

Supporting Information

Swietemahalactone, a rearranged phragmalin-type limonoid with anti-bacterial effect, from *Swietenia mahagoni*

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Experimental Section

1.1 General Experimental Procedures.

Optical rotations were obtained on JASCO P-1020 digital polarimeter. IR spectra were recorded on a BRUKER TENSOR27 spectrometer. UV spectra were taken on Shimadzu 2401PC spectrophotometer. FAB-MS was determined on a VG Auto Spec-3000 mass spectrometer. Regular 1D-NMR and 2D-NMR spectra were measured on either a Bruker AV-400 or an Avance III 600 instrument with TMS as internal standard, and chemical shifts (δ) were reported using Pyridine- d_5 or $CDCl_3$ as the solvent. X-ray diffraction was realized on a Bruker SMART APEX CCD crystallography system.

1.2 Plant Material.

The leaves and branches of *S. mahagoni* were collected from Xishuangbanna Prefecture of Yunnan province, China, in July 2010 and identified by Prof. Chen Shukun. (Kunming Institute of Botany, CAS).

1.3 Extraction and isolation.

The powder and dried leaves and branches of *S. mahagoni* (5 kg) were extracted with MeOH by refluxing (80 °C, 10 L \times 3, 4 h each time) and concentrated in vacuo to give a crude extract (500 g), which was then partitioned in succession between H_2O and n-hexane, EtOAc, and then n-BuOH. The EtOAc fraction (100 g) was chromatographed on silica gel with $CHCl_3/MeOH$ gradient elution (100:1 \rightarrow 1:1) to afford five fractions: *Fr. 1* (100:1), *Fr.2* (50:1), *Fr.3* (20:1), *Fr.4* (5:1), *Fr.5* (1:1). *Fr.3* was on chromatography over silica gel with $CHCl_3$ —MeOH (50:1, 20:1, and 10:1) to yield three fractions (*Fr.3-1*, *Fr.3-2*, and *Fr.3-3*). Combination of *Fr.3-3* and *Fr.4* was further chromatographed on an ODS column gradient eluting with MeOH- H_2O (45% \rightarrow 65%) to obtain swietemahalactone A (**1**, 250 mg) and 1-*O*-deacetyl-2a-hydroxykhayanolide E (**2**, 100 mg).

Swietemahalactone (1): white, amorphous powder; $[\alpha]_D^{25} +89.6$ (c 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ): 207 (4.11) nm; IR (KBr) ν_{max} : 3444, 2951, 2884, 1728, 1460,

1382, 1256, 1244, 1162, 1125, 1086, 1026 cm^{-1} . Positive ESIMS m/z 537 $[\text{M} + \text{Na}]^+$; positive HRESIMS m/z 515.1910 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{27}\text{H}_{31}\text{O}_{10}$ $[\text{M} + \text{H}]^+$, 515.1917).

1.4 1,3,6-triacetyl swietemahalactone (1a).

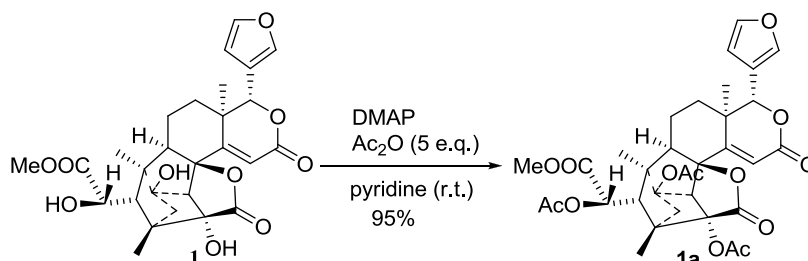


Table 2. ^1H and ^{13}C NMR-DEPT data for **1a** (400 Hz and 100 Hz).

1a (in CDCl_3)					
No.	δ_{C} multi	δ_{H} (multi; J in Hz)	No.	δ_{C} multi	δ_{H} (multi; J in Hz)
1	93.9 s		16	164.6 s	
2	170.0 s		17	82.3 d	5.24 (s)
3	88.5 s		18	20.1 q	1.22 (s)
4	48.5 s		19	15.1 q	1.07 (s)
5	42.5 d	2.51 (d; 2.7)	20	119.6 s	
6	70.9 d	5.39 (d; 3.0)	21	141.2 d	7.43 (s)
7	170.0 s		22	109.8 d	6.40 (d; 1.0)
8	89.4 s		23	143.0 d	7.42 (t; 1.6)
9	48.2 d	2.27 (dd; 11.6, 8.4)	28	40.7 t	2.31 (d; 10.5); 2.93 (dd; 10.5, 1.1)
10	53.7 s		29	11.1 q	1.13 (s)
11	15.4 t	1.61-1.72 (m); 1.85-1.89 (m)	30	55.1 d	3.76 (s)
12	25.9 t	1.92-1.99 (m); 1.41 (dd; 13.1, 9.3)	7-OMe	52.7 q	3.73 (s)
13	39.3 s		1-OAc	170 s	
				21.6 q	2.03 (s)
14	161.5 s		3-OAc	169 s	
				20.5 q	2.20 (s)
15	116.2 d	6.25 (s)	6-OAc	169 s	
				20.9 q	2.20 (s)

1.5 1,3,6-tris(4-bromobenzoyl)swietemahalactone (**1b**).

A sample of **1** (20.6 mg, 0.04 mmol), *p*-bromobenzoyl chloride (36.4 mg; 0.168 mmol), and DMAP (35.6 mg; 0.292 mmol) were dissolved in pyridine (16 mL), and the solution was stirred at room temperature under Ar. TLC analysis of the solution after 20 h indicated near completion of the reaction. The solution was combined with saturated aqueous NaHCO₃ solution (8 mL) and H₂O (4 mL), and the resulting mixture was extracted with CH₂Cl₂ (12 × 3 mL). The combined organic extract was dried under airflow, and the residue was subjected to PLTC with CHCl₃ elution to afford 1,3,6-tris(4-bromobenzoyl)swietemahalactone (**1b**).

X-ray data of 1b: C₄₈H₃₉Br₃O₁₃, M = 1063.52, colorless prism, space group P2₁, *a* = 11.446 (2) Å, *b* = 14.770 (3) Å, *c* = 14.492 (2) Å, $\alpha = \gamma = 90^\circ$, $\beta = 96.02^\circ$, *V* = 2436.4 (7) Å³, *Z* = 2, *d* = 1.450 g/cm³, crystal dimensions 0.50×0.14×0.11 mm was used for measurements on a Bruker APEX DUO diffractometer with a graphite monochromator (Φ/ω scans), Mo K α radiation. The total number of independent reflections measured was 11778, of which 8207 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices: *R*₁ = 0.0723, *wR*₂ = 0.2020 (*w* = 1/ $\sigma|F|^2$), *S* = 1.010, Flack parameter 0.021(11). The crystal structure of **1b** was solved by direct method SHELXS-97 (Sheldrick, G. M. University of Gottingen: Gottingen, Germany, 1997) and the full-matrix least-squares calculations. Crystallographic data for the structure of **1b** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 900980). Copies of these data can be obtained free of charge via the Internet at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, U.K.; fax (+44) 1223-336-033; or deposit @ccdc.cam.ac.uk).

