Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information for "The cleavage of perylenequinones through photochemical oxidation acts as a detoxification mechanism for the generator"

Xiao-Bin Li,[ab] Wen-Qiang Chang,[ab] Sha Zheng,[a] Wei Li[a] and Hong-Xiang Lou*[a]

[a]*Department of Natural Product Chemistry, Key Lab of Chemical Biology of Ministry of Education, School of Pharmaceutical Sciences, Shandong University, 44 West Wenhua Rood, Jinan 250012, P. R. China.*

[b]*Xiao-Bin Li and Wen-Qiang Chang contributed equally to this work.*

**Corresponding author.*

Fax: +86-531-88382019; Tel: +86-531-88382012

E-mail: louhongxiang@sdu.edu.cn

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Experimental section

1. General experimental procedures.

A daylight lamp with light intensity of 30 W/m^2 was used for the visible light irradiation. The apparatus used for ultraviolet (UV) light irradiation was a Rayonet RPR-350 (Southern New England UV Co.) equipped with 16 fluorescent lamps and a merry-go-round. High-performance liquid chromatography (HPLC) was carried out on an Agilent Technologies 1260 infinity equipped with a ZORBAX SB-C_{18} 5 μm column (9.4 × 250 mm). TLC was performed with glass precoated silica gel GF_{254} plates (Qingdao Marine Chemical Co. Ltd.). UV-visible data were obtained on a UV-2450 spectrophotometer (Shimadzu, Japan). The compounds were visualized under UV (254 nm) light and by spraying with H_2SO_4/EtOH (1:9, v/v) followed by heating. NMR spectra

were recorded by a Bruker Avance-DRX-600 spectrometer operating at 600 (^{1}H) and 150 (^{13}C) MHz with TMS as an internal standard. MS were performed on a Finnigan LC-Q^{DECA} mass spectrometer.

2. Perylenequinones preparation.

Previously isolated endolichenic fungus identified as *Phaeosphaeria* sp. (GenBank: HQ324780) that generated perylenequinones (PQs)[1] was cultured on potato dextrose agar (PDA) plates. The colony was inoculated into liquid potato dextrose broth (PDB) and cultured in the darkness at 25 °C on a rotary shaker (110 rpm) for 20 days. The fermentation culture of *Phaeosphaeria* sp. was extracted by EtOAc. Hypocrellin A (HA) and calphostin D were obtained from *Phaeosphaeria* sp., which has been reported previously.[1]

3. CLSM observation.

The mycelium was picked up from the culture and observed under a LSM700 confocal laser scanning microscopy (CLSM). 488 nm argon laser was used to excite the perylenequinones with 570–700 nm emission spectrum recorded.

4. Photochemical reaction.

The crude EtOAc extracts were equally divided into three parts. They were respectively treated by visible light irradiation for 4 days, UV irradiation for 5 h or in the

darkness following with HPLC analysis.

A solution of HA (5 mg) in acetonitrile (5 mL) was exposed to a daylight lamp for a total of 48 h, or UV (8 fluorescent lamps) for 4 h. At indicated time, the samples were collected and confirmed by a combined experimental approach utilizing HPLC, TLC, and UV absorption. After evaporation of the solvent, compound **1** (2.5 mg) was separated from the reaction mixture by HPLC (90% MeOH/H_2O, 1.8 mL/min).

Acetonitrile (15 mL) containing calphostin D (15 mg) was irradiated by UV (8 fluorescent lamps) for 9 h. The experimental approach was same as that used for HA. Compounds **2** (6.5 mg) and **3** (1.6 mg) were isolated from the reaction mixture using HPLC (75% MeOH/H_2O, 1.8 mL/min).

5. Antimicrobial assays.

C. albicans strain ATCC10231 was propagated in yeast-peptone dextrose (YPD) medium in an orbital shaker at 30 °C. *Staphylococcus aureus* ATCC6538 and *Bacillus subtilis* ATCC9372 strain were cultured in LB medium at 37 °C. The organisms were assayed in their culture conditions. One milliliter of microbial suspensions (2×10^6 CFUs/mL) were incubated in glass tubes with perylenequinones extracts, HA, calphostin D or their photochemical products at a serial of concentrations ranging from 0.125 to 16 μg/mL. For *C. albicans*, the mixtures were placed under a 9 W fluorescent lamp with light intensity of 10 W/m^2 for 20 min. For *Bacillus subtilis*, the organisms were incubated with the tested agents for 1 h in the darkness following with another 1h light irradiation.

