Three Anthraquinone Derivatives from the Marine Mangrove Endophytic Fungus *Alternaria* sp. ZJ9-6B from the South China Sea

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Description of the Anti-cancer Activity Assay

Preparation of Compounds **1** and **2**

Compounds **1** and **2** were dissolved in 0.5% dimethylsulphoxide (DMSO) at a concentration of 1 mM as stock solution and diluted according to experimental requirements when used.

Cell culture

Human breast cancer cell lines MCF-7 and MDA-MB-435 were cultured in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA, USA) supplemented with 5% fetal bovine serum (Hyclone, Logan, UT, USA), 2mM L-glutamine, 100 $mg \cdot mL^{-1}$ streptomycin and 100 $units \cdot mL^{-1}$ penicillin (Invitrogen). The cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2. Cell Viability assay

Cells were seeded in 96-well flat-bottom plates at a density of 1×10^4 cells per well and cultured in a humidified incubator for 24 h, followed by exposure to various concentrations of compound 1 and 2 for 48 h respectively. Subsequently, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reagent (Genview, Houston, TX, USA) dissolved in phosphate-buffered saline (PBS) (pH 7.4) at a concentration of 5 $mg \cdot mL^{-1}$ was added to each well, and the cells were incubated for additional 4 h. The MTT-formazan crystals formed were dissolved in 150 μL DMSO (Sangon Biotech, Shanghai, China), and the absorbance was measured at 570 nm with a reference wavelength of 630 nm using a microplate reader. Cell growth inhibition was determined using the following formula according to a previously published method: growth inhibition (%) = (1-OD of treated cells/OD of control cells) ×100% (Moon et al., 2000). The half maximal inhibitory concentration (IC_{50}) was calculated by Bliss's software (Bliss, 1935), and the data were analysed by SPSS. For all tests, the inhibition assays were performed in triplicate.

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Figure S1. ^{1}H spectrum of 1 (500 MHz, DMSO-d_6**).**