1.6 Antibacterial Test.

The in vitro antibacterial activities against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (CMCC 10104), *Staphylococcus epidermidis* (CMCC 26069), and *Bacillus subtilis* (ACCC 11060) were evaluated using the agar diffusion method, according to the NCCLS protocol with slight modifications.¹ To the well-prepared agar plates, microbial cells along with the compounds were suspended in Mueller Hinton broth at the density of 10⁸ CFU/ml

and incubated at 37 °C for 18 h under aerobic conditions. The compound or positive control (norfloxacin) have been dissolved in DMSO to give an end concentration of 1 µg/ml, injected 200 µl to oxford cup with the diameter of 8 mm. The blank controls of microbial culture were incubated with limited DMSO under the same condition. The DMSO was determined not to be toxic at a limited amount under the experimental conditions. The sensitivity was recorded by measuring the clear zone of growth inhibition around the wells (mm diameter). Each set was tested in triplicate. The MIC value of the compound was determined by using the microdilution method in 96-well plates.

1. National Committee for Clinical Laboratory Standards (NCCLS), 2002. Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically, third ed. Approved standard NCCLS document M100-812, Wayne, PA.

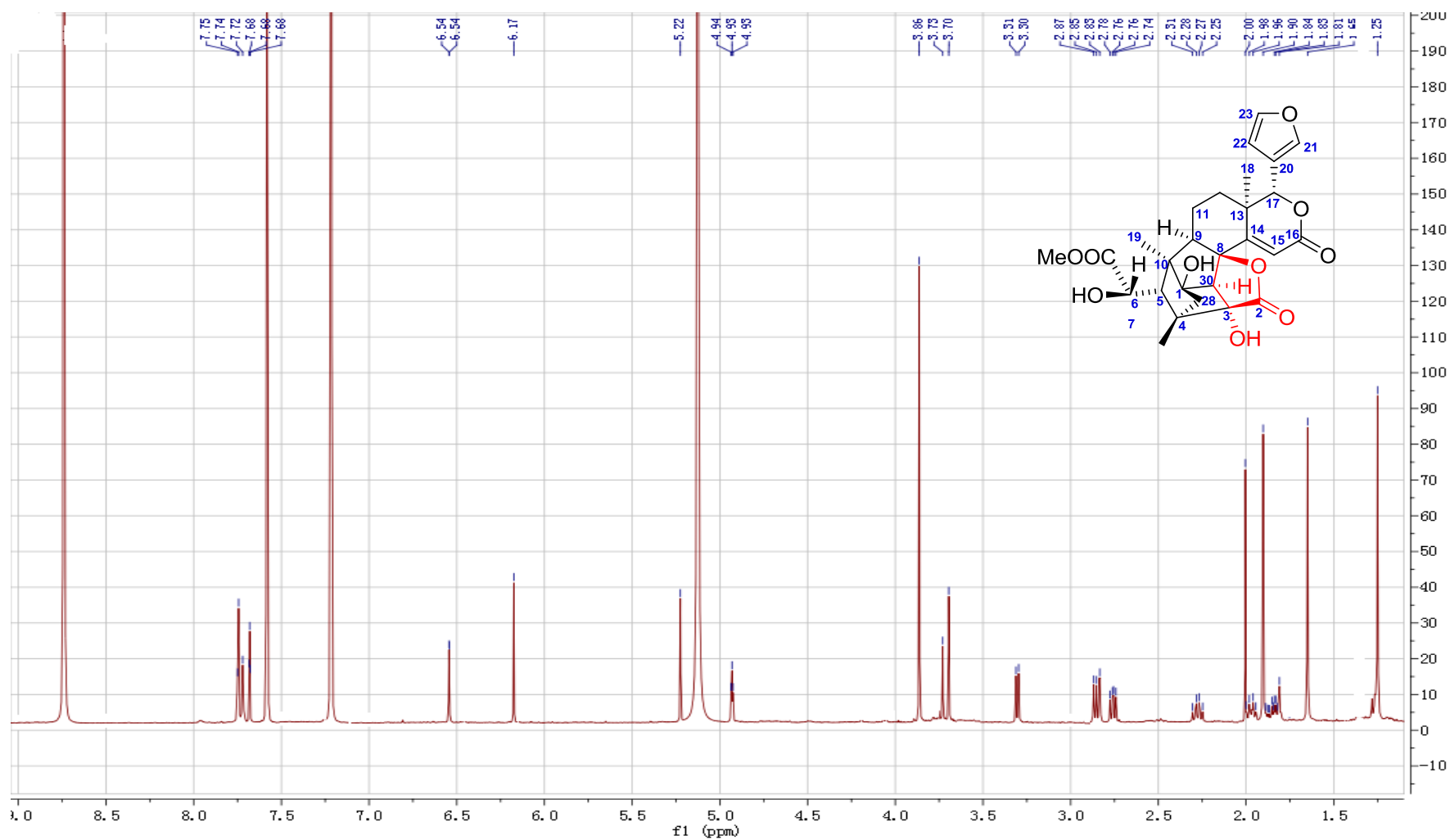
1.7 Brine shrimp (*Artemia salina*) toxicity assay.

Brine shrimp eggs (*Artemia salina*) obtained locally (ASK, Japan) were hatched in artificial seawater prepared from sea salt (ASK, Japan). After 48 h of incubation at 28 °C, nauplii were prepared for the following tests. The assays were performed in duplicate on a microtiter plate with more than 15 (15–25) nauplii in seawater and pure compounds in DMSO to give the end concentrations of 2, 10, or 100 µg/ml. DMSO was used for blank control. Toxicity (T) was determined after 24 h of exposure at room temperature under the microscope according to the formula $T = (A - N - B) * Z^{-1} * 100\%$ with A = number of dead nauplii after 24 h, N = number of dead nauplii before the addition of compound or extract, B = average number of dead nauplii in the blind sample, Z = total number of nauplii.