After photodynamic treatments, aliquots of 100 μL were taken out to determine the surviving CFUs.

Fig. S1 The color alteration of fermentation extracts when exposed to ultraviolet irradiation.

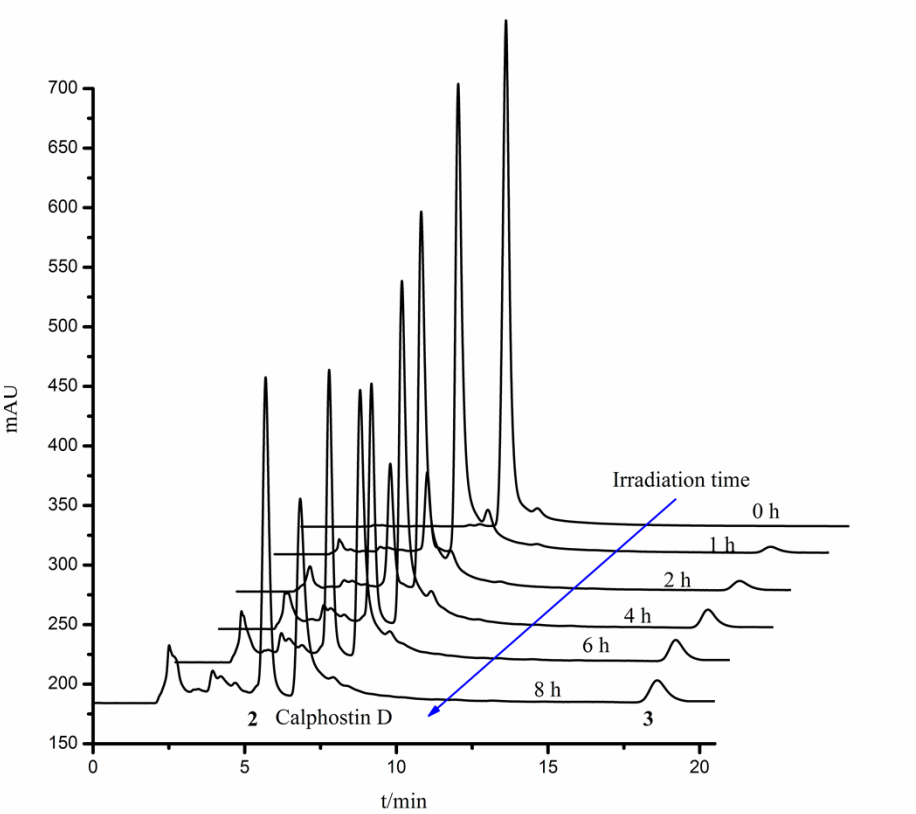

Fig. S2 HPLC chromatograms (67% MeOH-H_2O; 0.8 mL/min; 280 nm) as a function of irradiation time obtained for the irradiation of calphostin D in acetonitrile solution (350 nm).

Fig. S3 UV-visible absorption spectra of MeOH solution for HA and calphostin D.

Fig. S4 UV-visible absorption spectra of MeOH solution for compounds **1**–**3**.