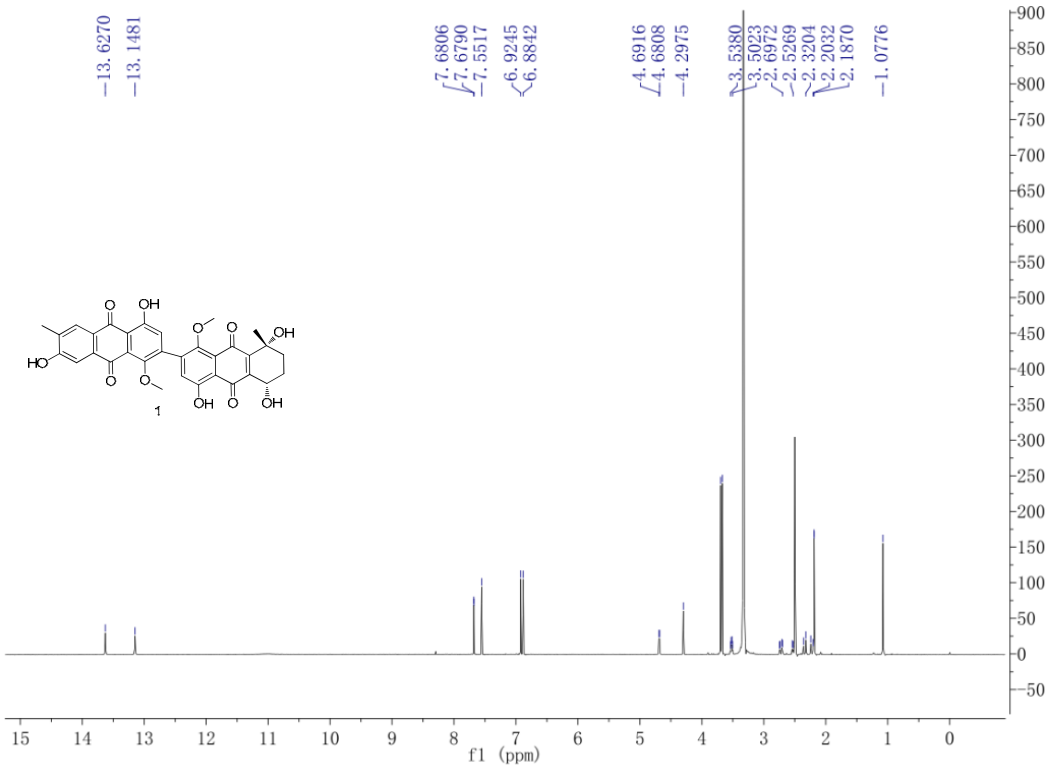


Figure S2. ^{13}C spectrum of 1 (125 MHz, DMSO-d_6**).**

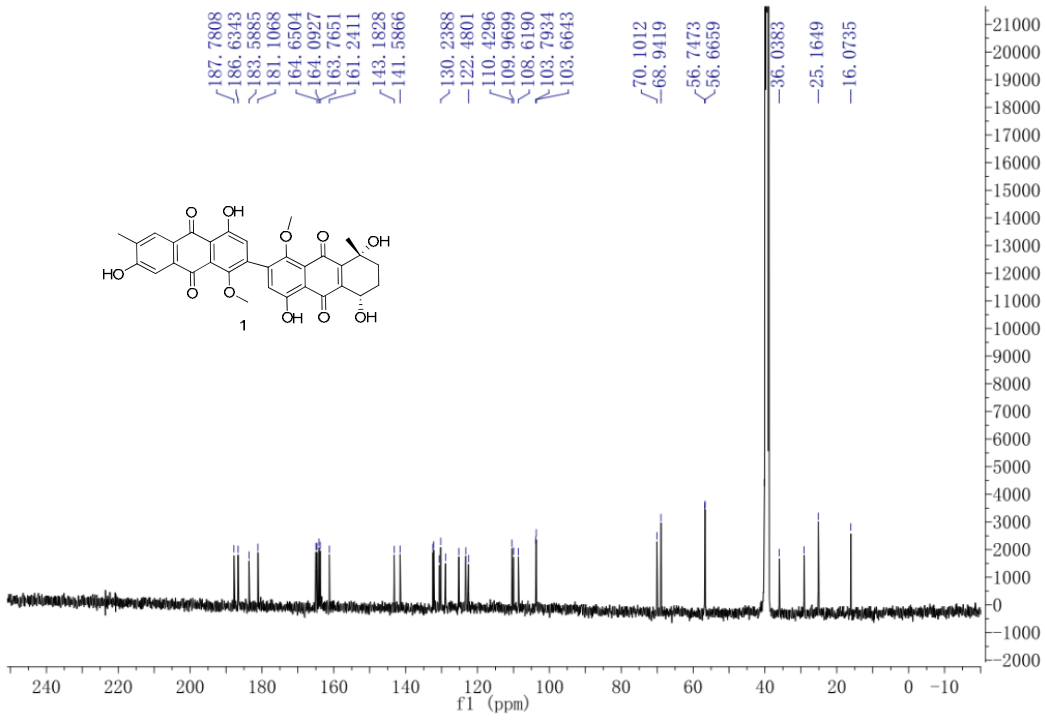


Figure S3. HMQC spectrum of 1 (500/125 MHz, DMSO-d_6**).**

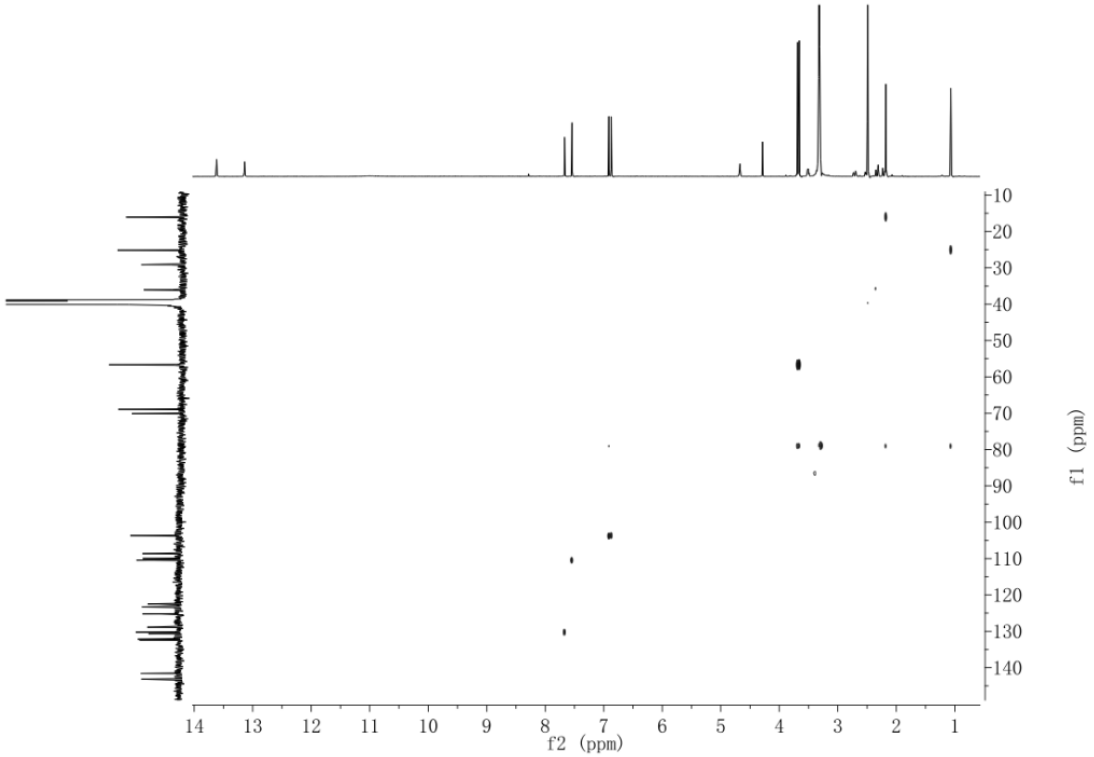


Figure S4. HMBC spectrum of 1 (500/125 MHz, DMSO-d_6**).**

