Supporting Information

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Streptolactone A, A New Antibiofilm Lactone Derivative from *Streptomyces* sp. A31

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S1: Experimental Section

S.1.1. General Experimental Procedures

The optical rotations were recorded using methanol as the solvent on a digital polarimeter named AUTOPOL. UV and IR spectra were acquired utilizing a Shimadzu UV-2600 spectrophotometer and a Bruker Tensor Π spectrophotometer, respectively. 1D and 2D NMR spectra were obtained using a JEOL JNM-ECZR instrument (600 MHz), with tetramethyl silane serving as the standard reference. High-resolution mass data were gathered utilizing an Agilent 1260 HPLC-6230 TOF tandem spectrometer. Sephadex LH-20 from Amersham Pharmacia Biotech and silica gel CC (100−200 or 200−300 mesh) from Qingdao Haiyang Chemical Company were utilized for column chromatography. Preparative HPLC, employing an Agilent Pursuit C-18 column (10 μm, 20×250 mm) on a Beijing Auno P-2050 system, was utilized for further purification.

S.1.2. Antibiofilm Assay

The static biofilm formation assay followed established protocols with some adjustments. Initially, all compounds were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 0.1 M to create a stock solution, which was then diluted to a final concentration of 1 mM. Biofilm development occurred in a customized M63 medium composed of 10 g $(NH_4)_2SO_4$, 15 g KH_2PO_4, 35 g K_2HPO_4, 0.2% glycerol, and 0.12 g $MgSO_4$ in 1 L of distilled water. A single colony of P. aeruginosa PA01 was initially cultured in LB medium at 37 ℃ on a shaker (180 rpm) overnight, followed by further dilution with M63 medium. Then, 80 μL of OD600 = 0.02 bacterial suspension and 80 μL of treatment compounds were successively added to a 96-microtiter plate. DMSO was used as the blank control. The plate was then incubated for 12–16 hours at 37 °C without shaking. After measuring at OD_{600}, the spent growth medium was removed. Biofilms were stained with crystal violet (0.1%, wt./vol) and dissolved in 30% acetic acid for 10 minutes. The absorbance at 595 nm was recorded to quantify biofilm formation, with data normalized by cell growth (OD_{600}). The experiment was conducted in six replicate wells and repeated twice.

S.1.3. Antimicrobial Assay

All compounds were assessed for antimicrobial activity against *P. aeruginosa* using a single dose (1 mM) for initial screening, followed by determination of the Minimum Inhibitory Concentration (MIC) through the broth microdilution method, as per a previously outlined procedure. Ciprofloxacin served as the positive control drug, while DMSO was utilized as the blank control.