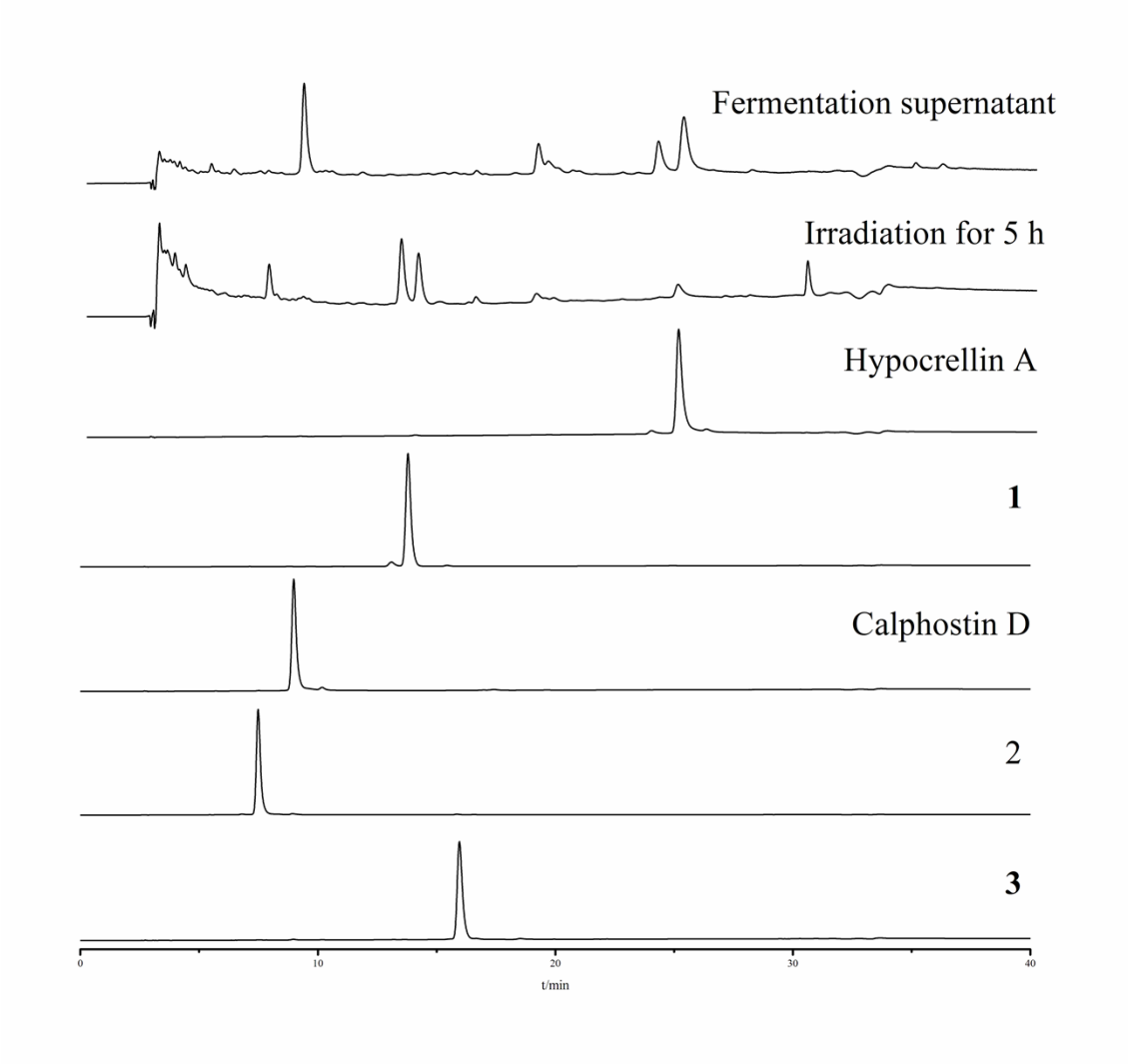

Fig. S5 HPLC analysis (60−100% MeOH-H_2O; 0.8 mL/min; 280 nm) of HA, calphostin D, and the fermentation supernatants of *Phaeosphaeria* sp. together with their photo-oxidation products.