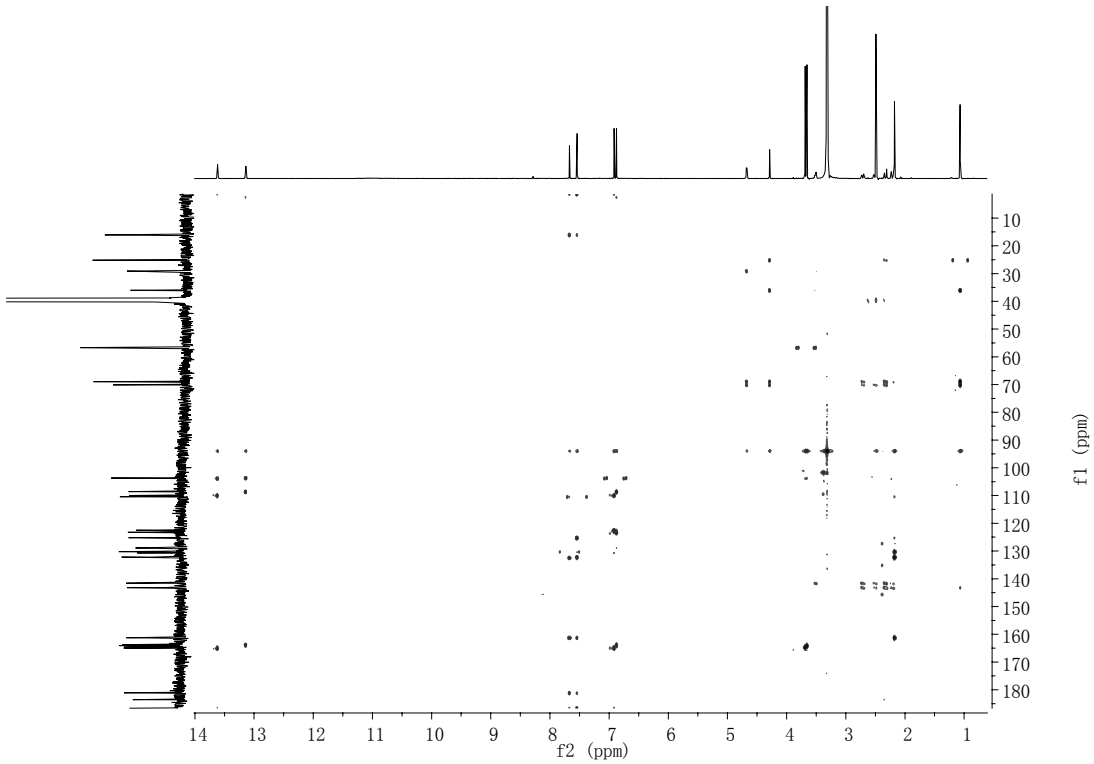


Figure S5. Expansion of HMBC spectrum of 1 (500 MHz, DMSO-d_6**).**

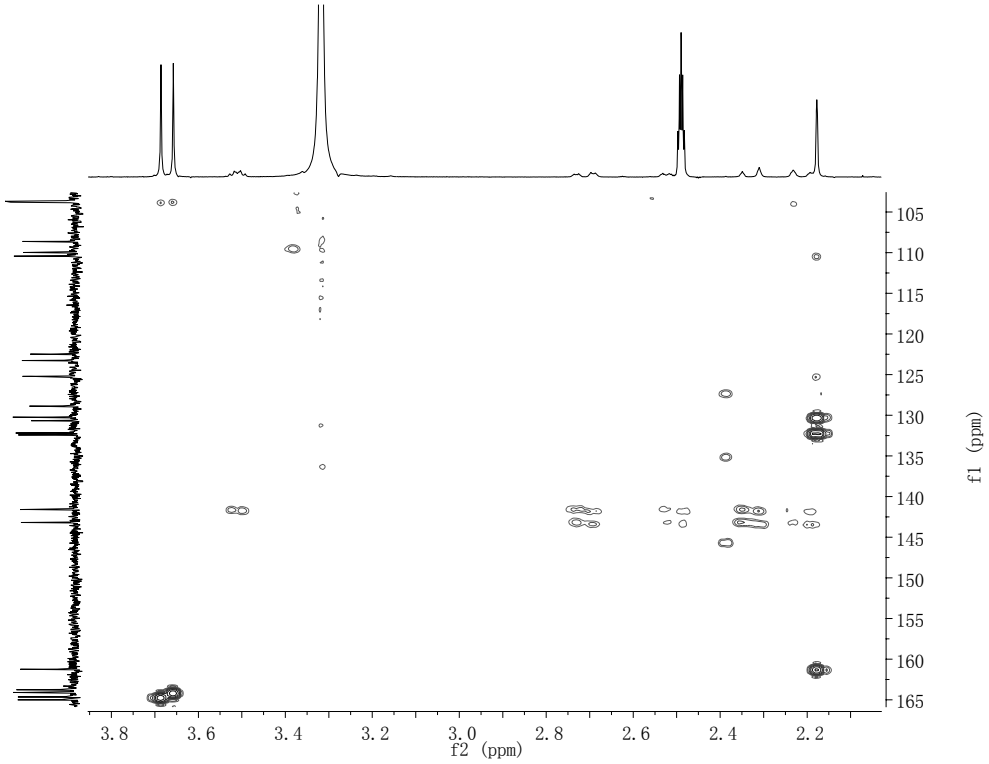


Figure S6. ^{1}H-^{1}H COSY spectrum of 1 (500 MHz, DMSO-d_6**).**

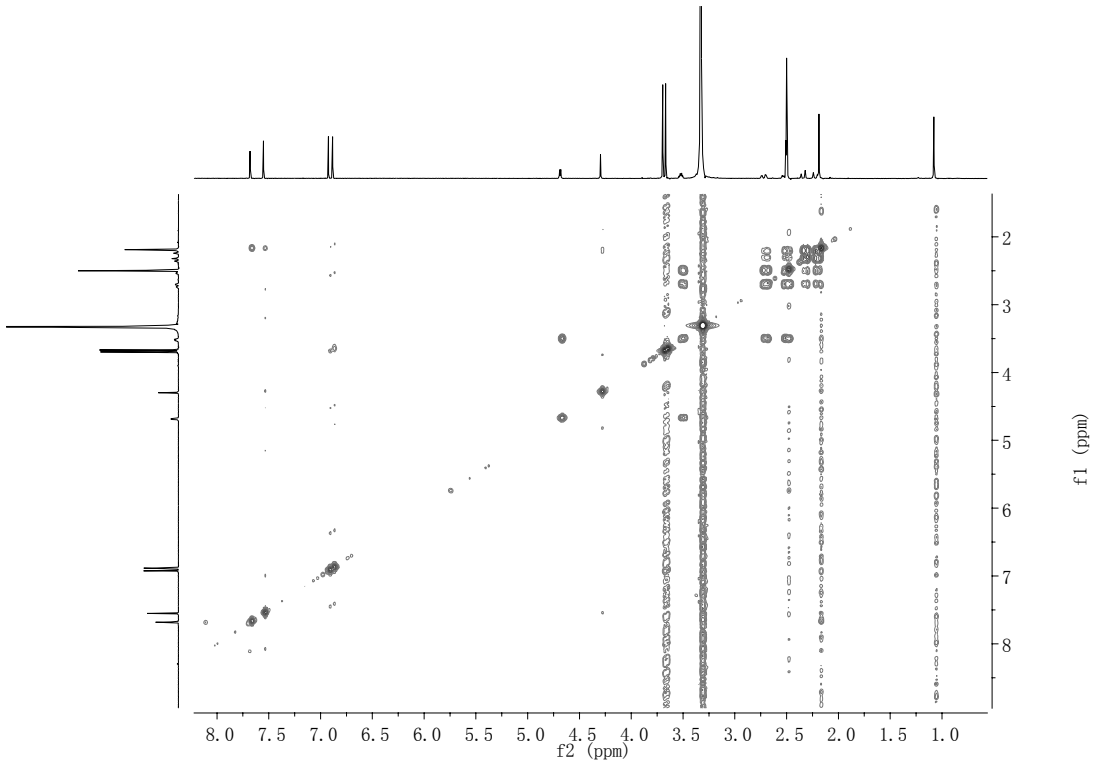


Figure S7. HR-EIMS spectrum of 1.