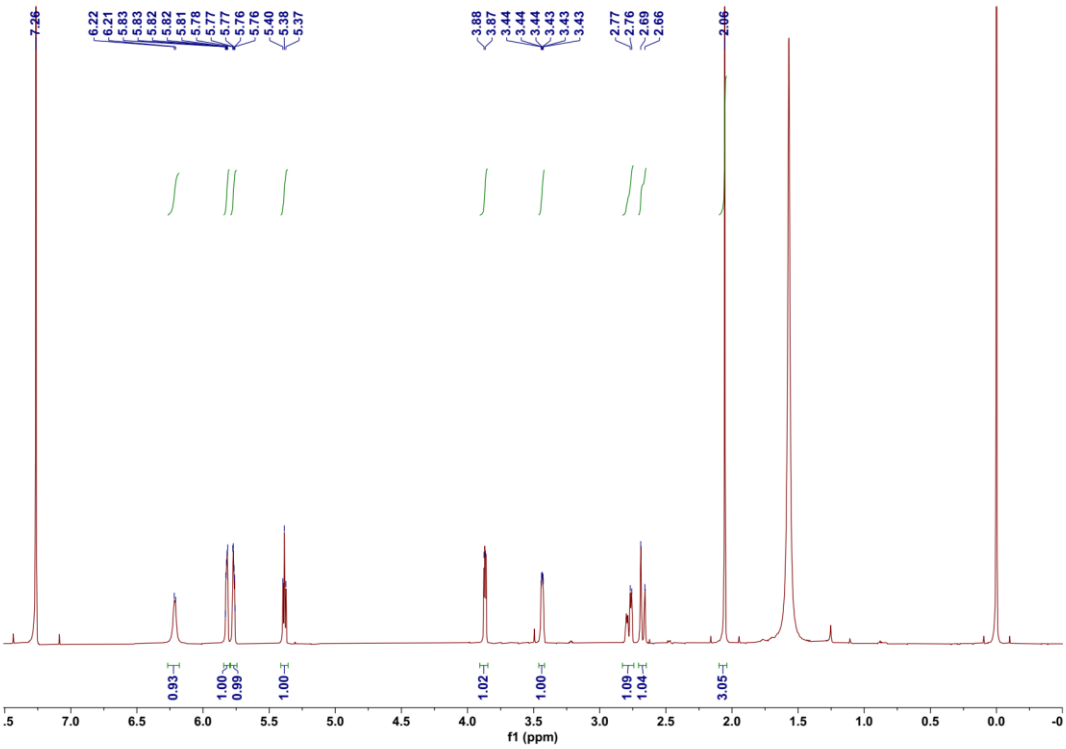

Figure S1: ^{1}H NMR spectrum of compound **1** in $CDCl_3$

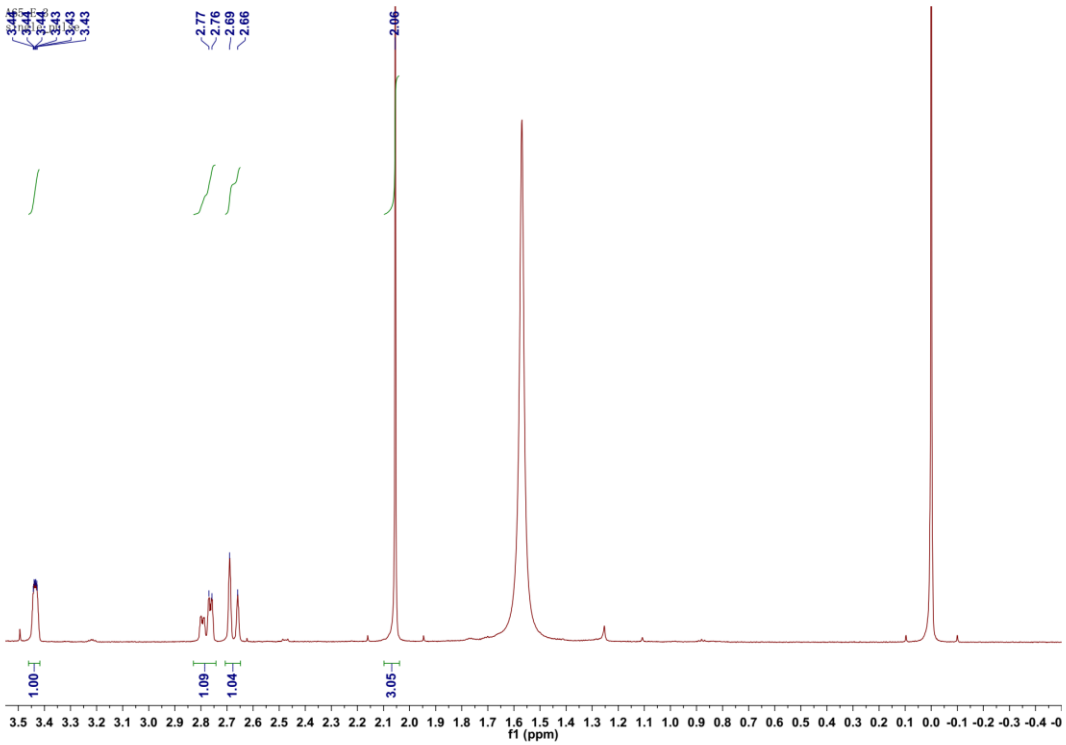

Figure S2: Enlarge ^{1}H NMR spectrum of compound **1** in $CDCl_3$ (-0.5-3.55 ppm)

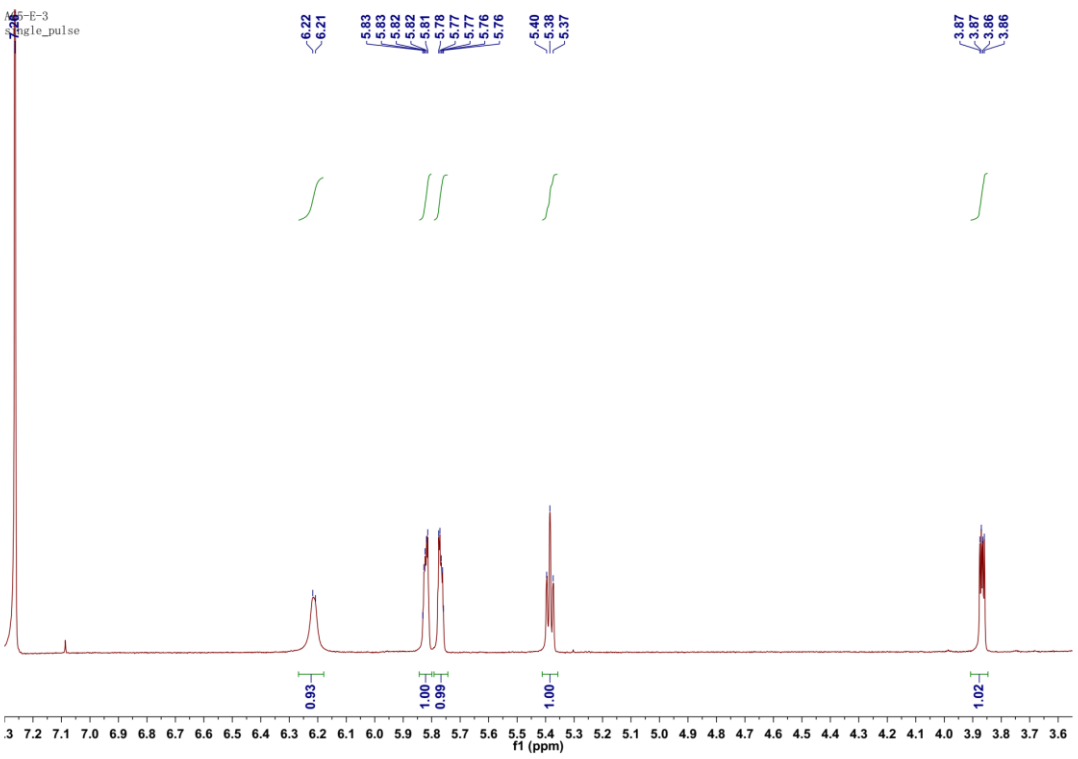

Figure S3: Enlarge ^{1}H NMR spectrum of compound **1** in $CDCl_3$(3.55-7.25 ppm)