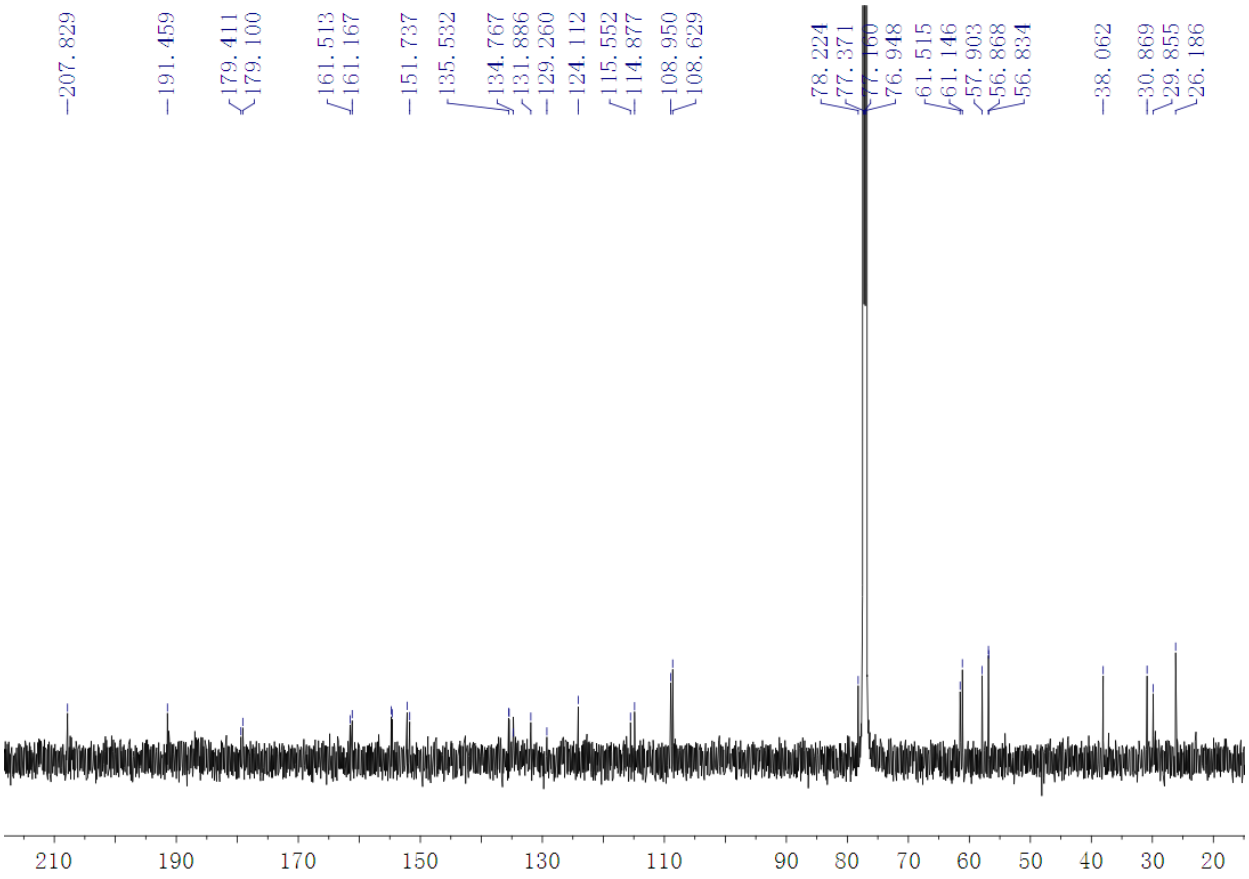

Fig. S6 The ^{13}C NMR spectrum (150 MHz) of compound **1** in $CDCl_3$.