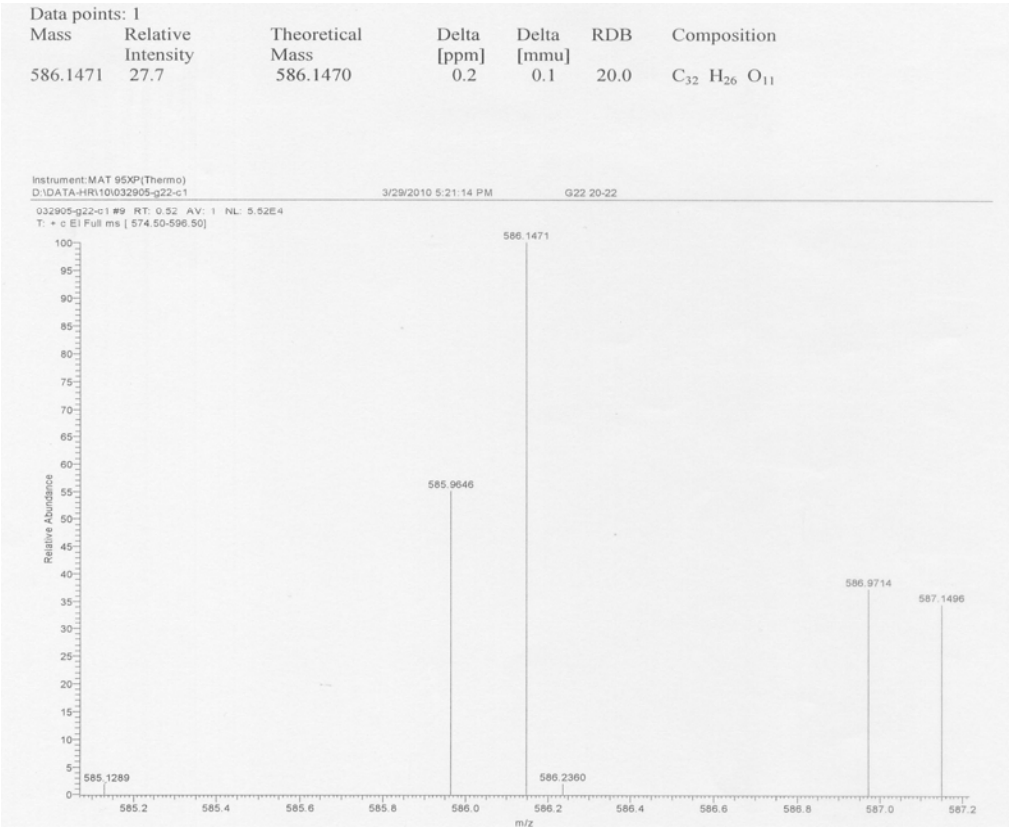


Figure S8. ^{1}H spectrum of 2 (500 MHz, DMSO-d_6**).**

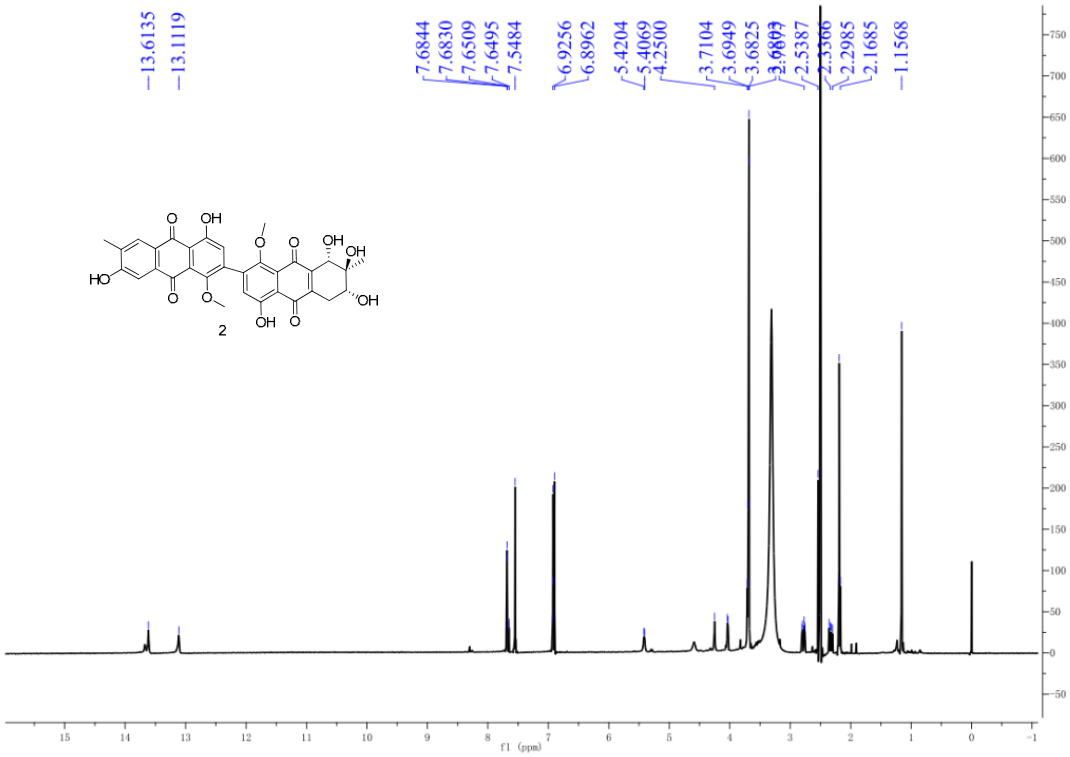


Figure S9. ^{13}C spectrum of 2 (125 MHz, DMSO-d_6**).**

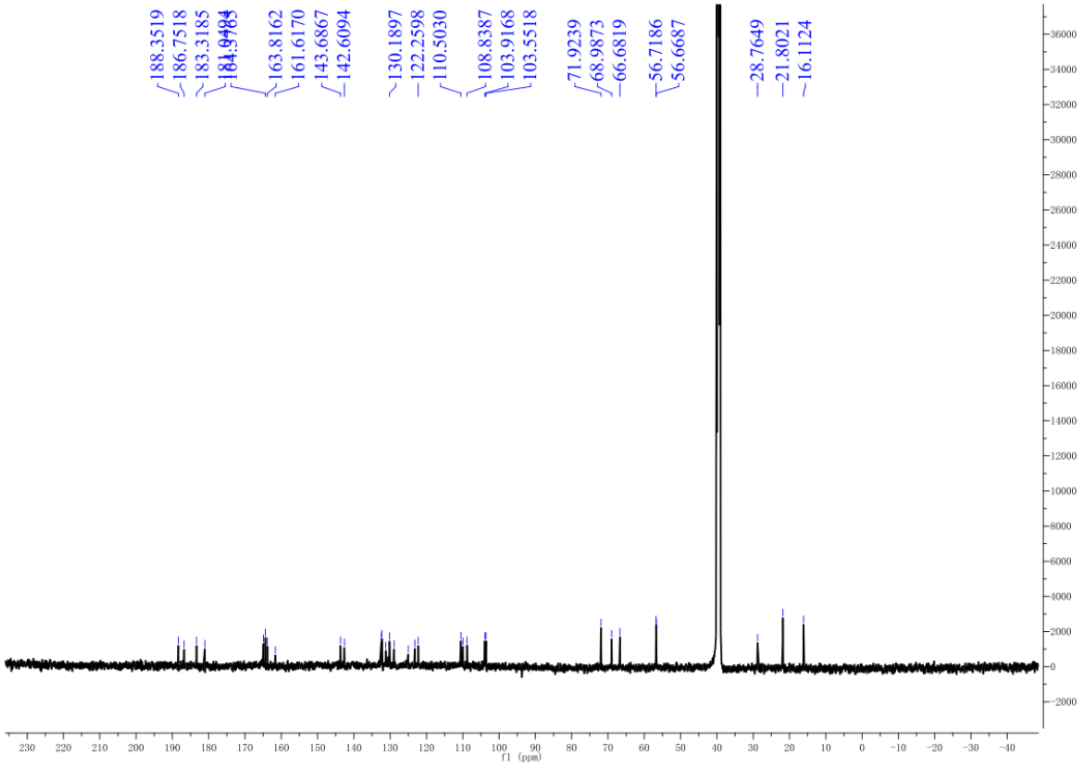


