genomic DNA was isolated from fresh mycelium and the nuclear ribosomal internal transcribed spacers and 5.8s gene (ITS rDNA; ca. 600 base pairs [bp]) and the adjacent portion of the nuclear ribosomal large subunit (LSU rDNA) was amplified as single fragment by PCR.[1] The positive amplicon was cleaned, normalized, and sequenced as described previously.[1] Basecalls were made by *phred*[2] and *phrap*[3] with orchestration by Mesquite,[4] followed by manual editing in Sequencher (Gene Codes Corp.). The resulting sequence has been deposited in GenBank under the accession number KM679364.

Because the isolate did not produce diagnostic fruiting structures in culture, two methods were used to tentatively identify isolate FL1581 using molecular sequence data. First, the LSUrDNA portion of the sequence was evaluated using the naïve Bayesian classifier for fungi available through the Ribosomal Database Project (http://rdp.cme.msu.edu/).[5] The Bayesian classifier estimated placement within the Ascomycota with high support, but placement at finer taxonomic levels was not possible. Second, we compared the entire sequence against the GenBank database using BLAST.[6] The top BLAST matches were primarily to unidentified, endophytic Dothideomycetes, as well as diverse *Massaria* spp., *Gloniopsis* spp., *Hysterium* spp., and *Delitschia* spp. (particularly *D. winteri*). To clarify the phylogenetic placement of the strain, we downloaded the top 100 BLAST matches from GenBank and aligned the query sequence and the resulting data set automatically using MUSCLE (http://www.ebi.ac.uk/Tools/msa/muscle/) with default parameters. The alignment was trimmed to consistent starting/ending points in the LSUrDNA to permit broad taxon sampling and adjusted manually in MacClade prior to analysis.[7] Taxon sampling was particularly rich in *Massaria* and in diverse Dothideomycetes that were obtained in BLAST searches. A review of the literature led to designation of several basal clades as outgroup taxa [data not shown]. The data set was analyzed using maximum likelihood in GARLI[8] using the GTR+I+G model of evolution as determined by ModelTest,[9] followed by a bootstrap analysis with 100 replicates. The resulting analysis indicated placement within *Delitschia* with strong support. Within *Delitschia*, FL1581 was reconstructed with strong support as sister to a clade of previously unidentified endophytic fungi, with which it forms a clade subtended by *Delitschia didyma.* Importantly, BLAST results alone would have led to estimation of the taxonomic placement for this strain in *Massaria*, but phylogenetic analyses were markedly

more informative. Pending morphological analysis to evaluate appropriate taxonomic placement relative to currently known species, we designate the strain *Delitschia* sp. FL1581 (Figure **S1**).

Culturing of *Delitschia sp.* FL1581 and Isolation of Metabolites The fungus was cultured in 20 T-flasks (800 mL), each containing 135 mL of PDA coated on five sides of the flasks, maximizing the surfacearea for fungal growth (total surface area/flask ca. 400 cm^2). After incubation for 21 days at 28 °C, MeOH (250 mL/T-flask) was added, the flasks were shaken in an ultrasonic bath for 1 h at 25 °C, and the resulting extract was filtered through a layer of Celite 545. The filtratewas concentrated to about one-third of its volume in vacuo below 40°C and was extracted with EtOAc (3 × 1500 mL). The EtOAc extract was concentrated to afford the crude extract (2.68 g). A portion (2.08 g) of this extract was partitioned between hexane and 80% aqueous MeOH. The cytotoxicity-active 80% aqueous MeOH fraction was diluted to 50% aqueous MeOH by addition of water and extracted with $CHCl_3$. The $CHCl_3$ fraction (1.76 g) was then subjected to gel permeation chromatography over a column of Sephadex LH-20 (30 g) made up in hexanes/CH_2Cl_2 (1:4) and eluted with hexanes/CH_2Cl_2 (1:4, 350 mL), CH_2Cl_2/acetone (4:1, 250 mL), CH_2Cl_2/acetone (2:3, 300 mL), and finally MeOH (300 mL). The fractions having similar TLC patterns were combined to give 4 major fractions [A (1.0155 mg), B (304.9 mg), C (447.1 mg), and D (19.8 mg). The cytotoxicity-fraction A was chromatographed on a reversed-phase (RP) C18 (40 μm; 20.0 g) open column. The column was eluted sequentially with 50% MeOH (aq) (300 mL), 70% MeOH (aq) (300 mL), and 100% MeOH (500 mL), and the resulting fractions were combined based on their TLC (SiO_2; $CHCl_3$−MeOH, 10:1) profiles to afford eight combined fractions A1−A8. Of these, fractions A_1 and A_5 were found to be cytotoxic. Fractions A1 (24.5 mg) was further purified on a column of silica gel (10.0 g) by elution with hexanes/ CH_2Cl_2 (1:20) to give **3**. Fraction A5 (83.0 mg) was chromatographed over a column of silica gel (10.0 g) eluted with hexane/Acetone (3:1) to give six subfractions (A5A−A5F). Subfraction A5F (19.2 mg) was purified by RP-HPLC using 55% aqueous MeOH to give **2** (0.8 mg, t_R = 20 min) and **1** (2.0 mg, t_R = 40 min), respectively.