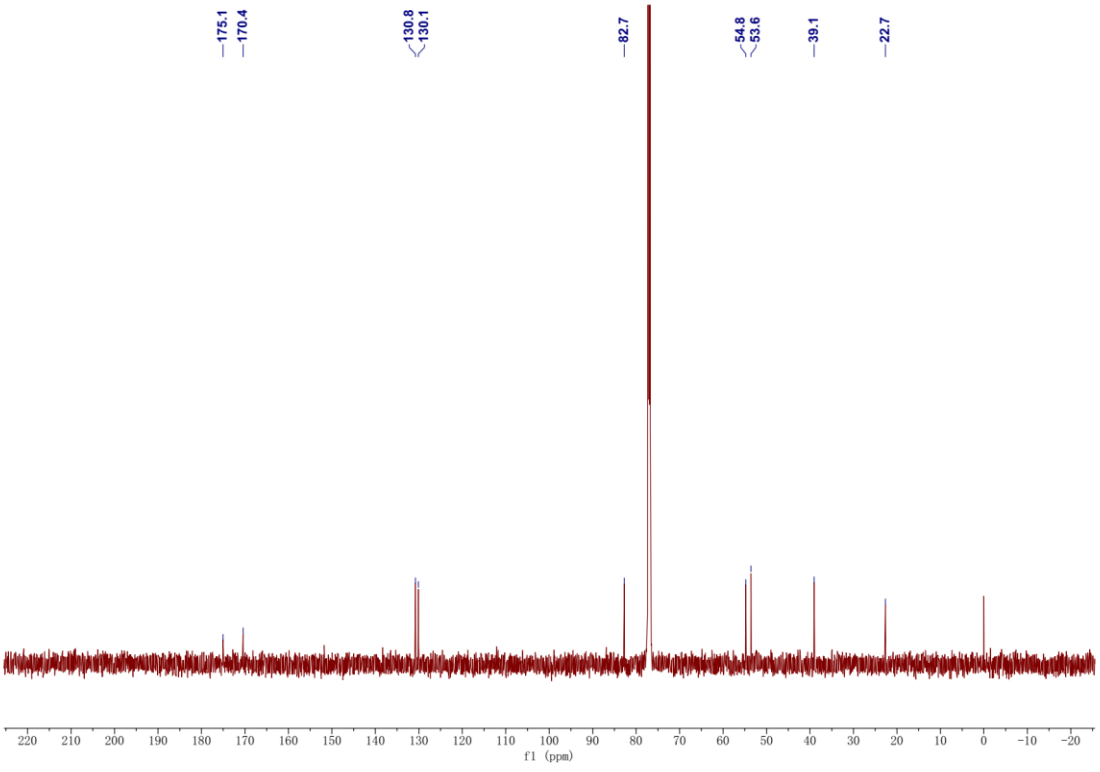

Figure S4: ^{13}C NMR spectrum of compound **1** in $CDCl_3$

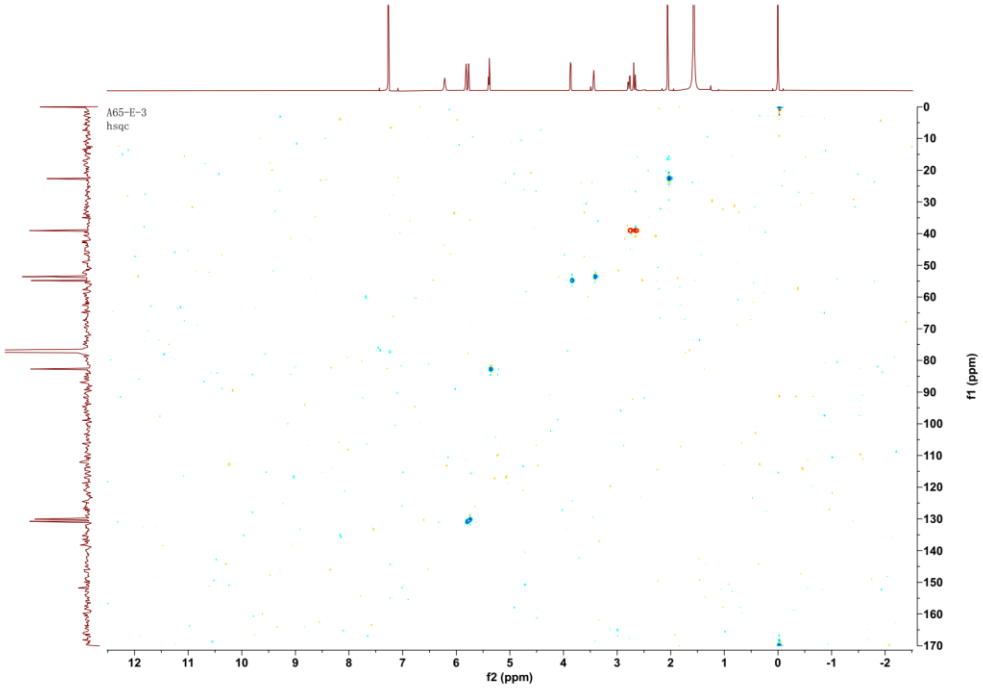

Figure S5: HSQC spectrum of compound **1** in $CDCl_3$

Figure S6: Enlarge HSQC spectrum of compound **1** in $CDCl_3$

Figure S7: Enlarge HSQC spectrum of compound **1** in $CDCl_3$

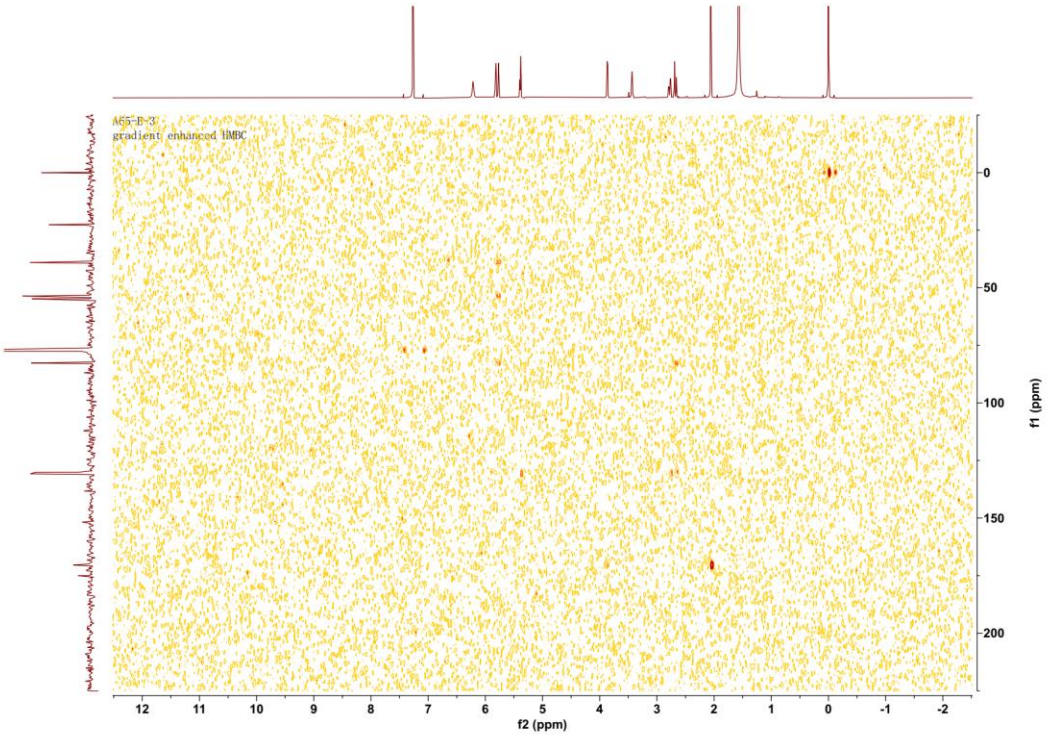

Figure S8: HMBC spectrum of compound **1** in $CDCl_3$