Figure S10. HMQC spectrum of 2 (500/125 MHz, DMSO-d_6**).**

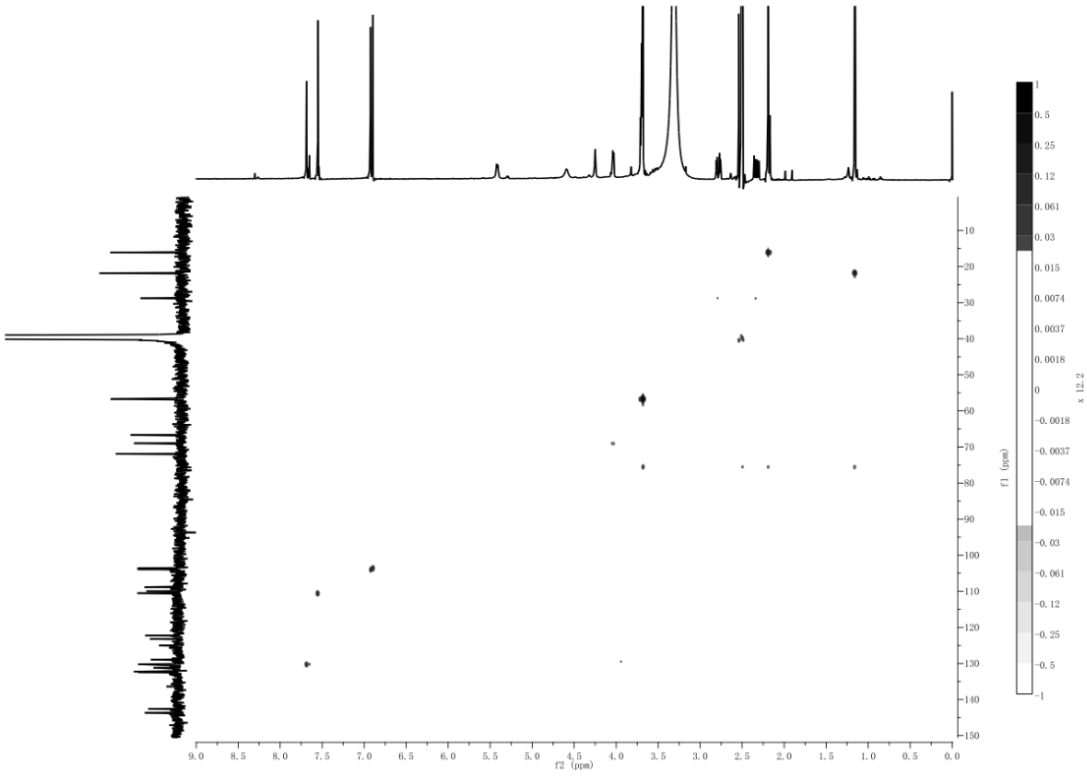


Figure S11. HMBC spectrum of 2 (500/125 MHz, DMSO-d_6**).**

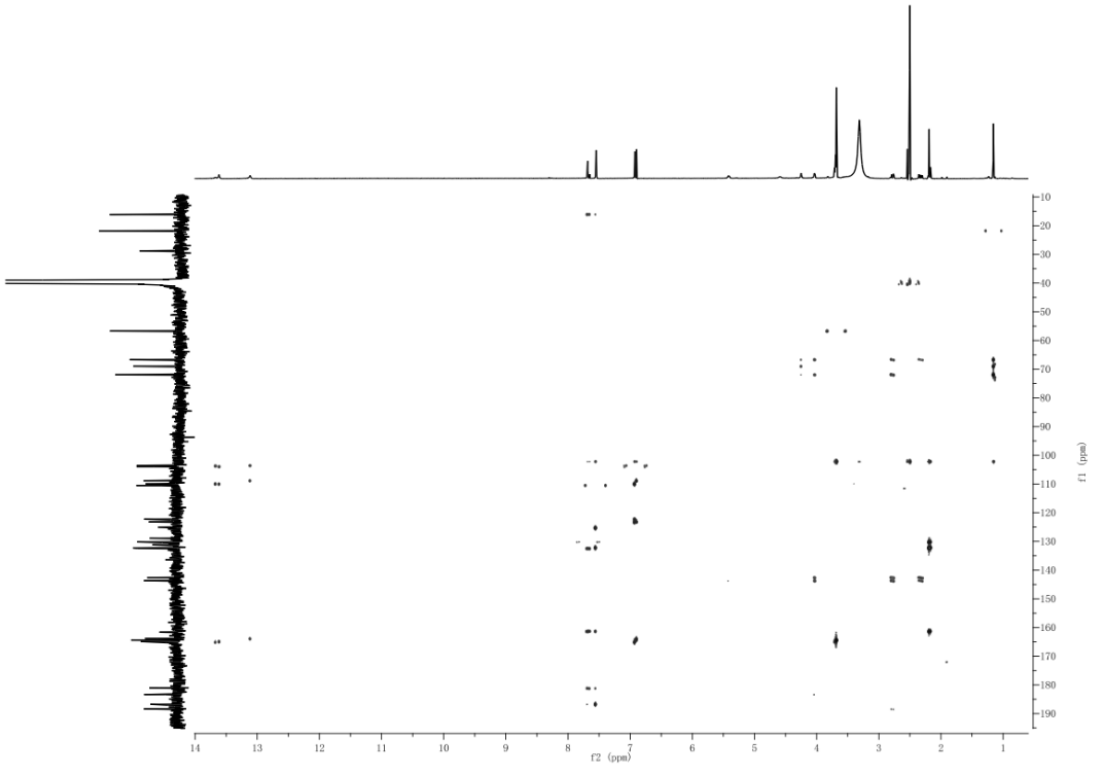


Figure S12. ^{1}H-^{1}H COSY spectrum of 2 (500 MHz, DMSO-d_6**).**