X-ray Crystallographic Analysis of 1. Upon crystallization from EtOH/H_2O (9:1) using the vapor diffusion method, pale yellow crystals of **1** were obtained. A crystal (0.50 × 0.10 × 0.04 mm) was separated from the sample and mounted on a glass fiber, and data were collected using

a Bruker Kappa APEX-II Duo diffractometer with graphite-monochromated Mo Kα radiation, λ = 0.71073 Å at 119.99 K. Crystal data: $C_{24}H_{27}O_{9.5}$, M = 467.45, space group orthorhombic, $P2_12_12$; unit cell dimensions a =14.1928 (13) Å, b = 17.7023 (16) Å, c = 10.7282 (10) Å, V = 2695.4 (4) $Å^3$, Z = 4, ρcalcd = 1.152 mg/mm^3, μ = 0.089 mm^{-1}, F(000) = 988. The structure was solved by direct methods using ShelXT and refined by using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. Absorption corrections were performed using the Siemens Area Detector Absorption final refinement gave R_1 = 0.0774 and wR_2 = 0.1827 [I > 2σ(I)].

(1′R) -2′, 3′-Dihydropyrenocine C (**2**): $[\alpha]_D^{25}$+15.7 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 284 nm (3.52); ESI-MS *m/z*: 213 $[M+H]^+$, 195 $[M+H-H_2O]^+$; ^1H and ^{13}C NMR data were consistent with literature values.[10]

6-Ethyl-2,7-dimethoxyjuglone (3): ^1H, ^{13}C NMR, and the MS data were consistent with literature values.[11,12]

Cytotoxicity Assay. The resazurin-based colorometric (AlamarBlue) assay[13] was used for evaluating in vitro cytotoxicity of samples against human lung carcinoma (H460), human hepatocarcinoma (HepG2), human breast carcinoma (MCF-7), human osteosarcoma (U2OS) cell lines. Cisplatin and DMSO were used as positive and negative controls, respectively.

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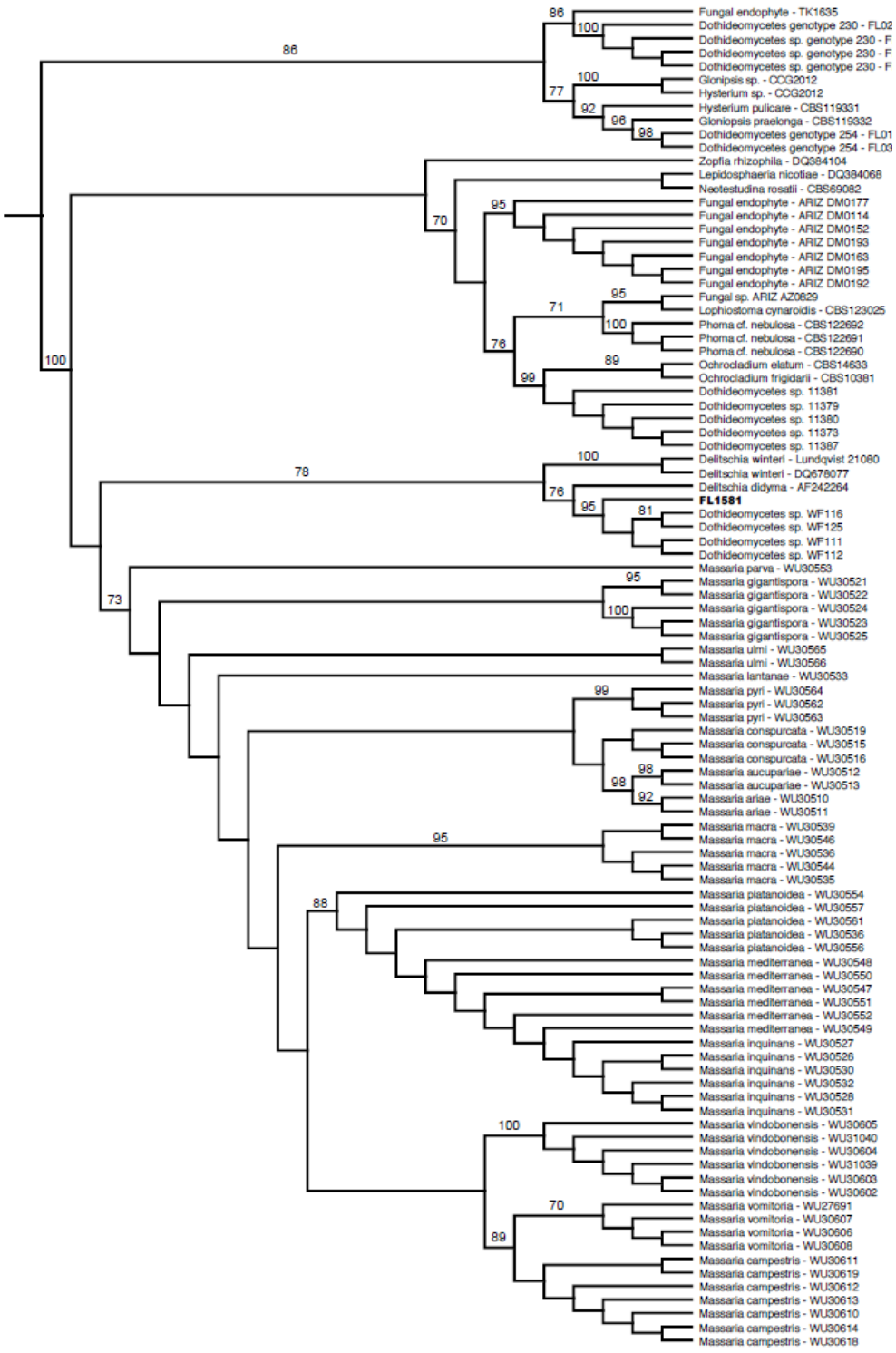
Figure S1. Phylogenetic Analysis of *Delitschia sp*. FL1581