Figure S9: Enlarge HMBC spectrum of compound **1** in $CDCl_3$

Figure S10: Enlarge HMBC spectrum of compound **1** in $CDCl_3$

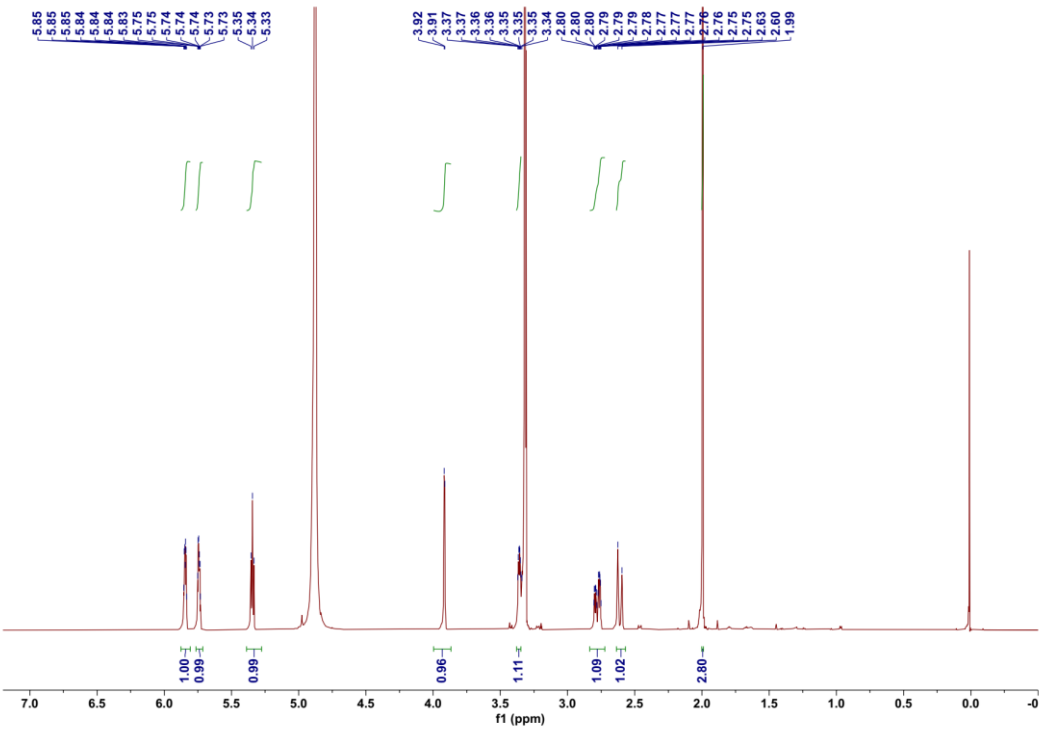

Figure S11: ^{1}H NMR spectrum of compound **1** in Methanol-d_4

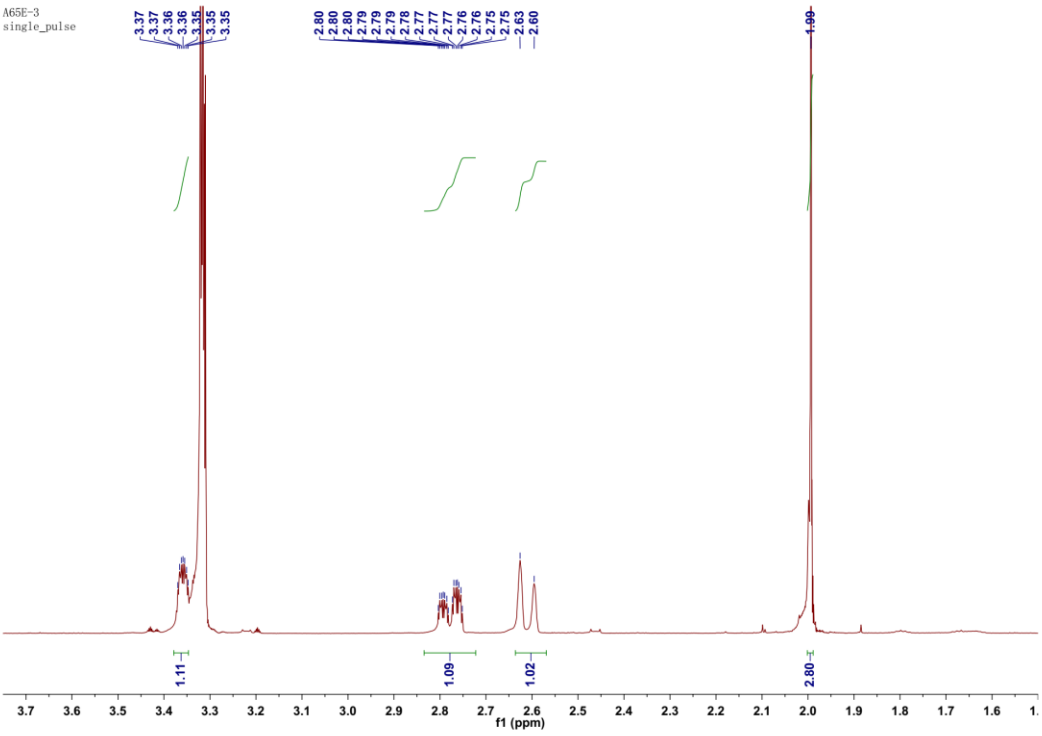

Figure S12: Enlarge ^{1}H NMR spectrum of compound **1** in Methanol-d_4

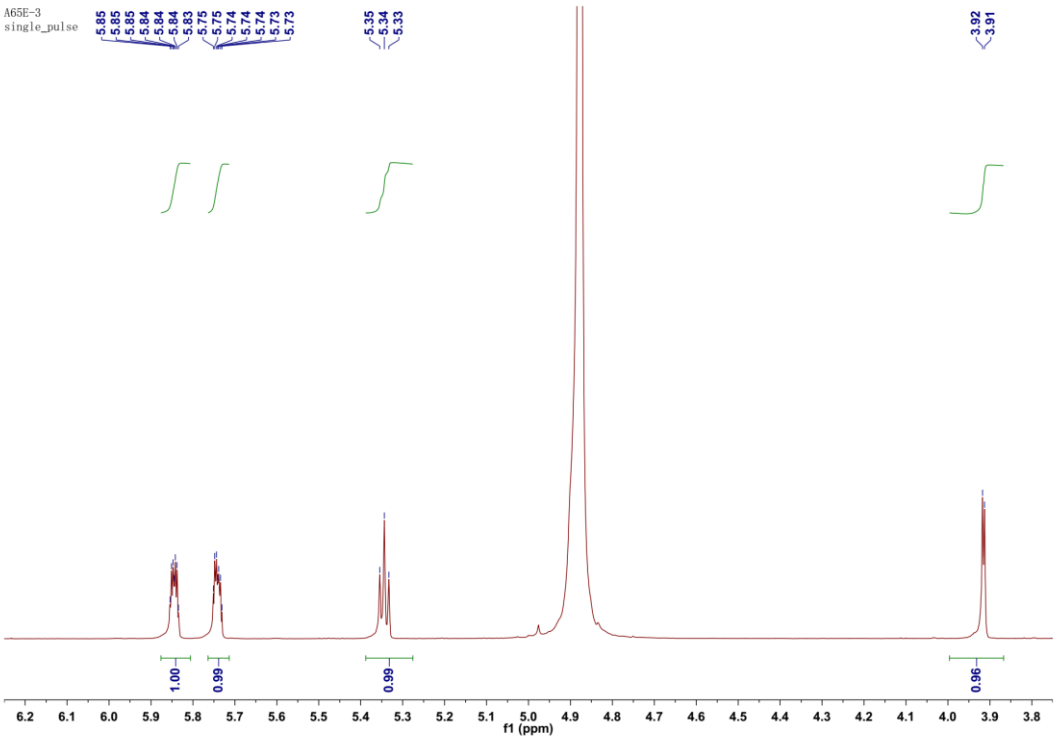