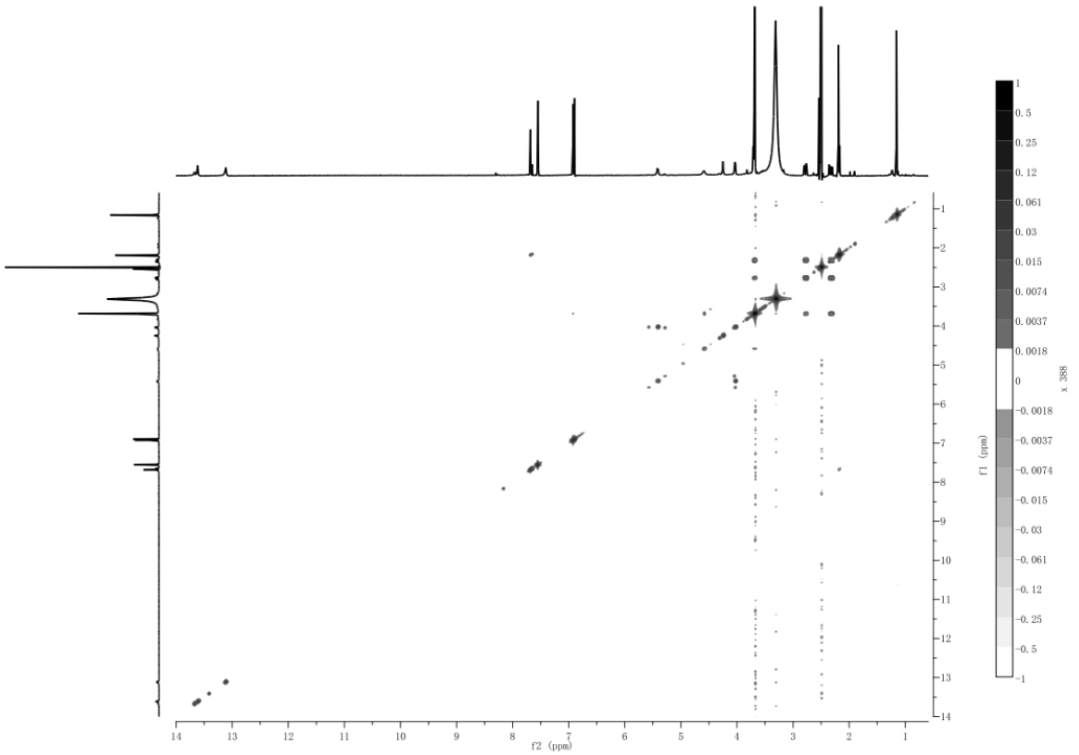
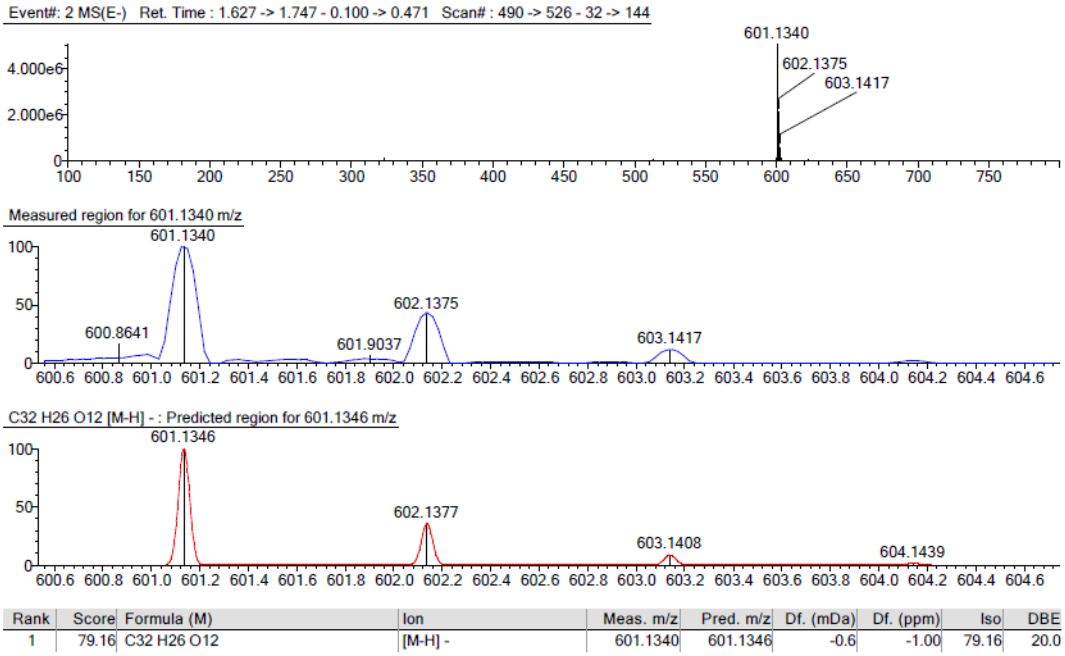


Figure S13. HR-ESIMS spectrum of 2.



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	79.16	C32 H26 O12	[M-H] -	601.1340	601.1346	-0.6	-1.00	79.16	20.0