Results of maximum likelihood analysis revealing placement of FL1581 within *Delitschia*. Values after taxon names are GenBank accession numbers or strain identifiers. Numbers above branches indicate bootstrap support; values ≥69 are shown.

Figure S2. ^{1}H NMR Spectrum of Delitschiapyrone A (**1**) in Acetone-d_6 (400 MHz)

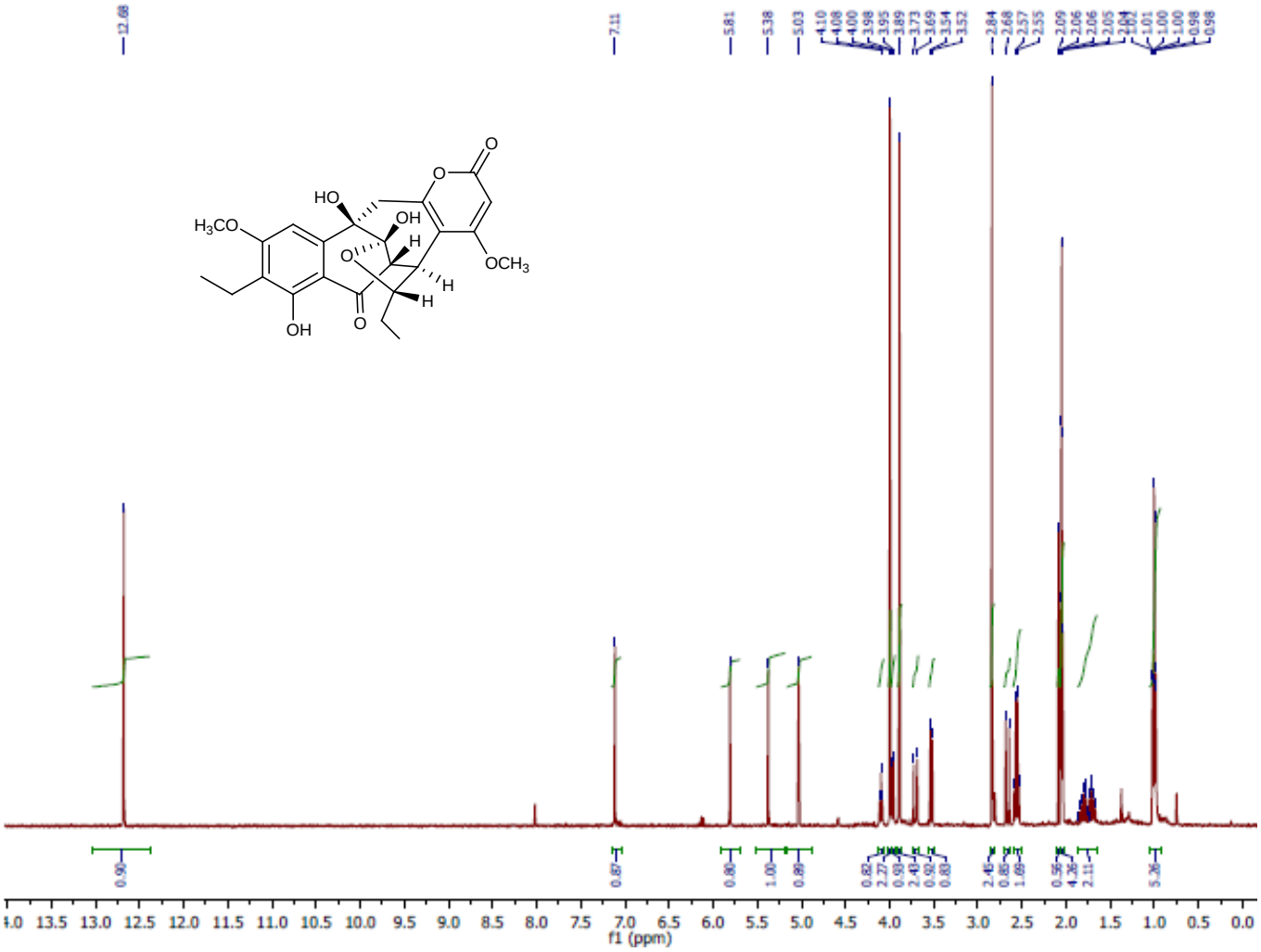


Figure S3. ^{13}C NMR Spectrum of Delitschiapyrone A (**1**) in Acetone-d_6 (100 MHz)

