SUPPLEMENTARY MATERIAL

A New Lactone from Mangrove Endophytic Fungus *Aspergillus* sp. GXNU-A9

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ABSTRACT: A new lactone, asperlactone A (**1**), and four known lactone derivatives **2-5** were isolated from the mangrove endophytic fungus *Aspergillus* sp. GXNU-A9. Their structures were elucidated based on high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) datum, extensive nuclear magnetic resonance (NMR) spectroscopic analysis, and comparison with literature data. The structure of **1** was further confirmed by single-crystal X-ray diffraction analysis, and the absolute configuration of **1** was established. Compounds **1-5** were evaluated for their anti-inflammatory activities against nitric oxide (NO) production, and compounds **1-5** showed moderate inhibitory activities with IC_{50} values ranging from 15.87 to 30.48 μM.

KEYWORDS: *Aspergillus* sp.; mangrove endophytic fungus; asperlactone A; anti-inflammatory effects

List of supporting information

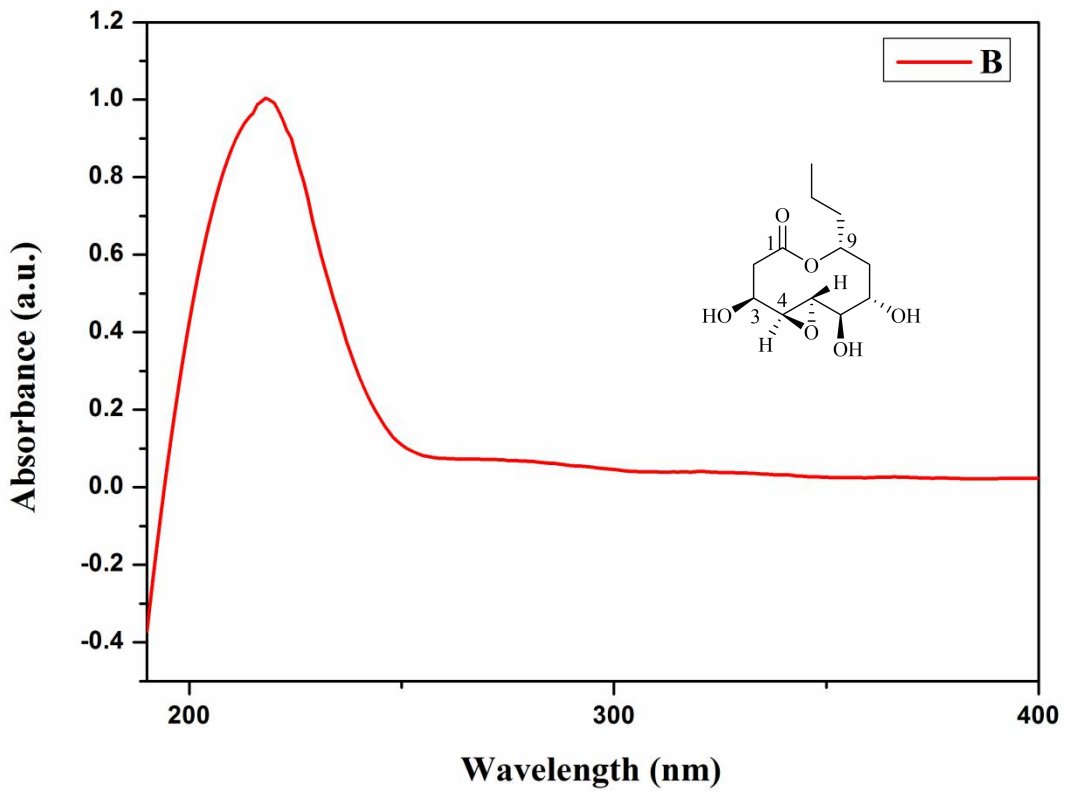