Figure S13: Enlarge ^{1}H NMR spectrum of compound **1** in Methanol-*d*$_4$

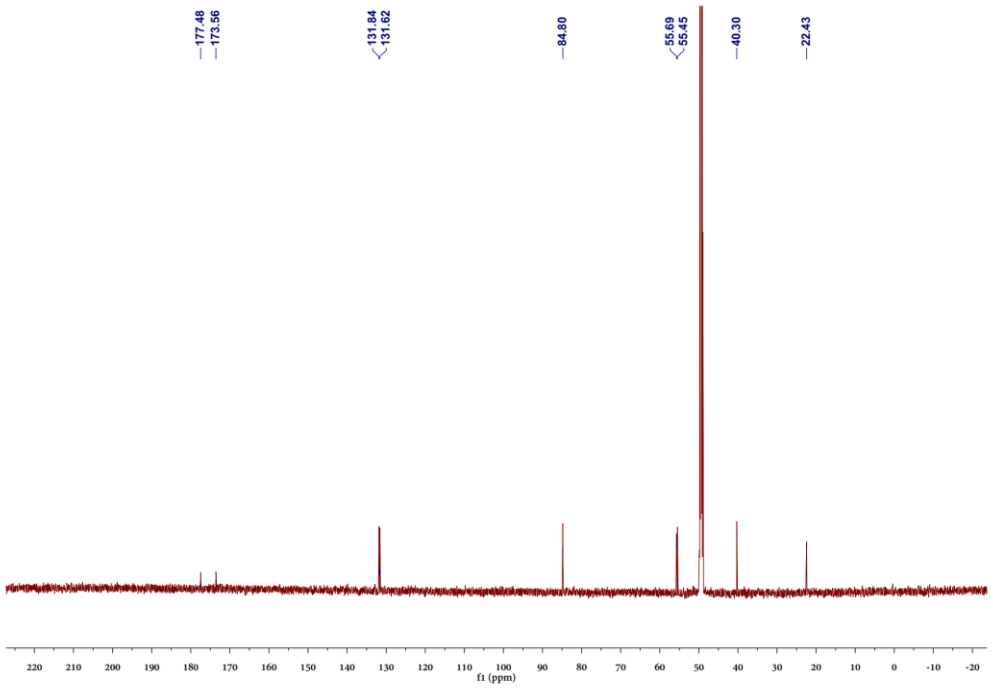

Figure S14: ^{13}C NMR spectrum of compound **1** in Methanol-*d*$_4$

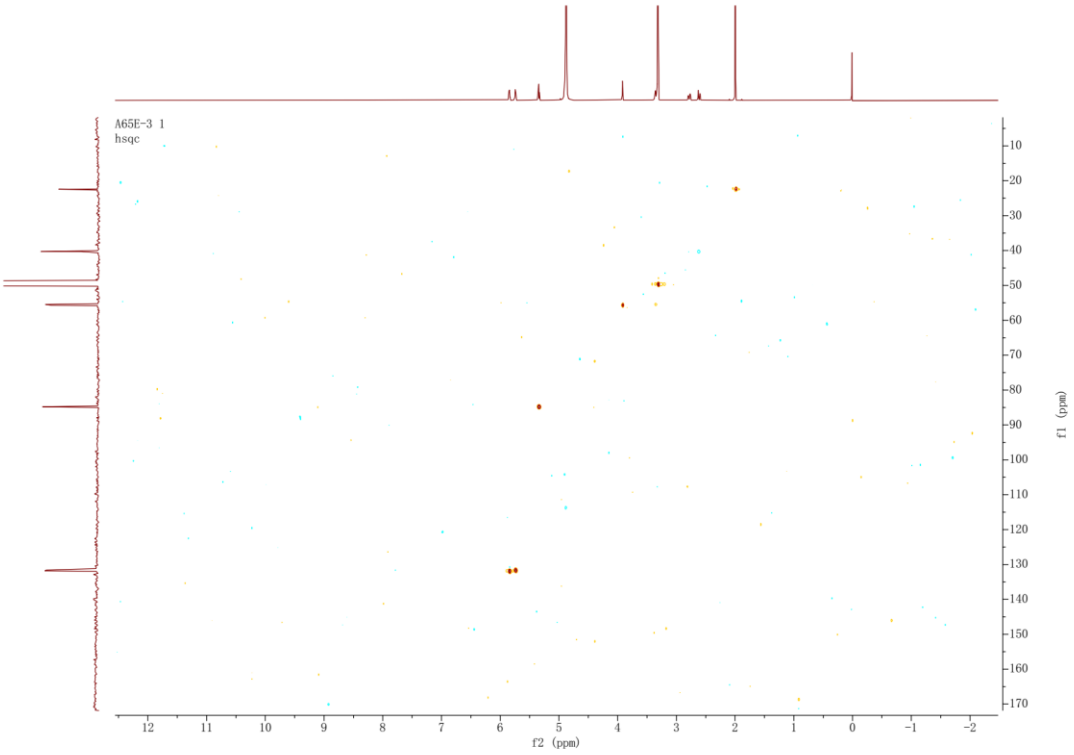

Figure S15: HSQC spectrum of compound **1** in Methanol-d_4

Figure S16: Enlarge HSQC spectrum of compound **1** in Methanol-d_4

Figure S17: Enlarge HSQC spectrum of compound **1** in Methanol-*d*$_4$

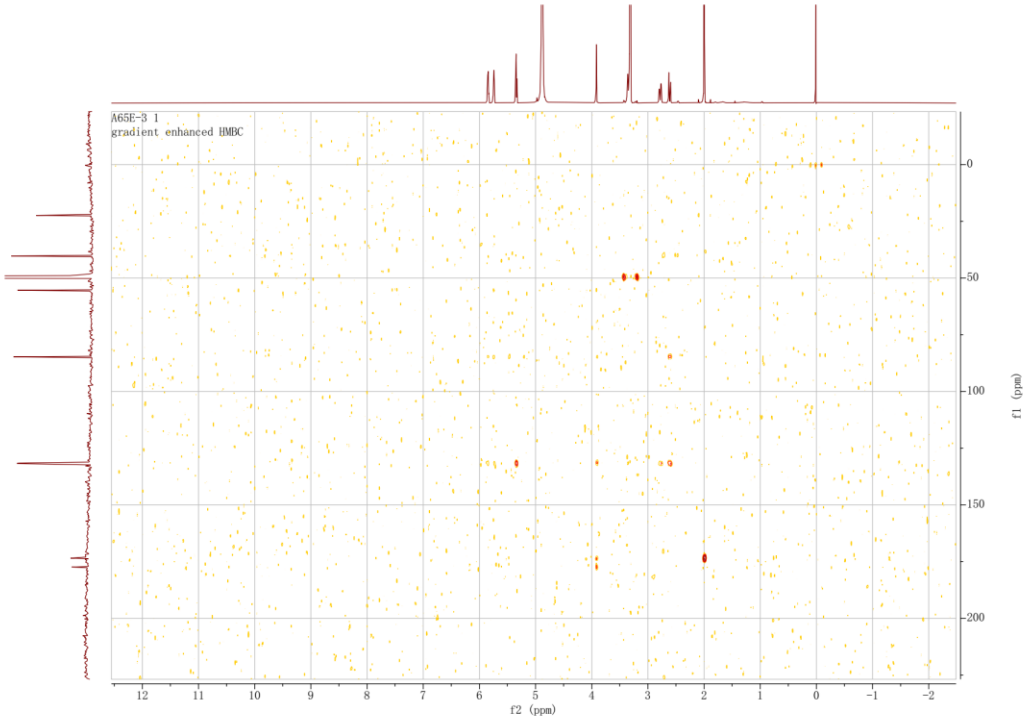