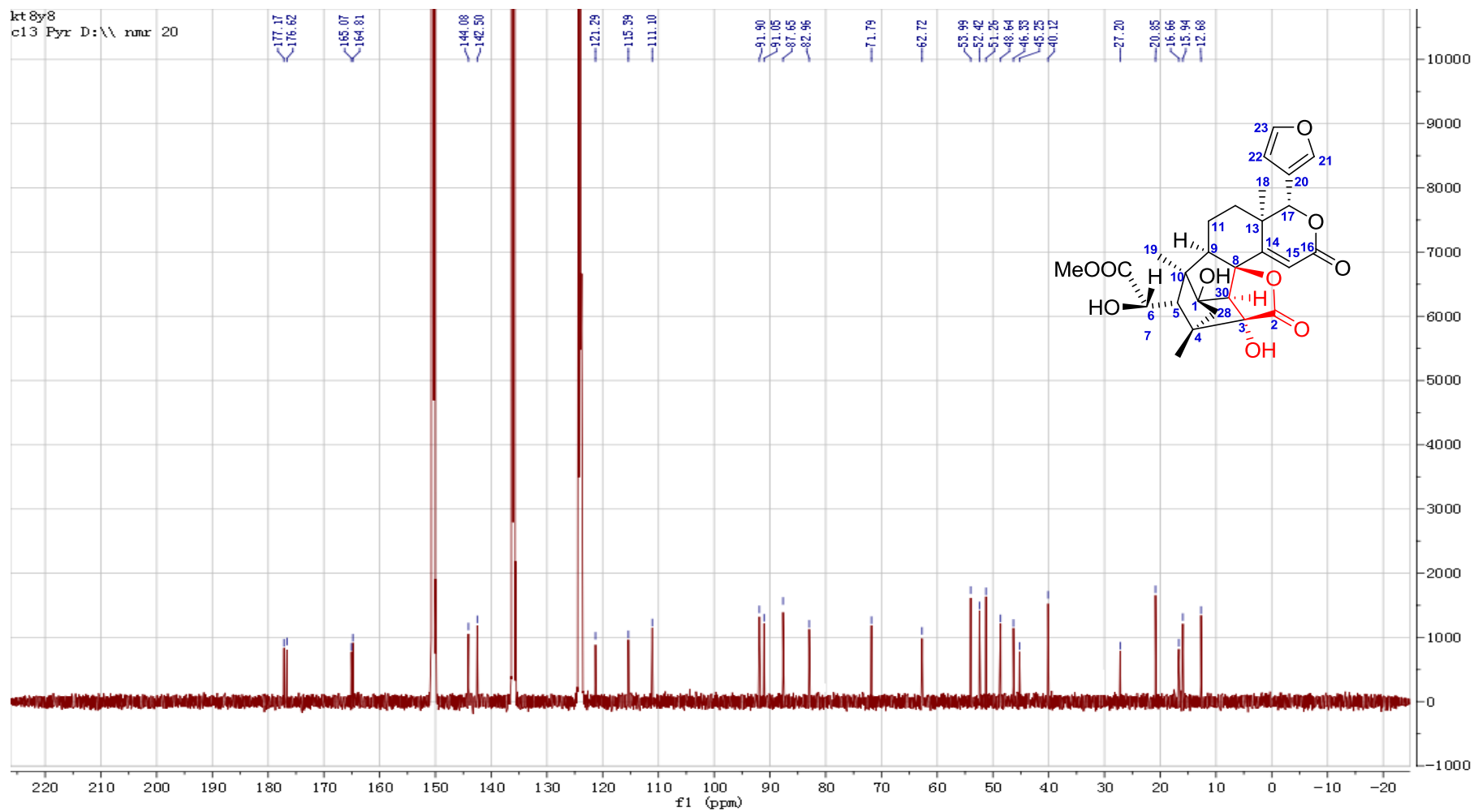
1.8 Cytotoxicity Assay.

MCF-7 human breast cancer, SMMC7721 human hepatocellular carcinoma, HL-60 human myeloid leukemia, SW480 colon cancer and A549 lung cancer were obtained from Shanghai cell bank in China. Cells were cultured in either RPMI-1640 or DMEM medium (Hyclone, USA), containing 10% fetal bovine serum (Hyclone, USA) at 37°C in a humidified atmosphere of 5% CO₂. The cytotoxicity was determined by a standard colorimetric MTT (2-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay in 96-well microplates. After compound treatment, cell viability was detected and a cell growth curve was graphed. IC₅₀ values were calculated by Reed and Muench's method.

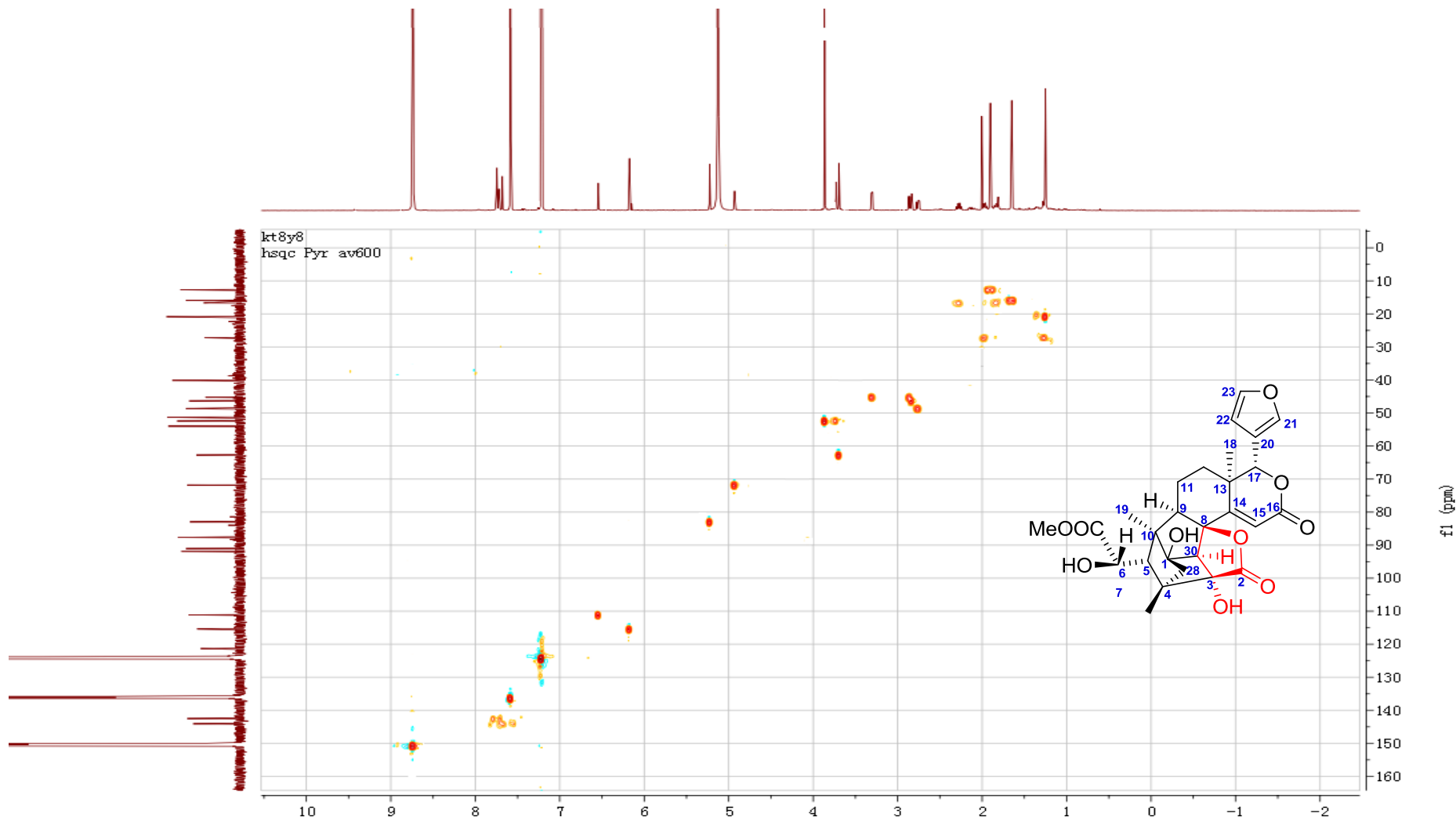
● Figure S1. ¹H NMR Spectrum of Swietemahalactone (**1**)