Figure S1. UV (MeOH) spectrum of compound **1**

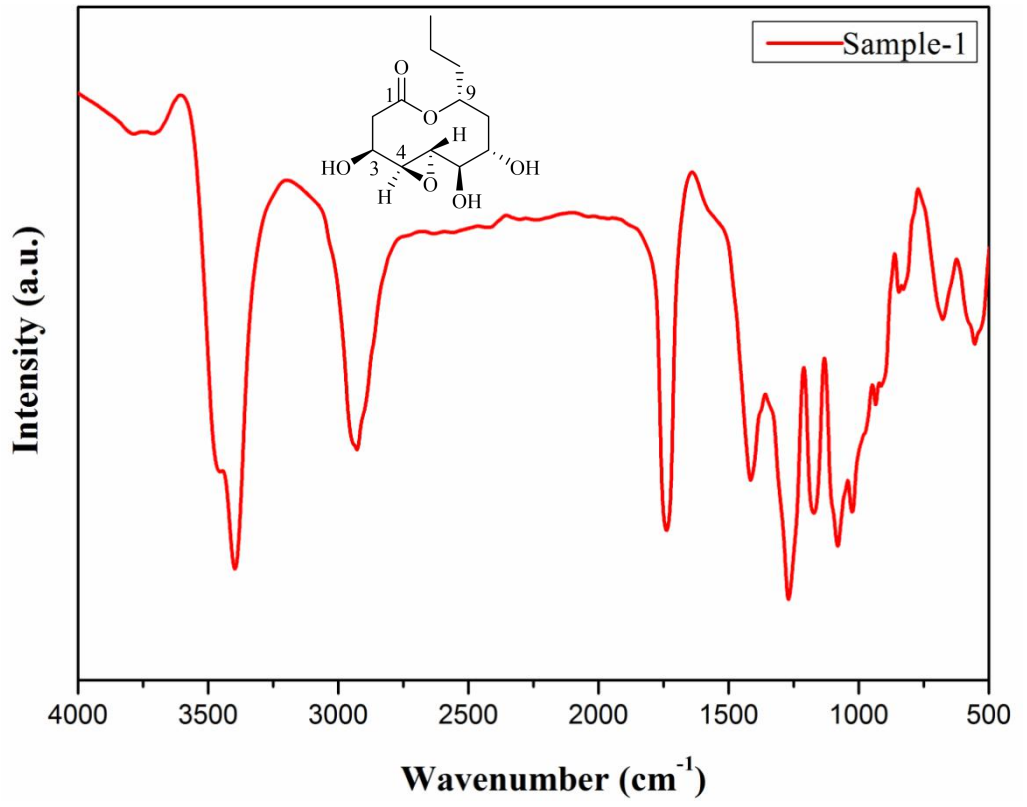

Figure S2. IR (KBr) spectrum of compound **1**

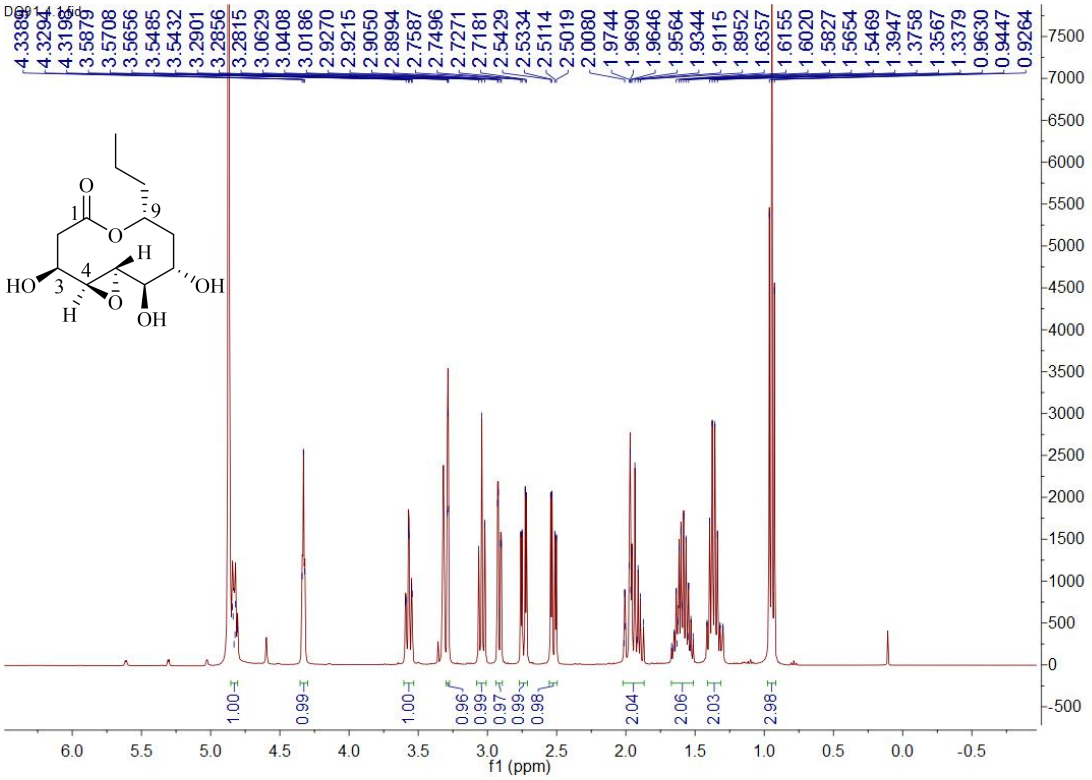

Figure S3. ^{1}H NMR (400 MHz, methanol-d_4) spectrum of compound **1**

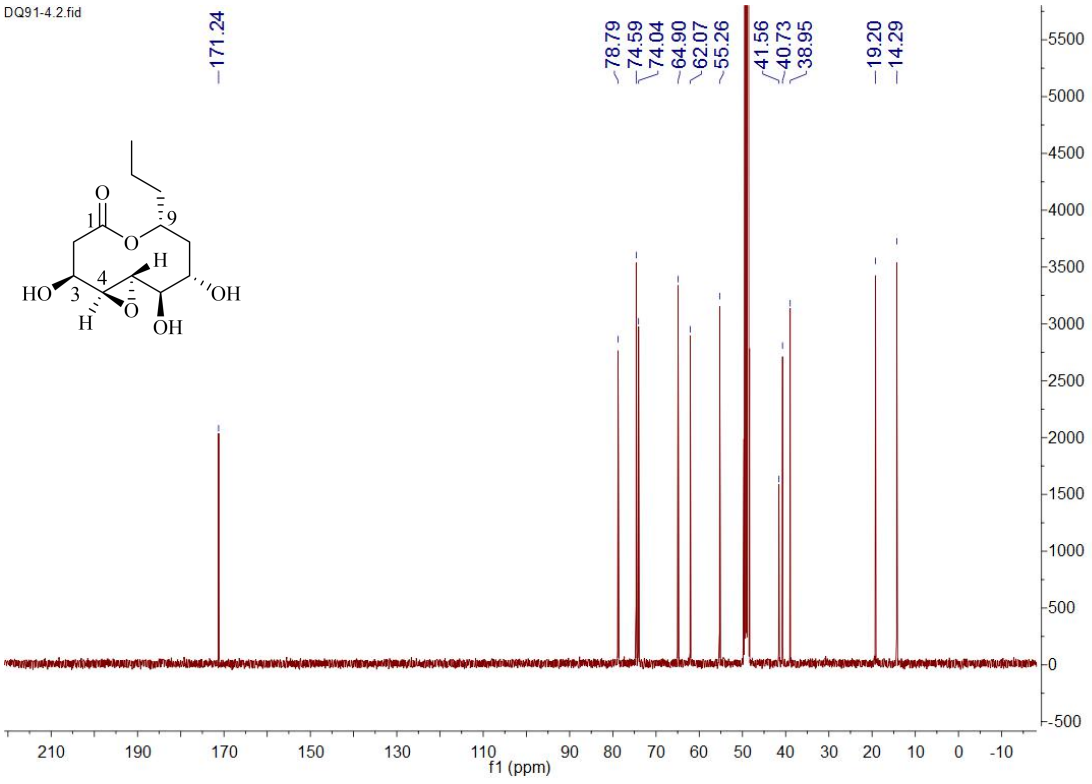

Figure S4. ^{13}C NMR (100 MHz, methanol-d_4) spectrum of compound

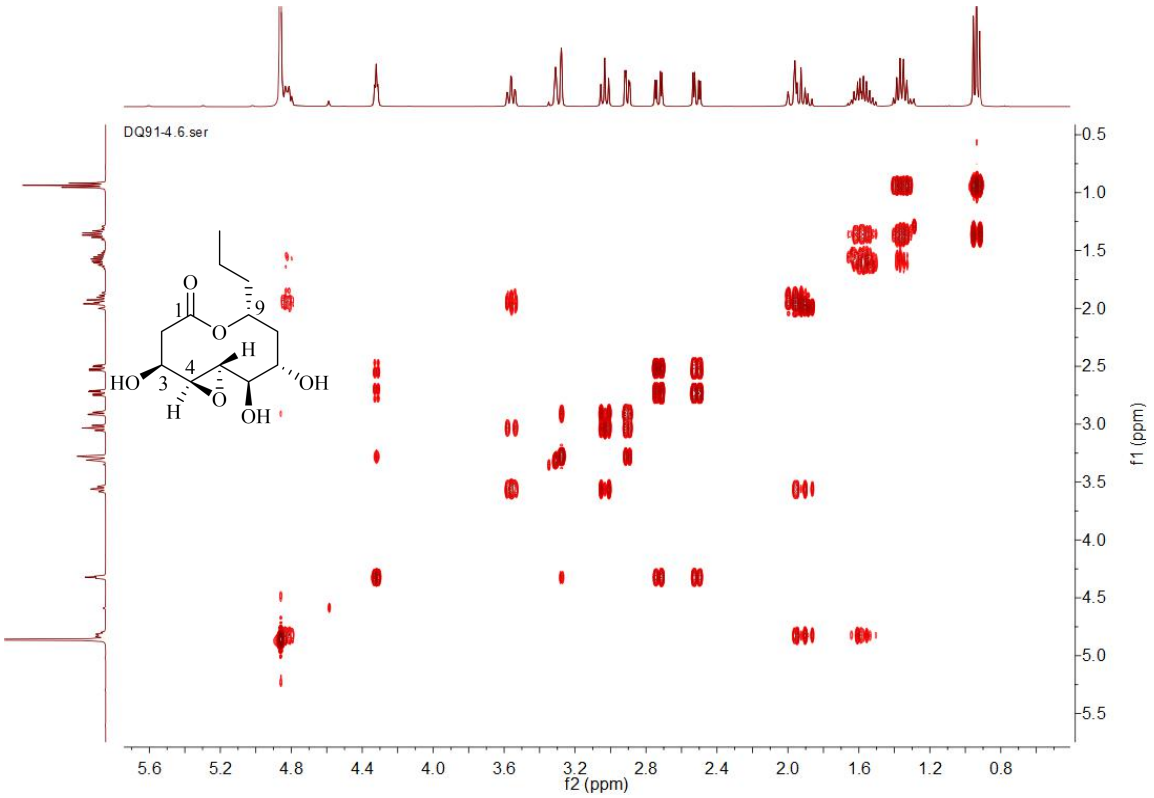

Figure S5. ^{1}H-^{1}H COSY (methanol-d_4) spectrum of compound **1**

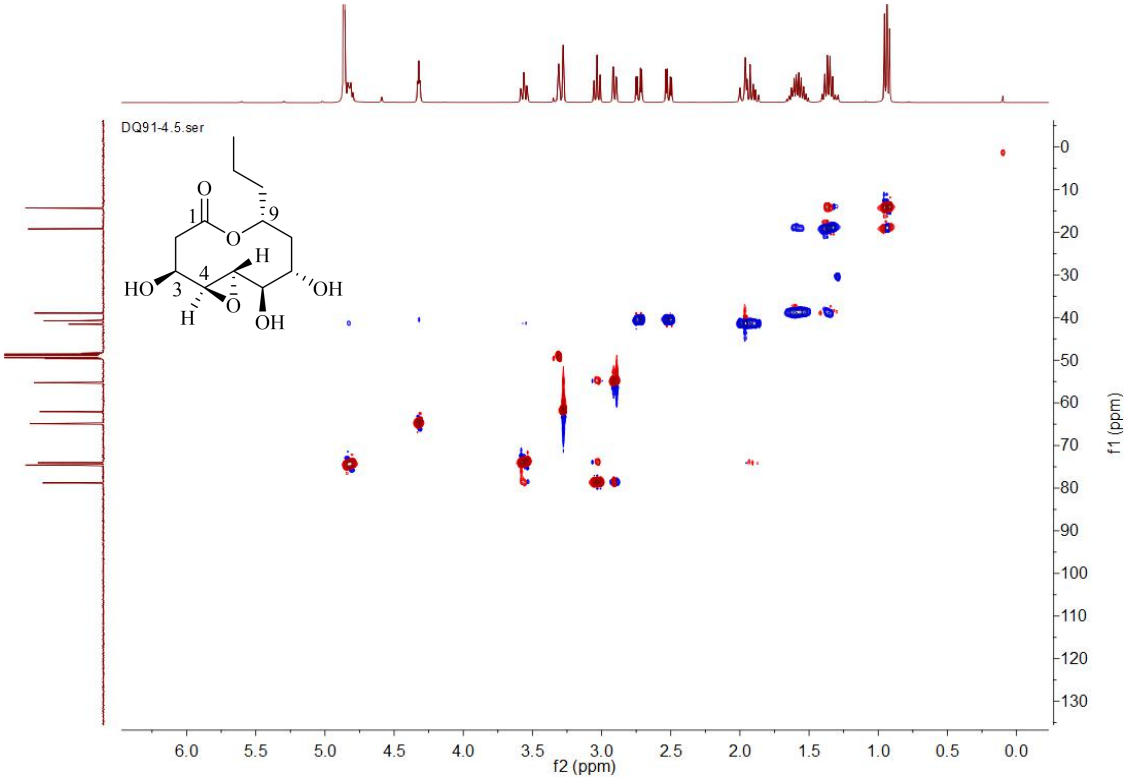

Figure S6. HMQC (methanol-d_4) spectrum of compound