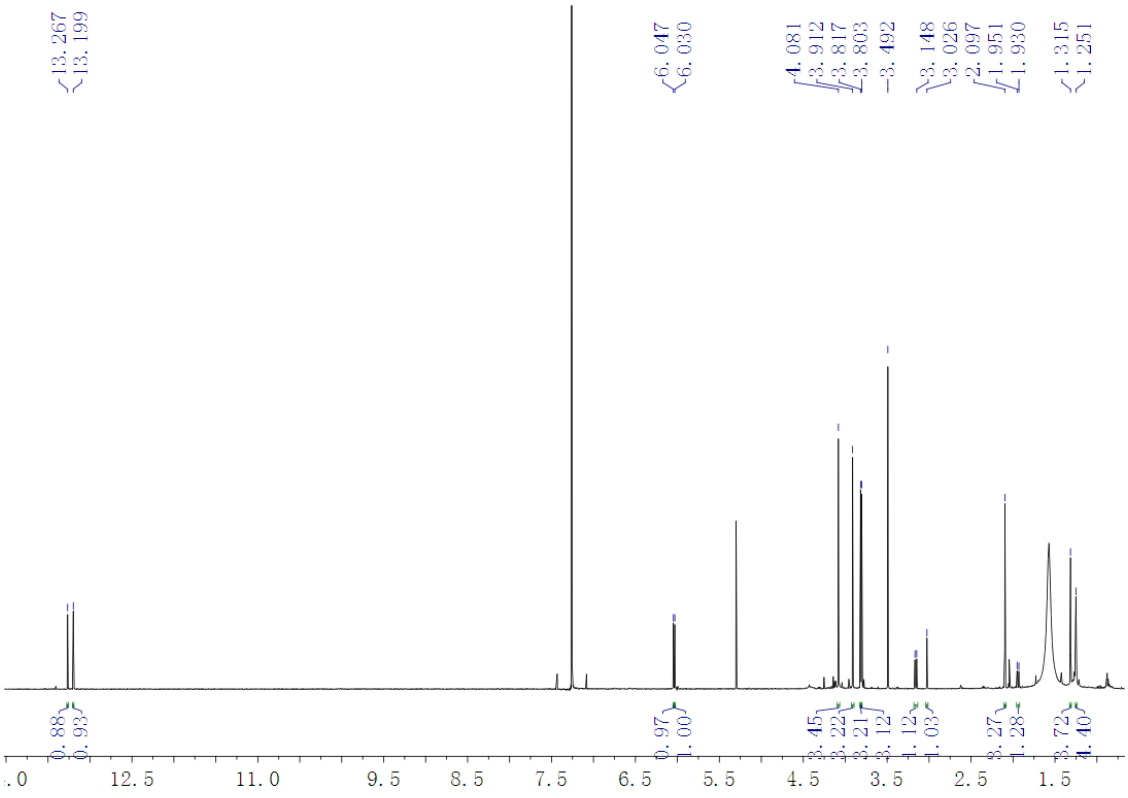

Fig. S7 The ^{1}H NMR spectrum (600 MHz) of compound **1** in $CDCl_3$.

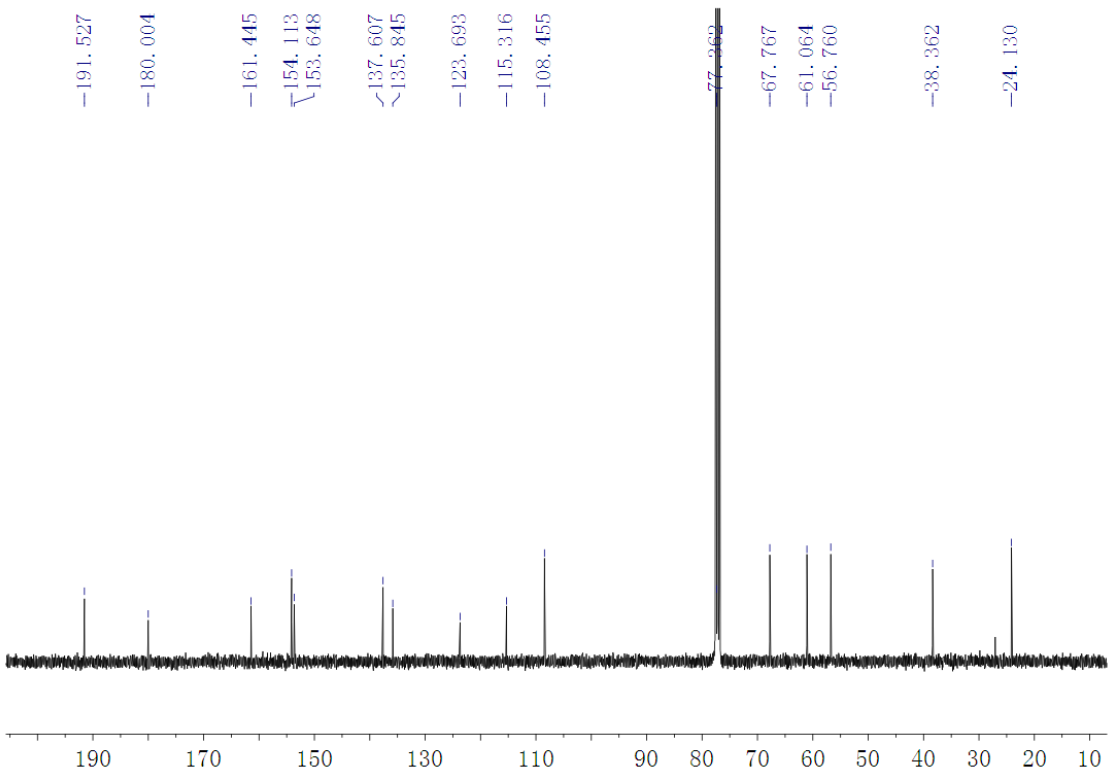

Fig. S8 The ^{13}C NMR spectrum (150 MHz) of compound **2** in $CDCl_3$.