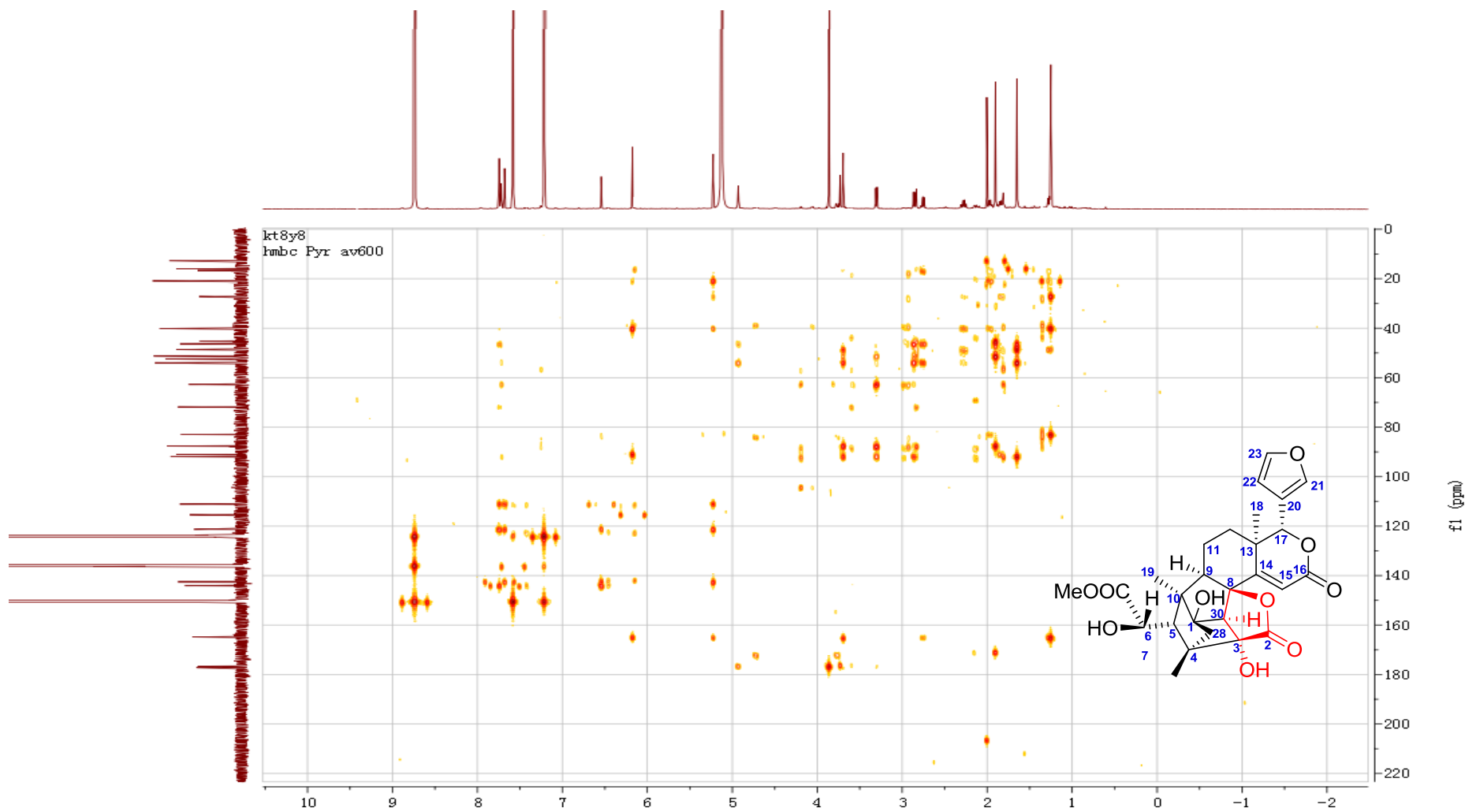
● Figure S2. ¹³C NMR Spectrum of Swietemahalactone (**1**)