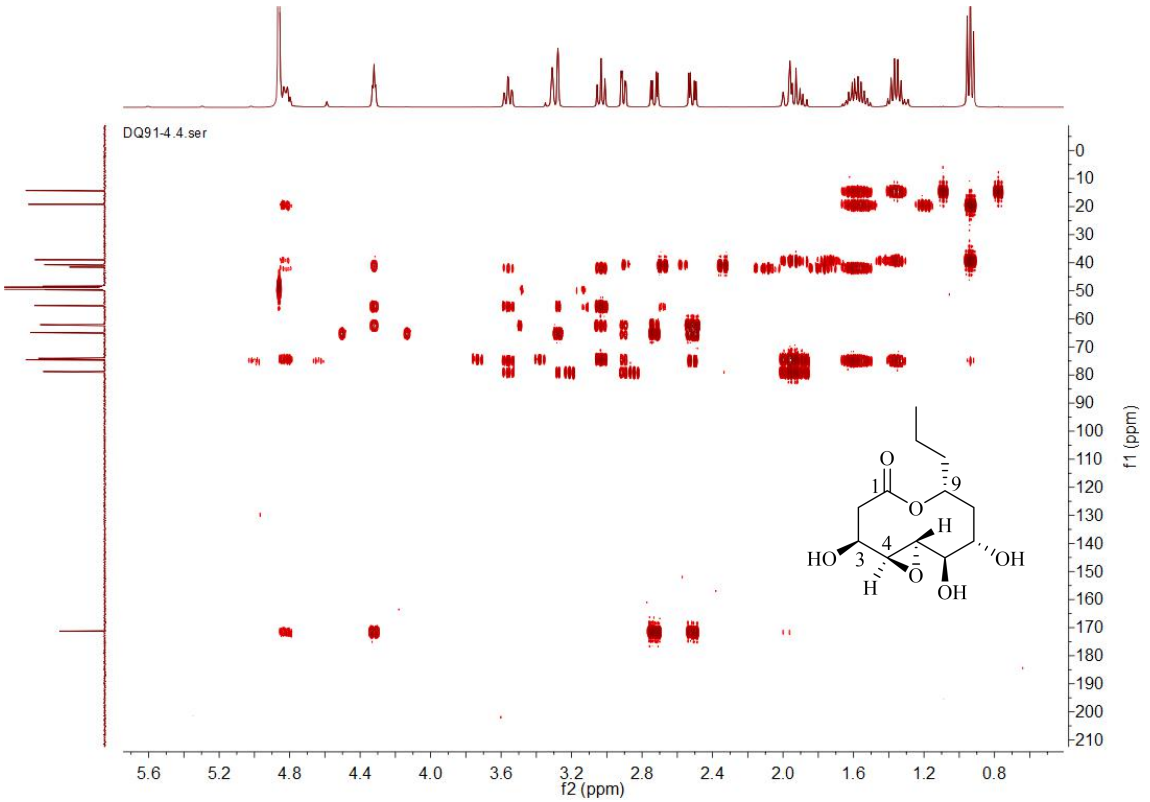

Figure S7. HMBC (methanol-d_4) spectrum of compound **1**

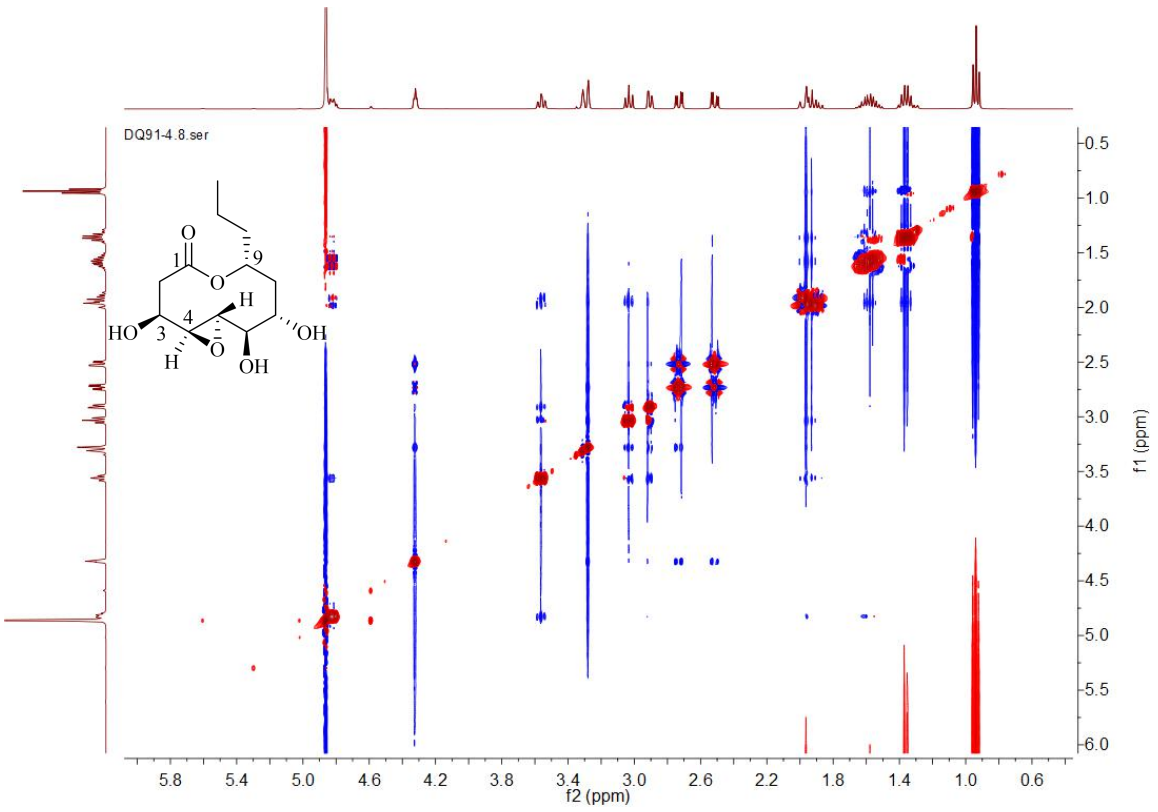

Figure S8. NOESY spectrum of compound **1**

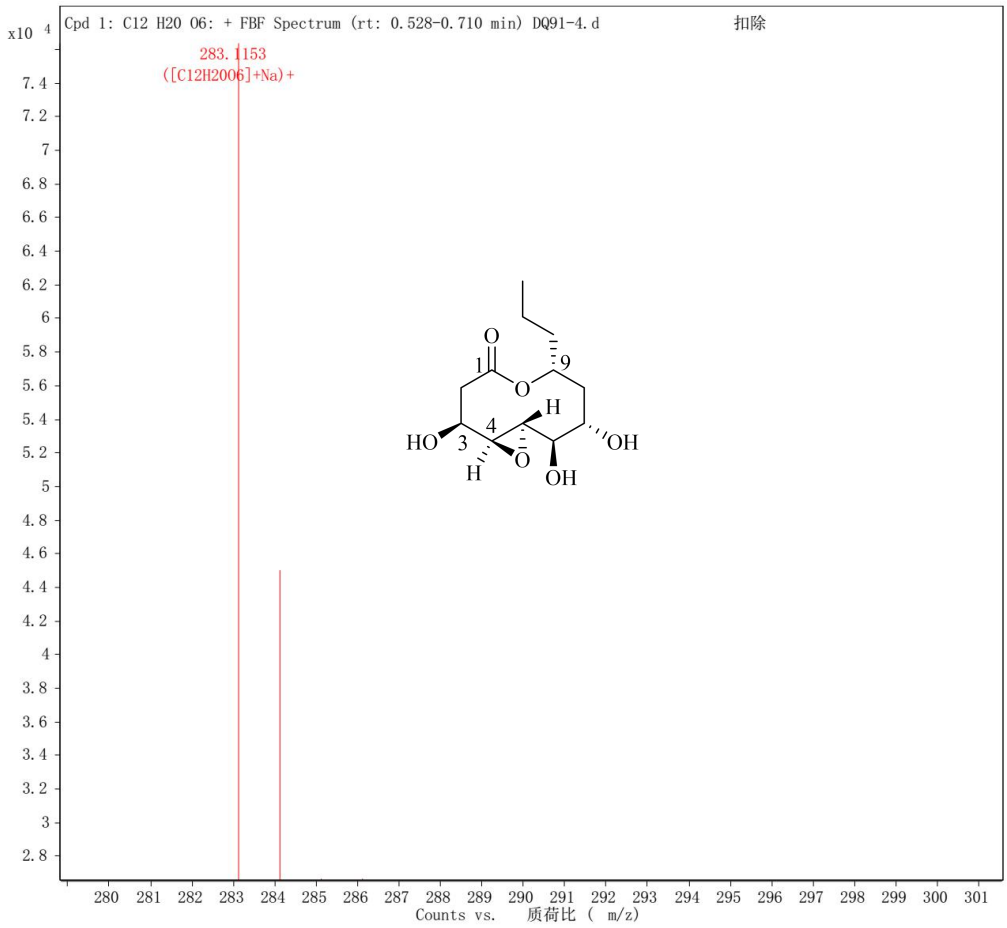

Figure S9. HR-ESI-MS spectrum of compound **1**

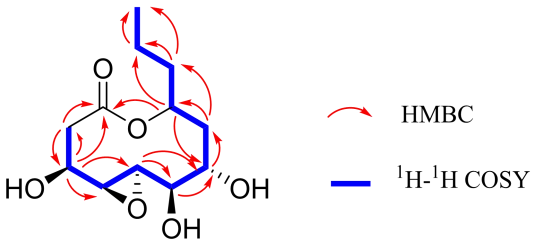

Figure S10. Key COSY and HMBC correlations of **1**

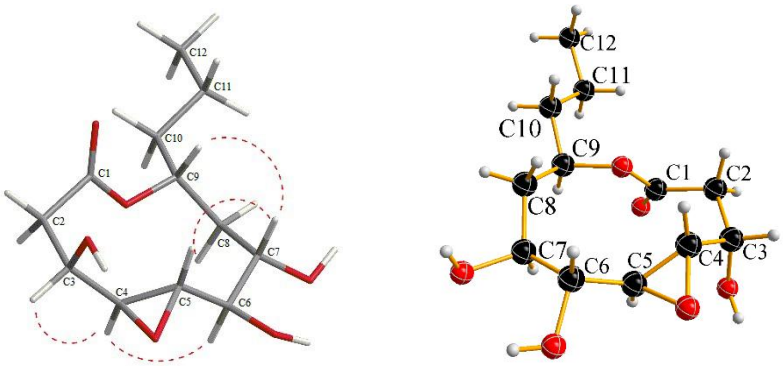

Figure S11. Key NOESY correlations observed for compound **1** and ORTEP diagram showing the structure of **1** in the crystal