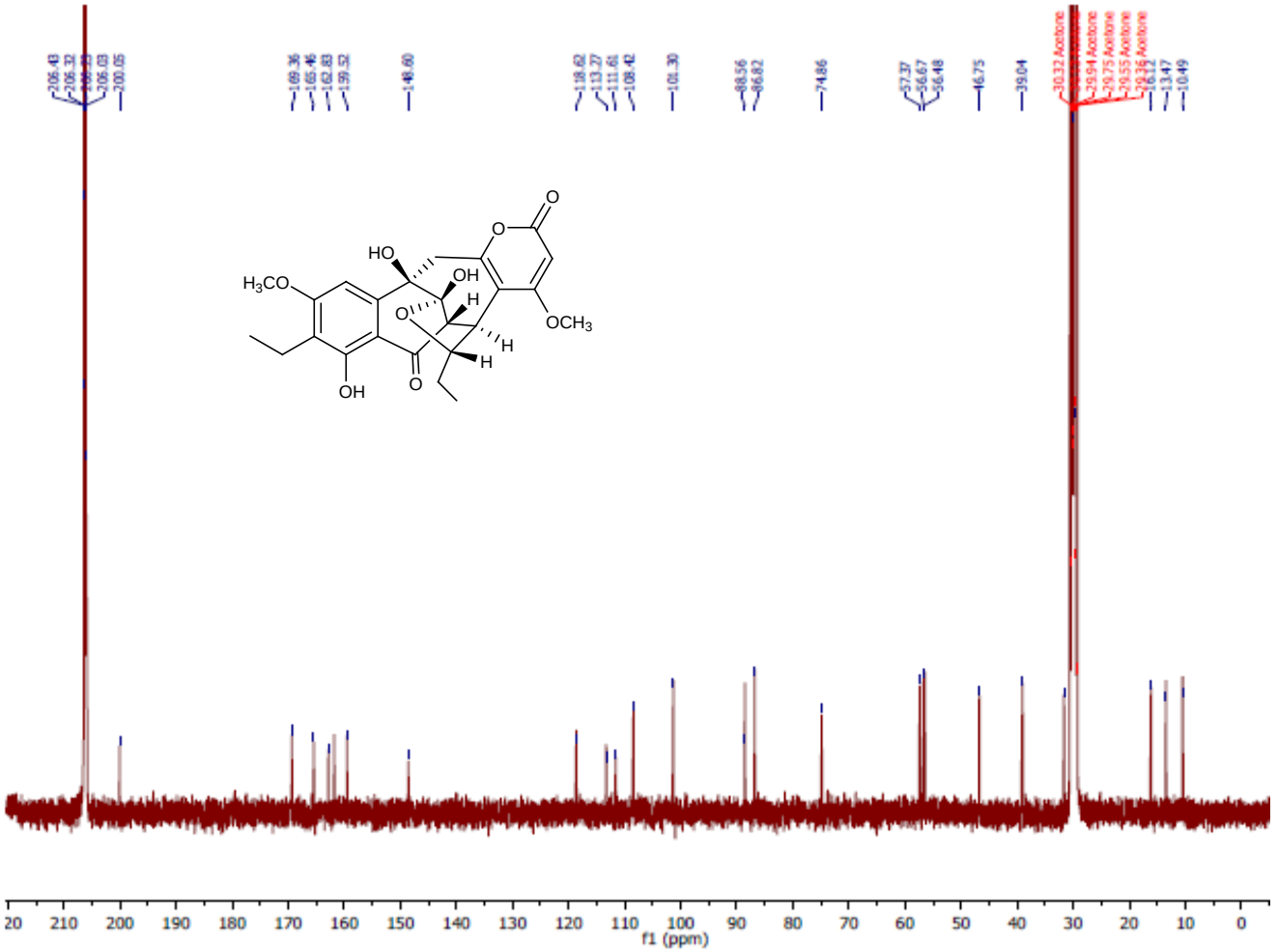


Figure S4. DEPT Spectrum of Delitschiapyrone A (**1**) in Acetone-d_6 (100 MHz)