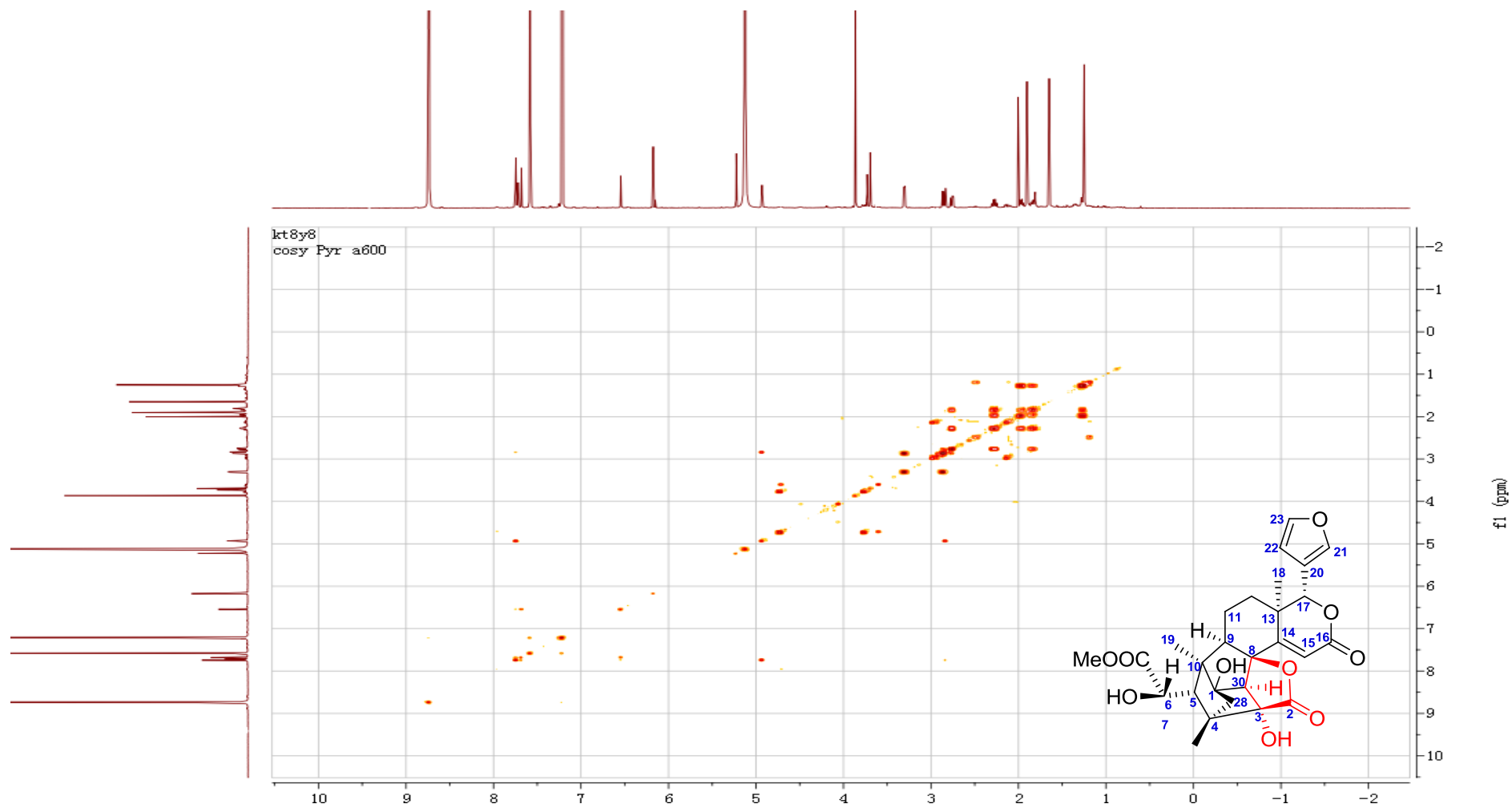
● Figure S3. HSQC Spectrum of Swietemahalactone (1)



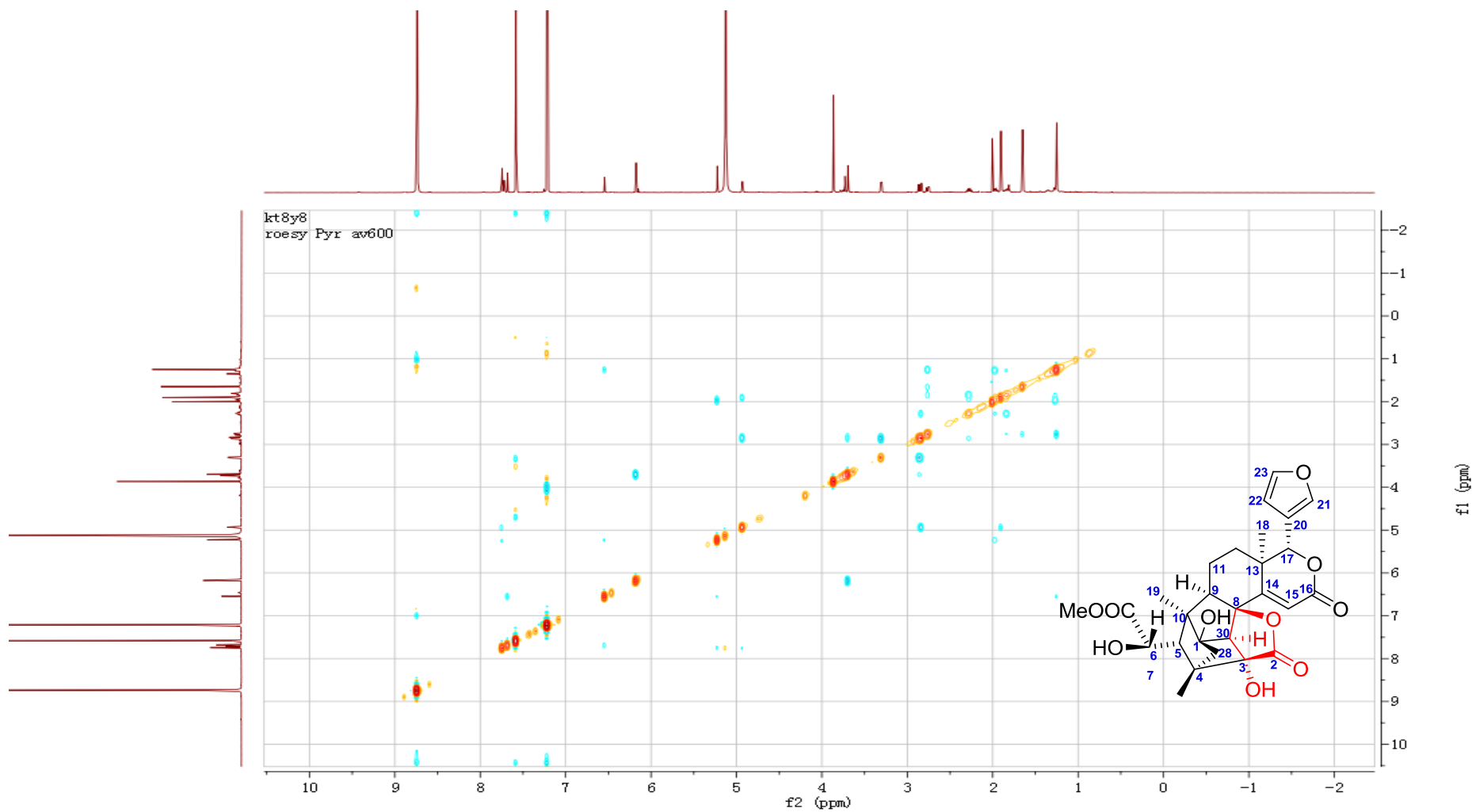
● Figure S4. HMBC Spectrum of Swietemahalactone (**1**)