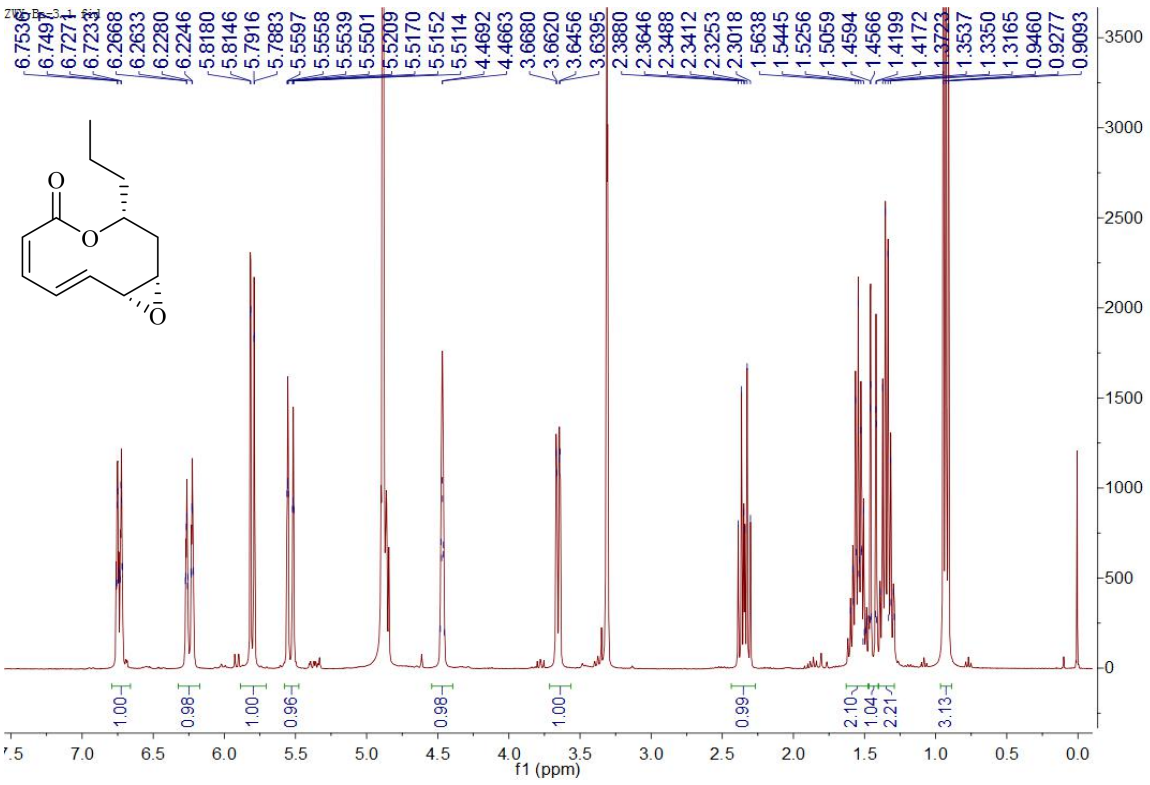

Figure S12. ^{1}H NMR (400 MHz, DMSO-d_6) spectrum of compound

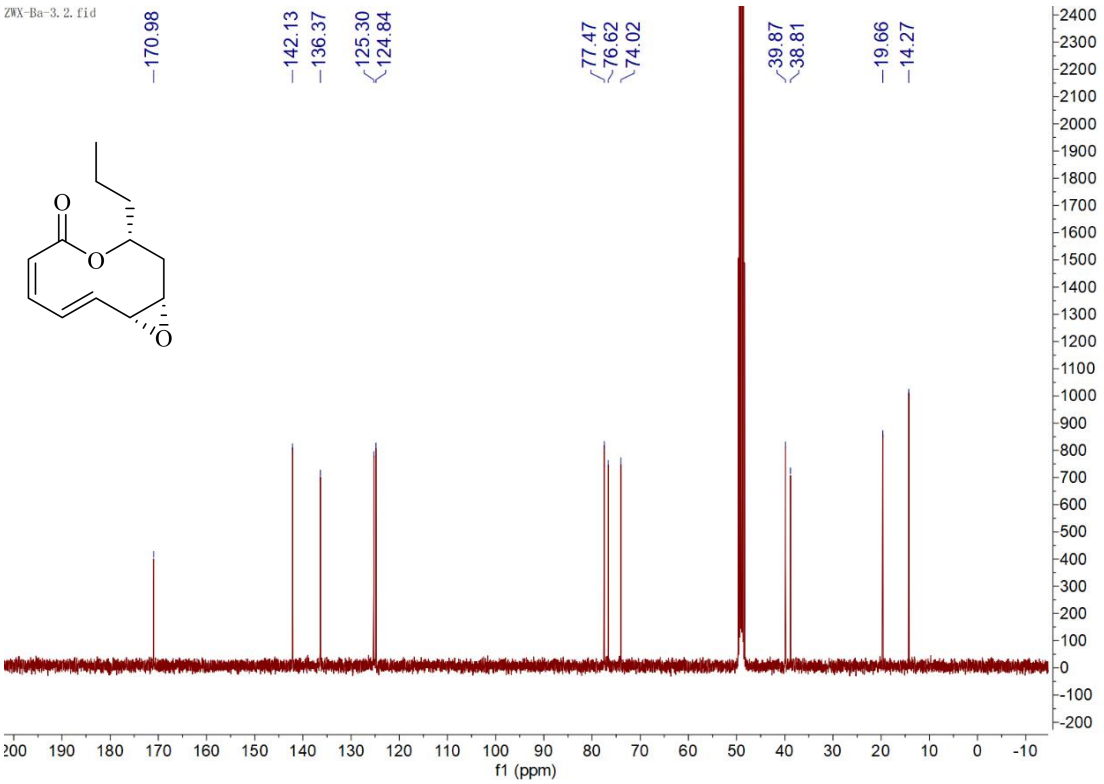

Figure S13. ^{13}C NMR (100 MHz, DMSO-d_6) spectrum of compound **2**

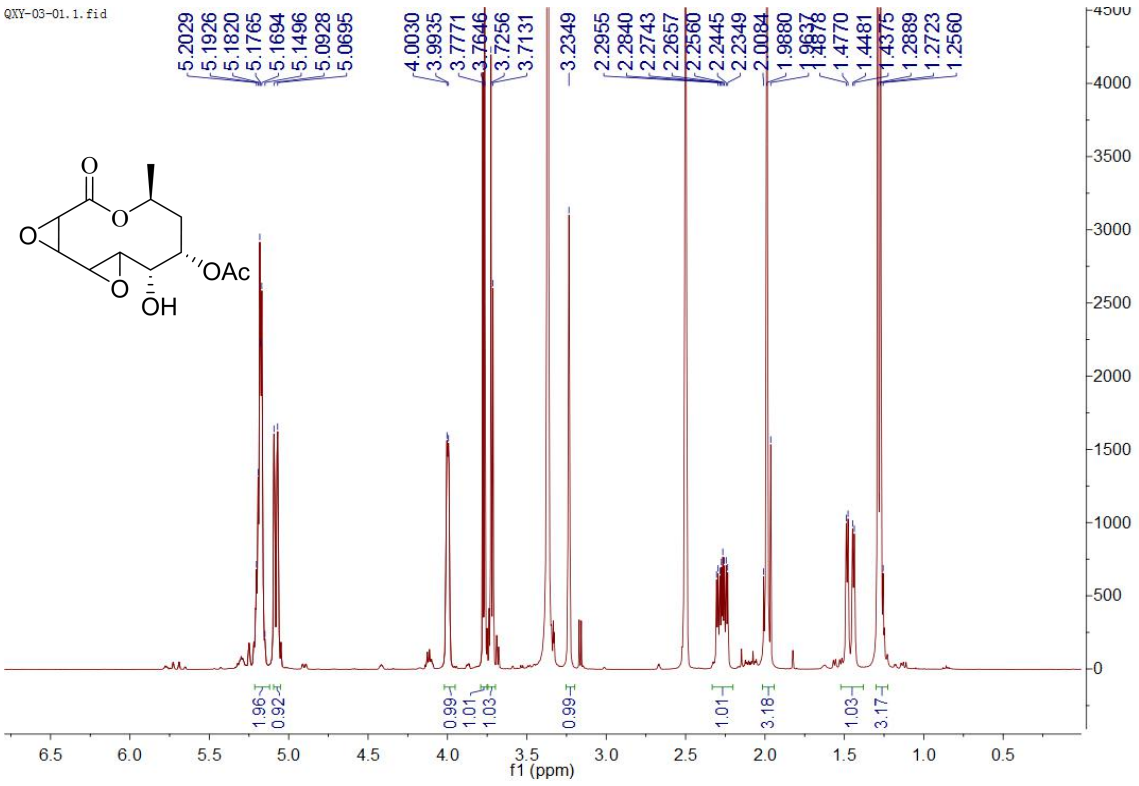

Figure S14. ^{1}H NMR (400 MHz, methanol-d_4) spectrum of compound **3**

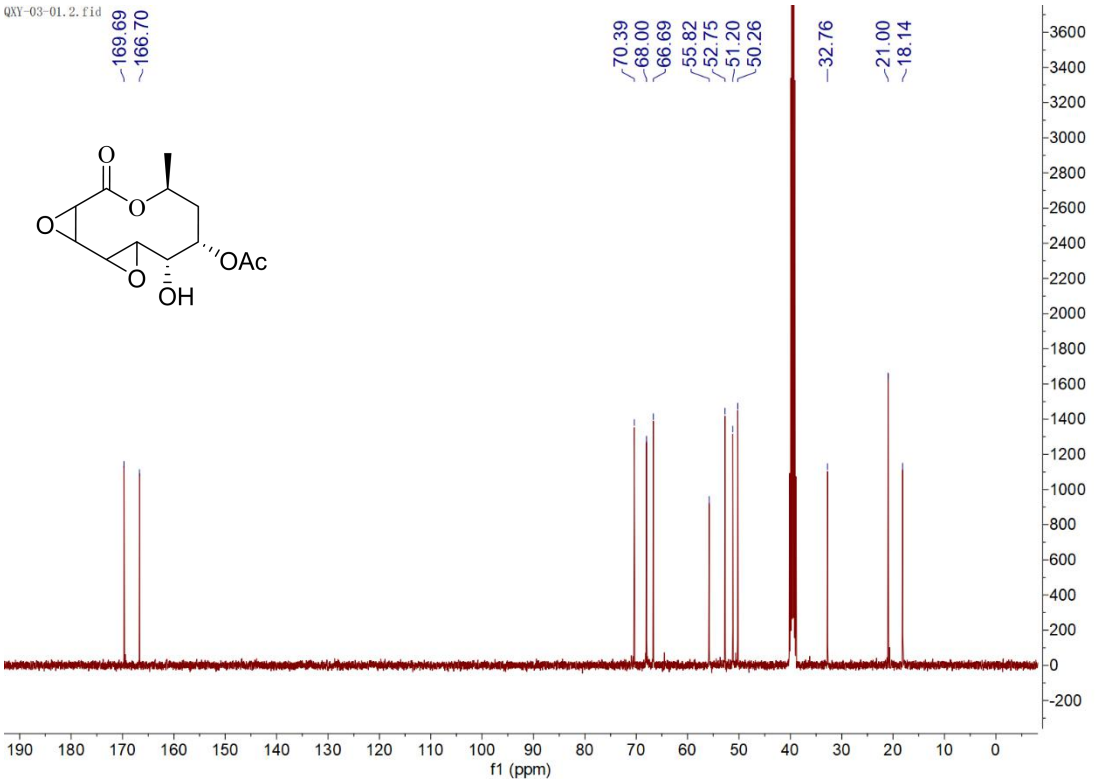

Figure S15. ^{13}C NMR (100 MHz, methanol-d_4) spectrum of compound **3**

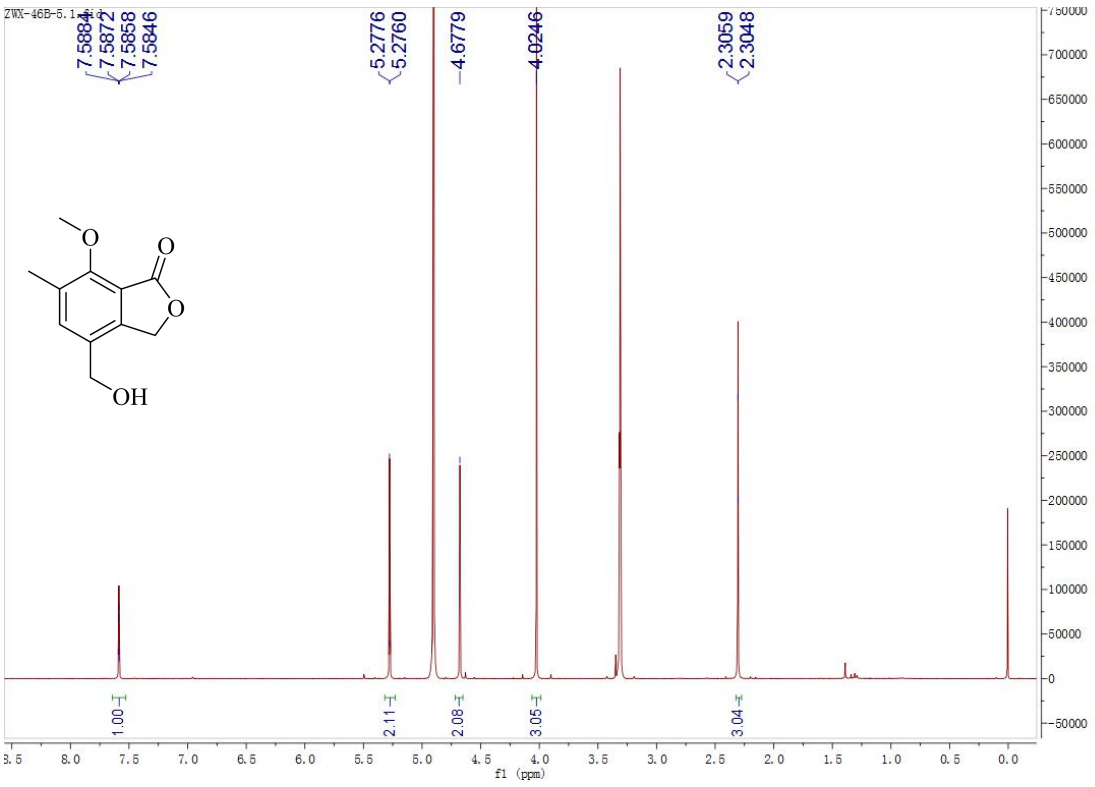

Figure S16. ^{1}H NMR (600 MHz, methanol-d_4) spectrum of compound **4**

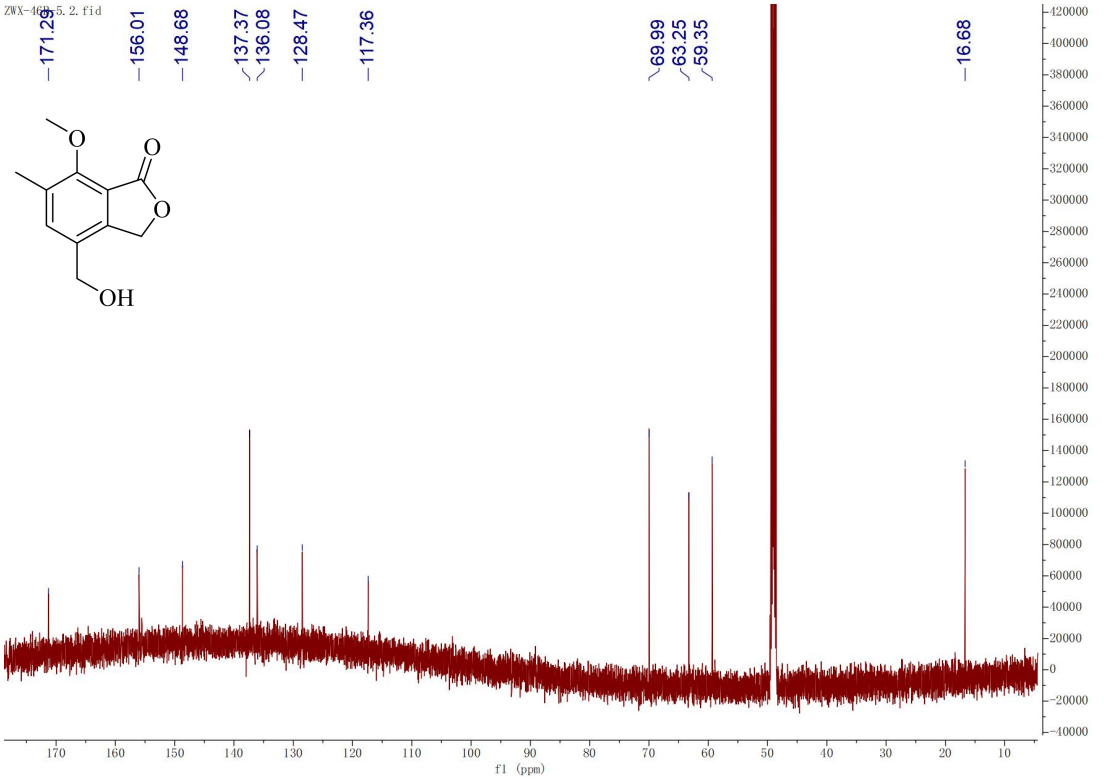

Figure S17. ^{13}C NMR (150 MHz, methanol-d_4) spectrum of compound **4**

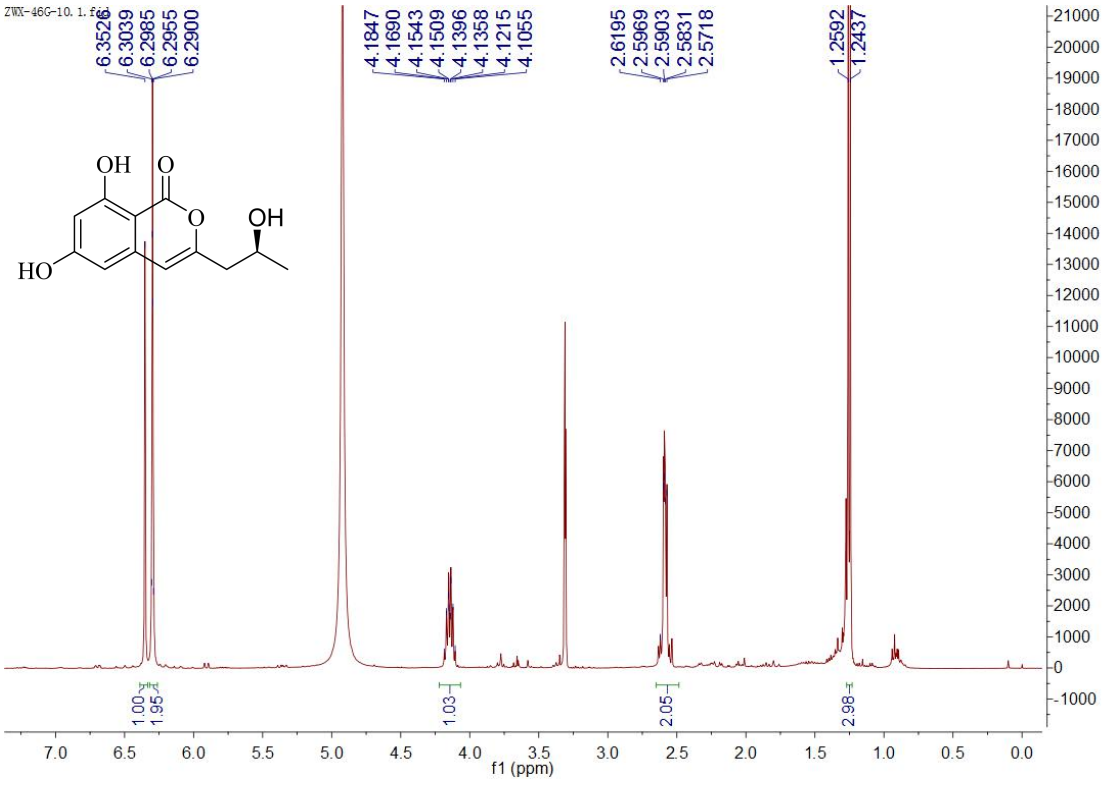

Figure S18. ^{1}H NMR (400 MHz, methanol-d_4) spectrum of compound **5**

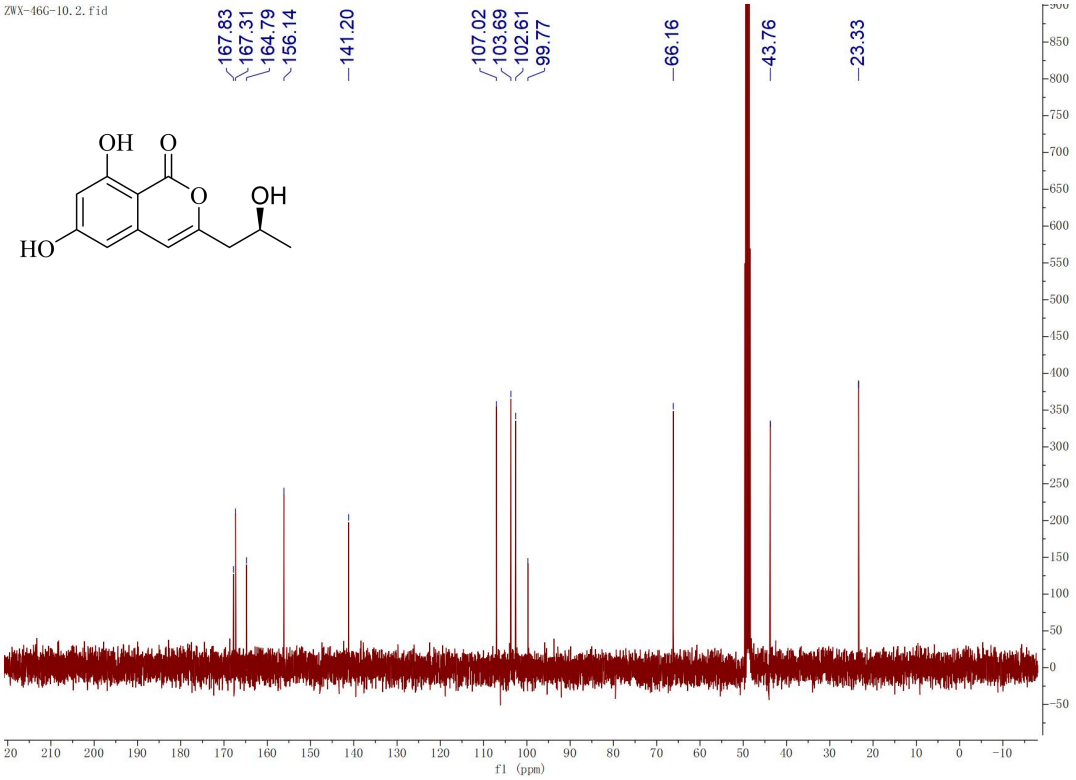

Figure S19. ^{13}C NMR (100 MHz, methanol-d_4) spectrum of compound **5**

Table S1. NMR data of compound **1** (400 MHz, Methanol-d_4, δ in ppm)