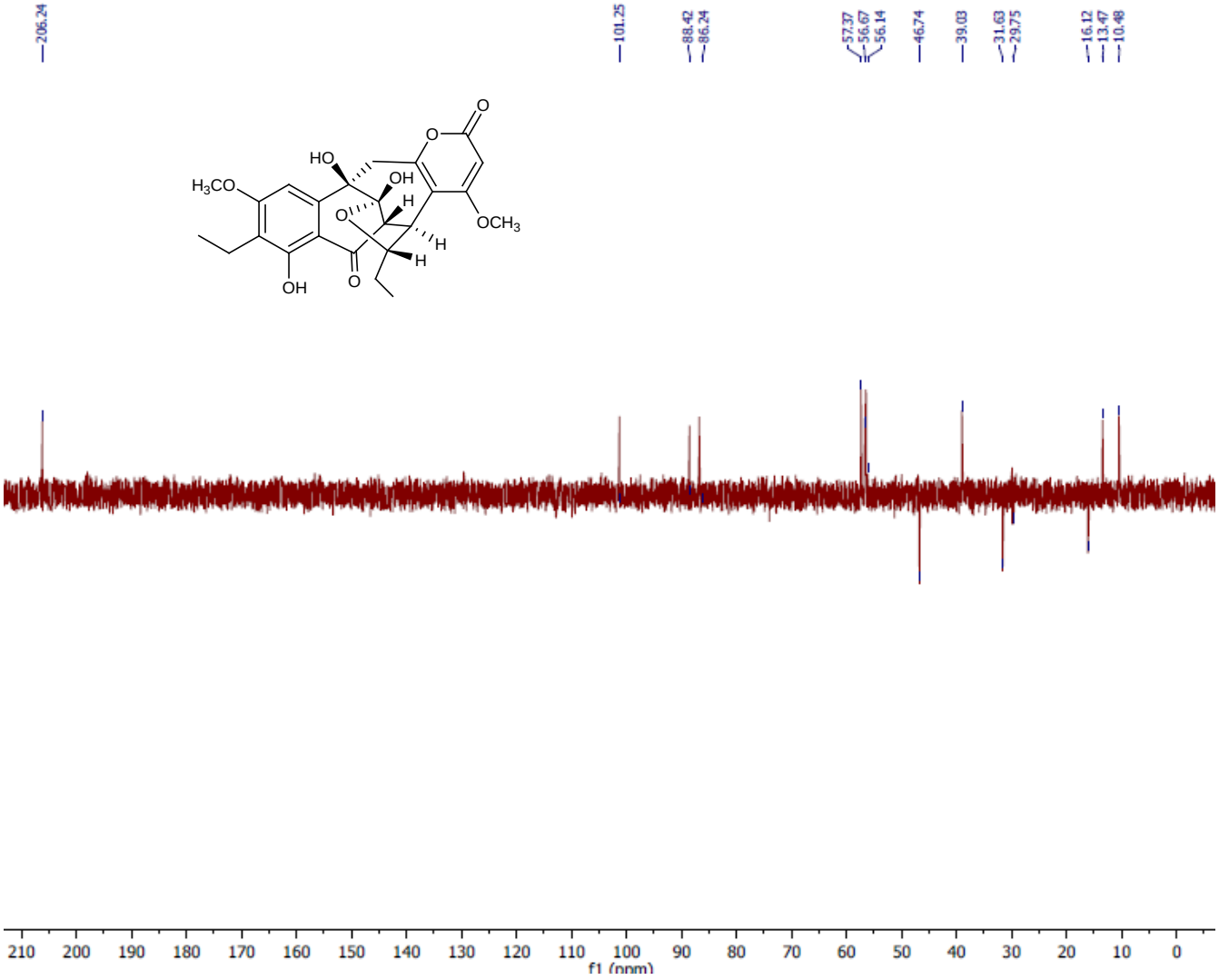


Figure S5. HSQC Spectrum of Delitschiapyrone A (**1**) in Acetone-d_6

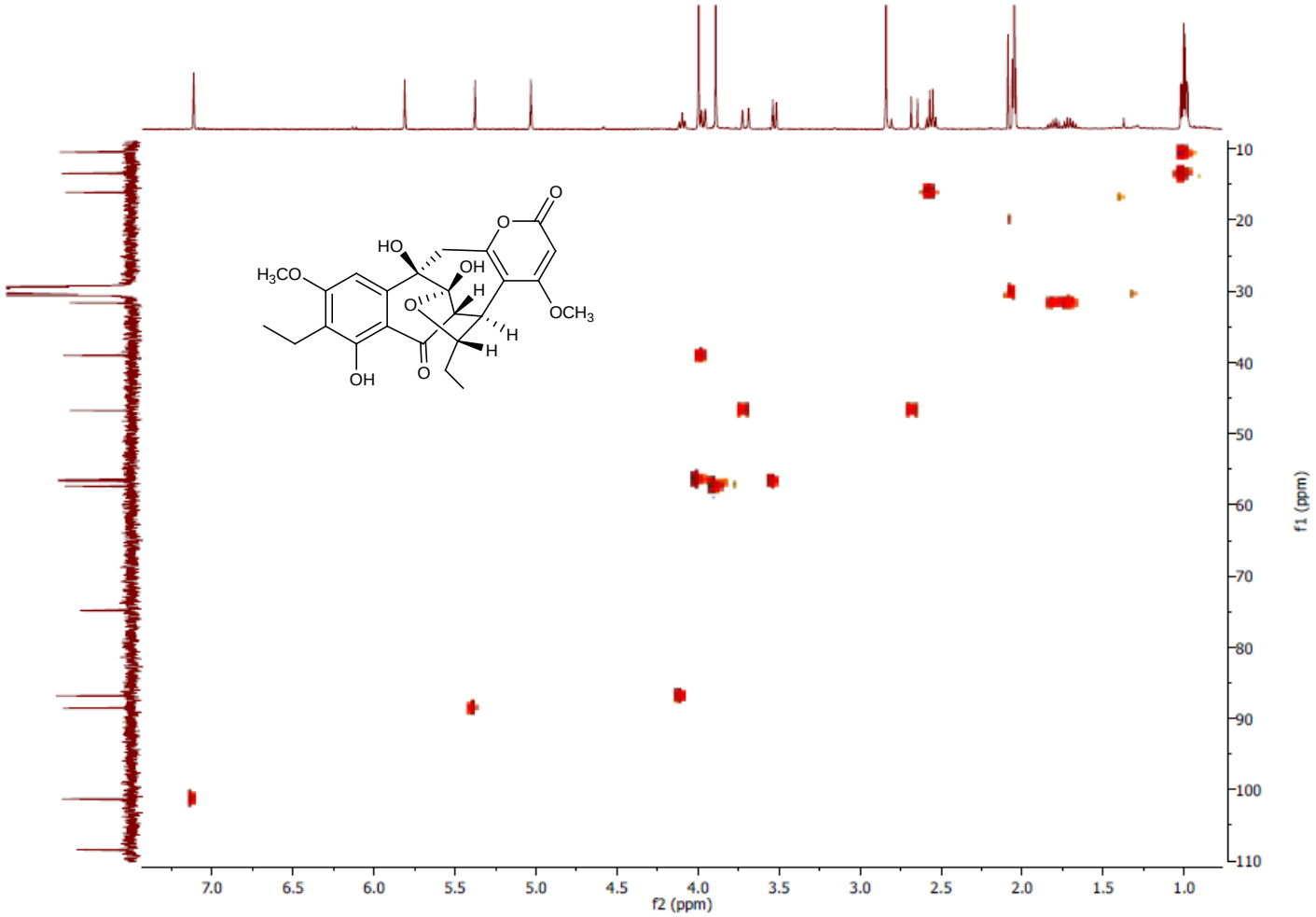


Figure S6. TOCSY Spectrum of Delitschiapyrone A (**1**) in Acetone-d_6