Figure S18: HMBC spectrum of compound **1** in Methanol-*d*$_4$

Figure S19: Enlarge HMBC spectrum of compound **1** in Methanol-d_4

Figure S20: Enlarge HMBC spectrum of compound **1** in Methanol-d_4

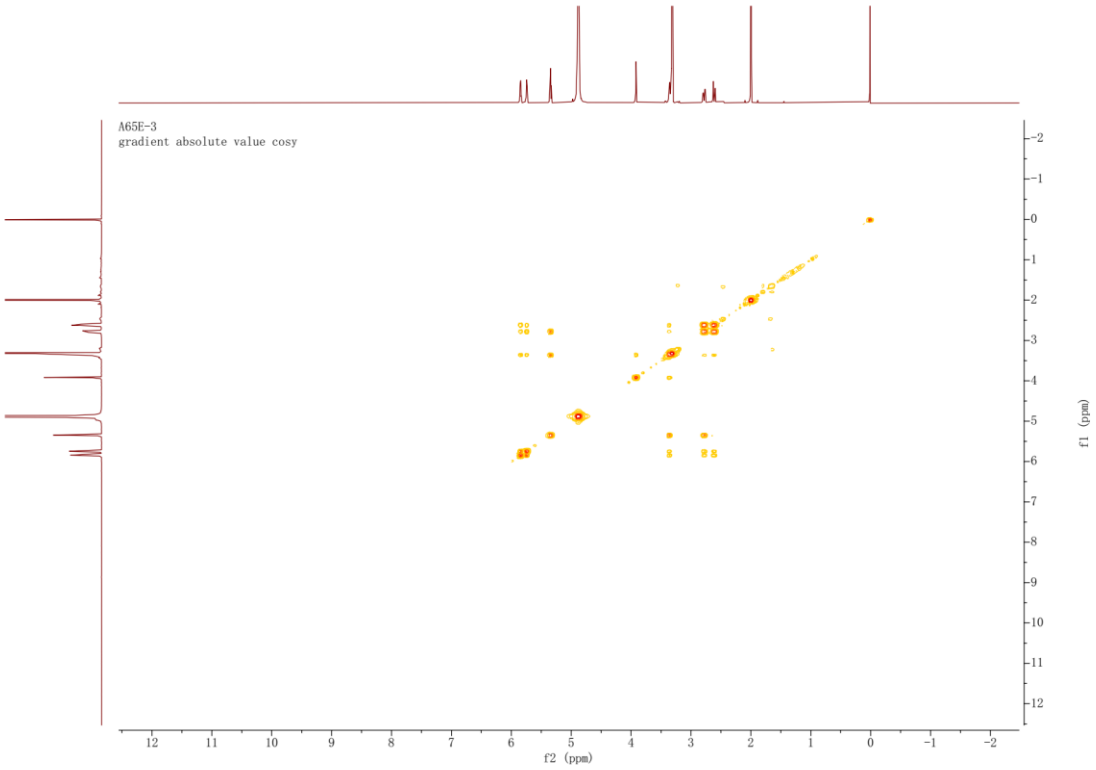

Figure S21: ^{1}H-^{1}H COSY spectrum of compound **1** in Methanol-*d*$_4$

Figure S22: Enlarge ^{1}H-^{1}H COSY spectrum of compound **1** in Methanol-*d*$_4$