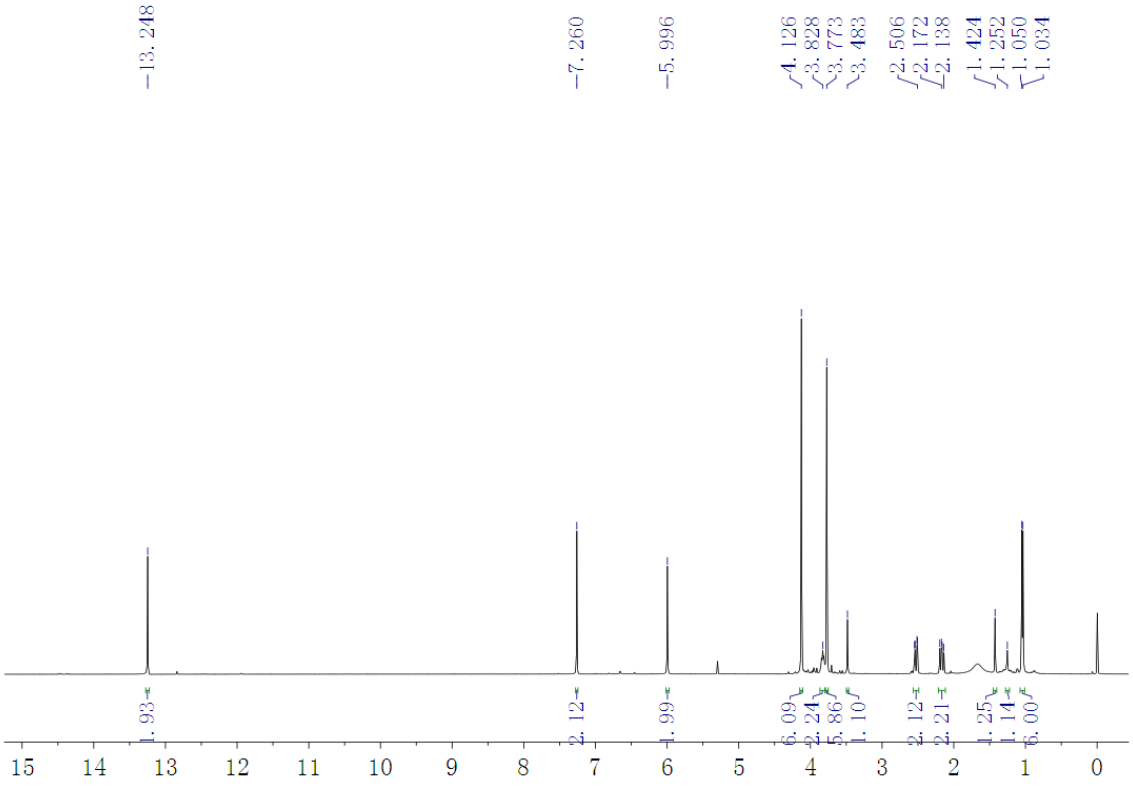

Fig. S9 The ^{1}H NMR spectrum (600 MHz) of compound **2** in $CDCl_3$.