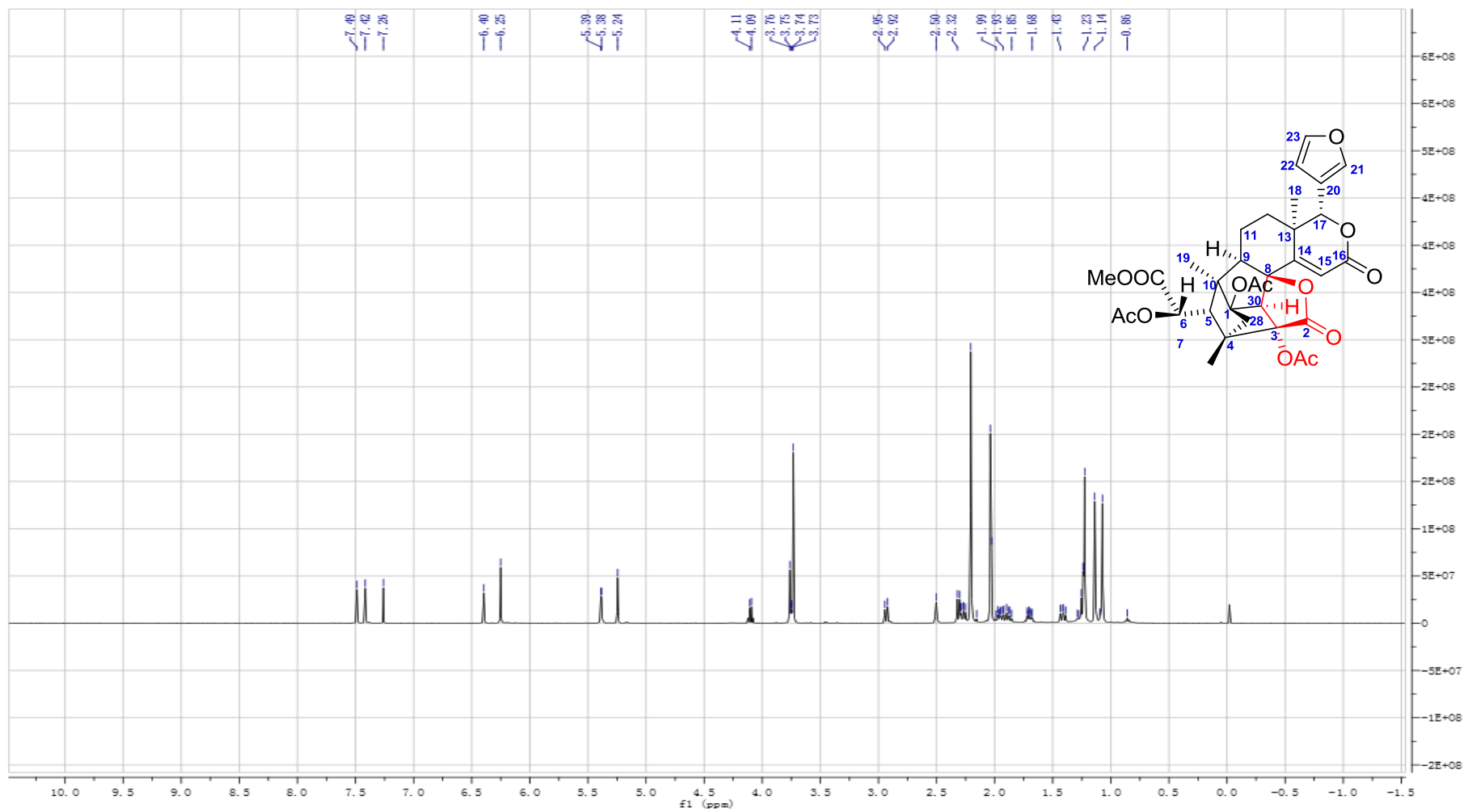
● Figure S5. ^1H - ^1H COSY Spectrum of Swietemahalactone (**1**)



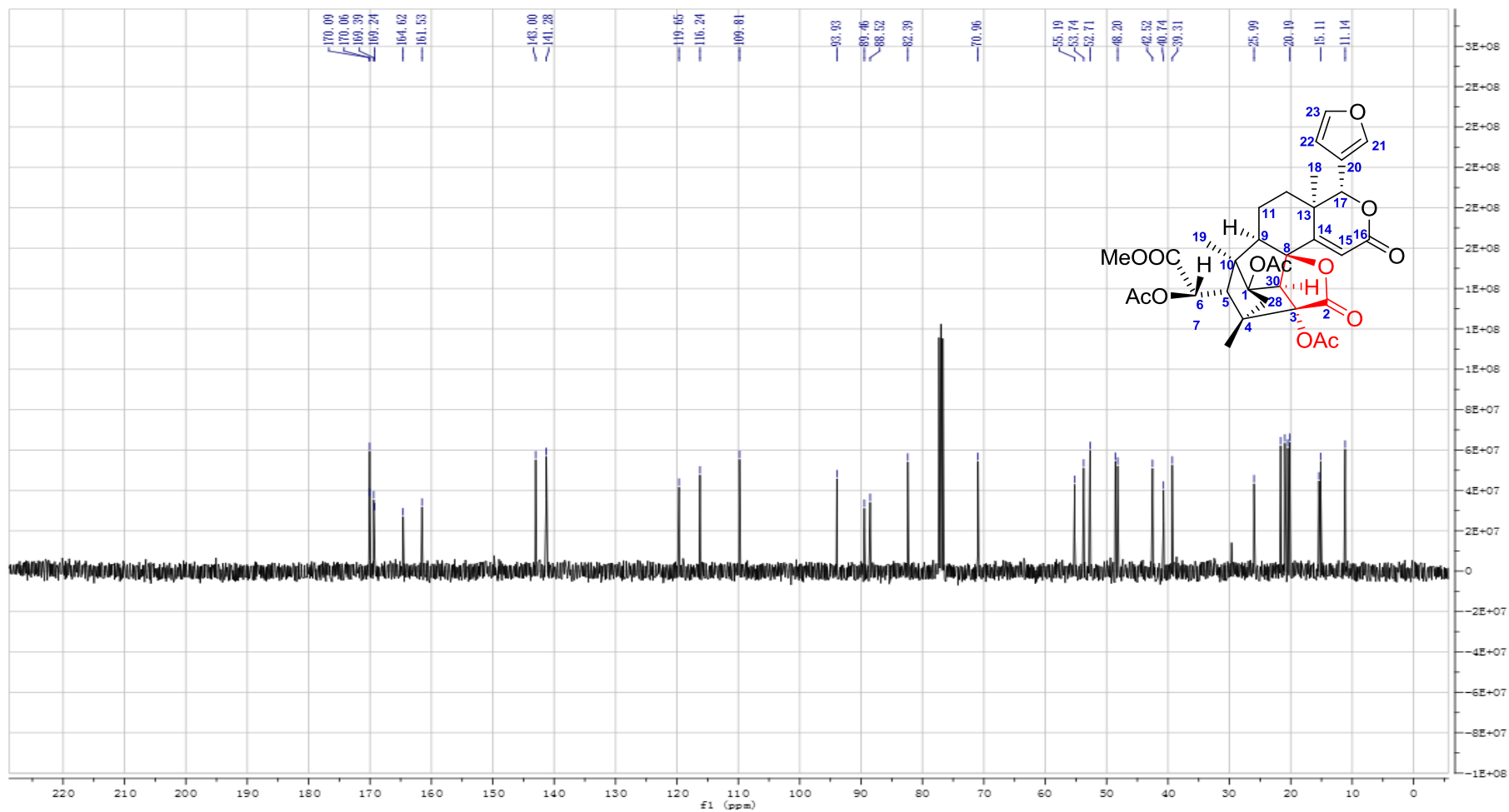
● Figure S6. ROESY Spectrum of Swietemahalactone (**1**)