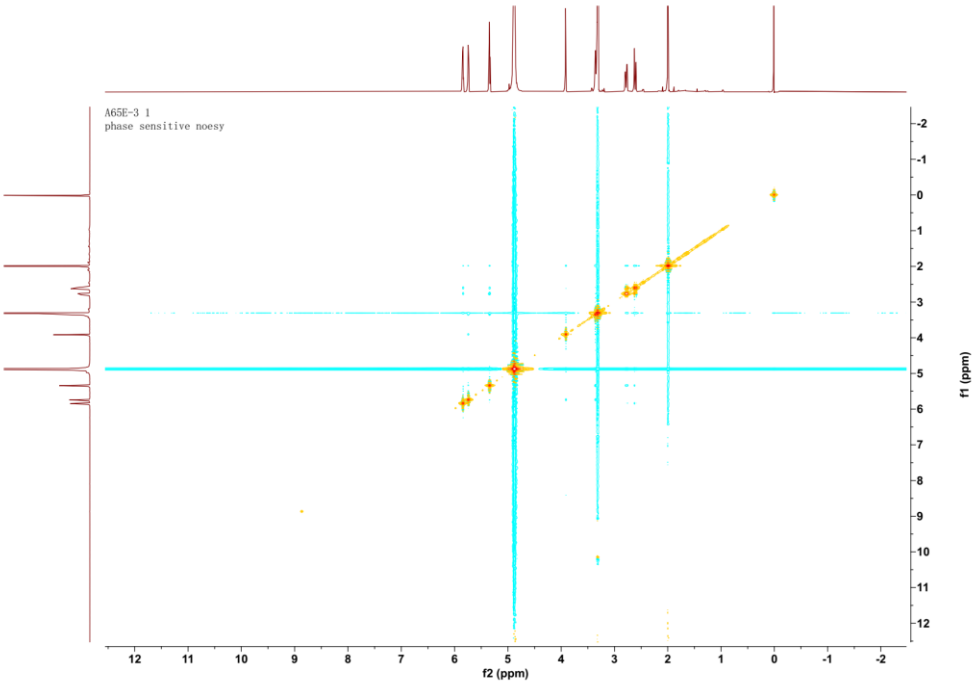

Figure S23: NOESY spectrum of compound **1** in Methanol-d_4

Figure S24: Enlarge NOESY spectrum of compound **1** in Methanol-d_4

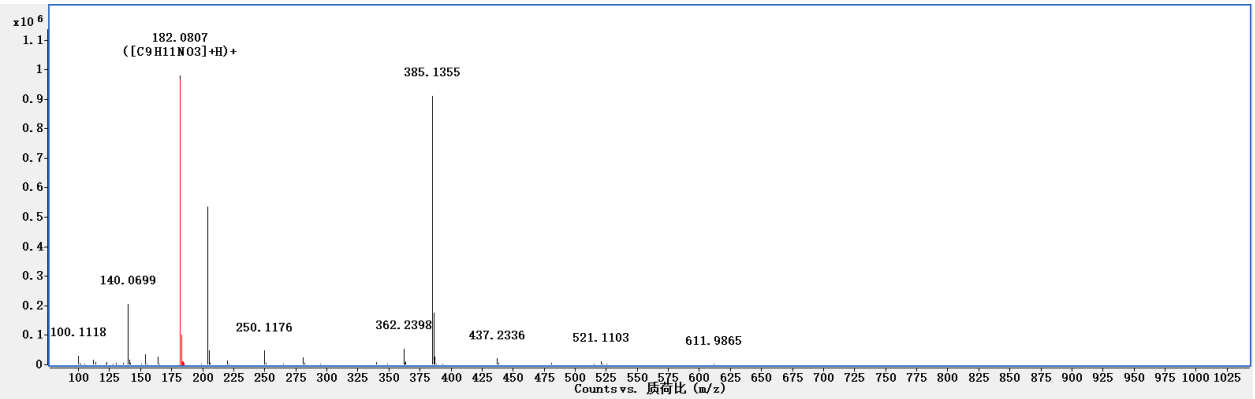

Figure S25: HR-ESI-MS spectrum of compound **1**