Figure S14. ^{1}H spectrum of 3 (500 MHz, DMSO-d_6**).**

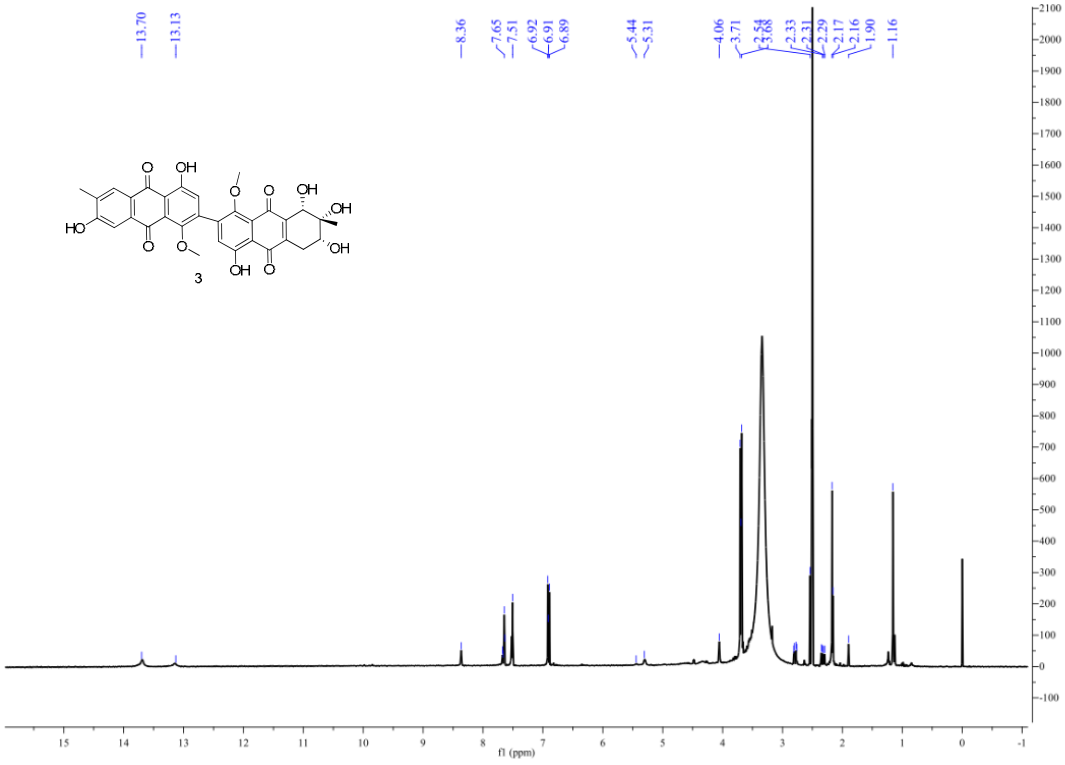


Figure S15. ^{13}C spectrum of 3 (125 MHz, DMSO-d_6**).**

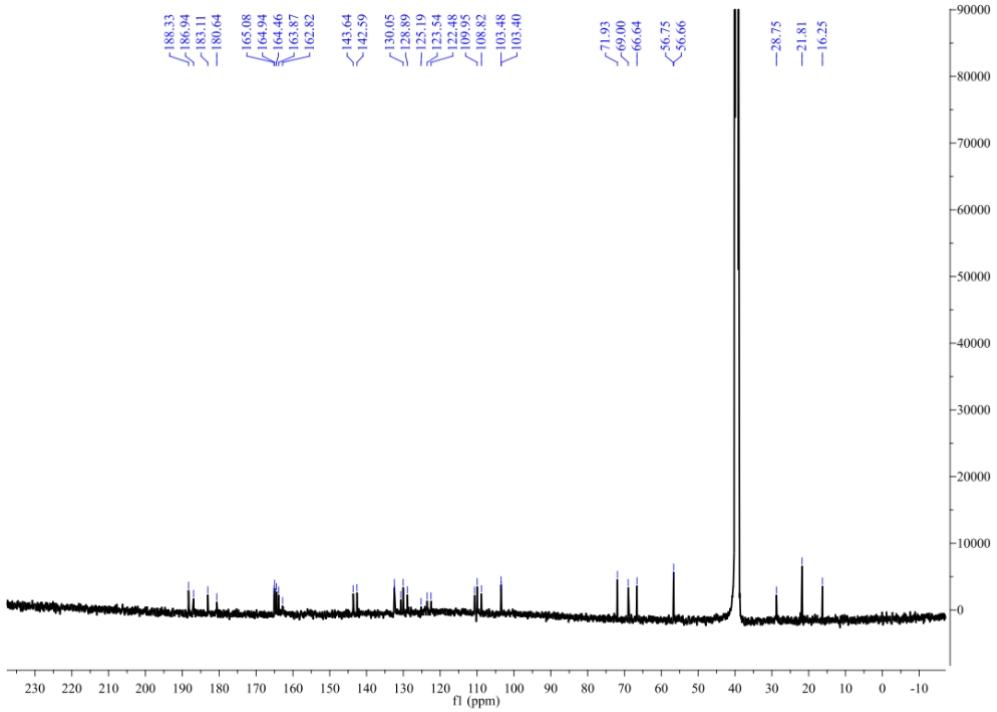


Figure S16. HMQC spectrum of 3 (500/125 MHz, DMSO-d_6**).**

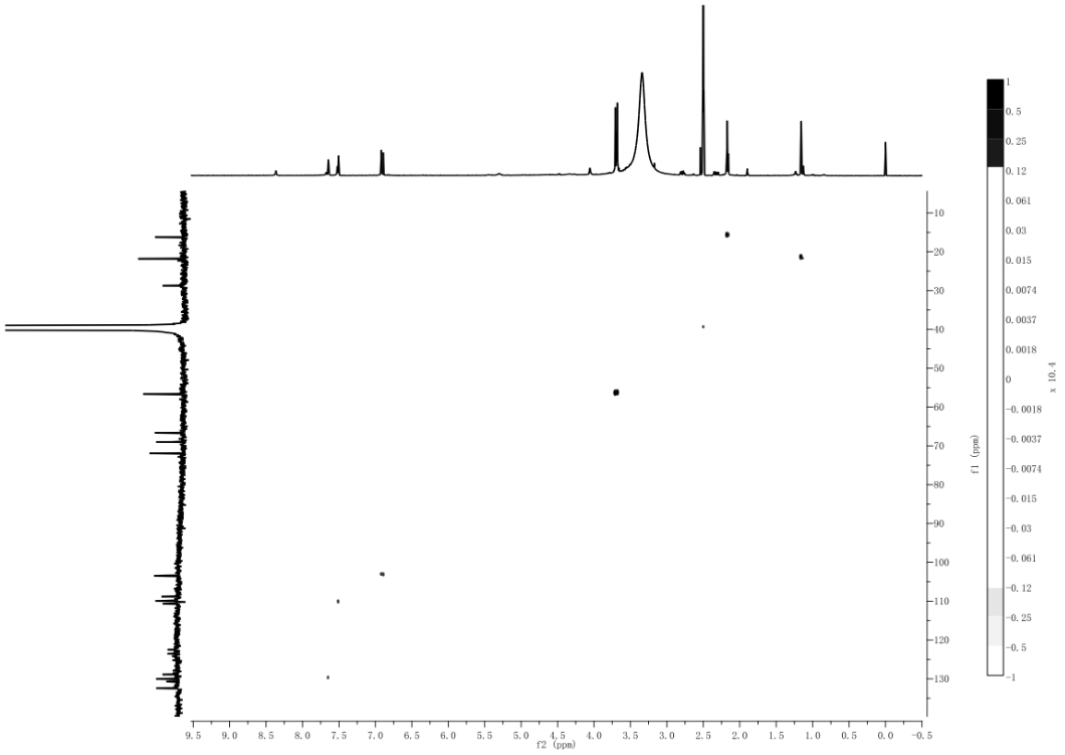


Figure S17. HMBC spectrum of 3 (500/125 MHz, DMSO-d_6**).**