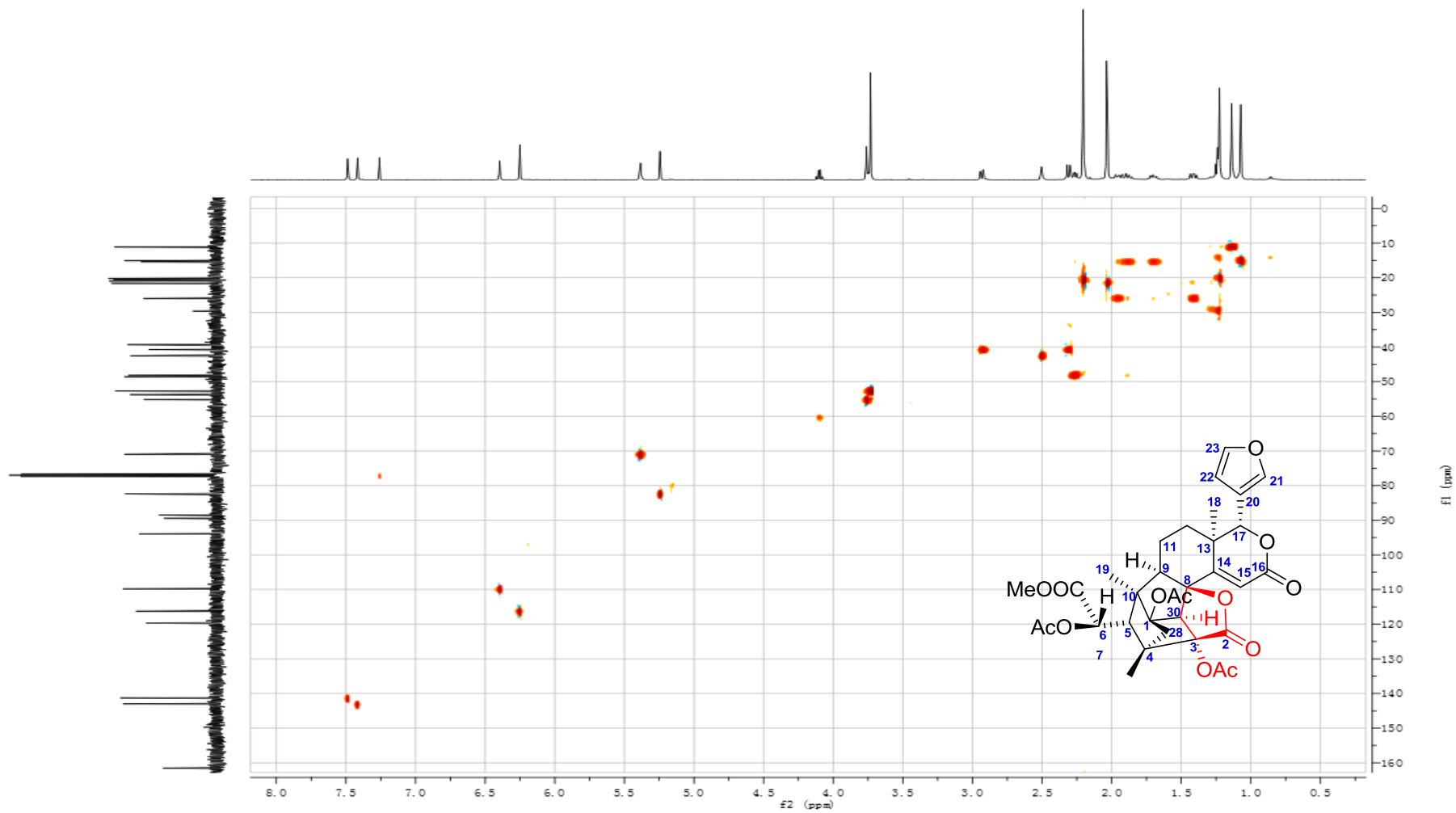
● Figure S7. ^1H NMR Spectrum of 1,3,6-triacetylsvietemahalactone (**1a**)