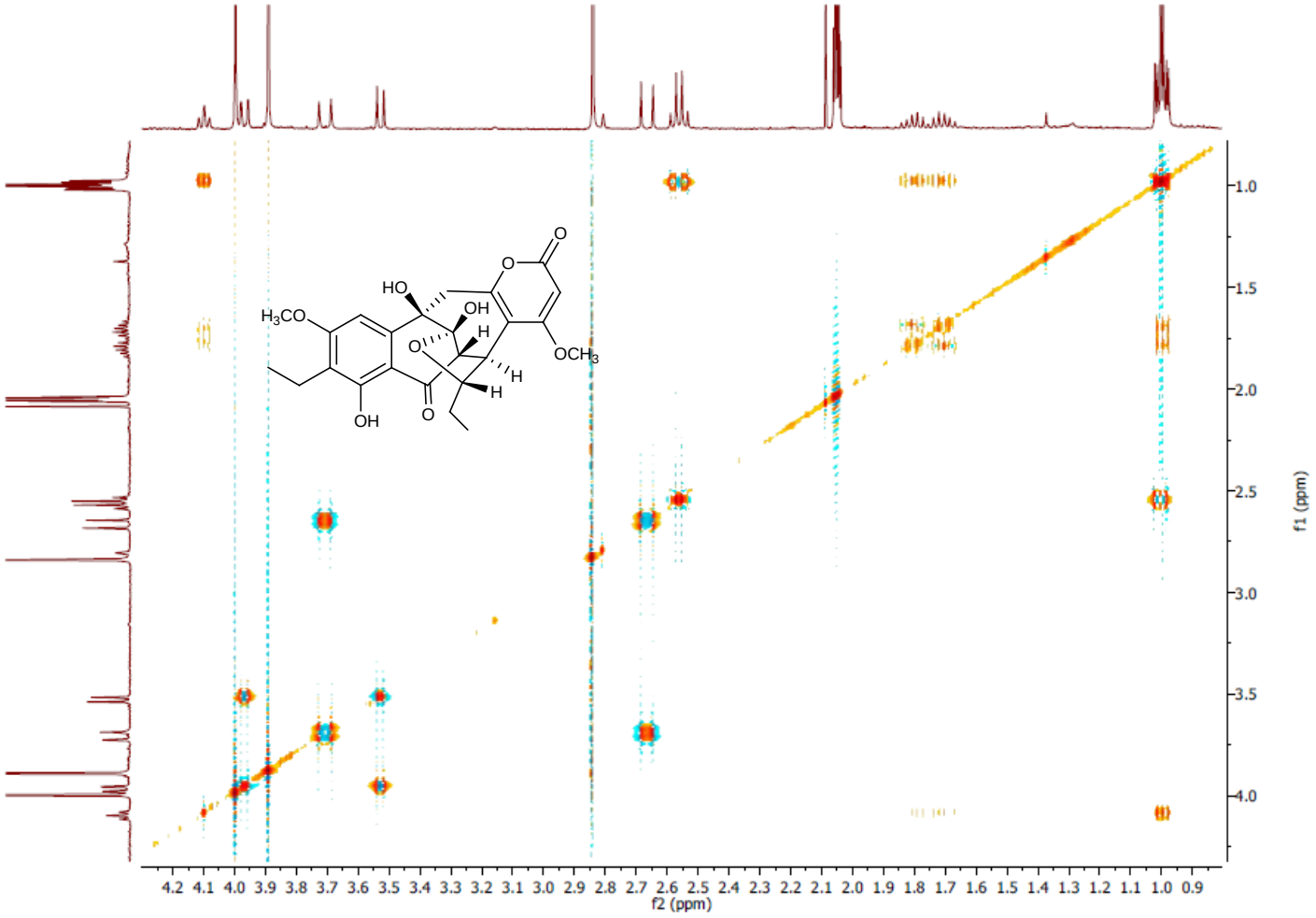


Figure S7. HMBC Spectrum of Delitschiapyrone A 6 (**1**) in Acetone-d_6

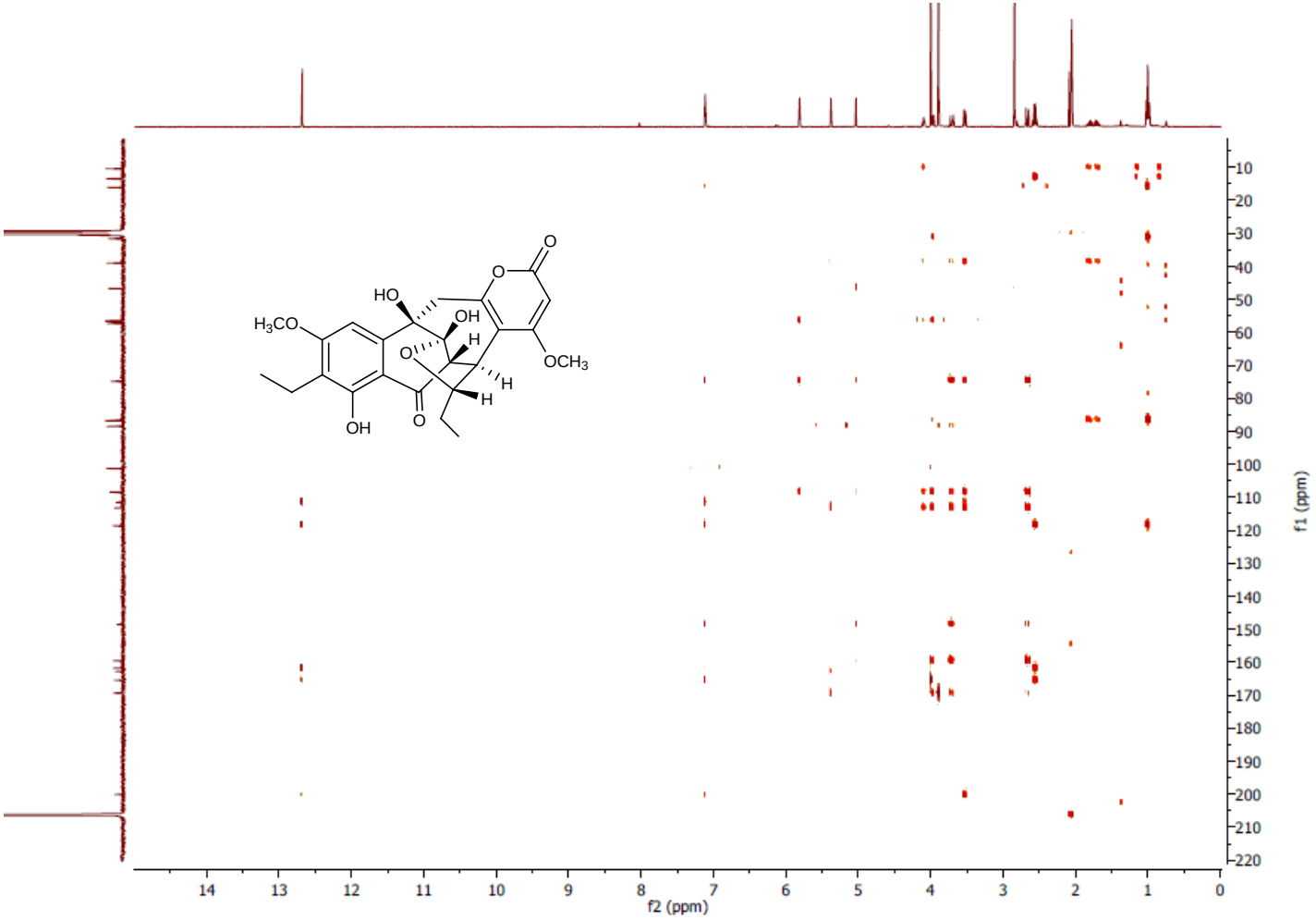


Figure S8. NOESY Spectrum of Delitschiapyrone A (**1**) in Acetone-d_6