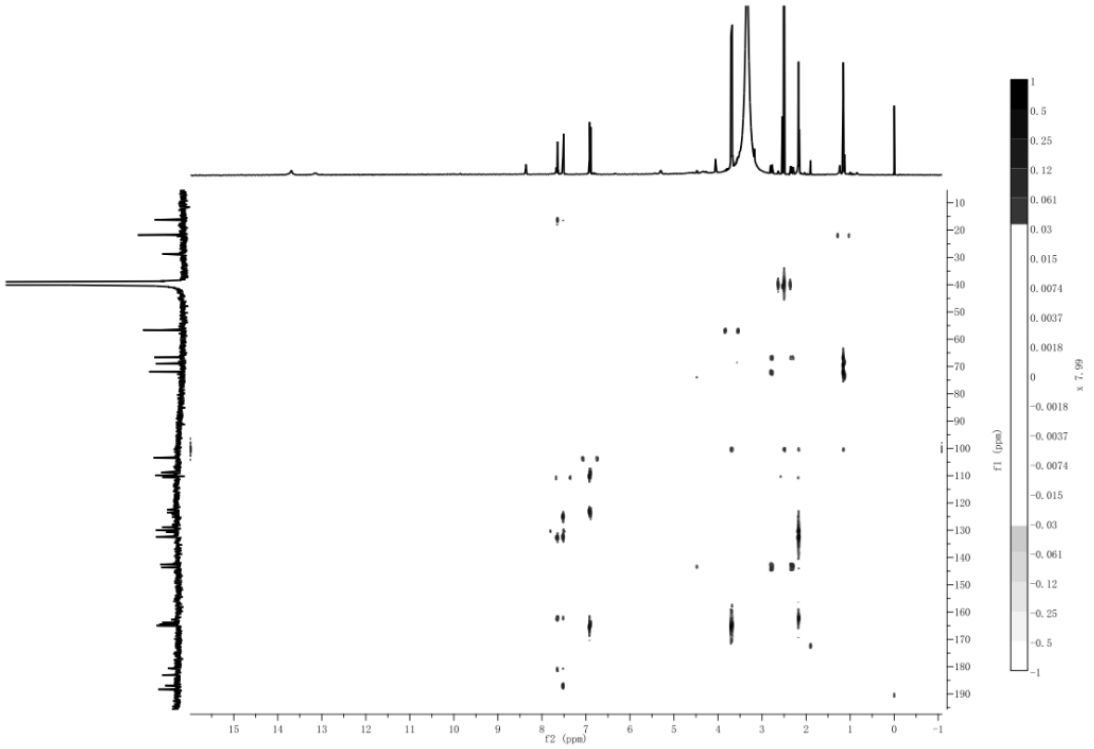


Figure S18. ^{1}H-^{1}H COSY spectrum of 3 (500 MHz, DMSO-d_6**).**

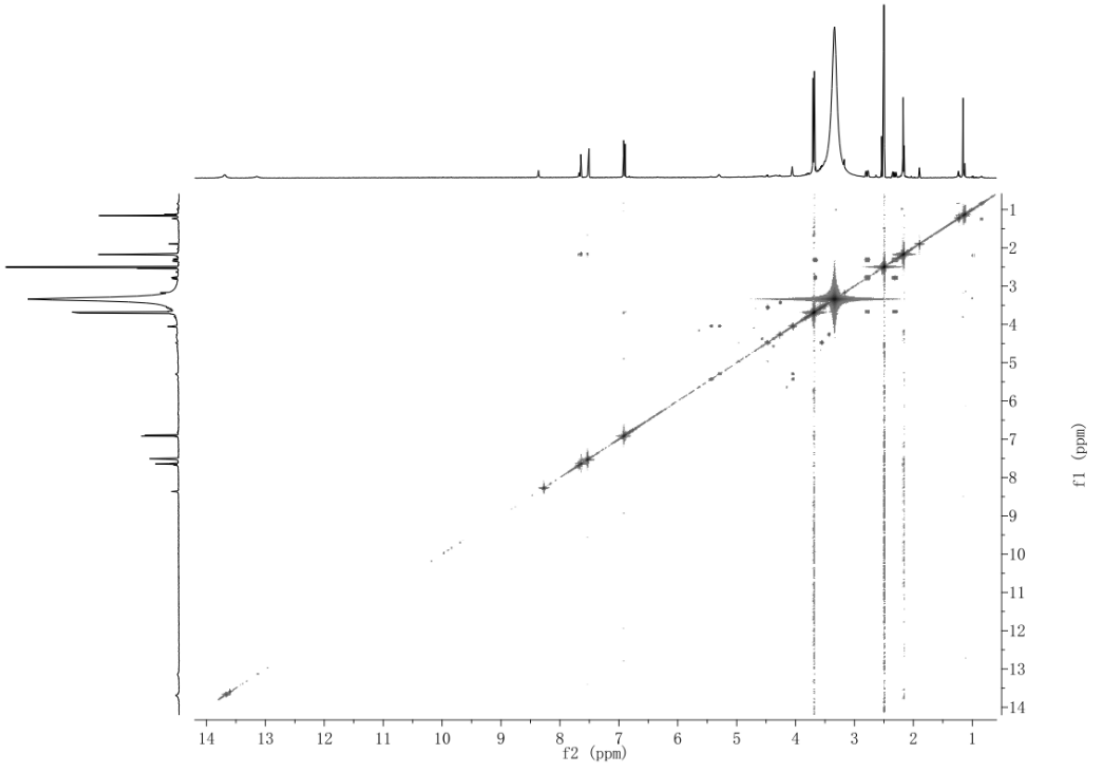
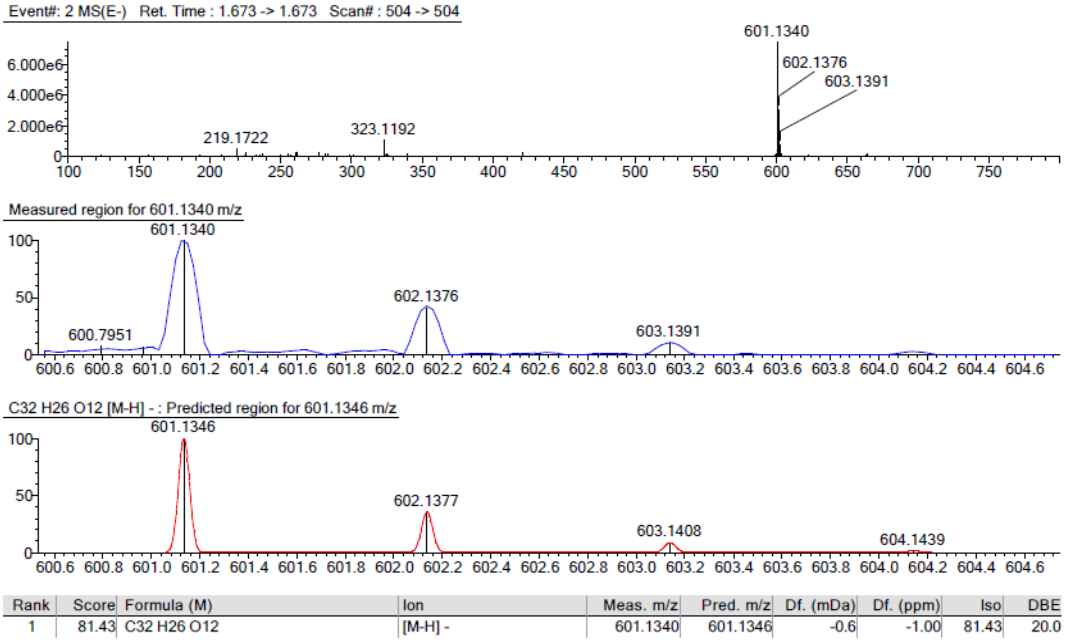


Figure S19. HR-ESIMS spectrum of 3.



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	81.43	C32 H26 O12	[M-H] -	601.1340	601.1346	-0.6	-1.00	81.43	20.0