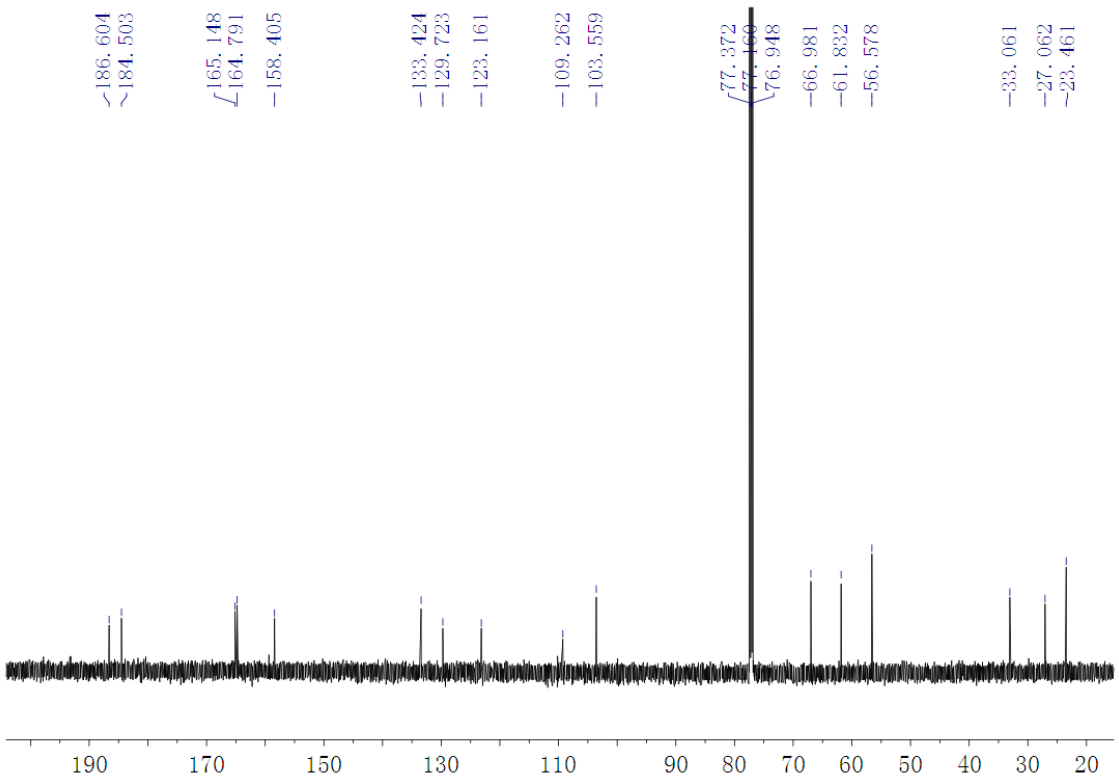

Fig. S10 The ^{13}C NMR spectrum (150 MHz) of compound **3** in $CDCl_3$.