NO.	δ_C, Type	δ_H (J in Hz)
1	171.2, C	
2	40.7, CH_2	*a* 2.74, dd (12.6, 3.6)
		b 2.52, dd (12.6, 3.8)
3	64.9, CH	4.33, dd (3.8, 3.6)
4	62.1, CH	3.29, br s
5	55.3, CH	2.91, dd (8.8, 2.2)
6	78.8, CH	3.04, t (8.9)
7	74.0, CH	3.57, td (8.9, 2.2)
8	41.6, CH_2	1.95, m
9	74.6, CH	4.83, m
10	39.0, CH_2	1.59, m
11	19.2, CH_2	1.36, dq (7.6, 7.3)
12	14.3, CH_3	0.94, t (7.3)

Table S2. Inhibitory activities on NO of compounds **1-5** in LPS-induced RAW 264.7 cells [a]

Compounds	IC_{50} (μM)
1	16.69± 0.21
2	15.87 ± 0.23
3	30.48 ± 0.11
4	30.12 ± 1.02
5	20.95 ± 0.88
Dexamethasone [b]	4.12 ± 1.41

[a] Values present mean ± SD of triplicate experiments.
[b] Dexamethasone was used as a positive control.

Table S3. Crystal data for compound **1**

Identification code	Compound **1**
Empirical formula	$C_{12}H_{20}O_6$
Formula weight	260.28
Temperature/K	100.15
Crystal system	monoclinic
Space group	$P2_1$
a/Å	5.04170(10)
b/Å	8.3524(2)
c/Å	14.5892(3)
α/°	90
β/°	96.413(2)