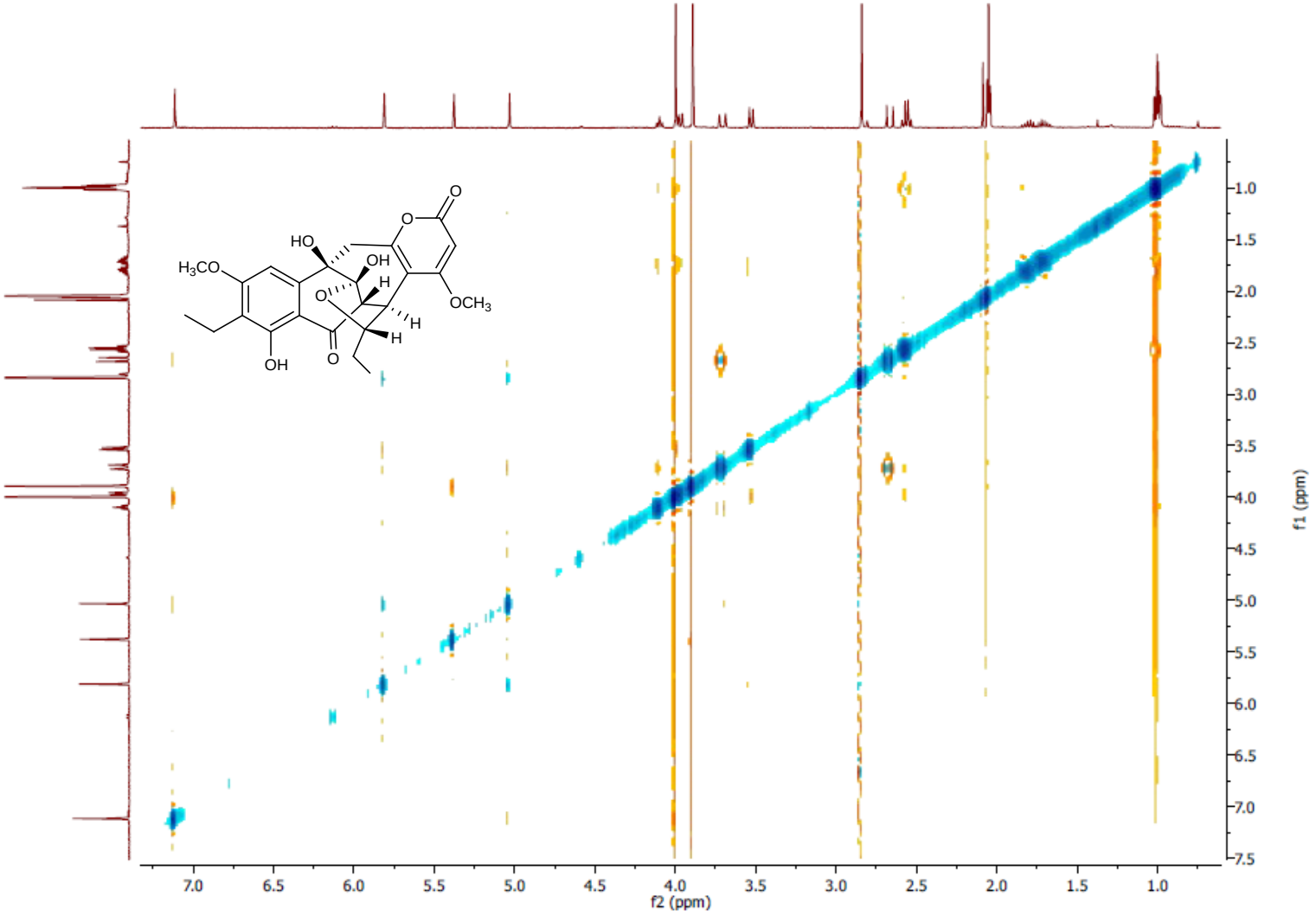


Table S1. IC_{50} Values (μM) of Compounds 1–3 and Cisplatin Against Human Tumor Cell Lines

Compound	MCF-7	H460	HepG2	U2OS
1	35.5 ± 2.3	12.9 ± 2.4	12.3 ± 0.6	22.3 ± 0.9
2	46.6 ± 4.5	22.0 ± 2.5	12.4 ± 0.9	25.6 ± 4.4
3	36.5 ± 1.0	33.4 ± 0.9	81.4 ± 0.8	26.7 ± 2.2
Cisplatin[a]	9.4 ± 0.3	2.2 ± 0.3	8.3 ± 0.6	6.4 ± 0.1

[a]Cisplatin was used as the positive control.