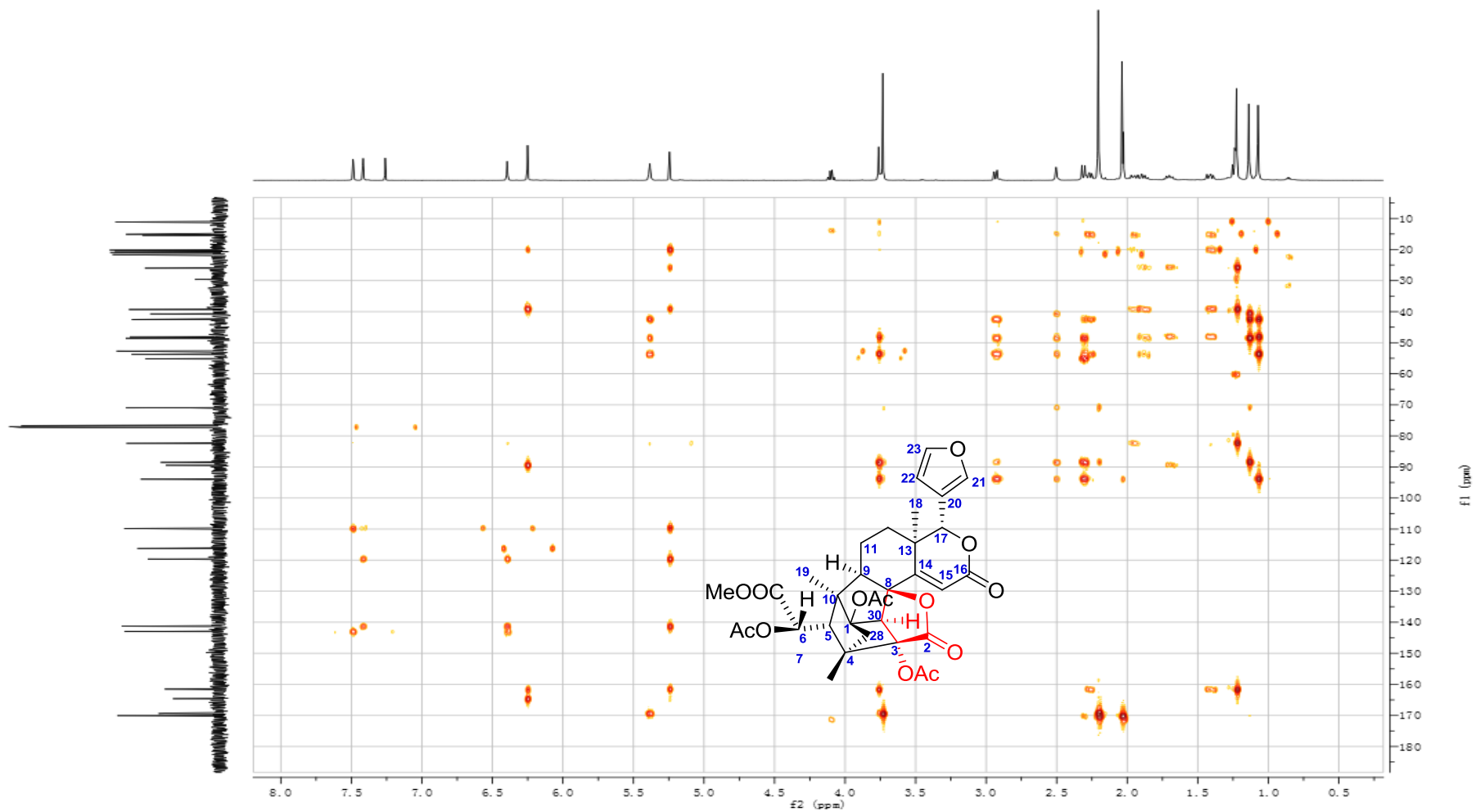
● Figure S8. ¹³C NMR Spectrum of 1,3,6-triacetylsvietemahalactone (**1a**)



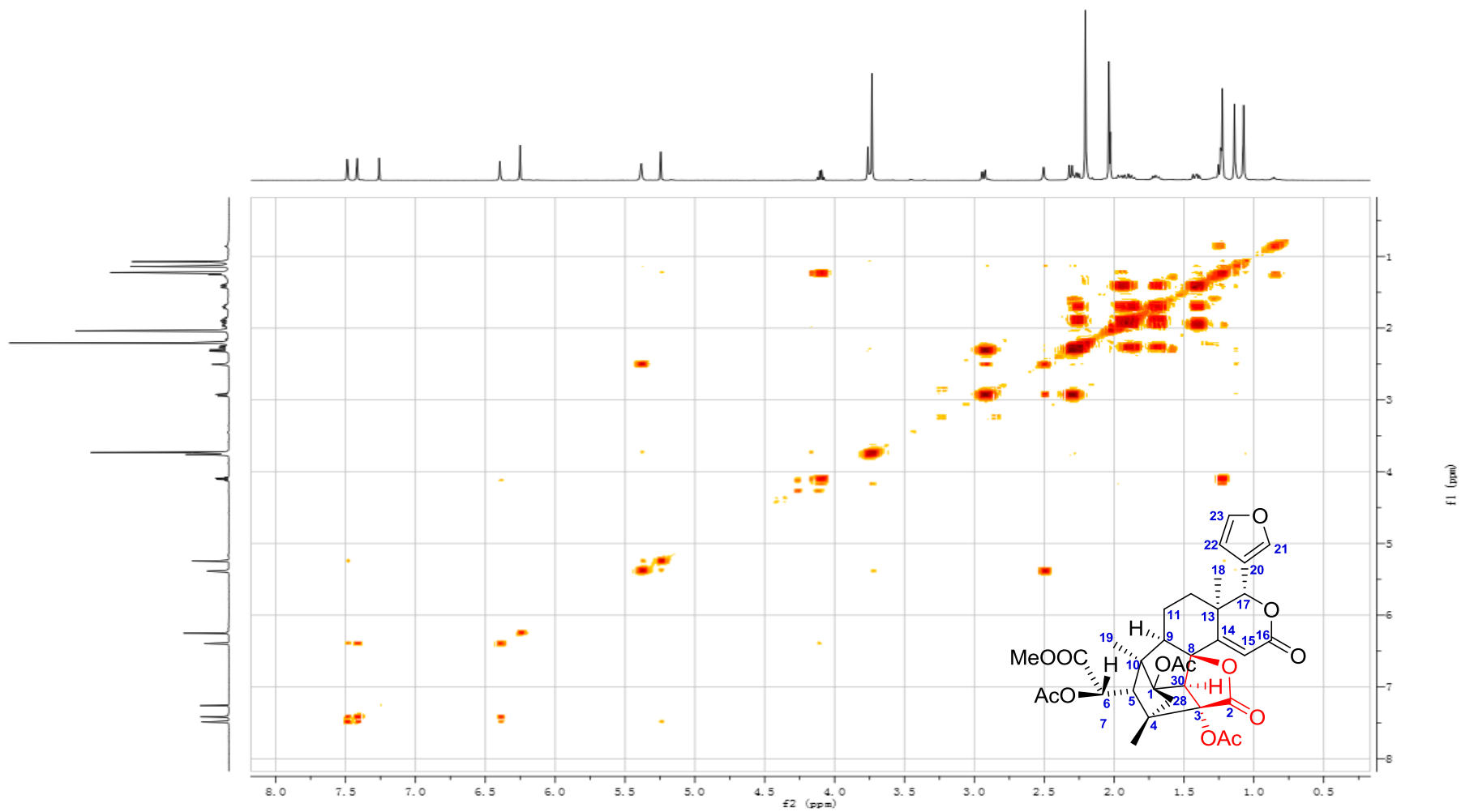
● Figure S9. HSQC Spectrum of 1,3,6-triacetylsvietemahalactone (**1a**)



● Figure S10. HMBC Spectrum of 1,3,6-triacetylsvietemahalactone (**1a**)



● Figure S11. ^1H - ^1H COSY Spectrum of 1,3,6-triacetylsvietemahalactone (**1a**)



● Figure S12. ROESY Spectrum of 1,3,6-triacetylsvietemahalactone (**1a**)