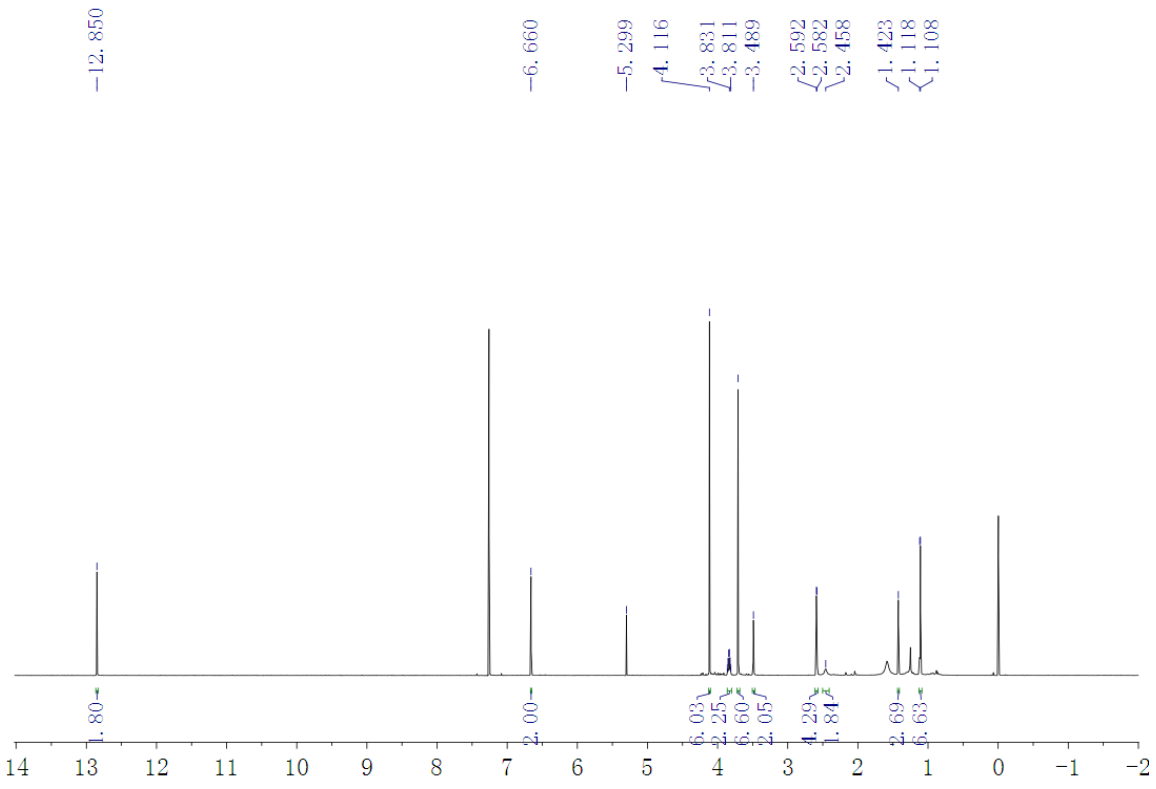

Fig. S11 The ^{1}H NMR spectrum (600 MHz) of compound **3** in $CDCl_3$.

Table S1 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compound **1** in $CDCl_3$.

Position	δ_C, type	δ_H, mult. (*J* in Hz)	Position	δ_C, type	δ_H, mult. (*J* in Hz)
1	131.9, C		11	151.7, C	
1a	135.5, C		12	134.7, C	
2	152.2, C		12a	135.4, C	
3	154.8, C		13	38.1, CH_2	3.15, d (12.6)
3a	114.9, C				1.94, d (12.6)
3b	129.3, C		14	78.2, C	
4	191.5, C		15	57.9, CH	3.03, s
5	108.9, CH	6.05, s	16	26.2, CH_3	1.32, s
6	161.2, C		17	207.8, C	
6a	179.3, C		18	30.9, CH_3	2.10, s
7	161.5, C		'2-OMe	61.5, CH_3	4.08, s
7a	179.1, C		6-OMe	56.9, CH_3	3.82, s
8	108.6, CH	6.03, s	7-OMe	56.8, CH_3	3.80, s
9	191.2, C		11-OMe	61.1, CH_3	3.91, s
9a	115.6, C		3-OH		13.20, s
9b	124.1		10-OH		13.27, s
10	154.6, C				

Table S2 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compounds **2** and **3** in $CDCl_3$.

Position	**2**		**3**	
	δ_C, type	δ_H, mult. (*J* in Hz)	δ_C, type	δ_H, mult. (*J* in Hz)
1, 12	137.6, C		133.4, C	
1a, 12a	135.8, C		186.6, C	
2, 11	153.6, C		158.4, C	
3, 10	154.1, C		184.5, C	
3a, 9a	115.3, C		109.3, C	
3b, 9b	123.7, C		129.7, C	
4, 9	191.5, C		165.1, C	
5, 8	108.5, CH	6.00, s	103.6, CH	6.66, s
6, 7	161.4, C		164.8, C	
6a, 7a	180.0, C		123.2, C	
13, 16	38.4, CH_2	2.53, dd (13.2, 3.0)	33.1, CH_2	2.58, d (6.0)
		2.17, dd (13.6, 9.2)		
14, 17	67.8, CH	3.83, m	67.0, CH	3.83, m
15, 18	24.1, CH_3	1.04, d (6.4)	23.5, CH_3	1.11, d (6.0)
2-OMe, 11-OMe	61.1, CH_3	4.13, s	61.8, CH_3	4.12, s
6-OMe, 7-OMe	56.8, CH_3	3.77, s	56.6, CH_3	3.71, s
3-OH, 10-OH		13.25, s		
4-OH, 9-OH				12.85, s
14-OH, 17-OH				2.46, br. s

References

1 G. Li, H. Y. Wang, R. X. Zhu, L. M. Sun, L. N. Wang, M. Li, Y. Y. Li, Y. Q. Liu, Z. T. Zhao and H. X. Lou, *J. Nat. Prod.*, 2012, **75**, 142–147.