γ/°	90
Volume/Å^3	610.51(2)
Z	2
ρ_{calc}g/cm^3	1.416
μ/mm^{-1}	0.955
F(000)	280.0
Crystal size/mm^3	0.34 × 0.26 × 0.22
Radiation	CuKα (λ = 1.54178)
2Θ range for data collection/°	6.096 to 148.482
Index ranges	-6 ≤ h ≤ 6, -10 ≤ k ≤ 10, -17 ≤ l ≤ 14
Reflections collected	5316
Independent reflections	2369 [R_{int} = 0.0313, R_{sigma} = 0.0358]
Data/restraints/parameters	2369/1/167
Goodness-of-fit on F^2	1.089
Final R indexes [I>=2σ (I)]	R_1 = 0.0321, wR_2 = 0.0857
Final R indexes [all data]	R_1 = 0.0332, wR_2 = 0.0864
Largest diff. peak/hole / e Å^{-3}	0.17/-0.16

Crystal Data for $C_{12}H_{20}O_6$ (M =260.28 g/mol): monoclinic, space group $P2_1$, a = 5.04170(10) Å, b = 8.3524(2) Å, c = 14.5892(3) Å, V = 610.51(2) Å^3, Z = 2, T = 100.15 K, μ(CuKα) = 0.955 mm^{-1}, $\rho calc$ = 1.416 g/cm^3, 5316 reflections measured (6.096° ≤ 2Θ ≤ 148.482°), 2369 unique (R_{int} = 0.0597, R_{sigma} = 0.0586) which were used in all calculations. Flack parameter [-0.09 (14)]. The final R_1 was 0.0321 (>2sigma(I)) and wR_2 was 0.0864 (all data). The CCDC number is 2026571.

Cell viability assay

RAW 264.7 cells viability was tested after treatment with the isolates and dexamethasone (positive control, Sigma) and measured using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Briefly, RAW 264.7 cells were cultured in 96-well plates at a density of 1×10^5 cells per well (180 μL per well) and incubated for 12 h. Then, the cells were treated with a series of compounds or dexamethasone for 24 h and then cultured with the MTT reagent for 4 h. Cell viability was measured by determining the absorbance at 560 nm using a microplate reader.

NO production assay

NO production was detected by the Griess assay. RAW 264.7 cells were seeded in 96-well plates at 20000 cells/well in triplicate. The next day, a series of diluted compounds or dexamethasone were added to the cells for 2 h, and then the cells were treated with 20 μL of 1 μg/mL LPS for 24 h.