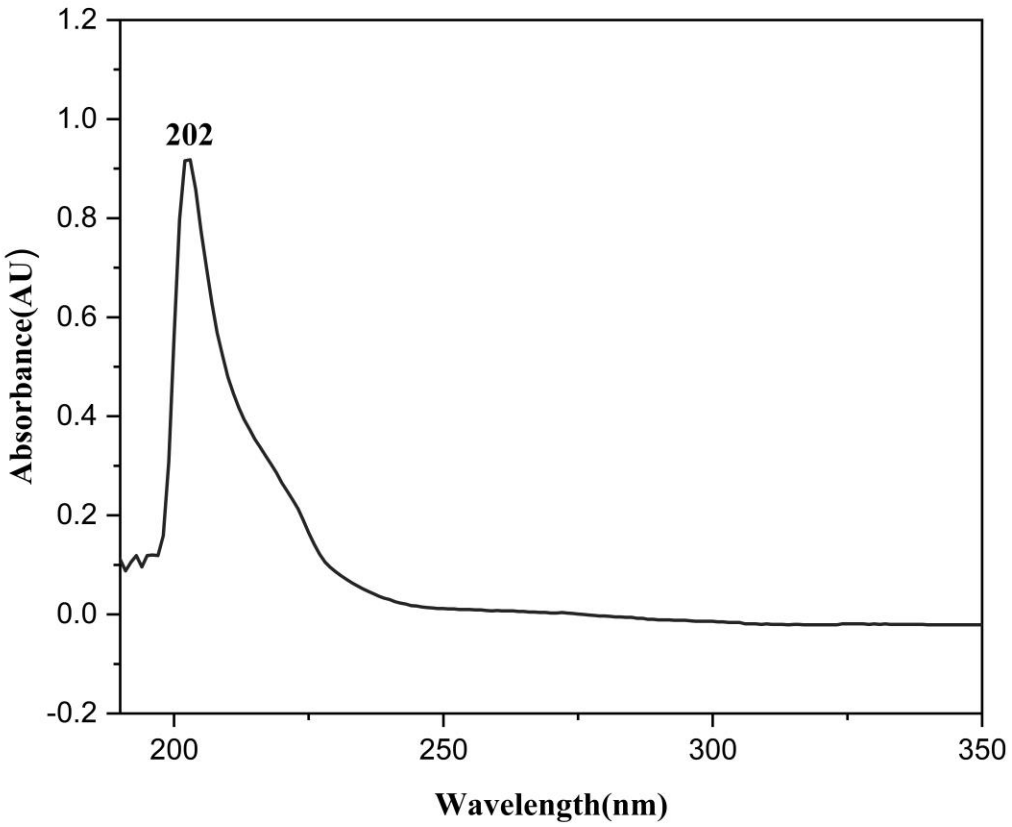

Figure S26: UV spectrum of compound **1**

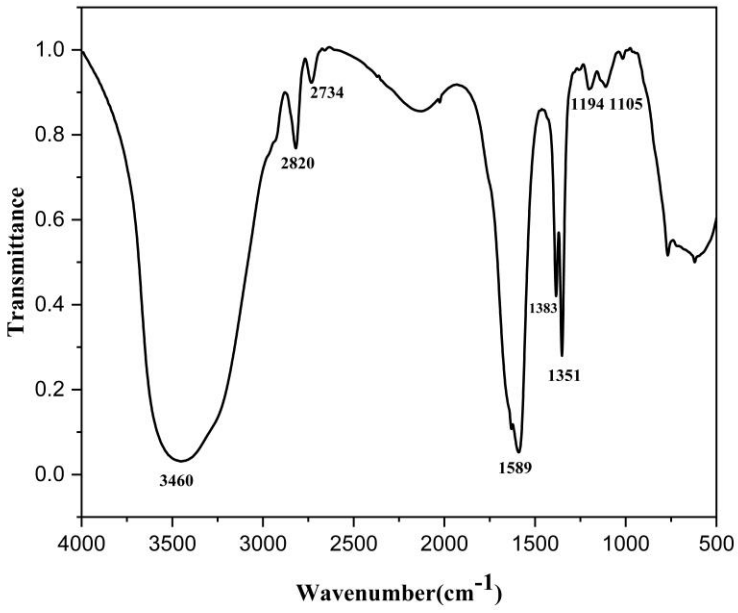

Figure S27: IR spectrum of compound **1**

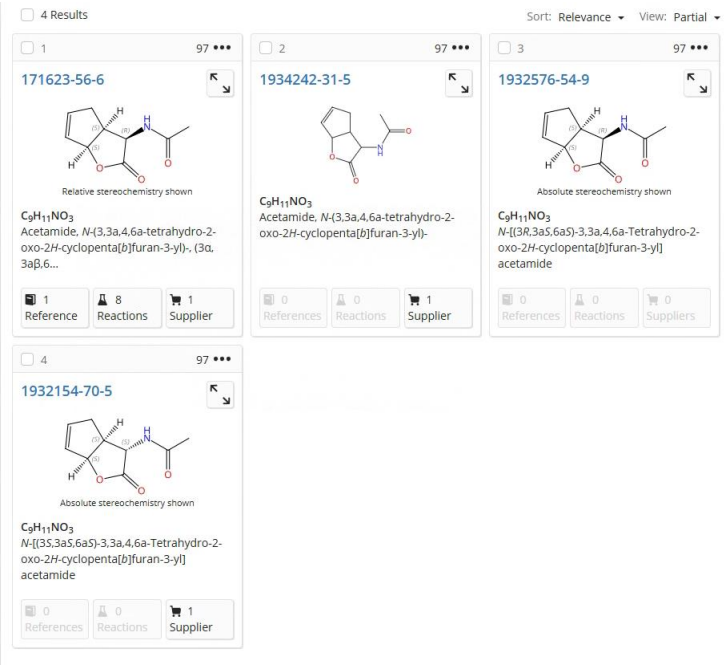

Figure S28: Scifinder search results of **1**